CRANFIELD UNIVERSITY

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Effects and influence of the urea component of an organomineral fertiliser on phosphorus mineralisation in a low-P index arable and grassland soil.

School of Energy, Environment and Agrifood.

MPhil

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This thesis is submitted in partial fulfilment of the requirements for the degree of MPhil

ABSTRACT

Addition of organic waste such as sewage sludge (biosolids) has been recognised as one of the cost effective method of waste recycling practice of maintaining levels of organic matter in agriculture. Biosolids are a slow-release nutrient (N and P) which also improves soil physical and microbiological properties and as such offer a promising plant nutrient with less impact on soil than mineral fertilisers. Implementation of wastewater treatment directive 91/271//EC has caused large amount of sewage sludge production and availability. Hence, management of biosolids to provide available phosphorus through soil recycling would reduce reliance on finite rock phosphates (over 85% P-fertilisers) extraction, protect or minimise environmental problems such as eutrophication and also help the resource-limited farmers particularly in developing countries, to mitigate P-fertiliser limitation in the soil. More awareness of the impact of different agricultural fertiliser management practices on soil quality and sustainability has led to more interest of combining organic residuals with inorganic fertilisers to prevent further fertility decline and degradation of soil. Amongst recent nutrient integration, was the nutrient-balanced sludge-based (biosolids with urea and potash) called organomineral fertiliser (OMF), which shows agronomic efficiency of phosphorus management when applied to the field crops such as winter wheat.

However, to manage phosphorus mineralisation effectively in the organomineral fertiliser treated soil, it is important to understand the effects of urea components in OMF during mineralisation of phosphorus in soil. This research aimed to understand the influence that the urea component of an organomineral fertiliser (OMF) has during phosphorus mineralisation in soil. Therefore urea granules were grounded into powdered form and mixed with grounded biosolids pellets as a source of phosphorus to obtain organomineral fertilisers.

This whole research included two different control soil incubation experiments (1a & 1b) both observed over a 60 days period. Incubation experiment 1a (involved mixing various rates of biosolids and urea in soil) had two different soil samples from the grassland (sandy clay loam) and arable (clay loam) sites. In

terms of initial soil phosphorus content, the grassland and arable soils are classified as P-index 1 and 2 respectively. Soil samples were analysed for pH, mineralisable nitrogen (NH_4^+, NO_3^-), available phosphorus, microbial biomass carbon and phosphorus and phospholipids fatty acids profiles during 0, 6, 15, 20, 35, 45 and 60 days incubation period. While in the incubation experiment 1b (mixing different rates of urea with fixed quantity of biosolids in soil), soil from the same grassland, were being sampled at 10 day intervals (10, 20, 30, 40, 50, and 60) after taking the initial sample of day 0 and analysed for pH, mineralisable P and phosphomonoesterase enzyme activities.

The incubation experiments showed that, mineralisation of available phosphorus were significantly ($p<0.001$) higher in the biosolids and organomineral (OMF) fertiliser treated soils compared to control during 20 and 30 days period. The mean values of mineralisable P from OMF treatment for the incubation experiment 1a were 14.5 and 19.5 mg/kg in the grass and arable soils respectively. The mineralisation rates of biosolids-P from organomineral fertiliser (OMF) amended soils were also reduced significantly, as the dosages of urea component were increased according to the 50, 150 and 250Kg/N equivalents during the 60 days incubation experiment 1b. The mean available P values from the OMF amendments were 28.0, 25.7 and 23.4mg/kg respectively; according to the increasing amount of urea content at 50, 150 and 250Kg/N equivalents respectively. The overall trend of organomineral fertiliser phosphorus (OMF-P) mineralisation rate have shown significant ($p<0.001$) reduction with increased doses of urea components, with the fixed biosolids equivalent rate of 250Kg/ha during the incubation experiment 1b. There were no significant ($p>0.01$) changes in pH from both soil incubation experiment 1a and 1b, except for the urea only treated samples at the 250KgN/ha equivalent rate from the sandy clay loam grassland soil, which shows up to 1 unit increase (pH=8.1) compared to other treatments, just a day after kick-starting the incubation experiment, but eventually becomes reduced to the original pH (6.9) during the incubation period.

Microbial community change in both arable and grassland soil from initial day zero showed distinct and consistent shifts in trends through the 20 and 45 days respectively, irrespective of their treatments, and then gradually shifted towards the original starting point at the final incubation study of day 60. This was however attributed to the function of change with time, since it could not be categorically assigned to the OMF application effects only, but perhaps community change effects with time could be the main factor. Even though there was no any important patterns or trends observed between the indigenous treatments, but the wide spreading and shift distances amongst treatments during 20 and 45 days were higher compared to the 0 and 60 days, and this is probably because there were more phosphorus mineralisation when microorganisms were able to access more dissolved organic carbon as shown by high biomass carbon during 20 and 35 days incubation time, and utilise it to generate energy that kept them more active within those period before it gradually becomes exhausted, since there was no external source of energy being added. Similarly, phosphomonoesterase enzyme activities in the soil treatments except for the urea only amended samples, showed significant ($p<0.01$) differences between days 20, 30 and 40 compared to days 0, 50 and 60 and the phosphatase activities in the OMF amendments had significantly higher acid than alkaline phosphatase activities. Organomineral phosphorus (OMF-P) mineralisation in soil during 60 days incubation in this short-term study have shown potential P release in both soils, and the OMF-P mineralisation rate was highest in the formulation having fixed biosolids with urea at 150KgN/ha equivalent compared to other formulations (50KgN/ha and 250KgN/ha) and therefore effects of urea component of the organomineral fertiliser is an important factor when considering OMF as a promising P alternative or source in low-P soil during phosphorus management. Appropriate product formulation depending on the crop needs is therefore very important for soil phosphorus nutrient management and sustainability.

Keywords: Organomineral fertiliser, Phospholipids fatty acid analysis, microbial biomass carbon and phosphorus, available phosphorus, total oxide of nitrogen phosphomonoesterases, and enzyme activity.

To my beloved parents Alhaji Musa Ahmad, Hajiya Zahra Yakubu and my lovely wife Amina Muhammad Auwal for their patience, care and affection all the way during my programme. And to my daughters Mariya Hanan and Fatima Zahra.

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LIST OF ABBREVIATIONS

BS	Biosolids
C:N	Carbon-Nitrogen ratio
FAO	Food and agricultural organisation
Ha	Hectares
NH_4-N	Ammonium
NO_3-N	Nitrate
MBC	Microbial biomass carbon
MBP	Microbial biomass phosphorus
OMF	Organomineral fertiliser
N	Nitrogen
P	Phosphorus
PLFA	Phospholipids fatty acids
MUF-P	Methylumbelliferylphosphate
MUB	Modified universal buffer
MUF	Methylumbelliferone
NVZ	Nitrogen vulnerable zone

1 LITERATURE REVIEW

1.1 Introduction to biosolids

Biosolids are semi-solid (sewage sludge) wastewater treatment byproducts (Shaheen and Tsadilas, 2013; Zaleski *et al*., 2004) generated by waste water treatment processes. This process involves various treatment steps including digestion, thermal lime stabilisation, thickening, dewatering and drying in order to achieve about 99-100% pathogen free granules (**Figure1-1**). Raw sludge is transferred to a primary or a series of settling tanks to enable separation of solid particulates from the water fraction, the primary sludge is further transferred to either aerobic or anaerobic digester where organic materials are broken down to gas or incorporated into cellular biomass (Xu *et al*., 2014). Digested sludge is then passed into the secondary digester in the presence of alkali for thickening and finally dewatered to obtain biosolids cake, while the liquid portion or water is further treated with chlorine before disposal into rivers (Hogan *et al*., 2001; Metcalf and Eddy, 1972).

These physical, chemical and biological processes undergone by wastewater in the treatment plant ultimately clean the wastewater and remove the solids which is further treated to an acceptable standard for beneficial soil amendments and hence termed Biosolids. These treated residuals are known to be useful as a soil amendment in agricultural fields (Chinault and O'Connor, 2008; Smith *et al*., 2005), recreational parks and even home gardens (Zaleski *et al*., 2004). The application of biosolids are considered to improve soil organic matter, moisture content and provided essential nutrients such as nitrogen and phosphorus in arable land as potential plant nutrients supplements for crop optimum yield (Chinault and O'Connor, 2008). Incubation studies on the biosolids amended soil have shown significant increases of soil phosphorus content being released as plant available P in the (Shober and Sims, 2003; Smith, 1996) and therefore could be a good source of phosphorus in a deficient native phosphorus soils. Field scale experiments on wheat grown with sewage sludge have also shown yield production

comparable to mineral fertiliser treated soils (Deeks *et al*., 2013; Zarabi and Jalali, 2013; Ippolito *et al.,* 2007).

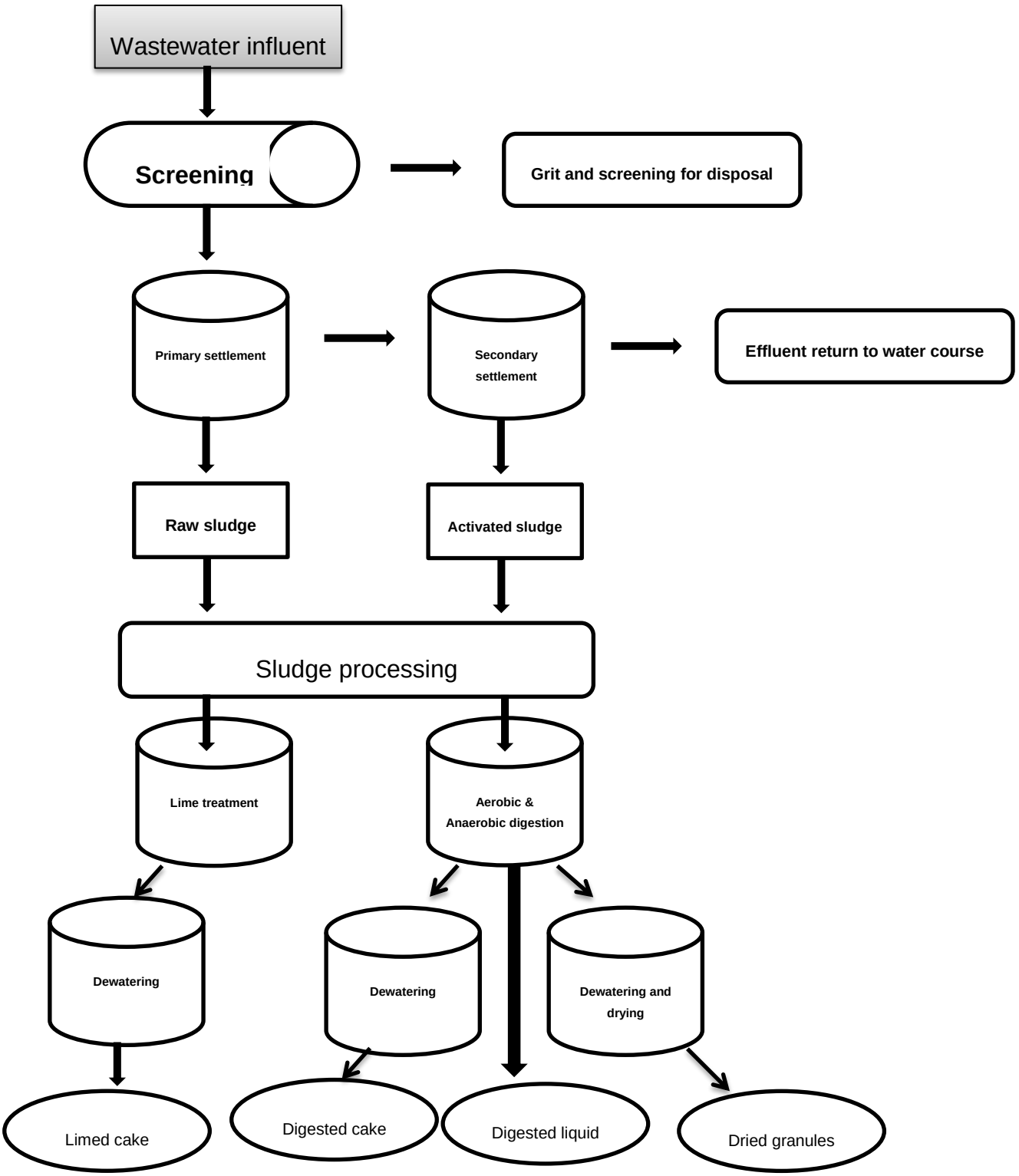


Figure 1-1 Production of biosolids in water industries

(Source: Water utility UK, 2010)

1.1.1 Origin and sources of biosolids

The main sources of biosolids are derived from municipal solid waste collected through the sewerage systems by the water companies during wastewater treatment and then using different (Figure1-1) processing methods to finally obtain the desired product. Biosolids like other sources of organic waste (manure and green compost) can be applied in soil to provide essential plant nutrients such as phosphorus and nitrogen (Shober and Sims, 2003; Smith, 1996). Phytoavailability of phosphorus in soil treated with biosolids is governed by several factors including the soil characteristic and sources of biosolids phosphorus removal (Penn and Sims, 2002; Hogan *et al*., 2001). For example biologically nutrient phosphorus removal (BNPR) produces more plant available phosphorus in soil than the chemically derived biosolids (Chinault and O'Connor, 2008; Penn and Sims, 2002). Thus, different sludge treatment processes could have an impact on mineralisation characteristics of the phosphorus present when applied in soil (Smith *et al.,* 2005). Phosphorus in biosolids is not necessarily as labile as P in mineral fertilisers or manure as their lability is greatly influenced by the wastewater treatment process (Metcalf and Eddy, 2003; Maguire *et al.,* 2000). Thermal drying significantly reduces P lability compared with the conventionally produced cake (Smith and Durham 2002). The biologically nutrient phosphorus removal (BNPR) for example, provides phosphorus uptake by microorganisms above normal levels and as such the surplus P is used for cell maintenance, synthesis and energy transport through the conversion of wastewater P to microbial biomass P (Powlson *et al.,* 1987).

1.1.2 Classification of biosolids

Biosolids vary in their inorganic, readily mineralisable and recalcitrant nitrogen and phosphorus contents, which is particularly dependent on the sewage sludge treatment methods of production as well as their pathogen level. They are classified either as class A or B digested sludge.

Table 1-1 Part 503 Pathogen density limits (USEPA, 2000).

MPN= most probable numbers. PFU= plaque forming units

Pathogens/Indicator and Class	Standard density limit (dry wt)
CLASS A	
Salmonellae	<3 MPN/4g of total solids
Faecal coliforms	< 1000 MPN/g
Enteric viruses	<1PFU/4g of total solids
Viable helminths ova	<1PFU/4g of total solids
CLASS B	
Faecal coliform density	<2000,000 MPN/g of total solids

Class A digested sludge consist of, chemically or biologically treated sludges that has a level of enterococci and thermotolerant coliforms per gram of dry matter below (<1000 cfu/g) and is without any detection of Salmonella spp (Zaleski *et al.,* 2004), according to the set standard of the 40 code of federal regulation part 503 biosolids rule (**Table 1-1**), established by United States Environmental Protection Agency. Class A products are suitable as fertiliser on lawns and gardens (Cogger *et al.,* 2013) and recommended for application to agricultural land as it is assumed to be essentially pathogen free (Smith, 1996). In contrast, produced class B

digested sludge, can contain an estimated faecal coliform density of over (>1000 cfu/g) (Zaleski *et al.,* 2004). The use of class B biosolids, products are more restricted because they contain detectable level of pathogens.

1.2 Biosolids in Agriculture and Environment

The beneficial use of biosolids in recycling to improve soil organic matter and crop nutrients in agriculture has increased since the implementation of the Sewage Sludge Directive 86/278/EEC by the European Union in the year 1989. As a result of the implementation of directives 91/271/EC large amount of sewage sludge were generated by water companies in the United Kingdom (DEFRA, 2002) and other European countries such as Spain, Denmark and France. An estimated annual production of over 9.4 million dry tonnes of biosolids is being generated by the European Union (DEFRA, 2002). While in the United States, over 7 million dry tonnes were generated by the wastewater treatment plants each year and up to 60% were recycled on agricultural land (Chinault and O'Connor, 2008).

Increase in the mineral fertilisers prices during the year 2008 (**Figure 1-2**) in the United Kingdom have also contributed towards the increased sludge production and demand for biosolids applications. Over 80% of biosolids are nowadays directly or indirectly used on land in England (Evans, 2012). Even though the application of biosolids in agriculture and environment have beneficial effects, there have been several concerns about their short and long term effects on agricultural soil and environment due to the presence of potential contaminated substances such as heavy metals or other pathogens if not properly treated and could be dangerous and toxic to human health and environment (Li *et al.,* 2012; Coker and Carlton-Smith, 1986).

Nonpoint- source pollution of surface water or eutrophication by agricultural phosphorus have been a major environmental concern worldwide (Haygarth and Jarvis, 1999) and significant fraction of this phosphorus mostly originated from organic waste such as manure and biosolids used during soil amendments (Sharpley *et al.,* 1994).

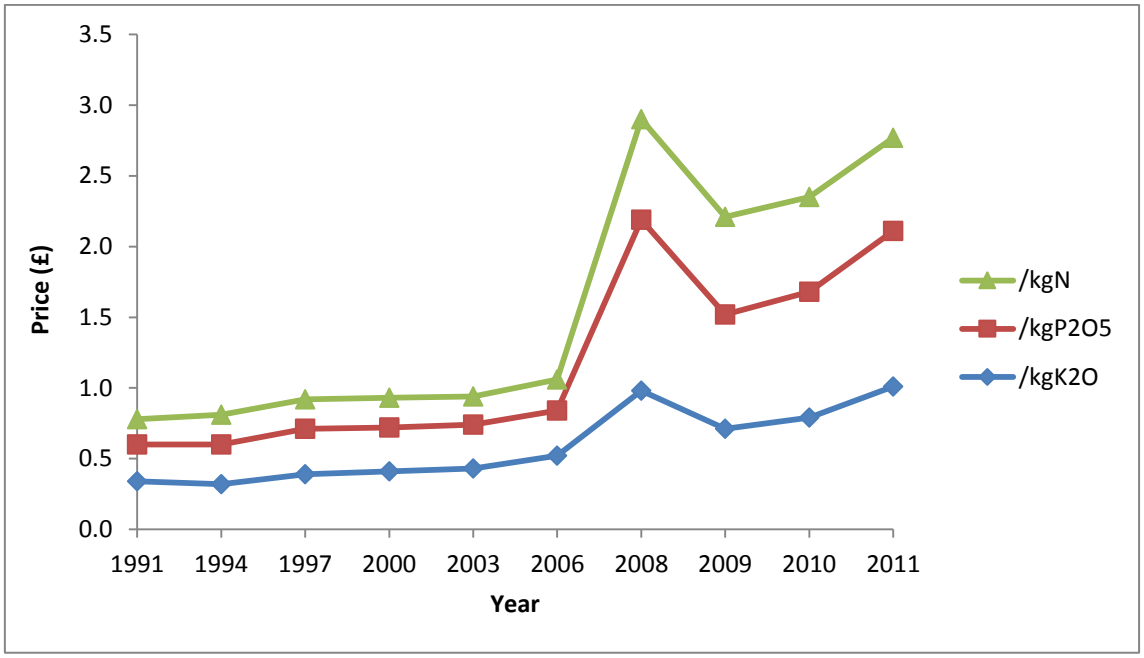


Figure 1-2 UK Fertiliser prices (1991-2011)

(Source: dairyco.net as modified by Evans, 2012)

1.2.1 Nutrients composition of biosolids

Biosolids like other sources of organic fertiliser can provide essential (nitrogen and phosphorus) nutrients to agricultural soil. There are different nutrient compositions of mainly phosphorus and nitrogen along with few micronutrients such as iron, copper, and zinc (Smith, 1996). The nitrogen and phosphorus contents are typically in the ranges of 2.8-3.8% and 1.2-3.0% respectively (Cooper *et al.,* 2011). Higher quantity of available nitrogen and phosphorus is found in dry digested cake (**Table 1-2**). However, there is large variability in the phosphorus contents present in biosolids, depending on the treatments methods (Penn and Sims, 2002; Maguire *et al.,* 2000) and the relative effectiveness of biosolids-P is 50% compared to soluble fertiliser P according to the recommendations in both United Kingdom and United States (MAFF, 1994). Potassium content of biosolids is very low (0.15-0.40%) because most of the compounds are water soluble and remains in the sewage effluent or aqueous fraction during sludge dewatering (Haynes *et al.,* 2009).

Table 1-2 A typical nitrogen and phosphorus content of sewage sludges

(Smith, 1996)

Sludge type	Dry matter (%)	Total Nitrogen	Total phosphorus	Available N	Available P
Liquid undigested (Kg/m^3)	5	1.8	0.6	0.6	0.3
Liquid digested (Kg/m^3)	4	2.0	0.7	1.2	0.3
Undigested cake (Kg/t)	25	7.5	2.8	1.5	1.4
Digested cake (Kg/t)	25	7.5	3.9	1.1	2.0

1.2.2 Chemical

The chemical form of phosphorus in biosolids influences the environmental chemistry and plant availability of soil P (Penn and Sims, 2002). Most of the biosolids-P produced through chemical treatment of wastewater during phosphorus removal is associated with inorganic iron bound (Fe-bound) or aluminium bound (Al-bound) phosphates (Sarkar and O'Connor, 2004). In a greenhouse study, Sarkar and O'Connor (2004), evaluated the effects of biosolids amendments on P availability in two sandy pasture soils with medium and very high native P content respectively. Four months after treatment, the grown cropping season bahiagrass yields did not show significance with P-sources or application rates, particularly in the very high native P soil. This was mainly due to the excessive high soil-P with high P retention capacity as a result it has masked the effect of biosolids added phosphorus (Sarkar and O'Connor, 2004; Sharpley, 1995).

1.2.3 Microbiology

Soil organic carbon is the main source of energy for soil microorganisms (Wardle, 1992). Microorganisms in soil are able to obtain available phosphorus upon hydrolysis of organic P catalysed by soil extracellular phosphatase enzymes. Extracellular phosphatases are those enzymes released into soil from active or non-proliferating cells such as spores, cysts, seeds, endospores, that becomes attached to dead cells or cell debris and absorbed to clay and humic colloids that play important role in catalysing the organic phosphorus hydrolysis reaction to release inorganic phosphorus (Dick, 2011). Organic matter content in biosolids would also provide energy which could contribute towards sustaining biological activity during nutrient mineralisation in soil (Madejón *et al.,* 2001). Therefore understanding of biological processes such as microbial biomass carbon and enzyme activities during the mineralisation of organic matter and nutrient turnover is very important (Melero *et al.,* 2007). Microbial biomass are those cells of living microorganisms notably, bacteria, actinomycetes and fungi that play vital roles in nutrient cycling and soil aggregation (Elliot and O'Connor, 2007). Biomass also functions as a sink for nutrients such as phosphorus and nitrogen under conditions of net immobilisation (Bünemann *et al.,* 2012) depending on the state of the whole system. During a 4 years study of crop rotation system with sunflower, winter wheat, lentil and winter wheat as the grown crops, when matured composts of vegetal and animal sources were consecutively added as source of organic matter in soil, microbial biomass carbon was observed to increase with increase in soil total organic carbon (Melero *et al.,* 2007), even though other factors such as soil moisture, pH and temperature could have an effect on this relationship (Melero *et al.,* 2007; Madejón *et al.,* 2001).

Microbial phosphorus immobilisation can affects P availability by removing inorganic P from soil solution particularly when soluble carbon is available for microbial growth (Bünemann *et al.,* 2012). Phosphorus in soil interacts with other essential nutrients such as carbon and nitrogen in regulating biological processes (Hui *et al.,* 2013) and as such, the ratio of C:N:P is an important indicator for estimating carbon and nutrient fluxes during the global circulation

models. Simple index measurement of the ratio of microbial biomass carbon to total organic carbon content (Cmic/TOC) is used as an indicator of carbon availability to microorganisms, conversion efficiency, losses of carbon and carbon stabilisation of soil (Melero *et al.,*2007). Extracellular enzymatic activity of phosphatase showed positive correlation with microbial biomass carbon, and the available phosphorus results were also correlated with the alkaline phosphatase activity in the matured compost treated soil (Melero *et al.,* 2007). The soil enzymes secreted by the microorganisms, to initially cleave or hydrolyse organic matter into smaller molecules (Burns *et al.,* 2013: Tabatabai and Bremner , 1969) are also potential indicators of soil quality as they play role in soil management, providing information on the biochemical processes occurring in soil (Madejon *et al.,* 2001). Phosphatase enzymes activities for example, are important during mineralisation of organic phosphorus in soil (Huang *et al.,* 2012).

1.2.4 Regulation and Global perspectives

Disposal of sewage sludges to sea was banned by the European Union (EU) under the water treatment directive 91/271/EEC in the year 1998 (CEC, 1999). This have resulted in generation of large amount of sewage sludges by water companies in the United Kingdom with over 9.4 million tonnes of dry weight produced annually in the European Union (CEC, 1999). The implementation of directives and other legislative measures in the European Union concerning collection, treatment and discharge of wastewater as well as advancement in the technological upgrading of wastewater treatment plants (WWTPs) have causes more sludge production, and even expected to increase up to 13 million tonnes in all EU member states by 2020 (European Commission, 2010). Safe disposal of biosolids is vital, as it is a major environmental concern throughout the world (Haynes *et al.,* 2009) which presents a major challenge in the wastewater management industries (UK Water, 2010).

In the United Kingdom (UK), biosolids land application and recycling is considered the best practicable options (EC, 1986). About 80% of sludges, goes to land in the UK (Evans, 2012). While in the USA up to 60% biosolids are

mostly recycled to agricultural soils to supply farmers with economic alternative of chemical fertilisers (Elliot and O'Connor, 2007). Other disposal options include landfilling and incineration.

1.2.5 Environmental risks

Even though, dewatered end products of the wastewater treatment companies is highly nutrient enriched (nitrogen and phosphorus) and amendable to use as agricultural fertiliser (Smith, 1996) or as mine waste covers (Zaleski *et al.,* 2004), they are recognised as repositories of organic pollutants and heavy metals (Smith *et al.,* 2005). Thus there are public concerns of biosolids application with regards to these potentially toxic elements or organic compounds effects over time. For example the application of biosolids to agricultural soils in excess of crop needs, when an N-based approach is used to determine land application rates, would results in the build-up of soil P, which is also amongst the direct similarity with manure (Shober and Sims, 2003) and that pose significant risk to surface and ground water during erosion and surface run-off in soil (Haygarth and Jarvis, 1999). However, environmental risk of biosolids application is minimal to both humans and environment (microorganisms) if properly managed according to the strict measures and regulations by the European Union directives (EC, 1986). Moreover, compared to manure (dairy cattle slurry), biosolids do not pose a greater risk in terms of losses along the runoff pathway in grassland soil (Haygarth and Jarvis, 1999).

1.2.6 Quantity of biosolids application in agricultural soils

There are major concerns, particularly regarding the long-term effects of biosolids application in agriculture and consequence on the soil and water quality. Biosolids are mostly applied to soil to meet nitrogen requirements in most agronomic crops with little regard to the phosphorus content, and this can results in the build-up of phosphorus in the soil (Antille *et al.,* 2014; O'Connor *et al.,* 2004). In order to mitigate soil phosphorus build –up, several State and Federal agencies such as

Ministry of Agriculture, Fisheries and Food (MAFF) and Department for Environment, Food and Rural Affairs (DEFRA) have recommended P-based nutrient management strategies depending on characteristics of native P of soils and other factors such as pH, soil texture, organic matter, soil moisture content and microbial activity (Haynes *et al.,* 2009).

1.3 Biosolids in soil

Application of biosolids to soil provides dissolved organic matter source, that causes initial degradation of decomposable fractions, accompanied by increased microbial activity in the sludge-amended soil (Haynes *et al.,* 2009) which may leads to a priming effect that can results in concomitant decomposition of native soil organic carbon (Marschner and Kalbitz, 2002). Biosolids induced positive priming effects, increases the decomposition of native soil carbon as such there is increase in the energy sources of microbial populations and subsequent increase in microbial activity in the soil (Thangarajan *et al.,* 2014). Negative priming effects where decomposition of the native carbon in soil is reduced upon addition of organic residuals such as biosolids or biochar would instead promote the immobilisation of carbon (Thangarajan *et al.,* 2014). Reactions of soil such as sorption-desorption, precipitation or metal speciation play critical roles in nutrient availability which often depends on the soil pH as one of the key factors (Stevenson and Cole, 1999). Residuals from biosolids have significant influence on solubility and speciation of soil nutrients. Buffering capacity which is the ability of soil solution to resist change in concentration of phosphorus when phosphorus is removed during plant uptake or added as fertiliser P and other amendments such as manure and biosolids (Holford, 1997), can be an important soil characteristics controlling relationship of solid phase P and it is concentration in soil solution (Lindsay, 1979; Holford, 1997). For example temporary induced increases or decreases of soil pH upon amendments could be restored after sometimes, perhaps due to soil buffering capacity (Stevenson and Cole, 1999). The solubility of iron bound biosolids-P was shown to be lower in biosolids-amended soils in terms of the P release or phytoavailability, compared to the thermally lime-stabilised biosolids or poultry litter (Penn and Sims, 2002).

1.3.1 Chemical and physical parameters influencing biosolids mineralisation in soil

1.3.2 pH

Soil pH has been an important factor affecting bioavailability of phosphorus for plant uptake (Hinsinger, 2001). In treated sludge, pH is a key factor towards controlling the phosphorus chemistry (Garau *et al.,* 1986). Application of biosolids in soil affected pH by either increasing or decreasing it depending on the initial soil pH and application rates. As a result it affects solubility and availability of nutrients and the soil microbial activities (Shaheen and Tsadilas, 2013). At extreme pH (> 10 or < 4), microbial activity is inhibited and that can affects mineralisation of P in soil (Aciego *et al.,* 2008). The dissolution and solution equilibrium reactions for the availability of inorganic phosphate in soil is largely achieved based on soil pH, phosphate minerals (iron, aluminium and calcium) and their organic matter content (Stevenson and Cole, 1999). For example, soluble phosphorus in oxides and hydroxides of aluminium or iron in the soil increases with pH levels up to about 6.5 and then, decreases significantly above the neutral pH or in high calcium phosphate compounds soils (Haynes *et al.,* 2009).

Dihydrogen phosphate ion ($H_2PO_4^-$) generally dominates at pH ranges between 2.2-7.2. While at 7.2-12.4 pH ranges, monohydrogen phosphate ion (HPO_4^{2-}) are the dominant species. In calcareous soil, calcium ($CaCO_3$) will react with phosphate (HPO_4^{2-}) to precipitate phosphate ion (Smith, 1996).

The optimum pH for P availability to crops in the soil is between 6-7 (Stevenson and Cole, 1999). Lowering soil pH (4.5-5.1) causes decreased soil microbial activities and can leads to subsequent changes in the substrate utilisation (Aciego *et al.,* 2008; Stevenson and Cole, 1999).

1.3.3 Organic matter

The organic matter content presence in soil controls the dynamics of phosphorus in soil (Troeh and Thompson, 1993). It plays a vital role, as it affects many important soil properties (Kirkby *et al.,* 2014). For example decomposing organic matter releases an acid that increases solubility of calcium sulphate which causes increase in the amount of available P. Organic matter forms two complex matrix associated with particles and other nutrients in the soil, mostly referred as coarse and fine fractions of soil organic matter (Kirkby *et al.,* 2014; Shaheen and Tsadilas, 2013). The coarse fraction of soil organic matter (CF-SOM) is that organic material (CF>0.4mm) composed of un-decayed plant and animal residues and recognised as highly labile material due to fast rates of organic matter breakdown (Paul and Clark, 1996). In contrast, fine fraction soil organic matter (FF-SOM < 0.4mm) is considered to be more stabilise and slowly decomposing pool of soil organic material (Shaheen and Tsadilas, 2013).

Application of organic residuals such as manure, green compost or biosolids as sources of organic matter to improve soil physical, chemical and biochemical properties has been practiced for a long time (Bernal *et al.,* 1998). The addition of an organic substrate generally results in an increase in the size and activity of the soil microbial community as well as activities of extracellular soil enzymes (Skujins, 1976). The residuals from biosolids originate mainly from human faeces and bacterial cells during primary and secondary sludge treatments respectively (Haynes *et al.,* 2009). Their organic carbon (C) ranges mostly between 20-50% and overall organic fraction of biosolids consists of a mixture of fats, carbohydrates, protein, lignin, amino acids, cellulose, sugars, humic materials and fatty acids (Haynes *et al.,* 2009). There were increases in the concentration of dissolved organic matter in soil solution following biosolids application, which subsequently decreases over time as the added biosolids organic matter decomposes (Smith *et al.,* 2005: Clapp *et al.,* 1986). Organic matter and pH in soil stimulate microbial biomass growth and this is beneficial to majority of microbes as it provides more surface area in addition to carbon or energy sources for microbial activities such as, effective nutrients degradation and mineralisation.

1.3.4 Moisture content

Soil moisture is amongst one of the key controlling factor for the available phosphorus in soil. The drying and re-wetting of arable and grassland soils therefore have an effect on the release of biosolids-P (Melero *et al.,* 2007). The decrease in microbial biomass carbon with an increase in extractable phosphorus in an air-dried soils during drying cycle, were shown (Agbenin and Adeniyi, 2005). However a constant microbial biomass carbon was observed with seasonal changes in microbial P content and organic P mineralisation during spring and then P immobilisation in the early winter months (Oberson and Joner, 2005). Under optimal soil moisture and temperature, a significant immobilisation-remobilisation sequence occurs upon addition of organic materials to soils and the pattern and dynamic of phosphorus turn over in such situation depend on the substrate, the microbial biomass (size, activity, composition), soil properties and community structure of soil (Oberson and Joner, 2005). Immobilisation of P in microorganisms also increases with increase in the proportion of soluble carbon in the added substrates (biosolids) and the initial size of the microbial biomass (Bünemann *et al.,* 2012).

1.3.5 Temperature

The solubility of phosphate ions in soil is also governed by temperature in addition to moisture content (Tisdale *et al.,* 1990). The impacts of temperature on biosolids-treated soil phosphorus release and mineralisation are minimal because phosphorus is typically immobile in most soil (Silveira and O'Connor, 2013). During a 90 days soil incubation study by Silveira and O'Connor (2013), it shows that an increase in the temperature from 20^0C to 32^0C have caused increased soil P retention which results in the low release of phosphorus into soil solution. Even though the distribution of phosphorus amongst various fractions were not significantly affected by the changes in temperature. Treatments of soil with biosolids, play little role in terms of differences of soil biosolids-P release or concentrations particularly in a high phosphorus content soil, or in those soil with

high affinity to retain P, as it is being easily masked by P-enriched soils at the surfaces (Sarkar and O'Connor, 2004).

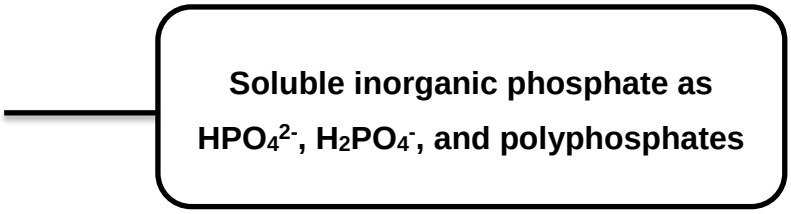
1.3.6 Mineralisation of biosolids phosphorus in soil

Mineralisation of P is the process by which soil microbes break down soluble and insoluble P nutrients present in organic matter through extracellular phosphatase enzymes secretion that becomes available to both plant and microorganisms (Quiquampoix and Mousain, 2005). Phosphorus mineralised in soil after organic sources input of manure, such as cow dung or biosolids is an important factor in determining overall P availability in soil (Haygarth and Jarvis, 1999). Soil native P increase, during biosolids-P mineralisation may leads to modification in the distribution of P in various pools, and can subsequently increase the soil total P over time (Shober and Sims, 2003). About 90% of applied phosphorus from biosolids in soil is not taken up by the plants. Rather it is retained (locked-up) as insoluble or fixed P, so that residual P can be used by subsequent growing crops (Stevenson and Cole, 1999).

1.3.7 Phosphorus cycle

Phosphorus is an essential element and its availability contributes in controlling some aspects of global biogeochemical processes (Haygarth and Jarvis, 1999) such as soil genesis (Castillo *et al.,* 2011; Richardson *et al.,* 2009).

Phosphorus cycle in soil are generally controlled by inorganic adsorption-desorption reactions, biologically controlled mobilisation and immobilisation by the microbial biomass, and the native P forms, principally, whether it is in an organic or inorganic form (Haygarth and Jarvis, 1999). In natural ecosystems, phosphorus is usually a scarce resource and is efficiently being recycled; whereas in agricultural systems, P is removed in crops or animal products (Haygarth and Jarvis, 1999). Figure 1-3 shows phosphorus cycling in soil.



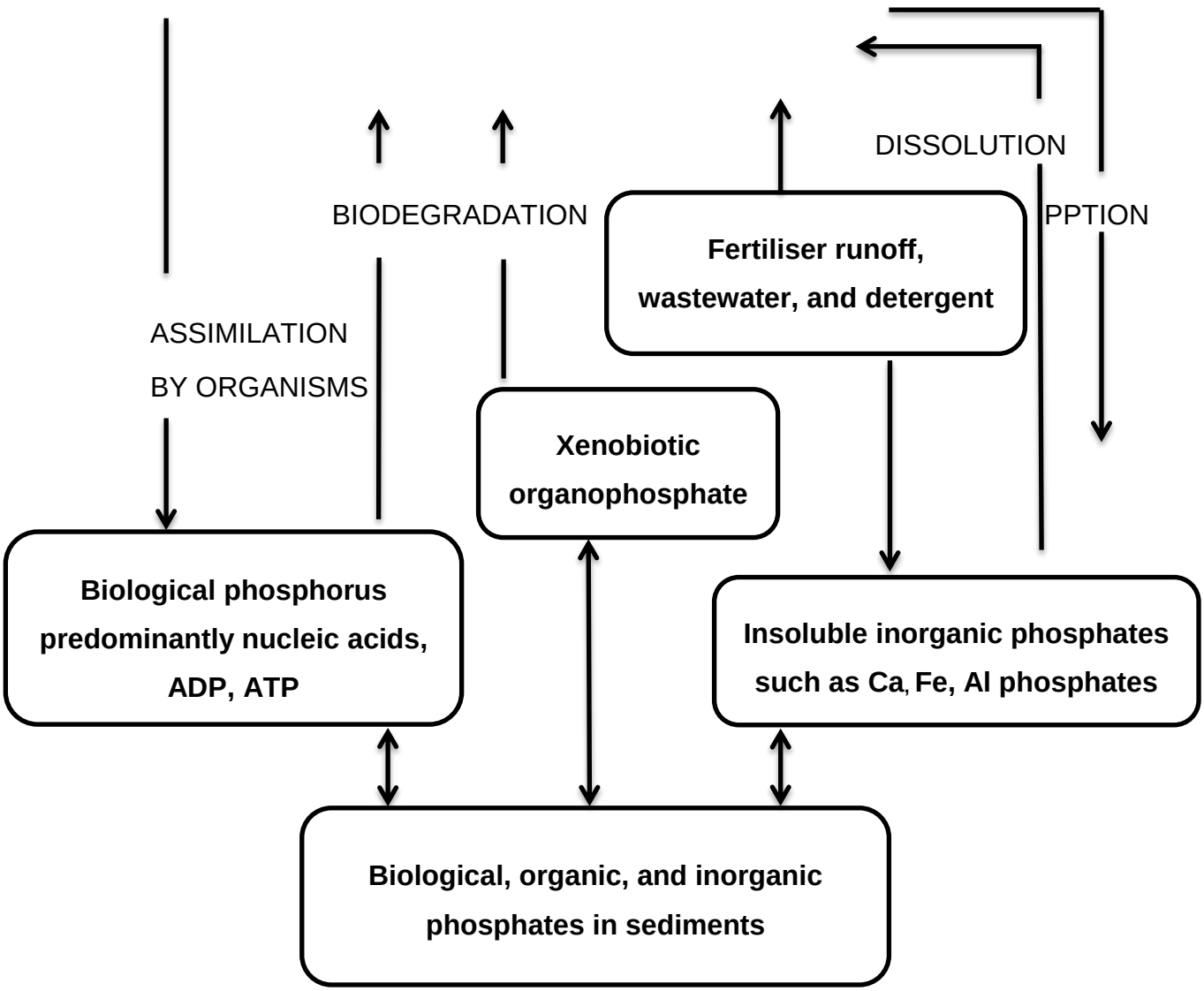


Figure 1-3 Phosphorus cycle in soil

(Adapted from Stevenson and Cole, 1999).

This cycling of phosphorus in soil provides an ecosystem services, such as plant nutrition and productivity, incorporation of inorganic P from the geochemical pool into biological organic pool by soil biota and also P sorption and desorption (**Figure 1-3**) as influenced by the nature of solid phase matrix, soil pH and biological activity (Pierzynski *et al.,* 2005). Inorganic P is the main form of P uptake by the plants and soil organisms (Stevenson and Cole, 1999). The transformation of soil inorganic and organic phosphorus is interrelated, because

organic P can replenish solution inorganic P upon depletion as a result of plant or microbial uptake, through enzyme hydrolysis. Organic P can account for a large proportion of total phosphorus in soil solution, drainage water, run-off water and stream (Fraser *et al.,* 2015; Withers and Haygarth, 2007). It is therefore, also an important P source for plants and microorganisms, but must be converted into inorganic P before it can be utilised (Fraser *et al.,* 2015). Soil microorganisms play key role during the biogeochemical cycling of P through excretion of extracellular enzymes such as phosphatase to convert organic P into phosphate (Burns, 1982; Skujins, 1976).

Phosphorus is a key nutrient to all living organisms which forms the primary component of essential biomolecules such as nucleic acids, adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), phospholipids and inositol hexaphosphate or phytin (**Figure 1-3**). Phosphorus is therefore is a requirement for energy, growth and development for plants in both managed and natural ecosystems (Withers and Haygarth, 2007). For example, reactions within cells where ATP is hydrolysed to ADP and inorganic phosphate (Stevenson and Cole, 1999) involves, energy metabolism. Therefore, presence of organic matter or organic residuals in soil controls the dynamics of phosphorus in soil.

1.3.8 Chemical form of phosphorus in soil

The chemical stability, relative immobility and low concentration (1µM) in the soil solution of phosphate in soil, determine the productivity of many ecosystems (Quiquampoix and Mousain, 2005). The chemistry of phosphorus in soil is very complex and most of the P occurs in inorganic forms with the exception of peaty soils, where it is mostly in an organic form (Stevenson and Cole, 1999; Tisdale *et al.,* 1990). In soil, phosphorus are chemically complexed as either aluminium or iron phosphates, they can be occluded iron or aluminium phosphorus oxides or calcium phosphates depending on soil pH (Stevenson and Cole, 1999). Typically a ranges of 50-3000mg/kg of total P content is present at the top (0-15cm) soils based on the soil parent material, type of soil, vegetation cover and soil

management, even though about 35-70% are inorganic P (Bünemann *et al.,* 2012).

1.3.9 Biological form of phosphorus in soil

Biological form or organic phosphorus (Po) in soil are those phosphate esterified with hydroxyl groups of sugars, alcohols or bound as pyrophosphate bond to another phosphate group. They are generally considered as that phosphorus containing carbon-hydrogen bond (Turner *et al.,* 2005). Organic phosphorus account for up to 90% of the total soil P (Dalal, 1977), particularly in high organic matter content soils such as peaty soil (Bünemann *et al.,* 2012). In natural ecosystem or managed soil, organic P can be an important source of P for plants during mineralisation in poor phosphate solubility soils, containing significant quantity of organic P (Tiessen *et al.,* 1984). The higher the organic matter content in soil the more organic P, which decreases with soil depth just like organic carbon (Rigby *et al.,* 2009). Primary organic phosphorus compounds found in soil include inositol phosphates, phospholipids and nucleic acids (Dalal, 1977). Phytins or inositol phosphates usually have the greatest quantities making up to 80% of the total soil organic P fraction found in polymeric states, and this makes it relatively resistant to biodegradation and decomposition (Quiquampoix and Mousain, 2005). Sequence of different phosphate monoesters such as inositol monophosphate and inositol hexakisphosphate in various (myo, scyllo, neo and D-chiro) stereoisomers can be present in soil. Phospholipids (0.5-7%) and nucleic acid (<3%) constitute small amount of soil organic P (Dalal, 1977), and the microbial mineralisation of these compounds contribute towards replenishment of depleted soil solution P in soils (Bünemann *et al.,* 2012).

1.4 Mineralisation and Immobilisation of phosphorus in soil

Phosphorus nutrients in soil organic matter are mostly present in organic form (Dalal,1977). They are converted to inorganic forms through the process of

mineralisation in order to become available to crops. Phosphorus mineralisation in soil is simply the release of orthophosphate during the decomposition of phosphorus nutrient containing organic materials, facilitated by extracellular phosphatase enzymes released by the soil microbes. Soil organic P is derived from combination of plant, animal and microbial residues and provides important P turnover during mineralisation both in the organic and microbial biomass pools that forms vital component of P cycling in soil-plant systems (Condron *et al.,* 2005). Mineralisation of phosphorus in soil is partly regulated by the carbon-phosphorus ratio (C:P) of substrates (Withers and Haygarth, 2007). Carbon-phosphorus ratio (C:P) greater than 100 for example, indicates high requirements of P (1.5-2.5% of dry weight) by soil microorganisms (Haygarth *et al.,* 1998), and as such, P would rather be immobilised by microbes. Microbes therefore, compete with plants for available P in soil. Mineralisation of phosphorus from microorganisms is also affected by soil texture

Immobilisation involves the uptake of the organic forms of phosphorus into the microbial biomass and it is a reaction catalysed by those active microbial biomass fractions in soil in order to acquire energy (Hinsinger, 2001). Microbial immobilisation of P is a vital process that provides an important source of available P, as it can often contain up to 20-30% of the total soil organic P pool, which is even significantly higher compared to carbon (1-2%) and nitrogen (2-10%) proportions in the soil microbes (Haygarth *et al.,* 2013). Thus biomass in this situation acts as a labile pool of P which is protected from fixation, but is rather plant-available during biomass turnover (Ayaga *et al.,*2006). Both mineralisation and immobilisation are depicted in Figure 1-4. Microbial P immobilisation makes soil P temporarily unavailable to plants at some points but eventually becomes available upon complete microbial decomposition due to the simultaneous mineralisation-immobilisation processes during phosphorus turnover (Condron *et al.,* 2005; Stevenson and Cole, 1999). Due to the fact that, mineralisation of soil organic phosphate pools is achieved through extracellular phosphatase (Skujins, 1976) enzymes activities, several factors, such as organic materials ratio (C:N:P), pH, moisture and fertiliser P amendments affects

phosphatase activities which could subsequently have effects on the organic P mineralisation (Stevenson and Cole, 1999).

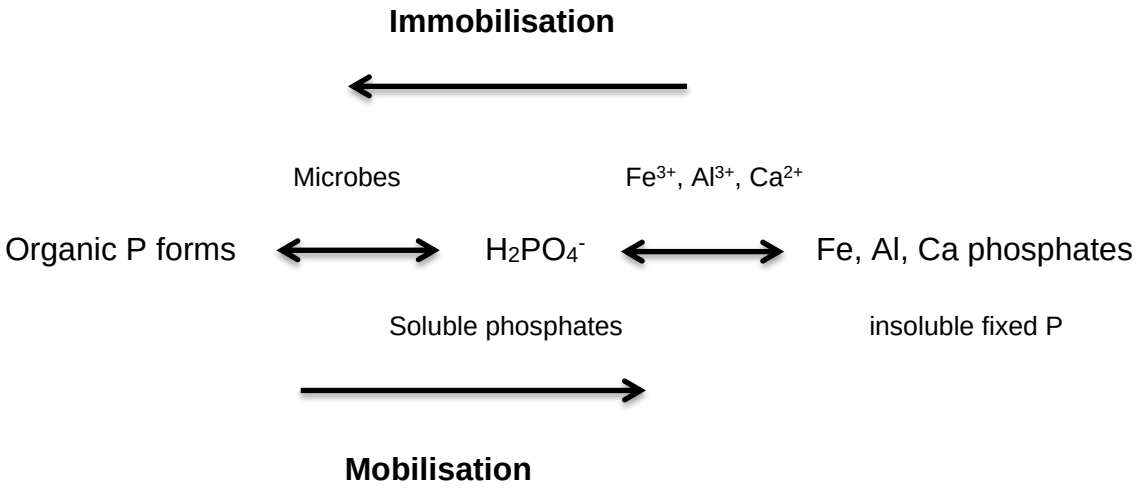


Figure 1-4 Phosphorus transformation in soil

1.4.1 Enzymes

Enzymes are biological catalyst or protein molecule which brings about or enhances one particular chemical reaction but itself remains unchanged at the end of the reaction, and all biological activities in soil proceed through enzymatic processes (Skujins, 1976). They are made up of 20 different amino acid species linked together to form one or more long chains or large molecules (molecular weight of up to 10000 Daltons in a small enzyme). These long chain amino acids are folded in three-dimensional structure, such that the enzyme active site is located on the surface which easily makes it fits with the substrate (Acosta-Martinez and Tabatabai, 2011). Enzyme active centre or catalytic site occupies a very small part of the protein in most cases, which is capable of binding with substrates. Substrate binding depends on the enzyme specificity with the interacting ligand which could be reversibly non-covalent or weak bonds (Burns, 1982). Thousands of biochemical reactions are catalysed by separate enzymes and in special situations, where multifunctional enzymes having several catalytic activities on the same molecule interacts to yield final products in accordance with the kinetics of soil enzyme reactions (Dick, 2011). Enzymes are therefore so efficient in lowering the energy barrier so that reactions proceeds at a reduced

energy expense unlike in the situation of un-catalysed reactions (Figure 1-5). Now based on the specific-ligand binding (Ligand is any combining molecule), substrates binds/attaches reversibly by a non-covalent or weak bonds. Interactions depend on the enzyme specificity with the interacting substrates.

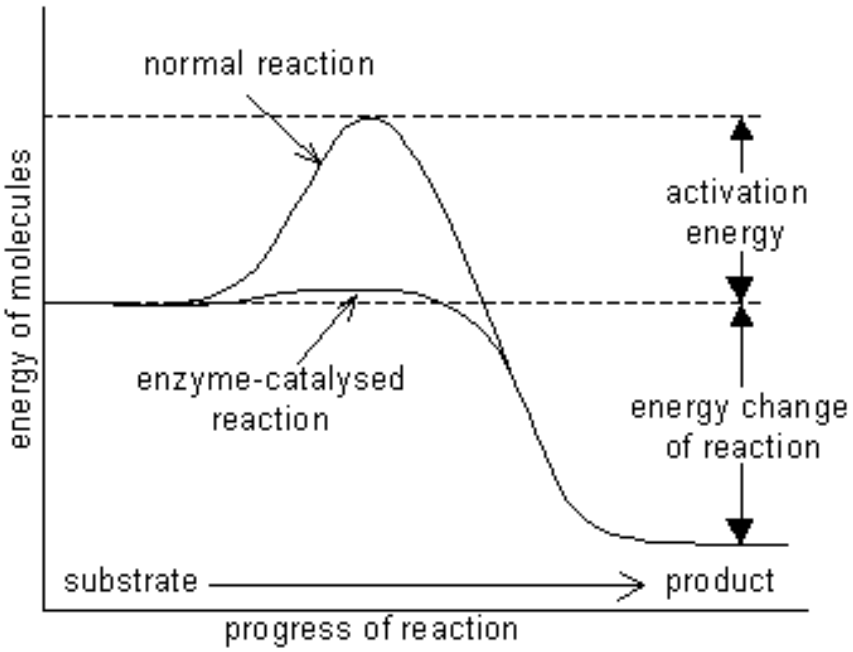


Figure 1-5 Enzyme activation energy efficiency

Additional features and characteristics of enzyme during reactions are the phenomena of specificity and saturation. The initial velocity of the reaction has been observed to follow first-order kinetics with respect to substrate concentration as the substrate concentration increases from zero (**Figure 1-5**).

The rate of increase in initial velocity becomes less and less with each unit increase in substrate concentration and eventually a point is reached where no any further increase of initial velocity is observed, and the reaction can be described by the zero-order kinetics with regards to the substrate concentration. At this point, the reaction rate is no longer a function of both substrate concentration and enzyme concentration but is rather a function of only enzyme concentration (Dick, 2011).

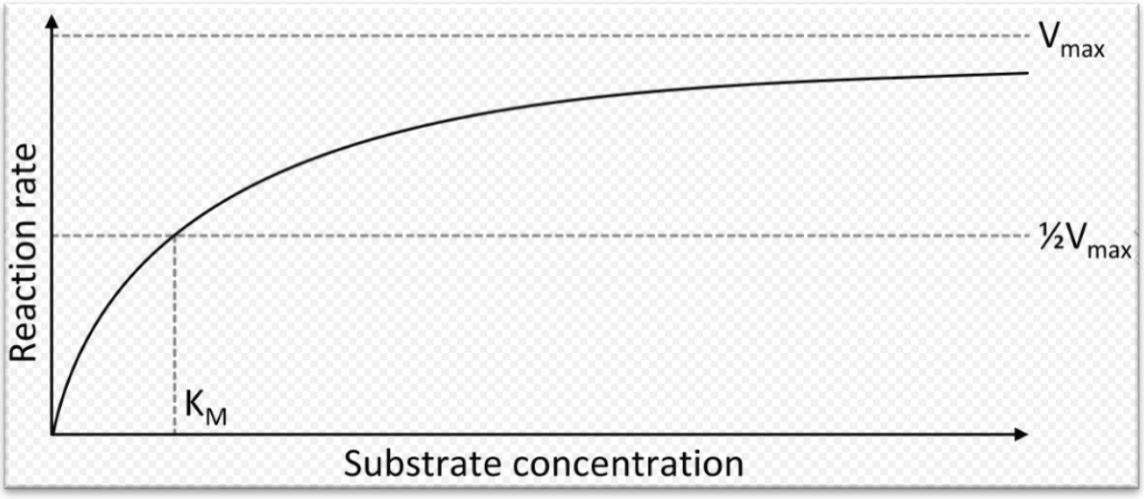


Figure 1-6 The initial reaction velocity against the substrate concentration of Michaelis-Menten plot.

1.4.2 Michaelis-Menten Kinetics of Enzyme-catalysed Reactions

The general theory of enzyme kinetics, and the equations for an enzyme initially reacting reversibly, with its substrate to form an enzyme-substrate complex, was formulated by Leonor Michaelis and Maud Menten called the Michaelis-Menten equation (Equation 1).

$$\boldsymbol{v = Vmax[S]/(Km + [S])} \qquad \textbf{[1]}$$

v = measured initial reaction rate or velocity

[S] = substrate concentration

V max = maximum rate of reaction or velocity

Km = Michaelis constant

The relationship between initial velocity (v) and substrate concentration ([S]) were shown as a hyperbolic graph (**Figure 1-5**).

Michaelis-Menten equation has been widely used to characterize extracellular enzyme kinetics in soils and to determine the Michaelis constant (Km) and the maximum rate of reaction (Vmax), for the single substrate enzyme-catalysed reactions (Dick, 2011). The classical model for an enzyme-catalysed reaction in its simplest form can be presented below

$$\mathrm{E}+\mathrm{S} \underset{\boldsymbol{k2}}{\overset{\boldsymbol{k1}}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{\boldsymbol{k3}} \mathrm{E}+\mathrm{P} \qquad \textbf{[2]}$$

Where E, S, ES and P refer to enzyme, substrate, enzyme-substrate complex and reaction products, respectively; k_1, k_2, and k_3 are the corresponding velocity constants of the assumed processes. Several assumptions were made in derivation of the Michaelis-Menten equation and as such the rate of formation of ES can be expressed as:

$$\frac{\mathrm{d[ES]}}{\mathrm{dt}} = \mathrm{k_1}([\mathrm{E_t}]\text{-}[\mathrm{ES}])([\mathrm{S}]) \qquad \textbf{[3]}$$

Note: $[E_t]$ is the concentration of total enzyme which is defined as the sum of concentration of enzyme in the free state $[E_f]$ and enzyme-substrate complex $[ES]$, i.e. $[E_t] = [E_f] + [ES]$.

The rate of breakdown of ES is given as:

$$-\frac{\boldsymbol{d[ES]}}{\boldsymbol{dt}} = \boldsymbol{k2}[\boldsymbol{ES}] + \boldsymbol{K3}[\boldsymbol{ES}] \qquad \textbf{[4]}$$

Setting the rates equal to each other gives:

$$\frac{\boldsymbol{d[ES]}}{\boldsymbol{dt}} = -\frac{\boldsymbol{d[ES]}}{\boldsymbol{dt}} \boldsymbol{or}\ \boldsymbol{k1}\ ([\boldsymbol{Et}] - [\boldsymbol{ES}])([\boldsymbol{S}]) = \boldsymbol{K2}\ [\boldsymbol{ES}] + \boldsymbol{K3}\ [\boldsymbol{ES}] \qquad \textbf{[5]}$$

Rearranging the above equations yields:

$$\frac{[\boldsymbol{S}]([\boldsymbol{Et}]-[\boldsymbol{ES}])}{[\boldsymbol{ES}]} = \frac{\boldsymbol{K2+K3}}{\boldsymbol{K1}} = \boldsymbol{Km} \qquad \textbf{[6]}$$

Further rearrangement yields

$$[\boldsymbol{ES}] = ([\boldsymbol{Et}][\boldsymbol{S}]/(\boldsymbol{Km} + [\boldsymbol{S}]) \qquad \textbf{[7]}$$

But K_3 [ES] = v (initial velocity) and K_3 [E_t] = Vmax (maximum rate of reaction) and therefore substituting these equations gives the classical derived form of the Michaelis-Menten (equation 1) formula.

Michaelis constant (Km) which forms the combination of the individual reaction constants k_1, k_2, and k_3 of the enzyme-catalysed reaction (Equation 6) is often used as a way to easily characterised the activity of an enzyme in relation with its substrate concentration. Thus Km value provides useful information because the smaller the Km value the greater the affinity of the enzyme for its substrate and consequently lower concentration of substrate is required to achieve 50% of the maximum rate of reaction (Dick, 2011). Therefore comparing Km values provides a rapid assessment of the interaction of an enzyme with its substrate and the amount of substrate concentration in soil needed to result in an enzyme having an ecological meaningful level of activity. In soil, not all the derivation of the Michaelis-Menten equation assumptions can be strictly maintained, for example the Km value of a pure enzyme catalysing the same reaction in a solution of a test tube is often different from that in soil (Dick, 2011).

1.4.3 Determination of Kinetic Constants Km and Vmax

The Michaelis constant Km is indicative of enzyme-substrate affinity, the higher the value the lower the affinity and the unit for Km is the same as that of substrate concentration (Deng, 2012; Dick, 2011). While maximum velocity (Vmax) during the reaction, describes the rate of substrate conversion at the time when the enzyme active site is saturated with substrate. Several linear transformations of Michaelis-Menten equation (Equation 1) have been proposed to facilitate determination of Km and Vmax. Amongst these is the Line weaver-Burk double-reciprocal equation (Equation 8) which is the most commonly used linear transformation.

$$\frac{\mathbf{1}}{\boldsymbol{v}} = \left(\frac{\boldsymbol{Km}}{\boldsymbol{Vmax}}\right)\left(\frac{\mathbf{1}}{[\boldsymbol{S}]}\right) + \mathbf{1}/\boldsymbol{Vmax} \qquad \textbf{[8]}$$

Plotting the inverse of the initial velocity of an enzyme-catalysed reaction on the y-axis against the inverse of the substrate concentration on the x-axis, the y-intercept value is 1/Vmax, the slope is equal to Km/Vmax and finally the x-intercept is -1/Km. Other linear transformations include single reciprocal form of Eadie-Hofstee (Equation 9) and Hanes-Woolf (Equation 10) respectively.

$$\boldsymbol{v} = -\boldsymbol{Km}\left(\frac{\boldsymbol{v}}{[\boldsymbol{S}]}\right) + \boldsymbol{Vmax} \qquad \textbf{[9]}$$

$$\frac{[\boldsymbol{S}]}{\boldsymbol{v}} = \left(\frac{\mathbf{1}}{\boldsymbol{Vmax}}\right)([\boldsymbol{S}]) + \boldsymbol{Km}/\boldsymbol{Vmax} \qquad \textbf{[10]}$$

1.4.4 Phosphatase enzymes

Phosphatases play a major role in the transformation of soil organic P forms (Dalal, 1977). Phosphatases are extracellular enzymes, catalysing the hydrolysis of both esters and anhydrides of phosphoric acid and their activities mediate the transformation and recycling of P forms in soil, applied P from fertiliser, organic residuals (manure) and biosolids, into free phosphates (PO_4^{3-}), and this can be taken up by plants and soil microorganisms (Quiquampoix and Mousain, 2005). Phosphatase enzymes have been extensively studied in soil (Tabatabai, 1994). Generally, the decomposition processes in soil are largely catalysed by extracellular enzymes, and their activity is often related to the type of clay mineral that is dominantly present in the soil system (Skujins, 1976). Biotic and abiotic soil composite fractions such as clay minerals, humic colloids, proliferating cells or cell debris and soil aqueous phase, can therefore, be used to determine activity of any particular enzyme in soil. The physical and chemical nature of soil colloids, size and solubility of substrate could localise enzymes in soil, even though enzymes may change location with time (Burns, 1982).

Phosphatase enzymes can be generally classified as phosphoric monoester hydrolases or phosphoric monoesterase (EC 3.1.3), phosphoric diester hydrolases or phosphodiesterase (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5); also enzymes acting on phosphoryl-containing anhydrides (EC 3.6.1) and on P-N bonds (EC 3.9) (Theng, 2012; Acosta-Martinez and Tabatabai, 2011). Soil microbes are closely involved in the cycling of P during solubilisation and mineralisation of inorganic and organic P respectively, through the release of those P acquiring phosphatase enzymes, capable of hydrolysing variety of phosphomonoesters (Tabatabai, 1994). Phosphomonoesterase activities are often measured as an index of the fertility status of soil ecosystems related to P cycling (Dick and Tabatabai, 1993). For example changes in the activities of phosphatases were sensitive to cropping systems, land use and tillage and application of organic amendments (Acosta-Martinez and Tabatabai, 2011; Wallenius *et al.,* 2011). Enzymes production by microorganisms during

geochemical processes or soil organic residuals amendments would therefore influences decomposition and stabilisation processes of phosphorus nutrient cycling (Skujin, 1976). Microorganisms in some soils were shown to be co-limited by carbon, nitrogen and phosphorus (Kunito *et al.,* 2012). For example the effect of addition of C, N and P on phosphomonoesterase and dehydrogenase activities in soil shows that P addition increases dehydrogenase activities as well as the acid phosphatase activities in the low pH soil (Kunito *et al.,* 2012).

1.4.4.1 Acid and Alkaline Phosphatase

Phosphoesterases in soil contribute to organic phosphate mineralisation (Skujins,1976). Alkaline phosphatase (E.C. 3.1.3.1) and acid phosphatase (E.C. 3.1.3.2) are the two recognised orthophosphoric monoester phosphohydrolases, classified according to their optimum pH activities, which varies towards alkaline and acid ranges respectively (Skujins,1976). Therefore phosphatases when released into the soil may apparently have different pH optima. Soil phosphomonoesterase activity mostly appears to have two pH optima of about pH 4-6 and 8-10 (Speir and Ross, 1978). Assays are usually carried out at pH 6.5 and 11 to determine acid phosphatase and alkaline phosphatase activities, respectively (Tabatabai, 1994). Ideally the whole range (acid, neutral and alkaline) of pH values and activity measurements within each individual soil should be considered (McLaren and Packer, 1970). Several assays of soil phosphatase enzymes have been set up using different substrates and detection methods. Most widely used assays are those based on spectrophotometric and fluorometric techniques.

Phosphatase enzymes generally act by cleaving ester bonds of organic phosphorus within organic matter to liberate phosphate (PO_4^{3-}) which is being taken up by the plant or microbial biomass (Bünemann *et al.,* 2012). When the carbon: phosphorus (C:P) ratio of approximately 100 is obtained, then P immobilisation by microbes is obtained, because of the relatively high P requirement by soil microorganisms compared to plants (Haygarth *et al.,* 2013; Dalal, 1997). Microbes would therefore in this situation compete aggressively

with plants for available P in soil. Management practices such as organomineral fertiliser amendments can influence soil structure and indirectly affects enzyme activities and kinetics during the adsorption of extracellular enzymes in the organomineral complexes (Quiquampoix and Mousain, 2005). Assessing derived kinetic parameters such as maximum velocity and Michaelis-Menten constant (Km) would therefore effectively highlight short-term sensitive changes in the soil treated with different fertiliser. Microbial P immobilisation can often contain as much organic P, about 20-30% of the total soil organic P pool, which is even larger than the proportion of both C (1-2%) and N (2-10%) held in microbes (Dalal, 1997).

1.5 Nitrogen cycle

Nitrogen is a group 15 essential element required by plant and forms a vital component of fertilisers and foods (Haygarth *et al.,* 2013). Unlike phosphorus, nitrogen (N) is highly mobile circulating between the atmosphere, soil and living organisms. Liquid and gaseous nitrogen are colourless and odourless, with the gaseous being generally inert making up about 78% of atmospheric volume (Stevenson and Cole, 1999). The greatest source of available nitrogen is the atmospheric dinitrogen (N_2), which is relatively stable and can be easily fixed from the atmosphere by free-living prokaryotic bacteria and symbiotic bacteria, that associates with roots of certain legume plants such as clover, as well as non-leguminous plants like alder (Haygarth *et al.,* 2013). As such N cycle has larger and more significant atmospheric component, and also biologically renewable via fixation.

Plants are known to access organic form of nitrogen, however most N in agricultural soil is taken up by plants as ammonium (NH_4^+) or nitrate (NO_3^-) ions and then becomes reduced to amino acids mainly in green leaf when synthesised with carbohydrate and this gives extra level of N compared to other nutrients, the extra protein produced allows the plant leaves to grow larger and have a larger surface area for photosynthesis, as such in many crops the amount of leaf area

available for photosynthesis is proportional to the amount or quantity of N supplied (Haygarth *et al.,* 2013).

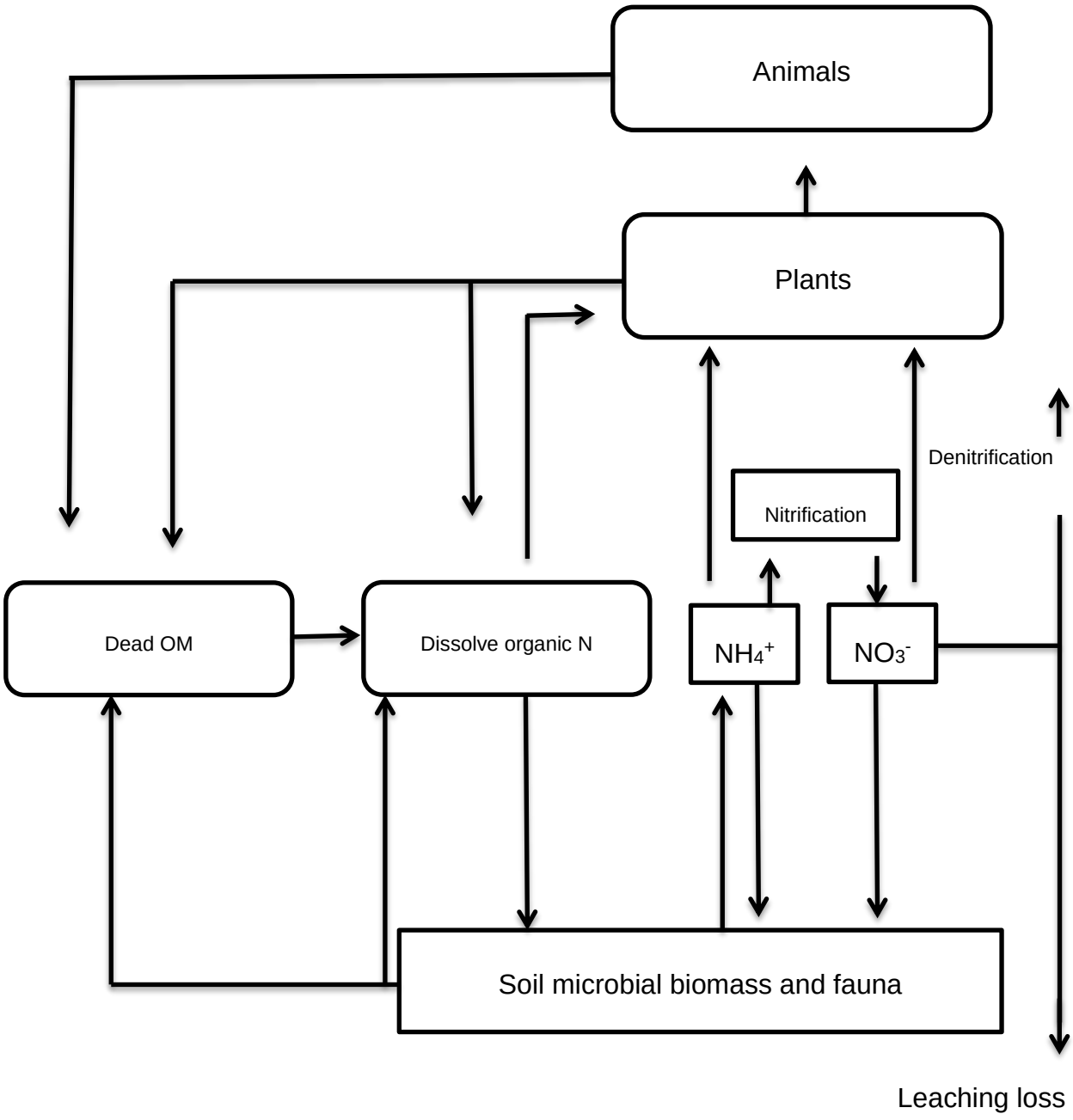


Figure 1-7 Nitrogen cycle in managed agricultural system.

Source: Bardgett (2005).

In soil the largest pool of N is in the organic matter as nitrogenous compounds mainly derived from the decomposition of plant material, microorganisms and added organic matter (manure) and residuals such as biosolids during agricultural systems (Figure 1-7).

1.5.1 Nitrogen mineralisation in soil

Organic nitrogen becomes available to plants through process of mineralisation by microorganisms (Stevenson and Cole, 1999). This results in the production of ammonium ion (NH_4^+), before it is further oxidised to nitrite and the nitrate (Equation 11), which is the most preferable plant available nitrogen in soil (Smith *et al.,* 1998). The rates of nitrogen mineralisation/immobilisation are therefore very important in controlling soil N cycle and thus governing the transfer of N to the wider environment (Haygarth *et al.,* 2013).

Mineralisation of nitrogen in soil generally involves three stages carried out by microorganisms and controlled by several factors such as soil moisture, temperature and size of available substrate (Mengel and Kirby, 1987). The ammonium ion (NH_4^+) produced during mineralisation process is converted to nitrate (NO_3^-). Firstly the NH_4^+ is oxidized to nitrite (NO_2^-) by the nitrosomonas bacteria and then, nitrite is further oxidized to nitrate (NO_3^-) by the nitrobacter as shown in the equation 11.

Organic N $\longrightarrow$ NH_4^+ $\longrightarrow$ NO_2^- $\longrightarrow$ NO_3^- **[11]**

1.6 Urea

Urea is an inorganic form of nitrogen fertiliser derived or synthesised from ammonia mainly through the Haber-Bosch process. It provides the readily available nitrogen in soil upon hydrolysis and is considered the cheapest and most commonly used inorganic fertiliser N accounting for over 50% worldwide in terms of application compared to other nitrogen fertiliser such as ammonium nitrate and calcium ammonium nitrate (Dampney *et al.,* 2003).

1.6.1 Urea in soil

Urea in soil is either volatilised to ammonia near the soil surface or percolates/diffuses into the soil as leachate. Applied urea at the soil surface will dissolve, within a few days. The dissolved urea then reacts to form NH_4^+ and HCO_3^- catalysed by soil urease enzymes (Equation 13). The ammonium carbonate produces, hydroxyl ion (OH^-) during ammonification which can causes increase in the soil pH; while on the contrary, nitrification caused by the release of twice the amount of hydrogen ion (H^+) can subsequently decrease soil pH (Bremner and Douglas, 1971). Thus ammonification of urea nitrogen causes temporary increase of soil pH, while nitrification causes decrease of soil pH (Garcia *et al.,* 2014; Kissel and Cabrera, 2005).

$$CO(NH_2)_2 + 2H_2O + H^+ \longrightarrow 2\,NH_4^+ + HCO_3^- \qquad [13]$$

$$HCO_3^- + H^+ \longrightarrow CO_2 + H_2O \qquad [14]$$

Efficiency of urea hydrolysis is govern by soil pH and greatly affected by the enzyme urease (Cabrera *et al.,* 1991). It is estimated that up to 30% of urea nitrogen is lost as volatilised ammonia when applied in soil surface (Mendoza *et al.,* 2006).

1.6.2 Factor influencing urea mineralisation in soil

Several factors influence the mineralisation of urea which includes; soil moisture, temperature, microbial activity, pH and buffering effect of soil and organic matter content (Kissel and Cabrera, 2005). However only a few microorganisms in soil provides the necessary conditons such as substrate, pH and water content for their growth and survival (Nannipieri *et al.,* 1983). In controlled experimental conditions, soil is regarded as distinctive system with determine total level of biomass.

A higher content of microbial biomass could only be maintained as long as energy supplies are available (Nannipieri *et al.,* 1983)

Long-term treatment of soil with organic manure and other residuals such as sewage sludge, have caused increase in soil pH (Cai *et al.,* 2014).

1.6.3 Use of urea in Agriculture

The world consumption of fertiliser nitrogen has doubled in the past three decades and over 75% of this nitrogen is in the form of ammoniacal N, or in forms of N such as urea (about 40%) that react in soil to produce ammoniacal N (Kissel and Cabrera, 2005). Urea (as a source of readily available nitrogen fertiliser) consumption has dominated the world nitrogen use in agriculture and other commercial purposes for many years with almost a 5-fold increase compared to ammonium nitrate (Figure 1-8). In comparison, the other forms of N have shown only a small increase in consumption over the same time period.

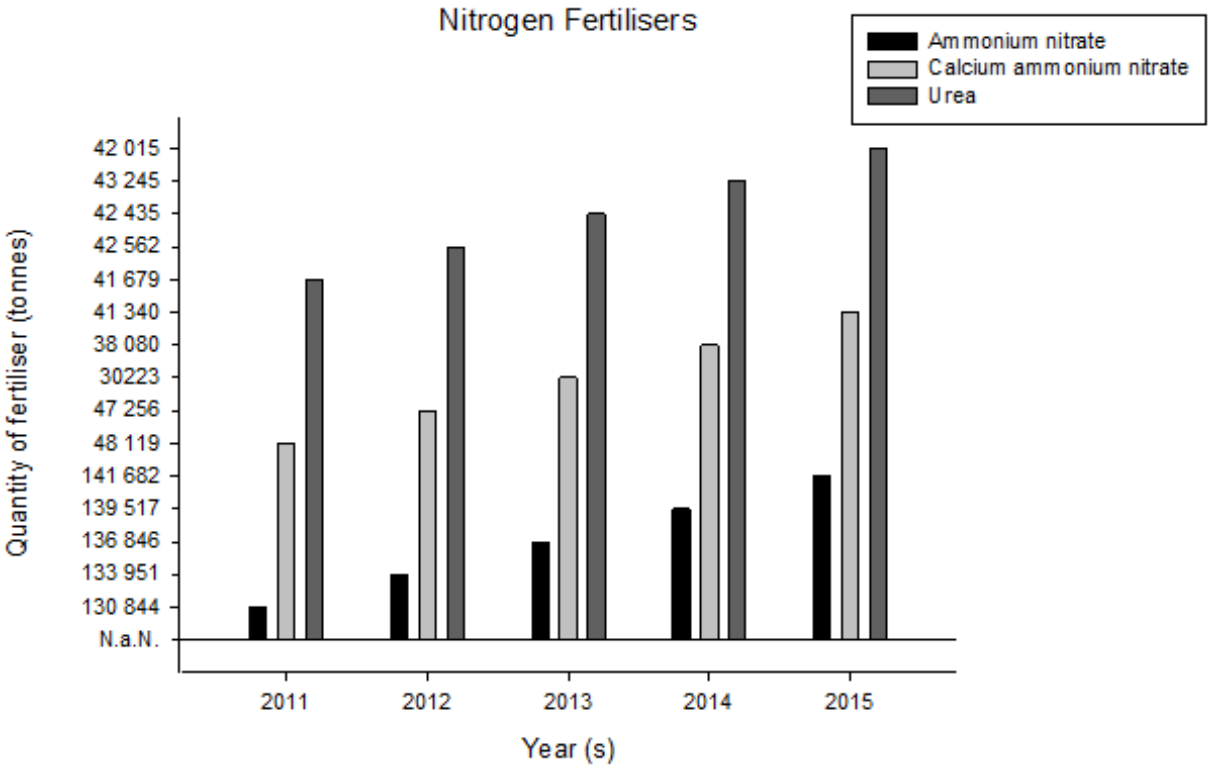


Figure 1-8 World Consumption of readily available nitrogen fertilisers

(Source: FAO, 2011).

1.6.4 Problems of urea in soil

Urea is release to the atmosphere from soil surface via volatilisation, which results in ammonia gas release, considered a greenhouse gas because it forms transport aerosols in the atmosphere (Mendoza *et al.,* 2006). Urea inform of NH_4^+/ NO_3^- in soil becomes readily available to plants according to their needs, but when it is in excess, it can accumulate in the soil or dissolve and become part of the leachate (Kissel and Cabrera, 2005). Application of urea in agricultural soil above certain levels (250KgN/ha) becomes toxic causing damage and loss of plants due to high production of ammonia (Kissel and Cabrera, 2005). It can also causes pollution of groundwater as nitrate which is of particular in nitrogen vulnerable zone (NVZ) areas. Safety levels of nitrate should be less than 10 mg/L (10ppm) in drinking water. Nitrate above the recommended levels are known to cause a potentially fatal blood disorder in infants under the age of 6 months called methemoglobinemia, popularly known as the “blue-baby” syndrome (Addiscott, 1999). This causes a reduction in the oxygen blood-carrying capacity in the babies’ blood which can leads to vomiting, diarrhoea and difficulty in breathing and even death in severe situations.

1.7 Integrated use of mineral and biosolids fertilisers

The use of biosolids as a potential source of fertiliser has been demonstrated for many years (Smith, 2006). Like other organic sources (manure and green compost), biosolids contain slow-release nitrogen and phosphorus fertiliser that can provide essential nutrients in agricultural soil. Studies by Huang *et al.,* (2012) on mineralisation rates of biosolids treated soils, incubated for 48 weeks, showed significant increase (0.047-0.075 mg/kg) of nitrogen per week. In another study by Smith *et al.* (2006) during which different rate (4, 8 and 16t/ha dry solids) of sewage sludges, were mixed with sandy loam soil to grow perennial rye-grass during 151 days incubation, there were significant nutritional benefits of phosphorus addition found in the grasses (Smith *et al.,* 2006).

Field scale experiments on wheat grown with biosolids, have also produced yields comparable to mineral fertiliser treated soils (Zarabi and Jalali, 2013;

Ippolito *et al.,* 2007). Therefore using different appropriate formulations and applications of biosolids with mineral fertiliser, that factor in the carbon to nitrogen (30:1) ratio, moisture content (50-60%), and timing, is important for optimum crop yield. Mixing organic residuals with the inorganic fertilisers in soil to enhance effective release of available plant nitrogen or phosphorus to achieve optimum crop yield in an environmentally friendly way have been carried out by several scientist. Sikora and Enkiri (2000) for example used biosolids combinations with ammonium nitrate to grow fescue grasses in their incubation studies, where they showed linear increase (9.1, 10.5, 11.4 mg/kg) in those blended pots compared to the un-amended pots. In another field-scale study using blended biosolids with urea formulations to grow rye-grasses, have also shown an important agronomic efficiencies in the range of 26-35 kg/g, which almost double the amount with only biosolids application by comparison (Antille, 2011.) Furthermore nutrient uptake efficiency by grass crops were comparable with mineral nitrogen fertiliser and have been suggested as being less damaging with regards to eutrophication effects (Antille *et al.,* 2013).

1.7.1 Biosolids and urea integration as organomineral fertilisers

The integration of organic residuals with mineral sources of fertiliser (**Section 1.7**) for good crop yield is promising, and therefore biosolids integration with urea to form organomineral fertiliser in order to provide essential soil available phosphorus for optimum plant growth is very important. This project establishes the potential of biosolids integrated with urea to release available phosphorus and the effects of urea component on the mineralisation rate of the biosolids P during short-term (60 days) control condition of soil incubation. The soil phosphorus index values which determine the initial soil native phosphorus content were considered. There are several ranges of soil index phosphorus values as classified according to the ADAS classification (RB 209). For example a soil with available phosphorus content 0-9mg/L is an index 0; 10-25 mg/L is P index 1; 16-25mg/L is P-index 2 and so on. In this project, P index values of 1 and 2 soil samples from grassland and arable sites respectively were used so as

to be able to notice the P mineralisation effectively during the 60 days incubation period.

1.7.2 Factors influencing the mineralisation of phosphorus in OMF

Several factors that are important during the mineralisation of phosphorus in the organomineral fertiliser includes: soil moisture content, organic matter, pH and also microbial active community. Hydrolysis of urea component in soil, results in the pH changes depending on the soil buffering capacity, ammonification as well as nitrification rates (Cai *et al.,* 2014; Moore *et al.,*2010) and would consequently influences P mineralisation. A high urea content can be toxic to important microbes present in the biosolids fraction and can decreases mobilisation and immobilisation processes of phosphorus. Also organic matter affects the energy flow for microbial growth and enzyme production (Theng, 2012).

1.8 Aim and Objectives

1.8.1 Aim

The aim of this research is to understand the influence that the urea component of an organomineral fertiliser (OMF) has on the mineralisation of phosphorus from the biosolids component.

This study will therefore focus on using integrated biosolids mixed with urea to form organomineral fertiliser in order to contribute to the understanding of short-term (60 days incubation periods) phosphorus mineralisation in arable and grassland soils compared to the urea, biosolids and control treatments respectively.

1.8.2 Objectives

The main objectives of this project are:

- To evaluate the influence of increasing urea component on the mineralisation rate of phosphorus in soil treated with OMF.

- To evaluate the effects of OMF addition on soil microbial parameters such as microbial biomass C and P, phosphatase enzymes activities and the phenotypic community changes of the phospholipids fatty acids profiles (PLFA) in order to elucidate potential mechanisms of the rate of P mineralisation.

- To compare the characteristic of OMF-P mineralisation trends and pattern to that of biosolids, urea and control treatments.

1.9 Research hypothesis

The following hypotheses were tested in this research study;

Hypothesis 1

High doses of urea component in the OMF influences the mineralisation rates of OMF-P during soil incubation period.

Hypothesis 2

The most highly dosed urea component (**250/250**) of OMF formulations this experimental application threshold will have the least OMF-P mineralisation rate in soil compared to the other OMF (**50/250**) and OMF (**150/250**) formulations respectively.

Hypothesis 3

The indigenous soil microorganisms would interact with the organomineral fertiliser, thereby causing changes in their community phenotypic identity compared to control.

2 Materials and Methods

2.1 Description of soil samples

The soil samples used for the incubation experiment were grassland and arable. The grassland (diary) was sandy clay loam with an initial P-index 1from the Broxton site while the arable was clay loam with a P-index 2 from the Lincolnshire area (Figure 2-1). Soil were air dried and ground to pass through a 2mm sieve and then their texture (Table 3-1) were verified by analysing the soil samples using the pipette method (Avery and Bascomb, 1982; BSI, 1990).

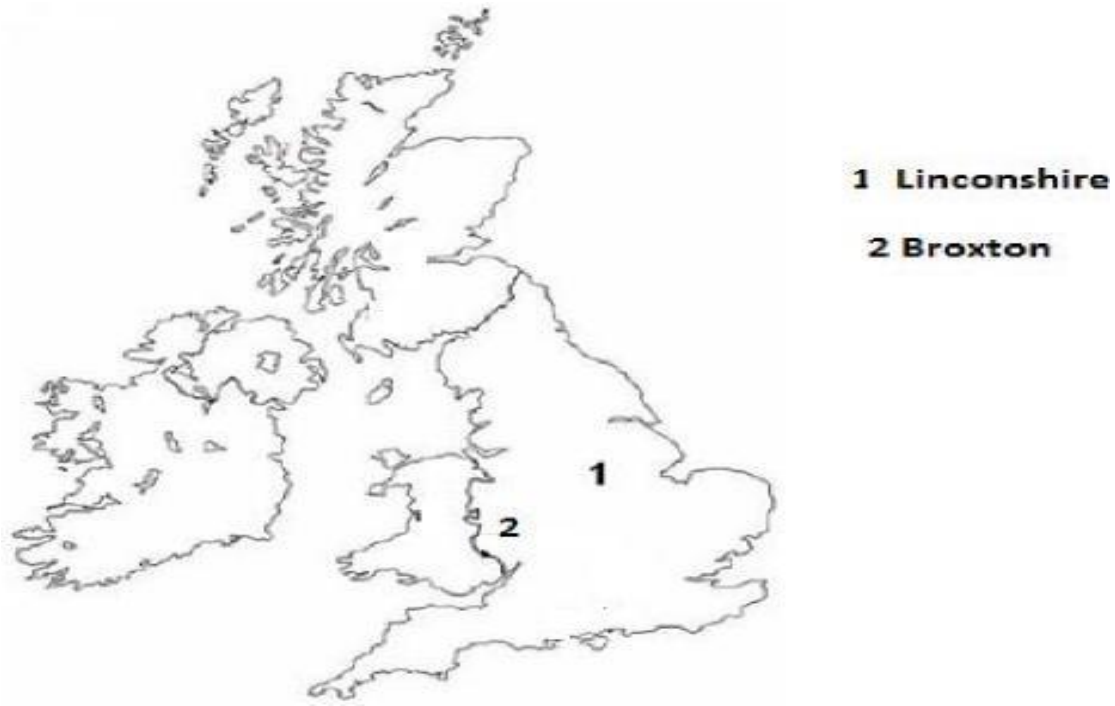


Figure 2-1 Location of soil sampling sites

2.2 Soil incubation

The first soil incubation (experiment 1a) process involved the use of triplicate soil samples in a randomised design of either sandy clay loam or clay loam soils. 520g of dry soil was initially mixed with distilled water to attain field capacity (50-55% moisture) in a 400ml pot. The soil in the pot was packed to a bulk density of 1300kg/m^3 and then stored at a temperature of ca. 25^{o}C. A total of 36 pots for each trial were pre-incubated for up to 2 weeks prior to adding any treatment, to restore equilibrium. During that time incubated pots were routinely monitored and deionised water were added to each pot to maintain field capacity range of 50-55% moisture content. The soil samples were then treated with different amounts of biosolids (BS), urea (U) and mixed quantity of biosolids and urea (BS+U) to obtain two application rates (100 and 250KgN/ha) base on nitrogen equivalent. The soils were properly mixed with the added amendments, covered with a perforated aluminium foil to allow for gaseous exchange and then incubated in the dark room at 25^{o}C for a period of 60 days. The actual amounts of BS, U and BS+U added to each pots are shown (Table 2-1) including the representative control which has no applied fertiliser treatments. Soil samples were taken from the pot by scooping from the soil surface and all the way down to the bottom of the pot. About 80-90g of soil is taken each time and the pot is re-weigh in order to account for equivalent soil moisture content by adding deionised water using water sprayer. The soils were sampled on days 0, 6, 15, 20, 35, 45 and 60 for the analysis of extractable P, mineralisable nitrogen, pH, microbial biomass carbon and biomass phosphorus for both 100KgN and 250KgN. While some of the soil samples obtained at intervals of 0, 20, 45 and 60 days for the 250KgN equivalent application rates were further used for phospholipids fatty acids analysis (PLFA).

In the incubation experiment 1b, only grassland soil (sandy clay loam) was used and initially treated as in the incubation experiment 1a except that, all the amendments had a fixed quantity of biosolids (250KgN/ha) added with various quantity of urea to analyse pH, available P and phosphatase enzymes activities as shown in table (2-1). In this incubation experiment a total of 15 pots including 3 replicates were randomly treated accordingly.

Table 2-1 Fixed amount of biosolids added with various quantity of urea for incubation experiment 1b. BS = biosolids @ 250KgN/ha; U = urea@ 250KgN/ha; BS+U(50KgN/ha) = indicates fixed amount of biosolids@250KgN mixed with 50KgN equivalent of urea; BS+U(150KgN/ha)= fixed amount of biosolids@250KgN mixed with 150KgN equivalent of urea; BS+U(150KgN/ha) = fixed amount of biosolids@250KgN mixed with 150KgN equivalent of urea; BS+U(250KgN/ha) = fixed amount of biosolids@250KgN mixed with 250KgN equivalent of urea.

Treatments	**Biosolids (g/pot) (mg)**	**Urea (g/pot) (mg)**	**50KgN/ha Equivalent (mg)**	**150KgN/ha Equivalent (mg)**	**250KgN/ha Equivalent (mg)**
BS	1206.8				✓
U	287				✓
BS+U (50KgN/ha)	1206.8	57.4	✓		
BS+U (150KgN/ha)	1206.8	172		✓	
BS+U (250KgN/ha)	1206.8	143.5			✓

Table 2-2 Amounts of biosolids and urea supply in the pots for the incubation experiment 1a. BS = biosolids; U = urea; BS+U = mixture of biosolids and urea. BS+U (90/10) = indicates 90% of mixture contain biosolids with 10% urea; BS+U (70/30) = 70% of mixture is biosolids with 30% urea; BS+U (50/50) = contain 50% from biosolids and 50% from urea fractions.

Treatments	**Biosolids** (g/pot)	**Urea** (g/pot)	**Application rates** 100KgN/ha	250KgN/ha
Control	**0**	**0**		
Urea	**0**	**0.115**	✓	
Urea	**0**	**0.287**		✓
Biosolids	**0.914**	**0**	✓	
Biosolids	**2.285**	**0**		✓
BS+U(90/10)	**0.883**	**0.012**	✓	
BS+U(90/10)	**2.057**	**0.029**		✓
BS+U(70/30)	**0.641**	**0.034**	✓	
BS+U(70/30)	**1.61**	**0.086**		✓
BS+U(50/50)	**0.457**	**0.057**	✓	
BS+U(50/50)	**1.143**	**0.144**		✓

2.3 Measurements and analysis

Soil samples were taken from the incubated pot seven times at different time point for a period of 60 days in order to analyse soil available phosphorus, pH, mineralisable nitrogen, biomass carbon and biomass phosphorus and phospholipids fatty acids profiling (PLFA).

2.3.1 Analysis of phosphorus and soil pH

Available phosphorus in soil was determined in accordance with British Standard (BS 7755: Section 3.6:1995). 5g of air-dried soil was extracted in 100ml $NaHCO_3$ by agitating on side to side shaker for 30 min±1(300 min^{-1}) and filtered using Whatman no.2 filter paper. Soil extracts were measured using Molybdenum-blue method (1.5 mol/l H_2SO_4, 20ml 0.15% m/v, 5ml ascorbic acid) and allowed to develop colour for 30 minutes before measuring absorbance at 880nm wavelength on spectrophotometer. Soil pH was determined in accordance with BS ISO: 10390:2005. 10ml scoop of air-dried soil was agitated in 50ml deionised water (1:5 suspension of soil: water) for 60min. The solution was left to stand for another 60 min and then the pH was measured using pH meter.

2.3.2 Biomass carbon

The microbial biomass carbon in the soil was determined in accordance with BS 7755: Section 4.4.2, 1997. Moist soils at field capacity were weighed at equivalent of 12.5g of dry masses of soil for the fumigated and non-fumigated extractions. Fumigated samples were placed in a desiccator with a beaker containing 25ml of ethanol free chloroform and also about 25ml of soda lime in a separate small crucible. Air was evacuated and chloroform was boiled for 2 minutes and left overnight (24 hours ± 1 hour) in the fume cupboard. The fumigated samples were extracted after 24 hours by the addition of 50ml 0.5 mol/l K_2SO_4 solution and shaked on side shaker for 30 min (300min^{-1}) and filtered to obtain the organic extracts. Non-fumigated samples were also agitated in 50ml 0.5 mol/l K_2SO_4 solution and extracted. Both fumigated and non-fumigated were then prepared for analyser and the differences between them were obtained as the biomass carbon values as described by Jenkinson and Powlson(1976).

2.3.3 Biomass phosphorus

Biomass phosphorus was determined by (**Section 2.3.2**) measuring moist soil sample at equivalent weight of 12.5g dry masses of soil. Fumigated samples were chloroform fumigated overnight before extraction with 100ml $NaHCO_3$ by agitating on side to side shaker for 30 min±1(300 min^{-1}) and filtered using Whatman no.2 filter paper. Extracts were then treated with Molybdenum-blue method (1.5 mol/l H_2SO_4, 20ml 0.15% m/v, 5ml ascorbic acid) before measuring absorbance at 880nm wavelength on spectrophotometer. Non-fumigated samples were also $NaHCO_3$ extracted, and measured by ammonium molybdate-ascorbic acid method described by Murphy and Riley (1962). Biomass P was then calculated from the relationship:

Biomass P = [inorganic P (Pi) extracted by 0.5M $NaHCO_3$ from 24h $CHCl_3$ fumigated soil] – [Pi extracted from non-fumigated soil]/ Kp. Conversion factor (Kp) of 0.4 was used for the biomass calculation (Brookes *et al.,* 1982).

2.3.4 Phospholipids fatty acids analysis

The soil samples collected at the interval days (0, 20, 45 and 60) for only 250KgN/ha equivalent that had been frozen (-80^{o}C) for the phospholipids analysis were freeze-dried prior to extraction. Subsamples of 8-10g of soil were extracted with a one-phase solvent extractant, using a modification of the Bligh and Dyer (1959).

2.3.4.1 Soil extraction procedure for PLFA analysis

About 15-20ml of prepared Bligh and Dyer reagent (0.8:1:2 volume ratio mix of citrate buffer: Chloroform: Methanol) was added to 8-10g freeze-dried soil in a glass bottle, sonicated for 30 minutes and centrifuged at 2000 rev/min (700RCF) for 10 minutes followed by the removal of settled organic upper layer. 4ml of chloroform and citrate buffer were added respectively and left over night in the fridge in order to separate the organic layer into 2 phases for fractionation phase.

2.3.4.2 Fractionation

Aqueous (upper) layer were discarded the following morning using a suction pump, while the organic lower layer were dried in a stream of nitrogen under 37^0C. Fractionation of the lipid (neutral, glycol and polar lipids) was carried out through solid phase extraction using commercially prepared solid phase extraction (SPE) cartridges. 0.5g of sodium sulphate was added to the top of arranged SPE cartridge, and then washed with 4ml (2 x 2ml) chloroform. Nitrogen dried lipid extract was then resuspended by pouring 1ml of chloroform, followed by the addition of few (0.5ml) amount of methanol to re-dry the sample. Lipid extract was then loaded onto the SPE cartridges, allowed to soak for about 2-3 minutes, before further adding 1ml chloroform to wash the sample and then connect vacuum pump to the SPE manifold. Neutral lipids was eluted first with 5ml of chloroform, then glycol lipids with 12ml of acetone and finally the polar lipids (containing the phospholipids) with 8ml of methanol in a clean glass media bottle. The phospholipid was then evaporated to dryness under a stream of nitrogen in a water bath set at 37^{o}C ready for next step (methanolysis).

2.3.4.3 Alkaline methanolysis

The resulting polar lipid fraction was then subjected to mild alkaline methanolysis. Polar lipid were reconstituted with 1ml toluene:methanol (1:1ratio) and then hydrolysed using 0.2M methanolic potassium hydroxide, swirled (mixed) and then incubated at 37^{o}C for 30 minutes before stopping the reaction by adding 0.25ml of 1M acetic acid. 5ml of hexane: chloroform (4:1) were added and mixed thoroughly before adding deionised water. Sodium hydroxide was used as the clean-up reagents in the clean-up procedure by discarding the aqueous lower layer, then adding 3ml of 0.3M sodium hydroxide, centrifuged and filtered through sodium sulphate into a clean glass media bottle. A stream of nitrogen, at 20^{o}C- 25^{o}C was then used to evaporate the sample (FAMEs) to dryness, ready for gas chromatography analysis.

2.3.4.4 Gas chromatography preparation

The resulting dried fatty acid methyl esters (FAMEs) were reconstituted with 200µl hexane and transferred into the GC vials and labelled accordingly and then ready for the gas chromatography run in the GC machines.

2.3.4.5 Gas chromatography machine and method

The gas chromatography machine is an Agilent Technologies with software agilent G2070 ChemStation for G.C systems fitted with a split/splitless injector and a HP-5 capillary column (30m length, 0.32 mm ID, and 0.25um film). The carrier gas (1ml/min) was Helium and the FAMEs are separated according to temperature programmed for starting at 50^{o}C per minute (split less hold time), increasing at 25^{o}C per minute until 160^{o}C and then 2^{o}C per minute increase until 240^{o}C and now 25^{o}C increase per minute again until it reaches 250^{o}C.Samples were loaded on to the GC machine. 1µl of the samples were being injected by the auto sampler and the fatty acids methyl esters were detected by the flame ionisation detector (FID).

2.4 Phosphatase enzymes assay

Phosphatases (acid and alkaline) enzymes activities in only grassland soil were determined with the 4-Methylumbelliferyl-phosphate substrate assay (Bremner and Tabatabai, 1976) using a micro-plate fluorimetric procedure according to Marx *et al.,* (2001).

2.4.1 Micro-plate fluorimetric enzyme assay procedure

Soil (1g) was mixed with 100ml sterile deionized water in a 300ml Pyrex beaker for 30 min using a 2.5cm magnetic stir bar at a speed that was sufficient for complete homogenization of the soil suspension. Aliquots (20µl each) of the soil suspension were taken during continues mixing using a 0-50µl multi-channel

pipette with appropriate tips and were placed into microplate wells each that contained different quantity of methylumbelliferone. Subsequently, respective 4-methylumbelliferyl phosphate substrates (2000, 200 and 20µM) was added to each microplate well. The reaction solution was mixed very well by pipetting up and down several times before incubating the microplate at 25^{o}C. Two pH ranges (6.5 and 11) of modified universal buffer were used for the acid and alkaline phosphatase enzymes assay respectively. Other buffers may be used to optimise sensitivity. The fluorescence intensity was measured using a fluorometric micro-plate reader (SpectraMAX-GeminiEM, Molecular devices) connected to a PC running SoftMax® Pro software (v5.0.1). The measurements were set to record readings every 2 minutes (120 seconds) over a period of 30 minutes at the excitation and emission wavelengths of 365 and 460nm respectively. Relative fluorescence increased intensity (RFU) were plotted against substrate concentrations (Figure2-2).

This method of enzyme assays has the advantage of analysing multiple enzymes activities within a short time and using a small quantity of soil samples. It is also very sensitive, as such making it possible to even quantify picomoles of methylumbelliferylphosphate (MUF-P) in 200-300 microliter solutions. However a major concern for the MUF-based methods is the significant quenching in detection of MUF in soil (Freeman *et al.,* 2005). To account for that in soil, calibration of the microplate is therefore very important. Although in this case only grassland soil were used for the experiment.

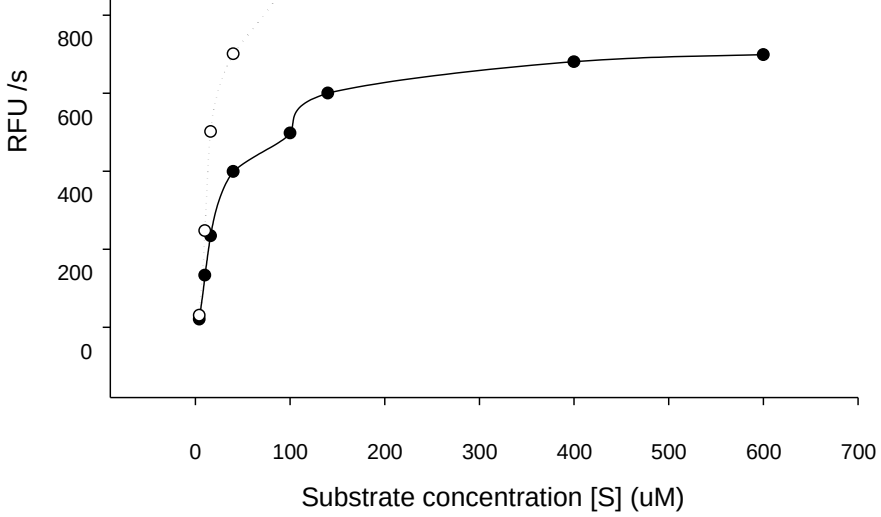


Figure 2-2 Fluorescence response at different substrate concentrations. Acid (pH= 6.5) and alkaline (pH= 11) phosphatases response.

2.4.2 Micro-plate calibration

Two separates calibration graphs were produced at pH 6.5 and 11 respectively to account for the quenching effects of the soil sample as follows: 10mM stock solution (0.176g 4-Methylumbelliferone in 100ml methanol) was initially prepared, from which 2.5µl was diluted to 25ml final volume with the modified universal buffer according to the required pH to give 1µM standard solution. Aliquots (n = 3) of 0 10, 20, 30, 40, 50, 60 and 70µl were dispensed into the microplate and made up to a total volume of 200ul with buffer. These make the final calibration concentrations equivalent of 0, 10, 20, 30, 40, 50, 60 and 70 pico mole 4-MUF 200µl. Fluorescence of 4-MUF was then measured per minutes (60 seconds) for a period of 30 minutes cycle (1800 seconds). Fluorescence reading (RFU) for both the blank (deionised water, 4-MUF and buffer) and soil samples (sample, 4MUF and buffer) in the 96 wells microplate were calculated. Each relative fluorescence unit (RFU) value was initially background corrected by subtracting the mean RFU measured at 0 pmol 4-MUF 200µl and the RFU was then plotted against 4-MUF concentration and fluorescence quenching was determined by comparing blank calibration with the soil calibration curves. Figure 2-3 shows the quenching effects of grassland soil sample during acid phosphatase (pH 6.5) activities. The effects on the rate of fluorescence efficiency were not significant (less than 10%) in both acid and alkaline phosphatase enzyme activities.

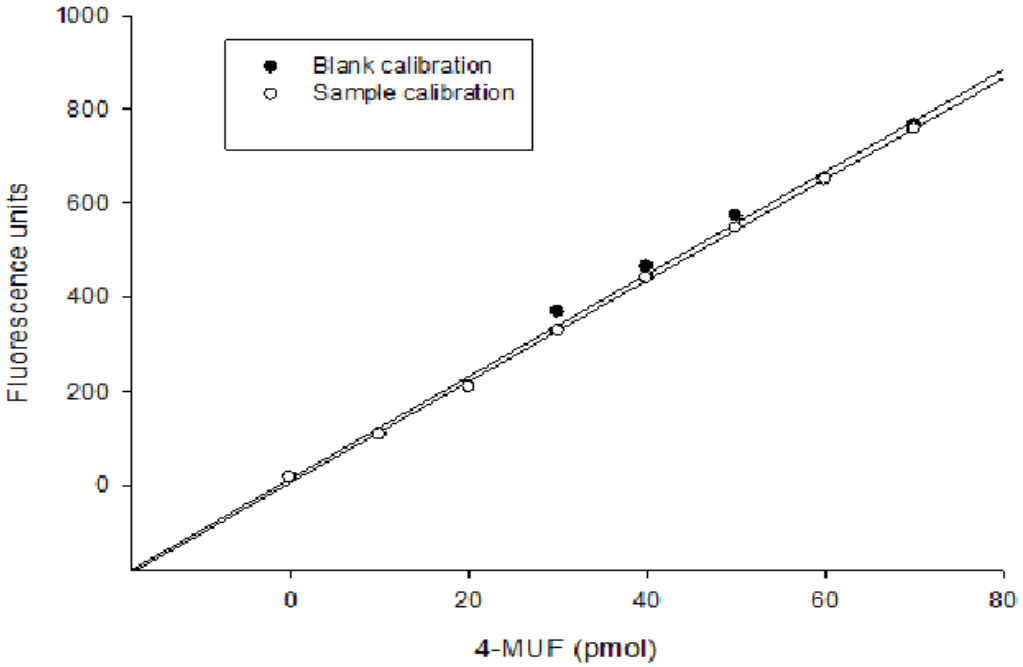


Figure 2-3 Percentage quenching of grassland soil sample on the relative fluorescence efficiency.

2.5 Statistical analysis

The effect of each treatment under variable addition of biosolids and urea and then fixed addition of biosolids with and without urea as well as the overall influence of urea on the measured variables such as pH, biomass carbon and phosphorus during P-mineralisation with time in soils were assessed by repeated measures analysis of ANOVA (General Linear Models) in Statistica 12.0, followed by Fischer test when least significant differences ($p<0.05$) were indicated. Highest factor loadings of the selected fatty acids methyl esters were analysed with principal component analysis (PCA) and the community changes were shown in ordination plots (Figure 3-7) and (Figure 3-14).

3 Results

3.1 Incubation experiment 1a

This incubation study, involves the use of grassland and arable soil samples treated as in **Section 2.2** and then amended at the application rates of 100 and

250KgN/ha equivalents using various quantity of biosolids and urea to give the following combinations [(BS, U, BS+U (90/10), BS+U (70/30), BS+U (50/50)] and the control samples as shown in Table 2-2.

3.1.1 Grassland and arable soil and biosolids characteristics

The initial characteristics of grassland (sandy clay loam) and arable (clay loam) prior to the start of the experiment were shown (Table 3-1). The soil pH for sandy clay loam and clay loam was 6.9 and 8.2 respectively. The total organic carbon in biosolids (35.9%) was significantly high compared to sandy clay loam (2.14%) and clay loam (1.9%) soils. Biomass carbon and phosphorus from the sandy clay loam soil where higher than the clay loam sample. The C/N ratios of the sandy clay loam from grassland and clay loam from the arable soils were 9.2 and 13.7 respectively. A summary of results of analyses conducted on the soils and biosolids prior to the incubation experiment has been presented in Table 3-1.

Table 3-1 General characteristic of soils and biosolids analysed prior to incubation experiment. Mean n=3 ± SE: standard error; nd=not detected. TOC=total organic carbon; C:N= carbon-nitrogen ratio.

Variables	Grassland	Arable	Biosolids
Total carbon (%)	2.19 (0.1)	2.47 (0.1)	37.15 (0.8)

Total nitrogen (%)	0.24 (0.1)	0.18 (0.1)	5.36 (0.2)
C:N	9.2 (0.2)	13.7 (0.2)	6.9 (0.1)
TOC (%)	2.14 (0.1)	1.9 (0.2)	35. 87 (0.1)
Total P (mg/kg)	853.7 (0.1)	733.5	-
Olsen P (mg/kg)	12.23 (0.8)	18.61 (1.3)	-
P index	1	2	-
TON-N (mg/kg)	7.97 (0.4)	34.49 (1.3)	7.5 (0.3)
pH	6.9 (0.1)	8.2 (0.1)	
Biomass C (µg-C/g)	547.9	368.2	-
Biomass P (µg-C/g)	52.3	nd	
Sand (%)	50.9	47.9	
Silt (%)	29.1	30.0	
Clay (%)	20.0	22.1	

3.1.2 Grassland soil

The following results are presented for the different analysis carried out on the amended grassland soil samples during 60 days incubation to determine pH, the available phosphorus mineralisation, soil mineralisable nitrogen, biomass carbon and phosphorus and the phospholipids fatty acids profiles.

3.1.2.1 Soil pH

This soil showed initial pH of 6.9 (Table 3-1). After amendments with different combinations of urea, biosolids and biosolids mixed with urea (OMF) and incubated for 60 days, changes in the soil pH analysed at the selected days interval during the incubation period were not significantly different between the various treatments as well as when compared to the control irrespective of the application rates (100KgN and 250KgN/ha) equivalents (Figure 3-1), except for those treatments containing only urea, that shows increased pH values than the other treatments at the initial day 0 and during 6 days period. This treatment (urea only) shows significant difference ($p<0.05$) of up to 1 unit increase in pH value compared to control, but during 35 days incubation time, it had the lowest pH value compared to other treatments including the control (Figure 3-2) even though during the subsequent days (45 and 60), the pH values were more or less of the same values.

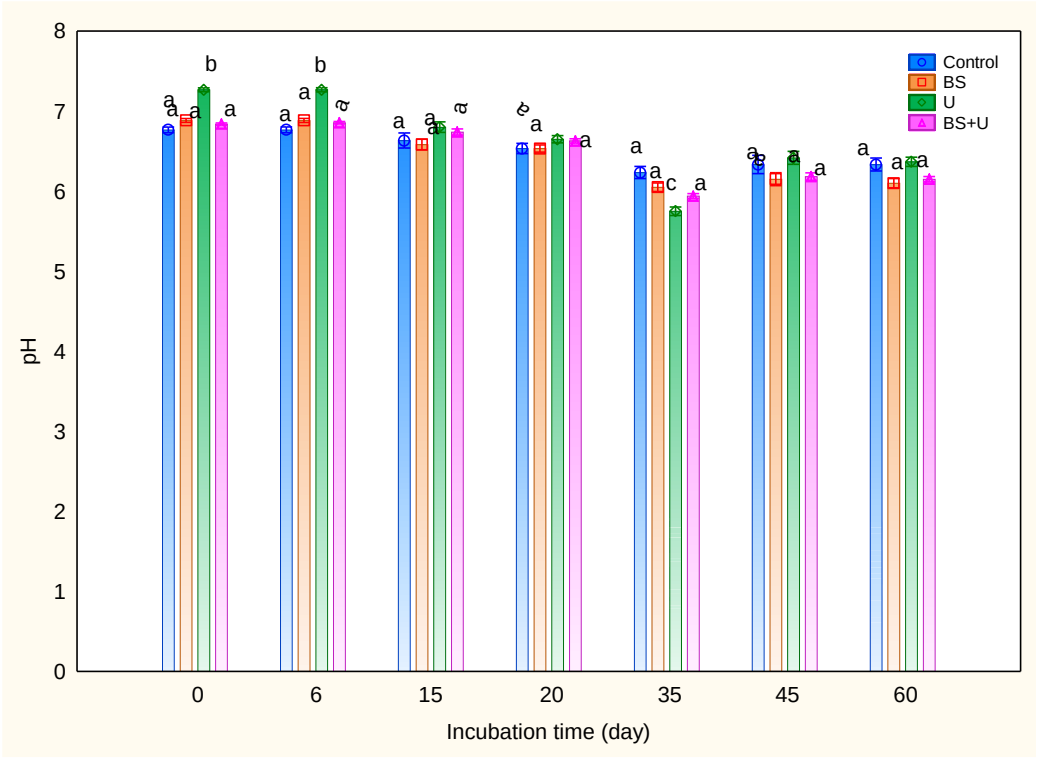


Figure 3-1 Mean pH values in different treatment during 60 days incubation. (SE= standard error; n=3) BS=biosolids; U=urea; BS+U= mixed biosolids and urea or can be refered as organomineral fertiliser (OMF). Different letters above the columns indicate significant difference between incubation time (days) at p<0.05.

3.1.2.2 Phosphorus mineralisation

As shown in Table 3-1, the initial soil P content was 12.2 mg/kg prior to amendment and as such is classified as Index 1 available P soil. Mineralisation of phosphorus in the amended soil was slow initially, from day 0 up to 15 days. However, during 20 days incubation time, there were significant increases in the mean values of soil available phosphorus, particularly in the biosolids (BS) treatments, mixed biosolids with urea (BS+U 70/30: BS+U 90/10: BS+U 50/50), for both 100KgN/ha (Figure 3-2) and 250KgN/ha (Figure3-3) equivalents respectively. It was also noticed that mixed combinations of BS+U 70/30 shows significant difference ($p<0.01$) with the control and only urea treatments at the

twentieth day, for the 100KgN/ha equivalent fertiliser, during the 60 days incubation period.

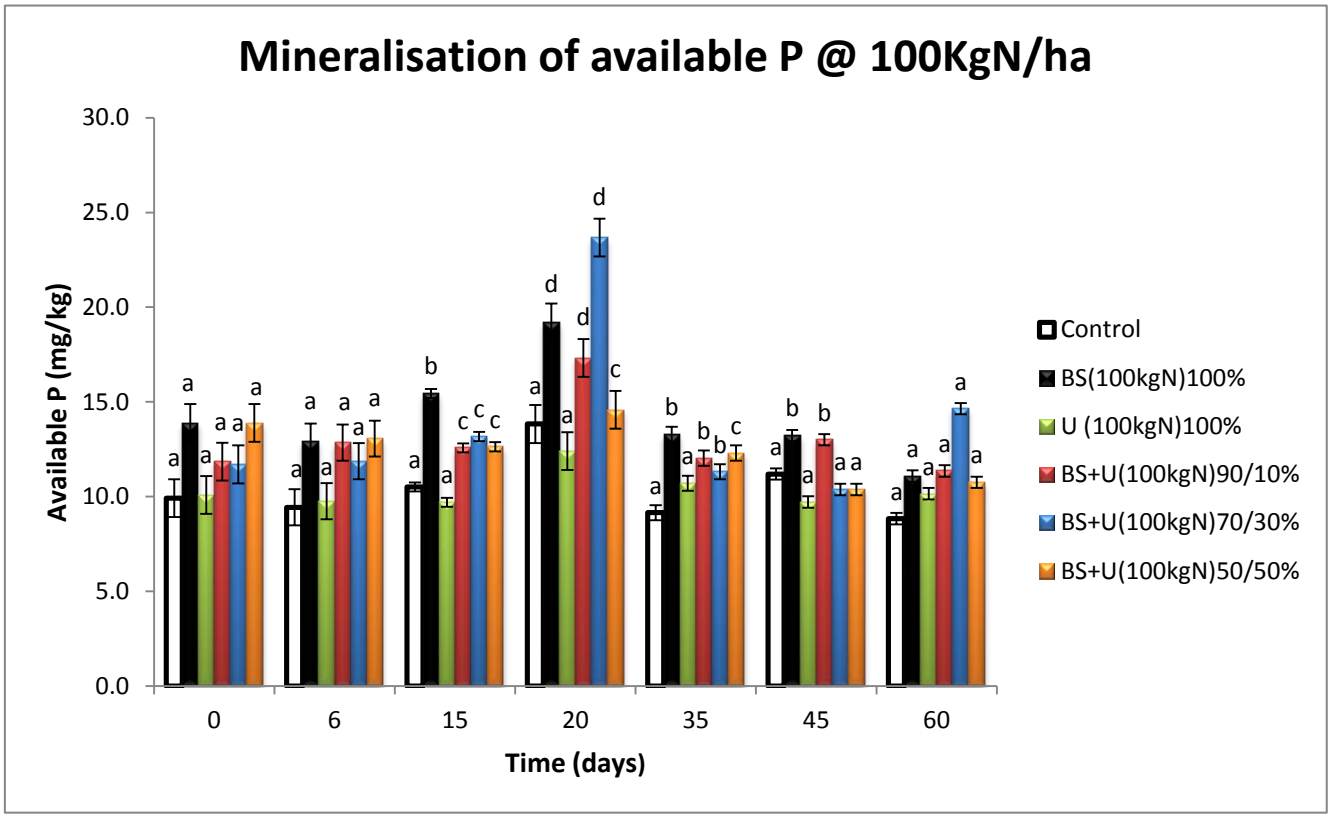


Figure 3-2 Phosphorus mineralisation in amended grassland soil during 60 days incubation. BS= biosolids; U=urea; BS+U=mixed biosolids and urea; BS+U 90/10=mixed biosolids and urea containing 90% from BS and 10% from U; BS+U=mixed biosolids and urea containing 70% of BS and 30% urea; BS+U 50/50=mixed biosolids and urea containing 50% BS and 50% U. ±SE, n=3. Different letters above the columns indicate significant difference between incubation time (days) at p<0.01).

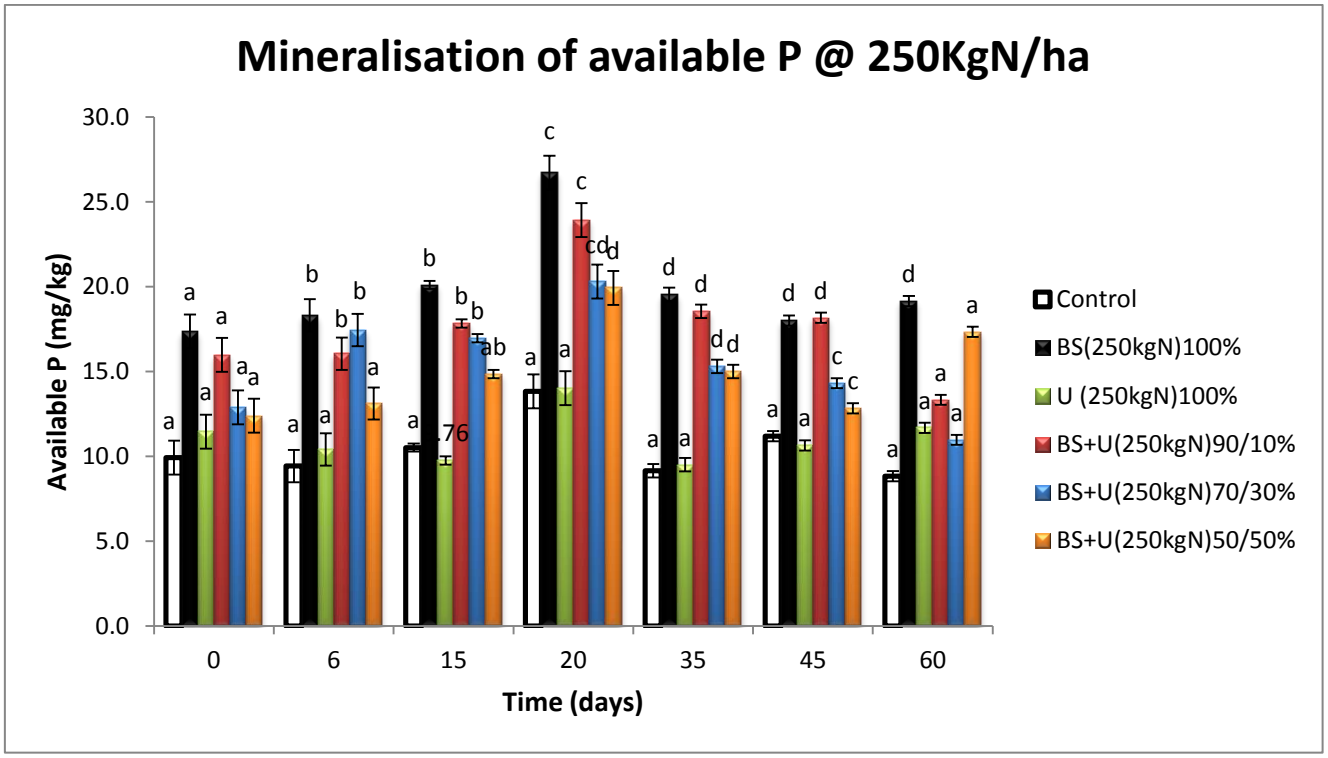


Figure 3-3 Phosphorus mineralisation in amended grassland soil during 60 days incubation. BS= biosolids; U=urea; BS+U=mixed biosolids and urea; BS+U 90/10=mixed biosolids and urea containing 90% from BS and 10% from U; BS+U=mixed biosolids and urea containing 70% of BS and 30% urea; BS+U 50/50=mixed biosolids and urea containing 50% BS and 50% U. ±SE, n=3. Different letters above the columns indicate significant difference between incubation time (days) at p<0.05.

The overall calculated mean values for the available phosphorus in the biosolids (BS) treatments for the 60 days period, were significantly different ($p<0.05$) with up to 60% compared to control and urea treatments. And up to 40% difference with those mixed treatments containing biosolids and urea (BS+U) at various application ratios (Figure 3-4; Appendix III).

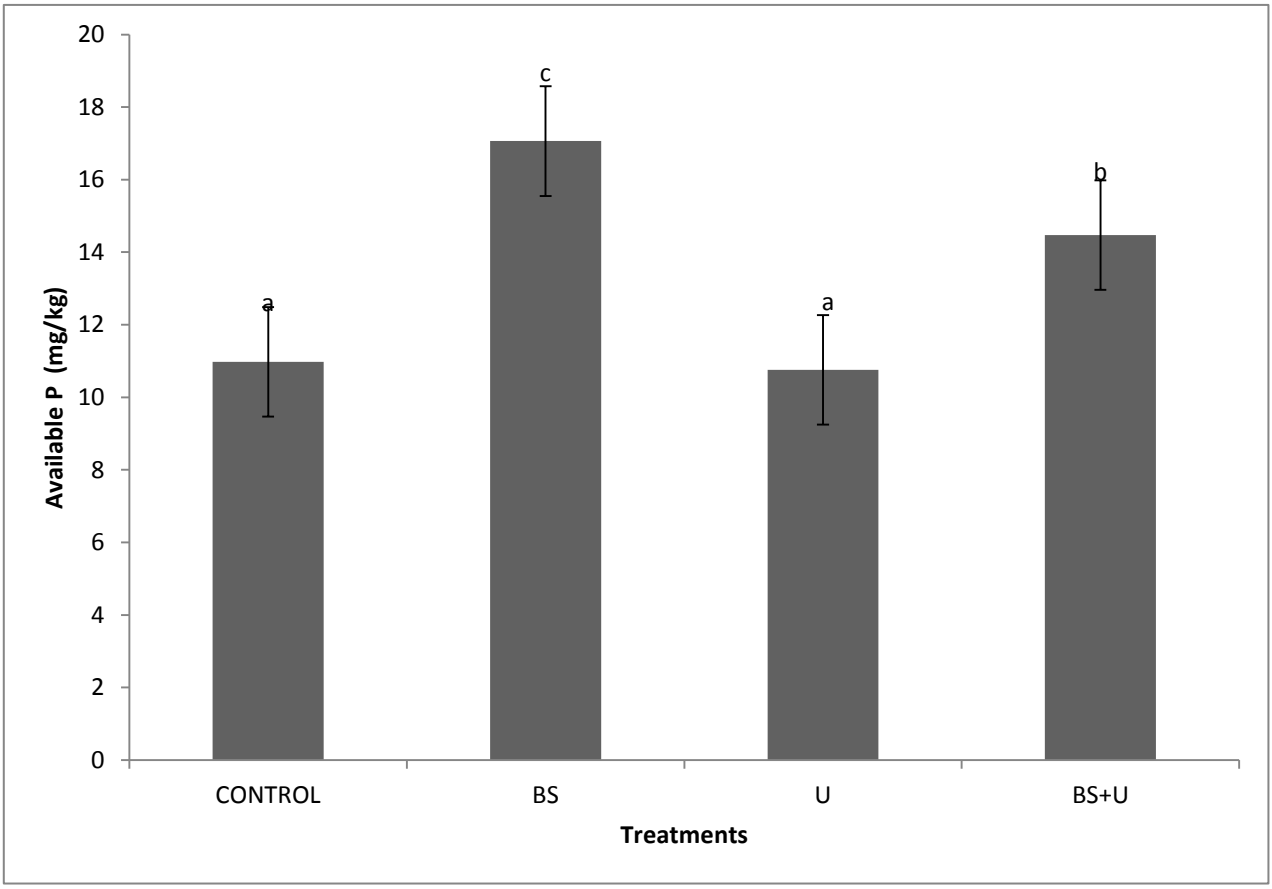


Figure 3-4 Mean values of available P release for the 60 days incubation period. BS= biosolids; U=urea; BS+U= mixed biosolids and urea. ±SE n=3. Different letters above the columns indicate significant difference between treatments for the incubation time (days) at p<0.05.

3.1.2.3 Nitrogen mineralisation

Unlike phosphorus, the nitrogen mineralisation in urea amended sample, shows significant released of total oxidised nitrogen (NO_3+NH_4) particularly in the urea (U) and mixed biosolids and urea (BS+U 70/30) treatments, just within 6 days of the incubation period (Figure 3-5). Total oxide of nitrogen which represents sum of the mineralised ammonium and nitrates, for the urea amended samples at the 250KgN/ha equivalent, have shown significant difference ($p<0.01$) compared to other treatments including control, particularly at day 20 of the soil incubation period, in which the TON-N concentration in the urea amendment had up to 60% higher than biosolids (BS), or mixed biosolids and urea (BS+U) and

up to 80% when compared with the control (C) during the 20 days period. Oxidised nitrogen finally becomes mineralised and reduced to more or less the same amount in all the treated and control samples during the remaining days 35, 45 and 60 respectively (Appendix IV and V). The overall mean values of urea, mixed biosolids and urea and the control after 60 days incubation were 382 mg/kg, 275 mg/kg, and 217mg/kg respectively.

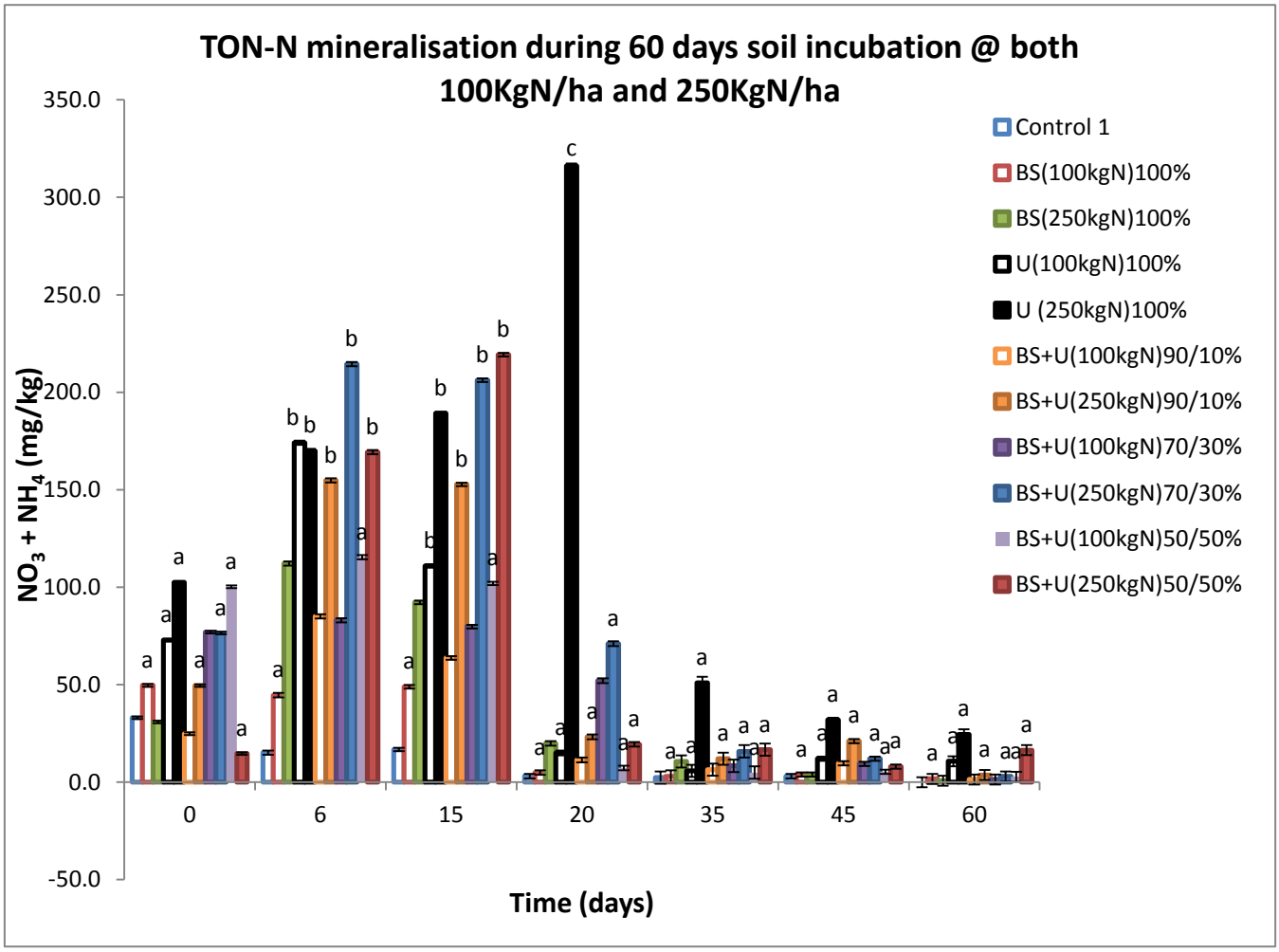


Figure 3-5 Changes in TON-N during 60 days soil incubation treated with different fertilisers at nitrogen equivalent rate of 100KgN/ha and 250KgN/ha respectively.(mean ±SE n=3). BS=biosolids; Control (no added fertiliser); U=urea; BS+U=mixed of biosolids and urea. Columns labelled with the same letter are not significantly different ($p<0.05$).

3.1.2.4 Microbial biomass carbon

Soil microbial activities are important for organic matter transformations, and microbial biomass carbon is an essential source and sink of nutrients in the terrestrial ecosystem (Caldwell, 2005: Nannipieri *et al.,* 1983). The changes in soil biomass carbon containing biosolids (BS) and mixed biosolids with the urea treatments (BS+U) showed significant differences ($p<0.01$) compared to control (C) and urea amendments at day 20 of the 60 days incubation period (Appendix I and II). Biomass carbon showed an increase trend from day 0 up to 20 days (Figure 3-6) before it gradually started decreasing during 35 days of incubation and then become further reduced to more or less the same amount as in the initial time (Day 0) at the final incubation period.

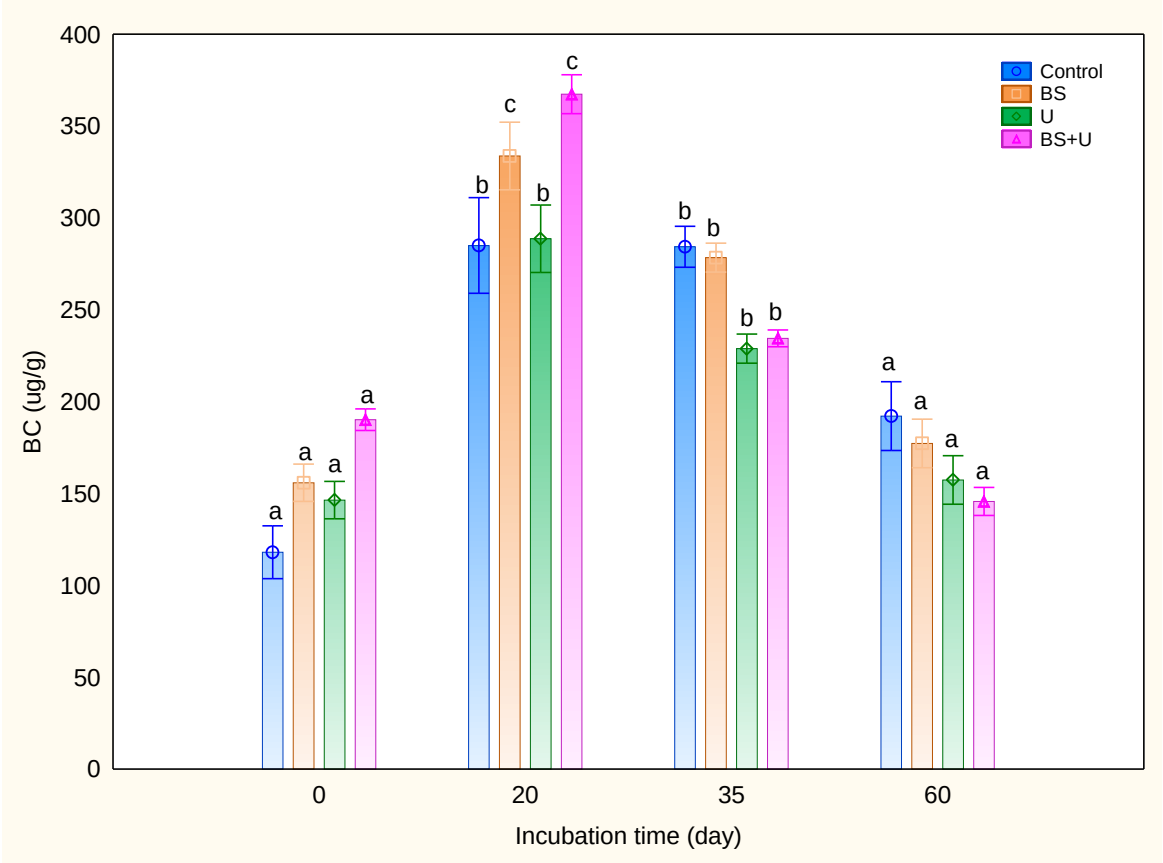


Figure 3-6 Biomass carbon in the amended soil during 60 days incubation. (mean ±SE n=3). BS=biosolids; Control (no added fertiliser); U=urea;

BS+U=mixed of biosolids and urea. Columns labelled with the same letter are not significantly different (p<0.05)

3.1.2.5 Biomass phosphorus

Phosphorus in the soil microbial biomass analysed on the selected days (0, 20, 45 and 60) during the 60 days incubation were not detected. The facts that the amount of biomass P is very small (2-5%) in soil, there could have been overlap in the ranges of signals for the detection of biomass P which is below the detection limits.

3.1.2.6 Phospholipids fatty acids (PLFA)

Soil treated as in **Section 2.3.4** were used for phospholipids fatty acid analysis to identify and examined the microbial community profile changes. The percentage mole data and those high factor loadings were calculated for the principal component analysis (PCA) and ordination plots (Figure 3-7). The results for the ordination plots of PCA, primarily according to the different amendment had 30.8% and 20.6% total variation on the first (PC1) and second (PC2) axis respectively. There were significant ($p<0.05$) effects on the community shift with time. At the initial incubation period (day 0), those treatments containing biosolids (BS) and mixed biosolids (BS+U 90/10) were significantly ($p<0.001$) pulled away from component axis (PC1) towards the second (PC2) axis compared to other treatments, i.e. control; mixed BS+U (70/30) and mixed BS+U (50/50) respectively (Figure 3-7). However, 20 days incubation period shows all the treatments now pulled towards first axis (PC1), except control located just on the second axis (PC2). Interestingly, a significant shift between the mixed BS+U (50/50) and control were seen during 45 days period before all the treatments, becomes clustered around PC1 including control at the final incubation time of day 60.

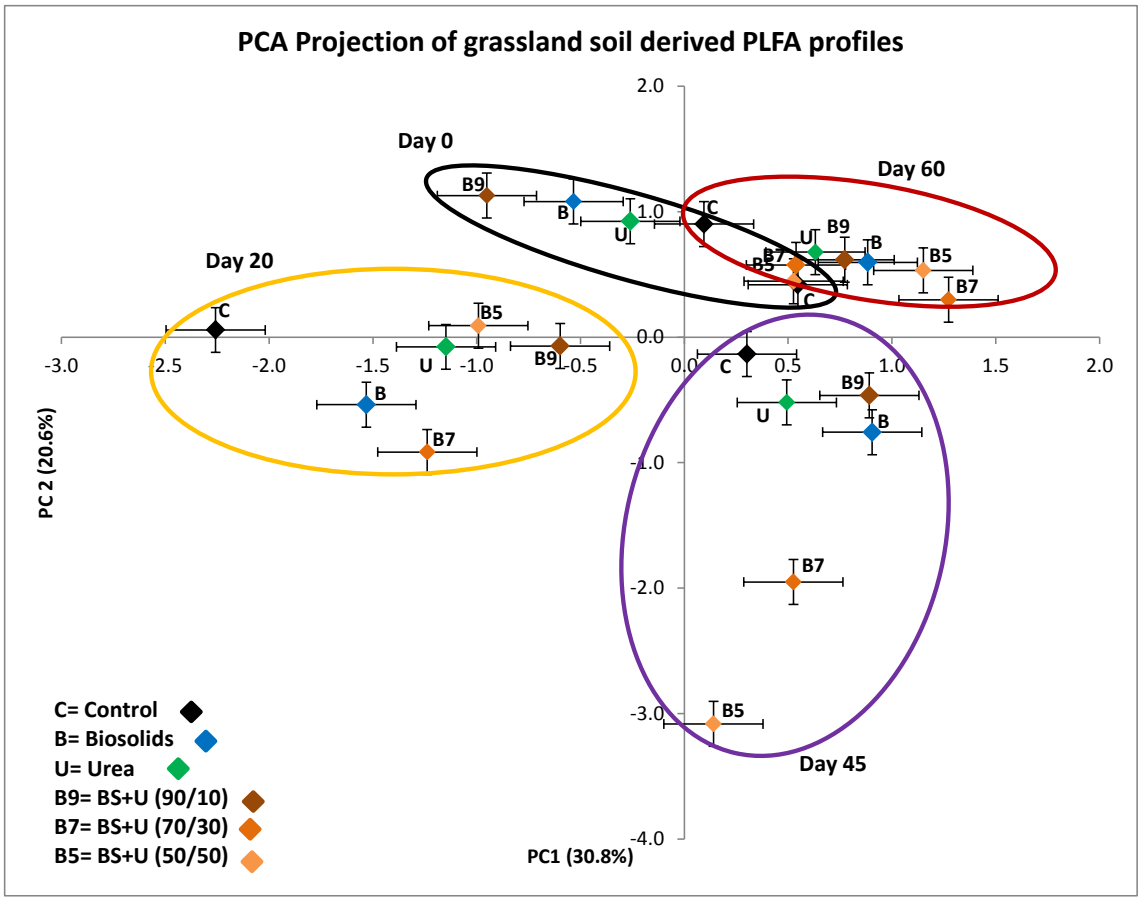


Figure 3-7 PCA ordination plot of grassland soil phospholipids fatty acids profiles during 60 days incubation. BS= biosolids; U=urea; BS+U=mixed biosolids and urea; BS+U 90/10=mixed biosolids and urea containing 90% from BS and 10% from U; BS+U=mixed biosolids and urea containing 70% of BS and 30% urea; BS+U 50/50=mixed biosolids and urea containing 50% BS and 50% U

3.1.2.7 Discussion

In general the amount of mineralised available phosphorus shows significant ($p<0.001$) differences (Appendix III and VI) in both biosolids and mixed biosolids with urea treatments during 20 days incubation compared to the urea and control irrespective of the two fertiliser application rates (100KgN/ha and 250KgN/ha). This has also corresponded with the analysed biomass carbon, which were highest in the biosolids and the mixed biosolids with urea amended soil at the 20

period (Appendix II). This indicates that as dissolved organic carbon becomes available in soil, it provides the energy required by microorganisms to be able to decompose organic matter, and further mineralisation of organic phosphorus present in the organic matter into inorganic phosphorus (Withers and Haygarth, 2007). Even though phospholipids fatty acids profiling have shown changes in all the treatments with time, however during the 20 day period all the treatments were located on the principal component 2 (PC2) axis (Figure 3-7), except the biosolids (BS) and one of the mixed biosolids with urea treatments ratio (BS+U 70/30). This shows that significant variation in the microbial activities was taking place during that time point of the whole incubation experiment when dissolved carbon was readily available to the microorganisms to synthesize extracellular phosphatases capable of hydrolysing organic phosphorus or access cell structures (cytoplasm, cell wall) protected phosphate in organic materials present in the added organic residuals or amendments (Richardson *et al.,* 2011). It has been shown that mineralisation of phosphorus in soil is partly regulated by the carbon-phosphorus ratio (C:P) of substrates (Withers and Haygarth, 2007). Due to initial high carbon content of 37.2 in biosolids (**Table 3-1**) and as such important in providing dissolve organic carbon during the incubation period for the microbial turnover of phosphorus in the soil.

Contrary to the P- mineralisation, urea amended soil samples produced significantly larger amount of the total oxide of nitrogen (NO_3 +NH_4) compared with biosolids, mixed biosolids with urea and control ($p<0.01$) at 20 day period of incubation time. Studies on nitrogen mineralisation indicate biosolids treated soils produced small amount of mineralised nitrogen (Smith *et al.,* 1998), and base on the initial soil nitrogen content (**Table 3-1**) present in both biosolids substrate and soil is small compared to readily available urea (46%N) nitrogen treated soil. Complete declined in the soil total oxide nitrogen (TON-N) was observed (Figure 3-5) after 60 days incubation. Probably at this point in time urea had been completely hydrolysed, converted to NH_4-N, NO_3-N, and NO_2 accordingly (Smith *et al.,* 1998). The initial pH value of the grassland soil was 6.9 and is believed not to have any negative effect on the mineralisation of phosphorus being within the normal ranges (Hinsinger, 2001; Stevenson and

Cole, 1999), and shows only marginal changes in both biosolids and mixed biosolids with urea treatments during incubation period.

3.1.3 Arable soil

The following results are presented for the different analysis carried out on the amended arable soil samples during 60 days control incubation to determine pH, the available phosphorus mineralisation, soil mineralisable nitrogen, biomass carbon and the phospholipids fatty acids profiles.

3.1.3.1 Soil pH

The analysed pH values in the arable soil before amendments was 8.2 (Table 3-1) and did not shows any significant changes after being treated with the various combinations (BS, U and BS+U) of fertiliser during the whole incubation period including the control (Figure 3-8)

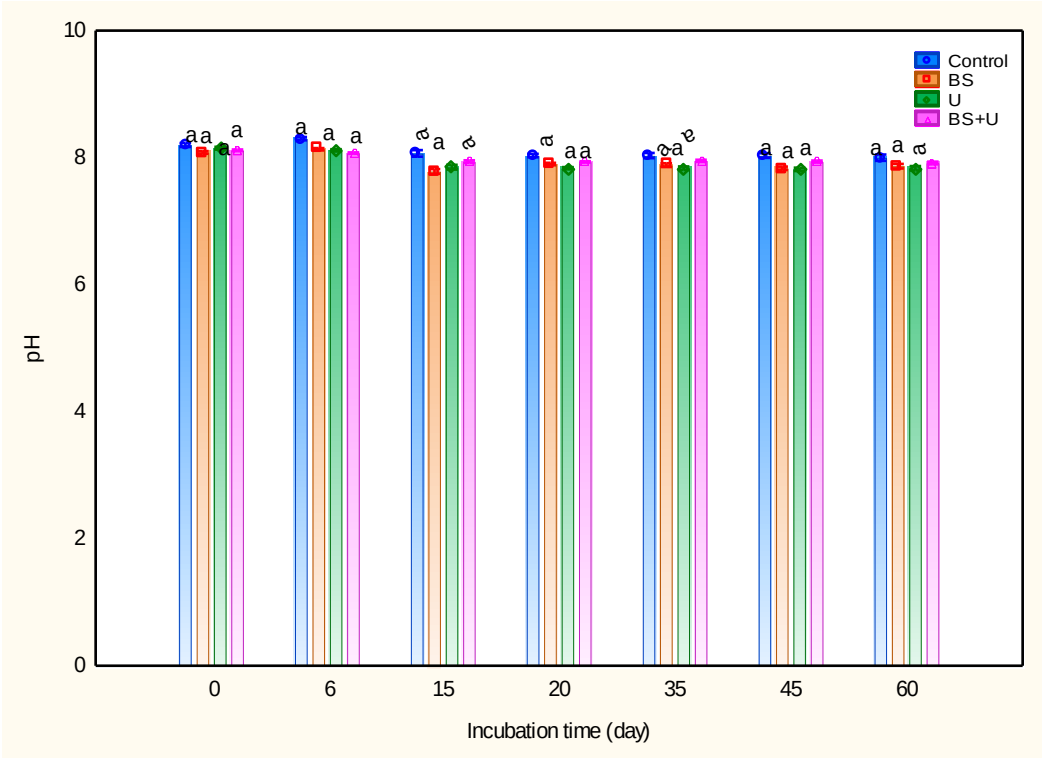


Figure 3-8 The mean pH values in soil amended with different amount of fertiliser during 60 days incubation. (SE= standard error; n=3). BS=biosolids; U=urea; BS+U= mixed biosolids and urea. Different letters above the columns indicate significant difference between incubation time (days).

3.1.3.2 Phosphorus mineralisation

The initial analysed phosphorus content of this arable soil was 18.61 mg/kg (Table 2-1). During 60 days incubation (Section 2.2; Table 2-2), there was no significant changes in soil phosphorus in any treatment at the initial day 0 and up to 6 day period, until after 6 days where a steady increase in P mineralisation rate and release of available phosphorus began, which shows a significant ($p<0.05$) difference of up to 50% in those biosolids (BS) and mixed biosolids and urea (BS+U) treatments compared to urea and control treatments, at the period of 15 and 20 day (Figure 3-9). However there was decline in P mineralisation rate in all the amended samples, which started after 15 days incubation, until all the mineralised P becomes reduced during days 35, 45 and 60 to more or less the same quantity of phosphorus at the initial incubation time.

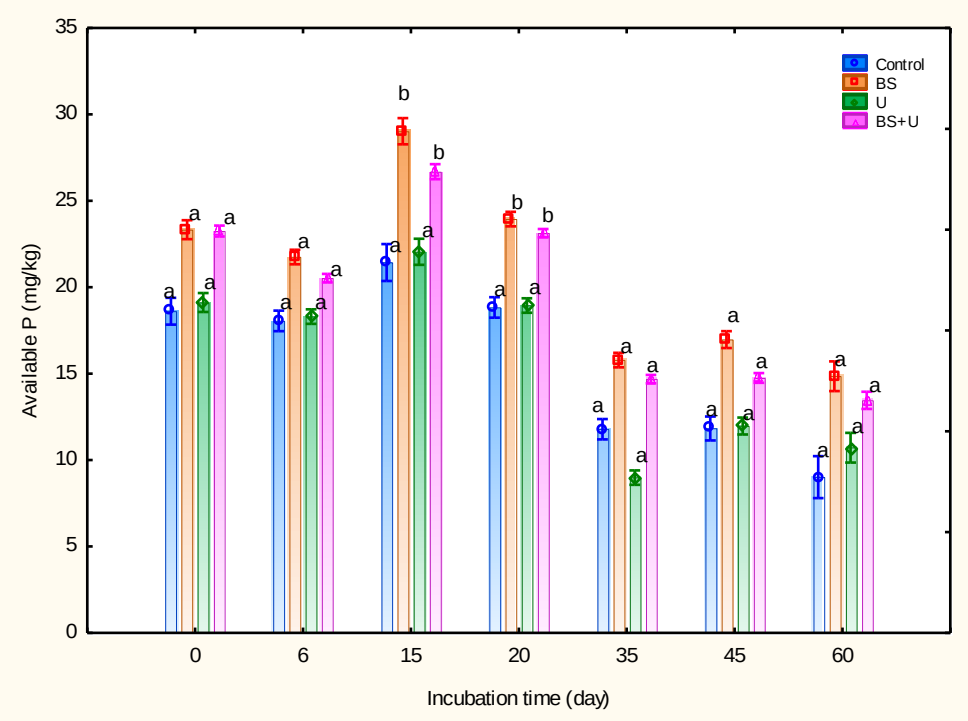


Figure 3-9 Phosphorus mineralisation in arable soil amended with different fertiliser during 60 days incubation. BS=biosolids; U=urea; BS+U= mixed biosolids and urea.

The overall mean values of mineralise available phosphorus in the arable soil during 60 days incubation shows significant ($p<0.001$) increase of up to 65% phosphorus in biosolids treated sample compared to control and urea treatments (Appendix VI). Mixed biosolids and urea treated samples also shows significant difference in mean available P compared with control or urea amendments (Figure 3-10).

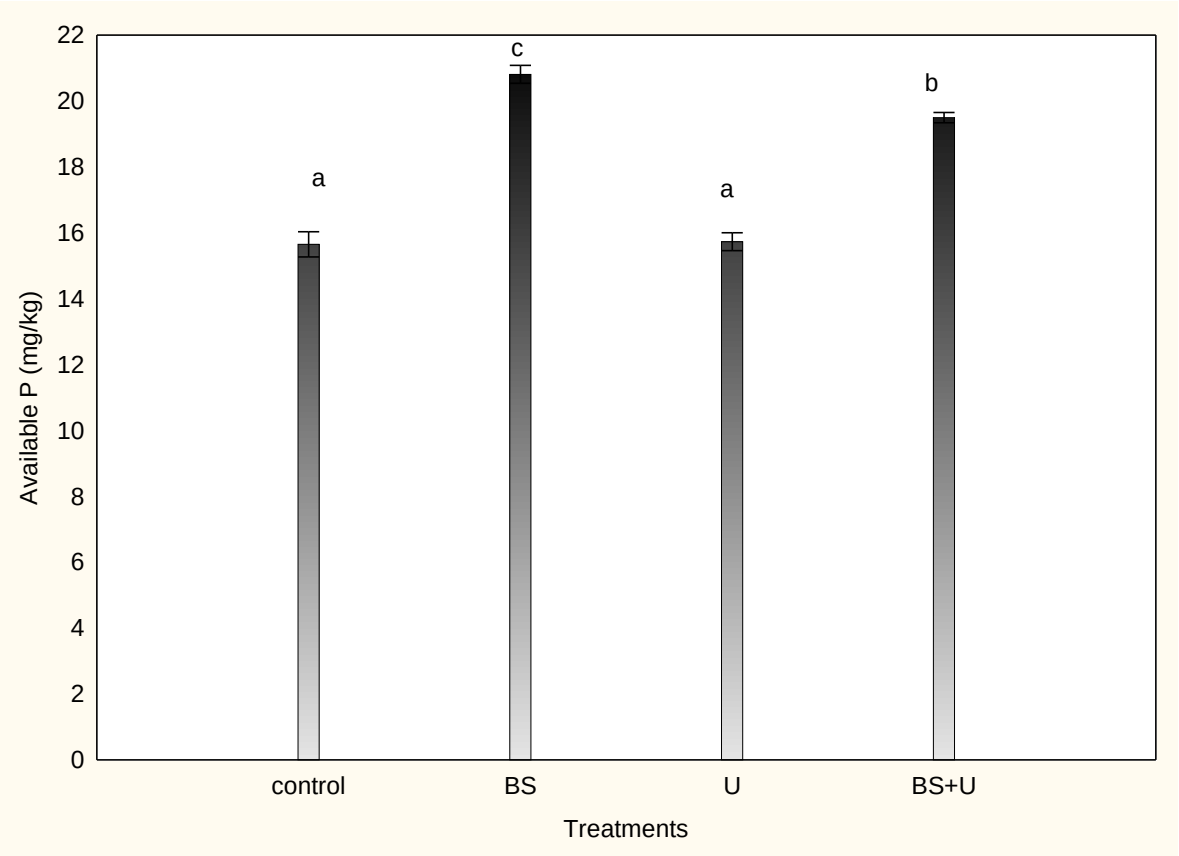


Figure 3-10 Mean values of available P mineralisation during 60 days incubation period. BS= biosolids; U=urea; BS+U= mixed biosolids and urea. ±SE n=3. Different letters above the columns indicate significant difference between treatments.

3.1.3.3 Nitrogen mineralisation

The rate of mineralisation of nitrogen from the urea amended soil sample shows significant released in total oxidised nitrogen (NO_3+NH_4) during day 6 of the incubation experiment. The amounts of total released oxidised nitrogen were significantly higher compared to other treatments through the whole incubation period (Figure 3-11).

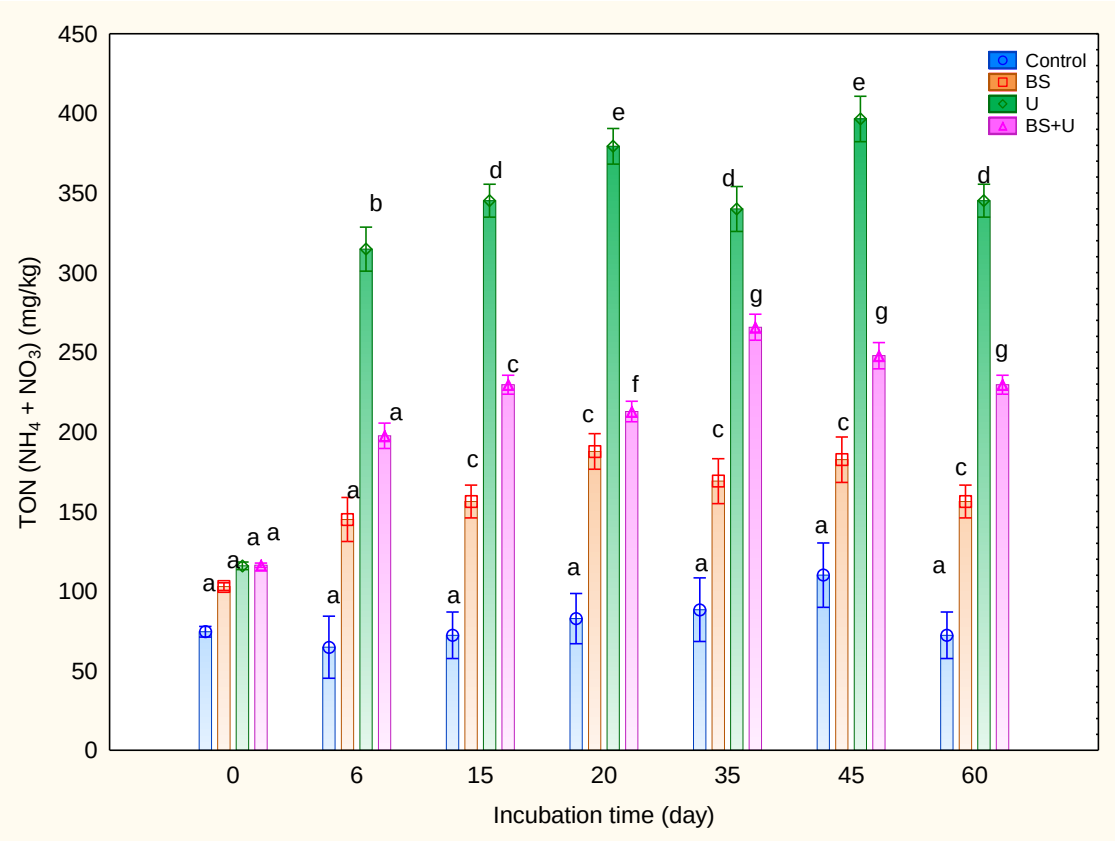


Figure 3-11 Nitrogen mineralisation in arable soil during 60 days incubation time. (SE= standard error; n=3). BS=biosolids; U=urea; BS+U= mixed biosolids and urea. Different letters above the columns indicate significant difference between incubation time (days).

The changes in the urea treatment particularly during day 20 and 45 were over 80% compared to control and up to 60% and 50% in the biosolids (BS) and mixed biosolids and urea (BS+U) treatments respectively. A steady increase in nitrogen mineralisation rate was observed in the urea treated sample from day 6 through to days 15 and 20, and then it gradually decreases as it reaches 35 days, after

which an increase trend continued until 45 days before it reduces to the same amount as in day 35 during the final day 60. Interestingly, the final amount of mineralised nitrogen in the urea amendments after 60 days incubation still shows significant difference ($p<0.001$) compared to control and biosolids treatments.

A significant ($p<0.001$) amount of total oxidised nitrogen was released from the amended arable soil during 60 days incubation. The overall mean value of urea (319.53 mg/kg) amended samples shows significant increases of over 60% TON-N compared to the control (80.69 mg/kg). There were also significant increases of up to 40% and 50% total oxidised nitrogen in the urea treatment compared to the mixed BS+U and BS amendments respectively (Figure 3-12).

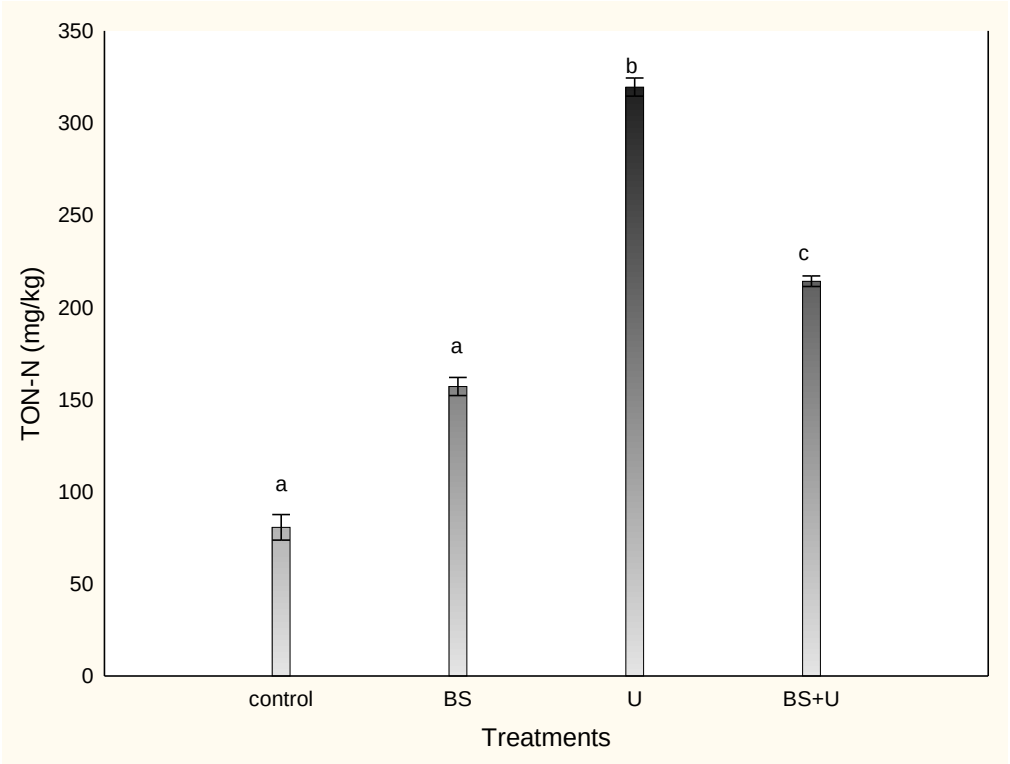


Figure 3-12 Mean values of mineralisable nitrogen during 60 days incubation. BS= biosolids; U=urea; BS+U= mixed biosolids and urea. ±SE n=3. Different letters above the columns indicate significant difference between treatments.

3.1.3.4 Microbial biomass carbon

There were significant changes in biomass carbon during 60 days incubation time (Figure 3-13). These changes occurred during the 20 and 35 days period in which biosolids (BS) treated soil samples shows significant amount of up to 60% of biomass carbon more than control or urea treatments, but no significant difference between the mixed biosolids and urea (BS+U) samples (Figure 3-13). There was a sharp reduction in biomass carbon after the initial incubation period in the control samples all the way to day 20 of the incubation period before it then became steady and constant between 20 and 35 days. However, between 35 and 60 days there was no further reduction.

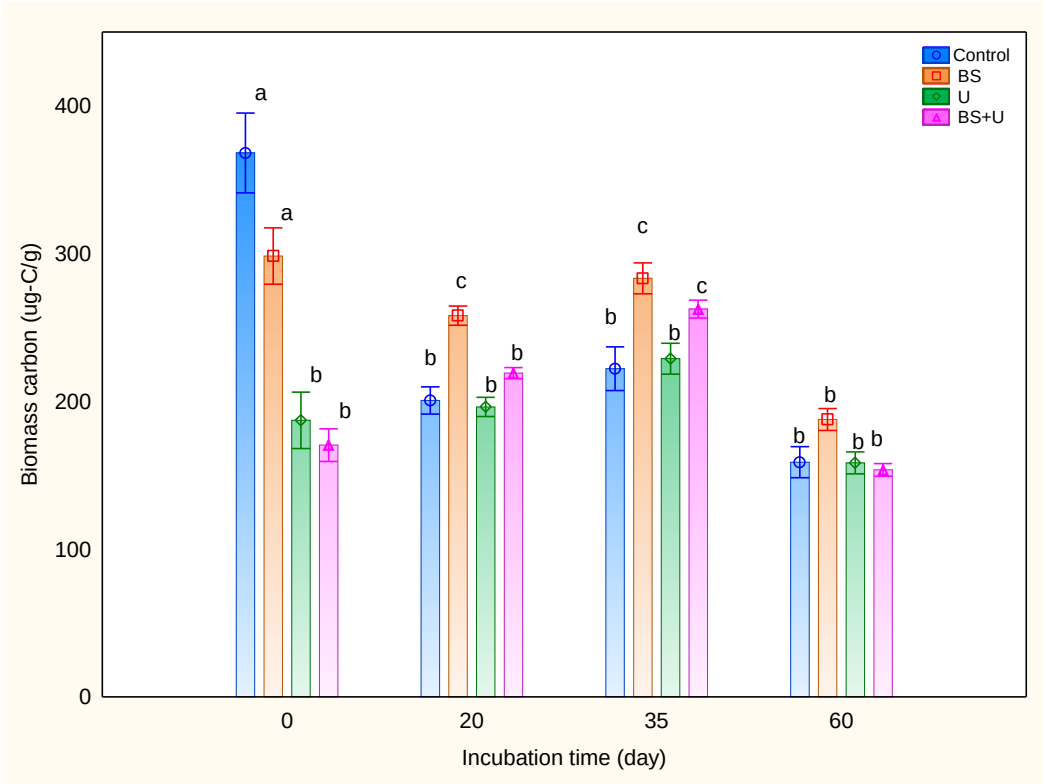


Figure 3-13 Changes in biomass carbon during 60 days soil incubation. BS=biosolids; Control (no added fertiliser); U=urea; BS+U=mixed of biosolids and urea. Columns labelled with the same letter are not significantly different (p<0.05).

3.1.3.5 Phospholipids fatty acids (PLFA)

The community changes in arable soil shows shift in trends with effects of time during 60 days incubation period. There were no significant ($p<0.001$) differences in all the various treatments at the initial day 0, until at 20 days period, in which mixed biosolids and urea (BS+U 90/10 and BS+U 50/50) shows their trends moved to the second axis (PC2) while control and the other treatments (**BS, U and BS+U 70/30**) were further away from the axis. By the end of the 45 days period, only urea amended trends was seen on the second principal axis (PC2), while all the other treatments were away from both PC1 and PC2 clustered together, with significant difference between the urea and control treatments (Figure 3-14). At day 60 all the treatments formed more or less trend inclined towards the initial starting point (day 0).

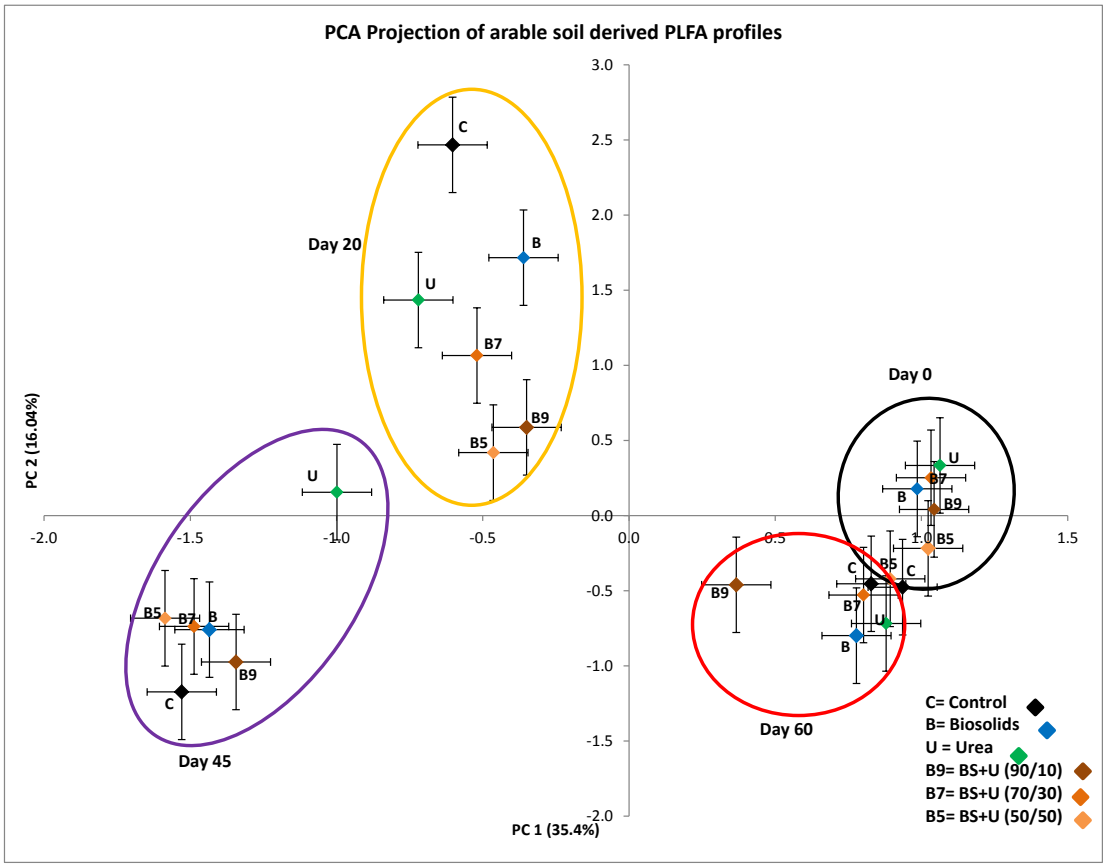


Figure 3-14 PCA ordination plot of arable soil phospholipids fatty acids profiles during 60 days incubation. BS= biosolids; U=urea; BS+U=mixed biosolids and urea; BS+U 90/10=mixed biosolids and urea containing 90%

from BS and 10% from U; BS+U=mixed biosolids and urea containing 70% of BS and 30% urea; BS+U 50/50=mixed biosolids and urea containing 50% BS and 50% U

3.1.3.6 Discussion

Soil mineralisable phosphorus in all the different treatments during the incubation period as presented in **Figure 3-9** shows significant amount of phosphorus mineralisation rates in the biosolids (BS) and mixed biosolids with urea treatments during 15 and 20 day time points compared to urea and control amendments. Since soil microorganisms primarily need to oxidised organic substrates to gain energy which is accompanied by the released of nutrients contained in the oxidised compound (Bünemann *et al.,* 2012), it is therefore during this period there is sufficiently dissolved carbon available to the organisms for complete oxidation as high biomass carbon (**Figure 3-13**) in biosolids amended sample was also observed during the 20 and 35 days compared to the other treatments. Highest P mineralisation rate in soil during incubation is shown to occur mainly within the 30 days period (Antille, 2011). During a 90 days incubation experiment by Antille (2011), he showed the highest P mineralisation rate in biosolids and organomineral fertiliser treated soil were encountered during 30 day time point compared to 60 and 90 days incubation period.

Even though the availability of phosphorus in arable soil amended with biosolids is mainly controlled by the sorption-desorption reactions of P as determine by soil pH (Garau *et al.,* 1986) and mineralisation of organic-P by soil microorganisms (Quiquampoix and Mousain, 2005). The changes in soil pH (Figure 3-8) were not significantly ($p<0.001$) different in all the treatments during the incubation period. Hence the mineralisation of available P were affected more by the soil microbial activities, as the overall community profile changes for the two component axes (**Figure 3-14**), describes 51.4% variation of the different amendments with a distinct clusters observed during 20 and 45 days incubation time. Although studies by Drenovsky *et al.,* (2004) on the agricultural soil samples after incubation described up to 64.9% variation for the two axes, based on the fatty

acids composition. Possible reason could be due to the initial soil organic matter and type and sources of biosolids (O'Connor *et al.,* 2004) as they all have textural soil of clay loam. A Significantly ($p<0.01$) large amount of the total oxide of nitrogen (NO_3 +NH_4) in urea (Figure 3-11) compared to biosolids and mixed biosolids with urea or control observed at 20 and 45 days period of incubation time also implies the need for favourable carbon: nitrogen ratio (C:P) for microbial activities. This process, therefore further affects the mineralisation of phosphorus, during the time when ammonium ion (NH_4^+) is oxidised to nitrate ion (NO_3^-) as a result, hydrogen ion (H^+) is released that cause changes in pH which would subsequently have effect on the rate of phosphorus mineralisation (Stevenson and Cole, 1999)

3.1.4 Discussion and conclusion of the incubation experiment 1a

Based on the incubation studies for 60 days, on grassland and arable soils amended with biosolids, urea or mixed biosolids with urea, highest rate of phosphorus mineralisation occurred during 20-30 days period in both soil type and this has been shown in previous studies (Antille, 2011; Tisdale *et al.,* 1990). Similar studies by Pare *et al.,* (2010) on the variation of organomineral fertilisers incubated with proportion of solid pig slurry, have shown the most biosolids-P mineralisation in the first 30 days. The mean P values in biosolids and mixed biosolids with urea from arable were 21.1mg/kg and 19.5 mg/kg respectively **(Figure 3-10).** While in grassland, the mean P values of the biosolids and mixed biosolids with urea were 18.2 mg/kg and 14.5 mg/kg respectively **(Figure 3-4).** This difference is due to the textural, site or location and land use management as well as initial phosphorus indexes **(Table 3-1)**. The changes in pH from both grassland and arable soils were marginal during the incubation experiment. Therefore overall main conclusions were summarised as follows:

- There was no significant difference in soil pH changes during 60 days incubation between the treatments.

- The greatest rate of nitrogen release from both urea and biosolids mixed with urea occurs during the 20-30 days period of the 60 days incubation experiment.
- The greatest rate of available phosphorus also occurs during 20 and 30 days period for the 60 days incubation experiment
- Presence of urea in the mixed combination shows reduction in the phosphorus mineralisation rate.
- Both grassland and arable soils had overall of 51.4% variation based on the fatty acid composition for the combined two principal component axes and shows community change effects with time.

3.2 Incubation experiment 1b

This experiment presents a soil incubation study of 60 days on grassland (sandy clay loam) soil samples that were treated with a fixed amount of biosolids (250Kg/ha equivalent) mixed with various ratios (50, 150 & 250KgN/ha) of urea to form an organomineral fertiliser (OMF) as an amendments as shown in Table 2-1. Initial soil sampling was carried out at day 0 to analyse the pH, available phosphorus and phosphatase enzymes activity in the soil and thereafter, samples were collected at 10 day intervals for 60 days during the incubation period. These samples were also analysed for pH, available phosphorus and phosphatase enzymes. Results were presented.

3.2.1 Soil pH

The analysis of soil pH at day 0, 10, 20, 30, 40, 50 and 60 on all the different treatments during 60 days incubation period did not shows significant ($p>0.001$) difference between the various treatments compared to the control. However, during day 1 incubation period, the urea (U [250KgN/ha]) and (BS+U [250KgN/ha]) amendments shows significant increase in pH values of more than one unit increase (Figure 3-15) compared to the control (BS [250KgN/ha]).

It is important to note that the control in the whole of this incubation experiment 1b is the biosolids, treated soil samples at 250KgN/ha equivalent rate application without added urea.

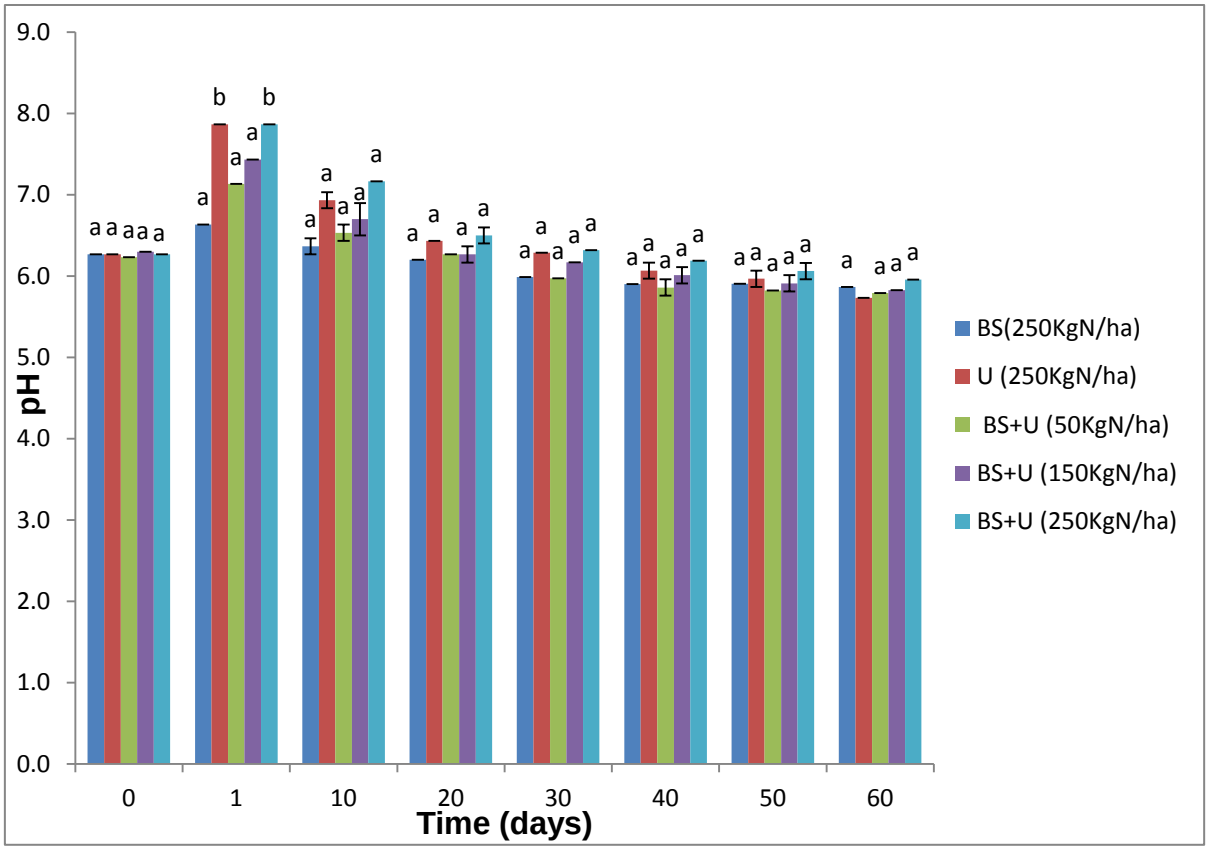


Figure 3-15 Changes in pH during 60 days incubation of organomineral treated grassland soil samples. (SE= standard error; n=3). BS (250KgN) = biosolids application @ 250KgN equivalent; U (250KgN/ha) = urea application @ 250KgN equivalent; BS+U (50KgN/ha) = fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent; BS+U (150KgN/ha) = fixed biosolids @ 250KgN/ha mixed with urea @150KgN equivalent; BS+U (250KgN/ha) = fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent. Different letters above the columns indicate significant difference during incubation period.

3.2.2 Phosphorus mineralisation

There were significant differences ($p<0.001$) in the amount of released available phosphorous between the urea and the mixed of biosolids with various quantity of urea that forms organomineral fertiliser (OMF), during the 60 day incubation period. Furthermore the rate of organomineral P (OMF-P) mineralisation between the individual fixed biosolids with different amount of urea shows significant ($p<0.05$) differences during the 20 and 30 days incubation period. The mineralisation of P was observed to have significantly decrease ($p<0.001$) with increase addition of urea component during the 20 and 30 days incubation period, before the mineralisation rate stabilizes to more or less the same level amongst the OMF treatments during the remaining 40, 50 and 60 days. Soil samples containing urea treatments showed the same amount of P across the whole different days during the incubation period (Figure 3-16).

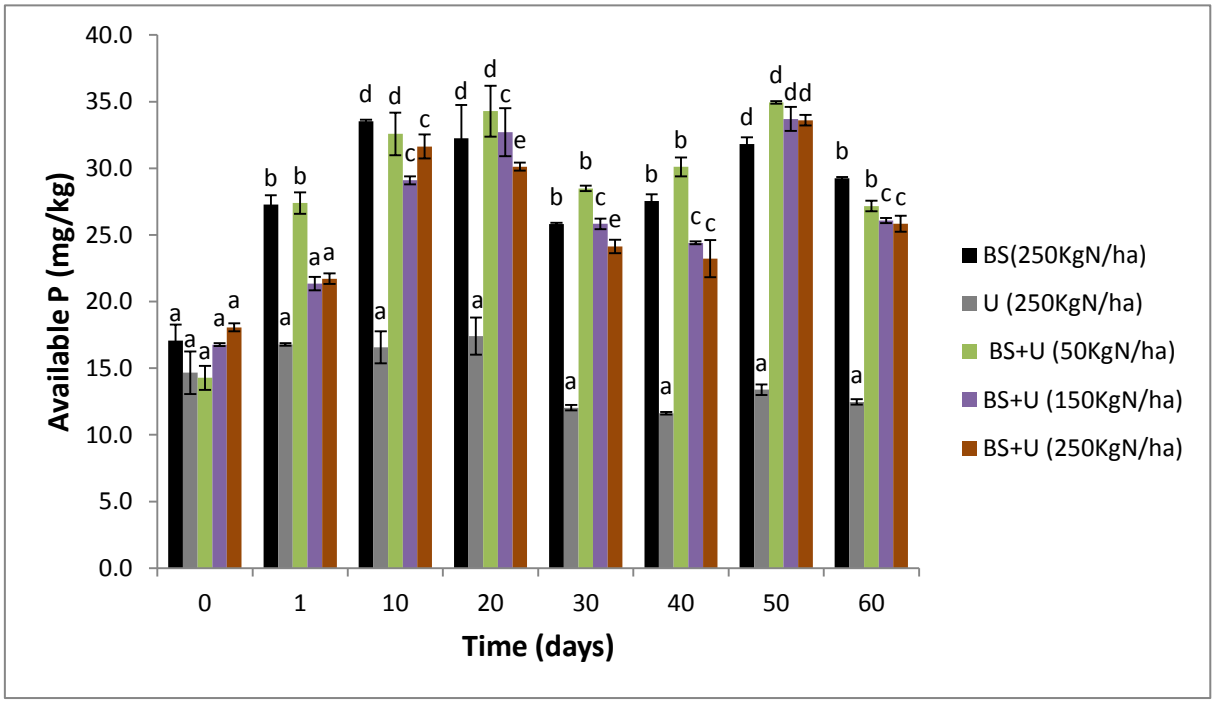


Figure 3-16 Phosphorus mineralisation during 60 days incubation of grassland soil amended with organomineral fertiliser. (SE= standard error; n=3). BS (250KgN) = biosolids application @ 250KgN equivalent; U (250KgN/ha) = urea application @ 250KgN equivalent; BS+U (50KgN/ha) = fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent; BS+U

(150KgN/ha) = fixed biosolids @ 250KgN/ha mixed with urea @150KgN equivalent; BS+U (250KgN/ha) = fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent. Different letters above the columns indicate significant difference during incubation period.

The overall mean values of mineralised available P from all the sampling days for the whole duration of 60 days in the fixed biosolids and various ratio of urea (50, 150 and 250KgN/ha) referred as organomineral (OMF) treatments were 28.0, 25.7 and 23.4 mg/kg respectively, and this shows significant reduction in the OMF-P mineralisation rate (Appendix XI) accordingly with the addition of more urea component to the biosolids fertiliser (Figure 3-17).

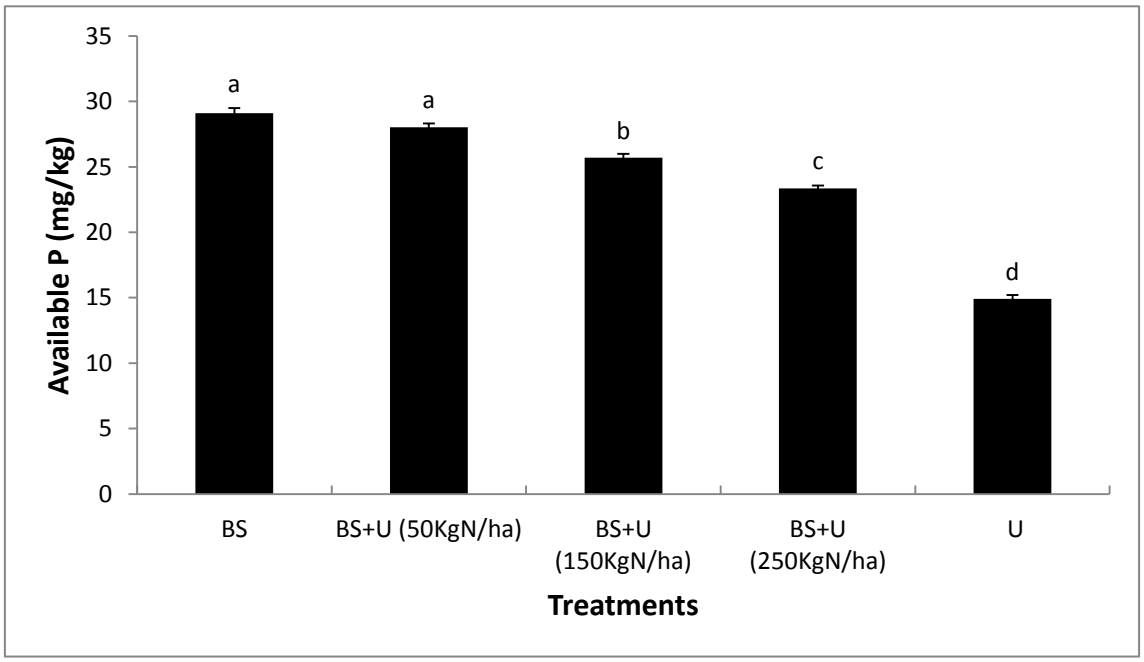


Figure 3-17 Mean values of available phosphorus mineralised for all the sampling days during 60 days incubation. SE+/- (n=3). BS=biosolids; BS+U (50KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent; BS+U (150KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @150KgN equivalent; BS+U (250KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @250KgN equivalent; U= urea

application @ 250KgN equivalent. Different letters above the columns indicate significant difference.

3.2.3 Phosphomonoesterase enzymes activity.

Fluorescence reading at different substrate concentrations in acidic (pH 6.5) and alkaline (pH 11) media were initially measured (**Section 2.4.1**) to attain substrate-saturation curves of the enzymes.

3.2.3.1 Acid phosphatase activity

The changes in acid phosphatase enzyme activities in all the treatments began slowly and were even more or less the same between initial day 0 and 10 day period. Enzyme, activities then started to gradually increase between 10 and 20 days, with the treatment that had fixed biosolids mixed with urea (BS+U 250KgN/ha) showing a significant difference ($p<0.001$) of phosphatase activities compared to the urea treated samples. Even though, the highest (60%) acid phosphatase enzyme activities was observed in the biosolids (BS) treatment as compared to all the other treated samples at the day 30 of incubation period, it become reduced to more or less the same value to the fixed biosolids mixed with urea amendments (BS+U (150KgN/ha) and (250KgN/ha), during 40 and 50 days incubation time (Figure 3-18). These two different combinations (BS+U (150KgN/ha) and BS+U (250KgN/ha) showed significantly high phosphatase activities of up to 55% and 50% respectively, compared to urea at the day 40. The activities in all the treatments eventually became as the same values, as the initial incubation time at the day 50, even though it began to show gradual increase between 50 and final incubation time of day 60.

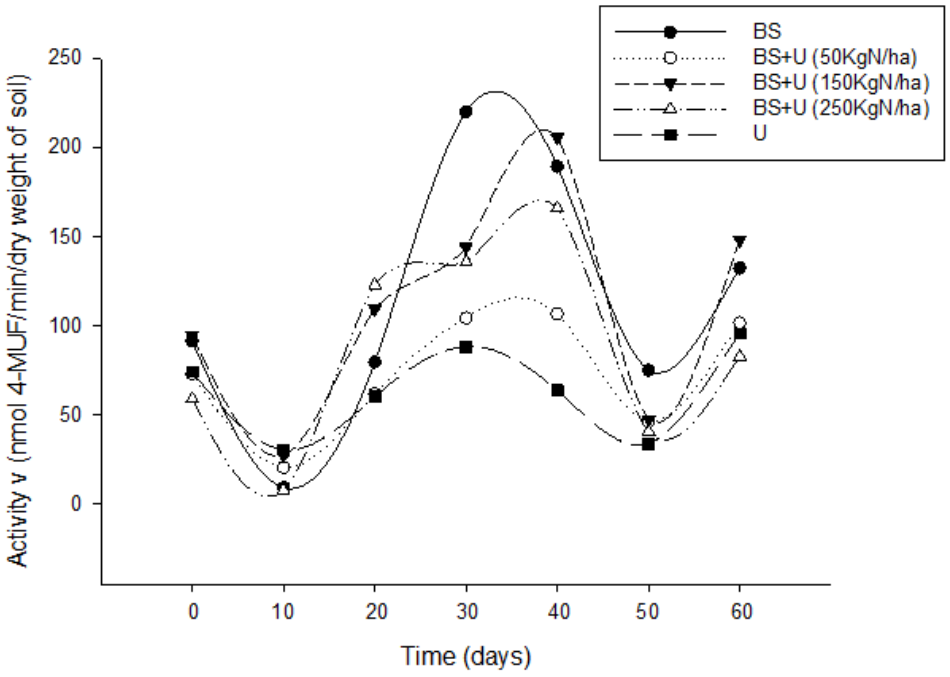


Figure 3-18 Changes in acid phosphatase enzyme activities in organomineral fertiliser amended soil during 60 days incubation. SE+/- (n=3). BS=biosolids; BS+U (50KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent; BS+U (150KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @150KgN equivalent; BS+U (250KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @250KgN equivalent; U= urea application @ 250KgN equivalent.

The overall mean values of activities during 60 days incubation, shows biosolids (BS) treatments had the highest mean activities value of 23.45 nmol 4-MUF-P/g of soil/min. While urea (U) treatments had the lowest mean activities value of 11.22 nmol 4-MUF-P/g of soil/min (Figure 3-19). Mixed combinations of BS+U (50KgN/ha), BS+U (150KgN/ha) and BS+U (250KgN/ha) had mean activities of

16.33 nmol 4-MUF-P, 20.91 nmol 4-MUF-P and 18.61 nmol 4-MUF-P/g of soil/min respectively.

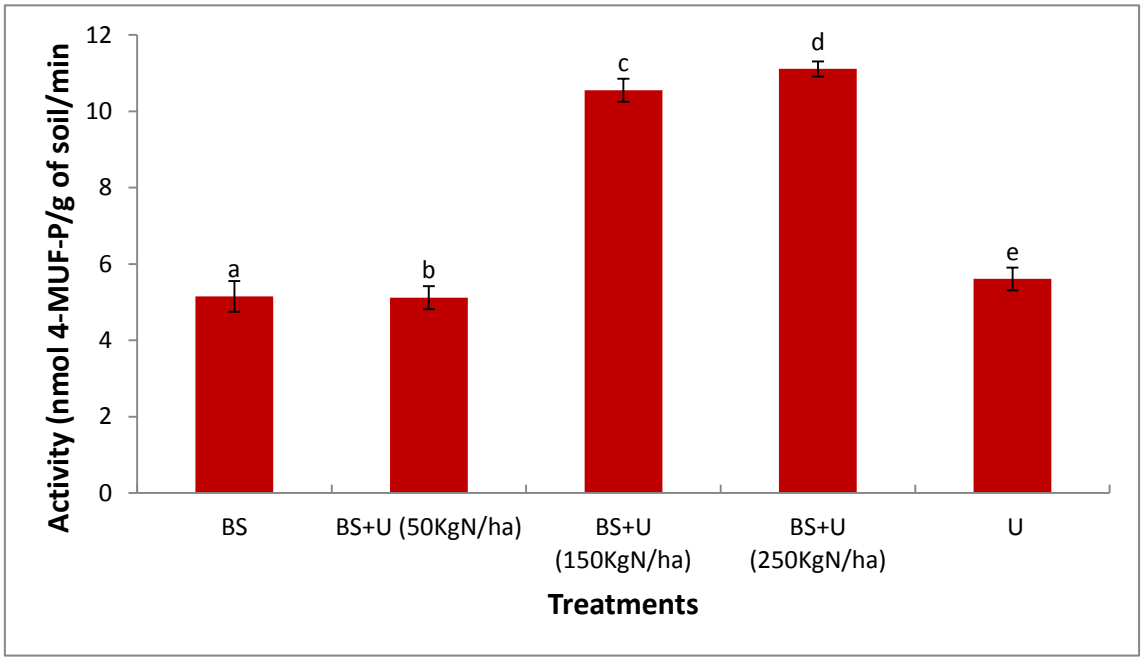


Figure 3-19 Mean values of acid phosphatase enzyme activities in organomineral fertiliser amended soil during 60 days incubation. SE+/- (n=3). BS=biosolids; BS+U (50KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent; BS+U (150KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @150KgN equivalent; BS+U (250KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @250KgN equivalent; U= urea application @ 250KgN equivalent. Different letters above the columns indicate significant difference.

3.2.3.2 Alkaline phosphatase enzyme activity

Alkaline phosphatase enzyme activities amongst the treatments shows a trends of two separate clusters of stable and rising activities at the initial day 0. The urea, mixed BS+U (250KgN/ha) and BS+U (150KgN/ha) were the clustered treatments showing immediate increase in activities while mixed BS+U (50KgN/ha) and biosolids (BS) were those showing slow response in enzyme activities change and the significant differences ($p<0.001$) in those enzyme activities were only seen between the mixed BS+U (250KgN/ha) and biosolids (BS) treatments at this day. A steady decrease in enzyme activity in the urea treatment during 20 days incubation and increased activities in biosolids amendments during 30 days incubation were noticed. A significantly high enzyme activity from all the treatments were recorded between 30 and 40 days incubation periods particularly, the mixed BS+U (150KgN/ha) which had the highest activity (Figure 3-20). And the between 50 and 60 days incubation, the enzyme activities in all the treatments reduces to virtually the same amount.

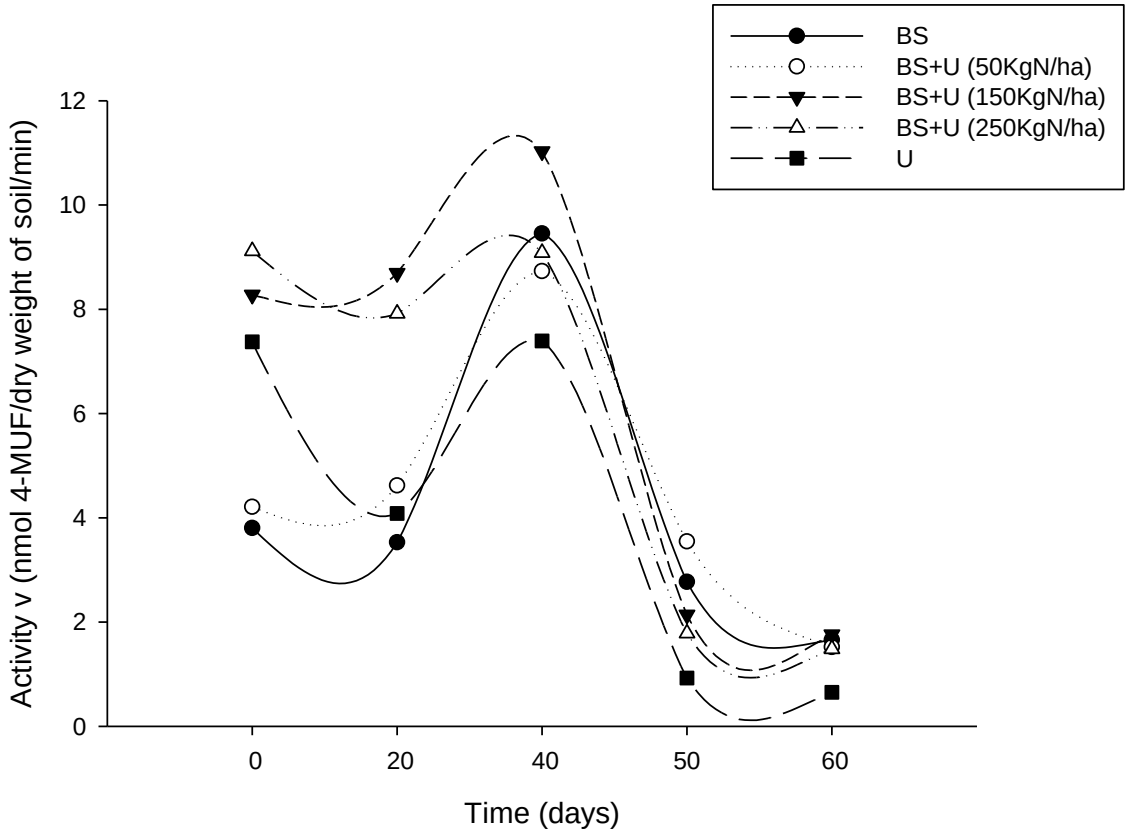


Figure 3-20 Changes in alkaline phosphatase enzyme activites in organomineral fertiliser treated soil during 60 days incubation. SE+/- (n=3). BS=biosolids; BS+U (50KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent; BS+U (150KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @150KgN equivalent; BS+U (250KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @250KgN equivalent; U= urea application @ 250KgN equivalent.

The overall mean alkaline phosphatase enzyme activities during 60 days incubation (Figure 3-21), shows mixed BS+U (250KgN/ha) and (150KgN/ha) having a value of 11.33nmol 4-MUF-P/g of soil/min and 10.55nmol 4-MUF-P/g of soil/min respectively, and significantly ($p<0.005$) higher than those other treatments of urea, biosolids and mixed BS+U (50KgN/ha).

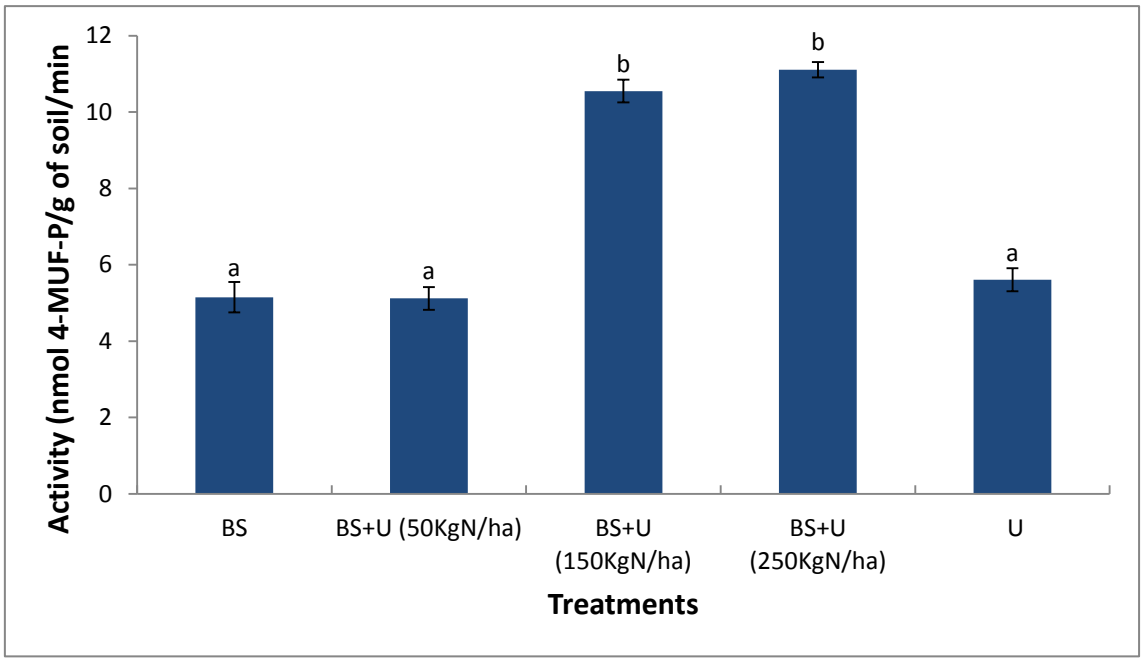


Figure 3-21 Mean alkaline phosphatase enzyme activities in the organomineral fertiliser amended soil during 60 days incubation. SE+/- (n=3). BS=biosolids; BS+U (50KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @50KgN equivalent; BS+U (150KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @150KgN equivalent; BS+U (250KgN/ha) =organomineral fertiliser with fixed biosolids @ 250KgN/ha mixed with urea @250KgN equivalent; U= urea application @ 250KgN equivalent. Different letters above the columns indicate significant difference.

3.2.4 Discussion and conclusion of incubation experiment 1b

This incubation experiments was carried out with only grassland soil to establish the mechanisms by which urea presence in the biosolids (when mixed to form organomineral fertiliser) influenced the available phosphorus mineralisation rate during a 60 day incubation period. Reduction in the rate of organomineral fertiliser phosphorus mineralisation with increases in urea doses were significantly observed **(Figure 3-16)** between 20 and 30 days period. The overall mean phosphorus mineralisation in the BS+U (50KgN/ha), BS+U (150KgN/ha) and

BS+U (250KgN/ha) formulations after 60 days incubation time were 28.0 mg/kg, 25.7mg/kg and 23.4mg/kg respectively **(Figure 3-17)** and these values showed statistically significant differences in the Fischer $LSD_{\alpha=0.5}$ analysis (Appendix IX). Soil pH, an important factor affecting bioavailability of phosphorus (Haynes *et al.,* 2009; Hinsinger, 2001) and regulator of soil microbial enzyme activities (Sinsabaugh *et al.,* 2008) did not show significant changes between the treatments **(Figure 3-15).** This indicates that pH plays little role with regards to reduction in OMF-P mineralisation rates, perhaps, the decrease in phosphorus mineralisation rates could be attributed to the nitrogen: phosphorus (N:P) ratio, which is also governed by the microbial turnover (Oberson and Joner, 2005). As such fractional increases of urea doses to some extent would leads to the reduction of net P mineralisation rates, because there is possible increase in the concentrations of nitrite due to high amount of total oxide nitrogen **(Figures 3-5)** and this nitrite have toxic effects on the soil microbial activities (Zhang *et al.,* 2013; Zhang *et al.,* 2008) and consequently, affects the secretion of vital enzymes such as phosphomoesterase in this case. Soil microorganisms have shown response to nitrogen enrichment in soil and also the microorganisms with the highest turnover rate can easily be changed by the nitrogen addition in a relatively short time (Zhang *et al.,* 2008). The changes in phosphomonoesterase enzymes activities between those treatments having fixed mixed quantity of biosolids with various increasing doses of urea have also shown significant mean differences ($p<0.01$) in the acid and alkaline phosphatase enzymes activities **(Figures 3-19 and 3-21)** during 60 days incubation experiment.

4 Integrated discussion

The overall result presented for this incubation experiments 1a and 1b on the mineralisation of available phosphorus when mixed with urea have all shown consistent reduction in the mineralisation rates of the organomineral fertiliser phosphorus (OMF-P) as the fractional urea doses is increased to the experimental threshold quantity and this has corresponded with the hypothesis I. Even though there are numerous factors such as organic matter content, pH, temperature and moisture (Shaheen and Tsadilas, 2013; Tisdale *et al.,* 1990) and the microbial enzymes (Bünemann *et al.,* 2012; Oberson and Joner, 2005) that may be affecting the mineralisation of phosphorus in soil. In this instance pH changes were marginal during the incubation period, and the other conditions (temperature and moisture) were being under controlled, therefore it could be argue with caution that main effects on OMF-P mineralisation rates reduction is inclined more to the presence of urea in the organic matter residuals (biosolids). The possible mechanisms involve were, as the urea breaks down and hydrolyses to release ammonium ion that is oxidised to form nitrite and then nitrate, during the processes, this would have an effects on the carbon: phosphorus (C:P) of substrates (Withers and Haygarth, 2007), that would now affects available phosphorus release so that is being gradually broken down by microorganisms as more dissolved carbon becomes available. Therefore as more urea is added it accumulates high amount of nitrites as more ammonium becomes oxidised, and this nitrite though not stable, may have toxic effects on microbial activity in soil (Sinsabaugh *et al.,* 2008).

It was found that all the significant changes for the analysed parameters such as available phosphorus, microbial biomass carbon, and acid and alkaline phosphatase enzyme activities were occurring during 20 and 30 days period during the incubation time. This could be that at that particularly days, fully dissolved carbon is available for microbial activities. This incubation studies, particularly with regards to highest phosphorus mineralisation time scale of 30 days is within the range suggested and reported in literature. For example Antille (2011) and Pare *et al.,* (2010) observed the mineralisation of phosphorus from

biosolids and urea amended soil for 90 days incubation, where they noticed that highest mineralisation took place during the first 30 days before it declined during the 90 day period.

Phospholipids fatty acids profiles in both grassland and arable soils showed over 50% variation for the two principal component axes based on their fatty acids composition (**Figures 3-7 and 3-14**). Even though community shift trends with time were observed, the effects of treatments did not show any significantly important clue or information, perhaps the application rates were not enough to affects the organic content, since phospholipids fractions is very small between 0.5-7% (Quiquampoix and Mousain, 2005). However, sensitive response and changes in phosphatase enzymes activities measured in grassland soil that were significantly different during 30 and 40 days corresponded with the greatest variation of the phenotypic shift with time during those particular time points. This indicates that even though the individual PLFA analysed treatments did not show significant differences, soils microbial community must have been affected, but not very effective in this type of incubation experiment probably because of the wetting and drying (Oberson and Joner, 2005) or maybe the incubation period is not long enough for more trends to prevail. Oberson and Joner (2005) have shown that flush effects such as sequences of drying and wetting or freezing and thawing leads to death and decomposition of microbial cells as a results only small viable cells are present.

5 Conclusion and Implications

The main conclusions which were drawn for this incubation experiment were:

1. There were no significant changes in the soil pH during the 60 days incubation irrespective of treatments in grassland (sandy clay loam) and arable (clay loam) soils. Both soils have shown strong buffering capacity which is perhaps due to their texture.

2. Greatest organomineral fertiliser phosphorus mineralisation were achieved between the 20 and 30 day incubation and this has corresponded with the several suggestion and reports in the literature.

3. Consistent and significant reduction of the organomineral fertiliser phosphorus mineralisation rate with increased doses of urea fractions were observed during the short-term of 60 days incubation experiment.

5.1 Implications and limitations of the study

As this research focussed on the short-term soil incubation for 60 days to determine the effects of urea component (added to biosolids to form organomineral related fertiliser) on the mineralisation of phosphorus. It was also important that other parameters such as pH, microbial biomass carbon and phosphorus and enzyme activities were measured as they are important in the soil during phosphorus mineralisation.

However this experiment is regarded as not long enough for those parameters to fully prevail. Hence a long-term study of effects of urea on the mineralisation of available phosphorus is essential. Higher application rates of amendments should also be considered so that analysis like the phospholipids fatty acids and biomass phosphorus can be fully established.

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Appendices

Appendix I ANOVA For the microbial biomass in grassland soil during 60 days incubation.

Effect	Repeated Measures Analysis of Variance for Biomass carbon of grassland soil samples				
	SS	Degr. of Freedom	MS	F	p
Intercept	5342116	1	5342116	4261.724	0.000000
Fertiliser	17749	3	5916	4.720	0.009985

Application rate (fertiliser)	2478	3	826	0.659	0.585362
Percentage mix (fertiliser)	33135	2	16568	13.217	0.000135
Error	30084	24	1254		
TIME	496323	3	165441	176.029	0.000000
TIME*Fertiliser	64080	9	7120	7.576	0.000000
TIME*Application rate (fertiliser)	12679	9	1409	1.499	0.164857
TIME*Percentage mix (fertiliser)	23028	6	3838	4.084	0.001398
Error	67669	72	940		

Appendix II Fischer LSD values of biomass carbon from grassland soil showing significance difference during 20 days incubation period.

LSD test; variable DV_1 (Grassland BC .stw) Homogenous Groups, alpha = .05000 (Non-Exhaustive Search) Error: Between; Within; Pooled MSE = 1018.3, df = 94.322											
Fertiliser	TIME (days)	Mean	1	2	3	4	5	6	7	8	9
control	BC_day0	118.0608	****								
BS+U	BC_day60	145.7032	****								
U	BC_day0	146.4312	****	****							
BS	BC_day0	155.8922	****	****		****					
U	BC_day60	157.3871	****	****		****					

BS	BC_day60	177.2789		****	****	****					
BS+U	BC_day0	190.1851			****						
control	BC_day60	192.1679			****	****	****				
U	BC_day35	228.8834					****	****			
BS+U	BC_day35	234.4885						****			
BS	BC_day35	278.4171							****		
control	BC_day35	284.3119							****		
control	BC_day20	285.0210							****		
U	BC_day20	288.6996							****		
BS	BC_day20	333.7373								****	
BS+U	BC_day20	367.3795									****

Appendix III ANOVA For the mineralisation of available phosphorus in grassland soil treated with various quantities of biosolids and urea during 60 days incubation.

Effect	Repeated Measures Analysis of Variance for Available phosphorus in grassland soil samples				
	SS	Degr. of Freedom	MS	F	p
Intercept	33685.84	1	33685.84	5597.085	0.000000
Fertiliser	1053.78	3	351.26	58.364	0.000000
Application rate(Type)	715.56	3	238.52	39.632	0.000000
Percentage mix (Type)	58.18	2	29.09	4.833	0.017224
Error	144.44	24	6.02		
TIME	631.22	6	105.20	13.005	0.000000
TIME*Fertiliser	171.17	18	9.51	1.176	0.288834
TIME*Application rate(Type)	74.24	18	4.12	0.510	0.950207
TIME*Percentage mix (Type)	267.92	12	22.33	2.760	0.002099
Error	1164.85	144	8.09		

Appendix IV ANOVA For the total oxidised ammonium and nitrate in grassland soil during 60 days incubation.

Effect	Repeated Measures Analysis of Variance; Std. Error of Estimate: 188.6251				
	SS	Degr. of Freedom	MS	F	p
Intercept	15763681	1	15763681	443.0558	0.000000
Fertiliser	496048	3	165349	4.6473	0.010650

Application rate(fertiliser)	167622	3	55874	1.5704	0.222466
Percentage mix (fertiliser)	14210	2	7105	0.1997	0.820323
Error	853907	24	35579		
TIME	6936631	6	1156105	124.1952	0.000000
TIME*Fertiliser	544667	18	30259	3.2506	0.000041
TIME*Application rate (fertiliser)	429281	18	23849	2.5620	0.001053
TIME*Percentage mix (fertiliser)	121164	12	10097	1.0847	0.377290
Error	1340463	144	9309		

Appendix V Fischer LSD values of mineral nitrogen from grassland soil showing significance difference between urea compared to other treatments during incubation period.

LSD test; variable Homogenous Groups, alpha = .05000 Error: Between MSE = 35579., df = 24.000			
Fertiliser	Mean	1	2
control	216.9193	****	
BS+U	275.0020	****	
BS	288.7580	****	
U	382.0851		****

Appendix VI ANOVA For the mineralisation of available phosphorus in arable soil treated with various quantities of biosolids and urea during 60 days incubation.

Effect	Analysis of variance for Available P in arable soil sample

	SS	Degr. of Freedom	MS	F	p
Intercept	60789.91	1	60789.91	19682.08	0.000000
Fertiliser	833.03	3	277.68	89.90	0.000000
Application rate (fertiliser)	539.44	3	179.81	58.22	0.000000
Percentage Mixed Ratio (fertiliser)	177.73	2	88.87	28.77	0.000000
Error	74.13	24	3.09		
TIME	3965.67	6	660.94	355.51	0.000000
TIME*FERTILISER	77.06	18	4.28	2.30	0.003441
TIME*APPLICATION RATE (fertiliser)	69.56	18	3.86	2.08	0.009311
TIME*MIXED RATIO (fertiliser)	90.20	12	7.52	4.04	0.000021
Error	267.72	144	1.86		

Appendix VII ANOVA For the microbial biomass in arable soil during 60 days incubation.

Effect	Repeated ANOVA for Biomass Carbon in arable soil samples				
	SS	Degr. of Freedom	MS	F	p
Intercept	5091002	1	5091002	3832.557	0.000000
Type	72970	3	24323	18.311	0.000002
Application rate(fertiliser)	21297	3	7099	5.344	0.005798
% Mixed ratio (fertiliser)	10205	2	5102	3.841	0.035707
Error	31881	24	1328		
TIME	124295	3	41432	59.139	0.000000
TIME*FERTILISER	111129	9	12348	17.625	0.000000
TIME*APPLICATION RATE (fertiliser)	23035	9	2559	3.653	0.000836
TIME*% MIXED RATIO (fertiliser)	17458	6	2910	4.153	0.001224
Error	50442	72	701		

Appendix VIII ANOVA For the mineralisation of available phosphorus in grassland soil treated with fixed quantity of biosolids and varied quantities of urea during 60 days incubation.

Effect	ANOVA for available phosphorus in grassland soil samples				
	SS	Degr. of Freedom	MS	F	p
Intercept	7842.727	1	7842.727	258461.3	0.000000
Fertiliser	1.038	3	0.346	11.4	0.000077
Application rate (fertiliser)	2.594	3	0.865	28.5	0.000000
Percentage mix (fertiliser)	22.009	6	3.668	239.2	0.000000
Error	0.728	24	0.030		
TIME	20.009	6	3.668	229.2	0.000000
TIME*Fertiliser	2.184	18	0.121	7.9	0.000000
TIME*Application rate (fertiliser)	1.669	18	0.093	6.0	0.000000
Error	2.208	144	0.015		

Appendix IX Fischer LSD values of available phosphorus mineralisation rates in grassland soil showing significant differences between various treatments of fixed biosolids with various quantity of urea component during incubation period.

	LSD test; variable Homogen alpha = .05000 Error: Between MSE = 35579., df = 21.000				
Fertiliser	Mean	1	2	3	4
BS	29.51	****			
BS+U (50KgN/ha)	28.00		****		
BS+U (150KgN/ha)	25.70			****	
BS+U (250KgN/ha)	23.40				****