CRANFIELD UNIVERSITY

SCHOOL OF APPLIED SCIENCES

DOCTOR OF PHILOSOPHY

ACADEMIC YEAR 2011-2012

PAUL ANDREW MASSEY

INTERACTIONS BETWEEN PHOSPHORUS FERTILISATION AND SOIL BIOTA IN MANAGED GRASSLAND SYSTEMS

SUPERVISED BY DR. RACHEL CREAMER AND PROF. KARL RITZ

MAY 2012

THIS THESIS IS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Abstract

The application of phosphorus (P) fertilisers to grassland systems is a common practice to increase and sustain grassland productivity. This is requisite for satisfying the nutritional needs of grazing animals and increasing dairy and livestock output. The costs of such fertilisers are increasing and the demands for such fertiliser will also most likely rise following governmental targets set in Ireland to increase national agricultural output. However, the application of P fertiliser to grassland systems can contribute to the eutrophication of water-courses, since fertiliser applications can result in the accumulation of P at the soil surface. One potential way to facilitate plant P acquisition in grasslands may be associated with the soil biota. In particular, the soil microbial biomass is recognised as a potential P pool that can provide a source of bioavailable P to the plant community. The soil biota may also facilitate the incorporation of P from the soil surface into the soil profile, since earthworms can actively increase the transport of P-rich soil material from the surface belowground. This project thus aimed to discern how P fertilisation affects microbial biomass nutrient pools and biologically-mediated P incorporation in grassland systems, and how this relates to plant P yields. To investigate this aim, two research questions were proposed: (i) is the soil biota affected by commonly adopted P fertiliser strategies in grassland systems?; (ii) what consequence does this hold for P acquisition by the plant community?

An experiment was conducted to examine how the soil biota responded to different rates of inorganic P fertilisation in two grassland sites of contrasting soil types over an 18 month period. This revealed that increasing P fertilisation did not affect microbial biomass P concentrations in the soil. However, an effect was observed upon plant P yield, in which greater plant P yields were obtained proportional to the P fertiliser rate. Two laboratory experiments were conducted to further investigate this lack of effect. These utilised soil from the same grassland sites and examined how nutrient additions to the soil affected microbial biomass nutrient pools and activity. Results from these experiments supported evidence from the field experiment, since the application of P fertiliser did not affect microbial biomass nutrient pools following fertiliser application, and supplementation of carbon (C) + P substrate to the soil did not invoke respiratory responses between P fertiliser treatments. Nevertheless,

supplementation with C + nitrogen (N) and C+N+P substrates was found to suppress microbial respiration. This was attributed to greater C assimilation by the microbial community in these particular substrate-induced respiration treatments.

In order to investigate biologically-mediated P incorporation, a glasshouse-based mesocosm scale experiment was carried out using two contrasting soils. Bulk soil (1 – 30 cm depth range) was derived from a nutrient poor grassland system, whereas the soil for the 0 – 1 cm depth range was taken from an intensive system that was seven times greater in labile inorganic P concentration. Three treatments were applied to mesocosms in an incomplete factorial design, involving the inclusion of earthworms, different botanical diversities (unplanted, monoculture or mixed plant community) and different fertiliser types (organic or inorganic). The absent factorial combinations involved the application of earthworms to unplanted mesocosms. With respect to the earthworm treatment, results revealed that the presence of earthworms reduced labile P concentrations in the 0 – 1 cm depth range of soil. The presence of different botanical diversities or fertiliser types did not affect microbial biomass nutrient pools, whilst the presence of mixed plant communities did increase plant P yields. However, microbial and nematode community structures were affected in an idiosyncratic manner by both botanical diversity and fertiliser type.

This project demonstrated the significance of grassland management regimes in governing microbial biomass P concentrations. In particular, it was revealed that the frequent defoliation of the sward appeared to uncouple the microbial community from both fertiliser inputs and possibly plant P yields. The fact that an increase in plant P yield with increasing P fertilisation was noted in the absence of microbial responses suggests that the soil biota may not be crucial for plant P acquisition in such intensive inorganic-fertiliser based regimes. This suggestion was also supported by the mesocosm experiment, since plant P yields differed between botanical diversities but no effects were observed on microbial biomass P concentrations. Furthermore, this project showed the potential of the earthworm community to reduce P concentrations in the volume of soil which poses the greatest risk to water quality. The collective evidence highlights the need for further understanding of the consequences of inorganic-based fertiliser management systems, since current strategies may not adequately account for management effects on soil biological P cycling.

Acknowledgments

I would like to thank both of my supervisors, Dr. Rachel Creamer and Prof. Karl Ritz, for their great insight and support throughout the course of this work. I would also like to thank them for keeping me level-headed and helping me focus upon what needed to be done. I will not forget their advice both scientifically and personally. Thanks to Dr. Mick Whelan for this support whilst establishing myself at Cranfield University and always throwing in a thought about something that I might have missed. I also thank Prof. Bryan Griffiths for his input into my work, organising Walsh Fellowship activities and tolerating my excessive work-related rambling (mainly at badminton). I am also very thankful for Dr. Rogier Schulte, particularly for his extensive modelling guidance and patience over the years.

I am grateful towards the technical and research staff and students at Johnstown Castle and Cranfield University for their help along the way. In particular, I am extremely gratefully towards Nicola Rochford, Jennifer Cookman, Dr. Dote Stone, Pat Sills and all the students that I marched into the dairy farm for all their laboratory and field support. I would like to thank Teagasc and the Walsh Fellowship Scheme for financial support during the course of this work.

Thanks to the other members of the 'Cluster', Dr. Emma Moynihan, Niamh Rogers and Fred Bourdin for being around when I needed them the most and reminding me that we were all in the same boat. I especially thank all my family for being very understanding and truly coming through for me when I needed them. Their words and wisdom have truly kept me going during this period of my life. I am also thankful towards my old and new friends for their patience, support and understanding.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ATP	Adenosine tri-phosphate
Al	Aluminium
ANOVA	Analysis of variance
C	Carbon
Ca	Calcium
CO_2	Carbon dioxide
DNA	Deoxyribose nucleic acid
DM	Dry matter
EC	Enzyme Commission
EMS	Error mean squares
Fe	Iron
ln	Natural log
ISO	International Standardization Organization
K	Potassium
K_2SO_4	Potassium sulphate
LOI	Loss-on-ignition
Mg	Magnesium
MIT	Mineralisation-immobilisation turnover
N	Nitrogen
$NaHCO_3$	Sodium hydrogen carbonate

NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
N_2O	Nitrous oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
OH_2	Hydroxyl group
P	Phosphorus
PC	Principal component
PCA	Principal component analysis
PLFA	Phospholipid fatty acid
PO_4	Phosphate group
P0	0 kg P ha^{-1} y^{-1} P fertilisation regime
P15	15 kg P ha^{-1} y^{-1} P fertilisation regime
P30	30 kg P ha^{-1} y^{-1} P fertilisation regime
P45	45 kg P ha^{-1} y^{-1} P fertilisation regime
Q1	Model parameter used to assess differences in nutrient concentration
Q2	Model parameter used to assess the evenness of nutrient depth distributions
RNA	Ribonucleic acid
S	Sulphur
Zn	Zinc

1. Introduction and literature review

1.1 Agriculture in Ireland

In 2007, the agricultural exports out of Ireland reached over 5 billion euros (Department of Agriculture, Fisheries and Food. and Central Statistics Office., 2011). In Ireland, almost 5 million hectares are used for both agricultural and forestry purposes, and of the agricultural area 90% is devoted to grassland (silage, hay and pasture), and 10% to crop production (Department of Agriculture, Fisheries and Food. and Central Statistics Office., 2011).

To improve the grazing quality of agricultural grassland, farmers amend the soil nutrient status through fertiliser applications. Between October 2010 and September 2011, Irish farmers were estimated to have used 1.27M tonnes of fertiliser (Department of Agriculture, Fisheries and Food. and Central Statistics Office., 2011). Of this, approximately 345k, 73k and 29k tonnes were applied to amend the N, K and P statuses of the agricultural systems, respectively. Fertiliser use in Ireland is likely to increase following the targets set for the agricultural, fisheries and forestry sectors in the Food Harvest 2020 report (Donnellan et al., 2012). The Food Harvest 2020 report has established a primary target of increasing the value of primary output by 1.5 billion euros by 2020 in these sectors, whilst promoting environmentally-friendly practices. Therefore, projections indicate 50% increase in milk and 20% increase in beef production compared to 2007 - 2009. With such an increase in production, grassland management is likely to be modified to accommodate changes in production intensity and in the composition and size of the Irish national cattle herd. If these are realised, then the usage of fertilisers will also increase to meet the demands of these productive systems (Donnellan et al., 2012).

1.1.1 Phosphorus fertiliser management in Irish grassland systems

Grasslands systems in Ireland are a major component of the agricultural sector. One reason for this is that Ireland has a major competitive advantage in this respect over most countries in the European Union, since a potential grassland productivity of between 12 to 16 t DM ha^{-1} can be achieved over a long growing season (Horan, 2009). This production can be achieved due to Ireland's damp temperate climate, which provides favourable conditions for grassland growth. When this is coupled with the long growing seasons of 200 d $year^{-1}$ in the northwest of the country and 235 d $year^{-1}$ in the southwest, Ireland has a competitive edge over most of its European counterparts in terms of maintaining low production costs because livestock can be situated in the grassland for longer periods of time. With livestock feeding from the grassland, the cost incurred from supplementing expensive food stuffs to the livestock is reduced, which lower production costs (Richards et al., 2009). However, to maximise the efficiency of the grassland system, different management strategies have been developed, for example maintaining grazing to a mean sward height of approximately 3.5 cm (which is designated as the optimum height for ensuring light reaches the primary growing points in newly formed tillers) and reseeding grasslands when productivity is below 15% of their potential yield. Nevertheless, one of the primary management considerations in such systems is nutrient management (Coulter and Lalor, 2008; Horan, 2009). Nutrient management is encouraged to follow the code of good agricultural environmental conditions (GAEC), which designates that nutrient supply should match nutrient demand by the crop and that nutrient applications in excess of this should not occur, since excess applications can contribute to harmful effects on the environment. Such logic is applied to P fertiliser applications to grasslands. The application of P is required to maintain a plant P yield between 3.7 and 4 g P kg^{-1} dry plant material and is crucial since P is often a growth-limiting nutrient in agricultural systems (Schulte and Herlihy, 2007; Bunemann et al., 2011). Furthermore, maintenance at this range of herbage P is required to meet the dietary requires of cattle, which would duly maintain high output. In particular, the demand for high herbage P contents is more acute in the dairy industry, since large quantities of P are required to compensate for the removal of high P concentrations in milk and to sustain animal health (Wu et al., 2001; Bunemann et al., 2011). With respect to animal health, high P concentrations are particularly required to ensure the

integrity and development of the skeletal system and to maintain reproductive performance.

The currently (2012) adopted management strategy for ensuring adequate P nutrition for the plant community involves building up P concentrations in the soil through repeated fertiliser applications (Coulter and Lalor, 2008). Once optimum concentrations are established, fertiliser inputs are managed on a supplementary basis. Supplementary in this context denotes that fertiliser applications are equivalent to nutrient concentrations removed from the grassland system during grazing or cutting. Therefore, frequent soil testing is an important tool to direct grassland management practices and to establish guidelines for future fertiliser applications (Plunkett, 2012). A review of soil testing on Irish farms by Teagasc (the agricultural and food development authority in Ireland) showed a decline in soil P fertility over the last 12 years, which was most notable over the last three to four years (Plunkett, 2012). These results were expressed in terms of the Irish soil P index system and showed that the percentage of soil tests, which were below the recommended Index 3 level, have increased from 40% in 2001 to 55% in 2011. This index is commonly employed as a management tool to portray the concentration ranges of P that are required to support optimum plant dry matter yields and plant P yields within the above mentioned range (Coulter and Lalor, 2008). In particular, this index is divided into four levels. Soil Indices 1, 2, 3 and 4 correspond to Morgan's P concentration ranges of 0 – 3, 3.1 – 5, 5.1 – 8 and > 8 mg P l^{-1} of dry soil, respectively. Farmers are encouraged to maintain Morgan's P concentrations at Index 3, which will ensure that the grassland community will achieve optimum yields. Therefore, maintenance at Index 3 level will meet nutritional requirements of the livestock. If a soil is determined to be at Index 4, then it is advised that no chemical fertilisers are added. If a soil is either Index 1 or 2, the chemical fertilisers should be applied at a rate of 20 kg P ha^{-1} and 10 kg P ha^{-1}, respectively, until an Index 3 status is obtained. Ensuring that Index 3 P concentrations are maintained is crucial, since the over-application of such fertilisers can pose potential risks to water quality and environmental health.

1.1.2 Phosphorus loss from grassland systems

A skewed distribution of P is typically observed vertically down the soil profile in grassland systems. In particular, the greatest concentrations of P are found near the soil surface, concentrations then substantially decline with depth (Daly, 2005; Owens et al., 2008; Costa et al., 2010). In intensively-managed grassland systems, the application of inorganic P fertiliser directly to the soil surface promotes this accumulation. When such fertilisers are applied, P is released in a water-soluble form, thus increasing P concentrations in the mobile phase. This increase disrupts the equilibrium between P in the mobile phase and soil phase (bound to the soil matrix). Therefore, this flush of water-soluble P is adsorbed to the soil matrix until the natural equilibrium is restored. This renders a large proportion of applied P immobile, and results in its accumulation at the soil surface.

The intensive use of P fertilisers in agricultural systems and its accumulation at the soil surface has led to concerns about fertiliser pollution of water courses (Dougherty et al., 2004; Delgado and Scalenghe, 2008). The repeated application of P fertilisers to the soil surface in agricultural systems develops into the accumulation of large quantities of P in an area that is highly susceptible to erosion. With respect to impacts on water quality, P bound to soil particles near the surface can be mobilised during heavy rain events, since overland flow can develop and soil erosion can occur (Hart et al., 2004; Mulqueen et al., 2004). Overland flow is generated when the infiltration rate of water into the soil matrix is exceeded by the volume of water deposited on the soil surface. Soil erosion is promoted when the velocity of the overland water flow dislodges soil material from the surface, thereby suspending it in rain water flowing over the soil surface. Soil erosion is also facilitated by the physical impact of water on the soil surface. The kinetic energy generated when rain drops hit the surface can dislodge soil material, which increases the mobility potential of the dislodged material on the surface if overland flow develops. Once suspended, soil material is transported out of the grassland system and deposited in the water course. The incidence of P transport out of grassland systems is not as high as other agricultural systems, for example in tilled systems (Dougherty et al., 2004; Delgado and Scalenghe, 2008). This is due to the presence of plants which intercept the rain drops before they impact on the surface, which reduces both the kinetic energy exerted and rain water

accumulated on the soil surface. By reducing both of these properties, soil erosion and overland flow development are consequently reduced. However, P losses from grassland systems still occur and can have a detrimental effect on the environment (Dougherty et al., 2004; Dorioz et al., 2006; Delgado and Scalenghe, 2008). Such drastic impact on the environment arises since the naturally P-limited conditions of water courses are removed following soil erosion from the agricultural system. With the removal of P limitation, eutrophication ultimately develops which negatively impacts water quality. The process of eutrophication specifically occurs when a surplus of P allows algae and bacteria to rapidly multiply in the water (Purves et al., 2004). The rapid expansion of the microbial community results in the stimulation of blooms, which visually changes the colour of the water to green. The death and decomposition of algae and bacteria depletes available oxygen in the water body, thereby promoting the growth of anaerobic microorganisms and the death of aquatic fauna and flora species. Such processes in the water ultimately reduce water quality and pose risks to public and environmental health.

1.2 Phosphorus in the soil environment

P cycling in the soil environment is a dynamic system that primarily exists in either inorganic or organic fractions of varying lability (Dixon et al., 1977; Sims and Sharpley, 2005 and Figure 1.1). Changes in inorganic and organic P lability are governed by soil biological activity and soil chemistry (Sims and Sharpley, 2005). Soil biological effects on P fractions are driven by plant and microbial demand for water-soluble P, since this is the only fraction of P that is readily available for acquisition (Figure 1.1). This demand is intensified by the fact that water-soluble P usually only comprises of less than 1% of total soil P (Sims and Sharpley, 2005). Water-soluble P occurs in many different species dependent upon the pH of the soil solution (Stevenson and Cole, 1999). In acidic solutions P is present as phosphoric acid (H_3PO_4). Under more neutral soil conditions (pH 6 – 8), P in the soil solution is present as orthophosphate ($H_2PO_4^-$, HPO_4^{2-}.) The orthophosphate derivatives $H_2PO_4^-$ and HPO_4^{2-} are both present at equal concentrations at pH 7.2 (Dixon et al., 1977). However, $H_2PO_4^-$ is more prevalent than HPO_4^{2-} at pH concentrations less than 7.21 and hence, HPO_4^{2-} is prevalent compared to $H_2PO_4^-$ at pH concentrations greater than

7.21. Under greatly alkaline conditions, phosphorus is present in the form of phosphate (PO_4^{3-}). The most common forms of phosphorus present within the soil solution are $H_2PO_4^-$, HPO_4^-, this is due to the fact that most soils have a pH ranging between 5 and 8 (Stevenson and Cole, 1999).

Whilst water-soluble P concentrations tend to be low in the soil solution, P from this fraction can be replenished from both labile inorganic and organic P pools (Figure 1.1). Replenishment of P into the soil solution from organic sources occurs through mineralisation of organic compounds by hydrolytic enzymes (Dick and Tabatabai, 1978; Beever and Burns, 1980; Turner et al., 2002; Turner and Haygarth, 2005). However, the ability of the soil biota to mineralise different P fractions is dependent on the stabilisation of organic compounds in the soil matrix. Recalcitrant organic P compounds, for example, inositol phosphates and humic P compounds are highly resistant to enzyme hydrolysis and are thus unavailable sources of P to the soil biological community. However, other P-containing compounds like phospholipids and DNA represent an active organic pool of P that can be potentially mineralised by the soil biota. This gradient of potentially-labile organic P shares many similarities to the dynamics of inorganic P in the soil.

Whilst labile organic P compounds denote a P fraction that can be readily mineralised by the soil biological community, labile inorganic P fractions are affected by both biological and chemical processes (Sims and Sharpley, 2005). Labile inorganic P fractions are those where inorganically-bound P (solid phase) can readily equilibrate with water-soluble P concentrations in the soil solution (mobile phase). Hence, non-labile inorganic P fractions are those that slowly equilibrate with water-soluble P concentrations (Sims and Sharpley, 2005). Consequently, depending on the shift in the equilibrium between labile bound P and water-soluble P, the latter can also be removed and become bound within the inorganic P fractions in the soil. The movement of P from a water-soluble P to bound inorganic P is one of the main contributing factors as to why P is biological limiting in many soil systems.

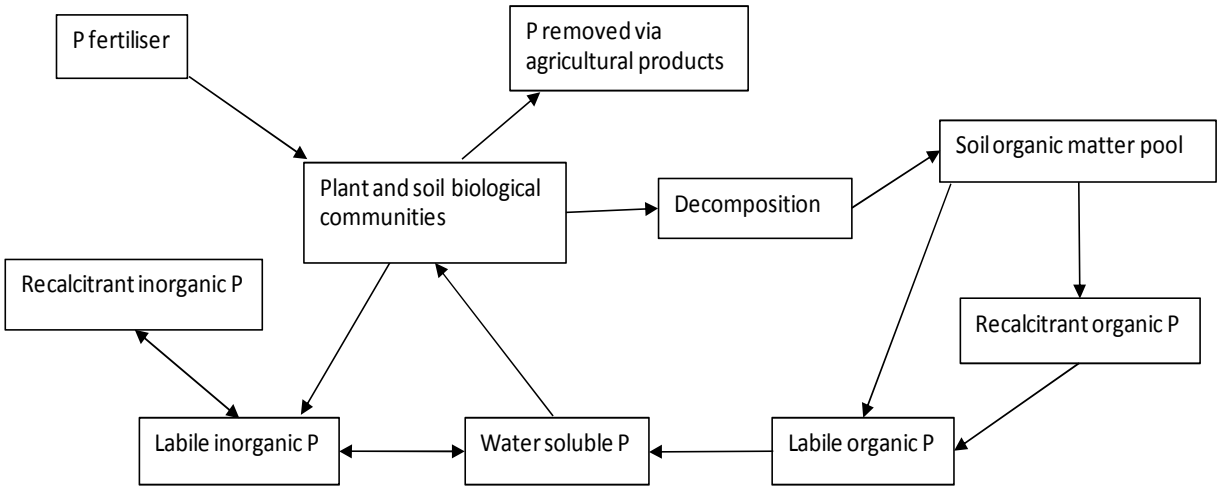

Figure 1.1 Generalised conceptual diagram of the soil P cycle.

1.2.1 Inorganic phosphorus fractions

The equilibrium between P in the mobile phase and in the solid phase of soil can occur via two different processes, *viz.* P adsorption to the soil and desorption into the soil solution, or P precipitation into labile complexes and dissolution back into the soil solution (Stewart, 1991; Sims and Sharpley, 2005). With respect to adsorption, water-soluble P can be adsorbed onto the surface of soil material through interactions with Al or Fe oxides. In some instances, absorbed P can penetrate into the absorbent body of the soil material and promote further adsorption to such material. When P adsorbs with the inner and outer areas of the soil matrix simultaneously, the process is defined as sorption. However, the adsorption of P to the soil material is dependent on soil pH (Stevenson and Cole, 1999), since greater adsorption to Al and Fe oxides occurs at lower pH. When sorption does occur, it is recognised that there are three ligand-binding mechanisms that are involved (Ryden et al., 1977b; Ryden et al., 1977a). Two ligand-binding mechanisms involve the chemisorption of P to hydrous oxide surfaces and the third mechanism involves a potential determining sorption that occurs at high P concentrations. However, other mechanisms have been recognised that include the

direct exchange between ligands, for example carbonates, and water-soluble P for reactive areas on the soil surface (Stewart, 1991; Sims and Sharpley, 2005).

The sorption of P through chemisorption and ligand exchange heavily affects the availability of P to plants (Stevenson and Cole, 1999). The binding of P to Fe and Al oxides through chemisorption results in the release of either a water or a hydroxyl group. One chemisorption mechanism involves a reaction between the negatively charged oxygen in water-soluble P species and a hydroxyl group on the hydrous oxide surface. This causes the release of a hydroxyl group into the surrounding environment. The second chemisorption mechanism occurs in a more acidic environment, where the hydroxyl group on the Fe oxide surface becomes protonated ($Fe - OH_2^+$) (Ryden et al., 1977b). This protonated site attracts the negatively charged water-soluble P species, which results in the binding of P to the Fe oxide surface and release of water into the soil solution.

P can form either single or multiple bonds with Fe (Ryden et al., 1977b; Stevenson and Cole, 1999). The first bond formed is referred to as a single co-ordinate linkage (monodentate bonding) and results in the formation of labile P compounds (Stevenson and Cole, 1999; Sims and Sharpley, 2005). The formation of the first bond is dependent on the water-soluble P species present, since a difference in ionic charge between species will determine which ligand-binding mechanism occurs. The formation of a single co-ordinate linkage on the surface of the hydrous oxide gel causes the surface potential to become close to zero. A lower surface potential causes further sorption of the already attached P through the potential determining mechanism (Ryden et al., 1977b), which is the third ligand binding mechanism. This results in orthophosphate sharing multiple bonds (bidentate bonding) with both Fe and Al complexes and becoming less available to the soil biological community (Sims and Sharpley, 2005).

Whilst sorption can bind P present in the soil solution to the surface of soil material, desorption can occur where labile P is exchanged from the soil material surface into solution. Desorption occurs by the exchange of labile P in the soil material by ligands present in the soil solution (Russell, 1988); hence these ligands consequently become bound to such material. This desorption of orthophosphate is also linked to changes in pH (Russell, 1988). This relationship shows that as soil pH increases, there is an

increase in the rate of desorption from the soil material, which may be associated with an increase in hydroxyl concentrations in the soil solution. An increase in hydroxyl concentrations would act as a ligand and displace P from the soil material into solution.

Unlike sorption, precipitation occurs when water-soluble P reacts with dissolved Fe, Al and Mg in acidic and Ca in alkaline soil solutions (Stevenson and Cole, 1999; Sims and Sharpley, 2005). Precipitation processes most commonly dominate sorption processes at high concentrations of water-soluble P in the presence of high concentrations of metallic cations, where as sorption is most dominant at smaller water-soluble P concentrations (Tunesi et al., 1999). For example, following the application of fertiliser, the localised decrease in pH coupled with a substantial increase in water-soluble P and dissolved cation concentrations, as the fertiliser granule dissolves, would promote greater precipitation of P complexes. These precipitated complexes may precipitate further in the presence of high P concentration into more stable products, which are more unavailable to the soil biota (Stevenson and Cole, 1999; Sims and Sharpley, 2005). Nevertheless, like P desorption from soil material, precipitated soil material may dissolve and increase the concentrations of P released into the soil solution. Dissolution of P precipitated complexes is based on equilibrium between precipitated P complexes and concentrations of water-soluble P and dissolved cations in the soil solution (Sims and Sharpley, 2005). Therefore, precipitated P equilibrates through dissolution when concentrations in the soil solution are not sufficient to maintain the equilibrium.

Both the sorption and precipitation of P into labile compounds decreases P availability to the soil biota. Nevertheless, the soil biota is also able to mineralise P from organic sources through the cycling of organic matter, thus making a significant contribution to the P nutrition component of the plant community (Oehl et al., 2001b).

1.2.2 Organic phosphorus fractions

The organic P content of soils range from trace amounts in arid regions to several hundred ppm in forest soils (Stewart, 1991) and can contribute to between 30 and

65% of total soil P (Sims and Sharpley, 2005). Organic P is synthesised through biological activity in the soil, since the uptake of water-soluble P is subsequently bonded to organic compounds through phosphorylation within the living biomass. Organic P accumulation is particularly evident in the microbial biomass, since data suggests that more than 90% of the total P is in an organic form (Sims and Sharpley, 2005). Like organic P concentrations in the microbial biomass, a high proportion of P present in plant tissue occurs in organic forms, between 30 to 60% (Sims and Sharpley, 2005). Therefore, due to this high proportion of P in plant tissue, the most common input of organic P into the soil arises from plant residues, which vary in quantity and quality and ultimately affect organic P fractions and concentrations in the soil.

One such fraction of organic P in the soil is the orthophosphate esters (Turner et al., 2005). These include low molecular weight compounds that contain P, for example phospholipids, DNA and ATP. Due to the large diversity of orthophosphate esters in the soil, they are categorised into different groups depending on the number of bonds that P shares with the organic compound. One main category is referred to as the orthophosphate monoesters, as a single ester bond binds the P to its organic counterpart. Orthophosphate monoesters are the dominant fraction of organic P in most agricultural grassland systems, but most tend to be easily mineralised by the soil biota (Turner et al., 2002; Murphy et al., 2009). It is the presence of one particular high order orthophosphate monoester that contributes to their dominance in grassland systems; this is the inositol phosphate group (Murphy et al., 2009). Inositol phosphates have a large charge density compared to other organic P compounds and are thus more strongly sorbed by Fe and Al oxides. This sorption may protect such compounds from hydrolysis by phosphorus-specific enzymes, which can develop into the accumulation of orthophosphate monoesters in the soil (Lung and Lim, 2006).

Another category of orthophosphate esters is denoted as the orthophosphate diesters, since P is covalently bound by two ester bonds in these compounds (Sims and Sharpley, 2005). These compounds typically comprise of around 10% of the organic P in the soil and consist of compounds like DNA and phospholipids. Nevertheless, these orthophosphate diesters are critically linked to P biological turnover since most organic P inputs into the soil are derived from this category (Turner and Haygarth,

2005; Turner et al., 2005). Orthophosphate monoesters and diesters are regarded as labile in the soil because they can be hydrolysed through enzyme activity. Water-soluble P is a product of this hydrolysis, which can be utilised by the soil biota.

1.3 Phosphorus cycling by the soil micro-biota

Whilst chemical processes in the soil reduce the bio-availability of P, the soil biota can modulate these processes by releasing P bound in labile inorganic and organic complexes. This modulation stems mainly via the use of P mineralising enzymes and organic anions, which ultimately increase P solubilisation into the soil solution.

Both microbial and plant components of the soil biota produce enzymes that are capable of hydrolysing organic compounds to release products that are necessary for activity and growth. Enzymes are globular proteins that are produced by all living organisms (Gobat et al., 2004). With respect to P cycling, there are enzymes that specifically hydrolyse bonds between P and an organic compound. These enzymes are referred to as phosphatases and have been classified into five groups by the Commission on Enzymes of the International Union of Biochemistry (Eivazi and Tabatabai, 1977). These include phosphoric monoester hydrolases (EC 3.1.3), phosphoric diester hydrolases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5), enzymes that act upon phosphoryl-containing anhydrides (EC 3.6.1) and phosphatases that act on P-N bonds (EC 3.9).

Out of these five groups of enzyme, most phosphatases in the soil occur as either phosphoric monoester hydrolases (phosphomonoesterases) or phosphoric diester hydrolases (phosphodiesterases) (Beever and Burns, 1980; Turner et al., 2002; Turner and Haygarth, 2005; Bunemann, 2008). Phosphomonoesterases and phosphodiesterases are specific to orthophosphate monoesters and diesters, respectively, but have a broad spectrum with regards to substrate specificity within their organic P substratum group (Bunemann, 2008). However, in the case of hydrolysing phosphate diesters, this substrate firstly needs to be modified by a phosphodiesterase to hydrolyse one of the bonds joining P to the organic compound. After the hydrolysis of the first ester bond, the second ester bond is hydrolysed by a

phosphomonoesterase and water-soluble P is released from the organic compound (Turner and Haygarth, 2005). Through the activity of soil phosphatases, the soil biological community is able to increase water-soluble P concentrations, which will ultimately increase P uptake.

The soil biota is also able to liberate P bound within labile inorganic fractions. More specifically, this ability to displace P is dependent on the binding strength between P and the counterpart inorganic material. For example, the ability of the soil biota to displace P in a labile state is greater compared to P bound in an occluded state. Nevertheless, both plants and microorganisms can also solubilise P that has either been sorbed into the soil material or precipitated into Al, Fe and Ca complexes by either exuding organic anions or changing soil pH (Cunningham and Kuiack, 1992; Illmer and Schinner, 1995a; Illmer et al., 1995b; Chuang et al., 2007; Bunemann, 2008). In the study conducted by Immer et al. (1995b), organic anion production in the presence of inoculated P solubilising microorganisms were shown to both solubilise Al – PO_4 complexes and increase Al^{3+} and water-soluble P concentrations in the soil. In particular, this study reported that the production of citrate and a decrease in pH by these organisms facilitated this solubilisation and that an increase in citrate anion concentrations could chelate with the Al – PO_4 complexes and increase water-soluble P concentrations.

Whist the production of citrate anions in the study by Immer et al. (1995b) was shown to increase P solubilisation; the presence of other organic anions did not invoke such a response. The soil biota is able to exude a variety of organic anions into the soil environment; these include oxalate, gluconate, malic and fumaric acids (Illmer et al., 1995b; Hinsinger, 2001). However, the majority of organic anions are low molecular weight molecules that contain one or more carboxylic acid groups. The diversity of these chelating organic anions is evident as viewed from a plant species perspective. For example, citric acid exudation is the dominate organic anion released from a plant species like white lupin, malic acid exudation has been reported in maize and oil seed rape and oxalic is of particular importance with respect to sugar beet (Hinsinger, 2001). All of these organic anions are capable of displacing P from labile inorganic fractions by chelating with the Fe, Al or Ca central atom in the complex and forming a

stable bond between the organic acid and the Fe, Al or Ca atom. Through the formation of this bond, P is solubilised and is available for biological uptake.

Like organic acid exudation, biologically induced changes in soil pH have also been reported to increase P availability (Illmer and Schinner, 1995a; Sims and Sharpley, 2005). Both plant and microorganisms release cations and anions into the soil that facilitate the uptake of nutrients. Such cations and anions include H^+ or OH^- / $HCO3^-$, which act to counterbalance the net excess of cations and anions entering the organism (Hinsinger, 2001). When released into the soil, these can greatly change the soil pH which can promote the dissolution of P precipitates. For example, the study by Cunningham and Kuiack (1992), revealed that the supplementation of $CaHPO_4$ powder to an agar medium induced an acidification of the medium, as visualised in the presence of Alizarin Red S, which complemented the solubilisation and dissolution of the $Ca - PO_4$ complex.

The use of phosphatases, organic anions and shifts in pH are mechanisms that the soil biota as a whole employ to increase soil P availability, as described above. However, with respect to plant P acquisition, the soil microbial biomass is recognised as an important source of P to facilitate plant uptake.

1.3.1 Soil microbial nutrient cycling

The soil microbial biomass includes all soil organisms with a volume less that $5x10^3$ μm^3, not including plant tissue (Brookes, 2001). Therefore, microbial biomass comprises of both bacterial and fungal components, both of which are considered to be a labile pool of essential nutrients, like N and P, for plant growth (Richardson and Simpson, 2011). For example, the Highfield grasslands in the Broadbalk Continuous Wheat Experiment at Rothamsted (UK) reportedly contain microbial biomass N and P concentrations of 130 and 65 kg ha^{-1}, respectively (Brookes, 2001). The nutrients held in the microbial biomass are held in forms that are resistant to leaching or fixation. With respect to P, immobilised P in the microbial biomass is protected from sorption and precipitation processes and is thus capable to maintaining a liable source of organic P in the soil.

Microbial biomass P consists of approximately 60% as nucleic acids, 20% as acid-soluble inorganic P and <10% as phospholipids (Oehl et al., 2001a). However, it is through microbial biomass turnover that these cellular components are potentially mineralised and available to the plant community. Work concerning the turnover of microbial biomass P has concentrated on shifts in the isotopic exchange of P between soil amendment material and the flush of P produced following chloroform treatment (Buenemann et al., 2012). Results from these studies indicate that microbial biomass P turnover is much greater than microbial biomass C turnover, since a large proportion of C in the microbial biomass occurs in resistant forms (for example in cell walls) and P is located mainly in labile forms (Achat et al., 2010). However, microbial biomass turnover has been reported to differ between different fertiliser management types and soil types (Buenemann et al., 2012). With respect to soil type, larger microbial biomass C and N pools occur in soil with greater clay contents (Gregorich et al., 1991; Hassink, 1994), hence smaller microbial biomass pools occur in soils with greater sand contents. Such difference between these soil textures is associated with the availability of decomposable soil material, since an increase in clay content increases the stability of the organic matter and promotes greater water retention (Gregorich et al., 1991; Thomsen et al., 2003). Both of these factors contribute to a reduction in organic matter decomposition by the soil microbial community, since greater organic matter stability reduces the decomposition of such material by the microbial community and greater water retention can limit the number of aerobic soil environments. Therefore, the actual nutrient content of the microbial biomass is much greater because microbial biomass turnover is reduced. By reducing the turnover rate, greater nutrient concentrations are retained in the biomass. Whilst the microbial biomass is critically linked to P cycling and plant P uptake, such biomass also actively participates in the cycling of other nutrients, namely C and N. The ability of the plant community to supply C to the soil enables the microbial community to mineralise and oxidise N into forms that support plant N acquisition in the soil.

Nitrogen is regarded as one of the main limiting nutrients in many ecosystems (Vitousek et al., 1997; Knops et al., 2002; Ollivier et al., 2011). Therefore, in agricultural systems, N fertilisers are commonly applied to increase the N status of the soil. Whilst N can be supplied to the soil as fertiliser, N can also be fixed into a biologically available form through a symbiotic relationship between specific plant

and soil microbial species. This symbiosis involves the fixation of nitrogen in the atmosphere into NH_3, which is then utilised by both the plant and microbial community (Knops et al., 2002 and Figure 1.2). Particular plant species have evolved such relationship and these are commonly from the *Fabaceae* family (legumes). The microbial species that forms the symbiosis is also specific and these include bacterial species such as those from the *Rhizobia* genus. With respect to the manner in which N enters the soil matrix, there is a trend that shows a reduction in nitrogen fixation with increasing N fertilisation (Knops et al., 2002). In soils that receive N fertilisation, the development of plant communities that contain a legume species is reduced. With a reduction of legume abundance, there is less nitrogen fixation and an increase in the reliance on fertilisers by the biological community.

Following N assimilation by the soil biota, N is cycled by the death and decomposition of plant and soil biological material (Ollivier et al., 2011; Veresoglou et al., 2012 and Figure 1.2). The rate and manner of decomposition is governed by the production of specialised enzymes by the soil biota. For larger and more complex organic compounds containing N, the microbial community is able to produce depolymerases that function extracellularly and hydrolyse structurally complex compounds like proteins and chitin (Ollivier et al., 2011). Hydrolysis of such compounds is critically linked to the turnover of N in soils since these organic compounds contain large proportions of N (Ollivier et al., 2011). By hydrolysing these complex compounds, relatively simpler bi-products are produced that can be assimilated by the soil biota. Likewise, low molecular weight organic compounds that naturally occur with the decomposing cell can also be readily utilised and re-incorporated into soil biomass upon release into the soil environment. When N is utilised directly from the soil organic matter pool and incorporated into biomass as described above, such turnover is referred to as the direct pathway (Ollivier et al., 2011 and Figure 1.2). Soil animals also promote the turnover of N through the predatory behaviour they exhibit towards soil microorganisms. For example, soil nematodes are intricately linked to the turnover of N since they actively feed on soil bacterial and fungal communities and consequentially release bioavailable N into the soil environment (Ingham et al., 1985; Yeates, 2003).

Whilst the microbial community can assimilate N-containing organic compounds as a source of N nutrition, the soil biota can also mineralise N from the non-living soil organic matter pool (Figure 1.2). The hydrolysis of these compounds results in the mineralisation of organic N into NH_4^+ through the process of ammonification (Knops et al., 2002; Ollivier et al., 2011). Ammonium production in the soil is important with respect to microbial N nutrition as this form of N is readily assimilated by soil microbial communities (Knops et al., 2002). Under aerobic conditions, NH_4^+ can be oxidised by the soil microbial community through a process called nitrification (Ollivier et al., 2011 and Figure 1.2). Such oxidation occurs rapidly, and results in the oxidation of NH_4^+ to NO_2^- and then further oxidation of NO_2^- to NO_3^- dependent upon the presence of particular bacterial species. However, the transformation step from NO_2^- to NO_3^- is crucial with respect to biological assimilation, since NO_2^- is toxic in high concentrations and NO_3^- is a bioavailable form of N that can be assimilated. Nevertheless, NO_3^- can also leach into the ground-water and the transfer of high concentrations into water-bodies can be detrimental to the environment (Knops et al., 2002). When N is mineralised from the soil organic matter pool and an inorganic form of N is in turn assimilated by the soil biota, this process of N turnover is referred to as the mineralisation-immobilisation-turnover pathway (Ollivier et al., 2011 and Figure 1.2).

The transformations of NO_3^- are not solely linked to leaching and biological assimilation, NO_3^- can also be reduced to N_2 through the process of denitrification (Hiscock et al., 1991; Wrage et al., 2001 and Figure 1.2). Denitrification is a stepwise process that requires multiple stages of reduction until N_2 is ultimately produced (Wrage et al., 2001). The intermediate steps include the reduction of NO_3^- to NO_2^-, then further reduction to NO, then to N_2O and the final reduction to N_2. The denitrification process occurs under anaerobic conditions and is conducted by soil bacterial called denitrifiers. Denitrifiers perform such process to utilise the oxygen present in the nitrogen-oxygen compound as an electron carrier for respiration (Wrage et al., 2001). Like the nitrogen cycle, the carbon cycle is also largely governed by biological processes (Janzen, 2004; Jones et al., 2009 and Figure 1.3).

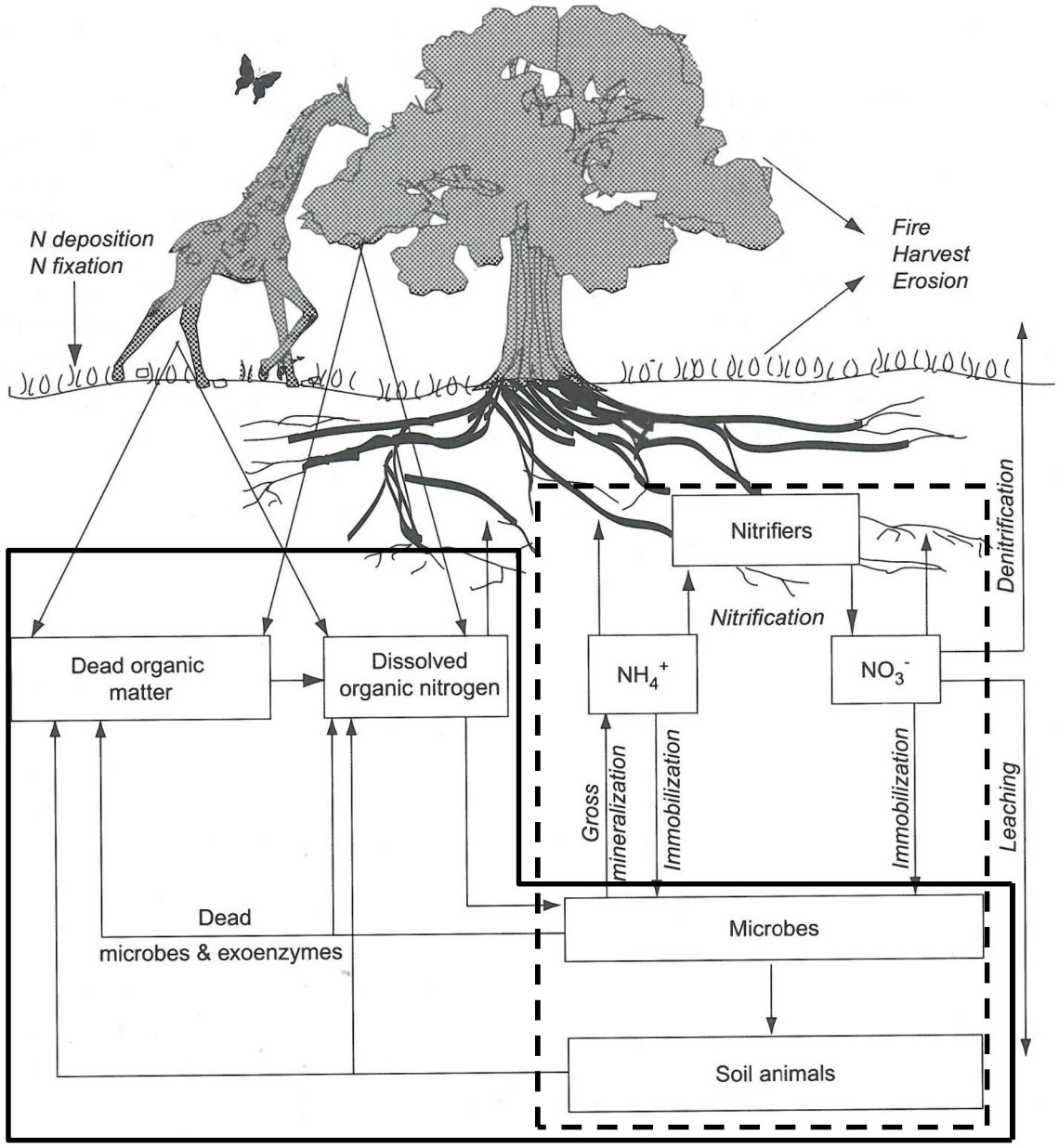

Figure 1.2 Conceptual diagram of the N cycle in terrestrial ecosystems. The area surrounded by a thick full line denotes the direct pathway of N turnover in the soil. The area surrounded by a dashed line shows the mineralisation-immobilisation turnover pathway. Image sourced from Chapin III et al., 2011.

One similarity between these cycles is that biological processes can actively immobilise and incorporate either nutrient from the atmosphere into biomass. For example, the plant community utilises CO_2 through the photoautotrophic process of photosynthesis to produce glucose and oxygen (Janzen, 2004; Jones et al., 2009). Synthesised C is transported around the plant via the phloem and is integral to plant activity. Plant-mediated C entry into the soil occurs through two mechanisms (Bardgett, 2005; Jones et al., 2009). These mechanisms include the senescence of aboveground plant material, which is consequently deposited onto the soil surface and incorporated into the soil matrix through the activity of saprophytic organisms (Bardgett, 2005). The other mechanism is termed rhizodeposition, which includes a wide range of plant processes belowground. Rhizodeposition includes the C entry into the soil through the death and lysis of root cells (i.e, death of border cap cells and root hairs), the flow of C into root symbionts (i.e mycorrhizal fungi) and the exudation of C from the root itself (Rees et al., 2002; Jones et al., 2009). Carbon released into the soil via these mechanisms is either directly assimilated by the soil microbial community or incorporated into the non-living soil organic matter pool (Jones et al., 2009 and Figure 1.3).

The turnover of C between non-living soil organic matter and living soil biomass is governed by the rate of decomposition (Rees et al., 2002; Cambardella, 2005). One factor that governs the rate of decomposition is the composition of the non-living organic matter pool. The soil organic matter pool is composed of organic compounds of varying bioavailability and quality (Knabner, 2002; Rees et al., 2002; Cambardella, 2005). More labile organic compounds are readily assimilated by the soil microbial community. Once assimilated, C is used as a source of energy or to synthesis biomass, both of which supports microbial growth and activity in the soil. However, other organic compounds are not readily bioavailable and require large amounts of energy to be hydrolysed into more bio-available products (Knabner, 2002; Cambardella, 2005). These compounds are thus regarded as being recalcitrant and require specialised enzymes produced by particular soil organisms to be decomposed. Therefore, another factor governing decomposition is the structure of the soil biological community (Cambardella, 2005). It is the presence of particular fungal species that are able to produce the necessary enzymes to hydrolyse these recalcitrant organic compounds, whereas, the soil bacterial species readily assimilate C present in

more labile states. Other soil organisms are also critically linked to the decomposition process, and these include both macro and mesofaunal organisms (Cambardella, 2005). It is the feeding activity of organisms like earthworms and collembola that ingest and digest complex plant material before excreting such digested material throughout the soil matrix. Once excreted, further decomposition occurs through the activity of the microbial community. Despite the activities of the soil biota, non-living organic material in the soil still persists since it is highly resistant to decomposition due to complex chemical and physical interactions with the soil matrix. Thus, this material, referred to as humus, accumulates in the soil and can account for 60 – 80% of the total organic matter content (Cambardella, 2005).

Organic C that is decomposed and utilised by the soil biota can be mineralised through the process of respiration (Janzen, 2004). Respiration is a critical process performed by all living organisms, which produces ATP through the hydrolysis of glucose. Through this process, CO_2 is evolved and is consequentially removed from the organisms and transferred to the atmosphere, where after is can be utilised by the plant community via photosynthesis (Janzen, 2004 and Figure 1.3) .

With respect to the P, N and C cycles, there is one major similarity between them. This similarity is that all three are requisite for soil biological growth and activity in the soil, which is evident in studies that have investigated nutrient limitations to the soil microbial community (Dilly, 1999; Smith, 2005; Gichangi et al., 2010). The common findings between these studies are that the availability of one nutrient will limit the assimilation of others and that nutrient limitation can constrain both the activity and size of the soil biological community. To increase nutrient availability, the efficient cycling of all these nutrients is necessary to maintain required bioavailable concentrations in the soil. With such dependencies on C, N and P availability, and thus the cycling of these nutrients, intricate biologically-mediated interactions between these nutrients exist (Tipping et al., 2012; Whitehead and Crossman, 2012). One such interaction affects the manner in which litter is decomposed, since the C to N ratio of decomposing litter affects the rate at which such material is decomposed.

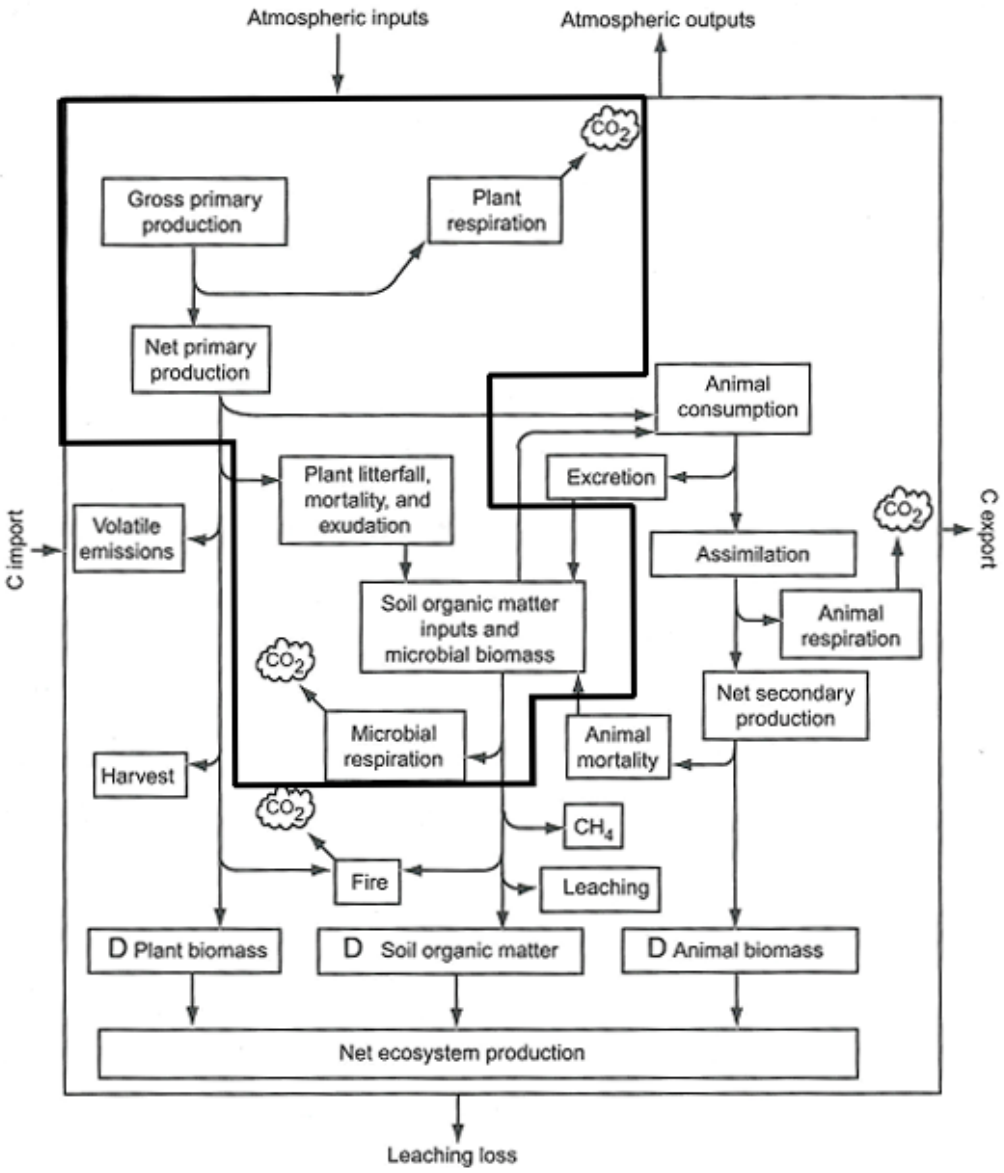

Figure 1.3 Conceptual diagram of the C cycle in terrestrial ecosystems. The area surrounded by a thick full line denotes the section of the C cycle that is mediated by plant and microbial communities. Image sourced from Chapin III et al., 2011.

Litter containing low C to N ratios have been reported to decompose much quicker than litter containing high C to N ratios, which was linked to N requirements for the synthesis of biomass (Zhang et al., 2008; Tipping et al., 2012). Whilst C and N availability in the soil can govern the decomposition rates of soil organic matter, C availability can also affect N transformations and thus N cycling. With greater plant-mediated C input, greater N fixation can be stimulated because limitations imposed by

C availability are removed (He et al., 2010). However, greater C input may also reduce nitrification in the soil (Hiscock et al., 1991). Nitrification is reduced due to the stimulation of the soil heterotrophic community by elevated concentrations of bioavailable C. Such stimulation decreases the availability of N to the nitrifier community and ultimately reduces their abundance.

Whilst the C cycle can interact with the N cycle, increasing P availability through the application of fertilisers can have profound effects on C cycling, namely on C assimilation by the plant community (Mackenzie et al., 2002; Purakayastha et al., 2008; Gong et al., 2012). The application of P fertilisers is well known to increase plant yields in agricultural systems and is the reason why they are applied. However, by increasing plant yields, there is also an effect on the C cycle, since greater organic C is synthesised by the plant community as P limitation to the plant community has been removed after fertiliser application (Mackenzie et al., 2002). Therefore, the transfer of C to the soil matrix through leaf senescence and rhizodeposition increases, which will ultimately affect the turnover of C by the soil biota, storage of C in the non-living organic matter pool and the transfer of C to the atmosphere through increased respiration (Gong et al., 2012). Like the effect that P fertiliser has on both the plant community and the C cycle, the application of fertilisers can also profoundly affect the soil microbial community and their ability to assimilate P.

With respect to fertiliser effects on microbial biomass P, the study conducted by Oehl et al. (2001) showed that more rapid microbial biomass P turnover rate occurred in systems that received organic fertiliser compared those that received mineral fertiliser. More specifically, the complete turnover of isotopic P in the microbial biomass derived from tillage soils receiving farmyard manure was 120 days compared to 160 days in soil receiving mineral fertiliser. This difference in microbial biomass turnover was also coupled with a larger microbial biomass P pool in the organic fertilised system compared to the mineral fertilised system. Therefore, it was concluded that fertiliser management affects microbial parameters in the soil, particularly with P cycling dynamics.

1.3.2 Phosphorus fertiliser management and the soil biota

The effect of P fertilisation on the microbial community is equivocal in the literature, with different studies highlighting the importance of contrasting management and fertilisation regimes on P fertiliser effects or the absence of an effect all together (Bunemann et al., 2004; Ayaga et al., 2006; Liu et al., 2012). Microbial biomass responses to inorganic P fertilisation have been reported to exhibit temporal variation. In the study conducted by Ross et al. (1995), microbial biomass P concentrations in a New Zealand grassland site were shown to fluctuate, in terms of fertiliser response, across the experimental sampling period. To further support this, a very significant effect of sampling date ($p<0.001$) was reported on microbial biomass P concentrations in an Argentinean pasture (Picone et al., 2003). This effect revealed that microbial biomass P increased over winter and started to decrease in the spring and summer. It was also noted that the length of time following P application also affected the microbial biomass response, since an increase in microbial biomass P with increasing P fertilisation was reported 16 days after P fertiliser was applied.

The majority of studies that have not observed an effect on microbial biomass P relate this to nutrient limitation other than for P. Therefore, in studies that have included the application of organic fertilisers, other nutrient limitations are removed and a response to P fertiliser is observed. In laboratory-based studies focused on increased microbial biomass P in African soils, soil amendment through the application of manure was reported to increase microbial biomass P concentrations more than the application of inorganic P fertiliser (Ayaga et al., 2006; Gichangi et al., 2009; Khan and Joergensen, 2009). Furthermore, the application of inorganic and organic fertilisers together promoted the greatest microbial biomass P concentrations. These studies explained that the application of such organic material stimulates the synthesis of biomass which will create a demand for P. Such demand increases P acquisition from the inorganic source, hence increasing P immobilisation in the microbial biomass. Another explanation presented by Ayaga et al. (2006) indicates that applied organic material may compete for adsorption sites on the soil material, thus decreasing P fixation. Such competition would ultimately increase water-soluble P concentrations, hence increasing P availability to the soil biota. Such effects of organic fertilisation have also been observed in the field. These field-based studies also indicate that organic

and inorganic P fertilisation had the greatest stimulatory effect on microbial biomass nutrient pools (Liu et al., 2010; Chakraborty et al., 2011). Furthermore, these field-based studies were also able to assess fertiliser type effects on plant yield parameters. The effect of fertiliser type on plant yield has also been reported to complement those observed on microbial parameters, since dual inorganic and organic fertiliser applications also promote the greatest plant yield response (Liu et al., 2010).

Whilst the effects of organic input on microbial responses to P fertilisation have been noted, the input of organic material from the plant community has also been shown to regulate such response. For example, in the study conducted by Bunemann et al. (2004), it was shown that soil amended with 50 kg ha^{-1} y^{-1} of triple superphosphate was not the overarching factor determining microbial acquisition of P in a Swiss crop rotation system. In fact it was the presence of different crops in this rotation and the supplementation of different C substrates that had the greatest affect on microbial biomass P concentrations. At high levels of labile C, the microbial community responded to P fertiliser applications and thus greater microbial biomass P pools were achieved. Therefore, this study concluded by stressing the importance of labile C in plant residues with respect to microbial responses to P fertilisation. The importance of plant community residues on microbial acquisition of fertiliser P has also been reported in the study by Liu et al. (2012). This study looked at the effects of P fertilisation on the soil microbial community in three Chinese forests that have different land-use histories. These land-use histories involved one forest that had been protected from anthropomorphic activity and two that were under continuous disturbance. Microbial biomass P, activity and phenotypic structure in the undisturbed forest were affected by P fertilisation, where as no such effects were observed in one disturbed forest treatment and only microbial activity and phenotype were affected by P fertilisation in the other disturbed forest. It was concluded that the microbial community in the undisturbed forest was P limited due to insufficient substrate supply from the forest, whilst C was most limiting in the disturbed forest. Therefore, the application of P increased microbial biomass P concentrations since a readily available supply of plant derived C was present in the soil. These studies indicate the importance of the plant community to regulate nutrient acquisition by the microbial community. The major interface between soil microbial and plant communities is via

the root system. Therefore the development of root networks in the soil governs the distribution and activity of the soil microbial community.

1.4 Plant and soil microbial interactions

The functions of the root system are to provide structural support for the shoot and to acquire nutrients from the soil. Despite this commonality of root function between species, its development is highly variable between species (Hodge et al., 2009). Differences between plant rooting networks and elongation strategies are evident between monocotyledonous and dicotyledonous plants (Hodge et al., 2009). In particular, the root architecture of monocotyledonous is referred to as adventitious or fibrous, because of the primary root axis that initially develops. An example of a plant family that develops a monocotyledonous rooting network includes the Gramineae (Grasses). In dicotyledonous plants, the root system consists of a primary root that develops only lateral branchings. Plant species that produce a dicotyledonous root system include herbaceous plants and shrubs. In some dicotyledonous species, this primary root thickens and becomes woody in appearance: this type of primary root is referred to as a taproot. Despite the morphological differences between adventitious and primary root systems, both support large microbial communities in close proximity to the root surface (Forde and Lorenzo, 2001).

It is the activity of the root that stimulates these microbial communities, since the root releases organic compounds into the surrounding soil matrix that microorganisms readily utilise. This area around the plant root typically stems from one millimetre to several millimetres based on soil nutrient concentration gradients, the diffusion capability of exuded organic compounds and microbial community structure (Bertin et al., 2003; van Elsas et al., 2007). This area immediately surrounding the plant root is referred to as the rhizosphere since it is vastly different from the soil environment typically observed in the bulk soil. For example, the rhizosphere has been shown to be 10 times more acidic than the bulk soil, which creates a very different environment in terms of nutrient availability and microbial community structure (Hinsinger, 2001; Bertin et al., 2003). Due to the release of organic compounds from the root into the soil, the rhizosphere typically contains large and diverse microbial populations, which

ultimately increase nutrient cycling capacity in this small volume of soil surrounding the plant root. With respect to biological P cycling within and beyond the rhizosphere, the relationship that exists between fungi and the plant root are particularly important.

Fungi share important mutualistic symbiotic relationships with plants through the rhizosphere and their roots. These relationships result in the formation of mycorrhiza between the fungal mycelium and the roots of a plant, with direct interface between such organisms either on the rhizoplane (the soil volume in contact with the root epidermis) or physically within the root (Smith and Read, 2007; Bunemann et al., 2011). As these mycorrhizal relationships develop, they promote the movement of essential nutrients between the plant and the fungal mycelium. In particular, plants supply photosynthetic derived C sources directly to the fungi and, in turn, fungi supply acquired water-soluble P directly to the plant (Smith and Read, 2007; Bunemann et al., 2011).

There are different types of mycorrhizae, defined by their morphology and whether the fungal biomass is predominantly located outside or inside the root. Arbuscular mycorrhizae are the most widespread form of fungal symbiosis with plants (Vladimir and Bucher, 2005; Gregory, 2006). Arbuscular mycorrhizae can be formed "with an estimated 250,000 species" (van Elsas et al., 2007). Arbuscular hyphae grow both within the intra and intercellular areas of the cortex cells within the root and do not penetrate their plasma membranes (Vladimir and Bucher, 2005; van Elsas et al., 2007). When the hyphae penetrate the cell walls of the cortex cells, the hyphae form branched arbuscules. These arbuscules are the main area in which nutrient transfer occurs between the plant cells and the fungal cells and have a structure that increases their surface area to volume ratio. This increase in surface area to volume ratio increases the rate of nutrient transfer.

In terms of P acquisition, the development of mycorrhizae effectively increases the surface area of the root system, since the developing hyphal network becomes an extension of the root network and actively transports the acquired nutrient directly into the root system (Rousseau et al., 1994; Aquino and Plassard, 2004). Furthermore, the smaller size of fungal hyphae enables the hyphal network to penetrate into micro-pore spaces with the soil matrix, which are spaces that plant roots cannot usually access. By accessing such spaces, the fungal hyphal network is also able to supply

nutrients to the plant from areas that the plant root system would not usually be able to access. Whilst the plant community shares these essential symbioses with specific soil fungal groups, plants also interact with the whole soil microbial community on a broader scale through multiple mechanisms.

1.4.1 General plant interactions with the soil microbial community

The plant community maintains these large microbial populations in the rhizosphere by releasing organic compounds into the soil. One of the main beneficial inputs from these organic compounds is the input of C, which is often regarded as limiting in many soil systems (Maillard and Pinheiro, 2008; Paterson et al., 2011). The input of C as plant derived organic compounds mainly occurs through two mechanisms (Bardgett, 2005). These mechanisms include leaf litter senescence and rhizodeposition. Between these two mechanisms, litter senescence is considered to be the largest contributor of plant inputs into the soil (Millard and Singh, 2010). The leaf deposition process occurs when dead components of the plant community are released into the surrounding environment. Following litter deposition onto the soil surface, an array of saprophytic organisms starts to breakdown such material. The presence of larger saprophytic organisms, like earthworms, feed on such material and promotes the re-distribution of leaf litter into the soil matrix from the soil surface. By feeding on this material, the earthworm community is able to breakdown chemically complex litter into organic residues that are more easily utilised by the soil microbial community. Therefore, the input of organic residues from plant senescence into the soil can have stimulatory effects on soil microbial activity.

Plants can also interact with the microbial community through rhizodeposition (Paterson et al., 2007; Millard and Singh, 2010). Rhizodeposition includes both the turnover of root material in the soil and the exudation of organic compounds directly from the root. Since rhizodeposition includes both exudation and root turnover, the quantities and qualities of supplied C are highly variable. Such variation in plant derived C can profoundly affect microbial community structure, as different organic compounds can have positive and negative effects on different microorganisms that constitute part of the overall community (Millard and Singh, 2010; Paterson et al.,

2011). The importance of rhizodeposition is also emphasised by the quantities of organic substrate released from the plant into the soil, since plants can exude between 10 to 40% of their photosynthate into the soil (van Elsas et al., 2007). However, the contribution of rhizodeposition into the soil can be manipulated through management practices. For example, mowing the aboveground proportion of the plant community can stimulate rhizodeposition on a short-term basis, but hinder rhizodeposition over a long-term period (Ilmarinen and Mikola, 2009; Ilmarinen et al., 2009). Such manipulation of the plant community may also affect plant responses to nutrient availability in the soil (Cayley and Hannah, 1995; Simpson et al., 2012). Over a short-term period, mowing can promote the exudation of C-rich organic compounds into the soil that consequently stimulates the soil saprophytic community. Rhizodeposition in this context is the primary support for the microbial community, since little senescence occurs due to mowing activity. However, over a long-term period, mowing can reduce the photosynthetic capacity of the plant, thus limiting rhizodeposition, and reducing the accumulation of leaf litter on the soil surface. Both of these negative effects reduce C supply to the soil and ultimately reduce soil saprophytic activity. With such effects on leaf litter input and rhizodeposition, mowing has also been reported to affect botanical diversity. For example, mowing effects on botanical diversity can be beneficial, since mowing two times a year has been shown to facilitate greater species richness (Schaffers, 2002). Mowing can induce such a beneficial effect due to an increase in light availability lower in the sward canopy, thereby promoting the growth of species that would not usually be present.

1.4.2 Botanical diversity interactions with microbial and nematode communities

Whilst management practices affect botanical diversity, the interactions between botanical diversity and soil microbial communities are less well defined, since evidence from the literature suggests that botanical diversity interacts with the soil biota in a wide variety of ways (Spehn et al., 2000; Bezemer et al., 2006; Millard and Singh, 2010; Scherber et al., 2010). When no synergy is observed between plant and

microbial diversities, the attributable explanation appears to be linked with complex interactions with other soil properties, for example nutrient status, sample location and management regime (Chabrerie et al., 2003; Hedlund et al., 2003; Bezemer et al., 2006). When looking at soil type effects on the relationship between botanical diversity and microbial community, Bezemer et al. (2006) showed that soil type can be the overarching factor involved with plant biomass synthesis rather than soil biotic properties in the rhizosphere. Using sites in the Netherlands and in the United Kingdom, plant-soil biota interactions were shown to be more correlated with soil nutrient status in the Dutch sandy soil. In contrast, plant biomass synthesis appeared to be more correlated with soil microbial community in the British chalk soil. Therefore, the relationship between botanical diversity and microbial diversity seemed to be applicable to certain soil types and nutrient statuses. In another study focusing on chalk soils of north-western Europe, the succession of plant species from agricultural grassland to forest was shown to have little effect on microbial functional and genetic structure, despite changes in plant community composition (Chabrerie et al., 2003). In addition, the absence of an interaction between plant succession and microbial properties was also observed by Hedlund et al. (2003). This particular study spanned several European countries and focused on the secondary succession of plant species on sites that were previously arable land. Changes in plant productivity, induced by succession, did not affect microbial biomass properties across all countries. However, shifts in plant species richness were shown to affect microbial community structure, but this was only evident in a Swedish soil and not in other sites present in other countries.

Whilst some studies have revealed the absence of an interaction between botanical diversity and soil microorganisms, other studies have reported such interactions (Bardgett and McAlister, 1999; Bardgett and Shine, 1999; Spehn et al., 2000). Microbial biomass size was reported to decrease with decreasing botanical diversity in the study conducted by Sphen et al. (2000). This decrease was attributed to a reduction in plant productivity, which was induced by a decrease in botanical diversity. By decreasing diversity in this manner, plant inputs into the soil were also reduced. Therefore the availability of nutrients to the microbial community decreased, which ultimately affected the size of the microbial biomass. When botanical diversity is increased, there can be a positive effect on the microbial biomass size. By

increasing plant litter diversity in the study conducted by Bardgett and Shine (1999), a non-additive response occurred in which the greatest microbial biomass C concentrations were observed when five to six litter types were present. However, a decrease in microbial biomass C concentrations was observed in the presence of two to four litter types when compared to single litter type addition. This effect of five to six litter types on the microbial community was also supported by a decrease in the metabolic quotient at such plant litter diversities. This decrease in metabolic quotient revealed that more efficient biological decomposition processes occurred when litter diversity was high. With respect to changes in microbial community structure, the study conducted by Bardgett and McAlister (1999) showed that changes in grassland botanical diversity induced such effects. More specifically, this study reported that a synergy between botanical diversity and microbial diversity occurs based on the land management practice employed. In particular, in systems which received intensive fertilisation, the fertiliser-induced development of less species-rich swards ultimately promoted a more bacterial dominated community. On the other hand, in unfertilised grasslands that represented a more natural self-sufficient system in this study, a more fungal dominated community was reported. Botanical diversity effects on bacterial or fungal dominance can consequently impact on other soil aspects of the soil biota. For example, Yeates et al. (1997) explained that the stimulation of a more fungal dominated community through changes in land management practices can shift the structure of the soil biota towards an overall more fungal-dominated system. Such a shift would envisage an increase in the number of fungal feeding fauna, such as nematodes, since the nematode community structure is intricately linked to the primary producing trophic components of the soil biota, like the soil microbial community.

1.5 Soil nematode communities

Nematodes are defined as unsegmented roundworms that are present in many different habitats around the world, these habitats range from the tops of mountains to the ocean depths (Weaver et al., 1994). At certain grassland sites around the world, nematode abundance is greater than 3 million per m^2 with community assemblages that consist of more than 200 species (Yeates, 2003). The ability of large numbers of

nematodes to coexist within a specific area is attributed not only to diversification into different niches within the soil matrix, but also to variations in reproductive behaviour and responses to environmental stresses. With such prevalence in many soil ecosystems, they are regarding as intricate components of the soil biota and are thus linked to soil biological nutrient cycling.

Nematode diversity and capability to influence biological nutrient cycling in the soil can be expressed through their feeding habits (Ritz and Trudgill, 1999). These feeding habits include the predation of plant roots, bacteria, fungi, algae, diatoms and other nematodes (Yeates et al., 1993). This diversity in feeding habits is further amplified by high host specificity that is exhibited by many of the nematode species that feed on plant roots. This is because certain species of plant-feeding nematodes will only feed on one species of plant (Yeates, 1999). Nematodes are usually split into five trophic groups; this is based upon the nature of their food. These are defined as plant-feeding, fungal-feeding, bacterial-feeding, carnivorous and omnivorous trophic groups (Bernard, 1992). The specialised feeding habits of nematodes are apparent in their morphology (Figure 1.1). In particular, the structure of the nematode buccal cavity is highly specialised and is commonly used to distinguish between trophic groups. Plant-feeding nematodes tend to have very distinct spear like stylets as their buccal cavity and are able to penetrate vulnerable plant root cells in the epidermal region. Fungal-feeding nematodes are also defined by having a spear like stylet, however, it tends to be smaller and finer compared to plant feeding stylets. The buccal cavities present in bacterial-feeding types are capable of ingesting bacteria whole. It is recognised, however, that not all nematode species can be clearly defined, since they exhibit multiple feeding habits (Weaver et al., 1994). Examples of such nematode families include the *Tripylidae*, which are reported to feed on other nematodes and on bacteria. Therefore, this nematode can be classified as both carnivorous and bacterial-feeding types.

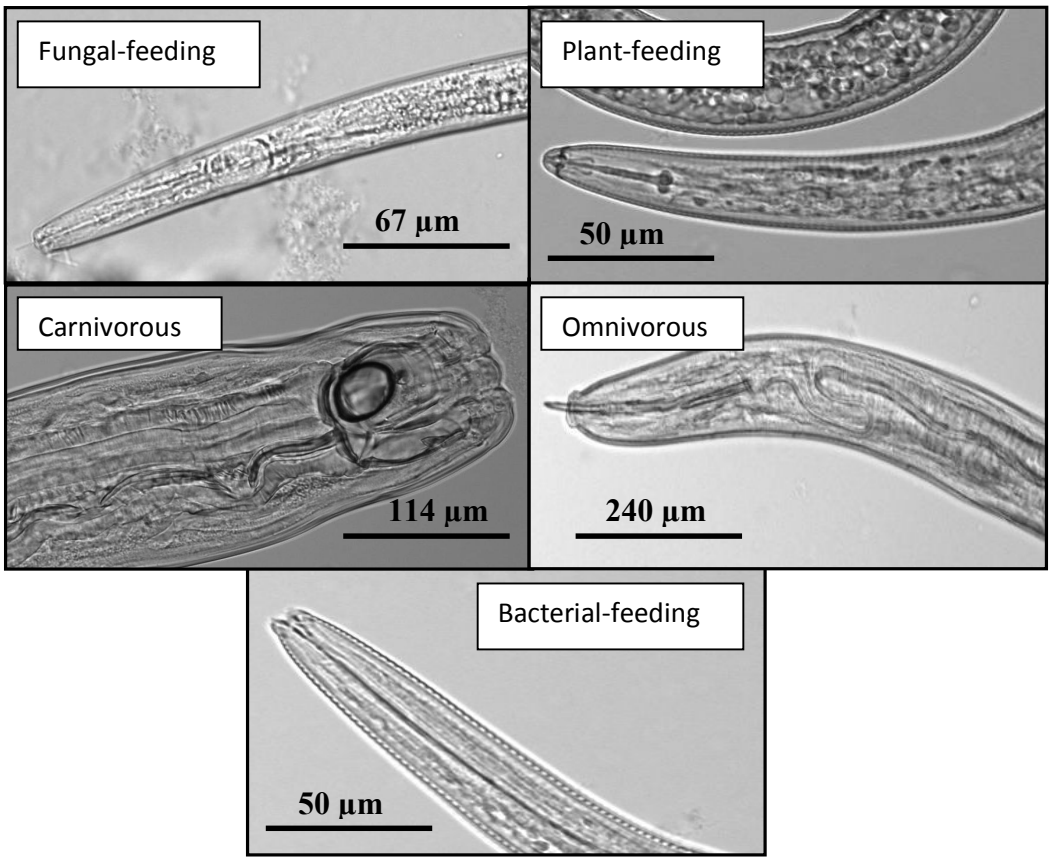

Figure 1.4 The main five nematode trophic feeding groups. Each morphological illustration is labelled with its appropriate specialised feeding group. Bars are 2 cm long and represent actual illustration lengths denoted above the bar.

Nematode activities in the soil are intricately linked to nutrient cycling. Their consumption of both living and dead components of the soil organic matter pool, promotes the turnover of nutrients in the soil environment (Murray et al., 2009). Such turnover is more readily associated with N in the literature, but some studies also considered nematode mediated P turnover (Ingham et al., 1985; Perez-Moreno and Read, 2001; Irshad et al., 2011). In the study conducted by Ingham (1985), the inoculation of agar media with nematodes in the presence of plant seedlings was shown to promote the mineralisation of N and P compared to a nematode absent treatment. Particularly, in the case of N, the presence of nematodes increased N mineralisation above the N requirement of the plant seedlings, which was attributed to grazing on the microbial community. With respect to P, the greatest rates of mineralisation were apparent in when nematodes were applied with fungi. The explanation as to why is not discussed in this study, since the focus was on N

dynamics. However, fungal presence is known to greatly affect P dynamics in soils and increase P mineralisation. Therefore, the data reported by Ingham (1985) could be interpreted as an effect of nematode grazing on fungal activity and P turnover. Fungal P turnover in this circumstance may facilitate greater P mineralisation, since grazing may have stimulated greater fungal activity, which could potentially increase P acquisition and fungal biomass turnover. Similarly, the study conducted by Perez-Moreno and Read (2001) looked into the relationship between fungal mycelial networks and soil nematodes in terms of P cycling. This study highlighted that the presence of dead nematodes provides a nutrient source for mycorrhizal fungi in the soil. Consequently, the presence of dead nematodes increased total P concentrations in the plant shoot and root biomass, via the symbiosis between the mycorrhizal mycelium and the plant. However, this study also showed that nematode presence also increased total P concentrations in the shoot of non-mycorrhizal plants, emphasising the importance of nematodes as a source of soil P for plant growth.

Whilst the importance of nematodes has been showed with respect to fungal P acquisition, bacterial-feeding nematodes have also been reported to increase P availability. In another laboratory-based media inoculation study, Irshad et al. (2011) showed that plant growth in media containing inoculated bacteria and bacterial-feeding nematodes increased P availability, whereas the bacterial only treatment did not reveal an increase in P availability compared to the treatment where no nematodes or bacteria were inoculated. One possible explanation for this increase in availability may be linked to bacterial grazing by the nematode community, since nematodes may have facilitated the mineralisation of organic P following its release from the bacterial biomass.

1.5.1 Nematode community dynamics in grassland systems

Nematode population sizes in grassland systems tend to be very large, but are also highly variable (Bardgett et al., 1998). These populations can range from 10 million per m^2 in productive grassland (Yeates et al., 1997) to less than 1 million per m^2 in acidic upland grassland. When looking at the trophic components of these nematode communities, it is recognised that plant and bacterial-feeding types are most common,

with fungal and omnivorous types similar in proportion but only contributing to small proportions of the community. Carnivorous nematodes only compose a small part of the overall community.

Whilst nematode trophic composition in grassland systems is viewed in this light, grassland management regimes can have large affects on nematode communities. For example, the application of P fertilisers to grasslands has been shown to both affect nematode population size and community structure (Yeates, 1976; Parfitt et al., 2010). However, such effects of fertiliser application appear to either coincide with livestock presence or plant productivity. With respect to studies looking at P fertilisation in the presence of livestock, the study conducted by Yeates (1976) showed that the application of superphosphate at rates of 50 and 100 kg P ha^{-1} increased the total number of nematodes in a New Zealand grassland site. The application of such fertiliser specifically affected the number of plant-feeding nematodes from the *Pratylenchus* genus, since their numbers increased from 29 thousand m^2 in the 50 kg P ha^{-1} treatment to 75 thousand m^2 in the presence of 100 kg P ha^{-1}. Similar results were observed in the study conducted by Parfitt et al. (2010), as the application of a "high fertility" treatment increased the number of nematodes from the *Pratylenchus* genus compared to the "low fertility" treatment. The high fertility treatment in this instance denoted the application of superphosphate coupled with a high stocking density of sheep; whereas the low fertility treatment was associated with no recent additions of superphosphate and a low stocking rate. This study also investigated the relationship between nematode feeding groups and herbage production. Analysis of this relationship revealed that plant and bacterial-feeding nematodes were positively correlated with high fertility grass species, whereas fungal-feeding species were negatively correlated with these grass species.

The coupling of plant productivity with the nematode community following fertiliser application generally shows that as plant productivity increases, so does nematode population size (Yeates and Bongers, 1999; Verschoor et al., 2001; van Eekeren et al., 2009a). In particular, an increase in plant productivity, either induced by shifts in sward composition or fertiliser application, can increase the abundance of herbivorous nematodes. When grassland management affects the structure of the plant community, shifts in nematode community composition are commonly observed. The review by

Yeates (1999) provides information indicating that plant-feeding nematode diversity is reduced in the presence of plant monocultures compared to mixed plant assemblages. This is due to the specific feeding nature of herbivorous nematodes to specific plant species.

With such effects of land management on nematode numbers and community composition, these soil organisms are recognised as suitable indicators of soil fertility and health (Yeates and Bongers, 1999). Generally, the underlying assumption regarding nematodes and soil fertility is that a more diverse assemblage represents a more stable and fertile soil. However, this assumption is not straightforward, since the nematode community is affected by many properties in the soil, for example other soil fauna, aboveground diversity and habitat structure. Therefore, there is no unambiguous evidence to support the hypothesis that increasing nematode diversity will be related to stability of soil ecosystem properties or processes (Yeates, 2003). To help ascertain shifts in soil fertility, a number of indices, ratios and indexes have been conceived using soil nematodes. One such ratio used to discern soil decomposition pathways is the nematode channel ratio (Yeates and Bongers, 1999; Li et al., 2007; Viketoft, 2008). The nematode channel ratio indicates whether the biotic component of the soil is either modulated by fungi or by bacteria at the primary trophic level, which ultimately provides information about soil decomposition pathways. Knowledge on decomposition pathways is particularly useful for predicting nutrient turnover and soil nutrient availability (Yeates and Bongers, 1999; Li et al., 2007; Viketoft, 2008). The nematode channel ratio is expressed as B/(B+F), where B and F are the proportions of the bacterial and fungal nematodes in the community respectively. The values generated are between 0 and 1, where 1 indicates a bacterial dominated energy channel and 0 indicates a fungal dominated energy channel.

1.6 Earthworm communities

Earthworms are segmented metazoans that are important components of the soil biota all around the world. They belong to the class Oligochaeta and it has been estimated that there are 6000 species world-wide (Edwards and Lofty, 1977; Sims and Gerard, 1999; Lavelle and Spain, 2001). In an Irish context, earthworm abundance ranges

from 100 to 400 individuals m^2 in Irish grassland systems and approximately 30 earthworm species have been recorded in the whole of Ireland (Curry et al., 2008). Earthworms thrive in moist soils as they extract water continuously from their environment and must ensure that their cuticles remain saturated to facilitate essential gas exchanges. Earthworms are very important in soil nutrient cycling. This is due to their saprophytic nature, their relationships with microorganisms and their relationships between different earthworm ecological groups. In particular, earthworms have been designated into three different ecological groups (Edwards and Lofty, 1977; Sims and Gerard, 1999; Lavelle and Spain, 2001). These groups denoted as epigeic, anecic and endogeic.

Species from the epigeic group are typically found within the leaf litter of the soil horizon (Edwards and Lofty, 1977; Sims and Gerard, 1999; Lavelle and Spain, 2001). The life cycles of epigeic earthworms involves the consumption of large amount of surface litter and are thus important decomposers of organic matter on the soil surface. The dietary behaviours of epigeic earthworms have been shown to decrease litter quantities but have been shown to increase microbial activity and nutrient leaching into the top soil (Edwards and Lofty, 1977; Sims and Gerard, 1999; Lavelle and Spain, 2001). Examples of epigeic earthworms include *Lumbricus rubellus* and *Lumbricus castaneus*.

The endogeic group of earthworms exhibit a life cycle that is completely subterranean and is sub-divided into polyhumic, mesohumic and oligohumic endogeic groups (Edwards and Lofty, 1977; Sims and Gerard, 1999; Lavelle and Spain, 2001). The distribution of resources within the soil profile has lead to the development of these three endogeic groups. However, all three sub-groups develop horizontal burrows that are not permanent, since these earthworms typically excrete large amounts of soil material whilst moving through the soil fabric. Polyhumic earthworms feed upon the organic matter within the 0 – 10 cm depth range of the soil profile. Mesohumic earthworms ingest large quantities of soil in approximately the 10 – 15 cm depth range (Edwards and Lofty, 1977; Sims and Gerard, 1999; Lavelle and Spain, 2001). The oligohumic are large earthworms that live deep in the profile compared to the polyhumic and mesohumic earthworms, and ingest large quantities of soil to

compensate for the nutrient deficiency of soils at lower depths. Examples of endogeic earthworms include *Apporectodae caliginosa* and *Apporectodae rosea*.

The third group of earthworms are the anecic group. Earthworms from the anecic group feed on surface litter but spend most of their time underground in vertical semi-permanent burrows (Edwards and Lofty, 1977; Sims and Gerard, 1999; Lavelle and Spain, 2001). Anecic species of earthworm feed on plant residues and organic matter from the surface and then deposit this organic material belowground. Anecic earthworms are also characterised by a behavioural response to defecated material. In particular earthworms from this ecological group typically excrete material at the entrance to their burrow on the soil surface. Accumulation of this material at the burrow entrance is called a cast. Examples of anecic earthworms include *Lumbricus terrestrius* and *Aporrectodae longa*.

1.6.1 Earthworm effects on phosphorus cycling

Whilst these different earthworm ecological groups exist, all can greatly impact on nutrient availability in the soils they inhabit. For example, the excretion of polysaccharides and faecal material by earthworms can increase P availability (Sharpley and Syers, 1976; Sharpley and Syers, 1977; Lopez- Hernandez et al., 1993). These polysaccharides are excreted through pores located in the earthworm furrows located between segments. In addition, exudates can either be released by glandular cells in the earthworm epidermis or by pores that directly release fluid from the coelom. These exudates are thought to provide protection for the earthworm by acting as a lubricant for travel through the soil (Sims and Gerard, 1999). The release of such compounds into the soil also increases the incorporation of P into the soil profile (MacKay et al., 1983). This incorporation occurs by both indirect and direct mechanisms. The indirect movement of P down the profile develops when earthworms physically move rock phosphate along the burrow wall. This physical movement of rock phosphate occurs, since it can become attached to the epidermal coating that covers the earthworm and this can facilitate the downward movement of P. The ingestion of rock phosphate by earthworms directly incorporates P into the soil. Rock phosphate ingested by earthworms can be dissolved through the acidic

environment found within the earthworm gut and be excreted in forms that are potential labile. Increasing the distribution of P through the activities of earthworms increases the amount of P available to plant community.

Literature regarding the role of earthworms in the incorporation of P into the soil fabric is sparse, for example the study conducted by Sharpley et al. (2011) appears to be the only one looking at earthworm facilitated P-incorporation following the application of different fertiliser types to a grassland system. More specifically, this study revealed that earthworms facilitated the incorporation of P into the soil profile to a depth of 30 cm. Earthworms were also reported to increase P incorporation following manure and compost application to the soil surface compared to the application of inorganic fertilisers. This increase in P incorporation following organic fertiliser application was attributed to a stimulation of earthworm activity by increasing the quantity of feeding material on the soil surface. However, whilst earthworm activity promoted the incorporation of P following organic amendment, data was also obtained that indicated the accumulation of P into earthworm faecal material on the soil surface (Sharpley et al., 2011). P concentrations in the cast material increased with increasing P fertiliser rate for both inorganic and organic soil amendments. This effect occurred since earthworms were consuming material containing high concentrations of P, which was the direct result of increasing P fertiliser rates. Other studies have also noted that earthworms promote P incorporation into casts (Sharpley and Syers, 1976; Sharpley and Syers, 1977). Results from the study conducted by Sharpley and Syers (1977) indicate that earthworm casts can contribute to the P-enrichment of surface waters in several ways. In particular, cast material was shown to be more susceptible to mobilisation than the underlying soil and also released more P in the water flowing over the soil surface compared to soil material. This was linked to both greater P concentrations and finer particle contents in cast material compared to the underlying soil. Cast material not only included large concentrations of inorganic P, but also contained large concentrations of organic P, which were mineralised by the presence of phosphatase activity in the cast following defecation. When comparing the redistribution of P through earthworm activity, Bayon and Binet (2006) showed that P was distributed both into the soil fabric and in cast material. Concentrations of labile inorganic P were greater in the cast material than the burrowing network throughout the study. However, total P concentrations

were initially greater in burrows two and four weeks following the application of soil and litter material, where as concentrations in the cast material were greater after four weeks. This observation in total P behaviour was ascribed to the use of *L. terrestris* in these experimental mesocosms, since this species is typed as anecic and would actively incorporate organic material from the soil surface into the burrow network. Once incorporated into the burrow network, organic material stimulates microbial proliferation along the burrow walls. These microbial communities also utilise the mucus produced by the earthworm whilst it moves through the burrow. Both of these properties lead to the development of a specific soil niche, which supports microbial communities that differ from those present in the bulk soil.

1.6.2 Earthworm effects on microbial and nematode communities

The ability of earthworms to manipulate both soil nutrient and biological properties is large when compared to the bulk soil (Devliegher and Verstraete, 1996; Devliegher and Verstraete, 1997; Sheehan et al., 2008). Such differences between earthworm manipulated and bulk soil has led to the distinct naming of the earthworm developed environment, the name ascribed to this environment is the drilosphere. The term drilosphere includes the internal environment of the burrow lining, the earthworm gut, the earthworm epidermis and cast material (Lavelle and Spain, 2001). What makes the drilosphere so distinct from the bulk soil is that the drilosphere is enriched with C and N, since earthworm activity promotes the dispersal of exudates, mucus and faecal material along the burrow network (Stromberger et al., 2012). Stromberger et al. (2012) found that the presence of *Lumbricus terrestris* facilitated the incorporation of C and N labelled plant material into the burrow network, which ultimately manifested as an isotopic C signature in one particular phospholipid fatty acid (PLFA) marker present in the drilosphere. The particular PLFA marker was 18:1ω9c fatty acid, which is associated with fungal presence. Therefore, increased nutrient availability in the drilosphere can directly impact microbial phenotypic structures (Stromberger et al., 2012). However, great variability was observed in microbial phenotypes between individual earthworms and their burrow networks, since some individuals promoted communities that were similar to the surrounding bulk soil and others did not. This was attributed to a difference in feeding and burrowing habits between individual

earthworms and the consequential effect this may have on drilosphere nutrient enrichment. Due to the large quantity of labile nutrients in the drilosphere, bacterial dominated communities are typically associated with such areas (Tiunov and Dobrovolskaya, 2002; Devliegher and Verstraete, 1997). However, an instance has been reported where the fungal to bacterial ratio was equivalent between the bulk soil and the drilosphere (Stromberger et al., 2012). This was not only associated with the availability of nutrients in these particular earthworm burrows, but also to the presence of grazing fauna. Indeed, soil fauna, for example nematodes, have been reported to occur in large numbers in the drilosphere and consequently contributes to a large grazing pressure on the microbial community (Tiunov et al., 2001). This grazing pressure has such an effect on the microbial community that it was postulated that a more bacterial dominated community is commonly observed in the drilosphere since the large grazing pressure exerted on the fungal component of the community greatly limits fungal growth (Tiunov and Scheu, 1999).

1.7 Summary

The dynamic of P in agricultural grassland systems is dynamic and complex. Such dynamics are modulated through several biological processes that ultimately affect P distribution and utilisation in these systems. These processes are summarised in the conceptual diagram present below (Figure 1.5). The application of P-based fertilisers to the system accumulates P at the soil surface. P accumulation at the soil surface is susceptible to overland flow and soil erosion, which can contribute to the eutrophication of water-courses. However, accumulated P can also be incorporated into the soil matrix. Such incorporation can be facilitated by the earthworm community, since earthworms can physically incorporate soil material from the surface whilst moving through the burrow network and excrete P rich material formally present at the soil surface deeper within the soil. By incorporating surface material, earthworms can promote a greater distribution of P in the soil matrix. More specifically, greater P distribution includes a greater distribution of water-soluble, labile inorganic and labile organic P. P cycling in the soil is dynamic and P is mainly present in both inorganic and organic forms, both of which can affect the availability

of P to the soil biota. For example, there is equilibrium between water-soluble and labile inorganic P concentrations in the soil, since P is readily adsorbed to and desorbed from soil material or precipitated into and dissolved from liable inorganic complexes. These labile inorganic states can react further and develop into recalcitrant forms that are highly unavailable to the soil biota because the biota can only assimilate P in a solubilised state. Labile organic P can also contribute to biological P acquisition, since these organic forms can be hydrolysed by soil phosphatases. By hydrolysing such organic compounds, P is released into the soil solution which can be readily assimilated by the biota.

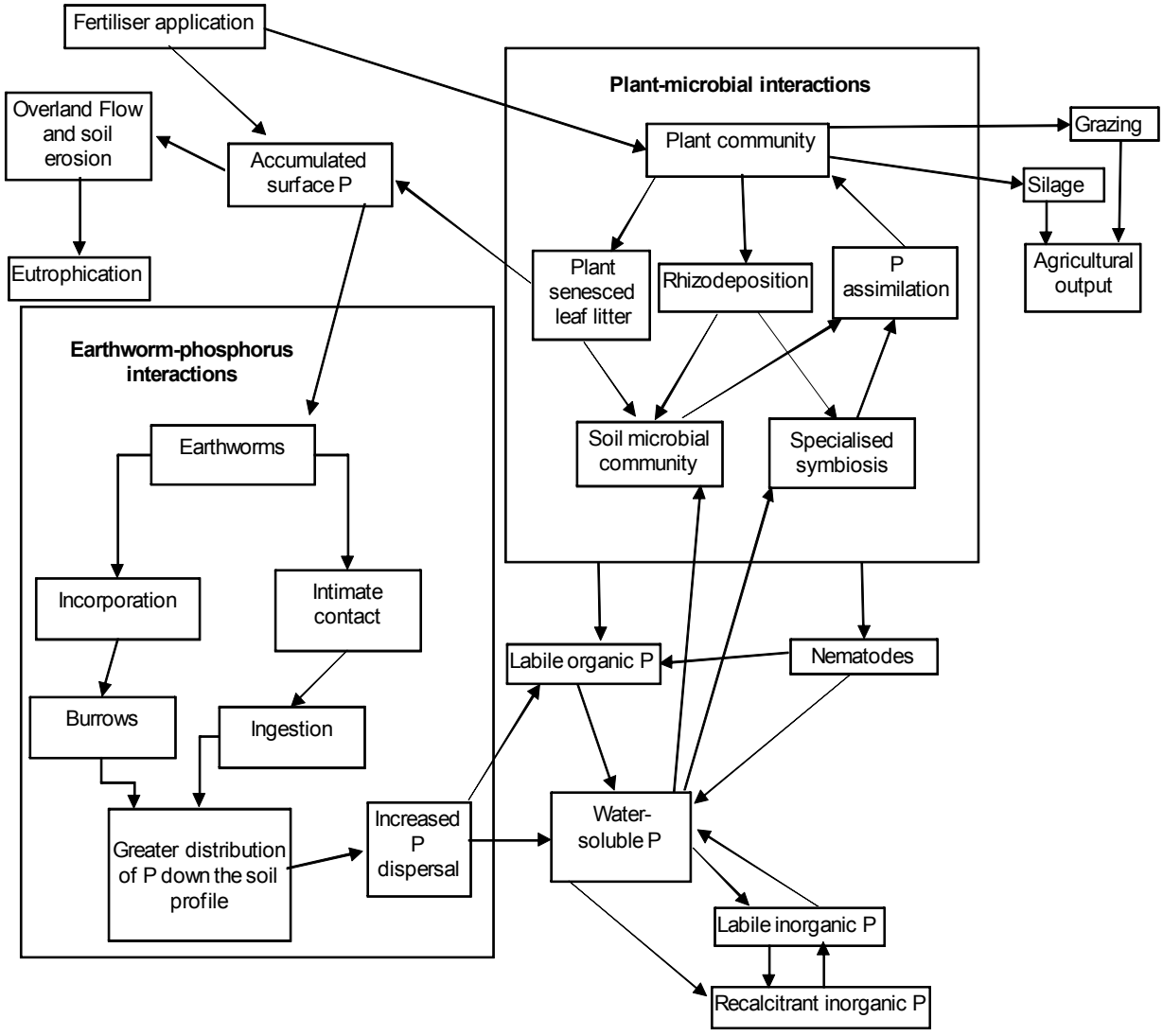

Figure 1.5 Conceptual diagram showing the incorporation and utilisation of P by the soil biota following fertiliser application to the soil surface.

With respect to plant P assimilation, the soil microbial community contributes to a significant quantity of P assimilated by the plant. Through microbial biomass turnover and the development of symbiotic mycorrhizae, the plant community can increase its assimilation of P. High P concentrations in the plant material are requisite for meeting and sustaining the high P demands of intensive agricultural practices, like dairy farming. Whilst the microbial community can aid the plant community, the plant community can also aid microbial growth and activity through the release of C into the soil. C release occurs through both rhizodeposition and litter senescence. Rhizodeposition is a process in which C is inputted into the soil as exudates or root material, which can have direct effects on the soil microbial community. Whilst such interactions between plants and microbial communities facilitate P cycling in the soil, the nematode community also promotes P cycling through their grazing behaviour. By grazing on bacterial, fungal and plant components of the soil biota, the nematode community can increase organic P concentrations in the soil and facilitate the mineralisation of P into a water-soluble form. The senesce of leaf litter onto the soil surface can stimulate saprophytic activity. In particular, saprophytic organisms like earthworms are stimulated and graze on such organic material deposited onto the soil surface. By grazing on this material, earthworms actively incorporate plant and soil material into the soil fabric, which can affect the distribution of P in the soil, as highlighted above.

1.8 Project aims

Given the challenging task to increase grassland productivity whilst ensuring that P fertiliser inputs remain at optimum levels to be economically viable and to minimise environmental impacts, this study ultimately aimed to examine how P fertilisation affected microbial biomass nutrient pools and biologically-mediated P incorporation in grassland systems, and how this related to plant P yields. By examining this, an insight into the best management options to maximise soil biological P cycling in grassland systems may be achieved. By maximising soil biological P cycling, it was envisaged that high grassland productivity could be maintained whilst reducing fertiliser dependency. Indeed, the soil microbial community is recognised as a potential source of P to the plant community and interactions with other soil

organisms mediate the release of such P from the microbial biomass. Furthermore, soil macrofauna may also assist this task by increasing grassland productivity and minimising fertiliser effects on the environment through their ability to facilitate P incorporation into the soil. Thus, the research questions proposed for this project were:

1. Is the soil biota affected by commonly adopted P fertiliser strategies in grassland systems?
2. What consequence does this hold for P acquisition by the plant community?

To answer these research questions, the following overarching hypotheses were set:

- Greater plant P yields will be coupled with greater microbial biomass P concentrations in the soil.
- The application of P-based fertilisers to grassland systems will increase microbial biomass P concentrations and modulate the structure of soil biological communities.
- The presence of earthworms will facilitate the incorporation of localised P-rich material into the soil fabric.

Three experiments were devised which collectively tested these hypotheses. More specifically, these experiments utilised different P fertilisation rates and botanical diversities in field, glass-house and laboratory settings to investigate soil biological P assimilation and incorporation in relation to plant P yields and P distribution in the soil.

2. Materials and Methods

2.1 Microbiological methodologies

2.1.1 Microbial biomass

The C, N and P content of the microbial biomass was determined using the chloroform fumigation extraction method (Brookes et al., 1982; Vance et al., 1987). This methodology uses chloroform to induce cellular lysis of the soil microbial community and causes a flush of microbial-derived nutrients into the soil. The extractable C, N and P content of the soil after fumigation is compared to a control that was not fumigated. The difference between fumigated and unfumigated samples represents microbial-derived nutrients and is then corrected using conversion factors which accounts for the proportion of the C, N and P that is not extracted.

2.1.1 Microbial biomass carbon and nitrogen

Microbial biomass C was measured using a modified procedure based on the extraction method proposed by Vance et al. (1987). Prior to extraction, all soil samples were incubated at 21^{o}C and field moisture content for 7 days to allow equilibration of the biomass following disturbance induced by sampling. After incubation, soil samples were weighed out in duplicate into two glass bottles. One subsample was fumigated using ethanol-free chloroform in a vacuum desiccator for 24 h and then extracted using 0.5 M K_2SO_4 (1:4 soil solution ratio) on a side-to-side shaker for 30 minutes. The other subsample was not fumigated using chloroform but was extracted using 0.5 M K_2SO_4 under the same conditions as the fumigated subsamples.

After extraction, all subsamples were filtered using Whatman No. 2 filter paper. Carbon concentrations were then determined using a Shimadzu TOC-V measuring unit (Shimadzu Corporation, Japan) (McDowell et al., 2006). The principle of this system was to convert K_2SO_4 extractable organic C present in the subsample into CO_2

through the acidification (to less than pH 2) and combustion (at 700°C) of the subsample in the presence of a platinum oxidising catalyst. Following combustion, CO_2 produced was transported in CO_2-free air into a non-dispersive infrared gas analyser and C concentrations were measured. Microbial biomass C was derived using the equation:

$$(FC - NFC) / 0.45 \quad (1)$$

where FC was organic C concentrations extracted from fumigated soil material and NFC was organic C concentrations extracted from non-fumigated soil material, and expressed as μg C g^{-1} dry soil (Vance et al., 1987).

Microbial biomass N was measured using the same fumigation and extraction procedure as microbial biomass C (Brookes et al., 1985). K_2SO_4 extractable nitrogen concentrations were determined using a Shimadzu TNM-1 Total Nitrogen Measuring unit (Shimadzu Corporation, Japan). This system functioned through the acidification (to less than pH 2) and combustion (at 700°C) of the subsample, which produced a flush of nitrous oxide. Following combustion, nitrous oxide was transported to a chemi-luminescence module and N concentrations measured. Microbial biomass N was then determined using the equation:

$$(FN - NFN) / 0.54 \quad (2)$$

where FN was N concentrations extracted from fumigated soil material and NFN was N concentrations extracted from non-fumigated soil material and expressed as μg N g^{-1} dry soil, (Brookes et al., 1985).

2.1.2 Microbial biomass phosphorus

Microbial biomass P measurement was based on the method proposed by Brookes et al. (1982). Before extraction, all soil samples were incubated for 7 days at 21°C. Soil subsamples were then fumigated using ethanol-free chloroform for 24 h using a vacuum desiccator. After fumigation, P was extracted from fumigated and non-fumigated subsamples using 0.5 M $NaHCO_3$ solution (adjusted to pH 8.5) in a 1:20 soil solution ratio and 1 ml of deionised water. A third subsample was also used to

correct for the apparent adsorption of P to the soil after fumigation. With this subsample, the addition of the 0.5 M $NaHCO_3$ (pH 8.5) solution was also coupled with the addition of 1 ml of 25 µg P g^{-1} dry soil weight, where P was applied as potassium dihydrogen orthophosphate (KH_2P0_4). All subsamples were then mixed on a side-to-side shaker for 30 minutes and filtered using Whatman No. 42 filter paper. Orthophosphate concentrations were then manually quantified using the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962). Microbial biomass P was derived using the equation:

$$[25 * (b - a) / 0.4 * (c - a)] \quad (3)$$

where a was the P concentration extracted from the non-fumigated subsample, b was the P concentration extracted from the fumigated subsample and c was the subsample spiked with 25 µg P ml^{-1} (Brookes et al., 1982).

2.1.3 Phospholipid fatty acid analysis

Phospholipid fatty acid analysis (PLFA) was used to determine the phenotypic structure of the microbial community. More specifically, this method used phospholipids derived from the cellular membrane to identify different groups of soil microorganisms. The nomenclature used when quantifying phospholipids was based on the structure of fatty acids that were produced during alkaline methanolysis section of the method. The type of fatty acid nomenclature associated with PLFA analysis in this project was based on molecular structure (Zelles, 1999). The formula X:Y(ω/Δ) Z was used to name fatty acids. X refers to the number of carbon atoms in the molecule and Y denotes the number of double bonds (Frostegard et al., 1993). Using either ω or Δ indicates the position of the double bond, since these denote whether the aliphatic or carboxyl end of the fatty acid should be used to determine the bond position, respectively (Zelles, 1999). The use of a, i and cy as a prefix reflects whether the fatty acid contains anteiso, iso or cyclopropyl branching, respectively. Known PLFA markers were determined using an external standard containing 26 known fatty acids. The bacterial: fungal ratio was determined using these known PLFA markers. Bacterial markers used were i15:0, a15:0, 15:0, i16:0, 17:0, i17:0 cy17:0 and cy19:0

(Bardgett et al., 1999). PLFA 18:2ω6 was used as a fungal marker (Frostegard et al., 1993; Bardgett et al., 1999).

2.1.3.1 Procedure

2.1.3.2 Lipid extraction and fractionation

Prior to analysis, all soil samples were stored at -80^{o}C using an ultra low temperature freezer (New Brunswick Scientific, Edison, USA) and freeze dried (ScanLaf Coolsafe 55-4 freeze dryer, ScanLaf A/S , Denmark). PLFA extraction was determined using a modified procedure described by Frostegard et al. (1991), which was based on the method described by Bligh and Dyer (1959). Briefly, 7 g of freeze-dried soil was weighed out and lipids were extracted using mono-phase Bligh and Dyer reagent that consisted of chloroform, methanol and 0.15 M citrate buffer (1:2:0.8, v/v/v respectively). After the addition of this reagent, the sample was sonicated (Branson 5510 sonicator, Branson Ultrasonics) for 30 minutes, mixed on an end-to-end shaker (Gerhardt Rotoshake RS12, Gerhardt, Germany) for 30 minutes and centrifuged at 700 RCF for 10 minutes using a Sigma Laboratory Centrifuge 4-15 (Sigma, Germany.) The organic top layer of the sample was removed and placed into a new glass media vial. This organic layer was further separated using chloroform and 0.15 M citrate buffer added at a 1:1 ratio. Samples were then centrifuged at 700 RCF for 10 minutes and the aqueous top layer of the sample was removed. The remaining organic layer was dried under a stream of nitrogen at 37^{o}C using a sample concentrator and Dri-Block DB3 (Techne, Germany). Lipids were then divided into fractions using solid-phase extraction. Using SPE cartridges (Silica Cartridge Sep Pak Vac 3 cc (500 mg), Waters Scientific (Product code, WAT020810)), lipids were divided into neutral, glyco- and polar-lipids using chloroform, acetone and methanol, respectively. The polar lipids, including phospholipids, were retained and methyl nonadecanoate was added to each sample as an internal standard. Samples were dried under a stream of nitrogen at 37^{o}C.

2.1.3.3 Mild alkaline methanolysis

Samples were re-constituted using toluene and methanol (1:1 ratio). Methanolysis was initiated using 0.2 M methanolic potassium hydroxide. Samples were incubated for an hour at 37^{o}C. The reaction was terminated using 1 M acetic acid and then hexane and chloroform (4:1 ratio) was added. After the termination of the reaction, samples were sonicated for 30 minutes and the lower aqueous layer was removed. Samples were then filtered through Whatman No. 2 filter paper with 0.5 g sodium sulphate placed onto the filter paper to remove excess water from the sample. Chloroform was used to facilitate the movement of the sample through the filter paper. The sample was then dried under a stream of nitrogen and store at 4^{o}C until further analysis could be carried out.

Fatty acid methyl esters were then separated using gas chromatographer (Varian, USA) and detected using a flame ionisation detector set to 310^{o}C. The column used was a HP-5 (Agilent Technologies) capillary column (30 m in length, 0.320 mm internal diameter and 0.25 μm film.) The initial oven temperature was set to 50^{o}C for 1 minute and then increased at a rate of 25^{o}C min^{-1} until 160^{o}C was achieved. From 160^{o}C, the temperature was increased at 2^{o}C min^{-1} until 240^{o}C was achieved and then increased at 25^{o}C min^{-1} until the temperature reached 310^{o}C. Samples were injected (5 μL) using a CP-8400 autosampler (Varian, USA) set to 310^{o}C. A 26 Bacterial acid methyl ester (BAME) mix external standard (Sigma-aldrich (product code, 47080-U)) was analysed at the beginning of each sample run to identify the common PLFA markers within samples.

2.2 Soil faunal methodologies

2.2.1 Nematode abundance and identification

Once fresh soil was collected (approximately 3 kg) and homogenised by hand, 100 g was elutriated (Seinhorst, 1962) but using a modified procedure with an Oostenbrink elutriator (MEKU Erich Pollähne GmbH, Germany). This form of elutriation involves suspending the sample in solution and passing it through a series of sieves with 180, 120, 95 and 53 μm apertures. Material retained on the 180 and 120 μm sieves was

washed into a 100 μm sieve. Material caught on the 95 and 53 μm sieve was transferred into a 100 μm sieve lined with wet tissue paper. Both of these sieves were placed onto separate Baermann funnels for 48 h, which were pre-filled with water to ensure that soil material was fully suspended. Nematodes were collected by releasing and capturing 50 ml of water from the bottom of the Baermann funnel after this 48 h period.

Samples were left for a day before removing 45 ml of water from the top of the sample. The remaining 5 ml sample was mixed until nematodes were re-suspended and 2 ml transferred to a counting dish, to generate a representative measure of the total number of nematodes in the sample. The number of nematodes within 2 ml was counted using a light microscope. The abundance of nematodes (number g^{-1} dry soil) within the sample was then estimated based upon this representative subsample.

Once counted, nematodes were transferred to a 2 ml microtube and allowed to settle for an hour. After settling, 0.5 ml of water was removed from the microtube and 0.5 ml of 4% formalin solution added and heated at 65^{o}C for 4 minutes (Griffiths et al., 2006) using a BD53 binder oven. After nematodes were preserved, 1 ml of solution was removed. The nematode sample was shaken by hand and a 0.4 ml subsample was wet mounted on a microscope slide (VWR International, Product code, 631-0113)) with cover glass (22 x 32 mm) (VWR International, Product code, 631-0133)). The first 50 located nematodes were then identified to family on an Olympus BX15 compound microscope. Once designated to family (Bongers, 1988), they were classified into trophic groups (Yeates et al., 1993).

2.3 Plant methodologies

2.3.1 Plant dry matter, nitrogen and phosphorus content

Plant material was dried at 70^{o}C for 72 h and weighed to determine plant dry matter. Plant N and P contents were measured by digesting dried plant subsamples using a Gerhardt Kjeldatherm block digester and analysed using a continuous flow peak height analyser (McCormack, 2002). Briefly, one No. 80 ‘Kjeltabs’ tablet (Carl Stuart Limited, Ireland) was added to the dried plant sample, 5 ml of 95% sulphuric acid and

3 ml of hydrogen peroxide were then added to the sample, heated at 150^{o}C for 1 h and then at 390^{o}C for 1.5 h using the block digester. When digested, samples were diluted with deionised water to make the sample volume up to 50 ml. Samples were then filtered using No. 2 Whatman filter paper and a 4 ml aliquot prepared for N and P analysis.

Plant N content was determined based on the ammonia concentrations produced during digestion. Ammonia concentrations were determined by the indophenol method and quantified colourimetrically by reading absorbance at 650 nm (McCormack, 2002).

Plant P content was determined based on orthophosphate concentrations produced during digestion. Orthophosphate concentrations were determined by reacting orthophosphate with a molybdovanadate reagent, which were then quantified colourimetrically by reading absorbance at 420 nm (McCormack, 2002).

2.4 Phosphorus determination methodologies

2.4.1 Labile inorganic phosphorus

A variety of extraction methods exist to extract labile inorganic P from the soil. In this project, two extraction methods were used. One extraction method followed the Morgan's P methodology to determine labile inorganic P as proposed by Peech and English (1944). This method is commonly used to measure nominally labile P concentrations in Irish soils for agricultural advisory purposes (Daly, 2005). The Morgan's extractant is also used to measure labile concentrations of other elements, such as K, Mg and Ca (McCormack, 2002). The other extraction method adopted here was the Olsen P methodology. This extractant is commonly reported in the literature to determine microbial biomass P concentrations, which is based on the original study by Brookes et al. (1982).

2.4.2 Morgan's phosphorus method

Labile inorganic P was extracted from 3 g dry soil using 15 ml of a 0.62 M NaOH and 1.25 M CH_3COOH solution adjusted to pH 4.8 at a 1:5 soil to solution ratio (Peech and English, 1944). The extract was then shaken for 30 minutes on a Brunswick Gyratory shaker and filtered through Whatman No. 2 filter paper. A subsample was taken for labile inorganic P quantification by colourimetry using a Camspec M330B-UV-Visable Spectrophotometer, (Camspec, UK). Another subsample was taken for labile inorganic K quantification by flame emission spectroscopy using a Sherwood Model 420 flame photometer (Sherwood, UK).

2.4.3 Olsen phosphorus method

Labile inorganic P was extracted from fresh soil (equal to 5 g dry weight) using 100 ml of a 0.5 M $NaHCO_3$ solution that had been adjusted to pH 8.5 at a 1:20 soil solution ratio (Brookes et al., 1982). Extractant and soil was mixed on a side-to-side shaker for 30 minutes and filtered using Whatman No. 2 filter paper. Labile inorganic P concentrations were then determined by colourimetry using a Cary 50 conc UV-visible spectrophotometer (Varian, USA).

2.4.4 Phosphorus measurement

Phosphorus concentrations in both extractants mentioned above were determined using the molybdate-ascorbic acid method (Murphy and Riley, 1962). This reaction uses potassium antimonyl tartrate to stabilise and induce a rapid reaction between molybdenum cations and orthophosphate anions. The reaction between the molybdenum and orthophosphate reduces the molybdenum ions. Such reduction results in a blue coloured solution which was then quantified colourimetrically by measuring absorbance at 880 nm.

2.5 Generic soil property methodologies

2.5.1 Soil moisture and dry matter content

The soil moisture content was determined by adding 10 g of fresh soil to a pre-weighted crucible. Samples were then oven dried at 105^{o}C for 24 h. After drying, samples were re-weighed. The moisture and dry matter contents were determined using the following equations:

Moisture content; (M1 - M2) / (M1 – M0) = g water mass g^{-1} fresh soil (4)

Dry matter content; (M2 – M0) / (M1 – M0) = g dry soil g^{-1} fresh soil (5)

Where M1 was the weight of fresh sample and container, M2 was the weight of dried sample and container and M0 was the weight of the container.

2.5.2 Soil water holding capacity

Soil water holding capacity was measured by placing 50 g fresh soil into glass filter funnels fitted with rubber tubing, a clamp and glass-wool (Watwood et al., 1991). Once soil was added, 100 ml of water was added to the soil and covered to prevent evaporation. After 30 minutes, the clamp was opened and the water drained for 24 h. Soil subsamples were collected after this time period and 10 g of fresh soil was weighed out into a pre-weighed container. These subsamples were dried at 105^{o}C for 24 h and re-weighed. The water holding capacity was calculated as the moisture content per gram of fresh soil (Watwood et al., 1991) using the calculation (4) stated above.

2.5.3 Organic matter content

The organic matter content of soils was estimated using the loss-on-ignition method (Rowell, 1994). Briefly, 4 g dry weight of soil was weighed into a pre-weighted crucible and dried at 105^{o}C for 24h. After drying, soil was re-weighed to check that the sample contained no water, placed into a muffle furnace and combusted at 500^{o}C

overnight. Soil was cooled in a desiccator containing silica gel before being weighed again. Soil organic matter content was determined by:

$$\text{Loss-on-ignition (\%)} = ((R1 - R2) / R1) * 100 \quad (6)$$

Where R1 was the pre-combustion soil weight (g dry soil) and R2 was the post-combustion soil weight (g combusted soil)

2.5.4 Soil pH

The pH of the soil was estimated by adding 10 g of dried soil to 20 ml of deionised water (1:2 soil solution ratio) (McCormack, 2002). Samples were stirred prior and during pH determination. The pH was then measured using an Aqua-analyser automated sampling system (Gilson Inc, USA) fitted with a Mettler Toledo electrode (Mettler Toledo, Switzerland/USA).

2.5.5 Total carbon and nitrogen analysis

Total C and N were measured using a LECO TrueSpec C N elemental analyser following ISO 10694-1995 (International Standardization Organization, 1995) regarding soil preparation and then analysed by combustion following the manufacturer's procedure (LECO Corporation, 2010). Soil samples were dried at 105^{o}C, milled to < 0.1 mm and aliquots (0.2 g) of material were prepared. The determination principle is based upon the combustion of soil at 950^{o}C followed by further combustion using an after-burner set to 850°C. Total C concentrations were quantified by the infrared detection of CO_2 concentrations produced from the combustion process. Total N concentrations were quantified from the production of N_2O during the combustion process. N_2O produced by the combustion process was reduced using a copper filter and quantified by thermal conductivity using He as a carrier gas.

3. Impacts of inorganic phosphorus fertilisation on the soil biota in an ungrazed pasture

3.1 Introduction

The application of fertiliser to grassland systems is a common practise that aims to increase growth and productivity of the vegetation. There are different fertiliser types that function by supplying specific nutrients to the soil. One such type includes P-based fertilisers that are applied to adjust the P status of the grassland soil. P can be inputted into the grassland system through the use of both organic and inorganic P fertilisers (Bunemann et al., 2011). The application of organic fertiliser typically includes both organic and inorganic P-pools that are both bioavailable or labile in behaviour, or more recalcitrant in terms of bioavailability (Bunemann et al., 2011). Inorganic P fertilisers solely contribute an inorganic P addition to the soil in which a flush of bioavailable P occurs within a short timeframe after application (Stewart, 1991; Stevenson and Cole, 1999). However, bioavailable P amendment of the soil disrupts the equilibrium between P in the mobile phase (water-soluble bioavailable P) and the solid phase (bound P), which ultimately develops into the adsorption of water-soluble P to the soil fabric, rendering it unavailable for biological assimilation. This inability of the plant community to readily assimilate P that has become bound to the soil matrix results in either the impairment of grassland growth or the over compensation of applied fertiliser P by the farmer to ensure that enough bioavailable P is supplied to the sward (Stewart, 1991). Furthermore, the adsorption of P to the soil matrix can also influence the distribution of P in grassland systems. Owens et al. (2008) showed that soil P in grassland systems exhibits a skewed spatial distribution towards greater concentrations of P at the soil surface, relative to concentrations further down the soil profile. One possible explanation for this observation is that the application of inorganic P fertiliser to the soil surface in a system that is rarely ploughed and mixed can lead to P accumulation in this zone, since applied P is readily adsorbed by the soil fabric in such area (Owens et al., 2008).

Whilst soil chemical processes can affect P availability, the soil microbial biomass is also regarded as an important factor governing the availability of P to the plant community (Brookes, 2001; Achat et al., 2010). The turnover of microbial biomass has been shown to release labile pools of organic P which can be mineralised and ultimately be utilised by the plant community. Based on this P-release as the microbial biomass turns over, the biomass can be viewed as a potential slow release pool of labile organic P into the soil (Brookes, 2001). Nevertheless, the actual size and activity of the microbial biomass is dependent on multiple soil factors, such as the availability of other soil nutrients, moisture content and soil type (Gregorich et al., 1991; Thomsen et al., 2003; Demoling et al., 2007). Soil moisture content and soil type affect microbial biomass turnover and therefore biomass size. In particular, increased clay content and moisture content has been reported to increase the size of the nutrient pool of the microbial community due to a reduction in biomass turnover (Gregorich et al., 1991; Thomsen et al., 2003). Microbial biomass turnover under such circumstances occurs since increasing the clay content increases the resistance of soil organic matter to decomposition and increases soil moisture contents. Therefore, with increasing moisture content and protected organic matter, the soil microbial community is less active and biomass turnover occurs more slowly. With respect to soil nutrient status and its effect on soil microbial communities, one of the main nutrients known to be limiting microbial growth is C, which in turn limits assimilation of P (and other elements) (Demoling et al., 2007; Bunemann et al., 2011). The plant community is a primary source of C to the soil microbial community (Bardgett, 2005). Through rhizodeposition and plant senescence, plants supply different forms of C both directly to the soil surface via litter deposition and within the soil matrix via rhizodeposition. Such input of C stimulates the soil heterotrophic community and aids in nutrient cycling.

Other soil organisms are also associated with regulating the activity of the soil microbial community. Such organisms include nematodes, which feed on bacterial, fungal and plant components of the biota have been shown to stimulate the microbial community through grazing (Ingham et al., 1985). By grazing on the microbial community, the turnover rate of microbial biomass increases and released nutrients aid in the synthesis of both microbial and plant biomass. Due to the extensive trophic behaviours observed across all species of soil nematodes, they are often regarded as

bio-indicators (Yeates, 2003). These indicators have been used to detect indirect shifts in microbial and plant communities. One concept that has been commonly applied to investigating shifts between bacterial and fungal components of the microbial community is the 'nematode channel index' (Yeates and Bongers, 1999; Li et al., 2007; Viketoft, 2008). This index is commonly used to indicate decomposition processes in the soil as fungi and bacteria utilise different strategies to cycle soil nutrients. In soils which are regarded as being more stable and dominated with greater recalcitrant organic matter contents, fungal dominated microbial communities occur (Bardgett and McAlister, 1999). Bacterially-dominated communities are associated with soils which contain greater labile substrate concentrations.

Earthworms also play an important role in the decomposition of soil organic matter (Lavelle and Spain, 2001). More specifically, the ingestion and excretion of organic material results in the breakdown of complex organic substrates and thus increases nutrient availability to other soil organisms (Lopez-Hernandez et al., 1993). By increasing nutrient availability, earthworms are an important group of organisms that promote nutrient transformations and contribute to the soil fertility. The population size and activity of earthworms is typically dependent on the soil organic matter content of grassland systems (Fraser et al., 1994; Curry et al., 2008). Therefore, the management regime of grasslands is of particular importance with respect to the earthworm community. More specifically, the application of organic fertilisers to the soil surface may act to stimulate the growth of the earthworm community, since this organic material may provide a source of food (Curry et al., 2008).

Unlike earthworms, the effects of fertiliser application on the microbial and nematode communities are not so clear in the literature (Yeates, 1976; Ross et al., 1995; Sarathchandra et al., 2001; Gichangi et al., 2009), as mixed results of P fertilisation on microbial and nematode communities have been reported. One common theme observed between these studies is linked to the joint application of both organic and inorganic fertiliser (Gichangi et al., 2009). Application of both inorganic and organic fertiliser has been shown to increase microbial biomass P concentrations above those observed from a sole application of inorganic fertiliser. But other studies show either inconsistent effects of P application on microbial biomass P over time or no effects at all (Ross et al., 1995; Sarathchandra et al., 2001). With respect to nematode

communities, responses to P fertilisation appeared to be coupled with livestock grazing, which would result in greater nutrient input back into the soil as faecal material. Such a return of nutrients stimulates microbial growth in the soil and in turn increases nematode numbers (Yeates, 1976). P fertiliser responses have been observed on particular nematode genera despite the management regime adopted (Yeates, 1976; Sarathchandra et al., 2001). However, the nematode genera affected differs between studies; hence a clear effect on community structure is hard to conclude.

With equivocal data from the literature regarding P fertilisation effects on soil biology, an experiment was conducted on different soil types that had received long-term applications of inorganic P fertiliser (>15 years). The aim of this experiment, denoted the main experiment hereafter, was to investigate how the soil biological community was affected by inorganic P fertiliser applications. The application of inorganic fertilisers only was chosen in this experiment since a coupling between plant productivity and microbial community dynamics is a theme for the project as a whole and the application of organic fertilisers may mask such coupling. Furthermore, by using two soil types that had received fertiliser applications for longer than 15 years, it was envisaged that an established response could be ascertained which would ultimately provide strong evidence for any observed effects. To achieve this aim, the following objectives and respective hypotheses were established:

Objective 1. To investigate how increasing P fertiliser rates affect aspects soil biomass

- Increasing P fertilisation will increase microbial biomass P concentrations in the soil.
- Increasing P fertilisation will increase earthworm biomass.

Objective 2. To examine how P fertilisation affects biological community structure in the soil

- The phenotypic structure of the soil microbial community will be responsive to P fertilisation rate.
- The application of P fertiliser will affect nematode community structure, which will manifest as changes in the relative proportions of trophic groups.

A preliminary study was conducted to initially establish the vertical distribution of P in both of these studied grassland sites in order to inform the prescription of an appropriate sampling strategy. It was posited that sampling to a depth of 10 cm, which is typically used in studies looking at biological properties in a field setting, may have been the incorrect strategy to use since P fertilisation effects may have been localised to the 0 - 1 cm depth range in this experiment due to P accumulation dynamics in fertilised grasslands as stated above. Therefore, sampling to a depth of 10 cm may dilute any P fertiliser effect and may lead to potentially erroneous conclusions about biological responses to P fertilisation. Therefore, the aim of the preliminary study was to validate the sampling depth adopted to sample the two grassland sites used during the course of the main experiment. The specific objective of this study was to establish how labile inorganic P concentrations change with depth in grassland soils that have been fertilised at contrasting rates of P application to the soil surface. Thus, the specific hypotheses were:

- There will be a decreasing concentration gradient of labile inorganic P with respect to depth, which is reflective of the application rate of applied P to the soil surface during fertilisation.
- Concentrations of labile inorganic P will be greater in the 0 - 1 cm depth of the soil profile relative to the 1 - 5 and 5 - 10 cm depths.

3.2 Materials and Methods

3.2.1 Field sites and sampling

All sampling was carried out on two long term sites located in the dairy farm area at Johnstown Castle, Wexford, County Wexford, Ireland (latitude 52 ^{o}17 N, longitude 06^{o}30 W) (Daly, 2005). Site 1 is founded on a sandy loam soil (32% coarse sand, 28% fine sand, 33% silt and 14% clay) and Site 2 on a loamy sand soil (70% coarse sand, 23% fine sand, 4% silt and 3% clay). Both of these sites received four different P application rates, *viz*. 0, 15, 30 and 45 kg P ha^{-1} y^{-1} of 16% superphosphate (2 $CaSO_4$ + $Ca(H_2PO_4)_2$) (hereafter denoted P0, P15, P30 and P45, respectively). P was applied to each plot in February each year. There were four replicates of each treatment within

a site and, therefore, 16 plots per site. Each plot had the dimension 10 x 2 m^2. Different P application rates were randomly positioned within a site according to a randomised block design (Appendix Figure A_1A). These trials were set up in February 1995 and thus had been established for 17 years prior to the onset of this study. Before the establishment of these trials, both sites were used for dairy farming. The available data is present in Appendix 1. For further background information on these sites before the experiment conducted for this project was commenced, see Appendix Table A_1A, A_1B and Figure A_1B.

Aboveground plant material in both sites was cut eight times per year to a height of 5/6 cm using a plot harvester. All cuttings were removed and sampled for dry matter yield and plant P yield; these methods have been described in Chapter 2. Data pertaining to these properties from October 2009 to April 2011 have been noted in this study. After harvesting plant material in both sites, all plots received 40 kg N ha^{-1}, as calcium ammonium nitrate (5 $Ca(NO_3)_2$ NH_4NO_3), and K as muriate of potash (KCl) was also applied to site at a rate of 125 kg K ha^{-1} y^{-1} to compensate for potassium removal from the system.

3.2.2 Preliminary study: Soil phosphorus distribution in a grassland system

The sampling sites and their associated management were described above. In this study, five cores were taken using a hand auger (3 cm diameter) from each plot at the nodes of a W-of-best-fit sampling design to ensure representative non-biased sampling in February 2010. Each core was taken to a depth of 10 cm and sub-divided into 0 - 1, 1 - 5 and 5 - 10 cm subsamples in the field. The samples from each respective depth were then bulked to achieve three composite depth-based samples per plot. Samples were sieved to pass through an aperture of 2 mm, air dried and labile inorganic P concentrations were then determined using the Morgan's P procedure as described in Chapter 2.

3.2.3 Main experiment: Phosphorus fertilisation effects on the soil biota

Soil samples were taken from plots in October 2009, April 2010, October 2010 and April 2011. Soil and air temperatures and rainfall from October 2009 to April 2011 were collected from the Johnstown Castle weather station. At each sampling occasion, five cores were taken from each plot at the nodes of a W-best-of-fit sampling design. Each core was taken to a depth of 10 cm. After extracting cores from each plot, these cores were bulked to form a composite sample. Soil samples were firstly broken up by hand, homogenised and then 100 g fresh weight of soil was subsampled for nematode analysis. Remaining soil was coned, quartered and subsampled for 4 mm and 2 mm sieving. Soil subsampled to 4 mm was subsampled for PLFA analysis and the soil that was sieved to 2 mm was subsampled for microbial biomass C, N and P. During the course of these experiments, all fresh soil samples were stored at 4^{o}C. Different sieve sizes were used in this experiment since the aim was not to relate such properties but to examine how they were affected by P fertilisation. By sieving to 4 mm for PLFA analysis, less disturbance of the soil matrix would occur compared to 2 mm sieving (Petersen and Klug, 1994). By comparing 2 mm and 4 mm sieving, Petersen and Klug (1994) showed sieving below 4 mm decreased the fungal marker 18:2ω6 compared to other markers, which would ultimately influence statistical analysis of the community structure. Sieving to 2 mm was chosen for all other parameters because this would enable for more comprehensive comparisons with the literature, especially in the case of microbial biomass C, N and P concentrations, since sieving to 2 mm is a more common practice for determining such concentrations (Sarathchandra et al., 2001; Turner et al., 2001; Liu et al., 2012).

Remaining soil was air dried, sieved to 2 mm and used to determine pH, organic matter, labile inorganic K and Morgan's P. All determinations above were as described in Chapter 2. Total P concentrations were determined in soil collected in April 2011 using the aqua regia digestion method. Soil samples were dried at 105^{o}C, milled <0.1 mm and 2 g of milled material were weighed into digestion tubes. Total P was only measured at one time interval since it was judged that concentrations would not significantly change during the experimental period. Both 16 ml of hydrochloric acid and 4 ml of nitric acid were then added to these digestion tubes, which were then

placed into a digestion block. Samples were digested at 140^{o}C for 2 h. After digestion, samples were filtered using No 2 Whatman filter paper and the filtered extract made up to a final volume of 100 ml using 2 M nitric acid. Extracted P concentrations were then measured using inductively coupled plasma optical emission spectroscopy.

Earthworms were extracted using the Octet method (Schmidt, 2001) in April and October 2010. Vegetation was clipped to the soil surface and eight stainless steel electrodes were pushed into the soil (40 cm deep) at marked positions around a sampling ring (area 0.125 m^2). The stainless steel rods were joined to the sampling ring using the designated connector clips and the octet machine attached to a 105 AHR 12-V car battery. The machine was turned on and voltage increased from 200/250 V to 500/600 V at 5 minute intervals for the first four steps and 10 minutes for the remaining two steps. Earthworms were collected from within the ring when they fully emerged. After extraction, the area within the ring was checked to ensure the recovery of individual earthworms that were missed. Live earthworms were then weighed, inclusive of gut content.

3.2.4 Statistical analyses

For the preliminary study, data was analysed by 3-way factorial ANOVA (Stats Soft, 1984 - 2011) using Site, P fertilisation rate and Sampling depth as main terms. A *post hoc* Fisher's least significance test was applied to establish homogeneous groups of means at the 95% level of significance.

For the main experiment, data were analysed using factorial repeated measures ANOVA (Stats Soft, 1984 - 2011). Site, P fertilisation rate and sampling occasion as main effects, with sampling occasion as the repeated measure level. Total P data was analysed using factorial two-way ANOVA since data was only obtained in April 2011 and not a repeated measure. Microbial community structure and nematode community structure profiles were analysed using principal component analysis (PCA). Resultant principal components (PC) were subsequently analysed using factorial repeated measures ANOVA as above.

3.3 Results

3.3.1 Preliminary study: Soil phosphorus distribution in a grassland system

There were no third-order interactions in the ANOVA on Morgan's P concentrations in the soil, but two second order interactions were apparent (Table 3.1).

Table 3.1 ANOVA table relating to significance terms in soil labile inorganic P concentration (µg P g^{-1}) with respect to site, P fertilisation rate (P treatment) and depth (Sampling depth). Significance terms denote *** $p<0.001$; * $p<0.05$; blank space $p>0.05$.

	Morgan's P (µg P g^{-1})
Site	***
P treatment	***
Sampling depth	***
Site x P treatment	*
Site x Sampling depth	
P treatment x Sampling depth	***
Site x P treatment x Sampling depth	

A significant interaction between P fertiliser rate and sampling depth was observed, where P concentrations increased with increasing P fertilisation rate at each sampling depth. There was a greater increase in P concentration with P fertilisation in the 0 - 1 cm depth compared to 1 - 5 and 5 - 10 cm (Figure 3.1). This was further supported by the *post hoc* analysis as there was a significant difference between all fertiliser rates in the 0 – 1 cm depth, whereas differences between all fertiliser rates were attenuated at the other depths (Figure 3.1).

This interaction also showed that P concentrations decreased with depth in the presence of all P fertiliser regimes including the P0 treatment (Figure 3.1). Decreases in P concentration with depth differed between treatments, with the largest decrease in the P45 treatment and smallest decrease in the P0 treatment.

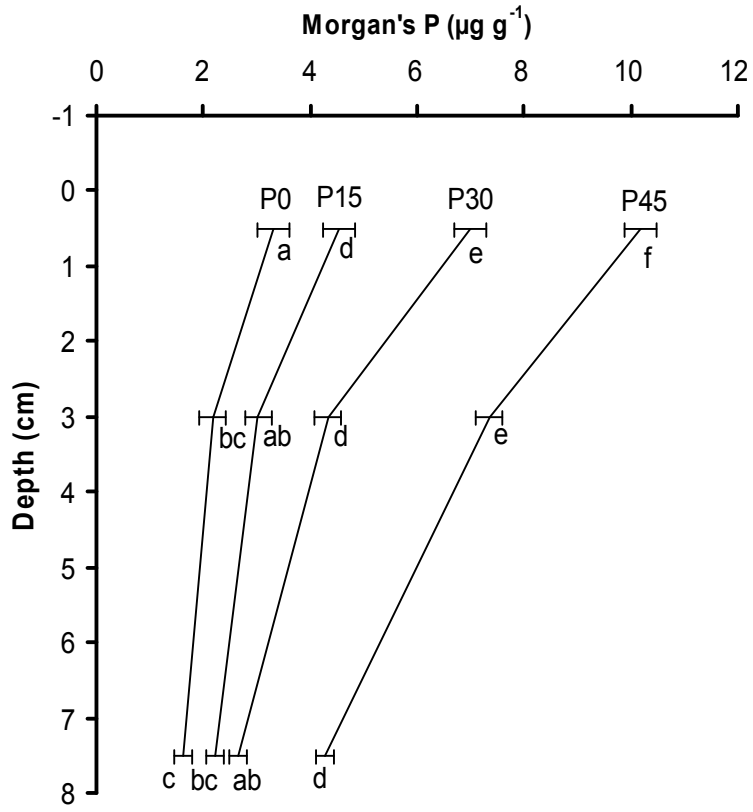

Figure 3.1 Mean concentrations of labile inorganic P sampled at three depths in the presence of four P fertilisation regimes. P treatment is displayed above respective curve. Means pooled across sites. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Bars denote standard error**.**

There was also a significant site by P treatment interaction (Table 3.1), which showed that higher concentrations of P were apparent in Site 1 compared to Site 2 with a greater increase in P with increasing P fertilisation rate observed in Site 1 (Figure 3.2).

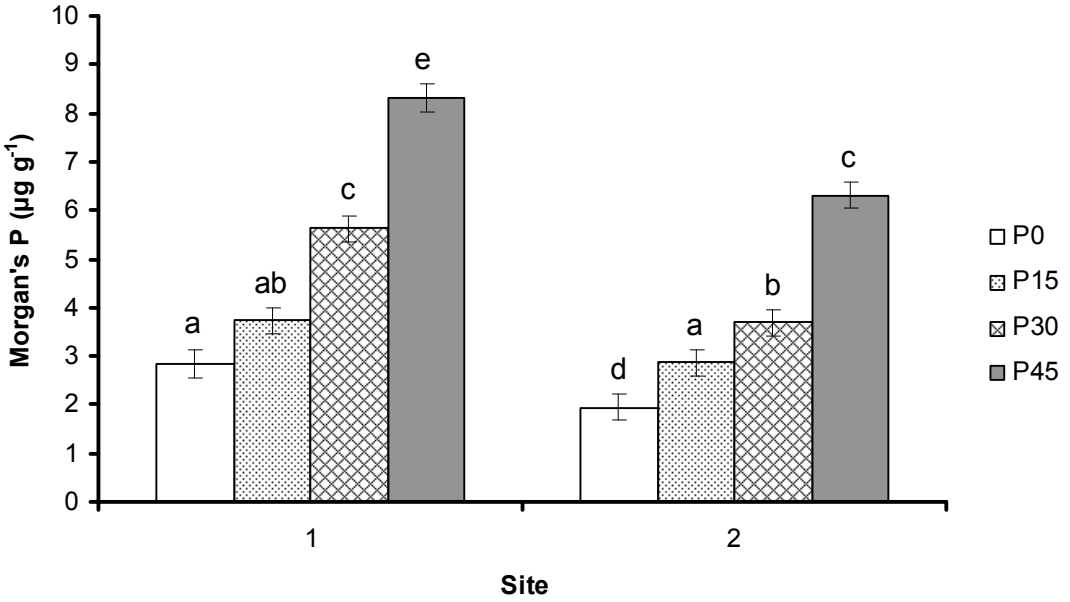

Figure 3.2 Mean labile inorganic P concentrations in two sites in the presence of four P fertiliser regimes. Means pooled across depths. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

3.3.2 Main experiment: Phosphorus fertilisation effects on the soil biota

3.3.2.1 Weather conditions

With respect to the sampling occasions adopted for this experiment, the soil and air temperatures were similar at all four occasions (Figure 3.3(a)). The range of soil temperatures observed for these occasions were between 12.8 ± 0.17 and 10.3^{o}C ± 0.31 S.E. Air temperature during these occasions ranged from 12.1 ± 0.26 to 8.5^{o}C ± 0.36 S.E. The greatest difference between soil and air temperatures occurred in April 2010 (soil temperature; 10.3 ± 0.31, air temperature; 8.5^{o}C ± 0.36 S.E., whereas soil and air temperatures in October 2009, October 2010 and April 2011 were similar.

Unlike soil and air temperatures, greater rainfall was observed in October 2009 and 2010 (5.2 ± 1.8 and 3 mm ± 0.8 S.E., respectively) compared to April 2010 and 2011 (0.9 mm ± 0.5 S.E. for both dates) (Figure 3.3(b)).

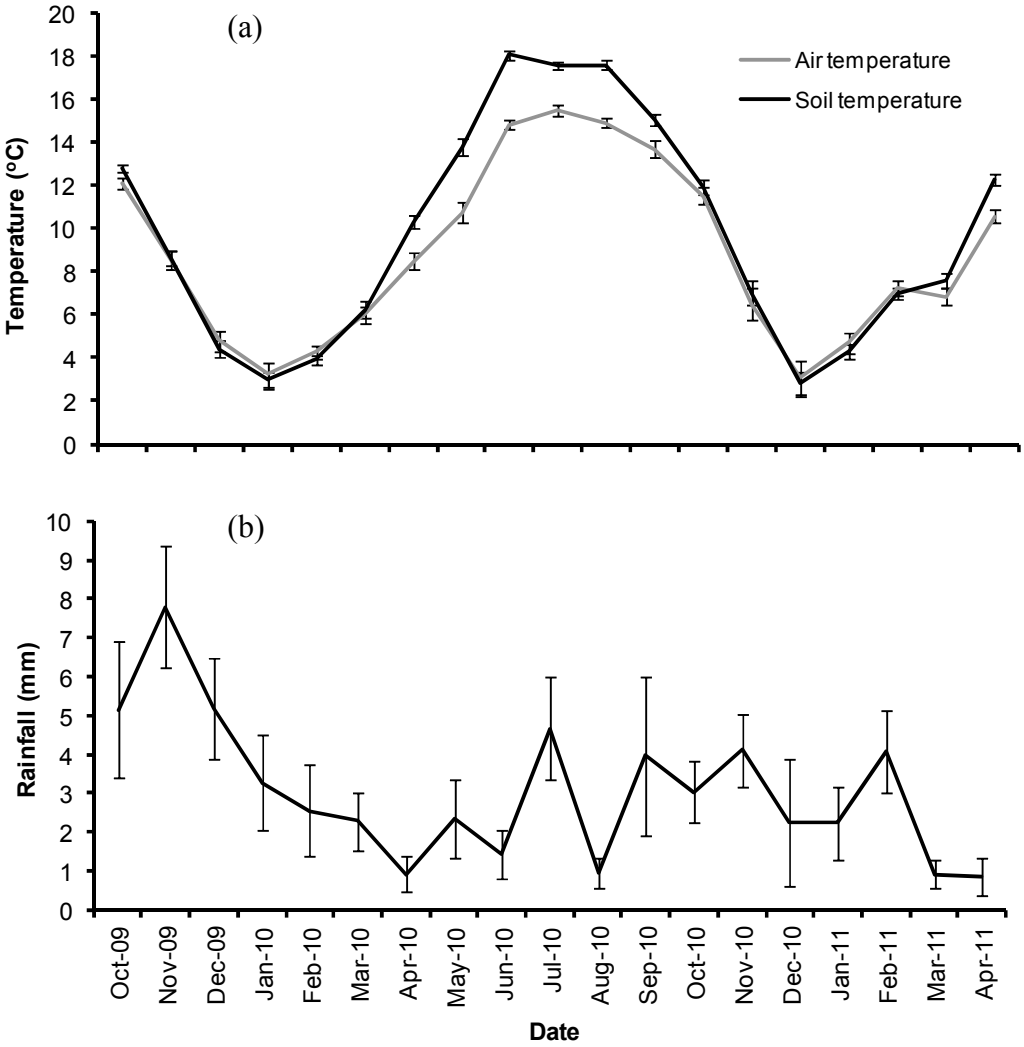

Figure 3.3 Environmental conditions in the Johnstown Castle estate from October 2009 to April 2011, (a) denotes air and soil temperature and (b) denotes rainfall. Error bars are standard error.

3.3.2.2 Plant properties

No second-order interactions were apparent on accumulated plant dry matter and P contents (Table 3.2). Nevertheless, a P fertilisation effect was observed on the total amount of plant dry matter that accumulated over the experimental period (Table 3.2). This effect showed an increase in dry matter with increasing P fertilisation, but a plateau between P30 and P45 treatments (Table 3.3). The amount of P removed by the plant community over the experimental period was affected by both P fertilisation and Site (Table 3.2). Increasing P fertilisation increased the total amount of plant P

removed from the system (Table 3.3). More plant P was removed in Site 1 compared to Site 2 (Table 3.4).

Table 3.2 ANOVA table showing the significance terms of total plant dry matter and total plant P over the course of this is experiment with Site and P treatment as main effects and its interaction. Significance terms denote *** $p<0.001$; ** $p<0.01$; blank space $p>0.05$.

	(kg m^2)	(g m^2)
	Total dry matter	**Total plant P**
Site		**
P treatment	***	***
Site x P treatment		

Table 3.3 Mean total plant dry matter and total plant P over the course of this experiment in the presence of four P fertiliser regimes. Different Letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Standard error is represented as error mean squares (EMS).

	(kg m^2)	(g m^2)
P fertilisation rate (kg P ha^{-1} y^{-1})	**Total dry matter**	**Total plant P**
0	1.38 a	1.87 a
15	1.67 b	2.93 b
30	1.79 c	3.86 c
45	1.79 c	4.42 d
EMS	0.01	0.07

Table 3.4 Mean total plant dry matter and total plant P yields over the course of this experiment in two sites. Standard error is represented as error mean squares (EMS).

Site	(kg m^2) Total dry matter	(g m^2) Total plant P
1	1.67	3.44
2	1.64	3.10
EMS	0.01	0.07

3.3.2.3 General soil properties

A third-order interaction was observed on labile inorganic P concentrations measured using the Morgan's P procedure (Table 3.5). This related to the subtle and complex interactions between the factors for which no clear trend could be identified. There were also more significant second-order interactions between P fertilisation rate and Time, and Site and Time (Table 3.5). When looking at the interaction between P fertilisation and Time, there was a general increase in Morgan's P with increasing P fertilisation rate but the change in P over time differs in the P45 treatment compared to all other treatments (Figure 3.4(a)). The interaction between sites over time was founded on increased P concentrations apparent in Site 1 compared to Site 2 on three sampling occasions, but there was a decrease in Site 1 P concentrations in October 2010, which were similar to concentrations in Site 2 (Figure 3.4(b)).

Table 3.5 ANOVA table showing the effects and interactions of P fertilisation, site and sampling occasion (Time) on general soil properties. Significance terms denote *** p<0.001; ** p<0.01; * p<0.05; blank space p>0.05.

	(µg g^{-1})					
	Morgan's P	Olsen P	K	Extractable OC	LOI (%)	pH
Site	***	***			***	
P treatment	***	***	***			
Time	***	***	***	***	***	***
Site x Time	**	*			***	*
P treatment x Time	***	***				
Site x P treatment						
Site x P treatment x Time	*					

No third-order interaction was observed on the concentrations of labile inorganic P when measured using the Olsen P procedure (Table 3.5). Nevertheless, a significant interaction between P fertilisation and sampling occasion was observed which showed an increase in Olsen P with increasing P fertilisation but changes in P concentration over the experimental period differed in the P45 treatment compared to all other treatments (Figure 3.5(a)). A Site x Time interaction was also observed with greater P concentration in Site 1 compared to Site 2. In Site 1, there was a decrease in P concentration over time until October 2010: P concentrations between sites were similar in October 2010. This was followed by a greater increase in P in Site 1 compared to Site 2 (Figure 3.5(b)). A strong positive correlation was observed between Olsen P and Morgan P concentrations ($R^2 = 0.76$) (Figure 3.6).

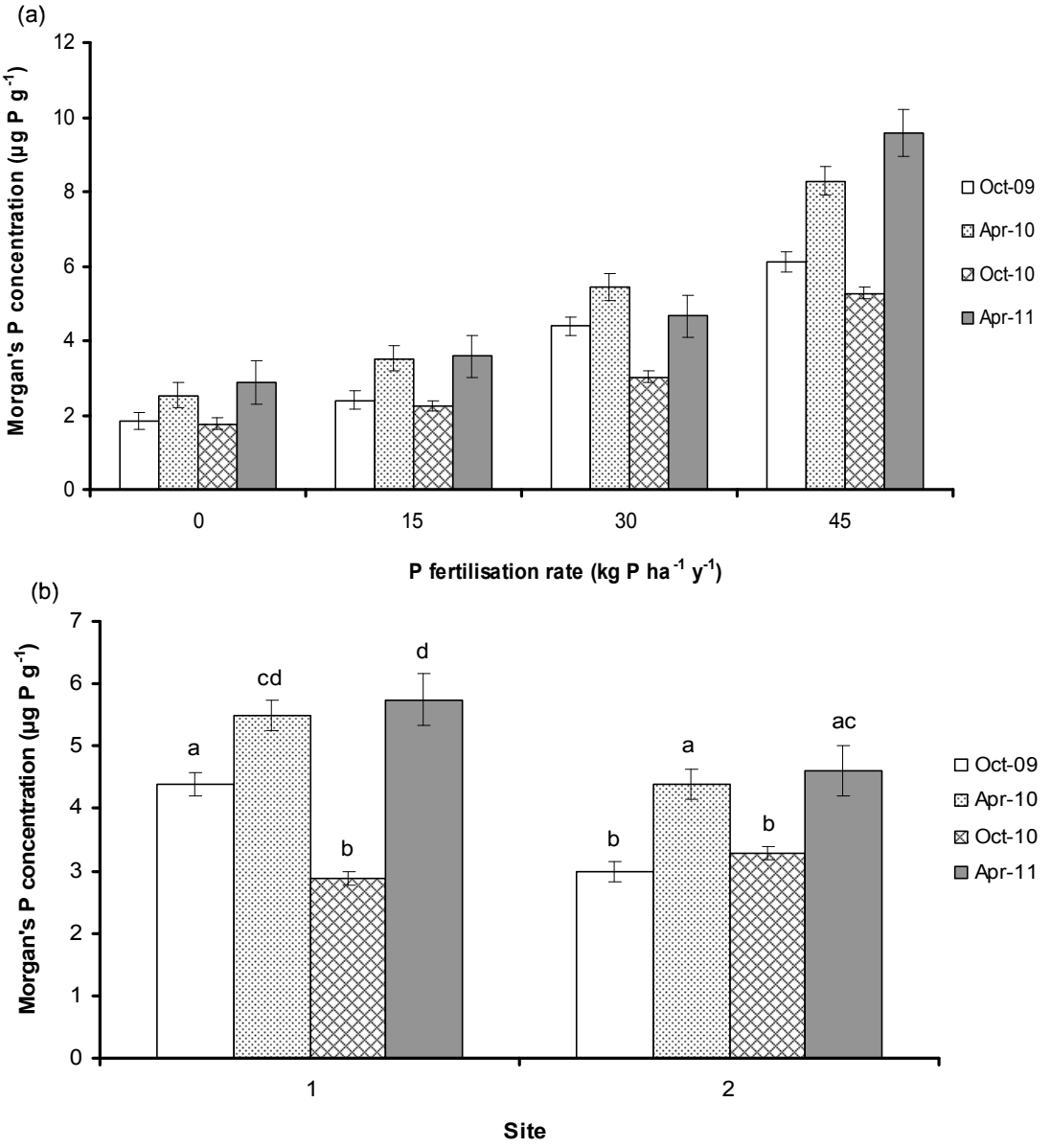

Figure 3.4 Mean Morgan's P concentrations expressed by (a) the interaction between P fertilisation and sampling occasion and (b) the interaction between site and sampling occasion. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

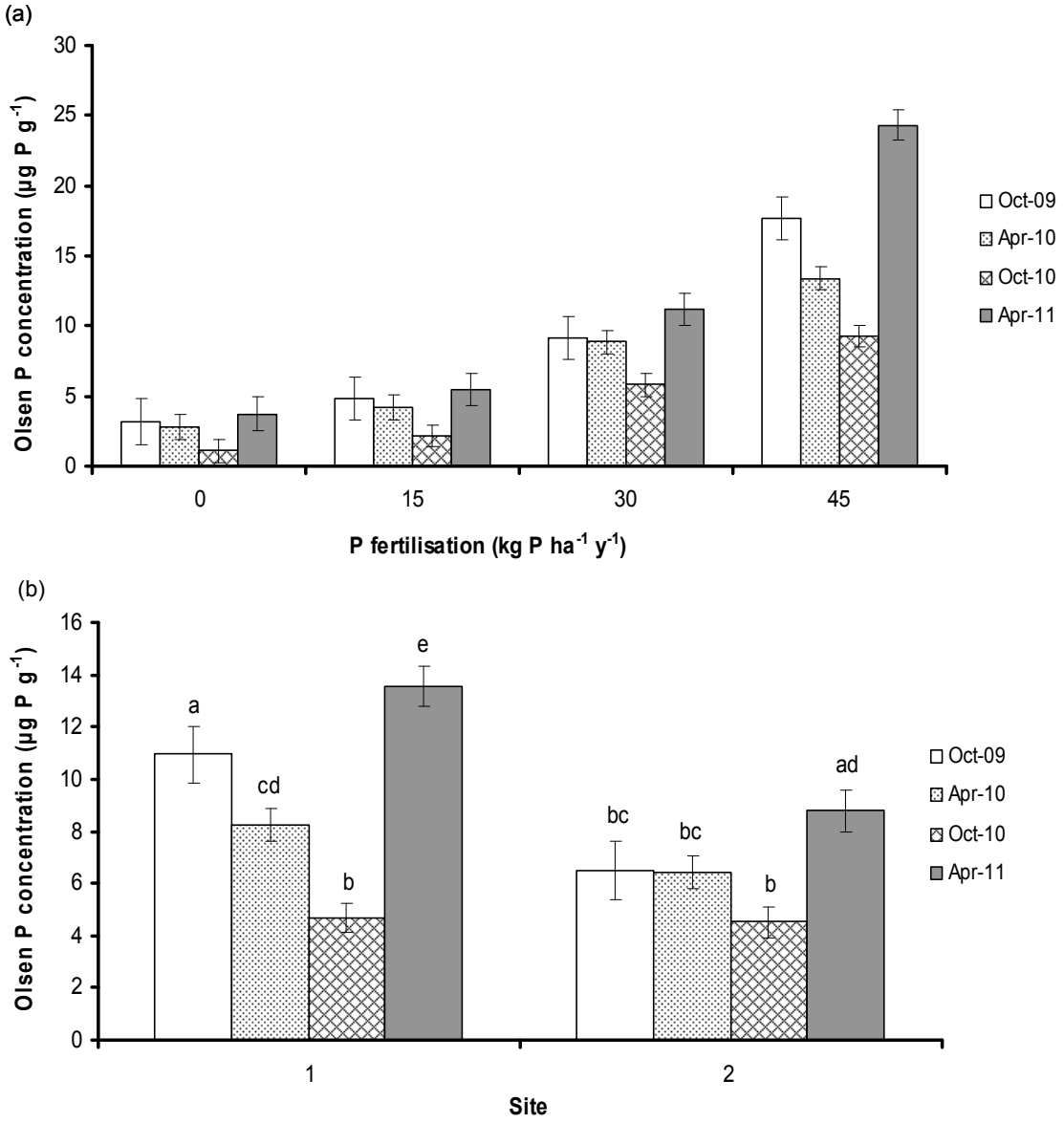

Figure 3.5 Mean Olsen P concentrations expressed by (a) the interaction between P fertilisation and sampling occasion and (b) the interaction between site and sampling occasion. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

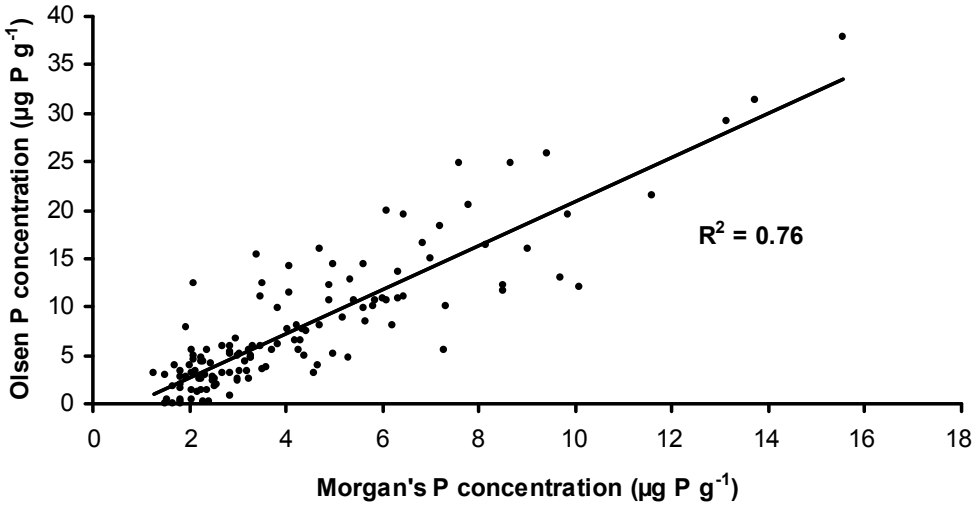

Figure 3.6 The relationship between Olsen P and Morgan's P concentrations. Concentrations have been pooled from the whole sampling period. Correlation coefficient is displayed.

No significant interaction between P fertilisation and Site was apparent on total P concentrations ($p>0.05$). There were significant P fertilisation and Site effects on these concentrations (P fertilisation effect; $p<0.001$, Site effect; $p<0.001$). The P fertilisation effect revealed that total P concentrations generally increased with increasing P fertilisation rate (P0; 504, P15; 566, P30; 590, P45; 714 mg kg^{-1} ± 20). More specifically, *post hoc* analysis showed that total P concentrations at the end of the sampling period in the P0 treatment were significantly lower than all other treatments, whereas concentrations in the P45 treatment were significantly greater than all other treatments. No significant difference between the P15 and P30 treatments was apparent. The Site effect showed that total P concentrations in Site 1 were greater than concentrations in Site 2 (Site 1; 670, Site 2; 517 mg kg^{-1} ± 14.2).

A significant P fertilisation effect was observed on the concentrations of K (Table 3.5). There were greater concentrations in the P0 treatment compared to the treatments that had received P fertilisation (P0; 110, P15; 71.8, P30; 67.3, P45; 60.7 µg g^{-1} (back–transformed from logs, i.e. geometric means) ± 0.03 (pooled S.E of log transformed data). There were no differences in K concentration between P fertilised treatments. In addition, there was a Time effect (Table 3.5) that showed a gradual increase in K concentration from 65.3 µg g^{-1} in October 2009 to 88.3 µg g^{-1} in April

2011 (Back-transformed means as stated above). No P fertilisation effects were observed on the material loss-on-ignition contents of the soil (Table 3.5). However, a significant interaction between sites over time was observed on organic matter contents, as measured by loss-on-ignition (Figure 3.7). This interaction showed that Site 1 had a greater organic matter content compared to Site 2 and there was a general trend which showed that greater organic matter contents occurred in April compared to October in both sites. No P fertilisation effects were observed on K_2SO_4 extractable organic C concentrations (Table 3.5). These concentrations were affected by Time, which revealed significantly greater concentrations in October 2009 (164 μg g^{-1} ± 4.9 S.E) than both April sampling dates (April 2010; 89 ± 1.9, April 2011; 92 ± 2.3 μg g^{-1}). Concentrations in October 2010 were significantly lower than all other sampling dates (79 μg g^{-1} ± 2.7).

No P fertilisation effects were observed on soil pH (Table 3.5) but a significant interaction between Site and Time was observed. This interaction showed that both sites were at a similar pH in October 2009, April 2010 and April 2011 but in October 2010 there was a significantly lower pH in Site 1 compared to Site 2 (Figure 3.8). In April 2011, significantly lower pH was observed in both sites compared to all other sampling occasions.

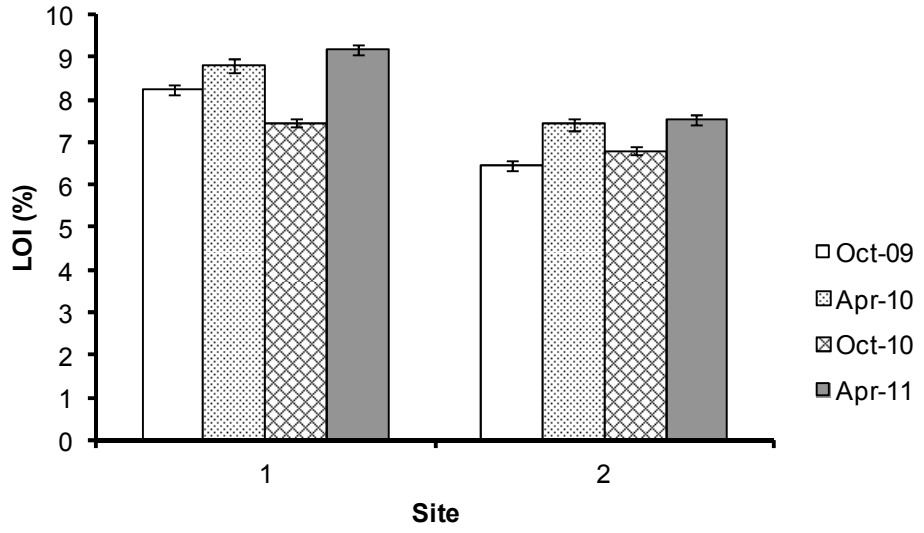

Figure 3.7 The interaction between site and sampling occasion on LOI contents. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

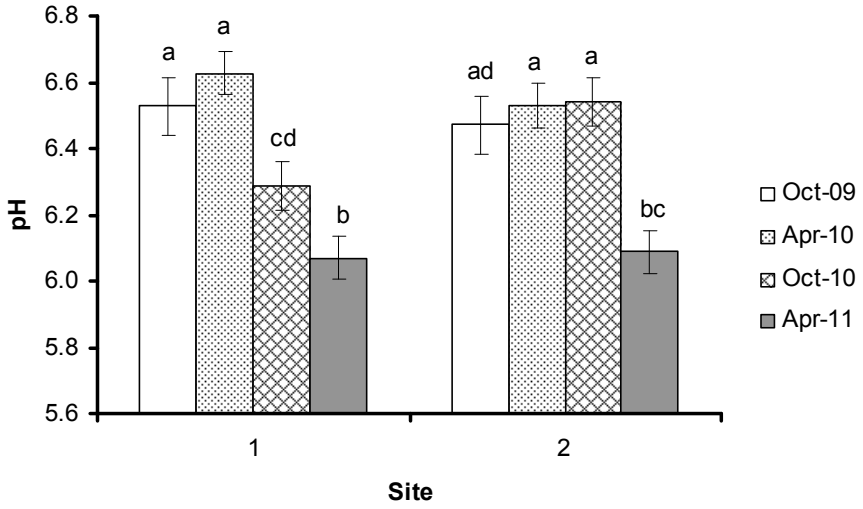

Figure 3.8 The interaction between site and sampling occasion on soil pH. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

3.3.2.4 Soil microbial properties

The only third-order interaction observed on microbial properties related to microbial biomass C (Table 3.6), whilst various second-order interactions occurred in relation to microbial biomass N, P and microbial community structure.

Table 3.6 ANOVA table showing the effects and interactions of site, P fertilisation and sampling date (Time) on measured microbial properties. Significance terms denote *** $p<0.001$; ** $p<0.01$; * $p<0.05$; blank space $p>0.05$.

	Microbial biomass (µg g^{-1})			PLFA			
	C	N	P	PC1 (38%)	PC2 (16%)	PC3 (11%)	Fungal: Bacterial Ratio
Site	***	***	***		***	***	
P treatment	**			*			
Time	***	***	***	***	***	***	***
Site x Time	***	***	***				
P treatment x Time	**			*	**	*	*
Site x P treatment							
Site x P treatment x Time	***						

A significant third-order interaction between Site, Time and P fertilisation was observed on microbial biomass C (Table 3.6). No clear trends were observed in this interaction between variables. However, there were generally greater concentrations of microbial biomass C in Site 1 compared to Site 2. With regard to changes over time, greatest concentrations were observed in April 2011. With respect to P fertilisation, an inconsistent effect was observed since this effect behaved differently over time and between sites (Figure 3.9).

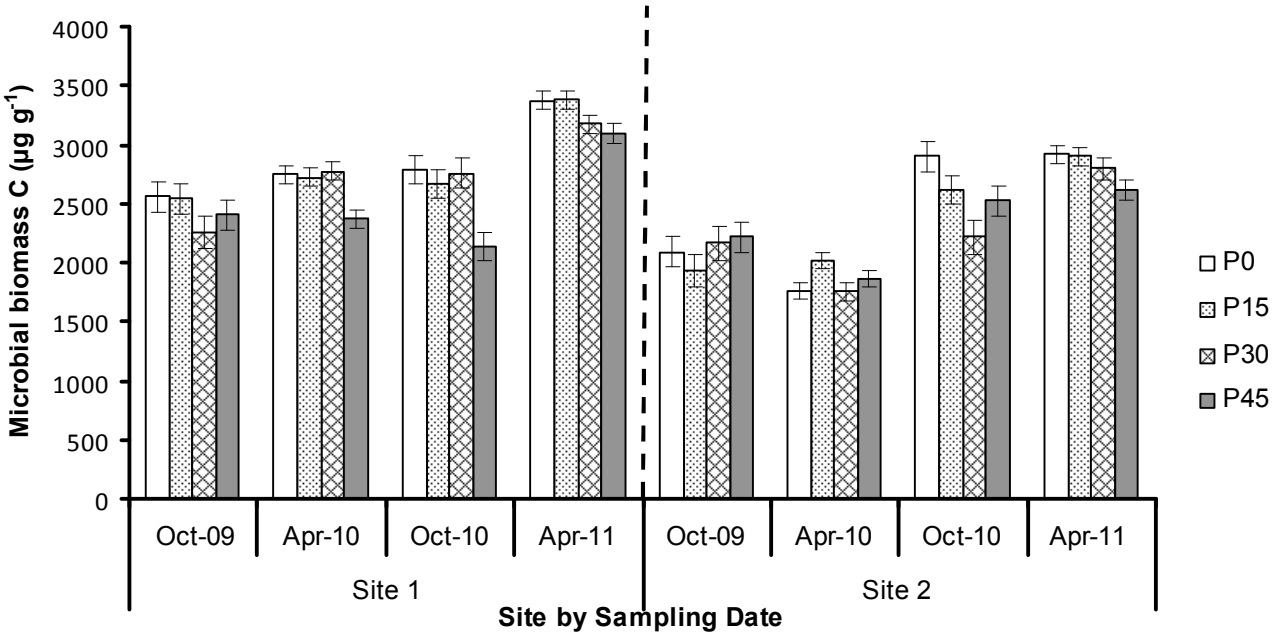

Figure 3.9 The interaction between site, sampling occasion and P fertilisation on microbial biomass C concentrations. The vertical dotted line is used for visual purposes and to separate sites. Error bars denote standard error.

A second-order interaction between site and sampling occasion was observed for both microbial biomass N and P (Table 3.6). With regard to microbial biomass N, this interaction showed differences between sites only occurred in October 2009 and April 2011 (Figure 3.10). When differences did occur, greater microbial biomass N concentrations were apparent in Site 1 compared to Site 2. No effects of P fertilisation were observed on microbial biomass N.

The interaction observed on microbial biomass P concentrations showed a different trend compared to microbial biomass N. There was a gradual increase in microbial biomass P over time in Site 1 (Figure 3.11) which increases from 47.4 μg g^{-1} ± 3.4 in October 2009 to 90.4 μg g^{-1} ± 2.9 in April 2011. In Site 2, there was an increase in microbial P from October 2009 to April 2010 but concentrations of microbial P then remained constant for October 2010 and April 2011. No effects of P fertilisation were observed on microbial biomass P.

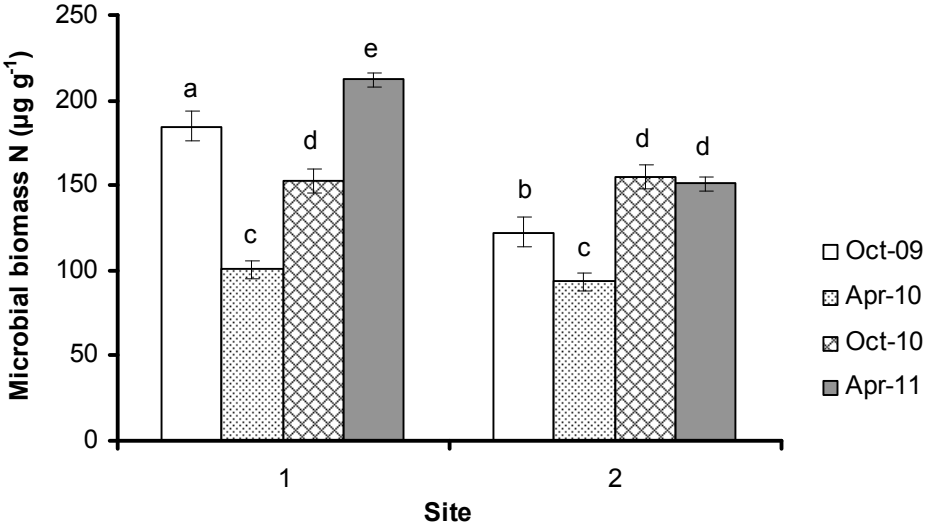

Figure 3.10 The interaction between site and sampling occasion on microbial biomass N concentrations. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

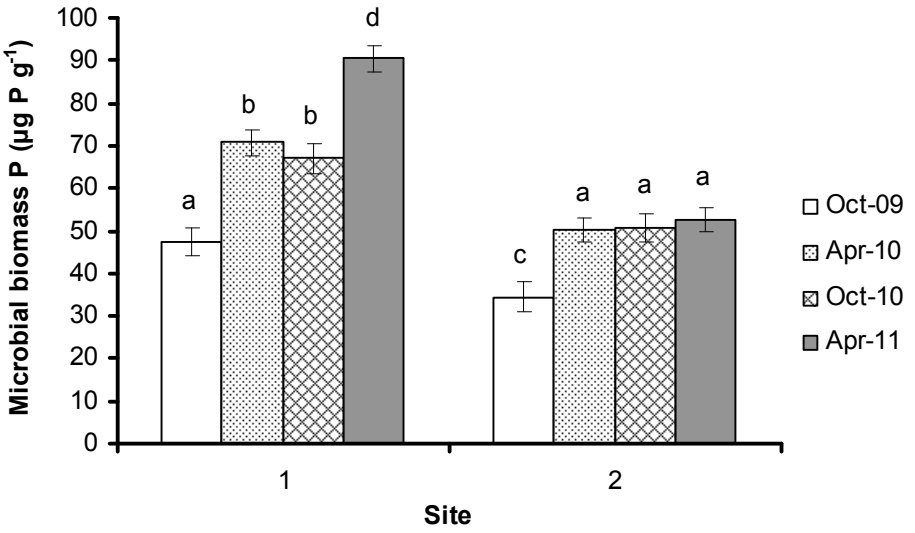

Figure 3.11 The interaction between site and sampling occasion on microbial biomass P concentrations. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

The phenotypic structure of the microbial community was significantly affected by Site, apparent via both PC2 and PC3 (Table 3.6): These differences were attributable to a range of multiple PLFA signatures. There were significant interactions between P fertilisation and sampling occasion on PC1, PC2 and PC3 (Table 3.6). These interactions showed that there was a strong effect of sampling date on each PC. With respect to the interaction between P fertilisation and sampling occasion, no differences in community structure were observed between October 2009 and April 2010, but then shifts in phenotypic structure became apparent in October 2010 and April 2011 (Figure 3.12(a)). When PC2 was plotted against PC3, there was a significant difference in community structure between all sampling occasions. With regard to P fertilisation, no single P treatment was consistently different from other treatments between sampling times in both Figures 3.12(a) and (b). All raw PLFA data for this experiment are presented in Appendix 1, Tables A_1C(a) to A_1F(e).

Changes in the fungal to bacterial ratio of the microbial community were observed mainly in the April sampling periods (Figure 3.13). A greater proportion of fungi were present in the microbial community in April 2010 compared to all other sampling dates. Within the April 2010 sampling date, a significantly greater proportion of fungi were observed in the presence of the P30 treatment compared to P0 and P15.

However, changes induced by the P30 treatment were not significantly different from P45. In contrast, in April 2011, differences in microbial community structure occurred between P30 and P45 treatments: where greater fungal proportions were present in P45 compared to P30. In both October sampling times this ratio was not significantly affected by P fertilisation.

3.3.2.5 Soil fauna properties

No significant P fertilisation effects were observed upon soil faunal properties (Table 3.7). However, earthworm biomass and nematode community dynamics differed during the course of this experiment, since Time effects were observed (Table 3.7). Site effects were also apparent on these properties. With respect to earthworm biomass, greater biomass was observed in Site 1 compared to Site 2 (77.3 g m^2 and 55.4 g m^2 respectively ± 4.9 pooled S.E). With respect to shifts in biomass over time, greater earthworm biomass was revealed in October 2010 (77.4 g m^2 ± 5.3 S.E) compared to April 2010 (55.3 g m^2 ± 4 S.E).

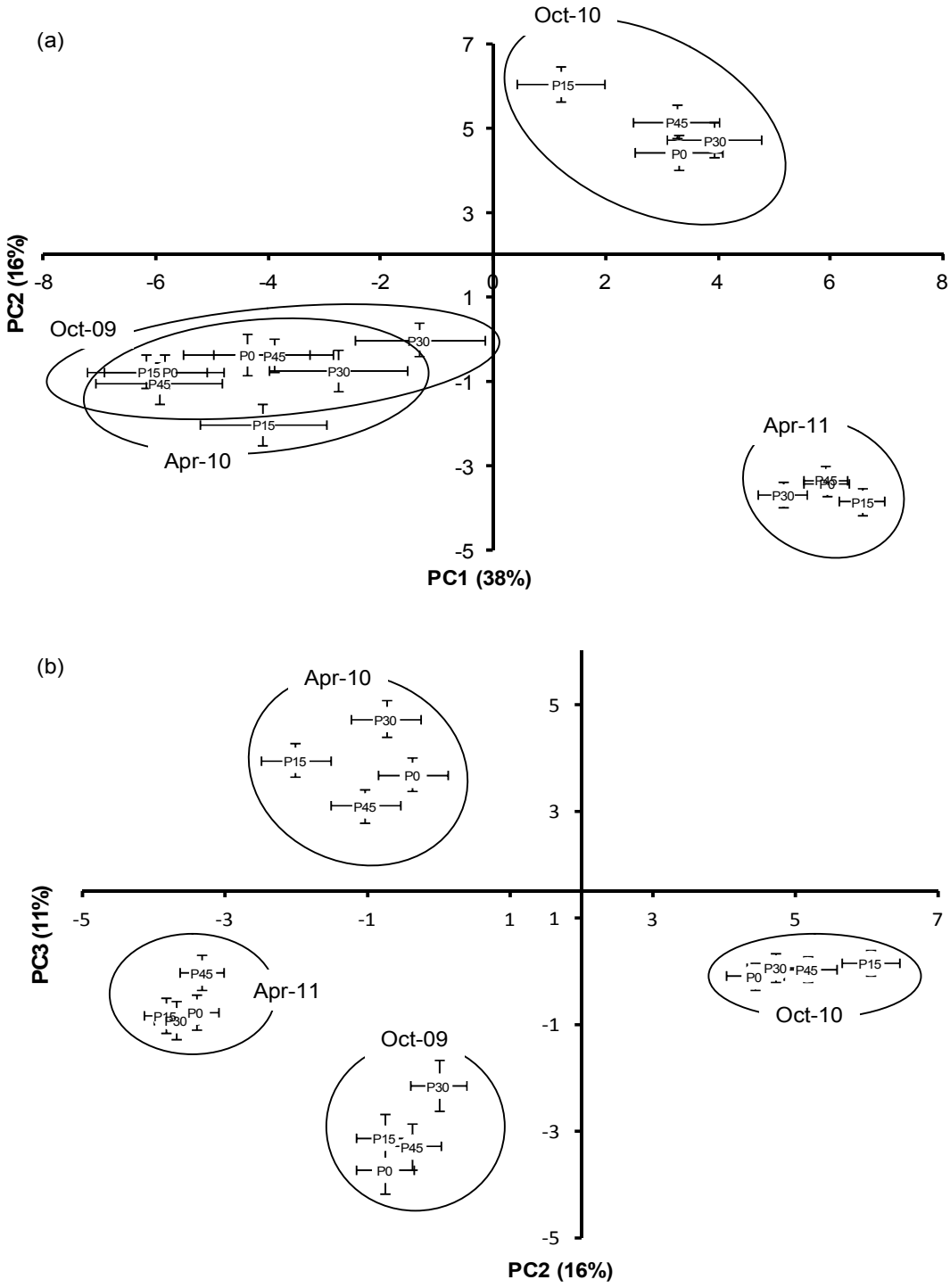

Figure 3.12 Differences in phenotypic microbial community structure in the presence of four different P fertilisation regimes at 4 sampling date as expressed by (a) PC1 and PC2 and (b) PC2 and PC3. Percentage variation is displayed in the parenthesis.

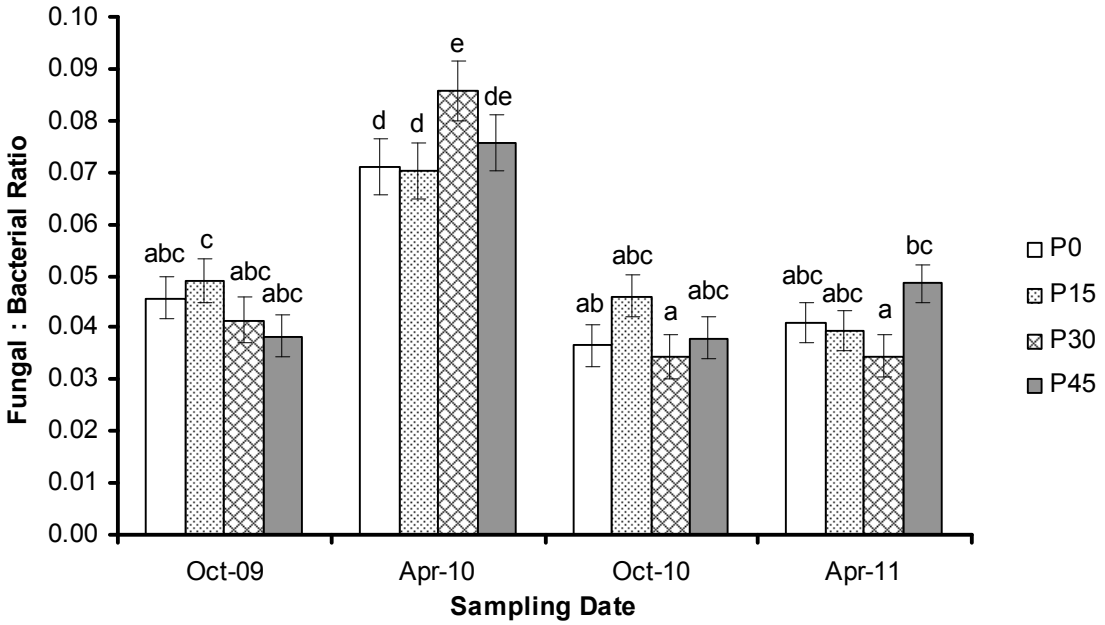

Figure 3.13 The interaction between P fertilisation rate and sampling occasion on the microbial fungal: bacterial ratio. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

Table 3.7 ANOVA table showing the effects and interactions of site, P fertilisation and sampling date (Time) on soil nematode and earthworm communities. Significance terms denote *** $p<0.001$; ** $p<0.01$; * $p<0.05$; blank space $p>0.05$.

			Nematode community structure					
			Family level			Trophic level		
	Earthworm biomass (g m^2)	Nematode abundance (number g^{-1})	PC1 (13%)	PC2 (11%)	PC3 (9%)	PC1 (37%)	PC2 (30%)	PC3 (18%)
Site	**				***			
P treatment								
Time	**	***	***	*	*	***	***	***
Site x Time		***	*					*
P treatment x Time								
Site x P treatment								
Site x P treatment x Time								

Nematode abundance was significantly affected by an interaction between Site and Time (Table 3.7). This interaction showed seasonal effects on nematode abundance in Site 1 but not in Site 2 (Figure 3.14). In Site 1, nematode abundance was greater in April compared to October. In Site 2, nematode abundance was significantly reduced in October 2009 compared to all other sampling occasions: From April 2010, nematode abundance remained consistent for the rest of the experiment.

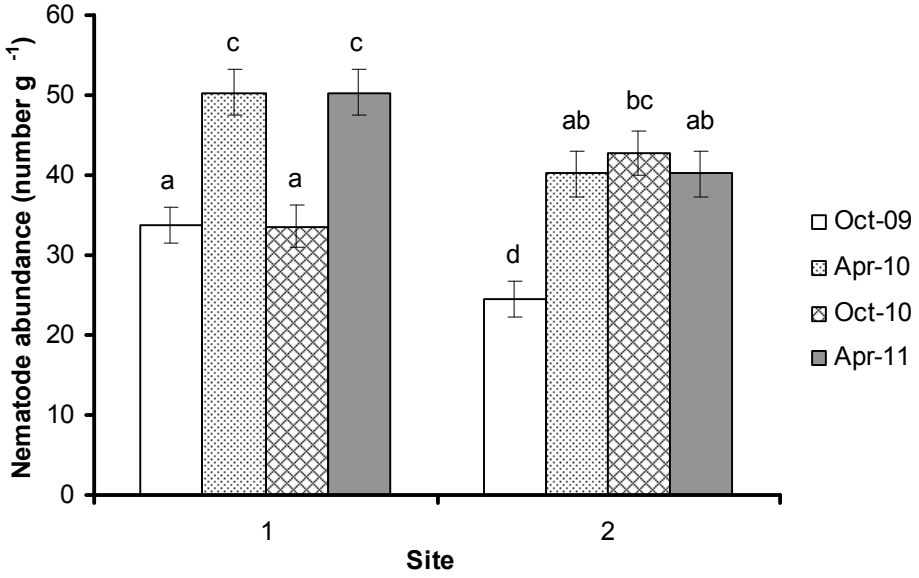

Figure 3.14 The interaction between site and sampling occasion on nematode abundance. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

An interaction between site and sampling occasion was observed on nematode community structure when structured at a family level (Table 3.7). Community structure was significantly affected by Site in October 2009 but not at any other sampling occasion (Figure 3.15). No P fertilisation effects were observed on nematode community structure. A subtle interaction between Site and Time occurred on nematode trophic structure on PC3 (Table 3.7). This interaction showed that sites did affect nematode trophic composition in April but not in October. However, significant time effects were observed on PC1 and PC2 (Table 3.7). When PC1 was plotted against PC2, these times effects showed shifts in nematode trophic structure at each sampling occasion (Figure 3.16(a) and (b)).

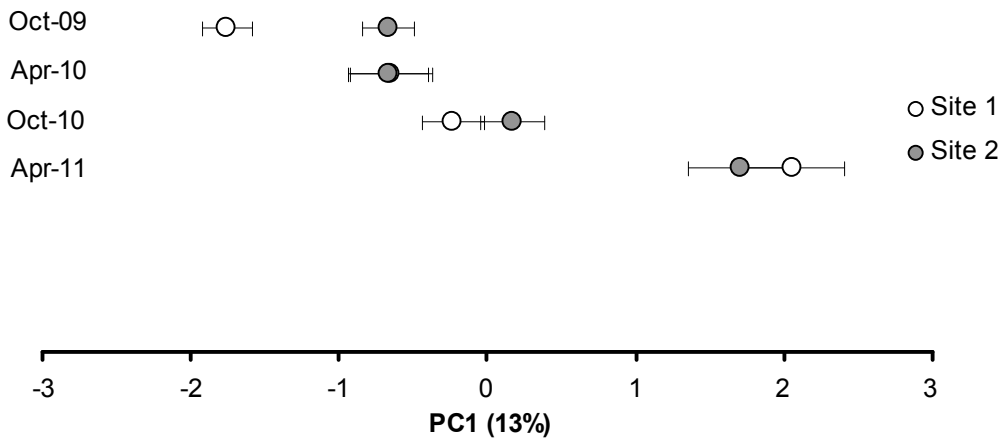

Figure 3.15 The interaction between site and sampling occasion on nematode community structure, from a family level, as expressed by PC1. PC variation accounted for and significance of the interaction is displayed in parenthesis. Placement along the Y axis is for visual purposes and denotes different sampling occasions. Error bars denote standard error.

A P fertilisation effect was observed on the nematode channel ratio ($p<0.05$). This effect showed a more fungal dominated nematode community in the P0 treatment compared to all other treatments (Figure 3.17). No significant differences were observed between nematode channel ratios that received P fertiliser.

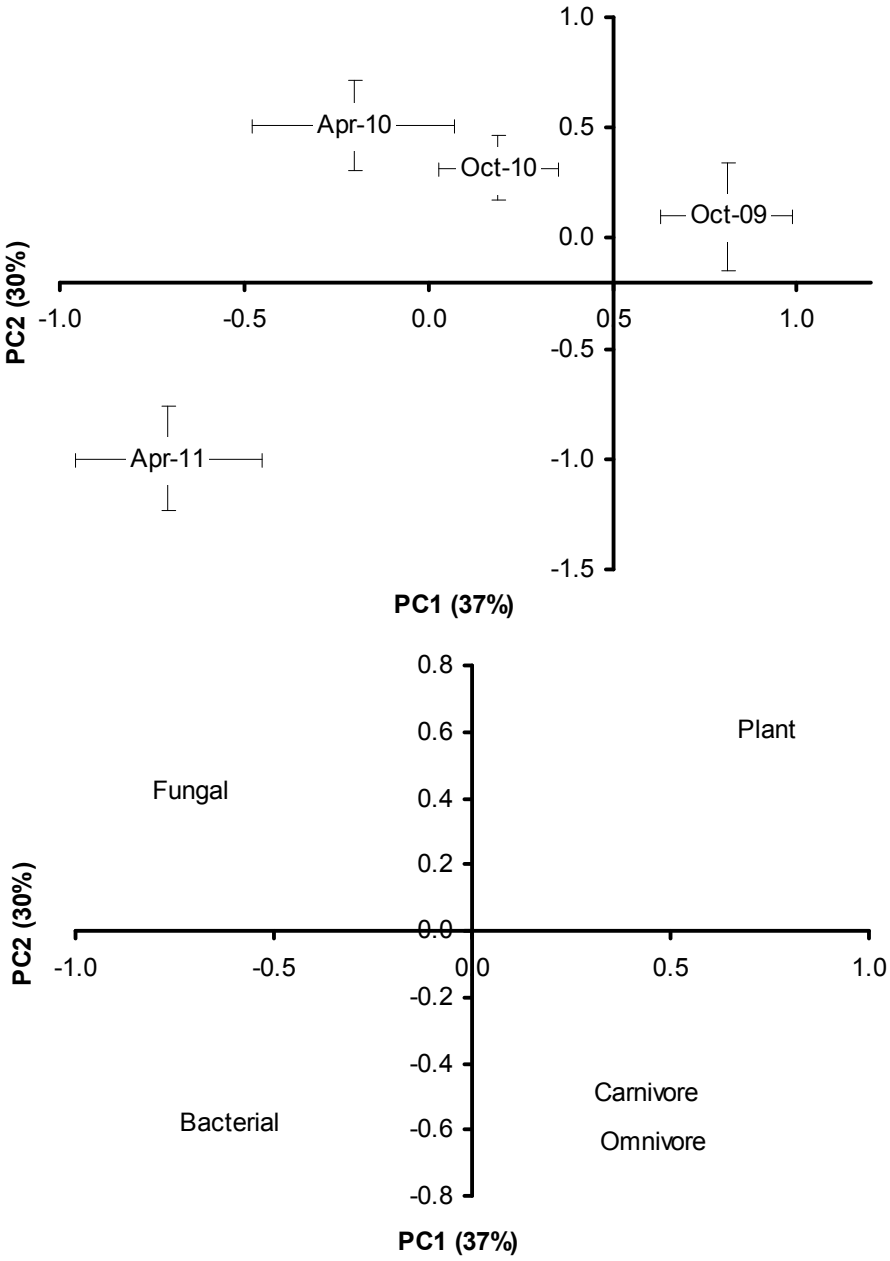

Figure 3.16 Changes in nematode trophic structure at (a) different sampling occasions as expressed by PC1 and PC2. (b) is a loadings plot which shows the separation of different nematode trophic groups by PC1 and PC2. Percentage variation and significance is denoted in the parenthesis. Error bars are standard error.

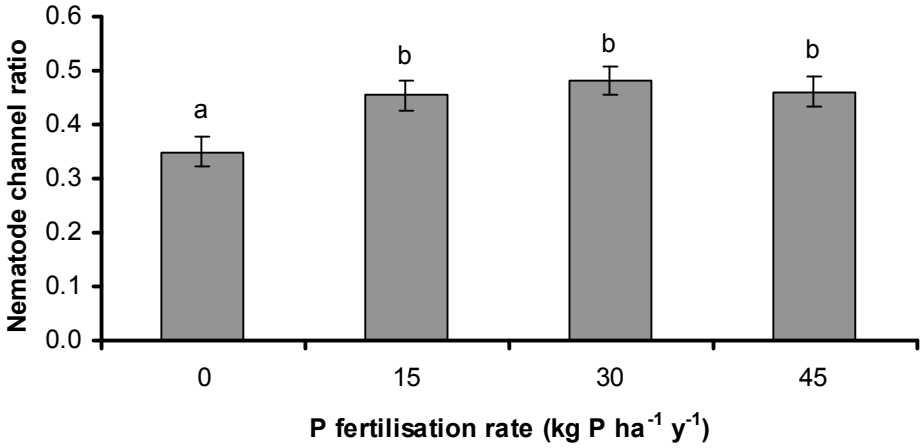

Figure 3.17 Main effect of P fertilisation on the nematode channel index. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

3.4 Discussion

3.4.1 Preliminary study: Soil phosphorus distribution in a grassland system

An interaction between P fertilisation rate and depth showed a P fertilisation effect at each depth, with the largest differences occurring in the 0 - 1 cm depth and the smallest in the 5 - 10 cm depth. Both of these results support the primary hypothesis and this validates using the 0 - 10 cm depth range in the field trial experiment looking at how P fertilisation affects the soil biota, as all the soil collected from 0 - 10 cm would be reflective of the respective P fertiliser rates applied to the surface. If P was localised predominately at the soil surface, then the interaction between P fertilisation and sampling depth would only show a P fertilisation effect at the 0 - 1 cm depth and not at the 1 - 5 and 5 - 10 cm depths.

In addition, a significant interaction between P fertilisation and depth occurred that showed a concentration gradient of P with depth, where P decreased with depth. This finding supports the ancillary hypothesis as there was a decrease in P concentration with depth for each P fertiliser rate. This occurred due to the direct application of

inorganic P fertiliser to the soil surface, which ultimately leads to P accumulation at the surface over time (Owens et al., 2008).

Validating the primary hypothesis was important with respect to measuring soil biological properties as the possibility of a dilution effect by bulking soil from 0 - 10 cm could then be minimised, since a P fertiliser effect was not exclusively localised to the soil surface but also present at other depth ranges.

3.4.2 Main experiment: Phosphorus fertilisation effects on the soil biota

3.4.2.1 Phosphorus fertilisation effects on plant and soil properties

Concentrations of labile inorganic P increased relative to the amount of P fertiliser applied. This was supported by both extraction procedures (Olsen and Morgan's P) and occurred at each sampling occasion in both sites. This is not surprising as the intent of adding P fertiliser was to increase the bioavailable (from an agronomic perspective, specifically plant-available) P content of the soil. However, when looking at both of these extraction methods, there were subtle differences in their response to P fertilisation between sites over time. For example, a third-order interaction between such interactions was observed on Morgan's P concentrations, where as a second-order interaction was observed on Olsen P concentrations. There was a strong positive linear correlation between both extraction procedures (R^2 = 0.76) and same general trend in the data as both extractions report an increase in labile inorganic P with increasing P fertilisation. Therefore, both tests seem suitable indicators of labile inorganic P. But the subtle difference may stem from the nature of the extraction procedures, since the Olsen P procedure involves the use of an alkaline extraction where as the Morgan's P procedure is acidic based (Humphreys et al., 1999). These differences may extract P from the soil fabric differently and target different P forms, for example P precipitated into calcium complexes or P that has been adsorbed onto the soil fabric. In the Irish context, Morgan's P is the extraction procedure commonly employed to detect labile inorganic P concentrations. Thus, the Morgan's P extraction would be expected to provide the most accurate picture of labile P concentrations in

this field experiment compared to Olsen P extraction and explains why a third order interaction was observed on Morgan P and not Olsen P concentrations. Despite these subtle differences, this data still confirms the establishment of a distinct gradient of P availability concomitant with P fertiliser rate, which provides the essential basis for testing the effects of P fertilisation on the soil biota in this experiment.

The distinct concentration gradient of P following the application of different P fertilisers is also supported by the total P data. The total P ranges observed in this experiment complement concentrations observed in other Irish grasslands and highlights that total P concentrations in the unfertilised P0 treatment were not diminished after 17 years of constant plant P removal (Daly et al., 2001). This suggests that large quantities of P were present in these sites before the establishment of the field experiment and were great enough to suffice the microbial and plant communities in unfertilised plots over this 17 year period.

In addition, an increase in cumulative plant P yield was apparent, commensurate with P fertilisation rate. Again, this would be entirely expected, but this data provides evidence that the plant community did respond to P fertilisation in this study, which sets a context for the nature of the microbial responses which were observed. In addition, the application of different rates of P fertiliser in these sites has affected the structure of the plant community (J. Murphy pers. comm.). In particular, the plant species composition in the 0 kg P ha^{-1} y^{-1} treatment differed compared to the fertilised treatments. In the 0 kg P ha^{-1} y^{-1} treatment, grass species from the *Agrostis* genus were most dominant and in fertilised treatments, *Lolium perenne* was dominant. Species richness at all P fertilisation rates was the same since five grass species were observed in all treatments. Therefore, the application of different rates of P fertiliser had an impact on the plant communities in these sites.

3.4.2.2 Phosphorus fertilisation effects on the soil biomass

With respect to Objective 1 in this experiment, no P fertilisation effects were observed on microbial biomass P concentrations. In particular, both the primary and ancillary hypotheses of this objective were rejected as no effects of P fertilisation were observed on microbial biomass P or on earthworm biomass. Other studies assessing

the effect of P fertilisation on soil microbial biomass have found mixed responses. Some studies focusing on the long-term application of inorganic fertiliser P to grassland systems have found no effects on microbial biomass (Sarathchandra et al., 1993; Sarathchandra et al., 2001). Ross et al. (1995) presented results that showed inconsistent effects of rock phosphate on microbial biomass P concentrations in a New Zealand pasture, in a study analysing microbial biomass measurements over a two year period. It was found that microbial biomass P did respond to P fertilisation on some sampling occasions but not all. The application of inorganic P fertiliser has also been shown to decrease microbial biomass C (Parfitt et al., 2010). The explanation for this observation was attributed to a shift in microbial community structure since a more bacterial dominated community occurred in the presence of P fertiliser. Consequently, a more bacterial dominated community contributed to a lower microbial biomass C concentration due to a more rapid turnover of such biomass due to grazing by micro-fauna. In the field experiment conducted for this project, no P fertilisation effects were observed on the soil biota. Therefore, the absence of an effect in this instance does complement results from other grassland systems that have focused on the sole application of inorganic P fertilisers.

Other studies have considered the fate of microbial responses to P immediately following fertiliser application. In particular, a study by Picone et al. (2003) showed that microbial biomass P did not significantly increase with increasing P fertilisation. However, there was a general trend in the data that suggested that the microbial community may have responded within a short time-frame after fertiliser application. It was concluded that sampling time after fertiliser application may be important as the turnover of microbial biomass P may be rapid and had reverted back to initial concentrations. Indeed, other studies looking at microbial P acquisition following P fertilisation over an approximate two month period found microbial responses irrespective of fertiliser type (He et al., 1997a; Gichangi et al., 2009; Khan and Joergensen, 2009). These incubation studies showed that the greatest increase in microbial biomass P occurred with the dual application of inorganic and organic fertilisers. This dual application was important as the application of an organic fertiliser increases soil labile organic C, N and P concentrations and the inorganic fertiliser directly increases bio-available P concentrations (Khan and Joergensen, 2009). The addition of labile organic C and N then stimulates the growth and

synthesis of microbial biomass, which in turn increases P demand (Gichangi et al., 2009). Therefore, the stimulatory effects of P fertilisation on microbial community seemingly occur within an approximate two month time-frame following application. This response immediately after the application of P was not investigated in the field experiment, since P fertilisation occurred annually in February and both sites were sampled in late April and October. Sampling in both April and October was chosen as these months occur within the periods of the year which exhibit heightened soil biological activity in temperate climates.

However, other studies have reported stimulatory effects of P fertilisation on the microbial biomass P concentrations. In a maize-wheat crop rotation system, Lui et al. (2010) reported that the application of inorganic P fertiliser combined with inorganic N fertiliser increased microbial biomass P concentrations. It was, however, highlighted that the greatest increase in microbial biomass occurred when inorganic and organic fertiliser were applied together. In another study conducted by Parfitt et al. (2005), P fertilisation effects were also observed on microbial biomass P concentrations in New Zealand pastures that were grazed by sheep under both conventional and organic fertiliser management. The P effect was attributed to P limitation in the control treatment, whilst no differences were observed between fertiliser management types. One common theme that appears to be associated with the P fertiliser effects on the soil biota is the importance of fertiliser type and management. When effects are observed, they were seemingly stimulated by the input of organic material. In contrast, the fertiliser type and grassland management regime adopted in the field experiment did not involve the input of organic matter into the system, since only inorganic fertilisers were applied and no livestock were present. Furthermore, the intensive cutting regime adopted in this experiment would have also impeded plant litter inputs into the soil and would have limited plant-mediated inputs into the soil. With respect to the importance of plant litter inputs to stimulate P demand in the microbial community, a study by Liu et al. (2012) investigated how P addition to forest systems under different management regimes affected microbial biomass P. Lui et al. (2012) showed that the addition of P to a forest system that was not disturbed increased both microbial biomass C and P, whereas P addition to a disturbed forest system failed to invoke a response. It was concluded that C and N limitation in the disturbed forest scenario may have limited P acquisition due to an

absence of nutrient accumulation. Therefore, one possible explanation for microbial biomass P not responding to P fertilisation in this experiment may be linked to the management of these grassland sites, since such management may govern the availability of other soil nutrients.

The intensive cutting regime adopted in the field experiment conducted for this project operated by removing all cut plant material from both sites after each harvest. The removal of these cuttings over a 17 year period would have constantly been removing C from the system and manipulated plant behaviour, particularly over a long-term period (Ilmarinen and Mikola, 2009; Ilmarinen et al., 2009): cutting in this manner may have promoted plant shoot growth and limited root growth and photosynthetic capacity. Therefore, this intensive cutting regime across all P treatments may have been affecting the microbial community, as the plant community is intricately linked to soil microorganisms (Bardgett, 2005). More specifically, plant senescence is an important process that stimulates the soil decomposer community (Bardgett, 2005). This has been supported by other studies, for example Bardgett and Shine (1999) showed that the input of litter from multiple plant species was linked to an increase in microbial biomass C. Furthermore, increased botanical diversity associated with less intensive grassland management was shown to affect microbial community structure by stimulating a more fungal dominated community (Bardgett and Shine, 1999). This stimulation of different phenotypic microbial profiles between management regimes was associated with varying quantities and qualities of organic material entering the soil matrix. Indeed the importance of organic matter inputs to the microbial community has also been proven in another field experiment on the same research site at the Johnstown Castle Estate (hereafter referred to as the 'Cowlands' experiment). The Cowlands experiment studied the effects of P fertilisation on the soil biota and reported that P fertilisation effects were observed on microbial nutrient concentrations (Chen, 2012). The experimental design of the Cowlands experiment consists of a long-term (>30 years) inorganic P fertilisation trial using plots that were also grazed at different beef cow stocking densities. In particular, three P fertilisation rates (0, 15 and 30 kg P ha^{-1} y^{-1}) were applied to plots, with two stocking densities of 2200 and 3300 kg of stock ha^{-1} and were managed on a rotational grazing scheme in which cattle were situated in designated plots for 18 – 24 day intervals. Results from this experiment revealed that microbial biomass C, N and P concentrations increased

with increasing P fertilisation on multiple sampling dates. Therefore, a consistent effect of P fertilisation was observed in the Cowlands experiment, whereas either no effect or inconsistent effects were observed microbial biomass properties in this project. One of the main differences between both experiments was the adopted management regime for their grassland sites. In the Cowlands experiment, grassland plots were grazed by livestock at different stocking densities, which is in contrast to the intensive cutting regime in this project. Therefore, like the input of plant material, the supply of endogenous organic material from the livestock would have been re-supplying the soil microbial community with labile substrates, which would have promoted their activity and growth in the soil. Such activity may have been impaired when these forms of input were not present, for example in the presence of an intensive cutting regime. Indeed, the only forms of input into this cutting system would have been primarily inorganic, which would not have been sufficient to stimulate microbial activity since the supply of labile organic substrates would be limited.

Despite the absence of P fertiliser effects on microbial biomass P concentrations in the field experiment conducted for this project, microbial biomass C concentrations were affected by P fertilisation, but in an inconsistent manner with different P fertilisation effects occurring at different sampling periods and between sites. In addition, the phenotypic structure of the microbial community also showed an inconsistent effect to P fertilisation over time. Therefore, the primary hypothesis associated with Objective 2 was rejected, since no clearly defined P fertiliser effect was observed on microbial phenotypic structure. These responses may be indicative of suppression on microbial P assimilation derived from fertiliser input due to an absence of additional nutrient input from the plant community. However, despite intensive C harvesting from these sites, microbial biomass C concentrations were not low compared to other studies in grassland systems (Turner et al., 2001). Turner et al. (2001) looked at microbial biomass C concentrations in a range of soil types in 29 UK permanent grasslands. These concentrations ranged from 412 ± 19 to 3412 μg g^{-1} ± 21. The largest concentrations observed were derived from soils with low percentage sand contents and high clay contents. Larger concentrations of microbial biomass C in the two grassland sites studied in this experiment indicated that C may not be the limiting nutrient. Further evidence for this was supported by soil loss-on-ignition contents,

which was similar to those observed in other Irish grasslands (Bourke et al., 2008). In this study conducted by Bourke et al. (2008), loss-on-ignition soil contents of sampled grassland on the Johnstown Castle Estate revealed a mean content of 8.22%, ranging from 6 - 10.9%. Thus, the accumulation of organic material in these two grassland site were also not reduced. This may indicate that it was the presence of a specific nutrient in the plant material rather than the quantity of material that was limiting microbial P acquisition in these sites.

Earthworm biomass was also not affected by P fertilisation in this experiment. Other studies that have looked at fertiliser effects on earthworm communities have reported increases in earthworm biomass. Using field sites located on the Johnstown Castle estate, Curry et al. (2008) found an N fertiliser effect on earthworm biomass. It was interpreted that an increase in management intensity increased the plant productivity, which increased the substrate available to the native earthworm population. In addition, the findings reported by Fraser et al. (1994) also found that earthworm biomass increased following the application of superphosphate to New Zealand pastures that had been receiving P fertilisation over 37 years. However, it is important to note that the lowest P application rate used in this study was 188 kg P ha^{-1} y^{-1}, which greatly exceeds that application rates used in this experiment. Fraser et al. (1994) also explained that the increase in earthworm biomass was linked to aboveground production, with increasing P fertilisation tied to increased plant productivity. Despite P fertiliser effects on earthworm biomass in these above mentioned studies, similar weights of biomass were observed in those studies to weights ascertained in these two grassland sites. Such biomass between treatments may be linked to similar loss-on-ignition contents between soils in these sites, since similar contents of organic material may indicate that earthworm populations were exposed to similar nutritional environments between treatments. Therefore, with similar nutritional environments between P fertiliser regimes, no effects were observed on earthworm biomass.

3.4.2.3 Phosphorus fertilisation effects on nematode community dynamics

The ancillary hypothesis associated with Objective 2 stated that P fertilisation would affect the trophic structure of the nematode community. Results from this experiment mainly provide data that rejects this hypothesis as no effects were observed on nematode abundance and community structure. Sarathchandra et al. (2001) observed no effects of P fertilisation on nematode abundance and on general community structure in a New Zealand grassland system. No effects of P fertilisation on nematode abundance and general community structure were also observed in the study conducted by Parfitt et al. (2010). However, this experiment did show specific effects on particular nematode genera. Most noticeably, an increase in the number of plant feeding nematodes from the *Pratylenchus* genus with increasing P fertilisation was reported. This observation complements the study by Yeates (1976), who also observed an increase in *Pratylenchus* plant feeding nematodes with increasing P fertilisation. But an increase in the nematode abundance was also apparent with increasing P fertilisation, which contradicts the other two studies. No effects of P fertilisation were observed on the nematode numbers from the *Pratylenchus* genera in this experiment, since no P fertilisation effects were observed on nematode community structure. This may be an indicator of belowground plant productivity in these sites as nematodes from this genus are dependent on roots as a source of nutrition. More specifically, no effects on nematodes from the *Pratylenchus* genera may indicate that P fertilisation effects on the plant community may be localised to aboveground growth, rather than root growth in this experiment. This possible lack of P fertilisation effect on the root biomass is also supported by the absence of a P fertilisation effect on microbial biomass C and P concentrations, as discussed above.

An effect of P fertilisation was observed on the nematode channel ratio, which indicates whether the nematode community was either fungal- or bacterial-feeder dominated (Yeates and Bongers, 1999). This effect showed a nematode community that was more fungal dominated in the P0 treatment compared to treatments where P fertiliser was applied. This result does support findings that state a more fungal microbial community would typically be associated with less intensive management (Bardgett and McAlister, 1999); therefore, a more fungal dominated nematode community would be expected. However, this result does not complement the PLFA

data as no specific P fertiliser effect on the principal fungal biomarker PLFA (18:2ω6) was observed.

When viewed from an overall trophic perspective, including bacterial-feeding, fungal-feeding, plant-feeding, omnivorous and carnivorous nematodes, the shift in trophic structure across sampling occasions does support the microbial fungal : bacterial ratio. This was indicated by the trajectory of the factor scores from the principal component analysis when nematode trophic structure was orientated by sampling data. This trajectory showed that a more fungal dominated nematode community occurred in April 2010, which was aligned with a more fungal dominated microbial community at this specific time period. In addition, the absence of P fertilisation effect on nematode community structure, as investigated from a family and trophic perspective, was not surprising when no consistent P fertiliser effects were observed on the microbial phenotypic structure.

3.4.2.4 Seasonal and soil type effects on the soil biota

Seasonal effects were observed on all biological properties in this study, with microbial biomass C, N and P differing between all sampling periods and no consistent trends between them. Furthermore, the weather data collected showed that different amounts of rainfall occurred during the experimental period, whilst temperature was similar between sampling occasions. Other studies have reported large variation in microbial properties on a temporal scale (He et al., 1997b; Krämer and Green, 2000; Chen et al., 2003). Seasonal variations in microbial nutrient pools has been associated with changes in soil moisture, soil temperature, root growth and activity (rhizodeposition) and organic matter input through plant defoliation (Chen et al., 2003). In the study conducted by Chen et al. (2003), microbial biomass C and P were shown to vary temporally, with greater variability observed in microbial biomass P than C. It appeared that microbial biomass P was more sensitive to plant growth (thus plant P demand) and soil moisture content compared to microbial biomass C. This result has also been observed in another study by Tate et al. (1991). In particular, moisture deficit in the soil may disrupt the diffusion of water-soluble P to the microbial community, thus affecting microbial assimilation of P on a temporal scale

(He et al., 1997b). He et al. (1997) explained that since a deficit in soil moisture content did not affect microbial biomass C, but coincided with a decrease in microbial biomass P, then this may represent a loss of P during microbial biomass P turnover and an inefficiency to then immobilise P from the soil. This effect of moisture deficiency on microbial biomass P may also be heightened by the activity of the plant community. The evapotranspiration induced by the plant community would not only hinder the diffusion of water-soluble P to the microbial community but could also be actively competing with the microbial community for smaller concentrations of orthophosphate in the soil solution (He et al., 1997b). Therefore, the amount of P available to the microbial biomass may be severely limited at specific times of the year and may explain why microbial biomass P concentrations fluctuated during the experimental sampling period. This conclusion is supported by rainfall data, since the amount of rainfall also fluctuated between sampling occasions.

In this experiment, greater microbial biomass C, N and P was generally observed in Site 1 (sandy loam soil) compared to Site 2 (loamy sand soil). This finding complements previous studies focusing on textural effects upon C and N mineralisation and microbial biomass C and N (Gregorich et al., 1991; Hassink, 1994). Furthermore, an increase in soil organic matter with increasing clay content has also been reported (Gregorich et al., 1991). This complements soil organic matter behaviour in this experiment, since Site 1 contained both greater organic matter contents and proportionally more silt and clay compared to Site 2 (29% and 11% more silt and clay, respectively). Soil organic matter was shown to be greater with increasing clay content due to the greater sorption of organic compounds to the clay components of the soil fabric (Gregorich et al., 1991). Such sorption would increase the stability of organic matter and retard soil decomposition processes. With reduced organic matter decomposition, the microbial biomass becomes less active which results in a slower biomass turnover rate. Therefore, greater microbial biomass C, N and P pools were observed in the Site 1 as this reflects a slower turnover and less active microbial community compared to the microbial biomass in Site 2 (Gregorich et al., 1991; Hassink, 1994).

3.4.3 Conclusions

This experiment showed that the application of P fertiliser to a grassland system does not necessarily increase the nutrient pools associated with the microbial biomass, or affect microbial community structure, even when an increase in plant dry matter and plant P yield with increasing P fertilisation was manifest. P fertilisation also did not affect earthworm biomass or nematode community dynamics. However, sampling occasion and site effects were observed on all soil biological measurements. The explanation for these observations may be linked to the management regime of both sites, since both have been intensively cut for a 17 year period and fertilised with appropriate amounts of inorganic P, N and K fertiliser. Therefore, under such conditions, the soil biota did not seem to be particularly associated with plant P acquisition. This highlights that the adopted management regime is very relevant for soil biological P acquisition, as the literature indicates that microbial biomass and nematode responses in the fertilised grassland setting is more responsive to the type of fertiliser and grazing regime adopted, with P fertiliser effects being observed with the application of both organic and inorganic fertiliser together and with livestock grazing.

4. Soil microbial responses to inorganic nutrient application

4.1 Introduction

The soil microbial biomass is an important component of soils in relation to nutrient cycling (Brookes, 2001). The turnover of the microbial biomass can act as both a source and sink of nutrients for the plant community (Brookes, 2001; Achat et al., 2010). In the context of P cycling, the ability of the microbial community to retain labile P pools within its biomass can be an important nutrient source for plant growth, especially considering that the fixation of P to the abiotic components of the soil fabric can lead to the sequestration of P in fractions that are recalcitrant and unavailable to plants (Ayaga et al., 2006).

Previous studies have shown that the addition of P-containing fertiliser to incubated soil in laboratory-based experiments increased microbial biomass P concentrations (He et al., 1997a; Gichangi et al., 2009; Khan and Joergensen, 2009). One such study conducted by Gichangi et al. (2009) looked at microbial responses to both inorganic and organic P fertilisation in African soils over 84 days. The response of the microbial community revealed an increase in microbial biomass P concentrations up to Day 28 in the presence of both inorganic and organic fertiliser types, where after a decline was observed to levels similar to initial P concentrations at the beginning of the experiment. However, the greatest increase in microbial biomass P was shown to occur when organic and inorganic P fertilisers were applied together (Khan and Joergensen, 2009; Gichangi et al., 2010). This was associated with the introduction of other nutrients that are essential for microbial growth, particularly C and N. After the addition of labile C, N and P, the microbial community was thus stimulated to grow and synthesised biomass P (Hinsinger, 2001; Gichangi et al., 2010). The study conducted by Khan and Joergensen (2009) showed that the supplementation of inorganic P fertiliser to compost stimulated microbial P acquisition. However, the incorporation of P into biomass was reported to only occur immediately after inorganic P fertilisation, and that greater microbial biomass turnover in compost

compared to soils led to lower incorporation rates at the end of the incubation period relative to other studies. This increase in microbial biomass P was coupled to a large increase in $NaHCO_3$ concentrations in the compost immediately following application, since 50% of the applied P was present in this fraction. Further support for a microbial response to P fertilisation occurred in the study conducted by He et al. (1997), who focused on rock phosphate additions to P-deficient forest soils over a 60 day period, and reported that the addition of such material stimulated microbial P acquisition in this time frame. Such a response by the microbial community was also coupled to a decrease in soil organic C concentrations since P limitation was removed from the system and the microbial community was able to utilise organic C stocks in the soil. It was concluded that by removing this P limitation, the microbial community was stimulated to facilitate greater C cycling in these forest soils.

One of the main factors limiting microbial acquisition of any particular nutrient in soils can be the relative availability of other soil nutrients. In many agricultural systems, the main primary limiting nutrients are recognised as C, N and P, typically in that order (Demoling et al., 2007; Bunemann et al., 2011). Nutrient ratios of the microbial biomass can provide an indicator of the nature of any nutrient limitation in the microbial community. In particular, ratios of C:N, C:P and N:P can be used to indicate which of these nutrients may be limiting microbial growth based on variations from nominal ratio thresholds (Elser et al., 2003; Cleveland and Liptzin, 2007; Chen et al., 2010). In the study conducted by Cleveland and Liptzin (2007), such estimated ratios of C:N, C:P and N:P in the soil microbial biomass were 8.6, 59.5 and 6.9, respectively. These ratios were determined through an extensive literature review of 48 other studies that had looked at microbial biomass nutrient concentrations in the soil. Overall 186 observations were used to produce a dataset that contained values derived from a large range of soils, plant communities and management regimes. Despite such a large range of data, microbial ratios were apparently similar at the global level, with similarities between microbial and soil nutrient ratios observed in most studies. The growth rate of the microbial community has been linked to these nutrient ratios since appropriate concentrations of N and P are essential for protein synthesis and for growth. A concept that has been used to explore nutrient limitation based on the role of P and N in microbial growth is the so-called growth rate hypothesis (Chen et al., 2010). This hypothesis states that higher growth

rates are associated with lower C:N, C:P and N:P ratios, and hence lower growth rates are linked to relatively high ratios. This variation in P concentration between soil organisms, as reflected by shifts in nutrient ratios, was hypothesised to be linked to the allocation of P to ribosomal RNA which in turn drives protein production within the cell (Chen et al., 2010).

Microbial activity, indicated by shifts in soil microbial respiration, has also been used to investigate microbial nutrient limitation by several authors (Nordgen, 1992; Amador and Jones, 1993; Dilly, 1999; Smith, 2005; Demoling et al., 2007). For example, Nordgen (1992) developed a method for investigating how N and P limited microbial activity, based on the responses of their respiration rate following supplementation with glucose, glucose + N, or glucose + P, over a 70 hour period. By supplementing soils with concentration gradients of glucose + N and glucose + P, this experiment showed that the resultant respiration rate was proportional to the amount of limiting nutrient added, whereas, the respiration rate ceased to increase if the applied nutrient was not limiting. Based upon the respiration methodology discussed above, other studies have shown that soil microbial communities are in general primarily limited by C availability (Teklay et al., 2006; Demoling et al., 2007; Ilstedt et al., 2007). For example, Teklay et al. (2006) showed across different land-use types (cultivated and forest use) in an African soil that C was the primary limiting nutrient. This was due to a general respiratory response after the addition of C compared to glucose combinations with N and P: this was measured over a 600 hour period using an automated respirometer. Another observation in this study was that soil microbial communities can be co-limited by more than one nutrient. The addition of C+N invoked a greater respiratory response than C+P which indicated that N was more limiting than P. This was attributed to high concentrations of indigenous labile P in the young andisol soils used in that study (Teklay et al., 2006). Indeed, the universal extent of labile C limitation in soils is suggested by the SIR procedure, since the basis of this method is to induce microbial activity through glucose supplementation (Anderson and Domsch, 1978; Nordgen, 1992). Evidence for such limitation was pioneered in the study conducted by Anderson and Domsch (1978), which revealed that the application of glucose increased microbial respiration three to five times greater than the basal respiration rate in a wide range of soils. Therefore, the application of glucose stimulated microbial activity because labile C or energy

limitations imposed on the microbial community were removed. This stimulation was further developed in the study by Nordgen (1992), as glucose induced respiration was coupled with a nutrient hypothesised to be limiting microbial growth and activity in the soil, as described above. Glucose was supplemented in that study to remove the overarching labile C or energy limitation on microbial activity, so that the other nutrient limitations could be identified.

Smith (2005) detected that microbial respiration greatly increased after nutrient amendment in sub-Antarctic soils that were nutrient deficient. In particular, both the addition of N and P without glucose over a 7 day period stimulated microbial respiration in nutrient poor soils but failed to invoke such a respiratory response in soils with sufficient concentrations of N and P to stimulate microbial activity without labile C amendment. With respect to P concentrations in the soil, the microbial community has been shown to exhibit a respiratory response which was commensurate with native total P concentrations (Amador and Jones, 1993). This study focused on microbial respiration over a 21 day period in incubated peat soils and showed that the manipulation of C:P ratios in the soil by P additions affected microbial respiration. More specifically, at low total P concentrations (high C:P ratio), a greater respiratory response was observed compared to soils with high total P concentrations (low C:P ratio) following P application.

Dilly (1999) showed that the effect of such nutrient supplementation on the microbial community derived from agricultural and forest soil varies with time. Over a short time frame (0 – 8 h), changes in microbial respiration after supplementation with combinations of C, C+N and C+P may have been stimulated by microbial growth or a change in metabolic processes of the resident biomass. For example, there was a reduction in microbial respiration after the addition of C+N in the initial 8 hours of the experiment compared to the addition of C only (Dilly, 1999). This was attributed to microbial growth through C assimilation rather than solely respiration due to a rapid increase in N availability. However, over longer time frames, microbial respiration greatly increased in all treatments. In particular, over a 48 hour period before glucose depletion, the availability of N and P in the soil was truly expressed since greater respiration rates were observed in soils that were amended with a previously unavailable soil nutrient.

In Chapter 3, it was found in the field experiment conducted for this project, that microbial biomass P did not respond to different rates of P fertilisation. One possible reason may have been associated with the availability of other soil nutrients in that circumstance. Therefore, to test whether P was truly a limiting nutrient in the field experiment, laboratory experiments were conducted that focused on microbial responses to inorganic nutrient application. These experiments differed from each other in terms of the microbial property measured, the length of the incubation period following nutrient supplementation and the number of nutrients added. More specifically, Experiment 1 focused upon microbial biomass C, N and P pools over a 56 day period after P supplementation, whereas Experiment 2 focused upon microbial respiration over a 6 h incubation period following supplementation by factorial combinations of C, N and P. Despite these differences, both of these experiments shared a common aim, which was to investigate whether the microbial community resident in the soils derived from the field experiment were P limited. Based on this aim, the specific hypotheses were:

1. Microbial biomass P will be increased by the addition of P fertiliser to the soil, concomitant with the fertiliser application rate.

2. Supplementary P will increase glucose-induced respiration in soils not fertilised with P, relative to those that had received P fertiliser.

4.2 Materials and methods

The sample sites and management regime used in the field experiment have been described in Chapter 3 Section 3.2. In summary, the field experiment consisted of two sites that represented two soil types. Site 1 was situated on a sandy loam and Site 2 on a loamy sand soil. Both of these sites have been receiving four different application rates of 16% superphosphate (2 $CaSO_4$ + $Ca(H_2PO_4)_2$) since February 1995. These application rates include 0, 15, 30 and 45 kg P ha^{-1} y^{-1} (denoted P0, P15, P30 and P45, respectively), with four replicates of each treatment within a site. Vegetation in these sites was cut eight times a year and cuttings were removed. After each cutting event, 40 kg N ha^{-1} was applied to each plot as calcium ammonium nitrate. Muriate of potash

was also applied at a rate of 125 kg K ha^{-1} y^{-1}. When sampling these sites, five soil cores were collected from each plot using a Dutch auger (10 cm depth, diameter 4 cm) at the nodes of a W-best-of-fit transect.

4.2.1 Experiment 1: Phosphorus supplementation effects on the microbial biomass

Soil samples for this experiment were collected from Site 1 only in October 2010. Once collected, samples were sieved to <2 mm and field replicates bulked to form single composite samples associated with each of the fertilisation rates. Replicates of a single treatment were bulked in this manner to remove variability induced by field conditions and provide uniform soil properties for the laboratory study. The soil moisture content was determined for each bulked sample and adjusted to, on average, 50% water holding capacity using the method described in Chapter 2 Section 2.5. Composite samples were pre-incubated for 7 days at 21^{o}C before P fertiliser application. Soil moisture contents were maintained using a wet-wick paper towel, which was secured to the sample bag to ensure no contact with the soil. This method of maintaining moisture content was used in both Experiment 1 and 2. Composite samples (mean weight; 1.9 kg dry weight ± 0.006 S.E.) were divided into four laboratory replicates using the coning and quartering procedure (mean weight; 479 g dry weight ± 1.46 S.E.). Each laboratory replicate was treated equivalent to their respective field-based P fertiliser regime that was applied annually in February. Therefore, 0, 115, 231 and 346 µg P g^{-1} dry weight were applied to replicates derived from field plots treated with 0, 15, 30 and 45 kg P ha^{-1} y^{-1} respectively, which are equivalent rates assuming a mean bulk density of 1.3 g cm^3 for soil collected from this 0 – 10 cm depth range (R. Creamer, pers. comm.). P was applied to designated replicates in the form of 16% superphosphate (sieved <0.1 mm). P fertiliser was mixed with 20 g coarse sand prior to application to promote a more even dispersal of P throughout the replicate. Samples were then incubated at 21^{o}C for the duration of the incubation. Microbial biomass C, N and P were determined 0, 7, 14, 28 and 56 days after application (denoted Day 0, 7, 14, 28 and 56 hereafter): the procedures for these methods are described in Chapter 2 Section 2.1. Unfumigated samples from

microbial biomass C, N and P measurements were used to determine K_2SO_4 extractable organic C, K_2SO_4 extractable N and Olsen P concentrations in the soil, respectively.

4.2.2 Experiment 2: Nutrient supplementation effects on microbial activity

Soil for this experiment was collected from both Site 1 and 2 in April 2010. Once collected and sieved to < 2 mm, soil was pre-incubated at 21^{o}C for 7 days and maintained at field moisture contents for this period. Microbial respiration in the presence of different substrates was then determined using a modified Microresp^{tm} assay (Campbell et al., 2003). The substrates used were a factorial combination of C, N and P applied in the ratio 10:2:1 [w:w:w], respectively (Dilly, 1999). This ratio was chosen as it nominally reflects the nutrient status of microbial cells (Anderson and Domsch, 1980). Carbon was applied to soils in the form of D-glucose, N as equal N proportions of ammonium chloride (NH_4Cl) and potassium nitrate (KNO_3) and P as potassium dihydrogen orthophosphate (KH_2PO_4).

These substrates were added to give final concentrations of 12 for C, 2.4 for N and 1.2 for P (all mg g^{-1} soil water), via 25 µl of solution per well in the Microresp plates. This concentration of C was based on 30 mg glucose g^{-1} soil water used in previous studies that utilised the Microresp method (Campbell et al., 2003; Creamer et al., 2009). Each substrate combination was allocated in blocks of three wells and randomly positioned within the deep-well plate to compensate for any edge effects that would affect colourimetric quantification. Therefore, four blocks were realised for each of the eight substrates used: this included a basal (control) measure using only deionised water. Consequently, each deep-well plate represented a single plot and thus 32 plates were constructed. These plates were arranged randomly in the incubator during the incubation period.

To detect changes in CO_2 concentrations, a colourmetric gel detector plate was constructed 10 days before experimental use and stored, covered with Parafilm, within a desiccator with soda lime and water (Campbell et al., 2003). The colourmetric gel

was prepared using Cresol Red (12.5 ppm w/w), potassium chloride (150 mM) and sodium bicarbonate (2.5 mM) set in 150 µl of purified agar (1%). Before using a plate, the absorbance was determined for each well and the coefficient of variance calculated to measure variability between all wells within a plate: absorbance was measured at 570 nm using a Modulus^{tm} Microplate Multimode Reader (Turner BioSystems Inc, USA). Only plates with a coefficient of variation in background absorbance less than 10% across all wells were used.

Soil was added to each deep well plate using a bespoke filling device (Campbell et al., 2003), which ensured that equal volumes of soil were added to each well. After the addition of soil, a rubber seal was set in place followed by a colourmetric gel detector plate. The whole assemblage was secured using metallic clamps and incubated for 6 h at 25^{o}C. Colourmetric plates were then removed and absorbance measured.

Microbial respiration rate was calculated by relating absorbance from the detector plates to CO_2 concentrations that were correlated to absorbance prior to the experiment. This calibration was performed by taking gas samples from, and incubating detector plates in sealed glass vials with soil supplemented with glucose (30 mg glucose g^{-1} soil water) or water (basal measure). In this experiment, a CO_2 concentration gradient was achieved by using five different soils at 30, 20 and 10 ml soil volumes, with three replicates of each treatment. Glucose or water was added as 10% of the soil volume; for example, 3 ml was added to a 30 ml soil volume. Soil used in this calibration was incubated for 6 h at 25^{o}C. Gas samples were analysed using gas chromatography and CO_2 concentrations measured as ppm. Corresponding detector plates were analysed using colourimetry immediately after the incubation period. CO_2 concentration was plotted against absorbance as a rectangular hyperbola as a smaller wavelength reflects greater CO_2 concentrations. A respiration rate was deduced for each well used in this experiment from the conversion of 6 h CO_2 ppm to µg g^{-1} h^{-1} CO_2-C using gas constants, headspace volume in wells (945 µl), incubation time, soil fresh weight per well (g) and soil percentage dry weight (g) (Creamer et al., 2009).

4.2.3 Statistical analysis

Statistical analyses for Experiment 1 were conducted using repeated measures ANOVA on all measured parameters (Stats Soft, 1984 - 2011). Data obtained in Experiment 2 was analysed by 3-way factorial ANOVA using Site, P fertilisation rate and SIR treatment as main terms. For both experiments, *post hoc* Fisher's least significance tests were applied to establish homogeneous groups of means at the 95% level of significance.

4.3 Results

4.3.1 Experiment 1: Phosphorus supplementation effects on the microbial biomass

Significant main time and P fertilisation effects were observed on extractable organic C concentrations, with no interaction (Table 4.1). The P fertilisation effect (pooled across all sampling dates) showed greater C concentrations generally associated with the highest rates of P fertilisation (Figure 4.1(a)). Extractable C concentrations were significantly greater after 56 days, showing an overall increase of 28% during the incubation period (Figure 4.1(b)).

Table 4.1 ANOVA table showing the effects and interaction of P fertilisation (P treatment) and sampling time (Time) on soil chemical and biological parameters.

	Soil parameters (μg g^{-1})			Microbial biomass (μg g^{-1})			Microbial biomass nutrient ratio		
	Extractable C	Olsen P	Extractable N	C	P	N	C:N	C:P	N:P
P treatment	**	***	***			***	**		*
Time	***	***	***	***	***	***	***	***	***
P treatment x Time		***	***						

Significance terms denote *** p<0.001; ** p<0.01; * p<0.05; blank space p>0.05.

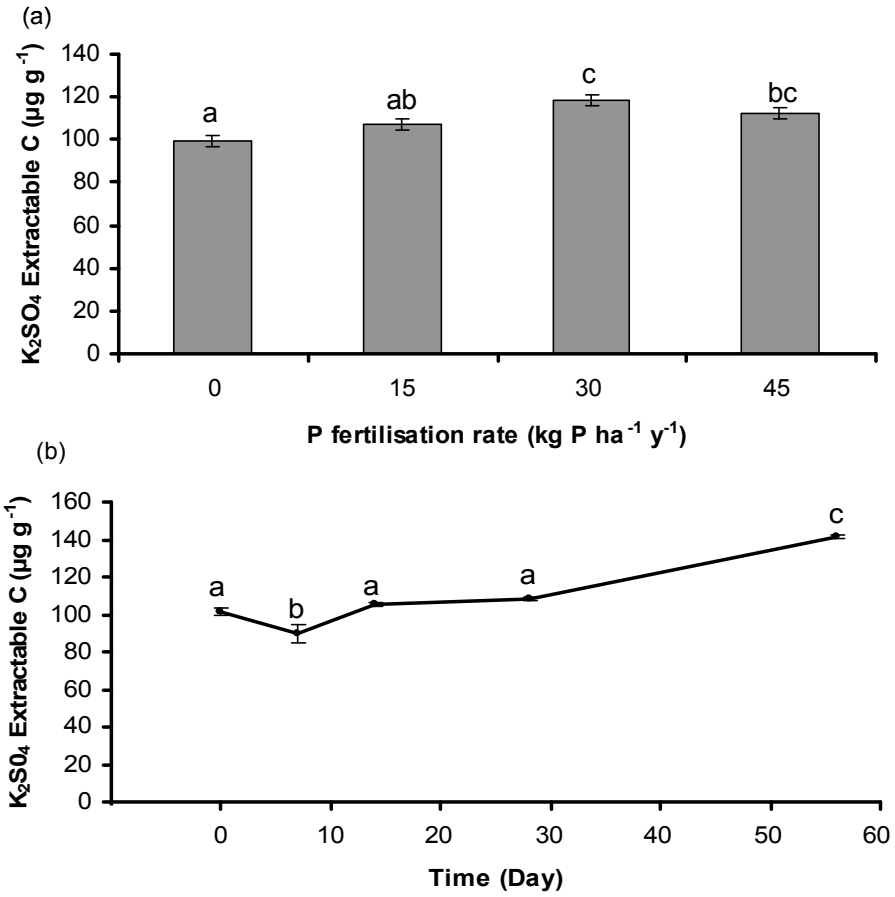

Figure 4.1 Main effects of (a) P fertilisation and (b) time following P application on K_2SO_4 extractable C concentrations. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test.

A significant interaction between P fertilisation and time was observed with respect to Olsen P concentrations (Table 4.1). This interaction showed that there was greater temporal variation in treatments fertilised with P compared to the P0 treatment (Figure 4.2(a)), with declines in P concentration over the first 7 days which was commensurate with the P application rate. Nevertheless, there was a general concentration gradient of labile inorganic P which showed an increase in P with increasing P fertilisation.

A significant interaction between P fertilisation and time was also observed on extractable N concentrations (Table 4.1). This interaction showed that N concentrations were congruent until Day 14, after which they diverged with greater

concentrations apparent in the higher P fertilisation treatments (Figure 4.2(b)). The greatest N concentrations were observed at Day 56 compared to other sampling dates, with *post hoc* analysis revealing that all treatments were significantly different from one another at this sampling time.

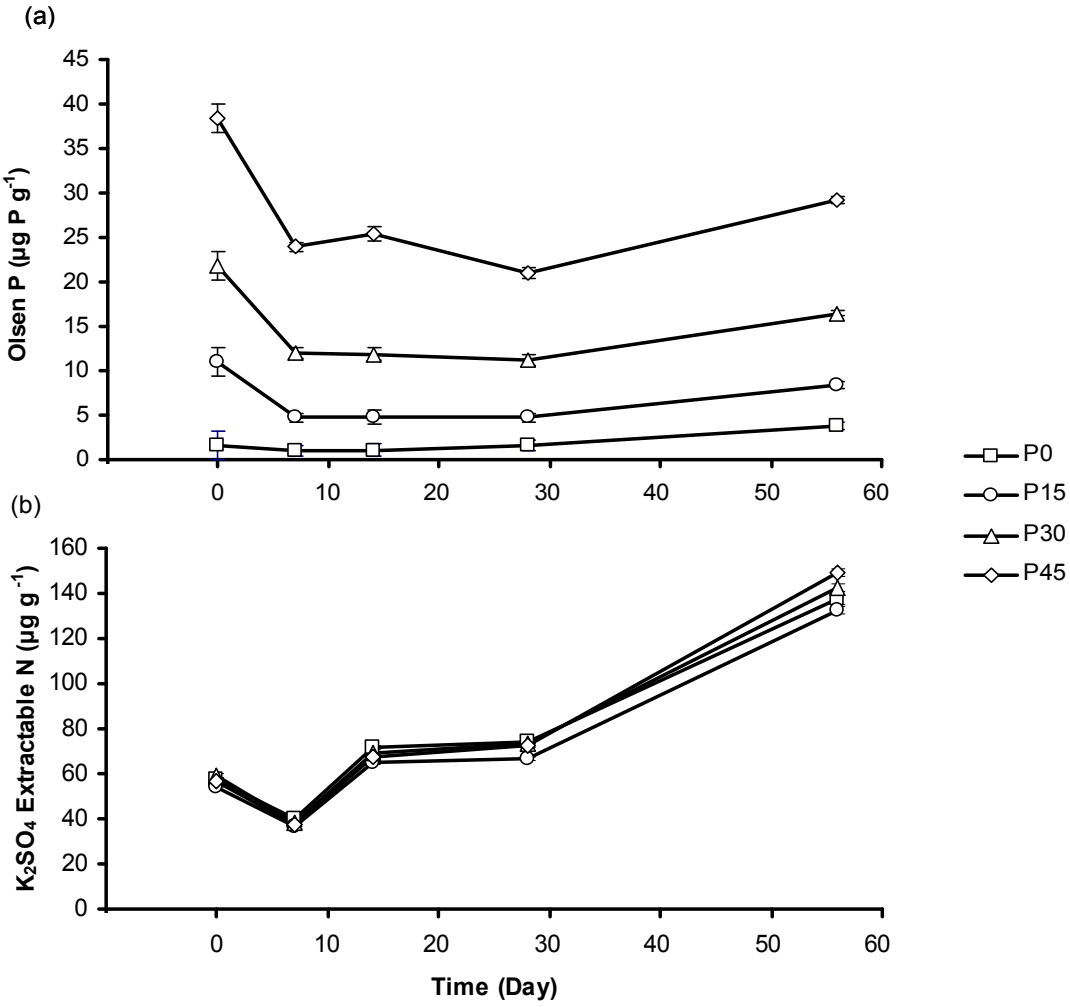

Figure 4.2 The interaction between P fertilisation rate and sampling time for (a) Olsen P concentrations and (b) K_2SO_4 extractable N concentrations. Error bars denote standard error.

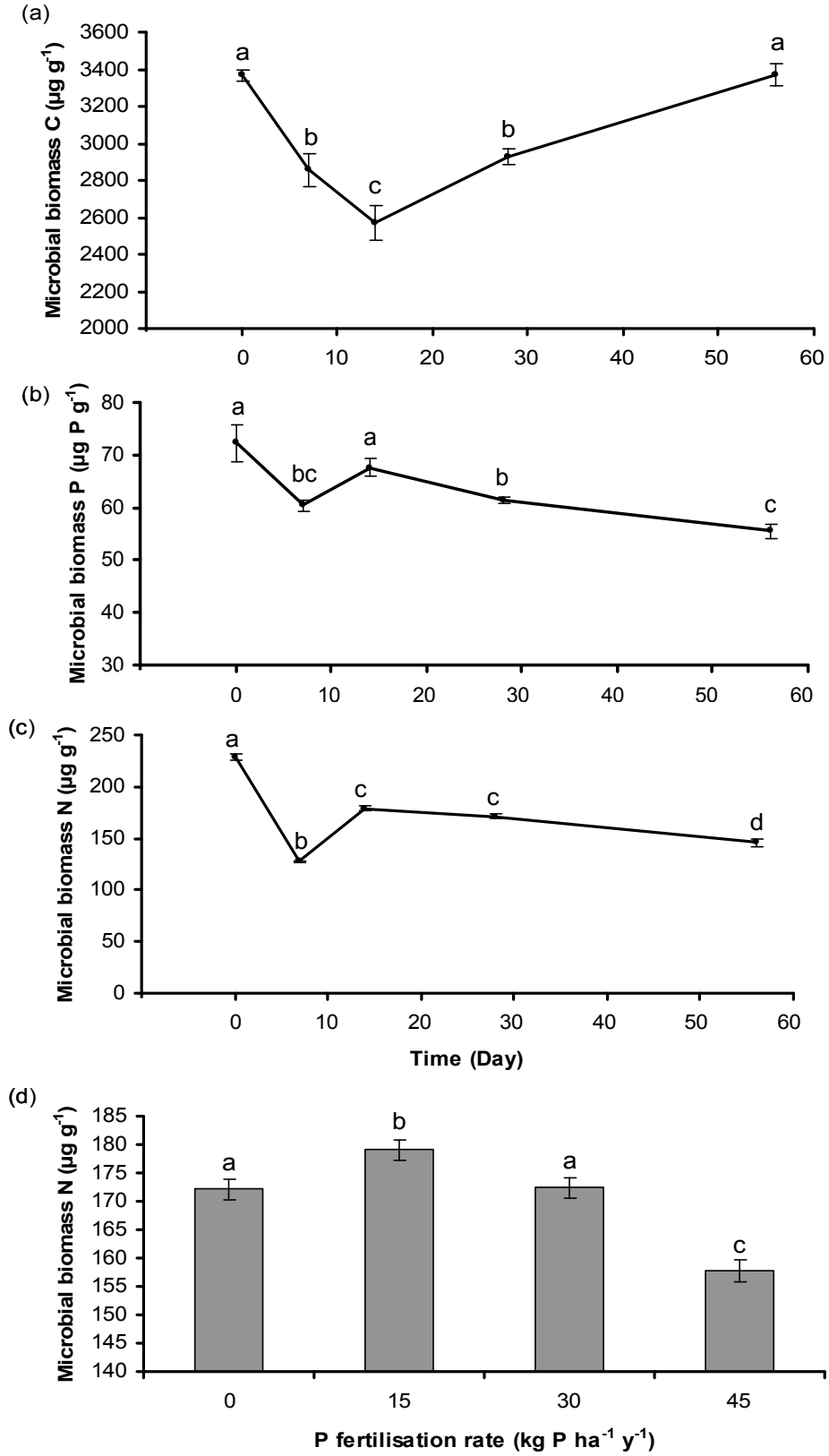

Figure 4.3 Main effects of time following P application on microbial biomass (a) C, (b) P and (c) N, (d) shows the effect of P fertilisation on microbial biomass N. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars represent standard error.

With regard to microbial biomass measurements, there were no significant interaction terms in the ANOVA. Main time effects were observed on C, N and P biomass concentrations after P application. Microbial biomass C concentrations decreased from Day 0 to Day 14 increasing thereafter to Day 56: this increase in the later part of incubation period resulted in microbial biomass C concentrations at Day 56 that were equivalent to concentrations at Day 0 (Figure 4.3(a)). Microbial biomass P concentrations decreased generally being 23% lower after 56 days (Figure 4.3(b)). The response of microbial biomass N over time following P application was similar to the profile observed for microbial biomass P (Table 4.1). This effect showed a 36% decrease in microbial biomass N concentrations over the incubation period (Figure 4.3(c)). A significant P fertilisation effect was observed on microbial biomass N (Table 4.1). This effect showed that microbial biomass N concentrations were greatest in the P15 treatment and lowest in the P45 treatment, whilst concentrations in P0 and 30 were similar (Figure 4.3(d)).

P fertilisation effects were observed on microbial biomass C:N and N:P ratios, but not the C:P ratio (Table 4.1). The microbial biomass C:N ratio was significantly higher in the P45 treatment compared to all other P treatments which were otherwise similar (Figure 4.4(a)). With regard to the microbial biomass N:P ratio, there was a general decline in N:P ratio with increasing P fertilisation (Figure 4.4(b)).

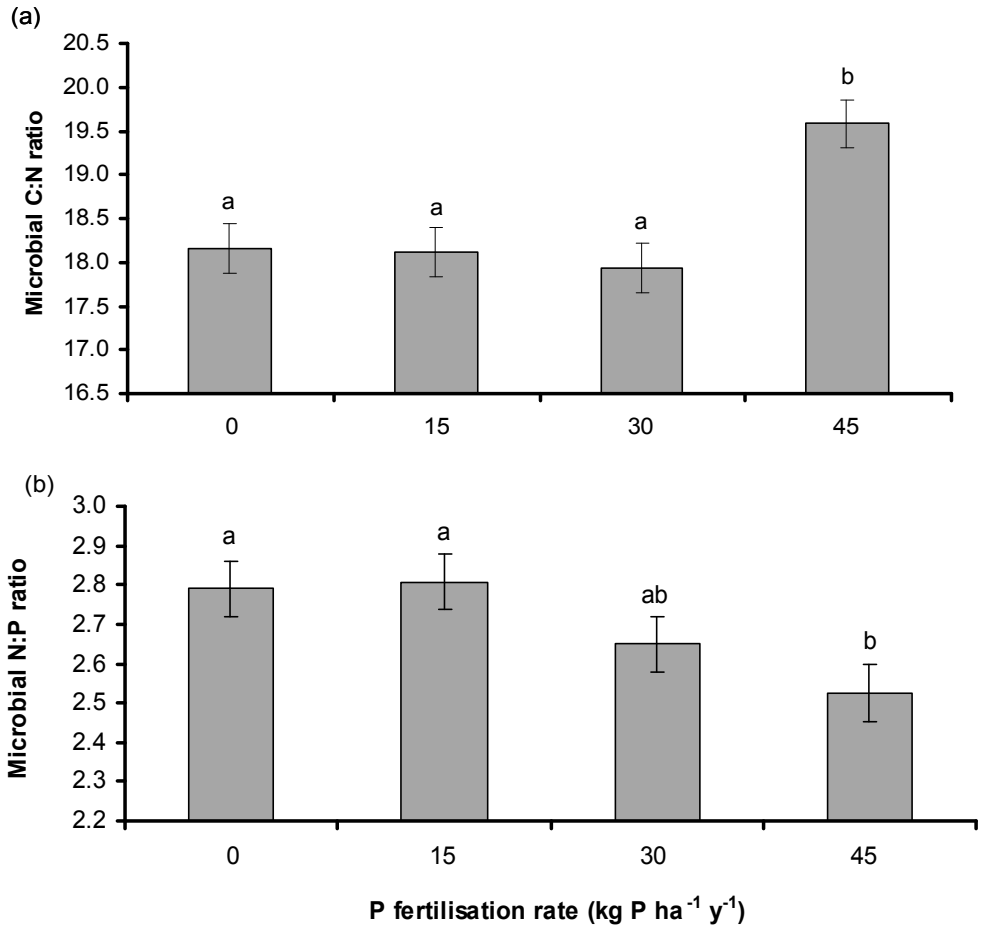

Figure 4.4 Main effects of P fertilisation on microbial (a) C:N ratios and (b) N:P ratios. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

Significant time effects were observed on microbial C:P, C:N and N:P ratios (Table 4.1). The mean microbial C:P ratio increased by 21% over the 56 day incubation period (Figure 4.5(a)). The microbial biomass C:N ratio also increased over time, with an increase of 37% from Day 0 to Day 56 (Figure 4.5(b)). However, the microbial biomass N:P ratio decrease by 23% from Day 0 to Day 14, then remained stable for the duration of the study (Figure 4.5(c)).

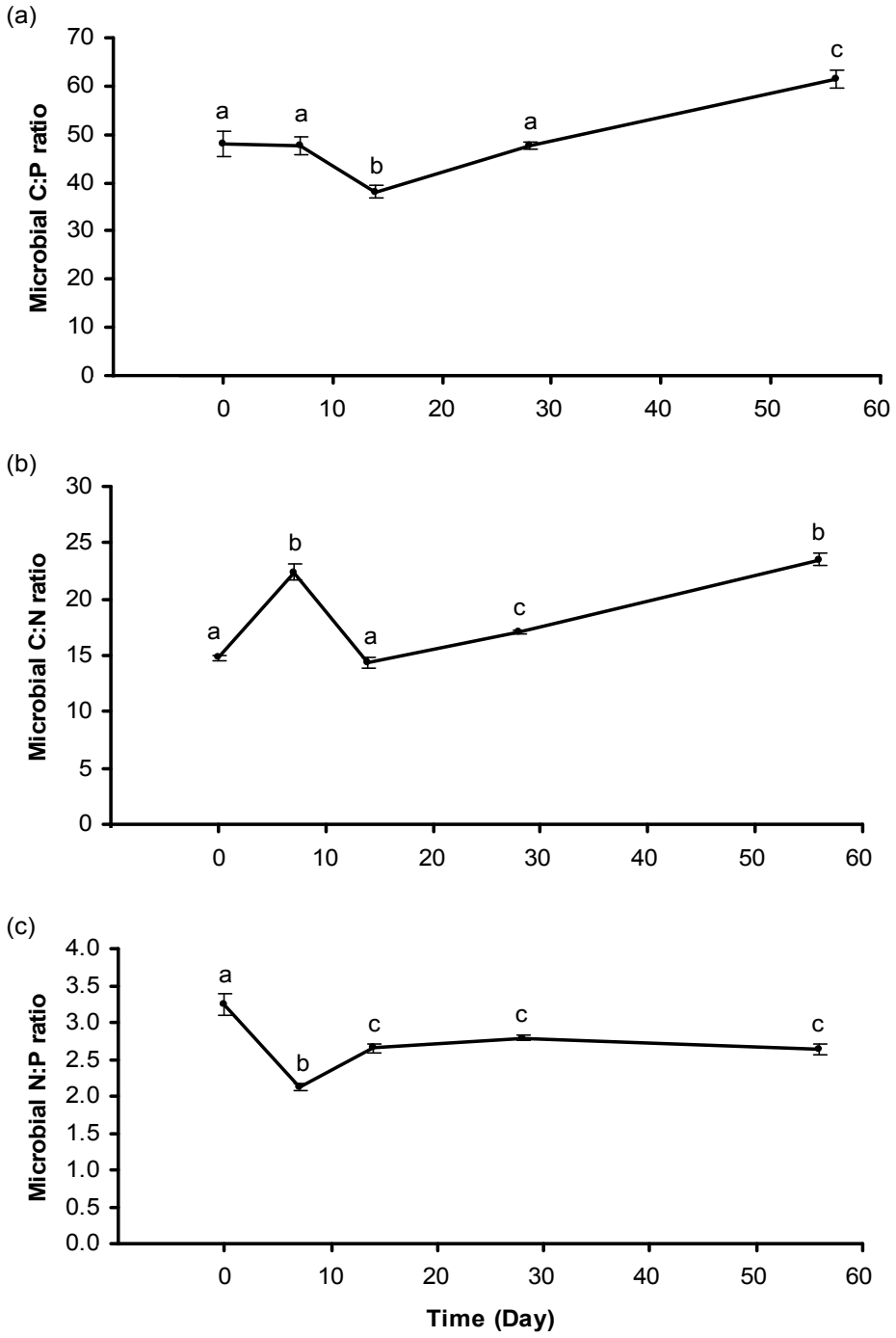

Figure 4.5 Main effect of time after P fertilisation on microbial (a) C:N ratios, (b) C:P ratios and (c) N:P ratios. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

4.3.2 Experiment 2: Nutrient supplementation effects on microbial activity

The basal respiration rate for P0, P15 and P30 treatments were below the limits of detection, whilst for the P45 treatment it was 0.49 ± 0.32 (S.E.) µg CO_2-C g^{-1} h^{-1}. Substrates that did not contain a C source failed in invoke a respiratory response greater than the basal respiration. Therefore, N, P and N+P substrate treatments were excluded from the ANOVA. Likewise, the basal respiration rate was not included in this analysis.

No significant interactions between P fertilisation, Site or SIR treatment were observed on microbial respiration when amended with C, C+N, C+P or C+N+P, but there were significant effects for each of these main terms (Table 4.2).

Table 4.2 ANOVA table showing the effects and interactions of Site, P fertilisation (P treatment) and substrate induced respiration (SIR) treatment on the rate of microbial respiration.

Source	Microbial respiration rate (ug $C0_2$-C g^{-1} h^{-1})
Site	***
P treatment	*
SIR treatment	***
Site x P treatment	
Site x SIR treatment	
P treatment x SIR treatment	
Site x P treatment x SIR treatment	

Significance terms denote *** $p<0.001$; * $p<0.05$; blank space $p>0.05$.

The respiration rate was not affected by supplementation with P (Figure 4.6). However, the presence of N resulted in a significant decrease in respiration, both in the presence and absence of P (Figure 4.6).

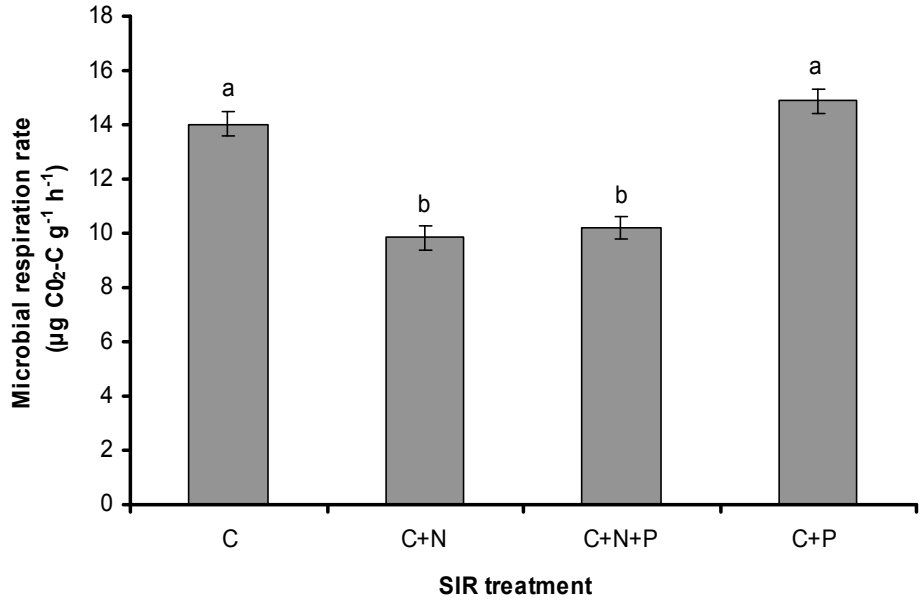

Figure 4.6 Main effect of the substrate induced respiration (SIR) treatment (C, C+N, C+P and C+N+P substrates) on microbial respiration. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

A greater mean respiration rate (pooled-mean across SIR treatments) was observed in soil derived from the P45 regime compared to the P0, P15 and P30 regimes, with no difference between these latter regimes (Figure 4.7).

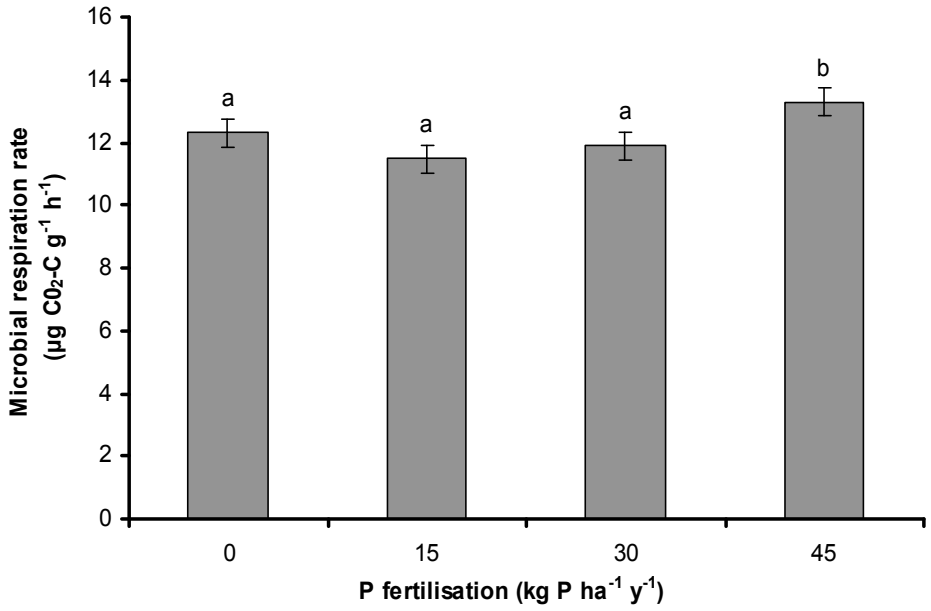

Figure 4.7 Main effect of P fertilisation on microbial respiration (pooled-mean across SIR treatments). Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

4.4 Discussion

4.4.1 Experiment 1: Phosphorus supplementation effects on the microbial biomass

4.4.1.1 Phosphorus fertilisation effects on labile inorganic phosphorus

Concentrations of labile inorganic P exhibited a gradient response to P fertilisation after application, where an increase in P was observed with increasing P fertilisation. This result would be expected under such circumstances, and such responses have been reported by several other authors (Stewart, 1991; Yang et al., 2002; Bunemann et al., 2011). The decrease after application for all treatments that received P was linked to the adsorption of P to the soil matrix. This was due to a shift in the equilibrium between P in the mobile phase (water soluble bioavailable P) and solid phase (bound P) after P application, since this application would greatly increase concentrations present in the mobile phase. In addition, the decrease in P from Day 0

to 7 was commensurate to the quantity of P supplied at Day 0. This may be associated with the concentration gradient of water-soluble P induced by increasing rates of P fertilisation. This concentration gradient would affect P adsorption to the soil fabric and thus affect the equilibrium between water-soluble and bound P. Nevertheless, the effect of P fertilisation on labile inorganic P concentrations would have promoted a concentration gradient of potentially bioavailable P, and hence provided a sound basis to test the hypothesis of this experiment.

4.4.1.2 Microbial growth rate stoichiometry during incubation

During the course of the incubation period, changes in microbial C:N, C:P and N:P ratios were observed. It is important to stress that the microbial biomass ratios reported in Experiment 1 were derived from microbial biomass concentrations that had been corrected using factors from the literature (Brookes et al., 1982; Brookes et al., 1985; Vance et al., 1987). Therefore, whilst these ratios are comparable to those reported in the literature, they may not accurately predict actual nutrient concentrations in diverse microbial communities (Cleveland and Liptzin, 2007). Nevertheless, there was an increase in the microbial C:N and C:P ratio over time and a decrease in the N:P ratio. According to the growth rate hypothesis (Elser et al., 2003; Chen et al., 2010) higher growth rates are coupled with lower C:N, C:P and N:P ratios as increased N and P concentrations in the microbial biomass indicate a faster growth rate due to greater protein synthesis linked to an increase in P-rich ribosomal RNA and N saturation (Hessen et al., 2007). In Experiment 1, an increase in the microbial C:N and C:P ratio over time indicated that the growth rate of the microbial community may have decreased during the incubation period. However, the microbial N:P ratio also decreased, which is in contradiction to the proposed hypothesis. The decrease in N:P ratio in this instance was due to a greater decrease in microbial biomass N compared to biomass P, a decrease of 36 and 23%, respectively. Therefore, this ratio was founded on a decrease in microbial biomass N and could be associated with N limitation in the microbial biomass (Cleveland and Liptzin, 2007; Chen et al., 2010).

4.4.1.3 Phosphorus fertilisation effects on the microbial biomass

In Experiment 1, the P content of the microbial biomass did not respond to the addition of P fertiliser. Therefore, the hypothesis stating that microbial biomass P would increase proportionally to the application rate of P was rejected. One possible explanation for this observation may be linked to the availability of other soil nutrients. Bunemann et al. (2004) showed that P availability did affect the P content of the microbial biomass, but only when adequate concentrations of labile C and N were also available. In addition, other laboratory studies that have looked at temporal patterns in microbial biomass P following fertiliser application have noted that the greatest increase occurs when both inorganic and organic sources of P were applied together (Ayaga et al., 2006; Gichangi et al., 2010; Gichangi et al., 2009). The explanation for this increase was linked to an increase in labile C and N with the application of manure. Increased labile C and N concentrations would then stimulate the microbial community to assimilate applied bioavailable P. With respect to Experiment 1, no C was added to the soils before or during the incubation period. However, concentrations of extractable organic C increased over the incubation period whilst microbial biomass C recovered to initial concentrations by the end of the experiment. This build-up of extractable C during the incubation period then suggests that a factor other than C was limiting.

A P fertilisation effect was observed on microbial biomass N: where there was a significant reduction in microbial biomass N in the P45 treatment, thus stimulating a significantly higher C:N and lower N:P ratio compared to other P treatments. This significant reduction may indicate that the limiting factor in these soils was most limiting in the presence of the highest P fertiliser rate. In this instance the microbial biomass appeared unable to assimilate N, as concentrations of extractable N generally increased during the 56 day incubation period, with a divergence between P treatments towards the end of the experiment in which N concentrations were greatest in the P45 treatment. Extractable N concentrations measured in Experiment 1 were reflective of total N concentrations in the K_2SO_4 extract. Thus, this increase in extractable N seems likely to reflect an increase in bioavailable N. Therefore, it seems unlikely that the microbial biomass was C, N or P limited in Experiment 1.

If none of these nutrients were limiting in this experiment, then it seems probable that nutrients not measured, like K or S, may have been limiting to the microbial biomass. In the field experiment (Chapter 3), both K and to a less extent S were applied to both grassland sites, since K was applied at the recommend rate and S is present in the superphosphate fertiliser. Therefore, in Experiment 1, S was applied during the superphosphate application at the beginning of the experiment, thus it seems unlikely that S may have been limiting to the microbial biomass. With respect to K, few studies have investigated microbial biomass K limitation in the presence of different fertiliser amendments (Perrott et al., 1990; Chowdhury et al., 2000; Chu et al., 2007). There is evidence, however, indicating that the addition of K can be beneficial in terms of facilitating soil decomposition processes and plant growth when coupled with the addition of other nutrients. In a study focusing on the importance of N, P and K nutritional balance in a crop system, it was shown that the greatest increase in microbial biomass C and metabolic activity occurred when K was applied with N and P compared to the applications of N+P, P+K and N+K (Chu et al., 2007). The application of N, P and K has also been shown to increase crop yield relative to the application of other inorganic fertiliser combinations (Cai and Qin, 2006). In Experiment 1, no K was applied at the beginning of the experiment and a P fertiliser effect was observed on K concentrations in the field experiment. More specifically, the P fertiliser effect on K concentrations revealed that such concentrations were greater in the P0 treatment compared to fertilised treatments. Therefore, initial soil K concentrations in Experiment 1 may have limited P acquisition by the microbial communities derived from fertilised treatments during the entire incubation period.

Whilst K may have been limiting to the microbial community, it is important to note that other elements that were not measured may have affected P acquisition. One commonly over-looked group of nutrients are the micro-nutrients and few studies have shown the importance of such nutrients in microbial decomposition processes (Kaspari et al., 2008; Powers and Salute, 2011). These studies have tended to focus on decomposition processes in tropical ecosystems; however, there does not appear to be any published studies that investigate micro-nutrient limitation in microbial communities derived from temperate grassland systems. The rare studies that did focus on micro-nutrient limitation and included grassland sites are focused upon heavy metal contamination (Khan et al., 2009). Nevertheless, these studies from the

tropics showed the complexity of microbial nutrient limitation due to the broad range of contrasting nutrients that can impose limitation, with particular reference to their importance in enzyme functionality and the necessity of multiple soil enzymes to breakdown chemically complex leaf litter (Kaspari et al., 2008; Powers and Salute, 2011). Powers and Salute (2011) focused on the role of macro and micro-nutrients on microbial decomposition of leaf litter and showed that the addition of P and Zn to one litter type increased CO_2 evolution, whereas the addition of N and Mg suppressed respiration on a different type of leaf litter. To further support the importance of micro-nutrients to microbial decomposition processes, the combination of multiple micro-nutrients was shown to increase leaf litter decomposition rates by 82% compared to the addition of P only that induced a 30% increase (Kaspari et al., 2008). The field sites used in this study by Kaspari et al. (2008) focused on fertilising forest plots which were situated on a low P fertility Oxisol soil. Therefore, these sites were P deficient and a P fertilisation effect was observed on decomposition rates. However, the 82% increase with micro-nutrient fertilisation was not fully discussed; thus the explanation for this observation was not entirely clear. However, such observation may indicate that micro-nutrient availability in the soil could potentially have substantial effects on litter decomposition processes. Highlighting the potential of micro-nutrient limitation in Experiment 1, and thus the field experiment, is pertinent due to the intensive grassland cutting regime (eight cuts per year) adopted in the field experiment. By adopting this regime, the majority of aboveground plant material, and all associated nutrients, would have been constantly harvested from both grassland sites. More specifically, by adopting this cutting regime, micro-nutrient availability may be low as large quantities of nutrient have been consistently removed from the field experiment for over 17 years and have not apparently been replenished, particularly since pure forms of inorganic fertiliser have been applied.

Whilst the results from Experiment 1 indicated that the microbial biomass was nutrient limited by a nutrient not measured, the plant data from the field experiment indicates no such limitation of biological nutrient acquisition. Indeed, the fact that an increase in plant P yield occurred with increasing P fertilisation supports this absence of a nutrient limitation to the plant community. Therefore, data from Experiment 1 provided supporting evidence that nutrient cycling by both microbial and plant

communities in the field experiment were seemingly uncoupled and were acting independently from one another.

4.4.2 Experiment 2: Nutrient supplementation effects on microbial activity

4.4.2.1 Nutrient effects on microbial respiration

Data collected in Experiment 2 rejects the primary hypothesis which stated that the microbial communities in the P0 treatment were P limited compared to communities that received P fertilisation. This was supported by the absence of any increase in microbial respiration between the addition of C only and C+P SIR treatments. This indicates that P was not a limiting nutrient to microbial activity in these soils. Amador and Jones (1993) conducted a study looking at nutrient limitation in peat soils, it was shown that the addition of P to soil that contained low total P concentrations increased microbial respiration, whereas the addition of P to soils with high total P concentrations did not alter respiration. One explanation for this result was that P additions to soils with already high P contents may have promoted greater C assimilation rather than respiration due to a lower C:P ratio. Furthermore, Raiesi and Ghollarta (2006) explained that the addition of P to a high P-fixing soil may stimulate microbial respiration based on soil C availability and that both nutrients may be concurrently limiting based on the P status of the soil. This was also supported by evidence that showed increases in microbial respiration after the addition of N and P (coupled with glucose) on arctic soils that were N and P deficient (Smith, 2005). Therefore, in Experiment 2, if P was a limiting nutrient in these soils, the addition of C+P to the P0 treatment should have invoked a greater respiratory response compared to the P45 treatment, because P was hypothesised to be more of a limiting factor in P0 compared to the regimes where P was added. Such hypothesis is based upon Morgan P concentrations obtained from the field experiment. In the field experiment, Morgan P concentrations in the P0 treatment fell within the P Index 1 classification. Therefore, P concentrations are regarded as being very low in the presence of this treatment compared to the optimum concentrations required to maintain sufficient plant P yields. However, the P30 and P45 treatments both fell within the P Index 3

classification and therefore were at the targeted P index classification for Irish grassland soils. Despite these differences in Morgan P concentrations, Experiment 2 showed similar microbial respiration rates between P treatments following C+P supplementation. The presence of P also, did not increase respiration in the presence of N, which further supports the absence of P limitation across P fertilisation treatments in this experiment.

The supplementation of C+N and C+N+P as SIR treatments was shown to decrease microbial respiration across all P fertiliser treatments compared to C and C+P treatments. This decrease may be attributed to a more efficient use of C that was added along with N as part of these SIR treatments (Dilly, 1999). This more efficient use of C denoted greater microbial assimilation of C into biomass rather than catabolised and expressed as increased respiration. Such assimilation may have been attributed to a shift in the microbial C:N ratio, which would have been lower following supplementation with N. By lowering the C:N ratio in this manner, the microbial community may have been stimulated to assimilate supplemented C to restore initial C:N ratios in the biomass.

4.4.2.2 Effects of phosphorus fertilisation on microbial respiration

Microbial communities derived from all P fertilisation treatments (including the P0 treatment) universally exhibited greater respiration after the addition of C. This indicates that labile C or energy was the overarching factor limiting to microbial activity in these soils. Labile C or energy limitation is a well-established phenomenon in the majority of soil systems (Ilstedt and Singh, 2005; Teklay et al., 2006; Demoling et al., 2007). Such a phenomenon is universally exhibited in the SIR method and forms its basis. Therefore, an increase in respiration following labile C supplementation was expected when compared to basal respiration rates in Experiment 2.

The P45 treatment also had significantly greater basal respiration and SIR rates compared to other treatments. Greater basal respiration in the P45 treatment indicates that C availability is greater in this treatment compared to other P fertiliser treatments. Indeed, contradicting results have been obtained on K_2SO_4 extractable organic carbon

concentrations in this project. In the field experiment, no effects of P fertilisation were observed on such concentrations, whereas, in Experiment 1 an effect was observed. Therefore, extractable organic carbon concentrations in the grassland sites used in this field experiment appear to be inconsistent during the sampling period. Concentrations of dissolved organic carbon (DOC) have been reported to exhibit great temporal variability and are readily affected by temperature and soil pH (Bonnett et al., 2006). Furthermore, DOC concentrations in the soil are also governed by biological processes, which in turn exhibit temporal variability. Therefore, such shifts in K_2SO_4 extractable organic carbon concentrations in this project may reflect climatic-induced effects during the sampling period in the field experiment and the effects of incubation in both laboratory experiments. Whilst greater basal respiration rates in the P45 treatment indicate greater C availability, a significant increase in the SIR rate following C supplementation indicates a more energy dependent system due to the greater availability of other soil nutrients in this treatment. However, as stated above, no differences between P treatments occurred following supplementation with other SIR treatments. Therefore, this response following the application of C only seemingly contradicts the effects of other SIR treatments on microbial respiration in Experiment 2.

4.4.3 Conclusions

Both Experiment 1 and 2 revealed that P was not particularly limiting to microbial growth and activity, thus these experiments indicate that a nutrient other than P may be limiting to the microbial community. Therefore, the overarching aim to investigate whether P was a limiting factor in the field experiment (Chapter 3) has shown that there was no effective supporting evidence that the microbial communities in these soils were P limited with respect to pool size or activity. Experiment 1 revealed that C and N were unlikely to be limiting in this experiment, as concentrations of extractable organic C and extractable N increased during a 56 day incubation period, despite an apparent decrease in the growth rate of the microbial community. In addition, Experiment 2 showed that C and P supplementation did not invoke a respiratory response in the presence of any P fertiliser regime. Such result indicated that P was not particularly limiting to microbial activity in the field experiment. Therefore, the

limiting factor in the grassland soils used in Experiment 1 and 2 and thus the field experiment, remains elusive.

Results from the field experiment showed a P fertilisation effect on labile K concentrations. Concentrations of labile K were specifically shown to be significantly greater in the P0 treatment compared to treatments where P was applied. Therefore, K concentrations may have been limiting microbial nutrient assimilation and respiration in both Experiments 1 and 2. In addition, other soil nutrients, like trace elements, that were not measured in either this experiment or the field experiment may have imposed nutrient limitation to the microbial community. Despite the supporting evidence in both Experiment 1 and 2 for nutrient limitation to the microbial community in the field experiment, no such limitation is apparent in the field experiment plant communities. The absence of limitation in the plant community is supported by an increase in plant P yield with increasing P fertilisation rate in the field experiment. Therefore, results from both Experiment 1 and 2 provided supporting evidence that nutrient cycling in the microbial and plant communities were independent from one another in the field experiment.

5. Phosphorus dispersal and acquisition by the soil biota

5.1 Introduction

5.1.1 Phosphorus application

In the preceding chapters, emphasis was given to the effects of inorganic P and N supplementation on the soil biota, with particular emphasis how P supplementation affects microbial and nematode communities. This particular experiment was aimed to discern how nutrient supplementation from different plant communities and fertiliser types affect soil microbial P acquisition and how the soil biota affects P incorporation into the soil matrix. By investigating soil biological P dynamics in systems receiving both inorganic and organic forms of fertiliser input to stimulate the microbial community, it was postulated that a more comprehensive view of soil biological P acquisition would be developed upon the results already obtained from previous chapters.

Both fertiliser application and efficient nutrient cycling are required to meet plant P demands in grassland systems. Once fertiliser has been applied, soil organisms play an important role in cycling these nutrients and increasing P availability to plants (Yeates, 1976; Brookes, 2001). However, P availability following application to the soil is notably dependent on the type of fertiliser used. This chapter focuses on the application of two commonly-applied forms of fertiliser P with the intent to fertilise the system, *viz*. slurry and superphosphate. Slurry in many grassland systems is derived from cattle, particularly via the dairy industry. Due to the heavy fertiliser inputs within typical dairy farming systems, dairy cattle slurry tends to have a high P content relative to other based P fertilisers. Typical concentrations are hard to predict as P concentrations in faecal material are reflective of factors like dietary intake and animal health (Bunemann et al., 2011). Nevertheless, intensive system dairy cows have been reported to excrete 43 – 88 g P day^{-1} in their faeces (Wu et al., 2001), in this instance, variability between these faecal concentrations was primarily dependent

on cow dietary intake. In contrast to organic fertiliser use, superphosphate (typically of 16% P concentration) is an inorganic P fertiliser that is manufactured by dissolving rock phosphate in 65 - 75% sulphuric acid (Sims and Sharpley, 2005). The application of this inorganic based P fertiliser increases water-soluble P concentrations in the soil solution. This increase in water-soluble P disrupts the equilibrium between P in the mobile phase (water-soluble bioavailable P) and the solid phase (bound P). Such disruption in the equilibrium is counter-balanced and water-soluble P is adsorbed into the soil matrix: which increases P concentrations in the solid phase. In a grassland setting, the application of inorganic P fertiliser leads to the accumulation of immobile bound P at the soil surface (Owens et al., 2008). Whilst the application of inorganic P fertiliser generally leads to the accumulation of P at the soil surface, the activity of the soil biota can act to promote the incorporation of P in the soil profile.

5.1.2 Phosphorus dispersal by the soil biota

Earthworms are intricately linked to nutrient cycling through their ability to engineer the soil matrix (Edwards and Lofty, 1977; Devliegher and Verstraete, 1997; Devliegher and Verstraete, 1996; Lavelle and Spain, 2001). Soil engineering in this context denotes their ability to manipulate soil macropore density, increase soil water retention and increase soil structural stability (Lavelle and Spain, 2001). However, another earthworm engineering function that is not commonly studied is their ability to redistribute and mix nutrients throughout the soil (MacKay et al., 1983). MacKay et al. (1983) reported that earthworm activity promotes the incorporation of P into the soil matrix through both the physical displacement and ingestion of soil, which may ultimately increase P availability to the plant community. Other studies indicate that earthworm activity can redistribute P from the soil onto the soil surface zones via their casts (Sharpley and Syers, 1977; Sharpley and Syers, 1976). The accumulation of P into earthworm casts can pose a potential risk to water quality, since P present in the cast is susceptible to mobilisation by overland flow, or the P-rich cast itself can be eroded by the flow of water over the soil surface (Sharpley and Syers, 1977). Once mobilised, P can be deposited into a water-course which can promote eutrophication. Despite the risk to water quality that P present in earthworm casts can pose, earthworm activity can also promote the incorporation of P into the soil matrix

(Sharpley et al., 2011). More specifically, following the application of inorganic and organic fertilisers, Sharpley et al. (2011) reported that anecic earthworm activity increased labile inorganic P concentrations below the soil surface in the presence of organic fertilisation, whereas no such effect occurred following inorganic fertilisation. Inorganic fertilisation resulted in P accumulation at the soil surface in far greater concentrations compared to all organic treatments. The application of organic fertiliser has also been shown to affect the structure of the nematode community (van Eekeren et al., 2009a). The study conducted by van Eekeren et al. (2009) reported a greater number of bacterial-feeding nematodes in sites that were organically fertilised compared to those that were inorganically fertilised. In comparison, inorganically fertilised sites revealed a greater number of plant-feeding nematodes. These differences in nematode community structure were coupled with N fertiliser effects on plant and microbial parameters and facilitated N cycling in these systems.

5.1.3 Nutrient cycling by the soil biota

Nematodes contribute to soil nutrient cycling by grazing on other soil organisms, which, has been shown to consequently stimulate microbial activity in the soil (Ingham et al., 1985). Grazing by the nematode community is extensive as nematodes feed on many different types of soil organism and thus exhibit a range of feeding habits (Yeates et al., 1993; Yeates, 2003). These feeding habits are commonly divided into five feeding groups; plant-feeding, fungal-feeding, bacterial-feeding, omnivorous and carnivorous nematodes. Due to this association with many components of the soil biota, they are often regarded as bio-indicators and reflect the fertility of soils (Ritz and Trudgill, 1999; Yeates, 2003). In addition, changes in nematode community structure have been used to assess shifts in microbial and plant communities. Numerous indices, indexes and ratios have been developed to quantify shifts in nematode communities and how this reflects indirect changes in other soil communities. One such measurement used by nematologists to assess soil biological changes is the so-called nematode channel ratio (Yeates and Bongers, 1999; Li et al., 2007; Viketoft, 2008). The nematode channel ratio (calculated as (B / (B+F)), where B is the number of bacterial-feeding and F is the number of fungal-feeding nematodes) is a measure of the change in dominance between fungal- and bacterial-

feeding nematodes in the nematode community. This shift between these microbial components of the community reflects changes in soil decomposition pathways. Fungal-dominated communities have been reported in environments which are dominated by recalcitrant substrate (Bardgett and McAlister, 1999). By contrast, bacterial-dominated communities thrive in environments where labile substrates are prevalent.

Shifts in microbial decomposition pathways are important to investigate as the microbial biomass is regarded as an important source of nutrition for the plant community. More specifically, the turnover of the microbial biomass releases P, both in labile organic and water-soluble forms, that plants can utilise (Brookes, 2001; Achat et al., 2010). Conversely, the plant community also supplies nutrients to the microbial community; of these nutrients, C and N have received particular attention in the literature, for example (Denef et al., 2009; Pietikainen et al., 2009). Soil nutrient amendment from the plant community occurs by two main mechanisms (van Elsas et al., 2007; Orwin et al., 2010). One mechanism includes the senescence of leaf litter onto the soil surface, which provides substrate for the soil heterotrophic community. The other mechanism includes the exudation of C from plant roots directly into the rhizosphere, where after the microbial community utilises these labile C substrates as sources of energy and synthesising biomass. Previous studies have shown that plant species differ in the quantity and quality of organic material released into the soil (Bardgett and McAlister, 1999; Bardgett and Shine, 1999). These studies have shown that such contrasts in organic amendment through litter deposition can readily shape the structure of the microbial community, hence affecting microbial functionality and biomass size. Therefore, different botanical diversities can shape the microbial community due to the input of a diverse range of litter material (Bardgett and McAlister, 1999; Bardgett et al., 1999; Innes et al., 2004). For example, a stimulatory effect of botanical diversity was reported on microbial measurements when multiple leaf litter types were incubated during a laboratory based experiment (Bardgett and Shine, 1999). Bardgett and Shine (1999) focused on soil which had been amended by different litter diversities and showed an increase in microbial biomass C with greater litter diversity. The application of organic material to the soil as a fertiliser also has stimulatory effects on microbial biomass pools (Gichangi et al., 2009; Ge et al., 2010). During an incubation experiment, greater concentrations of microbial biomass

P were observed with the application of manure compared to inorganic P application (Gichangi et al., 2009). However, the greatest increase in microbial biomass P was observed when both inorganic and organic fertilisers were applied together. Organic fertilisation using manure fertilisers was also shown to increase microbial biomass C concentrations compared to inorganic urea fertilisation (Ge et al., 2010). This effect on microbial biomass C was attributed to an increase in soil organic C due to the continuous applications of manure. Whilst effects on microbial biomass C and P have been reported in the presence of organic fertilisers, information on the effects of botanical diversity on microbial biomass P is scare.

This experiment was conducted in order to establish how the form of P fertilisation, earthworms and botanical diversity affect the incorporation of P into the soil matrix, its partitioning between soil and biotic pools, and microbial and nematode community structure.

The specific aims were:

1. To investigate how contrasting fertiliser amendments affect P incorporation and assimilation by the soil biota.

Hypotheses;

a) The application of slurry will promote a more even vertical distribution of labile inorganic P within the soil, compared to inorganic applications of superphosphate P.

b) The application of fertilisers will induce shifts in the structure of the microbial and nematode communities.

2. To examine whether earthworm activity can promote the incorporation of surface applied P into the soil matrix.

Hypotheses;

a) The presence of earthworms will promote a more even vertical distribution of labile inorganic P in the soil matrix.

3. To investigate botanical diversity effects on biological P assimilation and belowground communities.

Hypotheses:

a) An increase in botanical diversity will increase microbial biomass P.

b) Greater plant P yields will be harvested from treatments with greater botanical diversity.

5.2 Materials and Methods

Experimental mesocosms were constructed using polypropylene containers (27 x 38 cm, 35 cm deep), filled to an average depth of 27 cm with two layers of contrasting soils. The soil used as the lower layer (30 cm) was derived from a low-fertility pasture situated on a sandy loam soil. The soil used to construct the upper layer (1 cm) was from a high-fertility pasture also situated on a sandy loam soil. Both of these soils were located at Johnstown Castle, Wexford, Ireland; the initial characteristics of both are described in Table 5.1. To examine these initial characteristics, five soil cores were sampled from both pastures in a W-sampling strategy to account for variability within either sampling plot. Soil used to construct the mesocosm lower layer was collected using a mechanical digger to strip the surface vegetation and then collect soil from between 10 – 50 cm within the whole sampling area. With respect to the upper layer, all soil was collected from between 0 – 10 cm from the high-fertility sampling area. Once collected, both soils were air dried for 1 week, and homogenised whilst sieving to 4 mm. Sieving was conducted to removal earthworms and their cocoons from both soils. The homogenised lower layer soil was added on top of a drainage layer of stone chippings (3 cm). After initial settling of this soil, the average bulk density of the lower layer soil was 1.02 g cm^{-3} ± 0.004 SE. To ensure that no earthworms escaped from the drainage holes in the bottom of each mesocosm, wire mesh with an aperture of 0.5 mm was placed in the bottom of the mesocosms. Soil used for the upper layer was added to each mesocosm until a 1 cm depth was achieved; this made an overall depth of 31 cm for each mesocosm. After the addition

of this soil, the actual exposed surface in each mesocosm had an effective surface area of 0.1 m^2 (Figure 5.1).

Each mesocosm was then placed upon wooden supports and different treatments were randomly allocated to each mesocosm to generate an incomplete randomised block design for the factors P fertiliser form, earthworm inoculation and botanical diversity (Table 5.2). This design was incomplete since earthworms were not added to unplanted mesocosms, since unplanted mesocosms were judged to be unsuitable for earthworms to thrive due to a lack of food source.

All mesocosms were watered daily at a rate of 398 ml day^{-1} (1500 mm y^{-1}) until November 2010 and then changed to a rate of 286 ml day^{-1} (1000 mm y^{-1}). From March 2011 till the end of the experiment, mesocosms were watered at 398 ml day^{-1}; these rates were used to reflect average rainfall for Ireland. Water was applied using an irrigation system that releases water directly into the soil via an automated timed system that regulated the water application. Water flow was checked across all mesocosms to ensure that it was consistent throughout the whole watering system.

Table 5.1 The initial mean soil characteristics for two different soils used in the construction of experimental mesocosms. Standard error is displayed in the parenthesis.

		Soil Position	
	Property	**Top soil**	**Bottom soil**
Particle size (%)	Coarse sand (2 - 0.2 mm)	25	32
	Fine sand (0.2 - 0.05 mm)	29	30
	Silt (0.05 - 0.002 mm)	32	25
	Clay (<0.002 mm)	14	13
	Morgan's P (µg g^{-1})	19.1 (0.2)	2.4 (0.06)
	Olsen P (µg P g^{-1})	29.2 (0.47)	3.9 (0.45)
	K^+ (µg g^{-1})	122 (0.7)	119 (1.34)
	LOI (%)	10.5 (0.8)	4.7 (0.07)
	pH	5.7 (0.01)	5.9 (0.01)
Microbial biomass	C (µg g^{-1})	1670 (58.3)	322 (6.5)
	N (µg g^{-1})	184 (5.7)	58.4 (2.1)
	P (µg P g^{-1})	123 (6.9)	28.4 (1.3)
	Microbial Fungal: Bacterial ratio	0.37 (0.016)	0.023 (0.001)
	Total number of nematodes (g^{-1} dry weight)	8 (0.35)	4.7 (0.59)
	Nematode Channel Ratio	0.93 (0.02)	0.88 (0.03)

Figure 5.1 Mesocosms set within an incomplete randomised block design in a glasshouse based at Teagasc Environmental Research Centre at Johnstown Castle, Wexford, Ireland.

Table 5.2 The experimental treatments for this study; where + is presence and – is absence.

	Treatments		
Factorial combinations	**Plant**	**Fertilisation**	**Earthworm**
1	Bare	Inorganic	-
2	Bare	Organic	-
3	Monoculture	Inorganic	+
4	Monoculture	Inorganic	-
5	Monoculture	Organic	+
6	Monoculture	Organic	-
7	Polyculture	Inorganic	+
8	Polyculture	Inorganic	-
9	Polyculture	Organic	+
10	Polyculture	Organic	-

Four grassland plant species representing three different functional groups were used to create the mixed plant community: one grass species, *Lolium perenne*; two forb species *Plantago lanceolata* and *Achillea millefolium*; and one legume species, *Lotus*

pedunculatus. Plant monocultures consisted of *Lolium perenne* only. Either plant monocultures or polycultures were applied to their respective mesocosm in equal total number of seeds that was predicted to germinate based on preliminary germination studies, adopting a seeding rate of 4 g m^2. These were applied in May 2010. In polycultures, each species was equally represented, thus each species represented 25% of the community structure at the beginning of the experiment. Before applying seeds, each seed mixture was mixed with 50 g of sand to facilitate the uniform dispersal of seeds on the soil surface.

5.2.1 Earthworm treatment

Two ecological groups of earthworm were used; an anecic species and an epigeic/endogeic species. The anecic earthworm species used was *Aporrectodea longa* and the epigeic/endogeic species was *Allolobophora chlorotica*. When applying earthworms, three *Aporrectodea longa*, with a mean total weight of 5.67 ± 0.16 g, and six *Allolobophora chlorotica*, with a mean total weight of 1.84 ± 0.19 g was used. These earthworm species were chosen as they reflect commonly occurring species in grassland systems at Johnstown Castle (Curry et al., 2008). The earthworm densities used in this experiment for these two species were greater than the densities observed in grassland sites at Johnstown Castle (Curry et al., 2008). This was to compensate for potential earthworm mortality during the course of the experiment.

All earthworms were applied to their respective mesocosm in September 2010 and were collected from the surrounding farmland areas at the research centre. Earthworm casts were counted in every mesocosm after plant yield samples were taken. Labile inorganic P concentrations were also extracted in earthworm casts using the Morgan's P method as stated in Chapter 2.

5.2.2 Fertiliser amendments

After cutting each mesocosm, N and K were applied to compensate for the loss of these nutrients from the system. Nitrogen was applied in the form of urea ($CO(NH_2)_2$) at a rate of 125 kg N ha^{-1} for the second cutting period and at a rate of 100 kg N ha^{-1} for subsequent cut (Coulter and Lalor, 2008). Therefore, 2.67 g of urea was applied during the second cutting period and 2.14 g of urea was added for all following cuts to each mesocosm. Potassium was applied as potassium sulphate (K_2SO_4) at a rate of 150 kg K ha^{-1} for the second cutting period and was applied at 50 kg K ha^{-1} for following cutting periods (Coulter and Lalor, 2008). Thus, 6.88 g of potassium sulphate was applied to each mesocosm in the second cutting period and 2.3 g in following cutting periods. When fertilisers were applied to each mesocosm following cutting, they were dissolved in 1 L of deionised water prior to application. This is due to the nature of the irrigation system used to supply water to each mesocosm, as this system directly supplied water into the soil rather than disperse water over the soil surface.

The application of fertilisers that are an experimental treatment occurred in September 2010. Organic fertilisation of mesocosms involved the application of cattle slurry to the soil surface and inorganic fertilisation involved adding N, P and K in reciprocal concentrations as those observed within the applied slurry. The application of cattle slurry (containing 3.27 g N, 0.69 g P and 3.08 g K $mesocosm^{-1}$) to their respective mesocosms was based on a slurry application rate of 25 m^3 ha^{-1}, and thus 250 ml of slurry was applied to each mesocosm. As the inorganic fertilisation treatment, 7.01 g of urea, 2.65 g of 16% superphosphate (2 $CaSO_4$ + $Ca(H_2PO_4)_2$) and 14.13 g of potassium sulphate were added to fertilise mesocosms with N, P and K, respectively, equivalent to concentrations applied with cattle slurry. Both potassium sulphate and urea were dissolved in 1 L of deionised water before application. Superphosphate was ground, sieved (< 0.1 mm aperture,) and applied as powder over the soil surface.

5.2.3 Plant analyses

All plants were cut back to 5 cm and the cuttings analysed for dry matter yield, P and N content every six weeks. The methods followed for this procedure are described in Chapter 2 Section 2.3.

5.2.4 Mesocosm sampling procedure

Deconstruction of all mesocosms occurred in May 2011 immediately after plants were harvested from the relevant mesocosms. Mesocosms were prepared for deconstruction by marking out the walls at each depth, based on the distance from the soil surface. These depths were 0 - 1, 1 - 5, 5 - 10, 10 - 20 and 20 - 30 cm. Mesocosms were then hand-sawn and soil collected at each depth range. During this process, earthworms were collected as found, including an associated check in mesocosms that did not originally contain them. Thus, the abundance of juvenile, adult *Allolobophora chlorotica*, adult *Aporrectodea longa* and the total weight of all earthworms were determined. Subsamples were then taken from the 0 - 1, 1 - 5 and 5 - 10 cm depths, coned, quartered and then bulked to form a composite sample for nematode analysis. From this bulked sample, 100 g fresh soil was weighed out and elutriated following the method previously stated in Chapter 2 Section 2.2. The remaining soil was sieved to 4 mm for each depth and then subsampled, by coning and quartering, for moisture content and PLFA. PLFA samples were immediately store at -80^{o}C until further sample preparation was carried out, as described in Chapter 2 Section 2.3. Soil was further subsampled, by coning and quartering and sieved to 2 mm. This soil was pre-incubated for a week at 21^{o}C and then analysed for microbial biomass C, N and P using the methods stated in Chapter 2 Section 2.1.

The remaining soil from all five depths was oven dried at 40^{o}C and sieved to 2 mm for soil elemental analysis. Labile inorganic P and K^{+} were extracted using the Morgan's extraction procedure. Total C and N were analysed by combustion using a LECO elemental analyser. Soil pH was also measured at all depths. All these methods have been described in Chapter 2. A summary of the measured parameters and the depths they were measured at is provided in Table 5.3.

Table 5.3 A summary of measured chemical and biological parameters and the depths they were measured at. * refers to a taken measurement. Nematodes were measured over a composite 0-10 cm samples. Earthworms were collected from the entire mesocosm.

	Chemical property					Biological property					
Depth (cm)	Morgan's P	K^+	Total N	Total C	pH	Microbial C	Microbial N	Microbial P	PLFA	Nematodes	Earthworms
0 - 1	*	*	*	*	*			*	*	0-10	0-30
1 - 5	*	*	*	*	*	*	*	*	*		
5 - 10	*	*	*	*	*	*	*	*	*		
10 - 20	*	*	*	*	*						
20 -30	*	*	*	*	*						
Earthworm casts	*	*	*	*	*						

5.2.5 Statistics

Data was checked for normality was natural log transformed when necessary for all ANOVA analyses (Stats Soft, 1984 - 2011). For plant analyses at different sampling occasions, plant yield, N and P measurements were analysed using repeated measures ANOVA. Total N and P yields from plant cuttings were analysed using one-way ANOVA.

Earthworm cast abundance at each plant sampling event was analysed using repeated measures ANOVA. The abundance and weight of earthworms at the end of the experiment were analysed using a factorial ANOVA between fertiliser amendment and plant community structure.

All other soil biological parameters (microbial biomass, PLFA, nematode abundance and community structure) and two chemical parameters (Olsen P and P recovery rate) were analysed using a factorial nested ANOVA design. This nested design was achieved by designating all earthworm and plant treatments containing plants as one denominator referred to as planted and no plant containing treatments as unplanted. By doing this, it was possible to overcome the incomplete factorial design and compare all treatments and their interactions by treating unplanted circumstances as their own treatment and weighting the means derived from the imbalanced number of

observations. Slight variations in the implementation of this design occurred based on the number of depth ranges each parameter was measured at. Depth was treated as an exploratory variable for analysis and was not nested. PLFA and nematode community structure (either at family or trophic level) were firstly analysed by principal components analysis to derive factor scores. These principal components were analysed using the factorial nested ANOVA design.

Nutrient data collected at all five depth ranges was modelled based on an exponential decay curve between the nutrient in question and depth. Data was ln-transformed, as the variation in nutrient concentration between replicates increased moderately with overall increasing concentrations (Mead et al., 2003). The term applied to achieve this relationship was nutrient concentration (NC) = $Q1 \bullet e^{-Q2 \bullet d}$. Thus ln(NC) = ln(Q1) + -Q2 ln(d), where ln(NC) is the natural log of the nutrient concentration, ln(Q1) is the intercept, -Q2 is the curvature and ln(d) is the natural log of depth (cm). By plotting the ln(NC) against the ln(d), both ln(Q1) and –Q2 were obtained from linear regression and used to assess differences in nutrient concentrations with different treatments (ln(Ql)) or evenness of the distribution (-Q2; Figure 5.2). To determine whether both ln(Q1) or –Q2 were affected by treatments, stepwise regression was applied to this data which specifically compared main treatments against the baseline treatments (inorganic fertilised, unplanted and not containing earthworms). With respect to comparisons between fertiliser types, then the comparison was made between organic and inorganic fertilisation by using the inorganic treatment in all unplanted or planted circumstances as a baseline measure.

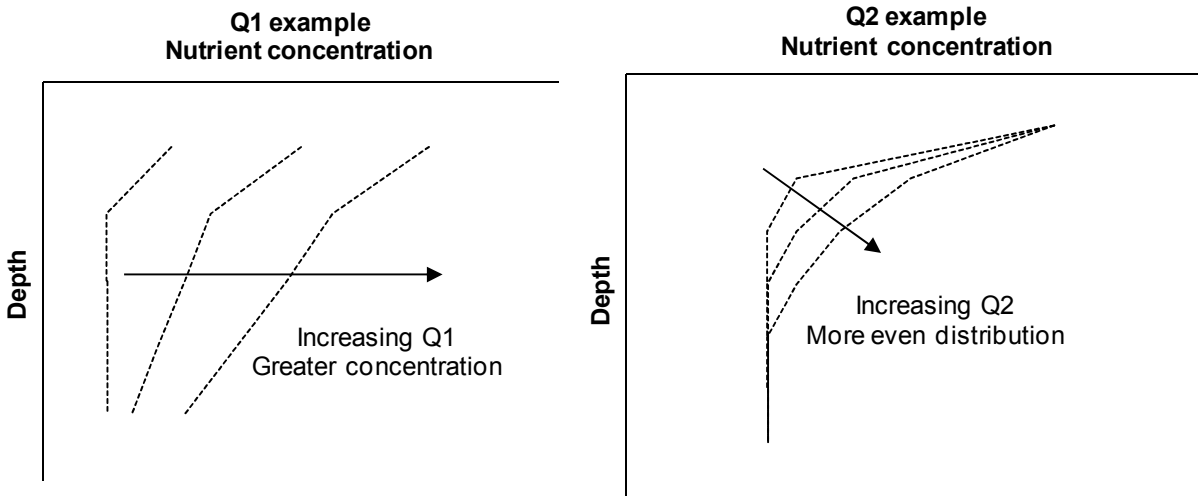

Figure 5.2 Diagram explaining how changes in the two calculated parameters relate to changes in nutrient concentration and distribution. (a) denotes a change in Q1, which explains increasing or decreasing nutrient concentrations and (b) denotes Q2, which explains how changes in curvature relate to nutrient mixing in the soil, thus relating to distribution.

5.3 Results

5.3.1 Herbage data

Table 5.4 details the significance terms pre- and post- earthworm and fertiliser treatment on all plant measurements; second-order interactions between plant composition and time were manifest in relation to plant dry matter and N yield. After earthworm and fertiliser treatments were applied, second- order interactions between plant composition and time were observed on plant P yields. Interactions between fertiliser amendment and time following application were observed on plant dry matter, N yield and P yield.

Table 5.4 ANOVA table showing the effects and interactions that occurred on the plant dry matter, N and P yield before (Pre-treatment) and after (Post-treatment) the application of earthworms and fertiliser to respective mesocosms. Missing interactions were not included as they were not significant.

	Plant measurment (g m^2)		
	Dry matter	**P yield**	**N yield**
Pre-treatment			
Plant	***	***	***
Time	***	***	***
Plant x Time	***		***
Post-treatment			
Plant	***	***	***
Earthworm			
Fertiliser	***	***	***
Time	***	***	***
Plant x Time	***	***	***
Fertiliser x Time	***	***	***
Plant x Earthworm x Time	*		

Significance terms denote *** $p<0.001$; * $p<0.05$; blank space $p>0.05$.

Before the application of earthworms and fertilisers to their respective mesocosms, greater plant dry matter yields were obtained from mixed plant communities compared the monocultures (Figure 5.3(a)). There was an increase in overall plant yield as the plant community developed prior to the fertilisation treatment being applied. A significant interaction between plant community and sampling date on harvested plant dry matter occurred after the application of earthworms and fertiliser treatments (Table 5.4). This interaction showed that both mono and mixed plant communities produced the same quantity of dry matter for both the November and March sampling dates, but for April and May before deconstruction, the composition of the plant community significantly affected the production of plant dry matter.

The plant P yield removed from each mesocosm per harvest was constantly and significantly greater in mixed plant communities compared to monoculture communities prior to the application of fertiliser and earthworm treatments (Table 5.4). Whereas afterward, similar P yields between plant communities were observed in both November and March. Consequently, a fertiliser effect was observed in November and March, in which greater P yields were observed in the presence of inorganic fertilisation compared to organic fertiliser ($p<0.001$). In April, P yield was affected by plant community composition again, where greater concentrations were observed in mixed plant communities compared to monoculture (Figure 5.3(b)), and the fertiliser effect ceased to affect P yield.

There was a significant interaction between plant community composition and harvest occasion on the N yields prior to the application of fertiliser and earthworm treatments (Table 5.4), where greater N yields were obtained in the presence of mixed plant communities compared to monocultures. After fertiliser application, the difference in N yield between the plant communities was removed for both November and March sampling occasions but then resumed from April until mesocosm deconstruction (Figure 5.3(c)). The application of the fertiliser treatment removed the botanical effect for November and March: where inorganically fertilised treatments increased the accumulation of N in plant material compared to organically fertilised treatments (Table 5.4).

There was a significant plant community effect on the cumulative amount of P and N removed, with greater yields from mixed plant communities for both nutrients (Table 5.5).

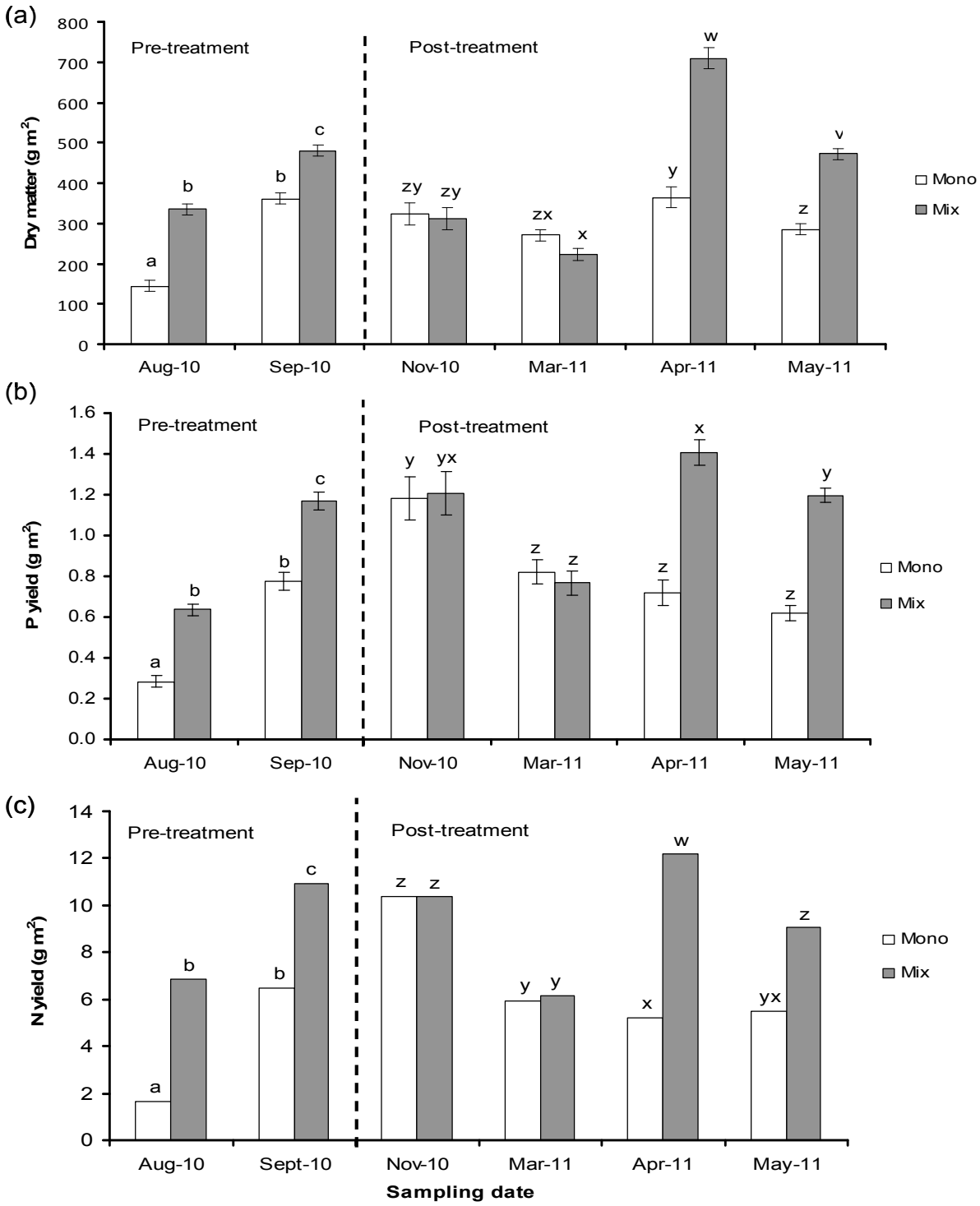

Figure 5.3 Mean (a) dry matter, (b) P and (c) N yields in the aboveground plant material collected from each mesocosm at specific sampling dates during the whole course of the experiment in the presence of two botanical diversities. The dotted vertical line symbolises the time when earthworms and fertilisers were applied to the experiment. Error bars in (a) and (b) denote the standard error; (c) data ln-transformed for analysis. Letters denote significant differences in either pre-treatment or post-treatment data using Fisher least significant difference test.

Table 5.5 Mean total P and N yields removed in plant material harvested during the course of the experiment.

	Nutrient yield (g m^2)	
Plant treatment	**P**	**N**
Mono	4.4	34.2
Mix	6.4	55.1
EMS	1.07	0.04
Plant	***	***

Plant N data was analysed using ln – transformation thus the error mean squared (EMS) was derived from this analysis. Plant N means have been back-transformed. *** denotes a significance of $p<0.001$.

No effects of fertiliser treatment or earthworm presence were observed on the species-specific yield of *Plantago lanceolata, Lotus penunculatus* and *Achillea millefolium* harvested on the last sampling occasion before mesocosm deconstruction, thus no effects were observed on the total weight of plant material. However, the mass of *Lolium perenne* collected was affected by both fertiliser treatment and earthworm presence (Table 5.6). With respect to fertiliser treatment, greater weights occurred with inorganic fertiliser (mean = 6.4 g ± 0.73) as opposed to organic fertilisation (mean 2.9 g ± 0.73). The biomass of *Lolium perenne* was greater in the presence of earthworms (mean = 6 g ± 0.73) compared to earthworm absence (mean = 3.3 g ± 0.73.) No significant interaction between earthworm presence and fertiliser treatment occurred for any plant species.

Table 5.6 Mixed plant community composition data which shows the mean weights of each plant species as a function of the different combination of treatments applied. EMS refers to the error mean squared. Significance; * p<0.05; blank space p>0.05.

		Dry matter (g)				
Fertilisation treatment	**Earthworm treatment**	***Lolium perenne***	***Plantago lanceolata***	***Lotus pedunculatus***	***Achillea millefolium***	**Total weight**
Organic	Present	3.3	26.3	1.8	3.1	34.5
Organic	Absent	2.4	30.4	1.4	2.9	37.1
Inorganic	Present	8.6	26.5	1.1	2.7	38.9
Inorganic	Absent	4.1	30.3	1.2	1.7	37.2
EMS		3.21	21.15	0.74	1.05	20.69
Fertilisation		*				
Earthworm		*				

5.3.2 Plant effects on nutrient distribution

There were mixed effects of plant community structure on the distribution of nutrients within the soil matrix as analysed using Q1 and Q2 derived from plotted nutrient distribution curves (Figure 5.5 and Table 5.7).

Table 5.7 Summary table showing the statistical terms for mixed plant and monoculture communities when both have been compared to the unplanted treatment on Q1 and Q2 for measured soil nutrients.

Model parameter	**Plant treatment**	**ln nutrient concentration (µg g^{-1})**			
		P	**C**	**N**	**K**
Q1	**Mono**				
Q1	**Mix**		** (+)	** (+)	** (-)
Q2	**Mono**				** (-)
Q2	**Mix**	* (-)	** (-)		*** (-)

Significance terms denote *** p<0.001; ** p<0.01; * p<0.05; blank space p>0.05.
(+) and (-) for Q1 denote an increase and a decrease in concentration, respectively.
(-) for Q2 denotes a less even nutirent distribution.

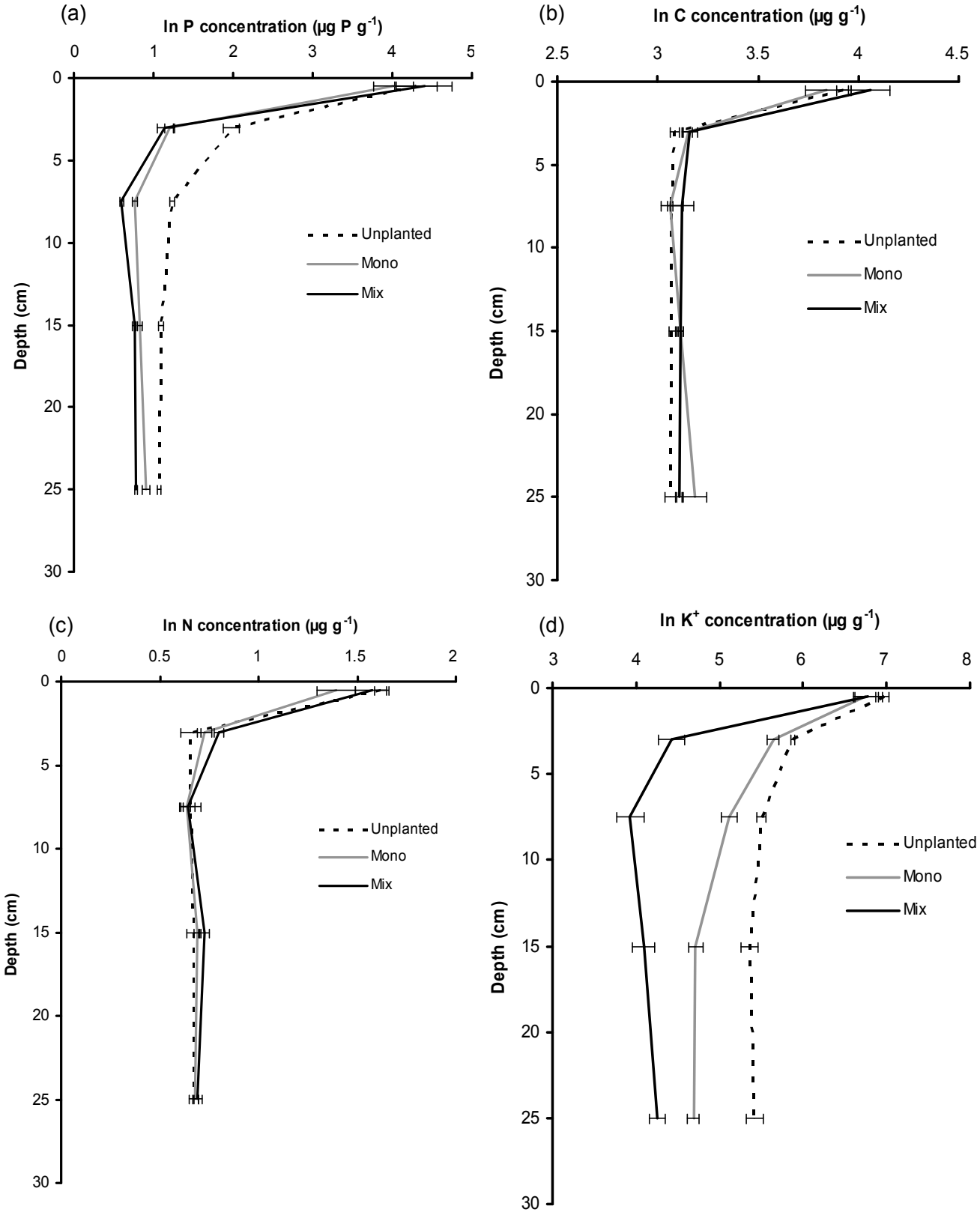

Figure 5.4 Nutrient distributions in the presence of unplanted, monoculture and mixed plant communities to a soil depth of 30 cm for (a) labile inorganic P, (b) total C, (c) total N and (d) labile K. Error bars denote standard error. All displayed concentrations have been natural log transformed.

There was a significant difference in the distribution of Morgan's P between monocultures and mixed plant communities (Figure 5.5(a)) as determined by Q2 ($p<0.05$). This difference showed a more even distribution of P in the presence of monocultures compared to mixed plant communities but not compared to the unplanted treatment (Table 5.7). Therefore, a significantly more even distribution of P was observed in the unplanted treatment compared to the mixed plant community but not to the monoculture P distribution. C was also more evenly distributed in the unplanted compared to the mixed plant community but not to the monoculture (Table 5.7 and Figure 5.5(b)). There was a significant difference in K distribution; with a more even distribution observed in the presence of monocultures compared to mixed plant communities ($p<0.01$). Labile K was more evenly distributed in the unplanted treatment compared to planted treatments, (Figure 5.5(d)). There was no significant effect of plant community on the distribution of total N.

Q1 also revealed that there was an increase in total C and N in the presence of mixed plant communities compared to unplanted treatments (Table 5.7) but not compared to monocultures ($p>0.05$). There were greater concentrations of K in the unplanted treatments compared to the mixed plant communities (Table 5.7), but not the monocultures ($p>0.05$). However, no effect of botanical diversity was observed on P concentrations in the soil.

5.3.3 Plant effects on microbial and nematode communities

With respect to microbial biomass pools, no interactions were observed on microbial biomass C and P, but only on N (Table 5.8). This interaction between plant community composition and depth showed that microbial biomass N was greater at a depth of 1 - 5 cm in the presence of a mixed plant community compared to N concentrations in the presence of a monoculture, whilst similar concentrations between the two plant community types occurred in the 5 – 10 cm depth (Table 5.9). Microbial biomass N concentrations were significantly lower in the unplanted treatments compared to both planted treatments. A nesting effect (a significant difference between planted treatments and those not containing plants) occurred with respect to microbial biomass P concentrations (Table 5.8). In particular, this effect

showed greater concentrations of microbial biomass P in planted treatments compared to unplanted (Table 5.9). A depth effect was also observed on microbial biomass P, since greater concentrations were observed in the 0 – 1 cm depth compared to both 1 – 5 and 5 – 10 cm: P concentrations were similar between 1 – 5 and 5 – 10 cm depth ranges. No plant community effects were observed on microbial biomass C concentrations (Table 5.8).

Table 5.9 Mean concentrations of microbial biomass P, N and C under different plant treatments with respect to depth. Microbial biomass N and C concentrations for the 0 - 1 cm depth were not determined.

		Microbial biomass		
Depth (cm)	**Plant treatment**	**P (µg P g^{-1})**	**N (µg g^{-1})**	**C (µg g^{-1})**
0 - 1	Unplanted	76	-	-
0 - 1	Mono	78	-	-
0 - 1	Mix	81	-	-
1 - 5	Unplanted	14	56	1975
1 - 5	Mono	25	99	1853
1 - 5	Mix	24	114	2002
5 - 10	Unplanted	17	57	1973
5 - 10	Mono	24	88	1996
5 - 10	Mix	21	84	1827
	EMS	0.139	155	141962

Microbial biomass P values were statistically analysed after natural log transformation and the values displayed here are back-transformed: the EMS value is derived from natural log transformed data.

Table 5.8 ANOVA table showing the effects and interactions of treatments on the microbial properties measured in this experiment. Significance terms denote *** p<0.001; ** p<0.01: * p<0.05; blank space p>0.05. (N) denotes treatments and interaction terms in which treatments were nested within the ANOVA design.

	Microbial biomass (µg g^{-1})			PLFA				
	C	N	P	PC1 (31%)	PC2 (21%)	PC3 (8%)	PC4 (6%)	Fungal: Bacterial Ratio
Nesting		***	**				***	
Plant (N)					**			
Earthworm (N)							***	
Fertiliser								*
Depth		***	***	***	***		***	**
Depth x Fertilisation					*			*
Depth x Plant (N)		*					**	
Fertilisation x Plant (N)								
Depth x Earthworm (N)					**		***	
Fertilisation x Earthworm (N)					*			
Plant x Earthworm (N)								
Depth x Fertilisation x Plant (N)								
Depth x Fertilisation x Earthworm (N)								
Depth x Plant x Earthworm (N)							*	
Fertiliser x Plant x Earthworm (N)								
Fertilisation x Plant x Earthworm x Depth (N)								

With respect to the microbial phenotype, a depth effect was observed on PC 1 (Table 5.8). Significant depth effects were also observed on PC 2 and PC 3. When factor scores were ordinated between PC 1 and 2, a clear separation was observed between the phenotype in the 0 - 1 cm depth and those in the 1 – 5 and 5 – 10 cm depth ranges (Figure 5.6). Along the PC 2 plane, a difference was apparent between all three depth ranges. All raw PLFA data for this experiment are presented in Appendix 2, Tables A_2A(a) to A_2C(e).

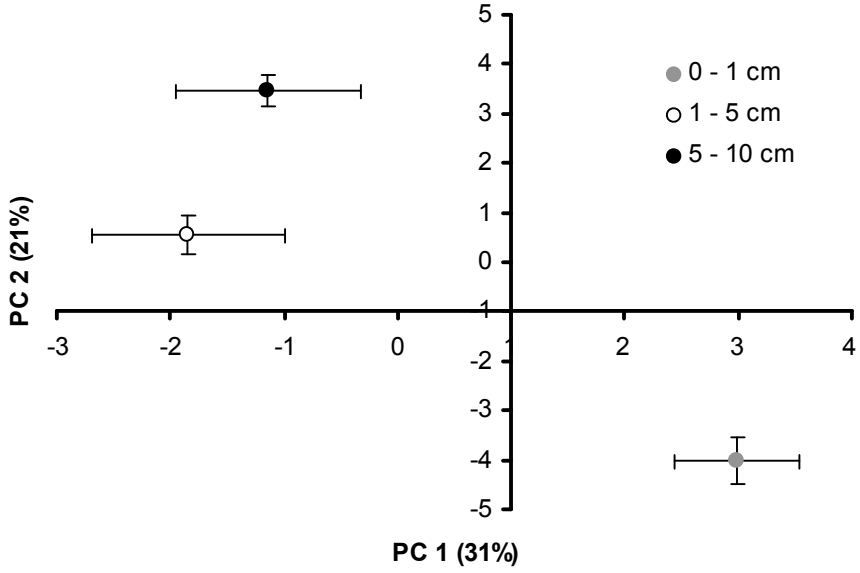

Figure 5.5 Shifts in microbial community structure with depth as expressed by PC 1 and 2. Accounted principal component variation displayed in parenthesis. Error bars denote standard error.

With respect to plant community impacts on microbial community structure, an effect was observed on PC 2 (Table 5.8). There was a difference between the monoculture and mixed plant communities with the unplanted treatment supporting microbial communities similar to both planted treatments. In addition, an interaction between plant community composition and depth occurred in PC 4 (Table 5.8), in which shifts in the microbial phenotype were apparent between the two planted communities in the 0 – 1 and 1 - 5 cm depth range, but not in the 5 – 10 cm depth range (Figure 5.7).

Microbial community structure in the unplanted treatment was different to both planted treatments.

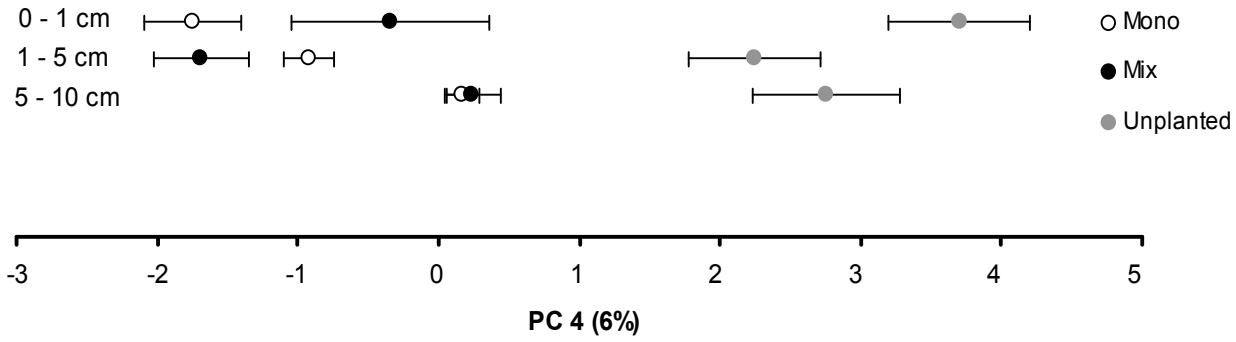

Figure 5.6 The interaction between plant community composition and depth on microbial community structure as expressed by PC 4. Accounted principal component variation is displayed in parenthesis. Placement along the nominal Y axis does not represent difference but is for visualisation only.

Nematode abundance was significantly different between planted treatments (33.6 ± 2.62 g^{-1} dry soil weight) and those which were unplanted (10.2 ± 1.87 g^{-1} dry soil weight) (Table 5.10). Nematode community structure, when aggregated at a family level, changed with plant community composition in relation to the type of fertiliser treatment applied (Table 5.10). This change was manifest in PC 1 and showed that nematode community structure in the presence of a monoculture that had received inorganic P fertiliser differed from all other planted treatments irrespective of fertiliser amendment (Figure 5.8). Both mixed plant communities that received either organic or inorganic fertiliser supported nematode communities that were similar. Communities in the unplanted circumstance differed from both planted treatments, but the type of fertiliser applied to these unplanted treatments did not affect community structure.

Table 5.10 ANOVA table showing the effects and interactions on measured nematode properties. Significance terms denote *** p<0.001; ** p<0.01; * p<0.05; blank space p>0.05. (N) denotes treatments and interaction terms in which treatments were nested within the ANOVA design.

		Community Structure								
		Family level				Trophic level				
	Nematode abundance (number g^{-1})	PC1 (18%)	PC2 (15%)	PC3 (12%)	PC4 (10%)	PC1 (46%)	PC2 (29%)	PC3 (15%)	PC4 (9%)	Nematode Channel Index
Nesting	***	***				***				
Plant (N)		***			**			*		*
Earthworm (N)										
Fertiliser		*	*			*	*			*
Plant x Earthworm (N)										
Plant x Fertiliser (N)		*								*
Earthworm x Fertiliser (N)										*
Plant x Fertiliser x Earthworm (N)										

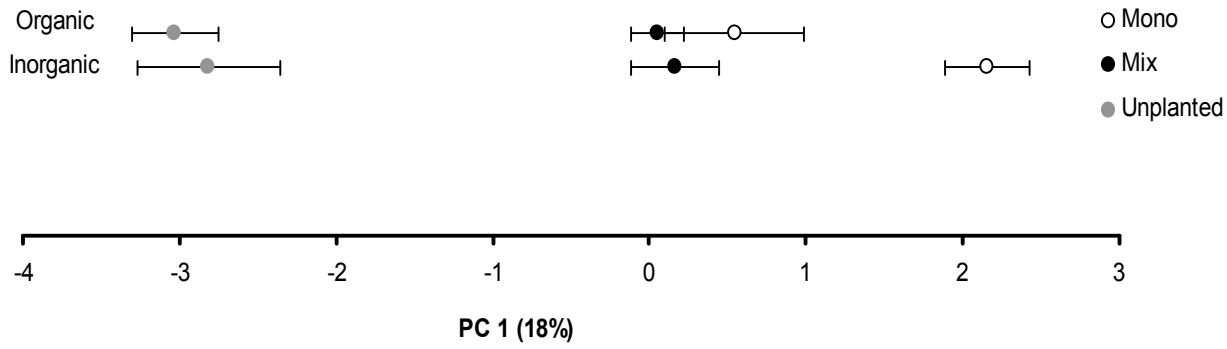

Figure 5.7 Interaction between plant community composition and fertiliser amendment on nematode community structure, depicted from a family level, as displayed by PC 1. PC percentage variation displayed in parenthesis. Placement along the nominal Y axis does not represent difference but is for visualisation only.

When nematode community structure was aggregated at a trophic level, a nesting effect (a significant difference between planted and unplanted treatments) and a plant effect were manifest by PC 1 and PC 3, respectively (Table 5.10). The presence of plants changed the trophic composition of the nematode community compared to unplanted treatments along the PC 1 plane (Figure 5.9(a)). This divergence occurred as plant feeding nematodes were associated with planted treatments and bacterial and omnivorous nematodes in the unplanted treatment. The plant effect in PC 3 related to a difference in the nematode community structure between mixed and monoculture plant communities, with the unplanted treatment similar to both plant treatments along this plane (Figure 5.9(b)).

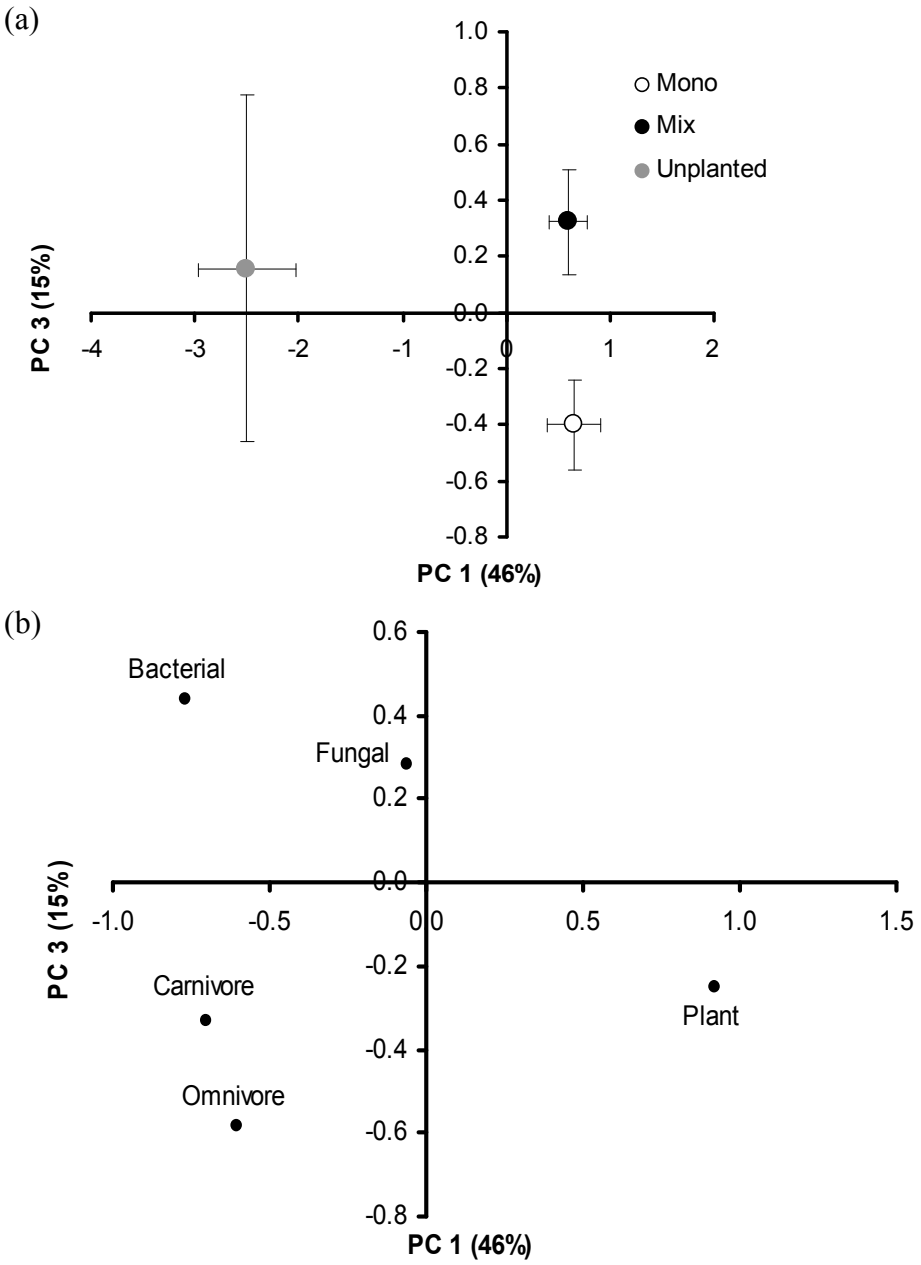

Figure 5.8. Changes in nematode community structure from a trophic level, (a) shows the effects of 3 plant treatments on nematode trophic structure and (b) loadings plot which displays how 5 trophic groups are separated by PC 1 and PC 3. PC percentage variation displayed in parenthesis. Error bars denote standard error.

5.3.4 Earthworm effects on nutrient distribution

Within one month of earthworm addition, earthworm casts were present in mesocosms in which they were applied, whilst no casts were present in mesocosms that did not receive earthworm application. There were no plant community or fertiliser amendment effects on the number of casts. However, there was a significant time effect following the application of earthworms which showed an increase in cast number from October 2010 to November 2010, where after cast numbers remained similar across the remaining sampling dates (Figure 5.10).

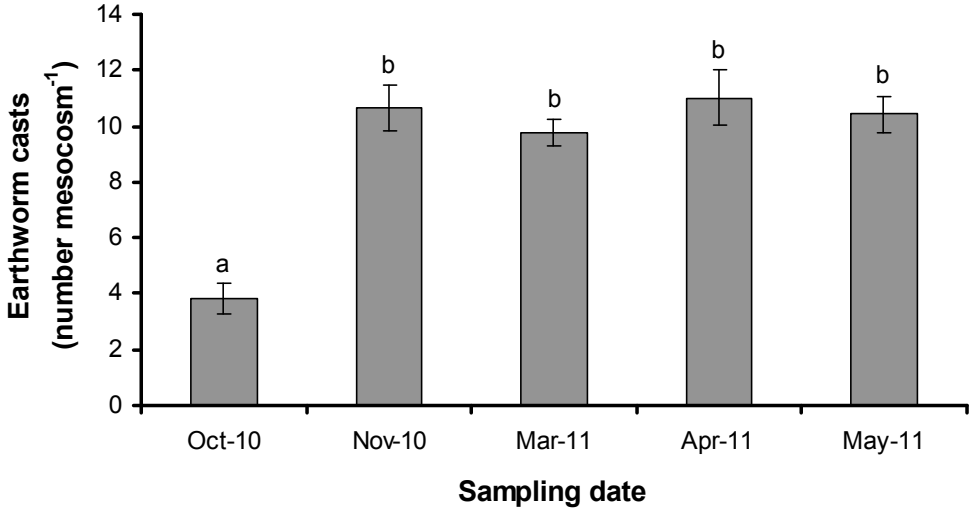

Figure 5.9 Time effect following earthworm addition on the number of earthworm casts present. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Error bars denote standard error.

Labile inorganic P concentrations in earthworm surface cast material ranged from 50 – 100 μg P g^{-1}. However, labile inorganic P concentrations in such cast material were not affected by any treatment ($p>0.05$).

When mesocosms were deconstructed, earthworms were only present in mesocosms to which they had been applied at the outset of the experiment. Both adult *Allolbophora chlorotica* and *Aporrectodea longa* were present with *Allolbophora chlorotica* typically in the top soil to a depth of 5 cm and *Aporrectodea longa* deeper within the soil. Juvenile earthworms were also present and were greater in numbers than adult earthworms (Table 5.11). No significant effects of plant community structure or fertiliser treatment were observed on earthworm abundance or total weights.

Table 5.11 Mean earthworm abundances and total weights under different combinations of experimental treatments. No significant interaction was observed. A blank space denotes p>0.05.

		Abundance				
		Juvenile	Adult *Allolobophora chlorotica*	Adult *Aporrectodea longa*	Total	Total weight (g)
Mono	Inorganic	26.3	4.7	3.3	34.3	8.7
Mono	Organic	41.7	6.0	3.0	50.7	8.5
Mix	Inorganic	35.7	3.7	2.3	41.7	8.3
Mix	Organic	43.0	2.7	2.3	48.0	5.3
	EMS Plant Fertiliser	414.0	6.3	0.5	492.8	8.1

Earthworm presence promoted a significantly more even distribution of labile P, labile K, total C and total N (Figure 5.11), as expressed by Q2 (Table 5.12), compared to both earthworm absence and the unplanted treatments.

Earthworm presence also decreased overall concentrations of labile P, labile K, total C and total N compared to earthworm absence and unplanted treatments (Figure 5.11), which was expressed by Q1 (Table 5.12).

Table 5.12 The statistical terms for earthworm presence when compared to both earthworm absence and unplanted treatments on Q1 and Q2 for measured soil nutrients.

Model parameter	ln nutrient concentration (μg g^{-1})			
	P	C	N	K
Q1	*** (-)	*** (-)	*** (-)	* (-)
Q2	** (+)	*** (+)	*** (+)	* (+)

Significance terms denote *** p<0.001; ** p<0.01; * p<0.05; blank space p>0.05. (-) for Q1 denotes a decrease in concentration. (+) for Q2 denotes a more even nutirent distribution.

In addition to the decrease of Morgan's P in the top 0 – 1 cm, as shown in Figure 5.11, this effect was also shown by Olsen P concentrations within the 0 - 10 cm of the soil matrix ($p<0.001$). The presence of earthworms decreased the concentrations of Olsen P in the 0 – 1 cm of soil with no difference in the 1 - 5 and 5 – 10 cm depth ranges. Concentrations of Olsen P within the unplanted treatment were much greater than either of the earthworm treatments in planted circumstances.

Earthworm presence also significantly affected the recovery rate of P from the soil ($p<0.001$). In their presence, there was a reduction in the amount of labile inorganic P recovered after a known amount of P was added to the soil (69.4% ± 0.029 [ln – transformed]) compared to their absence (85.1% ± 0.045.) The recovery rate of labile inorganic P in unplanted mesocosms was not different from planted mesocosms irrespective of earthworm presence or absence.

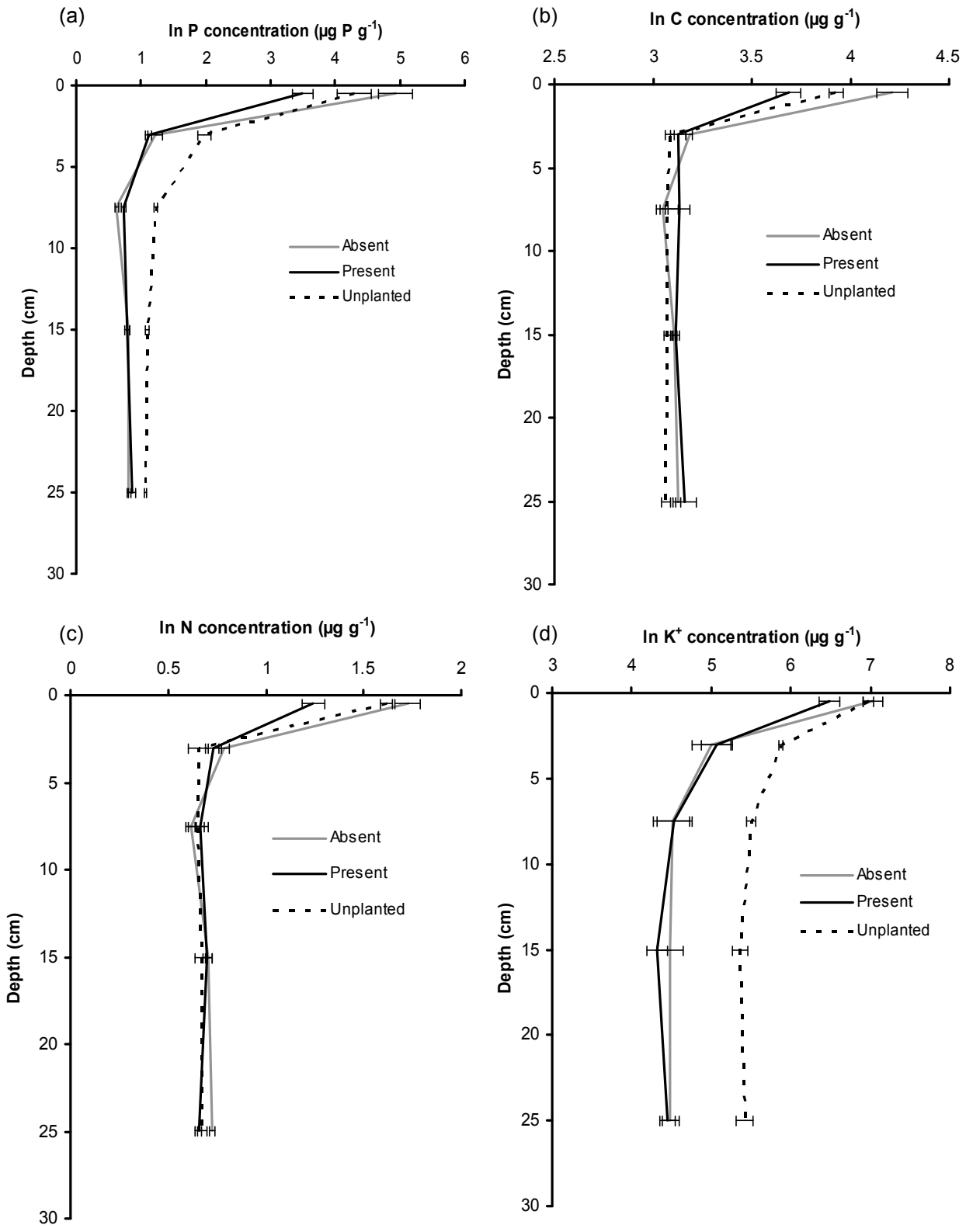

Figure 5.10 Nutrient distributions to a soil depth of 30 cm in earthworm present, earthworm absent and unplanted treatments for (a) labile P, (b) total C, (c) total N and (d) labile K. Error bars denote standard error. All displayed concentrations have been natural log transformed.

5.3.5 Earthworm effects on microbial and nematode communities

Earthworm presence did not affect microbial biomass C, N or P pools (Table 5.8). Nevertheless, their presence did significantly affect the phenotypic structure of the microbial community between depths on PC 2 and PC 4 (Table 5.8). This interaction between depth and earthworm presence showed the microbial phenotype was different between all treatments in the 0 – 1 cm depth (Figure 5.12). In the 1 – 5 cm and 5 – 10 cm depths, the phenotype was similar between planted treatments with or without earthworm, both of which were different from the unplanted circumstance.

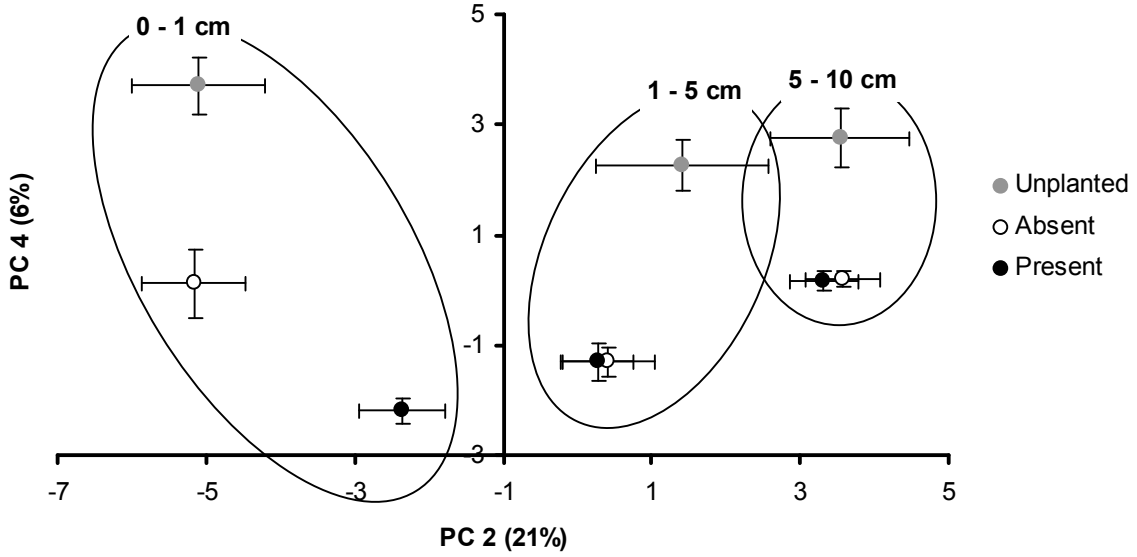

Figure 5.11 The interaction between earthworm presence or absence and depth on microbial community structure along PC 2 and 4. PC percentage variation displayed in parenthesis. Error bars denote standard error.

Neither nematode abundance nor community structure was affected by the presence of earthworms (Table 5.10). However, the nematode channel ratio, which measures the difference between fungal and bacterial feeding dominance in the nematode community, was affected by an interaction between plant community structure, fertiliser amendment and earthworm presence (Figure 5.13).

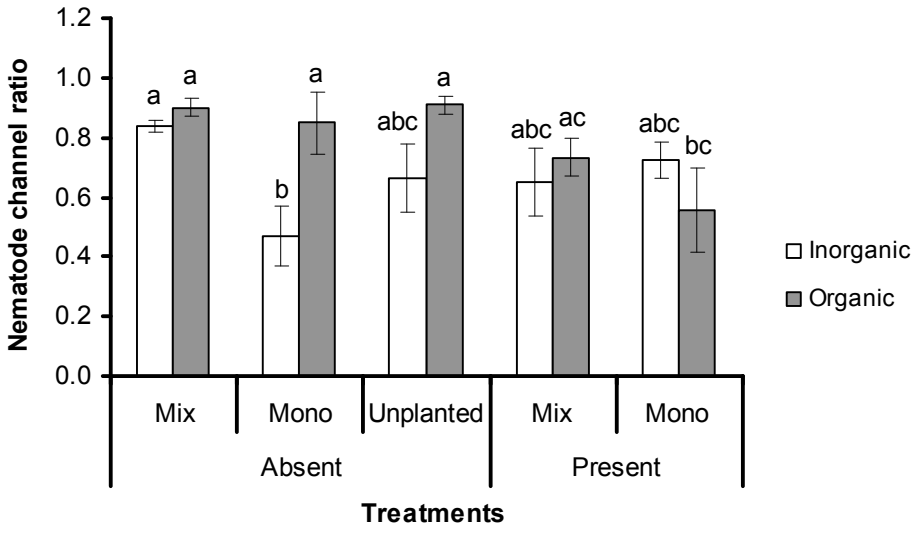

Figure 5.12 The interaction between earthworm presence, plant community structure and fertiliser amendment on the nematode channel index. Error bars denote standard error and different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test.

With respect to earthworms, this interaction showed that earthworm presence removed the effect of the fertilisation treatment and plant community structure on the nematode channel ratio. In the absence of earthworms, there was a shift towards a fungal dominated system when inorganic fertiliser was applied to monocultures. Thus a relatively bacterial dominated system was observed when organic fertiliser was applied to this plant treatment. The application of different fertiliser treatments to mixed plant communities did not have an effect.

5.3.6 Fertiliser treatment effects on nutrient distribution

The application of fertiliser treatments to the soil had mixed effects on the distribution of nutrients (Table 5.13). No difference in the distributions of labile K, total C and total N were observed when soil was amended with organic or inorganic fertiliser (Figure 5.14(b), (c) and (d)) as expressed by Q2 (Table 5.13). The application of organic

fertiliser as a treatment promoted a more even distribution of P compared to the application of inorganic fertiliser (Figure 5.14(a)).

A decrease in labile P and K concentration was observed in the presence of the organic soil amendment (Figure 5.14(a) and (d)) as expressed by Q1 (Table 5.13). This difference in P concentration was most evident within the 0 – 1 and 1 – 5 cm depth ranges where there was less P in the organic amendment, but with respect to K, there was a distinct decrease in concentration at all depths following organic amendment contributing to the overall decrease in this treatment (Figure 5.14(a) and (d)). There were no fertiliser effects on total C and N concentrations.

Table 5.13 Summary table showing the effect of different fertiliser amendments on Q1 and Q2 for measured soil nutrients.

Model parameter	In nutrient concentration (μg g^{-1})			
	P	C	N	K
Q1	*** (-)			** (-)
Q2	** (+)			

Significance terms denote *** p<0.001; ** p<0.01; blank space p>0.05. (-) for Q1 denotes a decrease in concentration. (+) for Q2 denotes a more even nutirent distribution

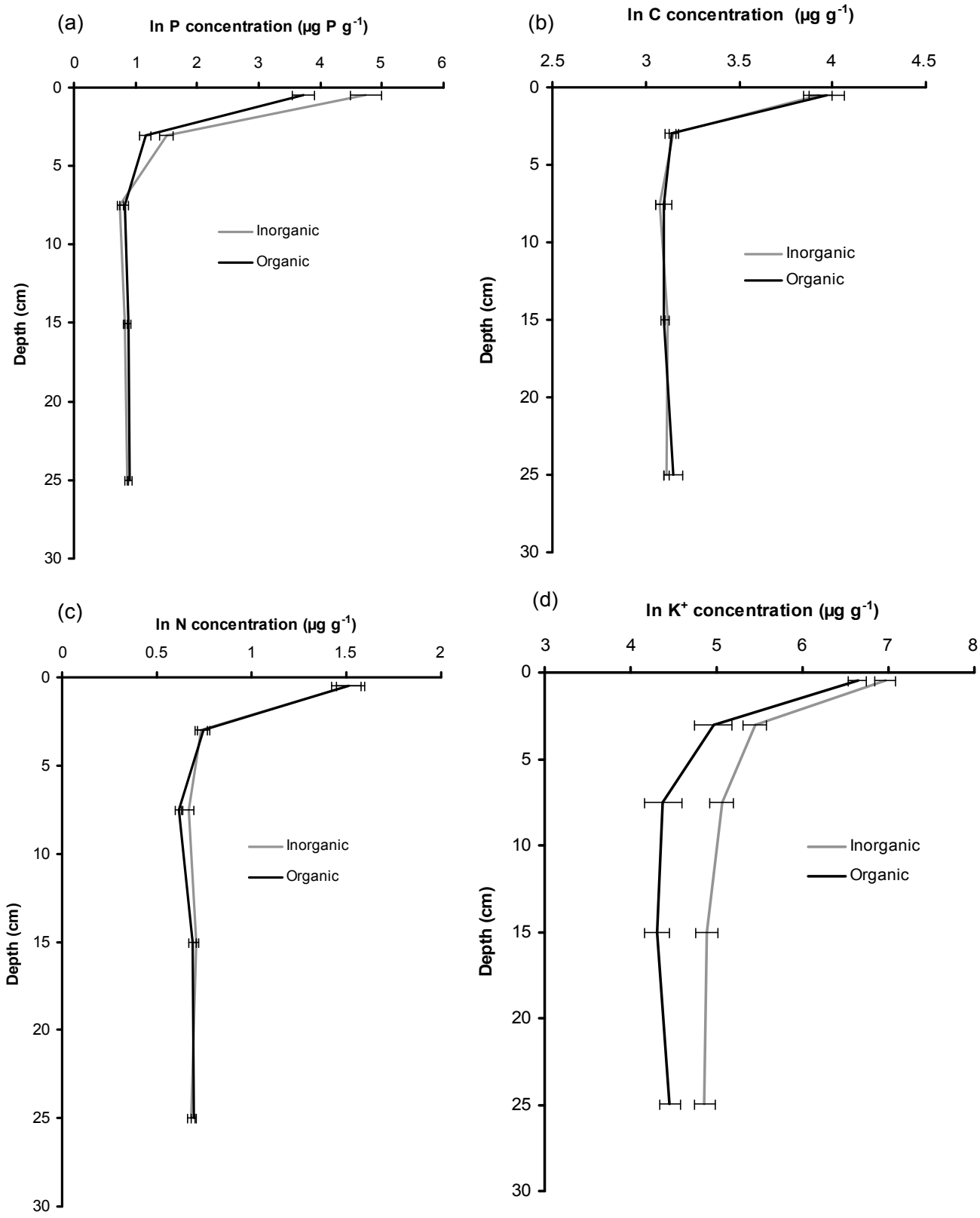

Figure 5.13 The distribution of (a) labile P, (b) total C, (c) total N and (d) labile K down 30 cm of the soil matrix under organic and inorganic soil amendment. Error bars denote the standard error. Nutrient concentrations have been natural logged transformed.

A significant interaction between fertiliser amendment, plant community composition and depth was observed on labile P concentrations, as determined by the Olsen P method ($p<0.01$) (Figure 5.16). In the 0 - 1 cm depth, there was generally lower P with the application of the organic fertiliser amendment for both planted treatments; monoculture and mixed, respectively. From 1 - 5 cm, there was less P in the presence of the organic fertiliser amendment and mixed plant communities but the opposite occurred within monocultures. In monocultures, there was less P associated with the inorganic fertiliser amendment compared to organic. At 5 – 10 cm, there was no significant difference between plant communities receiving different types of fertilisation.

In the unplanted treatments, the Olsen P concentrations were greater than those observed in planted treatments with no effects of the fertiliser amendment apart from in the 0 – 1 cm depth in which greater concentrations of P were observed after the inorganic fertiliser amendment was applied.

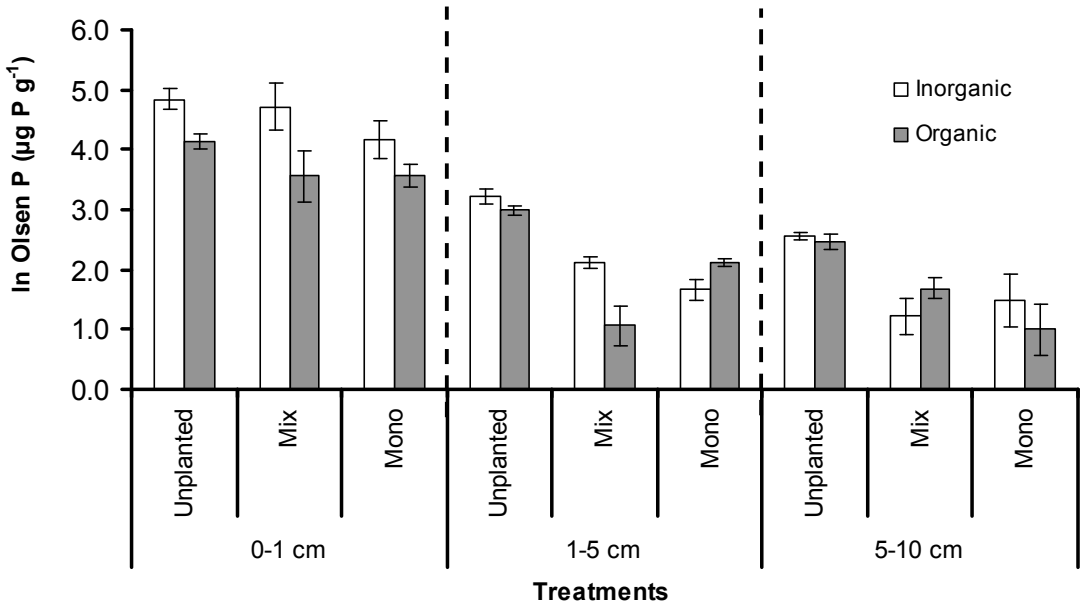

Figure 5.14 The interaction between fertiliser amendment, plant community composition and depth on concentrations of naturally logged labile P. Error bars denote standard error.

5.3.7 Fertiliser effects on microbial and nematode communities

The addition of different fertiliser amendments did not affect the nutrient content of the microbial community (Table 5.8). However, fertiliser amendment did affect microbial phenotypic structure. An interaction between fertiliser amendment and depth was observed on PC 2 (Table 5.8), which revealed changes between the two fertiliser amendments in the 0 - 1 cm depth but not in the 1 – 5 and 5 - 10 cm depths. However, microbial community structure was different at each depth range. This interaction between fertiliser treatment and depth was related to shifts in multiple PLFA signatures. Another second order interaction was observed between fertiliser treatment and earthworm presence on PC 2 (Table 5.8). This interaction showed that earthworm presence did affect microbial community structure after inorganic fertiliser was applied but not following organic fertilisation (Figure 5.17): unplanted mesocosms, which contained no earthworms, supported microbial communities that were similar to all fertiliser by earthworm combinations.

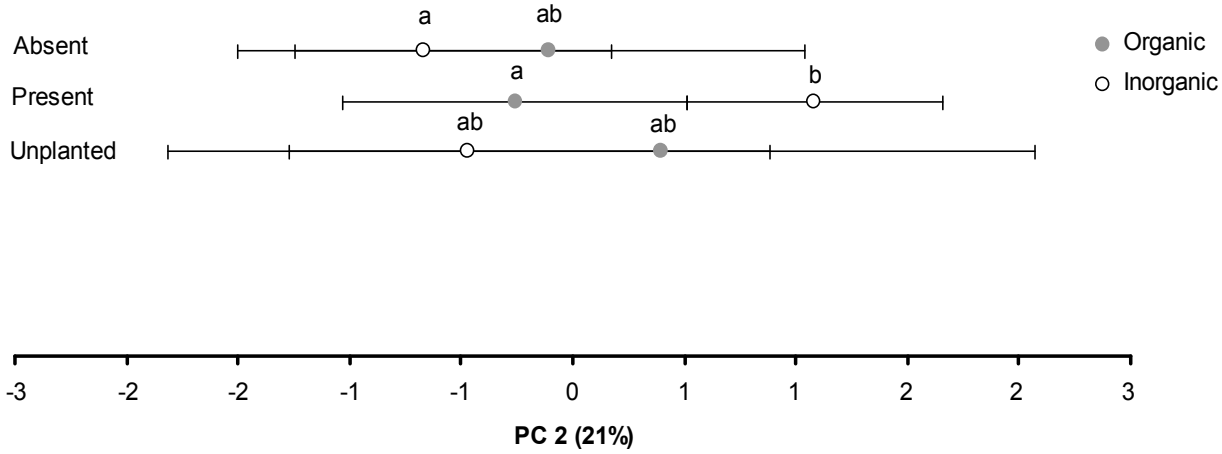

Figure 5.15 The interaction between fertiliser amendment and earthworm presence on the microbial phenotype as displayed by PC 2. PC percentage variation is denoted in the parenthesis. Error bars denote standard error. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test. Placement along the Y axis does not represent difference but is for visual purposes only.

An interaction between fertiliser amendment and depth was also observed on the fungal: bacterial ratio of the microbial community (Table 5.8). The most pronounced difference occurred in the 1 – 5 cm depth where a more fungal dominated community occurred following inorganic amendment (Figure 5.18). Both the 0 – 1 and 5 – 10 cm depths were not significantly affected by contrasting fertiliser amendments.

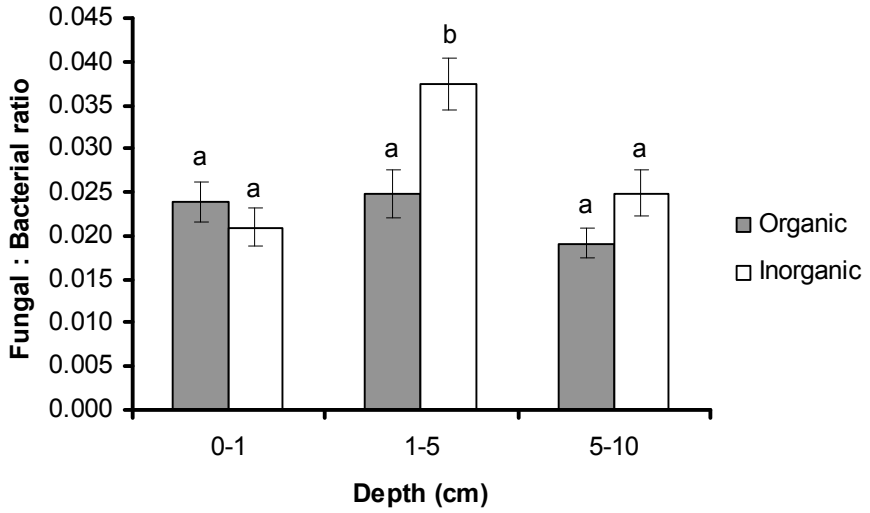

Figure 5.16 The interaction between fertiliser amendment and depth on microbial fungal: bacterial ratios. Error bars denote standard error. Different letters denote homogeneous means ($p<0.05$) using Fisher least significant difference test.

Nematode community structure was affected following the application of different fertiliser treatments, on both family and trophic levels (Table 5.10). The fertiliser treatment effect on nematode families occurred on PC 2 and was caused by changes in multiple families. Consequently, significant fertiliser treatment effects on trophic structure were manifested by both PC 1 and PC 2 (Table 5.10). PC 1 revealed that this shift was attributed to a link between inorganic amendment and plant feeding nematodes, whereas organic amendment was associated with omnivorous and bacterial feeding nematodes (Figure 5.19(a) and (b)). On PC 2, however, the omnivorous, bacterial and plant feeding nematodes were associated with organic fertilisation and fungal feeding nematodes were related to inorganic fertilisation.

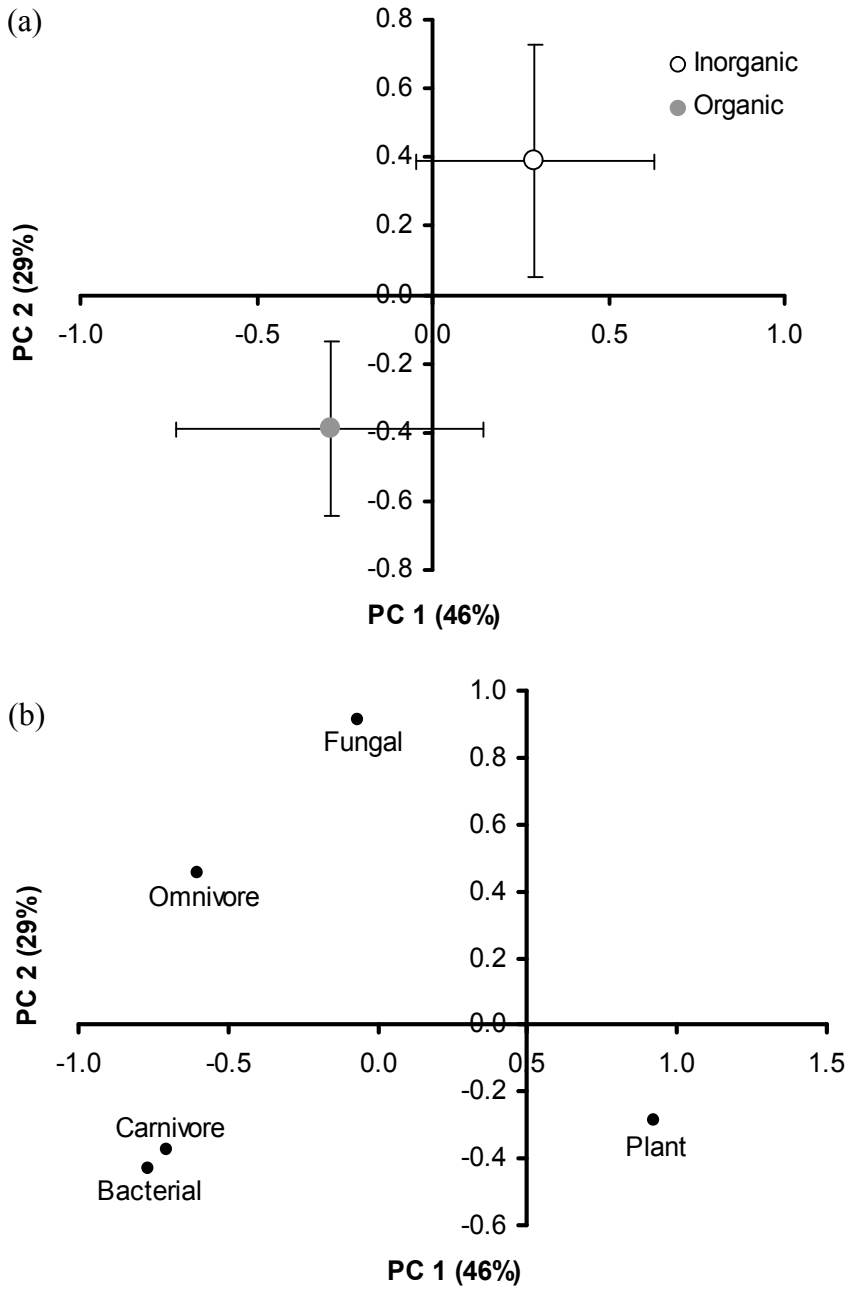

Figure 5.17 Changes in nematode trophic structure (a) in the presence of 2 fertiliser amendments (b) loadings plot as displayed by PC 1 and PC 2. PC percentage variance is presented in parenthesis. Error bars denote standard error.

5.4 Discussion

5.4.1 Experiment summary

There were 3 main aims of this experiment; these aims and their associated hypotheses are revised below:

1. To investigate the how contrasting fertiliser amendments affect P incorporation and assimilation by the soil biota.

    a) The application of slurry will promote a more even vertical distribution of labile inorganic P within the soil, compared to inorganic applications of superphosphate P.

    b) The application of fertilisers will induce shifts in the structure of the microbial and nematode communities.

2. To examine whether earthworm activity can promote the incorporation of surface applied P into the soil matrix.

    a) The presence of earthworms will promote a more even vertical distribution of labile inorganic P in the soil matrix.

3. To investigate botanical diversity effects on biological P assimilation and belowground communities.

    a) An increase in botanical diversity will increase microbial biomass P.

    b) Greater plant P yields will be harvested from treatments with greater botanical diversity.

To achieve the aims of this experiment an incomplete factorial combination of botanical diversity, earthworm presence and fertiliser type were allocated to different glass-house based mesocosms. By analysing a broad range of biological and chemical parameters in the presence of these different treatment combinations, a comprehensive system view of

soil biology and P cycling was accomplished. Greater precision in this experiment was achieved by analysing all soil properties at different depth ranges. This was pertinent, since different soil types were used to construct the vertical concentration gradient of P. This concentration gradient was used to assess the P incorporation potential of the earthworm community. A four-factor factorial design was applied to assess biological and chemical responses at different depth ranges.

5.4.2 Slurry effects on phosphorus distribution and biological communities

Data obtained in this experiment supports Hypothesis a) associated with Aim 1, which stated that the application of slurry will promote a more uniform distribution of P within the soil. Greater mixing of labile P with organic fertilisation may be indicative of the mobilisation capability of organic P pools, since slurry application has been shown to increase the infiltration rate of nutrients following application (Vadas, 2006; Chardon et al., 2007). This would not be the case following the application of an inorganic P-based fertiliser, as the release of P from superphosphate will quickly undergo adsorption, which will remove the flush of water-soluble P derived from fertiliser application into labile solid phases. This removal acts to restore the equilibrium between P concentrations in mobile and solid phases to its initial state. Through adsorption, P becomes highly immobile and accumulates in the 0 – 1 cm depth range, which skews the vertical distribution of P in the soil (Owens et al., 2008). Therefore, in this mesocosm experiment, the less uniform distribution of P following superphosphate application would have been attributed to the adsorption of P to soil material at the soil surface, whereas a more uniform distribution of P following slurry application was observed since greater infiltration rates of organic, P containing, material would have occurred.

A difference in microbial and nematode community structures were observed between the two fertiliser types, which supports Aim 1, Hypothesis b). Studies that have looked at organic and inorganic fertiliser amendments appear to have mainly focused on microbial biomass concentrations and activity (Gichangi et al., 2009; Ge et al., 2010).

These indicate that microbial biomass C increases with organic fertiliser application and decreases with inorganic application. Greater microbial biomass P concentrations were reported when inorganic and organic fertilisers were applied together in both of these studies. However, in this mesocosm experiment, fertiliser type effects only occurred on microbial phenotypic structure and not on biomass nutrient concentrations. With respect to the shift in microbial community structure, a more fungal dominated community was observed following the application of inorganic P fertiliser in the 1 – 5 cm, but not in the 0 – 1 and 5 – 10 cm depth ranges. It was not entirely clear why such an interaction did not reveal a response in the 0 – 1 cm depth, since this was exposed to the greatest quantity of fertiliser. This may indicate that other factors had a larger impact on community structure over such a depth range rather than fertiliser type. Further evidence for a fungal dominated microbial community after inorganic P fertilisation was indicated by a shift towards a fungal feeding nematode community. However, it was also not clear why fungal feeding nematodes dominated the nematode community in the presence of *L. perenne* after inorganic fertilisation, particularly when the same interaction did not occur with respect to PLFA-based fungal : bacterial ratio. This may indicate that nematode community analysis in this experiment was a more sensitive measure of biological community structure compared to the microbial phenotype. Nevertheless, fungal dominance in mesocosms that had been inorganically fertilised may provide an indication of nutrient availability and organic substrate quality in the soil (Bardgett et al., 1999). In the study conducted by Bardgett et al. (1999), it was reported that fungi were more dominant in unfertilised grassland systems compared to those that were fertilised. This was attributed to an increase in recalcitrant plant material entering the soil matrix, and to lower concentrations of available nutrients that would have been supplied through the application of fertilisers. In the mesocosm experiment conducted for this project, amendment with slurry would have increased labile C, N and P concentrations in the soil, whereas the supply of labile C in systems that received only inorganic fertilisers was dependent on the plant community. Therefore, fungal dominance on multiple trophic levels may indicate limited labile nutrient concentrations in systems that were only inorganically fertilised and a dependency by the microbial community on plant derived C. However, such an increase in labile C, N and P and its consequential effects on microbial phenotypic structure seemingly contradicts the lack

of a fertiliser type effect on microbial biomass C, N and P. This is a contradiction since an increase in labile C, N and P should increase microbial biomass concentrations compared to the application of inorganic fertiliser, as stated above. In this experiment, the inorganic fertiliser treatment included N and P fertilisation in equal concentrations to those applied in the organic treatment. Therefore, N and P should have been equally available to the microbial community in both fertiliser treatments. However, C availability would have been expected to be different between fertiliser treatments, with greater C availability following organic fertilisation. One possibility why such a difference in C availability might not have affected microbial biomass C may be linked to the chosen deconstructive sampling date. Mesocosms were sampled seven months following the application of both fertiliser types. Therefore, the effect of applied labile C following organic fertilisation may have ceased by this sampling date, since a large proportion of applied labile C may had been utilised by the soil biota before mesocosms were deconstructed.

5.4.3 Phosphorus incorporation through earthworm activity

Analysis of the model parameter Q2 revealed that a more uniform distribution of labile inorganic P occurred with earthworm presence. This supports Hypothesis (a associated with Aim 2 of this experiment. Furthermore, the presence of earthworms significantly reduced P concentrations in the 0 - 1 cm soil depth range. It is important to note that earthworm presence was shown to facilitate the dispersal of P but not necessarily incorporate P, since a more uniform distribution of P in this experiment was driven by decreasing P concentrations in the 0 – 1 cm depth range rather than increasing concentrations below this depth. Earthworms have been shown to facilitate the dispersal of P through two mechanisms (MacKay et al., 1983; Mackay et al., 1982). One mechanism involves the ingestion and excretion of soil material. By ingesting such material, it is transported and dispersed within the soil matrix. The other mechanism is linked to the physical movement of P bound material through earthworm activity. The mucus coating the earthworm epidermis may trap soil material along the surface of the earthworm, which in turn is then redistributed within the soil. With these mechanisms in

mind, earthworm presence has been shown to facilitate the vertical movement of P into the soil matrix (MacKay et al., 1983; Sharpley et al., 2011), but information on this subject is still sparse. The studies here revealed that P incorporation was enhanced following the application of organic fertilisers compared to inorganic types. Greater activity following organic fertilisation would be expected, since this organic material provided a food source for the earthworm community in contrast to the inorganic fertiliser. However, such a finding could not be proven in this mesocosm experiment since a limited number of degrees of freedom inhibited the analysis of interactions between treatments used in the nutrient distribution models.

Whilst earthworm activity was shown to facilitate P dispersal in this mesocosm experiment, P incorporation was not proven. If earthworm mediated P incorporation did occur, there should have been an increase in P concentrations from 1 – 30 cm in the earthworm present treatment compared to their absence. There are a number of reasons why such incorporation might not have occurred. Firstly, the time-frame for this experiment may not have been long enough to have invoked a substantial movement of a highly immobile nutrient like P into the soil matrix. More specifically, these mesocosms were seeded in May 2011, earthworms were applied in September 2011 and mesocosms were deconstructed in May 2012. Therefore, earthworms were present in their respective mesocosms for eight months. In the study conducted by Sharpley et al. (2011), earthworm incorporation of P appeared to occur after a five year period of annual fertiliser applications. However, this was due to the sampling nature of the study and not constant P distribution sampling on an annual basis. However, the study conducted by Sharpley et al. (2011) also indicated that P dispersal into surface casts appeared in the first year following fertiliser application.

Secondly, a lack of apparent incorporation may be linked to the dilution of P-rich material from the 0 – 1 cm layer into the substantially larger soil volumes in the lower layer. The soil used to construct the upper layer was approximately seven times greater in labile inorganic P concentration compared to the soil used for the lower layer. In addition, one application of P (69 kg P ha^{-1}) was applied to the soil surface of these systems as either slurry or superphosphate. Therefore, substantially greater P concentrations were present in the 0 – 1 cm depth range compared to the 1 – 30 cm

depth range. Despite this difference in P concentration, the incorporation of small amounts of P – rich material from the 0 – 1 cm depth into the larger soil volumes present in the 1 – 5, 5 – 10, 10 – 20 and 20 – 30 cm depth ranges may not have been detectable using conventional colourimetric analysis.

Another possibility for no apparent increase in labile inorganic P below the 0 – 1 cm depth range may have been linked to the re-distribution of P into surface cast material (Sharpley and Syers, 1977; Le Bayon and Binet, 2006). Both of these studies reported that cast material can contain substantially greater concentrations of P than the surrounding grassland soil. More specifically, the microcosm study conducted by Bayon and Binet (2006) reported that labile inorganic P concentrations were much greater in earthworm casts rather than in their burrows. More specifically, $NaHCO_3$ extracted organic P concentrations ranged from 103 - 168 μg g^{-1} in their cast material compared to 76 - 94 μg g^{-1} along their burrow walls, this finding emphasises the earthworm's ability to deposit large concentrations of P to the soil surface rather than the burrow network. Greater concentrations of P in cast material were also revealed in the study conducted by Sharpley and Syers (1997), since earthworm casts collected from a New Zealand pasture in their study were reported to contain much greater concentrations of inorganic and organic P compared to soil collected from a 0 – 5 cm depth range. Inorganic and organic P concentrations reported by Shapley and Syers (1997) in earthworm cast material were 461 and 353 μg g^{-1}, respectively (no standard error was reported by these authors). In comparison, inorganic and organic P concentrations in the 0 – 5 cm depth range of soil were 257 and 246 μg g^{-1}, respectively. The mesocosm experiment showed that large concentrations of Morgan's inorganic P were measured in earthworm casts (between 50 - 100 μg P g^{-1}), albeit not as great as the P concentrations reported in the above mentioned studies. In comparison, Morgan extracted inorganic P concentrations in the 0 – 1 cm depth range in mesocosms which contained earthworms ranged from 15 – 52 μg P g^{-1}, which was lower than the concentrations observed in earthworm cast material. Therefore, earthworm activity in this experiment did redistribute P to the soil surface, which is in contrast to P incorporation into the soil matrix.

5.4.4 The effects of botanical diversity on microbial biomass phosphorus

No effects of botanical diversity were observed on microbial biomass P concentrations in this mesocosm experiment. Therefore, Hypothesis a) associated with Aim 3 was rejected; since it was hypothesised that greater botanical diversity will increase microbial biomass P concentrations in the soil. It was not entirely clear as to why no botanical diversity effect was observed on microbial biomass P concentrations. An effect would have been expected since the plant community is recognised as able to modulate the development and activity microbial populations in the soil (Bardgett and Shine, 1999; Spehn et al., 2000; van Elsas et al., 2007). In particular, the plant community can affect microbial communities through the input of C into the soil by rhizodeposition (root exudation and root biomass turnover) and leaf litter senescence (litter deposition to the soil surface). In botanical diverse assemblages, the quality and quantity of inputted C varies, since different species within the plant community produce different quantities and types of root exudate, different rooting architectures and different types of leaf litter (Hodge et al., 2009; Millard and Singh, 2010). Therefore, botanical diversity can promote the development of diverse and large microbial communities, compared to more homogenous plant community assemblages, through their C inputs into the soil (Bardgett and McAlister, 1999; Millard and Singh, 2010). With the greater acquisition of plant-derived C by the microbial community, it could be hypothesised that the microbial demand for P would increase and ultimately develop into greater microbial biomass P concentrations in the soil. However, it is also important to note that other soil properties can affect the relationship between botanical diversity and microbial community dynamics, for example soil type and geographical location (Chabrerie et al., 2003; Bezemer et al., 2006). Nevertheless, despite the recognised effects of botanical diversity on C supplementation to the microbial community in the literature, no botanical diversity effect on microbial biomass C concentrations were observed in this mesocosm experiment. It seemed unlikely that other soil properties affected the relationship between plant and microbial communities in this experiment, since all mesocosms were constructed in the same manner and were situated under the same conditions within a randomised block design in a glass-house.

Therefore, one possible explanation for the similar microbial biomass C concentrations between plant communities may be linked to a factor limiting the input of plant C into the soil. All planted mesocosms in this experiment were intensively defoliated (seven cuttings over an 11 month period) and such intensity may have affected plant C inputs into the soil. Indeed, Medina-Roldan and Bardgett (2011) reported that the constant defoliation of the plant community may have limited leaf litter senescence, consequential litter deposition to the soil surface and affected C exudation into the soil matrix. Such a reduction in exudation may have occurred because aboveground defoliation can preferentially partition newly-produced photosynthates to promote aboveground growth (Paterson and Sim, 1999). However, it is important to recognise that there is evidence to suggest that defoliation can increase photosynthate allocation to the roots and potentially increase C exudation (Pietikainen et al., 2009). Therefore, in this mesocosm experiment, the adopted cutting regime may have induced a situation where the quantity of plant C inputted into the soil was similar between mixed and mono plant communities. Such imposed C input may then have affected C acquisition by microbial community, which in turn may have induced similar microbial biomass P concentrations between plant treatments.

Despite the absence of a botanical diversity effect on microbial biomass P concentrations in the soil, the presence of different plant communities did affect the structure of the microbial community. Such effect was manifested as a difference in microbial phenotypic structure between plant communities, which was attributed to a general difference between the PLFA profiles rather than a specific difference in individual PLFA markers. The way that the plant community primarily modulates the structure of the microbial community is through litter deposition and rhizodeposition, as detailed in the above paragraph. However, the fact that no effect was apparent on microbial biomass C concentrations despite an effect on community structure suggests that it may have been the type, rather than the quantity, of plant C that affected the microbial community in this experiment. Whilst the type of plant C may have affected the microbial community structure, another explanation may be linked to plant N inputs. Indeed, greater concentrations of microbial biomass N were observed in the presence of a mixed plant community, which would have been attributed to the presence of a legume in this plant treatment. With such an effect on microbial biomass N, the

presence of a legume may have also attributed to the differences in microbial community structure between plant treatments (van Eekeren et al., 2009b). Legumes develop specialised symbiotic relationships with particular bacterial species, namely bacteria from the *Rhizobium* genus and the presence of bacteria from this genus may have contributed to this difference in microbial community structure between plant treatments in this experiment. However, this explanation could not be definitively proven in this experiment since the adopted PLFA methodology is not a specific tool for explicitly identifying groups of microorganism and only provides broader details about the living structural components of the microbial community (Zelles, 1999).

5.4.5 Botanical diversity effects on productivity and nutrient distribution

Greater plant P yields were observed in mixed plant communities compared to monocultures. This supported Aim 3, Hypothesis b) as this hypothesis stated that increasing botanical diversity will increase plant P yield. The greater yields observed in these mixed plant communities may be explained by a difference in resource acquisition strategies employed by different plant species (van Ruijven and Berendse, 2005). Furthermore, this observation may have also been promoted by the interactions plant species share with one another. One such interaction includes N fixation (Orwin et al., 2010). N fixation occurred in this mesocosm experiment because the legume *Lotus pedunculatus* was present in the mixed plant communities. With greater plant N yields in this mesocosm experiment due to the presence of a legume, there may have been a greater demand for other essential nutrients like P in the mixed plant communities.

Plant species may also interact through complementary resource usage of nutrients that are present in different forms. A proposed hypothesis based on complementary resource use, with respect to plant P utilisation, states that different plant species are able to utilise P from different fractions (Turner, 2008). For example, some species may acquire P present as phosphate monoesters, other species from phosphate diesters and some from labile inorganic pools. This may be a reason why elevated plant P yields were harvested in the presence of a mixed plant community, since each individual

species may have been utilising P from different fractions in the soil. To develop this point further, resource partitioning in the presence of mixed plant communities may also be complemented by the development of different rooting architectures, which would predominately occupy different areas in the soil (Forde and Lorenzo, 2001; Hodge et al., 2009). By occupying different areas of the soil, these rooting systems would increase the total volume of root biomass present within the soil matrix. This larger presence would ultimately enable the entire community to exploit different P fractions in this volume of soil. In this experiment, the presence of two taproot species *Plantago lanceolata* and *Achillea millefolium* would develop lateral branching from a primary root axis that would penetrate deeper into the soil than a grass species with a shallow distribution of roots in comparison. Evidence for greater P utilisation in this experiment occurred since less uniform distributions of labile inorganic P were observed in the presence of a mixed plant community compared to monocultures.

5.4.6 Conclusion

The principal findings in this experiment were the following:

- Microbial community structure differed between botanical diversities; but botanical diversity had no effect on microbial biomass P concentrations.
- Nematode community structure was also affected by botanical diversity.
- Greater botanical diversity increased plant dry matter and P yields.
- Earthworm presence was shown to facilitate P dispersal into the soil matrix, since a decrease in P concentrations were apparent in the 0 – 1 cm depth range when earthworms were present.
- Soil amendment with organic fertiliser promoted a more uniform vertical distribution of P, compared to amendment with inorganic fertiliser only.

- Different microbial community structures were observed between fertiliser types, but no differences in microbial biomass P were apparent.

This experiment highlights the complexity of soil biological interactions with respect to P cycling and dispersal. When these interactions are viewed together, they indicate that the microbial biomass is unresponsive to differences in botanical diversity and fertiliser type. However, despite similar microbial biomass C and P concentrations between treatments, differences were observed in plant P yield and microbial phenotypic structure. These effects may indicate limited C input into the soil from the plant community and utilisation of labile C stocks following organic fertilisation, both of which have limited microbial P acquisition. Finally, the macrofaunal aspect of the soil biota was shown to facilitate P dispersal in the soil matrix, but precisely where P was dispersed was unclear and further work is required to discern how earthworms influence P incorporation.

6. General Discussion

6.1 Project summary

Sustainable agriculture is particularly dependent upon the efficient use and cycling of soil nutrients. Of these nutrients, the behaviour of P makes it one of the primary limiting nutrients to biological growth and productivity in many agricultural systems. In particular, the sorption of P to the soil matrix and the precipitation of P complexes in the soil can reduce bioavailability, which can ultimately limit grassland productivity. In the Irish context, 90% of agricultural land is under grassland and appropriate management strategies are employed to try and ensure the efficient use of P in these systems. These management strategies are aimed to meet the great demand for P in these grassland systems and ultimately maximise output whilst trying to reduce the agricultural impact on environmental health. In addition, the price of P-based fertiliser is increasing and therefore sustainable management of this resource is vital. One recognised way to potentially facilitate grassland P requirements may be linked to maximising P cycling by the soil biota. Furthermore, soil biological activity may also hold the potential to increase P storage in grasslands, since they may reduce P accumulation at the soil surface, increase P concentrations down the soil profile and increase P availability to other components of the grassland biota. Therefore, this project aimed to discern how multiple aspects of the soil biota contributed to P fertiliser assimilation by the plant community. Particular emphasis was given to studying P incorporation in the context of earthworm activity, nematode community dynamics and the effect of P fertilisation and botanical diversity on microbial biomass nutrient pools. The key findings were:

- Greater plant P yields were obtained by increasing the rate at which inorganic P fertilisers were applied, and were also associated with increased botanical diversity of the sward.
- Microbial biomass P concentrations were generally not affected by inorganic P fertilisation rates or botanical diversity.

- Inorganic P fertilisation and botanical diversity both affected the associated soil microbial phenotypes, in idiosyncratic ways since no consistent effect of P fertilisation was observed across the four sampling occasions and the botanical diversity effect was only present in the 0 – 5 cm depth range.

- Nematode community structure was not affected by inorganic P fertilisation, but was altered by botanical diversity.

- Earthworm presence redistributed P from the volume of soil containing the greatest concentrations of P.

- Organic and inorganic fertiliser types affected microbial and nematode community structures in different ways, but had no effect on microbial biomass C, N and P pools.

To better understand the complexity of the P cycle as affected by the soil biota based upon information from the literature, a conceptual diagram was proposed in Chapter 1 Section 1.7. In light of the key findings of this project, this conceptual scheme was modified to account for the results obtained during the project (Figure 6.1). There are two main changes to the initial concept. The first set of revisions was with respect to earthworm effects on phosphorus redistribution. One major change was the removal of the incorporation concept, which was consequentially updated with dispersal. Dispersal in this case was used since results from Chapter 5 only indicated that P concentrations were reduced in the 0 - 1 cm depth range, with no apparent effects at other depth ranges. Thus the box indicating greater distributions of P was removed as no incorporation of P could be proved. The other change to this section involved generalising P redistribution to the entire drilosphere rather than just the burrow network. This was pertinent since analysis of earthworm surface cast material indicated the deposition of P onto the soil surface, which is indicated in the figure as an output from the earthworm-phosphorus interactions box.

The other set of revisions was associated with the plant-microbial interactions box (Figure 6.1). The main modifications in this box were linked to the primary acquisition of fertiliser P by the plant community in the presence of a management regime that removes plant material from the system: indicated by thicker arrows.

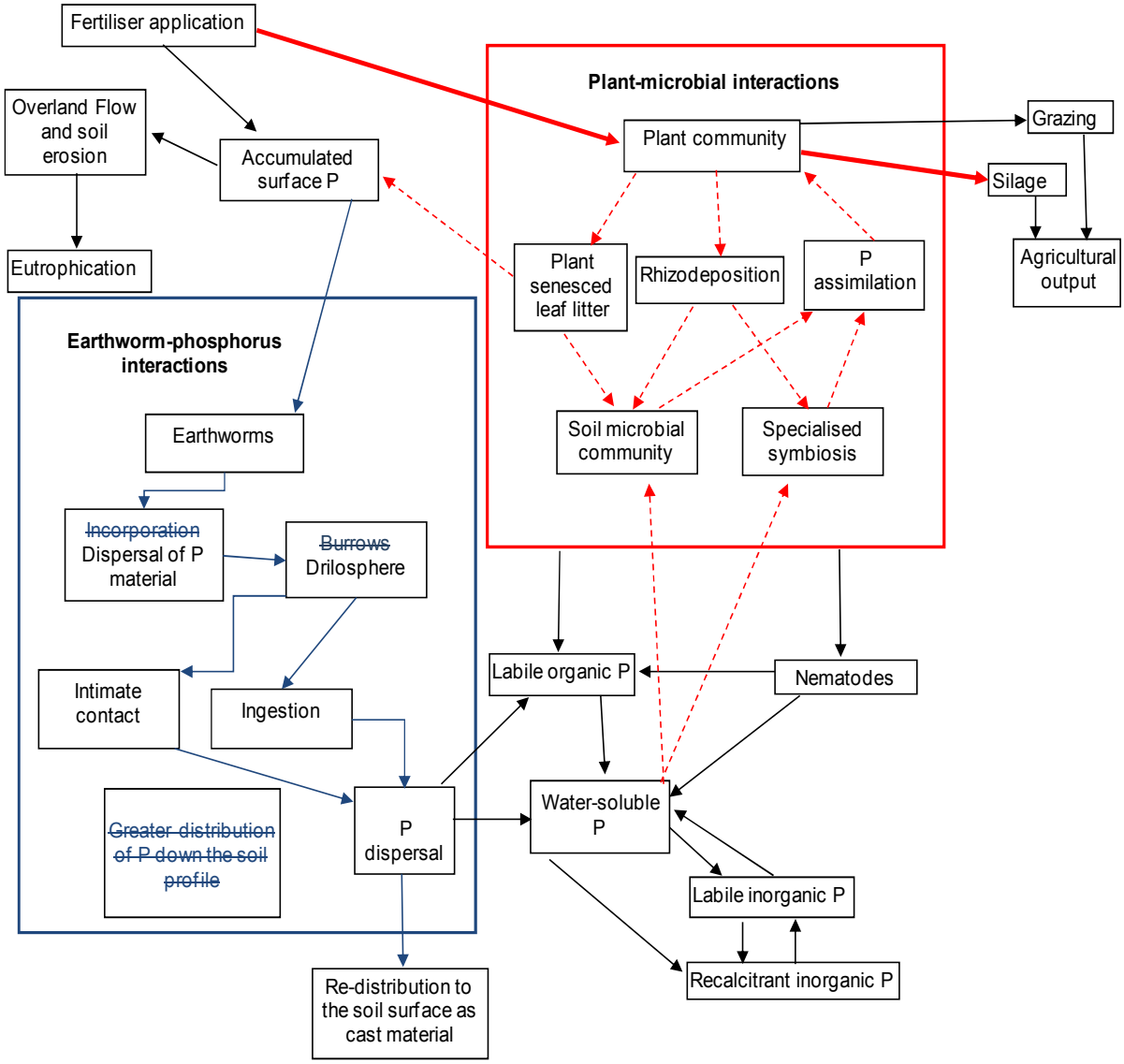

Figure 6.1 Revised conceptual diagram based upon the initial conceptual diagram proposed in Chapter 1 Section 1.7. This conceptual diagram is focused upon the interactions between P and the soil biological community. The two sets of modifications are displayed, which are primary linked to the Earthworm-phosphorus interactions box and the Plant-microbial interactions box. Boxes that have strikethrough text indicate the removal of text present in the initial conceptual diagram (Figure 1.1). Thicker red arrows indicate a primary direction of flow and the dashed red arrows represent a hypothesised limited flow in the system.

Due to the direct removal of P via the plant community, the belowground microbial community was hypothesised to become nutrient limited; indicated by dashed arrows. Such nutrient limitation was also hypothesised to limit P assimilation by the microbial community and thus P assimilation by the plant community via microbial sources. Such hypotheses were incorporated into this conceptual diagram since results from Chapter 3 showed plant responses to P fertilisation, whereas no microbial biomass P effects were apparent. In addition, further investigation into microbial responses to nutrient supplementation (Chapter 4) using soil from the experiment in Chapter 3 revealed no effects of P supplementation on microbial biomass P. This finding supported the hypothesis that microbial communities investigated in Chapter 3 were limited by a nutrient other than P.

6.2 Plant phosphorus yield as affected by soil biological phosphorus cycling

It was hypothesised that greater plant P yields would be coupled to greater microbial biomass P concentrations in the soil. In this project, plant P yields were unequivocally affected by different rates of inorganic P fertiliser in the field experiment, since greater plant P yields were obtained with increasing P fertilisation (Chapter 3). Furthermore, different fertiliser applications in this field experiment promoted subtle differences in plant community structure. Such differences arose from shifts between the dominance of different grass species, since the botanical composition in the P0 treatment differed from those observed in plots that had been fertilised. Plant P yields were also shown to significantly differ between different botanical diversities of the model swards in the mesocosm experiment (Chapter 5). In this experiment, both monocultures and mixed plant communities were constructed, these consisted *of L. perenne* and *L. perenne*, *P. lanceolata*, *A. millefolium* and *L. pedunculatus*, respectively. However, no such effects were observed on microbial biomass P concentrations in the soil in either experimental circumstance. Such contrasting differences between microbial and plant responses to P fertilisation and botanical diversity in these experiments were unanticipated, since the soil microbial biomass can store applied P following fertiliser application and is

regarded as an important source of bio-available P to the plant community (Nziguheba et al., 1998; Brookes, 2001; Achat et al., 2010). Since the microbial biomass is regarded as an important sink and source of P to the plant community, it was expected that a similar trend would have occurred between both plant and microbial P contents. However, the absence of a P fertiliser effect on microbial biomass P, yet manifestation of an effect on plant P yield, in the field experiment indicated that the microbial biomass may not necessarily be such a significant source of P to the plant community. One possible explanation for such a phenomenon may be linked to the plant community itself. Studies indicate that the plant community is able to promote P availability via the release of organic acids and P mineralising enzymes (Richardson et al., 2009). Through the exudation of such organic acids and enzymes, P in labile inorganic and organic forms are either desorbed or mineralised to water-soluble P, whereafter P can be utilised by the plants. Thus, one possible explanation for the plant responses observed may be that the plant communities in these experiments were acquiring P through mechanisms that were independent of the microbial community (Figure 6.1). To further develop this point, different plant species have developed different methods to acquire nutrients from the soil fabric (Forde and Lorenzo, 2001). For example, white lupin (*L. albus*) increases P bioavailability in the soil via a cluster root architecture and mass exudation of large quantities of organic acids, which actively displace P bound to the soil fabric (Forde and Lorenzo, 2001). Building upon this example, expressing multiple rooting architectures through botanical diversity may also facilitate plant P removal without interaction with the microbial community. With respect to the mesocosm experiment, botanical diversity did increase plant P yield, whereas no botanical effects were observed on microbial biomass P. The presence of multiple root architectures in the botanically diverse treatment may have increased the number of effective plant-based P acquisition strategies in the soil (Turner, 2008). Turner (2008) proposed the hypothesis that different plant species have the ability to occupy different niches in the soil. By occupying different niches, individual species exploit different sources of P, which can result in the coexistence between plant species. Based upon this proposed hypothesis, the mixed plant communities in the mesocosm experiment may have acquired P from multiple soil sources, which may have reduced plant community dependence on the microbial community. In the field experiment, the presence of *Lolium perenne* may be

more suited to acquiring P compared the presence of a plant species from the *Agrostis* genus. With the above in mind, one possible way to develop this concept of increasing plant P nutrition further may include focusing on the incorporation of particular, complementary, plant species into the grassland community rather than simply increasing botanical diversity.

In addition to a difference in plant community structure between experiments, the plant P yield obtained from both was also different. In the field experiment, the range of P removed in aboveground plant material over the 19 month experimental period ranged from 1.2 to 2.8 g m^2 y^{-1} between the 0 kg P ha^{-1} y^{-1} and 45 kg P ha^{-1} y^{-1} treatments, respectively. In contrast, the P yields removed in the mesocosm experiment ranged from 4.4 g m^2 y^{-1} in monocultures and 6.4 g m^2 y^{-1} in mixed plant communities. Thus, substantially less P was removed in plant material in the field experiment compared to the mesocosm experiment. There are a number of potential explanations for such observations, since greater yield in the mesocosm experiment may be associated with the constant watering regimes which were applied, the situation in a glasshouse and the application of substantially larger quantities of P in both inorganic and organic treatments, since the equivalent to 69 kg P ha^{-1} was applied to mesocosms as P. Nevertheless, the removal of larger quantities of P in the mixed plant communities in the mesocosm may also be attributed to greater (plant) physiological diversity compared to the field experiment. In the field experiment, grass species were dominant, where as in the mesocosm experiment, two forbs, a grass and a legume species were all present. The presence of all of these physiological groups may have increased the volume of soil explored since both forbs and the legume species would have produced a deep rooting network to explore the soil medium and the grass species would have developed a shallow rooting network in comparison. These different strategies would have exposed these different plant species to different P sources throughout the soil matrix.

Another difference that arose between the mesocosm experiment and field experiment was the effect of botanical diversity on microbial phenotypic structure. In the mesocosm experiment, strong botanical diversity effects were apparent on the microbial phenotype, whereas inconsistent effects were observed between different P fertiliser treatments. One important aspect of the microbial community with respect to plant P acquisition

that would be expected to differ between botanical diversities would be the arbuscular mycorrhizae (Smith and Read, 2007). The symbiotic relationship between plant and arbuscular mycorrhizae is particularly focused upon fungal P acquisition and transportation to the plant and plant C exudation directly to the fungal hyphae. Nevertheless, the shift in microbial community structure between botanical diversities in the mesocosm experiment was manifested through shifts in multiple PLFA markers, with no particular contribution from any single PLFA marker including the arbuscular mycorrhizal PLFA marker 16:1ω5. Furthermore, an inconsistent effect of P fertilisation in the field experiment occurred on microbial community structure, therefore it may be considered that no consistent effect of plant community structure was observed on any specific aspect of the microbial community. Thus, in both circumstances, it seems unlikely that arbuscular mycorrhizae were pivotally active in plant P acquisition, since no specific PLFA markers were greatly manifested in the PCA analysis conducted for both experiments. No further investigation of arbuscular mycorrhizae populations were conducted in this project since they have been the focus of many other studies and are not considered relevant to nutrient cycling in highly fertilised systems, since fungal dominated communities are typically associated with low fertility conditions (Bardgett and McAlister, 1999).

6.3 The role of earthworms in phosphorus soil re-distribution

The presence of earthworms was hypothesised to redistribute P from the P-rich upper zone to deeper within the soil profile. To test this hypothesis, experimental mesocosms were specifically constructed to produce a concentration gradient of P with depth (Chapter 5). More specifically, two contrasting soils were used to produce two different soil layers in the mesocosms, with an approximate seven fold difference in P concentration between layers. This concentration gradient was produced to represent P spatial distributions typically observed in grassland systems (Owens et al., 2008). By producing such a steep gradient between layers, it was envisaged that the application of earthworms and their consequential activity would produce detectable changes in P concentrations between depths. The earthworm application consisted of earthworms

from two ecological groups. In designated mesocosms, three *Ap. longa* (an anecic form) and six *Al. chlorotica* (an epigeic/endogeic form) were applied. Both of these species were chosen since they interact with the P-rich top soil, and with both species active in the top soil, it was hypothesised that greater mixing would occur, which would ultimately facilitate P incorporation.

The presence of both *Ap. longa* and *Al. chlorotica* in this mesocosm experiment was shown to reduce P concentrations in the 0 – 1 cm compared to mesocosms where they were absent. However, no significant increases in P concentration within the 1 – 30 cm depth range were observed when earthworms were present. Such reduction provides evidence that earthworms can actively contribute to P removal in the top soil, which suggests that earthworms can apparently disperse P from the volume of soil containing the greatest concentrations of P in many grassland systems (Figure 6.1). The dispersal and potential mitigation P loss is important since the accumulation of P at the soil surface is susceptible to soil erosion, which can have detrimental effects on water quality. However, it was unclear precisely where the P was redistributed in this experiment (Figure 6.1). Data from the literature suggests that P may accumulate on the soil surface, via association with surface earthworm cast material (Sharpley and Syers, 1977; Sharpley and Syers, 1976). However, it was also possible that an increase in P below this depth was below the limits of detection and therefore inconclusive. In particular, possible dilution effects of incorporating P into large volumes of soil that were homogenised before laboratory analysis may have also promoted conditions where any increase in P was below detection limits. To further elucidate where P was redistributed, further experimentation should focus on discerning the specific areas in the soil in which P ingested by earthworms is deposited. In particular, a more practical way to assess P incorporation into the soil fabric from the surface may be to measure P concentrations in spatially prescribed zones of the soil such as the drilosphere. By measuring in this manner, it may be feasible to detect changes of P concentration in key locations and indicate whether P incorporation is isolated to the burrow network. However, this approach would only be suited to experiments focused on anecic earthworm types, as the presence of endogeic earthworms could promote greater horizontal mixing of soil that might affect the architecture of the anecic earthworm burrow network (Capowiez and Belzunces, 2001; Felten and Emmerling, 2009), which

may re-distribute P concentrations present in the anecic earthworm burrow into the surrounding soil and decrease P concentrations present along the burrow wall. Such ecological synergy between earthworm behaviours was considered in this experiment; however, it was not the focus and was not fully investigated. Future work on earthworm mediated P-incorporation should also be targeted on the relationships that earthworms exhibit towards each other and the consequences this may have on P incorporation into the soil profile. For example, can greater P incorporation and mixing into the soil matrix be promoted by coupling anecic earthworms with strictly endogeic types, or does the combination of epigeic/endogeic and anecic earthworms provide the best coupling to reduce P concentrations in the top soil and mix incorporated P into the soil fabric?

6.4 How phosphorus fertilisers affect soil microbial communities

One of the hypotheses tested in this project was that soil microbial communities would respond to inorganic P fertiliser inputs. To test this hypothesis, two grassland sites were utilised that had been receiving constant applications of inorganic P fertiliser for 17 years at four different application rates (0, 15, 30 and 45 kg P ha^{-1} y^{-1}) (Chapter 3). The results from this experiment revealed that microbial biomass P concentrations did not respond to such P fertiliser inputs. Therefore, two additional laboratory experiments were conducted to further investigate the possible basis for this absence of P fertiliser effect (Chapter 4). In these experiments, neither microbial activity nor microbial biomass P concentrations were affected by inorganic P supplementation, which provided supporting evidence for data collected from the field experiment that the application of P fertiliser did not affect the soil microbial community in the studied grassland sites. The experimental design of the field experiment simulated an intensive silage-based system in which little organic material was returned to the soil, since no organic fertilisers were added, no animal grazing occurred and no harvested material was returned to the plots. The system was therefore being heavily 'cropped' for biomass (and hence associated C) and energy returns to the soil would have consequently been limited (Figure 6.1). One possibility was that the microbial communities in both the field and laboratory experiments were limited by a nutrient other then P, and hence were

not responsive to additions of this element. In another P fertiliser field experiment established on the Johnstown Castle Estate (referred to as the Cowlands experiment), microbial biomass P concentrations increased with increasing P fertilisation, which was in contrast to the results obtained in the field experiment conducted for this project. One of the main differences between both fertiliser experiments was that organic material was being returned to the soil in the Cowlands experiment (Chen, 2012). Therefore, results from the Cowlands experiment may be interpreted as being supportive of the conclusion drawn for the field experiment conducted for this project. In particular, results from the Cowlands experiment supported the hypothesis that organic material inputs into the soil are intricately linked to microbial P acquisition. Furthermore, when no organic material is inputted, the microbial biomass becomes seemingly uncoupled from P fertiliser applications and does not necessarily acquire P despite increasing water-soluble P concentrations in the soil.

Both management regimes described above may also have implications for C and N cycling in the soil. With respect to the C cycle, the intensively cropped system would have limited the senescence of aboveground plant material and consequentially limit the amount of organic C being deposited onto the soil surface. Such limited input would have large implications on the decomposition process, since a reduced quantity of substrate would be made available to the soil heterotrophic community (Rees et al., 2002; Camberdella, 2005). By limiting plant inputs, it could also be hypothesised that the accumulation of C in the soil organic matter pool may be impaired. Evidence for this impairment was present during the field experiment, since no effects of P fertilisation were observed on estimated organic matter contents and dissolved organic C concentrations (Chapter 3 Section 3.3.2.2). If C was being cycled efficiently in this cropped system, then greater organic matter contents and dissolved organic C concentrations would be envisaged in the presence of the highest P fertiliser regime: since plant dry matter yield increased with increasing P fertilisation in this experiment. In the contrasting grazed system, the input of organic material through animal excretion would supply a variety of C compounds to the soil. The input of such material would stimulate the soil heterotrophic community more and promote a greater storage of C in the soil organic matter pool compared to the cropped system.

With respect to N, both management regimes could have a large effect on N transformations in the soil. In the field experiment conducted for this project, the intensive cutting regime resulted in the application of inorganic N fertiliser (applied as calcium ammonium nitrate) following each harvest to compensate for N transfer from the system. In the grazing system, the input of animal faecal material would increase organic N concentrations alongside the application of inorganic N fertiliser. The input of different forms of N into these systems would have large effects on the cycling of N in these systems (Knops et al., 2002; Ollivier et al., 2011. Firstly, the input of N mainly as inorganic fertiliser could be hypothesised to mainly stimulate the MIT pathway (Ollivier et al., 2011). Stimulation of this pathway is due to the assimilation of NH_4^+ and NO_3^- following fertiliser application. Furthermore, amending the soil with NH_4^+ could also stimulate greater rates of nitrification; however, this would be affected by the structure of the microbial community and on environmental conditions. Therefore, in the cropped system, the primary pathway of N turnover may be through the MIT pathway. In the grazed circumstance, the direct pathway would be hypothesised as the primary N turnover mechanism in the soil (Ollivier et al., 2011). This is due to the additions of organic material, which would stimulate the soil heterotrophic community. Following such decomposition, the biological community could utilise the flush of low molecular weight organic compounds as a source of N to facilitate growth.

Despite the potential effects on the cycling of P, C and N, an unexpected observation between both management regimes was that microbial biomass C concentrations were similar. This was unexpected since the microbial community in the intensively cut field experiment appeared to be limited by a nutrient other than P, whereas no such limitation was apparent in the grazed system. More specifically, in the Cowlands experiment, microbial biomass C concentrations ranged from 1325 – 2888 μg g^{-1} and in the field experiment conducted for this project, microbial biomass C concentrations ranged from 1324 – 3547 μg g^{-1}. However, microbial biomass P and N concentrations were greater in the Cowlands experiment compared to the experiment conducted for this project. Therefore, the microbial biomass in Cowlands experiment would have consisted of lower C: P and C: N ratios, which would indicate that the microbial community in that experiment would have a greater growth rate compared to the microbial communities studied in this project (Elser et al., 2003; Chen et al., 2010). To conclude, utilising the

soil microbial community to mobilise and acquire P following fertiliser application seems to be most efficient when coupled with organic matter input. In systems where seemingly little organic matter input occurs (for example in the field experiment), the microbial community becomes limited and is thus less responsive to such applications. More specifically, in the field experiment, the microbial community may have been K limited, since results showed significantly lower concentrations in P fertilised (P15, P30 and P45) treatments compared to the control (P0) treatment, despite the input of the recommended inorganic K fertiliser rates (125 kg K ha^{-1} y^{-1}) to both grassland sites.

Based on this conclusion, a further hypothesis can be set which tests whether greater microbial nutrient acquisition can be stimulated following P fertiliser applications to the 'inorganic intensive' grassland sites investigated in this project via a change in management. More specifically, that following the onset of the application of organic material to these grassland systems, microbial biomass nutrient concentrations will increase compared to circumstances where such material is not applied.

Based on this hypothesis, future work on the effects of P fertilisation on microbial biomass could focus on the cutting regime currently adopted in these grassland sites. This would discern whether grassland harvesting is linked to P fertiliser assimilation by the microbial community. To undertake this, one proposed experimental update would be to split existing grassland plots from the field experiment into either eight cuts $year^{-1}$ or three – four cuts $year^{-1}$ treatments and continue monitoring the soil biota, with more consideration of K dynamics in both sites. By reducing the intensity of the cutting regime, the supply of C, and possibly K, into the soil may increase and could be hypothesised to induce a P fertiliser effect on microbial biomass P concentrations. With little effect of P fertilisation on the microbial community, these grassland sites could also be used to assess fertiliser type effects on the soil biota and whether fertiliser type can stimulate the microbial community to respond to P fertilisation. For example, incubation studies have reported fertiliser type effects on microbial biomass P (Gichangi et al., 2009; Ge et al., 2010). These studies report that greater microbial biomass P concentrations were achieved when both organic and inorganic fertiliser were applied together. This was attributed to the addition of labile C and N through organic fertiliser application and the consequential synthesis of microbial biomass. Therefore, it would be

appropriate to investigate whether changing fertiliser type in this field experiment would invoke a microbial biomass P response where previously it was absent. By changing fertiliser types in this experiment, an external source of labile C, N and K can be supplied to the soil, which may ultimately stimulate microbial P acquisition in both of these sites commensurate with the quantities of fertiliser P applied.

6.5 Practical implications

The apparent ability of earthworms to reduce P concentrations in the topsoil holds potential for utilising the soil biota as a tool to facilitate sustainable P cycling. More specifically, the ability of earthworms to reduce P concentrations in the volume of soil that is most susceptible to erosion may be potentially utilised to help reduce agricultural P pollution in water courses and storage of P in grassland systems. Therefore, this data highlights the importance for maintaining earthworm activity in such systems from a P mitigation perspective. One way to practically maintain and perhaps manipulate such activity at the soil surface may be through the application of organic fertilisers. This project utilised two earthworm species that are active at the soil surface and it is activity in this area that facilitated a reduction of P. Therefore, the application of organic material may stimulate greater earthworm activity in this area compared to the application of inorganic fertilisers (Sharpley et al., 2011). By promoting greater activity, greater quantities of P may be mixed into the soil matrix below the top soil, which would reduce the risk of P loss from the grassland system. With respect to earthworm mediated P incorporation, future work should investigate whether organic matter inputs can be used to regulate earthworm community dynamics and whether this regulation can aid in P incorporation into the soil. By investigating the relationship between organic matter inputs and earthworm P incorporation, the potential to develop a practical management tool to aid in P mitigation in agricultural systems may be achieved.

From an agricultural perspective, the use of cutting regimes in an experimental context has been proven to stimulate similar but relative plant yield responses to P fertilisation rates as those observed in a grazed system (Cayley and Hannah, 1995; Morton and Roberts, 2001). With similar responses revealed to P fertiliser applications, the

justification for using cheaper and more practical cutting regimes to reflect plant responses to P fertilisation in a grazed system is proven for experimental purposes. Nevertheless, emphasis on the similarity in these studies was in fact relative, since greater plant dry matter yields were typically observed in grazed systems. Thus, when viewed from the maximum yield potential of the grassland system between regimes, the rate of P fertiliser required to achieve a given proportion of the potential yield in either circumstance was similar, despite the fact that different dry matter yields were produced. With respect to this project, such differences in dry matter production coupled with actual nutrient return in grazed systems when compared to cut systems was revealed to have substantial effects on the soil biology. Therefore, data obtained in this project suggests that whilst similar plant P responses can be obtained by adopting these management regimes (as shown in the literature), the consequential effects on the soil biota and their ability to cycle P may have been largely overlooked in current grassland P management advice. Nevertheless, the fact that similar plant P responses can be obtained in the presence of both management regimes indicates that nutrient cycling by the soil biota may not be important with respect to plant P acquisition, since the plant community was still able to acquire P whilst an unresponsive microbial community was present. However, this ability of the plant community to respond to P fertilisation in systems with little organic matter return is based upon regular and considerable inorganic fertilisation regimes, which is not the economical solution and questions whether the plant community can sustain high productivity when fertiliser applications are reduced. Therefore, this project stresses that the adopted grassland management regime plays a critical role in governing the ability of the soil biota to supply P to the plant community, and also raises the question whether intensive defoliation of the grassland community is truly reflective of plant P acquisition in a grazed system. An applied future work consideration is that attention should be paid to investigating the importance of the soil biota in situations where P fertiliser applications have ceased, especially where they have been intensive, and how plants acquire P in these systems thereafter. Based on data from this project, the previous management history would apparently be particularly important in determining the ability of the soil microbial biomass to supply P to the plant community when P fertiliser inputs have ceased.

6.6 Project conclusions

In this project, two research questions were devised to investigate the overarching aim. The first question was: Is the soil biota affected by commonly adopted P fertiliser strategies in grassland systems? This project investigated the soil biota in the presence of different inorganic P fertilisation rates. It was found that the soil biota is seemingly uncoupled from these P fertiliser applications, since no increase in microbial biomass P with increasing P fertilisation was observed in a field experiment and in two laboratory experiments. Furthermore, no consistent effect of P fertilisation was observed on other soil biological properties, which strengthened the absence of an association between P fertiliser strategies and the soil biota. This lack of association was attributed to the grassland management regime, since the intensive cutting regime adopted permanently removed large quantities of plant material from the system, which ultimately may have limited the activity and size of the microbial community. In addition, this project also showed that earthworm activity in a planted system did facilitate the removal of P in the 0 – 1 cm depth range of soil. This finding was observed in a glass-housed based mesocosm experiment, which focused on how multiple aspects of the soil biota are affected by the application of P fertiliser and different botanical diversities. Thus, the soil biota can be affected by fertiliser strategies, but this is seemingly dependent upon the management of the grassland system.

The second research question was: what consequence does this hold for P acquisition by the plant community? In both the field based and mesocosm experiments, the application of P fertilisers had an effect on plant P yield. More specifically, an increase in plant P yield with increasing P fertiliser rate was observed in the field experiment and greater plant P yields were obtained with increasing botanical diversity following fertiliser application. Similar to the field experiment, no botanical diversity effects were observed on microbial biomass P concentrations in the mesocosm experiment as well. Therefore, the response by the soil biota to P fertiliser strategies seemingly has little consequence on P acquisition by the plant community under this defoliation management system. In particular, it appeared that the microbial community is also uncoupled from the plant community, since the plant community responded to P fertiliser applications and the soil biota did not. Thus, the application of inorganic P

fertiliser was more important for plant P acquisition than the soil biota. However, solely relying on inorganic P fertilisers for sufficient plant P nutrition is not a sustainable option and may not be economically viable in the long-term. Therefore, future work derived from this project should fully investigate the importance of grassland management regimes, and whether adjusting such regimes in relation to the provision of energy to the soil biota via organic materials may stimulate microbial responses to fertiliser application and increase microbial biomass P concentrations in the soil. By increasing microbial biomass P concentrations in the soil, dependency on inorganic P fertiliser to sustain high plant P yields may be reduced.

Reference list

Achat, D. L., Morel, C., Bakker, M. R., Augusto, L., Pellerin, S., Gallet-Budynek, A. and Gonzalez, M. (2010), "Assessing turnover of microbial biomass phosphorus: Combination of an isotopic dilution method with a mass balance model", *Soil Biology and Biochemistry,* vol. 42, pp. 2231-2240.

Amador, J. A. and Jones, R. D. (1993), "Nutrient limitations on microbial respiration in peat soils with different total phosphorus content", *Soil Biology and Biochemistry,* vol. 25, pp. 793-801.

Anderson, J. P. E., and Domsch, K . H. (1980), "Quantities of plant nutrients in the microbial biomass of selected soils", *Soil Science*, vol. 130, pp. 212-216.

Anderson, J. P. E. and Domsch, K. H. (1978), "A physiological method for the quantitative measurement of microbial biomass in soils", *Soil Biology and Biochemistry,* vol. 10, pp. 215-221.

Aquino, M. T. and Plassard, C. (2004), "Dynamics of ectomycorrhizal mycelial growth and P transfer to the host plant in response to low and high soil P availability", *FEMS Microbiology Ecology,* vol. 48, pp. 149-156.

Ayaga, G., Todd, A. and Brookes, P. C. (2006), "Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems", *Soil Biology and Biochemistry,* vol. 38, pp. 81-90.

Bardgett, R. D. (2005), *The Biology of Soil: A Community and Ecosystem Approach,* Oxford University Press, United Kingdom.

Bardgett, R. D., Wardle, D. A. and Yeates, G. W. (1998), "Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influences soil organisms", *Soil Biology and Biochemistry,* vol. 30, pp. 1867-1878.

Bardgett, R. D., Lovell, R. D., Hobbs, P. J. and Jarvis, S. C. (1999), "Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands", *Soil Biology and Biochemistry,* vol. 31, pp. 1021-1030.

Bardgett, R. D. and McAlister, E. (1999), "The measurement of soil fungal : bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands", *Biology and Fertility of Soils,* vol. 29, pp. 282-290.

Bardgett, R. D. and Shine, A. (1999), "Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands", *Soil Biology and Biochemistry,* vol. 31, pp. 317-321.

Beever, R. E. and Burns, D. J. W. (1980), "Phosphorus uptake, storage and utilization by fungi.", *Advances in Botanical Research,* vol. 8, pp. 128-219.

Bernard, E. C. (1992), "Soil nematode biodiversity", *Biology and Fertility of Soils,* vol. 14, pp. 99-103.

Bertin, C., Yang, X. and Weston, L. A. (2003), "The role of root exudates and allelochemicals in the rhizosphere", *Plant and Soil,* vol. 256, pp. 67-83.

Bezemer, T. M., Lawson, C. S., Hedlund, K., Edwards, A. R., Brook, A. J., Igual, J. M., Mortimer, S. R. and Van der Putten, W. H. (2006), "Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands", *Journal of Ecology,* vol. 94, pp. 893-904.

Bligh, E. G. and Dyer, W. J. (1959), "A rapid method of total lipid extraction and purification", *Canadian Journal of Biochemistry and Physiology,* vol. 37, pp. 911.

Bongers, T. (1988), *De nematoden van Nederland,* Stichting Uitgeverij Koninklijke Nederlandse Natuurhistorische Vereniging.

Bonnett, S. A. F., Ostle, N. and Freeman, C. (2006), "Seasonal variations in decomposition processes in a valley-bottom riparian peatland", *Science of The Total Environment,* vol. 370, pp. 561-573.

Bourke, D., Dowding, P., Tunney, H., O'Brien, J. and Jeffrey, D. W. (2008), "The organic phosphorus composition of an irish grassland soil", *Biology and Environment: Proceedings of the Royal Irish Academy,* vol. 108B, pp. 17-28.

Brookes, P. C. (2001), "The Soil Microbial Biomass: Concept, Measurment and Applications in Soil Ecosystem Research", *Microbes and Environment,* vol. 16, pp. 131-140.

Brookes, P. C., Powlson, D. S. and Jenkinson, D. S. (1982), "Measurement of microbial biomass phosphorus in soil", *Soil Biology and Biochemistry,* vol. 14, pp. 319-329.

Brookes, P. C., Landman, A., Pruden, G. and Jenkinson, D. S. (1985), "Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil", *Soil Biology and Biochemistry,* vol. 17, pp. 837-842.

Bunemann, E. K. (2008), "Enzyme additions as a tool to assess the potential bioavailability of organically bound nutrients.", *Soil Biology and Biochemistry,* vol. 40, pp. 2116-2129.

Bunemann, E. K., Bossio, D. A., Smithson, P. C., Frossard, E. and Oberson, A. (2004), "Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization.", *Soil Biology and Biochemistry,* vol. 36, pp. 889-901.

Bunemann, E. K., Oberson, A. and Frossard, E. (eds.) (2011), *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling.* Springer-Verlag, Berlin Heidelberg.

Buenemann, E. K., Oberson, A., Liebisch, F., Keller, F., Annaheim, K. E., Huguenin-Elie, O. and Frossard, E. (2012), "Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a grassland soil with low inorganic phosphorus availability", *Soil Biology and Biochemistry,* vol. 51, pp. 84-95.

Cai, Z. C. and Qin, S. W. (2006), "Dynamics of crop yields and soil organic carbon in a long-term fertilization experiment in the Huang-Huai-Hai Plain of China", *Geoderma,* vol. 136, pp. 708-715.

Cambardella, C. A. (2005), "CARBON CYCLE IN SOILS; Formation and Decomposition", in Editor-in-Chief: Daniel Hillel (ed.) *Encyclopedia of Soils in the Environment,* Elsevier, Oxford, United Kingdom: pp. 170-175.

Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S. and Potts, J. M. (2003), "A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil.", *Applied and Environmental Microbiology,* vol. 69, pp. 3593-3599.

Capowiez, Y. and Belzunces, L. (2001), "Dynamic study of the burrowing behaviour of Aporrectodea nocturna and Allolobophora chlorotica: interactions between earthworms and spatial avoidance of burrows", *Biology and Fertility of Soils,* vol. 33, pp. 310-316.

Cayley, J. W. D. and Hannah, M. C. (1995), "Response to phosphorus fertilizer compared under grazing and mowing", *Australian Journal of Agricultural Research,* vol. 46, pp. 1601-1619.

Chabrerie, O., Laval, K., Puget, P., Desaire, S. and Alard, D. (2003), "Relationship between plant and soil microbial communities along a successional gradient in a chalk grassland in north-western France", *Applied Soil Ecology,* vol. 24, pp. 43-56.

Chakraborty, A., Chakrabarti, K., Chakraborty, A. and Ghosh, S. (2011), "Effect of long-term fertilizers and manure application on microbial biomass and microbial activity of a tropical agricultural soil", *Biology and Fertility of Soils,* vol. 47, pp. 227-233.

Chapin III, F. S., Matson, P. A. and Mooney, H. A. (2011), *Principles of Terrestrial Ecosystem Ecology*, 2 ed, Springer Science and Business Media Inc, United States of America.

Chardon, W. J., Aalderink, G. H. and van der Salm, C. (2007), "Phosphorus leaching from cow manure patches on soil columns", *Journal of Environmental Quality,* vol. 36, pp. 17-22.

Chen, X. (2012), *Nematode response to nitrogen and phosphorus in grasslands, assessed by microscopy and molecular methods* (PhD thesis), National University of Galway, Ireland.

Chen, C., Condron, L., Sinaj, S., Davis, M., Sherlock, R. and Frossard, E. (2003), "Effects of plant species on phosphorus availability in a range of grassland soils", *Plant and Soil,* vol. 256, pp. 115-130.

Chen, M. M., Yin, H. B., O'Connor, P., Wang, Y. S. and Zhu, Y. G. (2010), "C: N: P stoichiometry and specific growth rate of clover colonized by arbuscular mycorrhizal fungi", *Plant & Soil,* vol. 326, pp. 21-29.

Chowdhury, M. H., Kouno, K., Ando, T. and Nagaoka, T. (2000), "Microbial biomass, S mineralization and S uptake by African millet from soil amended with various composts", *Soil Biology and Biochemistry,* vol. 32, pp. 845-852.

Chu, H., Lin, X., Fujii, T., Morimoto, S., Yagi, K., Hu, J. and Zhang, J. (2007), "Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management", *Soil Biology and Biochemistry,* vol. 39, pp. 2971-2976.

Chuang, C., Kuo, Y., Chao, C. and Chao, W. (2007), "Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger"*, *Biology and Fertility of Soils,* vol. 43, pp. 575-584.

Cleveland, C. C. and Liptzin, D. (2007), "C : N : P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass?", *Biogeochemistry,* vol. 85, pp. 235-252.

Costa, S. E. V. G. A., Souza, E. D., Anghinoni, I., Flores, J. P. C., Vieira, F. C. B., Martins, A. P. and Ferreira, E. V. O. (2010), "Patterns in phosphorus and corn root

distribution and yield in long-term tillage systems with fertilizer application", *Soil and Tillage Research,* vol. 109, pp. 41-49.

Coulter, B. S. and Lalor, S. (2008), *Major and micro nutrient advice for productive agricultural crops.* Third ed, Teagasc: Agriculture and Food Development Authority, Teagasc, Oak Park, Co Carlow, Ireland.

Creamer, R. E., Bellamy, P., Black, H. I. J., Cameron, C. M., Campbell, C. D., Chamberlain, P., Harris, J., Parekh, N., Pawlett, M., Poskitt, J., Stone, D. and Ritz, K. (2009), "An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring", *Biology and Fertility of Soils,* vol. 45, pp. 623-633.

Cunningham, J. E. and Kuiack, C. (1992), "Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium balaii*", *Applied and Environmental Microbiology,* vol. 58, pp. 1451-1458.

Curry, J. P., Doherty, P., Purvis, and Schmidt, O. (2008), "Relationships between earthworm populations and management intensity in cattle-grazed pastures in Ireland", *Applied Soil Ecology,* vol. 39, pp. 58-64.

Daly, K. (2005), *Evaluating Morgan's Phosphorus Test as an Environmental Indicator,* RMIS 4976, Teagasc, Johnstown Castle Research Centre Wexford.

Daly, K., Jeffrey, D. and Tunney, H. (2001), "The effect of soil type on phosphorus sorption capacity and desorption dynamics in Irish grassland soils", *Soil Use and Management,* vol. 17, pp. 12-20.

Delgado, A. and Scalenghe, R. (2008), "Aspects of phosphorus transfer from soils in Europe", *Journal of Plant Nutrition and Soil Science,* vol. 171, pp. 552-575.

Demoling, F., Figueroa, D. and Baath, E. (2007), "Comparison of factors limiting bacterial growth in different soils", *Soil Biology and Biochemistry,* vol. 39, pp. 2485-2495.

Denef, K., Roobroeck, D., Manimel Wadu, M. C. W., Lootens, P. and Boeckx, P. (2009), "Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils", *Soil Biology and Biochemistry,* vol. 41, pp. 144-153.

Department of Agriculture, Fisheries and Food and Central Statistics Office. (2011), Compendium of Irish Agricultural Statistics 2008*, Department of Agriculture, Fisheries and Food*, Ireland.

Devliegher, W. and Verstraete, W. (1996), "Lumbricus Terrestris in a soil core experiment: Effects of nutrient-enrichment processes (NEP) and gut-associated processes (GAP) on the availability of plant nutrients and heavy metals", *Soil Biology and Biochemistry,* vol. 28, pp. 489-496.

Devliegher, W. and Verstraete, W. (1997), "Microorganisms and soil physico-chemical conditions in the drilosphere of Lumbricus Terrestris", *Soil Biology and Biochemistry,* vol. 29, pp. 1721-1729.

Dick, W. A. and Tabatabai, M. A. (1978), "Hydrolysis of organic and inorganic phosphorus compounds added to soils", *Geoderma,* vol. 21, pp. 175-182.

Dilly, O. (1999), "Nitrogen and phosphorus requirement of the microbiota in soils of the Bornhoved Lake district", *Plant and Soil,* vol. 212, pp. 175-183.

Dixon, J. B., Weed, S. B., Kittrick, J. A., Milford, M. H. and White, J. L. (eds.) (1977), *Minerals in Soil Environments,* Soil Science Society of America, United States of America.

Donnellan, T., Hanrahan, K. and Lalor, S. (2012), "Food Harvest 2020 and the Demand for Fertilizer", Lalor, S. (ed.), in: *Role of fertilizer and soil fertility in achieving Food Harvest 2020 targets,* Vol. 47, 7th February, Ireland, The Fertilizer Association of Ireland, Ireland, pp. 3.

Dorioz, J. M., Wang, D., Poulenard, J. and Trevisan, D. (2006), "The effect of grass buffer strips on phosphorus dynamics - A critical review and synthesis as a basis

for application in agricultural landscapes in France", *Agriculture, Ecosystems & Environment,* vol. 117, pp. 4-21.

Dougherty, W. J., Fleming, N. K., Cox, J. W. and Chittleborough, D. J. (2004), "Phosphorus transfer in surface runoff from intensive pasture systems at various scales: A review", *Journal of Environmental Quality,* vol. 33, pp. 1973-1988.

Edwards, C. A. and Lofty, J. R. (1977), *Biology of Earthworms,* 2 ed, Richard Clay Group, United Kingdom.

Eivazi, F. and Tabatabai, M. A. (1977), "Phosphatases in Soil", *Soil Biology and Biochemistry,* vol. 9, pp. 167-172.

Elser, J. J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W., Schade, J., Hood, J. and Sterner, R. W. (2003), "Growth rate-stoichiometry couplings in diverse biota", *Ecology Letters,* vol. 6, pp. 936-943.

Felten, D. and Emmerling, C. (2009), "Earthworm burrowing behaviour in 2D terraria with single- and multi-species assemblages", *Biology and Fertility of Soils,* vol. 45, pp. 789-797.

Forde, B. and Lorenzo, H. (2001), "The nutritional control of root development", *Plant and Soil,* vol. 232, pp. 51-68.

Fraser, P. M., Haynes, R. J. and Williams, P. H. (1994), "Effects of pasture improvement and intensive cultivation on microbial biomass, enzyme-activities, and composition and size of earthworm populations", *Biology and Fertility of Soils,* vol. 17, pp. 185-190.

Frostegard, A., Baath, E. and Tunlid, A. (1993), "Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis", *Soil Biology and Biochemistry,* vol. 25, pp. 723-730.

Frostegard, A., Tunlid, A. and Baath, E. (1991), "Microbial biomass measured as total lipid phosphate in soils of different organic content", *Journal of Microbiological Methods,* vol. 14, pp. 151-163.

Ge, G., Li, Z., Fan, F., Chu, G., Hou, Z. and Liang, Y. (2010), "Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers", *Plant and Soil,* vol. 326, pp. 31-44.

Gichangi, E. M., Mnkeni, P. N. S. and Brookes, P. C. (2009), "Efects of goat manure and inorganic phosphate addition on soil inorganic and microbial biomass phosphorus fractions under laboratory incubation conditions", *Soil Science and Plant Nutrition,* vol. 55, pp. 764-771.

Gichangi, E. M., Mnkeni, P. N. S. and Brookes, P. C. (2010), "Goat manure application improves phosphate fertilizer effectiveness through enhanced biological cycling of phosphorus", *Soil Science and Plant Nutrition,* vol. 56, pp. 853-860.

Gobat, J. M., Aragno, M. and Matthey, W. (2004), *The Living Soil: Fundamentals of Soil Science and Soil Biology.* Science Publishers Inc, USA.

Gong, W., Yan, X. and Wang, J. (2012), "The effect of chemical fertilizer application on carbon input and export in soil—A pot experiment with wheat using natural 13C abundance method", *Geoderma,* vol. 189–190, pp. 170-175.

Gregorich, E. G., Voroney, R. P. and Kachanoski, R. G. (1991), "Turnover of carbon through the microbial biomass in soils with different texture", *Soil Biology and Biochemistry,* vol. 23, pp. 799-805.

Gregory, P. J. (2006), "The Rhizosphere", in *Plant roots: their growth, activity, and interaction with soils,* Blackwell Publishing Ltd, United Kingdom, pp. 216-247.

Griffiths, B. S., Donn, S., Neilson, R. and Daniell, T. J. (2006), "Molecular sequencing and morphological analysis of a nematode community", *Applied Soil Ecology,* vol. 32, pp. 325-337.

Hart, M. R., Quin, B. F. and Nguyen, M. L. (2004), "Phosphorus runoff from agricultural land and direct fertilizer effects: A review", *Journal of Environmental Quality,* vol. 33, pp. 1954-1972.

Hassink, J. (1994), "Effect of soil texture on the size of the microbial biomass and on the amount of C and N mineralized per unit of microbial biomass in Dutch grassland soils", *Soil Biology and Biochemistry,* vol. 26, pp. 1573-1581.

He, Z. L., Baligar, V. C., Martens, D. C. and Ritchey, K. D., (1997a), "Effect of phosphate rock, lime and cellulose on soil microbial biomass in acidic forest soil and its significance in carbon cycling", *Plant and Soil*, vol. 195, pp. 129-136.

He, Z. L., Wu, J., ODonnell, A. G. and Syers, J. K. (1997b), "Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture", *Biology and Fertility of Soils,* vol. 24, pp. 421-428.

He, Z. L., Xu, M. Y., Deng, Y., Kang, S. H., Kellogg, L., Wu, L. Y., Van Nostrand, J. D., Hobbie, S. E., Reich, P. B. and Zhou, J. K. (2010), "Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO2", *Ecological Letters,* vol. 13, pp. 564-575.

Hedlund, K., Santa-Regina, I., Van der Putten, W. H., Leps, J., Diaz, T., Korthals, G. W., Lavorel, S., Brown, V. K., Gormsen, D., Mortimer, S. R., Barrueco, C. R., Roy, J., Smilauer, P., Smilauerova, M. and Van Dijk, C. (2003), "Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncracy or above-belowground time lags", *Oikos,* vol. 103, pp. 45-58.

Hessen, D. O., Jensen, T. C., Kyle, M. and Elser, J. J., (2007), "RNA responses to N- and P-limitation; reciprocal regulation of stoichiometry and growth rate in Brachionus", *Functional Ecology*, vol. 21, pp. 956-962.

Hinsinger, P. (2001), "Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review", *Plant and Soil,* vol. 237, pp. 173-195.

Hiscock, K. M., Lloyd, J. W. and Lerner, D. N. (1991), "Review of natural and artificial denitrification of groundwater", *Water Research,* vol. 9, pp. 1099-1111.

Hodge, A., Berta, G., Doussan, C., Merchan, F. and Crespi, M. (2009), "Plant root growth, architecture and function", *Plant and Soil,* vol. 321, pp. 153-187.

Horan, B. (2009), "Grassland managment and fertilizer use on intensive diary farms", Lalor, S. (ed.), in: *Fertilizer in 2009 - Economics and Agronomy,* Vol. 44, 3rd February, Ireland, The Fertilizer Association of Ireland, Ireland, pp. 2.

Humphreys, J., Tunney, H. and Duggan, P. (1999), Soil analyses and comparison of soil phosphorus tests for the Bellsgrove catchment, Cavan*, ARMIS 4366*, Teagasc, Johnstown Castle Research Centre, Wexford, Ireland.

Illmer, P., Barbato, A. and Schinner, F. (1995b), "Solubilization of hardly-soluble $AlPO_4$ with P-solubilizing microorganisms", *Soil Biology and Biochemistry,* vol. 27, pp. 265-270.

Illmer, P. and Schinner, F. (1995a), "Solubilization of inorganic calcium phosphates-solubilization mechanisms", *Soil Biology and Biochemistry,* vol. 27, pp. 257-263.

Ilmarinen, K. and Mikola, J. (2009), "Soil feedback does not explain mowing effects on vegetation structure in a semi-natural grassland", *Acta Oecologica-International Journal of Ecology,* vol. 35, pp. 838-848.

Ilmarinen, K., Mikola, J., Nissinen, K. and Vestberg, M. (2009), "Role of Soil Organisms in the Maintenance of Species-Rich Seminatural Grasslands through Mowing", *Restoration Ecology,* vol. 17, pp. 78-88.

Ilstedt, U. and Singh, S. (2005), "Nitrogen and phosphorus limitations of microbial respiration in a tropical phosphorus-fixing acrisol (ultisol) compared with organic compost", *Soil Biology and Biochemistry,* vol. 37, pp. 1407-1410.

Ilstedt, U., Singh, S. and Nordgren, A. (2007), "Using perlite as a substrate carrier for measuring microbial available phosphorus by respiration kinetics in soils", *Biology and Fertility of Soils,* vol. 43, pp. 503-510.

Ingham, R. E., Trofymow, J. A., Ingham, E. R. and Coleman, D. C. (1985), "Interactions of bacteria, fungi, and their nematode grazers-effects on nutrient cycling and plant-growth", *Ecological Monographs,* vol. 55, pp. 119-140.

Innes, L., Hobbs, P. J. and Bardgett, R. D. (2004), "The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility", *Biology and Fertility of Soils,* vol. 40, pp. 7-13.

International Standardization Organization. "Soil Quality- Part 3: Chemical method- Section 3.8: Determination of organic and total carbon after dry combustion elementary analysis", *International Standardization Organization*, ISO 10694: 1995.

Irshad, U., Villenave, C., Brauman, A. and Plassard, C. (2011), "Grazing by nematodes on rhizosphere bacteria enhances nitrate and phosphorus availability to *Pinus pinaster* seedlings", *Soil Biology and Biochemistry,* vol. 43, pp. 2121-2126.

Janzen, H. H. (2004), "Carbon cycling in earth systems - a soil science perspective", *Agriculture, Ecosystems and Environment,* vol. 104, pp. 399-417.

Jones, D. L., Nguyen, C. and Finlay, R. D. (2009), "Carbon flow in the rhizosphere: carbon trading at the soil-root interface", *Plant and Soil,* vol. 321, pp. 5-33.

Kaspari, M., Garcia, M. N., Harms, K. E., Santana, M., Wright, S. J. and Yavitt, J. B. (2008), "Multiple nutrients limit litterfall and decomposition in a tropical forest", *Ecology Letters,* vol. 11, pp. 35-43.

Khan, K. S. and Joergensen, R. G. (2009), "Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilisers", *Bioresource Technology,* vol. 100, pp. 303-309.

Khan, K. S., Heinze, S. and Joergensen, R. G. (2009), "Simultaneous measurement of S, macronutrients, and heavy metals in the soil microbial biomass with $CHCl_3$ fumigation and NH_4NO_3 extraction", *Soil Biology and Biochemistry,* vol. 41, pp. 309-314.

Knabner, I. K. (2002), "The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter", *Soil Biology and Biochemistry*, vol. 34, pp 139-162.

Knops, J., Bradley, K. and Wedin, D. (2002), "Mechanisms of plant species impacts on ecosystem nitrogen cycling", *Ecology Letters,* vol. 5, pp. 454-466.

Krämer, S. and Green, D. M. (2000), "Acid and alkaline phosphatase dynamics and their relationship to soil microclimate in a semiarid woodland", *Soil Biology and Biochemistry,* vol. 32, pp. 179-188.

Lavelle, P. and Spain, A. V. (2001), *Soil Ecology,* Kluwer Academic Publishers, The Netherlands.

Le Bayon, R. C. and Binet, F. (2006), "Earthworms change the distribution and availability of phosphorous in organic substrates", *Soil Biology and Biochemistry,* vol. 38, pp. 235-246.

LECO Corporation (2010), LECO TruSpec CN carbon nitrogen determinator instruction manual*, LECO Corporation*, United States of America.

Li, Q., Liang, W., Jiang, Y., Shi, Y., Zhu, J. and Neher, D. A. (2007), "Effect of elevated CO_2 and N fertilisation on soil nematode abundance and diversity in a wheat field", *Applied Soil Ecology,* vol. 36, pp. 63-69.

Liu, E., Yan, C., Mei, X., He, W., Bing, S. H., Ding, L., Liu, Q., Liu, S. and Fan, T. (2010), "Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China", *Geoderma,* vol. 158, pp. 173-180.

Liu, L., Gundersen, P., Zhang, T. and Mo, J. (2012), "Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China", *Soil Biology and Biochemistry,* vol. 44, pp. 31-38.

Lopez-Hernandez, D., Lavelle, P., Fardeau, J. C. and Niño, M. (1993), "Phosphorus transformations in two P-sorption contrasting tropical soils during transit through *Pontoscolex corethrurus* (*Glossoscolecidae*: *Oligochaeta*)", *Soil Biology and Biochemistry,* vol. 25, no. 6, pp. 789-792.

Lung, S. C. and Lim, B. L. (2006), "Assimilation of phytate-phosphorus by the extracellular phytase activity of tobacco (*Nicotiana tabacum*) is affected by the availability of soluble phytate", *Plant and Soil,* vol. 279, pp. 187-199.

Mackay, A. D., Syers, J. K., Springett, J. A. and Gregg, P. E. H. (1982), "Plant availability of phosphorus in superphosphate and a phosphate rock as influenced by earthworms", *Soil Biology and Biochemistry,* vol. 14, pp. 281-287.

MacKay, A. D., Springett, J. A., Syers, J. K. and Gregg, P. E. H. (1983), "Origin of the effect of earthworms on the availability of phosphorus in a phosphate rock", *Soil Biology and Biochemistry,* vol. 15, pp. 63-73.

Mackenzie, F. T., Ver, L. M. and Lerman, A. (2002), "Century-scale nitrogen and phosphorus controls of the carbon cycle", *Chemical Geology,* vol. 190, pp. 13-32.

Maillard, P. and Pinheiro, S. N. A. (2008), "A spatial-statistical approach for modelling the effect of non-point source pollution on different water quality parameters in the Velhas river watershed - Brazil", *Journal of Environmental Management,* vol. 86, pp. 158-170.

McCormack, S. (2002), "Analysis of Agricultural Materials: *Methods used at Johnstown Castle Research Centre*, Johnstown Castle Research Centre, Wexford, Ireland", vol. 2.

McDowell, W. H., Zsolnay, A., Aitkenhead-Peterson, J. A., Gregorich, E. G., Jones, D. L., Jödemann, D., Kalbitz, K., Marschner, B. and Schwesig, D. (2006), "A

comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources", *Soil Biology and Biochemistry,* vol. 38, pp. 1933-1942.

Mead, R., Curnow, R. N. and Hasted, A. M. (2003), *Statistical methods in agriculture and environmental biology,* 3 ed, Chapman and Hall CRC press, United States of America.

Millard, P. and Singh, B. K. (2010), "Does grassland vegetation drive soil microbial diversity?", *Nutrient Cycling in Agroecosystems,* vol. 88, pp. 147-158.

Morton, J. D. and Roberts, A. H. C. (2001), "Pasture response to soil phosphorus levels measured under mowing and dairy grazing", *New Zealand Journal of Agricultural Research,* vol. 44, pp. 259-268.

Mulqueen, J., Rodgers, M. and Scally, P. (2004), "Phosphorus transfer from soil to surface waters", *Agricultural Water Management,* vol. 68, pp. 91-105.

Murphy, J. and Riley, J. P. (1962), "A modified single-solution method for the determination of phosphate in natural waters", *Analytica Chimica Acta.,* vol. 27, pp. 31-36.

Murphy, P. N. C., Bell, A. and Turner, B. L. (2009), "Phosphorus speciation in temperate basaltic grassland soils by solution (31)P NMR spectroscopy", *European Journal of Soil Science,* vol. 60, pp. 638-651.

Murray, P. J., Clegg, C. D., Crotty, F. V., de la, F. M., Williams, J. K. and Blackshaw, R. P. (2009), "Dissipation of bacterially derived C and N through the meso- and macrofauna of a grassland soil", *Soil Biology and Biochemistry,* vol. 41, pp. 1146-1150.

Nordgen, A. (1992), "A method for determining microbially available-N and available-P in an organic soil", *Biology and Fertility of Soils,* vol. 13, pp. 195-199.

Nziguheba, G., Palm, C. A., Buresh, R. J. and Smithson, P. C. (1998), "Soil phosphorus fractions and adsorption as affected by organic and inorganic sources", *Plant and Soil,* vol. 198, no. 2, pp. 159-168.

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H. and Frossard, E. (2001a), "Kinetics of microbial phosphorus uptake in cultivated soils", *Biology and Fertility of Soils,* vol. 34, pp. 31-41.

Oehl, F., Oberson, A., Sinaj, S. and Frossard, E. (2001b), "Organic phosphorus mineralization studies using isotopic dilution techniques", *Soil Science Society of America Journal,* vol. 65, pp. 780-787.

Ollivier, J., Towe, S., Bannert, A., Hai, B., Kastl, E. M., Meyer, A., Su, M. X., Kleineidam, K. and Schloter, M. (2011), "Nitrogen turnover in soil and global change", *FEMS Microbiology Ecology,* vol. 78, pp. 3-16.

Orwin, K. H., Buckland, S. M., Johnson, D., Turner, B. L., Smart, S., Oakley, S. and Bardgett, R. D. (2010), "Linkages of plant traits to soil properties and the functioning of temperate grassland", *Journal of Ecology,* vol. 98, pp. 1074-1083.

Owens, P. N., Deeks, L. K., Wood, G. A., Betson, M. J., Lord, E. I. and Davison, P. S. (2008), "Variations in the depth distribution of phosphorus in soil profiles and implications for model-based catachment-scale predictions of phosphorus delivery to surface waters", *Journal of Hydrology,* vol. 350, pp. 317-328.

Parfitt, R. L., Yeates, G. W., Ross, D. J., Schon, N. L., Mackay, A. D. and Wardle, D. A. (2010), "Effect of fertilizer, herbicide and grazing management of pastures on plant and soil communities", *Applied Soil Ecology,* vol. 45, pp. 175-186.

Paterson, E., Gebbing, T., Abel, C., Sim, A. and Telfer, G. (2007), "Rhizodeposition shapes rhizosphere microbial community structure in organic soil", *New Phytologist,* vol. 173, pp. 600-610.

Paterson, E. and Sim, A. (1999), "Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation", *Plant and Soil,* vol. 216, pp. 155-164.

Paterson, E., Sim, A., Osborne, S. M. and Murray, P. J. (2011), "Long-term exclusion of plant-inputs to soil reduces the functional capacity of microbial communities to mineralise recalcitrant root-derived carbon sources", *Soil Biology and Biochemistry; 19th International Symposium on Environmental Biogeochemistry,* vol. 43, pp. 1873-1880.

Peech, M. and English, L. (1944), "Rapid microbiological soil tests", *Soil Science,* vol. 57, pp. 167-195.

Perez-Moreno, J. and Read, D. J. (2001), "Nutrient transfer from soil nematodes to plants: a direct pathway provided by the mycorrhizal mycelial network", *Plant, Cell and Environment,* vol. 24, pp. 1219-1226.

Perrott, K. W., Sarathchandra, S. U. and Waller, J. E. (1990), "Seasonal storage and release of phosphorus and potassium by organic matter and the microbial biomass in a high producing pastoral soil", *Australian Journal of Soil Research,* vol. 28, pp. 593.

Petersen, S. O. and Klug, M. J. (1994), "Effects of sieving, storage, and incubation-temperature on the phospholipid fatty-acid profile of a soil microbial community", *Applied and Environmental Microbiology,* vol. 60, pp. 2421-2430.

Picone, L. I., Zamuner, E. C., Berardo, A. and Marino, M. A. (2003), "Phosphorus transformations as affected by sampling date, fertilizer rate and phosphorus uptake in a soil under pasture", *Nutrient Cycling in Agroecosystems,* vol. 67, pp. 225-232.

Pietikainen, A., Mikola, J., Vestberg, M. and Setala, H. (2009), "Defoliation effects on *Plantago lanceolata* resource allocation and soil decomposers in relation to AM symbiosis and fertilization", *Soil Biology and Biochemistry,* vol. 41, pp. 2328-2335.

Plunkett, M. (2012), "Using Soil Test Results for Soil Fertility Management", Lalor, S. (ed.), in: *Role of fertilizer and soil fertility in achieving Food Harvest 2020 targets,* Vol. 47, 7th February, Ireland, The Fertilizer Association of Ireland, Ireland, pp. 21.

Powers, J. and Salute, S., (2011), ″Macro- and micronutrient effects on decomposition of leaf litter from two tropical tree species: inferences from a short-term laboratory incubation", *Plant and Soil*, vol. 34, pp. 245-257.

Purakayastha, T. J., Rudrappa, L., Singh, D., Swarup, A. and Bhadraray, S. (2008), "Long-term impact of fertilizers on soil organic carbon pools and sequestration rates in maize–wheat–cowpea cropping system", *Geoderma,* vol. 144, pp. 370-378.

Purves, W. K., Sadava, D., Orians, G. H. and Heller, H. C. (2004), "Earth Systems Science", in *Life: The science of biology,* 7 ed, Sinauer Associates Inc, USA, pp. 1107-1120.

Rees, R. M., Bingham, I. J., Baddeley, J. A.and Watson, C. A. (2005), " The role of plants and land managment in sequestering soil carbon in temperate arable and grassland ecosystems", *Geoderma*, vol. 128, pp. 130-154

Richards, K. E., Fenton, O., Khalil, M. I., Haria, A. H., Humphreys, J., Doody, D., Moles, R., Morgan, G. and Jordan, P. (2009), "Good water status: The integration of sustainable grassland production and water resources in Ireland", *Tearmann: The Journal of Agri-Environmental Research,* vol. 7, pp. 143-160.

Richardson, A. E., Hocking, P. J., Simpson, R. J. and George, T. S. (2009), "Plant mechanisms to optimise access to soil phosphorus", *Crop and Pasture Science,* vol. 60, pp. 124-143.

Richardson, A. E. and Simpson, R. J. (2011), "Soil microorganisms mediating phosphorus availability", *Plant Physiology,* vol. 156, pp. 989-996.

Ritz, K. and Trudgill, D. L. (1999), "Utility of nematode community analysis as an integrated measure of the functional state of soils: perspectives and challenges - Discussion paper", *Plant and Soil,* vol. 212, pp. 1-11.

Ross, D. J., Speir, T. W., Kettles, H. A. and Mackay, A. D. (1995), "Soil microbial biomass, C and N mineralization and enzyme activities in a hill pasture: Influence of season and slow-release P and S fertilizer", *Soil Biology and Biochemistry,* vol. 27, pp. 1431-1443.

Rousseau, J. V. D., Sylvia, D. M. and Fox, A. J. (1994), "Contribution of Ectomycorrhiza to the potential nutrient-absorbing surface of pine", *New Phytologist,* vol. 128, pp. 639-644.

Rowell, D. L. (1994), *Soil Science: Methods and Applications,* Longman Ltd, United Kingdom.

Russell, E. W. (1988), *Russell's Soil Conditions & Plant Growth,* 11 ed, Longman Scientific & Technical, United Kingdom.

Ryden, J. C., McLaughlin, J. R. and Syers, J. K. (1977b), "Mechanisms of phosphate sorption by soils and hydrous ferric oxide gels", *Journal of Soil Science,* vol. 28, pp. 72-92.

Ryden, J. C., Syers, J. K. and McLaughlin, J. R. (1977a), "Effects of ionic strength on chemisorption and potential-determining sorption of phosphate by soils", *Journal of Soil Science,* vol. 28, pp. 62-71.

Sarathchandra, S. U., Ghani, A., Yeates, G. W., Burch, G. and Cox, N. R. (2001), "Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils", *Soil Biology and Biochemistry,* vol. 33, pp. 953-964.

Sarathchandra, S. U., Lee, A., Perrott, K. W., Rajan, S. S. S., Oliver, E. H. A. and Gravett, I. M. (1993), "Effects of phosphate fertilizer applications on microorganisms in pastoral soil", *Australian Journal of Soil Research,* vol. 31, pp. 299-309.

Schaffers, A. P. (2002), "Soil, biomass, and management of semi-natural vegetation", *Plant Ecology,* vol. 158, pp. 247-268.

Scherber, C., Eisenhauer, N., Weisser, W. W., Schmid, B., Voigt, W., Fischer, M., Schulze, E., Roscher, C., Weigelt, A., Allan, E., Beszler, H., Bonkowski, M., Buchmann, N., Buscot, F., Clement, L. W., Ebeling, A., Engels, C., Halle, S., Kertscher, I., Klein, A., Koller, R., Konig, S., Kowalski, E., Kummer, V., Kuu, A., Lange, M., Lauterbach, D., Middelhoff, C., Migunova, V. D., Milcu, A., Muller, R., Partsch, S., Petermann, J. S., Renker, C., Rottstock, T., Sabais, A., Scheu, S., Schumacher, J., Temperton, V. M. and Tscharntke, T. (2010), "Bottom-up effects of plant diversity on multi-trophic interactions in a biodiversity experiment", *Nature,* vol. 468, pp. 553-556.

Schmidt, O. (2001), "Appraisal of the electrical octet method for estimating earthworm populations in arable land", *Annals of Applied Biology,* vol. 138, pp. 231-241.

Schulte, R. P. O. and Herlihy, M. (2007), "Quantifying responses to phosphorus in Irish grasslands: Interactions of soil and fertiliser with yield and P concentration", *European Journal of Agronomy,* vol. 26, pp. 144-153.

Seinhorst, J. W. (1962), "Modifications of the elutriation method for extracting nematodes from soil", *Nematologica,* vol. 8, pp. 117-128.

Sharpley, A., McDowell, R., Moyer, B. and Littlejohn, R. (2011), "Land application of manure can influence earthworm activity and soil phosphorus distribution", *Communications in Soil Science and Plant Analysis,* vol. 42, pp. 194-207.

Sharpley, A. N. and Syers, J. K. (1976), "Potential role of earthworm casts for phosphorus enrichment of run-off waters", *Soil Biology and Biochemistry,* vol. 8, pp. 341-346.

Sharpley, A. N. and Syers, J. K. (1977), "Seasonal-variation in casting activity and in amounts and release to solution of phosphorus forms in earthworm casts", *Soil Biology and Biochemistry,* vol. 9, pp. 227-231.

Sheehan, C., Kirwan, L., Connolly, J. and Bolger, T. (2008), "The effects of earthworm functional diversity on mircobial biomass and the microbial community level physiological profile of soils", *European Journal of Soil Biology,* vol. 44, pp. 65-70.

Simpson, M., McLenaghen, R. D., Chirino-Valle, I. and Condron, L. M. (2012), "Effects of long-term grassland management on the chemical nature and bioavailability of soil phosphorus", *Biology and Fertility of Soils,* vol. 48, pp. 607-611.

Sims, J. T. and Sharpley, A. N. (eds.) (2005), *Phosphorus: Agriculture and the Environment.* Agronomy monograph no. 46, American Society of Agronomy, Inc.; Crop Science Society of America, Inc.; Soil Science Society of America, Inc., United States of America.

Sims, R. W. and Gerard, B. M. (1999), *Earthworms,* Field Studies Council Publications, United Kingdom.

Smith, S. E. and Read, D. J. (2007), *Mycorrhizal Symbiosis,* Academic Press, United Kingdom.

Smith, V. R. (2005), "Moisture, carbon and inorganic nutrient controls of soil respiration at a sub-Antarctic island", *Soil Biology and Biochemistry,* vol. 37, pp. 81-91.

Spehn, E. M., Joshi, J., Schmid, B., Alphei, J. and Korner, C. (2000), "Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems", *Plant and Soil,* vol. 224, pp. 217-230.

Stats Soft Inc., (1984 - 2011), Statistica 9, *Stats Soft Inc*, United States of America.

Stevenson, F. J. and Cole, M. A. (1999), "The Phosphorus Cycle", in *Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients.* 2 ed, John Wiley & Sons Inc, United States of America, pp. 279-329.

Stewart, B. A. (ed.) (1991), *Advances in Soil Science,* Springer-Verlag New York Inc, United States of America.

Stromberger, M. E., Keith, A. M. and Schmidt, O. (2012), "Distinct microbial and faunal communities and translocated carbon in *Lumbricus terrestris* drilospheres", *Soil Biology and Biochemistry,* vol. 46, pp. 155-162.

Teklay, T., Nordgren, A. and Malmer, A. (2006), "Soil respiration characteristics of tropical soils from agricultural and forestry land-uses at Wondo Genet (Ethiopia) in response to C, N and P amendments", *Soil Biology and Biochemistry,* vol. 38, pp. 125-133.

Thomsen, I. K., Schjønning, P., Olesen, J. E. and Christensen, B. T. (2003), "C and N turnover in structurally intact soils of different texture", *Soil Biology and Biochemistry,* vol. 35, pp. 765-774.

Tipping, E., Rowe, E. C., Evans, C. D., Mills, R. T. E., Emmett, B. A., Chaplow, J. S. and Hall, J. R. (2012), "N14C: A plant-soil nitrogen and carbon cycling model to simulate terrestrial ecosystem responses to atmosphere nitrogen deposition", *Ecological Modelling,* vol. 247, pp. 11-26.

Tiunov, A. V., Bonkowski, M., Tiunov, J. A. and Scheu, S. (2001), "Microflora, protozoa and nematoda in *Lumbricus terrestris* burrow walls: a laboratory experiment", *Pedobiologia,* vol. 45, pp. 46-60.

Tiunov, A. V. and Dobrovolskaya, T. G. (2002), "Fungal and bacterial communities in *Lumbricus terrestris* burrow walls: a laboratory experiment", *Pedobiologia,* vol. 46, pp. 595-605.

Tiunov, A. V. and Scheu, S. (1999), "Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lumbricus terrestris* (*Lumbricidae*)", *Soil Biology and Biochemistry,* vol. 31, pp. 2039-2048.

Tunesi, S., Poggi, V. and Gessa, C. (1999), "Phosphate adsorption and precipitation in calcareous soils: the role of calcium ions in solution and carbonate minerals", *Nutrient Cycling in Agroecosystems,* vol. 53, pp. 219-227.

Turner, B. L. (2008), "Resource partitioning for soil phosphorus: a hypothesis", *Journal of Ecology,* vol. 96, pp. 698-702.

Turner, B. L., Cade-Menun, B. J., Condron, L. M. and Newman, S. (2005), "Extraction of soil organic phosphorus", *Talanta,* vol. 66, pp. 294-306.

Turner, B. L. and Haygarth, P. M. (2005), "Phosphatase activity in temperate pasture soils: Potential regulation of labile organic phosphorus turnover by phosphodiesterase activity", *Science of the Total Environment,* vol. 344, pp. 27-36.

Turner, B. L., Bristow, A. W. and Haygarth, P. M. (2001), "Rapid estimation of microbial biomass in grassland soils by ultra-violet absorbance", *Soil Biology and Biochemistry,* vol. 33, pp. 913-919.

Turner, B. L., McKelvie, I. D. and Haygarth, P. M. (2002), "Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis", *Soil Biology and Biochemistry,* vol. 34, pp. 27-35.

Vadas, P. A. (2006), "Distribution of phosphorus in manure slurry and its infiltration after application to soils", *Journal of Environmental Quality,* vol. 35, pp. 542-547.

van Eekeren, N., de Boer, H., Bloem, J., Schouten, T., Rutgers, M., de Goede, R. and Brussaard, L. (2009a), "Soil biological quality of grassland fertilized with adjusted cattle manure slurries in comparison with organic and inorganic fertilizers", *Biology and Fertility of Soils,* vol. 45, pp. 595-608.

van Eekeren, N., van Liere, D., de Vries, F., Rutgers, M., de Goede, R. and Brussaard, L. (2009b), "A mixture of grass and clover combines the positive effects of both plant species on selected soil biota", *Applied Soil Ecology,* vol. 42, pp. 254-263.

van Elsas, D. J., Jansson, J. K. and Trevors, J. T. (2007), *Modern Soil Microbiology,* 2 ed, CRC Press, Taylor and Francis Group, United States of America.

van Ruijven, J. and Berendse, F. (2005), "Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 102, pp. 695-700.

Vance, E. D., Brookes, P. C. and Jenkinson, D. S. (1987), "An extraction method for measuring soil microbial biomass C", *Soil Biology and Biochemistry,* vol. 19, pp. 703-707.

Veresoglou, S. D., Chen, B. and Rillig, M. (2012), "Arbuscular mycorrhiza and soil nitrogen cycling", *Soil Biology and Biochemistry,* vol. 46, pp. 53-62.

Verschoor, B. C., de Goede, R. G. M., de Vries, F. W. and Brussaard, L. (2001), "Changes in the composition of the plant-feeding nematode community in grasslands after cessation of fertiliser application", *Applied Soil Ecology,* vol. 17, pp. 1-17.

Viketoft, M. (2008), "Effects of six grassland plant species on soil nematodes: A glasshouse experiment", *Soil Biology and Biochemistry,* vol. 40, pp. 906-915.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H. and Tilman, D. G. (1997), "Human Alteration of the Global Nitrogen Cycle: Sources and Consequences", *Ecological Applications,* vol. 7, pp. 737-750.

Vladimir, K. and Bucher, M. (2005), "Symbiotic phosphate transport in the arbuscular mycorrhizas", *Trends in Plant Science,* vol. 10, pp. 22-29.

Watwood, M. E., White, C. S. and Dahm, C. N. (1991), "Methodological Modifications for Accurate and Efficient Determination of Contaminant Biodegradation in Unsaturated Calcareous Soils", *Applied and Environmental Microbiology,* vol. 57, pp. 717-720.

Weaver, R. W., Angle, S., Bottomley, P., Bezdicek , D., Smith, S., Tabatabai, A. and Wollum, A. (1994), *Methods of Soil Analysis. Part 2 - Microbiological and Biochemical Properties,* Soil Science Society of America, United States of America.

Whitehead, P. G. and Crossman, J. (2012), "Macronutrient cycles and climate change: Key science areas and an international perspective", *Science of the Total Environment,* vol. 434, pp. 13-17.

Wrage, N., Velthof, G. L., van Beusichem, M. L. and Oenema, O. (2001), "Role of nitrifier denitrification in the production of nitrous oxide", *Soil Biology and Biochemistry,* vol. 33, pp. 1723-1732.

Wu, Z., Satter, L. D., Blohowiak, A. J., Stauffacher, R. H. and Wilson, J. H. (2001), "Milk production, estimated phosphorus excretion, and bone characteristics of dairy cows fed different amounts of phosphorus for two or three years", *Journal of Dairy Science,* vol. 84, pp. 1738-1748.

Yang, J. E., Jones, C. A., Kim, H. J. and Jacobsen, J. S. (2002), "Soil inorganic phosphorus fractions and Olsen-P in phosphorus-responsive calcareous soils: Effect of fertilizer amount and incubation time", *Communications in Soil Science and Plant Analysis,* vol. 33, pp. 855-871.

Yeates, G.W., (1976), "Effect of fertiliser treatment and stocking rate on pasture nematode populations in a yellow-grey earth", *New Zealand Journal of Agricultural Research*, vol. 19, pp. 405-408.

Yeates, G. W. (1999), "Effects of plants on nematode community structure", *Annual review of phytopathology,* vol. 37, pp. 127-149.

Yeates, G. W. (2003), "Nematodes as soil indicators: functional and biodiversity aspects", *Biology and Fertility of Soils,* vol. 37, pp. 199-210.

Yeates, G. W., Bongers, T., De Goede, R. G. M., Freckman, D. W. and Georgieva, G. G. (1993), "Feeding habits in soil nematode families and genera - An outline for soil ecologists", *Journal of Nematology,* vol. 25, pp. 315-331.

Yeates, G. W., Bardgett, R. D., Cook, R., Hobbs, R., Bowling, P. J. and Potter, J. F. (1997), "Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes", *Journal of Applied Ecology,* vol. 34, pp. 453-470.

Yeates, G. W. and Bongers, T. (1999), "Nematode diversity in agroecosystems", *Agriculture, Ecosystems and Environment,* vol. 74, pp. 113-135.

Zelles, L. (1999), "Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review", *Biology and Fertility of Soils,* vol. 29, pp. 111-129.

Zhang, D., Hui, D., Luo, Y. and Zhou, Z. (2008), "Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors", *Journal of Plant Ecology,* vol. 1, pp. 85-93.

Appendix 1

Site 1

P30	P15	P45	P15
P45	P0	P30	P0
P0	P30	P15	P45
P15	P45	P0	P30

Gate

Site 2

P45	P0	P15	P30
P15	P30	P45	P0
P0	P45	P30	P45
P30	P15	P0	P15

Gate

Figure A_1A The randomised layout of P treatments in both Site 1 and 2. P0, P15, P30 and P45 represent P fertilisation regimes 0, 15, 30 and 45 kg P ha^{-1} y^{-1}. The Gate denotes the position of the gate on the fenced area surrounding each site and is stated to aid in orientation.

Table A_1A The soil chemical data available for two sites in the presence of four P treatments when the field trials were initiated in February 1995. Data source from Teagasc Environmental Research Centre, Johnstown Castle, Wexford, Co Wexford, Ireland.

Site	P treatment (kg P ha^{-1} y^{-1})	Morgan's P concentration (µg g^{-1})	Morgan's K concentration (µg g^{-1})	pH
1	0	5.2	114	6.4
1	15	5.2	114	6.4
1	30	5.2	114	6.4
1	45	5.2	114	6.4
2	0	3.6	99	6.2
2	15	3.6	99	6.2
2	30	3.6	99	6.2
2	45	3.6	99	6.2

Table A_1B Total plant dry matter and P yields harvested in the presence of four P treatments across two sites. Data includes accumulated yields from April 1995 to September 2009. Standard errors are reported in parentheses. Data source from Teagasc Environmental Research Centre, Johnstown Castle, Wexford, Co Wexford, Ireland.

		t m^{-2}	kg m^{-2}
Site	P treatment (kg P ha^{-1} y^{-1})	Total dry matter	Total plant P
1	0	1.62 (0.02)	31.2 (2.1)
1	15	1.64 (0.04)	32.9 (2.6)
1	30	1.61 (0.06)	33.2 (3.4)
1	45	1.70 (0.03)	38.5 (2.1)
2	0	1.50 (0.02)	22.1 (1.6)
2	15	1.58 (0.08)	28.8 (3.6)
2	30	1.60 (0.05)	29.7 (3.2)
2	45	1.64 (0.03)	32.4 (2.5)

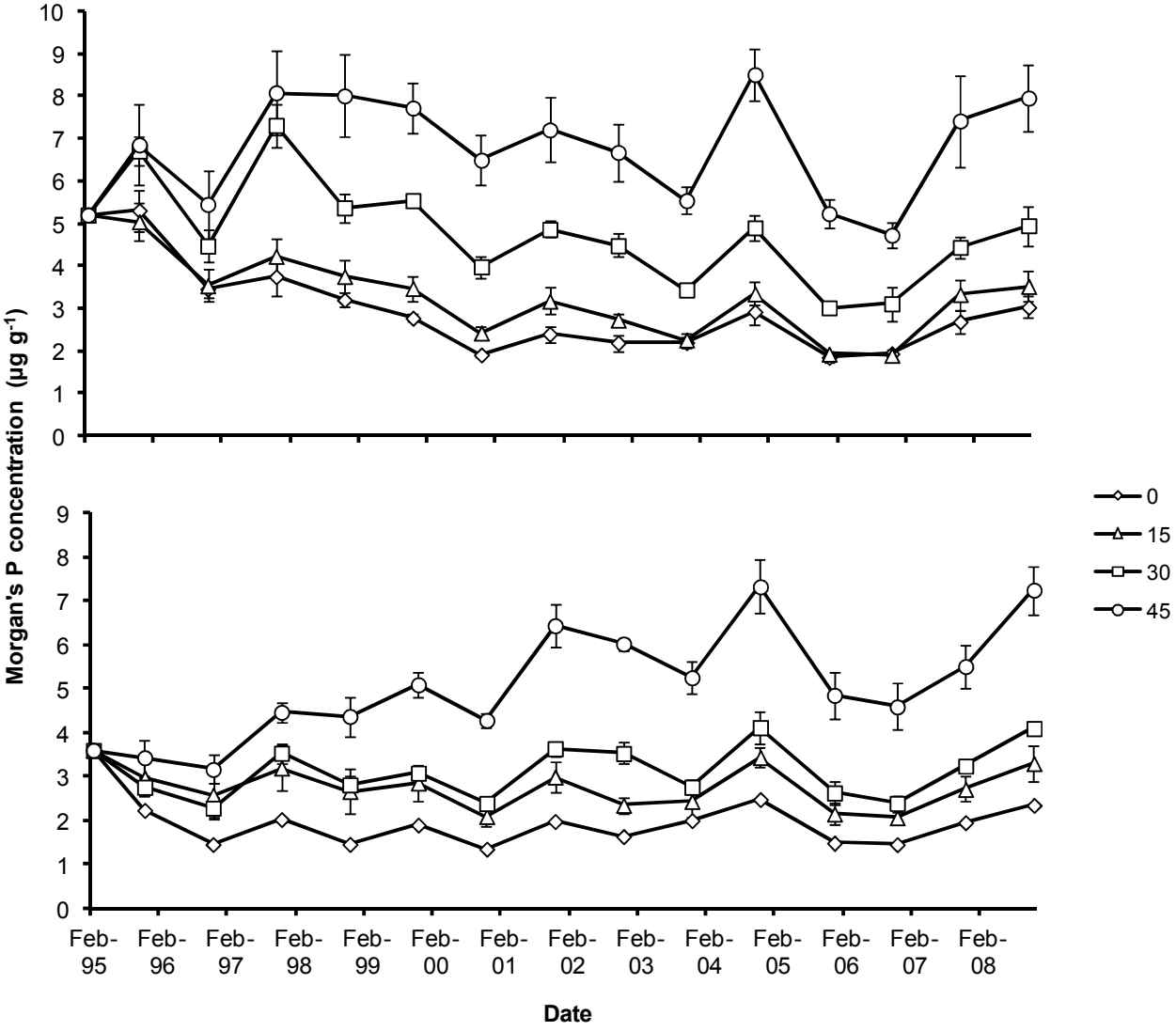

Figure A_1B Changes in Morgan's P concentration from February 1995 to February 2009 in the presence of four P treatments. (a) denotes changes in Site 1 and (b) in Site 2. 0, 15, 30 and 45 represent P treatments 0, 15, 30 and 45 kg P ha-1 y-1. Error bars are standard error. Data source from Teagasc Environmental Research Centre, Johnstown Castle, Wexford, Co Wexford, Ireland.

Table A_1C(a) Raw phospholipid fatty acid (PLFA) analysis profile data for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2009. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 1	PLFA 2	12:0	PLFA 3	PLFA 4	14:0	PLFA 5	PLFA 6	PLFA 7	PLFA 8	PLFA 9	i15:0	a15:0	PLFA 10	15:0	PLFA 11	PLFA 12
1	0	3	0.00	0.00	0.00	0.32	0.00	0.35	0.15	0.15	0.71	0.00	0.25	4.36	5.06	0.00	0.37	0.45	0.11
		5	0.00	0.12	0.08	1.22	0.00	0.88	0.33	0.27	1.14	0.19	0.34	7.29	7.66	0.00	0.52	0.64	0.17
		11	0.00	0.00	0.00	0.26	0.00	0.28	0.12	0.11	0.39	0.11	0.16	3.29	4.10	0.00	0.28	0.28	0.07
		14	0.00	0.10	0.09	0.81	0.00	0.60	0.24	0.24	0.72	0.21	0.28	4.92	6.06	0.00	0.35	0.33	0.08
1	15	4	0.00	0.00	0.00	0.39	0.00	0.37	0.16	0.13	0.53	0.13	0.19	4.21	4.59	0.00	0.33	0.36	0.09
		6	0.00	0.00	0.00	0.38	0.00	0.38	0.14	0.09	0.51	0.10	0.20	3.55	3.84	0.00	0.32	0.30	0.10
		12	0.00	0.00	0.00	0.36	0.00	0.34	0.14	0.11	0.52	0.12	0.18	3.99	4.43	0.00	0.36	0.35	0.10
		13	0.00	0.00	0.00	0.35	0.00	0.34	0.14	0.11	0.51	0.11	0.20	3.70	4.40	0.00	0.33	0.37	0.08
1	30	1	0.00	0.08	0.00	0.84	0.00	0.61	0.26	0.20	0.88	0.15	0.33	5.24	5.44	0.00	0.35	0.37	0.10
		7	0.00	0.81	0.13	1.02	0.00	0.74	0.30	0.18	0.91	0.15	0.33	5.40	5.42	0.00	0.40	0.34	0.12
		10	0.00	0.00	0.00	0.42	0.00	0.37	0.15	0.14	0.56	0.12	0.22	3.93	4.48	0.00	0.33	0.33	0.09
		16	0.00	0.07	0.00	0.56	0.00	0.44	0.19	0.13	0.60	0.13	0.24	4.02	4.81	0.00	0.32	0.37	0.08
1	45	2	0.00	0.11	0.10	0.97	0.00	0.67	0.26	0.19	0.78	0.18	0.31	5.09	5.59	0.00	0.39	0.49	0.11
		8	0.00	0.00	0.00	0.30	0.00	0.32	0.13	0.09	0.45	0.12	0.18	3.66	4.03	0.00	0.31	0.33	0.09
		9	0.00	0.09	0.08	0.91	0.00	0.70	0.29	0.22	0.87	0.19	0.32	5.67	6.35	0.00	0.45	0.48	0.09
		15	0.00	2.98	0.18	1.13	0.00	0.82	0.36	0.33	0.96	0.28	0.37	6.86	7.85	0.00	0.52	0.67	0.12
2	0	19	0.00	0.00	0.00	0.17	0.00	0.29	0.00	0.00	0.43	0.00	0.14	3.31	3.24	0.00	0.32	0.19	0.00
		21	0.00	1.31	0.00	0.66	0.00	0.69	0.26	0.21	0.90	0.21	0.34	5.67	5.66	0.00	0.43	0.33	0.12
		28	0.00	0.20	0.05	0.64	0.00	0.63	0.24	0.18	0.98	0.13	0.35	5.28	5.06	0.00	0.43	0.24	0.12
		30	0.00	0.21	0.16	0.99	0.00	0.81	0.34	0.25	1.17	0.23	0.40	6.19	5.85	0.00	0.47	0.25	0.16
2	15	17	0.00	1.48	0.06	0.63	0.00	0.75	0.23	0.23	0.99	0.21	0.36	5.56	5.15	0.00	0.50	0.31	0.11
		24	0.00	0.14	0.10	0.91	0.00	0.94	0.33	0.32	1.55	0.00	0.47	7.52	7.42	0.00	0.63	0.43	0.15
		25	0.00	0.51	0.09	0.65	0.00	0.69	0.26	0.22	0.93	0.20	0.33	5.55	5.34	0.00	0.44	0.28	0.11
		31	0.00	0.08	0.05	0.76	0.00	0.63	0.26	0.17	1.00	0.18	0.37	5.27	5.04	0.00	0.41	0.22	0.12
2	30	20	0.00	0.00	0.00	0.63	0.00	0.68	0.27	0.22	0.96	0.19	0.32	5.29	4.91	0.00	0.48	0.21	0.11
		22	0.00	0.11	0.07	0.59	0.00	0.68	0.20	0.85	0.18	0.00	0.32	5.03	5.08	0.00	0.50	0.33	0.09
		27	0.00	0.09	0.07	0.64	0.00	0.64	0.20	0.99	0.20	0.00	0.34	5.29	5.20	0.00	0.45	0.27	0.12
		29	0.00	0.00	0.00	0.21	0.00	0.30	0.13	0.15	0.46	0.13	0.18	3.81	3.60	0.00	0.37	0.23	0.00
2	45	18	0.00	0.37	0.06	0.64	0.00	0.66	0.26	0.22	0.89	0.19	0.30	5.26	5.29	0.00	0.46	0.26	0.12
		23	0.00	0.08	0.07	0.63	0.00	0.69	0.24	0.22	0.89	0.22	0.33	5.24	5.56	0.00	0.46	0.31	0.11
		26	0.00	0.00	0.00	0.22	0.00	0.30	0.11	0.55	0.12	0.00	0.21	3.62	3.74	0.00	0.37	0.23	0.10
		32	0.00	0.06	0.00	0.30	0.00	0.36	0.14	0.11	0.57	0.13	0.23	3.71	3.62	0.00	0.37	0.27	0.08

Table A_1C(b) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1C(a) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2009. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 13	3-OH 14:0	PLFA 14	i16:0	PLFA 15	16:1w7c	PLFA 16	PLFA 17	16:0	PLFA 18	PLFA 19	PLFA 20	PLFA 21	PLFA 22	PLFA 23	PLFA 24	i17:0
1	0	3	0.52	0.33	0.00	2.36	2.04	7.57	0.78	5.36	13.62	0.40	0.30	0.40	2.56	4.28	0.70	0.55	1.62
		5	0.61	0.24	0.00	2.64	2.46	9.09	0.84	6.18	12.88	0.34	0.25	0.42	2.58	3.73	0.72	0.49	1.44
		11	0.36	0.17	0.00	1.99	1.55	5.88	0.59	4.11	10.29	0.25	0.23	0.41	1.94	3.35	0.54	0.45	1.38
		14	0.41	0.15	0.00	2.02	1.73	6.40	0.67	4.28	10.52	0.26	0.19	0.40	2.04	3.17	0.51	0.44	1.29
1	15	4	0.46	0.17	0.00	2.19	1.86	7.18	0.67	4.76	11.12	0.34	0.30	0.41	2.30	4.01	0.58	0.47	1.46
		6	0.39	0.15	0.00	1.84	1.82	6.94	0.57	4.84	10.89	0.27	0.21	0.36	2.09	3.51	0.63	0.46	1.35
		12	0.42	0.24	0.00	2.03	1.78	6.67	0.68	4.59	11.74	0.29	0.29	0.35	2.16	3.50	0.62	0.46	1.45
		13	0.39	0.21	0.00	1.90	1.61	6.00	0.66	4.31	11.35	0.27	0.26	0.40	1.98	3.16	0.58	0.45	1.38
1	30	1	0.48	0.17	0.00	2.03	1.89	7.12	0.59	4.53	10.26	0.35	0.25	0.37	2.21	3.65	0.58	0.46	1.33
		7	0.45	0.14	0.00	1.96	1.99	7.66	0.53	4.77	9.99	0.27	0.25	0.42	2.16	3.51	0.65	0.45	1.28
		10	0.42	0.19	0.00	2.00	1.84	6.74	0.57	4.51	10.91	0.27	0.27	0.40	2.11	3.51	0.62	0.48	1.41
		16	0.45	0.21	0.00	2.00	1.81	6.44	0.55	4.62	11.39	0.28	0.35	0.39	2.01	3.39	0.61	0.50	1.43
1	45	2	0.49	0.17	0.00	2.02	1.82	8.86	0.65	4.32	10.41	0.29	0.29	0.38	2.00	3.57	0.59	0.47	1.33
		8	0.42	0.17	0.00	1.95	1.67	6.73	0.59	4.28	10.52	0.31	0.33	0.38	2.11	3.81	0.65	0.49	1.43
		9	0.48	0.18	0.00	2.19	1.86	6.93	0.65	4.54	11.18	0.29	0.28	0.45	2.26	3.49	0.58	0.53	1.46
		15	0.53	0.25	0.00	2.48	2.20	7.55	0.83	4.86	12.08	0.35	0.23	0.41	2.33	3.69	0.61	0.44	1.39
2	0	19	0.34	0.20	0.13	1.80	1.58	7.02	0.50	4.34	12.87	0.20	0.38	0.32	1.95	3.05	0.48	0.43	1.37
		21	0.43	0.14	0.11	1.98	1.86	7.97	0.62	4.75	12.07	0.20	0.24	0.41	2.25	3.28	0.63	0.50	1.41
		28	0.38	0.16	0.14	1.72	1.75	8.62	0.64	4.94	11.16	0.18	0.27	0.39	2.16	2.91	0.55	0.45	1.25
		30	0.43	0.16	0.13	1.90	2.36	9.94	0.64	6.04	12.58	0.22	0.23	0.44	2.33	3.21	0.71	0.49	1.37
2	15	17	0.44	0.20	0.14	2.00	1.80	8.26	0.67	4.72	11.99	0.16	0.23	0.29	2.13	2.96	0.41	0.45	1.26
		24	0.58	0.20	0.13	2.44	2.23	9.67	0.78	5.46	13.98	0.21	0.39	0.46	2.37	3.30	0.57	0.56	1.36
		25	0.40	0.19	0.12	1.81	1.76	8.07	0.63	4.66	11.45	0.19	0.29	0.42	2.14	2.95	0.53	0.48	1.28
		31	0.38	0.15	0.10	1.82	1.92	8.34	0.72	5.19	11.26	0.20	0.19	0.38	2.04	2.64	0.56	0.47	1.27
2	30	20	0.32	0.17	0.13	1.74	1.81	8.01	0.69	4.67	11.98	0.15	0.27	0.33	2.04	2.69	0.47	0.45	1.21
		22	0.42	0.22	0.13	1.92	1.68	7.34	0.58	4.47	12.58	0.17	0.31	0.40	2.01	2.98	0.47	0.50	1.32
		27	0.35	0.17	0.11	1.81	1.80	8.04	0.74	4.71	11.61	0.16	0.26	0.38	2.06	2.87	0.53	0.47	1.27
		29	0.32	0.21	0.17	1.74	1.46	6.66	0.68	4.20	11.64	0.18	0.26	0.31	2.08	2.86	0.42	0.45	1.33
2	45	18	0.38	0.14	0.13	1.79	1.90	7.42	0.65	4.62	11.36	0.15	0.28	0.39	2.11	2.92	0.54	0.44	1.24
		23	0.42	0.20	0.15	1.93	1.77	7.73	0.67	4.65	10.96	0.17	0.31	0.28	2.14	2.95	0.44	0.44	1.23
		26	0.32	0.20	0.18	1.72	1.64	7.40	0.62	4.42	12.08	0.18	0.27	0.32	2.15	3.15	0.58	0.48	1.39
		32	0.38	0.22	0.13	1.77	1.68	7.07	0.51	4.46	12.30	0.20	0.31	0.34	1.94	2.84	0.57	0.53	1.33

Table A_1C(c) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1C(b) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2009. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 25	PLFA 26	17:0c	PLFA 27	17:0	PLFA 28	PLFA 29	2-OH 16:0	PLFA 30	PLFA 31	PLFA 32	PLFA 33	18:2w6c	18:1w9c	18:1w9t	PLFA 34	PLFA 35
1	0	3	1.60	0.37	3.25	0.27	0.42	0.80	0.10	0.00	0.00	0.41	0.55	0.00	0.93	6.67	13.58	0.48	0.76
		5	1.36	0.38	2.99	0.22	0.34	0.64	0.08	0.00	0.00	0.40	0.33	0.00	0.70	5.10	10.43	0.37	0.59
		11	1.48	0.50	2.79	0.29	0.39	0.67	0.08	0.08	0.00	0.60	0.55	0.00	0.94	8.23	15.41	0.63	0.80
		14	1.44	0.49	2.77	0.24	0.35	0.57	0.08	0.08	0.00	0.52	0.54	0.00	0.87	6.88	13.71	0.56	0.72
1	15	4	1.42	0.40	2.91	0.23	0.40	0.75	0.09	0.08	0.00	0.56	0.51	0.00	0.96	7.89	14.21	0.49	0.77
		6	1.29	0.40	2.84	0.20	0.36	0.61	0.09	0.06	0.00	0.48	0.59	0.00	1.56	9.88	14.86	0.54	0.98
		12	1.46	0.48	2.92	0.22	0.38	0.72	0.10	0.00	0.00	0.58	0.60	0.00	1.23	7.42	15.00	0.58	0.77
		13	1.45	0.45	2.66	0.23	0.38	0.65	0.08	0.08	0.00	0.55	0.69	0.00	1.24	7.85	14.61	0.68	0.95
1	30	1	1.28	0.37	2.71	0.20	0.35	0.71	0.09	0.08	0.00	0.47	0.59	0.00	0.95	7.36	13.00	0.51	0.86
		7	1.19	0.38	2.73	0.18	0.34	0.66	0.08	0.06	0.00	0.42	0.51	0.00	1.07	7.55	12.68	0.51	0.87
		10	1.45	0.48	2.82	0.26	0.39	0.67	0.08	0.09	0.00	0.55	0.55	0.00	1.25	9.62	14.41	0.51	1.04
		16	1.48	0.49	2.50	0.23	0.37	0.62	0.08	0.08	0.00	0.44	0.59	0.00	1.28	9.73	12.91	0.53	0.97
1	45	2	1.33	0.55	2.93	0.19	0.35	0.68	0.09	0.08	0.00	0.45	0.70	0.00	1.00	7.19	15.13	0.50	0.76
		8	1.43	0.50	3.15	0.23	0.40	0.80	0.10	0.08	0.00	0.55	0.63	0.00	1.03	7.40	14.96	0.67	0.87
		9	1.48	0.47	2.82	0.26	0.37	0.67	0.08	0.08	0.00	0.52	0.45	0.00	0.81	6.46	12.65	0.54	0.69
		15	1.36	0.32	2.56	0.24	0.31	0.69	0.08	0.00	0.00	0.42	0.31	0.00	0.68	5.39	10.62	0.44	0.57
2	0	19	1.40	0.64	3.03	0.19	0.47	0.56	0.00	0.00	0.00	0.61	0.78	0.00	1.36	8.34	13.33	0.75	0.61
		21	1.43	0.55	3.20	0.20	0.42	0.57	0.07	0.09	0.00	0.53	0.53	0.00	0.96	7.09	11.43	0.63	0.60
		28	1.28	0.55	3.03	0.15	0.37	0.44	0.05	0.07	0.00	0.43	0.61	0.00	1.05	6.74	12.05	0.80	0.58
		30	1.35	0.56	3.23	0.17	0.36	0.43	0.00	0.07	0.00	0.37	0.50	0.00	1.17	7.27	10.71	0.46	0.70
2	15	17	1.29	0.57	2.85	0.20	0.41	0.47	0.00	0.08	0.00	0.53	0.58	0.00	1.14	8.46	12.17	0.67	0.75
		24	1.38	0.57	3.09	0.19	0.37	0.47	0.00	0.00	0.00	0.47	0.40	0.00	0.78	5.97	9.20	0.42	0.50
		25	1.29	0.49	2.89	0.16	0.39	0.44	0.06	0.08	0.00	0.45	0.59	0.00	1.02	6.72	11.86	0.65	0.80
		31	1.27	0.55	2.98	0.16	0.33	0.38	0.06	0.07	0.00	0.43	0.60	0.00	1.08	6.65	12.62	0.68	0.72
2	30	20	1.23	0.56	2.89	0.17	0.39	0.35	0.00	0.08	0.00	0.45	0.57	0.00	1.05	7.67	11.72	0.71	0.68
		22	1.36	0.55	2.87	0.19	0.43	0.48	0.00	0.08	0.00	0.56	0.53	0.00	1.05	8.55	11.86	0.67	0.74
		27	1.27	0.53	3.06	0.18	0.35	0.44	0.06	0.07	0.00	0.49	0.62	0.00	1.11	6.88	12.06	0.63	0.59
		29	1.35	0.46	2.79	0.20	0.42	0.49	0.00	0.08	0.00	0.57	0.62	0.00	1.05	7.57	13.72	0.79	0.89
2	45	18	1.25	0.51	2.86	0.20	0.39	0.48	0.05	0.07	0.00	0.47	0.52	0.00	1.02	7.56	11.73	0.77	0.69
		23	1.30	0.51	2.94	0.19	0.41	0.49	0.05	0.08	0.00	0.50	0.51	0.00	1.02	7.54	12.48	0.74	0.76
		26	1.40	0.56	3.22	0.20	0.44	0.56	0.00	0.09	0.00	0.56	0.14	0.00	0.62	8.07	14.17	0.91	0.81
		32	1.34	0.52	2.63	0.18	0.41	0.51	0.06	0.08	0.00	0.53	0.11	0.00	0.65	9.93	13.29	0.63	0.98

Table A_1C(d) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1C(c) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2009. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	18:0	PLFA 36	PLFA 37	PLFA 38	PLFA 39	PLFA 40	PLFA 41	PLFA 42	PLFA 43	19:0c	19:0	PLFA 44	PLFA 45	PLFA 46	PLFA 47	PLFA 48	PLFA 49
1	0	3	2.25	0.60	1.65	0.23	0.00	0.00	0.15	0.00	0.00	5.19	2.79	0.00	0.20	0.12	0.12	0.19	0.15
		5	1.60	0.43	1.34	0.16	0.00	0.00	0.11	0.00	0.00	3.76	1.70	0.00	0.23	0.17	0.08	0.15	0.12
		11	2.60	0.78	2.39	0.29	0.00	0.15	0.22	0.12	0.00	9.10	3.83	0.21	0.70	0.27	0.40	0.43	0.24
		14	2.39	0.72	2.21	0.25	0.00	0.13	0.20	0.12	0.00	8.01	3.96	0.08	0.57	0.19	0.36	0.39	0.21
1	15	4	2.30	0.65	2.16	0.27	0.00	0.11	0.19	0.10	0.00	6.65	3.00	0.00	0.54	0.16	0.30	0.36	0.25
		6	2.50	0.67	2.43	0.28	0.00	0.14	0.21	0.11	0.00	6.76	3.22	0.16	0.60	0.27	0.18	0.33	0.18
		12	2.60	0.69	2.42	0.28	0.00	0.13	0.20	0.11	0.00	7.01	3.39	0.14	0.46	0.14	0.22	0.32	0.17
		13	2.59	0.67	2.60	0.30	0.00	0.14	0.20	0.11	0.00	7.93	3.90	0.09	0.60	0.26	0.31	0.39	0.24
1	30	1	2.18	0.62	2.17	0.24	0.00	0.10	0.22	0.14	0.00	6.61	3.42	0.07	0.52	0.20	0.32	0.39	0.25
		7	1.97	0.60	2.05	0.24	0.00	0.12	0.21	0.13	0.00	6.41	2.50	0.22	0.55	0.25	0.26	0.44	0.24
		10	2.44	0.64	2.16	0.27	0.00	0.11	0.19	0.10	0.00	6.87	3.01	0.17	0.49	0.17	0.24	0.34	0.17
		16	2.24	0.59	2.23	0.25	0.00	0.14	0.18	0.08	0.00	6.70	3.06	0.12	0.59	0.26	0.38	0.38	0.25
1	45	2	2.16	0.61	0.10	0.26	0.00	0.11	0.20	0.11	0.00	5.99	3.09	0.04	0.40	0.13	0.26	0.34	0.20
		8	2.58	0.71	2.44	0.29	0.00	0.10	0.24	0.13	0.00	7.56	3.80	0.23	0.54	0.17	0.31	0.44	0.27
		9	2.02	0.60	1.88	0.19	0.00	0.12	0.17	0.08	0.00	6.69	2.65	0.25	0.51	0.14	0.25	0.33	0.21
		15	1.76	0.49	1.62	0.21	0.00	0.09	0.12	0.00	0.00	4.55	2.12	0.00	0.31	0.11	0.16	0.18	0.10
2	0	19	3.25	0.75	2.25	0.32	0.00	0.13	0.24	0.13	0.00	6.84	5.73	0.18	0.52	0.23	0.25	0.35	0.17
		21	2.28	0.61	1.78	0.30	0.00	0.11	0.17	0.10	0.00	5.90	2.83	0.08	0.35	0.09	0.20	0.26	0.13
		28	2.23	0.66	1.94	0.20	0.00	0.14	0.20	0.14	0.00	6.52	3.71	0.14	0.60	0.27	0.28	0.47	0.20
		30	1.88	0.49	1.41	0.13	0.00	0.08	0.13	0.07	0.00	4.25	2.13	0.00	0.35	0.17	0.10	0.22	0.07
2	15	17	2.35	0.68	1.77	0.19	0.00	0.13	0.16	0.09	0.00	5.61	2.37	0.08	0.37	0.15	0.15	0.21	0.10
		24	1.66	0.46	1.22	0.14	0.00	0.07	0.11	0.07	0.00	3.97	1.73	0.00	0.26	0.10	0.11	0.15	0.10
		25	2.38	0.64	1.98	0.20	0.00	0.26	0.18	0.12	0.00	6.70	3.77	0.12	0.46	0.15	0.23	0.34	0.17
		31	2.37	0.68	2.13	0.19	0.00	0.00	0.20	0.13	0.00	6.49	4.14	0.10	0.49	0.17	0.26	0.46	0.19
2	30	20	2.41	0.69	1.92	0.19	0.00	0.13	0.19	0.13	0.00	7.12	3.92	0.13	0.57	0.20	0.28	0.43	0.18
		22	2.47	0.65	1.91	0.22	0.00	0.14	0.19	0.12	0.00	6.74	3.35	0.12	0.42	0.14	0.25	0.29	0.16
		27	2.33	0.65	2.03	0.20	0.00	0.14	0.18	0.10	0.00	6.76	4.01	0.10	0.57	0.20	0.25	0.43	0.20
		29	2.69	0.74	2.50	0.25	0.00	0.16	0.22	0.16	0.00	8.75	4.68	0.23	0.64	0.25	0.39	0.53	0.29
2	45	18	2.28	0.64	2.18	0.09	0.00	0.14	0.18	0.14	0.00	7.76	3.35	0.13	0.58	0.24	0.26	0.35	0.16
		23	2.27	0.68	2.04	0.08	0.00	0.13	0.18	0.10	0.00	6.97	3.50	0.09	0.55	0.19	0.29	0.34	0.15
		26	2.76	0.74	2.31	0.08	0.00	0.16	0.23	0.15	0.00	8.04	3.98	0.11	0.52	0.15	0.30	0.43	0.21
		32	2.73	0.70	2.27	0.14	0.00	0.17	0.25	0.15	0.00	7.44	4.29	0.13	0.62	0.29	0.27	0.44	0.19

Table A_1C(e) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1C(d) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2009. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area				
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	20:0	PLFA 50	PLFA 51	PLFA 52	Total Area (%)
1	0	3	0.16	0.00	0.15	0.21	100
		5	0.12	0.00	0.15	0.21	100
		11	0.36	0.57	0.37	0.57	100
		14	0.28	0.00	0.25	0.37	100
1	15	4	0.34	0.41	0.30	0.46	100
		6	0.19	0.16	0.27	0.36	100
		12	0.23	0.22	0.24	0.32	100
		13	0.33	0.40	0.31	0.49	100
1	30	1	0.35	0.49	0.44	0.60	100
		7	0.38	0.37	0.42	0.69	100
		10	0.27	0.27	0.21	0.29	100
		16	0.34	0.42	0.49	0.63	100
1	45	2	0.24	0.31	0.26	0.35	100
		8	0.38	0.44	0.29	0.41	100
		9	0.24	0.41	0.34	0.53	100
		15	0.12	0.17	0.10	0.14	100
2	0	19	0.41	0.48	0.23	0.41	100
		21	0.21	0.23	0.18	0.26	100
		28	0.40	0.62	0.30	0.59	100
		30	0.13	0.15	0.09	0.15	100
2	15	17	0.20	0.21	0.11	0.21	100
		24	0.13	0.12	0.08	0.10	100
		25	0.34	0.52	0.25	0.35	100
		31	0.30	0.44	0.23	0.29	100
2	30	20	0.33	0.60	0.23	0.44	100
		22	0.33	0.46	0.19	0.40	100
		27	0.32	0.59	0.28	0.50	100
		29	0.41	0.78	0.32	0.47	100
2	45	18	0.36	0.56	0.21	0.41	100
		23	0.31	0.63	0.20	0.26	100
		26	0.32	0.47	0.31	0.31	100
		32	0.40	0.45	0.25	0.43	100

Table A_1D(a) Raw phospholipid fatty acid (PLFA) analysis profile data for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 1	PLFA 2	12:0	PLFA 3	PLFA 4	14:0	PLFA 5	PLFA 6	PLFA 7	PLFA 8	PLFA 9	i15:0	a15:0	PLFA 10	15:0	PLFA 11	PLFA 12
1	0	3	0.00	0.00	0.00	0.63	0.00	0.46	0.17	0.11	0.68	0.10	0.25	3.73	4.54	0.00	0.35	0.25	0.00
		5	0.00	0.00	0.00	0.76	0.00	0.57	0.26	0.19	0.80	0.18	0.31	5.26	6.03	0.00	0.40	0.38	0.00
		11	0.00	0.00	0.00	0.65	0.00	0.49	0.19	0.14	0.74	0.13	0.26	4.28	5.60	0.00	0.37	0.29	0.00
		14	0.00	0.00	0.00	0.77	0.00	0.54	0.21	0.14	0.77	0.13	0.26	4.06	5.33	0.00	0.38	0.28	0.00
1	15	4	0.00	0.00	0.00	0.75	0.00	0.55	0.23	0.16	0.85	0.15	0.31	4.90	5.83	0.00	0.41	0.39	0.00
		6	0.00	0.00	0.00	1.06	0.00	0.77	0.27	0.14	1.04	0.17	0.42	5.45	6.14	0.00	0.47	0.35	0.00
		12	0.00	0.00	0.00	0.82	0.00	0.59	0.26	0.15	1.02	0.17	0.36	5.35	6.15	0.00	0.45	0.40	0.00
		13	0.00	0.00	0.00	0.66	0.00	0.46	0.17	0.10	0.68	0.10	0.29	3.68	4.58	0.00	0.35	0.24	0.00
1	30	1	0.00	0.00	0.00	0.53	0.00	0.42	0.19	0.12	0.62	0.12	0.25	4.00	4.43	0.00	0.33	0.23	0.00
		7	0.00	0.00	0.00	1.06	0.00	0.77	0.32	0.21	1.03	0.21	0.40	6.00	6.82	0.00	0.49	0.37	0.00
		10	0.00	0.00	0.00	0.58	0.00	0.46	0.18	0.14	0.69	0.11	0.25	3.91	4.71	0.00	0.35	0.26	0.00
		16	0.00	0.00	0.00	1.03	0.00	0.67	0.29	0.17	0.95	0.19	0.37	5.30	6.66	0.00	0.48	0.47	0.00
1	45	2	0.00	0.00	0.00	0.76	0.00	0.55	0.21	0.12	0.70	0.13	0.28	4.10	4.72	0.00	0.37	0.29	0.00
		8	0.00	0.00	0.00	0.96	0.00	0.71	0.28	0.21	0.90	0.20	0.36	5.47	6.33	0.00	0.49	0.42	0.00
		9	0.00	0.00	0.00	0.52	0.00	0.40	0.18	0.14	0.64	0.13	0.24	3.96	4.75	0.00	0.33	0.34	0.00
		15	0.00	0.00	0.00	0.50	0.00	0.41	0.17	0.12	0.62	0.11	0.24	3.71	4.43	0.00	0.36	0.37	0.00
2	0	19	0.00	0.00	0.00	0.80	0.00	0.89	0.29	0.28	1.25	0.25	0.43	6.94	6.90	0.00	0.72	0.41	0.00
		21	0.00	0.00	0.00	0.56	0.00	0.57	0.19	0.15	0.85	0.14	0.30	4.49	4.53	0.00	0.47	0.24	0.00
		28	0.00	0.00	0.00	0.32	0.00	0.37	0.00	0.00	0.79	0.00	0.28	4.13	4.10	0.00	0.39	0.18	0.00
		30	0.00	0.00	0.00	0.44	0.00	0.45	0.18	0.11	0.83	0.12	0.28	4.26	4.25	0.00	0.42	0.21	0.00
2	15	17	0.00	0.00	0.00	0.41	0.00	0.57	0.17	0.19	0.78	0.17	0.28	4.57	4.31	0.00	0.49	0.24	0.00
		24	0.00	0.00	0.00	0.62	0.00	0.65	0.25	0.17	1.03	0.16	0.36	0.00	5.40	0.00	0.54	0.25	0.00
		25	0.00	0.00	0.00	0.24	0.00	0.31	0.13	0.00	0.55	0.00	0.20	3.33	3.37	0.00	0.36	0.20	0.00
		31	0.00	0.00	0.00	0.55	0.00	0.54	0.22	0.15	0.85	0.13	0.32	4.62	4.63	0.00	0.41	0.19	0.00
2	30	20	0.00	0.00	0.00	0.53	0.00	0.58	0.18	0.14	0.84	0.16	0.29	4.17	4.20	0.00	0.46	0.15	0.00
		22	0.00	0.00	0.00	0.43	0.00	0.45	0.18	0.14	0.80	0.12	0.27	4.19	4.37	0.00	0.39	0.22	0.00
		27	0.00	0.00	0.00	0.86	0.00	0.83	0.30	0.22	1.37	0.20	0.47	6.46	6.63	0.00	0.62	0.24	0.00
		29	0.00	0.00	0.00	0.30	0.00	0.36	0.13	0.11	0.61	0.12	0.23	3.54	3.71	0.00	0.38	0.18	0.00
2	45	18	0.00	0.00	0.00	0.91	0.00	0.92	0.29	0.26	1.28	0.25	0.45	6.79	6.96	0.00	0.68	0.29	0.00
		23	0.00	0.00	0.00	0.45	0.00	0.56	0.18	0.19	0.81	0.14	0.28	4.53	5.11	0.00	0.48	0.25	0.00
		26	0.00	0.00	0.00	0.49	0.00	0.52	0.18	0.15	0.87	0.13	0.30	4.30	4.48	0.00	0.42	0.21	0.00
		32	0.00	0.00	0.00	0.60	0.00	0.58	0.25	0.23	0.86	0.19	0.32	5.19	5.11	0.00	0.46	0.31	0.00

Table A_1D(b) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1D(a) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 13	3-OH 14:0	PLFA 14	i16:0	PLFA 15	16:1w7c	PLFA 16	PLFA 17	16:0	PLFA 18	PLFA 19	PLFA 20	PLFA 21	PLFA 22	PLFA 23	PLFA 24	i17:0
1	0	3	0.00	0.40	0.27	1.82	1.78	7.22	0.50	4.68	10.79	0.23	0.17	0.44	1.73	3.10	0.65	0.45	1.28
		5	0.00	0.57	0.30	2.62	2.24	9.09	0.61	5.67	11.17	0.31	0.20	0.45	2.49	3.95	0.82	0.55	1.38
		11	0.00	0.46	0.26	2.22	2.13	9.16	0.63	5.39	10.61	0.25	0.21	0.39	2.16	3.57	0.67	0.51	1.39
		14	0.00	0.38	0.19	1.81	1.96	7.54	0.53	4.84	10.58	0.21	0.27	0.34	1.84	2.96	0.63	0.44	1.27
1	15	4	0.00	0.52	0.28	2.38	2.47	9.80	0.71	5.94	11.28	0.31	0.22	0.49	2.28	3.85	0.75	0.49	1.35
		6	0.00	0.51	0.28	2.29	2.44	9.72	0.69	5.76	12.58	0.27	0.33	0.42	2.01	3.30	0.62	0.43	1.19
		12	0.17	0.54	0.00	2.34	2.62	10.19	0.71	6.19	11.07	0.29	0.22	0.46	2.21	3.65	0.75	0.49	1.32
		13	0.12	0.39	0.00	1.74	1.89	7.39	0.53	4.92	11.22	0.25	0.28	0.30	1.88	3.07	0.61	0.42	1.22
1	30	1	0.09	0.44	0.00	1.88	1.99	7.60	0.45	4.81	10.75	0.31	0.20	0.41	2.10	3.81	0.70	0.47	1.37
		7	0.17	0.54	0.28	2.39	2.62	10.07	0.24	5.77	12.64	0.29	0.38	0.42	2.24	3.63	0.74	0.47	1.33
		10	0.11	0.38	0.23	1.81	1.93	7.58	0.54	4.75	10.54	0.22	0.30	0.37	1.96	3.11	0.62	0.44	1.29
		16	0.14	0.50	0.27	2.25	2.37	9.03	0.67	5.45	12.25	0.24	0.31	0.46	2.00	3.30	0.71	0.46	1.30
1	45	2	0.11	0.42	0.22	1.91	2.02	8.55	0.13	4.91	10.42	0.27	0.16	0.39	1.92	3.53	0.68	0.44	1.29
		8	0.13	0.53	0.27	2.38	2.40	9.28	0.63	5.29	11.78	0.28	0.19	0.39	2.27	3.69	0.70	0.50	1.39
		9	0.08	0.41	0.24	1.91	1.87	7.46	0.49	4.58	9.06	0.26	0.15	0.40	2.17	3.47	0.69	0.49	1.36
		15	0.09	0.42	0.24	1.99	1.70	7.12	0.54	4.56	11.82	0.24	0.21	0.41	2.00	3.33	0.65	0.48	1.41
2	0	19	0.15	0.55	0.36	2.43	1.93	10.52	0.45	5.72	14.93	0.00	0.27	0.33	2.38	3.17	0.64	0.49	1.31
		21	0.13	0.33	1.01	1.65	1.61	8.69	0.40	4.92	12.21	0.16	0.26	0.36	2.08	2.87	0.63	0.45	1.22
		28	0.00	0.38	0.39	1.83	2.17	11.20	0.67	5.82	11.37	0.19	0.29	0.42	2.51	3.36	0.76	0.53	1.39
		30	0.14	0.41	0.27	1.80	2.24	9.69	0.61	5.86	13.27	0.21	0.26	0.38	2.16	3.15	0.74	0.48	1.35
2	15	17	0.10	0.40	0.35	1.90	1.77	8.95	0.58	4.66	13.22	0.14	0.27	0.31	2.13	2.97	0.46	0.51	1.26
		24	0.15	0.44	0.33	1.98	2.33	10.60	0.61	5.75	14.73	0.16	0.32	0.44	2.28	3.11	0.61	0.50	1.29
		25	0.00	0.33	0.29	1.51	1.70	7.65	0.48	4.59	12.17	0.17	0.32	0.38	1.95	2.87	0.59	0.45	1.24
		31	0.13	0.31	1.68	1.70	2.04	9.08	0.58	5.04	12.35	0.19	0.30	0.39	2.25	2.87	0.61	0.46	1.24
2	30	20	0.10	0.25	1.36	1.55	1.92	8.42	0.57	4.94	12.07	0.12	0.27	0.32	1.92	2.54	0.54	0.43	1.12
		22	0.13	0.37	0.25	1.75	2.08	9.10	0.54	5.48	10.24	0.16	0.26	0.40	2.17	3.03	0.65	0.48	1.29
		27	0.20	0.48	0.33	2.09	2.67	11.95	0.82	6.51	15.21	0.16	0.26	0.30	2.31	2.97	0.66	0.45	1.23
		29	0.00	0.36	0.27	1.66	1.75	8.25	0.60	4.80	11.40	0.16	0.21	0.37	1.98	2.84	0.57	0.44	1.24
2	45	18	0.18	0.50	0.30	2.27	2.52	11.00	0.70	6.38	14.95	0.15	0.30	0.43	2.11	2.89	0.65	0.47	1.26
		23	0.13	0.40	0.33	1.90	2.09	9.24	0.61	5.09	12.89	0.16	0.34	0.35	2.19	3.10	0.54	0.51	1.31
		26	0.12	0.35	0.27	1.69	1.88	9.06	0.55	4.79	11.91	0.16	0.32	0.42	1.96	2.82	0.58	0.43	1.21
		32	0.13	0.45	0.32	2.02	2.19	9.23	0.63	5.52	13.55	0.23	0.43	0.42	2.33	3.19	0.69	0.52	1.37

Table A_1D(c) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1D(b) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 25	PLFA 26	17:0c	PLFA 27	17:0	PLFA 28	PLFA 29	2-OH 16:0	PLFA 30	PLFA 31	PLFA 32	PLFA 33	18:2w6c	18:1w9c	18:1w9t	PLFA 34	PLFA 35
1	0	3	1.34	0.52	2.53	0.21	0.35	0.00	0.59	0.00	0.00	0.40	0.44	0.00	1.93	9.71	14.49	0.52	0.83
		5	1.49	0.50	3.01	0.24	0.31	0.00	0.63	0.00	0.00	0.40	0.34	0.00	1.07	8.22	12.12	0.15	0.75
		11	1.52	0.52	2.82	0.25	0.36	0.00	0.54	0.00	0.00	0.48	0.48	0.00	1.36	9.43	13.62	0.39	0.89
		14	1.38	0.47	2.57	0.23	0.37	0.00	0.47	0.00	0.00	0.40	0.47	0.00	1.33	9.49	14.02	0.42	0.84
1	15	4	1.40	0.45	2.78	0.19	0.34	0.00	0.54	0.00	0.00	0.44	0.46	0.00	1.32	8.75	12.80	0.34	0.90
		6	1.26	0.50	2.54	0.16	0.34	0.00	0.49	0.00	0.00	0.38	0.13	0.00	1.68	8.52	11.86	0.39	0.81
		12	1.38	0.51	2.69	0.19	0.32	0.00	0.49	0.00	0.00	0.41	0.50	0.00	1.53	7.85	12.29	0.28	0.81
		13	1.32	0.49	2.34	0.18	0.36	0.00	0.52	0.00	0.00	0.40	0.59	0.00	2.04	9.91	14.14	0.60	0.96
1	30	1	1.35	0.53	2.74	0.19	0.37	0.00	0.68	0.00	0.00	0.45	0.54	0.00	1.82	9.92	14.19	0.43	0.86
		7	1.32	0.50	2.70	0.18	0.36	0.00	0.56	0.00	0.00	0.39	0.42	0.00	1.40	7.68	10.91	0.37	0.76
		10	1.35	0.49	2.59	0.22	0.37	0.00	0.53	0.00	0.00	0.45	0.53	0.00	1.49	9.79	14.57	0.55	1.01
		16	1.37	0.47	2.51	0.18	0.31	0.00	0.49	0.00	0.00	0.38	0.46	0.00	1.40	8.36	11.78	0.37	0.70
1	45	2	1.28	0.55	2.75	0.17	0.32	0.00	0.58	0.00	0.00	0.41	0.49	0.00	1.52	9.54	13.82	0.48	0.82
		8	1.40	0.53	2.79	0.20	0.35	0.00	0.60	0.00	0.00	0.44	0.44	0.00	1.26	8.02	12.06	0.37	0.70
		9	1.43	0.53	2.87	0.25	0.36	0.00	0.61	0.00	0.00	0.49	0.51	0.00	1.44	8.90	14.19	0.36	0.84
		15	1.44	0.51	2.47	0.24	0.36	0.00	0.67	0.00	0.00	0.51	0.52	0.00	1.85	10.30	14.52	0.51	0.86
2	0	19	1.31	0.65	2.75	0.17	0.42	0.00	0.42	0.00	0.00	0.43	0.40	0.00	1.22	7.65	9.39	0.38	0.52
		21	1.28	0.56	2.89	0.15	0.40	0.00	0.45	0.00	0.00	0.42	0.63	0.00	1.68	9.31	11.85	0.57	0.64
		28	1.46	0.76	3.56	0.17	0.42	0.00	0.41	0.00	0.00	0.45	0.66	0.00	1.54	9.21	13.65	0.50	0.79
		30	1.33	0.59	2.91	0.16	0.41	0.00	0.44	0.00	0.00	0.42	0.61	0.00	2.17	8.69	12.76	0.50	0.74
2	15	17	1.30	0.63	2.68	0.17	0.43	0.00	0.40	0.00	0.00	0.54	0.60	0.00	1.80	10.62	12.54	0.54	0.67
		24	1.34	0.65	3.04	0.16	0.41	0.00	0.38	0.00	0.00	0.32	0.53	0.00	1.91	9.31	11.39	0.46	0.62
		25	1.25	0.54	2.67	0.16	0.40	0.00	0.40	0.00	0.00	0.44	0.65	0.00	2.03	10.01	13.24	0.59	0.80
		31	1.28	0.53	2.66	0.15	0.34	0.00	0.35	0.00	0.00	0.40	0.95	0.00	1.76	8.17	11.87	0.47	0.73
2	30	20	1.17	0.62	2.65	0.16	0.43	0.00	0.31	0.00	0.00	0.41	0.57	0.00	1.68	10.29	12.22	0.63	0.77
		22	1.36	0.69	3.09	0.17	0.41	0.00	0.41	0.00	0.00	0.49	0.57	0.00	1.53	10.54	13.04	0.47	0.80
		27	1.23	0.57	2.82	0.14	0.36	0.00	0.33	0.00	0.00	0.34	0.42	0.00	1.58	6.99	9.15	0.36	0.50
		29	1.26	0.55	2.75	0.19	0.40	0.00	0.40	0.00	0.00	0.52	0.64	0.00	1.71	9.16	14.08	0.65	0.72
2	45	18	1.25	0.56	2.67	0.17	0.39	0.00	0.34	0.00	0.00	0.37	0.36	0.00	1.56	7.19	9.04	0.41	0.51
		23	1.41	0.61	2.88	0.19	0.44	0.00	0.41	0.00	0.00	0.45	0.51	0.00	1.80	9.67	12.01	0.53	0.67
		26	1.25	0.55	2.80	0.16	0.40	0.00	0.41	0.00	0.00	0.43	0.58	0.00	1.82	9.38	13.06	0.56	0.77
		32	1.36	0.51	2.76	0.18	0.38	0.00	0.44	0.00	0.00	0.44	0.47	0.00	1.44	7.90	11.24	0.35	0.68

Table A_1D(d) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1D(c) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	18:0	PLFA 36	PLFA 37	PLFA 38	PLFA 39	PLFA 40	PLFA 41	PLFA 42	PLFA 43	19:0c	19:0	PLFA 44	PLFA 45	PLFA 46	PLFA 47	PLFA 48	PLFA 49
1	0	3	2.33	0.62	1.99	0.23	0.00	0.00	0.00	0.19	0.14	6.85	2.93	0.00	0.96	0.53	0.41	0.47	0.28
		5	1.81	0.54	1.89	0.26	0.00	0.00	0.00	0.14	0.08	4.74	1.90	0.00	0.41	0.18	0.16	0.21	0.13
		11	1.98	0.59	1.87	0.24	0.00	0.00	0.00	0.16	0.10	5.33	2.09	0.00	0.48	0.23	0.23	0.25	0.13
		14	2.11	0.66	2.08	0.24	0.00	0.00	0.00	0.16	0.10	7.14	2.46	0.00	0.89	0.58	0.42	0.47	0.26
1	15	4	1.70	0.50	1.56	0.19	0.00	0.00	0.00	0.13	0.00	4.17	1.76	0.00	0.36	0.22	0.16	0.20	0.13
		6	1.70	0.46	1.70	0.17	0.00	0.00	0.00	0.13	0.00	4.14	1.60	0.00	0.49	0.25	0.18	0.23	0.13
		12	1.74	0.47	1.52	0.16	0.00	0.00	0.00	0.13	0.00	4.11	1.97	0.00	0.40	0.25	0.13	0.23	0.13
		13	2.30	0.59	2.02	0.20	0.00	0.00	0.00	0.19	0.11	6.84	2.95	0.00	0.79	0.40	0.33	0.43	0.20
1	30	1	2.22	0.59	2.14	0.29	0.00	0.00	0.00	0.20	0.11	6.45	2.50	0.00	0.65	0.30	0.29	0.38	0.20
		7	1.55	0.41	1.49	0.16	0.00	0.00	0.00	0.11	0.00	3.77	1.39	0.00	0.38	0.21	0.16	0.20	0.13
		10	2.26	0.62	2.16	0.28	0.00	0.00	0.00	0.20	0.13	6.65	2.73	0.00	0.68	0.34	0.32	0.39	0.24
		16	1.74	0.45	1.71	0.20	0.00	0.00	0.00	0.13	0.00	4.54	1.81	0.00	0.50	0.25	0.23	0.26	0.17
1	45	2	2.09	0.58	2.05	0.25	0.00	0.00	0.00	0.22	0.14	6.15	2.55	0.00	0.79	0.39	0.30	0.41	0.20
		8	1.73	0.50	1.60	0.20	0.00	0.00	0.00	0.15	0.10	4.61	1.59	0.00	0.41	0.23	0.17	0.22	0.11
		9	2.15	0.62	2.25	0.28	0.00	0.00	0.00	0.19	0.11	7.44	3.13	0.00	0.85	0.45	0.38	0.44	0.23
		15	2.20	0.61	2.20	0.28	0.00	0.00	0.00	0.18	0.12	6.35	2.36	0.00	0.60	0.31	0.27	0.31	0.19
2	0	19	1.65	0.44	1.11	0.13	0.00	0.00	0.00	0.00	0.00	3.49	1.43	0.00	0.31	0.17	0.12	0.16	0.00
		21	2.43	0.57	1.76	0.23	0.00	0.00	0.00	0.22	0.12	5.75	3.50	0.00	0.69	0.36	0.23	0.38	0.17
		28	2.21	0.61	1.70	0.17	0.00	0.00	0.00	0.00	0.00	4.40	2.60	0.00	0.39	0.27	0.23	0.00	0.00
		30	2.21	0.57	1.65	0.17	0.00	0.00	0.00	0.14	0.00	4.46	2.26	0.00	0.57	0.36	0.20	0.30	0.15
2	15	17	2.35	0.64	1.66	0.17	0.00	0.00	0.00	0.15	0.09	5.11	2.74	0.00	0.48	0.20	0.17	0.26	0.14
		24	2.07	0.53	1.45	0.20	0.00	0.00	0.00	0.14	0.10	5.07	2.43	0.00	0.55	0.26	0.23	0.30	0.16
		25	2.65	0.66	2.03	0.21	0.00	0.00	0.00	0.19	0.00	7.18	3.93	0.00	0.91	0.39	0.35	0.56	0.26
		31	2.26	0.58	1.76	0.21	0.00	0.00	0.00	0.24	0.11	5.31	3.27	0.00	0.58	0.29	0.31	0.34	0.17
2	30	20	2.24	0.62	1.93	0.23	0.00	0.00	0.00	0.21	0.14	6.36	2.78	0.00	0.74	0.39	0.30	0.44	0.22
		22	2.31	0.65	1.88	0.18	0.00	0.00	0.00	0.17	0.11	5.85	2.92	0.00	0.60	0.30	0.19	0.27	0.12
		27	1.56	0.39	1.14	0.11	0.00	0.00	0.00	0.00	0.00	2.98	1.49	0.00	0.28	0.16	0.15	0.00	0.00
		29	2.44	0.71	2.22	0.21	0.00	0.00	0.00	0.21	0.15	7.33	3.25	0.00	0.85	0.40	0.36	0.48	0.26
2	45	18	1.54	0.43	1.15	0.13	0.00	0.00	0.00	0.09	0.00	3.32	1.27	0.00	0.35	0.18	0.10	0.15	0.00
		23	2.15	0.59	1.51	0.14	0.00	0.00	0.00	0.15	0.10	5.18	2.51	0.00	0.47	0.19	0.19	0.25	0.11
		26	2.24	0.62	1.84	0.21	0.00	0.00	0.00	0.18	0.13	6.22	2.82	0.00	0.68	0.34	0.29	0.40	0.20
		32	1.98	0.55	1.67	0.22	0.00	0.00	0.00	0.15	0.09	5.03	2.39	0.00	0.54	0.26	0.19	0.32	0.16

Table A_1D(e) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1D(d) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area				
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	20:0	PLFA 50	PLFA 51	PLFA 52	Total Area (%)
1	0	3	0.33	0.39	0.31	0.41	100
		5	0.15	0.19	0.19	0.27	100
		11	0.13	0.00	0.15	0.23	100
		14	0.26	0.45	0.26	0.38	100
1	15	4	0.11	0.10	0.13	0.21	100
		6	0.15	0.14	0.15	0.19	100
		12	0.12	0.15	0.14	0.19	100
		13	0.36	0.33	0.27	0.31	100
1	30	1	0.23	0.23	0.21	0.27	100
		7	0.14	0.12	0.16	0.17	100
		10	0.30	0.33	0.26	0.36	100
		16	0.20	0.23	0.24	0.27	100
1	45	2	0.34	0.42	0.31	0.48	100
		8	0.19	0.22	0.15	0.24	100
		9	0.32	0.63	0.41	0.63	100
		15	0.25	0.26	0.25	0.23	100
2	0	19	0.13	0.11	0.10	0.12	100
		21	0.33	0.37	0.22	0.37	100
		28	0.00	0.00	0.00	0.00	100
		30	0.20	0.17	0.15	0.16	100
2	15	17	0.23	0.23	0.11	0.18	100
		24	0.23	0.32	0.14	0.20	100
		25	0.47	0.70	0.42	0.55	100
		31	0.27	0.35	0.16	0.26	100
2	30	20	0.35	0.44	0.24	0.32	100
		22	0.19	0.36	0.16	0.24	100
		27	0.10	0.00	0.00	0.00	100
		29	0.38	0.51	0.31	0.34	100
2	45	18	0.12	0.12	0.00	0.09	100
		23	0.21	0.30	0.12	0.15	100
		26	0.30	0.34	0.17	0.28	100
		32	0.24	0.31	0.21	0.25	100

Table A_1E(a) Raw phospholipid fatty acid (PLFA) analysis profile data for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 1	PLFA 2	12:0	PLFA 3	PLFA 4	14:0	PLFA 5	PLFA 6	PLFA 7	PLFA 8	PLFA 9	i15:0	a15:0	PLFA 10	15:0	PLFA 11	PLFA 12
1	0	3	0.00	0.00	0.05	0.51	0.10	0.43	0.19	0.21	0.58	0.17	0.23	5.64	6.67	0.00	0.40	0.53	0.13
		5	0.00	0.00	0.08	1.14	0.08	0.72	0.32	0.24	0.97	0.18	0.30	6.69	7.29	0.09	0.44	0.51	0.17
		11	0.00	0.00	0.05	0.49	0.05	0.41	0.18	0.17	0.64	0.16	0.25	4.97	6.29	0.07	0.39	0.42	0.14
		14	0.00	0.00	0.05	0.41	0.03	0.32	0.13	0.14	0.53	0.14	0.20	4.31	5.69	0.05	0.36	0.36	0.13
1	15	4	0.00	0.00	0.04	0.42	0.07	0.34	0.16	0.15	0.51	0.15	0.21	4.44	5.23	0.05	0.36	0.37	0.13
		6	0.00	0.00	0.04	0.34	0.08	0.33	0.17	0.14	0.61	0.15	0.25	5.18	5.60	0.06	0.40	0.38	0.16
		12	0.00	0.00	0.12	1.37	0.00	0.82	0.35	0.24	1.16	0.25	0.45	7.37	8.01	0.11	0.49	0.43	0.20
		13	0.00	0.00	0.05	0.57	0.05	0.43	0.20	0.16	0.73	0.16	0.28	5.20	6.32	0.07	0.39	0.40	0.16
1	30	1	0.00	0.00	0.08	1.28	0.09	0.78	0.37	0.28	0.94	0.24	0.35	7.02	7.24	0.10	0.46	0.46	0.17
		7	0.00	0.00	0.09	1.25	0.08	0.79	0.36	0.28	0.94	0.32	0.38	7.23	7.85	0.10	0.47	0.48	0.18
		10	0.00	0.00	0.09	1.26	0.07	0.84	0.36	0.30	1.03	0.24	0.37	7.12	8.15	0.10	0.48	0.51	0.17
		16	0.00	0.00	0.06	0.62	0.08	0.46	0.22	0.19	0.70	0.18	0.29	5.72	6.95	0.06	0.42	0.52	0.16
1	45	2	0.00	0.00	0.08	0.80	0.12	0.59	0.24	0.17	0.73	0.21	0.33	6.46	6.90	0.08	0.48	0.59	0.13
		8	0.00	0.00	0.11	1.40	0.09	0.89	0.42	0.33	1.12	0.27	0.43	8.33	9.30	0.11	0.56	0.66	0.20
		9	0.00	0.00	0.10	1.51	0.10	0.98	0.41	0.34	1.43	0.31	0.53	8.38	9.22	0.13	0.53	0.63	0.19
		15	0.00	0.00	0.10	1.44	0.09	0.88	0.39	0.36	1.06	0.30	0.43	7.78	9.03	0.10	0.52	0.62	0.16
2	0	19	0.00	0.00	0.05	0.43	0.00	0.47	0.20	0.12	0.93	0.16	0.32	6.00	5.92	0.09	0.49	0.34	0.19
		21	0.00	0.00	0.06	0.49	0.02	0.56	0.26	0.22	0.95	0.24	0.35	6.90	7.06	0.09	0.51	0.43	0.19
		28	0.00	0.00	0.04	0.38	0.03	0.42	0.00	0.13	0.81	0.15	0.27	5.74	5.74	0.09	0.45	0.25	0.20
		30	0.00	0.00	0.08	0.64	0.04	0.58	0.30	0.21	0.99	0.25	0.34	6.58	6.35	0.10	0.43	0.28	0.21
2	15	17	0.00	0.00	0.09	0.89	0.04	0.91	0.34	0.29	1.21	0.27	0.39	7.22	7.08	0.11	0.55	0.36	0.17
		24	0.00	0.00	0.11	1.09	0.05	0.95	0.43	0.26	1.50	0.24	0.48	8.27	8.31	0.13	0.55	0.31	0.26
		25	0.00	0.00	0.09	0.94	0.05	0.76	0.34	0.25	1.32	0.23	0.42	6.80	6.61	0.11	0.46	0.31	0.20
		31	0.00	0.00	0.10	0.90	0.09	0.72	0.37	0.26	1.19	0.26	0.38	6.91	6.74	0.11	0.46	0.30	0.19
2	30	20	0.00	0.00	0.03	0.21	0.02	0.29	0.13	0.10	0.61	0.11	0.19	4.11	4.24	0.05	0.37	0.17	0.15
		22	0.00	0.00	0.04	0.33	0.03	0.40	0.19	0.15	0.68	0.16	0.23	4.94	5.10	0.07	0.41	0.28	0.16
		27	0.00	0.00	0.06	0.42	0.09	0.49	0.21	0.18	0.91	0.20	0.32	6.07	6.41	0.09	0.48	0.31	0.18
		29	0.00	0.00	0.14	1.00	0.21	0.88	0.38	0.38	1.28	0.28	0.41	8.15	8.28	0.12	0.55	0.41	0.18
2	45	18	0.00	0.00	0.05	0.11	0.00	0.16	0.08	0.05	0.30	0.07	0.10	2.34	2.66	0.00	0.23	0.17	0.07
		23	0.00	0.00	0.10	1.08	0.08	1.03	0.39	0.36	1.29	0.33	0.42	8.11	8.66	0.14	0.63	0.48	0.18
		26	0.00	0.00	0.04	0.27	0.03	0.39	0.18	0.16	0.72	0.17	0.27	5.19	5.27	0.08	0.42	0.32	0.16
		32	0.00	0.00	0.11	1.10	0.07	0.87	0.37	0.34	1.32	0.27	0.47	7.88	7.43	0.11	0.50	0.46	0.20

Table A_1E(b) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1E(a) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 13	3-OH 14:0	PLFA 14	i16:0	PLFA 15	16:1w7c	PLFA 16	PLFA 17	16:0	PLFA 18	PLFA 19	PLFA 20	PLFA 21	PLFA 22	PLFA 23	PLFA 24	i17:0
1	0	3	0.56	0.29	0.00	2.84	2.30	7.87	0.69	5.38	11.40	0.49	0.22	0.42	2.64	4.54	0.70	0.58	1.60
		5	0.56	0.25	0.00	2.67	2.51	8.63	0.66	5.96	10.97	0.43	0.19	0.46	2.54	4.11	0.77	0.54	1.51
		11	0.51	0.22	0.00	2.52	2.29	8.32	0.69	5.62	11.22	0.37	0.24	0.42	2.52	4.26	0.74	0.56	1.55
		14	0.50	0.29	0.00	2.68	2.29	7.98	0.75	5.95	11.87	0.44	0.24	0.47	2.61	4.62	0.75	0.61	1.71
1	15	4	0.50	0.22	0.00	2.43	2.28	7.93	0.68	5.63	11.03	0.44	0.22	0.44	2.47	4.46	0.77	0.56	1.60
		6	0.56	0.35	0.00	2.65	2.46	8.98	0.69	6.07	11.44	0.47	0.22	0.49	2.75	4.78	0.83	0.58	1.58
		12	0.62	0.29	0.00	2.65	2.71	9.79	0.73	6.67	11.39	0.41	0.28	0.43	2.49	4.06	0.75	0.50	1.38
		13	0.52	0.28	0.00	2.38	2.39	8.52	0.66	6.13	11.05	0.42	0.23	0.43	2.42	4.29	0.75	0.53	1.48
1	30	1	0.60	0.29	0.00	2.49	2.54	8.58	0.62	5.66	10.64	0.50	0.19	0.49	2.60	4.76	0.81	0.56	1.52
		7	0.61	0.26	0.00	2.59	2.33	8.83	0.63	5.62	10.64	0.37	0.17	0.44	2.62	4.34	0.78	0.55	1.49
		10	0.58	0.31	0.00	2.70	2.56	9.02	0.76	6.04	10.79	0.35	0.18	0.40	2.55	4.11	0.69	0.53	1.45
		16	0.56	0.29	0.00	2.76	2.47	8.28	0.75	5.73	11.64	0.43	0.25	0.46	2.52	4.63	0.70	0.54	1.59
1	45	2	0.62	0.28	0.00	2.93	2.36	8.11	0.75	5.53	11.25	0.45	0.21	0.40	2.42	4.85	0.76	0.60	1.61
		8	0.68	0.30	0.00	2.89	2.71	9.88	0.72	6.12	11.57	0.42	0.22	0.42	2.73	0.04	0.74	0.60	1.51
		9	0.61	0.28	0.00	2.72	2.53	9.29	0.65	5.74	10.73	0.39	0.21	0.41	2.49	3.93	0.69	0.51	1.38
		15	0.61	0.24	0.00	2.74	2.52	8.32	0.75	5.56	10.96	0.43	0.18	0.43	2.48	4.13	0.72	0.55	1.46
2	0	19	0.55	0.22	0.00	2.51	2.13	10.39	0.50	7.00	12.41	0.29	0.31	0.36	2.57	3.78	0.81	0.53	1.48
		21	0.58	0.24	0.00	2.59	2.16	9.74	0.52	6.68	12.25	0.29	0.24	0.43	2.67	3.92	0.82	0.61	1.57
		28	0.49	0.21	0.00	2.31	2.38	11.02	0.62	7.70	12.73	0.27	0.27	0.40	2.66	3.86	0.86	0.53	1.53
		30	0.52	0.27	0.00	2.38	2.18	10.71	0.57	7.23	12.16	0.35	0.24	0.46	2.73	4.01	0.95	0.56	1.52
2	15	17	0.52	0.19	0.00	2.52	2.40	9.73	0.75	6.18	11.64	0.19	0.21	0.36	2.40	3.32	0.57	0.54	1.37
		24	0.54	0.22	0.00	2.32	2.41	10.99	0.62	6.97	11.78	0.25	0.27	0.42	2.49	3.52	0.74	0.50	1.35
		25	0.49	0.24	0.00	2.20	2.11	10.23	0.61	6.98	11.41	0.26	0.22	0.43	2.70	3.66	0.83	0.55	1.47
		31	0.48	0.73	0.00	2.31	2.10	9.64	0.51	6.67	11.49	0.27	0.24	0.41	2.67	3.59	0.85	0.58	1.49
2	30	20	0.38	0.21	0.00	1.98	1.79	8.92	0.54	6.39	12.00	0.20	0.29	0.37	2.60	3.77	0.84	0.56	1.60
		22	0.44	0.21	0.00	2.22	2.00	8.87	0.58	6.28	11.95	0.21	0.25	0.41	2.68	3.86	0.82	0.61	1.64
		27	0.48	0.22	0.00	2.47	2.10	10.08	0.69	6.85	12.44	0.24	0.25	0.46	2.64	3.83	0.82	0.60	1.57
		29	0.57	0.26	0.00	2.59	2.64	9.89	0.89	6.58	11.74	0.22	0.24	0.46	2.43	3.39	0.72	0.55	1.40
2	45	18	0.25	0.52	0.00	1.40	1.45	5.91	0.40	4.80	11.43	0.23	0.39	0.32	2.28	3.48	0.54	0.56	1.37
		23	0.52	0.27	0.00	2.50	2.05	9.20	0.63	5.86	11.30	0.22	0.16	0.36	2.52	3.48	0.64	0.57	1.46
		26	0.48	0.25	0.00	2.49	2.04	10.08	0.68	6.67	12.75	0.25	0.26	0.40	2.67	3.80	0.80	0.65	1.63
		32	0.56	0.31	0.00	2.61	2.20	10.20	0.61	6.70	12.42	0.34	0.26	0.49	2.80	3.79	0.82	0.61	1.53

Table A_1E(c) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1E(b) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 25	PLFA 26	17:0c	PLFA 27	17:0	PLFA 28	PLFA 29	2-OH 16:0	PLFA 30	PLFA 31	PLFA 32	PLFA 33	18:2w6c	18:1w9c	18:1w9t	PLFA 34	PLFA 35
1	0	3	1.68	0.46	2.95	0.26	0.35	0.71	0.11	0.00	0.47	0.07	0.35	0.08	0.75	8.53	11.99	0.31	0.92
		5	1.51	0.45	2.88	0.23	0.28	0.66	0.10	0.00	0.42	0.06	0.35	0.08	0.91	7.51	10.67	0.25	0.85
		11	1.66	0.49	3.09	0.30	0.34	0.62	0.10	0.00	0.46	0.06	0.43	0.08	1.19	8.31	12.02	0.34	0.84
		14	1.91	0.52	3.20	0.33	0.35	0.67	0.10	0.00	0.50	0.07	0.34	0.07	1.34	8.69	12.55	0.37	0.94
1	15	4	1.66	0.50	3.06	0.23	0.37	0.66	0.11	0.00	0.45	0.07	0.43	0.06	1.18	9.38	13.04	0.30	1.07
		6	1.63	0.49	3.18	0.21	0.35	0.63	0.10	0.00	0.46	0.07	0.34	0.06	1.06	8.07	11.92	0.29	0.97
		12	1.40	0.53	2.76	0.19	0.26	0.55	0.09	0.00	0.37	0.05	0.37	0.04	0.99	6.09	9.46	0.24	0.72
		13	1.56	0.46	2.94	0.21	0.29	0.57	0.10	0.00	0.41	0.05	0.41	0.06	1.30	7.80	11.93	0.33	0.93
1	30	1	1.47	0.49	2.96	0.21	0.31	0.70	0.12	0.00	0.39	0.07	0.36	0.08	0.91	7.15	10.26	0.26	0.88
		7	1.46	0.53	2.99	0.21	0.31	0.67	0.09	0.00	0.41	0.07	0.36	0.03	0.89	6.96	10.44	0.21	0.75
		10	1.46	0.44	2.95	0.24	0.29	0.65	0.08	0.00	0.41	0.06	0.32	0.06	0.82	6.66	10.23	0.25	0.77
		16	1.63	0.47	2.96	0.27	0.32	0.65	0.10	0.00	0.44	0.06	0.34	0.03	1.04	7.83	11.20	0.29	0.83
1	45	2	1.56	0.55	3.10	0.22	0.34	0.73	0.11	0.00	0.46	0.08	0.31	0.07	0.56	7.52	11.32	0.31	0.84
		8	1.48	0.57	3.04	0.21	0.31	0.67	0.09	0.00	0.40	0.06	0.30	0.03	0.79	6.46	9.85	0.24	0.70
		9	1.38	0.51	2.81	0.24	0.28	0.60	0.08	0.00	0.36	0.05	0.29	0.05	0.87	5.92	9.27	0.25	0.66
		15	1.47	0.42	2.57	0.25	0.28	0.64	0.09	0.00	0.38	0.05	0.30	0.07	0.75	6.78	9.58	0.27	0.79
2	0	19	1.48	0.73	3.63	0.00	0.36	0.52	0.07	0.00	0.42	0.04	0.41	0.05	1.28	8.04	10.47	0.36	0.65
		21	1.56	0.67	3.60	0.21	0.34	0.51	0.07	0.00	0.42	0.04	0.35	0.04	0.65	7.26	9.97	0.35	0.68
		28	1.49	0.61	3.87	0.18	0.34	0.46	0.06	0.00	0.37	0.04	0.10	0.00	0.40	7.25	10.99	0.40	0.68
		30	1.49	0.55	3.62	0.17	0.33	0.49	0.08	0.00	0.36	0.05	0.06	0.04	0.87	6.29	10.39	0.32	0.75
2	15	17	1.39	0.62	3.06	0.20	0.33	0.45	0.05	0.00	0.41	0.05	0.15	0.05	1.14	7.80	9.78	0.34	0.65
		24	1.36	0.64	3.59	0.18	0.33	0.42	0.06	0.00	0.32	0.03	0.05	0.03	0.83	6.36	8.89	0.32	0.59
		25	1.45	0.57	3.41	0.19	0.33	0.47	0.07	0.00	0.37	0.05	0.10	0.04	1.20	7.13	10.39	0.32	0.72
		31	1.47	0.54	3.39	0.20	0.32	0.45	0.07	0.00	0.41	0.05	0.08	0.04	1.05	6.77	10.70	0.31	0.79
2	30	20	1.61	0.70	3.94	0.24	0.43	0.46	0.06	0.00	0.47	0.06	0.12	0.51	1.47	9.96	12.65	0.50	0.87
		22	1.65	0.73	3.79	0.24	0.00	0.54	0.07	0.00	0.49	0.05	0.08	0.47	1.10	9.62	11.70	0.41	0.83
		27	1.56	0.66	3.64	0.21	0.33	0.48	0.07	0.00	0.45	0.06	0.11	0.41	1.16	7.29	10.72	0.37	0.74
		29	1.38	0.54	3.02	0.22	0.32	0.43	0.05	0.00	0.38	0.05	0.12	0.28	0.96	6.03	9.04	0.29	0.67
2	45	18	1.44	0.75	2.67	0.33	0.41	0.39	0.07	0.00	0.29	0.00	0.09	0.68	1.93	13.35	16.08	0.63	1.02
		23	1.49	0.51	3.10	0.26	0.32	0.49	0.07	0.00	0.40	0.04	0.11	0.33	1.15	7.31	9.44	0.22	0.55
		26	1.64	0.67	3.48	0.22	0.38	0.51	0.06	0.00	0.43	0.04	0.07	0.44	0.96	8.36	11.80	0.37	0.86
		32	1.47	0.60	3.18	0.20	0.30	0.50	0.07	0.00	0.35	0.05	0.00	0.40	0.82	6.30	9.31	0.18	0.61

Table A_1E(d) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1E(c) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	18:0	PLFA 36	PLFA 37	PLFA 38	PLFA 39	PLFA 40	PLFA 41	PLFA 42	PLFA 43	19:0c	19:0	PLFA 44	PLFA 45	PLFA 46	PLFA 47	PLFA 48	PLFA 49
1	0	3	1.84	0.57	0.07	1.46	0.26	0.07	0.11	0.07	0.00	4.87	1.61	0.00	0.19	0.12	0.00	0.00	0.11
		5	1.63	0.52	0.06	1.45	0.18	0.06	0.11	0.06	0.00	4.20	1.74	0.00	0.30	0.16	0.00	0.00	0.10
		11	1.98	0.60	0.07	1.58	0.19	0.08	0.12	0.07	0.00	5.21	1.93	0.00	0.36	0.18	0.00	0.00	0.13
		14	1.84	0.62	0.06	1.56	0.16	0.08	0.10	0.06	0.00	5.04	1.58	0.00	0.00	0.20	0.00	0.00	0.08
1	15	4	2.05	0.61	0.08	1.76	0.21	0.08	0.14	0.08	0.00	5.04	1.97	0.00	0.33	0.17	0.00	0.00	0.13
		6	1.77	0.54	0.07	1.48	0.27	0.06	0.10	0.06	0.00	4.30	2.01	0.00	0.33	0.00	0.00	0.00	0.10
		12	1.49	0.42	0.05	1.20	0.16	0.05	0.10	0.05	0.00	3.43	1.76	0.00	0.22	0.16	0.00	0.00	0.08
		13	1.82	0.56	0.07	1.59	0.25	0.09	0.12	0.08	0.00	5.17	2.05	0.00	0.39	0.22	0.00	0.00	0.13
1	30	1	1.61	0.48	0.08	1.40	0.21	0.06	0.12	0.06	0.00	4.07	1.79	0.00	0.26	0.16	0.00	0.00	0.10
		7	1.57	0.48	0.08	1.41	0.18	0.05	0.11	0.06	0.00	4.05	1.59	0.00	0.32	0.19	0.00	0.00	0.10
		10	1.51	0.49	0.07	1.37	0.16	0.06	0.10	0.06	0.00	4.03	1.49	0.00	0.27	0.14	0.00	0.00	0.11
		16	1.76	0.53	0.06	1.50	0.19	0.07	0.11	0.07	0.00	4.54	1.87	0.00	0.18	0.11	0.00	0.00	0.09
1	45	2	1.71	0.55	0.08	1.38	0.20	0.06	0.14	0.06	0.00	4.32	1.74	0.00	0.17	0.09	0.00	0.00	0.12
		8	1.45	0.50	0.06	1.19	0.24	0.05	0.09	0.05	0.00	3.56	1.42	0.00	0.08	0.11	0.00	0.00	0.08
		9	1.42	0.44	0.05	1.14	0.24	0.05	0.09	0.05	0.00	3.53	1.38	0.00	0.17	0.14	0.00	0.00	0.09
		15	1.52	0.46	0.05	1.34	0.15	0.05	0.09	0.06	0.00	3.94	1.60	0.00	0.19	0.14	0.00	0.00	0.08
2	0	19	1.73	0.45	0.00	1.24	0.18	0.07	0.10	0.05	0.00	3.62	1.63	0.00	0.29	0.15	0.00	0.00	0.11
		21	1.69	0.53	0.06	1.16	0.15	0.08	0.09	0.05	0.00	3.89	1.52	0.00	0.08	0.00	0.00	0.00	0.09
		28	1.71	0.58	0.06	1.34	0.23	0.08	0.10	0.06	0.00	3.75	1.61	0.00	0.25	0.14	0.00	0.00	0.09
		30	1.65	0.55	0.06	1.25	0.18	0.06	0.09	0.05	0.00	3.46	2.16	0.00	0.14	0.00	0.00	0.00	0.07
2	15	17	1.66	0.63	0.02	1.33	0.26	0.07	0.11	0.06	0.00	3.91	1.70	0.00	0.30	0.23	0.00	0.00	0.11
		24	1.53	0.45	0.06	0.03	0.15	0.06	0.08	0.04	0.00	3.26	1.58	0.00	0.18	0.08	0.00	0.00	0.00
		25	1.74	0.55	0.07	0.05	0.23	0.08	0.10	0.05	0.00	4.01	1.90	0.00	0.41	0.29	0.00	0.00	0.11
		31	1.77	0.60	0.07	0.05	0.19	0.07	0.11	0.05	0.00	3.95	2.49	0.00	0.35	0.22	0.00	0.00	0.10
2	30	20	2.24	0.74	0.08	0.07	0.29	0.10	0.13	0.08	0.00	5.33	2.40	0.00	0.44	0.34	0.00	0.00	0.08
		22	2.12	0.65	0.09	0.06	0.27	0.10	0.12	0.07	0.00	5.15	2.26	0.00	0.39	0.25	0.00	0.00	0.05
		27	1.82	0.56	0.07	0.04	0.20	0.08	0.10	0.05	0.00	4.09	1.83	0.00	0.27	0.16	0.00	0.00	0.00
		29	1.51	0.42	1.25	0.00	0.14	0.06	0.08	0.04	0.00	3.48	1.69	0.00	0.00	0.23	0.00	0.00	0.00
2	45	18	2.85	0.90	0.00	0.00	1.18	0.17	0.15	0.07	0.00	8.54	4.20	0.00	0.07	0.00	0.00	0.00	0.00
		23	1.55	0.49	0.03	0.02	0.21	0.08	0.12	0.07	0.00	4.17	1.62	0.00	0.36	0.14	0.00	0.00	0.05
		26	1.94	0.62	0.06	0.04	0.28	0.08	0.11	0.06	0.00	4.37	1.82	0.00	0.24	0.15	0.00	0.00	0.00
		32	1.57	0.52	0.05	0.03	0.21	0.06	0.09	0.03	0.00	3.35	1.76	0.00	0.09	0.00	0.00	0.00	0.00

Table A_1E(e) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1E(d) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in October 2010. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area				
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	20:0	PLFA 50	PLFA 51	PLFA 52	Total Area (%)
1	0	3	0.05	0.00	0.08	0.13	100
		5	0.07	0.00	0.08	0.10	100
		11	0.09	0.00	0.14	0.20	100
		14	0.03	0.00	0.00	0.06	100
1	15	4	0.10	0.00	0.16	0.21	100
		6	0.05	0.00	0.08	0.15	100
		12	0.04	0.00	0.08	0.09	100
		13	0.10	0.00	0.15	0.19	100
1	30	1	0.08	0.00	0.09	0.11	100
		7	0.08	0.00	0.12	0.13	100
		10	0.09	0.00	0.12	0.15	100
		16	0.04	0.00	0.08	0.12	100
1	45	2	0.04	0.00	0.07	0.17	100
		8	0.04	0.00	0.04	0.06	100
		9	0.06	0.00	0.08	0.10	100
		15	0.06	0.00	0.10	0.13	100
2	0	19	0.08	0.00	0.11	0.18	100
		21	0.04	0.00	0.07	0.14	100
		28	0.03	0.00	0.06	0.13	100
		30	0.05	0.00	0.05	0.09	100
2	15	17	0.09	0.00	0.10	0.13	100
		24	0.03	0.00	0.04	0.07	100
		25	0.08	0.00	0.09	0.10	100
		31	0.09	0.00	0.09	0.12	100
2	30	20	0.09	0.00	0.13	0.18	100
		22	0.12	0.00	0.15	0.20	100
		27	0.04	0.00	0.10	0.14	100
		29	0.03	0.00	0.03	0.05	100
2	45	18	0.00	0.00	0.00	0.00	100
		23	0.09	0.00	0.09	0.12	100
		26	0.08	0.00	0.10	0.15	100
		32	0.05	0.00	0.05	0.07	100

Table A_1F(a) Raw phospholipid fatty acid (PLFA) analysis profile data for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2011. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area															
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 1	PLFA 2	12:0	PLFA 3	PLFA 4	14:0	PLFA 5	PLFA 6	PLFA 7	PLFA 8	PLFA 9	i15:0	a15:0	PLFA 10	15:0	PLFA 11	PLFA 12
1	0	3	0.51	0.00	0.00	1.53	0.13	1.00	0.31	0.22	0.91	0.25	0.39	5.94	7.88	0.00	0.47	0.50	0.16
		5	0.45	0.62	0.10	1.88	0.17	1.13	0.43	0.26	1.39	0.26	0.50	7.28	8.44	0.00	0.49	0.46	0.21
		11	0.25	0.61	0.14	1.90	0.27	1.17	0.38	0.26	1.35	0.28	0.47	7.00	9.30	0.00	0.54	0.51	0.19
		14	0.12	0.63	0.13	1.98	0.20	1.15	0.35	0.26	1.17	0.33	0.47	6.87	9.52	0.00	0.51	0.50	0.16
1	15	4	0.00	0.57	0.08	1.86	0.24	1.16	0.39	0.28	1.22	0.26	0.45	6.98	8.16	0.00	0.54	0.48	0.18
		6	0.09	0.40	0.06	1.30	0.17	0.88	0.36	0.22	1.10	0.23	0.39	6.36	7.24	0.00	0.45	0.45	0.20
		12	0.10	0.45	0.07	1.70	0.10	1.03	0.38	0.21	1.53	0.22	0.52	7.13	7.70	0.00	0.49	0.39	0.23
		13	2.17	0.41	0.08	1.62	0.12	0.93	0.36	0.20	1.51	0.00	0.44	6.33	7.74	0.00	0.47	0.43	0.22
1	30	1	0.00	0.46	0.00	1.51	0.23	0.99	0.38	0.20	1.21	0.26	0.46	7.03	7.84	0.00	0.54	0.49	0.22
		7	0.23	0.52	0.07	1.69	0.16	1.11	0.35	0.22	1.19	0.30	0.49	6.92	8.34	0.00	0.51	0.57	0.17
		10	0.08	0.41	0.08	1.68	0.23	1.01	0.33	0.23	1.20	0.28	0.47	6.80	8.19	0.00	0.50	0.46	0.20
		16	0.15	0.49	0.09	2.08	0.28	1.15	0.39	0.23	1.39	0.33	0.44	7.54	9.35	0.00	0.54	0.53	0.20
1	45	2	0.18	0.43	0.06	1.84	0.21	1.12	0.42	0.25	1.23	0.27	0.46	7.19	8.55	0.00	0.55	0.65	0.20
		8	0.07	0.47	0.10	1.97	0.18	1.19	0.41	0.22	1.35	0.24	0.47	7.34	8.57	0.00	0.54	0.51	0.22
		9	0.37	0.44	0.08	1.63	0.12	1.02	0.36	0.27	1.40	0.25	0.47	6.77	8.03	0.00	0.51	0.54	0.19
		15	8.36	0.00	0.09	1.54	0.22	0.93	0.26	0.20	0.78	0.26	0.36	5.31	7.32	0.00	0.43	0.60	0.13
2	0	19	0.09	0.66	0.12	1.33	0.21	1.27	0.38	0.27	1.46	0.31	0.53	7.37	7.15	0.00	0.64	0.39	0.20
		21	0.06	0.48	0.09	1.13	0.16	1.10	0.46	0.31	1.35	0.32	0.45	7.69	7.90	0.00	0.55	0.48	0.22
		28	0.06	0.52	0.07	1.13	0.44	1.08	0.34	0.26	1.33	0.27	0.47	7.16	7.22	0.00	0.56	0.37	0.20
		30	0.70	0.51	0.12	1.35	0.48	1.13	0.46	0.31	1.36	0.33	0.47	7.69	7.63	0.00	0.54	0.38	0.22
2	15	17	0.33	0.58	0.09	1.05	0.09	1.17	0.34	0.29	1.20	0.27	0.43	7.29	7.26	0.00	0.58	0.40	0.18
		24	0.11	0.57	0.07	1.12	0.09	1.01	0.39	0.26	1.35	0.25	0.45	7.06	7.29	0.00	0.51	0.31	0.24
		25	0.13	0.54	0.06	1.17	0.35	1.06	0.34	0.24	1.72	0.30	0.59	7.69	7.27	0.00	0.56	0.28	0.23
		31	0.22	0.86	0.18	1.45	0.71	1.24	0.51	0.39	1.61	0.35	0.50	8.28	8.26	0.00	0.55	0.35	0.24
2	30	20	0.33	0.63	0.09	1.26	0.13	1.22	0.41	0.30	1.51	0.26	0.45	7.55	7.71	0.00	0.57	0.24	0.24
		22	0.55	0.54	0.08	1.22	0.17	1.12	0.35	0.24	1.65	0.23	0.56	6.98	6.80	0.00	0.55	0.32	0.20
		27	0.22	0.66	0.09	1.20	0.15	1.13	0.34	0.27	1.25	0.23	0.48	6.91	7.12	0.00	0.53	0.31	0.20
		29	0.17	0.56	0.05	0.98	0.41	1.01	0.33	0.31	1.19	0.27	0.42	7.31	7.84	0.00	0.57	0.36	0.21
2	45	18	0.13	0.66	0.13	1.62	0.21	1.30	0.45	0.31	1.73	0.31	0.56	8.18	8.26	0.00	0.59	0.31	0.25
		23	0.13	0.64	0.11	1.07	0.17	1.09	0.34	0.36	1.21	0.31	0.41	6.95	7.64	0.00	0.55	0.40	0.19
		26	0.19	0.69	0.14	1.13	0.24	1.23	0.33	0.25	1.14	0.29	0.43	6.63	7.25	0.00	0.54	0.39	0.17
		32	0.84	0.47	0.09	1.22	0.26	0.99	0.34	0.24	1.51	0.25	0.50	6.68	6.22	0.00	0.48	0.34	0.20

Table A_1F(b) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1F(a) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2011. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 13	3-OH 14:0	PLFA 14	i16:0	PLFA 15	16:1w7c	PLFA 16	PLFA 17	16:0	PLFA 18	PLFA 19	PLFA 20	PLFA 21	PLFA 22	PLFA 23	PLFA 24	i17:0
1	0	3	0.62	0.24	0.00	2.45	1.81	6.87	0.72	4.89	11.94	0.30	0.74	0.35	1.69	3.41	0.45	0.46	1.35
		5	0.69	0.21	0.00	2.50	2.36	9.01	0.69	5.63	11.57	0.28	0.63	0.35	2.00	3.29	0.65	0.44	1.26
		11	0.64	0.28	0.00	2.43	2.03	9.00	0.90	5.53	12.26	0.25	0.59	0.32	1.87	3.28	0.52	0.39	1.18
		14	0.65	0.20	0.00	2.48	1.95	7.97	1.00	5.28	11.69	0.24	0.58	0.28	1.70	3.22	0.49	0.39	1.17
1	15	4	0.67	0.19	0.00	2.54	2.28	9.11	0.77	5.37	11.76	0.26	0.57	0.32	1.95	3.39	0.52	0.43	1.26
		6	0.67	0.18	0.00	2.38	2.34	9.21	0.65	5.75	11.28	0.26	0.58	0.39	2.09	3.54	0.66	0.47	1.32
		12	0.64	0.18	0.00	2.31	2.53	10.27	0.68	6.61	11.10	0.25	0.56	0.37	2.12	3.28	0.73	0.42	1.23
		13	0.63	0.28	0.00	2.29	2.50	9.68	0.71	6.41	11.55	0.28	0.68	0.37	2.01	3.41	0.74	0.42	1.14
1	30	1	0.71	0.20	0.00	2.46	2.46	9.37	0.67	6.07	13.20	0.33	0.57	0.36	2.06	3.77	0.72	0.43	1.31
		7	0.67	0.28	0.00	2.57	2.35	8.33	1.29	5.47	11.87	0.29	0.63	0.34	1.82	3.65	0.63	0.49	1.32
		10	0.63	0.26	0.00	2.47	2.34	9.35	1.15	5.99	11.63	0.25	0.54	0.33	1.98	3.47	0.71	0.47	1.28
		16	0.73	0.22	0.00	2.67	2.37	8.69	1.31	6.04	11.38	0.27	0.61	0.36	1.81	4.17	0.47	0.17	1.26
1	45	2	0.68	0.21	0.00	2.54	2.43	9.08	0.63	5.69	11.92	0.31	0.48	0.36	1.95	3.74	0.44	0.07	1.31
		8	0.71	0.18	0.00	2.60	2.54	10.26	0.71	6.12	11.80	0.26	0.49	0.35	2.06	3.69	0.44	0.06	1.30
		9	0.65	0.18	0.00	2.52	2.36	9.59	0.64	5.52	10.97	0.25	0.43	0.34	2.13	3.29	0.43	0.08	1.26
		15	0.51	0.28	0.00	2.17	1.85	5.87	1.17	4.26	11.02	0.29	0.70	0.28	1.47	3.26	0.41	0.12	1.23
2	0	19	0.59	0.22	0.00	2.16	2.27	10.80	0.59	5.74	14.28	0.19	0.64	0.31	1.99	2.88	0.41	0.08	1.16
		21	0.66	0.22	0.00	2.43	2.39	10.10	0.58	5.92	11.75	0.20	0.68	0.39	2.19	3.30	0.52	0.07	1.31
		28	0.62	0.23	0.00	2.40	2.32	10.90	1.16	5.86	12.87	0.19	0.59	0.33	2.04	3.13	0.48	0.09	1.22
		30	0.64	0.22	0.00	2.27	2.39	9.85	0.56	6.08	12.24	0.20	0.62	0.39	2.01	3.13	0.49	0.04	1.25
2	15	17	0.64	0.24	0.00	2.36	1.95	9.62	0.73	5.54	12.83	0.14	0.59	0.32	2.05	3.15	0.51	0.04	1.26
		24	0.56	0.17	0.00	2.04	2.46	10.35	0.57	6.42	12.08	0.18	0.62	0.38	2.11	3.06	0.43	0.04	1.16
		25	0.56	0.24	0.00	2.09	2.41	11.79	1.21	6.33	12.14	0.16	0.70	0.30	2.08	3.04	0.41	0.17	1.14
		31	0.65	0.29	0.00	2.21	2.51	10.98	0.68	6.40	12.12	0.19	0.75	0.40	2.14	3.00	0.44	0.08	1.19
2	30	20	0.55	0.19	0.00	2.06	2.52	10.96	0.69	6.49	12.46	0.14	0.62	0.34	2.03	2.92	0.39	0.03	1.12
		22	0.55	0.19	0.00	2.10	2.24	10.95	0.57	5.89	12.08	0.14	0.71	0.32	2.10	2.72	0.48	0.09	1.21
		27	0.57	0.21	0.00	2.11	2.21	9.74	0.79	5.89	12.19	0.17	0.68	0.34	2.08	3.23	0.41	0.04	1.26
		29	0.57	0.31	0.00	2.26	2.25	9.77	1.10	6.07	13.92	0.18	0.62	0.33	2.07	3.16	0.45	0.09	1.31
2	45	18	0.58	0.22	0.00	2.46	2.64	10.94	1.13	6.79	12.03	0.15	0.64	0.31	2.03	3.00	0.42	0.12	1.15
		23	0.55	0.48	0.00	2.31	2.19	10.00	0.67	5.55	12.26	0.15	0.63	0.38	2.15	0.49	0.47	0.03	1.24
		26	0.59	0.24	0.00	2.42	2.15	8.99	0.88	5.36	12.65	0.17	0.76	0.31	1.81	3.26	0.46	0.49	1.29
		32	0.50	0.77	0.00	2.16	2.28	10.70	0.69	5.95	11.51	0.18	0.67	0.32	2.08	2.86	0.75	0.42	1.22

Table A_1F(c) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1F(b) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2011. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	PLFA 25	PLFA 26	17:0c	PLFA 27	17:0	PLFA 28	PLFA 29	2-OH 16:0	PLFA 30	PLFA 31	PLFA 32	PLFA 33	18:2w6c	18:1w9c	18:1w9t	PLFA 34	PLFA 35
1	0	3	1.38	0.43	2.49	0.27	0.29	0.53	0.00	0.00	0.00	0.34	0.00	2.12	0.58	7.66	12.02	0.28	0.83
		5	1.32	0.42	2.38	0.23	0.26	0.49	0.00	0.00	0.00	0.28	0.00	1.18	0.91	6.70	10.42	0.19	0.71
		11	1.39	0.41	2.36	0.23	0.27	0.46	0.00	0.00	0.00	0.28	0.00	1.05	0.97	6.48	10.21	0.50	0.63
		14	1.44	0.39	2.43	0.25	0.27	0.43	0.00	0.00	0.00	0.33	0.00	2.30	0.68	6.31	10.63	0.58	0.66
1	15	4	1.26	0.51	2.38	0.22	0.29	0.50	0.00	0.00	0.00	0.37	0.00	1.16	1.09	7.43	10.73	0.13	0.63
		6	1.32	0.50	2.65	0.22	0.28	0.48	0.00	0.00	0.00	0.37	0.00	2.05	1.22	7.08	11.08	0.20	0.83
		12	1.25	0.51	2.53	0.19	0.28	0.46	0.00	0.00	0.00	0.32	0.00	1.39	1.29	6.46	10.42	0.17	0.68
		13	1.24	0.45	2.40	0.21	0.27	0.45	0.00	0.00	0.00	0.30	0.00	1.32	1.22	6.35	10.17	0.19	0.67
1	30	1	1.32	0.52	2.62	0.20	0.30	0.60	0.00	0.00	0.00	0.31	0.00	1.01	1.22	6.64	9.89	0.26	0.63
		7	1.56	0.48	2.70	0.22	0.30	0.56	0.00	0.00	0.00	0.37	0.00	1.22	0.60	6.36	10.58	0.76	0.69
		10	1.54	0.44	2.74	0.21	0.29	0.51	0.00	0.00	0.00	0.33	0.00	1.00	0.99	6.37	10.41	0.72	0.71
		16	1.56	0.38	2.33	0.23	0.26	0.49	0.00	0.00	0.00	0.31	0.00	2.70	0.76	5.43	8.81	0.80	0.60
1	45	2	1.31	0.47	2.53	0.22	0.30	0.59	0.00	0.00	0.00	0.35	0.00	0.95	0.95	6.91	10.65	0.19	0.61
		8	1.27	0.49	2.65	0.21	0.29	0.56	0.00	0.00	0.00	0.35	0.00	0.96	1.00	6.34	10.16	0.20	0.67
		9	1.27	0.51	2.60	0.25	0.29	0.52	0.00	0.00	0.00	0.39	0.00	1.36	1.22	6.99	11.18	0.22	0.72
		15	1.68	0.33	2.24	0.26	0.26	0.58	0.00	0.00	0.00	0.37	0.00	1.53	0.42	6.54	10.54	1.07	0.78
2	0	19	1.13	0.59	2.42	0.18	0.33	0.39	0.00	0.00	0.00	0.28	0.00	1.20	1.38	7.24	8.94	0.31	0.57
		21	1.30	0.57	2.68	0.22	0.32	0.45	0.00	0.00	0.00	0.39	0.00	2.05	0.98	6.95	9.09	0.26	0.64
		28	1.43	0.53	2.67	0.16	0.30	0.40	0.00	0.00	0.00	0.34	0.00	1.23	1.17	6.36	9.78	0.69	0.62
		30	1.21	0.51	2.54	0.18	0.30	0.39	0.00	0.00	0.00	0.34	0.00	1.35	1.06	5.99	9.71	0.26	0.66
2	15	17	1.32	0.59	2.73	0.20	0.32	0.40	0.00	0.00	0.00	0.39	0.00	1.68	0.95	7.31	9.96	0.36	0.64
		24	1.21	0.54	2.80	0.20	0.31	0.36	0.00	0.00	0.00	0.30	0.00	2.39	1.20	7.14	9.40	0.29	0.65
		25	1.67	0.54	2.68	0.15	0.28	0.33	0.00	0.00	0.00	0.25	0.00	1.75	1.00	5.92	8.79	0.63	0.49
		31	1.19	0.50	2.50	0.18	0.27	0.32	0.00	0.00	0.00	0.27	0.00	1.50	1.15	5.42	8.56	0.12	0.51
2	30	20	1.12	0.53	2.78	0.19	0.31	0.29	0.00	0.00	0.00	0.27	0.00	1.73	1.16	7.03	8.98	0.15	0.49
		22	1.32	0.63	2.45	0.16	0.33	0.40	0.00	0.00	0.00	0.33	0.00	1.37	1.45	7.59	9.84	0.27	0.59
		27	1.21	0.54	2.96	0.19	0.32	0.40	0.00	0.00	0.00	0.36	0.00	1.60	0.71	6.79	10.55	0.31	0.66
		29	1.36	0.45	2.71	0.21	0.31	0.40	0.00	0.00	0.00	0.32	0.00	1.21	0.93	5.94	9.55	0.56	0.63
2	45	18	1.40	0.50	2.65	0.19	0.29	0.34	0.00	0.00	0.00	0.27	0.00	2.76	0.97	5.59	7.85	0.54	0.45
		23	1.32	0.55	2.64	0.23	0.33	0.43	0.00	0.00	0.00	0.36	0.00	1.80	1.27	8.03	10.66	0.30	0.65
		26	1.25	0.52	2.66	0.28	0.32	0.46	0.00	0.00	0.00	0.39	0.00	3.28	0.64	6.79	10.40	0.35	0.67
		32	1.19	0.55	2.61	0.18	0.29	0.41	0.00	0.00	0.00	0.38	0.00	1.72	1.44	6.70	10.48	0.24	0.67

Table A_1F(d) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1F(c) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2011. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area																
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	18:0	PLFA 36	PLFA 37	PLFA 38	PLFA 39	PLFA 40	PLFA 41	PLFA 42	PLFA 43	19:0c	19:0	PLFA 44	PLFA 45	PLFA 46	PLFA 47	PLFA 48	PLFA 49
1	0	3	2.59	0.48	0.00	1.66	0.18	0.00	0.00	0.30	0.00	4.38	1.18	0.34	0.00	0.14	0.00	0.00	0.13
		5	1.81	0.39	0.00	1.24	0.14	0.00	0.00	0.27	0.00	3.28	0.72	0.37	0.00	0.16	0.00	0.00	0.09
		11	1.80	0.39	0.00	1.12	0.13	0.00	0.00	0.31	0.00	3.38	0.66	0.29	0.00	0.00	0.00	0.00	0.09
		14	1.98	0.45	0.00	1.33	0.15	0.00	0.00	0.18	0.00	3.67	0.77	0.29	0.00	0.12	0.00	0.00	0.10
1	15	4	1.76	0.45	0.00	1.34	0.17	0.00	0.00	0.24	0.00	3.34	0.70	0.33	0.00	0.15	0.00	0.00	0.09
		6	2.07	0.46	0.00	1.44	0.18	0.00	0.00	0.24	0.00	3.36	0.86	0.48	0.00	0.19	0.00	0.00	0.10
		12	1.81	0.37	0.00	1.25	0.13	0.00	0.00	0.20	0.00	2.89	0.69	0.44	0.00	0.21	0.00	0.00	0.09
		13	1.88	0.39	0.00	1.24	0.12	0.00	0.00	0.20	0.00	2.93	0.76	0.41	0.00	0.20	0.00	0.00	0.08
1	30	1	1.68	0.35	0.00	1.09	0.15	0.00	0.00	0.29	0.00	2.86	0.55	0.33	0.00	0.16	0.00	0.00	0.09
		7	1.80	0.42	0.00	1.28	0.13	0.00	0.00	0.44	0.00	3.22	0.67	0.24	0.00	0.08	0.00	0.00	0.00
		10	1.69	0.42	0.00	1.20	0.16	0.00	0.00	0.45	0.00	3.18	0.60	0.29	0.00	0.10	0.00	0.00	0.10
		16	1.57	0.32	0.00	1.13	0.13	0.00	0.00	0.45	0.00	2.63	0.57	0.21	0.00	0.09	0.00	0.00	0.09
1	45	2	1.71	0.44	0.00	0.06	1.12	0.00	0.00	0.26	0.00	3.27	0.60	0.32	0.00	0.14	0.00	0.00	0.10
		8	1.60	0.39	0.00	1.09	0.12	0.00	0.00	0.20	0.00	2.70	0.54	0.29	0.00	0.11	0.00	0.00	0.07
		9	1.74	0.43	0.00	1.27	0.15	0.00	0.00	0.21	0.00	3.59	0.65	0.45	0.00	0.19	0.00	0.00	0.11
		15	2.30	0.50	0.00	1.58	0.18	0.00	0.00	0.79	0.00	4.26	0.96	0.22	0.00	0.06	0.00	0.00	0.16
2	0	19	2.00	0.38	0.00	0.94	0.12	0.00	0.00	0.16	0.00	2.90	0.75	0.39	0.00	0.21	0.00	0.00	0.08
		21	1.83	0.44	0.00	1.17	0.16	0.00	0.00	0.14	0.00	3.15	0.69	0.36	0.00	0.16	0.00	0.00	0.08
		28	1.85	0.47	0.00	1.12	0.14	0.00	0.00	0.34	0.00	2.87	0.69	0.27	0.00	0.11	0.00	0.00	0.08
		30	1.98	0.46	0.00	1.23	0.11	0.00	0.00	0.16	0.00	3.30	0.82	0.47	0.00	0.23	0.00	0.00	0.09
2	15	17	2.06	0.49	0.00	1.22	0.15	0.00	0.00	0.22	0.00	3.52	0.86	0.33	0.00	0.13	0.00	0.00	0.08
		24	2.10	0.47	0.00	0.07	1.12	0.00	0.00	0.15	0.00	3.39	0.91	0.41	0.00	0.19	0.00	0.00	0.09
		25	1.85	0.34	0.00	0.00	1.02	0.00	0.00	0.65	0.00	2.59	0.77	0.26	0.00	0.13	0.00	0.00	0.07
		31	1.89	0.37	0.00	0.02	1.02	0.00	0.00	0.13	0.00	2.67	0.81	0.28	0.00	0.15	0.00	0.00	0.06
2	30	20	1.98	0.42	0.00	0.03	1.08	0.00	0.00	0.11	0.00	3.14	0.77	0.36	0.00	0.17	0.00	0.00	0.07
		22	2.04	0.43	0.00	0.07	1.16	0.00	0.00	0.28	0.00	3.15	0.84	0.45	0.00	0.26	0.00	0.00	0.10
		27	2.27	0.51	0.00	0.07	1.48	0.00	0.00	0.15	0.00	3.73	0.95	0.33	0.00	0.13	0.00	0.00	0.11
		29	1.92	0.42	0.00	0.03	1.20	0.00	0.00	0.30	0.00	3.40	0.68	0.32	0.00	0.14	0.00	0.00	0.08
2	45	18	1.52	0.36	0.00	0.02	0.91	0.00	0.00	0.30	0.00	2.27	0.54	0.21	0.00	0.10	0.00	0.00	0.06
		23	2.15	0.48	0.00	0.03	1.25	0.00	0.00	0.16	0.00	3.93	0.93	0.40	0.00	0.16	0.00	0.00	0.17
		26	2.17	0.49	0.00	0.07	1.29	0.00	0.00	0.13	0.00	3.38	0.79	0.22	0.00	0.09	0.00	0.00	0.11
		32	2.05	0.40	0.00	0.03	1.39	0.00	0.00	0.19	0.00	2.92	0.89	0.46	0.00	0.23	0.00	0.00	0.32

Table A_1F(e) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 1F(d) for all field plots in the presence four P fertilisation regimes across two sites, which was collected in April 2011. Unidentified PLFA peaks are denoted as PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

			% Peak Area				
Site	P treatment (kg P ha^{-1} y^{-1})	Plot number	20:0	PLFA 50	PLFA 51	PLFA 52	Total Area (%)
1	0	3	0.15	0.26	0.21	0.28	100
		5	0.07	0.13	0.11	0.13	100
		11	0.08	0.13	0.13	0.14	100
		14	0.08	0.21	0.15	0.16	100
1	15	4	0.08	0.13	0.13	0.14	100
		6	0.10	0.17	0.14	0.20	100
		12	0.08	0.12	0.10	0.13	100
		13	0.07	0.13	0.11	0.13	100
1	30	1	0.09	0.11	0.10	0.14	100
		7	0.07	0.12	0.15	0.13	100
		10	0.08	0.15	0.19	0.16	100
		16	0.06	0.12	0.11	0.12	100
1	45	2	0.09	0.13	0.12	0.14	100
		8	0.06	0.09	0.08	0.10	100
		9	0.09	0.19	0.15	0.18	100
		15	0.10	0.23	0.13	0.24	100
2	0	19	0.10	0.12	0.09	0.12	100
		21	0.08	0.17	0.09	0.12	100
		28	0.08	0.13	0.13	0.13	100
		30	0.11	0.22	0.11	0.16	100
2	15	17	0.10	0.20	0.13	0.15	100
		24	0.10	0.21	0.14	0.17	100
		25	0.07	0.14	0.20	0.09	100
		31	0.07	0.14	0.07	0.10	100
2	30	20	0.08	0.19	0.08	0.10	100
		22	0.10	0.19	0.18	0.15	100
		27	0.11	0.24	0.14	0.19	100
		29	0.10	0.16	0.13	0.12	100
2	45	18	0.05	0.12	0.06	0.07	100
		23	0.12	0.25	0.10	0.14	100
		26	0.07	0.16	0.07	0.11	100
		32	0.10	0.15	0.15	0.17	100

Appendix 2

Table A_2A(a) Raw phospholipid fatty acid (PLFA) analysis profile data for all mesocosms. These were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 0 – 1 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area														
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	12:0	PLFA 1	PLFA 2	14:0	PLFA 3	PLFA 4	PLFA 5	PLFA 6	PLFA 7	i15:0	a15:0	15:0	PLFA 8	PLFA 9	PLFA 10
Unplanted	Inorganic	Absent	8	0.17	0.34	0.00	0.58	0.00	0.00	0.00	0.73	0.27	4.48	4.01	0.56	0.35	0.00	0.00
			20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	2.86	2.94	0.35	0.26	0.00	0.00
			27	0.35	0.72	0.21	0.78	0.27	0.30	0.87	0.39	0.35	8.01	8.46	0.58	0.67	0.00	0.22
Unplanted	Organic	Absent	10	0.17	0.29	0.00	0.58	0.00	0.00	0.00	0.44	0.31	4.00	3.88	0.64	0.33	0.38	0.00
			14	0.17	0.23	0.00	0.43	0.12	0.24	0.16	0.00	0.18	4.53	5.17	0.56	0.55	0.16	0.00
			29	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	3.59	4.34	0.36	0.48	0.00	0.00
Monoculture	Inorganic	Absent	11	0.12	0.31	0.00	0.46	0.00	0.00	0.00	0.70	0.51	4.29	4.14	0.62	0.24	0.26	0.11
			17	0.43	1.64	0.24	1.53	0.26	0.40	1.76	0.33	0.52	10.12	9.60	0.78	0.67	0.00	0.21
			28	0.33	0.66	0.00	0.70	0.27	0.32	0.76	0.41	0.32	7.90	8.36	0.56	0.77	0.00	0.22
Monoculture	Inorganic	Present	4	0.29	1.23	0.00	1.31	0.09	0.12	1.23	0.17	0.71	6.19	5.62	0.82	0.34	0.24	0.09
			6	0.18	0.00	0.00	0.25	0.00	0.00	0.41	0.00	0.00	3.78	3.74	0.52	0.40	0.00	0.00
			21	0.42	1.01	0.24	0.99	0.30	0.38	0.72	0.45	0.37	8.71	9.46	0.66	0.78	0.00	0.20
Monoculture	Organic	Absent	1	0.00	0.00	0.00	0.28	0.00	0.00	0.00	0.24	0.19	2.91	2.91	0.58	0.29	0.29	0.00
			3	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.19	0.00	3.30	3.51	0.48	0.48	0.00	0.00
			23	0.29	0.26	0.00	0.47	0.00	0.25	0.00	0.31	0.23	0.00	6.31	0.59	0.76	0.00	0.00
Monoculture	Organic	Present	12	0.00	0.35	0.00	0.48	0.00	0.00	0.00	0.77	0.40	3.68	3.71	0.59	0.15	0.15	0.15
			18	0.27	0.41	0.00	0.60	0.17	0.00	1.10	0.14	0.29	4.75	4.28	0.52	0.29	0.16	0.15
			26	0.34	0.90	0.19	1.05	0.32	0.28	1.39	0.37	0.50	8.72	8.73	0.68	0.60	0.00	0.23
Polyculture	Inorganic	Absent	15	0.27	1.01	0.19	1.81	0.00	0.17	1.14	0.19	0.74	7.68	8.33	0.88	0.63	0.23	0.00
			16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.16	1.21	0.00	0.00	0.00	0.00
			22	0.34	0.24	0.23	0.60	0.00	0.38	0.00	0.20	0.26	5.65	5.56	0.72	0.64	0.25	0.00
Polyculture	Inorganic	Present	5	0.23	0.61	0.00	1.00	0.00	0.10	1.14	0.12	0.83	5.60	5.32	0.72	0.25	0.18	0.11
			9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00	2.90	2.64	0.37	0.33	0.00	0.00
			30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.12	2.14	0.26	0.37	0.00	0.00
Polyculture	Organic	Absent	2	0.13	0.31	0.00	0.51	0.00	0.00	0.00	0.54	0.43	3.56	3.94	0.78	0.17	0.28	0.00
			7	0.60	0.81	0.25	1.01	0.00	0.29	0.85	0.36	0.37	7.46	8.02	0.75	0.83	0.00	0.00
			13	0.28	0.23	0.00	0.46	0.18	0.32	0.00	0.33	0.23	6.00	5.98	0.56	0.73	0.00	0.18
Polyculture	Organic	Present	19	0.23	2.27	0.15	1.84	0.11	0.15	1.83	0.20	1.49	7.12	7.25	0.92	0.27	0.24	0.11
			24	0.23	0.40	0.00	0.61	0.16	0.67	0.20	0.28	0.00	5.89	5.64	0.69	0.65	0.21	0.00
			25	0.21	0.25	0.00	0.43	0.17	0.40	0.23	0.00	0.21	5.13	5.34	0.49	0.67	0.00	0.00

Table A_2A(b) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2A(a). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 0 – 1 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area													
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 11	3-OH 14:0	i16:0	PLFA 12	16:1w7c	PLFA 13	PLFA 14	PLFA 15	16:0	PLFA 16	PLFA 17	PLFA 18	PLFA 19	PLFA 20	PLFA 21
Unplanted	Inorganic	Absent	8	0.59	0.28	3.10	2.18	9.57	0.84	5.23	0.00	13.63	0.21	0.38	0.25	2.01	2.49	0.40
			20	0.44	0.44	2.47	2.15	7.95	0.74	4.96	0.00	13.41	0.34	0.62	0.41	2.44	3.90	0.55
			27	0.64	0.79	3.13	2.70	8.54	0.76	5.20	0.00	11.74	0.50	0.31	0.55	2.76	4.61	0.68
Unplanted	Organic	Absent	10	0.47	0.22	3.61	1.42	6.37	0.73	3.81	0.25	16.09	0.18	0.36	0.24	1.61	2.34	0.31
			14	0.53	0.43	3.40	1.64	6.07	0.87	4.23	0.00	16.14	0.42	0.63	0.30	2.08	3.96	0.49
			29	0.44	0.45	2.54	1.58	4.89	0.61	3.76	0.00	12.21	0.41	0.54	0.41	2.46	5.20	0.67
Monoculture	Inorganic	Absent	11	0.57	0.24	3.47	2.01	8.80	0.81	4.89	0.00	13.37	0.14	0.45	0.21	2.45	2.49	0.37
			17	0.70	0.48	3.03	2.43	8.61	0.78	5.31	0.00	12.44	0.28	0.25	0.27	2.63	3.01	0.49
			28	0.71	0.73	3.17	2.64	7.92	0.74	4.79	0.00	11.42	0.53	0.34	0.60	3.05	5.07	0.62
Monoculture	Inorganic	Present	4	0.61	0.23	3.49	2.21	9.74	0.93	5.56	0.00	14.64	0.15	0.28	0.29	1.67	1.96	0.32
			6	0.54	0.36	2.95	2.05	8.10	0.85	5.29	0.00	13.50	0.26	0.53	0.37	2.50	3.12	0.52
			21	0.65	0.70	3.11	2.35	7.36	0.71	5.07	0.00	11.66	0.48	0.00	0.43	2.61	4.43	0.47
Monoculture	Organic	Absent	1	0.47	0.28	3.40	1.37	6.04	0.91	4.45	0.19	15.75	0.18	0.39	0.22	1.60	2.41	0.33
			3	0.53	0.42	3.13	1.71	5.90	0.86	4.32	0.00	15.07	0.34	0.53	0.00	2.54	4.22	0.00
			23	0.59	0.59	3.35	1.98	5.63	0.75	4.30	0.00	16.23	0.56	0.69	0.41	2.58	5.25	0.61
Monoculture	Organic	Present	12	0.50	0.16	2.56	2.31	11.66	0.60	6.88	0.00	15.12	0.12	0.33	0.27	1.71	1.85	0.39
			18	0.51	0.37	2.25	2.52	9.80	0.57	6.13	0.00	12.89	0.27	0.60	0.47	1.73	2.90	0.54
			26	0.70	0.58	2.70	2.76	9.34	0.59	6.13	0.00	12.54	0.50	0.26	0.49	2.18	4.22	0.65
Polyculture	Inorganic	Absent	15	0.55	0.35	2.83	2.06	7.35	0.50	2.69	0.59	16.78	0.22	0.15	0.17	2.33	2.17	0.38
			16	0.00	1.01	2.08	1.20	4.47	0.51	2.69	0.00	12.24	0.45	0.75	0.41	2.65	4.90	0.81
			22	0.51	0.57	2.76	1.82	5.64	0.66	2.60	0.00	18.55	0.44	0.68	0.33	2.26	4.05	0.58
Polyculture	Inorganic	Present	5	0.62	0.19	2.84	1.87	10.26	0.77	4.22	0.17	14.32	0.11	0.35	0.25	2.10	1.76	0.26
			9	0.56	0.32	2.67	1.65	7.47	0.58	4.64	0.00	13.83	0.31	0.55	0.54	2.68	3.93	0.51
			30	0.47	0.39	2.59	1.45	5.72	0.42	3.94	0.00	12.74	0.51	0.71	0.56	3.13	5.86	0.82
Polyculture	Organic	Absent	2	0.34	0.22	1.95	1.33	7.29	0.43	3.19	0.18	19.88	0.00	0.28	0.16	1.95	1.72	0.28
			7	0.53	0.97	2.81	2.22	7.45	0.72	4.02	0.00	13.69	0.35	0.00	0.44	2.31	3.53	0.59
			13	0.56	0.58	2.98	2.16	6.07	0.69	3.82	0.00	14.91	0.63	0.77	0.56	2.68	5.53	0.81
Polyculture	Organic	Present	19	0.61	0.22	3.31	1.88	11.43	0.75	4.36	0.15	12.45	0.11	0.15	0.17	1.78	1.50	0.25
			24	0.64	0.42	3.23	2.12	8.08	0.76	4.87	0.00	14.84	0.32	0.39	0.36	2.14	3.35	0.45
			25	0.57	0.42	2.74	1.92	6.39	0.63	4.04	0.00	13.09	0.47	0.55	0.43	2.58	4.69	0.61

Table A_2A(c) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2A(b). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 0 – 1 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area														
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 22	i17:0	PLFA 23	PLFA 24	17:0c	PLFA 25	PLFA 26	17:0	18:2w6c	PLFA 27	PLFA 28	PLFA 29	18:1w9c	18:1w9t	PLFA 30
Unplanted	Inorganic	Absent	8	0.40	1.47	1.39	0.91	0.28	2.08	0.45	0.95	0.57	0.00	0.62	4.12	10.70	12.02	0.28
			20	0.47	1.81	1.67	0.68	0.00	2.61	0.45	1.02	0.61	0.00	0.94	2.73	9.50	13.92	0.36
			27	0.49	1.71	1.58	0.52	2.40	0.20	0.32	0.81	0.41	0.00	0.33	1.13	5.83	8.73	0.32
Unplanted	Organic	Absent	10	0.32	1.47	1.47	1.18	0.29	1.95	0.60	1.01	0.66	0.15	0.60	6.09	12.23	11.16	0.69
			14	0.50	1.92	1.69	0.89	2.64	0.21	0.47	1.28	0.70	0.20	1.04	2.09	8.19	10.88	0.38
			29	0.53	2.04	1.92	0.46	2.73	0.29	0.43	1.20	0.62	0.00	1.58	1.59	7.93	11.84	0.51
Monoculture	Inorganic	Absent	11	0.51	1.40	1.54	0.99	0.27	2.04	0.48	0.82	0.62	0.00	0.84	4.18	9.80	12.48	0.43
			17	0.45	1.43	1.28	0.56	2.03	0.15	0.29	0.80	0.43	0.00	0.41	1.12	5.26	8.49	0.24
			28	0.52	1.74	1.64	0.52	2.50	0.23	0.32	0.97	0.48	0.00	0.33	1.02	5.84	8.81	0.34
Monoculture	Inorganic	Present	4	0.40	1.20	1.30	0.94	1.95	0.11	0.43	0.74	0.47	0.00	0.69	3.38	8.33	10.70	0.25
			6	0.45	1.66	1.54	0.86	0.00	2.58	0.45	1.05	0.66	0.16	0.80	2.74	8.83	13.28	0.33
			21	0.44	1.57	1.46	0.45	2.29	0.22	0.31	0.85	0.41	0.00	0.28	1.47	5.55	8.50	0.29
Monoculture	Organic	Absent	1	0.40	1.45	1.57	1.21	0.30	2.02	0.58	1.14	0.74	0.00	0.00	7.96	13.33	11.47	0.49
			3	0.47	1.80	1.63	0.94	0.24	2.46	0.48	1.31	0.79	0.20	0.67	3.03	9.55	12.11	0.36
			23	0.63	2.07	1.85	0.55	2.75	0.32	0.46	1.23	0.72	0.00	1.20	1.99	7.44	10.28	0.39
Monoculture	Organic	Present	12	0.43	1.07	1.32	0.76	0.00	2.19	0.45	0.42	0.26	0.00	0.42	6.58	10.26	11.68	0.15
			18	0.47	1.37	1.34	0.60	2.15	0.15	0.47	0.67	0.38	0.00	1.60	3.04	7.21	11.78	0.29
			26	0.43	1.45	1.38	0.44	2.23	0.15	0.33	0.73	0.30	0.00	0.51	1.65	4.81	8.20	0.25
Polyculture	Inorganic	Absent	15	0.38	1.61	1.25	0.56	1.75	0.00	0.36	0.81	0.43	0.38	0.52	4.67	8.69	7.12	0.49
			16	0.54	2.53	2.16	0.53	0.00	2.82	0.59	1.72	0.89	0.00	1.21	2.08	9.79	14.01	0.77
			22	0.50	2.13	1.58	0.33	2.21	0.23	0.46	1.02	0.50	0.39	1.23	3.36	7.09	7.67	0.61
Polyculture	Inorganic	Present	5	0.48	1.14	1.18	0.80	0.00	1.96	0.37	0.60	0.39	0.13	0.47	5.62	9.99	11.36	0.30
			9	0.51	1.84	1.63	0.70	0.00	2.78	0.43	1.29	0.69	0.20	0.86	2.62	9.52	14.52	0.32
			30	0.65	2.22	2.03	0.54	0.00	3.05	0.48	1.39	0.76	0.00	0.92	2.13	9.00	13.57	0.48
Polyculture	Organic	Absent	2	0.36	1.26	1.20	0.72	0.00	1.66	0.56	0.54	0.31	0.18	0.69	9.06	10.92	9.66	0.56
			7	0.39	1.52	1.43	0.52	2.01	0.00	0.35	0.88	0.43	0.00	0.58	1.95	7.14	8.73	0.37
			13	0.52	2.01	1.78	0.39	2.50	0.26	0.44	1.16	0.61	0.00	1.37	0.73	5.78	8.81	0.46
Polyculture	Organic	Present	19	0.37	0.92	1.15	0.78	1.68	0.00	0.35	0.52	0.39	0.11	0.39	4.53	8.83	9.65	0.37
			24	0.40	1.49	1.39	0.73	2.25	0.19	0.39	1.05	0.61	0.16	0.97	2.73	7.32	11.27	0.36
			25	0.51	1.76	1.59	0.48	2.51	0.26	0.40	1.10	0.63	0.17	1.24	2.02	7.40	10.59	0.42

Table A_2A(d) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2A(c). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 0 – 1 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area														
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 31	18:0	PLFA 32	PLFA 33	PLFA 34	PLFA 35	PLFA 36	PLFA 37	PLFA 38	19:0c	19:0	PLFA 39	PLFA 40	PLFA 41	PLFA 42
Unplanted	Inorganic	Absent	8	0.55	1.59	0.71	0.00	2.01	0.15	0.19	0.00	0.22	2.87	1.17	0.84	0.30	0.00	0.00
			20	0.66	1.85	0.81	0.00	2.19	0.00	0.00	0.00	0.31	5.26	2.71	1.05	0.38	0.00	0.00
			27	0.51	1.23	0.48	0.00	1.42	0.00	0.00	0.00	0.00	3.53	2.54	0.39	0.00	0.00	0.00
Unplanted	Organic	Absent	10	0.47	1.65	0.98	0.16	2.09	0.19	0.17	0.13	0.22	2.79	0.96	0.47	0.20	0.14	0.00
			14	0.47	1.79	0.77	0.15	1.93	0.19	0.16	0.12	0.32	4.17	1.32	0.63	0.23	0.13	0.00
			29	0.74	2.22	0.78	0.00	2.28	0.28	0.30	0.00	0.48	7.43	3.43	0.89	0.29	0.22	0.22
Monoculture	Inorganic	Absent	11	0.26	1.51	1.01	0.16	2.10	0.24	0.16	0.15	0.20	2.64	0.95	0.95	0.33	0.12	0.13
			17	0.37	1.00	0.46	0.00	1.19	0.00	0.00	0.00	0.17	2.55	1.23	0.65	0.22	0.00	0.00
			28	0.55	1.21	0.48	0.00	1.50	0.00	0.00	0.00	0.23	3.93	2.47	0.47	0.00	0.00	0.00
Monoculture	Inorganic	Present	4	0.33	1.27	0.69	0.14	1.59	0.11	0.17	0.10	0.17	1.99	0.83	0.46	0.24	0.10	0.09
			6	0.57	1.58	0.81	0.18	2.26	0.20	0.18	0.00	0.28	3.80	2.25	1.03	0.48	0.18	0.00
			21	0.47	1.22	0.48	0.00	1.42	0.20	0.00	0.00	0.22	3.96	2.45	0.55	0.21	0.00	0.00
Monoculture	Organic	Absent	1	0.46	1.76	1.03	0.16	2.38	0.00	0.20	0.00	0.23	2.79	1.21	0.49	0.26	0.15	0.16
			3	0.52	1.90	0.89	0.21	2.45	0.24	0.20	0.00	0.40	4.64	2.05	0.81	0.34	0.17	0.20
			23	0.51	1.99	0.82	0.22	2.12	0.29	0.00	0.00	0.39	5.34	1.77	0.64	0.23	0.00	0.19
Monoculture	Organic	Present	12	0.44	1.41	0.65	0.00	1.43	0.00	0.00	0.00	0.13	2.14	0.99	1.19	0.52	0.00	0.00
			18	0.62	1.68	0.60	0.16	1.53	0.17	0.16	0.00	0.23	3.23	1.87	1.37	0.72	0.17	0.16
			26	0.43	1.16	0.36	0.00	1.13	0.16	0.00	0.00	0.20	3.00	1.67	0.74	0.35	0.00	0.00
Polyculture	Inorganic	Absent	15	0.29	1.05	0.46	0.15	1.10	0.15	0.00	0.14	0.18	2.56	0.90	0.94	0.30	0.00	0.00
			16	0.64	2.71	0.91	0.00	2.99	0.39	0.00	0.00	0.58	8.15	5.02	1.52	0.44	0.00	0.00
			22	0.27	1.91	0.52	0.00	1.59	0.21	0.00	0.00	0.37	4.88	1.87	1.48	0.41	0.00	0.00
Polyculture	Inorganic	Present	5	0.37	1.20	0.79	0.12	1.39	0.13	0.12	0.12	0.12	2.04	0.77	0.75	0.36	0.00	0.12
			9	0.58	1.78	0.76	0.21	2.23	0.25	0.00	0.00	0.35	4.62	2.03	1.18	0.50	0.00	0.00
			30	0.75	2.18	0.74	0.00	2.53	0.38	0.00	0.00	0.39	6.54	2.88	1.19	0.45	0.00	0.00
Polyculture	Organic	Absent	2	0.42	1.56	0.70	0.11	1.07	0.13	0.11	0.14	0.13	2.16	0.93	3.18	0.79	0.19	0.12
			7	0.46	1.30	0.49	0.00	1.50	0.00	0.00	0.00	0.28	3.57	3.48	1.13	0.25	0.00	0.00
			13	0.49	1.91	0.50	0.00	2.09	0.29	0.00	0.00	0.34	5.43	2.39	0.51	0.00	0.00	0.00
Polyculture	Organic	Present	19	0.26	0.92	0.67	0.00	1.32	0.10	0.00	0.12	0.14	1.60	0.74	0.33	0.17	0.00	0.00
			24	0.51	1.49	0.68	0.00	1.71	0.20	0.15	0.00	0.25	3.39	1.59	0.67	0.29	0.00	0.00
			25	0.54	1.82	0.67	0.00	2.09	0.29	0.15	0.00	0.36	5.41	2.25	0.83	0.32	0.00	0.18

Table A_2A(e) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2A(d). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 0 – 1 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area									
Plant Community	**Fertilisation type**	**Earthworm presence**	**Mesocosm number**	**PLFA 43**	**PLFA 44**	**PLFA 45**	**PLFA 46**	**PLFA 47**	**20:0**	**PLFA 48**	**PLFA 49**	**PLFA 50**	**Total Area (%)**
Unplanted	Inorganic	Absent	8	0.00	0.00	0.22	0.00	0.00	0.14	0.00	0.00	0.15	100
			20	0.00	0.22	0.26	0.00	0.00	0.00	0.00	0.00	0.00	100
			27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Unplanted	Organic	Absent	10	0.00	0.00	0.00	0.17	0.00	0.14	0.00	0.00	0.13	100
			14	0.00	0.19	0.19	0.00	0.00	0.16	0.15	0.00	0.15	100
			29	0.00	0.40	0.28	0.00	0.00	0.26	0.29	0.18	0.23	100
Monoculture	Inorganic	Absent	11	0.00	0.00	0.17	0.00	0.11	0.13	0.14	0.00	0.13	100
			17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Monoculture	Inorganic	Present	4	0.00	0.09	0.13	0.00	0.00	0.11	0.00	0.00	0.00	100
			6	0.21	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.17	100
			21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Monoculture	Organic	Absent	1	0.00	0.00	0.19	0.00	0.00	0.17	0.00	0.00	0.00	100
			3	0.00	0.18	0.24	0.00	0.14	0.21	0.18	0.00	0.26	100
			23	0.00	0.21	0.22	0.00	0.00	0.21	0.00	0.00	0.00	100
Monoculture	Organic	Present	12	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			18	0.00	0.19	0.18	0.29	0.00	0.15	0.14	0.00	0.18	100
			26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Polyculture	Inorganic	Absent	15	0.00	0.13	0.00	0.00	0.00	0.00	0.13	0.00	0.14	100
			16	0.00	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			22	0.00	0.20	0.22	0.00	0.00	0.23	0.00	0.00	0.00	100
Polyculture	Inorganic	Present	5	0.14	0.16	0.00	0.00	0.00	0.11	0.10	0.00	0.00	100
			9	0.00	0.20	0.25	0.00	0.00	0.00	0.19	0.00	0.24	100
			30	0.00	0.00	0.26	0.28	0.00	0.00	0.00	0.00	0.00	100
Polyculture	Organic	Absent	2	0.00	0.29	0.12	0.19	0.00	0.00	0.12	0.00	0.00	100
			7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			13	0.00	0.20	0.20	0.00	0.00	0.22	0.21	0.00	0.19	100
Polyculture	Organic	Present	19	0.00	0.00	0.11	0.00	0.00	0.00	0.08	0.00	0.08	100
			24	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			25	0.26	0.26	0.00	0.00	0.00	0.23	0.21	0.15	0.25	100

Table A_2B(a) Raw phospholipid fatty acid (PLFA) analysis profile data for all mesocosms. These were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 1 – 5 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area													
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	12:0	PLFA 1	PLFA 2	14:0	PLFA 3	PLFA 4	PLFA 5	PLFA 6	PLFA 7	i15:0	a15:0	15:0	PLFA 8	PLFA 9	PLFA 10
Unplanted	Inorganic	Absent	8	0.17	1.50	0.12	2.03	0.15	0.19	1.56	0.16	1.13	7.36	6.32	0.86	0.18	0.19	0.11
			20	0.21	0.30	0.00	0.54	0.00	0.00	0.53	0.00	0.19	5.39	5.21	0.61	0.41	0.00	0.00
			27	0.28	0.57	0.00	0.77	0.23	0.27	0.53	0.36	0.30	7.61	8.97	0.68	0.58	0.00	0.00
Unplanted	Organic	Absent	10	0.24	0.78	0.00	0.99	0.00	0.09	1.11	0.10	0.98	4.98	4.81	0.87	0.10	0.29	0.00
			14	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.20	2.20	0.35	0.27	0.00	0.00
			29	0.40	0.93	0.20	1.01	0.28	0.36	0.69	0.46	0.37	8.65	9.69	0.71	0.67	0.00	0.20
Monoculture	Inorganic	Absent	11	0.26	0.64	0.00	0.97	0.00	0.12	1.02	0.11	0.56	5.46	4.56	0.70	0.24	0.17	0.14
			17	0.38	1.04	0.35	1.11	0.25	0.31	1.21	0.29	0.41	8.78	8.75	0.70	0.54	0.00	0.21
			28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	3.98	4.31	0.44	0.47	0.00	0.00
Monoculture	Inorganic	Present	4	0.24	1.97	0.12	1.83	0.14	0.23	1.70	0.27	0.74	9.02	7.75	0.76	0.52	0.14	0.12
			6	0.27	0.33	0.00	0.55	0.00	0.00	0.59	0.22	0.26	6.32	6.27	0.65	0.76	0.00	0.00
			21	0.31	0.35	0.00	0.56	0.19	0.48	0.28	0.00	0.26	6.31	6.30	0.61	0.79	0.00	0.00
Monoculture	Organic	Absent	1	0.31	2.25	0.21	2.21	0.15	0.19	2.12	0.24	0.63	8.40	7.57	0.84	0.50	0.20	0.18
			3	0.40	0.33	0.00	0.55	0.00	0.57	0.20	0.24	0.00	5.30	5.53	0.62	0.69	0.00	0.00
			23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	3.12	3.36	0.36	0.43	0.00	0.00
Monoculture	Organic	Present	12	0.36	1.94	0.18	1.93	0.23	0.19	1.79	0.25	0.77	8.79	7.85	0.76	0.51	0.13	0.20
			18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.99	1.92	0.31	0.31	0.00	0.00
			26	0.39	0.85	0.26	0.98	0.32	0.35	0.76	0.49	0.41	9.21	9.43	0.69	0.82	0.00	0.23
Polyculture	Inorganic	Absent	15	0.28	0.82	0.00	1.14	0.15	1.12	0.12	0.00	0.75	6.28	5.13	0.93	0.22	0.25	0.10
			16	0.27	0.66	0.00	0.81	0.00	0.00	0.26	0.82	0.23	7.48	7.35	0.72	0.66	0.00	0.00
			22	0.18	0.22	0.00	0.38	0.17	0.30	0.24	0.00	0.19	5.18	5.54	0.47	0.59	0.00	0.15
Polyculture	Inorganic	Present	5	0.20	0.30	0.00	0.74	0.00	0.12	0.00	0.71	0.34	5.38	4.84	0.74	0.52	0.38	0.00
			9	0.37	0.68	0.00	0.91	0.00	0.00	0.25	0.72	0.28	8.09	8.89	0.71	0.80	0.00	0.00
			30	0.36	0.73	0.24	0.88	0.28	0.33	0.56	0.43	0.32	7.86	8.17	0.62	0.73	0.00	0.20
Polyculture	Organic	Absent	2	0.16	0.25	0.00	0.44	0.00	0.00	0.00	0.60	0.30	4.52	4.44	0.63	0.46	0.25	0.00
			7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.18	3.36	0.42	0.46	0.00	0.00
			13	0.19	0.21	0.00	0.37	0.00	0.00	0.23	0.23	0.18	4.87	5.22	0.48	0.62	0.00	0.00
Polyculture	Organic	Present	19	0.24	1.25	0.11	1.74	0.16	0.21	1.34	0.17	0.75	7.90	7.81	0.99	0.54	0.28	0.12
			24	0.46	0.73	0.00	0.99	0.00	0.28	0.79	0.38	0.41	8.06	8.67	0.82	0.83	0.00	0.00
			25	0.34	0.42	0.00	0.57	0.00	0.00	0.00	0.00	0.32	6.63	6.87	0.54	0.63	0.00	0.00

Table A_2B(b) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2B(a). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 1 – 5 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area													
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 11	3-OH 14:0	i16:0	PLFA 12	16:1w7c	PLFA 13	PLFA 14	PLFA 15	16:0	PLFA 16	PLFA 17	PLFA 18	PLFA 19	PLFA 20	PLFA 21
Unplanted	Inorganic	Absent	8	0.59	0.17	3.01	2.31	9.54	0.72	4.28	0.13	14.07	0.07	0.18	0.17	1.90	1.65	0.24
			20	0.47	0.40	2.61	2.26	8.17	0.72	5.23	0.00	15.17	0.28	0.52	0.37	2.27	3.33	0.47
			27	0.60	0.71	3.08	2.65	7.69	0.77	4.98	0.00	14.26	0.58	0.00	0.47	2.42	4.57	0.65
Unplanted	Organic	Absent	10	0.48	0.16	2.74	1.36	10.94	0.49	4.13	0.29	15.12	0.11	0.28	0.14	1.83	1.32	0.17
			14	0.36	0.32	2.27	1.66	7.36	0.59	4.77	0.00	12.83	0.27	0.53	0.43	2.49	3.52	0.47
			29	0.61	0.66	2.95	2.39	7.86	0.67	5.15	0.00	13.07	0.50	0.24	0.48	2.31	4.27	0.56
Monoculture	Inorganic	Absent	11	0.60	0.20	2.99	2.12	8.88	0.63	5.20	0.12	14.02	0.12	0.32	0.27	1.87	1.90	0.29
			17	0.64	0.61	3.02	2.71	9.08	0.76	5.99	0.00	12.10	0.35	0.21	0.44	2.40	3.46	0.44
			28	0.49	0.50	2.85	2.17	6.53	0.65	4.62	0.00	14.84	0.60	0.63	0.50	2.60	5.21	0.76
Monoculture	Inorganic	Present	4	0.79	0.25	3.53	2.09	9.23	0.72	4.48	0.00	11.86	0.21	0.20	0.27	2.27	2.47	0.30
			6	0.68	0.46	3.46	1.80	7.80	0.71	4.77	0.00	14.95	0.42	0.55	0.34	2.48	3.97	0.55
			21	0.64	0.48	3.28	2.01	6.43	0.66	4.16	0.00	14.65	0.52	0.70	0.39	2.52	4.97	0.55
Monoculture	Organic	Absent	1	0.77	0.31	3.87	2.58	9.52	1.01	5.79	0.00	11.75	0.20	0.16	0.30	1.94	2.15	0.35
			3	0.64	0.37	3.21	1.86	6.98	0.84	4.41	0.00	13.85	0.37	0.53	0.31	2.16	3.60	0.46
			23	0.52	0.47	2.93	1.81	6.09	0.68	3.94	0.00	13.18	0.53	0.65	0.52	2.73	5.56	0.67
Monoculture	Organic	Present	12	0.82	0.31	3.55	2.42	9.73	0.73	5.62	0.00	12.15	0.22	0.18	0.33	1.93	2.33	0.43
			18	0.43	0.41	2.49	1.72	7.19	0.62	4.50	0.00	13.71	0.31	0.64	0.37	2.59	3.82	0.64
			26	0.63	1.10	3.29	2.45	7.44	0.69	4.90	0.00	12.48	0.54	0.28	0.46	2.61	4.49	0.68
Polyculture	Inorganic	Absent	15	0.61	0.20	3.12	1.89	8.58	0.69	4.99	0.26	16.65	0.08	0.35	0.27	1.66	1.67	0.29
			16	0.65	0.60	3.33	2.53	9.21	0.71	5.83	0.00	12.99	0.33	0.00	0.45	2.73	3.54	0.46
			22	0.52	0.45	2.73	1.96	6.17	0.60	4.59	0.00	12.92	0.52	0.57	0.46	2.57	5.00	0.60
Polyculture	Inorganic	Present	5	0.49	0.34	2.58	1.65	7.64	0.54	3.37	0.47	18.22	0.19	0.32	0.21	2.43	2.31	0.35
			9	0.58	0.77	3.23	2.43	7.90	0.78	4.13	0.00	13.60	0.43	0.00	0.00	2.66	3.92	0.63
			30	0.55	0.71	2.94	2.56	7.11	0.68	3.90	0.00	13.43	0.60	0.00	0.49	2.67	4.90	0.71
Polyculture	Organic	Absent	2	0.62	0.25	3.09	1.92	8.76	0.71	5.40	0.14	15.08	0.22	0.41	0.27	2.16	2.63	0.49
			7	0.54	0.39	2.88	2.01	7.95	0.66	4.83	0.00	13.72	0.43	0.61	0.44	2.82	4.49	0.60
			13	0.50	0.50	2.86	1.79	5.31	0.64	4.30	0.00	13.86	0.54	0.57	0.42	2.43	5.06	0.61
Polyculture	Organic	Present	19	0.57	0.33	3.09	1.91	9.18	0.68	4.50	0.16	16.70	0.19	0.19	0.25	2.21	2.46	0.50
			24	0.49	0.97	2.96	2.41	7.66	0.71	4.16	0.00	14.45	0.42	0.00	0.45	2.40	3.87	0.63
			25	0.50	0.90	2.91	2.50	7.44	0.71	4.38	0.00	13.26	0.64	0.00	0.63	3.06	5.70	0.91

Table A_2B(c) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2B(b). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 1 – 5 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area													
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 22	i17:0	PLFA 23	PLFA 24	17:0c	PLFA 25	PLFA 26	17:0	18:2w6c	PLFA 27	PLFA 28	PLFA 29	18:1w9c	18:1w9t	PLFA 30
Unplanted	Inorganic	Absent	8	0.33	1.08	1.12	0.77	1.84	0.07	0.38	0.38	0.33	0.09	0.51	6.41	9.34	8.74	0.28
			20	0.40	1.65	1.43	0.61	2.61	0.00	0.41	0.94	0.47	0.00	1.30	2.23	7.54	12.41	0.30
			27	0.48	1.70	1.62	0.41	2.55	0.00	0.35	0.78	0.40	0.00	0.27	1.33	5.79	9.53	0.33
Unplanted	Organic	Absent	10	0.36	1.02	1.19	0.80	0.00	2.11	0.49	0.28	0.31	0.11	0.74	7.95	9.11	11.34	0.51
			14	0.43	1.73	1.60	0.88	0.00	2.54	0.46	1.07	0.65	0.00	1.26	3.46	9.27	16.25	0.36
			29	0.43	1.54	1.44	0.41	2.17	0.19	0.32	0.74	0.38	0.00	0.36	1.28	5.12	8.70	0.28
Monoculture	Inorganic	Absent	11	0.45	1.27	1.34	0.82	0.24	1.66	0.42	0.58	0.36	0.00	0.75	6.26	10.34	10.78	0.27
			17	0.42	1.55	1.40	0.53	2.22	0.00	0.32	0.66	0.36	0.00	0.56	1.54	5.66	9.01	0.21
			28	0.58	2.10	1.90	0.47	0.00	2.61	0.48	1.09	0.57	0.00	0.67	1.55	7.16	11.62	0.41
Monoculture	Inorganic	Present	4	0.45	1.34	1.18	0.80	1.88	0.11	0.32	0.92	0.56	0.20	0.34	2.39	7.61	9.27	0.25
			6	0.53	1.84	1.61	0.67	2.44	0.22	0.40	1.13	0.68	0.20	1.06	1.83	6.68	10.14	0.28
			21	0.54	1.91	1.69	0.49	2.46	0.25	0.41	1.11	0.63	0.00	1.07	1.64	6.34	9.36	0.38
Monoculture	Organic	Absent	1	0.36	1.18	1.13	0.79	1.70	0.00	0.32	0.78	0.46	0.00	0.29	2.42	7.54	8.94	0.23
			3	0.44	1.63	1.49	0.69	2.24	0.23	0.48	1.06	0.65	0.00	1.08	3.02	8.08	10.89	0.33
			23	0.58	2.11	1.90	0.58	0.00	2.90	0.47	1.39	0.79	0.00	0.78	2.42	8.70	12.11	0.45
Monoculture	Organic	Present	12	0.44	1.23	1.23	0.74	1.95	0.00	0.31	0.74	0.42	0.00	0.36	2.77	7.43	8.80	0.18
			18	0.55	2.01	1.80	0.74	0.00	2.87	0.52	1.25	0.74	0.25	0.94	2.82	9.55	14.64	0.40
			26	0.50	1.69	1.54	0.42	2.26	0.21	0.35	0.89	0.41	0.00	0.21	1.27	5.13	7.96	0.28
Polyculture	Inorganic	Absent	15	0.40	1.14	1.22	0.87	1.73	0.09	0.45	0.51	0.35	0.09	0.87	7.01	8.69	9.80	0.38
			16	0.43	1.59	1.43	0.68	2.38	0.00	0.36	0.84	0.44	0.00	0.66	1.69	6.18	10.51	0.21
			22	0.51	1.85	1.79	0.44	2.55	0.27	0.40	1.03	0.52	0.00	1.27	1.56	6.34	10.45	0.42
Polyculture	Inorganic	Present	5	0.41	1.74	1.33	0.64	0.00	1.98	0.48	0.99	0.46	0.38	0.76	4.38	9.16	9.74	0.80
			9	0.52	1.82	1.54	0.41	2.20	0.00	0.38	1.02	0.50	0.00	0.53	1.36	6.53	8.48	0.41
			30	0.51	1.75	1.57	0.36	2.28	0.21	0.35	0.89	0.40	0.00	0.40	1.85	6.47	7.60	0.42
Polyculture	Organic	Absent	2	0.46	1.46	1.42	0.85	0.00	2.39	0.43	1.09	0.54	0.00	0.60	3.41	9.34	12.18	0.44
			7	0.57	1.92	1.74	0.77	0.00	2.89	0.43	1.19	0.70	0.00	0.86	2.41	8.15	13.43	0.41
			13	0.49	1.95	1.72	0.48	2.62	0.28	0.44	1.12	0.58	0.00	1.52	2.32	7.30	10.43	0.39
Polyculture	Organic	Present	19	0.39	1.57	1.37	0.77	2.09	0.11	0.47	0.81	0.44	0.21	0.52	4.39	9.12	0.00	0.51
			24	0.37	1.61	1.44	0.55	2.11	0.00	0.36	0.91	0.43	0.00	0.75	1.92	6.91	8.11	0.36
			25	0.54	1.99	1.84	0.42	2.62	0.00	0.40	1.04	0.44	0.00	0.29	1.55	6.76	8.54	0.44

Table A_2B(d) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2B(c). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 1 – 5 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area														
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 31	18:0	PLFA 32	PLFA 33	PLFA 34	PLFA 35	PLFA 36	PLFA 37	PLFA 38	19:0c	19:0	PLFA 39	PLFA 40	PLFA 41	PLFA 42
Unplanted	Inorganic	Absent	8	0.21	1.19	0.85	0.00	1.00	0.12	0.00	0.10	0.10	1.63	0.46	0.76	0.21	0.06	0.00
			20	0.53	1.61	0.70	0.00	1.64	0.00	0.00	0.00	0.24	3.93	1.81	1.08	0.33	0.00	0.00
			27	0.52	1.38	0.51	0.00	1.31	0.00	0.00	0.00	0.00	3.79	1.84	0.52	0.00	0.00	0.00
Unplanted	Organic	Absent	10	0.24	1.23	1.19	0.09	0.88	0.13	0.10	0.10	0.08	2.06	0.62	0.93	0.47	0.20	0.12
			14	0.66	1.82	0.92	0.00	2.14	0.00	0.00	0.00	0.33	4.95	2.73	1.68	0.71	0.29	0.23
			29	0.49	1.30	0.52	0.00	1.27	0.00	0.00	0.00	0.22	3.69	1.82	0.56	0.24	0.00	0.00
Monoculture	Inorganic	Absent	11	0.45	1.33	0.80	0.12	1.35	0.11	0.14	0.11	0.12	2.15	0.85	1.19	0.45	0.14	0.12
			17	0.50	1.17	0.50	0.00	1.26	0.00	0.00	0.00	0.00	2.98	1.70	0.67	0.20	0.00	0.00
			28	0.72	2.05	0.66	0.00	1.94	0.23	0.00	0.00	0.38	5.89	2.14	1.12	0.42	0.00	0.00
Monoculture	Inorganic	Present	4	0.34	1.02	0.66	0.14	1.50	0.14	0.00	0.11	0.18	2.24	0.73	0.42	0.19	0.00	0.00
			6	0.39	1.55	0.60	0.00	1.80	0.21	0.00	0.00	0.25	3.75	1.65	0.70	0.25	0.00	0.00
			21	0.45	1.77	0.53	0.00	1.87	0.25	0.18	0.00	0.35	4.62	1.71	0.63	0.20	0.00	0.00
Monoculture	Organic	Absent	1	0.28	0.93	0.60	0.00	1.62	0.00	0.00	0.13	0.18	2.06	0.89	0.46	0.00	0.00	0.00
			3	0.48	1.86	0.64	0.00	2.06	0.20	0.00	0.00	0.25	4.24	1.83	0.90	0.53	0.00	0.00
			23	0.60	2.00	0.69	0.00	2.47	0.35	0.00	0.00	0.36	6.13	2.52	0.75	0.27	0.00	0.00
Monoculture	Organic	Present	12	0.40	1.00	0.46	0.00	1.26	0.00	0.00	0.00	0.16	1.93	0.88	0.47	0.22	0.00	0.00
			18	0.74	2.11	0.81	0.00	2.35	0.28	0.00	0.00	0.40	5.14	2.99	1.26	0.59	0.00	0.00
			26	0.46	1.31	0.42	0.00	1.31	0.18	0.00	0.00	0.00	3.31	1.88	0.54	0.24	0.00	0.00
Polyculture	Inorganic	Absent	15	0.32	1.15	0.79	0.10	0.98	0.09	0.11	0.09	0.10	1.57	0.56	0.85	0.35	0.17	0.13
			16	0.47	1.25	0.59	0.00	1.46	0.00	0.00	0.00	0.22	3.13	2.15	0.71	0.00	0.00	0.00
			22	0.64	1.86	0.66	0.00	1.97	0.27	0.00	0.35	0.49	5.62	2.50	0.77	0.30	0.17	0.20
Polyculture	Inorganic	Present	5	0.37	1.53	0.71	0.21	1.24	0.17	0.11	0.14	0.20	3.04	0.83	1.28	0.43	0.18	0.00
			9	0.41	1.40	0.52	0.00	1.53	0.00	0.00	0.00	0.34	3.93	2.66	0.72	0.00	0.00	0.00
			30	0.48	1.34	0.45	0.00	1.48	0.20	0.00	0.00	0.26	4.23	2.64	0.70	0.17	0.00	0.00
Polyculture	Organic	Absent	2	0.53	1.44	0.73	0.18	1.81	0.17	0.16	0.00	0.25	2.71	1.07	0.77	0.37	0.12	0.13
			7	0.68	1.68	0.86	0.00	2.10	0.27	0.00	0.00	0.32	4.90	2.41	0.91	0.39	0.00	0.00
			13	0.61	2.04	0.64	0.00	2.06	0.28	0.00	0.00	0.37	5.64	2.59	0.91	0.31	0.00	0.00
Polyculture	Organic	Present	19	0.43	1.37	0.75	0.18	1.34	0.16	0.00	0.12	0.19	2.78	1.01	1.21	0.35	0.00	0.00
			24	0.40	1.20	0.52	0.00	1.33	0.00	0.00	0.00	0.25	3.33	2.30	0.86	0.00	0.00	0.00
			25	0.55	1.54	0.48	0.00	1.68	0.00	0.00	0.00	0.00	4.42	3.81	0.77	0.00	0.00	0.00

Table A_2B(e) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2B(d). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 1 – 5 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area									
Plant Community	**Fertilisation type**	**Earthworm presence**	**Mesocosm number**	**PLFA 43**	**PLFA 44**	**PLFA 45**	**PLFA 46**	**PLFA 47**	**20:0**	**PLFA 48**	**PLFA 49**	**PLFA 50**	**Total Area (%)**
Unplanted	Inorganic	Absent	8	0.00	0.09	0.18	0.00	0.07	0.09	0.10	0.00	0.07	100
			20	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	100
			27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Unplanted	Organic	Absent	10	0.12	0.12	0.00	0.00	0.00	0.10	0.00	0.00	0.00	100
			14	0.00	0.24	0.24	0.00	0.00	0.00	0.00	0.00	0.00	100
			29	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Monoculture	Inorganic	Absent	11	0.15	0.13	0.19	0.00	0.00	0.00	0.10	0.00	0.00	100
			17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			28	0.00	0.26	0.23	0.00	0.00	0.21	0.19	0.00	0.00	100
Monoculture	Inorganic	Present	4	0.00	0.00	0.12	0.00	0.09	0.00	0.11	0.00	0.13	100
			6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			21	0.00	0.19	0.22	0.00	0.00	0.20	0.00	0.00	0.16	100
Monoculture	Organic	Absent	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			3	0.00	0.31	0.20	0.00	0.00	0.19	0.00	0.00	0.00	100
			23	0.00	0.25	0.21	0.00	0.00	0.23	0.00	0.00	0.23	100
Monoculture	Organic	Present	12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			18	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Polyculture	Inorganic	Absent	15	0.11	0.15	0.08	0.00	0.00	0.00	0.08	0.00	0.00	100
			16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			22	0.00	0.34	0.27	0.00	0.00	0.22	0.23	0.21	0.25	100
Polyculture	Inorganic	Present	5	0.11	0.13	0.18	0.00	0.10	0.13	0.17	0.00	0.12	100
			9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Polyculture	Organic	Absent	2	0.00	0.18	0.00	0.00	0.13	0.15	0.14	0.00	0.18	100
			7	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			13	0.00	0.26	0.23	0.00	0.00	0.21	0.21	0.00	0.00	100
Polyculture	Organic	Present	19	0.12	0.15	0.13	0.00	0.10	0.00	0.17	0.00	0.15	100
			24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100

Table A_2C(a) Raw phospholipid fatty acid (PLFA) analysis profile data for all mesocosms. These were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 5 – 10 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area														
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	12:0	PLFA 1	PLFA 2	14:0	PLFA 3	PLFA 4	PLFA 5	PLFA 6	PLFA 7	i15:0	a15:0	15:0	PLFA 8	PLFA 9	PLFA 10
Unplanted	Inorganic	Absent	8	0.33	0.56	0.00	0.85	0.11	0.14	0.76	0.16	0.33	6.03	5.20	0.64	0.48	0.16	0.11
			20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.28	0.00	3.25	3.15	0.45	0.36	0.00	0.00
			27	0.21	0.18	0.00	0.32	0.00	0.00	0.28	0.19	0.16	4.63	4.81	0.47	0.54	0.00	0.00
Unplanted	Organic	Absent	10	0.23	0.83	0.00	1.11	0.14	0.16	1.05	0.16	0.46	6.19	5.34	0.77	0.35	0.20	0.09
			14	0.24	0.37	0.15	0.58	0.13	0.66	0.15	0.00	0.23	5.12	4.92	0.58	0.44	0.12	0.00
			29	0.25	0.21	0.00	0.35	0.00	0.00	0.35	0.19	0.18	4.62	5.43	0.45	0.40	0.00	0.00
Monoculture	Inorganic	Absent	11	0.24	0.13	0.00	0.31	0.00	0.00	0.00	0.25	0.63	2.58	3.50	0.58	0.00	0.40	0.00
			17	0.20	0.00	0.00	0.28	0.00	0.00	0.00	0.34	0.00	4.39	4.54	0.56	0.48	0.00	0.00
			28	0.43	1.30	0.25	1.27	0.38	0.50	0.79	0.60	0.42	10.25	11.09	0.71	0.93	0.21	0.22
Monoculture	Inorganic	Present	4	0.28	0.47	0.00	0.89	0.15	0.69	0.09	0.00	0.38	5.08	4.91	0.76	0.42	0.24	0.08
			6	0.20	0.00	0.00	0.21	0.00	0.18	0.00	0.00	0.00	2.69	3.37	0.34	0.30	0.21	0.00
			21	0.43	0.54	0.23	0.58	0.00	0.26	0.34	0.28	0.00	5.60	7.16	0.46	0.30	0.00	0.00
Monoculture	Organic	Absent	1	0.20	1.49	0.11	1.63	0.13	0.16	1.57	0.15	1.31	7.61	7.13	0.84	0.27	0.27	0.10
			3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	3.14	2.82	0.46	0.39	0.00	0.00
			23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.44	3.67	0.41	0.43	0.00	0.00
Monoculture	Organic	Present	12	0.20	0.69	0.00	1.29	0.00	0.94	0.09	0.00	1.31	5.48	5.76	0.84	0.21	0.30	0.00
			18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.39	2.37	0.37	0.36	0.00	0.00
			26	0.35	0.86	0.27	1.03	0.28	0.39	0.76	0.50	0.41	8.97	9.42	0.69	0.76	0.00	0.21
Polyculture	Inorganic	Absent	15	0.35	1.44	0.00	2.10	0.00	0.20	1.22	0.19	1.10	9.27	8.25	0.95	0.56	0.24	0.00
			16	0.27	0.35	0.00	0.58	0.00	0.00	0.53	0.19	0.24	6.13	5.91	0.65	0.69	0.17	0.00
			22	0.36	0.86	0.18	0.87	0.28	0.32	0.61	0.44	0.34	8.16	8.50	0.58	0.83	0.00	0.20
Polyculture	Inorganic	Present	5	0.21	0.60	0.09	0.85	0.11	0.87	0.12	0.00	0.58	5.09	4.78	0.77	0.31	0.21	0.09
			9	0.39	0.35	0.00	0.50	0.00	0.61	0.20	0.00	0.24	5.52	5.56	0.55	0.59	0.00	0.00
			30	0.00	0.00	0.00	0.27	0.00	0.00	0.31	0.00	0.00	4.76	5.01	0.46	0.64	0.00	0.00
Polyculture	Organic	Absent	2	0.22	1.35	0.13	1.57	0.00	0.13	0.94	0.18	0.62	6.55	6.41	0.88	0.47	0.23	0.08
			7	0.50	1.52	0.27	1.41	0.16	0.28	0.83	0.35	0.46	8.58	9.43	0.76	0.77	0.00	0.16
			13	0.20	0.20	0.15	0.37	0.15	0.34	0.00	0.23	0.20	4.85	4.96	0.49	0.58	0.00	0.15
Polyculture	Organic	Present	19	0.34	0.44	0.00	1.08	0.10	0.65	0.11	0.00	0.58	5.17	5.81	0.90	0.44	0.27	0.00
			24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.52	1.56	0.00	0.00	0.00	0.00
			25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.26	2.85	0.33	0.00	0.00	0.00

Table A_2C(b) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2C(a). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 5 – 10 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area													
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 11	3-OH 14:0	i16:0	PLFA 12	16:1w7c	PLFA 13	PLFA 14	PLFA 15	16:0	PLFA 16	PLFA 17	PLFA 18	PLFA 19	PLFA 20	PLFA 21
Unplanted	Inorganic	Absent	8	0.60	0.30	3.04	1.77	7.29	0.61	4.14	0.00	14.60	0.25	0.46	0.33	2.04	2.59	0.47
			20	0.41	0.71	2.68	1.94	7.72	0.67	4.64	0.00	15.35	0.31	0.68	0.38	2.97	3.68	0.53
			27	0.47	0.43	2.68	1.84	5.76	0.59	3.90	0.00	14.08	0.52	0.66	0.46	2.47	4.67	0.72
Unplanted	Organic	Absent	10	0.61	0.22	3.21	2.41	9.37	0.90	6.01	0.06	14.39	0.17	0.34	0.27	2.02	2.13	0.41
			14	0.50	0.43	2.82	2.00	7.57	0.78	4.94	0.00	15.26	0.31	0.65	0.32	2.24	3.20	0.50
			29	0.33	0.91	2.35	2.00	6.44	0.69	4.21	0.00	11.98	0.46	0.87	0.40	2.45	4.22	0.58
Monoculture	Inorganic	Absent	11	0.33	0.19	2.56	0.70	7.56	0.63	1.89	0.39	15.93	0.00	0.27	2.05	0.00	1.57	0.36
			17	0.60	0.42	3.17	2.10	7.36	0.79	4.66	0.00	14.48	0.33	0.57	0.40	2.64	3.79	0.49
			28	0.74	0.61	3.20	2.44	7.27	0.73	4.66	0.00	11.20	0.52	0.25	0.50	2.56	4.45	0.57
Monoculture	Inorganic	Present	4	0.43	0.26	2.55	1.47	6.96	0.51	3.61	0.24	17.91	0.15	0.37	0.17	1.95	2.23	0.41
			6	0.23	0.48	1.84	1.42	4.64	0.52	2.98	0.00	11.75	0.38	0.89	0.30	1.87	3.54	0.59
			21	0.35	0.84	2.43	2.19	5.97	0.60	3.75	0.00	12.21	0.61	0.00	0.60	2.24	4.14	0.66
Monoculture	Organic	Absent	1	0.65	0.19	3.42	1.76	10.61	0.54	3.84	0.20	13.39	0.00	0.00	0.29	1.80	1.67	0.28
			3	0.50	0.35	2.70	1.65	8.37	0.58	4.91	0.00	13.84	0.25	0.49	0.36	2.58	3.20	0.55
			23	0.58	0.46	2.88	1.74	6.86	0.56	4.59	0.00	14.68	0.51	0.57	0.51	2.98	5.50	0.68
Monoculture	Organic	Present	12	0.57	0.17	3.48	1.22	9.61	0.62	3.02	0.32	17.25	0.10	0.25	0.15	1.56	1.41	0.15
			18	0.44	0.31	2.67	1.52	7.34	0.58	4.53	0.00	13.25	0.30	0.52	0.38	2.51	3.64	0.53
			26	0.71	0.62	3.16	2.40	7.73	0.68	4.93	0.00	12.70	0.52	0.27	0.43	2.61	4.58	0.58
Polyculture	Inorganic	Absent	15	0.74	0.51	4.17	1.74	7.12	0.88	3.49	0.18	13.85	0.22	0.21	0.30	2.01	2.42	0.25
			16	0.64	0.44	3.27	1.87	7.29	0.69	4.39	0.00	15.33	0.37	0.60	0.38	2.33	3.59	0.54
			22	0.66	0.66	3.25	2.33	7.20	0.71	4.46	0.00	11.12	0.57	0.30	0.50	2.82	5.01	0.73
Polyculture	Inorganic	Present	5	0.57	0.21	2.94	1.78	9.25	0.74	4.49	0.00	14.49	0.15	0.41	0.19	2.28	2.15	0.34
			9	0.61	0.49	3.09	2.13	7.80	0.76	4.90	0.00	12.99	0.36	0.77	0.37	2.53	3.86	0.62
			30	0.54	0.52	2.91	2.08	6.57	0.69	4.25	0.00	13.01	0.52	0.66	0.48	2.89	5.18	0.82
Polyculture	Organic	Absent	2	0.63	0.30	3.73	1.73	6.74	0.92	5.38	0.15	15.50	0.18	0.19	0.21	1.50	2.22	0.35
			7	0.63	0.68	3.42	2.20	6.87	0.83	5.35	0.00	12.72	0.35	0.00	0.30	2.07	3.34	0.45
			13	0.54	0.46	2.66	1.82	6.17	0.48	4.08	0.00	13.66	0.50	0.62	0.46	2.53	4.79	0.69
Polyculture	Organic	Present	19	0.52	0.22	2.67	1.52	6.92	0.51	2.53	0.63	22.78	0.15	0.30	0.16	1.89	1.85	0.32
			24	0.00	0.43	1.93	1.02	4.57	0.50	2.37	0.00	13.82	0.00	0.69	0.00	2.49	3.54	0.55
			25	0.00	0.61	2.32	1.56	5.06	0.56	3.01	0.00	16.10	0.51	0.79	0.44	2.19	4.37	0.59

Table A_2C(c) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2C(b). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 5 – 10 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area													
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 22	i17:0	PLFA 23	PLFA 24	17:0c	PLFA 25	PLFA 26	17:0	18:2w6c	PLFA 27	PLFA 28	PLFA 29	18:1w9c	18:1w9t	PLFA 30
Unplanted	Inorganic	Absent	8	0.49	1.57	1.36	0.79	2.31	0.14	0.45	1.17	0.58	0.17	0.80	3.76	8.67	11.01	0.25
			20	0.51	1.93	1.67	0.74	0.00	2.62	0.52	1.03	0.72	0.00	1.03	2.73	8.73	12.72	0.36
			27	0.55	2.00	1.75	0.47	2.48	0.29	0.46	1.11	0.66	0.17	1.29	1.77	7.11	11.37	0.42
Unplanted	Organic	Absent	10	0.45	1.36	1.27	0.80	2.16	0.11	0.42	0.78	0.44	0.08	0.76	3.26	8.28	10.01	0.33
			14	0.44	1.69	1.46	0.64	2.20	0.17	0.42	0.92	0.56	0.13	1.16	2.09	7.61	11.13	0.36
			29	0.46	1.77	1.72	0.45	2.49	0.24	0.40	0.85	0.49	0.00	2.13	1.31	7.20	12.18	0.41
Monoculture	Inorganic	Absent	11	0.00	0.96	1.38	0.98	0.00	1.75	0.59	0.41	0.45	0.17	0.68	13.05	14.77	11.72	0.76
			17	0.44	1.74	1.59	0.76	0.00	2.48	0.44	1.13	0.70	0.21	0.87	2.58	8.49	12.93	0.43
			28	0.45	1.48	1.38	0.41	2.19	0.21	0.28	0.84	0.41	0.00	0.26	1.13	4.90	7.51	0.26
Monoculture	Inorganic	Present	4	0.37	1.54	1.27	0.73	1.88	0.11	0.49	0.81	0.45	0.25	1.13	5.96	9.41	9.12	0.57
			6	0.47	1.76	1.65	0.53	1.94	0.20	0.43	1.01	0.58	0.00	3.09	1.96	8.81	12.70	0.77
			21	0.54	1.91	1.96	0.40	2.17	0.00	0.40	0.86	0.46	0.00	0.40	1.31	7.74	11.32	0.51
Monoculture	Organic	Absent	1	0.34	1.02	1.15	0.81	1.78	0.00	0.41	0.57	0.41	0.11	0.54	5.05	8.95	9.66	0.38
			3	0.48	1.72	1.53	0.84	0.00	2.66	0.46	1.11	0.75	0.21	1.04	3.43	9.01	14.85	0.30
			23	0.59	2.01	1.83	0.56	0.00	2.86	0.45	1.18	0.67	0.00	0.71	2.04	8.08	12.39	0.38
Monoculture	Organic	Present	12	0.37	1.02	1.34	0.82	1.42	0.00	0.48	0.43	0.00	0.42	0.68	8.11	10.34	9.78	0.57
			18	0.54	1.86	1.67	0.85	0.00	2.82	0.45	1.36	0.78	0.21	0.97	3.23	9.77	14.88	0.36
			26	0.48	1.58	1.46	0.42	2.25	0.21	0.33	0.83	0.40	0.00	0.32	1.51	5.29	8.00	0.29
Polyculture	Inorganic	Absent	15	0.39	1.41	1.34	0.89	1.92	0.00	0.36	0.91	0.66	0.27	0.26	3.39	8.57	7.97	0.29
			16	0.50	1.82	1.55	0.71	2.53	0.21	0.45	1.25	0.65	0.20	1.10	2.15	7.15	10.22	0.23
			22	0.55	1.79	1.64	0.45	2.60	0.24	0.34	1.00	0.49	0.00	0.39	1.06	5.73	8.50	0.35
Polyculture	Inorganic	Present	5	0.56	1.34	1.42	0.92	2.18	0.10	0.50	0.89	0.54	0.11	0.87	4.68	9.17	11.02	0.37
			9	0.49	1.69	1.53	0.68	2.29	0.22	0.39	1.08	0.64	0.00	1.38	1.74	7.34	11.52	0.33
			30	0.55	2.02	1.85	0.47	2.81	0.29	0.42	1.18	0.61	0.00	1.28	1.21	7.02	11.24	0.40
Polyculture	Organic	Absent	2	0.34	1.28	1.33	0.99	1.81	0.12	0.46	0.90	0.52	0.08	0.35	4.43	9.31	9.15	0.39
			7	0.36	1.45	1.33	0.70	2.22	0.17	0.34	0.82	0.47	0.00	0.33	1.93	6.60	8.66	0.27
			13	0.62	1.85	1.65	0.50	2.45	0.29	0.43	1.03	0.63	0.16	1.34	1.94	6.78	10.79	0.43
Polyculture	Organic	Present	19	0.38	1.47	1.27	0.68	1.61	0.00	0.49	0.79	0.39	0.32	0.67	5.86	9.67	7.64	0.47
			24	0.42	2.39	1.93	0.59	0.00	2.86	0.61	1.51	0.83	0.54	0.00	3.83	12.20	14.64	1.11
			25	0.55	2.20	2.08	0.45	0.00	2.24	0.54	1.17	0.68	0.00	0.90	1.84	9.41	13.26	0.71

Table A_2C(d) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2C(c). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 5 – 10 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area														
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 31	18:0	PLFA 32	PLFA 33	PLFA 34	PLFA 35	PLFA 36	PLFA 37	PLFA 38	19:0c	19:0	PLFA 39	PLFA 40	PLFA 41	PLFA 42
			8	0.38	1.50	0.71	0.17	1.74	0.19	0.17	0.14	0.24	3.07	0.97	0.86	0.37	0.12	0.18
Unplanted	Inorganic	Absent	20	0.53	1.83	0.80	0.00	2.05	0.24	0.00	0.00	0.36	4.43	2.57	1.05	0.42	0.00	0.00
			27	0.58	2.10	0.75	0.21	2.18	0.31	0.00	0.00	0.37	6.01	1.99	0.93	0.33	0.00	0.00
			10	0.47	1.22	0.73	0.15	1.72	0.11	0.16	0.10	0.17	2.28	0.91	0.65	0.26	0.09	0.11
Unplanted	Organic	Absent	14	0.48	1.70	0.61	0.16	1.99	0.17	0.16	0.11	0.33	3.87	1.64	0.94	0.38	0.13	0.10
			29	0.65	1.86	0.73	0.00	1.91	0.19	0.24	0.00	0.32	6.10	2.82	0.93	0.33	0.00	0.21
			11	0.22	1.75	1.11	0.00	1.62	0.13	0.14	0.13	0.00	2.26	0.77	0.54	0.28	0.00	0.00
Monoculture	Inorganic	Absent	17	0.63	1.64	0.79	0.00	1.92	0.00	0.00	0.00	0.30	4.13	1.73	1.05	0.43	0.00	0.00
			28	0.41	1.09	0.53	0.00	1.25	0.17	0.00	0.00	0.22	3.47	1.54	0.42	0.16	0.00	0.00
			4	0.32	1.49	0.71	0.16	1.25	0.17	0.12	0.15	0.17	3.02	0.86	2.01	0.52	0.09	0.00
Monoculture	Inorganic	Present	6	0.78	2.34	0.95	0.19	2.46	0.27	0.21	0.16	0.56	7.78	5.08	1.60	0.34	0.00	0.21
			21	0.64	1.80	0.77	0.00	1.62	0.00	0.00	0.00	0.33	6.13	4.90	0.81	0.26	0.00	0.00
			1	0.23	1.08	0.66	0.09	1.13	0.11	0.10	0.10	0.13	1.50	0.55	0.66	0.30	0.10	0.10
Monoculture	Organic	Absent	3	0.52	1.65	1.01	0.22	2.11	0.23	0.00	0.00	0.30	3.69	1.85	1.16	0.61	0.21	0.19
			23	0.63	1.89	0.63	0.00	2.07	0.30	0.00	0.00	0.33	5.59	2.33	1.00	0.42	0.00	0.00
			12	0.23	1.22	0.77	0.10	1.27	0.10	0.12	0.11	0.09	1.56	0.59	0.53	0.22	0.00	0.00
Monoculture	Organic	Present	18	0.60	1.79	0.90	0.23	2.48	0.26	0.00	0.00	0.33	4.76	2.18	1.21	0.57	0.24	0.00
			26	0.48	1.21	0.41	0.00	1.23	0.18	0.00	0.00	0.19	3.40	1.63	0.62	0.25	0.00	0.00
			15	0.25	1.17	0.70	0.00	1.67	0.17	0.00	0.00	0.16	2.15	0.89	0.27	0.00	0.00	0.00
Polyculture	Inorganic	Absent	16	0.38	1.65	0.67	0.00	1.79	0.21	0.00	0.00	0.30	3.70	1.33	0.84	0.25	0.00	0.00
			22	0.52	1.34	0.49	0.00	1.58	0.24	0.00	0.00	0.29	4.24	2.19	0.53	0.17	0.00	0.00
			5	0.30	1.50	0.85	0.16	1.64	0.12	0.16	0.12	0.18	2.20	0.90	0.85	0.47	0.11	0.13
Polyculture	Inorganic	Present	9	0.50	1.58	0.66	0.18	1.90	0.21	0.00	0.00	0.29	4.24	1.90	0.77	0.28	0.00	0.00
			30	0.62	1.88	0.62	0.00	2.14	0.30	0.19	0.00	0.37	5.75	2.47	0.66	0.22	0.00	0.00
			2	0.36	1.28	0.83	0.14	1.59	0.12	0.16	0.10	0.18	2.09	0.82	0.38	0.22	0.12	0.12
Polyculture	Organic	Absent	7	0.42	1.20	0.58	0.00	1.49	0.00	0.00	0.00	0.24	3.13	1.71	0.60	0.27	0.00	0.00
			13	0.56	1.90	0.68	0.23	2.03	0.31	0.18	0.00	0.43	5.58	2.03	0.93	0.39	0.20	0.19
			19	0.23	1.33	0.49	0.10	0.96	0.14	0.10	0.10	0.11	2.29	0.71	1.75	0.49	0.18	0.00
Polyculture	Organic	Present	24	0.58	2.76	0.98	0.00	2.75	0.00	0.00	0.00	0.47	7.25	4.75	1.58	0.41	0.00	0.00
			25	0.62	2.66	0.85	0.00	2.24	0.00	0.00	0.00	0.53	7.61	4.06	1.14	0.00	0.00	0.00

Table A_2C(e) Raw phospholipid fatty acid (PLFA) analysis profile data continued from Table 2C(d). Mesocosms were treated with different plant communities and fertiliser types in the presence or absence of earthworms. Data collected from the 5 – 10 cm depth range. Unidentified PLFA peaks are denoted PLFA 1, 2 etc. Known PLFA peaks follow the nomenclature described in Chapter 2, Section 2.1.3.

				% Peak Area									
Plant Community	Fertilisation type	Earthworm presence	Mesocosm number	PLFA 43	PLFA 44	PLFA 45	PLFA 46	PLFA 47	20:0	PLFA 48	PLFA 49	PLFA 50	Total Area (%)
Unplanted	Inorganic	Absent	8	0.11	0.13	0.22	0.00	0.15	0.16	0.13	0.15	0.22	100
			20	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			27	0.00	0.00	0.30	0.29	0.00	0.23	0.22	0.00	0.24	100
Unplanted	Organic	Absent	10	0.06	0.10	0.18	0.00	0.09	0.10	0.11	0.06	0.10	100
			14	0.00	0.19	0.24	0.00	0.12	0.21	0.00	0.10	0.17	100
			29	0.00	0.37	0.25	0.00	0.00	0.22	0.26	0.00	0.16	100
Monoculture	Inorganic	Absent	11	0.00	0.18	0.00	0.00	0.00	0.16	0.00	0.00	0.00	100
			17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Monoculture	Inorganic	Present	4	0.17	0.23	0.17	0.00	0.09	0.11	0.20	0.08	0.15	100
			6	0.00	0.46	0.35	0.00	0.18	0.36	0.36	0.19	0.36	100
			21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Monoculture	Organic	Absent	1	0.00	0.12	0.00	0.08	0.00	0.00	0.08	0.00	0.09	100
			3	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.21	100
			23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Monoculture	Organic	Present	12	0.00	0.15	0.00	0.00	0.00	0.10	0.08	0.00	0.00	100
			18	0.00	0.24	0.23	0.00	0.00	0.00	0.00	0.00	0.24	100
			26	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
Polyculture	Inorganic	Absent	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			16	0.00	0.17	0.19	0.00	0.00	0.18	0.00	0.00	0.17	100
			22	0.00	0.00	0.16	0.15	0.00	0.00	0.16	0.00	0.00	100
Polyculture	Inorganic	Present	5	0.07	0.11	0.19	0.00	0.10	0.13	0.12	0.09	0.13	100
			9	0.00	0.19	0.20	0.00	0.00	0.00	0.00	0.00	0.00	100
			30	0.00	0.25	0.22	0.00	0.00	0.20	0.20	0.00	0.00	100
Polyculture	Organic	Absent	2	0.00	0.14	0.00	0.09	0.00	0.10	0.10	0.00	0.10	100
			7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			13	0.00	0.25	0.27	0.00	0.00	0.00	0.24	0.17	0.23	100
Polyculture	Organic	Present	19	0.19	0.12	0.16	0.00	0.00	0.00	0.00	0.00	0.00	100
			24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
			25	0.35	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100