CRANFIELD UNIVERSITY

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Effects of rhizosphere priming and microbial functions on soil carbon turnover

School of Environment Energy and Agrifood

Doctor of Philosophy
Academic Years: 2011 - 2014

Supervisors: Professor Guy Kirk and Professor Karl Ritz

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ABSTRACT

A major uncertainty in soil carbon studies is how inputs of fresh plant-derived carbon affect the turnover of existing soil organic matter (SOM) by so-called priming effects. Priming may occur directly as a result of nutrient mining by existing microbial communities, or indirectly via microbial population adjustments. Soil type and conditions may also influence the intensity and direction of priming effects. However the mechanisms are poorly understood. The objectives of this study were (1) to investigate how additions of labile C_4 substrate affected SOM turnover in two contrasting unplanted C_3 soils (clayey fertile from Temple Balsall, Warwickshire (TB) and sandy acid from Shuttleworth, Bedfordshire (SH) using ^{13}C isotope shifts; (2) to investigate the influence of rhizodeposition from plant roots on SOM turnover in the same two soils planted with a C_4 grass; (3) to assess an automated field system for measuring soil temperature, moisture and photosynthesis sensitivities of SOM turnover in the same two soils over diurnal to seasonal time scales. I used a combination of laboratory incubation, glasshouse and field experiments.

In the soil incubation experiment, I made daily applications of either a maize root extract or sucrose to soil microcosms at rates simulating grassland rhizodeposition, and followed soil respiration (R_s) and its $\delta^{13}C$ over 19 days. I inferred the extent of priming from the $\delta^{13}C$ of R_s and the $\delta^{13}C$ of substrate and soil end-members. There were positive priming effects in both soils in response to the two substrates. In the SH soil there were no differences in priming effects between the substrates. However in the TB soil, sucrose produced greater priming effects than maize root extract, and priming effects with sucrose increased over time whereas with maize root extract declined after the first week. I explain these effects in terms of the greater fertility of the TB soil and resulting greater microbial nitrogen mineralization induced by priming. Because the maize root extract contained some nitrogen, over time microbial nitrogen requirements were satisfied without priming whereas with sucrose the nitrogen demand increased over time.

In the glasshouse experiment, I planted C_4 Kikuyu grass (*Pennisetum clandestinum*) in pots with the same two soils. The extent of rhizodeposition by the plants was altered by intermittently clipping the grass in half the pots (there were also unplanted controls) and priming effects were inferred from the $\delta^{13}C$ of R_s and the $\delta^{13}C$ of plant and soil end-members. Unclipped plants in both soils generated positive priming effects, while clipping reduced priming in TB soil and produced negligible PEs in SH soil. Microbial nutrient mining of SOM again explained the observed PEs in this experiment. Photosynthesis was a major driver of priming effects in the planted systems.

In the third experiment, I found that the tested automated chamber system provided reliable measurements of R_s and net ecosystem exchange (NEE), and it was possible to draw relations for the dependency of R_s and NEE on key environmental drivers.

Collectively, the results contribute to a better understanding of the mechanisms of priming effects and highlight possibilities for further research. The methods developed here will allow high temporal and spatial resolution measurements of R_s and NEE under field conditions, using stable isotope methods to separate fluxes into plant- and soil-derived components.

Keywords: Soil respiration, soil moisture, soil temperature, Isotope ratio, maize root, flux chamber, climate change, organic matter, rhizodeposition

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I dedicate this work to the memory of my Dad, John Lloyd and to my children Tariq, Ayodele and Zariel.

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LIST OF ABBREVIATIONS

Al	aluminium
C	carbon
DAP	days after planting
DAT	days after treatment
DOC	dissolved organic carnon
EC	eddy covariance
GM	gradient method
GPP	gross primary productivity
Gt	gigaton
HPLC	high performance liquid chromatograph
IPCC	Intergovernmental Panel on Climate Change
IRGA	infrared gas analyser
IRMS	isotope ratio mass spectrometer
IT	information technology
LSD	least significant difference
N	nitrogen
NEE	net ecosystem exchange
NEP	net ecosystem productivity
NPP	net primary productivity
PAR	photosynthetically active radiation
PE	priming effect
Pg	petagram = 1 billion metric tons
PLFA	phospholipid fatty acids
R_{eco}	ecosystem respiration
R_{root}	root-derived respiration
RPE	rhizosphere priming effect
R_{rhiz}	microbial respiration in rhizosphere
R_{shoot}	shoot-derived respiration
R_{SOM}	soil organic matter decomposition
SEM	standard error of mean
SOM	soil organic matter
SOP	standard operating procedure
WFL	Wolfson Field Laboratory

Chapter 1: Introduction and literature review

1.1 Introduction

Soil carbon (C), principally in the form of soil organic matter (SOM), plays a crucial role in ecological soil functions and conservation of soil fertility, and on a global scale, soil is the main repository of terrestrial C stocks. The amount of C in the top metre of soil globally is estimated to be more than double that in the atmosphere or vegetation (Ciais et al., 2013). The annual flux of C between the land and the atmosphere is estimated to be 120 Pg C yr^{-1} (1 Pg = 1 petagram = 1 billion tonnes = 10^{15} grams), whereas the C flux from fossil fuel to the atmosphere is 8 Pg yr^{-1} and that between the ocean and atmosphere is 80 Pg yr^{-1} (Ciais et al., 2013). The land surface is thought to currently absorb 30% of the CO_2 emitted from fossil fuel combustion as vegetation grows faster with rising atmospheric CO_2, and the oceans absorb a further 20% (Ciais et al., 2013). However, according to the IPCC (Ciais et al., 2013), the future trend in the land-surface sink is one of the largest uncertainties in predicting how the global C balance will respond to climate change. Models predict that the current enhanced land sink will diminish as other factors limit vegetation growth, whereas emissions from soils will increase as the earth warms, so at some point in the future the land surface will become a net source of atmospheric C, resulting in a positive feedback loop (Friedlingstein et al., 2006)

In view of this, the potential for soil-based management interventions to mitigate anthropogenic CO_2 emissions through enhanced C sequestration is much debated (Royal Society, 2009; Powlson et al., 2011). The latest IPCC report recognizes the value of climate change adaptation strategies based on agricultural management that simultaneously preserve and increase SOM while continuing to meet production and productivity targets.

However the mechanisms controlling the exchange of C between soils, plants and the atmosphere are poorly understood. The SOM content of a soil depends on the balance between below-ground inputs -- primarily of detrital material and root exudates – versus outputs -- primarily as CO_2 to the atmosphere. The

coupling between plant and soil C dynamics remains one of the least understood components of the global carbon cycle (Reichstein et al., 2014; Hill et al., 2015). A particular knowledge gap is how the turnover of existing soil organic matter is affected by inputs of fresh (plant derived) organic matter, so-called ‘priming effects’ (Kuzyakov, 2002; Paterson and Sim, 2013; Chen et al., 2014; Cheng et al., 2014). Potentially, climate change feedback loops could enhance priming effects and increase soil C losses.

A major problem in studying plant and soil C dynamics is the difficulty in quantifying soil C fluxes and in distinguishing fluxes from live roots (autotrophic respiration) and from the decomposition of recent root debris and exudates and true soil organic matter (heterotrophic respiration) (Paterson et al., 2009). Most past research was done with laboratory soils in which the natural soil structure was destroyed, and in un-planted soils in which the linkages between plant inputs to the rhizosphere, microbial functions and soil carbon dynamics are lost (Conant et al., 2011). The influence of root activity on SOM decomposition is central to soil and ecosystem C dynamics (Paterson et al., 2009; Kuzyakov, 2010; Fontaine et al., 2011). In true field soils, the occlusion of SOM in soil aggregates, restricting access by roots, microbes and their mineralizing enzymes, contribute to the spatial and temporal variability of SOM turnover. This also complicates temperature and moisture sensitivities of SOM turnover (Davidson and Janssens, 2006), and understanding of the C sequestration potential of different soil types (Conant, 2010).

1.1.1 Terminology used in the thesis

Before the main literature review, I here summarise the terminology I use in the thesis to describe soil respiration and carbon turnover.

The components of plant and soil C budgets are summarised in Figure 1-1. Losses through leaching as dissolved organic carbon (DOC) were estimated to be negligible (Niklaus et al., 2000).

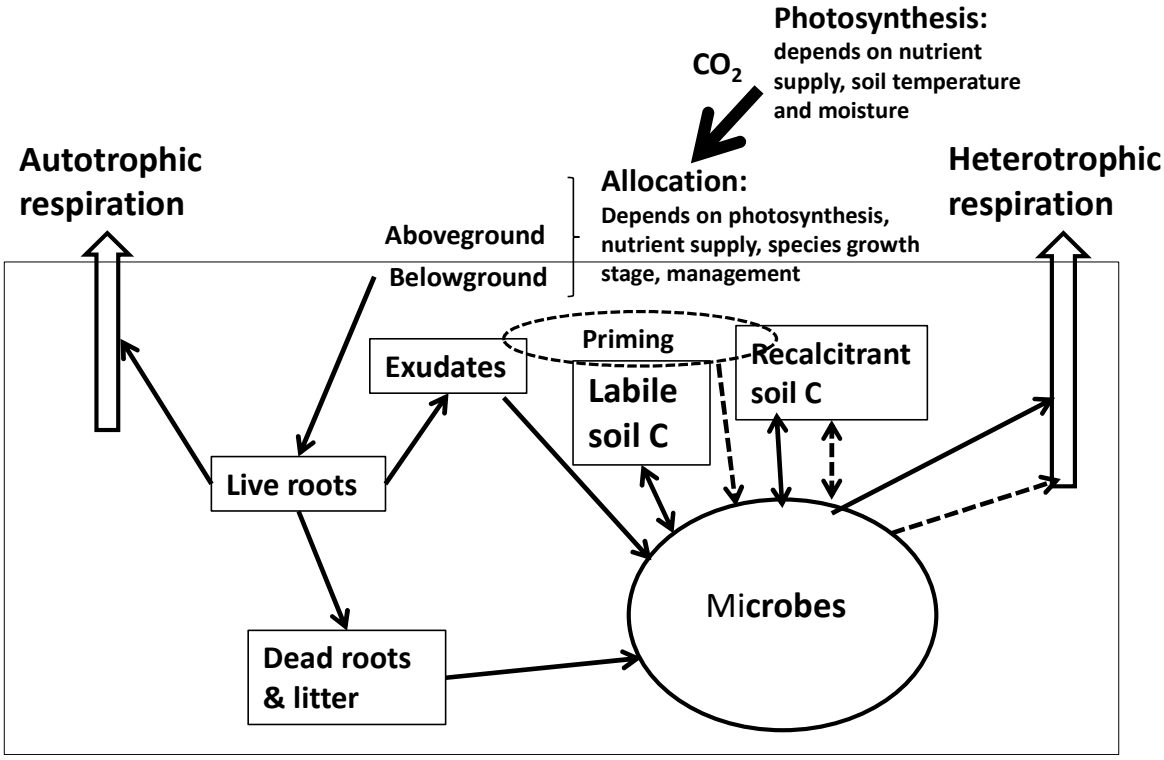

Figure 1-1 Conceptual model of the components of plant and soil carbon turnover. Both the autotrophic and heterotrophic components are strongly controlled by substrate availability – transport of carbohydrate supply for root respiration and dead organic material for microbial respiration. The dotted circle highlights rhizosphere priming and dotted arrows show the effect on enhanced decomposition of recalcitrant C and a consequential increase in heterotrophic respiration. Adapted from Ryan and Law (2005)

Plants assimilate atmospheric CO_2 through photosynthesis. The total rate of C fixation in photosynthesis in a particular ecosystem is the gross primary production (GPP). Some of this assimilated C is used to supply energy to plant shoots and roots, resulting in the release of CO_2 back to the atmosphere as

autotrophic respiration. The net C assimilated, after autotrophic losses, is referred to as the net primary production (NPP), and is a measure of the rate at which energy is stored by plants in the form of organic substances:

$$NPP = GPP - R_{shoot} - R_{root} \quad (1.1)$$

Several sources combine to produce the composite flux of CO_2 in a plant-soil system. These include: microbial respiration from SOM and litter decomposition, (R_{SOM}); soil microbial respiration in the rhizosphere, including mycorrhizal fungi (R_{rhiz}); and autotrophic, plant respiration (R_{shoot} + R_{root}). Collectively this is referred to as ecosystem respiration (R_{eco}). Hence

$$R_{eco} = R_{shoot} + R_{root} + R_{rhiz} + R_{SOM} \quad (1.2)$$

The processes of CO_2 fixation in photosynthesis and release in respiration drive ecosystem-level C exchange between the soil and the atmosphere. The total ecosystem level exchange of CO_2 is called the net ecosystem exchange (NEE):

$$NEE = GPP - R_{eco} \quad (1.3)$$

Total soil respiration (R_s) is regarded as the total release of CO_2 originating from different belowground sources: R_{SOM}, R_{root} and R_{Rhiz}. Hence

$$R_s = R_{SOM} + R_{root} + R_{rhiz} \quad (1.4)$$

Respiration processes

Total soil respiration (R_s) includes respiration from below-ground sources as shown in Equation 1.4 and depicted in Figure 1-1. Physiologically, respiration involves a series of metabolic processes that break down compounds containing organic C to produce energy, H_2O and CO_2. In soils, carbon-containing compounds are the substrates for R_s. These substrates are derived from organic materials that may be grouped as fresh, partially decomposed or fully decomposed in the form of humus, and their decomposability depends on physical and chemical protection within the soil matrix, as well as their chemical characteristics. Conventional models of SOM turnover subdivide SOM into

hypothetical pools with different mean residence times and accumulation rates, but in reality there is a continuum of reactivities and decomposition potentials across SOM.

1.2 Literature review

1.2.1 Priming effects

1.2.1.1 A definition of priming effects

The priming effect is commonly defined as the observed changes in SOM turnover following additions of C or nutrients to soil (Kuzyakov, 2010; Kuzyakov et al., 2000; Lavelle and Spain, 2001; Hütsch et al., 2002; Blagodatskaya et al., 2007; Nottingham et al., 2009). A range of responses can be obtained based on the type and quantities of substrate applied and prevailing soil conditions (Figure 1-2).

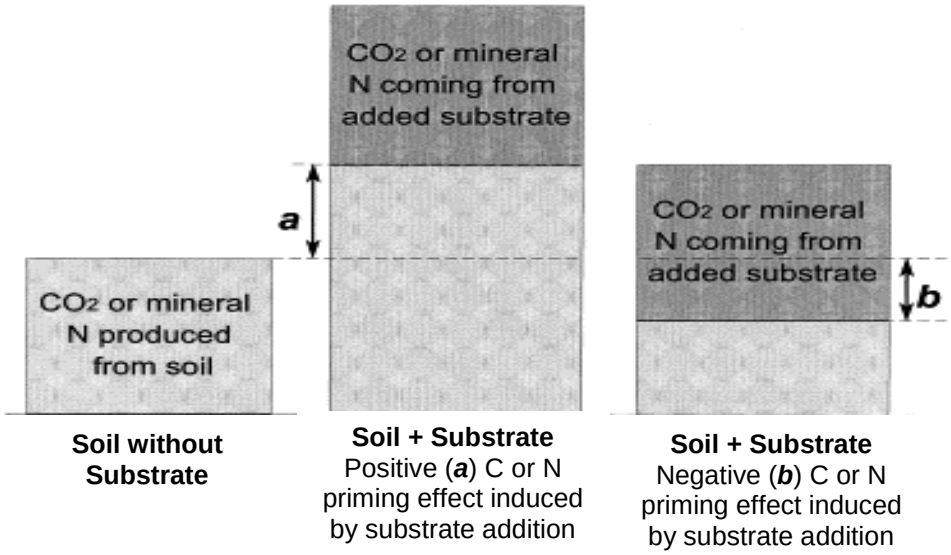

Figure 1-2 Schematic representation of the priming effect — non-additive interactions between decomposition of the added substrate and of soil organic matter (SOM): (a) acceleration of SOM decomposition — positive priming effect; (b) retardation of SOM decomposition — negative priming effect (Kuzyakov et al., 2000).

Kuzyakov (2010) noted two different approaches to the definition depending on whether C or nitrogen (N) was the focus. In cases where C was being studied priming effects would be the extra decomposition of organic C (released as CO_2) after the addition of an easily decomposable organic C substrate. In terms

of N it relates to the extra soil N taken up by plants (through mineralization) after the addition of mineral N fertilizer compared to plants not treated with (inorganic) N (Figure 1-2).

Although there is a tendency to consider priming effects only as positive, added substrates can also result in negative priming, where added substances contribute to reducing the total amount mineralised from SOM compared to that mineralised from basal respiration in untreated controls. According to Kuzyakov et al. (2000) negative priming effects may be of much greater significance to ecosystems than positive ones. This is primarily because negative priming ultimately results in conservation of stored C or N, since the added substrate replaces some or all the stored C or N that would otherwise be lost to the atmosphere through mineralisation. In comparison positive priming is associated with an accelerated rise in SOM and N mineralisation with an undesirable increase in atmospheric C or N (Figure 1-2).

While priming can be readily demonstrated artificially in the laboratory it is a naturally occurring phenomenon. For example, deposits of low molecular weight C compounds from plant roots into soils (rhizodeposits) may cause priming effects (Zhu et al., 2014; Shahzad et al., 2015). The quantity and quality of rhizodeposits varies spatially in the soil profile. There are also significant differences between plant species and across the different plant growth stages and in response to environmental impacts. The quantification of rhizodeposition and identification of individual compounds and their functionality is not very well developed (Reichstein et al., 2014; Hütsch et al., 2002). The critical link between vegetation and soil C and N cycling, where living roots stimulate the decomposition of SOM, is still poorly understood (Gärdenäs et al., 2011). Various mechanisms have been put forward to explain the wide range of reported effects but none are well established. A consequence is that priming effects are generally not included in current ecosystem and global scale models of C-cycling (Kuzyakov et al., 2000; Gärdenäs et al., 2011).

For the purpose of this study I define priming as the change in native SOM turnover in response to C substrate additions as rhizodeposits in planted soil

systems or as plant derived substrates specially prepared and applied to soil microcosms. The observed changes in native SOM were compared to unplanted and untreated controls, respectively, receiving only water.

1.2.1.2 Experimental evidence of priming effects in unplanted soils

Accelerated SOM decomposition in the presence of labile carbon has been demonstrated by many researchers. This was first noticed in 1929 by Lohnis, cited in Kuzyakov et al. (2000) in studies of green manure decomposition in soil. Lohnis observed an intensification of humus N mineralization when fresh OM residues were added to soils. Later in 1946 Broadbent and Norman, cited in Kuzyakov et al. (2000) found a four to 11 fold increase in CO_2 evolution from SOM after adding ^{13}C labelled residues.

Soil organic matter decomposition is usually determined by proxy from changes in CO_2 efflux rates or N mineralization rates. However, the total CO_2 efflux from a soil will be a composite derived from several sources. In addition to sources given in Equation 1.4, priming effects induced by roots or by addition of plant residues are also included (Kuzyakov, 2006). Since not all the sources contributing to the total soil CO_2 efflux are related to SOM decomposition, potentially misleading results could be obtained where only the total efflux is considered. Partitioning of the efflux according to its various sources is required to derive more accurate estimates (Millard et al., 2008). Kuzyakov et al. (2000) categorized priming effects based on the source of the CO_2 efflux rate change and its direction. Four categories of priming effects were identified:

(a) Real positive and (b) real negative priming effects – increase or decrease, respectively, in C or N mineralization in an unplanted soil subjected to a substrate compared to an untreated control.

(c) Apparent positive and (d) apparent negative priming effects – increase or decrease, respectively, in CO_2 efflux from soils in response to added substrate but this change in microbial activity is related to microbial turnover (in the case of C) and the substitution or exchange of nutrients (N) within the various pools existing in soil. There is no turnover or mineralization of resident soil C or N.

Apparent priming effects were reported by Jenkinson and Rayner (1985) when they explained observed interactions between applied ^{15}N fertilizers and resident soil N. They reported that real positive priming effects were commonly observed as increased native N uptake in plants treated with fertilizer N compared to controls not receiving fertilizers. Positive apparent priming effects are thought to relate to the release of soil microbial biomass N or C that is replaced by applied fertilizer N or applied C without any turnover of resident C or N (Jenkinson et al., 1985; Blagodatskaya and Kuzyakov, 2008). Apparent priming effects were linked to initial increases in microbial metabolism following additions of labile C substrates (Blagodatsky et al., 2010). The extent of real or apparent PEs in laboratory experiments is verified by analysis of microbial biomass C using isotopic techniques to determine its relative composition of SOM or substrate-derived C (Paterson and Sim, 2013; Ghee et al., 2013; Dimassi et al., 2014).

Removal of plant roots from the soil system eliminates the possibility of rhizosphere priming effects. Important interactions involving soil microbes and plant roots and soil processes related to C and nutrient turnover are either absent or less realistic in unplanted systems. Nonetheless, this approach brings simplicity and facilitates compartmentalisation of a highly complicated and diverse system. It allows for deliberate studies investigating the mechanisms of priming effects. In earlier studies root exclusion techniques, including trenching (where sections of roots particularly in forest trees were cut off and physically separated) were used to compare the CO_2 efflux from planted and unplanted soils (Hütsch et al., 2002; Fisher and Gosz, 1986; Lee et al., 2003). Such comparisons were used to partition root respiration and estimate the contribution of soil and its microbial constituents to the CO_2 efflux. However, this technique does not assess the dynamic effects of plant roots and their interactions and so only provides rough estimates of the root CO_2 efflux. Apart from the priming effects of root exudates, the presence of roots alters the soil moisture balance and temperature regimes compared to an unplanted soil. Also, dead roots, root hairs and sloughed off cells generate changes in the structure and activity of the soil microbial community. Hence several important

parameters impacting on the soil CO_2 efflux are different in a planted soil. However, unplanted soil systems can be valuable especially when used in combination with other methods, like isotope techniques.

Using an unplanted soil incubation experiment, Blagodatskaya et al. (2007) studied glucose and N induced priming effects relative to microbial growth strategies. They found positive and negative priming effects depending on the level of C and N added relative to the soil microbial biomass C. Likewise, Blagodatskaya and Kuzyakov (2011) using data from several previous studies, showed that low levels of C in added substrates (less than 15% of soil microbial biomass C) produced real positive priming effects (Figure 1-3) whereas at much greater levels of substrate C relative to microbial biomass C (greater than 200%) they found positive apparent, negative and zero priming effects (Figure 1-4).

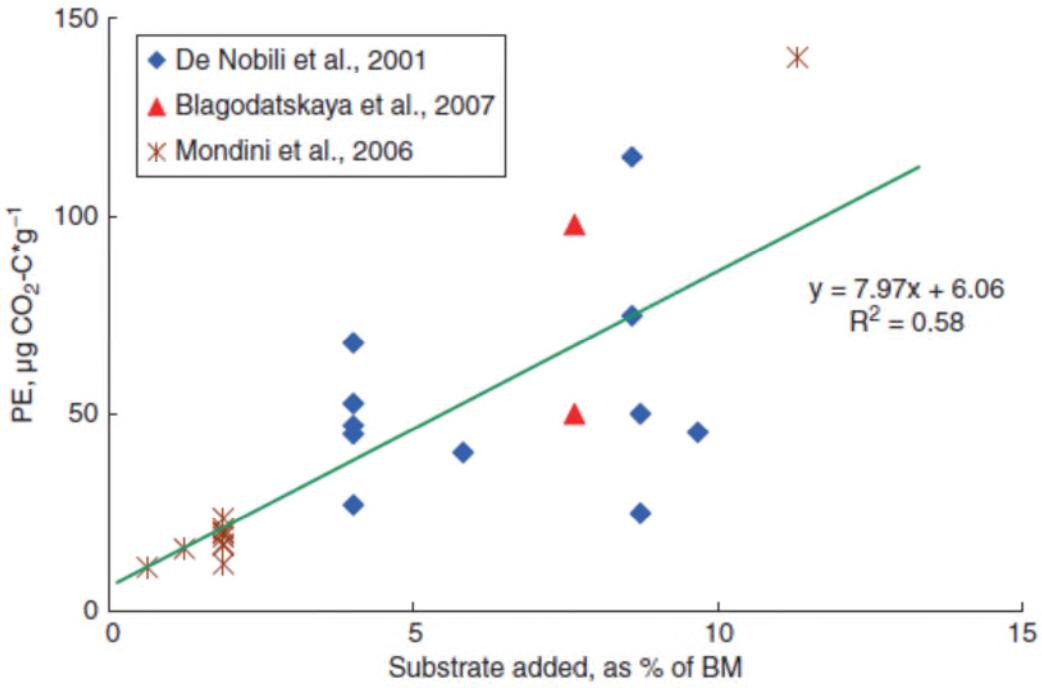

Figure 1-3 Primed CO_2 –C efflux as affected by the amount of easily available substrate C added as percentage of microbial biomass C. The added C is < 15% of microbial C (Blagodatskaya and Kuzyakov, 2011).

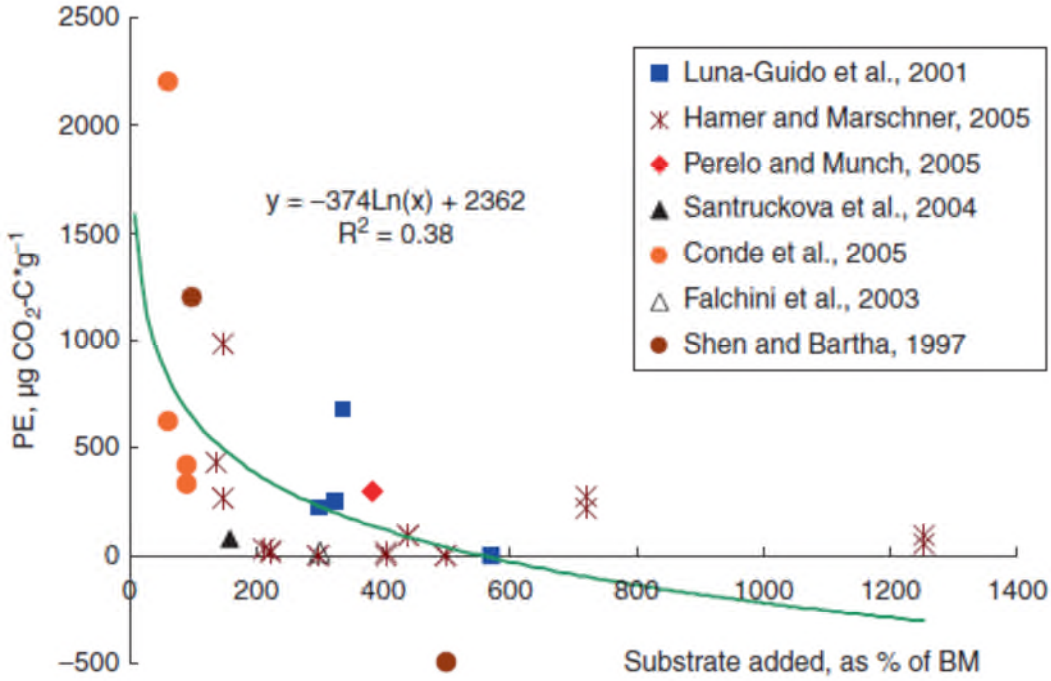

Figure 1-4 Primed CO_2-C efflux as affected by the amount easily available substrate C added and expressed as percent of microbial biomass C; added C is > 50% of microbial C (Blagodatskaya and Kuzyakov, 2011).

Nottingham et al. (2009) demonstrated real positive priming effects in response to additions of sucrose and maize leaf litter to a brown forest soil. Their study showed that the type and quality of added substrate were key determinants of the levels of real positive priming effects. Sucrose applied at 6 mg C g^{-1} of soil generated much greater positive priming effects compared to similar quantities of C added as maize leaf litter, either ground or chopped. Ground maize produced greater positive priming effect than chopped maize residue, indicating that the finer substrate was more readily decomposed.

According to Blagodatskaya and Kuzyakov (2011) carbon substrates of variable quality (especially in forms not readily utilised by the soil microbes) can produce similar priming effect responses (to that of glucose, for example) when applied to soil at sufficiently low quantities relative to soil biomass C. A study by Blagodatskaya et al. (2007) showed that substrates of different quality (glucose and plant residue) applied at the same rate (6 mg C g^{-1}) produced different

rates of priming. It can be concluded that the inherent qualities of the substrates used may have accounted for the observed differences in priming effects, even under similar conditions. The physical attributes like particle size and especially chemical composition, the type of C compounds and possibly other nutrient compounds involved can account for significant differences in decomposition and priming effects. For example, simple sugars exuded from plant roots would be readily decomposed with a high turnover rate and mean residence time (MRT) of minutes to hours, whereas plant residues with high contents of cellulose and lignin would decompose within weeks to months (Kuzyakov, 2006). These details are highly relevant for proper evaluation and comparison of priming studies. They are also likely to account for the high variability in available literature.

Notwithstanding, using soil samples from a drained Cambisol developed from granitic rock, Fontaine et al. (2007) showed that supplies of fresh plant-derived C (cellulose) could generate real priming effects by stimulating microbial turnover of old recalcitrant C age 2,567 ± 226 years. The old C was in soil samples collected at 0.6 to 0.8 m depth. Cellulose was applied at a rate representing one-quarter of the annual C litter loading from plant roots into the upper soil layer. This produced an increase in turnover rate of 72 mg C kg^{-1} for old recalcitrant C compared with the same subsoil incubated without added cellulose. This rate was comparable to the SOM turnover rates observed in the topsoil at the sample site. The SOM turnover rate of the topsoil is influenced by plant roots, which provide a continuous supply of fresh C that is not available at greater depths (0.6 to 0.8 m in this case). However, when fresh C was added to the subsoil samples the turnover of its stored, recalcitrant C proceeded at comparable rates to the topsoil. This led to the conclusion that the absence of fresh C supplies was crucial for C recalcitrance and hence sequestration. These effects need to be explored in contrasting soil types and general conditions.

While the above study (Fontaine et al., 2007) refers to laboratory experiments, there are important practical concerns for the sustainable management of farm lands and the natural environment. Decomposition of resident SOM at greater

depths could be facilitated inadvertently by poor soil management practices and land use changes. For instance, during deep ploughing, subsoil material is exposed to the surface, mixed with surface material rich in highly available C and a microbial community capable of rapidly decomposing the old, sequestered C. As conditions of aeration and moisture improve deeper into the soil profile root growth will also be facilitated. In essence, the rhizosphere may extend downwards and stimulate SOM decomposition in sections of the profile that would be otherwise physically protected.

1.2.2 Evidence of plant-induced priming effects

The important role of plants and particularly plant roots in SOM turnover was noted earlier. Cycling of C through plant roots is pivotal to ecosystem responses to climate change (Lavelle and Spain, 2001; Zobel, 2005; Bardgett, 2011). In the presence of sunlight during photosynthesis plants are the receptors and effective conduits of fixed atmospheric C to soils. While the mechanisms by which plant roots modulate important soil processes are not fully understood, their importance is obvious from comparisons of planted and unplanted soil systems and confirmed by the isotopic techniques now available (Kuzyakov, 2002; Fontaine et al., 2011; Hütsch et al., 2002; Paterson, 2003; Kuzyakov and Schneckenberger, 2004; Fontaine and Barot, 2005). Plant roots make a significant and direct contribution to the total CO_2 efflux from soils. Their exudates of easily available C-rich compounds nourish the soil microbial community by altering the soil physical and chemical environment (i.e. soil structure, water flow, pH). Consequently, they exert a controlling effect on SOM turnover. It can also be argued that the plant shoots rather than roots may ultimately be in control of SOM turnover in planted systems since roots are only able to transfer C compounds that were initially fixed in the green leaves of the plant. However, it is discussed later that the process of C partitioning within the plant into its root and shoot resources may actually be paramount in determining what proportion of fixed C actually reaches the soil.

1.2.3 Plant root dynamics and the rhizosphere

The zone of root and soil interaction in the soil close to root surfaces is known as the rhizosphere. Rhizosphere processes depend upon C inputs from root growth and turnover, rhizodeposition, and resulting microbial activity. Because the supply of nutrients and water are unevenly distributed in soils and varies greatly in time, roots typically possess a level of phenotypic plasticity that enables them to respond to changing soil conditions. The resulting root architecture therefore depends on a combination of inheritable traits and environmental conditions. To a large extent root architecture determines the spatial distribution of the rhizosphere within soils.

Within the rhizosphere, roots extract nutrients and water from soils and in turn release exudates and shed dead cells from root tips and mucilage. Roots penetrating through soil may be important for breaking down soil aggregates and exposing otherwise protected SOM particles to microbial attack which may increase decomposition (Kuzyakov, 2002). The role of roots in stimulating decomposition of SOM is seen as key to understanding soil carbon turnover and possible feedback loops to future climate change (Cheng and Kuzyakov, 2005; Kuzyakov and Gavrichkova, 2010; Phillips et al., 2011).

Kuzyakov (2010) distinguished the effects of a one-time or occasional pulse release of C into the soil and a permanent or continuous flow. Inputs from plants can occur both as pulses and continuously. The subsequent pattern of C release from root debris or exudate will depend on its inherent decomposability. Microbial breakdown of dead roots and the decomposition of aboveground plant parts can be relatively quick in some species; for example grass or pasture systems have relatively high turnover rates compared with forests. After decomposition any DOM is coupled with available root exudates and enters the soil solution, providing a one-time or pulse delivery of C to the soil. Such releases can occur at microsites which may join to form zones with high concentrations of readily available C substrates, referred to as ‘hotspots’ (Kuzyakov and Blagodatskaya, 2015).

Kuzyakov (2010) argued that continuous input is typical of the slow decomposition of dead roots, leaf and shoot residues. These decompose at a slower rate than root exudates and are available in very small amounts at a time. The extent or prevalence of pulse or continuous C inputs is highly dependent on environmental conditions, plant species, phenological stage and especially rooting densities (Cheng and Kuzyakov, 2005). In grasslands, where a dense mat of dead roots, leaves and other easily decomposed plant matter occupy the top few cm of soil, there can be a continuous supply of C from live roots and detrital material particularly during the growing season.

Kuzyakov (2010) noted that the possible differential effects of repeated pulses or continuous inputs are under-investigated. However it may be likely that differences in both the pattern and intensity of SOM decomposition and related PEs could vary just based on whether inputs are solitary or continuous. It is also likely that prevailing soil and environmental conditions could also play major roles in how PEs are manifested in either scenario. Most priming studies have been limited to simulation of a one-time or pulse C release. In one of the few studies that investigated repeated substrate additions on priming effects, using a 1-4 month incubation study Hamer and Marschner (2005) added ^{14}C labelled substrates (fructose, alanine, oxalic acid and catechol) in various combinations as pulse and repeated applications. They monitored SOM mineralization rates at hourly intervals and found that repeated (minimum 6-day interval) and combined additions of substrates generated greater priming effects than a one-time, pulse release. Although the important link between C assimilation by plants (photosynthesis) and rhizodeposition is well established (Bardgett, 2011; Kuzyakov and Gavrichkova, 2010; Kuzyakov and Cheng, 2001) it was demonstrated by Dilkes et al, (2004) using wheat plants in solution culture, that the internal partitioning of C between shoot and root had greater significance on the temporal exudation of C compounds than photosynthesis. The pattern of C flows as rhizodeposition could be of major significance. But as for studies on priming where the soil system is simplified to consider only a few factors at a time, there is the risk the experimental system does not adequately represent reality. Consequently the models and predictions of SOM turnover generated

from such studies may be of limited value. A way forward may be to link such studies (microcosm and mesocosm) directly with *in situ* field approaches where the systems are left undisturbed as much as possible and to develop composite models that assimilate the results.

1.2.3.1 Root exudates

As much as 50% of C fixed by plants in photosynthesis can be deposited in soils either as root exudates or respired as CO_2 (Lavelle and Spain, 2001; Kuzyakov and Gavrichkova, 2010; Bais et al., 2006). Jones et al. (2004) reasoned that root exudates may account for 2-4% of photo-assimilated C, however the variability of reported results suggest a dependency on context.

Root exudates comprise a mixture of carbohydrates (simple sugars and polysaccharides), amino compounds, organic acids, nucleotides, flavones, enzymes and growth factors. Bais et al. (2006) noted that root exudation represents a significant carbon cost to the plant. Another perspective may be one of a selfish trade-off or exchange of plant C for other valuable nutrients within SOM, which are made available after mineralization by C feeding microbes. Using a root-shoot separated chamber experiment with wheat and alfalfa, Hutch et al. (2002) demonstrated that up to 20% of photosynthetically fixed C was released into the soil during the vegetative phase and approximately 64% (alfalfa) and 86% (wheat) of the released C was rapidly respired by micro-organisms. This supply of C to the root zone is continuous and the fact that a large percentage is quickly used by soil microbes suggests the existence of a tight and continuous association between plant roots and soil microbiota.

Plant species, growing conditions, nutrient availability and stage of plant growth affect the nature and composition of exudates, the interactions with soil microbes and therefore associated priming effects. Differences at the plant species level and the impact of added nutrients, especially N, have been observed. Cheng (2009) found that there was a greater priming effect from soybean plants compared to wheat (251 and 116% in comparison to an unplanted control). Not only were there differences in the priming effects in

relation to plant species but plant phenology and time of sampling also exerted a significant effect on the levels of rhizosphere priming effects.

Though there is a basic understanding of rhizodeposition and the constituents of root exudates, we are still unclear about their mechanisms, environmental triggers, linkages to other soil processes and overall importance in nutrient cycling (Cheng et al., 2014; Gärdenäs et al., 2011). The tracking of C flows from plant roots to soils, including detailed *in situ* assessments of their cumulative effects over temporal scales (diurnal and seasonal), and their dependency on environmental drivers are lacking from previous studies in this area.

1.2.4 Possible mechanisms to account for priming in soils

1.2.4.1 Biologically-driven mechanisms

A fundamental argument still exists about whether the rates of SOM turnover are controlled by biotic or abiotic processes (Brookes et al., 2008; Kemmitt et al., 2008; Brookes et al., 2009; Kuzyakov et al., 2009). The lack of consensus reflects insufficient knowledge about the processes involved and the mechanism(s) driving priming effects. This is also indicated by the paucity of models of C and N dynamics allowing for priming effects (Kuzyakov et al., 2000).

The focus of earlier studies was on demonstrating the existence of priming effects rather than on establishing the mechanisms involved (Blagodatskaya and Kuzyakov, 2011). But an understanding of the mechanisms is crucial to a better understanding of the overall processes. Kuzyakov (2002) proposed seven possible mechanisms through which plants interact with soil components to stimulate SOM turnover.

(1) The growth of plants accentuates soil-drying and this affects the rate of SOM decomposition. This hypothesis considers that in planted soils the natural drying process will be accentuated by plant transpiration and water harvesting by roots. Rewetting a dry soil has long been known to create a rush of respiration known as the “Birch Effect”. However, as considered by Blagodatskaya and Kuzyakov (2011) soil moisture is the main driver of

microbial activity and lower priming effects can be expected from soil conditions where moisture is limiting. They noted that both the intensity and types of priming effects dominating in a soil also depended on the frequency and duration of drying-rewetting events. Plants themselves have a threshold beyond which they will be affected by moisture deficits. Microbes generally are more tolerant to moisture stresses and can survive beyond the wilting point for most plants. Furthermore, this mechanism is only relevant in planted soils and could not explain priming in unplanted systems or where adequate moisture is maintained constantly. These considerations suggest that this mechanism may not be very significant overall.

(2) Mechanical effects of growing roots assist in the release of SOM physically bound in soil aggregates and make it available for microbial attack. Several studies have demonstrated that a portion of labile SOM may be hidden within the structures of stable soil microaggregates (Beare et al., 1994; Denef et al., 2001; McCarthy et al., 2008). On a larger scale growing roots assist with the disaggregation of larger soil clods. However at the level of soil particles and micro-aggregates (2 to 250 µm in diameter) roots tend to lend more support to the aggregation rather than disaggregation thus forming stable macroaggregates (0.25 to 5 mm) (Brady and Weil, 2008). The pressure of growing roots actually forces nearby soil particles closer together and soil particles and micro-aggregates are bound by sticky root exudates to form macro-aggregates (Brady and Weil, 2008). The importance of this effect depends on the existing level of soil aggregation (Denef et al., 2001; Six and Jastrow, 2002). The mean residence time of C in macro-aggregates is shorter than that of C in micro-aggregates where organic C is bound much more tightly with greater stability and could potentially account for long-term C sequestration in soils (Six and Jastrow, 2002; Schlesinger and Lichter, 2001). Cheng and Kuzyakov (2005) noted that the effects of aggregate disturbance associated with roots may be due to the effects of intense wetting and drying accentuated by roots. The evidence is that roots do more for aggregate construction rather than destruction. However, the impact of roots on SOM decomposition via

aggregate destruction has not been thoroughly investigated or clearly understood.

(3) Uptake of exuded soluble organic substances by plant roots decreases the amount available for microbes. Exudates from plant roots are a primary source of highly available C-rich compounds for soil microbes. If these are substantially depleted in the rhizosphere through reabsorption by plant roots, then microbial activity could be adversely affected, causing a decrease in SOM decomposition. The bi-directional flow of C resources between roots and the rhizosphere was reviewed by Jones et al. (2009). They admitted that roots are ineffective competitors for these resources compared to the soil microbial biomass but proposed four hypotheses to explain root uptake of soluble organic substances. Nonetheless, as stated earlier the boundaries of available C pools in the rhizosphere are very porous with continuous exchange of resources between the various pools. Along with plant root secretions, other sources (including degraded plant and animal cells) simultaneously make contributions to the total available C pool in soils. The extent to which soil microbes are actually deprived of available C through direct competition from plants is not likely to be significant but could vary considerably with plant species, growing conditions, stage of plant development and plant health.

(4) Faunal grazing on microbes in the rhizosphere (especially by protozoa and nematodes) increases microbial turnover and thereby release nutrients and CO_2 (Bonkowski, 2004). However, the turnover is mainly of fresh highly available forms of C and other nutrients recently exuded from plant roots rather than of recalcitrant or stored SOM. The exudates are rapidly consumed by bacteria which are then preyed on by the higher trophic groups (including protozoa, nematodes and earthworms). The faunal grazers incorporate about 50 to 70 % of the ingested material with the balance being excreted and returned to the soil. The grazers eventually die or are preyed upon by other faunal groups, carnivorous nematodes and arthropods.

(5) Competition between plant roots and microbes for limited N resources in the rhizosphere contributes to a decline in SOM decomposition. Nitrogen is required for microbial growth in the rhizosphere. Although there are symbiotic relationships between microbes and the plant at various levels Bonkowski (2004) highlighted the selfishness of these relationships and suggested they be viewed in an evolutionary context. Essentially, the plant is trying to get what it needs and the bacteria and other microbes are similarly looking to fulfil their own needs. It is a case of the fittest surviving better. The ability to garner the mineral N on offer will differ with plant species, root architecture and growing conditions. Depending on the plant efficiency of acquiring the limited N on offer, there may or may not be a sufficient supply for microbial growth. A consequence of the latter is reduced microbial growth and thereby a decrease in SOM decomposition.

(6) Preferential utilization of available microbial substrate over SOM C. This theory states that where mineral nutrient resources are abundantly available in the soil, soil microbes will prefer the labile root - derived C to SOM C. This has the effect of reducing SOM turnover. On the other hand if soil mineral nutrients are limiting, soil microbes will prefer C derived from SOM with the associated nutrients (Cheng and Kuzyakov, 2005).

(7) Root exudates stimulate microbial activity and increase SOM decomposition. It was shown by De Nobli et al. (2001) that relatively small amounts of readily available C substrates can significantly increase SOM decomposition. Blagodatskya and Kuzyakov (2011, p 661) referred to this as signalling and triggering effects. They defined the triggering effect as "*an acceleration of internal microbial metabolism by trace amounts of substrate with an immediate (several minutes to several hours) increase in respiratory activity*". They further observed that in several studies under field conditions, the amount of C added to soil was relatively insignificant as an energy source but provided the impetus to changes requiring much more energy.

Based on available evidence, the most significant mechanisms are thought to be (5), (6) and (7): concurrence of demand for mineral N between plants roots and soil microbes (Fontaine et al., 2011; Zhu et al., 2014; Yin et al., 2013; Nottingham et al., 2015), preferential substrate utilization (Milcu et al., 2011) and microbial activation (Blagodatsky et al., 2010; Cheng and Kuzyakov, 2005). Cheng and kuzyakov (2005) argued that the operationalization of a particular mechanism was dependent on the relative abundance of available soil C and N. Where both C and N were limiting, competition between the plant roots and microbes would dominate, leading to decreases in microbial activity and SOM decomposition. Where available C is limiting and N is in abundance, preferential substrate utilization will be the dominant mechanism. After available C is consumed, microbes capable of mining the more recalcitrant C will dominate and SOM decomposition will be enhanced. Where N is limiting and available C remains in abundance, microbial activation will be dominant. The available C will stimulate growth of microbial populations which will initially consume and immobilize available nutrients. The nutrients are released again by faunal grazing or microbial turnover.

1.2.4.2 Bank theory

Fontaine and Barot (2005) proposed a ‘bank’ mechanism whereby sequestration of nutrients and carbon in SOM are adjusted according to the availability of nutrients in the soil solution. This is based on the assumption that the SOM priming is controlled by the relative concentrations of nutrients in the soil solution (Fontaine et al., 2011). It follows that where nutrients are in short supply, microbial species with the ability to seek out these nutrients in SOM (k-strategists) would be stimulated to mine for them in preference over species feeding only on fresh carbon substrates (r-strategists). In contrast, when soluble nutrients are in abundance microbial mining should decrease, resulting in more sequestration of nutrients (Fontaine et al., 2011).

The common and important central thread through these mechanisms is that they are biologically driven. The role of soil microbes is central at least to the three proposed mechanisms highlighted earlier as being the most significant for

explaining soil priming effects. Notwithstanding this, in recent years abiotic mechanisms have also been proposed.

1.2.4.3 Abiotically-driven mechanisms – the regulatory gate hypothesis

Jenkinson (1966), and Jenkinson and Powlson (1976) observed that SOM mineralization continued at the same rate after severe perturbations (chloroform fumigation) that resulted in the loss of most of the soil microbial biomass. Brookes et al. (2008) showed that even after 90% of the soil microbial biomass was removed, the process of SOM mineralization continued as if the microbial biomass was untouched. Considering these observations Brookes et al. (2008, p 718) concluded that "*the rate of soil organic matter mineralization is independent of both the size and community structure of the soil microbial biomass.*"

Building on these ideas Kemmitt et al. (2008) proposed the 'regulatory gate' hypothesis whereby the mineralization of the SOM occurs in two stages: (1) non-bioavailable humified SOM is altered by an abiotic process(es) to make it bioavailable; (2) the SOM that is now bioavailable is mineralized.

Kemmitt et al. (2008) proposed that the regulatory gate hypothesis explained the observation that both the depleted and intact microbial mass appeared to be mineralizing SOM at the same rate. Since Stage 1 is abiotic it proceeds unhindered by size and activity of the microbial biomass; because it is also slow compared to Stage 2, it limits the overall rate of mineralization and consequently the overall rate is insensitive to the size of the microbial biomass. It follows that additions of labile C in the context of the 'regulatory gate' may not generate any priming effects, since the initial access to less available SOM is abiotic.

1.2.5 Interactions with other drivers of soil C mineralization

1.2.5.1 Environmental impacts on rhizosphere priming

Global climate change impacts are of major concern and recent studies have focused on the possible feedback effects to soil processes linked to SOM decomposition (Kuzyakov and Gavrichkova, 2010; Gregory, 2006; Heimann and

Reichstein, 2008; Subke and Bahn, 2010; Carrillo et al., 2011). Rising atmospheric CO_2 levels is a predicted consequence of human perturbations of the C-cycle and several free-air CO_2 enrichment (FACE) experiments are being used to assess the impacts on various soil processes. Phillips et al. (2011) showed that root exudation in a pine forest under elevated CO_2 increased by 50% compared to control treatments.

There is mounting evidence from different ecosystems that plant traits have major influences on soil nutrients and C cycling, and that certain plants can select for particular groups of soil organisms that play key roles in biogeochemical processes, including SOM decomposition through rhizosphere priming effects. This suggests that climate adaptations such as changes in vegetation composition could potentially alter patterns of C dynamics (Bardgett, 2011; Paterson, 2003; Kuzyakov and Gavrichkova, 2010; Phillips et al., 2011; Gregory, 2006; Dijkstra et al., 2009).

Apart from the changes in plant species composition there are other environmental effects that can have direct and significant impacts on SOM turnover. Soil organic C content, photosynthesis intensity, soil mineral N content, N fertilisation regimes, N mineralisation by extra decomposition of SOM and elevated atmospheric CO_2 concentrations were reviewed by Kuzyakov (2002). The impact of soil moisture, soil temperature, aggregate and particle-size fractions and soil pH were reviewed later (Blagodatskaya and Kuzyakov, 2011). These are now considered in greater detail.

1.2.5.2 Soil organic C content

Kuzyakov (2002) considered two contrasting hypotheses to explain the effects of organic C content in a soil on SOM turnover. Firstly, large SOM levels provide for high quantities of readily utilizable C, which can be readily mineralized by soil microorganisms during rhizosphere priming. On the other hand, where organic soil C content is large, N and other nutrients are seldom limiting when compared to infertile soils with low organic C. Therefore, plants and microorganisms growing in these soils are not as dependent on enhanced SOM mineralization (priming effects) to obtain nutrients. Contrasting results

have been obtained from studies on the impacts of soil organic C content on rhizosphere priming effects, depending on incubation conditions and plant species used (Kuzyakov, 2002).

1.2.5.3 Photosynthesis intensity

There are several studies and reviews demonstrating the controlling effect of photosynthesis intensity on root exudation of C belowground as well as CO_2 efflux from soils (Kuzyakov, 2002; Kuzyakov and Gavrichkova, 2010; Kuzyakov and Cheng, 2001; Beylich et al., 2010). Kuzyakov and Cheng (2001) showed that the absence of light caused a decrease in root exudation and subsequent priming effects. They explained that this was caused by the interruption of the flow of easily decomposable substrates. Several studies have showed that apart from reduced light, other growth limiting factors, such as soil compaction can decrease photosynthesis. Clay and Worrall (2013) showed that compaction by grazing sheep on a peat soil significantly reduced photosynthesis. Tubeileh et al. (2003) reported that artificially compacted soils planted with maize significantly reduced C assimilation and increased C partitioning to roots and exudates compared to controls. They hypothesized that compaction-induced resistance to root penetration developed a sink limitation leading to accumulated C in roots. This resulted in a feed-back to shoots that regulated C assimilation. Another possible mechanism advanced was the effect of compaction on limiting soil water movement and uptake leading to moisture stress signals being sent to shoots. The plant responds by limiting stomata opening and therefore photo-assimilation. The two scenarios highlight the varied responses that are possible from different abiotic stresses to the plant-soil system. Another important factor for consideration is the effect of defoliation through clipping or herbivory on the photosynthetic potential of plants.

1.2.5.4 Effects of foliage clipping on plant C partitioning and soil C turnover

Defoliation through clipping or herbivory is common in grassland management systems. Clipping events can alter plant C partitioning and affect the quantity and timing of rhizodeposits and contribute to changes in soil respiration and

rhizosphere priming effects (Shahzad et al., 2012; Schmitt et al., 2013). Shahzad et al (2012) showed that artificial clipping in a potted grass experiment resulted in significantly reduced priming effects in a drained Cambisol. Generally, defoliation will result in reduced photosynthesis in plants. The intensity of clipping will determine the extent of photosynthesis reduction and a range of physical and chemical changes within the plant can follow clipping. These include, but are not limited to, aboveground effects, such as changes in growth rate, biomass production, plant height, photosynthetic activity, root to shoot ratio and C dynamics within plant tissues. Clipping has been shown to alter the distribution of C resources within the plant. After clipping C resources initially allocated to roots are reallocated to the shoot to support regrowth (Schmitt et al., 2013).

1.2.5.5 Soil nutrient status

Although soil C has been the focus of this review so far, the importance of N cycling to C turnover in soils is fundamental. The key mechanisms of soil priming effects noted earlier, microbial concurrence of demand for mineral N between plants roots and soil microbes, preferential substrate utilization and microbial activation all involve N cycling. There is general agreement that the amount of available soil N impacts the amount of C primed in soils. Decrease in priming effects was observed when available N was applied to soil with organic C (Blagodatskaya et al., 2007; Liljeroth et al., 1994).

Kuzyakov (2002) noted that N applications can have both direct and indirect effects on soil conditions and consequently SOM turnover. A direct effect of mineral N additions is the reduction of N limitations in the rhizosphere. This is thought to weaken the concurrence of N demand between soil microbes and roots and improve growing conditions for the microbial community. The resulting growth of the microbial community facilitates efficient use of root exudates and reduces the need for decomposition of SOM to mobilize additional N.

Indirectly, higher levels of soil mineral N positively impact plant growth and the assimilation of C through photosynthesis. However, there may be a simultaneous reduction in the allocation of assimilated C to roots (Coleman et

al., 1983; Paterson and Sim, 1999). Although the quantities of exudates may be the same for fertilized and unfertilized systems, the quality of the exudates may differ significantly to create differences in priming effects. Fertilizer application method (banded, broadcasted or fertigation) and inherent soil characteristics (hydraulic conductivity, leaching potential, organic matter content, water holding capacity) may influence priming effects of fertilizer applications.

1.2.5.6 Interaction between soil moisture and access to substrate

Soil moisture is a major driver of microbial activity therefore smaller priming effects may be expected under moisture limiting conditions. The frequency and duration of wetting and drying events can also have direct effects on the intensity of priming effects (Blagodatskaya and Kuzyakov, 2011). Rainfall events that break extended periods of drought result in a short-term activation of soil microbes that metabolize available substrates (the Birch effect). According to Blagodatskaya and Kuzyakov (2011), in such cases rapid moisture loss is the major limitation to microbial activity. Therefore both apparent positive priming effects, resulting from the triggering of the microbial community and pool substitution, and negative priming effects from preferential substrate utilization, would be expected. Dry conditions would therefore favour C sequestration. These observations agree with Lamparter et al. (2009) who reported that C sequestration is moisture dependent and concluded that physical soil properties, like aggregation, wettability and wetting dynamics were important to understand specific differences in the dynamics of carbon mineralization in soils.

1.2.5.7 Soil temperature

The relationship between temperature and the biochemical processes of soil C turnover or soil respiration (R_s) can be described by the Arrhenius equation (Arrhenius, 1898). The term ‘temperature sensitivity’ has been used to describe the temperature dependence of R_s (Davidson and Janssens, 2006); it defines the relative change in R_s in direct response to a temperature change over a specific range. Temperature sensitivity is usually shown as the Q_{10} value, representing the increase in R_s in response to a 10^oC rise in temperature.

Eq.1.5 below can be used to calculate Q_{10} of R_s if there is a significant rise in temperature. It assumes an equal Q_{10} over the range of the temperature increase.

$$Q_{10}=\left[\frac{R_2}{R_1}\right]^{10(T_2-T_2)} \quad (1.5)$$

where R_2 and R_1 represent the R_s observed at times T_2 and T_1, respectively.

There are several recommended variations to the Q_{10} function based on the context of use and underlying assumptions. For example (Heinemeyer et al., 2012) applied the Q_{10} Equation 1.2 from (Atkin et al., 2000)), to annual and seasonal periods using mean daily values of R_s and its components. In Equation 1.2, the regression slope (β) of the $\log_{10}$ (R_s) plotted against soil temperature, is used to calculate Q_{10}.

$$Q_{10}=\mathbf{10}^{(\mathbf{10}\times\boldsymbol{\beta})} \quad (1.6)$$

The temperature dependence of R_s is widely studied but there has not been consensus about the mechanisms involved. The temperature sensitivity of C turnover is thought to depend heavily on the molecular complexity of the substrates being mineralised. By comparison SOM with more complex molecular structures and also physical barriers that occlude them from degrading enzymes are likely to have higher temperature sensitivities compared to root exudates which comprise simple compounds that are readily available (Davidson and Janssens, 2006; Arrhenius, 1898; Knorr et al., 2005). It is therefore likely that relative combinations of different C pools in a soil could influence the overall temporal and spatial temperature dependence of R_s. Subke and Bahn (2010) argued that the temperature effect on soil C turnover was only transient, as substrate pools are exhausted and the soil microbial community acclimatize to warmer temperatures. Blagodatskaya and Kuzyakov (2011) contended that psychrophilic microorganisms thriving at low temperatures (less than 20^oC) have enzyme systems with higher affinity for low availability substrates like soil humus. They play an important role in SOM decomposition in cold environments (Gounot, 1986). Both increase and

decrease in priming effects were observed in different studies that compared low and high temperatures

The varied results from temperature dependence studies have been linked to the inherent variations in substrate quantity, quality and availability combined with the influence of environmental factors, all of which can change at micro scales within the soil profile (Davidson and Janssens, 2006). Soil C models that prescribe a one dimensional measure of SOM, without consideration for the variability of soil C and their diversity of kinetic properties are likely to produce misleading results. Either over or underestimating the impact of the predicted future rise in global temperatures and other climatic events.

Plausible approaches to investigating temperature dependence of SOM turnover may be to: (1) Use continuous high resolution flux chamber measurement systems that are field based, and designed to separately measure fluxes from the major C pools. This should be coupled with depth-resolved measurements of temperature and moisture. (2) Observe how mineralization of different C substrates as representatives of soil C pools, are influenced by various simultaneous perturbations of soil temperature, moisture and other important environmental impacts. A better understanding of how temperature changes affect soil C turnover is important to improve current model predictions about how future climate change might affect soil C stocks.

1.2.5.8 Soil acidity and pH

Blagodatskaya and Kuzyakov (2011) reviewed eight different studies conducted between 1997 and 2007 that demonstrated effects of soil pH on the intensity of priming effects. They reported that greater priming effects occurred in neutral soils in the pH range 6 to 8, when both easily available substrate and plant residues were added to soil. Rousk et al. (2010), among others, have shown that soil pH can have greater impacts on soil microbial community structures than biome types. The changes of soil microbial activity and community structure as well as enzyme synthesis are higher in soils with pH 5 - 8 compared to acidic soils (Blagodatskaya and Anderson, 1998). The possible mechanisms underlying effects of soil pH on the soil microbial phenotype was

explored by Zhang et al. (2015). They used soils from 17 different sites and worked with a pH range of 4 to 7 in a ten-year study. They concluded that counteractive effects of evolutionary filtering, (which promoted microbes that were already adapted to the current pH) and evolutionary dispersal of microbes (that evolved or acclimatised to the new pH) were responsible for the counteractive effects of evolutionary and dispersal attributes of microbial response to pH changes.

Priming studies are largely based on CO_2 measurements, and artefacts can occur where the pH dependent solubility of CO_2 is not considered (Blagodatskaya and Kuzyakov, 2011). It stems from the fact that the equilibrium of the different forms of carbonates in soils (H_2CO_3, CO_3^{2-}, and HCO_3^-, all derived from CO_2) is pH dependent. Thus the soil pH must be duly considered when interpreting results from CO_2 evolution. At lower soil pH, values below 5, the possibilities of artefacts are significantly reduced because H_2CO_3 would be the dominant carbonate form (about 98%), which dissociates immediately into CO_2 and H_2O. In this case all the CO_2 produced by soil microorganisms is freely evolved and the efflux is intact. As pH increases the relative proportion of H_2CO_3 decreases, yielding larger misestimates of the CO_2 efflux. The predominant forms of carbonate at pH values greater than 6.8 are HCO_3^- and CO_3^{2-}. The CO_2 produced by the soil microbes must first dissolve in soil water until the saturation capacity of the solution is reached. In such circumstances, CO_2 evolution will depend on the volume of the soil solution. Misestimates of between 10 and 100% at pH values 8 and 9, respectively, are possible. The pH status of soils is therefore a very important consideration for soil priming studies both from the perspective of the effects on the soil microbial community and the processes they mediate, and for the interpretation of soil respiration data.

The vast temporal and spatial diversity of soils makes it almost impossible to consider all variables at once. However, better synergy across working groups involved with different but related aspects of soil science maybe crucial to advance a more comprehensive understanding of the mechanisms driving soil C turnover. There is consensus that an understanding of ecosystem function

and response to global change requires due consideration of feedbacks between plants, microbes and soil processes (Bardgett, 2011; Paterson, 2003). However, more research is required.

1.2.6 Measuring soil respiration and priming effects

A review by Luo and Zhou (2010) provides a history of soil respiration (R_s) measurements and different approaches used over the years. I consider three commonly used approaches for measuring R_s, and then consider aspects of isotopic and non-isotopic methods for differentiating R_s into its component plant and SOM-derived components (Section 1.1.1), reviewed previously by Kuzyakov (2006).

1.2.6.1 Measuring soil respiration

Eddy-covariance

Among earlier approaches for measuring soil respiration, the eddy-covariance (EC) technique is widely used for ecosystem scale measurements (Baldocchi, 2008; Myklebust et al., 2008; Ward et al., 2012). It relies on a measure of the covariance between fluctuations in vertical wind velocity and CO_2 mixing ratio. Essentially, high frequency measurements of wind speed and direction (10-20 Hz) and measurements of CO_2 at a point above the area of interest (called the 'foot print'), using a sonic anemometer and a fast response infrared gas analyser, respectively. It assumes perfect turbulent air mixing and the measurements are integrated over periods from 30 to 60 minutes to build a basis for calculating C balances over daily to annual scales. Since turbulence is required for EC functioning, there can be considerable down time in periods of reduced turbulence. The technique is not well suited for understorey vegetation and when high resolution spatial variability needs to be assessed. Chamber measurements are more appropriate for such conditions.

Gas flux chambers

Gas flux chambers are widely used for measuring and monitoring soil respiration. They are usually in two modes, steady state and non-steady state (Davidson et al., 2002). In steady state systems the flux is calculated from the

difference in CO_2 concentration from the air flowing at a known rate between the inlet and outlet after the chamber air reaches equilibrium CO_2 concentration. In non-steady state systems the flux is calculated as a change in concentration in the chamber headspace of known volume shortly after the chamber is placed over the soil. Non-steady state systems are commonly linked with infrared gas analysers to provide accurate and relatively quick soil respiration and net ecosystem exchange measurements (Heinemeyer et al., 2012; Bahn et al., 2009; Ojanen et al., 2012; Thurgood et al., 2014). Chambers systems are associated with unnatural disturbances to the soil and roots and importantly they impose pressure gradient changes which can alter the flux being measured. Improvements have been made to address these issues and correction factors are applied to address the errors they may pose (Davidson et al., 2002; Livingston and Hutchinson, 1995).

Gradient method

The gradient method (GM) has grown in popularity and attempts to address the pressure gradient issues associated with chamber measurements (Subke et al., 2004b; Maier and Schack-Kirchner, 2014). Like chamber measurements this approach is suited for higher resolution measurements. The method uses Fick's first law to calculate soil CO_2 efflux (f_s)

$$f_s = D_s \frac{\partial C}{\partial z} \quad (1.7)$$

Where D_s is the soil gas diffusion coefficient, C is the soil CO_2 concentration and z is the depth below the soil surface. The method relies on measurements of both soil CO_2 profile concentrations and the coefficient of soil CO_2 diffusion which are both difficult to measure and model (Pingintha et al., 2010). The essential difference of the GM compared to the chamber method is that it does not alter conditions of the soil surface boundary as with chambers. It can also provide measurements of the profile dynamics of CO_2 concentrations (DeSutter et al., 2008). Performance comparisons of these flux measurement options (Myklebust et al., 2008) show that they all possess inherent advantages and limitations.

1.2.6.2 Separating soil respiration in soil and plant derived components

Natural abundance

The natural ^{13}C abundance technique has been used to evaluate rhizosphere impacts on SOM decomposition and critical aspects of soil C turnover (O'Leary, 1981; Kristiansen et al., 2005). It is based on the natural occurrence of two stable isotopes of C in the environment, the more abundant ^{12}C and ^{13}C. Atmospheric CO_2 contains about 1.1% of the heavier C isotope ^{13}C and 98.9% of the lighter isotope ^{12}C. Carbon isotope data are conventionally reported as $\delta^{13}C$ values: the $^{13}C/^{12}C$ ratio of the sample relative to the international Vienna PDB (Pee Dee Belemnite) standard. Typically $\delta^{13}C$ values are reported in parts per thousand (‰), i.e. Equation 1.8.

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{VPDB}} - \mathbf{1}\right] \times 1000 \qquad (1.8)$$

Plants discriminate against the heavier ^{13}C during photosynthesis in ways that reflect plant metabolism and the environment. The extent of the depletion reflects the photosynthetic pathway and specific ranges of $\delta^{13}C$. Plants with the Calvin Cycle or C_3 pathway have a lower $\delta^{13}C$ value, averaging about -27‰, while plants from the Hatch-Slack, C_4 pathway have a mean $\delta^{13}C$ value -12‰. By using a C_3 derived soil in a C_4 plant system or vice-versa, the C entering the rhizosphere through live roots will have a different $\delta^{13}C$ value from the $\delta^{13}C$ value of SOM. This allows for the separation of the total CO_2 efflux from the system into its SOM and plant derived components. The relative contribution of SOM-derived C to R_s is determined by a mass balance equation:

$$R_{SOM} = \frac{\boldsymbol{\delta}^{13}C - \boldsymbol{\delta}^{13}C_{SOM}}{\boldsymbol{\delta}^{13}C - \boldsymbol{\delta}^{13}C_{plant}} \qquad (1.9)$$

where R_{SOM} is the SOM-derived proportion of R_s. The plant-derived component (R_{plant}) is determined by the equation

$$R_{plant} = 1 - R_{SOM} \qquad (1.10)$$

This natural method eliminates some of the difficulties of earlier methods and provides a fairly simplistic way to study rhizosphere impacts on SOM

decomposition. However, where C_3 derived soil is used with C_4 plants and vice-versa, unnatural conditions may arise that could potentially compromise the rhizosphere influenced SOM decomposition that would have occurred naturally. For example the original microbial community in a C_4 derived soil with C_4 plant could be different from a C_3 derived soil with C_4 plants thus providing differences in the magnitude and direction of priming effects.

1.2.6.3 Pulse and continuous δ ^{14}C labelling

Plant labelling approaches are widely used for disaggregating R_s and to study the dynamics of soil C turnover belowground (Schmitt et al., 2013; Gamnitzer et al., 2009; Wu et al., 2013). This technique involves exposure of plants to a CO_2 gas of constant ratio of ^{13}C or ^{14}C and air. The labelling process can be introduced continuously or as a pulse. Continuous labelling required lengthy periods, usually starting from shoot initiation to the end of the experiment. This provides enough time for all C pools contributing to root exudation to be uniformly labelled. The proportion of plant and SOM-derived components in the flux is determined from the relative dilution of the labelled CO_2 from the plants by unlabelled CO_2 from SOM. In contrast, pulse labelling is characterised by labelling of shoots for short time periods. Repeated pulse labelling can also be done to evaluate effects of multiple substrate flows from plant to the soil system. The relative proportion of root-derived C in R_s following pulse labelling is calculated based on the percentage of assimilated ^{14}C or ^{13}C evolved as root-derived CO_2. As the total ^{14}C or ^{13}C absorbed by shoots is known the calculation is simple if the efflux can be sampled correctly to determine its labelled C composition. The technique involves several assumptions. Meharg (1994) reviewed the two methods and compared their various advantages and disadvantages. Other labelling approaches that have evolved include the labelling of soil (Dalenberg and Jager, 1989) and plant material (Subke et al., 2004a; Conde et al., 2005) and substrates (Nguyen and Guckert, 2001) for the conduct of priming experiments.

1.3 Summary

A considerable body of work on rhizosphere priming effects (RPEs) have been generated in the last 20 years. Current research trends highlight soil microbial nutrient demand, preferential substrate utilization and microbial activation induced by fresh labile substrates additions as leading explanations of mechanisms involved. There is not a standard approach to priming studies and many contradictory results are generated. Substrate quality, identity and diversity have been shown to influence changes in soil biological, physical and chemical properties that can alter RPEs. Simple polysaccharide substrates like glucose and sucrose are commonly used for priming studies. Multiple compound substrates that better resemble the diversity of root rhizodeposits could potentially produce more realistic results. In addition, systems that allow for high resolution measurements of soil respiration and their dependency on key drivers also hold tremendous value to contribute to a better understanding of the mechanisms involved with RPEs. This study uses a combination of laboratory incubation, glasshouse and field experiments to investigate RPEs.

1.4 Overall aim of the research

The overall aim of the study is to contribute to a better understanding of the processes regulating soil carbon balances, specifically the mechanisms of soil-plant interactions that release stored C from soils through rhizosphere priming effects.

1.5 Specific research objectives

The specific objectives of the study are:

1. To investigate the influence of labile substrate availability on SOM turnover in two contrasting unplanted soils, with rates of substrate addition simulating rhizodeposition.

2. To investigate the influence of labile substrate released from plant roots on SOM turnover (rhizosphere priming effects) in the same two contrasting soils planted with grass.

3. To assess an automated field system for measuring the soil temperature, moisture and photosynthesis sensitivities of SOM turnover in the same two soils over diurnal to seasonal time scales.

Chapter 2: General Methodologies

This chapter describes details of generic methods used at various stages of this study, and in some cases the process of their development. Specific methods are detailed in the chapters where they were used.

2.1 Maize root extract preparation

Planting

Maize seeds, of the Marai F1 hybrid early variety (Unwins GroSure, Huntingdon, Cambridge, UK) were planted in media of compost and horticultural sand mixed in a 3 to 1 (compost to sand) ratio. Seeds were planted in 1 litre pots at four seeds per pot. The plants were watered daily and fertilized weekly with an all-purpose soluble fertilizer containing supplemental iron. After 8 weeks there was is sufficient root growth to fully occupy the media and plants were harvested shortly thereafter.

Harvesting and cleaning

The pots were kept very moist just before harvest; since the drying media generated increased osmotic pressure from roots that made compost particles cling more tightly to roots and this made root-cleaning more difficult. The shoots were cut off at the base leaving an intact root ball which were soaked in water to remove adhering debris. Using 2 mm and 1 mm seives to contain the roots, both fine and larger roots were carefully cleaned with water. Roots were kept covered with moist tissue until extraction.

Root extract preparation

Cleaned roots were packed into the receiving column of a BNIB stainless steel compact mega power juicer 700W-2011. Compacted roots in the column were fed through the juicer. The machine extracted the liquid contents of the roots, which were collected. Root fragments remaining on the mesh/screen filter were then passed through the juicer for a second time for further extraction.

Filtration

The extract was centrifuged at 8000 rpm for 10 minutes to separate solid material from the liquid. The liquid was then filtered through a glass microfiber (1.2 µm) filter under suction. Filtered samples were immediately stored in 250 ml plastic bottles and frozen.

Freeze drying

Frozen samples were then freeze dried using a Christ Alpha 1-2LD drier, and stored in a desiccator until ready for use. In preparation for use, freeze-dried material was resuspended in ultra pure water. Several tests were conducted to determine the total organic and inorganic C content of reconstituted root extract using a total carbon (TOC) analyser, (Shimadzu TOC-V analyser, Tokyo, Japan). Applications in incubation treatments were based on the determined values of total C concentration.

2.2 Determination of $\delta^{13}C$ in air samples

Isotope ratio mass spectrometer (IRMS)

The $\delta^{13}C$ analysis used in the experiments relied solely on natural abundance differences of isotope ratios in the plants and soils used without any labelling for enrichment or depletion. I used a gas chromatograph coupled with an isotope ratio mass spectrometer (GC-IRMS) Sercon 20-22 (Sercon Limited, Crewe, UK) to analyse for differences in the $\delta^{13}C$ of collected air samples. Each sample was referenced to a pulse of standard gas, N5.0 grade 99.99999% CO_2 (BOC Limited, Surrey, UK) with a known isotopic value. Before use the $\delta^{13}C$ value of the standard was determined at Sercon Limited relative to the International Vienna PDB (Pee Dee Belemnite) standard. In brief, the IRMS operates by ionizing the gas sample, accelerating it over a potential in the kilovolt range and separating the resulting stream of ions according to their mass-to-charge ratio (m/z). Beams of lighter ions bend at a smaller radius than beams with heavier ions. The current of each ion beam is then measured using a multiplier detector (Figure 2-1). I analysed for isotopes of C and O in the air samples.

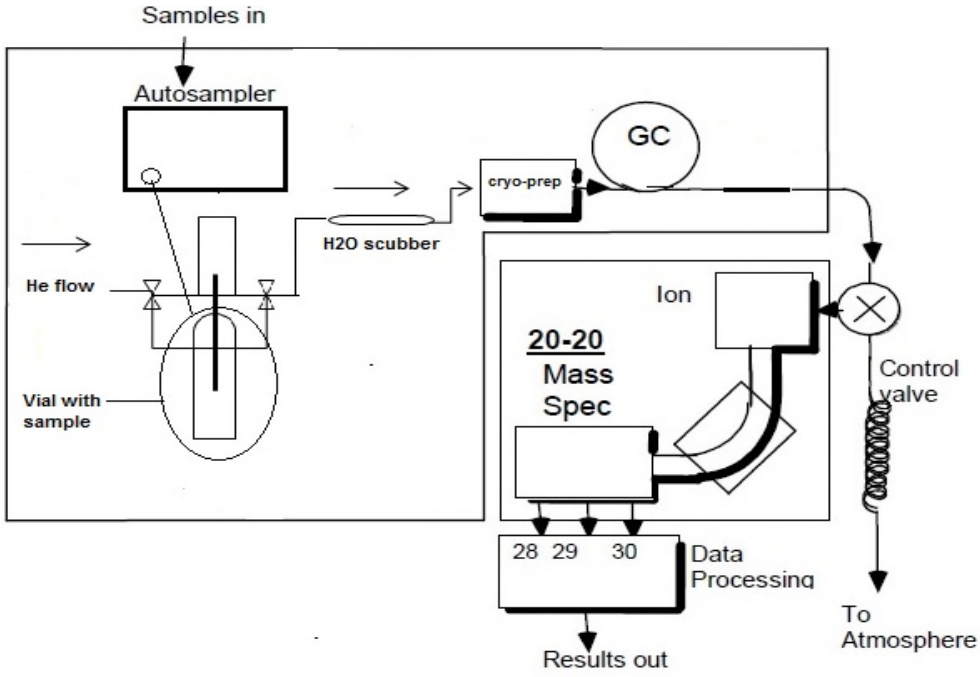

Figure 2-1 Schematic of IRMS analysis, arrows indicate sample flow with helium carrier gas

Under helium flow and through a double-holed needle, air samples from each vial were inserted in the aluminium pipeline, through a water trap, the liquid-N cryo-trap, the GC (Poropack QS column) and then to the IRMS (Table 2-1). A minimum of four sub-samples were used to arrive at a mean isotopic value per sample. Prior tests and quality control checks conducted showed that GC-IRMS had a precision of 0.20 ‰ ± standard deviation of the mean for measuring $\delta^{13}C$ of CO_2 in 5 ml samples.

Table 2-1 Results of air sample (5 ml) analysis to determine GC-IRMS accuracy

Sample	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
1	-13.51	-7.28
2	-13.3	-7.41
3	-12.98	-7.15
4	-13.26	-7.58
5	-13.4	-7.74
Mean	**-13.29**	**-7.43**
SD	**0.20**	**0.23**

Sample preparation

Only clean septum-capped 12 ml exetainers (Labco Ltd) were used. The caps were first fitted with new septa and heat-treated at 105^{o}C overnight. This removed any volatile compounds that might contaminate the samples. Pre-experiment trials showed that heat-treatment significantly improved measurement accuracy. Vials were the evacuated using a suction-pump, P6Z-101 Chemvac (Ilmvac Vogelherd, Germany) and purged with helium at 60 L min^{-1} using a Gilson autosampler attached to the GC-IRMS assembly. To maintain sample integrity they were analysed within 24 hours.

Control of the sampling process

The sampling system was controlled by the Callisto CF_IRMS software for stable isotope ratio analysis on SerCon mass spectrometers and prep systems (SerCon 2009). Callisto was used to control the available functions and interface of the auto sampler, the prep system and the IRMS. I used the system to perform periodic quality control checks and to manipulate data processing and collection to determine the δ^{13}C values of air samples. Sample processing was closely monitored as several problems, including prolonged freezing of cryo-traps, were encountered and urgently handled (Figure 2-2).

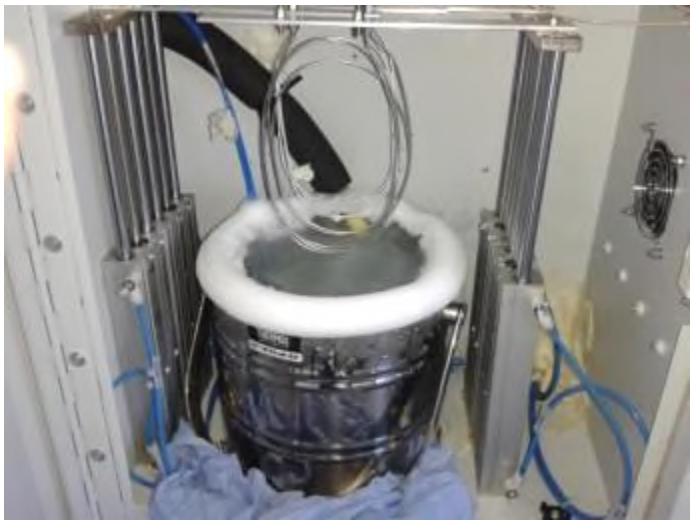

Figure 2-2 Dewar with frost and frozen cryo-traps during δ^{13}C analysis

Prolonged cryo trap freezing delayed the sample from getting to the GC. Since all related processing events, including reference gas pulses, are time based, this can produce skewed results and the loss of valuable samples.

Callisto is based on the Microsoft Windows operating system. Control and sequencing of switches and valves is made through Setup Groups, accessed via an icon in the master window toolbar. A setup group defines the experimental timings, gas species, integration windows, outputs, references and sequences for a particular analysis. It includes a sequence table, which determines the valves/switches to be operated and when. The table has three columns, Time: to specify when an event occurs; Events: to specify the operations to be performed; and L/R: to specify whether the event is associated with the prep system (Local) or with the mass spec (Remote).

2.3 Determination of microbial biomass carbon by fumigation extraction

The chloroform fumigation method was used to determine the microbial biomass C of the two soils in response to various experimental treatments. The results are reported in Chapters 3 and 4. The method is based on the British Standard BS 7755: Section 4.4.2:1997 Determination of soil microbial mass – fumigation-extraction method which is identical to ISO 14240-2:1997 first reported by Vance et al. (1987). The basic principle of the method involves the use of chloroform fumes to kill and lyse microbe cells to release their carbon. The released C can be recovered by extraction with a potassium sulphate solution. The extraction process does not affect inorganic soil C. Soil microbial biomass is estimated from the increase in extracted C flushed from fumigated soil samples compared to flushed C from non-fumigated soils.

Fumigation

The soils were first homogenized by sieving through a 2 mm sieve. Two aliquots of each sample (12.5g dry weight equivalent) were weighed into 200 ml glass jars, one for fumigated and the other for the non-fumigated control. Fumigation was conducted under a fume hood in implosion resistant desiccators. The desiccators were lined with moist paper jars with soil for fumigation placed in

the desiccator with a 100 ml beaker containing 25 ml ethanol-free chloroform and a few anti-bumping granules. A beaker containing 25 ml soda lime was also placed at the bottom. The desiccators were then sealed using a gasket seal and evacuated with a vacuum pump until the chloroform boiled for two minutes. The valve on the desiccator was then locked and the vacuum pump switched off. The desiccator was left under the fume hood for 24 hours. The tissue, beaker with chloroform and bumping granules were removed and the desiccator evacuated six times using a vacuum pump, each time for two-minutes

Extraction

The carbon from each sample was extracted by adding 50 ml (0.5mol/l) potassium sulphate solution. The jars were capped and loaded on a side-to-side shaker, set at 300 min^{-1} for 30 minutes. The suspensions where then filtered through Whatman No 42 filter paper into separate sample bottles and stored at -15^{o}C until ready for analysis.

Determination of carbon in extracts

A Burkard Scientific SFA-2000 Segmented Flow Analyser (Uxbridge, Middlesex) was used to analyse all the samples. The process involved a series of steps beginning with sparging of the extract with CO_2 –free air to remove inorganic C as CO_2. The sample was then mixed with acidified potassium persulphate and irradiated with UV light to oxidize the organic C in the extract to CO_2. The CO_2 concentration was determined by infra-red spectrometry.

Segmented flow analysis was carried out according to the standard operating procedure for the instrument. If the concentration reached higher than that of the concentration curve then the sample was diluted with 0.5 M K_2SO_4 solution.

Calculation

$$\mathrm{C}\ (\mu g/g) = (\text{sample - blank}) \times \left[\frac{\text{extract volume}}{\text{dry weight soil mass}}\right] \qquad (.2.1)$$

$$\text{Microbial C}\ (\mu g/g) = \text{fumigated organic C - non-fumigated organic C}$$

Microbial C was converted to biomass using the conversion factor of 0.45 (Jenkinson et al., 2004).

2.4 Phospholipid fatty acid (PLFA) profiling

Phospholipid fatty acids (PLFAs) were measured as described by Bligh and Dyer (1959) and as modified by White et al. (1979) and used by Bardgett et al., (1996). The PLFAs were measured on soil samples from each of the 24 lysimeters at the Wolfson Field Laboratory (WFL) described in Chapter 5. Chapter 5:Lipids were extracted from 5 g of fresh soil using a mix of chloroform, methanol and citrate buffer (1:2:0.8 by volume). The supernatant from this was split into two phases by adding chloroform and citrate buffer. The lower chloroform phase containing the lipids was recovered and evaporated under a stream of nitrogen (N_2) gas. These lipids were re-suspended in chloroform, and then separated into neutral lipids, glycolipids and phospholipids (eluted individually, with chloroform, acetone and methanol) by fractionation on silicic acid tubes, Strata® NH_2 (55 µm, 7 nm), 500 mg / 12 mL, Giga Tubes, supplied by Phenomenex, Cheshire, UK. The phospholipids were retained and evaporated under a stream of N_2 gas, and mild alkaline methanolysis was then performed to create methyl esters. These samples were also evaporated under N_2 gas and stored at -20^{o}C until analysis by gas chromatography (GC).

After GC analysis, peaks were identified by calculating retention times relative to an added internal standard (C19) and comparing this with peaks from a bacterial methyl ester standard (Supelco Bacterial Acid Methyl Esters CP Mix 47080-U). The results were normalised by expressing each peak as a percentage of the sum of all peaks. This gave results on percent mol (% mol) basis and were characterized by standard nomenclature (Tunlid et al., 1989). PLFAs used to represent bacteria were: cyclic fatty acids (cy-17:0, cy-19:0), branched fatty acids (i-15:0, a-15:0, i-16:0, i-17:0 and 15:0). A relative measure of the fungal:bacterial ratio was calculated by dividing fungal PLFA (18:2ω9,12) by bacterial PLFA.

Chapter 3: The effects of simulated plant exudates on soil carbon turnover in two unplanted and contrasting soils

3.1 Introduction

Additions of highly available C substrates to soils, like those from plant root exudates are sometimes found to stimulate the rapid turnover of soil organic matter, the so-called soil priming effect (PE) (Section 1.2.1.1). Decomposition of "old" sequestered C can be accelerated where there are favourable soil moisture and temperature conditions coupled with plant cover which provides a continuous supply of root exudates (Kuzyakov, 2002). There is still much uncertainty about the extent of such phenomena in different plant-soil systems and about the mechanisms driving them. Priming effects are expected to differ between soil types with different quantities and qualities of organic matter, and different physical, chemical and biological attributes. Differences between plant species, including root growth, turnover and exudation of labile substrates, and their interactions with soil microbes and the soil environment, are likely to also affect priming effects. Experiments on priming are often made excluding plant roots to simplify the system and isolate processes. Simple sugars like glucose and sucrose are commonly used as surrogates for root exudates, without the other compounds and nutrients that may be important for PE. Applications are usually made in single pulses, which are not reflective of field conditions because root exudates are thought to be continuously involved in mediating plant-soil interactions (Walker et al., 2003). Thus, such experiments may give a misleading picture of PE phenomena in true plant-soil systems.

In this chapter I report on an attempt to simulate the effects of root exudation and turnover by applying an extract of root material to a soil incubation system to study soil priming effects. I made continuous daily additions at C rates commensurate with 15 and 50% of soil microbial biomass and comparable to weekly net primary productivity of a grazed pasture system (UK). I used a prepared extract from roots of the C_4 plant, maize (Section 2.1) in C_3 soil and compared the extract to standard C_4 sucrose in two contrasting C_3 soils in order

to separate the turnover of the root extract or sucrose and soil organic matter based on $\delta^{13}C$ differences. To the best of my knowledge this type of root preparation has not been used in previous soil priming studies (Section 2.1).

3.1.1 Research objective

The primary objective of this experiment was to investigate soil C turnover responses and priming effects in two contrasting soil types in response to two substrates, maize extract and cane sugar, added repeatedly. I tested the following hypotheses:

1. The two substrates will produce different soil C turnover responses
2. The two soils will have different C turnover responses to the substrates
3. Priming effects will occur in both soils, sustained for the duration of the experiment

3.2 Materials and methods

3.2.1 Soils

Two soils were used in this study, collected from (0-5 cm) at two locations. Monoliths of these soils are also used in lysimeters at the Wolfson Field Laboratory, discussed in Chapter 5. They are:

(1). A poorly drained, seasonally waterlogged loamy clay soil from Temple Balsall, Warwickshire, England (276559 N, 420189E), sampled in March 2012, hereafter referred to as TB for Temple Balsall. It is classified (UK) as a typical stagnoley soil, Brockhurst series (Beard, 2010). The primary vegetation has been permanent pasture. Relevant properties are given in Table 3-1. Compared with the second soil, this soil has a higher pH, a smaller organic carbon content but with a greater N content and total phosphorus.

(2). A well-drained coarse loamy soil from Shuttleworth College, Bedfordshire, England (243867.30N 514421.09E), sampled in May 2012, hereafter referred to as SH for Shuttleworth. It is classified (UK) as a typical brown sand, Cottenham series (Beard, 2010). The sampled layer comprises large amounts of partly decomposed woody material originating from bracken growth. The area is a

remnant bracken heathland on the field edge. Relevant properties are given in Table 3-1 and the processing of collected is soil samples outlined in Figure 3-1.

Table 3-1 Selected properties of clay loam (TB) and sandy loam before pH adjustments (SH) (6 mm sieved –air dried except where indicated)

Soil	TB	SH
pH (KCl)	5.5	3.8
Total P (mg kg^{-1})*	609.6	801.6
N (%)	0.49 ± 0.01	0.42 ± 0.003
C (%)	4.62 ± 0.06	6.48 ± 0.02
C/N Ratio	9.5	15.3
Microbial Biomass C (mg kg^{-1})	704 ± 15	407 ± 30
Sand (%)	60	82
Silt (%)	18	8
Clay (%)	22	10

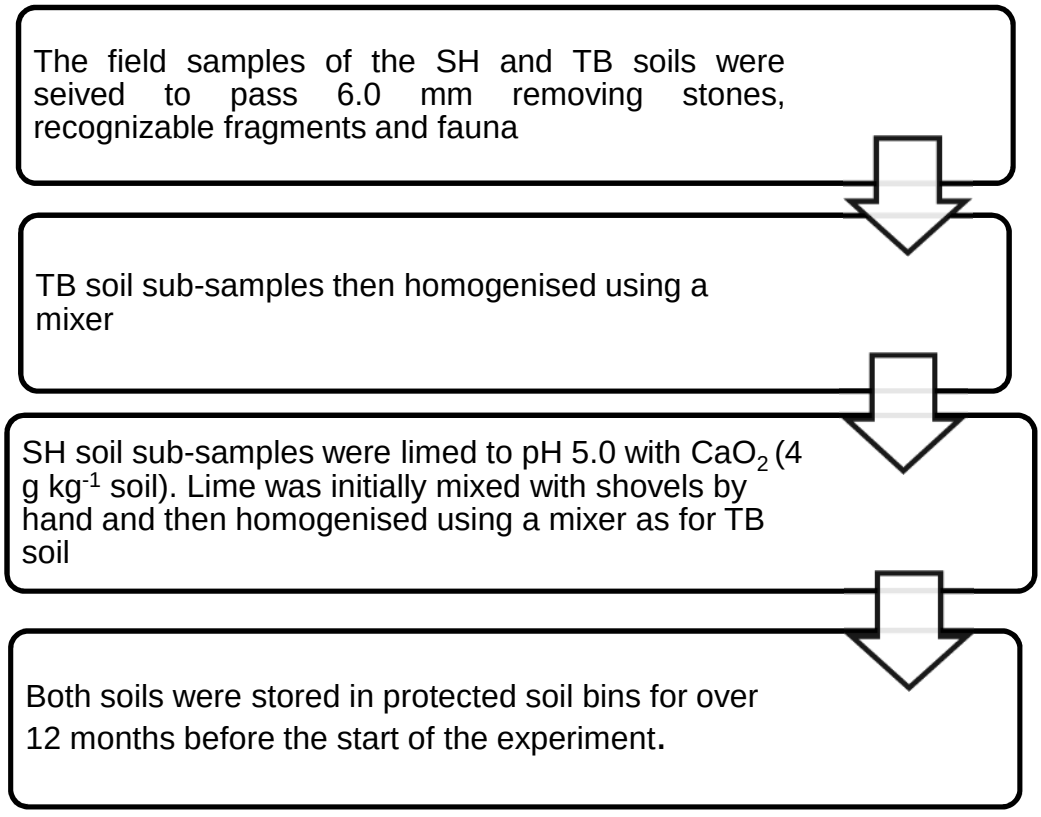

Figure 3-1 Flow diagram of soil processing operations prior to the start of the experiment.

Just before starting the experiment, samples of both soils were sieved to pass 2 mm, removing the remaining debris and larger soil fauna. The gravimetric moisture contents of the 2 mm sieved TB and SH soil were 20% and 27%, respectively.

For the experiment soil moisture was adjusted to 65% of field capacity, and samples were packed to an initial dry bulk density of 0.7 and 0.8 g cm^{-3}, respectively for TB and SH, in microcosms as shown in Figure 3-2 (Paterson et al., 2007). These comprised semi-circular sections of PVC pipe (120 mm length, 46 mm internal diameter) mounted on stands for stability (Figure 3-2). The volume of soil in each microcosm was 100 cm^3.

Figure 3-2 Incubation microcosm packed with soil

3.2.2 Experimental design

For each soil type, 12 microcosms were prepared to allow six treatments (2 soils x 2 substrates and a water control) with four replicate blocks, a total of (6 x 4) = 24 microcosms. The substrates used were a maize root extract and sugarcane (i.e. C_4) sucrose (Billington's Fairtrade light brown sugar, Tesco) applied at 0.35 and 0.2 mg C g^{-1} dry soil for the TB and SH soils, respectively. This represented 15 and 50% of the microbial biomass C in the TB and SH soils, respectively, and 140 and 80 % of the weekly estimated net primary productivity (NPP) (Equations 3.1 to 3.4).

The microcosms were pre-incubated in the dark at 20^{o}C and 80% humidity for 16 days before treatments were applied. During the pre-incubation phase, soil moisture was maintained by watering with a syringe to the original weight. During the incubation phase, treatments were applied daily in a 1 ml solution, predetermined as the minimum moisture loss from the microcosm in 1 day. Moisture requirement above 1 ml was applied as deionised water mixed with the respective treatment solution before application by syringe. Soil respiration and δ^{13}C measurements were made periodically as follows.

3.2.2.1 Calculation of net primary productivity (NPP)

The calculations were based on analysis of temperate grassland responses to climate change using the Pasture Hurley Model (Thornley and Cannell, 1997).

Under standard conditions in grazed pasture system in a Southern lowland (UK) the annual net primary productivity (NPP) was estimated as 0.57 kg C m^{-2}.

Thus

NPP = 0.57 kg C m^{-2} year^{-1} (3.1)

Assumptions: soil depth = 10 cm; bulk density =1.0 g cm^{-3}; 70% of annual NPP is deposited during 4 months of the year.

Then NPP = 0.57 kg C in (10,000 cm^{2} x 10 cm) (3.2)

and NPP = 570 g C in 100,000 cm^{3} x 1.0 g cm^{-3} soil

Therefore NPP = 0.0057 g C in 1 g soil or 5.7 mg C g^{-1} soil per year.

(3.3)

Weekly NPP loading during high production period =

$$\frac{5.7 \text{ mg C x } 0.7}{16 \text{ weeks}} = 0.25 \text{ mg C} \quad (3.4)$$

The substrates applied at 0.2 and 0.35 mg g^{-1} soil were 80 and 140% of the weekly NPP in the SH and TB soil, respectively.

3.2.3 Soil respiration and isotope measurements

The Kilner jars containing the microcosms were fitted with lids containing a 1 mm plastic mesh (Plastok Ltd Birkenhead, Merseyside, UK) that allowed gaseous exchange (Figure 3-3 A). Prior to respiration measurements the mesh lids were replaced with gastight lids containing inlet and outlet ports with Luer-lock valves (Figure 3-3 B).

Figure 3-3 (A) Lid with plastic mesh for incubation and storage (B) Airtight lid with inlet and outlet for respiration measurements and air sampling

The inlet and outlet valves were connected to the system shown in Figure 3-4, with which to scrub CO_2 from the system microcosm headspace and follow the subsequent accumulation of CO_2 released from the soil and sample for $\delta^{13}C$. To scrub CO_2, the system was flushed with CO_2-free air at a flow rate of approximately 1 L min^{-1}. The headspace concentration was measured with an infrared gas analyser (IRGA), (LICOR-820, Lincoln NE, USA). Purging continued until the CO_2 concentration at the outlet reached a minimum of <10 µmol mol^{-1} CO_2 and subsequently incubated for 2.5 hours. At the end of this period, a 10 ml gas syringe (SGE Europe Ltd) was attached (with Luer lock connections) to the outlet valve on the Kilner jar lid to extract three 5 ml samples from the headspace. Each sample was injected into separate evacuated and helium-purged 12 ml exetainers (Labco Ltd) capped with

gastight septa. Samples were analysed for $\delta^{13}C$ within 24 hours using a Sercon 20-22 IRMS with auto sampler as described in Chapter 2. After sampling the headspace, the jars were immediately re-connected to the IRGA and headspace circulation contained at approximately 1 L min^{-1} for 2 minutes to determine the soil respiration from the microcosm.

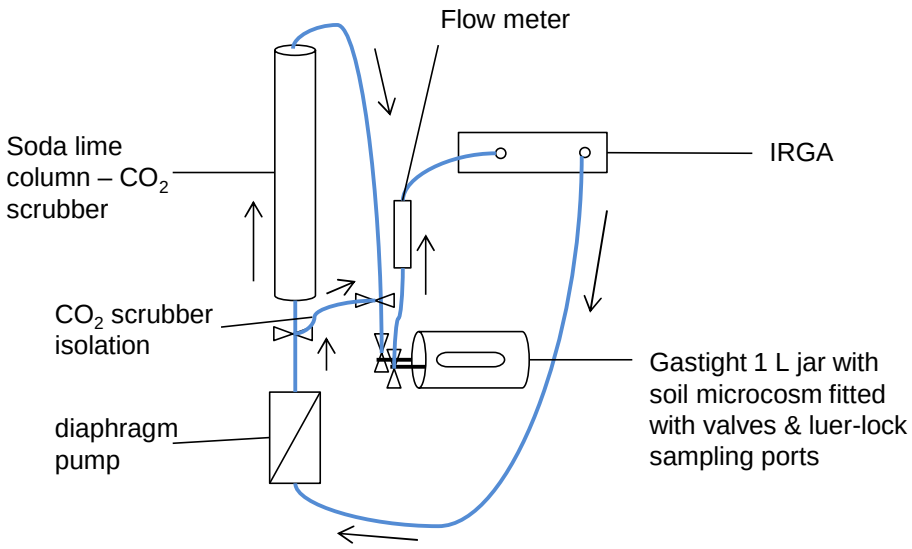

Figure 3-4 Diagram of assembly for scrubbing CO_2 from jars prior to incubation, measuring soil efflux, and air sampling for $\delta^{13}C$. Bev-A-line (2 mm I.D) with airtight connectors are shown in blue

3.2.3.1 Soil and substrate analysis

The maize extract was analysed for total C and N content using and Elementar Vario El elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The sugar composition of the maize extract was determined using an Agilent 1260 Infinity high performance liquid chromatograph with evaporative light scattering detector (HPLC-ELSD) (Agilent Technologies, California, USA).

3.2.3.2 Calculation of soil respiration rate

The soil respiration rate R_S (µg C g^{-1} soil h^{-1}), was calculated from the measured rate of change in CO_2 concentration in the microcosm headspace, d*C*/d*t* (µg C cm^{-3} headspace h^{-1}), using the equation

$$R_S = dC/dt \times V/ M \quad (3.5)$$

Where V is the volume of the headspace (cm^3) and M the mass of the soil in the microcosm (g).

The value of d*C*/d*t* over the measurement period is found from

$$dC/dt = \Delta C / \Delta t \quad (3.6)$$

where ΔC is the change in CO_2 concentration (µg C cm^{-3} headspace) over the measurement interval Δ*t* (2.5 h).

The value of C is related to the measured CO_2 concentration (44.0 g mol^{-1} air), by

$$C = \Delta C^* \times M_m / V_m \quad (3.7)$$

Where M_m is the molar mass of CO_2 (44.0 g mol^{-1}) and V_m is the molar volume of air (24.4 x 103 cm^3 mol^{-1} at 20^{o}C and 1 atm)

Combining Equations 3.5 to 3.7 gives

$$R_S = \frac{\Delta C^* M_m V}{\Delta t V_m M} \quad (3.8)$$

The value of V is found from

$$V = V_{jar} - V_{microcosm} \quad (3.9)$$

where V_{jar} and $V_{microcosm}$ are the volumes (cm^3) of the Kilner jar and microcosm, respectively.

3.2.4 Partitioning of the soil respiration between SOM and added substrate

I used the measured isotope data to partition the total soil respiration (R_S) between soil organic matter (SOM) and added substrate (maize root extract or sucrose) sources as follows.

Carbon isotope data are conventionally reported as $\delta^{13}C$ values: the $^{13}C/^{12}C$ ratio of the sample relative to the international Vienna PDB (Pee Dee Belemnite) standard. Typically $\delta^{13}C$ values are reported in parts per thousand (‰), i.e.

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{VPDB}} - \mathbf{1}\right] \times 1000 \qquad (3.10)$$

Hence, the more negative the $\delta^{13}C$ value, the more depleted the sample is in ^{13}C relative to the standard.

I used the measured $\delta^{13}C$ values to determine the relative proportions R_S derived from the applied substrates ($R_{substrate}$) and from the native soil organic matter (R_{SOM}) with the two end-member mixing model:

$$R_{SOM} = \frac{\boldsymbol{\delta}^{13}C - \boldsymbol{\delta}^{13}C_{SOM}}{\boldsymbol{\delta}^{13}C - \boldsymbol{\delta}^{13}C_{substrate}} \qquad (3.11)$$

and

$$R_{substrate} = 1\text{-}R_{SOM} \qquad (3.12)$$

where $\delta^{13}C_{SOM}$ and $\delta^{13}C_{substrate}$ are the values for SOM and the added substrates, respectively.

The $\delta^{13}C_{SOM}$ values were determined from the measured $\delta^{13}C$ of the control soils, unamended with substrate. The $\delta^{13}C_{substrate}$ values were determined on solid samples of the substrates (freeze dried maize root extract and granular cane sugar) burnt in a combustion coupled to IRMS at the James Hutton Institute, Aberdeen, Scotland (c/o Dr. Eric Paterson). The values were -12.20‰ and -11.52‰ (± 0.1 ‰), for maize and cane sugar, respectively.

A correction to the measured $\delta^{13}C$ values was necessary because of isotopic fractionation in the transport of respired CO_2 through the soil to the microcosm headspace by diffusion. This arises because the diffusion of the heavier $^{13}CO_2$ is slightly slower than that of $^{12}CO_2$. The ratio of the diffusion coefficients is

$$D(^{12}CO_2)/D(^{13}CO_2) = 1.0044 \qquad (3.13)$$

i.e. a difference of 4.4‰ (Cerling et al., 1991). This means that the CO_2 at the point of production within the soil pores is enriched in ^{13}C relative to CO_2 leaving the soil as R_S by at least 4.4 ‰, i.e. the $\delta^{13}C$ of soil air is less negative by at least 4.4‰.

In measurement systems in which the initial steady-state concentration gradient of CO_2 through the soil is maintained, all the CO_2 produced in soil respiration is transferred to the headspace and the measured $\delta^{13}C$ is that of the respired CO_2 with no diffusional fractionation. However, in the system used here, which is designed to equilibrate the headspace CO_2 with the CO_2 in the soil air, the measured headspace $\delta^{13}C$ is offset from the true $\delta^{13}C$ of the respired CO_2 by some 4.4 ‰.

A realistic value for the fractionation is therefore 4.4 ‰ (Cerling et al., 1991) and accordingly I corrected the measured $\delta^{13}C$ and $\delta^{13}C_{SOM}$ values by subtracting 4.4‰. Note there is no diffusional fractionation in the measurements of $\delta^{13}C_{substrate}$ obtained by combustion of the solid substrates. Other potential sources of error in chamber measurements of the $\delta^{13}C$ of respired CO_2 were discussed by others (Nickerson and Risk, 2009; Ohlsson, 2010; Midwood and Millard, 2011).

Having partitioned R_S in this way, I calculated priming effects (PE), defined as the change in the decomposition of native SOM as a result of substrate additions, as the difference between the basal respiration in controls and the SOM-derived respiration in substrate-amended soils (equation 3.8).

$$PE = (R_{SOM} \text{ in substrate amended soil}) - (R_{SOM} \text{ in unamended soil}) \qquad (3.14)$$

3.2.4.1 Determination of δ^{13}C and required adjustments

Natural abundance differences of δ^{13}C in C_3 soils and C_4 substrates were used to separate soil respiration into its substrate and soil-derived components. This formed the basis for determining PEs, their patterns and direction. A purged static chamber (PSC) design, depicted in Figure 3-4, was used for flux measurements and chamber air samples were collected for δ^{13}C analysis. This chamber design is used widely. However there are concerns about their accuracy and repeatability with respect to δ^{13}C and F_{CO2} measurements. These were studied by Nickerson and Risk (2009). They found that PSCs produce SOM δ^{13}C values that were more enriched than actual within a range of approximately 0 to 15 ‰, over all diffusivities and production rates evaluated. It was notable that the largest deviations occurred when soil respiration was low and soil diffusivity was high. In comparison to more common chamber systems (Subke et al., 2004b; Ekblad and Högberg, 2000) the surface area of the exposed soil (120 mm x 46 mm) of microcosms was relatively wide compared to soil depth (23 mm). This may have allowed for higher diffusivity of CO_2 through soil. When coupled with low CO_2 production within the soils, as evidence by low flux measurements in control treatments (data not shown), this could have accounted for the higher than expected δ^{13}C values. The SOM end member δ^{13}C values determined from the control treatments were adjusted by the minimum value, -4.4‰, recommended by Cerling et al. (1991). Similarly the δ^{13}C for the flux of treated microcosms were also adjusted by -4.4‰.

3.2.5 Statistics and data analyses

The software package Statistica version 12 (Statsoft Inc. Dell Inc. Aliso Viejo, USA) was used for statistical analyses. Repeated measures ANOVA was used to study the variation in R_S and its δ^{13}C over time, between the various treatments. One-way ANOVAs were used for determining treatment effects on soil microbial biomass. The differences between means were analysed using post hoc Fisher least significant difference (LSD).

3.3 Results

3.3.1.1 Soil equilibrium and basal respiration

During the 17 day stabilisation period, total respiration in both soils declined after disturbance for preparation of microcosms and acclimatisation to incubation conditions (Figure 3-5). Soil respiration rates in the clay loam (TB) was initially greater than in the sandy loam soil (SH) but decreased significantly near the end of the stabilisation phase. By Day 18 the mean respiration rates in each soil reflected a state of stabilised decline (Figure 3-5), indicating that the length of the stabilisation phase before applying treatments was adequate. The stable basal respiration in TB was less than that in SH (Figure 3-5).

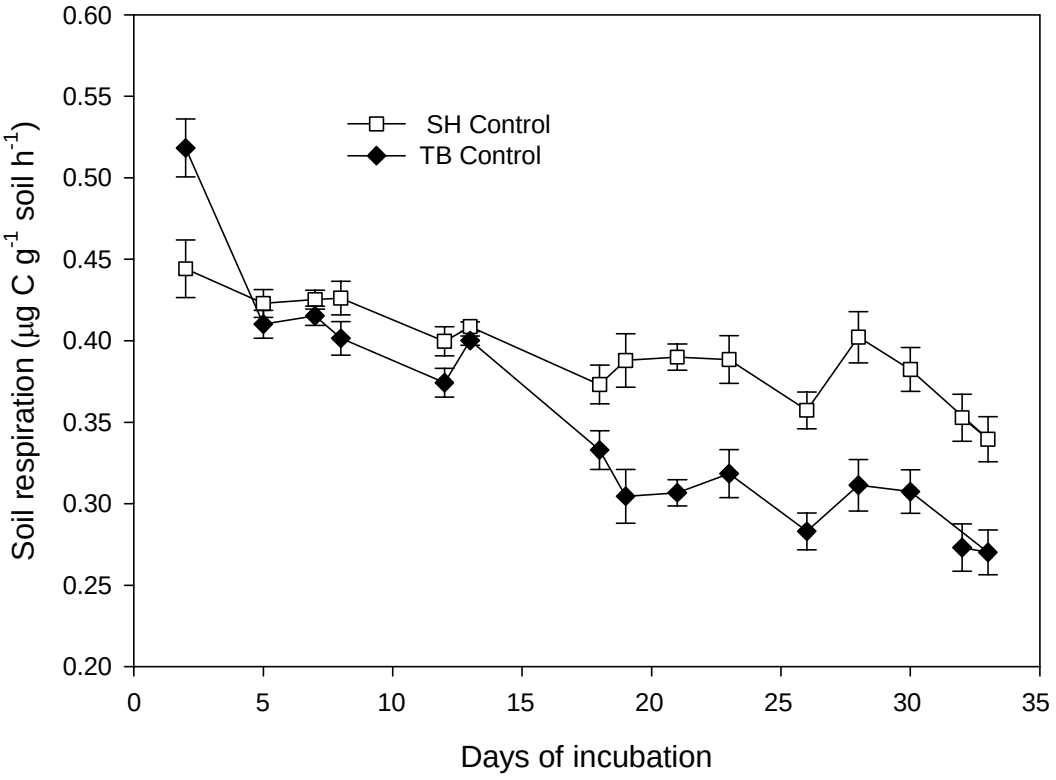

Figure 3-5 Measured soil respiration rates (R_S) in control treatments in TB and SH soils receiving only water over the duration of the experiment. Days 0-17 comprise the stabilisation phase after disturbance. Points show means (n=4), bars show standard errors.

3.3.1.2 Effects of added substrates on soil respiration (R_S)

In both soil types the added substrates (from maize roots and sugarcane) produced significant increases in total soil respiration (R_S) compared to basal soil respiration measured in controls treated only with water (Figure 3-6). Generally, added sucrose produced greater R_S than maize, markedly so in the clay loam, TB soil (the trend was generally insignificant ($P \leq 0.198$) in the sandy loam, SH soil). From the second day after treatment (DAT) the R_S from sucrose-treated TB soil was significantly greater than that from the maize treated TB soil and increased with time. At the end of the experiment 19 DAT, R_S in sucrose-treated TB soil was 4.6 ± 0.12 µg C g^{-1} dry soil h^{-1} compared to 1.90 ± 0.12 µg C g^{-1} soil h^{-1} in the maize-treated TB soil, a difference of over 240%.

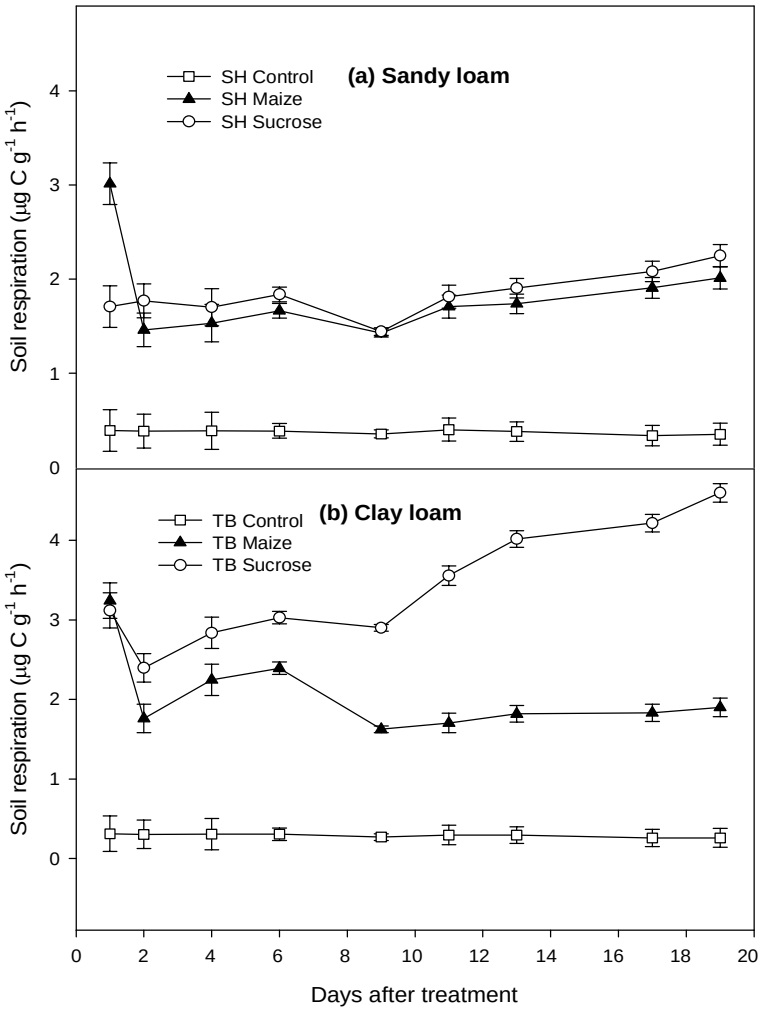

Figure 3-6 Effect of maize root extract and sucrose on soil CO_2 efflux in (a) sandy loam soil [SH] and (b) Clay loam soil [TB]. Points show means (n=4), bars show standard errors.

3.3.1.3 δ^{13}C of soil efflux

The δ^{13}C values of R_S in control treatments receiving only water were stable over the duration of the experiment and not significantly different between the two soils (Figure 3-7). However, the values were large (or less enriched) compared with typical temperate soils with a history of C_3 vegetation (O'Leary, 1981; Buchmann and Ehleringer, 1998; Flanagan and Ehleringer, 1998; Griffis et al., 2005).

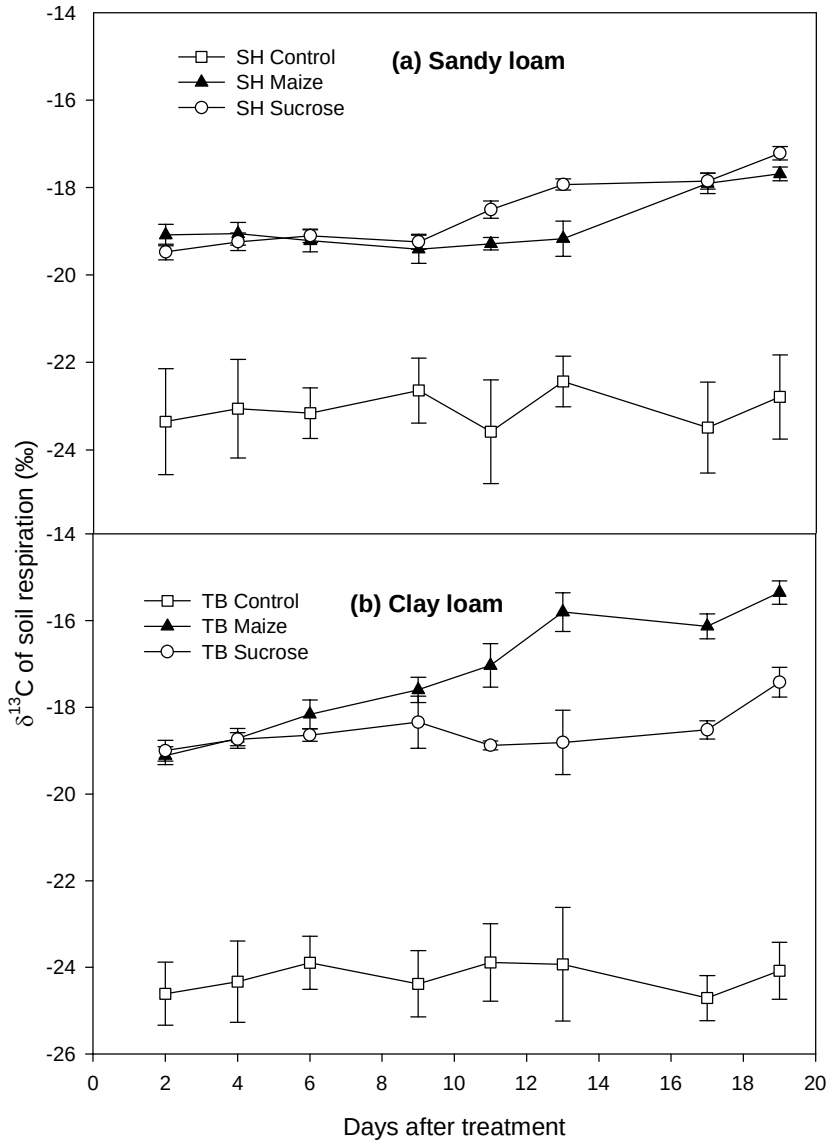

Figure 3-7 The measured δ^{13}C of respiration in microcosms treated with and without added substrates (a) sandy loam SH and (b) clay loam TB. Points show means (n=4), bars show standard errors.

Table 3-2 Comparison of $\delta^{13}C$ values of soil respiration measured for control treatments in SH and TB soils measured over 19 days. Data are means ± SE (n-4)

Time (DAT)	$\delta^{13}C$ of R_S in SH soil (‰)	$\delta^{13}C$ of R_S in TB soil (‰)
1	-20.29 ± 0.01	-22.92 ± 0.28
2	-23.35 ± 1.21	-24.61 ± 0.73
4	-23.06 ± 1.13	-24.33 ± 0.94
6	-23.16 ± 0.58	-23.89 ± 0.61
9	-22.64 ± 0.74	-24.38 ± 0.76
11	-23.59 ± 1.19	-23.89 ± 0.89
13	-22.44 ± 0.58	-23.93 ± 1.31
17	-23.49 ± 1.04	-24.71 ± 0.52
19	-22.79 ± 0.96	-24.08 ± 0.66

DAT - days after treatment

3.3.1.4 SOM contributions to total soil respiration and priming effects

The effect of substrate additions on the proportion of R_S derived from SOM (R_{SOM}) in the two soils is shown in Figure 3-8. In both soils the effect of substrates on R_{SOM} was always positive, i.e. SOM–derived respiration was above basal respiration, indicating positive priming effects. Sucrose caused significantly greater R_{SOM} values in both soils. In the TB soil, the increase in R_{SOM} with sucrose was greater than with the maize extract from 1 DAT. SOM decomposition was greatest in sucrose treated TB soil (2.4 ± 0.06 µg C g^{-1} soil h^{-1}) at 17 DAT. However, in SH soils R_{SOM} differences were only significant at 1 DAT when the maize treatment induced greater primed C and sucrose induced slightly greater but significant R_{SOM} at 6 and 17 DAT (Figure 3-8 a). There appears to be a consistent pattern of R_{SOM} in all treatments and soils until 6 DAT (Figure 3-9). Then from 9 DAT there appears to be divergence in both soil and treatment responses, particularly with sucrose (Figure 3-8 and Figure 3-9). The R_{SOM} reduction 9 DAT in the maize-treated TB soil was most dramatic and the only treatment where R_{SOM} continuously declined from that point, albeit gradually (Figure 3-8 b). In the maize treated SH soil R_{SOM} increased at 11 DAT and then gradually declined to the end, 19 DAT (Figure 3-8). The sucrose treatments were characterised by significant and persistent increase in R_{SOM}

until 17 DAT in both soils with a more significant and dramatic increase in the TB soil (Figure 3-8 and Figure 3-10).

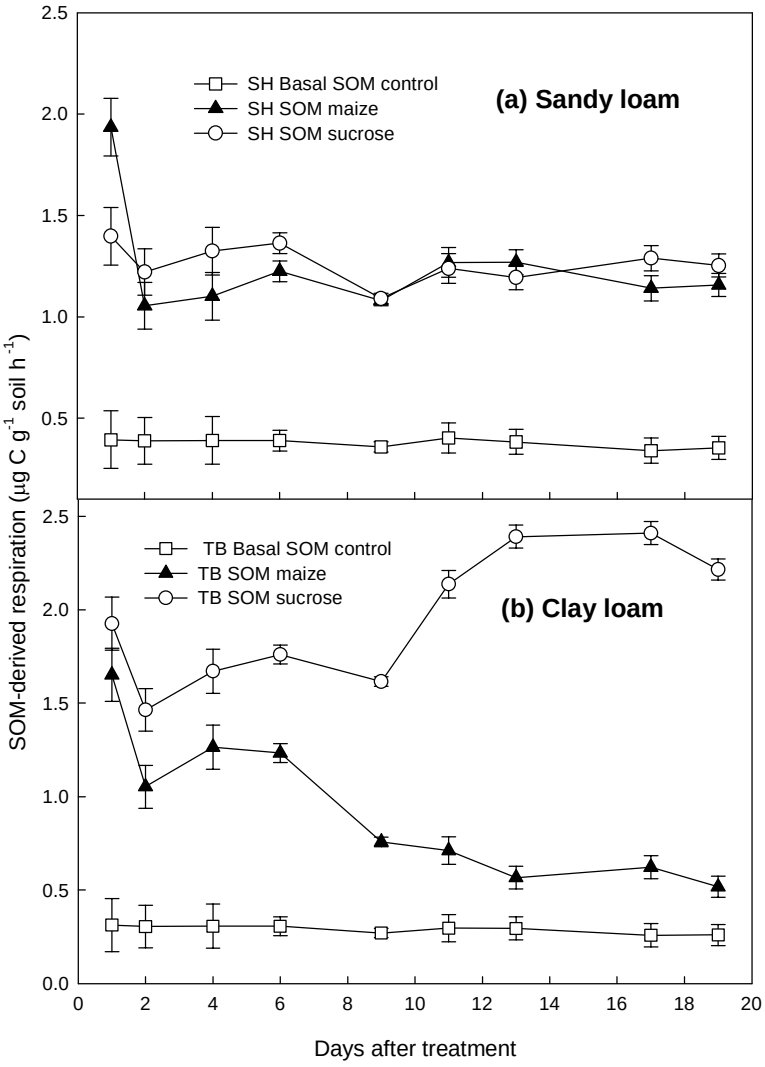

Figure 3-8 SOM-derived respiration in (a) SH soil and (b) TB soil treated with sucrose and maize. Points show means (n=4), bars show standard errors.

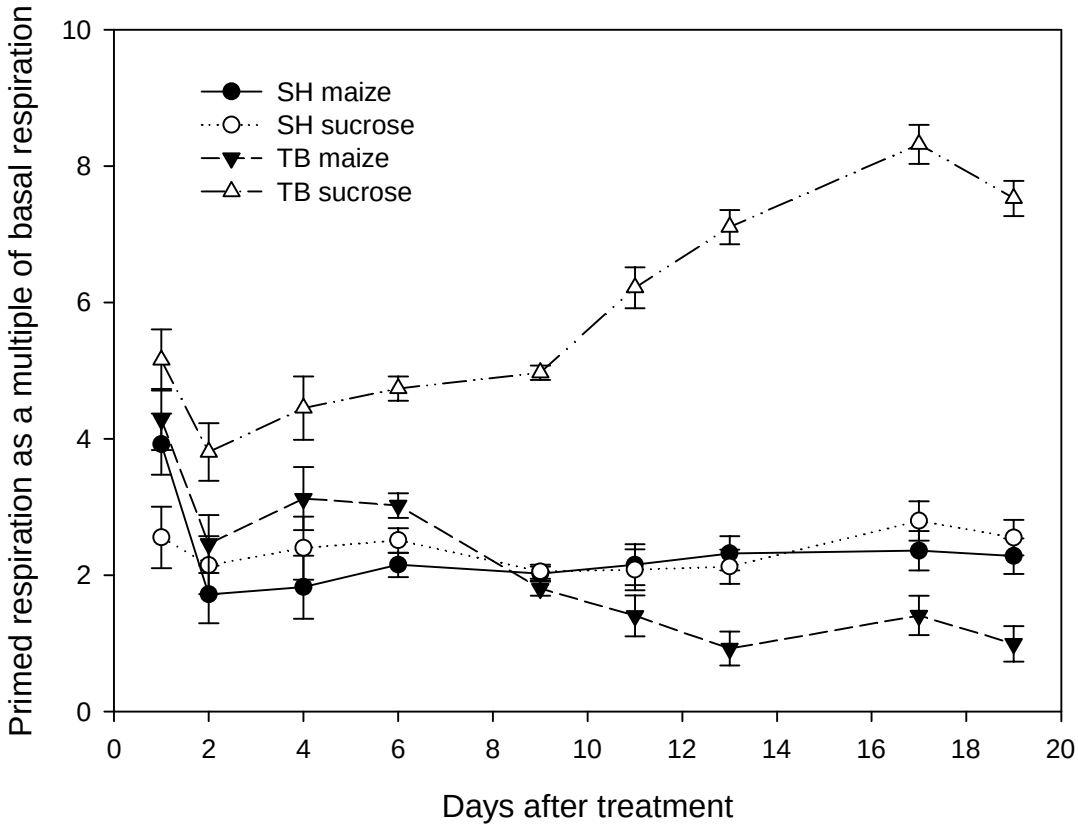

Figure 3-9 Pattern of priming effects in contrasting soils, captured by the primed respiration expressed as a multiple of basal $\left(\frac{\text{primed resp}}{\text{Basal resp}}\right)$ soil respiration at each sampling point in SH and TB soils. Points show means (n=4), bars show standard error.

Sucrose caused significantly greater priming effects in both soils (Table 3-3). The magnitude of maize-induced soil respiration (R_S) was greater in TB compared to SH (Table 3-3). However, primed soil C in maize treatments contributed more to R_S in SH soils, 48% compared to 29% in TB (Table 3-4). The relative significance of substrate induced priming was the same for both substrates in the SH soil, 48% of R_S. Sucrose induced priming effects accounted for 50% of R_S in the TB soil compared to 29% in maize treatments (Table 3-4).

Table 3-3 Cumulative respiration per microcosm (micr) during the treatment phase, partitioned into SOM, primed and substrate-derived components.

Soil type	Treatment	Total C (mg C micr^{-1})	Basal soil-derived C (mg C micr^{-1})	Primed soil-derived C (mg C micr^{-1})	Substrate-derived C (mg C micr^{-1})
SH	Maize	52.38 ± 0.63	11.44 ± 0.28	24.92 ± 0.42	16.02 ± 0.21
	Sucrose	55.93 ± 1.25		26.72 ± 0.87	17.76 ± 0.39
TB	Maize	67.98 ± 0.95	10.05 ± 0.25	19.86 ± 0.44	38.07 ± 0.52
	Sucrose	120.34 ± 5.66		59.43 ± 3.25	50.86 ± 2.41

Table 3-4 Relative proportions (%) of SOM, primed and substrate-derived respiration per microcosm during treatment phase.

Soil type	Treatment	Total C (% of R_S)	Basal soil-derived C (% R_S)	Primed soil-derived C (% R_S)	Substrate derived-C (% R_S)
SH	Maize	100	22	48	30
	Sucrose	100	20	48	32
TB	Maize	100	15	29	56
	Sucrose	100	8	50	42

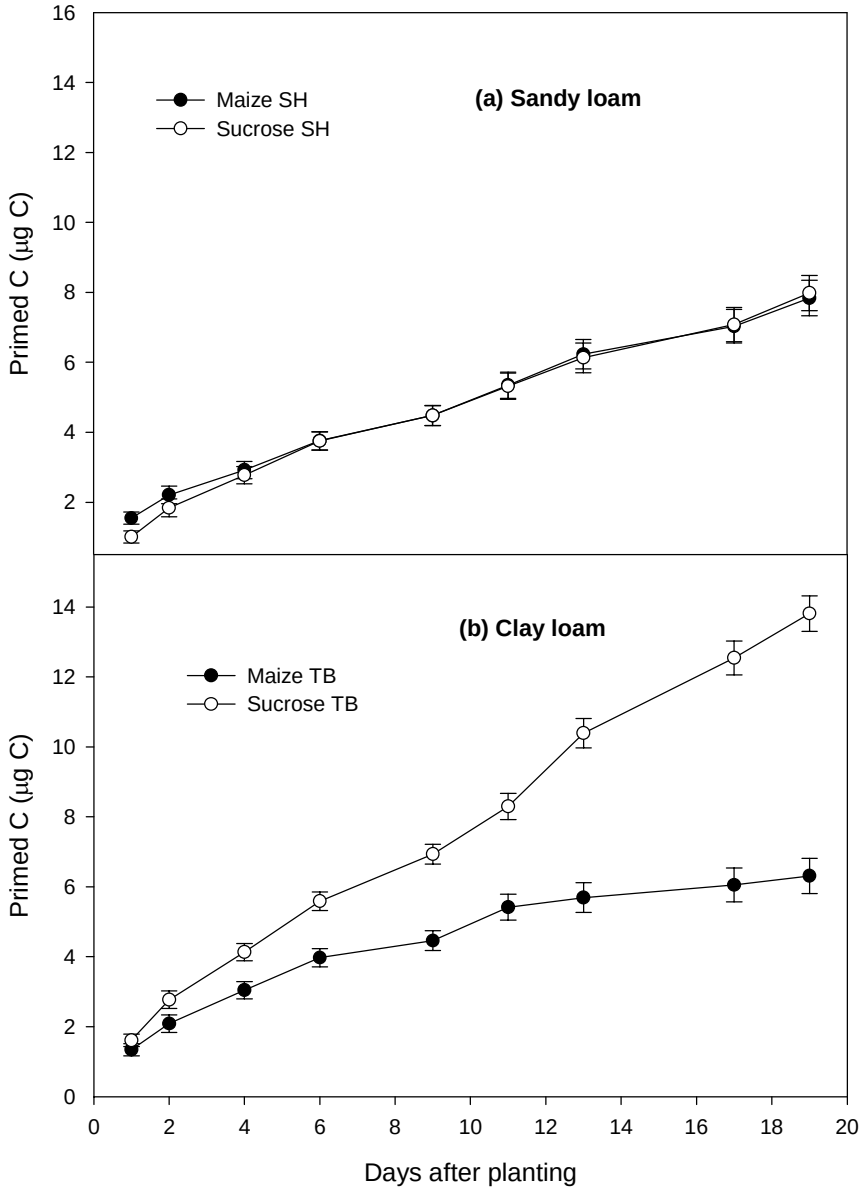

Figure 3-10 Cumulative priming effects in response to maize extract and sucrose additions in (a) SH and (b) TB soils. Points show means (n=4), bars show standard errors.

3.4 Discussion

3.4.1.1 Priming effects, intensity and patterns in contrasting soils

The results presented above met the objective of quantifying changes in SOM turnover in two contrasting soils in response to continuous additions of labile substrates. This study differed from most previous studies in that it (a) simulated root exudation in a temperate pasture with daily substrate additions, and (b) compared a multi-compound substrate with a single-compound substrate, rather than just single substrates.

In both soils positive priming effects were detected after the first day following maize and sucrose additions and continued with repeated substrate additions for the duration of the experiment. However, there were differences between the soils. In the sandy, low fertility soil (SH) maize and sucrose additions produced similar total soil respiration patterns and priming effects but in the more fertile clay loam soil (TB), sucrose produced consistently greater respiration rates and priming effects than maize extract. Also, in the TB soil the priming effect induced by maize declined after the first week, whereas the PE with sucrose increased.

Greater soil microbial activity and priming effects in response to single sucrose additions compared to maize were observed in similar studies (Nottingham et al., 2009; Engelking et al., 2007) and with repeated (26 days) substrate applications (Hamer and Marschner, 2005).This contrasts with the results of Blagodatskaya et al.(2007) who observed zero and negative PE, from applied glucose, 2 to 4 days after application both with and without added N.

My intention was to simulate natural continuous C additions in a grazed temperate grassland, during the most actively growing periods of the year. Due consideration was also given to the size of the microbial biomass (Blagodatskaya and Kuzyakov, 2011) with rates of additions comparable to root exudation in the rhizosphere.

Several studies investigating SOM turnover and PEs have used maize plant tissue (Liljeroth et al., 1994; Nguyen and Guckert, 2001; Baudoin et al., 2003;

Marx et al., 2007; Machinet et al., 2009). However these were mostly as solid dried plant material (Chen et al., 2014; Nottingham et al., 2009; Conde et al., 2005; Clemente et al., 2013). Use of liquid formulations are rare, however use of hydroponic-derived maize root mucilage (Benizri et al., 2007) and exudates (Marx et al., 2007) were reported. Comparison of a maize cellulose (from stems, added as powder) to C_4 sucrose (Engelking et al., 2007) was also reported for a priming experiment using a Haplic Luvisol. Nonetheless, to my knowledge, this is the first study comparing sucrose to a composite liquid maize root extract with repeated applications. The literature is replete with demonstrations of priming effects so a primary focus was to determine how my observations of continuous rather than pulse additions and mixed-composition substrate relate directly to the underlying mechanisms driving priming effects.

Possible priming mechanisms

There is a clear indication that the inherent qualities of both soil and substrate influenced the operating mechanisms. The differences in magnitude and time-course of priming effects following repeated substrate applications are of particular interest. The pattern of priming response between the soil types and substrates were initially similar but changed over time. Additions of easily available substrates to soils can energise the soil microbial community into activity well over basal soil respiration. An initial burst of microbial activity within hours of substrate additions is widely recognised (Paterson and Sim, 2013; Jenkinson et al., 1985). It is believed that the most active part of the microbial community, comprising so-called R-strategists, benefits most with activity and population surges (Chen et al., 2014; Fontaine et al., 2011). It could explain the initial activity observed following the first application of all substrates in both soils. It is likely the peak activity occurred before the initial measurement 19 hours after the first application as total soil respiration appears to be falling after 1 DAT. It is also thought that the early microbial stimulation is mostly associated with ‘apparent’ PE from the turnover of microbial biomass with the labile newly-available substrates (Blagodatskaya and Kuzyakov, 2008; Ghee et al., 2013). With a single substrate pulse, this may explain PE observations occurring within 1 to 2 days (Blagodatskaya et al., 2007). Repeated daily applications at the

rates used in this experiment are likely to rapidly satisfy the C-limitations of R-strategists. However, the significant and relatively high levels of priming effects observed throughout this experiment cannot be explained by the theory of microbial activation.

After the initial activation it is possible that subsequent growth and changes in the structure of the soil microbial community induced mineralisation of mostly the added substrates rather than the less decomposable SOM. This mechanism is referred to as preferential substrate utilization (Sparling et al., 1982). Repeated daily substrate applications made in this experiment would have enhanced preferential substrate utilization and possibly produced negative PE in the early incubation period. However, only positive priming effects were observed. Although PE significantly declined in the maize-treated TB soil after the first week, PE remained positive in all treatments. So it may still be reasonable to conclude that this mechanism was either not important or non-existent.

Relationships between rhizosphere N transformations, soil C dynamics and the release of fresh labile C from plant roots are well established (Yin et al., 2013; Norton and Firestone, 1996; Landi et al., 2006; Jackson et al., 2008). Mechanisms of priming related to the C and N composition of added substrates in comparison with existing soil C and N availability have been explored in planted systems (Cheng, 2009; Liljeroth et al., 1994) and microcosm studies (Chen et al., 2014; Dijkstra et al., 2009). Similarly the role of soil phosphorus (P) availability on SOM mineralisation (Bünemann et al., 2012) and the combined influence of N and P availability on the soil priming mechanisms were considered (Nottingham et al., 2015). The evidence suggests that depending on general fertility conditions PEs can be either amplified or reduced. The microbial N mining theory was advanced to explain observations of enhanced PEs in soils with low available N. It assumes that N-seeking microbes use added labile C substrates as energy sources to mine for N within SOM (Blagodatskaya and Kuzyakov, 2008). This is consistent with my results where sucrose and maize root extract stimulated positive PE in both soils throughout the incubation. I postulate that similar patterns of soil respiration and PE observed in response to

added sucrose and maize in the SH soil was due to the proliferation of N mining microbes. This could be a consequence of selection pressure placed on the biomass by a ready supply of C for respiration but little N/P/S for growth, hence the need to mine.

Similar patterns of PE in response to both substrates in the TB soil until 9 DAT is consistent with the N microbial mining theory. Significantly greater PE during that period in TB over the SH soil may be due to the greater substrate application rates (twice that in SH soil based on microbial biomass). Another factor could be the much greater fertility of the TB soil and the consequently greater return of mineralised N or other nutrients to the microbes from priming. Although SH had greater total C content compared to TB, a major component of C in the SH soil was recalcitrant woody fragments from its bracken cover, as shown by the much greater C:N ratio of the SOM. The greater fertility of TB was demonstrated by its higher microbial biomass (Table 3-1) and that it supported much better plant growth (Chapter 4).

From 9 DAT the pattern of PEs separated markedly with a rapid decline in maize-treated TB soil with a simultaneous rise in sucrose-treated TB soil. I attribute this decline in the maize extract treatment to an accumulation of N in the soil derived from the added material. The maize extract had a C/N ratio of 34. As a result 0.84 mg N was added daily to each microcosm giving soil a total of 7.56 mg N per microcosm by 9 DAT (i.e. approximately 0.1 mg N g^{-1}). As available N accumulated, microbial N mining became redundant, thus the rate of PE fell. I hypothesize that at 9 DAT a threshold of available N was reached in the maize-treated TB soil, which contributed to the dramatic fall in PE. However, there was still some demand for N, since although reduced, PE remained positive and above basal respiration in control treatments. In the sucrose treated TB soil, SOM decomposition continued to increase because the N-demand was still unfulfilled. It was not until 19 DAT that there was a significant decline in PE, possibly due to reduced N demand or exhaustion of available resources, including nutrients.

By contrast, the SH soil received a smaller substrate addition based on its smaller microbial biomass, 0.42 mg N daily. If the apparent N threshold of 7.56 mg N inferred for TB soil is applied per unit mass (0.1 mg N g^{-1} soil) of SH soil then that threshold would be reached approximately 20 DAT, after the experiment concluded. This is because the microcosms were packed to 80.8 and 70.7 g soil for SH and TB soil, respectively. Reduced SOM mineralisation in response to added N is well documented (Chen et al., 2014; Nottingham et al., 2015) however contrasting results with positive PE have also been reported (Conde et al., 2005).

It is well known that different C substrates applied to the same soil at similar rates can have different effects on the soil microbial community structure (Hamer and Marschner, 2005; Liljeroth et al., 1994; Landi et al., 2006). The simple sugars glucose and fructose at 121 and 87 mg g^{-1}, respectively, were found in the maize extract. However, a range of other water soluble compounds are also present in maize roots and exudates and would have also been present in the extract. Hütsch et al. (2002) found that maize root exudates comprised 79% water soluble compounds, which were partitioned into 64% carbohydrates, 22% amino acids or amides and 14% organic acids. Actual root material was found to have similar compounds and other nutrients (Clemente et al., 2013; Badri and Vivanco, 2009). I hypothesize that the diversity of compounds in this substrate would have stimulated changes in the soil microbial community compared to soils treated with sucrose. Changes in microbial community profiles (Orwin et al., 2006) and relative proportions of bacterial to fungal microbes (Jagadamma et al., 2014) in response to variable C substrates combinations were confirmed from laboratory incubations. Conceivably, sucrose was mostly stimulating to the N miners and may have contributed to the higher PE levels observed, especially in the TB soil. This is consistent with several studies using glucose or sucrose (Paterson and Sim, 2013; Nottingham et al., 2009; Hamer and Marschner, 2005).

3.5 Conclusions

An incubation study over 19 days represents a snap shot relative to complex soil processes continuing over millennia. Nonetheless, the results shed light on the processes governing root-soil interactions on seasonal time-scales. Sucrose additions stimulated consistently greater total soil respiration values across both soils and yielded positive priming effects over the duration of the experiment. Overall maize extract and sucrose both produced positive priming effects in both soils. It appears that substrate quality contributed most to the magnitude and direction of priming effects compared to the nature and properties of the two soils. The microbial N mining theory may be more significant to explain the SOM turnover dynamics observed in this experiment. Further research is required on the mechanisms and effects of C supply in relation to microbial community composition, substrate quality and soil fertility.

Chapter 4: The effects of plants and rhizodeposition on soil carbon turnover in two contrasting soils

4.1 Introduction

In this chapter I discuss the effects of plant growth and root interactions with microorganisms on soil C turnover. Compared to the incubation study reported in the previous chapter, planted systems provide more realistic conditions since they involve biological and physical interactions of roots, soil and the environment. Photosynthesis by plants is thought to exert a controlling effect on all plant processes including root-microbial interactions. Photosynthetic C is synthesised into plant tissues, sugars and other soluble compounds within the plant and up to 30% (Gregory, 2006) can be released into the soil as exudates and detrital plant material. While detrital plant material is thought to increase SOM and C storage, high energy C compounds in root exudates can accelerate the mineralisation of stored soil C through rhizosphere priming effects (RPE). It is desirable to have SOM function mostly as a store rather than a source of CO_2, as this can positively impact climate change by reducing atmospheric C. However, the evidence for RPE and how they differ between different plant - soil systems is equivocal.

A key methodological problem in studying RPE is the necessity to partition soil respiration into its component soil and plant-derived parts. This is necessary in order to quantify the effect of newly deposited plant C on existing soil C. The natural abundance method takes advantage of differences in the C isotope signature of C_3 and C_4 plant and soils, whether C_3 soil with C_4 plants or vice versa. The $\delta^{13}C$ of C_3 respiration is typically ≤ 10 ‰ more negative than C_4 respiration.

For the experiments reported in this chapter I used the C_4 Kikuyu grass (*Pennisetum clandestinum*) in pots containing soils from experiments discussed in chapter 3 with a history of C_3 vegetation. By following the $\delta^{13}C$ of the net soil CO_2 efflux and knowing the $\delta^{13}C$ of the plant and soil C sources in isolation, I could infer the separate plant and soil C fluxes and the extent of RPE. I sought

to vary the extent of rhizodeposition by clipping the grass leaves periodically. This should have varied the net photosynthesis, root:shoot partitioning of photosynthate and root exudation.

4.1.1 Objective

To investigate the role of plant-soil interactions on the turnover of soil C and possible rhizosphere priming effects in two contrasting soils.

The following hypotheses were tested:

1. Clipping will cause differences in root to shoot partitioning.
2. Resulting differences in rhizosphere C inputs will produce different soil turnover responses and priming effects.
3. The two soils will have different soil C turnover responses and priming effects.

4.2 Materials and methods

4.2.1 Experimental design

The clay loam (TB) and sandy loam (SH), the two soils used were described in section 3.2.1. The experiment was conducted in a glasshouse located within the compound of the Wolfson Field Lab at Cranfield University from May to July 2014.

A randomized block design was used with the two soils, the following three treatments and four replicates:

a) Planted soil (with grass) and unclipped
b) Planted soil (with grass) and clipped weekly to 2 cm
c) Unplanted control with soil only.

A total of 40 pots were prepared, including 16 planted pots that were destructively sampled at the midpoint of the experiment (31 DAP). The treatments were arranged in a randomized block design. The harvested material from clipping treatments was collected to determine dry matter production. Total root and shoot dry matter was determined at the end of the experiment. The soil CO_2 efflux and its $\delta^{13}C$ were measured periodically.

4.2.2 Preparation of pots and glasshouse operations

Sieved soils (> 6 mm) were moistened to 60% field capacity and packed into 30 cm long, 10.3 cm ID polyvinyl chloride (PVC) tubes to give 1.5 and 1.4 kg dry weight per pot for TB and SH, respectively. The base of each pot was capped with a weed block fabric to constrain root growth. Pots were fixed on table tops 2 m above a concrete floor. Seeds of Kikuyu grass (*Pennisetum clandestinum*) were obtained from Barenbrug Holland B.V., Nijmegen, Netherlands. They were sown 2-3 mm below the soil surface at a rate of 20 seeds per pot on May 16th 2014. Seeds were sown in a circular band approximately 3 cm from the edge of the pots. This ensured that there were no seeds at the centre of the pot which was reserved for the chamber headspace (next section) which was mounted shortly after germination. One fertilizer application was made soon after

germination with a water soluble NPK formulation (36-0-12 + trace elements; Vitax, Leicester, UK) at 0.16 g per pot (226.5 Kg ha^{-1}). Soil moisture was maintained at 60% of field capacity by daily weighing and watering. A fan was used to improve air circulation and to lower temperatures especially on hot days. Average daily temperature in the glasshouse for the duration of the experiment is given in Figure 4-1.

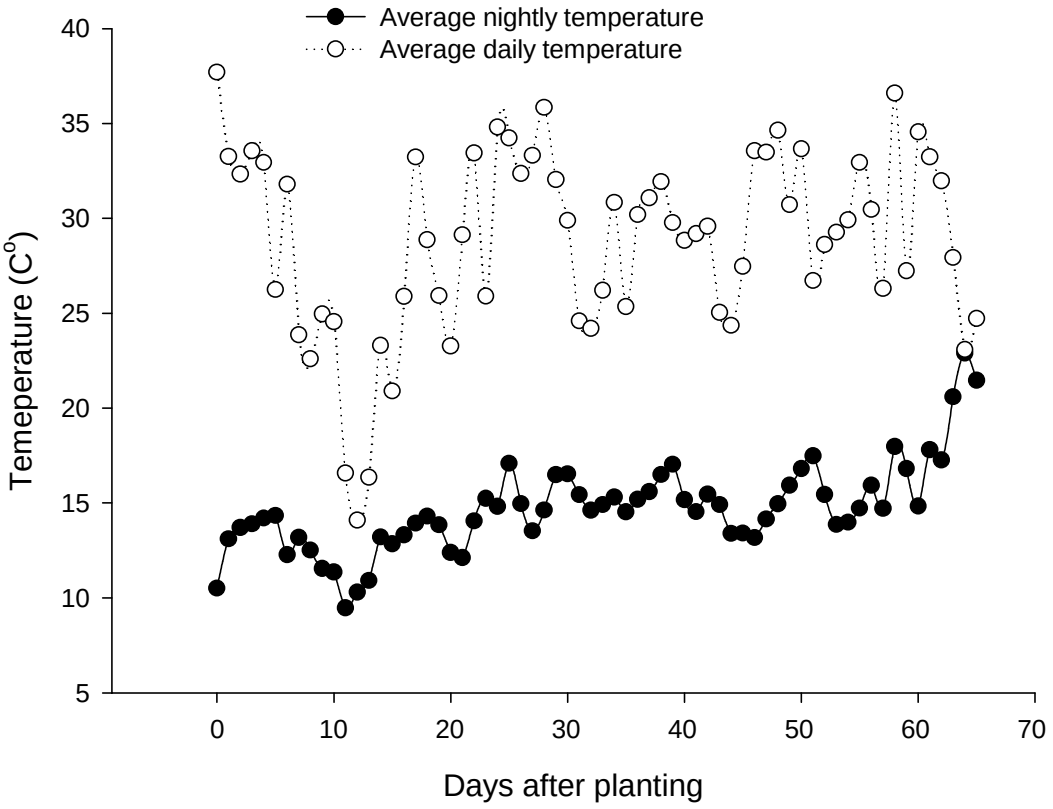

Figure 4-1 Recorded average nightly and daily temperatures (C^o) inside the glasshouse during the experiment

4.2.3 Soil respiration measurements

Soil respiration measurements were made from each pot using gastight head space made of tubes permanently fixed in the soil. The tubes were 100 mm long, 50 mm OD 46 mm ID lengths of PVC pipe (i.e. head space volume 166 cm^3). The top end was fitted with a cap containing gastight inlet and outlet with two way lock valves and luer lock fittings (Figure 4-2). The other end was inserted 30 mm into the soil in the centre of the pots where there were no grass seeds.

Figure 4-2 Headspace chamber in planted pot showing gastight inlet and outlet ports and luer lock valve

Before respiration measurements each chamber was connected to an infra-red gas analyser (IRGA, LICOR-820, Lincoln NE, USA) in an arrangement similar to the diagram in the previous chapter (Figure 3-4). Each headspace was flushed with CO_2-free air at approximately 1 L min^{-1} for two minutes. This was sufficient to reduce the chamber CO_2 concentration to ≤ 1 ppm. The chamber was left to incubate for 15 minutes which was sufficient to raise CO_2 concentrations well over ambient. 5 ml air samples were taken with a gastight syringe, four from the

headspace transferred to helium purged vials for analysis by IRMS as in Chapter 3.

4.2.4 Calculating soil respiration rate

The soil respiration rate R_s (µmol CO_2-C m^{-2} s^{-1}), was determined from the measured rate of change in CO_2 concentration - △C (µmol CO_2 mol^{-1} air) in the chamber headspace coming from the area of soil under the chamber - A (m^2) over a time interval △t (min) given by:

$$\text{Flux} = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \quad (4.1)$$

The chamber volume (in litres) was initially converted to moles (*n*) using equation 4.2 derived from the ideal gas law (equation 4.1).

$$RT = nPV \quad (4.2)$$

$$\text{moles (n)} = \frac{PV}{RT} \quad (4.3)$$

where R is the gas rate constant (0.80201 $\frac{\text{L-atm}}{\text{mol K}}$), T is the temperature 273 Kelvin(K), V is the chamber volume (L), n is moles air and P is pressure 1 atmosphere (atm).

Hence R_s (µmol CO_2-C m^{-2} s^{-1}) was calculated as

$$R_s = \frac{\triangle C\ (\mu\text{mol } CO_2\ \text{mol}^{-1}\ \text{air})}{\triangle t\ (\text{min})} \times \frac{\text{chamber headspace (mol air)}}{\text{soil surface area } (m^3)} \times \frac{\text{min}}{60\ \text{sec}} \quad (4.4)$$

Vial preparation for sampling

Prior to sampling, vial caps were fitted with new septa and heated overnight at 105°C. This removed any volatiles capable of diminishing the integrity of the samples. New unused vials were then tightly covered with the heat-treated caps and evacuated for 25 sec. They were immediately purged with helium for approximately 100 sec at a flow rate of approx. 60 ml min^{-1}. Vials were used on the day of purging.

4.2.5 Partitioning of the soil CO_2 flux

Plant end member isotope ratios were determined from freshly sampled plant roots. Samples of soil-free and soil-attached roots of approximately 0.3 g and 0.5 g, respectively were placed in 12 ml vials and incubated for 20 minutes after Midwood et al. (2006). At the end of the incubation a 5 ml sample of the vial headspace was transferred to a helium-purged septum-capped 12 ml vial followed by analysis by IRMS. Isotope ratio values were expressed as per equation. 3.10 in Chapter 3.

A two end-member mixing model described in equations 3.11 and 3.12 was used to determine the relative proportions of total soil respiration that were plant and soil-derived.

4.2.6 Determining plant biomass

Sixteen pots representing four replicates from each treatment were destructively sampled at 48 days after planting (DAP), this represented the midpoint of the clipping period, which lasted 31 days. Similarly, at the end of the experiment at 62 DAP, the remaining 24 pots were destructively sampled. Gas chambers were removed from each pot and any soil remaining in the chambers was collected and later homogenised with the bulk soil. In planted pots, shoots were cut at soil level, placed into paper bags and immediately oven-dried at 100^{o}C for 24 hours to determine the shoot biomass. The pots were transferred to the lab for further processing. The soil was emptied in a large tray and roots were extracted by hand and shaken lightly to remove loosely-attached soil. The roots were then thoroughly cleaned with water to remove soil material and then oven-dried to determine the root biomass.

Soil analyses

At the end of the experiment soil samples were collected for determination of microbial biomass, total C and N. Samples were also immediately frozen at -80 ^{o}C for 24 hours, then freeze dried for PLFA analysis.

4.2.7 Statistics and data analyses

The software package Statistica version 12.5 (Statsoft Inc. Dell Inc. Aliso Viejo, USA) was used for statistical analyses. Repeated measures ANOVA were used to assess the variation in total soil CO_2 efflux and its $\delta^{13}C$ between the two treatments and controls in both soil types. One-way ANOVAs were used to assess treatment effects on soil microbial biomass. Two-way ANOVAs were used to determine the significance of clipping effects on the two soils. The differences between means were analysed using post hoc Fisher least significant difference (LSD).

4.3 Results

4.3.1.1 Soil CO_2 efflux without plants

The measured basal soil SOM-derived efflux of soil respiration, (R_{SOM}) in unplanted control treatments was relatively constant throughout the experiment (Figure 4-3). The basal respiration was not statistically different in both soils. The TB soil produced the lowest $R_{SOM,}$ 0.73 ± 0.27 µmol CO_2-C m^{-2} soil s^{-1}, at 34 DAP, and the highest mean R_{SOM}, 2.36 ± 0.69 µmol CO_2-C m^{-2} soil s^{-1}, at 55 DAP.

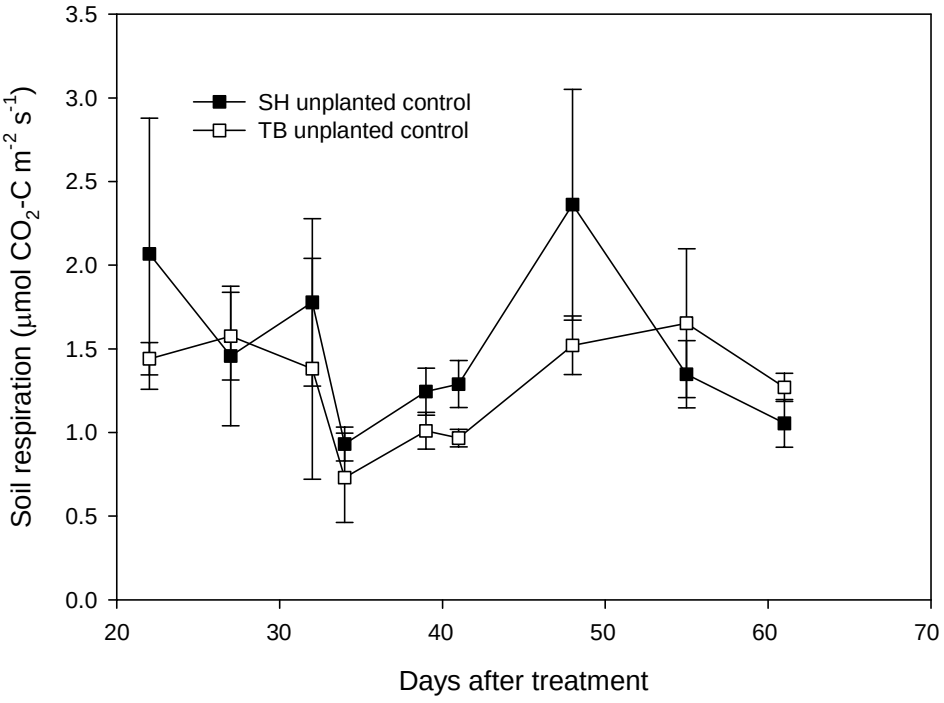

Figure 4-3 Comparison of basal soil respiration (R_{SOM}) in unplanted control treatments of TB and SH. Data are means and error bars indicate ±SEM**.**

4.3.1.2 Effect of plants on the total soil CO_2 efflux

The presence of unclipped plants significantly increased the total soil respiration (R_S) over R_{SOM} measured in unplanted controls in both soil types (Figure 4-4). The magnitude and time course of the R_S increase differed between the two soils. In the TB soil R_S was significantly greater than in the unplanted control 27 DAP, whereas in the SH soil this only happened 39 DAP. The increased R_S in TB soil was 5.95 times basal respiration and in the SH soil it was 1.95 times basal respiration.

Clipping commenced 31 DAP and had a dramatic effect on R_S in both soils. In the clipped SH soil treatment, the measured R_S was not significantly different from the basal soil respiration (Figure 4-4). The TB soil clipping treatments also produced depressed R_S values, these were generally greater than basal respiration but the differences were only statistically significant at 27 and 48 DAP (Figure 4-4).

At 32 DAP one day after the first clipping event, R_S in the TB soil decreased by 30 % and was further reduced by 48 % on the following day (Table 4-2). The decrease was less dramatic in the SH soil where R_S initially declined by 4 %, one day after clipping and then declined by 55 % when measured two days after clipping. Two days after the first clipping R_S in all clipped treatments irrespective of soil type was not significantly different from unplanted controls. There was a resurgence in R_S values in both soils 48 DAP with an increase of 80% and 138 % in the SH and TB soils respectively. This was exactly 10 days after the previous clipping event (Table 4-1 Table 4-2).

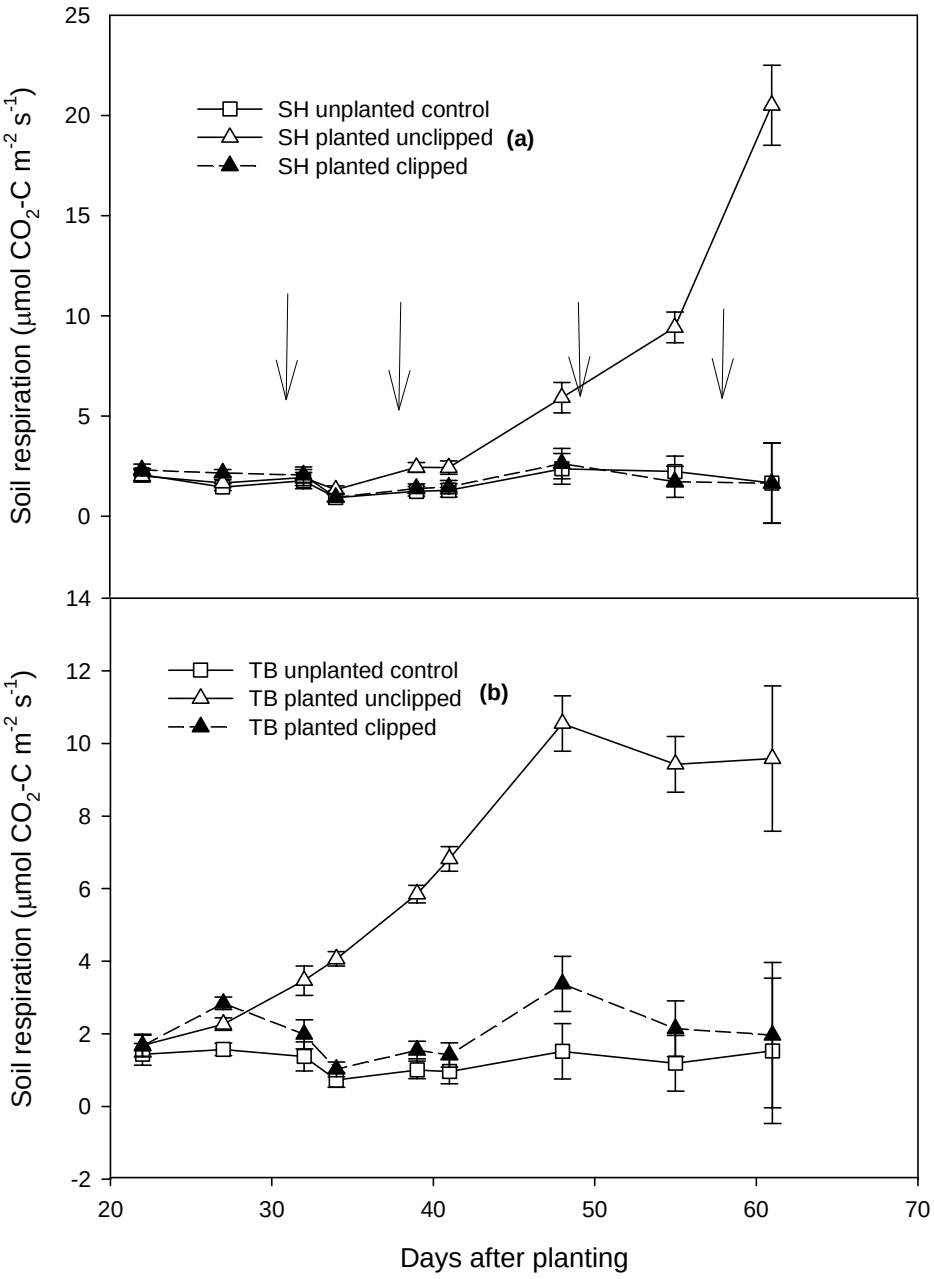

Figure 4-4 Soil respiration rates measured in two contrasting soils (a) SH - Sandy loam and (b) TB - clay loam that were either planted with Kikuyu grass and clipped weekly or remained unclipped, compared to unplanted controls. Data are means and error bars indicate ±SE (n=4). Arrows show clipping dates for both soils

Table 4-1 Clipping effects on mean R_S (µmol CO_2-C m^{-2} s^{-1}) ± SE per pot in SH soils and percentage changes relative to the timing of clipping events (DAP) and compared to R_S values in unclipped treatments.

Date of clipping event (DAP)	Date R_S measured (DAP)	SH unclipped mean RS ± SE	% Change in R_S	SH clipped mean R_S ± SE	% Change in R_S
	27	1.65 ± 0.17		2.15 ± 0.17	
31	32	1.93 ± 0.40	17	2.05 ± 0.40	-4
	34	1.31 ± 0.20	-32	0.93 ± 0.20	-55
	39	2.44 ± 0.24	85	1.38 ± 0.24	49
38	41	2.42 ± 0.34	0	1.46 ± 0.34	6
	48	5.91 ± 0.76	144	2.62 ± 0.76	80
49	55	9.42 ± 0.77	59	1.72 ± 0.77	-34
58	61	20.51 ± 2.0	118	1.65 ± 2.0	-4

Table 4-2 Clipping effects on mean R_S (µmol CO_2-C m^{-2} s^{-1}) ± SE per pot in TB soils and percentage changes relative to the timing of clipping events (DAP) and compared to R_S values in unclipped treatments.

Date of clipping event (DAP)	Date R_S measured (DAP)	TB unclipped mean R_S ± SE	% Change in R_S	TB clipped mean R_S ± SE	% Change in R_S
	27	2.27 ± 0.17		2.84 ± 0.17	
31	32	3.47 ± 0.40	53	1.99 ± 0.40	-30
	34	4.07 ± 0.20	17	1.03 ± 0.20	-48
	39	5.85 ± 0.24	44	1.55 ± 0.24	51
38	41	6.82 ± 0.34	17	1.42 ± 0.34	-9
	48	10.55 ± 0.76	55	3.38 ± 0.76	138
49	55	9.43 ± 0.77	-11	2.14 ± 0.77	-37
58	61	9.58 ± 2.0	2	1.97 ± 2.0	-8

4.3.1.3 The $\delta^{13}C$ values of root and rhizosphere respiration

The mean $\delta^{13}C$ values of root respiration obtained from freshly harvested soil-free roots and that of roots with adhering rhizosphere soil were all within the expected range for a C_4 Kikuyu grassland system, -11 to -16‰, (Figure 4-5). There were no significant differences (P=0.66) between the soils or clipping treatments (Figure 4-5).

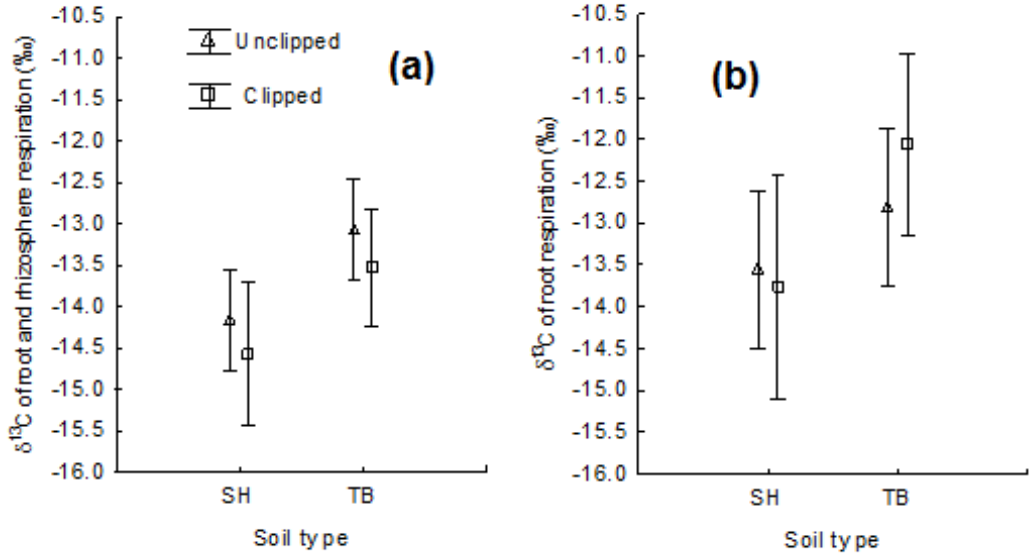

Figure 4-5 Measured $\delta^{13}C$ of (a) root and rhizosphere respiration and (b) root respiration, determined from fresh kikuyu grass roots grown in SH and TB soils with two clipping treatments. Data are means ±SE of mean (n=4)

4.3.1.4 Measured $\delta^{13}C$ of the soil CO_2 efflux

The $\delta^{13}C$ values of the soil used for partitioning the soil efflux into its respective C components differed significantly between soil types and clipping treatments and over time (Figure 4-6). The adjusted mean $\delta^{13}C$ values of R_s in the planted unclipped treatments were significantly greater than the control and clipped treatments in both soils. Generally the $\delta^{13}C$ values in the planted and unclipped treatments were more enriched (less negative) than the control and clipped treatments. The differences were highly significant in the TB soil but not in the SH soil (Figure 4-6).

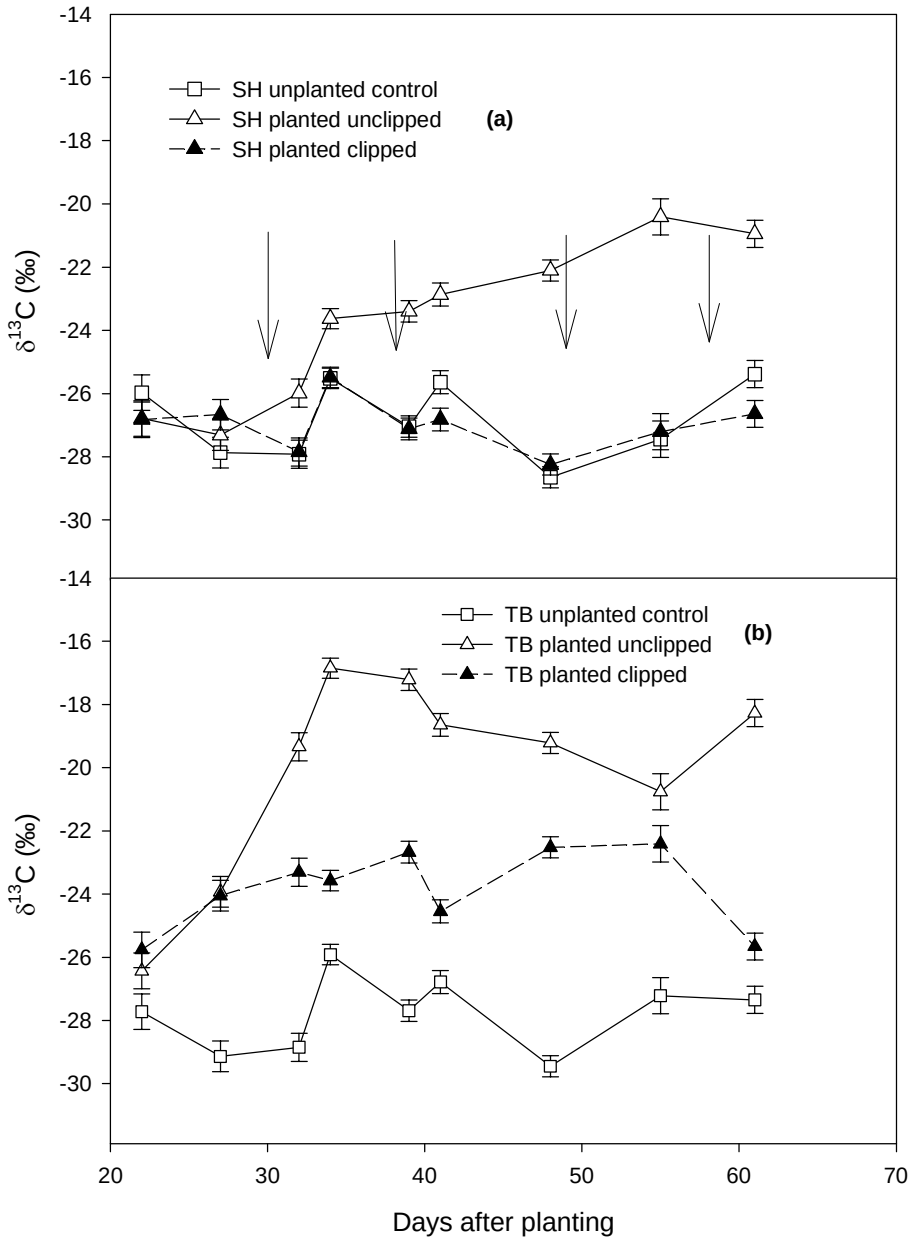

Figure 4-6 The $\delta^{13}C$ of R_s measured from planted (clipped and unclipped) and unplanted control pots with (a) SH soils and (b) TB soils Data are means ± SE (n=4), Arrows show clipping dates.

4.3.1.5 Calculated treatment effects on SOM turnover

Figure 4-7 shows the soil CO_2 efflux data from Figure 4-4 partitioned according to SOM and root-derived components. Turnover of SOM was stimulated in the unclipped treatments in both soils and the effect increased with time and greater plant biomass. In the SH soil SOM turnover increased 1.61-fold 48 DAP. In the TB soil the increase occurred 12 days earlier at 34 DAP and was 2.22-fold. At the end of the experiment (61 DAP) the increases were 6.9 and 3.3-fold in the TB and SH soils, respectively (Figure 4-7).

There was no stimulation of SOM turnover in the SH clipped treatment. In the TB soil, clipped treatments stimulated SOM turnover which was apparent throughout the clipping period but was not significant (Figure 4-7).

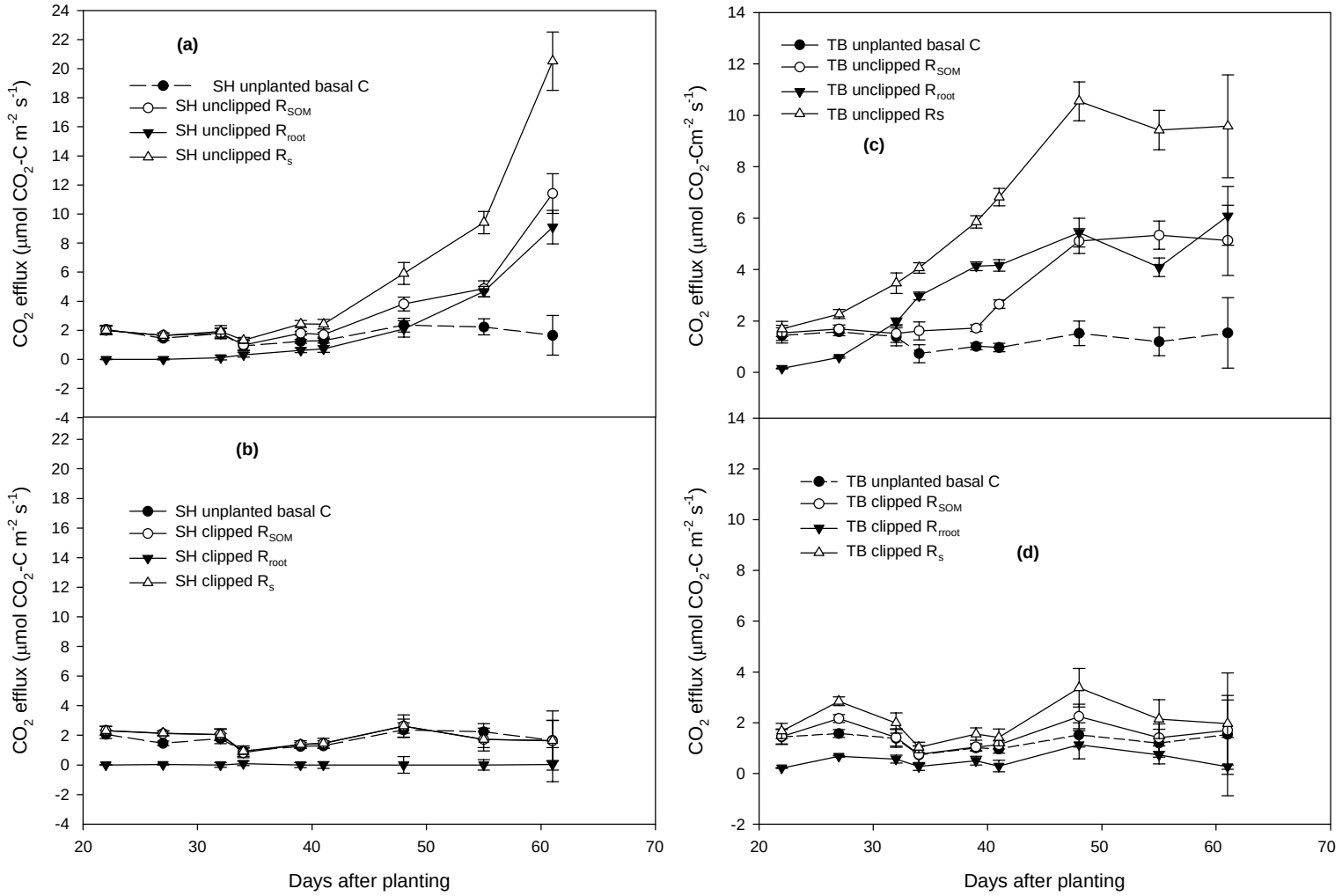

Figure 4-7 The partitioning of the soil CO_2-C efflux (R_s) into its soil-derived (R_{SOM}), root-derived (R_{root}) components compared to the basal soil treatment from unplanted controls in following treatments: SH soil unclipped (a) and clipped (b) and the TB soil unclipped (c) and clipped (d). Data are means ±SE (n=4).

The plant contribution to the total soil CO_2 efflux increased gradually with time in the unclipped treatments in both soils. The pattern of increase was different in the two soils with TB unclipped plants making an earlier significant contribution to R_S 22 DAP compared to 34 DAP in the SH soil. Related measurements began only 22 DAP, hence it is possible that the plant contribution to R_S in the TB unclipped could have started earlier. The overall plant contribution to R_S in the TB unclipped was 44.5% greater than the plant contribution to the SH unclipped treatment. However the single highest plant contribution, 9 µmol CO_2-C m^{-2} s^{-1}, was recorded in the SH unclipped treatment on the final sampling day, 61 DAP.

4.3.1.6 Rhizosphere priming effects

Unclipped planted treatments

Figure 4-8 shows the priming effects calculated from the results in Figure 4-7. Unclipped treatments in both soils produced positive priming effects. The amount of primed C significantly increased with time in both soils. The greatest priming effect, an additional 9.76 ± 1.66 µmol CO_2-C m^{-2} s^{-1} occurred in the SH soil. Overall the TB soils consistently produced greater priming effects than the SH soils, except at 61 DAP.

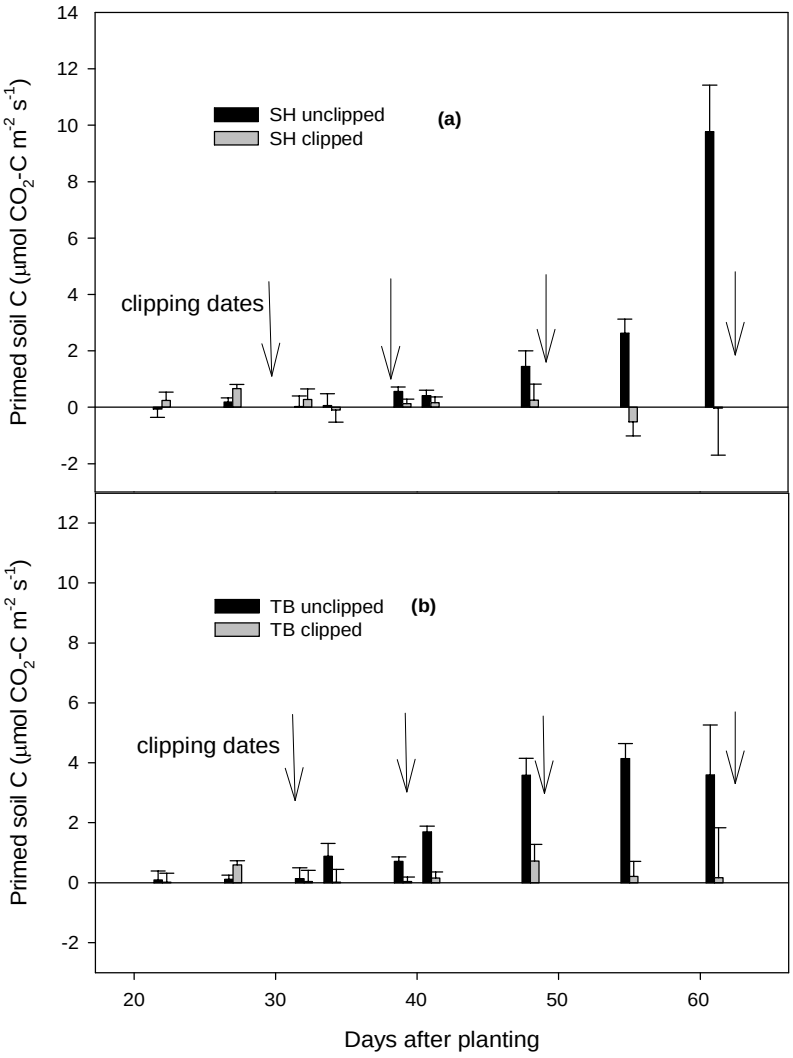

Figure 4-8 Total primed CO_2 – C from clipped and unclipped treatments in (a) SH soils and (b) TB soils. Data are means ± SE, (n=4). Arrows show clipping dates.

The clipping effect on priming

Clipping produced relatively weak priming effects in both soils. Four of the nine measurements made in the SH soil produced negative priming effects, while all measurements from clipping treatments in the TB soil produced positive priming effects (Figure 4-8). There was no significant priming in the clipping treatments in either soil. The timing of measurements after a clipping event 1, 2, 3 or 5 days did not significantly affect the intensity or direction of priming in both soils.

4.3.1.7 Treatment effects on plant biomass production

The treatment effects on plant biomass were measured by destructive sampling at 48 DAP and at the end of the experiment (62 DAP). Figures 4-9 and 4-10 and Tables 4-3 and 4-4 show the results.

Midpoint evaluation

At the midpoint evaluation shoot and root dry matter produced from the unclipped grass treatment in the TB soil was significantly greater ($P<0.001$) than in the unclipped treatment in the SH soil (Figure 4-9 and Table 4-3). Generally the clipped treatments in the TB soils produced greater shoot and root dry matter compared to SH soils, however there were no significant differences in shoot and root dry matter in TB clipped treatments, unclipped grass in SH soil and that of the clipped grass in the SH soils.

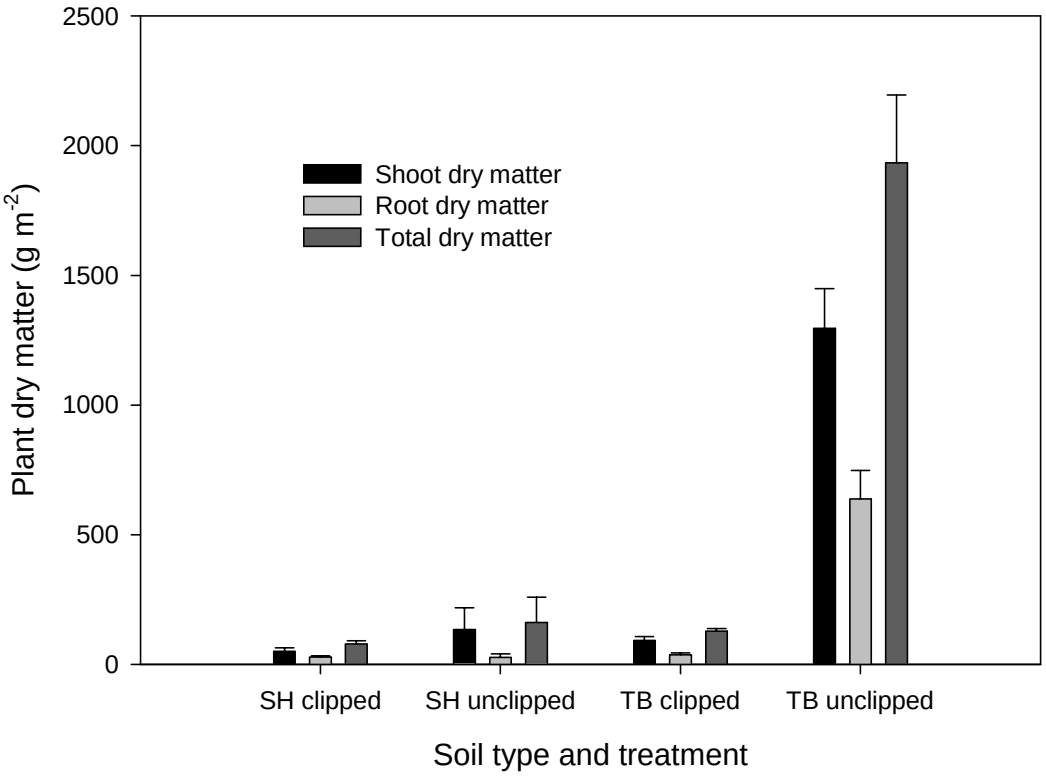

Figure 4-9 Total plant biomass production partitioned into shoots and roots, retrieved from the indicated treatments 48 DAP. Columns represent mean dry matter per pot expressed as g m^{-2} and error bars indicate SE, (n=4).

End point evaluation

At the end of the experiment the unclipped grass in the TB soil had accumulated the greatest plant biomass ($P<0.01$) with mean shoot and root biomass of 3368 ± 119 g m^{-2} and 4069 ± 347 kg g m^{-2}, respectively (Table 4-3). The unclipped treatment in the SH soil followed with shoot and root biomass of 2,887 ± 152 g m^{-2} and 1,370 ± 178 g m^{-2}, respectively (Figure 4-10 and Table 4-3). On comparison of the two destructive sampling events at 48 and 62 DAP, plant biomass accumulation was apparently greater in the SH unclipped treatment, where root and shoot biomass increased 50 and 20-fold, respectively. Overall biomass production was significantly decreased in the clipped plants in both soils. The TB clipped treatment produced significantly

greater shoot biomass than the SH clipped. However there was no significant difference in root biomass between the soils. Considering the total biomass, there was significant difference between all treatments in the order TB unclipped > SH unclipped > TB clipped > SH clipped. Comparing the effect of time and clipping on shoot to root partitioning, in both soils the root to shoot ratios in the clipped treatments decreased significantly while they increased significantly in the unclipped treatments.

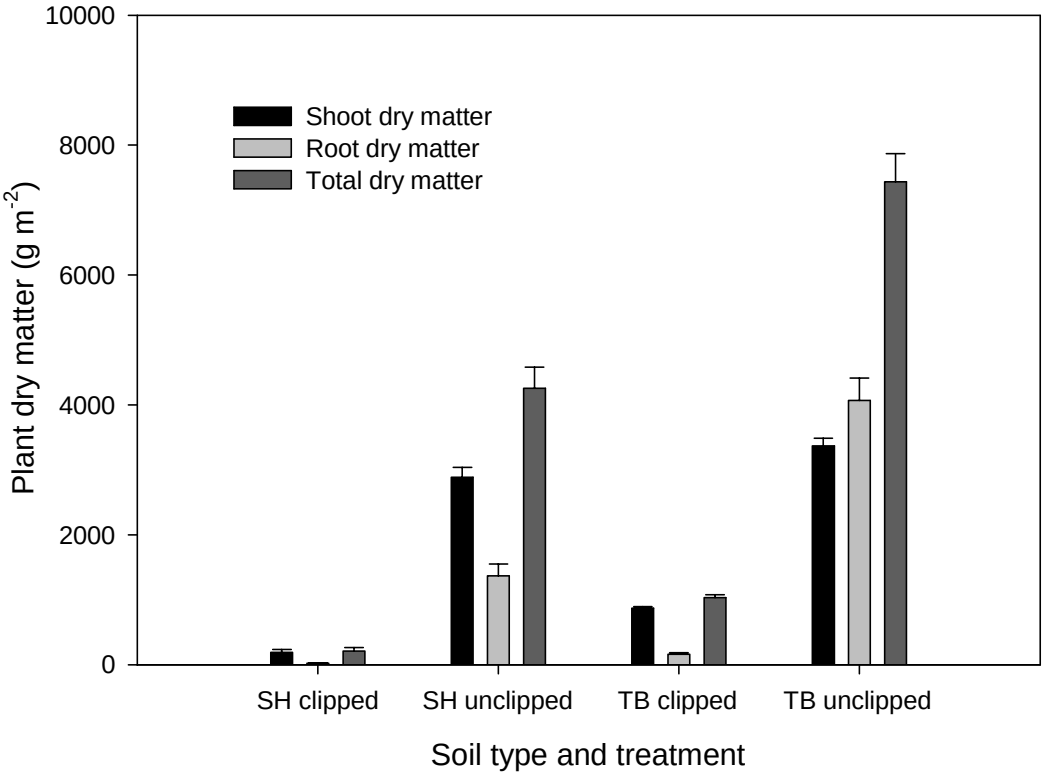

Figure 4-10 Total plant biomass partitioned into shoot and root components retrieved from destructively harvested pots from all planted treatments at the end of the experiment. Columns represent mean dry matter per pot expressed as g m^{-2} and error bars indicate SE (n=4).

Table 4-3 Root (rt) and shoot (sht) dry matter (DM) partitioning and total DM per pot for the respective treatments in SH and TB soils measured from destructive sampling at 48 and 62 DAP data are means ± SEM, (n=4).

Soil	Treatment	Sht DM (g)	Rt DM (g)	Rt:sht ratio	Total DM	Sht DM (g)	Rt DM (g)	Rt:sht ratio	Total DM
		48 DAP				62 DAP			
SH	Clipped	0.43 ± 0.10	0.23 ± 0.04	0.65 ± 0.21	0.66 ± 0.10	1.56 ± 0.41	0.17 ± 0.07	0.10 ± 0.02	1.73 ± 0.48
SH	Unclipped	1.13 ± 0.69	0.23 ± 0.12	0.23 ± 0.03	1.35 ± 0.81	24.04 ± 1.27	11.42 ± 1.48	0.47 ± 0.04	35.46 ± 2.68
TB	Clipped	0.77 ± 0.13	0.31 ± 0.07	0.46 ± 0.21	1.07 ± 0.07	7.25 ± 0.20	1.34 ± 0.20	0.18 ± 0.02	8.59 ± 0.38
TB	Unclipped	10.79 ± 1.27	5.31 ± 0.92	0.48 ± 0.03	16.10 ± 2.18	28.05 ± 0.99	33.89 ± 2.89	1.21 ± 0.08	61.94 ± 3.62

Table 4-4 The relative changes in root (rt) and shoot (sht) dry matter (DM) production from 48 to 62 DAP.

Soil	Treatment	% Δ rt DM	% Δ sht DM	Total DM
SH	Clipped	-26	266	164
SH	Unclipped	4974	2037	2527
TB	Clipped	336	847	701
TB	Unclipped	538	160	285

4.3.1.8 Soil microbial biomass C

Table 4-5 provides results of soil microbial biomass C measurements determined from soil samples collected at the end of the experiment. The treatments did not induce any significant change to the size of the microbial biomass in either soil. Microbial biomass in the TB soil was at least 6 times greater than in the SH soil.

Table 4-5 Microbial biomass C (µg g^{-1}) of soil samples from planted pots and unplanted controls measured at the end of the experiment. Data are means ± SE, (n=4).

Treatment	Microbial Biomass C (µg g^{-1})
SH clipped	382 ± 38
SH unclipped	400 ± 26
SH control	316 ± 75
TB clipped	2230 ± 57
TB unclipped	2178 ± 52
TB control	2235 ± 58

4.3.1.9 Nitrogen balance calculations

Typically N concentrations in productive pasture at low to high rates of N range from 2.5 to 5% of plant dry matter. Moir et al. (2013) studied N uptake in 13 temperate grass species in response to N rates (0 – 700 kg N ha^{-1}) and found N concentrations in shoots ranged from 2.47 to 4.55% N. Nitrogen concentrations in grass stubble and roots were generally 50% lower. Vellinga et al. (2010) demonstrated that different soil characteristics, well or poorly-drained and high sand or clay content did not affect N uptake or dry matter yield. I hypothesise that in this experiment N concentration in both soils was similar and concentrations in shoots and roots should be at least 2.5 and 1.25% N, respectively. Nitrogen leaching losses were negligible in this experiment as all applied water was contained within pots. Total soil N uptake by plants is inferred from Equation 4.5. Equations 4.6 and 4.7 provide the basis for calculating total plant-N uptake and the fertilizer-N percentage of total plant-N uptake.

Nitrogen balance for planted pots with SH an TB soils

$$\text{Total soil N uptake} = \text{Total N uptake} - \text{Fertilizer N} \quad (4.5)$$

Fertilizer N share (%)

$$\text{Fertilizer N share (\%)} = \frac{\text{Fertilizer N}}{\text{Total soil N uptake}} \times 100 \quad (4.6)$$

Total plant N uptake

$$\text{Total plant N uptake} = (\text{Shoot biomass} \times N_s\%) + (\text{Root biomass} \times N_r\%) \quad (4.7)$$

N_s = 2.5% =N concentration in shoots; N_r = 1.25% =N concentration in roots

Table 4-6 shows that total plant N-uptake was significantly different in the treatments in the order TB unclipped>SH unclipped>TB clipped>SH clipped. The total plant N-uptake per pot in the SH unclipped and TB clipped and unclipped treatments surpassed the amount of added fertilizer-N. However N demand in the SH clipped treatment remained very low and did not exhaust the applied fertilizer-N. The unclipped treatments in both soils had the greatest N-demand, which was fulfilled largely from the soil. The soil-N contribution was 6

and nearly 4-times greater than fertilizer-N, in the TB and SH unclipped treatments, respectively (Table 4-7).

Table 4-6 Dry matter and N uptake by shoots and roots data are means per pot ± SE, (n=4).

Treatment	Dry matter yield (g/pot)		N Uptake (mg/pot)		
	Shoot dry matter (g)	Root dry matter (g)	Shoot N uptake (mg)	Root N uptake (mg)	Total plant N uptake (mg)
SH clipped	1.6 ± 0.41	0.17 ± 0.07	39 ± 10.1	2 ± 0.99	41 ± 11.0
SH unclipped	24.04 ± 1.27	11.42 ± 1.48	601 ± 31.7	143 ± 18.5	744 ± 49.0
TB clipped	7.25 ± 0.20	1.34 ± 0.20	181 ± 5.0	17 ± 2.5	198 ± 7.2
TB unclipped	28.05 ± 0.99	34 ± 2.89	701 ± 24.7	424 ± 36.1	1124 ± 55.6

Table 4-7 Utilisation of fertilizer-N applied just after germination in planted pots with SH and TB soils.

Treatment	Fertilizer N (mg)	Soil N uptake (mg)	% Diff. (N_{soil} - N_{fert})	Fertilizer N share (%)
SH clipped	160	-119	-74	389
SH unclipped	160	584	365	22
TB clipped	160	38	24	81
TB unclipped	160	965	603	14

4.4 Discussion

The influence of plants and clipping on soil respiration (R_S)

The results showed clear differences in the extent and pattern of R_S between treatments and soil types. At the first measurement and prior to clipping, 22 DAP, R_s in all planted treatments was dominated by soil-derived C in both soils with negligible plant-derived contributions. There was no significant difference between R_s and basal soil respiration from unplanted controls. As plants grew, the relative contributions of plant-derived C increased primarily in the unclipped treatments in both soils. This was more acute in the unclipped TB soil, which initially supported plant growth much better than SH (Figures 4-9 and 4-10). The data indicated a positive relationship between plant biomass (shoot and root) and R_s over time. The observed plant influence on increasing R_S is consistent with the results of similar studies (Thurgood et al., 2014; Uchida et al., 2011) and entirely expected when root biomass is increasing, thus generating more root respiration. It is likely that greater shoot and root production in the TB soil allowed both greater C fixation through photosynthesis and greater rhizosphere (surface area) deposition, respectively. This would contribute to the different responses observed between the soils. The N balance calculations presented earlier showed the N fertilizer consumption correlated positively with plant biomass and was completely consumed in all treatments except the SH clipped treatment. The applied fertilizer apparently did not produce any measurable effect on R_s in the treatments. This is supported by the fact that the respective R_S in the control treatments of both soils and the SH clipped treatment remained relatively unchanged throughout the experiment (Figure 4-3 and Figure 4-4). Generally, there was a trend of increasing R_S values in both soils except for one occasion in either soil type when declining R_S values were observed, 34 and 55 DAP in the SH and TB soils, respectively (Table 4-1). Although the experimental design sought to minimise soil moisture differences by periodic watering to constant weight, it is possible that differences in soil properties and plant biomass production may have at times led to moisture differences between soils and treatments.

Overall clipping significantly reduced R_s in both soils compared to unclipped treatments. In response to the first clipping 31 DAP there was a comparable drop in mean R_S of 48 and 55% in TB and SH, respectively, two days after clipping. The effect seemed to occur gradually, since one day after that clipping event mean R_S had only declined by 17 and 4%, in TB and SH, respectively. The magnitude of R_S reduction within two days was lower than 65% reported by Shahzad et al. (2012) for clipped *Lolium perenne* over the same period. However, Hamilton et al. (2008) reported that clipping events were accompanied by a flush of root exudation that caused increased R_S, microbial activity and RPE. If R_S increased following clipping in this experiment, it would only be a transient effect, under 24 hours after clipping. This could have possibly contributed to the gradual decline in R_S observed. An important aspect of this study was to quantify the effect of plants and clipping on the mineralisation of existing SOM-derived C, rhizosphere priming effects.

Plant effects on the SOM mineralisation – rhizosphere priming effects

Planted treatments produced significantly greater levels of SOM mineralisation compared to unplanted controls in both soils (Figure 4-7). From 34 to 61 DAP the intensity of rhizosphere priming effects (RPE) ranged from 7 to 590% in SH and 122 to 235% in TB soil. The results are consistent with the results summarised in a review by Cheng et al. (2014), with the exception of the final measurement 61 DAP in the SH soil, which was unusually high. It was explained earlier in (Section 1.2.1.2) that RPE can be either (a) apparent - where an observed increase in R_{SOM} is caused mostly by turnover of microbial biomass or (b) real - where the increased R_{SOM} is caused primarily by enhanced turnover of stored soil C. The extent of real or apparent priming is usually confirmed by determining the isotope ratio of microbial biomass C after extraction by chloroform fumigation (Garcia-Pausas and Paterson, 2011). This is used to determine the relative proportions of the plant or SOM-derived components that make up the soil biomass (Paterson and Sim, 2013). Higher plant-derived proportions relative to SOM would indicate that microbial biomass

turnover was predominant and therefore apparent priming effects occurred. However, if the soil microbial biomass contained isotope ratios indicating that it was composed primarily of SOM, this would indicate that SOM mineralisation was the predominant process and therefore real priming effects had occurred. I was not able to make these analyses due to time constraints. However, the consistent increase in R_{OM} in the unclipped treatments is not characteristic of apparent PE, as progressive microbial biomass turnover rather the SOM decomposition would result in declining contributions of soil-derived C to soil respiration as microbial biomass became more plant-derived. The size of the microbial measured biomass at the end of the experiment, using the chloroform fumigation technique, showed that the size of microbial biomass was not significantly different across all treatments (Table 4-5). This was consistent with the findings of Tanvir et al. (2015) who assessed changes in the microbial biomass of soils from a 55-day experiment with three grass species compared to unplanted controls. They found that the size of the microbial biomass remained unchanged in the planted soil compared to unplanted controls and that the composition of the soil microbial biomass remained predominantly of SOM origin, indicating real priming effects. Based on the results shown in Table 4-5 above, there is evidence that the rhizosphere priming effects observed were real and reflected loss of pre-existing C from both soils as depicted in Figure 4-8 However, it was interesting to observe that the soils might have responded differently with respect to changes in the size of the soil microbial biomass.

Clipping effects and related changes in rhizodeposition

Rhizodeposition derived from fixed C during photosynthesis is considered a primary driver of SOM mineralisation and RPE in planted systems (Cheng et al., 2014). Plant biomass and the plant-derived component of R_S are recognised proxies for root exudation and photosynthesis (Bahn et al., 2009). The different clipping regimes and variable soil types used in this experiment were designed to produced variations in photosynthesis and thereby alter the rate and volume of root exudation and root turnover. The observed consequential effects on SOM mineralisation was expected to provide an improved understanding of the

mechanisms driving the processes involved. Clipping reduced plant biomass and importantly leaf area for photosynthesis and was associated with significantly lower root biomass. This is consistent with several previous studies summarised by Ferraro and Oesterheld (2002). The contribution of plant-derived C to R_S was either significantly reduced in the TB soils or negligible in SH soils for all clipped treatments. This agrees with Shahzad et al., (2012) who reported reduced SOM mineralisation in response to clipping. However their plants were grown for 190 days before a single experimental clipping with measurements 1 and 30 days after. They found that clipping produced much less drastic reductions in SOM mineralisation than I measured here.

In this experiment, soil type contributed to the variable clipping effect on SOM but the timing and frequency of clipping events were likely to be significant. Plants were clipped at four times at 7, 11, 7, and 4-day intervals and flux measurements taken 1, 2, 4 and 5 days after clipping. Based on the resolution of the dataset gathered and especially previous work assessing C partitioning in response to clipping (Kuzyakov, 2002; Schmitt et al., 2013) and specifically in Kikuyu grass (Roper et al., 2013), I inferred that root exudation and the measured plant-derived C component of SOM mineralisation had similar patterns of flow and magnitude.

The SH soils appeared to severely limit early plant growth compared to the TB soil. These limitations apparently restricted the regeneration process, after clipping events recovery in the TB soils was always faster. Clipping reduced the available labile C for energising the microbial community into SOM mineralisation and thus the nutrient limitations were not satisfied. Clipping changed the C partitioning within plants as clipped plants allocated more resources to regrowth. This is supported by a significant reduction in root to shoot ratio in response to successive clipping events from 48 to 62 DAP (Table 4-3). Compared to the clipped plants the root to shoot ratio in the unclipped treatments increased significantly over the same period indicating a greater allocation of C resources belowground which related to greater SOM mineralisation in both soils.

Interactions of plant and soil processes driving RPE

While RPE on SOM mineralisation is commonly demonstrated, the mechanisms driving this effect are not well understood (Cheng et al., 2014; Zhu et al., 2014). This study provides additional evidence supporting the microbial activation hypothesis and relevance of plant N demand as potential mechanisms underlying RPE. The results show a gradual increase in microbial activity that is consistent with the increase in plant biomass and increasing levels of rhizodeposits. This trend is consistent for the unclipped treatments in both soils. With the exception of a one off N application that would be consumed shortly after germination, all the resources required for plant growth were acquired from the soil. For all intents and purposes both soils were resource poor and especially N limited. The growing plants increasingly partitioned more resources below ground for root production, thus rhizodeposits would have increased. Consequently, energised microbes were able to mineralise SOM to release stored nutrients with potential for greater plant development. This process occurred faster in the TB soil possibly because it initially contained a microbial community better adapted and able to quickly decompose SOM in that soil. This is supported by the pattern of SOM decomposition in the microcosm study reported in the previous chapter and PLFA studies conducted on these soils confirmed that their microbial community phenotypes were distinctly different (Chapter 5). The SH soil was inherently acidic and sandy (82%) with less stable aggregates for occluding nutrients compared to TB. Apart from having a reduced overall nutrient supply it is possible that its low inherent pH (4.4) may have contributed to chemical bounding nutrients that could be otherwise available. These hurdles contributed to the late surge in biomass production and dramatic rise in RPE and SOM mineralisation in SH. The results depict the synchronised communication and alignment of plant requirements and processes (photosynthesis and rhizodeposition) with microbial support to mine existing soil resources for nutrients.

4.5 Conclusion

The study demonstrated that the presence of plants in a soil system is not necessarily sufficient to stimulate SOM turnover and priming effects. The plants must develop a certain productive capacity before these processes can set in. When left unclipped, plants generated sufficient root biomass to thoroughly colonise the soil. As plant biomass developed, the rate of photosynthesis and rhizodeposition increased and this stimulated greater plant demand for N and other nutrients. Rhizodeposits provided energy for the soil microbial biomass which allowed greater SOM mineralisation to most likely meet plant nutrient requirements. Soil type, growing conditions and management will influence the rate and intensity of RPE but not the direction. This study indicates the possibility for generating greater value from similar research efforts if soil nutrient dynamics are considered rather than the primary focus on soil carbon turnover with respect to climate change. It is important to understand these processes in relation to (a) the effects of increased atmospheric CO_2 and climate change on soil C turnover, and (b) agronomic management and plant breeding efforts for manipulating soil C and nutrient fluxes and enhancing nutrient use efficiency.

Chapter 5: An assessment of an automated field system for measuring NEE, plant and soil respiration and their dependence on key drivers

5.1 Introduction

In this Chapter I test an automated lysimeter system for measuring net ecosystem exchange (NEE) and plant and soil respiration (R_{eco}), and the sensitivities of these to diurnal and seasonal changes in soil temperature and moisture and photosynthesis.

The system I used is the Wolfson Field Laboratory (WFL) at Cranfield University. The WFL comprises 24 lysimeters containing intact soil monoliths and fitted with automated closing chambers for measuring gas fluxes (CO_2, CH_4 and N_2O) and their isotope composition near-continuously. The lysimeters contain two soil types (12 replicates of each): an infertile sandy loam (SH) and a more-fertile clay loam (TB). The soils are the same as the ones used for the incubation and glasshouse experiments reported in Chapters 3 and 4. Their main properties are given in Chapter 3.

The study was conducted over five months from May to September 2014. Periodic measurements of NEE were made in chambers with transparent lids, and of R_{eco} in chambers with opaque lids. Simultaneously, depth-resolved measurements of soil temperature and moisture were made in each lysimeter. Light energy, rainfall, ambient temperature and relative humidity were also continuously measured at the site. Multiple regression analysis was used to determine the extent to which the soil temperature and moisture and photoperiod – as a proxy for photosynthesis – contributed to the observed patterns of R_{eco} and NEE.

My original intention was to measure the $\delta^{13}C$ of the CO_2 efflux from plants and soil, so as to separate the flux into plant- and soil-derived components. However, the state of development of the isotope measurement system in the WFL at the time I made my study was such that this was not possible.

5.1.1 Research objective

The broad objective was to evaluate the WFL for measuring R_{eco} and NEE and their sensitivities to environmental drivers. To this end I tested the following specific hypotheses:

1. The WFL system can reliably measure R_{eco} and NEE so as to evaluate their relationships with key drivers (soil temperature and moisture, solar radiation and day length).
2. The system can accurately measure short-term changes in soil moisture and temperature and photosynthesis.
3. Short-term changes in soil moisture and temperature are different in the two soils.
4. Plant and soil respiration rates have different sensitivities to changes in soil moisture and temperature in the two soils.
5. Carbon balance estimates based on NEE fluxes match C stock inventory changes (principally from harvested dry matter).

5.2 Materials and methods

5.2.1 The Wolfson Field Laboratory

The experiment was performed between 10 April 2014 and 09 September 2014 at the Wolfson Field Laboratory (WFL) at Cranfield University. The WFL was constructed three years before the start of the experiment. It comprises a set of 24 lysimeters (0.8-m diameter and 1-m deep) containing intact soil monoliths, 12 each of the two soils described in Chapter 3. The lysimeters were buried so the soil level is flush with the surrounding soil. Each lysimeter was fitted with soil moisture and temperature sensors (Delta-T SM 300) at depths of 60, 120, 250 and 500 mm, connected to data loggers. Measurements were recorded every 30 minutes.

Each lysimeter is fitted with a gas flux chamber, with cylindrical walls and pneumatically-operated lids, made of 10-mm thick clear acrylic plastic (Perspex). The lid makes a gas-tight seal with the cylinder wall when closed, and contains a 5-cm diameter vent valve which closes a few seconds after the lid to dampen pressure changes. The chamber walls are 20 cm high, so the height of the lid above the soil surface is 26 cm and the chamber volume is approximately 130 L. Gases accumulating when the lids are closed are passed through a continuous sampling loop to an infrared gas analyser (IRGA; a Licor LI-840A), and an isotope ratio mass spectrometer (IRMS; a Sercon 20-22) housed in an instrument building (Figure 5-1). The flow through the sampling loop is at 10 L min^{-1}. The opening and closing of the chamber lids and the switching of valves in the sample loops directing the air flow is controlled by the IRMS software (Sercon Callisto), such that the chambers can be sampled in any sequence with pre-set times for the lids closing and opening.

There is a complete weather station at the WFL site, containing a Vaisala WXT520 weather transmitter for wind speed and direction, precipitation, atmospheric pressure, temperature and relative humidity and Delta-T ES2 energy flux sensors for solar radiation. During the experiment this data was measured at five-minute intervals and accumulated on data loggers along with

the soil moisture and temperature data from the lysimeters, and periodically transferred to web-accessible servers.

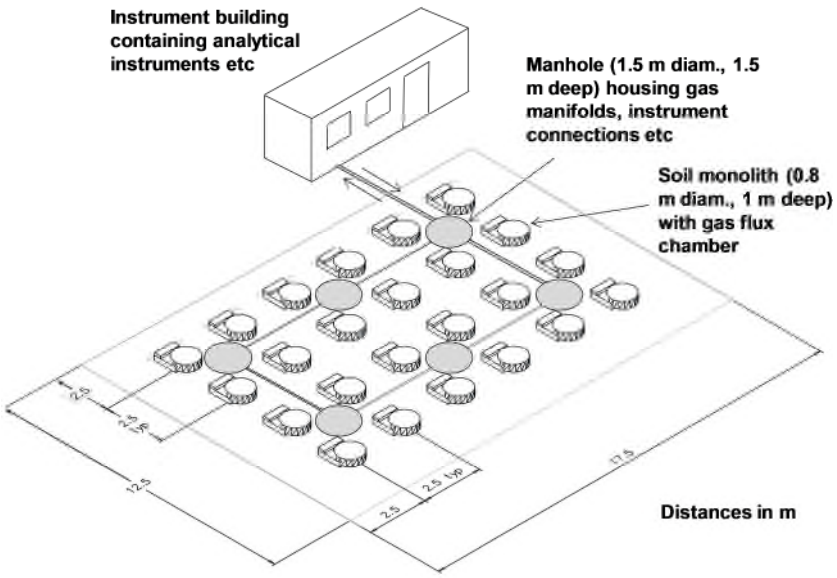

Figure 5-1 Plan of the Wolfson Field Lab site at Cranfield University.

5.2.2 Experimental design

A randomised block design was used for the experiment comprising four blocks of transparent and opaque chamber lids and three replicates each of the two soils, for a total of 24 lysimeters (Figure 5-2).

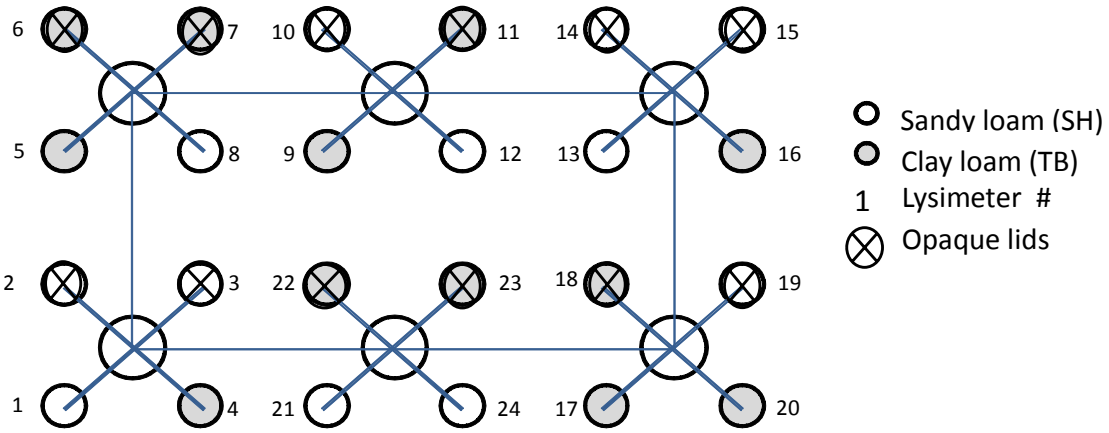

Figure 5-2 Layout of lysimeters in the Wolfson Field Laboratory, indicating soil type and arrangement of opaque and transparent chamber lids. The blue lines represent the gas sampling loops connecting individual chambers to the IRGA and IRMS in the instrument building.

5.2.3 Gas flux measurements

Since the lysimeters were continuously covered with grass, it was only possible to measure combined soil and plant-derived CO_2 fluxes. To measure plant and soil respiration (R_{eco}) and net ecosystem exchange (NEE), half of the chamber lids (six of each soil type) were covered with a reflective aluminium bubble insulation foil material (Multifoil Limited, Exeter, UK) making them opaque, and so preventing photosynthesis when the lids were closed. The other 12 lids were left transparent. The walls of both sets of chambers were covered with foil so that the grass in both sets of chambers had the same light regimes when the lids were open. Soil CO_2 flux measurements in opaque and transparent chambers allowed for disaggregation of the NEE flux into ecosystem C uptake (photosynthesis) and R_{eco}.

Flux rates were calculated from the linear increases in CO_2 concentration in the chambers over 2 min during a 15-min lid-closure period. Based on observed equilibration rates in the chambers, data from the first 5 min following lid closure was discarded, and fluxes calculated from data captured during the 5^{th} to 7^{th} minute after lid closure. The fluxes were then calculated as described in Section 4.2.4. Correlations of concentration vs time (sec) were conducted to ensure linearity of the concentration change over the selected measurement period (5-7 min). Another 2 min section of the sample period was chosen if the correlation values were less than 0.95

The sequence of lid closures and openings was randomized using the Callisto IRMS software (as described in Chapter 2). A batch of 24 lysimeters was sampled in approximately 7 h. On average, seven batches were completed in a single sampling event, which lasted approximately 48 h.

The actual time course of lid closure and opening was recorded with the Callisto software, whereas the time course of CO_2 concentration measurements was recorded with the Licor IRGA software. It was therefore necessary to synchronise them on a common time-scale, and this was done with a program written in Microsoft Excel VBA software. The environmental data (soil temperature and moisture, and weather) were also synchronised using this program. The sampling order within any batch of the 24 was randomized to prevent any bias. Hence any given lysimeter could be sampled at any time of the day. This meant that the interval between measurements for R_{eco} and NEE in the respective soils was variable, ranging from 0.3 to 3.75 h.

5.2.3.1 Solar radiation and photosynthesis

Solar radiation as light energy (kW m^{-2}) was recorded every 5 min. The day length was calculated as time interval between the first and the last recorded light measurement on a particular day. The calculated instantaneous flux measurement per lysimeter at the 6^{th} minute after lid closure was matched with the nearest light measurement (within 3 min). This represented the light intensity associated with the particular flux measurement. The minimum recorded light measurement of 0.01 kW m^{-2} was deemed to have wavelengths

within the photosynthetically active range and therefore consistent with photosynthesis.

5.2.3.2 Estimating photosynthesis

Photosynthesis C uptake or net primary productivity (NPP) was initially estimated for each soil using the global means of all efflux measurements (during the day) from opaque chambers (R_{eco}) less the actual measured efflux from the transparent chambers (NEE).

$$\text{Estimated NPP} = \text{Mean (All } R_{eco}) - \text{NEE} \quad (5.1)$$

The estimated NPP values were then compared with the light measurements associated with the respective NEE efflux using linear regression analysis. Strong relationships would provide a suitable model for predicting photosynthesis based on light measurements.

NPP was also estimated on a daily basis. For a particular day, flux measurements from the opaque chambers were used to determine the mean ecosystem respiration in the two soils. Flux measurements from transparent chambers representing the NEE were subtracted from the mean ecosystem respiration to determine the estimated NPP.

$$\text{Estimated NPP} = \text{Mean (daily } R_{eco}) - \text{NEE} \quad (5.2)$$

The estimated NPP was then plotted against the associated light intensity to determine the extent of correlations between NPP and light for the respective soils. These relationships were used to generate models using light data to estimate photosynthesis in the lysimeters. High quality models would allow for estimating NPP values over the experiment, May to September 2014 and to draw comparison with dry matter harvested from the lysimeters over the period in order to establish a soil carbon balance for the WFL system.

5.2.4 Measuring and modelling the influence of key drivers

Soil moisture and temperature measurements were made every 30 min and recorded over the duration of the experiment. Reliable measurements of soil temperature at depths of 60 mm and 120 mm, along with soil moisture

measurements at 250 mm, were used to evaluate patterns of temperature and moisture changes in both soils and the respective sensitivities of their R_{eco} to moisture and temperature changes. The R_{eco} measurements were matched to the corresponding or nearest records (within 5 to 15 min) of soil moisture and temperature, solar radiation and day length. Linear regression models were used to predict R_{eco} and NEE for the entire season.

5.2.5 Harvesting and processing dry matter

Grass from the lysimeters was harvested on four dates: 09/04/14, 29/05/14, 09/07/14 and 24/09/14. The last three harvests were used to calculate plant growth and C accumulation during the experiment. All material was collected, oven dried at 60 - 70 ^{o}C for 48 h and weighed to determine the net primary production during the experiment. The grass samples collected were analysed for total C and N. The results were used to determine the quantity of C removed from the lysimeter systems over the season.

5.2.6 Soil sampling and analyses

I took samples from each of the lysimeters to assess the soil properties. The soil processes related to soil CO_2 efflux are mediated by the soil microbes (Nannipieri et al., 2003). I therefore assessed the microbial community profiles of the two soils, as well as their basic soil properties.

Soil samples were collected from each lysimeter using a corer made from a single stainless-steel tine (9 mm OD) used for turf aeration. The corer had to be small enough to fit between the lattice of heating cables embedded in the top 1 cm of the lysimeter soils, and to minimise disturbance to the lysimeter soil system. Six sub-samples were taken from each lysimeter at the points shown in Figure 5-3. The collected samples were homogenised and sieved at 2 mm to remove debris and plant material and then separated into portions for chemical and microbial analysis. Samples for microbial analysis were stored in plastic zip-lock bags and frozen at 80^{o}C for 24 hours and then freeze-dried until ready for analysis. The other samples were air-dried and stored until processing.

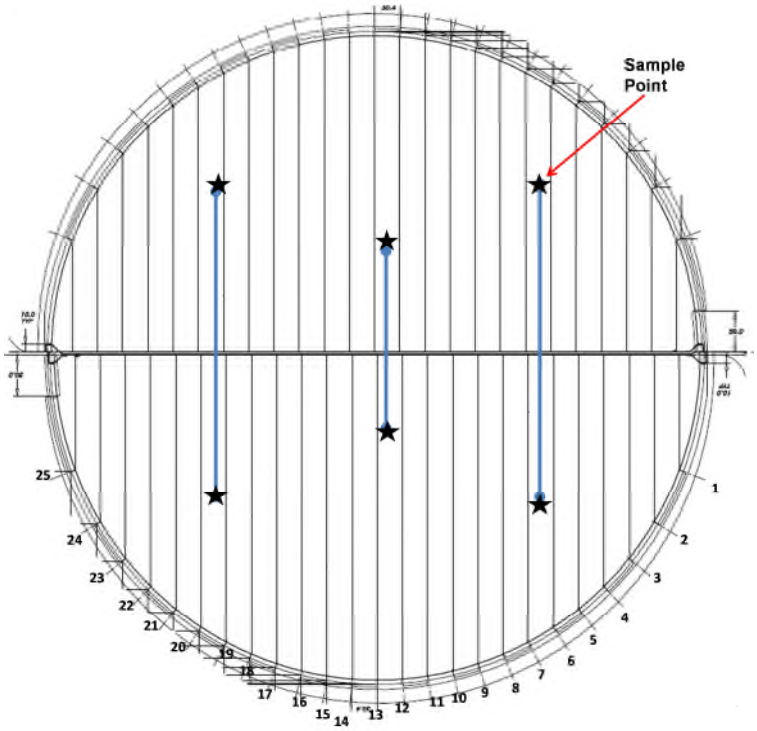

Figure 5-3 Depiction of the surface of a lysimeter showing the points used for soil sampling. The grey lines indicate heating cables embedded 1 cm below the soil surface, which had to be avoided.

5.2.6.1 Laboratory analyses

Phospholipid fatty acid (PLFA) extraction and analysis was used to investigate differences between microbial community profiles in the two soils, as described by Bligh and Dyer (1959) and as modified by White et al. (1979) and used by Bardgett et al. (1996). The samples were processed as described in Chapter 2.

5.2.7 Lysimeter carbon budget calculations

The dried plant material samples were finely ground and analysed for total C using an elemental analyser, (Vario EL III, Elementar Analysensysteme, Hanau, Germany). Mean total dry weight of the sample was 34 ± 4 mg.

5.2.8 Statistical analysis

Statistical analyses were carried out using Statistica version 12.5 (Statsoft Inc. Dell Inc. Aliso Viejo, USA). Initially, analysis of covariance (ANCOVA) was used to establish whether there were statistically significant differences in variates between the soils. Multiple linear regression analysis was then used to generate a model for fitting the data for each soil type. One-way ANOVA was used to assess differences between night and day time fluxes and the consistency of NEE estimates. Two-way ANOVA was used to assess differences in flux measurements from replicate lysimeters in the two soils.

5.3 Results

5.3.1 Respiration measurements

Figure 5.4 shows the measured daily R_{eco} in the two soils over the measurement period (17 May to 8 September 2014). The gas flux chamber system at the WFL provided measurements of both soil R_{eco} and NEE that allowed for the study and analysis of relationships with their key drivers over diurnal cycles. Soil ecosystem respiration was significantly greater ($p<0.0001$) in the more fertile clay loam (TB) soil compared to the infertile sandy (SH) soil. The greatest daily mean R_{eco} of 7.24 µmol CO_2-C m^{-2} s^{-1} was measured in the TB soil on May 18th, while the lowest of 2.45 µmol CO_2-C m^{-2} s^{-1} in the SH soil on September 8th. Peak R_{eco} appeared to have coincided with the period of maximum soil moisture content.

Figures 5.5 and 5.6 compare night- and day-time R_{eco} measurements in the two soils. Overall there was no significant diurnal difference in measured R_{eco} from opaque chambers in both soils (insets in Figs 5.5 and 5.6). However, this did not hold on every sampling day. Significant diurnal differences were observed for May 18th, 23rd, 25th June 20th, 28th, 29th and September 8th in the TB soil (Figure 5-5) and similarly during June 29th, July 12th and August 31st in the SH soil (Figure 5-6). At these points R_{eco} was significantly greater during the day than the night.

There were no significant differences in the measured NEE in the two soils (Figure 5-7). The NEE was sensitive to changes in the incident light during the daytime (Figure 5.7). Variable cloud cover during the day contributed to rapid and frequent short-term changes in light intensity during the experiment.

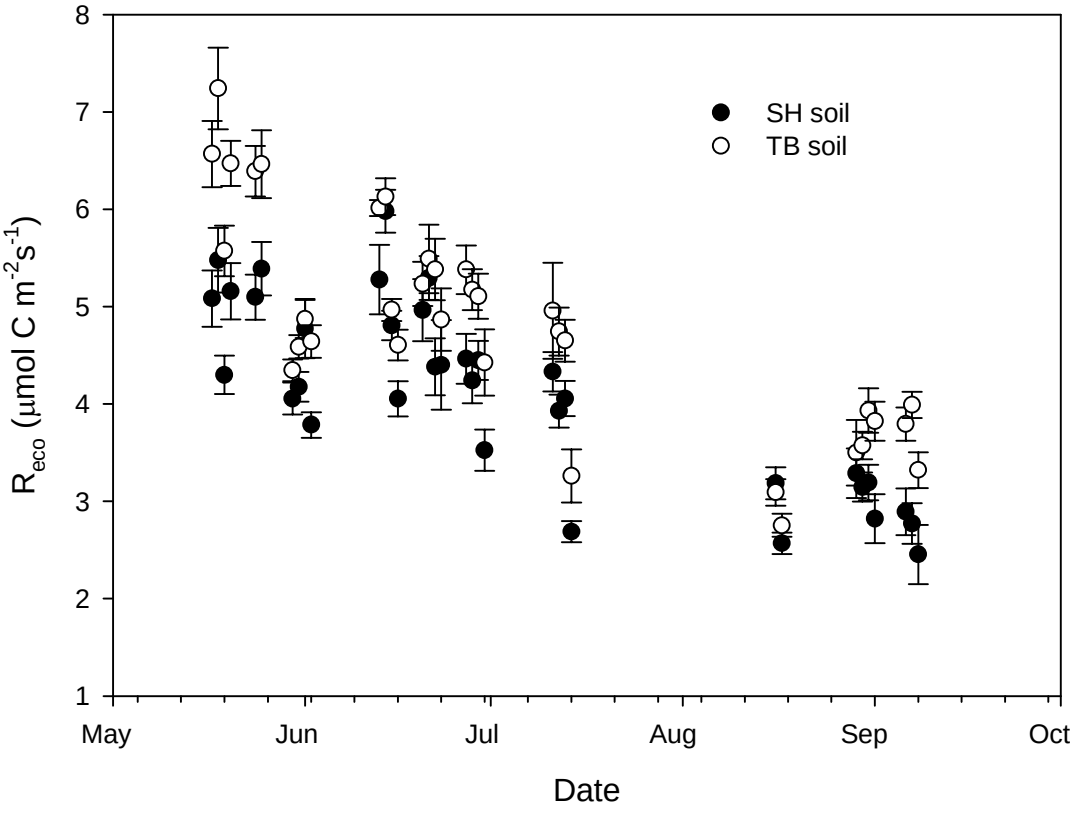

Figure 5-4 Mean daily R_{eco} measured in the opaque lysimeter chambers with SH and TB soils. Points show means of the six replicate chambers per soil (the actual number of measurements per day was randomly varied) and bars indicate standard errors.

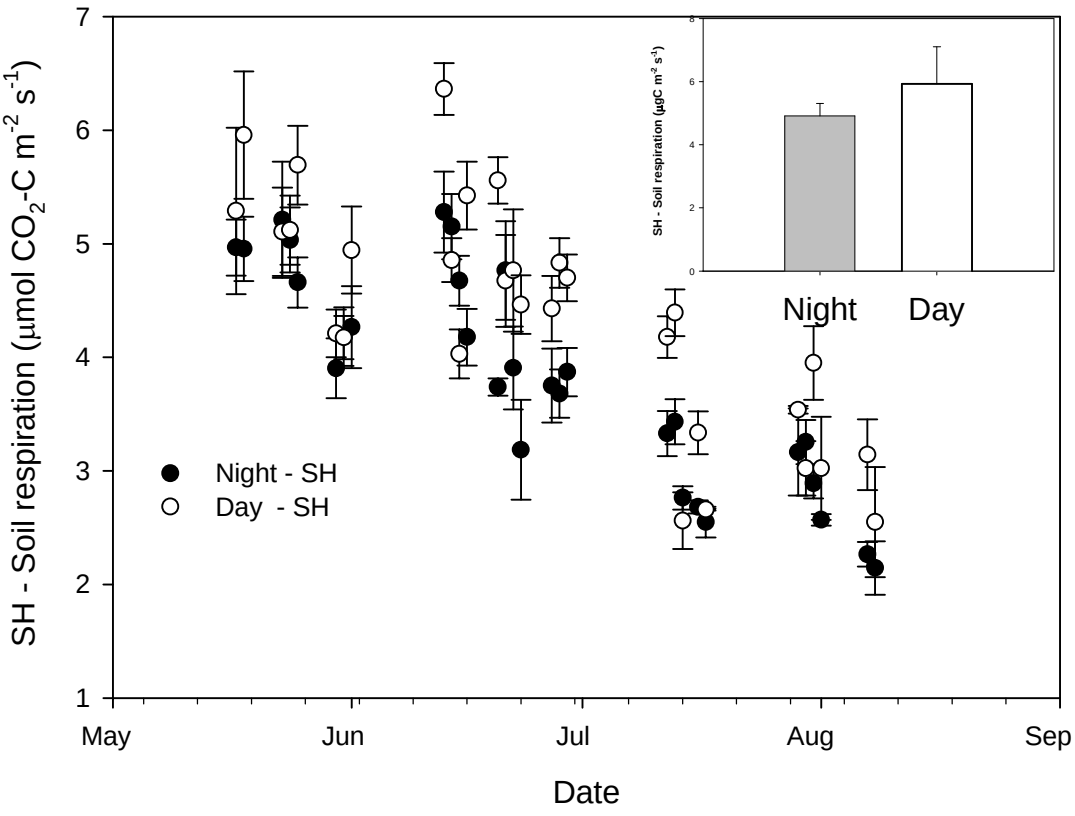

Figure 5-5 Comparison of night- and day-time R_{eco} measurements in lysimeter chambers with transparent lids and the sandy loam (SH) soil. Points show means of the six replicates (the actual number of measurements per day was randomly varied) and bars indicate standard errors. Columns in the inset graph show overall means and bars indicate standard errors. Both graphs have the same y-axis.

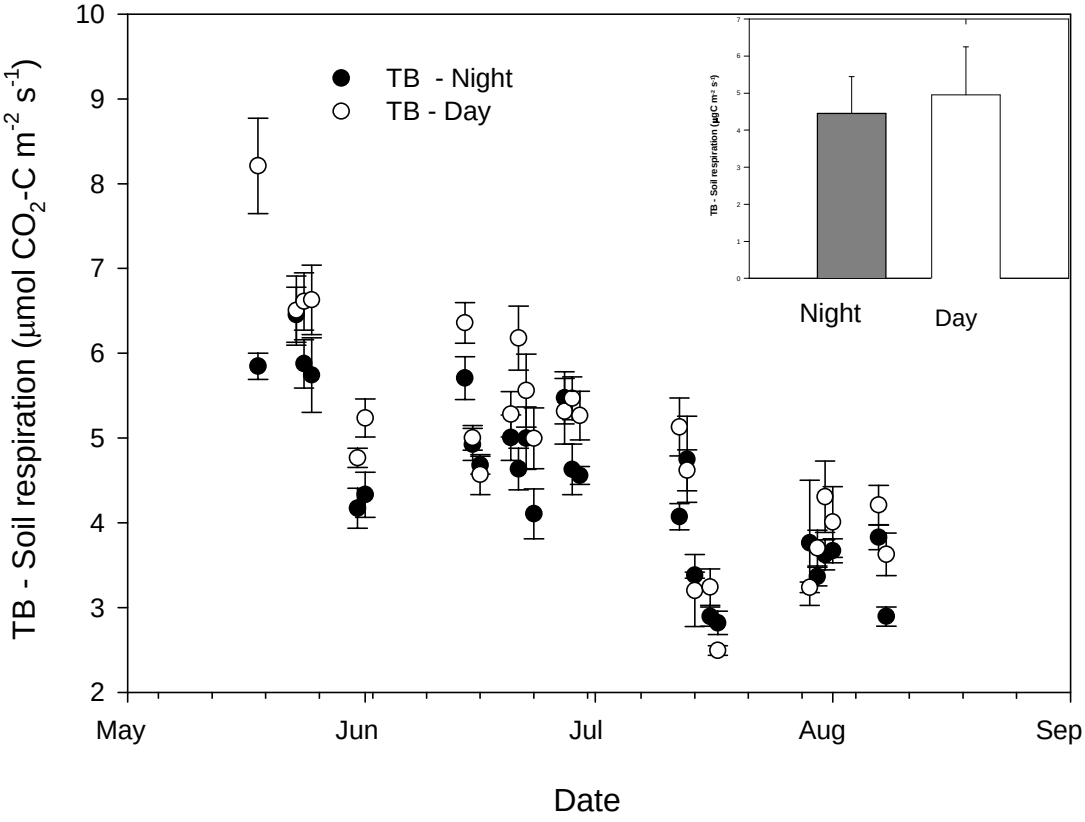

Figure 5-6 Comparison of night- and day-time R_{eco} measurements in lysimeter chambers with transparent lids and the clay loam (TB) soil. Points are daily means for measurements from six replicate chambers (the actual number of measurements per day was randomly varied) and bars show standard errors. Columns in the inset graph show overall means and bars indicate standard errors. Both graphs have the same y-axis.

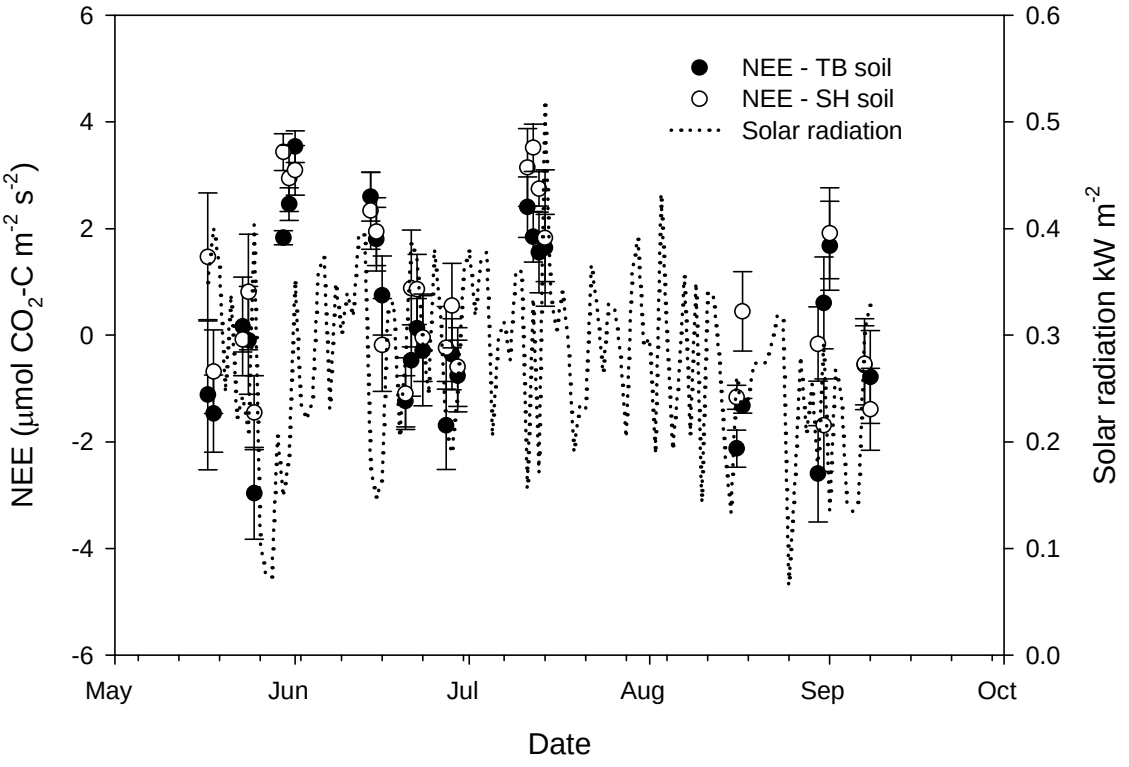

Figure 5-7 Measured NEE in opaque chambers with TB and SH soils and the corresponding solar radiation. Data points are daily mean NEE for six replicate chambers (the actual number of measurements per day was randomly varied) and bars show standard errors. Dotted line shows the daily mean solar radiation

5.3.2 Soil temperature and moisture measurements

The data retrieved from the moisture and temperature sensors at 60, 120, 250 and 500 mm were generally reliable with exceptions detailed below. Reliability was judged by their self-consistency over time, and by good correlations with rainfall and air temperature records. The exceptions were moisture readings at 60 and 120 mm which were very inconsistent and mostly erratic, particularly in the sandy loam, SH soil. Moisture records from 250 mm were most reliable throughout the experiment across all soil types and replicates.

Soil moisture content at 250 mm depth and daily rainfall for the duration of the experiment are shown in Figure 5-8. As expected the sandy SH soil was

significantly drier than the clay loam TB soil. At two time points, in May and August there were abrupt increases in soil moisture in both soils. Analysis of representative data for May 17th to June 23rd 2014 shows that the moisture fluctuations in the two soils were more variable at 60 and 120 mm compared to 250 and 500 mm (Figure 5-9).

Mean soil temperature values at 60 mm were not significantly different in the two soils. Maximum mean temperatures of 25.0 and 24.4 ^{o}C were observed in the SH and TB soils, respectively. The minimum mean temperature values in the SH and TB soils were 15.2 and 15.5 ^{o}C, respectively (Figure 5-10). Recorded air temperature during the experiment and total daylight hours are shown in Figure 5-11 and Figure 5-12, respectively.

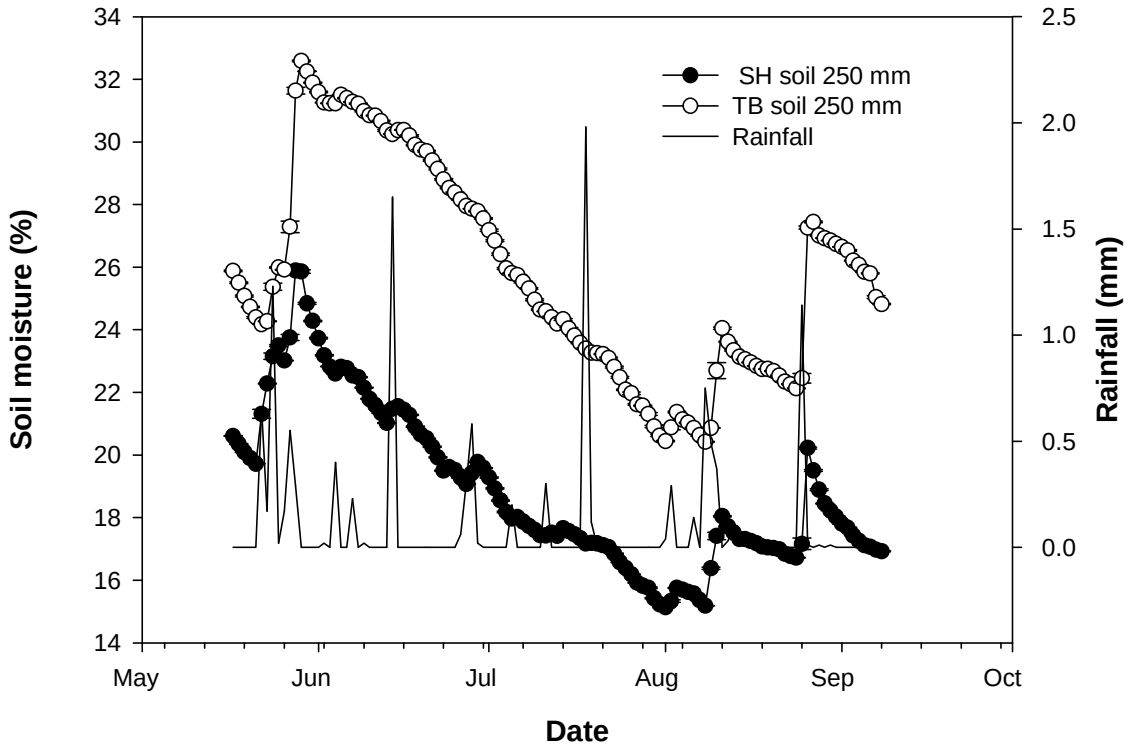

Figure 5-8 Volumetric soil moisture content in the lysimeter soils, SH and TB, at 250 mm depth and rainfall measured over the duration of the experiment (dates in 2014). Moisture data points show the means (n=12). Bars representing standard error are included but not visible.

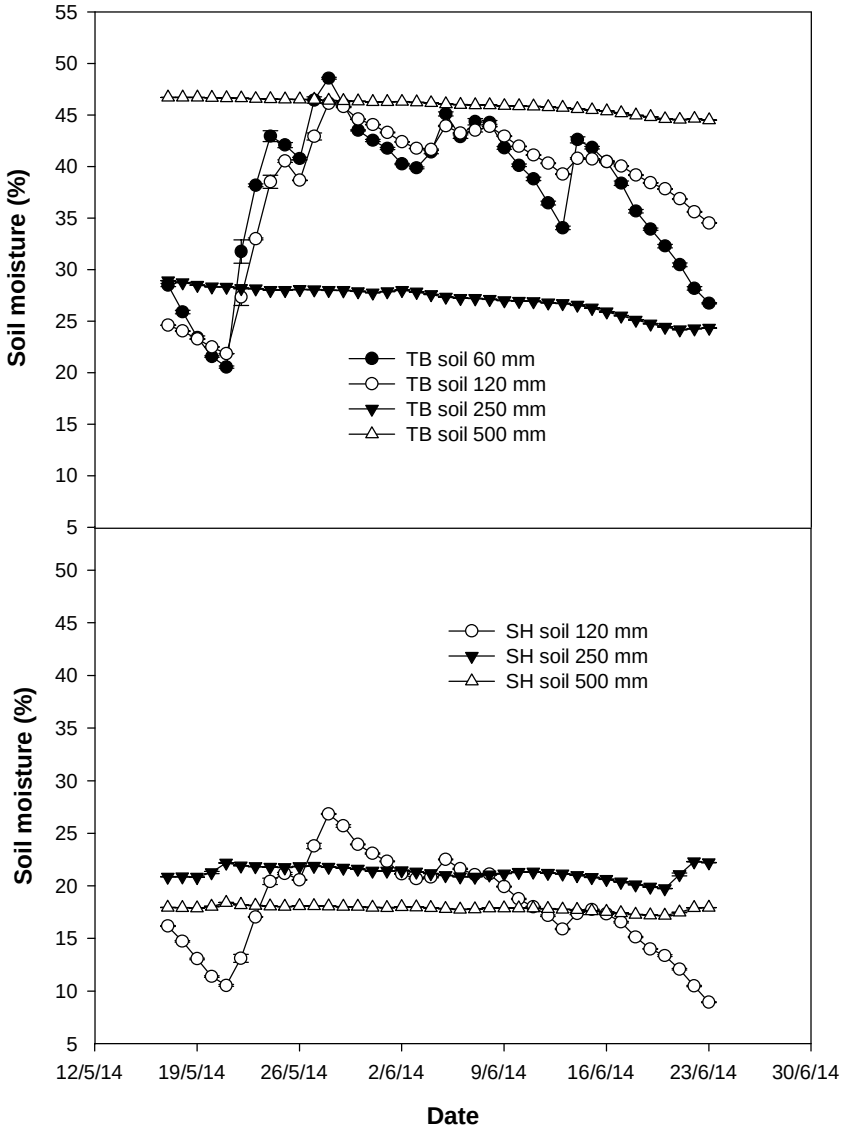

Figure 5-9 Comparison of daily volumetric soil moisture measurements from functional sensors in the representative lysimeters from TB and SH soils from May 17 to June 23, 2014. Points show means N=48 and bars show standard error.

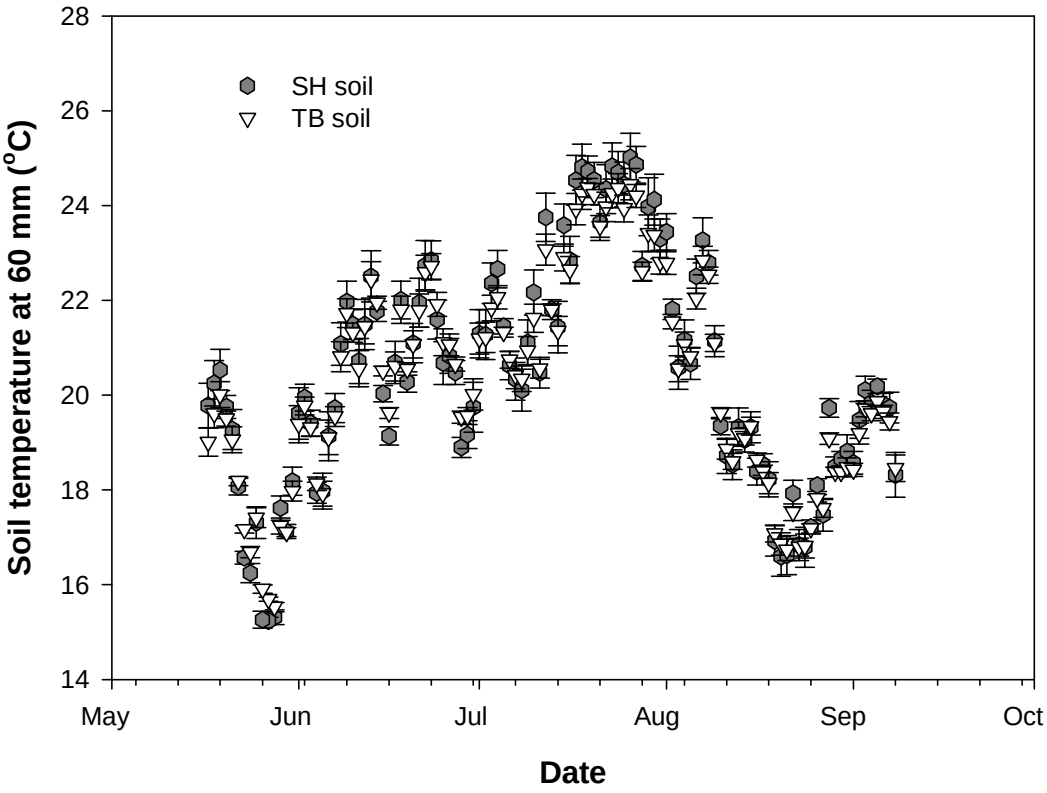

Figure 5-10 Mean daily soil temperature at 60 mm depth in lysimeters containing SH and TB soil. Data points are means of the 12 replicate lysimeters (except where sensors are faulty) and bars indicate standard errors.

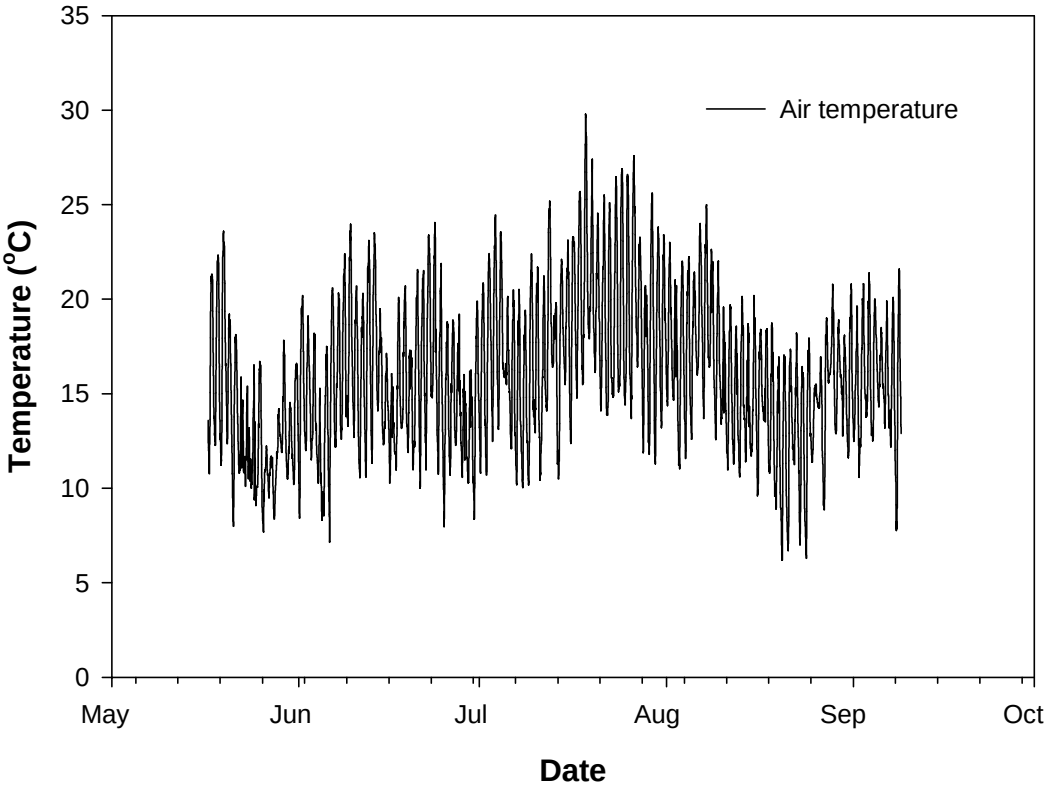

Figure 5-11 Recorded air temperature at the WFL at 30-minute intervals over the duration of the experiment

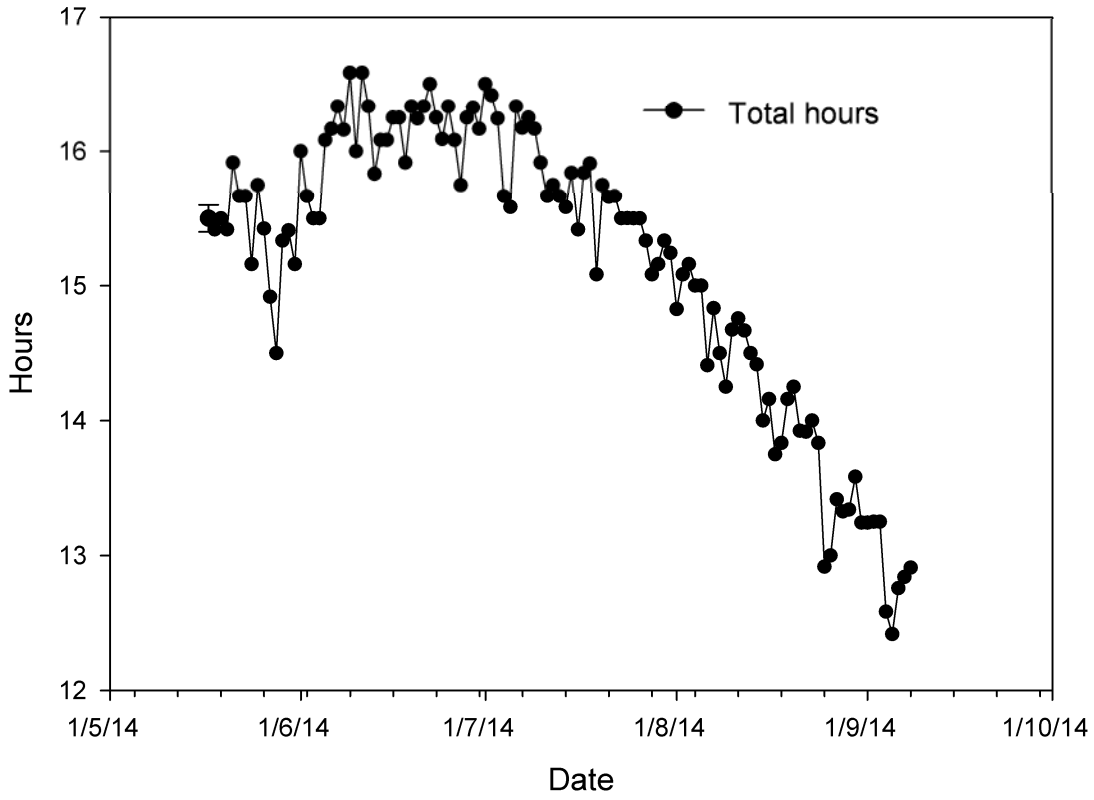

Figure 5-12 Total daily hours of measured light intensity greater than or equal to the minimum light energy measurement (0.01 kW m^{-2}) at the WFL. Points indicate actual period in hours.

5.3.3 The effect of environmental drivers on soil respiration

As was shown earlier in Figure 5-4 the analysis of covariance in Table 5-1 confirmed that the R_{eco} efflux patterns were different in the two soils. Overall, soil type interactions with moisture had the most significant impact on R_{eco} efflux (Table 5-1, $p<0.001$). When each soil was analysed separately using multiple linear regression analysis of the R_{eco} efflux against the drivers it showed that soil moisture and temperature had a significant positive linear relationship with R_{eco} in the sandy loam (SH) soils ($R^2 = 0.505$, $P<0.001$). Peak R_{eco} occurred when soil temperature (60 mm) was 25.42 ^{o}C and soil moisture at 250 mm was 20.24 %. In the clay loam (TB) soil temperature and day length had a significant and positive linear relationship with R_{eco}, with the peak R_{eco} occurring at soil temperature 25.42 ^{o}C and day length 15.42 hours ($R^2 = 0.185$, $p<0.001$).

Table 5-1 Analysis of covariance comparing the effect of selected drivers on R_{eco} in the two soils.

Effect	Univariate Tests of Significance				
	SS	Degr. of	MS	F	p
Intercept	4623.584	1	4623.584	2221.591	<0.0001
Soil	33.677	1	33.677	16.182	<0.0001
Soil*Soil temp 60 mm	6.367	1	6.367	3.059	0.08
Soil*Soil moisture 250 mm	23.541	1	23.541	11.311	0.00083
Soil*Light kW/h	1.377	1	1.377	0.662	0.416
Error	1109.282	533	2.081		

5.3.3.1 The effect of drivers on net ecosystem exchange (NEE)

There were no significant differences between the NEE measurements across the two soil types (p=0.941, n=538). Soil temperature and interactions between soil temperature and soil moisture appeared to have significant relationships with the NEE flux in both soils (Table 5-2). Multiple regression analysis showed a significant but weak positive relationship between the NEE flux and the combined effects of soil moisture and temperature (R^2 = 0.056, p<0.001).

Table 5-2 Results of multiple regression analysis indicating the drivers or their combinations, shown in red, having the strongest relationship with the NEE flux in both soils

	Level of	NEE Flux	NEE Flux	NEE Flux	NEE Flux	-95.00%	95.00%	Flux	Flux	-95.00%	95.00%
	Effect	Param.	Std.Err	t	p	Cnf.Lmt	Cnf.Lmt	Beta (ß)	St.Err.ß	Cnf.Lmt	Cnf.Lmt
Intercept		36.1082	14.59861	2.47340	0.013701	7.429	64.78746				
Soil type		-1.0651	14.59861	-0.07296	0.941865	-29.744	27.61410	-0.38213	5.237405	-10.6711	9.90685
Temp		-2.5077	0.75339	-3.32857	0.000935	-3.988	-1.02766	-2.70815	0.813606	-4.3065	-1.10980
Soil moisture		-1.2226	0.75325	-1.62311	0.105169	-2.702	0.25716	-0.67753	0.417429	-1.4976	0.14251
Solar R		-29.5177	43.00453	-0.68639	0.492774	-114.001	54.96550	-2.31035	3.365958	-8.9228	4.30214
Soil type * Temp	1	-0.0865	0.75339	-0.11480	0.908652	-1.567	1.39356	-0.63789	5.556777	-11.5543	10.27850
Soil type * moist	1	0.1314	0.75325	0.17450	0.861542	-1.348	1.61122	0.91600	5.249361	-9.3965	11.22847
Temp * moisture		0.1029	0.03814	2.69852	0.007190	0.028	0.17784	3.00594	1.113923	0.8176	5.19426
Soil type*Solar R	1	-43.7525	43.00453	-1.01739	0.309438	-128.236	40.73072	-5.43479	5.341877	-15.9290	5.05943
Temp *Solar R		3.3759	2.15717	1.56496	0.118197	-0.862	7.61371	5.77454	3.689890	-1.4743	13.02340
Moisture*Solar R		-0.7412	2.24907	-0.32956	0.741863	-5.160	3.67713	-1.10525	3.353702	-7.6937	5.48316
Soil type* Temp *Moisture	1	0.0011	0.03814	0.02771	0.977901	-0.074	0.07598	0.15349	5.538583	-10.7272	11.03415
Soil type*Temp * Solar R	1	2.5947	2.15717	1.20282	0.229590	-1.643	6.83251	6.83082	5.678983	-4.3257	17.98729
Soil type*moisture * Solar R	1	1.9061	2.24907	0.84753	0.397090	-2.512	6.32449	4.53257	5.347999	-5.9737	15.03882
Temp*Moisture*Solar R		-0.0813	0.11062	-0.73510	0.462610	-0.299	0.13600	-2.72290	3.704135	-9.9997	4.55394
Soil type*Temp *Moisture*Solar R	1	-0.1185	0.11062	-1.07082	0.284744	-0.336	0.09886	-6.06240	5.661440	-17.1844	5.05961

5.3.4 Estimating photosynthesis

Using the global mean R_{eco} values to estimate NPP a weak positive relationship was observed between estimated NPP and solar radiation in both soils (Figure 5-13, R^2 = 0.264 and R^2 = 0.227) in TB and SH, respectively. Using the daily mean values several strong relationships were observed on a few occasions. Some of the stronger relationships are given in Figure 5-14 (R^2 = 0.810, p<0.001), Figure 5-15 (R^2 = 0.671 p<0.001) and Figure 5-16 (R^2 = 0.818, p<0.001)

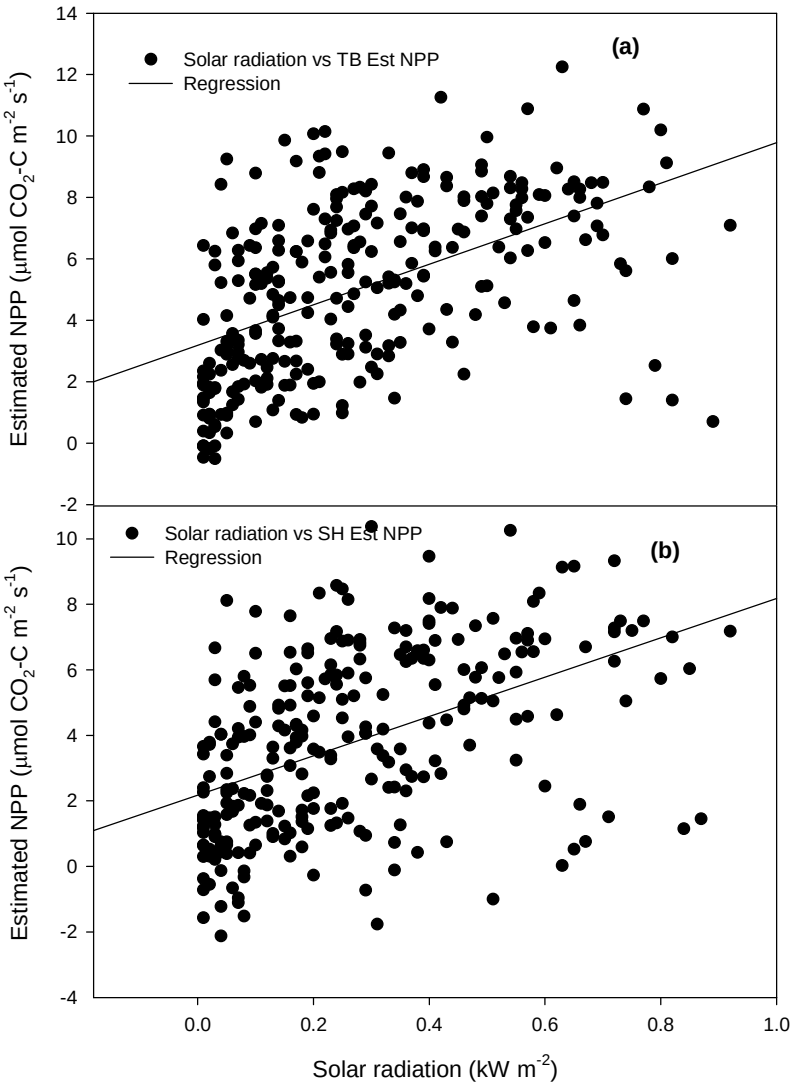

Figure 5-13 Regression of solar radiation vs estimated NPP based on the mean of all R_{eco} values in (a) the TB soil and (b) SH soil for the duration of the experiment

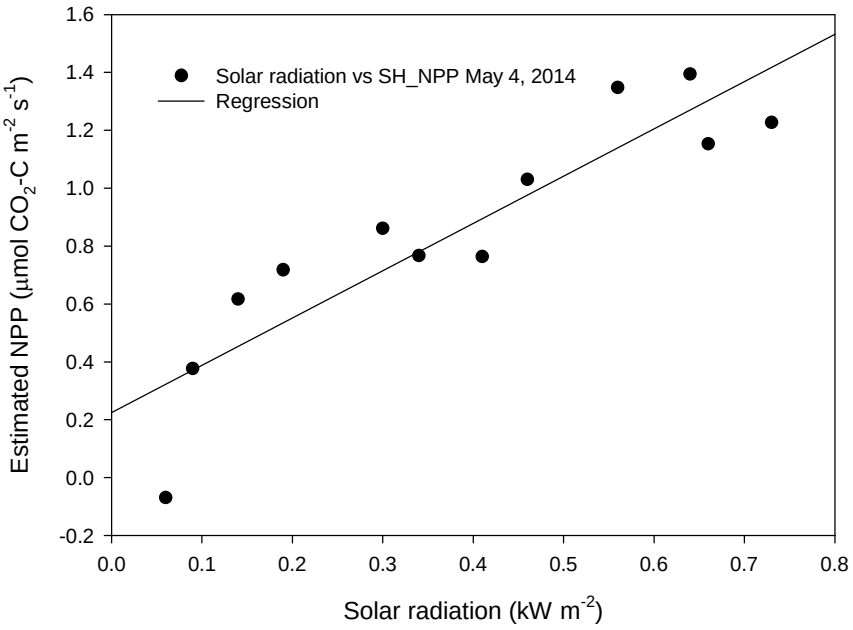

Figure 5-14 Regression of solar radiation vs estimated NPP values in SH soil for May 4th 2014 based on mean daily R_{eco} values

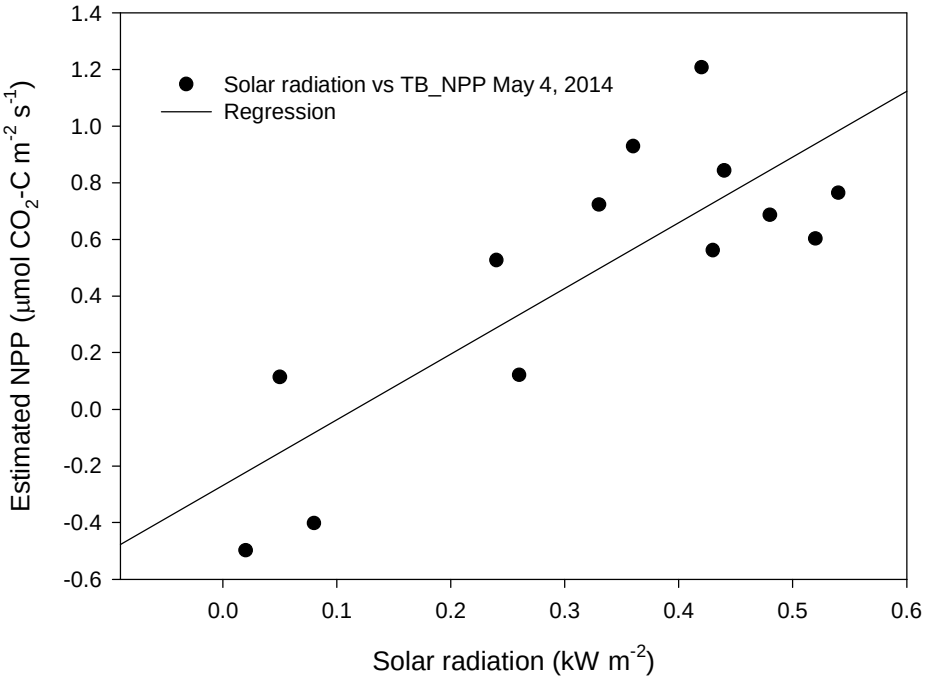

Figure 5-15 Regression of solar radiation vs estimated NPP values in TB soil for May 4th 2014 based on mean daily R_{eco} values

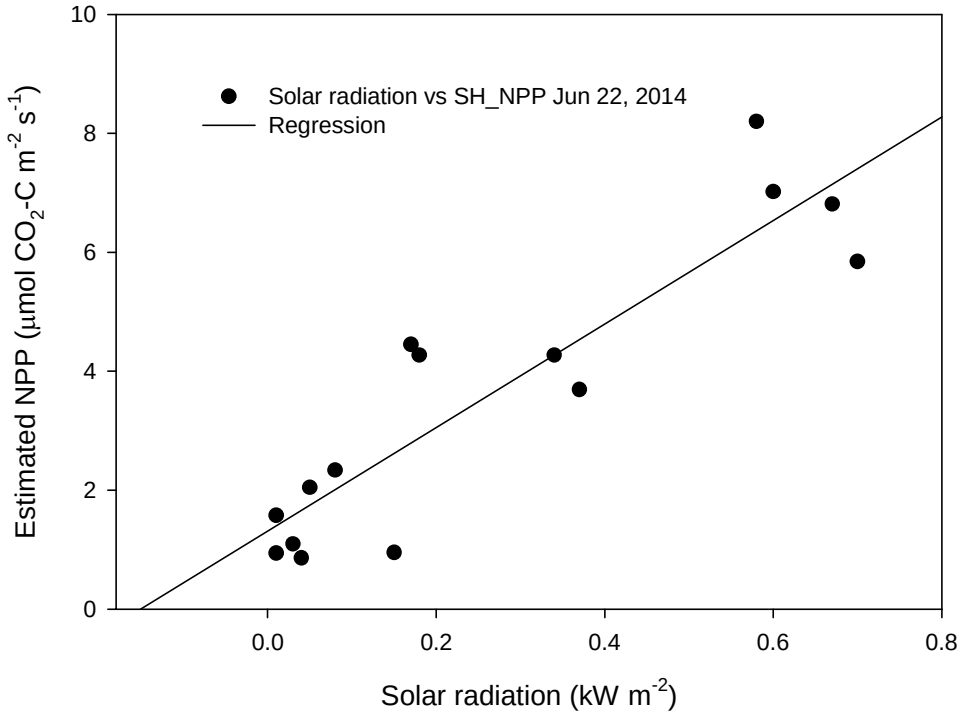

Figure 5-16 Regression of solar radiation vs estimated NPP values for June 22nd 2014 based on mean daily R_{eco} values

5.3.5 Dry matter and carbon content analysis

Total dry matter and carbon content and mean values per lysimeter and per unit area are shown in Table 5-3. The total harvested material coincides with a period which spans a slightly longer than the duration of the experimental measurements.

Table 5-3 Mean dry matter and C harvested per unit area in 12 of each soil type

Soil	dry matter ($g\,m^{-2}$)	St err	Total C ($g\,m^{-2}$)	Std err
SH	212.79	24.81	94.48	11.02
TB	300.40	16.75	133.38	7.44

5.3.6 Microbial community profile

Figure 5-17 shows distinct soil microbial phenotype differences between the SH and TB soils from the WFL lysimeters. Most of the variability (73.63%) was explained in principle component (PC) 1, while PC 2 accounted for 6.09% of the variability. Statistical significance of the differences between the soils is shown in Figure 5-17. The PLFAs 14:00, 15:00, 15:0ai 16:00, 16:0i, 16:1ω5, 17:0, ai17:0, cy17:00, 18:1ω9c and 18:2ω6, contributed most to differentiating the soil microbial phenotype. The SH soil had a significantly higher fungal to bacterial ratio compared to the TB soil (Figure 5-18).

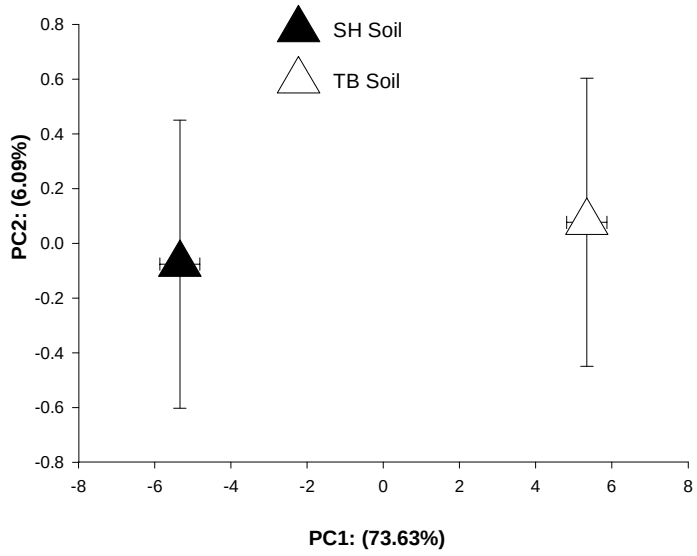

Figure 5-17 First and second principle components (PCs) of the phospholipid fatty-acid profiles derived from SH and TB lysimeter soil samples. The points show the mean value of coordinates for the PLFA profiles in the SH and TB soil. Bars show the standard error. Percent variation accounted for by PC shown in parentheses on each axis.

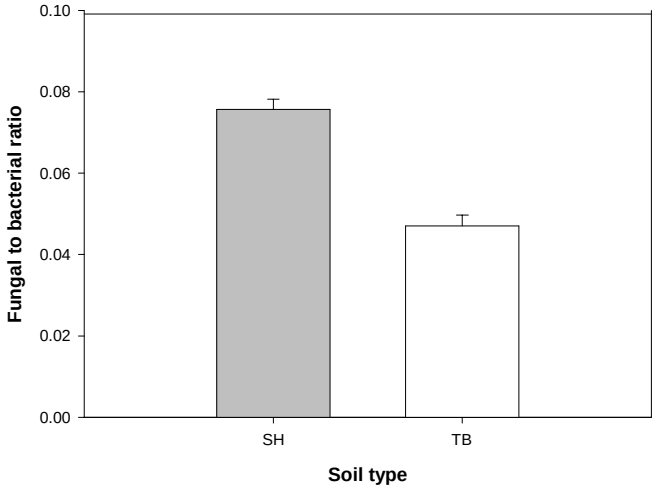

Figure 5-18 Fungal to bacterial ratio determined from the relative mole% of representative fungal and bacterial PLFA biomarkers. Columns show means and bars show standard error.

5.4 Discussion

Gas flux measurements

The findings in this study demonstrate the potential of the WFL system to measure and monitor soil respiration components and investigate relationships with key drivers on diurnal and seasonal scales. Overall, the system performed consistently without failures. Gaps in data collection were mainly due to alternative use of the system for concurrent experiments and analyses. However, there were important considerations that future work at this facility will benefit from. These are concerned with improving overall performance and particularly related to measuring and monitoring the key drivers of soil respiration. These are discussed in Chapter 6. I now focus on the significance of the results.

The range of values obtained for soil ecosystem respiration in this experiment was within the ranges reported for temperate grasslands systems (e.g. Bahn et al., 2008). Consistent with the incubation and glasshouse experiments, the more-fertile clay loam soil (TB) produced significantly greater levels of soil respiration (Figure 5-4). Although it was not possible to separate the flux into its soil and plant-derived components, it is likely that the greater R_{eco} values in the TB soil were due to greater contributions from plant respiration, and potentially also greater turnover of soil C through priming effects fuelled by plant C. This was supported by significantly greater dry matter production from the TB soil during the experimental period (Section 5.3.5).

Overall, there were no significant diurnal differences in the measured R_{eco} in either soil. On some days the day-time efflux was significantly greater than at the night-time efflux. These differences could have been due to variability in the number of measurements per day and the interval between measurements, as these were not standardised in this experiment (because the order of sampling the lysimeters was randomised). Varying diurnal responses with vegetation, climatic conditions and season are reported in the literature. Wood et al. (2013) found no diurnal differences in soil respiration measured in a humid tropical forest, while Barron-Gafford et al. (2011) reported significant diurnal differences

in soil respiration in a semi-arid temperate grassland, using chamber measurements.

Solar radiation and photosynthesis

Estimated NPP values showed positive correlations with light measurements when the NPP estimates were based on both global (all recorded values) or daily mean values of R_{eco}. However, high R^2 values, up to 0.818, were only found using daily estimates. The best relationships were found on days with at least 12 measurements of NEE efflux, with short time intervals between. The variability in the number of measurements per day and the time interval between measurements, in combination with available light on the respective days, could have account for the variable predictions of global and daily NPP.

Key drivers of R_{eco} and NEE

Temperature is highlighted in several studies as the primary driver of soil and ecosystem respiration (Thurgood et al., 2014; Lloyd and Taylor, 1994; Schimel, 1994; Yang et al., 2011). In this study, ecosystem respiration was consistently related to soil temperature measured at 60 mm depth in both soils. However, the maximum mean flux obtained for both soils did not correspond with the maximum temperature. This indicates that other drivers were also significant. In the sandy loam (SH) soil moisture was the other significant driver of respiration. Typically, a sandy soil would have lower moisture retention capacity compared to a clay loam. This is evident by the much lower available moisture in the SH soil compared to TB (Figures 5-8 and 5-9). It is likely that the sandy SH soil had moisture limitations not present in the clayey TB soil. Thus moisture was a more important driver of soil respiration in the SH soil. Moisture and temperature dependence of soil respiration has been explored by many researchers (e.g. Davidson and Janssens, 2006; Lamparter et al., 2009; Bahn et al., 2008; Wood et al., 2013; Barron-Gafford et al., 2011; Yang et al., 2011; Carbone et al., 2008). There is evidence that the temperature sensitivity of soil respiration can be affected by soil moisture availability, and conditions of both limited and excess moisture can complicate assessments of the temperature dependence of soil respiration (Davidson and Janssens, 2006; Yang et al., 2011). A

comparison of moisture data from functional sensors in Figure 5-9 indicates that moisture measured at 250 mm depth may have underestimated soil moisture at the zone of temperature measurements. The temperature dependence of soil respiration in field experiments has been shown to vary with soil depth (Graf et al., 2008) and accurate descriptions of moisture conditions at depths relevant to temperature are essential to reliably account for any covariance effects. However, analysis of moisture measurements at 120 mm in SH soil and 60 mm in TB soil using the limited dataset (Figure 5-9) did not improve the covariance and regression relationships with R_{eco} and NEE in either soil.

In the clay loam soil (TB), soil temperature and day length were the most significant drivers of R_{eco}. Unlike the SH soil, moisture was not as limiting and therefore did not feature as a major driver, although the soils were under the same climatic conditions. Photoperiod (day length) was taken as a surrogate for photosynthesis (NPP), consistent with its use as an integral component models predicting C assimilation in grasslands (Wulfes et al., 1999). Considering that the clay loam was more fertile and had greater dry matter production than the sandy soil, it is likely there was greater C assimilation with increasing day-length, and that this increased root exudation. The experiments reported in Chapters 3 and 4 clearly indicated that the TB soil had the greater potential for priming effects and this could have contributed to the observed effect of photoperiod on soil respiration. Priming effects probably did not occur in the SH soil or occurred at much lower levels because of soil moisture deficits and soil fertility limitations discussed in Chapters 3 and 4. Another consideration is the distinct difference in the microbial community profiles between the two soils shown by PLFA analysis (Section 5.3.6). The higher proportion of fungal to bacterial microbes in the SH relative to the TB soil are consistent with the greater acidity of the SH soil and the high volume of woody fragments in it, they being decomposed mostly by fungi. Greater bacterial dominance and microbial biomass in the TB soil could be indicative of greater capacity to utilise labile C for the production of enzymes for microbial decomposition (Bardgett et al., 1996).

There were no significant differences in NEE estimates between the two soils. NEE was most influenced by soil temperature and interactions between soil temperature and moisture. While the effect of moisture and temperature stood out from the covariate analysis, the regression analysis of NEE against moisture and temperature produced very weak results and the resulting models could not reliably predict NEE efflux for periods not measured.

Modelling R_{eco} and NEE

An objective of this study was to measure and model R_{eco} and NEE. There was success in measuring R_{eco} and NEE but not in generating valid models. This was largely related to the poor model fitting from the regression analysis. Since the values of carbon inputs (NPP) and outputs (R_{eco}) could not be reliably estimated over the growing season, it was not possible to compare this with the carbon balance predicted from harvested dry matter.

5.5 Conclusions

It was demonstrated that the automated chamber system at the WFL can be used to:

(1) Produce reliable measurements of R_{eco} and NEE for use in obtaining relationships with key drivers.
(2) Reliably measure short-term changes in soil temperature and moisture at different depths, though not soil moisture at depth <250 mm in the sandy (SH) soil.

The results also confirmed that:

(3) The short-term changes in soil moisture were significantly different in the two soils but there was no significant difference in their soil temperature variations.
(4) Soil temperature was a major diver of R_{eco} in both soils. In the clayey soil day length also featured as a driver of R_{eco} and in the sandy soil, moisture featured as a driver.
(5) It was not possible to obtain reliable estimates of NEE over the season from the data collected in this experiment.

5.5.1 Further considerations

The dates reflected in this thesis might suggest that most of the work was concentrated in a few months during 2014. However, it must be noted that the WFL was a newly constructed facility without any history or much background information. This was the first study at the WFL. Commissioning and validation of the IRMS was prolonged and thwarted by several teething issues. Operating procedures had to be developed and much of the work initially concentrated on method development and operationalising the WFL systems. There were several pre-experiment glasshouse trials to evaluate different grass species for suitability to growing conditions and susceptibility to shading. Shading then gave way to clipping as the option for altering rhizodeposition in the glasshouse experiment.

Chapter 6: Synthesis and recommendations for future work

6.1 Summary

This study was conducted to contribute to a better understanding of the processes regulating soil carbon balances, specifically the mechanisms of soil-plant interactions that release stored C from soils through rhizosphere priming effects (RPE), using two contrasting soils. The project also supported continuing work at the Wolfson Field Laboratory (WFL) at Cranfield to monitor and model greenhouse gas emissions from soils, initially CO_2. The initial plan was to use a combination of laboratory incubation, glasshouse (with potted plants) and field experiments to investigate RPEs in two contrasting soils. As the field system was not yet fully developed for making the isotope measurements required for priming studies, the RPE study was limited to the laboratory and the glasshouse (Chapters 3 and 4). Additionally, the capacity of the WFL to measure and monitor soil ecosystem respiration and net ecosystem exchange and their dependence on key drivers was evaluated (Chapter 5).

The results highlight characteristic differences in RPEs between the two soils in both the unplanted and planted systems used in Chapter 3 and 4, respectively. Generally, the direction of RPEs was the same in both soils but the amount and intensity of priming were different in the two soils. The WFL automated chamber system produced reliable measurements of ecosystem respiration and net ecosystem exchange that were useful for monitoring and evaluating their relationships with moisture, temperature and photosynthesis. The capacity of the WFL for high resolution measurements of the soil C turnover processes and associated drivers over diurnal and seasonal time scales is a tremendous platform for future research.

6.2 Rhizosphere priming effects in the unplanted system

The laboratory incubation experiment simulated rhizosphere priming effects in grassland systems by adding carbon substrates to two contrasting, unplanted soils at rates consistent with rhizodeposition in temperate grasslands at the

peak of the growing season. The substrates used were a maize root extract and sucrose, i.e. with C_4 isotope signatures, and the soils had C_3 isotope signatures, so it was possible to determine priming effects on the basis of the isotope composition of respired CO_2. The soils differed strongly in their physical, chemical and biological properties. As far as I am aware, no similar study has been made before, especially on contrasting soils.

The root extract represented a more realistic substrate having variable water soluble compounds and nutrients known to be largely responsible for RPEs. By comparison sucrose was expected to have a unidirectional high value impact as an energy source. I therefore hypothesised that the substrates would generate different priming effects in each soil and the soils would have different priming effects based on their inherent differences.

In the acidic sandy SH soil, the two substrates had similar patterns of priming. However in the fertile clayey TB soil, sucrose produced greater priming effects per unit of C applied than the maize root extract, and the priming increased over time with sucrose but decreased after one week with the maize root extract. I explain these differences in terms of microbial access to soil N. I hypothesise that in the more-fertile TB soil, provision of sucrose provided energy for growth of the microbial community while generating greater demand for N, which was met by increasing SOM turnover over time. Whereas in the TB soil with maize root extract, the supply of N increased over time with continuing addition of the substrate, which contained N, so the need for accessing soil N through priming declined. By contrast, in the infertile SH soil, the availability of mineralizable soil N was such that the N supply continued to limit microbes with either of the substrates, so SOM mining progressed at the same rate with either substrate. Because the addition of substrate was in proportion to the soil microbial biomass, which was smaller in the SH soil, the cumulative addition of N in the SH soil was smaller. The results in this study agree with the theory of microbial N mining generated by stoichiometric C:N imbalances in the soil microbial biomass.

6.3 Rhizosphere priming effects in the planted system

Results from the glasshouse experiment corroborated the findings in Chapter 3. In this experiment, a C_4 grass was grown in the two C_3 soils from Chapter 3, and priming effects assessed based on the isotope signature of soil respiration. To produce differences in rhizodeposition, the grass was either clipped or left unclipped.

The patterns of soil respiration (R_S) and rhizosphere priming effects (RPE) were strongly influenced by differences in gross primary production (GPP) between the soils and clipping treatments. Plant growth was much better in the TB soil and was associated with earlier onset of RPE and greater overall RPEs compared to the SH soil. Clipping the grass drastically reduced GPP, and resulted in reallocation of photo-assimilates to shoot regeneration, and so probably reduced rhizodeposition. Rhizosphere priming effects were negligible in the clipped treatment in the SH soil. The clipping frequency was such that grass growth in the SH soil was severely impaired. The results clearly show that GPP is a key driver of RPEs in these soils and potentially even greater losses of stored soil C could occur during the more active growing periods when GPP is highest.

6.4 Evaluation of the WFL automated chamber system

High resolution field measurements of soil respiration with simultaneous measurement and monitoring of key drivers are essential for improving understanding of soil C turnover. The automated chamber system at the WFL provides a platform that has now been validated for the conduct of future studies on soil carbon dynamics (Chapter 5).

Several issues have surfaced, particularly related to the experimental design and scheduling of sampling events to obtain data with sufficient frequency to characterise short- and medium-term patterns. The results showed that while continuous sampling is valuable, it is also important to maximize day-light hours for NEE flux measurements. The number of measurements made from each soil is also important; equal measurements will allow for more accurate analysis

of soil effects on flux measurements. As data is now available on the system's diurnal R_{eco} patterns, a way forward can be to focus on improving the linkages with drivers. Standardization of measurements is likely to improve outcomes and generate data suitable for modelling. This might be achieved by limiting measurements to daylight hours and setting a prescribed minimum number of samples from both opaque and transparent chamber measurements in each soil.

The assessment of the relative dependence of R_{eco} and NEE on the drivers explored in this study was hinged on the reliability of the measurements made. Overall, measurements of soil respiration and their drivers allowed for a deliberate evaluation of the sensitivity of the soil respiration to soil moisture and temperature changes in two contrasting soils. The availability of more data would mostly likely have improved the outcomes and enhanced the possibility of generating sensitivity models for the system. The malfunctioning of sensors during the experiment needs to be minimized and addressed properly to reduce the risk similar occurrences in the future. While routine checks were attempted, the system design does not allow for easy access to sensors that may need to be repaired or replaced. An appropriate maintenance regime that can minimize the risk of disruptions and malfunctioning components is essential going forward.

The lysimeters are now well into their fifth year and may need replacing at some point. Consideration should be given to replacing the current mixed-grass species (including 70% perennial rye grass) with a single cultivar. This could contribute to reducing variability across and within the soil types. A possibility could be the C_4 species Bermuda grass, *Cynodon dactylon*, which performed very well on both soils in glasshouse trials. It has also adapted to parts of Southern England and these locally available cultivars are likely to perform well at the WFL. The primary benefit being the ability to separate of soil- and plant-derived C in future lysimeter studies, using stable isotope techniques.

Overall, the study demonstrated the immense potential of the WFL, which must be harnessed to explore the functionality of the soil system in field conditions.

6.5 Future work

In Chapter 3, the quality of substrate in conjunction with soil characteristics appeared to influence how RPEs influenced soil C turnover in the two soils studied. The maize root extract was analysed for C and N and polysaccharides. However it is likely that the extract contained nutrients and other compounds that impacted on the experiment. These factors and nutrient mineralization rates in the soils need be studied further to test the priming mechanisms proposed here.

The results in Chapters 3 and 4 are consistent with several previous priming studies. It would have been helpful to have analysed the $\delta^{13}C$ of the soil microbial biomass during and immediately after incubation and upon destructive sampling in Chapters 3 and 4, respectively. This would have provided further confirmation of priming effects. Future work could include a larger experiment with more replicates to allow for periodic sampling to measure soil microbial changes, and to analyse for critical enzymes linked to SOM mineralisation and N release. The possible effects of added mineral N and P should be further explored by measuring N and P dynamics. It would be interesting to observe whether declines in PE would occur in the SH soil if the experiment was extended, and likewise in the TB soil, whether further additions of maize root extract would reduce priming to negative values. Hence this experiment could be repeated for an extended period. The results may provide further evidence in support of the priming mechanisms proposed.

In Chapter 4, the subtropical grass species Kikuyu grass (*Pennisetum clandestinum*) was selected as a C_4 plant with a growth habit similar to rye grass, *Lolium perenne*. However I was not able to determine whether this species induced changes to the microbial community phenotype relative to the microbial assessments of the WFL lysimeter soils, which contained mostly ryegrass species (Chapter 5). The possible consequences of C substrate identity and diversity on soil functions require investigation, particularly as

regards whether the different sources of fresh C in Chapter 3 and 4 induced significant changes in the soil microbial community profiles.

In Chapter 5, time constraints precluded the use of non-linear statistical methods to analyse the data generated. However it is possible that non-linear models could better account for the seasonal flux dynamics based on the drivers evaluated. Automation of chamber lid closure and opening relied on the IRMS software. Therefore measurements were only possible when the IRMS was not otherwise in use. This limited the frequency of measurements which could have improved outcomes. The light measurement system installed at the facility was not the most appropriate for this study. It measured the full spectrum of solar radiation as opposed to photosynthetically active radiation (PAR), which would have been more relevant for modelling NPP. The approximation that all measured light was within the PAR range could have contributed to the poor correlations between light and estimated NPP. Another contributing factor may have been reduced light incidence from the Perspex material of the chamber lids and the reflection of light from the chamber walls which had a white inner surface. Faulty moisture sensors particularly in the uppermost layers of the sandy soil profile precluded a more realistic assessment of the covariance of moisture and temperature with respiration measurements. Once the issues highlighted above are addressed, this experiment could be repeated and extended over an entire year or consecutive years, if possible, to investigate how C dynamics change in the systems during the cooler months and in transition periods between cooler to warmer seasons and vice-versa.

Once the capability of the WFL is improved to facilitate isotope labelling and PE studies in the field, the results generated can be compared with those reported in this study. This could potentially provide some guidelines as to how well the experimental conditions in the laboratory and glasshouse reflect actual field scale C dynamics and related processes.

Notwithstanding an expanding body of knowledge on rhizosphere priming effects, this study makes a unique contribution to elucidating the mechanisms underlying the related processes. It is rare to have multiple assessments of contrasting soils on the scales studied in this experiment. Use of a maize root extract and comparison with sucrose on contrasting soils has not been reported before. Studies reporting on the effects of substrate diversity on priming have deliberately excluded nutrients or nutrient containing compounds to avoid so-called 'confounding' effects. However, these confounding effects are not excluded in isotope (plant) labelling experiments, which have been helpful to advance current understanding of priming mechanisms. I advance that the use of plant root extracts as reported in this study may provide more realistic representations of rhizodeposition in laboratory incubation studies investigating rhizosphere priming effects. There are still many unknowns about the mechanisms driving RPEs and further research is required. However, microbial nutrient mining of SOM best explained my findings in the both priming experiments. The methods developed in this study will allow for high temporal and spatial resolution measurements of R_S and NEE in field conditions, using stable isotope methods to separate fluxes into plant- and soil-derived components.

Chapter 7: References

Arrhenius, S. (1898), "The effect of constant influences upon physiological relationships", *Scandinavian Archives of Physiology,* vol. 8, pp. 367-415.

Atkin, O. K., Edwards, E. J. and Loveys, B. R. (2000), "Response of root respiration to changes in temperature and its relevance to global warming", *New Phytologist,* vol. 147, no. 1, pp. 141-154.

Badri, D. V. and Vivanco, J. M. (2009), "Regulation and function of root exudates", *Plant, Cell & Environment,* vol. 32, no. 6, pp. 666-681.

Bahn, M., Rodeghiero, M., Anderson-Dunn, M., Dore, S., Gimeno, C., Drösler, M., Williams, M., Ammann, C., Berninger, F., Flechard, C., Jones, S., Balzarolo, M., Kumar, S., Newesely, C., Priwitzer, T., Raschi, A., Siegwolf, R., Susiluoto, S., Tenhunen, J. and Wohlfahrt, G. (2008), "Soil respiration in European grasslands in relation to climate and assimilate supply", *Ecosystems,* vol. 11, no. 8, pp. 1352-1367.

Bahn, M., Schmitt, M., Siegwolf, R., Richter, A. and Brüggemann, N. (2009), "Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on a diurnal timescale?", *New Phytologist,* vol. 182, no. 2, pp. 451-460.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, R. and Vivanco, J. M. (2006), "The role of root exudates in rhizosphere interactions with plants and other organisms", *Annual Review of Plant Biology,* vol. 57, pp. 233-266.

Baldocchi, D. D. (2008), "Turner Review no. 15. Breathing of the terrestial biosphere: lessons from a global network of carbon dioxide flux measurement systems", *Australian Journal of Botany,* vol. 56, no. 1, pp. 1-26.

Bardgett, R. D., Hobbs, P. J. and Frostegård, Å. (1996), "Changes in soil fungal: bacterial biomass ratios following reductions in the intensity of management of an upland grassland", *Biology and Fertility of Soils,* vol. 22, no. 3, pp. 261-264.

Bardgett, R. D. (2011), "Plant-soil interactions in a changing world", *F1000 Biology Reports,* [Online], vol. 3, no. 16, pp. 16/11/2011 available at: http://f1000.com/reports/b/3/16.

Barron-Gafford, G. A., Scott, R. L., Jenerette, G. D. and Huxman, T. E. (2011), "The relative controls of temperature, soil moisture, and plant functional group on soil CO2 efflux at diel, seasonal, and annual scales", *Journal of Geophysical Research: Biogeosciences,* vol. 116, no. G1.

Baudoin, E., Benizri, E. and Guckert, A. (2003), "Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere", *Soil Biology and Biochemistry,* vol. 35, no. 9, pp. 1183-1192.

Beare, M. H., Cabrera, M. L., Hendrix, P. F. and Coleman, D. C. (1994), "Aggregate-protected and unprotected organic matter pools in conventional-tillage and no-tillage soils", *Soil Science Society of America Journal,* vol. 58, pp. 787-795.

Benizri, E., Nguyen, C., Piutti, S., Slezack-Deschaumes, S. and Philippot, L. (2007), "Additions of maize root mucilage to soil changed the structure of the bacterial community", *Soil Biology and Biochemistry,* vol. 39, no. 5, pp. 1230-1233.

Beylich, A., Oberholzer, H., Schrader, S., Höper, H. and Wilke, B. (2010), "Evaluation of soil compaction effects on soil biota and soil biological processes in soils", *Soil and Tillage Research,* vol. 109, no. 2, pp. 133-143.

Blagodatskaya, E. and Kuzyakov, Y. (2008), "Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review", *Biology and Fertility of Soils,* vol. 45, pp. 115-131.

Blagodatskaya, E. and Kuzyakov, Y. (2011), "Priming effects in relation to soil conditions - mechanisms", in Glinski, J., Horabik, J. and Lipiec, J. (eds.) *Encyclopedia of Agrophysics,* Springer, Dordrecht, Germany, pp. 657-666.

Blagodatskaya, E., Blagodatsky, S., Anderson, T. H. and Kuzyakov, Y. (2007), "Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies", *Applied Soil Ecology,* vol. 37, no. 1-2, pp. 95-105.

Blagodatskaya, E. V. and Anderson, T. (1998), "Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO_2 of microbial communities in forest soils", *Soil Biology and Biochemistry,* vol. 30, no. 10–11, pp. 1269-1274.

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T. and Kuzyakov, Y. (2010), "Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition", *Soil Biology and Biochemistry,* vol. 42, no. 8, pp. 1275-1283.

Bligh, E. G. and Dyer, W. J. (1959), "A rapid method of total lipid extraction and purification", *Canadian Journal of Biochemistry and Physiology,* vol. 37, no. 8, pp. 911-917.

Bonkowski, M. (2004), "Protozoa and plant growth: the microbial loop in soil revisited", *New Phytologist,* vol. 162, no. 3, pp. 617-631.

Brady, N. C. and Weil, R. R. (2008), *The Nature and Properties of Soil,* 14th ed, Prentice Hall, Harlow, UK.

Brookes, P. C., Cayuela, M. L., Contin, M., De Nobili, M., Kemmitt, S. J. and Mondini, C. (2008), "The mineralisation of fresh and humified soil organic matter by the soil microbial biomass", *Waste Management,* vol. 28, no. 4, pp. 716-722.

Brookes, P. C., Kemmitt, S. J., Addiscott, T. M. and Bird, N. (2009), "Reply to Kuzyakov et al.'s comments on our paper: 'Kemmitt, S. Lanyon, C. V., Waite, I.S., Wen, Q., O'Donnell, A.G., Brookes, P.C., 2008. Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective. Soil Biology and Biochemistry 40, 61–73'", *Soil Biology and Biochemistry,* vol. 41, no. 2, pp. 440-443.

Buchmann, N. and Ehleringer, J. R. (1998), "CO_2 concentration profiles, and carbon and oxygen isotopes in C3 and C4 crop canopies", *Agricultural and Forest Meteorology,* vol. 89, no. 1, pp. 45-58.

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

Carbone, M. S., Winston, G. C. and Trumbore, S. E. (2008), "Soil respiration in perennial grass and shrub ecosystems: Linking environmental controls with plant and microbial sources on seasonal and diel timescales", *Journal of Geophysical Research: Biogeosciences,* vol. 113, no. G2.

Carrillo, Y., Pendall, E., Dijkstra, F. A., Morgan, J. A. and Newcomb, J. M. (2011), "Response of soil organic matter pools to elevated CO_2 and warming in a semi-arid grassland", *Plant and Soil,* vol. 347, no. 1, pp. 339-350.

Cerling, T. E., Solomon, D. K., Quade, J. and Bowman, J. R. (1991), "On the isotopic composition of carbon in soil carbon dioxide", *Geochimica et Cosmochimica Acta,* vol. 55, no. 11, pp. 3403-3405.

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E. and Kuzyakov, Y. (2014), "Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories", *Global Change Biology,* vol. 20, no. 7, pp. 2356-2367.

Cheng, W. and Kuzyakov, Y. (2005), "Root effects on soil organic matter decomposition", in Zobel, R. W. and Wright, S. F. (eds.) *Roots and soil*

management: interactions between roots and the soil, American Society of Agronomy, Madison, WI, pp. 119-143.

Cheng, W. (2009), "Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C–N budgets", *Soil Biology and Biochemistry,* vol. 41, no. 9, pp. 1795-1801.

Cheng, W., Parton, W. J., Gonzalez-Meler, M. A., Phillips, R., Asao, S., McNickle, G. G., Brzostek, E. and Jastrow, J. D. (2014), "Synthesis and modeling perspectives of rhizosphere priming", *New Phytologist,* vol. 201, no. 1, pp. 31-44.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R. B., Piao, S. and Thornton, P. (2013), "Carbon and Other Biogeochemical Cycles", in Stocker, T. F., Qin, D., Plattner, G. K., et al (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I tothe Fifth Assessment Report of the Intergovernmental Panel on Climate Change ,* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 465-570.

Clay, G. D. and Worrall, F. (2013), "The response of CO_2 fluxes from a peat soil to variation in simulated sheep trampling", *Geoderma,* vol. 197–198, pp. 59-66.

Clemente, J. S., Simpson, M. J., Simpson, A. J., Yanni, S. F. and Whalen, J. K. (2013), "Comparison of soil organic matter composition after incubation with maize leaves, roots, and stems", *Geoderma,* vol. 192, pp. 86-96.

Coleman, D. C., Reid, C. P. P. and Cole, C. V. (1983), "Biological strategies of nutrient cycling in soil systems", *Advances in Ecological Research,* vol. 13, pp. 1-55.

Conant, R. T. (2010), *Challenges and opportunities for carbon sequestration in grassland systems. A technical report on grassland management and climate change mitigation,* FAO, Rome.

Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., Evans, S. E., Frey, S. D., Giardina, C. P., Hopkins, F. M., Hyvönen, R., Kirschbaum, M. U. F., Lavallee, J. M., Leifeld, J., Parton, W. J., Megan Steinweg, J., Wallenstein, M. D., Martin Wetterstedt, J. A. and Bradford, M. A. (2011), "Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward", *Global Change Biology,* vol. 17, no. 11, pp. 3392-3404.

Conde, E., Cardenas, M., Ponce-Mendoza, A., Luna-Guido, M. L., Cruz-Mondragón, C. and Dendooven, L. (2005), "The impacts of inorganic nitrogen application on mineralization of ^{14}C-labelled maize and glucose,

and on priming effect in saline alkaline soil", *Soil Biology and Biochemistry,* vol. 37, no. 4, pp. 681-691.

Dalenberg, J. W. and Jager, G. (1989), "Priming effect of some organic additions to ^{14}C-labelled soil", *Soil Biology and Biochemistry,* vol. 21, no. 3, pp. 443-448.

Davidson, E. A. and Janssens, I. A. (2006), "Temperature sensitivity of soil carbon decomposition and feedbacks to climate change", *Nature,* vol. 440, no. 7081, pp. 165-173.

Davidson, E. A., Savage, K., Verchot, L. V. and Navarro, R. (2002), "Minimizing artifacts and biases in chamber-based measurements of soil respiration", *Agricultural and Forest Meteorology,* vol. 113, no. 1–4, pp. 21-37.

De Nobili, M., Contin, M., Mondini, C. and Brookes, P. (2001), "Soil microbial biomass is triggered into activity by trace amounts of substrate", *Soil Biology and Biochemistry,* vol. 33, no. 9, pp. 1163-1170.

Denef, K., Six, J., Paustian, K. and Merckx, R. (2001), "Importance of macroaggregate dynamics in controlling soil carbon stabilization: short-term effects of physical disturbance induced by dry–wet cycles", *Soil Biology and Biochemistry,* vol. 33, no. 15, pp. 2145-2153.

DeSutter, T., Sauer, T., Parkin, T. and Heitman, J. (2008), "A subsurface, closed-loop system for soil carbon dioxide and its application to the gradient efflux approach", *Soil Science Society of America Journal,* vol. 72, no. 1, pp. 126-134.

Dijkstra, F. A., Bader, N. E., Johnson, D. W. and Cheng, W. (2009), "Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability?", *Soil Biology and Biochemistry,* vol. 41, no. 6, pp. 1080-1087.

Dilkes, N. B., Jones, D. L. and Farrar, J. (2004), "Temporal dynamics of carbon partitioning and rhizodeposition in wheat", *Plant Physiology,* vol. 134, no. 2, pp. 706-715.

Dimassi, B., Mary, B., Fontaine, S., Perveen, N., Revaillot, S. and Cohan, J. (2014), "Effect of nutrients availability and long-term tillage on priming effect and soil C mineralization", *Soil Biology and Biochemistry,* vol. 78, pp. 332-339.

Ekblad, A. and Högberg, P. (2000), "Analysis of $\delta^{13}C$ of CO_2 distinguishes between microbial respiration of added C4-sucrose and other soil respiration in a C3-ecosystem", *Plant and Soil,* vol. 219, no. 1-2, pp. 197-209.

Engelking, B., Flessa, H. and Joergensen, R. G. (2007), "Microbial use of maize cellulose and sugarcane sucrose monitored by changes in the $^{13}C/^{12}C$ ratio", *Soil Biology and Biochemistry,* vol. 39, no. 8, pp. 1888-1896.

Ferraro, D. O. and Oesterheld, M. (2002), "Effect of defoliation on grass growth. A quantitative review", *Oikos,* vol. 98, no. 1, pp. 125.

Fisher, F. M. and Gosz, J. R. (1986), "Effects of trenching on soil processes and properties in a New Mexico mixed-conifer forest", *Biology and Fertility of Soils,* vol. 2, no. 1, pp. 35-42.

Flanagan, L. B. and Ehleringer, J. R. (1998), "Ecosystem-atmosphere CO_2 exchange: interpreting signals of change using stable isotope ratios", *Trends in Ecology & Evolution,* vol. 13, no. 1, pp. 10-14.

Fontaine, S. and Barot, S. (2005), "Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation", *Ecology Letters,* vol. 8, no. 10, pp. 1075-1087.

Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B. and Rumpel, C. (2007), "Stability of organic carbon in deep soil layers controlled by fresh carbon supply", *Nature,* vol. 450, pp. 277-280.

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J. M. G., Maire, V., Mary, B., Revaillot, S. and Maron, P. A. (2011), "Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect", *Soil Biology and Biochemistry,* vol. 43, no. 1, pp. 86-96.

Friedlingstein, P., Cox, P., Betts, R., Bopp, L., von Bloh, W., Brovkin, V., Cadule, P., Doney, S., Eby, M., Fung, I., Bala, G., John, J., Jones, C., Joos, F., Kato, T., Kawamiya, M., Knorr, W., Lindsay, K., Matthews, H. D. and Raddatz, T. (2006), "Climate–Carbon Cycle Feedback Analysis: Results from the C4MIP Model Intercomparison", *Journal of Climate,* vol. 19, no. 14, pp. 3337-3353.

Gamnitzer, U., Schäufele, R. and Schnyder, H. (2009), "Observing ^{13}C labelling kinetics in CO_2 respired by a temperate grassland ecosystem", *New Phytologist,* vol. 184, no. 2, pp. 376-386.

Garcia-Pausas, J. and Paterson, E. (2011), "Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon", *Soil Biology and Biochemistry,* vol. 43, no. 8, pp. 1705-1713.

Gärdenäs, A. I., Ågren, G. I., Bird, J. A., Clarholm, M., Hallin, S., Ineson, P., Kätterer, T., Knicker, H., Nilsson, S. I., Näsholm, T., Ogle, S., Paustian, K., Persson, T. and Stendahl, J. (2011), "Knowledge gaps in soil carbon and

nitrogen interactions – from molecular to global scale", *Soil Biology and Biochemistry,* vol. 43, no. 4, pp. 702-717.

Ghee, C., Neilson, R., Hallett, P. D., Robinson, D. and Paterson, E. (2013), "Priming of soil organic matter mineralisation is intrinsically insensitive to temperature", *Soil Biology and Biochemistry,* vol. 66, pp. 20-28.

Gounot, A. M. (1986), "Psychrophilic and psychrotrophic microorganisms", *Experientia,* vol. 42, no. 11, pp. 1192-1197.

Graf, A., Weihermüller, L., Huisman, J. A., Herbst, M., Bauer, J. and Vereecken, H. (2008), "Measurement depth effects on the apparent temperature sensitivity of soil respiration in field studies", *Biogeosciences,* vol. 5, no. 4, pp. 1175-1188.

Gregory, P. J. (2006), "Roots, rhizosphere and soil: the route to a better understanding of soil science?", *European Journal of Soil Science,* vol. 57, no. 1, pp. 2-12.

Griffis, T. J., Baker, J. M. and Zhang, J. (2005), "Seasonal dynamics and partitioning of isotopic CO_2 exchange in a C3/C4 managed ecosystem", *Agricultural and Forest Meteorology,* vol. 132, no. 1–2, pp. 1-19.

Hamer, U. and Marschner, B. (2005), "Priming effects in soils after combined and repeated substrate additions", *Geoderma,* vol. 128, no. 1–2, pp. 38-51.

Hamilton III, E. W., Frank, D. A., Hinchey, P. M. and Murray, T. R. (2008), "Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland", *Soil Biology and Biochemistry,* vol. 40, no. 11, pp. 2865-2873.

Heimann, M. and Reichstein, M. (2008), "Terrestial ecosystem dynamics and climate feedbacks", *Nature,* vol. 451, pp. 289-292.

Heinemeyer, A., Wilkinson, M., Vargas, R., Subke, J. A., Casella, E., Morison, J. I. L. and Ineson, P. (2012), "Exploring the overflow tap theory: linking forest soil CO_2 fluxes and individual mycorrhizosphere components to photosynthesis", *Biogeosciences,* vol. 9, no. 1, pp. 79-95.

Hill, P. W., Garnett, M. H., Farrar, J., Iqbal, Z., Khalid, M., Soleman, N. and Jones, D. L. (2015), "Living roots magnify the response of soil organic carbon decomposition to temperature in temperate grassland", *Global Change Biology,* vol. 21, no. 3, pp. 1368-1375.

Hütsch, B. W., Augustin, J. and Merbach, W. (2002), "Plant rhizodeposition—an important source for carbon turnover in soils", *Journal of Plant Nutrition and Soil Science,* vol. 165, no. 4, pp. 397-407.

IPCC (2014), "Terrestrial and Inland water systems", in Field, C. B., Barros, V. R., Dokken, D. J., et al (eds.) *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A:Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,* Cambridge University Press, Cambridge, pp. 271-360.

Jackson, L. E., Burger, M. and Cavagnaro, T. R. (2008), "Roots, nitrogen transformations, and ecosystem services", *Annual Review of Plant Biology,* vol. 59, pp. 341-363.

Jagadamma, S., Mayes, M. A., Steinweg, J. M. and Schaeffer, S. M. (2014), "Substrate quality alters the microbial mineralization of added substrate and soil organic carbon", *Biogeosciences,* vol. 11, no. 17, pp. 4665-4678.

Jenkinson, D. S., Brookes, P. C. and Powlson, D. S. (2004), "Measuring soil microbial biomass", *Soil Biology and Biochemistry,* vol. 36, no. 1, pp. 5-7.

Jenkinson, D. S. (1966), "Studies on the decomposition of plant material in soil", *Journal of Soil Science,* vol. 17, no. 2, pp. 280-302.

Jenkinson, D. S., Fox, R. H. and Rayner, J. H. (1985), "Interactions between fertilizer nitrogen and soil nitrogen - the so-called 'priming' effect", *Journal of Soil Science,* vol. 36, pp. 425-444.

Jenkinson, D. S. and Powlson, D. S. (1976), "The effects of biocidal treatments on metabolism in soil—I. Fumigation with chloroform", *Soil Biology and Biochemistry,* vol. 8, no. 3, pp. 167-177.

Jones, D. L., Nguyen, C. and Finlay, R. D. (2009), "Carbon flow in the rhizosphere: carbon trading at the soil–root interface", *Plant & Soil,* vol. 321, no. 1, pp. 5-33.

Jones, D. L., Hodge, A. and Kuzyakov, Y. (2004), "Plant and mycorrhizal regulation of rhizodeposition", *New Phytologist,* vol. 163, no. 3, pp. 459-480.

Kemmitt, S. J., Lanyon, C. V., Waite, I. S., Wen, Q., Addiscott, T. M., Bird, N. R. A., O'Donnell, A. G. and Brookes, P. C. (2008), "Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass—a new perspective", *Soil Biology and Biochemistry,* vol. 40, no. 1, pp. 61-73.

Knorr, W., Prentice, I. C., House, J. I. and Holland, E. A. (2005), "Long-term sensitivity of soil carbon to warming", *Nature,* vol. 433, pp. 298.

Kristiansen, S. M., Hansen, E. M., Jensen, L. S. and Christensen, B. T. (2005), "Natural 13C abundance and carbon storage in Danish soils under

continuous silage maize", *European Journal of Agronomy,* vol. 22, no. 2, pp. 107-117.

Kuzyakov, Y. (2006), "Sources of CO_2 efflux from soil and review of partitioning methods", *Soil Biology and Biochemistry,* vol. 38, no. 3, pp. 425-448.

Kuzyakov, Y. (2010), "Priming effects: interactions between living and dead organic matter", *Soil Biology and Biochemistry,* vol. 42, no. 9, pp. 1363-1371.

Kuzyakov, Y. and Cheng, W. (2001), "Photosynthesis controls of rhizosphere respiration and organic matter decomposition", *Soil Biology and Biochemistry,* vol. 33, no. 14, pp. 1915-1925.

Kuzyakov, Y., Friedel, J. K. and Stahr, K. (2000), "Review of mechanisms and quantification of priming effects", *Soil Biology and Biochemistry,* vol. 32, no. 11-12, pp. 1485-1498.

Kuzyakov, Y. and Gavrichkova, O. (2010), "Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls", *Global Change Biology,* vol. 16, no. 12, pp. 3386-3406.

Kuzyakov, Y. and Schneckenberger, K. (2004), "Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation", *Archives of Agronomy & Soil Science,* vol. 50, no. 1, pp. 115-132.

Kuzyakov, Y. (2002), "Review: Factors affecting rhizosphere priming effects", *Journal of Plant Nutrition and Soil Science,* vol. 165, no. 4, pp. 382-396.

Kuzyakov, Y. and Blagodatskaya, E. (2015), "Microbial hotspots and hot moments in soil: Concept & review", *Soil Biology and Biochemistry,* vol. 83, pp. 184-199.

Kuzyakov, Y., Blagodatskaya, E. and Blagodatsky, S. (2009), "Comments on the paper by Kemmitt et al. (2008) ‘Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass – a new perspective’ [Soil Biology & Biochemistry 40, 61–73]: The biology of the Regulatory Gate", *Soil Biology and Biochemistry,* vol. 41, no. 2, pp. 435-439.

Lamparter, A., Bachmann, J., Goebel, M. O. and Woche, S. K. (2009), "Carbon mineralization in soil: impact of wetting–drying, aggregation and water repellency", *Geoderma,* vol. 150, no. 3–4, pp. 324-333.

Landi, L., Valori, F., Ascher, J., Renella, G., Falchini, L. and Nannipieri, P. (2006), "Root exudate effects on the bacterial communities, CO2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils", *Soil Biology and Biochemistry,* vol. 38, no. 3, pp. 509-516.

Lavelle, P. and Spain, A. V. (2001), *Soil Ecology,* Kluwer, London.

Lee, M. S., Nakane, K., Nakatsubo, T. and Koizumi, H. (2003), "Seasonal changes in the contribution of root respiration to total soil respiration in a cool-temperate deciduous forest", *Plant and Soil,* vol. 255, no. 1, pp. 311-318.

Liljeroth, E., Kuikman, J. and Van Veen, J. A. (1994), "Carbon translocation to the rhizosphere of maize and wheat and influence in the turnover of native soil organic matter at different nitrogen levels", *Plant and Soil,* vol. 161, pp. 233-240.

Livingston, G. and Hutchinson, G. (1995), "Enclosure-based measurement of trace gas exchange: applications and sources of error", in Matson, P. A. and Harriss, P. A. (eds.) *Biogenic trace gases: measuring emissions from soil and water,* Blackwell Science Cambridge, , pp. 14-51.

Lloyd, J. and Taylor, J. A. (1994), "On the temperature dependence of soil respiration", *Functional Ecology,* vol. 8, no. 3, pp. 315.

Luo, Y. and Zhou, X. (2010), *Soil respiration and the environment,* Academic press, London.

Machinet, G. E., Bertrand, I., Chabbert, B., Watteau, F., Villemin, G. and Recous, S. (2009), "Soil biodegradation of maize root residues: Interaction between chemical characteristics and the presence of colonizing micro-organisms", *Soil Biology and Biochemistry,* vol. 41, no. 6, pp. 1253-1261.

Maier, M. and Schack-Kirchner, H. (2014), "Using the gradient method to determine soil gas flux: A review", *Agricultural and Forest Meteorology,* vol. 192–193, pp. 78-95.

Marx, M., Buegger, F., Gattinger, A., Zsolnay, Á. and Munch, J. C. (2007), "Determination of the fate of ^{13}C labelled maize and wheat exudates in an agricultural soil during a short-term incubation", *European Journal of Soil Science,* vol. 58, no. 5, pp. 1175-1185.

McCarthy, J. F., Ilavsky, J., Jastrow, J. D., Mayer, L. M. and Perfect, E. (2008), "Protection of organic carbon in soil microaggregates via restructuring of aggregate porosity and filling of pores with accumulating organic matter", *Geochimica et Cosmochimica,* vol. 72, pp. 4725-4744.

Meharg, A. A. (1994), "A critical review of labelling techniques used to quantify rhizosphere carbon-flow", *Plant and Soil,* vol. 166, no. 1, pp. 55-62.

Midwood, A. J., Gebbing, T., Wendler, R., Sommerkorn, M., Hunt, J. E. and Millard, P. (2006), "Collection and storage of CO_2 for ^{13}C analysis: an application to separate soil CO_2 efflux into root- and soil-derived

components", *Rapid Communications in Mass Spectrometry,* vol. 20, no. 22, pp. 3379-3384.

Midwood, A. J. and Millard, P. (2011), "Challenges in measuring the $d^{13}C$ of the soil surface CO_2 efflux", *Rapid Communications in Mass Spectrometry,* vol. 25, no. 1, pp. 232-242.

Milcu, A., Heim, A., Ellis, R. J., Scheu, S. and Manning, P. (2011), "Identification of general patterns of nutrient and labile carbon control on soil carbon dynamics across a successional gradient", *Ecosystems,* vol. 14, no. 5, pp. 710-719.

Millard, P., Midwood, A. J., Hunt, J. E., Whitehead, D. and Boutton, T. W. (2008), "Partitioning soil surface CO2 efflux into autotrophic and heterotrophic components, using natural gradients in soil [delta] 13C in an undisturbed savannah soil", *Soil Biology and Biochemistry,* vol. 40, no. 7, pp. 1575-1582.

Moir, J. L., Edwards, G. R. and Berry, L. N. (2013), "Nitrogen uptake and leaching loss of thirteen temperate grass species under high N loading", *Grass and Forage Science,* vol. 68, no. 2, pp. 313-325.

Myklebust, M. C., Hipps, L. E. and Ryel, R. J. (2008), "Comparison of eddy covariance, chamber, and gradient methods of measuring soil CO2 efflux in an annual semi-arid grass, *Bromus tectorum*", *Agricultural and Forest Meteorology,* vol. 148, no. 11, pp. 1894-1907.

Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G. and Renella, G. (2003), "Microbial diversity and soil functions", *European Journal of Soil Science,* vol. 54, no. 4, pp. 655-670.

Nguyen, C. and Guckert, A. (2001), "Short-term utilisation of ^{14}C-[U]glucose by soil microorganisms in relation to carbon availability", *Soil Biology and Biochemistry,* vol. 33, no. 1, pp. 53-60.

Nickerson, N. and Risk, D. (2009), "A numerical evaluation of chamber methodologies used in measuring the $\delta^{13}C$ of soil respiration", *Rapid Communications in Mass Spectrometry,* vol. 23, no. 17, pp. 2802-2810.

Niklaus, P. A., Stocker, R., Körner, C. and Leadley, P. W. (2000), "CO2 flux estimates tend to overestimate ecosystem C sequestration at elevated CO2", *Functional Ecology,* vol. 14, no. 5, pp. 546-559.

Norton, J. M. and Firestone, M. K. (1996), "N dynamics in the rhizosphere of *Pinus ponderosa* seedlings", *Soil Biology and Biochemistry,* vol. 28, no. 3, pp. 351-362.

Nottingham, A. T., Griffiths, H., Chamberlain, P. M., Stott, A. W. and Tanner, E. V. J. (2009), "Soil priming by sugar and leaf-litter substrates: a link to microbial groups", *Applied Soil Ecology,* vol. 42, no. 3, pp. 183-190.

Nottingham, A. T., Turner, B. L., Stott, A. W. and Tanner, E. V. J. (2015), "Nitrogen and phosphorus constrain labile and stable carbon turnover in lowland tropical forest soils", *Soil Biology and Biochemistry,* vol. 80, pp. 26-33.

Ohlsson, K. E. A. (2010), "Reduction of bias in static closed chamber measurement of $d^{13}C$ in soil CO_2 efflux", *Rapid Communications in Mass Spectrometry,* vol. 24, no. 2, pp. 180-184.

Ojanen, P., Minkkinen, K., Lohila, A., Badorek, T. and Penttilä, T. (2012), "Chamber measured soil respiration: A useful tool for estimating the carbon balance of peatland forest soils?", *Forest Ecology and Management,* vol. 277, pp. 132-140.

O'Leary, M. H. (1981), "Carbon isotope fractionation in plants", *Phytochemistry,* vol. 20, no. 4, pp. 553-567.

Orwin, K. H., Wardle, D. A. and Greenfield, L. G. (2006), "Ecological consequences of carbon substrate identity and diversity in a laboratory study", *Ecology,* vol. 87, no. 3, pp. 580-593.

Paterson, E., Midwood, A. J. and Millard, P. (2009), "Through the eye of the needle: a review of isotope appraches to quantify microbial processes mediating soil carbon balance", *New Phytologist,* vol. 184, pp. 19-33.

Paterson, E. (2003), "Importance of rhizodeposition in the coupling of plant and microbial productivity", *European Journal of Soil Science,* vol. 54, no. 4, pp. 741-750.

Paterson, E., Gebbing, T., Abel, C., Sim, A. and Telfer, G. (2007), "Rhizodeposition shapes rhizosphere microbial community structure in organic soil", *New Phytologist,* vol. 173, no. 3, pp. 600-610.

Paterson, E. and Sim, A. (1999), "Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation", *Plant and Soil,* vol. 216, no. 1-2, pp. 155-164.

Paterson, E. and Sim, A. (2013), "Soil-specific response functions of organic matter mineralization to the availability of labile carbon", *Global Change Biology,* vol. 19, no. 5, pp. 1562-1571.

Phillips, R. P., Finzi, A. C. and Bernhardt, E. S. (2011), "Enhanced root exudation induces microbial feedback to N cycling in a pine forest under long-term CO_2 fumigation", *Ecology Letters,* vol. 14, pp. 187-194.

Pingintha, N., Leclerc, M. Y., Beasley Jr, J., Zhang, G. and Senthong, C. (2010), "Assessment of the soil CO_2 gradient method for soil CO_2 efflux measurements: comparison of six models in the calculation of the relative gas diffusion coefficient", *Tellus B,* vol. 62, no. 1, pp. 47-58.

Powlson, D. S., Whitmore, A. P. and Goulding, K. W. T. (2011), "Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false", *European Journal of Soil Science,* vol. 62, no. 1, pp. 42-55.

Reichstein, M., Bahn, M., Mahecha, M. D., Kattge, J. and Baldocchi, D. D. (2014), "Linking plant and ecosystem functional biogeography", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 111, no. 38, pp. 13697-13702.

Roper, M. M., Fillery, I. R. P., Jongepier, R., Sanford, P., Macdonald, L. M., Sanderman, J. and Baldock, J. A. (2013), "Allocation into soil organic matter fractions of ^{14}C captured photosynthesis via photosynthesis by two perrenial grass pastures", *Soil Research,* vol. 51, pp. 748-759.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R. and Fierer, N. (2010), "Soil bacterial and fungal communities across a pH gradient in an arable soil", *ISME Journal: Multidisciplinary Journal of Microbial Ecology,* vol. 4, no. 10, pp. 1340-1351.

Royal Society (2009), *Geoengineering the climate: science, governance and uncertainty,* RS policy document 10/09, Royal Society, London.

Ryan, M. G. and Law, B. E. (2005), "Interpreting, measuring, and modeling soil respiration", *Biogeochemistry,* vol. 73, no. 1, pp. 3-27.

Schimel, D. S. (1994), "Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils", *Global Biogeochemical Cycles,* vol. 8, no. 3, pp. 279-293.

Schlesinger, W. H. and Lichter, J. (2001), "Limited carbon storage in soil of experimental forest plots under increased atmospheric CO_2", *Nature,* vol. 411, pp. 466-469.

Schmitt, A., Pausch, J. and Kuzyakov, Y. (2013), "Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by ^{14}C labelling", *Applied Soil Ecology,* vol. 64, pp. 228-236.

Shahzad, T., Chenu, C., Repinçay, C., Mougin, C., Ollier, J. L. and Fontaine, S. (2012), "Plant clipping decelerates the mineralization of recalcitrant soil organic matter under multiple grassland species", *Soil Biology and Biochemistry,* vol. 51, pp. 73-80.

Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C. and Fontaine, S. (2015), "Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species", *Soil Biology and Biochemistry,* vol. 80, pp. 146-155.

Six, J. and Jastrow, J. D. (2002), "Organic matter turnover", in Lal, R. (ed.) *Encyclopedia of soil science,* Marcel Dekker, New York, pp. 936-942.

Sparling, G. S., Cheshire, M. V. and Mundie, C. M. (1982), "Effect of barley plants on the dcomposition of ^{14}C-labelled soil organic matter", *Journal of Soil Science,* vol. 33, pp. 89-100.

Subke, J. A. and Bahn, M. (2010), "On the 'temperature sensitivity'of soil respiration: Can we use the immeasurable to predict the unknown?", *Soil Biology and Biochemistry,* vol. 42, no. 9, pp. 1653-1656.

Subke, J. A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N. and Cotrufo, M. F. (2004a), "Feedback interactions between needle litter decomposition and rhizosphere activity", *Oecologia,* vol. 139, no. 4, pp. 551-559.

Subke, J., Inglima, I., Peressotti, A., Delle Vedove, G. and Francesca Cotrufo, M. (2004b), "A new technique to measure soil CO_2 efflux at constant CO_2 concentration", *Soil Biology and Biochemistry,* vol. 36, no. 6, pp. 1013-1015.

Thornley, J. H. M. and Cannell, M. G. R. (1997), "Temperate grassland responses to climate change: an analysis using the Hurley Pasture Model", *Annals of Botany,* vol. 80, no. 2, pp. 205-221.

Thurgood, A., Singh, B., Jones, E. and Barbour, M. M. (2014), "Temperature sensitivity of soil and root respiration in contrasting soils", *Plant and Soil,* vol. 382, no. 1-2, pp. 253-267.

Tubeileh, A., Groleau-Renaud, V., Plantureux, S. and Guckert, A. (2003), "Effect of soil compaction on photosynthesis and carbon partitioning within a maize–soil system", *Soil and Tillage Research,* vol. 71, no. 2, pp. 151-161.

Tunlid, A., Hoitink, H. A. J., Low, C. and White, D. C. (1989), "Characterization of bacteria that suppress *Rhizoctonia* damping-off in bark compost media by analysis of fatty acid biomarkers", *Applied and Environmental Microbiology,* vol. 55, no. 6, pp. 1368-1374.

Uchida, Y., Clough, T. J., Kelliher, F. M., Hunt, J. E. and Sherlock, R. R. (2011), "Effects of bovine urine, plants and temperature on N_2O and CO_2 emissions from a sub-tropical soil", *Plant and Soil,* vol. 345, no. 1, pp. 171-186.

Vance, E. D., Brookes, P. C. and Jenkinson, D. S. (1987), "An extraction method for measuring soil microbial biomass C", *Soil Biology and Biochemistry,* vol. 19, no. 6, pp. 703-707.

Vellinga, T. V., André, G., Schils, R. L. M., Kraak, T. and Oenema, O. (2010), "Accounting for residual effects of previously applied nitrogen fertilizer on intensively managed grasslands", *Grass & Forage Science,* vol. 65, no. 1, pp. 58-75.

Walker, T. S., Bais, H. P., Grotewold, E. and Vivanco, J. M. (2003), "Root exudation and rhizosphere biology", *Plant Physiology,* vol. 132, no. 1, pp. 44-51.

Ward, P. R., Micin, S. F. and Fillery, I. R. P. (2012), "Application of eddy covariance to determine ecosystem-scale carbon balance and evapotranspiration in an agroforestry system", *Agricultural and Forest Meteorology,* vol. 152, pp. 178-188.

White, D., Davis, W., Nickels, J., King, J. and Bobbie, R. (1979), "Determination of the sedimentary microbial biomass by extractible lipid phosphate", *Oecologia,* vol. 40, no. 1, pp. 51-62.

Wood, T. E., Detto, M. and Silver, W. L. (2013), "Sensitivity of soil respiration to variability in soil moisture and temperature in a humid tropical forest", *PloS one,* vol. 8, no. 12.

Wu, N., Bai, E. and Xu, W. (2013), "Application of carbon stable isotope pulse labeling technique in research on carbon allocation in plant-soil system", *International Journal of Applied Environmental Sciences,* vol. 8, no. 16, pp. 2141-2147.

Wulfes, R., Nyman, P. and Kornher, A. (1999), "Modelling non-structural carbohydrates in forage grasses with weather data", *Agricultural Systems,* vol. 61, no. 1, pp. 1-16.

Yang, Q., Xu, M., Liu, H., Wang, J., Liu, L., Chi, Y. and Zheng, Y. (2011), "Impact factors and uncertainties of the temperature sensitivity of soil respiration", *Shengtai Xuebao/ Acta Ecologica Sinica,* vol. 31, no. 8, pp. 2301-2311.

Yin, H., Li, Y., Xiao, J., Xu, Z., Cheng, X. and Liu, Q. (2013), "Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming", *Global Change Biology,* vol. 19, no. 7, pp. 2158-2167.

Zhang, X., Liu, W., Zhang, G., Jiang, L. and Han, X. (2015), "Mechanisms of soil acidification reducing bacterial diversity", *Soil Biology and Biochemistry,* vol. 81.

Zhu, B., Gutknecht, J. L. M., Herman, D. J., Keck, D. C., Firestone, M. K. and Cheng, W. (2014), "Rhizosphere priming effects on soil carbon and nitrogen mineralization", *Soil Biology and Biochemistry,* vol. 76, pp. 183-192.

Zobel, R. W. (2005), "Primary and secondary root systems", in Zobel, R. W. and Wright, S. F. (eds.) *Roots and soil management: interactions between roots and the soil,* American Society of Agronomy, Madison, WI, pp. 3-14.