Investigating Rhizosphere Controls of Soil Organic Matter Dynamics in Forest Soils using a ^{13}C Labelling Approach

Oyindamola Ibitola Jackson

January 2019

Biological and Environmental Sciences
School of Natural Sciences
University of Stirling
Stirling
Scotland, UK

Thesis submitted for the degree of Doctor of Philosophy

UNIVERSITY of STIRLING

Declaration

I hereby declare that this thesis has been written by me and that it contains the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

……..

Oyindamola. I. Jackson

Abstract

Rising atmospheric CO_2 concentration may increase plant productivity through the "CO_2 fertilization effect", which may in turn increase the input of carbon (C) to soils through rhizodeposition or plant residues. However, whether this increase in C input to soils results in greater soil C storage is not clear, as the decomposition of different forms of organic matter and the role of the rhizosphere in the decomposition process remain poorly understood. In this thesis, I investigated the interactions between plant C dynamics and soil microbial processes, and how these interactions control C and nutrient cycling in forest soils. I manipulated soil carbon supply from trees to the rhizosphere both in mesocosms and in the field through either canopy shading or soil trenching. This allowed me to investigate the effect of assimilate C supply on the decomposition of ^{13}C-labelled substrates of varying chemical compositions and structural complexities (glucose, straw, fungal necromass or biochar), and their combined effect on soil organic matter (SOM) decomposition. I found that plant C supply to the rhizosphere had no significant effect on the decomposition of substrates. Similarly, the presence of roots and their associated mycorrhizal fungi had no significant effect on litter mass loss. However, it was found that supply of C from plant to the rhizosphere promoted SOM decomposition by up to two-fold in soils amended with substrates. Although, the addition of both simple and complex substrates stimulated the activities of C, N and P- degrading enzymes, I observed that the activities of these enzymes were significantly greater in soils where a labile substrate (glucose) had been added. The increased activities of C-degrading enzymes suggest that microorganisms were C limited, and the input of labile C substrate alleviated C and energy limitation of enzyme production, allowing microbial communities to mobilize nutrients from decomposition of native SOM. This thesis demonstrates that substrate quality influences SOM decomposition, and that increased availability of labile substrates to the rhizosphere may have implications on forest soil C stocks.

Acknowledgements

I would like to thank the Petroleum Technology Development Fund (PTDF), Abuja, Nigeria for funding my research. I also appreciate NERC Life Science Mass Spectroscopy Facility (LSMSF) for funding the isotopic analyses. I thank my employer, Forestry Research Institute of Nigeria (FRIN), Ibadan for the permission to take a leave of study.

First, I would like to thank the best mentor and supervisor any student can ever have – Jens-Arne Subke for his tremendous support throughout my years as a PhD student. I thank him for his patience, advice and assistance with fieldwork. I appreciate him for making my PhD enjoyable, as he eased off pressures whilst also ensuring that I was on point with tasks. I would also like to thank my 2nd supervisor, Richard Quilliam for his advice and support. I appreciate both of my supervisors for their prompt feedbacks on my thesis.

I specially appreciate my husband, Michael Jackson, and daughters Gabriella and Janelle. They have been very supportive and understanding during these past years, overlooking my shortcomings, as I have had to severally spend times away from them due to my research. I also thank my husband for his substantial assistance with fieldwork. I particularly remember him suffering from muscle pains for several days after hammering down PVC collars in the field. I would like to specially thank my mum, Dorcas Akinyemi for the sacrifices she has had to make for me every year for the past four years. I thank her for the prayers, support and encouragements. Also, to my dad, Arc. Olutosin Akinyemi, my brothers, sisters, aunties and uncles for their encouragement. A big thank to my big brother, Temitope Akinyemi for his financial assistance.

I would like to thank Dave Johnson for his advice and support, particularly with regards to the subject on ECM fungi. I appreciate him for providing the isolate of fungi that was used to produce the ^{13}C-labelled fungal necromass. I also thank him for his feedback on the formation of my lab protocol for the extracellular enzyme assays. I would like to appreciate Marc Buée for his advice and the lab protocols on enzyme assays. I am grateful to Kevin Petrie and Jenson Lim for their advice and assistance on enzyme assays. I would also like to thank James Weir for his patience and technical assistance in the construction and setup of mesocosms, collars and other handiworks. I also appreciate all technicians in BES, particularly Lorna English and Ian Washbourne for their advice and assistance with laboratory work. I would like to thank Scott Jackson and Ronald Balfour that are always

available for my requests and assistance. I appreciate Allan McPherson for his assistance with his assistance with gardening and field matters.

I would like to appreciate Timothy C. Paine for his tremendous advice and help, tackling questions and issues with R. I also appreciate my colleagues and friends Karen Zapata Carbonell, Robin Whytock, Tom Bradford Lawrence, Jonathan Fletcher, Tom Parker, Renee Hermans for their encouragement and friendship. Many people have contributed one way or the other to the success of my PhD; I hereby acknowledge and appreciate them.

Ultimately, I thank Almighty God for His very present help in my time of need. I thank Him for the strength and wisdom to finish this PhD. I thank him for His protection over me, my family and helpers, now and always.

Table of Contents

Chapter 1

General Introduction

Since the start of the industrial era at around 1750, there has been an increase in the atmospheric concentration of greenhouse gases (GHGs) - carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Anthropogenic emissions, mainly from the combustion of fossils fuel, cement production and land use change have increased atmospheric CO_2 concentrations by about 40% between 1750 and 2013 (Le Quéré *et al.* 2015). Although anthropogenic emissions of CO_2 are the main drivers of climate change, GHGs fluxes from plants, animals and microbial communities (collectively called biogenic emissions) due to natural or anthropogenic disturbances, also contribute significantly to climate change (Tian *et al.* 2016). More than half of these CO_2 emissions are absorbed by ocean and terrestrial sinks while the rest accumulate in the atmosphere (Fig. 1.1) (Le Quéré *et al.* 2015). Terrestrial C uptake is largely through increased plant productivity stimulated by rising atmospheric CO_2, increased nutrient availability, warming and rainfall changes (Ciais *et al.* 2013; Le Quéré *et al.* 2015).

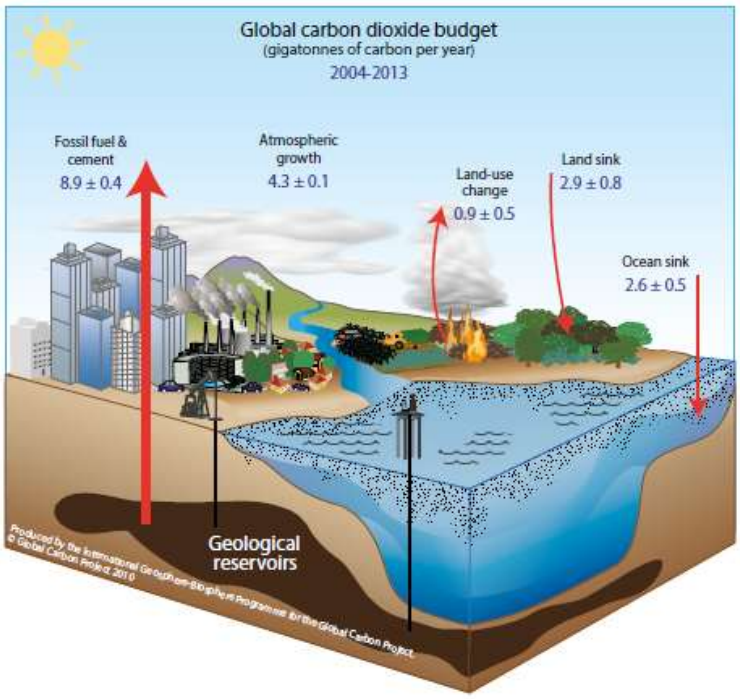

Figure 1.1 The global carbon cycle showing changes in carbon flows between the atmosphere, ocean and land caused by anthropogenic activities for the decade 2004 – 2013. Adapted from Le Quéré et al. (2015).

1.1 Forest C cycle and climate change

Forest ecosystems are the primary terrestrial C sink, storing about 45% of terrestrial C and sequestering large amounts of C annually mainly in soils as soil organic matter (SOM) (Bonan 2008; Pan *et al.* 2011). Forests cover about 4000 M ha, which is equivalent to 31% of global land cover (Keenan *et al.* 2015). Temperate forests exchange large amounts of CO_2 with the atmosphere through soil respiration (soil CO_2 efflux), which is the largest C flux between the soil and atmosphere and mainly driven by microbial decomposition of SOM (Bradford *et al.* 2016). Forests therefore play a major role in global C cycle; hence we need to understand the effects of changing environmental conditions on biogeochemical processes and soil C stocks in forest ecosystems.

Elevated atmospheric CO_2 resulting from increased GHG emissions has resulted in climate change. The direct link between increasing atmospheric CO_2 emissions and global temperature has been demonstrated by global climate models (Ciais *et al.* 2013). The global surface temperature has increased by an average of 0.78 ºC in the past century, and is projected to increase by up to 2 ºC by 2100 if GHG emissions continue to rise at the current rate (Ciais *et al.* 2013). In addition to global warming, elevated GHG emissions will also change precipitation events and heat extremes, resulting in stronger droughts and forest fires (Ciais *et al.* 2013). All of these events could influence ecosystem processes such as photosynthesis, belowground C allocation, respiration, and nutrient cycling, which in turn affect soil C stocks and the sequestration ability of these terrestrial sinks (DeAngelis *et al.* 2015; Peng *et al.* 2015; Pold *et al.* 2016; Chen *et al.* 2017, 2018).

Both empirical and modelling studies suggest that rising atmospheric CO_2 concentration increases photosynthesis and net primary productivity (NPP) of forests through the CO_2 fertilization effect (Norby *et al.* 2005; Piao *et al.* 2013). In a long term (12 years) Free Air CO_2 Enrichment (FACE) study, NPP was enhanced by about 23% per year after exposure to elevated CO_2 (Zak *et al.* 2011). In a mature eucalypt forest, no significant increase in NPP was observed despite the increases in photosynthesis after three years of exposure to elevated CO_2 (Ellsworth *et al.* 2017), which was attributed to phosphorus (P) limitation as NPP increased by 35% in P amended treatments. Similarly, (Norby *et al.* 2010) reported that N limitation constrained productivity in forests exposed to elevated CO_2. As plant productivity increases, C and nutrient cycling is also enhanced to support plant productivity, which may

result in minor changes in soil C stocks (Drake *et al.* 2011; Phillips *et al.* 2012). The extent to which the terrestrial C sink will offset climate change therefore still remains an important question (Sun *et al.* 2017; Liu *et al.* 2018).

1.2 Soil CO_2 efflux

Soil respiration (soil CO_2 efflux, R_S) is the main route by which C fixed by forest trees during photosynthesis returns back to the atmosphere (Janssens *et al.* 2001), contributing up to 60-80% of total ecosystem respiration (Matteucci *et al.* 2015). R_S can be partitioned into respiration by roots and associated rhizosphere microorganisms (autotrophic respiration, R_A) and the decomposition of dead organic matter by heterotrophic organisms (heterotrophic respiration, R_H) (Hanson *et al.* 2000; Subke, Inglima & Cotrufo 2006). The dynamics of these components of R_S vary within and among ecosystems, and these variations are driven by both abiotic and biotic factors such as temperature, moisture, N availability, aboveground plant community and photosynthetic activity (Subke *et al.* 2006). R_S and its components are likely to change due to the effects of global change factors such as increase in CO_2 concentrations, temperature, moisture content or N deposition (Wu *et al.* 2011; Hopkins *et al.* 2013; Moinet *et al.* 2016; Liu *et al.* 2018).

Decomposition is a key biogeochemical process underlying the cycling of C and nutrients within terrestrial ecosystems, and between the biosphere and the atmosphere (Schlesinger & Bernhardt 2013; Bradford *et al.* 2016). It arises from the activities of free-living saprotrophs and mycorrhizal fungi that break down complex organic matter in soils into simpler forms that can be utilized for growth and metabolism (Swift, Heal & Anderson 1979). Although climatic factors, litter quality and decomposers are considered to be the primary drivers of decomposition rates (k) (Swift *et al.* 1979; Bradford *et al.* 2016), decomposing organisms dynamics are not well represented in most ecosystem models. The rates of litter mass loss have therefore been extensively related to temperature, precipitation (using indices such as actual or potential evapotranspiration), C:N ratios, lignin contents and lignin-N ratios (Hobbie 1996; Moorhead *et al.* 1999; Zhang *et al.* 2008; Bradford *et al.* 2017; Sun *et al.* 2018). In a meta-analytical study of 293 k-values from 70 studies, litter quality factors (C:N ratio and total nutrient content of the litter) were found to be the dominant controls of decomposition rates (Zhang *et al.* 2008). Another synthesis of published studies demonstrated that decomposition was mainly influenced by plant functional traits (Cornwell *et al.* 2008).

There is evidence that plant functional traits influence the composition and functioning of soil microbial community through the nature of the litter, soils, mycorrhizal fungi associated with tree roots and the rhizosphere (reviewed by Prescott and Grayston, 2013). Plant species can determine litter quality and soil nutrient contents, and therefore influence decomposition rates. For instance, studies have shown that deciduous broadleaf litter decompose at a faster rate than evergreen coniferous litters (Gholz *et al.* 2000; Prescott *et al.* 2000), but this can be dependent on the interaction between litter type and the decomposer environment (Freschet, Aerts & Cornelissen 2012; Keiser *et al.* 2014). However, these interactions between plant and microbial communities remain elusive. Given that elevated CO_2 and its associated global warming influences plant-soil interactions (Phillips, Finzi & Bernhardt 2011; Terrer *et al.* 2018), it is expedient to understand the interactions between plant processes and microbial processes that mediate C input and decomposition of SOM in order to improve terrestrial ecosystem models (Paterson & Sim 2013).

Microorganisms carry out many ecosystem processes such as decomposition. However, the assumption that the influence of the composition and structure of decomposer community on decomposition rates are negligible at regional to global scales and only relevant at local scales (microsites) (Swift *et al.* 1979), and the poor understanding of the role and response of microorganisms to environmental changes confounds their inclusion in ecosystem models (Prescott & Grayston 2013). Earlier studies found no relationship between microbial diversity/composition and soil processes, and therefore suggested redundancy in microbial functions, whereby changes in microbial community composition had no effect on decomposition (Nannipieri *et al.* 2003; Wertz *et al.* 2006). However, this idea of 'functional similarity/equivalence' has been challenged, as differences in microbial community composition have been observed to affect soil processes such as decomposition (Vivanco & Austin 2008; Strickland *et al.* 2009a; b; Fontaine *et al.* 2011; Keiser *et al.* 2011, 2014). For example, litter decomposes at a faster rate in ecosystems where they naturally occur, termed 'home-field advantage' (Gholz *et al.* 2000; Vivanco & Austin 2008; Ayres *et al.* 2009; Keiser *et al.* 2011, 2014). The relationship between decomposer community composition and ecosystem functioning is relevant in the context of rapid global change, to predict the response of ecosystems to future environmental conditions.

1.3 Priming effect

Carbon enters into the soil mainly as plant detritus (litter), which is incorporated into SOM through litter decomposition (Swift *et al.* 1979; Cotrufo *et al.* 2015). C also enters into soils through living roots as soluble exudates, sloughed off root cells, and other secretions, a process referred to as rhizodeposition (Jones, Nguyen & Finlay 2009). Glucose is the predominant sugar in rhizodeposits (Derrien, Marol & Balesdent 2004) and also a decomposition product of the polysaccharides in plant litter (Kuzyakov 2010). The ready availability of rhizodeposits creates hotspots of microbial activities in soils, especially in the rhizosphere (Kuzyakov & Blagodatskaya 2015). Carbon inputs to forest soils are determined by NPP, the amount of litter, and microbial activities (Gómez-Guerrero & Doane 2018). Under elevated CO_2, C input to the soil is likely to increase as a result of enhanced primary productivity of plants and enhanced root growth (Zak *et al.* 2000, 2011; Norby *et al.* 2005; Phillips *et al.* 2011; Kuzyakov *et al.* 2019). Furthermore, pyrogenic C input is likely to increase in future due to increasing fire frequency and severity (Flannigan *et al.* 2013). This has great implication for forest soil C stocks.

The supply of fresh C into the soil may influence the decomposition of SOM, thereby affecting soil C stocks, a phenomenon referred to as "the priming effect". The priming effect (PE) is defined as the short-term changes in the turnover of SOM caused by the addition of labile C source, organic or mineral fertilizer, root exudation, or drying and rewetting of the soil (Kuzyakov, Friedel & Stahra 2000). The change can either be an acceleration of soil C and N mineralization (positive PE) or its reduction (negative PE) as illustrated in Fig. 1.2.

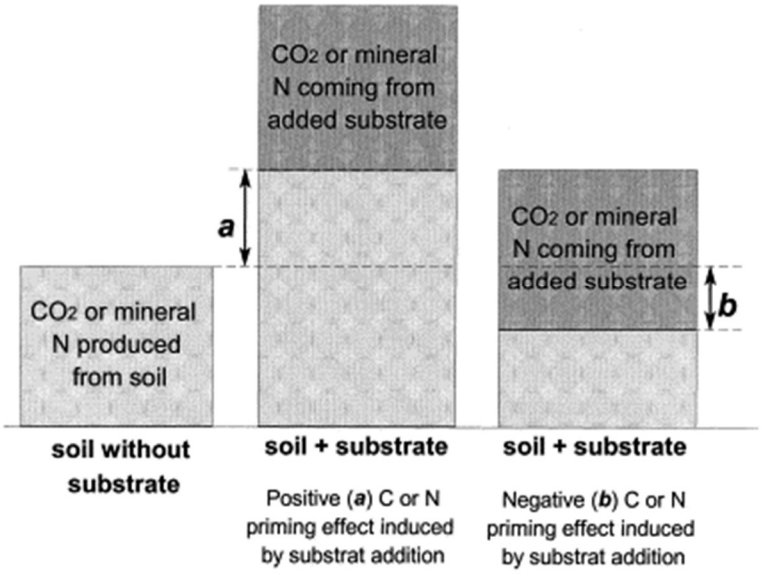

Figure 1.2 Priming effect of substrate addition on C or N mineralization in soils. (a) acceleration of C or N mineralization (positive priming effect), (b) retardation of C or N mineralization (negative priming effect). Diagram modified from Kuzyakov et al. (2000).

The source of the CO_2 released from the soil following the input of substrate C can be used to classify PEs into 'apparent' or 'real', which are indicated by the PE dynamics and the amount of the extra CO_2 released (Kuzyakov 2010). 'Apparent' PEs occur when the additional CO_2 is not related to the turnover of SOM but originates due to the activation of microbial metabolism and higher microbial biomass turnover. 'Real' PEs on the other hand, are caused by the accelerated activity of soil microorganisms, leading to enhanced turnover of SOM. Apparent PEs usually occur shortly (within the first 3 days) after the substrate input, or when the substrate is available in trace amounts (De Nobili *et al.* 2001), whereas real PEs occur over the longer term, when easily available substrates are added in amounts greater than microbial biomass (Blagodatskaya & Kuzyakov 2008; Blagodatskaya *et al.* 2011a). Real PEs can also be induced by the input of complex substrates, which require the synthesis of enzymes to decompose the complex compounds (Blagodatskaya *et al.* 2011b, 2014a). Synthesis of 170 PE experiments showed that the PE was more pronounced at the early stage of experiments, when PEs were as large as 67% in the first few days after C input, but reduced to about 10% by the third week and remained stable afterwards (Luo, Wang & Sun 2016). This indicates that PEs following the addition of easily available substrates such as glucose in short-term incubations may reflect 'apparent' rather than 'real' PEs. Thus, assessing the dynamics of PEs over an incubation period of several weeks after substrate

addition is required to distinguish real from apparent PEs, rather than focusing on the average PE observed over the period of incubation (Nottingham *et al.* 2009; Kuzyakov 2010). Furthermore, changes in microbial biomass C can be compared to the extra CO_2 released using three-source isotopic partitioning of C sources between substrate, soil and microbial biomass to separate apparent and real PEs (Kuzyakov & Bol 2004, 2006, Blagodatskaya *et al.* 2011a, 2014a; Wang *et al.* 2015b).

The magnitude and direction of the PE is dependent on the quantity and quality of fresh C inputs (Blagodatskaya *et al.* 2011a; Paterson & Sim 2013; Zhang, Wang & Wang 2013; Wang *et al.* 2015b; Liu *et al.* 2017; Shahbaz *et al.* 2017b) and nutrient availability (Blagodatskaya *et al.* 2007; Chen *et al.* 2014; Nottingham *et al.* 2015; Kumar, Kuzyakov & Pausch 2016; Fang *et al.* 2018). PEs generally increase with increasing concentration of C input towards a saturation point (Blagodatskaya & Kuzyakov 2008; Kuzyakov 2010). Positive, negative or no priming has been observed in the presence of a range of substrates, ranging from low molecular weight, readily available substrates to structurally complex, recalcitrant substrates (Subke *et al.* 2004; Blagodatskaya *et al.* 2009, 2014a; Luo *et al.* 2017b; Jackson *et al.* 2019). The quality of substrates is related to their accessibility to microorganisms, C:N ratio and the concentrations of recalcitrant fractions (Chen *et al.* 2014). A meta-analysis showed that PEs were not significantly different between treatments amended with either simple or complex substrates, where simple substrates referred to soluble, low molecular weight substrates that are readily available for microbial utilization, whereas complex substrates referred to polymerised C molecules that require more energy and nutrient investments for decomposition (Luo *et al.* 2016). However, other studies suggested that complex substrates will induce a greater 'real' PE than simple (high quality) substrates (Blagodatskaya & Kuzyakov 2008; Fontaine *et al.* 2011). In one incubation study, the addition of root residues that had high amounts of lignin, suberin and low N induced a rapid and stronger PE than leaves and stems, despite similar C:N ratio between roots and leaves (Shahbaz *et al.* 2017b). Since C enters the soil in different forms, it is imperative to compare the effects of the substrates of varying microbial availabilities and chemical structures on the release of C from soils.

Soil properties such as organic C and N content can influence the magnitude and direction of PEs. A meta-analysis showed that PE was greater in N-poor soils with high soil organic C (SOC) contents (i.e. a high C:N ratio), compared to soils with higher N content and lower C:N ratios (Zhang *et al.* 2013). Chen et al. (2014) observed a strong interaction of fresh C

input with mineral N additions; SOM decomposition was higher in soils amended with both sucrose and mineral N than in sucrose only treatments. However, they did not observe this dynamic when a more complex substrate – maize-straw was added, as SOM decomposition was not significantly different in maize straw only and maize+N treatments.

Forest burning or wildfires inputs C into soils as pyrogenic organic matter (hereafter called biochar). Biochar is also added to soils as an amendment to improve soil fertility and sequester C in order to mitigate climate change (Sohi *et al.* 2010). The priming potential of biochar has received increased attention in recent years. Although, the purported capability of biochar to mitigate climate change relies on its relative recalcitrance against microbial decomposition, contradictory results of positive, negative and absence of PE have been reported in the presence of biochar (Cross & Sohi 2011; Zimmerman, Gao & Ahn 2011; Stewart *et al.* 2013; Cheng *et al.* 2017; Luo *et al.* 2017b; Zimmerman & Ouyang 2019). By synthesizing data from biochar incubation studies, Maestrini, Nannipieri & Abiven (2015) reported that biochar amendment of soils induced positive PEs within the first 20 days of biochar addition, after which negative PEs occurred over the longer term. However, a recent study concluded that addition of biochar at the rate of 10 mg C $(g\ soil)^{-1}$ reduced SOM decomposition, which was observed as early as day two, but stimulated SOM decomposition when added at lower quantities (DeCiucies *et al.* 2018). Thus, the PE of biochar remains inconsistent, probably due to the highly variable chemistry of different types of biochar.

1.4 Rhizosphere and the priming effect

Whereas most priming effects have been conducted in root-free soils (e.g. Blagodatskaya et al. 2011a, 2014, Wang et al. 2015a), there is increasing evidence that the presence of plant roots significantly influences SOM decomposition (Huo, Luo & Cheng 2017). The stimulation or retardation of soil C decomposition in the presence of roots and their associated mycorrhizal fungi, when compared to root-free soils, is referred to as the rhizosphere priming effect (RPE) (Kuzyakov 2002). A significant fraction (up to 17%) of assimilated C enters into the soil as rhizodeposits, which are important energy sources for the microbial production of extracellular enzymes that break down SOM (Schimel & Weintraub 2003; Averill & Finzi 2011). Changes in SOM turnover from 50% retardation to about four-fold acceleration have been reported in the presence of plants compared to un-planted soils (Cheng *et al.* 2014; Yin *et al.* 2018). Roots affect SOM decomposition by releasing C into the

rhizosphere either as root exudates or through their symbiotic association with mycorrhizal fungi. The supply of these C inputs can either accelerate SOM decomposition by stimulating rhizospheric microbial growth and enzyme production (Drake *et al.* 2011; Brzostek *et al.* 2013, 2015) or retard SOM decomposition by inducing rhizosphere microbes to immobilize nutrients, thereby inhibiting the activities of free-living saprotrophs (Gadgil & Gadgil 1975; Lindahl, De Boer & Finlay 2010).

Most plant roots form symbiotic associations with mycorrhizal fungi, which are critical for terrestrial biogeochemical cycling and plant growth (Averill & Finzi 2011; Phillips *et al.* 2012; Averill, Turner & Finzi 2014; Yin, Wheeler & Phillips 2014; Brzostek *et al.* 2015). Ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi are the most prevalent mycorrhizal groups; ECM fungi dominate in most temperate and boreal forest ecosystems, whereas AM fungi dominate in grasslands and tropical forests (Smith & Read 2002). Plants allocate up to 22% of C assimilated during photosynthesis to the ECM fungi (Hobbie 2006). In return, ECM fungi improve the nutrient uptake of the host plants by releasing extracellular enzymes for the decomposition of SOM (Talbot, Allison & Treseder 2008; Talbot *et al.* 2015; Drake *et al.* 2011; Phillips *et al.* 2012). The mycorrhizal association of plants may affect ecosystem C and nutrient cycling, with feedbacks to global changes (Sulman *et al.* 2017; Terrer *et al.* 2018). Across a synthesis of plant biomass from 83 elevated CO_2 experiments, Terrer et al. (2016) reported that under low N availability, plants associated with ECM fungi were able to increase both aboveground and belowground biomass under elevated CO_2, whereas plants associated with AM fungi could not. ECM plants have also been reported to have higher exudation rates, which stimulates microbial activities and extracellular enzyme production, and thus induce stronger RPE than AM plants (Phillips & Fahey 2006; Yin *et al.* 2014; Sulman *et al.* 2017). Other studies have demonstrate greater C storage with ECM fungi, by competing with free-living saprotrophs for resource, thereby inhibiting SOM decomposition (Orwin *et al.* 2011; Averill & Hawkes 2016). Under a changing environment, elevated CO_2 conditions could therefore lead to loss or storage of soil C in ECM-dominated ecosystems and positive or negative feedback to climate. Thus, the role of ECM fungi in decomposition and priming is of increasing interest (Talbot *et al.* 2008, 2013; Phillips, Ward & Jones 2014; Lindahl & Tunlid 2015).

1.5 Mechanisms of priming effect

A range of effects of fresh C inputs on SOM decomposition have been reported, with positive, negative or no PE observed. This has led to the suggestion of several theories to explain the mechanisms of PE, such as microbial activation and cometabolism (Kuzyakov *et al.* 2000), preferential substrate utilization (Cheng 1999), shift in microbial communities (Fontaine, Mariotti & Abbadie 2003; Fontaine & Barot 2005), and microbial nutrient mining (Moorhead & Sinsabaugh 2006; Craine, Morrow & Fierer 2007).

The 'microbial activation' hypothesis (Kuzyakov *et al.* 2000) suggests that the input of easily available substrates stimulates microbial growth in soils and leads to the production of extracellular enzymes and co-metabolic decomposition of old SOM (Zhu *et al.* 2014). Low input of C may fail to induce priming, as the C input may not sufficient to supply energy required for microbial growth and activity (Blagodatskaya & Kuzyakov 2008).

The 'microbial nutrient mining' hypothesis (Craine *et al.* 2007) suggests that, as microbial demand for nutrients stored in SOM increases with the input of fresh C, microbes utilize the added C as energy sources for the production of extracellular enzymes to decompose SOM to release nutrients. In low-nutrient soils, the addition of labile, simple substrates further exacerbates microbial nutrient limitation and therefore may induce greater PE than the input of complex substrates of lower microbial availability (Chen *et al.* 2014; Wang *et al.* 2015a). As N is a limiting nutrient in temperate forest ecosystems (Ramirez, Craine & Fierer 2012), and microbes and plants compete for available N; trees will likely release more exudates into the rhizosphere to promote the mineralization of SOM by stimulating the growth and activities of microbes (Bengtson, Barker & Grayston 2012; Phillips *et al.* 2012; Zhu *et al.* 2014; Fang *et al.* 2018; Yin *et al.* 2018).

Priming effects have also been explained based on the conceptual view that microbial communities vary in their capacity to utilize SOM, and the competition between these microbial communities for the added C substrates (Fontaine *et al.* 2003, 2011). The addition of fresh labile C stimulates the activities of fast growing, microbial groups that are specialized in using the labile C (*r*-strategists), out-competing the slow-growing microorganisms (*K*-strategists). Upon substrate depletion, *r*-strategists lose their competitive ability leading to the dominance of *K*-strategists that are specialized in the utilization of complex, more recalcitrant SOM (Fontaine *et al.* 2003). Structural changes in microbial communities have been reported after the addition of substrates, where dominance of bacterial biomarkers was observed during early stages of substrate utilization, followed by

the dominance of fungal biomarkers in the late stages of priming (Fontaine *et al.* 2011; Blagodatskaya *et al.* 2014a). Chen et al. (2014) measured the specific growth rate of microbial communities to differentiate between fast-growing *r*- and slow-growing *K*-strategists in soils following the addition of sucrose or maize with or without mineral N. They reported that *r*-strategists were responsible for the PE observed in the presence of available C and higher N availability, whereas N limitation induced a shift in the microbial community from *r*- to *K*- strategists.

Negative PEs have often been explained as a result of 'preferential substrate utilization', i.e. microbes switch from the decomposition of less available SOM to the utilization of the added C substrate to obtain C and energy (Kuzyakov & Bol 2006; Blagodatskaya *et al.* 2007; Wang *et al.* 2016a). This may occur when *r*-strategists dominate the utilization of readily available, high quality substrates (Dorodnikov *et al.* 2009; Dijkstra *et al.* 2013). In an incubation experiment, a negative PE was observed when glucose was added into the soil in large amount (4.87 mg C (g soil)$^{-1}$) along with mineral N, whereas no PE occurred in the absence of N (Blagodatskaya *et al.* 2007). The addition of fresh organic matter (leaves and stalks) into forest soils characterized by recalcitrant litter that is not readily available for microbial utilization resulted in a strong negative PE in the first few days of substrate addition (Wang *et al.* 2015a).

In reality, the above mechanisms may occur in succession. Temporal dynamics of PE showed that the addition of cellulose to soil resulted in an initial negative PE in the first two weeks of substrate addition caused by a shift from SOM to cellulose decomposition, and subsequent significant increases in enzyme activities and changes in microbial community structure resulting in a positive PE in the later stages of decomposition (Blagodatskaya *et al.* 2014a).

1.6 Microbial enzyme activities

Soil microbes mediate decomposition of litter or SOM to acquire energy and release nutrients by releasing extracellular enzymes in to the soil to catalyse the rate-limiting step of decomposition (Stone, DeForest & Plante 2014; Allison, Chacon & German 2014; Chen *et al.* 2018). Understanding the dynamics of microbial communities, including the expression of extracellular enzymes, is pivotal for determining the mechanisms underlying PEs. A wide range of extracellular enzymes have been associated with C and nutrient cycling in soils,

including hydrolytic and oxidative enzymes that decompose substrates of varying composition and complexity (Sinsabaugh & Shah 2011). Cellulases are a group of hydrolytic enzymes produced for the decomposition of polysaccharides to acquire C; they include α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), cellobiohydrolase (CBH) and β-1,4-xylosidase (BX). Enzymes that target chitin, protein and urea to acquire N are β-1,4-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP) and urease respectively. Acid phosphatase (AP) and alkaline phosphatase are involved in P acquisition by cleaving phosphate ions from P- containing organic compounds (Jian *et al.* 2016). Phenol oxidase, also called laccase and peroxidase are the most commonly assayed oxidative enzymes involved in the degradation of lignin (Sinsabaugh 2010). Extracellular enzyme activities are linked to microbial community dynamics that drive SOM decomposition, however the role of belowground C flux in influencing the activities of different extracellular enzymes remains unclear.

The production and expression of extracellular enzymes is determined by the availability of resources (Sinsabaugh *et al.* 2008; Sinsabaugh, Hill & Follstad Shah 2009). Global changes may therefore influence the production and activities of extracellular enzymes. Under elevated atmospheric CO_2, (at the Duke forest FACE site; (Phillips *et al.* 2011), NAG was found to have a strong positive correlation with exudation rates in the rhizosphere of unfertilized soils but no association was observed in fertilized soils, which suggests that labile C from roots enhances N mining and increases SOM decomposition. The disruption of root C input to a pine forest soil in Sweden led to a shift in microbial community structure, whereby a major reduction in ECM fungi resulted in an increased abundance of free-living saprotrophs (Lindahl *et al.* 2010). This shift in fungal community was associated with increased activities of cellulases and laccase, indicative of C limitation of saprotrophs as they do not receive C from roots like ECM fungi do. Extracellular enzyme activities are therefore pivotal to understanding the fate of C in forest soils.

It has generally been assumed that saprotrophic fungi are the primary decomposers of SOM in soils (Bërg & McClaugherty 2008; García-Palacios *et al.* 2016). However, studies have shown that ECM fungi and saprotrophic fungi have complementary roles in the decomposition of SOM (Talbot *et al.* 2008, 2013). ECM fungi can produce a wide range of extracellular enzymes, comparable to those produced by saprophytic fungi (Burke *et al.* 2014; Phillips *et al.* 2014). Due to their competitive advantage, ECM fungi are consistently dominant in deeper soil horizons, whereas saprotrophic fungi dominate the litter layer in soils

(Lindahl *et al.* 2007; Talbot *et al.* 2013). Using mesh in-growth bags in a temperate forest to isolate the effect of roots, ECM fungi and free-living saprotrophs, Averill and Finzi (2011) observed that when fertilized with complex N (collagen), the activities of N-acquiring enzymes (NAG and LAP) and phenol oxidase increased in bags with roots but not in root-free treatments. This suggests that ECM roots may release more C into the rhizosphere that alleviates C limitation of N-acquiring enzyme production (Allison & Vitousek 2005; Phillips *et al.* 2011; Brzostek *et al.* 2013), which may explain long-term increases in plant productivity observed under elevated CO_2 (Drake *et al.* 2011).

1.7 Partitioning techniques

The partitioning of soil CO_2 efflux into rhizosphere (roots and ECM fungi) respiration and decomposition of SOM allows the investigation of these soil processes, and the prediction of their responses to environmental changes. Different partitioning methods such as root exclusion, physical separation of components and isotopic techniques have been extensively reviewed (Kuzyakov 2006; Subke *et al.* 2006).

Trenching is a root exclusion technique that have been widely used to partition total soil CO_2 efflux into the autotrophic and heterotrophic components (Ngao *et al.* 2007; Heinemeyer *et al.* 2012; Yan *et al.* 2015; Aubrey & Teskey 2018). Here, 'trenching' refers to all occasions where assimilate C supply to soils were altered by severing roots and mycorrhizal hyphae by cutting trenches or inserting deep PVC collars to a depth where majority of the roots are excluded. Surface CO_2 efflux from trenched collars or treatments is the R_H component, which is deducted from R_S from the adjacent un-trenched, control areas to derive the R_A. A commonly recognised disadvantage of trenching and any other root exclusion technique is the considerable increase in dead root biomass in the root-excluded treatments (Subke *et al.* 2006; Savage *et al.* 2018). The decomposition of dead roots contributes to R_H, thereby leading to an under-estimation of R_A when comparing CO_2 efflux from trenched and control areas (Subke *et al.* 2006). However, root decay constants in trenched soils can be estimated by fitting an exponential decay function to the mass loss of coarse (>2mm) and fine (<2mm) root biomass, sampled at the time of and after trenching (Epron *et al.* 1999). The decay constant is then used to obtain the C lost as CO_2 during root decomposition, which is subtracted from R_H to correct for decaying roots in trenched soils (Subke *et al.* 2006). In addition to the artefact caused by decaying roots, reduction in water uptake by plant roots in

root-excluded treatments may increase moisture content in trenched areas, compared to control areas (Subke *et al.* 2006). Since soil moisture has a significant influence on R_H (Moyano *et al.* 2012; Subke *et al.* 2018), differences in moisture contents between trenching treatments may have important implications therefore must be taken into consideration when interpreting the results (Comstedt, Boström & Ekblad 2011; Savage *et al.* 2018).

Shading of above-ground plant parts is a less invasive partitioning technique, compared to root exclusion. Shading partitions R_S into R_A and R_H by inhibiting photosynthesis, thus excluding fresh assimilate C transport to the roots. The advantage of shading over root exclusion is that transpiration rates are not affected by the shading treatment (Hasselquist *et al.* 2016), thereby maintaining similar moisture contents in shaded and un-shaded treatments. In a Swedish pine forest stand, photosynthetic C uptake was reduced by shading, which in turn resulted in a 30% decline in soil respiration and 25% reduction in ECM fungal biomass relative to the un-shaded treatments (Hasselquist *et al.* 2016). However, it is possible that plant root may continue to respire shortly after shading by using their starch reserves. Although shading is commonly used in grasslands and crop plants (Hartley, Heinemeyer & Ineson 2007; Bahn *et al.* 2009, 2013; Schmitt, Pausch & Kuzyakov 2013), others have also used shading to alter belowground C supply in forest ecosystems (Fischer *et al.* 2015; Mao *et al.* 2016; Hasselquist *et al.* 2016).

Isotopic techniques allow for non-intrusive measurements of CO_2 flux components. It involves the separation of the different sources of CO_2 fluxes based on differences in C isotope ratios. The ratio of C isotopes can be differentiated using stable (^{13}C) and radio- (^{14}C) isotopes of C via pulse or continuous labelling, natural abundance, or radiocarbon dating (Kuzyakov 2006; Subke *et al.* 2006). The basis of isotopic partitioning is to measure the isotopic signature of R_S, and other sources such as soil, roots or substrates separately, which form 'end members' for a mass balance model. The transfer of C within the ecosystem through assimilation, allocation and respiration can be traced by exposing the plant to an isotopic label (usually ^{13}C- or ^{14}C- labelled CO_2) for a period of time (Derrien *et al.* 2004; Högberg *et al.* 2008; Epron *et al.* 2011; Subke *et al.* 2012; Schmitt *et al.* 2013; Heinrich *et al.* 2015). The natural abundance technique of partitioning components of CO_2 efflux is therefore based on growing C_3 plants on C_4 soil and vice versa (e.g. Kuzyakov and Bol 2004, Kumar et al. 2016, Luo et al. 2017a). ^{13}C discrimination by C_3 and C_4 photosynthesis differ, such that greater discrimination by C_3 plants results in the formation of more depleted $\delta^{13}C$-SOM (-27‰) than C_4 plants (-13‰) (Farquhar, Ehleringer & Hubick 1989). In an incubation

study, CO_2 efflux from three sources was partitioned into CO_2 derived from added substrate, recent and old SOM following the input of a ^{14}C-glucose into soils that originated from a C_3 – C_4 vegetation change (Blagodatskaya *et al.* 2011a). The advantage of isotopic techniques is the minimal disturbance to soils and their applicability to a wide range of ecosystems.

1.8 Thesis aims and objectives

This thesis aims to investigate the interactions between plant C dynamics and soil microbial processes, and how these interactions control C and nutrient cycling in forest soils. These are important for understanding the consequence of environmental changes on forest soil C stocks. This study addresses the following questions:

1. What is the influence of an intact rhizosphere on soil CO_2 efflux and the decomposition of organic substrates that vary in their chemical composition and complexities?
2. What are the effects inputs of fresh C into soils in form of glucose, straw litter, fungal necromass or biochar on soil C decomposition?
3. Do the effects of substrate additions vary with structural complexity and chemical composition of the substrates?
4. How do the activities of extracellular enzymes respond to rhizosphere C supply from roots and substrates?
5. What are the interactions between the composition of decomposer communities and plant species that determine the quality of the resources?

Chapter 2 addresses questions 1 - 3 using forest mesocosms, where two-year birch (*Betula pendula*) trees were planted in 33 L boxes. Assimilate C supply from plant roots to the rhizosphere was altered by shading the trees, and ^{13}C- labelled substrates of varying structural complexity and quality was added to soils. These questions are also addressed under field conditions in Chapter 3, where incubation of ^{13}C- labelled substrates was done, in combination with soil trenching. Chapter 4 addressed questions 3 & 4, where the activities of six extracellular enzymes involved in the cycling of C, N and P were measured in relation to the input of organic materials and rhizosphere C supply from roots. In chapter 5, question 5 was addressed using litter bag incubations in a reciprocal transplant experiment.

Chapter 2

Rhizosphere carbon supply accelerates soil organic matter decomposition in the presence of fresh organic substrates

Oyindamola Jackson[a,b], Richard S. Quilliam[a], Andy Stott[c], Helen Grant[c], Jens-Arne Subke[a]

[a]Biological and Environmental Sciences, Faculty of Natural Sciences, University of Stirling, Stirling. FK9 4LA, UK

[b]Forestry Research Institute of Nigeria, Jericho, Ibadan, Nigeria

[c]Life Sciences Mass Spectrometry Facility, Natural Environment Research Council, Centre for Ecology and Hydrology, Lancaster, LA1 4AP. UK

2.1 Abstract

Belowground C supply from plant roots may accelerate the decomposition of SOM through the rhizosphere priming effect, but the detailed interaction between substrate quality and rhizosphere C supply is poorly understood. We hypothesize that decomposition of organic matter is enhanced by the combined effect of assimilate C supply to the rhizosphere and substrate amendments. Birch trees (*Betula pendula*) planted in experimental mesocosms were shaded to reduce the supply of assimilate C to roots and ECM fungi. Either ^{13}C-enriched glucose, straw, fungal necromass or C_4 biochar were subsequently added to each mesocosm. CO_2 efflux derived from substrates were separated from that derived from native SOM and roots based on the isotopic composition of total respired CO_2. The addition of all substrates increased fluxes in both un-shaded and shaded treatments, with greatest total CO_2 efflux observed in soils amended with straw. Increases in un-labelled CO_2 were observed to be greater in the presence of belowground C supply than in mesocosms with shaded trees. Turnover of SOM is closely linked to belowground C allocation. The biochemical quality and recalcitrance of litter entering the soil C pool is of critical importance to this priming, as is the interaction with rhizosphere-associated decomposition activity.

2.2 Introduction

Soil organic matter (SOM) is the largest carbon (C) pool in terrestrial ecosystems, and therefore plays an important role in the global C cycle (Ciais *et al.* 2013; Schlesinger & Bernhardt 2013). In addition, SOM improves the structure and fertility of soils (Six *et al.* 2000). SOM is mainly composed of a heterogeneous mixture of plant and microbial residues, which vary in chemical structures and decomposition rates (Kögel-Knabner 2002; Simpson & Simpson 2012). Soil CO_2 efflux (a combination of CO_2 respired by roots and their associated microorganisms (rhizomicrobial respiration) and the decomposition of SOM) is the primary pathway by which terrestrial C returns to the atmosphere. Due to the large amount of global C stored as SOM, changes in SOM decomposition can cause significant changes to the concentrations of atmospheric CO_2 (Lal 2004). Hence, it is important to understand the controls and drivers of SOM decomposition to forecast terrestrial ecosystem feedbacks, particularly under projected climate change scenarios.

Abiotic factors such as temperature and moisture are regarded as the major drivers of SOM decomposition (Bond-Lamberty & Thomson 2010; Moyano *et al.* 2012); however, there is increasing evidence that C supply to the rhizosphere, via plant roots, can directly drive SOM decomposition (Subke *et al.* 2011; Finzi *et al.* 2015). In addition to the input of litter to soils, plants release labile organic compounds into soils in the form of rhizodeposition, which includes root exudates and sloughed-off root cells (Jones *et al.* 2009; Pausch & Kuzyakov 2018). These organic compounds may act as energy source for microorganisms, and thereby increase microbial activity. As microbial activity increases, the demand for nutrients also increases which can stimulate the decomposition of SOM. Hence, changes in the productivity of plants can in turn affect rhizodeposition, which may influence the stability of soil C.

Roots of many temperate trees are often heavily colonised by ectomycorrhizal (ECM) fungi (Lang, Seven & Polle 2011). ECM fungi supply the host plant with nutrients derived from soils and in return receive up to 22% of photoassimilate C from the host plant (Smith & Read 2002; Hobbie 2006). This C supply allows the fungus to form extensive mycelial networks that can dominate organic horizons (Lindahl *et al.* 2007; Phillips *et al.* 2014), and stimulate the mobilization of nitrogen (N) in N-limited environments. ECM fungi also have the ability to produce a wide range of enzymes, e.g. β-glucosidase, cellobiohydrolase, N-acetyl glucosaminidase and leucine aminopeptidase, that allow them to mineralize C and N from SOM (Talbot *et al.* 2008; Brzostek *et al.* 2015). The majority of ECM fungi are found in

mineral horizons, with more decayed litter and humus whereas saprophytic fungi mainly colonize fresh litter found in the top layers of soil (Rosling *et al.* 2003; Lindahl *et al.* 2007). However, the ability of ECM fungi to successfully compete with saprophytic fungi and other soil microorganisms is dependent on a supply of C from host plant roots (Lindahl *et al.* 2010). The competition for limiting nutrients between these two fungal groups was hypothesized to retard SOM decomposition (Gadgil effect, (Gadgil & Gadgil 1971, 1975)). However, studies have shown that ECM fungi stimulate SOM decomposition as a result of C provisions from roots (Brzostek *et al.* 2015). Experimental reduction in C allocation to ECM fungi through girdling and tree shading results in significant decreases in soil respiration and fungal biomass (Högberg & Högberg 2002; Subke *et al.* 2004; Hasselquist *et al.* 2016), which implies that a reduction in assimilate C alters root respiration, but may also reduce the activities of ECM fungi, potentially reducing SOM decomposition.

The input of organic substrates into soils can either increase or decrease SOM decomposition via a 'priming effect, PE', defined as a short-term change in the decomposition of SOM caused by the input of a substrate, e.g. fertilizers (organic or mineral) or plant residues (Kuzyakov *et al.* 2000). The rhizosphere priming effect (RPE) is the change in SOM decomposition driven by the presence of plant roots (Kuzyakov 2002; Cheng & Kuzyakov 2005). Compared to soils without roots, a broad range of RPE from 50% reduction to 380% acceleration of SOM decomposition in the presence of roots has been reported (Cheng *et al.* 2014). Readily accessible C and plant residues may stimulate activities of microbial populations, thereby increasing SOM decomposition as a result of increased production of extracellular enzymes (Phillips & Fahey 2006; Yin *et al.* 2014). CO_2 evolution following the input of labile substrate can be used to classify PE into either a 'real' or an 'apparent' effect (Blagodatskaya & Kuzyakov 2008). The activation of microbial metabolism and acceleration of microbial biomass C turnover is referred to as 'apparent' PE (Blagodatskaya *et al.* 2007), as observed increases in CO_2 flux are not associated with decomposition of SOM. 'Real' priming effects require an actual acceleration of SOM decomposition caused by change in microbial community structure and extracellular enzyme production (Blagodatskaya & Kuzyakov 2008). Future predictions of elevated atmospheric CO_2 concentrations and higher temperature may increase net primary productivity through CO_2 fertilisation (Norby *et al.* 2005). Increased plant productivity results in higher rhizodeposition and plant litter production (Zak *et al.* 2011), thereby resulting in a net increase in C stock. However, due to

the potential priming effect of increased input of both labile and complex C into soils, the degree to which priming effect offsets this higher C input is uncertain.

The magnitude and direction of priming effects are influenced by nutrient availability, quality or quantity of substrate C. The quality of substrate is related to its susceptibility to microbial uptake and enzymatic degradation, for which the C to N ratio (C:N) and the concentration of recalcitrant fractions such as lignin, phenolics and tannins have been used as proxies (Chen *et al.* 2014; Wang *et al.* 2015a; Di Lonardo *et al.* 2017). Substrates of low recalcitrance and a higher availability of C (often considered to be of 'high quality') may induce higher PEs than substrates with less available C or more recalcitrant compounds (Blagodatskaya & Kuzyakov 2008). Soil microbial communities can influence the PE, as specific microbial groups (r versus K strategists) preferentially dominate decomposition of labile and recalcitrant C pools (Fontaine *et al.* 2003). However, our knowledge of the effects of substrate quality, and the interaction with the microbial community on PE is limited, and only a few studies have tested this theory (Chen *et al.* 2014; Wang *et al.* 2015a). Several studies have investigated the individual effects of C inputs from plants or substrates on organic matter decomposition (Zhang *et al.* 2013; Luo *et al.* 2016; Huo *et al.* 2017), but their interactions have not been widely explored. In a girdling study, (Subke *et al.* 2004) observed additional efflux in litter-amended, non-girdled plots, which was not significant in litter-amended, girdled plots or non-amended, non-girdled plots. Since organic matter enters the soil environment in different forms (for example, as rhizodeposits, litter or microbial necromass), and may differentially affect the PE, it is important to determine the range of potential effects on SOM decomposition in order to predict changes in C storage with different C inputs.

Here, we aim to determine the effect of an altered C supply to roots, and their associated ECM fungi, on the decomposition of a range of substrates, and to assess the decomposition of SOM with the input of ^{13}C-labelled substrates of different qualities. To address these aims, we tested the following hypotheses: (1) Decomposition of organic substrates is accelerated by the supply of assimilate C to the rhizosphere. (2) Decomposition of older native SOM is increased by the combined effect of substrate addition and rhizosphere C supply, (3) Decomposition of SOM is greater following the addition of readily available glucose compared to more complex organic substrates (i.e. straw, fungal necromass or biochar).

2.3 Materials and methods

2.3.1 Experimental design

Forty experimental mesocosms were constructed using 33 L boxes (71 x 44 x 16.5 cm, Really Useful Products Ltd, Normanton, UK). Mesocosms were assigned into four blocks in a randomised complete block design, each containing a shaded/not-shaded treatment, and a substrate treatment (4 blocks x 2 shading treatments x 5 substrate treatments). Organic-rich mineral soil (pH (H_2O) 4.04, C:N 14.9) was collected from the top 15 cm of a mixed broadleaf woodland in Stirling, Scotland, UK (56º8′ N, 3º54′ W), which was dominated by birch (*Betula pendula* Roth.) and beech (*Fagus sylvatica* L.) with little understory vegetation. The soil was a freely draining, brown earth formed from fluvioglacial sands and gravels derived mainly from carboniferous soil in the Dreghorn soil series of UK (National soil map of Scotland). The soil was air-dried, sieved (< 2 mm) and homogenised. A two-year old birch tree was planted in each mesocosm in August 2015 and allowed to establish for 8 months. Although relative amount of ECM fungi in the mesocosms were not determined directly, the presence of ECM was confirmed using in-growth nylon mesh bags (6 x 6 cm; 41µm mesh size, Normesh Ltd, Oldham, UK) filled with sterilized sand (Wallander *et al.* 2013). These in-growth bags had been used to differentiate between ectomycorrhizal hypha from that of saprotrophs, as ECM fungi are able to grow in sand while saprotrophic fungi cannot (Wallander *et al.* 2001; Ekblad *et al.* 2013). In spring 2016, collars (10 cm diameter, 2 cm high) were inserted (< 1 cm deep) into all mesocosms, taking care to minimise disturbance to roots. All mesocosms were kept outside, in the grounds of the University of Stirling, Scotland, where the mean annual air temperature is 9.2 ºC and the mean annual precipitation is *c*. 1019 mm (UK Met Office 2017).

On 2nd August 2016, nine days prior to the addition of the substrates, 20 trees were shaded to reduce photosynthesis and reduce the supply of C to roots and their fungal symbionts. Shading was achieved by using dark but air-permeable phormisol material (LBS Worldwide Ltd, Lancashire, UK) to cover each tree. This material limited irradiance by at least 90%, whilst allowing air exchange and avoiding excessive rises in air temperature for the shaded trees. Care was also taken to ensure that soils were not covered by shading material in order to minimise changes in soil temperature.

2.3.2. Preparation of ^{13}C-labelled substrates

Four substrates were selected to represent different forms of organic materials with varying structural composition and complexities: glucose, straw, fungal necromass and biochar.

^{13}C-glucose was obtained by diluting ^{13}C-D-glucose (99 atom%. Cambridge Isotope Laboratories, Inc., Andover, USA) with D-glucose of natural isotope abundance (Fisher Scientific UK Ltd, Loughborough, UK) with a dilution factor of 1:20, resulting in an enrichment in ^{13}C of c. 5 atom%.

^{13}C wheat straw (*Triticum aestivum* L.) was obtained from wheat grown in a chamber enriched with 10 atom% $^{13}CO_2$. The straw was rinsed in deionised water 4 times, air dried for several days, then shredded (<2 mm) using a grinder.

Mycelium of the basidiomycete *Hebeloma crustuliniforme* UP184 was grown on agar in Petri dishes containing 1/10 Modified Melin Norkans (MMN) growth medium (Marx 1969), with glucose reduced fom 10 g to 1 g. Based on the result of a pilot study to determine the medium composition that supports the most rapid fungal growth, a modified MMN growth medium containing 10 g of malt extract (instead of 3 g) was selected for growing ^{13}C-labelled fungal biomass (see Appendix 1 for full list of ingredients). In the liquid growth medium (100 cm^3), the fungal biomass was labelled by replacing 20% of the ^{12}C-glucose with 99 atom% ^{13}C-glucose (CK Isotopes Ltd, Leicester, UK). Flasks were incubated at 20 ^{o}C for 30 days, or until filled with mycelia. Mycelium was harvested from each flask, and rinsed with deionized water 4 - 5 times to remove any remaining media, air-dried and stored at -4˚C. Prior to use, the mycelium was homogenised using a grinder (< 2mm).

Biochar was obtained from the pyrolysis of *Miscanthus* (a C_4 species) at 450 ^{o}C and subsequently ground (<2 mm). The δ^{13}C values of all substrates were confirmed at Life Sciences Mass Spectrometry Facility (CEH, Lancaster, UK) using an automated elemental analyser NA1500 (Carlo Erba, Milan, Italy) coupled to an Isotope Ratio Mass-Spectrometer (Dennis Leigh Technology Ltd, Keele, UK).

Each treatment was represented in all blocks to ensure that all treatments were exposed to similar conditions. The four substrates with distinct ^{13}C signatures were applied as dry powder to the collars on 11th August 2016. Substrates were applied to collars at the rate of approximatly 3 mg C g^{-1} soil except for biochar, which was applied at rate of 4.7 mg C g^{-1} soil (collar area = 79 cm^2, depth = 2 cm). The amount of substrates added are within the range of quantities that can induce '*real*' PEs and the application rate of biochar currently

used in agriculture (Blagodatskaya & Kuzyakov 2008; Zimmerman & Ouyang 2019). The isotopic signatures and the exact amount of the added substrates are presented in Table 2.1. Substrates were thoroughly mixed into the top 2 cm of the soil within each collar, whereas control collars were also mixed although no substrate was added. 250 cm^3 of water was subsequently added to all treatments including control treatments.

Table 2.1 Total C, N, C:N ratio and $\delta^{13}C$ of substrates, and quantity of substrates and total ^{13}C label added to soils in the experiment

Substrate	Total C (%)	Total N (%)	C:N ratio	$\delta^{13}C$ (‰)	Amount of ^{13}C added (g)
Biochar	68.9 ± 0.221	0.222 ± 0.001	307 ± 0.669	-4.22 ± 0.066	0.008
Straw	41.9 ± 0.284	0.753 ± 0.008	55.7 ± 0.851	9320 ± 6.95	0.046
Fungal necromass	49.3 ± 2.11	1.718 ± 0.088	28.7 ± 0.461	2070 ± 24.7	0.017
Glucose	41.24 ± 1.01	N.A	N.A	5250 ± 0.591	0.029
Control	5.64 ± 0.367	0.377 ± 0.020	14.9 ± 0.198	-27.9 ± 0.088	N.A

2.3.3 Soil respiration and isotopic measurements

Soil respiration measurements were carried out using a portable EGM-4 infrared gas analyser (PP Systems, Amesbury, MA, USA) attached to a 15 cm diameter custom-built respiration chamber with a headspace volume of approximately 2,300 cm^3, inserted gently into soil (< 1 cm). Respiration rates were derived from linear rise in CO_2 concentration within the closed chamber system over a period of two minutes. Sampling for isotopic composition of soil-derived CO_2 was carried out at 2.5 and 5 h (for glucose, straw and control treatments only), 1, 3, 7, 15 and 30 d after substrate addition. On each sampling occasion, five 15 cm gas flux

chambers (15 cm diameter, 5 cm height collars with lids; Plastic Company, UK) were placed on the soil of all five treatments within an experimental block simultaneously, ensuring a good seal between soil and chamber. CO_2 was allowed to accumulate in these chambers for 15 minutes, resulting in concentrations between 490 and 7680 ppm of CO_2. Gas samples (20 ml) were then collected using 20 ml syringes through a septum in the chamber and injected under pressure into previously evacuated borosilicate exetainers (12 ml; Labco Ltd, UK) for isotopic analysis. The concentration of CO_2 in all gas samples was obtained using a gas chromatograph, GC-MS (Hewlett Packard 5890) coupled to a flame ionization detector. Isotopic measurements for $\delta^{13}C$ values of the gas samples were measured at the Life Sciences Mass Spectrometry Facility (CEH, Lancaster, UK) using an Isoprime Tracegas Preconcentrator coupled to an IsoPrime Isotope Ratio Mass Spectrometer (Elementar UK Ltd, Stockport, UK) at an analytical precision of ± 0.17‰.

2.3.4 Soil sampling and analysis

Soil samples were collected to a depth of 5 cm from each collar after gas sampling on the last day of sampling and stored in sealed plastic bags at 4 ºC until processing for soil microbial biomass C, which was determined using the fumigation extraction method (Vance, Brookes & Jenkinson 1987; Joergensen 1996). Fresh soil was passed through a 2 mm sieve from which 5 g each was weighed into two glass jars (20 ml). One jar was fumigated in a desiccator containing ethanol-free $CHCl_3$ and water (to avoid drying) for 24 h and evacuated using a vacuum pump, while the other jar was not fumigated. After fumigation for 24 h, $CHCl_3$ was removed from the soil by evacuating the desiccator three to four times using a vacuum pump. Both the fumigated and non-fumigated soils were extracted with 20 ml 0.5 M K_2SO_4 (1:4 w/v; soil:extractant) and the mixture shaken for 30 min at 300 rpm, before being filtered through Whatman no. 42 filter papers (pore size: 2.5 µm). Soil microbial biomass C was determined based on the difference between fumigated and non-fumigated soils using the *kec* factor of 0.45 (Wu et al. 1990). Total organic C (TOC) and total organic N (TON) content of the filtrates were determined using a TOC – VCSN analyzer (Shimadzu Corporation, Kyoto, Japan).

2.3.5 Calculations and statistical analysis

Isotopic abundance of CO_2 collected from chambers was corrected for atmospheric CO_2 using the isotopic ratio and concentration of atmospheric CO_2 present in the chambers at the start of measurements. A two end-member linear mixing model was formulated to partition total soil flux (F_{Total}) into substrate-derived CO_2 (F_{Sub}) and soil-derived CO_2 (F_{Soil}) based on the ^{13}C isotopic abundance between the substrates and the soil using the following mass balance equation:

$$F_T\delta_T = F_{Sub}\delta_{Sub} + F_{Soil}\delta_{Soil} \quad (2.1)$$

Where F_T, F_{Sub} and F_{Soil} are the total CO_2 flux, substrate derived CO_2 and soil derived CO_2 respectively, and δ_T, δ_{Sub} and δ_{Soil} are the $\delta^{13}C$ isotopic signatures for the total flux, substrates and soil, respectively. In this experimental setup, CO_2 from both heterotrophic and rhizomicrobial respiration are considered as one isotopic pool (indicated by "soil"), while the suffix "sub" represents the glucose, straw, fungal necromass or biochar treatments. The proportion of substrate in the total flux was calculated as:

$$f_{Sub} = \frac{\delta_T - \delta_{NS}}{\delta_{Sub} - \delta_{NS}} \quad (2.2)$$

$$1 = f_{Sub} + f_{Soil} \quad (2.3)$$

where δ_T, is $\delta^{13}C$ obtained from CO_2 samples collected from all soil collars after correction for atmospheric CO_2. δ_{Sub} is $\delta^{13}C$ of the labelled- glucose, straw, fungal necromass or biochar, while δ_{NS} is $\delta^{13}C$ obtained from CO_2 samples collected from control soils where no substrate was added. The CO_2 derived from substrates was calculated as:

$$F_{Sub} = f_{Sub} \times F_T \quad (2.4)$$

and the standard error for the proportion of substrate flux contribution (f_{Sub}) was calculated according to the method by Phillips & Gregg (2001), which accounts for variabilities in both the mixture ($\delta^{13}C_{Total}$) and the sources ($\delta^{13}C_{Sub}$ and $\delta^{13}C_{Soil}$) as:

$$SE(f_{Sub}) = \sqrt{\left(\frac{1}{\delta_{Sub} - \delta_{NS}}\right)^2 \left[\sigma^2{}_{\delta T} + {f_{Sub}}^2 \sigma^2{}_{\delta Sub} + (1 - f_{Sub})^2 \sigma^2{}_{\delta NS}\right]} \quad (2.5)$$

where σ^2 represent the square of the standard errors of the mean isotopic signatures for the components as indicated by the suffixes.

Priming effects were calculated in two categories. First, priming of soil C decomposition in un-shaded or shaded treatments induced by the addition of substrates was calculated as the difference of the CO_2 produced from control soils without substrate amendment from CO_2 derived from sources other than the substrate added (Eq 1.6):

$$PE(\%) = \frac{^{amended}F_{Soil} - ^{NS}F_{Soil}}{^{NS}F_{Soil}} \times 100 \quad (2.6)$$

Where $^{amended}F_{Soil}$ is the soil-derived CO_2 in soils amended with biochar, straw, fungal necromass or glucose of shaded or un-shaded treatments determined by $F_{Soil} = F_T - F_{sub}$; and $^{NS}F_{Soil}$ is the soil-derived CO_2 in soils without substrate addition for the respective light condition.

Second, priming of added substrate decomposition induced by rhizosphere input was calculated as the difference of substrate-derived CO_2 in shaded conditions from substrate-derived CO_2 from soils in un-shaded conditions. The rhizosphere priming effect of substrate decomposition (RPE_{Sub}) was calculated as:

$$RPE_{Sub}(\%) = (^{unshaded}F_{Sub} - ^{shade}F_{Sub})/^{shaded}F_{Sub} \times 100 \quad (2.7)$$

where $^{unshaded}F_{Sub}$ is the substrate-derived CO_2 in soils that receive autotrophic inputs from roots of un-shaded trees with added substrate, and $^{shaded}F_{Sub}$ is the substrate-derived CO_2 in soils without root input from shaded trees with added substrate.

Effects of shading and substrate additions on total soil CO_2 efflux, soil-derived CO_2, substrate-derived CO_2, PE and soil microbial biomass C were analysed using two-way analysis of variance (ANOVA) following linear mixed effect models, where 'mesocosm' nested within blocks and sampling dates were assigned as random factors. Data were log-transformed when necessary to meet the assumptions of normal distribution. When statistical significance was observed, Tukey post-hoc tests were used for pairwise comparisons at significance level of 0.05. All analyses were carried out using RStudio v0.99.903.

2.4 Results

2.4.1 Soil respiration

Prior to tree shading, there was no significant difference between CO_2 efflux from soil collars assigned as un-shaded and shaded treatments (Fig. 2.1). Nine days after shading, soil respiration decreased significantly by 63% relative to un-shaded treatments ($p < 0.001$) and this reduction in CO_2 efflux was significant for all subsequent sampling dates. Average soil CO_2 efflux from un-shaded mesocosms (2.39 ± 0.38 μmol m^{-2} s^{-1}) was significantly greater than that of shaded mesocosms (0.78 ± 0.08 μmol m^{-2} s^{-1}) for the period after shading ($P < 0.001$, Table 2.2)). The relative contribution of root-derived CO_2 (rhizomicrobial respiration) to the total cumulative soil CO_2 efflux was 67%, calculated as differences between un-shaded and shaded treatments in control (un-amended) soils.

The addition of substrates to soils significantly increased total CO_2 efflux ($P < 0.001$) from both un-shaded and shaded treatments throughout the sampling period of 30 days, relative to controls without substrate (Figs. 2.2 and 2.3). Whereas shading and substrate addition significantly affected total soil CO_2 efflux, there was no significant interaction between these two fixed effects (Table 2.2). Total soil CO_2 efflux increased significantly following the addition of straw, fungal necromass and biochar in un-shaded treatments. In shaded treatments on the other hand, the addition of biochar significantly increased soil CO_2 flux ($P < 0.05$, Tukey-post hoc test) whereas glucose, straw and fungal necromass had no effect on total soil CO_2 efflux.

Table 2.2 Analysis of variance (ANOVA) of light condition, substrates and their interactions on total soil CO_2, soil-derived CO_2 and substrate-derived CO_2 efflux. Significant values ($P < 0.05$) are in bold.

	Transformation	Fixed factors	*df*	*F*	*P value*
Total Soil CO_2 efflux	Log	Shading	1,35	82.3	**<0.001**
		Substrate	4,36	10.3	**0.001**
		Shading x Substrate	4,36	1.29	0.29
Soil-derived CO_2	Log	Shading	1,30	84.9	**<0.001**
		Substrate	4,30	9.77	**<0.001**

		Shading x Substrate	4,30	1.12	0.51
Substrate-derived CO_2	Log	Shading	1,20	0	0.99
		Substrate	3,24	20.03	**<0.001**
		Shading x Substrate	3,23	1.04	0.38

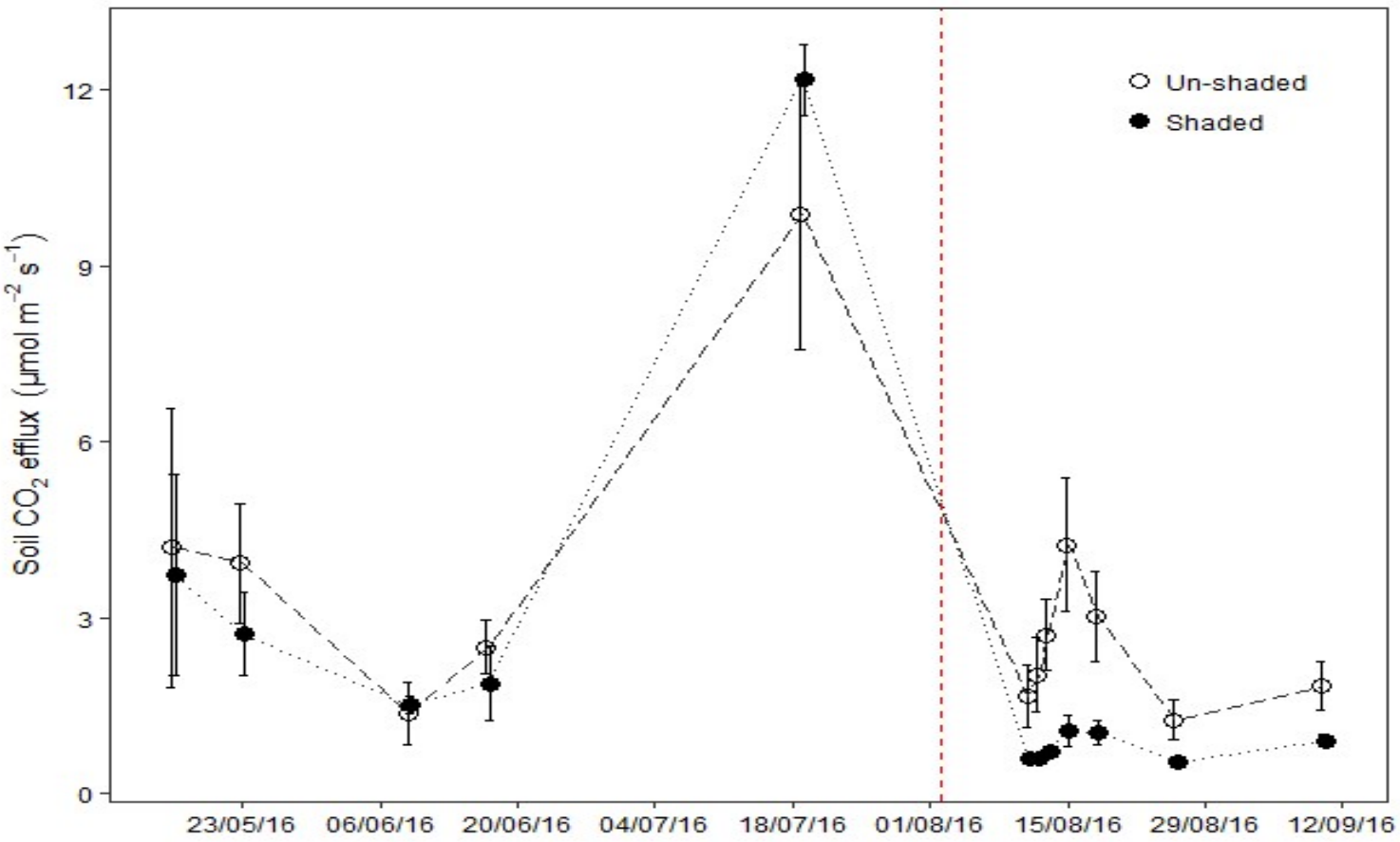

Figure 2.1 Average soil CO_2 efflux for the period of 16th May 2016 to 10th September 2016 in mesocosms without substrates added (control treatments). Soils were watered on 18th July 2016 prior to measurement on 19th July 2016 due to excessive dryness in the mesocosms. Trees

were shaded on 2nd August 2016, indicated by the red line. Error bars represent ± 1 SE (n =4). There was no significant difference between shading treatments prior to shading ($P = 0.6274$) but significantly different after shading ($P < 0.001$, paired t-test).

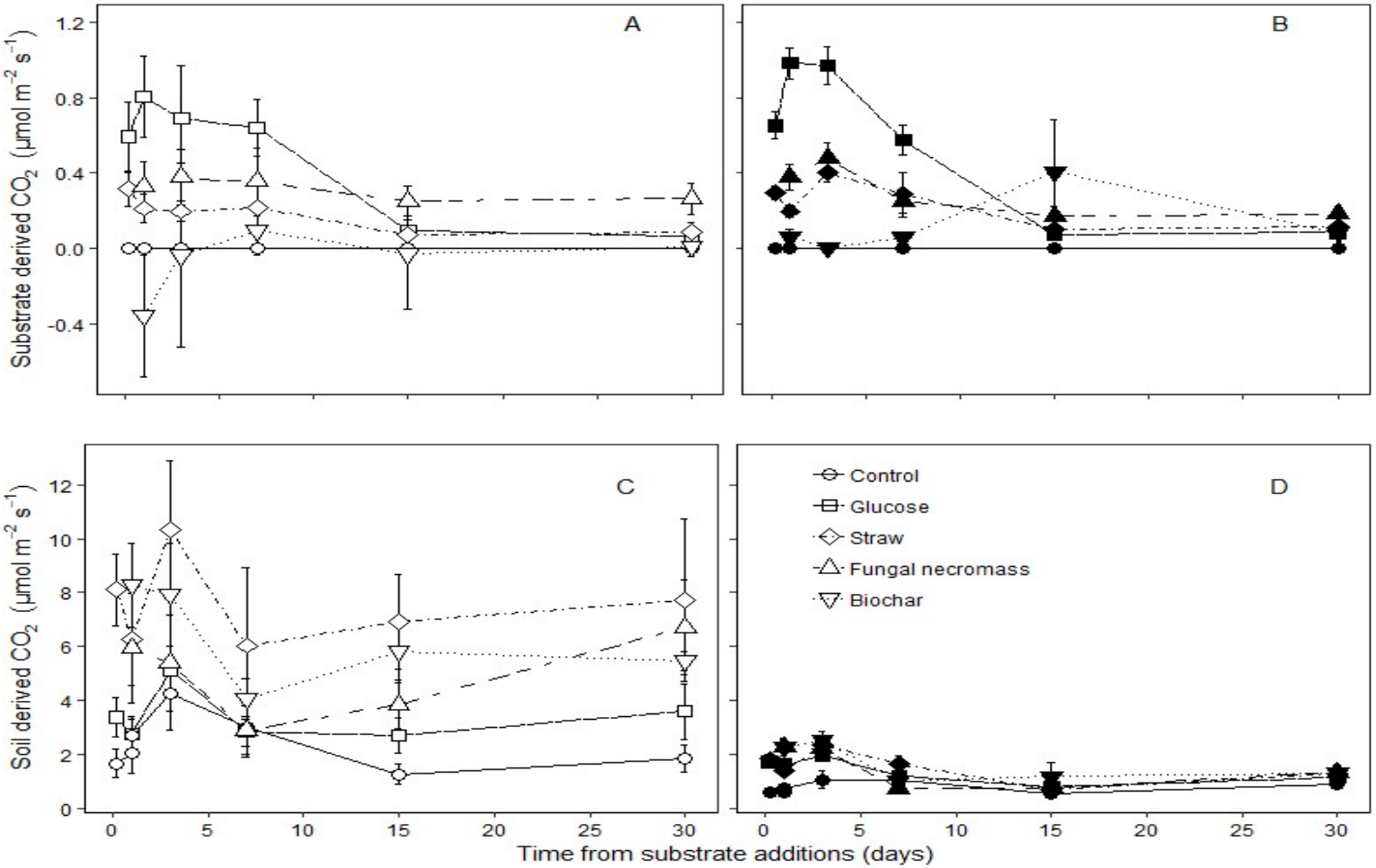

Figure 2.2 Soil CO_2 efflux in un-shaded (open shapes) and shaded (filled shapes) treatments derived from the added ^{13}C labelled substrate derived CO_2 (A & B) and un-labelled soil CO_2, (C & D) for the duration of 30 days after the addition of glucose, straw residues, fungal necromass and biochar. Values denote mean ± 1 SE (n = 4).

Increases in $\delta^{13}C$ of soils were observed within 5 hours of glucose and straw addition, and 24 hours of fungal necromass addition when the first measurements were taken (Fig. 2.4). The respiration of glucose was highest in the first 7 days after its addition in both shaded and un-shaded treatments after which decomposition of glucose declined, with a higher decomposition of glucose in the shaded treatment than the un-shaded treatment. The decomposition of straw and fungal necromass were greater in shaded treatments and continued throughout the sampling period whereas in un-shaded treatments, both, decomposition of straw and fungal necromass was highest on day 7. ^{13}C values in biochar treated soils were not different from those in control mesocosms that received only water.

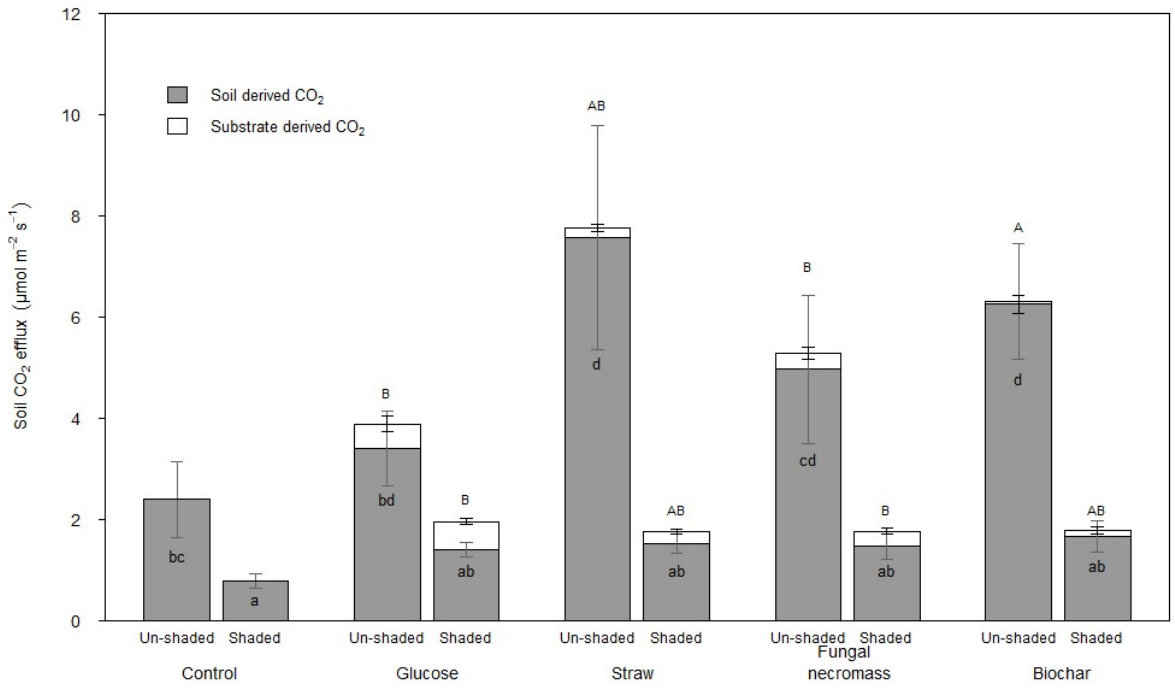

Figure 2.3 Partitioning of total CO_2 efflux into CO_2 derived from root and heterotrophic ("Soil derived CO_2") and from the addition of glucose, straw, fungal necromass and biochar ("Substrate-derived CO_2") in un-shaded and shaded treatments. Control treatments received water only. Error bars are 1SE. Different letters indicate significant interactions between shading and substrate factors ($P < 0.05$, Tukey *post hoc*) for soil-derived CO_2 efflux (small letters) and substrate-derived CO_2 (capital letters).

2.4.2 Source-partitioning and priming effects

CO_2 efflux derived from added substrate was significantly different among substrates ($P < 0.001$) but not between shading treatments (Table 2.2 and Fig. 2.3). In both un-shaded and shaded treatments, the majority of glucose-derived CO_2 evolved within seven days of glucose addition, with CO_2 flux rates from glucose-amended mesocosms with shaded trees (Fig. 2.2A, B). For the period of 30 days after substrate addition, CO_2 evolved from sources other than the added substrate ranged between 3.40 ± 0.74 and 7.56 ± 2.21 μmol m^{-2} s^{-1} in un-shaded treatments, whereas it ranged between 1.40 ± 0.15 and 1.65 ± 0.31 μmol m^{-2} s^{-1} in shaded treatments. Adding straw to un-shaded mesocosms resulted in soil-derived CO_2 that was approximately three times higher than control mesocosms (un-shaded, no substrates) and about 5 times that produced from the corresponding shaded treatment (shaded, straw added; Fig. 2.3). In un-shaded mesocosms, un-labelled CO_2 efflux increased immediately following the addition of straw by up to four times relative to un-shaded control mesocosms and the increases persisted until the end of sampling (Fig. 2.2C). For the shaded treatments, the addition of glucose, straw, fungal necromass and biochar increased un-labelled CO_2 during the first 3 days only (Fig. 2.2D). Glucose amended soils produced the least CO_2 from soil in both un-shaded and shaded treatments, but more CO_2 derived from the added substrate.

No significant priming of substrate decomposition by the supply of C to the rhizosphere (PE_{Sub}) as calculated by the difference between substrate derived CO_2 in un-shaded and shaded treatments was observed (Fig. 2.3). However, increases in un-labelled CO_2 (priming effects) after the addition of all substrates were observed in both shaded and un-shaded trees (Fig. 2.5). These priming effects were not different between soils with un-shaded and shaded trees, with the exception of straw treatments where the un-shaded treatment was significantly higher than the shaded treatment ($P < 0.01$). The addition of glucose generated the lowest PE over the experimental period in the shaded treatments (88 %) and in the un-shaded treatment (61 %). The PE was significantly higher immediately after the addition of ^{13}C-labelled straw and glucose (5 h) and fungal necromass and biochar (1 d) in both shading treatments (Fig. 2.5). Following this initial flush, no significant PE was observed until 15 days after substrate additions in un-shaded treatments.

2.4.3 Microbial biomass carbon

At the end of the sampling period, soil microbial biomass C was generally higher in un-shaded treatments than in shaded treatments ($P < 0.05$). However, there was no overall significant difference in soil microbial biomass C among substrates, nor between the interaction of shading and substrates (Fig. 2.6).

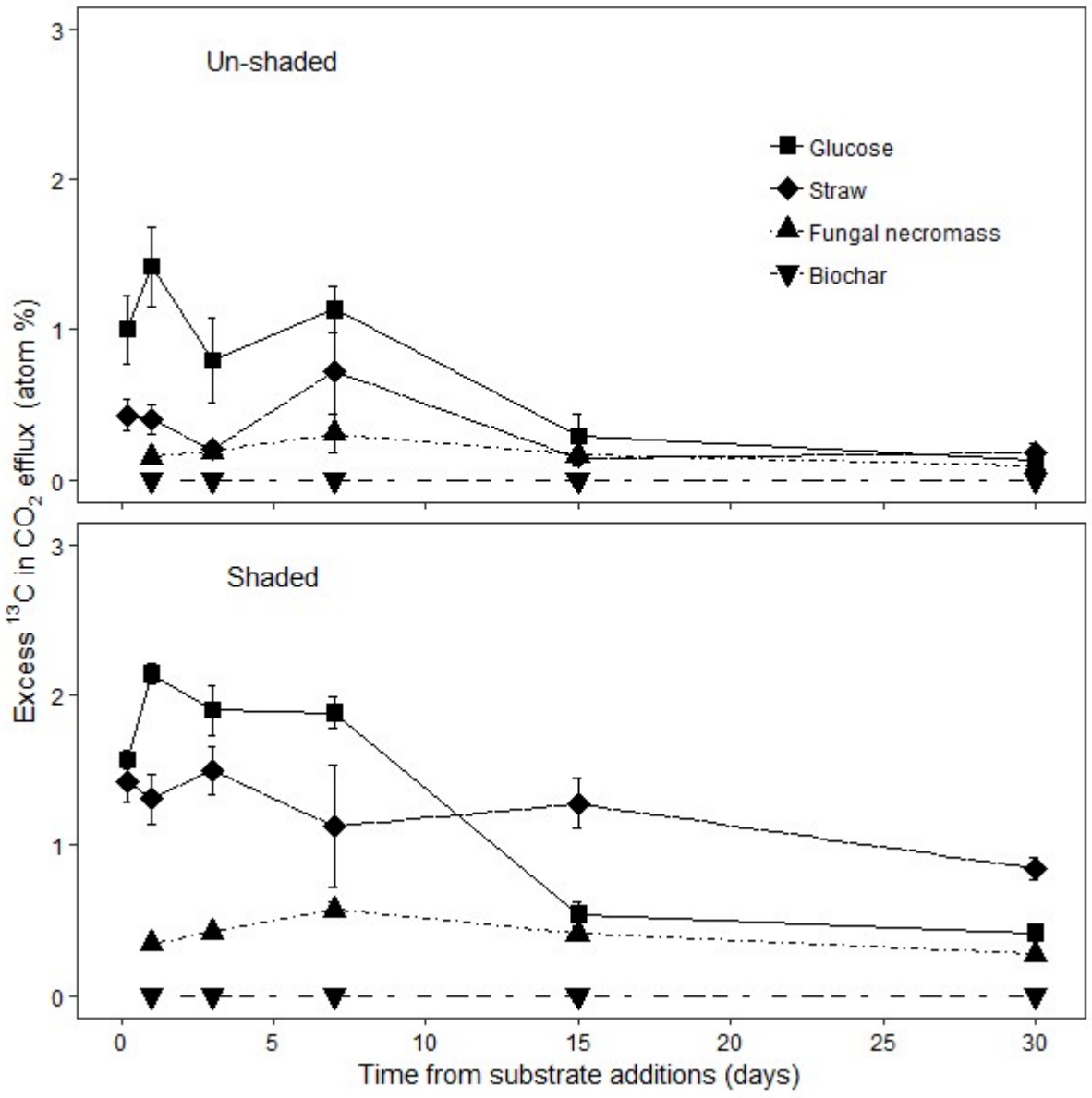

Figure 2.4 Excess of ^{13}C in soil CO_2 efflux relative to the natural abundance in un-labelled soil with time following the addition of ^{13}C-labelled glucose, straw, fungal necromass and

biochar into soils with un-shaded (top) and shaded (bottom) trees. The vertical bars represent 1 SE (n = 4).

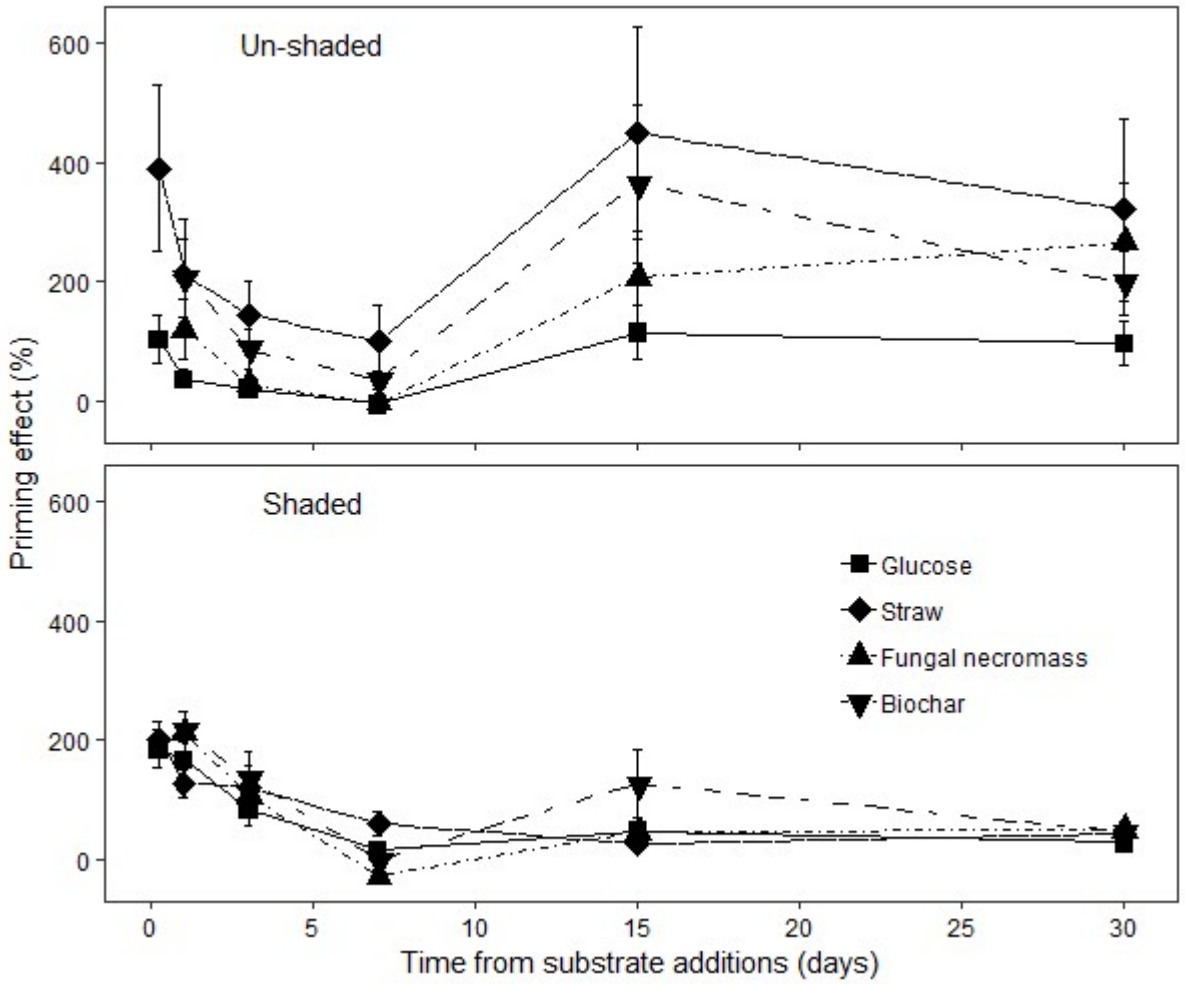

Figure 2.5 Unlabelled CO_2 efflux expressed as priming effect in percent of extra CO_2 above control levels, following the addition of glucose, straw, fungal necromass or biochar from un-shaded (top) and shaded (bottom) treatments. Values are mean ± 1 SE (n = 4).

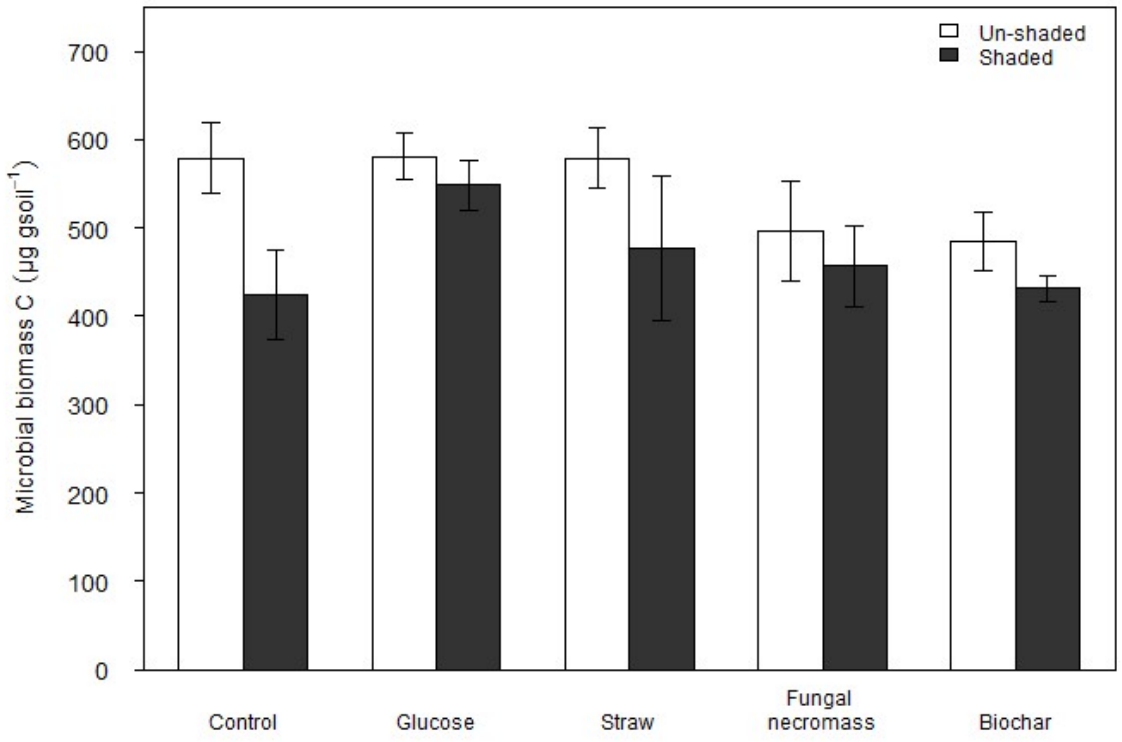

Figure 2.6 Soil microbial biomass C in un-shaded and shaded treatments following the addition of glucose, straw, fungal necromass and biochar. Control soils received only water. Error bars are SE of the mean (n = 4)

2.5 Discussion

The alteration of rhizosphere C supply through shading reduced total soil CO_2 efflux across all substrate treatments. The result also provides evidence that the combined effects of rhizosphere C supply and substrate amendments can increase decomposition of native SOM, the magnitude of which is dependent on the quality of the amendment. Tree shading reduced soil CO_2 efflux by about 67% for the duration of the experiment suggesting C supply to roots and their associated ECM fungi was strongly reduced, which suggests that assimilate C flux to roots is a major driver of soil CO_2 efflux. It is commonly reported that roots use their carbohydrate reserves after assimilate C supply is altered through girdling, trenching, defoliation or drought, thereby delaying the reduction in respiration rates and underestimating autotrophic respiration (Högberg *et al.* 2001; Díaz-Pinés *et al.* 2010; Hasibeder *et al.* 2015). However, plant age influences the availability of root carbohydrate reserves (Bahn *et al.* 2006); hence, in contrast to the mature forests reported in other studies, it is likely that the root C reserves were rapidly used up following shading in the young trees (3 years old) used in this study, resulting in rapid decline in root respiration. Shading is potentially an effective partitioning method with a number of benefits compared to root exclusion methods (such as trenching and other physical separation techniques). Some of these methods may significantly underestimate the contribution of roots due to limitations such as the physical disturbance to the soil structure and decomposition of decayed roots (Subke *et al.* 2006).

The addition of glucose, straw, fungal necromass and biochar, representing organic matter of varying structural complexities, resulted in increased microbial metabolic activities as indicated by higher CO_2 fluxes from soils of both shaded and un-shaded trees. As shown by the excess ^{13}C in soil CO_2 efflux, glucose, straw and fungal necromass were decomposed in both shaded and un-shaded treatments, whereas biochar was not decomposed in either shading treatments. The lack of biochar decomposition was likely because biochar, being a relatively recalcitrant substrate (Cross & Sohi 2011; Schmidt *et al.* 2011) is not readily available for microbial metabolism. Incubation studies have reported occurrences of biochar decomposition as well as a lack of decomposition (Zimmerman 2010, Stewart et al. 2013, Cui et al. 2017, Luo et al. 2017a), and the contrasting results may be attributed to differences in the combustion temperature, duration and biomass used for biochar production, which influence its lability (Zimmerman 2010). Use of natural abundance of C4 vegetation for

biochar contrasts with the much higher enrichment of other substrates. However, it is unlikely that the apparent lack of biochar decomposition is an artefact of the reduced isotopic range, as the contrast in C3 and C4 vegetation (c. -4‰ vs. c. -28‰) is the basis of many successful partitioning approaches (e.g. Yin et al. 2018).

The supply of assimilate C to the rhizosphere did not enhance the decomposition of substrates, as we did not observe any difference in the mineralization of glucose, straw, fungal necromass or biochar between shaded and non-shaded treatments. Previous studies that investigated the interaction between root activity and litter decomposition have either reported a positive (Subke *et al.* 2004, 2011; Trap *et al.* 2017) or negative (Gadgil & Gadgil 1971; Averill *et al.* 2014) effect of roots on litter decomposition. Since roots and ECM were present in both un-shaded and shaded treatments, it is possible that in addition to heterotrophic saprotrophs, ECM fungi contributed to the mineralization of the added substrates. Although, ECM fungi receive their C primarily from host plant photosynthates in return for nutrients, they may also access soil C either from the metabolism of low molecular weight compounds (Talbot *et al.* 2008) or through decomposition of complex organic compounds (Phillips *et al.* 2014) by investing in enzymes involved in the mobilization of C (Buée, Vairelles & Garbaye 2005; Buée *et al.* 2007; Courty, Bréda & Garbaye 2007).

As flux partitioning using the isotopic composition showed, the addition of fresh substrate increased CO_2 flux from sources other than the added substrate. This additional CO_2 efflux might have been caused by increased root-derived respiration, increased turnover of soil microbial biomass and/or accelerated SOM decomposition, all of which had similar isotopic compositions. We hypothesise that the additional un-labelled CO_2 production was caused by an initial increase in the turnover of microbial biomass C (apparent PE), followed by increased decomposition of SOM (real PE) rather than from higher rhizo-microbial respiration. It is possible that the proliferation of roots and associated mycorrhizal fungi in response to the addition of substrates also contributed to the observed increase in un-labelled CO_2 flux. However, the absence of a similar proliferation in shaded treatments suggests that the availability of labile C in the rhizosphere, not root growth causes the observed pattern. In a pot experiment, to test the contribution of ectomycorrhizal roots to the additional un-labelled CO_2 released following the addition of ^{13}C-labelled sucrose, Ekblad & Högberg (2000) found no increase in rhizomicrobial respiration with added sugar, corroborating our conclusion that the additional un-labelled CO_2 is from organic matter decomposition.

The rapid flush of un-labelled CO_2 efflux from both shaded and un-shaded treatments on or before 24 h was likely due to increased microbial turnover rates, and we propose that real PE involving the decomposition of SOM commenced after 1 day of substrate additions in the forest soil of our mesocosms. Therefore, this study demonstrates that the addition of complex substrates mostly causes real PE. Most likely due to the production of extracellular enzymes and subsequent co-metabolism of SOM (Blagodatskaya & Kuzyakov 2008; Fontaine *et al.* 2011; Blagodatskaya *et al.* 2014a). The PE occurred in two stages: short-term apparent PE occurring immediately after substrate addition followed by long-term real PE, which was predicted by the model of Blagodatsky et al. (2010). This temporal dynamic of PE has also been reported by others (Nottingham *et al.* 2009; Blagodatskaya *et al.* 2011a), and requires confirmation by distinct partitioning of rhizomicrobial respiration from microbial decomposition, whilst also tracing microbial biomass pools with a three-source labelling approach.

Positive priming effects of substrate on SOM, as reflected by increased soil derived CO_2 in soils amended with substrates relative to control soils, were generally lower in shaded treatments than un-shaded treatments. Glucose-amended soils were the exception; PE was greater in the absence of C supply to the rhizosphere. Supporting our second hypothesis, this demonstrates that rhizosphere C supply coupled with the input of substrate amendments accelerates SOM decomposition. In realistic field conditions, different forms of substrates are released into forest soils as litter or rhizodeposits (Kuzyakov 2010). However, our study is among the few that have investigated the combined effect of fresh substrate input and an intact rhizosphere in either forest (Subke *et al.* 2004) or agro-ecosystems (Mwafulirwa *et al.* 2017). A potential shortcoming of our simple forest system in this mesocosm study is the absence of the associations of ECM mycelium with more than one tree that may be found in natural forests (Lang *et al.* 2011), such that altered C supply from one tree host might be compensated by C supply from another tree host. Notwithstanding, our results suggest that in shaded treatments, microorganisms degraded the added substrate to derive energy for microbial growth, which led to co-metabolism of SOM. The PE observed in these shaded treatments was not significantly different among substrates, indicating that the acquired C from decomposition of labile fractions of substrates were not sufficient to sustain microbial activities for long. This suggests that the energy needed to metabolize SOC is greater than the energy acquired from the catabolism of the added substrate (Fontaine *et al.* 2007).

Soil priming after substrate additions was likely due to microbial activation (Cheng & Kuzyakov 2005), as we did not observe any difference in total microbial biomass C between shading treatments and among different substrates. Other studies have also attributed the increase in SOM decomposition to microbial activation in the presence of roots (Zhu *et al.* 2014; Kumar *et al.* 2016; Mwafulirwa *et al.* 2017) or substrates (Blagodatskaya *et al.* 2011a, 2014a; Shahbaz *et al.* 2017b). Owing to the low N soil content (0.35%), and no addition of fertiliser to the soils before or after planting, the soils were strongly N limited. This N limitation was further aggravated by adding substrates with high C to N ratio at concentrations (3 mg substrate C g^{-1} soil) high enough to induce SOM decomposition (Luo *et al.* 2016; Liu *et al.* 2017). Therefore, it is likely that in a bid to meet their nutrient demands, microorganisms utilized the rhizodeposits or the labile C fraction of substrates as an energy source to decompose the less available more stable SOM, and mobilize nutrients (i.e. the microbial mining hypothesis, (Fontaine *et al.* 2011)). Similar increased SOM decomposition was observed with the input of substrates with high C:N ratios into subtropical forest soils when compared to nutrient-rich soils (Qiao *et al.* 2016). This further supports the hypothesis that the direction and magnitude of PE is controlled by the compromise between energy and nutrient availability for SOM decomposition (Fontaine *et al.* 2003).

In general, the decomposition of complex substrates is dominated by fungi, whereas bacteria dominate decomposition of soluble substrates (sugars and amino acids) (Fontaine *et al.* 2011). In the presence of substrates, higher PEs were observed in un-shaded treatments than in shaded treatments with limited C supply to ECM fungi. This suggests that the supply of C by plants to roots and their associated ectomycorrhizae exerts a greater effect on SOM decomposition than the addition of substrates directly to the soil. However, a three-source partitioning of total CO_2 efflux is required for further confirmation. Ectomycorrhizal fungi therefore have a significant influence on soil C storage and the magnitude of this influence is dependent on plant productivity (Moore *et al.* 2015a).

Although both simple and complex substrates increased the decomposition of SOM, we observed higher priming effects in the presence of complex substrates of low microbial availability and higher C:N ratio than from glucose-amended soils. This result does not support our third hypothesis that SOM decomposition is higher following the addition of a readily available substrate (glucose) compared to complex substrates. Glucose is soluble and readily available for microbial utilization, whereas straw residue, fungal necromass and biochar have complex polymerised C molecules that require more energy for the production

of enzymes to decompose them (Luo *et al.* 2016). Our findings suggest that glucose addition stimulated only microorganisms that are specialised in decomposition of easily utilizable C (r-strategists; Fontaine et al. 2003), whereas straw, fungal necromass and biochar additions stimulated the activities of microorganisms that are responsible for the decomposition of complex organic molecules by synthesizing extracellular enzymes. Positive PE was therefore due to co-metabolism of SOM during the decomposition of the complex substrates (Kuzyakov *et al.* 2000; Paterson & Sim 2013).

Biochar has been shown to improve soil fertility as well as reduce soil nutrient losses (Sohi *et al.* 2010; Quilliam *et al.* 2012). However, biochar is a recalcitrant material, not readily available for microbial degradation, and the addition of biochar has been reported to have either a negative PE (Cross & Sohi 2011; Cheng *et al.* 2017), or a positive PE (Wardle, Nilsson & Zackrisson 2008; Cui *et al.* 2017). The feedstock, pyrolysis temperatures and retention duration used during biochar production, together with the age of the biochar and the duration of the experiment can greatly influence potential PE. In a recent study, (Zimmerman and Ouyang 2019) attributed the priming of organic matter by biochar to the presence of habitable surfaces on biochar that encouraged the growth and activities of microbes, hence co-metabolism of SOM. As no mineralization of biochar was observed in this study, positive PE induced by biochar obtained from *Miscanthus* biomass was likely due to the higher stability of biochar that changed the physico-chemical characteristics of soil (e.g. pH, porosity and bulk density), thus promoting SOM decomposition (Sohi et al. 2010, Luo et al. 2017b).

2.6 Conclusion

The combination of tree shading and addition of substrates in this forest soil mesocosm experiment enabled us to investigate the effects of rhizosphere C supply and fresh organic matter inputs on the decomposition of SOM. Although the addition of both simple and complex fresh organic matter increased SOM decomposition in shaded treatments, the supply of photoassimilate C to roots and ECM fungi further accelerated SOM decomposition in the presence of fresh organic matter as observed in un-shaded treatments. In nutrient-limited and ECM dominated systems such as temperate and boreal forests, interactions between increased rhizodeposition and litter input resulting from higher atmospheric CO_2 concentrations (CO_2 fertilization effect) may reduce soil C stocks and the sequestration ability of these systems. Better understanding of the mechanisms of PE and the net effect of fresh organic matter input

into forest soils might be obtained by measuring extracellular enzyme activities and estimating soil C budgets in forest ecosystems.

Chapter 3

Labile carbon limitation alters organic matter decomposition in a temperate forest soil

3.1 Abstract

Increased plant productivity as a result of elevated atmospheric CO_2 concentrations increases the supply of carbon (C) to the rhizosphere either as rhizodepositions or as litter input. These C inputs may in turn enhance decomposition of old SOM through the priming effect, resulting in increased soil CO_2 efflux and net loss of soil C. Although several priming studies have investigated the effects of adding substrates to soil, the effect of different substrates of ecological importance and their interactions with belowground C allocation from plants have not been well explored. We here investigate the effect of adding ^{13}C-labelled substrates of varying structural complexity and chemical composition (glucose, straw, fungal necromass or biochar) to soils whilst simultaneously manipulating C supply from plants in the rhizosphere, using trenching. We found that glucose and straw additions increased decomposition of native SOM by between approximately 27% and 42% irrespective of rhizosphere C supply, whereas fungal necromass and biochar had no significant effect on SOM decomposition. Belowground assimilate C supply had no significant impact on substrate decomposition. This study demonstrates that substrate quality influences PE, as a positive PE was induced by the addition of a labile substrate whereas complex substrates did not induce a PE, suggesting that microorganisms were C-limited. As the PE is linked to the availability of labile substrates, we speculate that root C supply to symbiotic mycorrhizal fungi alleviates C limitation of enzyme production, which may have implications for terrestrial C stocks.

3.2 Introduction

Temperate and boreal forests are regarded as the primary terrestrial carbon (C) sink for atmospheric carbon dioxide (CO_2), storing majority of this C in soils as organic matter (Pan *et al.* 2011). The extent to which these forests will remain as C sinks under changing climate remains uncertain, as the fate of forest C sinks is dependent on the balance between C gains from primary productivity and C losses through respiration. Although elevated atmospheric CO_2 conditions increases net primary productivity (NPP), only minor change in C stocks has been reported (Drake *et al.* 2011; Phillips *et al.* 2012). Increases in fresh plant derived C have been suggested to drive decomposition of old SOM through the priming effect. With the pending issue of climate change, caused as a result of rising atmospheric CO_2 concentrations, there is an urgent need to better understand how changes in environmental conditions with their accompanying effects such as increased plant productivity and litter production will affect soil C pools (Terrer *et al.* 2018).

Soil respiration (soil CO_2 efflux) is the primary route by which C stored in forests returns to the atmosphere. Sources of soil CO_2 efflux include: (a) root respiration, (b) decomposition of root derived C (rhizomicrobial respiration), (c) decomposition of fresh organic matter (FOM, e.g. litter and root residues), (d) decomposition of old soil organic matter (SOM), (e) additional decomposition of SOM by substrate input from root or litter (priming effect), and (f) weathering of soil carbonates (Subke *et al.* 2006). The dynamics of these components are regulated by abiotic factors (such as temperature and moisture), litter quality and biotic factors (microbial community composition and structure; Cleveland *et al.* 2014; Wang *et al.* 2014; Yu *et al.* 2015; Moinet *et al.* 2018; Subke *et al.* 2018). However, the relative influence of these factors differs. In recent years, there is increasing research on priming effect, in order to assess the potential increase in SOM decomposition caused by C input, which may result in positive feedback to the atmosphere (Vestergård *et al.* 2016a). Studies have suggested that increased C supply from rhizodeposition and litter input may stimulate old SOM decomposition thereby offsetting the increased C sinks under elevated CO_2 conditions (Phillips *et al.* 2012; Vestergård *et al.* 2016b; Terrer *et al.* 2018).

The priming effect (PE) is the short-term change in the decomposition of SOM caused by moderate substrate inputs to soils such as exudation of organic substances by roots (rhizosphere priming effect) or the addition of other labile substrates into soils (Kuzyakov *et al.* 2000). Priming effects can either be “apparent” or “real”. “Apparent” PEs occur when the addition of

substrate increases CO_2 efflux resulting from the activation of microbial metabolism (De Nobili *et al.* 2001). On the other hand, increased SOM decomposition due to co-metabolism and higher enzyme production following increased microbial activities caused by the addition of substrates is referred to as a "real" PE (Blagodatskaya & Kuzyakov 2008). The addition of substrates alters microbial community composition such that fast-growing microbes, generally referred to as r-strategists, may be stimulated immediately after labile C addition, whereas the slow growing K-strategists that are able to degrade complex substrates are involved in the later stages of decomposition (Fontaine *et al.* 2003).

It has been shown that the magnitude and direction of priming is dependent on soil variables such as N content (Fontaine *et al.* 2011; Paterson & Sim 2013; Kumar *et al.* 2016), and plant variables such as their mycorrhizal associations and root exudation (Yin *et al.* 2014, 2018; Blagodatskaya *et al.* 2014b). Compared to rootless soils, changes in SOM decomposition ranging from 50% decline to four-fold increase have been reported in planted soils (Cheng *et al.* 2014). Plant roots interact with soil microbes in several ways (Averill & Finzi 2011; Heinemeyer *et al.* 2012; Brzostek *et al.* 2015; Moore *et al.* 2015a), which in turn affect ecosystem processes such as soil respiration and nutrient cycling. For instance, living plant roots release organic C such as root exudates, sloughed-off root cells, mucilage etc., collectively called rhizodeposits into the soil (Jones *et al.* 2009), which are utilized by soil microbes for the production of extracellular enzymes that are involved in the decomposition of more complex organic matter to mobilize C and nutrients (Kuzyakov 2010). Plant roots may also compete with free-living saprotrophs for soil nutrients, making nutrients unavailable for saprotrophs (Lindahl *et al.* 2010).

Roots of temperate forests are often associated with ectomycorrhizal (ECM) fungi (Lang *et al.* 2011) that supply the host plant root with nutrients in exchange for energy supply (Smith & Read 2002). Plants allocate up to 22% of their assimilate C to ECM fungi (Hobbie 2006), which enables the ECM fungi to form extensive hyphal networks in soils, thereby colonizing the mineral horizons in contrast to saprophytic fungi that are mostly found in the surface horizons (Lindahl *et al.* 2007). ECM fungi also produce extracellular enzymes which enable them to decompose SOM (Talbot *et al.* 2008). This suggests that ECM fungi may play a significant part in SOM decomposition, although previous studies have contrasting reports on the effect of ECM fungi on SOM dynamics: Although some studies have reported that the presence of ECM fungi limits SOM decomposition by competing with other saprotrophs for nutrients, generally known as the "Gadgil effect" (Gadgil & Gadgil 1975; Averill *et al.* 2014; Averill &

Hawkes 2016). Others have shown that ECM fungi accelerate SOM decomposition by enhancing the availability of N for microbial and plant uptake (Phillips *et al.* 2012; Brzostek *et al.* 2013). This later concept fits into the paradigm of PE, where the supply of assimilate C to ECM fungi from host plant root stimulates microbial activities to mobilize nutrients from complex soil organic matter pools (Phillips *et al.* 2012; Moore *et al.* 2015b).

The majority of priming studies focus on the addition of either simple (Derrien *et al.* 2014; Qiao *et al.* 2014; Tian *et al.* 2015) or complex substrates (Fang *et al.* 2018; DeCiucies *et al.* 2018), or the effects of roots on SOM decomposition (Zhu *et al.* 2014; Kumar *et al.* 2016; Murphy *et al.* 2017; Yin *et al.* 2018). Carbon enters into the soil through different pathways such as rhizodeposition, litter fall, or as dead root and fungal biomass, which vary in their chemical compositions and microbial availabilities, and therefore may vary in their significance in observable PEs. Hence, it is imperative to investigate the potential effects of these different C inputs on SOM decomposition, to predict changes in C storage with different C inputs. One girdling study found a higher rate of litter decomposition in un-girdled plots than in girdled plots, suggesting an interaction between rhizosphere C supply and litter decomposition (Subke *et al.* 2004). However, the interactions between the decomposition of specific substrates and assimilate C supply to the rhizosphere from plants have not been well explored.

This study aims to investigate the interactions between organic substrates of varying chemical complexities and belowground assimilate C supply in the field. The use of mesocosm experiment enables the control of environmental factors in order to isolate interested factors to be investigated with minimum interference from external factors that may be found under a natural scenario. It is important to validate the findings of small-scale experiments in the field for better understanding of processes that are found in natural ecosystems. We test the hypothesis that the decomposition of organic substrates is promoted by the supply of assimilate C to roots and ECM fungi. We also hypothesize that the inputs of both simple that are readily available for microbial uptake and complex organic substrates promote the priming of native SOM, with greater priming in the presence of simple than complex substrates.

3.3 Methods

3.3.1 Study Site

The study was performed in a beech (*Fagus sylvatica* L) woodland, with trees planted at least 5 m apart located on the campus of the University of Stirling, Scotland, UK (56º08′ N, 3º54′ W). The forest stand had a closed canopy with sparse understory vegetation of shrubs (*Urtica dioica*). The mean annual precipitation and maximum temperature (1981 – 2010) are 1019 mm and 9.2 ^{o}C, respectively (UK Met Office 2017). The soil is brown earth soil formed from fluvioglacial sands and gravels in the Dreghorn series of UK (Soil Survey of Scotland Staff 1981). The soil is sandy loam soil with a pH of 4.0. The organic C and N contents are 4.5% and 0.4% respectively, and C/N ratio of 11.5.

3.3.2 Experimental design

To assess the influence of rhizosphere C supply, two treatments were established; one where rhizosphere C supply was altered (trenched treatment), and another with intact rhizosphere C input (untrenched treatment). Fifteen PVC collars (20 cm diameter) for each treatment were cut to a length of 45 cm to establish trenched treatments whereas shallow collars (untrenched treatments) were cut to a length of 5 cm.

Twenty collar locations (trees) within this beech stand were identified in May 2016 and soil CO_2 measurements were taken from these locations using an infra-red gas analyser (IRGA, PP systems, Germany) attached to a 15 cm custom-built chamber. Based on the CO_2 measurements, fifteen collar locations were ranked and assigned into three blocks, in a randomized block design, such that each block contained all treatments and substrates. One deep and shallow collar each was assigned to stand locations in a paired design making a total of 30 collars. Collars were positioned at least 50 cm but not more than 2 m from the base of the nearest tree and at least 1 m apart from the paired collar. Deep collars were hammered into the soils on the 10th of June 2016, using a bread knife to cut through large roots, ensuring that they cut beyond the root zones. All deep collars were cut through to at least a depth of 30 ± 2 cm. The shallow collars were inserted gently into the soil (1 - 2 cm), minimizing disturbance to the soil whilst ensuring also that a good seal with the soil surface is achieved.

3.3.3 Preparation and application of ^{13}C labelled substrates

Four substrates with distinct isotopic compositions and varying chemical structures were prepared as described in Chapter 2 (mesocosm study, Jackson *et al.* 2019): glucose, straw, fungal necromass and biochar. Chemical characteristics and isotopic signature of the substrates are also presented in Table 2.1. The substrates were applied into collars in their solid states on 24th August 2016, by mixing into the top 2 cm of soil, after which 250 cm^3 of water was added to all treatments. Glucose, straw and fungal necromass were applied at the rate of 3 mg C g^{-1} soil whereas biochar was applied at rate of 4.7 mg C g^{-1} soil (volume = 158 cm^3). Control treatments were exposed to the same conditions as other treatments, by mixing the top 2 cm and adding water only.

3.3.4 Soil CO_2 efflux and isotopic composition measurements

Prior to the addition of substrates on 24th August 2016, soil CO_2 efflux measurements were carried out using a portable EGM-4 infrared gas analyser (PP Systems, Amesbury, MA, USA) attached to a 15 cm diameter custom-built respiration chamber (volume of 2,300 cm^3), inserted gently into soil (< 1 cm). Following the addition of substrates, gas samples were collected for soil respiration measurement and isotopic analysis by inserting PVC gas flux chambers (15 cm diameter, 5 cm height) into soils (~ 1 cm depth) for 15 minutes to allow CO_2 to accumulate in the chambers. Gas samples were collected 2.5 and 5 h (for glucose, straw and control treatments only), 1 d, 3 d, 7 d, 21 d and 30 d after the addition of substrates. Ten chambers were placed on the soil on each occasion, whereby both trenched and untrenched treatments of all substrate treatments within an experimental block were sampled simultaneously. CO_2 samples were collected through a septum in the chamber using 20 ml syringes and injected under pressure into previously evacuated exetainers (12 ml, Labco Ltd, UK) for CO_2 measurements and isotopic analyses. The concentration of CO_2 was obtained using a gas chromatograph, GC-MS (Hewlett Packard 5890) coupled to a flame ionization detector. Isotopic $\delta^{13}C$ values of the gas samples were measured at the Life Sciences Mass Spectrometry Facility (CEH, Lancaster, UK) using an Isoprime trace gas preconcentration unit coupled to a Micromass IsoPrime Isotope Ratio Mass Spectrometer (Elemental Ltd, Stockport, UK) at an analytical precision of ± 0.17‰.

3.3.5 Soil analysis

Soil samples were collected from all collars up to a depth of 5 cm on 23rd September 2016, after the completion of gas sampling. The soils were stored in sealed plastic bags at 4 ºC until analysis. Soil microbial biomass carbon (MBC) was determined using the fumigation extraction method (Vance *et al.* 1987). Field moist soil was passed through a 2 mm sieve and 5 g were weighed into two 20 ml glass jars. One jar was fumigated in a desiccator with alcohol-free chloroform and water (to avoid drying) for 24 h, while the other jar was not fumigated. Both fumigated and non-fumigated soils were extracted with 20 ml of 0.5 M K_2SO_4 solution. The solution was shaken for 30 min at 300 rpm and filtered with Whatman no. 42 filter papers (pore size: 2.5 µm). The filtrate was analysed for total organic C (TOC) using a TOC – VCSN analyzer (Shimadzu Corporation, Kyoto, Japan). Microbial biomass C was calculated as the difference between fumigated and non-fumigated samples using a k_{EC} factor of 0.45 (Wu *et al.* 1990; Joergensen 1996).

Soil samples were also analysed for the isotopic signature after drying and grinding soil samples. The analysis was carried out at NERC Life Science Stable Isotope Facility (Lancaster, UK) using an automated Carlo Elba NA1500 elemental analyser coupled to a Dennis Leigh Technology isotope ratio mass spectrometer.

3.3.6 Root biomass and decay

Root biomass was determined by randomly collecting fifteen soil cores (5 cm diameter, 30 cm depth) close to the collar pairs from the site. Each core sample was divided into three 10 cm depth classes. Variations in the volume of soil collected from cores were observed, which might contribute to spatial variations in root biomass. The soil within each depth class was homogenized, and fine (< 2 mm) and coarse (> 2 mm) roots were picked using tweezers. All roots were thoroughly picked out from each depth class for a period of 50 min or less at 10 min intervals from eight of the soil core samples to obtain a calibrated estimate of root picking efficiency for these soils (Metcalfe *et al.* 2007). Based on this efficiency (Appendix 3), roots were picked from all other cores for only 10 min and total root biomass per root diameter class estimated. Root samples were dried at 70°C to constant mass and placed overnight in furnace at 550°C to obtain ash-free dry mass. Root ash-free dry mass was converted to root C using a root C content of 44% (Epron *et al.* 1999).

An exponential decay function was used to determine the contribution of dead decomposing roots, in order to correct soil efflux in trenched collars:

$$M_1 = M_0 e^{-kt} \quad (3.1)$$

Where M_1 and M_0 are the remaining and initial root dry mass respectively, t is the time elapsed after trenching (year), and *k* denotes the decay constant. Decay constant values used for fine and coarse beech roots were 0.38 and 0.22 year^{-1} respectively (Epron *et al.* 1999). CO_2 efflux from root decomposition (C_{Root}) was calculated (Epron *et al.* 1999):

$$C_{Root} = (1 - a)\, c\, M_0\, (1 - e^{-k}) \quad (3.2)$$

Where *c* and *a* are the initial C concentration in the root, and the fraction of initial C in root that was incorporated into SOM (44% and 0.22 respectively; Epron et al., 1999). The C_{Root} estimate was subtracted from soil CO_2 efflux in S collars to correct for decomposing fine and coarse roots.

3.3.7 Environmental parameters

Soil moisture was obtained from each collar at 10 cm depth at every gas sampling time using SM150 moisture sensor (Delta-T devices, Cambridge, England). Soil temperature at 5 cm depth was logged at 30 minutes intervals using three Tinytag Plus Two logger with PB-5001 thermistor probes (Gemini Data Loggers, Chichester, UK). Average soil temperature for the period of 09:00 to 17:00 on each sampling day was obtained from all three data loggers.

3.3.8 Calculations

The CO_2 concentrations of gas sampled from the collars were corrected using the average isotopic ratio of the atmospheric air samples collected above the soil surface during the sampling period according to:

$$\delta_{Respired}\, C_{Respired} = \delta_{Sample}\, C_{Sample} - \delta_{Air} C_{Air} \quad (3.3)$$

Where δ is the $\delta^{13}C$ isotopic signature and C is the CO_2 concentration of the respective components.

The total CO_2 respired was then partitioned into CO_2 derived from soil (C_{Soil}; i.e. including all heterotrophic and autotrophic sources of CO_2 except the labelled substrates) and substrate (C_{Sub}) using a two end-member mixing model:

$$\delta_{Respired}\, C_{Respired} = \delta_{Soil}\, C_{Soil} - \delta_{Sub} C_{Sub} \quad (3.4)$$

Where $C_{Respired}$ is the total CO_2 respired and $\delta_{Respired}$, δ_{Soil}, and δ_{Sub} are the $\delta^{13}C$ isotopic composition of the total CO_2, soil and labelled substrates respectively. Here, the suffix "soil" refers to the CO_2 derived from both rhizomicrobial (root + ECM) and heterotrophic respiration, whereas the suffix "sub" represents glucose, straw, fungal necromass and biochar treatments. The fraction of total soil CO_2 efflux derived from substrate (f_{Sub}) and soil (f_{Soil}) were calculated as:

$$f_{Sub} = \frac{\delta_{Respired} - \delta_{NS}}{\delta_{Sub} - \delta_{NS}} \quad (3.5)$$

$$f_{Soil} = 1 - f_{Sub} \quad (3.6)$$

Where δ_{NS} is the isotopic composition of gas samples collected from control collars without any added substrate. The CO_2 derived from the added substrate was calculated as:

$$C_{Sub} = f_{Sub} \times C_{Respired} \quad (3.7)$$

Standard errors of the fraction of substrate in total CO_2 efflux was calculated such that the variabilities of the sources (δ_{Soil} and δ_{Sub}) and mixture were accounted for according to the method by Phillips and Gregg (2001):

$$SE\ (f_{Sub}) = \sqrt{\left(\frac{1}{\delta_{Sub} - \delta_{NS}}\right)^2 \left[\sigma^2{}_{\delta_{Respired}} + {f_{Sub}}^2 \sigma^2{}_{\delta_{Sub}} + (1 - f_{sub})^2 \sigma^2{}_{\delta_{NS}}\right]} \quad (3.8)$$

Where σ represents the standard error of the mean isotopic compositions of the components as indicated by suffices.

Priming effect of soil C decomposition induced by the addition of substrates was calculated as the difference in the soil derived CO_2 efflux between control soils without substrates and substrate amended soils:

$$PE(\%) = \frac{{}^{amended}C_{Soil} - {}^{NS}C_{Soil}}{{}^{NS}C_{Soil}} \times 100 \quad (3.9)$$

Where ${}^{amended}C_{Soil}$ is the soil derived CO_2 in soils amended with biochar, straw, fungal necromass or glucose of untrenched and trenched treatment, as determined from $C_{Soil} = C_{Respired} - C_{Sub}$; and ${}^{NS}C_{Soil}$ is the soil derived CO_2 in control soils without substrate addition for the respective treatment.

Priming effect of substrate decomposition induced by rhizosphere C input from roots was calculated as the difference in the substrate derived CO_2 between untrenched and trenched treatments:

$$RPE_{Sub}(\%) = ({}^{RMS}C_{Sub} - {}^{S}C_{Sub})/{}^{S}C_{Sub} \times 100 \quad (3.10)$$

Where ${}^{RMS}C_{Sub}$ and ${}^{S}C_{Sub}$ are substrate derived CO_2 in untrenched and trenched treatments respectively.

3.3.9 Statistical analysis

A paired T-test was used to test for the significant differences between soil CO_2 efflux in the untrenched and trenched control treatments without substrate. Linear mixed-effect models were used to test the effects of rhizosphere C supply and substrate amendments, and their interactions separately on total CO_2 efflux rates, soil- and substrate- derived CO_2. Mixed effect model was fitted with collar treatment and substrate as fixed effects using *lmer* function in lme4 package. To account for the differences in individual trees within each block, and the repeated measurements during the sampling period, trees nested within each block and sampling days were included as random effects in all models. Models were fitted using maximum likelihood and the best parsimonious model selected by stepwise backward selection based on the lowest Akaike information criteria (AIC) (Pinheiro & Bates 2000). Tukey post-hoc tests were used for pairwise comparisons at statistical significance level of 0.05 using the *glht* function within the multcomp package (Hothorn, Bretz & Westfall 2008). The effects of trenching and substrate addition on microbial biomass was also analysed using ANOVA following a linear mixed effect model. The relationships between soil respiration and microbial biomass or environmental parameters (soil moisture, temperature) were also analysed with linear mixed effect model. In all analyses, data were log transformed when assumptions for parametric analysis was violated. All analyses were done with R Studio v1.0.143 (R Core Team 2017).

3.4 Results

Total fine and coarse root biomass (0 - 30 cm) for the study site were 409 ± 45.7 and 344 ± 115 g C m^{-2} respectively (Table 3.1). The remaining root biomass (fine and coarse) derived from a

simple exponential decay model, indicated that 10% of the dead fine and 6% of dead coarse roots had decayed in almost 3.5 months. Decaying fine and coarse roots contributed approximately 0.4 µmol m^{-2} s^{-1} to soil CO_2 efflux from collars. The average corrected CO_2 efflux from 25th May to 21st September 2016 was 1.85 ± 0.29 µmol m^{-2} s^{-1} from untrenched treatment and 1.29 ± 0.20 µmol m^{-2} s^{-1} from trenched treatments. The contribution of rhizomicrobial respiration to total soil CO_2 efflux for the sampling period was 31%, calculated as difference between untrenched and trenched treatments. Although, soil moisture differed between treatments with higher soil moisture in untrenched treatments than trenched treatments prior to substrate addition, there were no overall significant differences in the soil moisture and temperature between untrenched and trenched treatments ($P > 0.05$, Fig. 3.1A & B).

Table 3.1 Biomass C of fine (<2 mm) and coarse (> 2 mm) roots estimated from soil cores collected from *Fagus sylvatica* forest, Stirling, UK prior to trenching (mean ± 1 SE, n = 15). The carbon concentration of roots was estimated as 44% (Epron *et al.* 1999).

Depth	**Fine root (g C m^{-2})**	**Coarse root (g C m^{-2})**
0-10	254 ± 43.2	26.9 ± 0.01
10-20	107 ± 11.1	99.2 ± 22.3
20-30	47.1 ± 9.60	217 ± 113

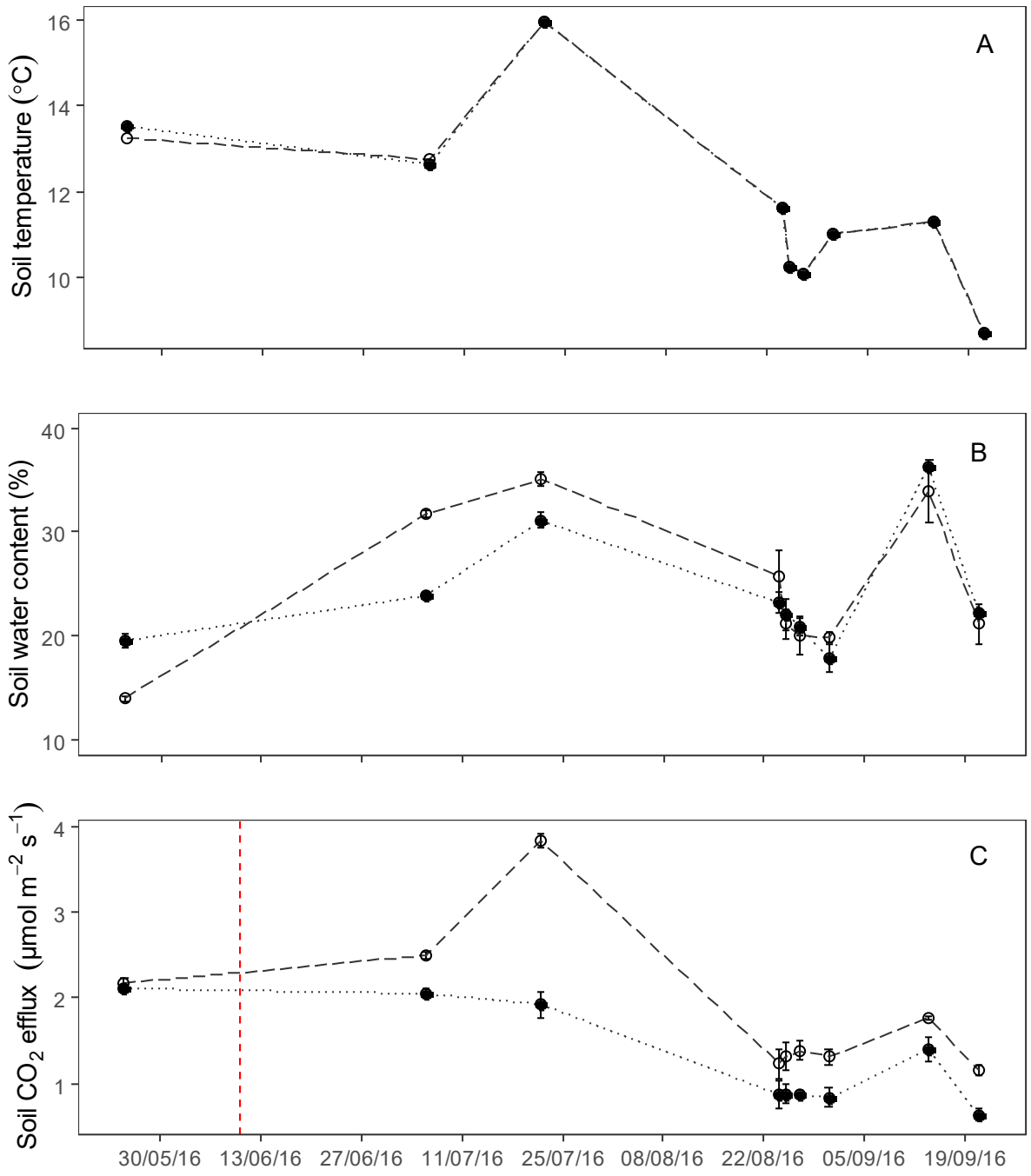

Figure 3.1 Soil temperature at 5 cm depth (A), moisture content at 10 cm depth (B), soil CO_2 efflux (C) of untrenched (open circles) and trenched (filled circles) treatments in a beech forest near Stirling, UK. Trenching was carried on 10th June 2016 as indicated by red broken lines. Values are mean ± 1 standard error. Prior to 24th August, soil respiration and moisture contents were measured from all collars in each treatment (n = 15). Measurements were taken from control collars without substrates after substrate addition (n = 3). Soil temperature was derived from the average of soil temperature from three data loggers between 9:00 and 17:00 on the days when soil respiration and moisture contents were measured.

Increases in ^{13}C in soil CO_2 efflux above the natural abundance were observed shortly after the addition of ^{13}C-labelled glucose, straw and fungal necromass in both treatments (Fig. 3.2). The mineralization of glucose was highest during the first week of glucose addition, which was followed by a rapid decline in glucose mineralization. Higher ^{13}C in soil by c. 0.5 atom% was observed 24 h after the addition of straw in untrenched and trenched treatments that persisted for the duration of 30 d, with a peak of c. 1 atom% observed on day 7 in trenched treatments. The addition of fungal necromass also increased the ^{13}C signature by c. 0.3 atom% throughout the sampling period in both treatments. The ^{13}C signature of soils amended with biochar, on the other hand, did not differ from natural abundance ($P > 0.05$).

The addition of substrates and trenching had significant effects on CO_2 efflux ($P < 0.001$), but there was no significant interaction between the two factors. Post hoc test showed that although, glucose and straw increased soil CO_2 significantly, the effects of fungal necromass and biochar were not significant. The addition of glucose led to the greatest increases in CO_2 efflux by 45% in untrenched (Tukey post-hoc, $P < 0.001$) and 67% in trenched treatments ($P < 0.05$), when compared to the respective control treatments without substrate (Fig. 3.3 & 3.4). Total CO_2 efflux in both trenching treatments was also higher following the addition of glucose than the addition of fungal necromass or biochar. However, total CO_2 efflux in both untrenched and trenched treatments was not significantly different between soils without substrate additions (control) and soils amended with straw, fungal necromass or biochar. Furthermore, CO_2 efflux was not significantly different between untrenched and trenched treatments for any substrates for the period of 30 days after substrate addition (Tukey post-hoc, $P> 0.05$).

The amount of CO_2 derived from substrates differed significantly between untrenched and trenched treatments ($P < 0.01$) and varied among substrates ($P < 0.001$). However, there was no significant interaction between treatments and substrates ($P > 0.05$). Substrate-derived CO_2 was significantly greater in glucose treated untrenched treatments than biochar untrenched treatments ($P < 0.001$). In trenched treatments, glucose also had significantly higher substrate derived CO_2 than straw ($P < 0.001$) and biochar ($P < 0.01$) (Fig. 3.3 & 3.4). For the period of 30 days after substrate addition, the substrate-derived CO_2 ranged between -0.042 ± 0.001 and 0.39 ± 0.14 µmol m^{-2} s^{-1} in untrenched treatments and 0.03 ± 0.003 and 0.49 ± 0.17 µmol m^{-2} s^{-1} in trenched treatments.

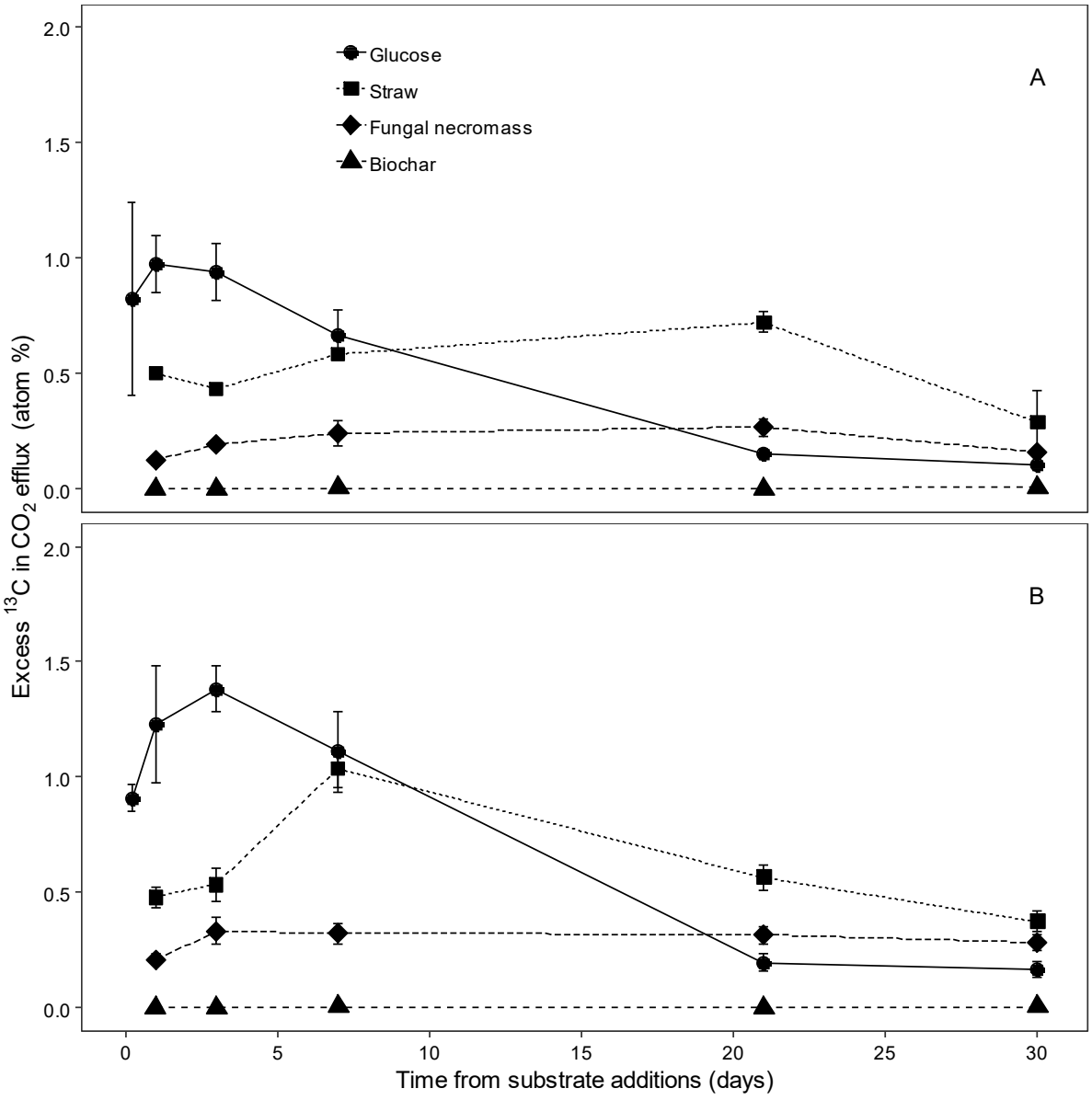

Figure 3.2 Excess of ^{13}C in CO_2 efflux relative to the natural abundance in un-labelled soil with time following the addition of ^{13}C- labelled glucose (circles), straw (squares), fungal necromass (diamonds) and biochar (triangles) into untrenched (A) and trenched (B) collars. Values are mean ± standard error (n =3).

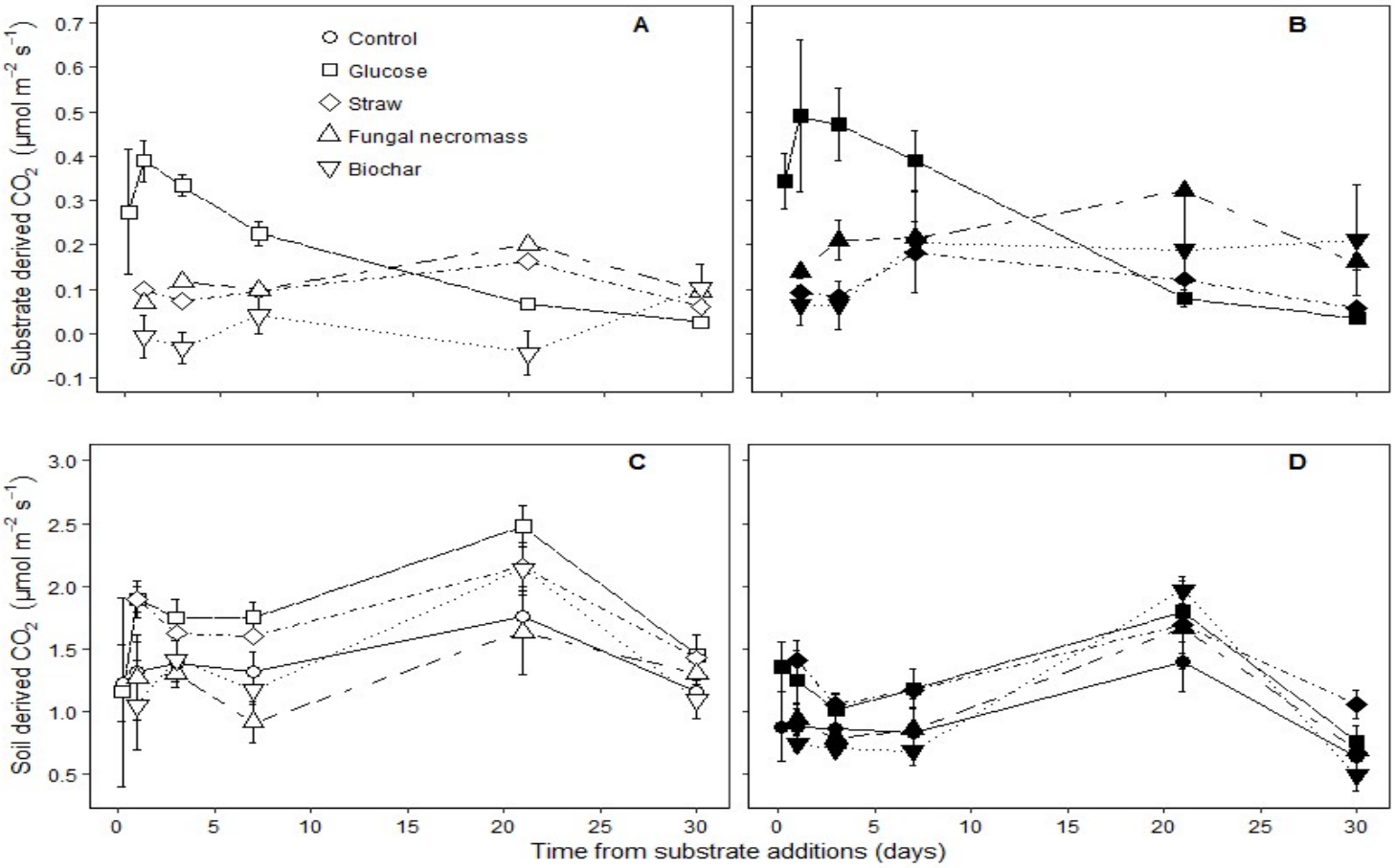

Figure 3.3 Soil CO_2 efflux from untrenched (open symbols, left) and trenched (filled symbols, right) treatments derived from the added ^{13}C-labelled substrates (A & B) and un-labelled soil (C & D) for the duration of 30 d after the addition of glucose, straw, fungal necromass or biochar. Error bars denote ± 1SE for n = 3.

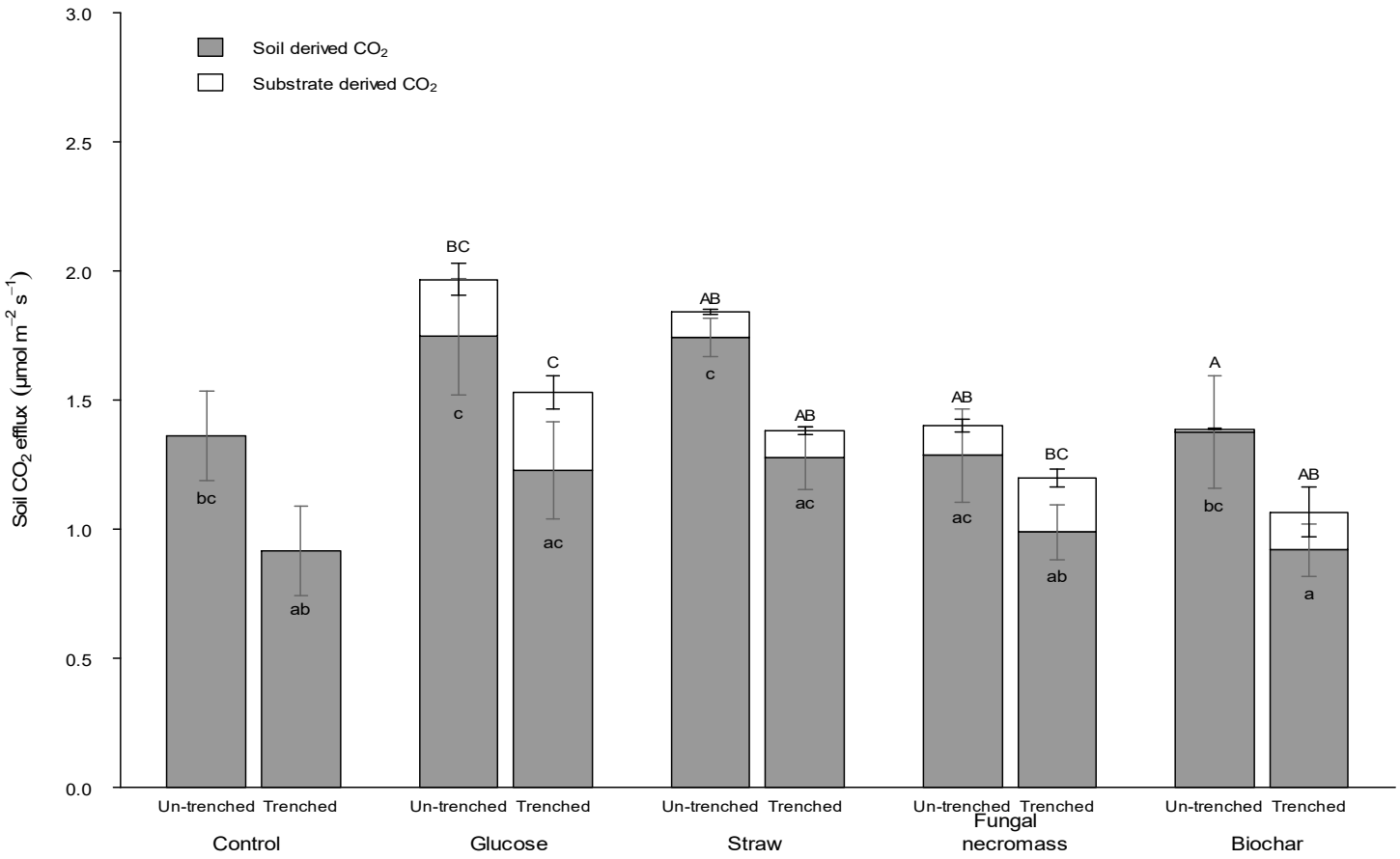

Figure 3.4 Total soil CO_2 efflux partitioned into CO_2 derived from the input of glucose, straw, fungal necromass and biochar (substrate derived CO_2) and CO_2 derived from roots and heterotrophic respiration (Soil derived CO_2) in un-trenched and trenched treatments. Error bars are standard errors of mean (n = 3). Difference among trenching and substrate treatments is indicated by different uppercase letters ($P < 0.05$, Tukey *post hoc*) for substrate-derived CO_2 and lowercase letters for soil-derived CO_2 efflux.

There was no significant priming of substrate decomposition by the input of C from the rhizosphere, as calculated by the difference in substrate derived CO_2 between untrenched and trenched soils (Fig. 3.4). The priming effect (PE) of substrate addition on SOM decomposition was calculated as the difference in the soil-derived CO_2 between substrate-amended treatments and control treatments without substrates. Soil derived CO_2 increased significantly during the period of 30 d after the addition of glucose and straw when compared to controls resulting in PE of c. 27% in untrenched treatments, and 34% and 42%, respectively, in trenched treatments. The dynamics of the PE as a result of the input of glucose was different between the treatments. Glucose resulted in a negative PE shortly (5 h) after its addition into untrenched collars, which was followed by a positive PE that persisted until the end of the study (Fig. 3.5). In trenched collars on the other hand, positive PE was observed throughout the sampling period following the addition of glucose, with the peak in the first 24 h of addition. In both treatments, straw induced a positive PE throughout the period of 30 days with the greatest PE observed 24 h after its addition. Fungal necromass and biochar on the other hand, did not significantly induce any significant priming effect over the 30-day period. For the first three weeks of fungal necromass additions to untrenched collars, no PE or negative PE were observed, which was followed by a positive PE of 13% on day 30, whereas no significant PE was observed in trenched treatment throughout the duration of sampling (Fig. 3.5). The addition of biochar resulted in negative PE in both untrenched and trenched treatments, with positive PE observed only three weeks following its additions.

Microbial biomass C did not significantly differ between untrenched and trenched treatments and among the different substrate treatments (Fig 3.6). Microbial biomass C was 547 ± 173 µg g $soil^{-1}$ in untrenched treatments and 477 ± 38.3 µg g $soil^{-1}$ observed in trenched treatments. Although the addition of substrates increased soil microbial biomass in both untrenched and trenched soils compared to soils that received only water (controls), these increases were not significant.

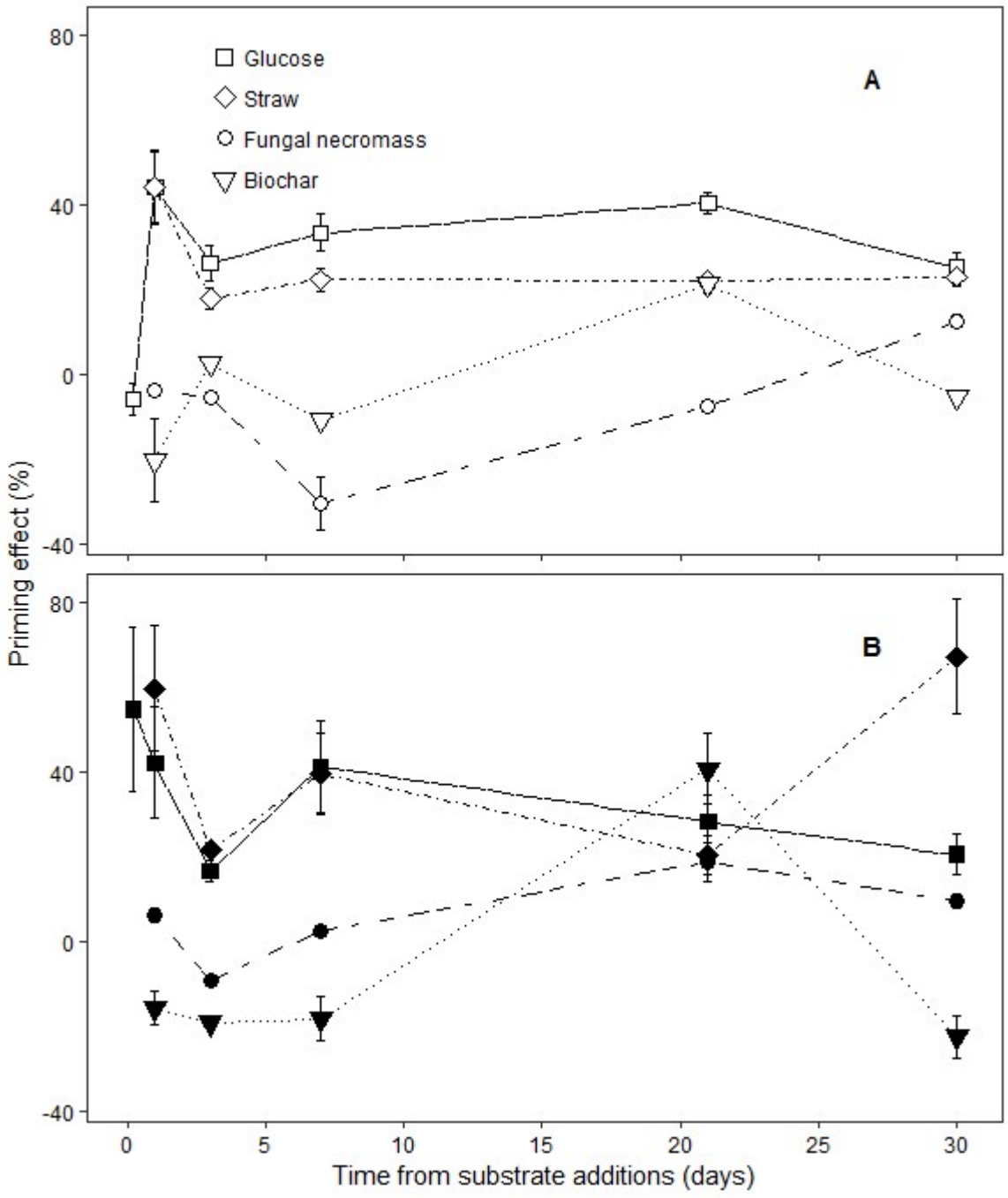

Figure 3.5 Priming effect of substrate addition on soil-derived CO_2 over the period of 30 days after the addition of ^{13}C-labelled glucose (squares), straw (diamonds), fungal necromass (circles) or biochar (triangles) into untrenched treatments (open symbols, A) and trenched treatments (filled symbols, B). Error bars are ±1 SE of mean (n = 3).

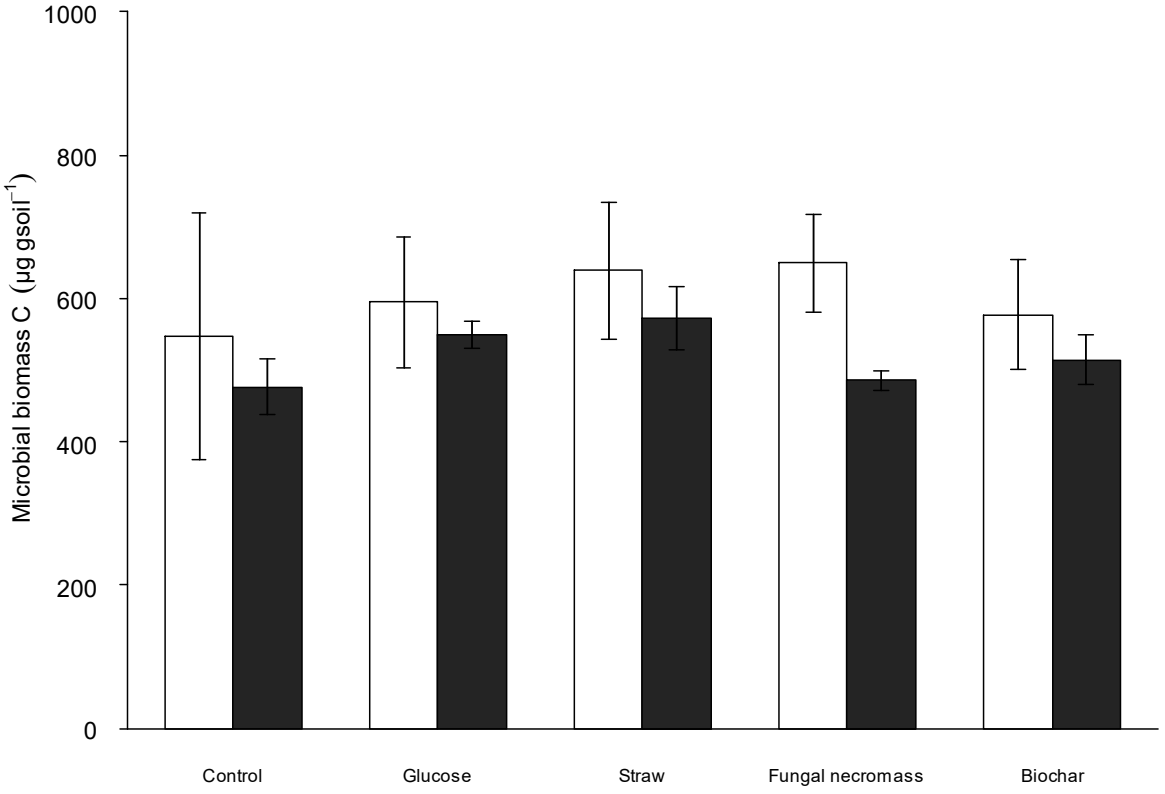

Figure 3.6 Soil microbial biomass C in untrenched (open) and untrenched (closed) treatments after the addition of glucose, straw, fungal necromass and biochar. Values are mean ± 1 SE (n = 3).

3.5 Discussion

This study shows that the input of organic C to soils promotes the decomposition of native SOM, with the magnitude of the decomposition determined by the quality of the substrate. However, the presence of roots and ECM fungi did not significantly stimulate the decomposition of the added organic substrates.

3.5.1 CO_2 efflux partitioning by trenching

Rhizomicrobial respiration, as estimated by trenching contributed 31% of total soil CO_2 efflux in this beech forest. This is similar to the 34% observed in a recent trenching study in the beech

stand at Harvard forest (Savage *et al.* 2018). Similarly, using the trenching method, rhizomicrobial respiration ranged from 30 to 60% in a beech forest in France (Epron *et al.* 2001). The autotrophic respiration observed in this study is on the lower side of the 10 - 90% range for autotrophic respiration (Hanson *et al.* 2000). The wide variation reported in studies are due to differences in ecosystems, method of partitioning, age of forest, and time-scale or season of study (Subke *et al.* 2006).

A common limitation of trenching methods is the increase in CO_2 efflux in trenched soils caused by soil disturbance, decaying dead roots and mycorrhizal hyphae, and increased moisture content (Subke *et al.* 2006; Comstedt *et al.* 2011). In this study, there was no significant difference in the soil moisture and temperature between untrenched and trenched treatments, suggesting that trenching had no effect on soil moisture content. This contrasts with finding by Comstedt et al., (2016), where differences in soil moisture contents between trenching treatments had a major effect on soil respiration estimation. Differences in the relative effects of soil water content in trenching studies may likely be due to varying water holding capacity of soils, as well as precipitation pattern of the study sites (Comstedt *et al.* 2011).

To minimize the effect of soil disturbance and decomposing roots, some studies waited for several months after trenching before commencing soil respiration measurements, with the assumption that dead root decomposition would have subsided after this period. Using decay constants of 0.38 $year^{-1}$ in the exponential decay function, we estimated that only 16% of roots were decomposed within 3.5 months of trenching, indicating that fine root decomposition may last for several years after trenching (Ngao *et al.* 2007). Therefore, an assumption that roots are decomposed within four months of trenching may likely lead to an underestimation of autotrophic respiration and overestimation of heterotrophic respiration.

Prior to correction for decaying roots, autotrophic respiration contributed 9% to total soil respiration for the period following trenching. After corrections for decaying roots, autotrophic contribution to soil respiration increased to 31%, indicating that additional CO_2 from decomposing dead roots contributed 22% of the CO_2 evolved in trenched treatments. Root decomposition therefore had a significant effect on the heterotrophic respiration in this study. This is in contrast to other studies that observed minor effects of dead root decomposition but a major effect of soil moisture in trenched plots (Comstedt *et al.* 2011; Savage *et al.* 2018). In a meta-analysis, (Subke *et al.* 2006) reported that studies that corrected for additional root

decay in trenched soils observed decreases in heterotrophic respiration ranging from 2 – 24%, depending on soil type, climate or quality of roots. Trenching remains an effective means of partitioning soil respiration into autotrophic respiration and heterotrophic, provided the artefacts arising from trenching are taken into account.

3.5.2 Substrate effects

Microorganisms utilize labile C substrates e.g. simple sugars as a source of energy for growth and for the production of extracellular enzymes that break down the complex soil organic matter to release the nutrients contained therein. Labile substrates are often released into the soil as the decomposition product of most natural polymers and also as rhizodeposits (Kuzyakov 2010), with glucose being the most released sugar in rhizodeposits (Derrien *et al.* 2004). The exclusion of assimilates from plants to soils through trenching alters the availability of labile C to microorganisms. In the absence of labile C e.g. in trenched treatments, the biosynthesis and growth of microorganisms are limited by the availability of C and energy (Schimel & Weintraub 2003). Microorganisms therefore switch to a dormant metabolic state, when they don't grow but maintain their metabolic activity by mineralizing soil organic C to CO_2 (basal respiration) in readiness for the input of labile C (Joergensen & Wichern 2018). These microbes are activated by the input of fresh substrates (De Nobili *et al.* 2001). In this study, the addition of glucose, straw, fungal necromass or biochar, which represent soil organic matter of varying quality, increased microbial metabolic activity in both untrenched and trenched treatments, as reflected by increased total soil CO_2 efflux in substrate-amended soils relative to control soils without substrates. The microbial switch from the dormant state to active state was rapid, occurring within a few hours (5h for glucose and 24h for straw, fungal necromass and biochar) of energy supply from the added substrate. It is also likely that decaying roots and ECM mycelia in trenched treatments may have served as C source that stimulated microbial activity within these soils. The activation of microbial activity following the input of fresh C increased the decomposition of organic matter. Although the addition of both readily available substrates and complex substrates increased total soil respiration, these increases were only significantly higher when compared to controls in soils amended with glucose from both untrenched and trenched treatments. This suggests that the magnitude of microbial metabolic activity is determined by the quantity and quality of the added substrate (Qiao *et al.* 2014; Liang *et al.* 2017).

The excess ^{13}C in CO_2 show that glucose, straw and fungal necromass were metabolised by microorganisms, with glucose being mineralized more than straw and fungal necromass in the first week of substrate additions. Biochar on the other hand was not decomposed in either untrenched or trenched treatments, due to its recalcitrance to microbial degradation. The rapid flush in CO_2 derived from the decomposition of glucose in the first three days has also been previously reported (De Nobili *et al.* 2001; Blagodatskaya *et al.* 2011a; Garcia-Pausas & Paterson 2011). The rapid mineralization of glucose observed in the first few days of addition was likely mediated by the r-strategists, which are fast-growing microorganisms that are mainly stimulated by the presence of readily available substrates like glucose (Blagodatskaya *et al.* 2009). In contrast, the decomposition of complex C was likely mediated by the slow-growing K strategists (Fontaine *et al.* 2011), hence the gradual, persistent mineralization of fungal necromass and straw observed over the sampling period.

Flux partitioning using isotopic composition shows that in addition to the added substrate, CO_2 originated from other sources (here referred to as soil-derived CO_2). The magnitude and direction of PE calculated as the difference in the soil derived CO_2 between substrate-amended and un-amended treatments was determined by the quality of the added substrate but not affected by the supply of assimilate C. For the period of 30 days after the addition of substrates, glucose and straw residues significantly resulted in a positive PE in both treatments, whereas there was no significant PE in soils amended with fungal necromass and biochar. The negative PE observed in untrenched treatment on the first sampling (5h) after glucose addition was likely due to the switch of microorganisms from utilizing complex SOM to the easily available glucose (preferential substrate utilization) (Cheng & Kuzyakov 2005; Kuzyakov & Bol 2006). This initial negative PE was not observed in trenched treatments likely due to reduced availability of C caused by the exclusion of root exudation. Therefore, microbial communities in trenched treatments were likely activated shortly after the addition of glucose, utilizing the added glucose as their primary C and energy source for growth and maintenance. In glucose-amended soils, the availability of C and energy increased microbial activities, which in turn increased their demand for nutrients, and hence mobilize nutrients through the decomposition of SOM (microbial mining hypothesis, (Chen *et al.* 2014)).

The dynamics and magnitude of the positive PE observed in soils amended with straw were similar in untrenched and trenched treatments indicating that straw residues had a similar effect on the microbial communities in both treatments. Here, priming was likely due to co-metabolism, whereby straw activated soil microorganisms to produce extracellular enzymes

that co-mineralize the added straw and SOM. This process was likely mediated by microbial communities that are better adapted to decomposing polymerised substrates (K-strategists, (Fontaine *et al.* 2011). We did not observe any significant PE following the addition of fungal necromass and biochar in either un-trenched or trenched soils. This suggests a cost/benefit trade-off of enzyme production, whereby the energy required to produce enzymes for the decomposition of these compounds was greater than the energy acquired from the degradation of the substrates (Fontaine *et al.* 2007).

Priming effects can either be from increased turnover of microbial biomass (apparent PE) or due to acceleration of SOM decomposition (real PE) (Blagodatskaya & Kuzyakov 2008). In this study, microbial biomass and their isotopic composition were not measured at each sampling time (Blagodatskaya *et al.* 2011a), to differentiate between real and apparent PE. However, using the dynamics of PE, quality and quantity of added substrate, we can make some inferences about the type of PE. It is likely that the PE observed in the first three days of glucose addition, a period of intensive glucose utilization was "apparent", resulting from intensified microbial metabolism (Blagodatsky *et al.* 2010; Blagodatskaya *et al.* 2011a). As no short-term intensive substrate utilization was observed with the addition of complex, less available substrates, (straw, fungal necromass and biochar), we suggest that the PE observed was "real", caused by extracellular enzymes produced during the decomposition of the complex substrates (Blagodatskaya & Kuzyakov 2008). However, it is possible that real PE also occurred in the first three days but their contribution to the total PE will probably be negligible (Blagodatskaya *et al.* 2011a).

3.5.3 Rhizosphere effects

In contrasts to our expectation, results showed that the presence of rhizosphere C input had no significant effect on the decomposition of substrates or native SOM. Previous studies have reported priming of SOM in the presence of the roots and their associated ECM (Subke *et al.* 2011; Parker *et al.* 2017) or arbuscular mycorrhizal fungi (Zhu *et al.* 2014; Kumar *et al.* 2016). Similarly, in a recent study (Yin *et al.* 2018) reported that decomposition of SOM was higher by 26% to 146% in soils where trees were planted when compared to unplanted soils, with the tree species influencing the magnitude of the rhizosphere PE. The lack of rhizosphere effects may be due to the apparent increase in microbial activity stimulated by decomposing root

materials in the trenched treatments. This was also reflected in the absence of difference in the microbial biomass C observed between trenching treatments and among substrate treatments.

3.6 Conclusion

Our study demonstrates that the presence of labile C substrates promotes the microbial decomposition of SOM, shown by positive PE in glucose-amended soils. Although straw promoted the decomposition of SOM, fungal necromass and biochar did not significantly alter SOM decomposition, thus indicating that the priming effect is determined by the trade-offs between the cost of enzyme production and the availability of C resources. It is likely that when energy-rich C resources are available in adequate amounts, this will stimulate microbes to continuously explore the soil to mobilize nutrients from SOM. This may result in positive feedback interaction between increased rhizodeposition and litter production resulting from CO_2 fertilization effect and the loss of soil C. Better understanding of this complex, plant-soil-microbe interactions is required in order improve C flux models and accurately predict the potential effects of environmental changes on terrestrial C stocks.

Chapter 4

Differential response of extracellular enzyme production to manipulation of rhizosphere carbon supply and contrasting SOM amendments

4.1 Abstract

Increased availability of carbon (C) caused by the input of plant residues or root exudations influences microbial activities and may accelerate or retard soil organic matter decomposition via the priming effect. The enzymes involved in the cycling of C, nitrogen (N) and phosphorus (P) would likely respond differently to the input of different organic materials, however this has not been well explored. We hypothesize that (a) the presence of readily available microbial substrate stimulates microbial enzyme activities to mobilize limited resources, but that (b) presence of less accessible substrates stimulates the production of enzymes involved in the degradation of complex organic molecules. We further hypothesize that these responses are enhanced in an intact rhizosphere due to the supply of assimilate C to microbes in the rhizosphere. Using forest soil mesocosms, we measured the activities of six extracellular enzymes in soils, which received a range of substrates in the presence of an intact rhizosphere or trenched soils. We found increased activities of C, N and P-degrading enzymes in the presence of glucose, especially when present in sufficient amounts. Similarly, less accessible substrates (straw and fungal necromass) increased activities of enzymes whereas no significant change in enzyme activities was observed in the presence of biochar. However, enzyme activities with or without substrates were not significantly promoted by rhizosphere C supply. Together, these results suggest that enzyme activity depends on the availability of C and microbial demand for limiting nutrients.

4.2 Introduction

Carbon (C) enters soil through various pathways, for example, via rhizodeposition, leaf and root litter, or forest fires (Godbold *et al.* 2006; Wardle *et al.* 2008; Jones *et al.* 2009), to form the largest pool of C in terrestrial ecosystems (Schlesinger & Bernhardt 2013). The pool of C in soil organic matter (SOM) is mostly regulated by the inputs of C from primary productivity and C outputs through microbial decomposition of organic matter (Schimel & Weintraub 2003). Much of SOM exists in chemically complex forms that require the production of extracellular enzymes by heterotrophic soil microorganisms in order to metabolize complex SOM polymers into bio-available forms (Read & Perez-Moreno 2003).

Increased availability of soil C can change microbial community size and structure resulting in acceleration or retardation of SOM decomposition via the so-called priming effect (PE) (Paterson, Midwood & Millard 2009; Kuzyakov 2010; Garcia-Pausas & Paterson 2011). Priming effects are strong short-term changes in the decomposition of SOM caused by moderate treatments of soils, such as the input of plant residues, fertilizers or root exudations (Kuzyakov *et al.* 2000). The magnitude and direction of PEs are dependent on the quality or quantity of organic substances (Chen *et al.* 2014; Liu *et al.* 2017; Shahbaz *et al.* 2017b), the structure and diversity of soil microbial communities (Brzostek *et al.* 2015), or soil variables such as the availability of nutrients and aggregate size (Dorodnikov *et al.* 2009; Tian *et al.* 2015). In this study, we describe 'availability' of substrates based on the susceptibility of organic compound to enzymatic degradation as well as the amount of energy derived from the organic compound (Chen *et al.* 2014). Readily available substrates from root exudates or plant residues often induce a positive PE due to higher availability of energy, thereby enhancing microbial metabolic activities (Cheng & Kuzyakov 2005; Di Lonardo *et al.* 2017). Negative or no PEs have also been observed following the input of labile substrates due to the preferential microbial utilization of the added substrate, rather than complex SOM (Blagodatskaya *et al.* 2007). Complex organic materials such as plant residues, which are less available due to their polymerised chemical structure, can also induce a positive PE due to the dominance of microbial groups that are adapted to degrading complex compounds (Chen *et al.* 2014; Fang *et al.* 2018). This suggests that the mechanisms regulating the occurrence, direction and magnitude of PEs are more complex, involving interactions between the acquisition of energy and nutrients and the dynamics of soil microbial communities.

Metabolism of labile C stimulates microbial growth (microbial activation hypothesis), but may also increase SOM decomposition in order to meet demand for limiting nutrients (Cheng & Kuzyakov 2005). The succession and competition between soil microbial groups of r- or K- strategies have also been suggested as a mechanism for PEs (Fontaine *et al.* 2003), whereby both microbial groups are activated by the addition of substrates for example via rhizodeposition or leaf litter (Fontaine & Barot 2005). As labile C is exhausted, rapidly growing r-strategists become less competitive within the microbial community, which favour the dominance of slower-growing K-strategists that are able to produce enzymes for the degradation of complex organic compounds. Due to these complex dynamics driving PEs, there is a need to further understand the dynamics of SOM decomposition and the importance of induced enzyme activity (Chen *et al.* 2014).

In temperate and boreal forests, roots are densely colonized by ectomycorrhizal (ECM) fungi, which receive a supply of C from their host in return for facilitating the transfer of nutrients to the roots (Smith & Read 2002). ECM fungi are able to produce a wide range of extracellular enzymes, which enables them to access nutrients from complex organic matter (Talbot *et al.* 2008). Although they receive their C from host plants, they may also act as saprotrophs, accessing C through SOM decomposition, and thus may contribute to the loss of soil C (Buée *et al.* 2007; Talbot *et al.* 2008; Lindahl & Tunlid 2015). Plants allocate a substantial amount of their assimilate C to their mycorrhizal partners (Hobbie 2006), which may enhance nutrient acquisition from SOM (Kaiser *et al.* 2010; Brzostek *et al.* 2013; Terrer *et al.* 2018). In contrast, the supply of assimilate C from host roots to mycorrhizal fungi may decrease SOM decomposition, as ECM fungi may induce or exacerbate nutrient limitation of free-living saprotrophs thereby retarding their growth and activities (Orwin *et al.* 2011; Averill *et al.* 2014; Averill & Hawkes 2016).

Previous studies have attributed increases in soil respiration following the input of organic substrates (including rhizodeposition) to increased enzyme activity (e.g. (Zhu *et al.* 2014; Kumar *et al.* 2016; Yin *et al.* 2018; Jackson *et al.* 2019)). However, a better understanding of the responses of specific enzymes involved in the cycling of C, N, and P to the input of organic materials of varying microbial availability (Chen *et al.* 2014; Allison *et al.* 2014) is required to clarify the mechanisms of priming effects (Blagodatskaya & Kuzyakov 2008). Due to the metabolic and nutritive costs of enzyme synthesis, the activity of extracellular enzymes is dependent on microbial demand and the amount of resource derived from soil substrates (Schimel & Weintraub 2003; Blagodatskaya *et al.* 2014a; Allison *et al.* 2014).

However, there is thus far only limited information that explains the mechanisms of the priming effect of organic substrates that vary in chemical composition and microbial degradability on enzyme activities.

This study aims to investigate the consequence of induced microbial enzyme production in response to the supply of assimilate C from plant roots, as well as additions of exogenous organic matter on old SOM. Specifically, we hypothesize that: (i) the addition of glucose enhances the activities of enzymes involved in the mobilization of limited resources; (ii) that more complex substrates (i.e. straw, fungal necromass and biochar) increase the production of enzymes specialised in the degradation of the complex substrates, and (iii) the supply of assimilate C from roots to the rhizosphere induces the production of extracellular enzymes involved in the degradation of organic matter to release nutrients (N and P) rather than C.

4.3 Methods

4.3.1 Experimental design

This study involved the use of forest mesocosms designed in Chapter 2, with details provided in section 2.3.1 (Chapter 2, (Jackson *et al.* 2019)). Based on pilot soil respiration measurements in August 2017, mesocosms were assigned into five blocks in a randomized block design containing all treatments. One mesocosm was excluded due to the significantly greater soil respiration observed from the mesocosm during the pilot survey, resulting in four replicates of one of the treatments.

4.3.2 Substrate preparation and application

There were six substrate treatments: water control (hereafter, control), high glucose, low glucose, straw, fungal necromass and biochar. The isotopic compositions of these substrates were differed from the C_3 forest soil (Table 4.1), thereby allowing for partitioning of CO_2 from the added substrate from other sources. These substrates were chosen to represent different constituents of organic matter present in forest soils with varying chemical structure and available C. Wheat straw (*Triticum aestivum* L.) was grown in a chamber enriched with 10 atom% $^{13}CO_2$, rinsed in deionised water four times, air-dried and ground into fine particles using a ball mill. Fungal necromass was obtained from mycelium of the ECM basidiomycete

Hebeloma crustuliniforme UP184. Cultures were grown on agar plates containing 1/10 Modified Melin Norkans growth medium (MMN, Marx, 1969), where 1 g (rather than 10 g) of glucose was used in the recipe. Fungal biomass was propagated in 500 ml flasks containing 200 ml of modified MMN liquid growth medium, which in a pilot study was found to support the most rapid fungal growth. In the modified MMN medium, 10 g of malt extract was used instead of 3 g. The fungal biomass was labelled by replacing 20% of ^{12}C-glucose in medium composition with 99 atom% ^{13}C- glucose (CK Isotopes, Leicester, UK). The flasks were incubated at 20 ^{o}C until mycelia covered the surface of the medium (approx. 30 days). After incubation, the mycelia were harvested from the media, rinsed with deionized water up to five times, air-dried and stored in a freezer at -4 ^{o}C prior to use. Biochar, obtained from pyrolysis of *Miscanthus* spp (a C_4 plant) biomass, was ground using a ball mill prior to use.

Substrates were homogenised and thoroughly mixed into the top 2 cm of the soil within collars in their solid states at concentration of 2.5 g C $(kg\ soil)^{-1}$ (volume = 157 cm^3). This rate was similar to that used in previous studies on priming effect (Blagodatskaya et al., 2009; Chen et al., 2014). It is higher than soil microbial biomass C (see Fig. 5), and therefore expected to induce *real* priming effects which involves the decomposition of SOM rather than turnover of microbial biomass C (*apparent* priming effect, *sensu* Blagodatskaya and Kuzyakov, 2008). The only exception was the *low glucose* treatment, where glucose was applied at concentration of 0.5 $gC\ kg^{-1}$ soil (i.e. 20% of the amount of C in other treatments), as priming has also been observed following the addition of readily available substrates at similar or even lower concentrations (Chen et al., 2014; Derrien et al., 2014; Tian et al., 2015). Control treatments were also mixed although no substrate was added. The chemical properties of substrates used in the experiment are presented in Table 4.1.

Table 4.1 Carbon (C) and nitrogen (N) content, C:N ratio and $\delta^{13}C$ expressed as ‰ relative to international standard Pee Dee Belemnite (PDB) of soil and substrates that were added to soils in the experiment.

	Total C (%)	Total N (%)	C : N ratio	$\delta^{13}C$ (‰)
Soil	5.06	0.31	15.85	-27.9
Glucose	41.2	n.a.	n.a.	5250
Straw	41.9	0.75	55.7	9320
Fungal necromass	49.3	1.72	28.7	2070
Biochar	68.1	0.22	307.2	-4.22

4.3.3 Soil respiration and isotopic measurements

Within each mesocosm, roots and hyphal networks were disrupted on 24th August 2017 by cutting through the entire depth of soil using a long knife, thus creating a treatment within the mesocosm that could not receive photosynthetic C from the birch trees. This "trenched" section (0.1 m^2) occupied a third of the entire area of the mesocosm. Two PVC collars (10 cm diameter, 2 cm high) were inserted (< 1 cm deep) into the trenched and un-trenched treatments of each mesocosm, taking care not to cut through roots. Substrates were thoroughly mixed into the top 2 cm of soils within the collars within three hours of trenching. Due to rainfall prior to substrate addition, only 20 cm^3 of water was added to each collar after addition of substrates, including no-substrate controls, avoiding saturation of soils. Soil respiration was measured from each collar after one and eight days of substrate addition and trenching by covering soils with cylindrical PVC gas flux chambers (15 cm diameter, 5 cm high), whilst ensuring a good seal between the soil and chamber. After 15 minutes of covering the soil, a gas sample (20 ml) was collected from each collar with a syringe through a septum in the chamber and injected under pressure into a previously evacuated Exetainer®

(12 ml; Labco Ltd, UK) for calculation of respiration rates and isotopic analysis. The amount of CO_2 accumulated in the headspace over 15 minutes was determined using a gas chromatograph coupled to a flame ionization detector (Hewlett Packard 5890). The CO_2 concentration (in ppm) obtained was converted to mass unit according to the ideal gas law and CO_2 efflux was presented as μmol m^{-2} s^{-1}. Isotopic measurements for $\delta^{13}C$ values of the gas samples were measured at the UC Davis Stable Isotope Facility (California, USA) using a ThermoScientific GasBench system interfaced to a Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, Germany), with certified reference CO_2 (NIST 8545), analysed with every ten samples resulting in an analytical precision of ±0.1‰. $\delta^{13}C$ (in ‰) values are expressed relative to the international standard Vienna Pee Dee Belemnite.

Soil CO_2 efflux was corrected for ambient CO_2 that was initially present in the chambers with isotopic mass balance approach, then partitioned into substrate-derived CO_2 (C_{Sub}) and soil-derived CO_2 (C_{Soil}) using a two-endmember mixing model:

$$C_T\delta_T = C_{Sub}\delta_{Sub} + C_{Soil}\delta_{Soil} \quad (4.1)$$

Where C_T, C_{Sub} and C_{Soil} are the total CO_2 flux, substrate-derived CO_2 and soil-derived CO_2 respectively. δ_T, δ_{Sub} and δ_{Soil} are the $\delta^{13}C$ isotopic signatures in ‰ for the total CO_2 flux, substrates and soil, respectively. Here, CO_2 from both heterotrophic decomposition of SOM and rhizosphere respiration are considered as one isotopic pool (indicated by "soil"), and the suffix "substrate" represents glucose, straw, fungal necromass or biochar treatments. The fraction of substrate-derived CO_2 in the total CO_2 flux (f_{Sub}) was calculated as:

$$f_{Sub} = \frac{\delta_T - \delta_{NS}}{\delta_{Sub} - \delta_{NS}} \quad (4.2)$$

$$1 = f_{Sub} + f_{Soil} \quad (4.3)$$

where δ_T, is $\delta^{13}C$ obtained from CO_2 samples collected from all soil collars after correction for atmospheric CO_2. δ_{NS} is $\delta^{13}C$ obtained from CO_2 samples collected from control soils where no substrate was added, whereas δ_{Sub} is $\delta^{13}C$ of the labelled glucose, straw, fungal necromass or biochar. f_{Soil} denotes the fraction of soil-derived CO_2 (i.e. SOM and rhizosphere) in the total CO_2 flux.

The CO_2 derived from substrates was then calculated as:

$$C_{Sub} = f_{Sub} \times C_T \quad (4.4)$$

and the standard error for the fraction of substrate flux (f_{Sub}) was calculated, accounting for variabilities in the $\delta^{13}C$ of mixtures and sources (Phillips & Gregg 2001):

$$SE(f_{Sub}) = \sqrt{\left(\frac{1}{\delta_{Sub}-\delta_{NS}}\right)^2 \left[\sigma^2{}_{\delta T} + {f_{Sub}}^2 \sigma^2{}_{\delta Sub} + (1-f_{Sub})^2 \sigma^2{}_{\delta NS}\right]} \qquad (4.5)$$

where σ^2 represents the square of the standard errors of the mean isotopic signatures for the component as indicated by the suffixes.

Priming effects of the addition of substrates on soil C decomposition in both un-trenched and trenched treatments were calculated as the difference of C_{Soil} between substrate-amended and control soils.

$$PE(\%) = ({}^{amended}C_{Soil} - {}^{NS}C_{Soil})/{}^{NS}C_{Soil} \times 100 \qquad (4.6)$$

where ${}^{amended}C_{Soil}$ is the soil-derived CO_2 in soils amended with biochar, fungal necromass, straw or glucose for a given trenching treatment; and ${}^{NS}C_{Soil}$ is the soil-derived CO_2 in corresponding soils without substrate addition.

The rhizosphere priming effect (RPE) on substrate decomposition was also calculated as the difference in C_{Sub} between untrenched and trenched mesocosms.

$$RPE_{Sub}(\%) = ({}^{untrenche}\ C_{Sub} - {}^{trenched}C_{Sub})/{}^{trenched}C_{Sub} \times 100 \qquad (4.7)$$

where ${}^{untrenched}C_{Sub}$ is the substrate-derived CO_2 in untrenched mesocosms, and ${}^{trenched}C_{Sub}$ is the substrate-derived CO_2 in trenched mesocosms without root input.

4.3.4 Extracellular enzyme assays

Soil samples were collected from each collar using a 1.5-cm diameter soil corer to a depth of 4 cm on the same days as CO_2 sampling. Two soil cores were collected from each collar and stored in zip-lock bags in a cooler box with ice packs, before being transported to the laboratory. Once in the laboratory, soil samples were stored at 4 ^{o}C and analysed within 72 h of collection. Prior to soil analysis, roots were removed by hand picking, and samples were homogenised by hand. Soil moisture was determined gravimetrically from a subsample by drying at 60 ^{o}C for 72 h.

Six enzymes involved in the decomposition of soil organic matter were assayed: β-glucosidase (BG) involved in sugar degradation; cellubiohydrolase (CBH) for cellulose degradation; leucine aminopeptidase (LAP) for protein degradation; *N*-acetyl-β-glucosaminidase (NAG) for chitin degradation; acid phosphatase (AP) for phosphorous mineralization; and laccase, which is involved with the degradation of aromatic compounds. These enzymes were further integrated into combined enzyme activities to represent proxies of resource acquisition. C-acquiring enzymes (BG + CBH) indicate the average enzyme activity of BG and CBH; and N-acquiring enzymes (NAG + LAP) indicate the average activity of NAG and LAP. With the exception of laccase, all enzyme assays were carried out fluorimetrically based on substrates containing either 4-methylumbelliferone (MU) or 7-amino-4-methylcoumarin (AMC): MU-phosphate was used for the detection of AP; MU-β-glucopyranoside for BG; MU-β-D-cellobioside for CBH; L-leucine-AMC for LAP; MU- *N*-acetyl-β-glucosaminide for NAG. Laccase was assayed photometrically using 2,2'-azino-bis-(3-ethylbenzothiazoline-6 sulfonic acid) (ABTS). All substrates were purchased from Sigma-Aldrich (St Louis, MO, USA).

Stock solutions of fluorogenic substrates (5 mM) were prepared in 2-methoxyethanol (Hoppe 1983), diluted with sterile deionised water to the desired working concentrations, and further diluted with 50 mM Tris-maleate buffer (pH 4.5 or pH 6.5) to the desired incubation concentration according to (Pritsch *et al.* 2011). All solutions were stored in the dark at -20 ^{o}C. Stock solutions of calibration standards (10 mM) of MU and AMC were prepared in 2-methoxyethanol and further diluted with sterile deionised water to concentrations of 0, 1, 2, 3, 4 and 5 µM. Photometric assay substrate was prepared as 2 mM ABTS in 50 mM Tris-maleate buffer pH 4.5 and further diluted with sterile deionised water for an incubation concentration of 667 µM. Calibration standards and ABTS solution were stored in the dark at 4 ^{o}C. Fluorometric and colorimetric assays were performed using the methods described by (Saiya-Cork, Sinsabaugh & Zak 2002; Pritsch *et al.* 2011).

A soil suspension was prepared by homogenising 1 g of fresh soil with 100 ml of sterile deionised water for 5 min, and 200 µl of the homogenate was pipetted into the wells of a clear flat-bottom 96-well incubation microplate. For fluorometric assays, 50 µl of substrate (sample assay), buffer (sample control), or different concentrations of MU/AMC calibration standards (sample quench) were added to sample wells. In each plate, reference standard wells contained 200 µl Tris-maleate buffer and 50 µl MU/AMC standards, which acted as negative controls. There were eight replicate wells each for assay, control and quench and

reference standard. Depending on the enzyme assay, incubation plates were incubated in the dark at 21 ºC on a microplate shaker for 10 min (AP), 20 min (BG and NAG), 40 min (CBH), 60 min (laccase) or 70 min (LAP) (Courty *et al.* 2005; Pritsch *et al.* 2011). Following incubation, fluorescence reactions were stopped by transferring 100 µl of the incubation solutions into black flat-bottom 96-well microplates (OptiPlate-96F, Perkin Elmer, USA) containing 100 µl 1M Tris pH 10-11 buffer. Fluorescence was measured immediately at 365 nm excitation and 450 nm emission wavelengths with a microplate reader (Tecan infinite M200, Austria). For the laccase assay, 100 µl of the incubation solution was transferred to a flat bottom transparent microplate and absorbance was immediately measured at 425 nm in a microplate reader (Tecan infinite M200, Austria). Enzyme activities based on fluorogenic substrates were expressed as MU or AMC release in nmol h^{-1} g^{-1} after correcting for quenching and auto-fluorescence. Laccase activity expressed as nmol h^{-1} g^{-1} was derived using the extinction coefficient for ABTS (ε_{425} = 3.6 x 104 M^{-1} cm^{-1}) and a path length of 0.29 cm.

4.3.5 Soil microbial biomass C

Microbial biomass C (MBC) of soil samples was determined using the fumigation extraction method (Vance *et al.* 1987; Joergensen 1996). Fresh soil (5g) was weighed into two glass jars (20 ml). One jar was fumigated in a dessicator with ethanol-free chloroform ($CHCl_3$) for 24 h, whereas the other jar was not fumigated. Both fumigated and non-fumigated soils were extracted with 20 ml 0.5 M K_2SO_4, shaken at 300 rpm for 30 min and filtered (Fisherbrand QT210 filter papers). Total organic C (TOC) and total N (TN) in the extracts were determined using TOC – VCSN analyzer (Shimadzu Corporation, Kyoto, Japan). Soil MBC and MBN were calculated from the difference between fumigated and un-fumigated soils using extraction efficiency factors (k_{EC} and k_{EN}) of 0.45 and 0.54 respectively (Wu *et al.* 1990).

4.3.6 Statistical analysis

Linear mixed effect models were used to test the effects of trenching, substrate additions, day of sampling and their interactions on soil characteristics such as enzyme activity, microbial biomass and total soil respiration separately. In the models, trenching, substrate and sampling

day were assigned as fixed effects, whereas blocks were assigned as a random effect using the *lme*r function in lme4 package. Response variables were log-transformed where data violated assumptions for linear models. Models were fitted using maximum likelihood and stepwise backward selection was used to select the best parsimonious model based on Akaike Information Criterion (AIC) (Pinheiro & Bates 2000). Post hoc Tukey test at a significant level of 0.05 was used to compare least mean squares of the interactions between trenching and substrate treatments for each sampling day using the *glht* function within the multcomp package (Hothorn *et al.* 2008). The relationship between soil respiration and microbial biomass, enzyme activities or C:N of soils collected at the last sampling point were analysed using Pearson-moment correlation test. All statistical analyses were performed using R studio v0.99.903 (R Core Team 2017).

4.4 Results

4.4.1 Soil respiration

Soil moisture did not differ significantly different between trenched and untrenched treatments, which eliminates any confounding effect of soil moisture on respiration between these treatments. Overall, soil CO_2 efflux differed between trenching treatments ($P < 0.001$), among substrates ($P < 0.001$) and with the day of sampling ($P < 0.001$) (Table 4.2). There was also a significant interaction between substrates and the day of sampling ($P < 0.01$). In control treatments, where no substrate was added, average soil respiration was 1.94 ± 0.33 μmol m^{-2} s^{-1} and 2.59 ± 0.48 μmol m^{-2} s^{-1} from trenched and un-trenched mesocosms respectively. Soil respiration was not affected by substrate addition on day one (Fig. 4.1a), although soil respiration was higher in un-trenched soils than trenched soils amended with fungal necromass (Fig. 4.1a). By day eight, soil respiration was not only 24 % higher in un-trenched mesocosms across all substrate treatments compared to trenched treatments ($P < 0.001$), but had also increased by 50 % in both trenching treatments with high glucose inputs relative to controls with water (Fig. 4.1b). Irrespective of trenching, CO_2 efflux was similar among soils amended with a low dose of glucose, straw, fungal necromass or biochar and with water (Fig 4.1b).

Isotopic analysis showed that the addition of glucose in high doses resulted in a positive PE, which increased the decomposition of SOM by 44 % ($P < 0.01$) in trenched mesocosms and

28% ($P < 0.1$) in untrenched mesocosms (Fig 4.2a). Low glucose marginally reduced SOM decomposition by 19% ($P = 0.1$) in un-trenched mesocosms but had no significant effect on SOM decomposition in trenched mesocosms. The addition of straw, fungal necromass and biochar had no significant effect on the decomposition of SOM in either trenching treatment. The decomposition of straw and fungal necromass was reduced in the presence of rhizosphere C, whereas the decomposition of biochar and glucose (at either concentration) were not different in un-trenched and trenched treatments (Fig. 4.2b). Soil respiration was negatively correlated to TN of soil (Table 4.3).

Table 4.2 Effects of trenching, substrate addition, day and their interactions on the activities of β-1,4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), β-1,4-glucosidase (BG), cellobiohydrolase (CBH), acid phosphatase (AP) and laccase. Values are *F* statistics (*P* value) of analysis of variance (ANOVA) tests following a mixed effect model with mesocosm nested within block as random factors.

Factor (df)	**NAG**	**LAP**	**BG**	**CBH**	**AP**	**LACCASE**
Trenching (1, 69)	1.14 (NS)	**4.19 (*)**	0.45 (NS)	0.64 (NS)	1.15 (NS)	0.00 (NS)
Substrate (5, 23)	**3.42 (*)**	**7.53 (***)**	2.23 (§)	**3.75 (*)**	**3.18 (*)**	0.88 (NS)
Day (1, 69)	3.8 (§)	**4.95 (*)**	**6.87 (*)**	0.96 (NS)	0.48 (NS)	1.87 (NS)
Trenching x Substrate (5, 69)	**4.29 (**)**	0.70 (NS)	0.30 (NS)	0.57 (NS)	0.64 (NS)	0.61 (NS)
Trenching x Day (1, 69)	0.15 (NS)	0.24 (NS)	0.30 (NS)	0.05 (NS)	0.01 (NS)	0.30 (NS)
Substrate x Day (5, 69)	1.02 (NS)	1.50 (NS)	**2.84 (*)**	1.45 (NS)	2.25 (§)	0.35 (NS)
Trenching x Substrate x Day (5, 69)	0.72 (NS)	0.32 (NS)	0.16 (NS)	0.18 (NS)	0.39 (NS)	0.37 (NS)

P > 0.1 (NS), P < 0.1 (§), P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***). Significant values (0.05) are in bold.

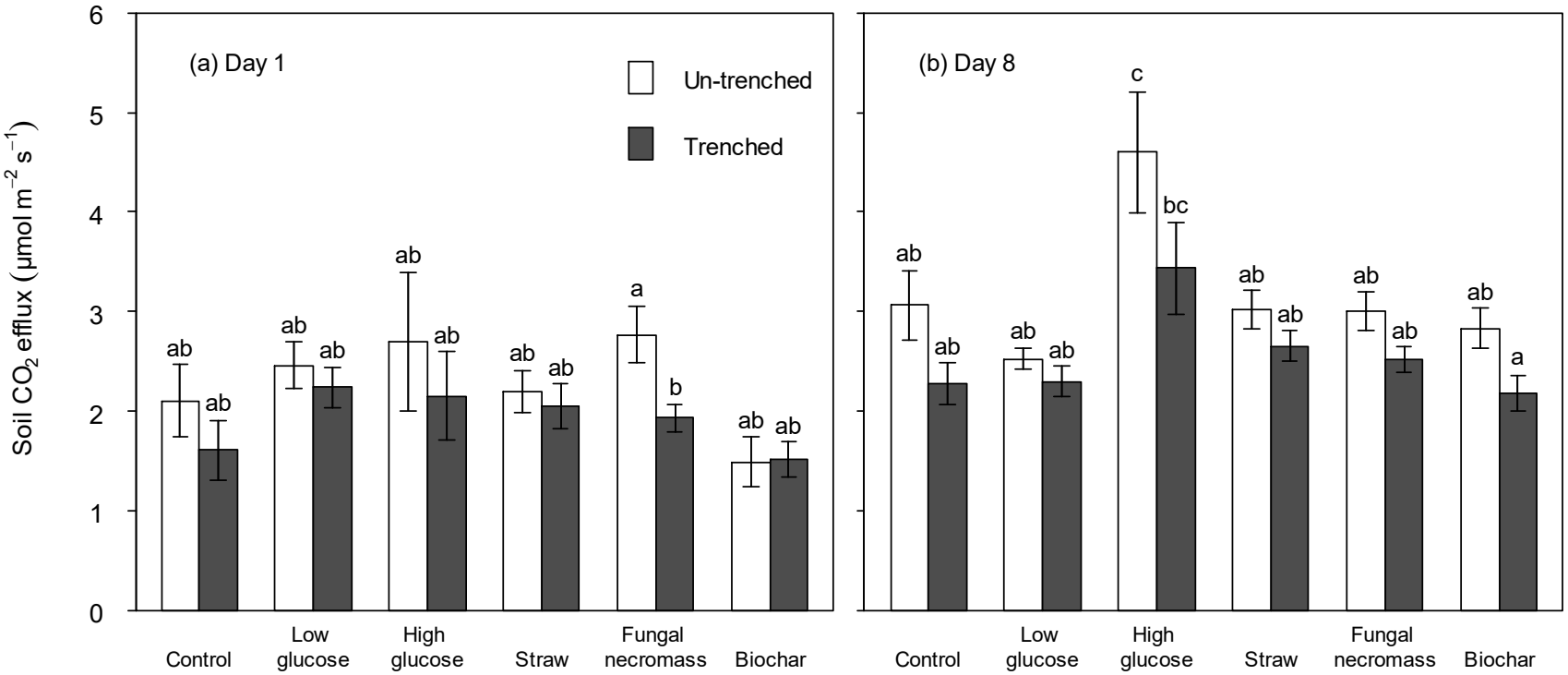

Figure 4.1 CO_2 efflux from un-trenched and trenched soils one (a) and eight (b) days after trenching and the addition of water (control), low glucose, high glucose, straw, fungal necromass and biochar. Means (bars) and standard errors (whiskers) are shown for n = 5, except for high glucose where n = 4 as one mesocosm was excluded due to unusually high CO_2 efflux. Different letters indicate significant differences among trenching and substrate treatments ($P < 0.05$).

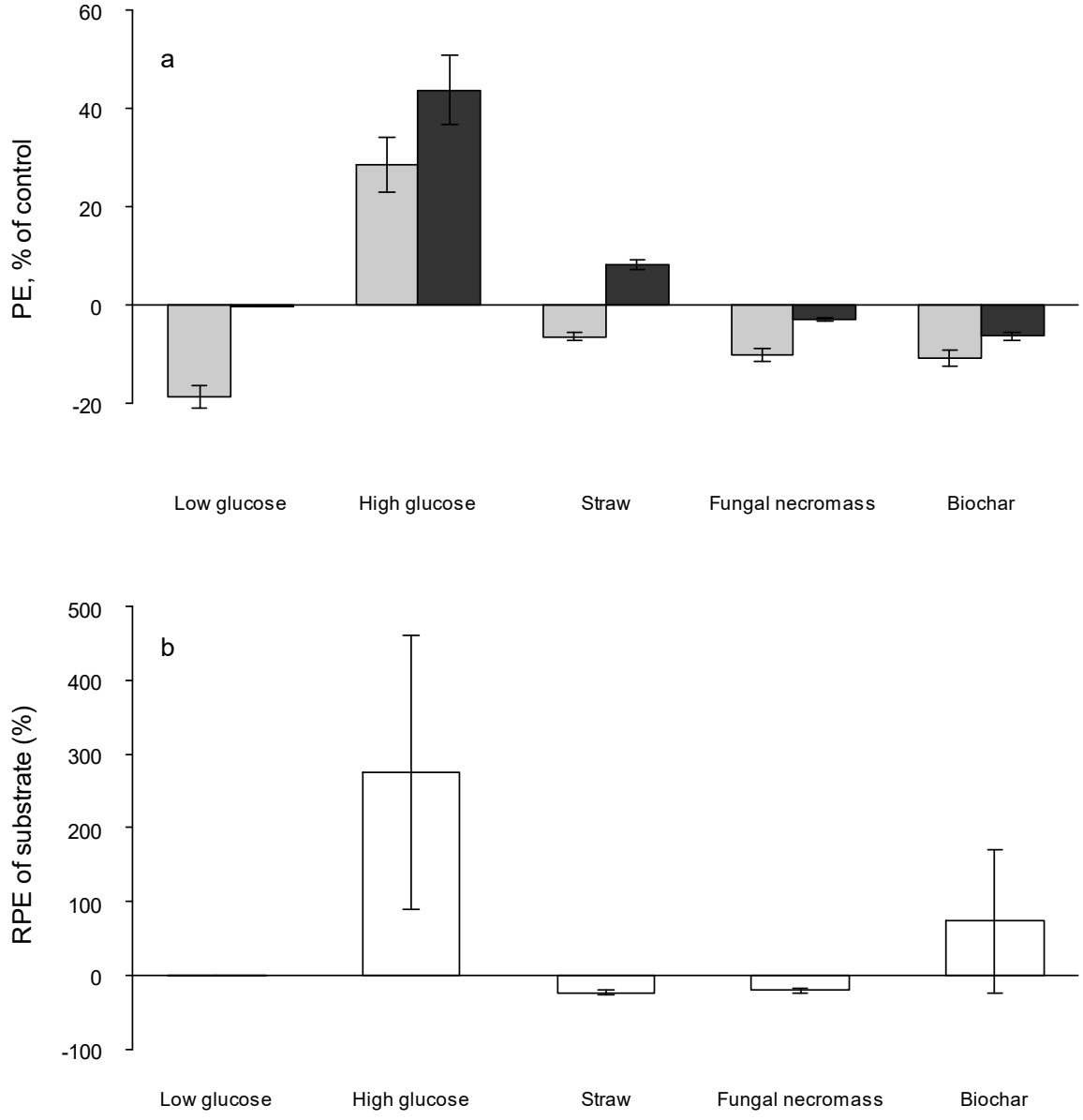

Figure 4.2 Priming effect of (a) substrate addition on SOM decomposition in un-trenched (light grey bars) and trenched (dark grey bars) mesocosms; and (b) rhizosphere C supply on the decomposition of low glucose, high glucose, straw, fungal necromass and biochar. Bars are means and standard errors (n = 5, except for high glucose where n = 4) of soil samples collected at the end of the sampling (eight days after substrate addition).

4.4.2 Enzyme activities

There was a significant difference in LAP activity between un-trenched and trenched mesocosms ($P < 0.05$, Table 4.2). The activities of NAG, LAP, CBH and AP differed significantly among substrate treatments, however BG and laccase activities were similar among the different substrate treatments (Table 4.2). LAP activities were significantly higher on day eight than day one ($P < 0.05$), whereas BG activities were significantly higher on day one, compared to day eight ($P < 0.05$). There was a significant interaction between trenching and substrate treatments on the activity of NAG, which was significantly higher in the trenched mesocosms following the addition of straw. On the other hand, NAG activity did not differ between un-trenched and trenched mesocosms amended with glucose, fungal necromass or biochar (Fig. 4.3). There was also a significant interaction between substrate and day on BG activities. BG activities were significantly higher on day one than day eight in soils amended with high glucose and fungal necromass, whereas BG activity was significantly higher on day eight compared to day one in straw-amended soils (Fig. 4.4).

The addition of glucose in small doses (0.5 mg g $soil^{-1}$) only significantly increased AP activities in both trenching treatments eight days after its addition but had no significant effect on its activity on day one (Fig. 4.4). High glucose addition on the other hand, increased the activities of all enzymes except laccase in un-trenched and trenched soils one day after its addition, and NAG, BG and AP activities eight days after the addition (Fig. 4.4). Straw significantly increased the activities of NAG and LAP on day one in trenched and un-trenched soils respectively. By day eight, NAG, LAP and AP activities were significantly higher in both trenched and un-trenched treatments than the respective controls, following the addition of straw. Fungal necromass significantly increased the activities of all enzymes except laccase in both trenching treatments shortly after its addition (Fig. 4.4). By day eight, CBH and LAP were significantly higher than the control in both un-trenched and trenched mesocosms but did not differ significantly on day one. There was no significant impact on enzyme activities following the addition of biochar. Overall, the activities of nutrient-mobilizing enzymes (NAG and LAP or AP) were higher than that of C-mobilizing enzymes (BG and CBH or laccase).

At the end of the sampling, the activities of LAP, NAG, AP, NAG + LAP were positively related to soil respiration (Table 4.3). BG was marginally correlated to soil respiration ($P < 0.1$) whereas no correlation was found between respiration and laccase or CBH. All enzyme

activities correlated positively with each other, with the exception of laccase that was not significantly correlated to BG (Table 4.3). Soil respiration was also positive correlated to C:N ratio ($P < 0.01$).

4.4.3 Microbial biomass C

MBC in un-trenched treatments did not differ significantly from the trenched treatments when pooled across all substrate treatments. In addition, MBC on day one did not differ from day eight. However, the addition of glucose (high/low doses), straw or fungal necromass increased MBC across trenching treatments during this period, compared to controls ($P < 0.05$; Fig. 4.5), whereas biochar addition had no significant effect on MBC. There was no significant interaction among factors (trenching, substrate, and sampling day). There was also no significant correlation between microbial biomass C and soil respiration at the end of sampling (Table 4.3). However, the activities of BG, CBH, LAP and laccase were positively related to MBC (Table 4.3).

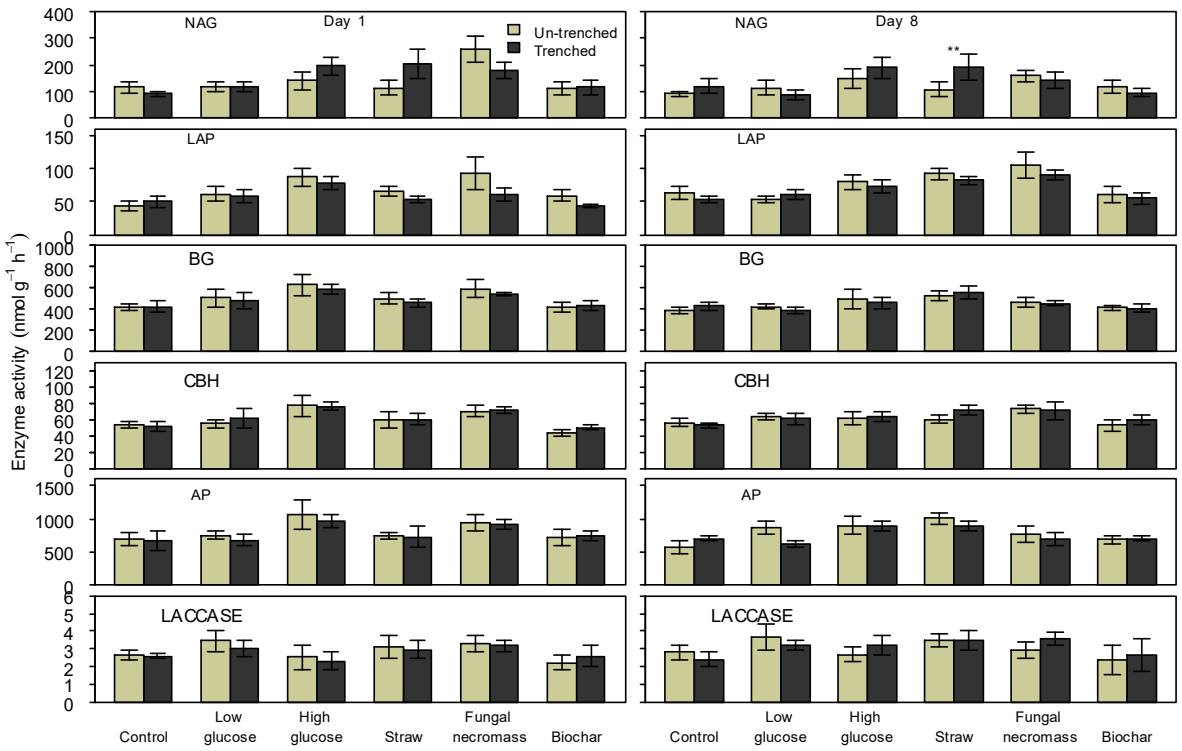

Figure 4.3 Activity of extracellular enzymes (a) N-acetyl glucosamindase (NAG), (b) leucine aminopetidase (LAP), (c) cellobiohydrolase (CBH), (d) β-glucosidase (BG), (e) acid phosphatase (AP) and (f) laccase and in un-trenched and trenched soils amended with water (control), low glucose, high glucose, straw, fungal necromass and biochar. Data are pooled means + 1SE (n = 5, except for high glucose where n = 4) for both sampling days. ** indicates significant difference ($P < 0.01$) between un-trenched and trenched for any particular substrate.

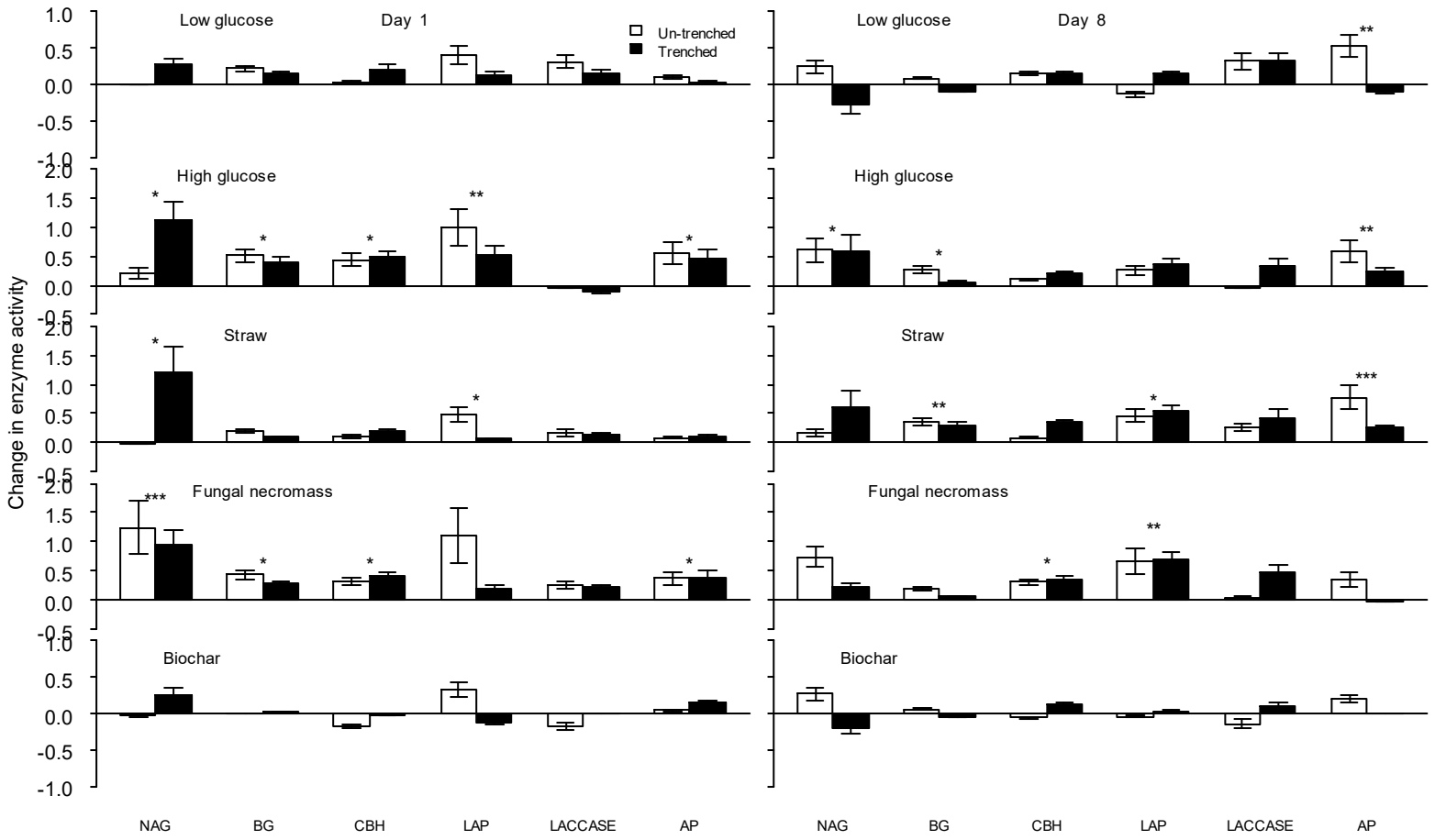

Figure 4.4 Change in activity of extracellular enzymes N-acetyl glucosamindase (NAG), β-glucosidase (BG), cellobiohydrolase (CBH), leucine aminopetidase (LAP), laccase and acid phosphatase (AP) in un-trenched and trenched soils after one (left panels) and eight (right panels) days of amendment with low glucose, high glucose, straw, fungal necromass and biochar, compared to water (control). Data are means of 5 replicates

(except for high glucose where n = 4). Vertical bars are ±1SE of means. Significant effects of substrate amendment on enzyme activities are denoted by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$)

Table 4.3 Pearson correlation coefficients of extracellular enzymes β-glucosidase (BG), cellobiohydrolase (CBH), N-acetyl glucosamindase (NAG), leucine aminopetidase (LAP), laccase and acid phosphatase (AP), C-acquiring enzymes (BG+CBH), N-acquiring enzymes (NAG+LAP), microbial biomass C (MBC), C:N ratio and soil respiration of soil samples at the end of incubation.

	CBH	NAG	LAP	AP	Laccase	BG + CBH	NAG + LAP	MBC	C:N ratio	Soil respiration
BG	0.56***	0.58***	0.56***	0.46***	0.28	0.99***	0.66***	0.32*	0.14	0.24
CBH		0.49***	0.61***	0.30*	0.53***	0.65***	0.59***	0.38**	0.18	0.14
NAG			0.47***	0.28*	0.31*	0.60***	0.95***	0.12	0.06	0.26*
LAP				0.26*	0.42***	0.60***	0.72***	0.38**	0.22	0.38**
AP					0.39*	0.46***	0.32*	0.24	0.14	0.36**
Laccase						0.32*	0.42**	0.31**	-0.02	0.21
BG + CBH							0.69***	0.34*	0.15	0.24
NAG + LAP								0.23	0.11	0.33*
MBC									0.32*	0.18
C:N ratio										0.35**

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$

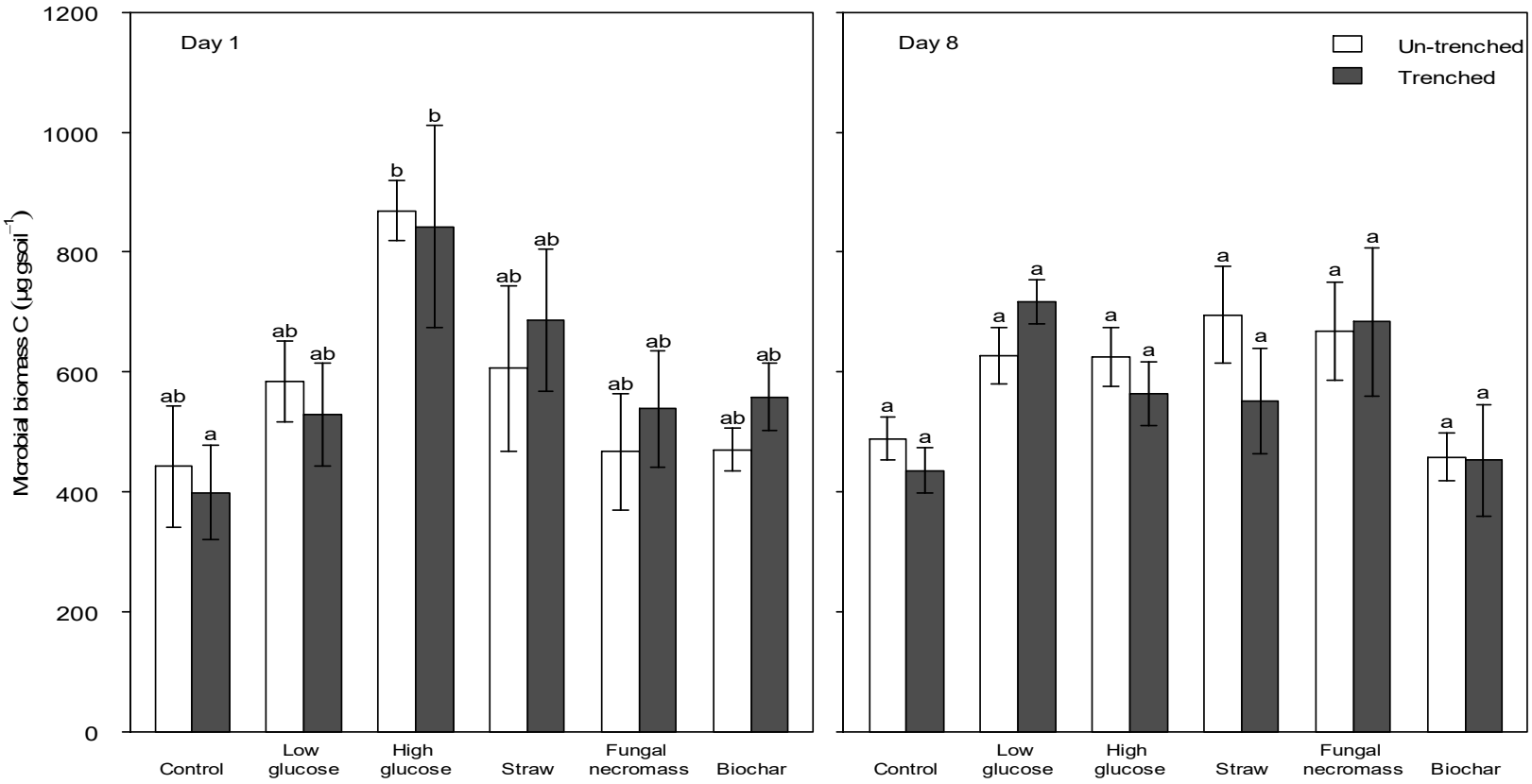

Figure 4.5 Soil microbial biomass C of un-trenched and trenched soils amended with water (control), low glucose, high glucose, straw, fungal necromass and biochar after one and eight days of trenching and substrate addition. Values are mean ± 1 SE (n = 5, except for glucose where n = 4). Different letters indicate significant differences among trenching and substrate treatments ($P < 0.05$).

4.5 Discussion

Our results show differential effects of substrates and rhizosphere C supply on microbial metabolism measured as extracellular enzyme activity and microbial biomass. The increases in activity of extracellular enzymes and microbial biomass following the addition of substrates to soils supports our hypotheses that the availability of C will promote enzyme activities. Secondly, the activities of enzymes involved in the mobilization of N and P were higher than the activities of C degrading enzymes, thus providing supporting evidence for microbial nutrient mining as the likely mechanism behind the increased soil respiration in the presence of rhizosphere C input and substrate additions (Craine *et al.* 2007). However, contrary to our hypothesis, assimilate C supply to the rhizosphere did not promote decomposition of organic substrates.

4.5.1 Effects of substrates

The substrates added in this study can be classified based on their susceptibility to microbial uptake and enzyme degradation into simple (glucose) and complex (straw, fungal necromass and biochar). Increases in enzyme activities indicate that the addition of both simple and complex substrates stimulated microbial activities, which produced enzymes based on microbial demand and the availability of C and nutrients for enzyme production (Schimel & Weintraub 2003; Allison & Vitousek 2005).

The addition of glucose rapidly increased the activities of BG and CBH that are involved in the acquisition of C. CBH and BG hydrolyse the β-1,4 glucosidic bonds in cellulose, with BG involved in terminal conversion of cellobiose to glucose (Courty *et al.* 2007). Increases in the activities of CBH and BG despite the addition of readily available C suggests that microbial community were C limited (Hernández & Hobbie 2010; Tian & Shi 2014). The activity of BG can be triggered by the input of a wide range of substrates including glucose and plant residues (Hernández & Hobbie 2010; Tian & Shi 2014; Wang *et al.* 2016a); however, the activities of these cellulases (BG and CBH) in this study were likely due to microbial demand for C (Schimel & Weintraub 2003). The addition of glucose, especially when available in high amounts, alleviated microbial C limitation of enzyme production, thereby allowing microorganisms to produce cellulases in order to meet their C demands and high growth rate

of microbial biomass through the decomposition of less available, complex organic matter. A low dose of glucose however, was not sufficient to meet the C demands for the production of all enzymes after eight days after its addition, hence the declines in enzyme activity (Fig. 4.4).

Laccase, an oxidative enzyme typically associated with plants and fungi for catalysing the degradation of lignin and other aromatic compounds such as phenols (Baldrian 2006; Sinsabaugh 2010), was not significantly induced by addition of all substrates (Fig. 4.4).. Lignin is an energy-poor, biochemically recalcitrant substrate (Talbot & Treseder 2012; Tian & Shi 2014), thus laccase is often produced when microbial activity is C limited to optimize C mobilization (Courty *et al.* 2007; Lindahl *et al.* 2010). We speculate that laccase was not expressed following the addition of substrates in trenched or un-trenched treatments due to the availability of C either as root exudates or substrates or both. It is also possible that other oxidative enzymes (e.g. phenol peroxidase), which were not measured in this study were expressed instead of laccase that we measured.

Demands for nutrients increases with higher microbial activities induced by the availability of C hence the investment into biochemical machinery to mobilize more nutrients (Allison *et al.* 2014). In addition to the increased activities of C-mobilizing enzymes in un-trenched or trenched mesocosms following the addition of glucose relative to the respective controls, other enzymes that were involved in the release of nutrients were also produced. The activities of LAP and NAG that are involved in the acquisition of N from proteins and chitin respectively, increased with the addition of glucose. This suggests rapid uptake and immobilization of N in biomass as a result of increased microbial activities following the input of labile C (Blagodatskaya & Kuzyakov 2008). The activity of AP that mineralizes phosphates from phospholipids and phosphosaccharides to acquire phosphorus (P) (Sinsabaugh & Shah 2011), was increased by the addition of high glucose on both days (Fig 4.4). Increases in AP activity were also observed following the addition of low glucose, fungal necromass and straw on either day one or day eight, thereby suggesting that production of AP is strongly determined by the availability of both C and N.

Higher enzyme activities observed in response to glucose addition might also be due to glucose effects on microbial community dynamics rather than enzyme activities directly. For example, fast growing r-strategists, which are better adapted to the utilization of energy-rich compounds, may have responded rapidly to the addition of glucose especially in high doses.

Thus, resulting in higher turnover of microbial biomass complemented by an increasing demand of nutrients such as N and P. Higher enzyme activities observed shortly after the addition of glucose suggests the dominance of r-strategists that utilized the simple substrate for the growth and production of extracellular enzymes released into the soil (Fontaine *et al.* 2003). Enzyme activities observed eight days after glucose additions were likely produced by K-strategists that grow slowly compared to r-strategists but better adapted to the degradation of more recalcitrant SOM (Blagodatskaya *et al.* 2009).

Straw and fungal necromass that are less readily available for microbial uptake, requiring the action of enzymes for degradation, also stimulated microbial activities in these forest mesocosms. Concurrent with the effect of glucose that increased both C and nutrient-acquiring enzymes, higher C and nutrient acquisition in the presence of complex substrates indicates increasing demand for these elements (C, N and P), with increasing microbial activities induced by the addition of the substrates (Kuzyakov 2010). However, unlike glucose that stimulated higher microbial activities shortly after its addition than eight days later, the addition of straw stimulated greater enzyme activities on day eight indicating the dominance of slow-growing K-strategists. The lack of significant change in enzyme activities following the addition of biochar indicates that the cost of resource acquisition from biochar was most likely greater than the acquired resources, hence microbes down-regulate investment in the production of enzymes (Schimel & Weintraub 2003; Allison *et al.* 2014). The absence of changes in the activities of some enzymes in the presence of biochar could also be as a result of sorption of DOC to biochar particles, thereby making it inaccessible for degradation (Zimmerman 2010; Zimmerman *et al.* 2011; DeCiucies *et al.* 2018).

These findings suggest that enzyme activity in soils depended on microbial elemental demand and the quality of substrates, which is in line with stoichiometric theory (Sinsabaugh *et al.* 2013). Although extracellular enzymes are stimulated by the presence of complex organic materials, microbial enzyme production was also stimulated in the presence of glucose that is readily available for microbial utilization. Others have also reported increased production of enzymes in the presence of labile C substrates (Allison & Vitousek 2005; Hernández & Hobbie 2010). Microbes regulated their production of extracellular enzymes, to optimize the decomposition of substrates and/or SOM to meet their demand for nutrients (Mooshammer *et al.* 2014).

The increases in microbial biomass following the addition of substrates confirms the microbial activation hypothesis (Cheng & Kuzyakov 2005). Increases in MBC and activities of extracellular enzymes by these activated microbes did not significantly increase soil CO_2 efflux except in soils amended with high amounts of glucose (Fig. 4.1). Although total soil CO_2 efflux did not significantly differ from the control, positive priming of SOM decomposition was observed in trenched mesocosms following the input of straw. This positive PE was likely due to co-metabolism of SOM during intensive straw degradation (Kuzyakov *et al.* 2000; Shahbaz *et al.* 2017b), whereby the enzymes produced may have resulted in the decomposition of SOM fractions with similar chemical structures (Shahbaz *et al.* 2017b). On the other hand, increased enzyme activities in un-trenched and trenched mesocosms following the addition of fungal necromass, low amounts of glucose and straw (un-trenched mesocosm only), with corresponding negative priming of SOM suggests preferential utilization of the added substrates versus SOM (Kuzyakov 2002; Cheng & Kuzyakov 2005). Microbial biomass was not affected by the addition of biochar, but SOM decomposition was reduced in both trenching treatments eight days after the addition of biochar; likely due to the sorption of DOC to biochar particles (DeCiucies *et al.* 2018). Other studies have also observed reductions in soil CO_2 emissions and SOM decomposition in the presence of biochar (Kuzyakov *et al.* 2009; Wang *et al.* 2016b; a). Further, the positive correlation between soil respiration and C:N ratio (Table 4.2) corroborates the stoichiometric theory that higher C mineralization occurs at high C:N ratio and vice versa (Mooshammer *et al.* 2014; Wild *et al.* 2017).

4.5.2 Effects of rhizosphere C supply

Plant roots supply an important source of labile C to soil microbes, enabling the formation of microbial communities (Lindahl *et al.* 2010), of which ectomycorrhizal fungi are a significant fraction in temperate forests (Read & Perez-Moreno 2003). No effect of plant C input to the rhizosphere on microbial activities (measured as enzyme production and microbial biomass) was evident in this study, although studies have reported increased microbial biomass and enzyme activities in the presence of plant C input compared to un-planted soils (Kumar *et al.* 2016; Loeppmann *et al.* 2016). Eliminating belowground assimilate C supply did not reduce enzyme activities in the trenched mesocosms in most substrate treatments. However, trenching resulted in decreases in soil CO_2 efflux ranging from 10% to 33% across all

treatments, confirming a general decline in rhizo-microbial respiration. Furthermore, rhizosphere C supply either reduced or did not significantly affect the decomposition of substrates.

Enzyme activities did not differ between un-trenched and trenched soils regardless of substrate addition, with the exception of NAG that was significantly higher in trenched soils of straw-amended mesocosms (Fig. 4.3). The experimental design in this study does not allow us to make conclusions as to the influence of ECM fungi on enzyme activity because ECM fungi was not distinctly isolated from roots in the treatments. However, we speculate that a switch to saprotrophy by ECM fungi in trenched soils may have been responsible for the similarities in enzyme activity after trenching, accessing C from the metabolism of low molecular weight compounds (Talbot et al. 2008). ECM fungi may invest in enzymes involved in the mobilization of C from SOM when supplies of assimilate C from host is low (Buée *et al.* 2005, 2007; Courty *et al.* 2007).

Since extracellular enzymes were measured from soil suspensions, enzyme activity is characteristic of both free-living saprotrophs and ECM fungi. N-acquiring enzyme (LAP+NAG) activity was marginally higher in un-trenched soils than trenched soils ($P = 0.08$) during the sampling period, which may explain the increase in soil respiration in the presence of fungal necromass. Increased NAG activity in trenched soils may indicate dominance of saprophytic fungi, which utilized the added straw as C source for the production of enzymes to mine N (Meier *et al.* 2015). Severing belowground C supply likely reduced the activities of ECM fungi (Högberg & Högberg 2002; Högberg, Högberg & Myrold 2006; Kaiser *et al.* 2010), which enhances N acquisition of free-living fungi. The decline in ECM fungal biomass or available C supply to ECM fungi may have reduced mycorrhizal competition in trenched soils, thereby increasing the biomass of free-living saprotrophs and their enzyme activities (Lindahl *et al.* 2010). Furthermore, the dead mycorrhizal necromass in trenched mesocosms probably acted as substrate for opportunistic saprotrophs, thus the significant increase in NAG activity in the presence of complex forms of C compared to un-trenched soils.

Put together, our result showing increased NAG activity in the absence of rhizosphere C to ECM fungi suggests that decomposition and uptake of N by ECM fungi may cause N limitation to free-living saprotrophs, hence retarding the decomposition of SOM (Orwin *et al.* 2011; Averill & Finzi 2011; Averill *et al.* 2014; Averill & Hawkes 2016). Although enzyme

activities and soil respiration were mostly similar in un-trenched and trenched soils, further longer-term study, where ECM fungi and root effects are separated, is required to assess the role of ECM fungi competition in soil enzyme and organic matter dynamics.

4.5.3 Interactions between enzyme activities and soil respiration

The activities of LAP, NAG, LAP+NAG and AP were significantly positively correlated with soil respiration eight days after trenching and substrate additions, whereas C degrading enzymes (BG, CBH and laccase) showed no correlation with soil respiration (Table 4.3). This provides additional evidence that the input of labile C stimulates enzymes that target SOM polymers to mobilize limiting nutrients particularly N and P. Higher decomposition of SOM in the presence of plant roots (rhizosphere) and/or input of labile C through the priming effect have been widely reported (Pausch *et al.* 2013; Cheng *et al.* 2014; Kumar *et al.* 2016; Shahbaz *et al.* 2017b). One mechanism proposed is the stimulation of microbial activity by the presence of labile C, leading to co-metabolism of less available substrates due to microbial N mining (Blagodatskaya & Kuzyakov 2008). The significant positive correlation between soil respiration and LAP, NAG, LAP + NAG and AP indicates that soil respiration is strongly linked to the mobilization of N and P (microbial nutrient-mining) and therefore may be related to positive priming effects (Chen *et al.* 2014; Shahbaz *et al.* 2017b). Due to the short sampling period, a fraction of the increase in soil respiration might have been due to accelerated microbial biomass C turnover (i.e. apparent priming) rather than accelerated SOM decomposition (i.e. real priming) (Blagodatskaya & Kuzyakov 2008). Nevertheless, the addition of organic materials to these forest mesocosms stimulated nutrient mining by increasing the production of enzymes involved in N and P acquisition.

4.6 Conclusion

We demonstrate that increased availability of labile C from rhizodeposition and substrates or their decomposition products to forest soils stimulates the activity of enzymes related to C and nutrient cycling, and thereby influences decomposition of SOM. Low substrate C availability on the other hand may limit microbial investment in enzyme production, resulting in reduced decomposition of the substrate or SOM. The positive relationship between N and P-acquiring enzymes and soil respiration suggests microbial nutrient mining as a driver of

priming effects. This will have a long-term effect on soil C sequestration under future climate predictions, where elevated atmospheric CO_2 concentrations may enhance plant productivity that will in turn increase rhizodeposition and litter inputs into soils. Future long-term studies integrating enzyme activities with microbial community characterization will provide further insights into the role of ECM fungi in the decomposition of SOM in forest ecosystems.

Chapter 5

Biotic environment controls litter decomposition in temperate forests

5.1 Abstract

Plant litter is the primary route by which carbon (C) enters the soil, and its decomposition determines the sequestration potential of soil. Climate, chemistry of the litter, and the physicochemical properties and decomposer community of the soil environment, where the decomposition takes place all determine litter decomposition rates. There are significant uncertainties about the role of roots and ECM fungi on decomposition rates, which can influence the dynamics of C and nutrient cycling in soils (Strickland *et al.* 2009b; Ball, Carrillo & Molina 2014). Using a beech (*Fagus sylvatica*) forest, we investigated if mass loss of broadleaved, beech litter increased in the presence of roots and/or ectomycorrhizal (ECM) fungi; and found that litter mass loss was not different among soils with or without roots and ECM fungi. This suggests that ECM fungi had no significant effect on the decomposition of fresh litter, and that litter decomposition in this forest was likely dominated by free-living saprotrophs.

5.2 Introduction

Litter decomposition in forest soil is a major ecosystem process that determines the sequestration of carbon (C) in soils, as well the cycling of C and nutrients within these ecosystems (Prescott 2010; Schlesinger *et al.* 2013; Bradford *et al.* 2016). Litter decomposition determines the formation of soil organic matter (SOM), which is formed through the incomplete decomposition and modification of plant residues by soil organisms (Cotrufo *et al.* 2015). Litter decomposition is also a key component of soil CO_2 efflux, as C contained in litter is released back to the atmosphere as CO_2. Soil CO_2 efflux comprises of respiration of roots and their associated rhizosphere microbes (autotrophic respiration), and the decomposition of dead organic matter in soil (heterotrophic respiration). Increased atmospheric CO_2 will likely increase plant productivity through the 'fertilization effect' (Norby *et al.* 2005), which may increase the allocation of C belowground, and stimulate microbial decomposition, hence creating a positive feedback to the climate change. The impending concerns about climate change caused by increasing atmospheric CO_2 concentrations therefore highlights the need for a better understanding of the decomposition processes, especially in temperate and boreal forests soils that store the majority of global terrestrial C (Pan *et al.* 2011).

Litter decomposition rates are controlled by both abiotic conditions (such as microclimate and soil physicochemical properties) and biotic factors that determine the quality and quantity of litter, as well as decomposer community composition (Gholz *et al.* 2000; Zhang *et al.* 2008; Prescott 2010). In a meta-analytical study of litter bag incubations across different terrestrial ecosystems, Zhang et al. (2008) demonstrated that litter quality was the key factor influencing mass loss accounting for at least 73% of the variation in litter decomposition rates globally. For example, high quality litter, with high concentrations of nitrogen (N), low C:N ratio and low concentration of chemically recalcitrant constituents often decompose faster than low quality litter, with low concentrations of N and high recalcitrant fractions (Gholz *et al.* 2000; Zhang *et al.* 2008). Decomposer communities (microbes and mesofauna) mediate litter decomposition, and therefore contribute to some of the variations in decomposition rates (Moorhead & Sinsabaugh 2006; Wall *et al.* 2008; Bray, Kitajima & Mack 2012). There is also increasing recognition of the role of plants in the decomposition of litter (Cornwell *et al.* 2008; Joly *et al.* 2017). Although the interactions between plant and soil microbial communities have been shown to influence decomposition rates (Kuzyakov 2010; Subke *et*

al. 2011), a better understanding of these interactions that therefore improve our predictions of litter decomposition under environmental change.

Plants may directly affect the abiotic and biotic environment, with feedbacks to C and N cycling in terrestrial ecosystems (Hobbie 1996, 2015; Vivanco & Austin 2008; Freschet *et al.* 2012; Churchland & Grayston 2014; Blagodatskaya *et al.* 2014b). Plant species can determine the quality and quantity of litter that enters the soil, soil nutrient contents, pH or microbial communities (Cornwell *et al.* 2008; Parker *et al.* 2018). For example, studies have shown that deciduous broadleaf litters decompose faster than evergreen coniferous litter (Gholz *et al.* 2000; Prescott *et al.* 2000), but this can be dependent on the interaction between litter type and the decomposer environment (Freschet *et al.* 2012; Keiser *et al.* 2014). The input of labile C in form of root exudates and other rhizodeposits that microbes can utilize as C source has increasingly been reported to influence SOM decomposition through the rhizosphere priming effect (RPE) (Cheng *et al.* 2014). Rhizosphere priming effect is defined as the stimulation or retardation of soil organic matter decomposition by living roots and their associated microorganisms when compared to unplanted soils exposed to the same environmental conditions (Kuzyakov 2002). Studies have demonstrated positive priming of SOM decomposition, following the input of assimilate C to the roots (Kuzyakov 2010; Pausch *et al.* 2013; Cheng *et al.* 2014). The priming effects of three tree species (larch, ash and Chinese fir) were shown to increase SOM decomposition, ranging from 26% to 1.5-fold (Yin *et al.* 2018). Increased decomposition of SOM can be accompanied by increased litter decomposition (Subke *et al.* 2004, 2011). Although the priming effect of the rhizosphere on soil CO_2 efflux has been extensively studied (Cheng *et al.* 2014), there remain uncertainties about the potential role of roots and ECM fungi on actual litter mass loss, which determines the formation of SOM (Cotrufo *et al.* 2015).

Plants may also influence microbial communities and ecosystem processes through their mycorrhizal fungal association (Courty *et al.* 2010; Buée *et al.* 2011). In most temperate and boreal forests, tree roots form symbiotic associations with ectomycorrhizal (ECM) fungi, which receive a significant allocation of assimilate C from their plant host and in return transfer nutrients to the host roots (Smith & Read 2002; Hobbie 2006). ECM fungi constitute more than half of the fungal species in temperate forest and have been reported to be negatively related to N mineralization (Buée *et al.* 2011). That is, as N availability increases, ECM fungal abundance decreases. This suggests that in low-nutrient ecosystems (e.g. temperate forests), the abundance of ECM fungi may stimulate the decomposition of litter to

mobilize nutrients (Subke *et al.* 2011; Brzostek *et al.* 2015; Trap *et al.* 2017). However, lower decomposition rates of litter have been reported in the presence of ECM (*Gadgil effect*, Gadgil and Gadgil (1971, 1975)). The input of C through root exudation and the supply of assimilate C to their symbiotic mycorrhizae and other rhizosphere microbes may therefore play a major role in litter decomposition.

The presence of plants may result in positive or negative or no effect on priming of heterotrophic decomposition, which has been shown in both mesocosms and in the field (Cheng *et al.* 2014; Huo *et al.* 2017; Jackson *et al.* 2019). However, the role of mycorrhizal fungi in the transfer of fresh C from roots to the rhizosphere is poorly understood, therefore more studies are required to further partition 'rhizosphere' effect on soil priming. This study uses a forest trenching experiment to separate the effects of root, ECM fungi and soil microbes on heterotrophic decomposition. We hypothesize that the presence of root and/or ECM fungi promotes litter mass loss in a temperate forest soil.

5.3 Methods

5.3.1 Site description

The forest site is a beech (*Fagus sylvatica* L) stand with sparse stinging nettle (*Urtica dioica*) as ground flora, located in Stirling, Scotland, United Kingdom (56º08′ N, 3º54′ W). The 10-year mean annual precipitation and temperature of the site are 1019 mm and 9.2 ºC respectively (UK Met Office 2017). The physical and chemical characteristics of the site are presented in Table 5.1.

5.3.2 Experimental set-up

Five experimental blocks were established in the forest by identifying five additional tree stands in May 2016 (see Chapter 3). Each block contained three collar treatments that allowed or excluded ECM hyphal in-growth and/or roots (five blocks, three collar treatments). The RMS treatment (root, mycorrhizal hyphae and heterotrophic soil organisms) was created using shallow PVC collars (20 cm diameter, 5 cm height) inserted 1 cm into the soil. The MS treatment which excludes the roots but allow the access of mycorrhizal hyphae was created using collars (20 cm diameter, 30 cm height) with four windows (width 6 cm,

height 4 cm) cut 5 cm from the top of the collar and covered with 41 µm mesh size (Normesh Ltd, Oldham, UK). The heterotrophic treatment (S) was created using "deep" collars (20 cm diameter, 45 cm height) to exclude both roots and mycorrhizal components. On 2nd July 2016, five "deep" collars were hammered into the soil in *F. sylvatica* stands to a depth of 30 ± 2 cm beyond the rooting system using a bread knife to cut through heavy roots. The MS collars were also inserted to a depth of 25 ± 2 cm by hammering down deep collars which were then carefully removed and replaced by the MS collars. The surface collars were inserted to a depth of 1 -2 cm to ensure a good seal with the soil without disturbing the soil significantly. All collars were placed at least 1 m apart, and between 0.5 m and 2 m from the nearest tree.

5.3.3 Litter bag incubations

Litter from *F. sylvatica* stands was collected in April 2016, washed to remove soil particles and air-dried for several weeks. Care was taken to ensure that only recently fallen *F. sylvatica* litter was used by sorting and removing senesced litter. Litter bags (9 cm x 9 cm) were created using mesh with 0.3 mm aperture size, which were sealed at two sides using hot melt adhesive. 1 g of dried beech litter was added to each litterbag along with a 9 mm embossing tape (Dymo, Cambridgeshire, UK) containing the sample bag label and sealed using hot melt adhesive.

During installation on the 22nd July 2016, all surface litter was removed from the collars, and three litterbags were placed in each collar, ensuring that bags were in contact with the soil. To ensure that similar dry mass of litter fall was replaced in the collars after litterbag installations, surface litter were randomly collected from three locations of each site on the 2nd of July 2016, prior to litter bag installation using a 0.5 m quadrat. After removing soil particles and drying the litter, the average dry mass of litter fall per unit area was calculated. Based on the calculation, 14.8 g of beech litter were moistened and placed in each collar after the insertion of litter bags. One bag from each of the collars in each collar was immediately harvested after installation, taken to the laboratory where litter was carefully extracted and oven-dried at 60 ºC for 72 hours. The average mass loss of litter calculated from harvested bags was used to correct for the effects of packaging, transit and installation. Litterbags were harvested on 22nd September 2016, 8th December 2016, 18th April 2017, 25th May 2017 and 25th September 2017. Two litter bags were collected from each collar on 25th May 2017.

Ingrown vegetation was carefully removed, and the litter was removed from the bags, oven-dried at 60 ºC for 72 hours, and the percentage of the remaining litter was calculated.

Table 5.1 Physico-chemical characteristics of *Fagus sylvatica* (means ± 1SE, n = 5). C and N denote carbon and nitrogen respectively.

Soil	
pH (1:2.5 H_2O)	5.51 ± 0.06
Texture	Loamy sand
Microbial biomass C (μg g_{soil}^{-1})	492 ± 15.1
Total C (%)	4.53 ± 0.26
Total N (%)	0.40 ± 0.03
C:N ratio	11.4 ± 0.51
Litter	
C (%)	44.4 ± 0.15
N (%)	1.07 ± 0.10
C:N ratio	42.9 ± 3.40

5.3.4 Chemical analysis

Litter collected at the first and last samplings were analysed for total organic C (TOC), total C (TC) and total N (TN) contents using a CHN analyser (Flash Smart, ThermoFisher Scientific, Bremen, Germany). Soil samples were collected from all collars up to a depth of 5 cm after collecting all litter bags from the collars. The soils were stored in sealed plastic bags at 4 °C until analysis. Soil pH was measured in 1:2.5 soil-water mixtures. Microbial biomass was determined using the chloroform fumigation extraction method (Vance *et al.* 1987). Briefly, 5 g of field moist soil was passed through a 2-mm sieve and weighed into two 20 ml jars. One sample was sealed in a vacuum desiccator containing alcohol-free chloroform for 24 h while the other was not fumigated with chloroform. All samples were extracted with 20 ml 0.5 M K_2SO_4, shaken at 300 rpm for 30 min and filtered (Fisherbrand QT210 filter paper, Fisher Scientific, UK). The filtrate was analysed for TOC using a TOC – VCSN analyzer (Shimadzu Corporation, Kyoto, Japan). Soil microbial biomass C was calculated as the

difference between fumigated and unfumigated samples using a k_{ec} factor of 0.45 (Wu *et al.* 1990).

5.3.5 Soil CO_2 efflux measurements

Soil CO_2 efflux was measured using a custom-built chamber (15 cm diameter, volume = 2,300 cm^3) connected to a portable EGM-4 infrared gas analyser (PP Systems, Amesbury, MA, USA). CO_2 efflux was measured as the linear rise in CO_2 concentration within the headspace over a period of two minutes.

5.3.6 Environmental parameters

Soil temperature was measured at 5 cm depth every 30 minutes using three Tinytag Plus Two logger with PB-5001 thermistor probe (Gemini Data Loggers, Chichester, UK). Soil temperature was also measured at 5 and 10 cm depths with a hand-held temperature probe on days when soil respiration was measured. Soil moisture was measured using an SM150 moisture sensor after soil respiration measurements were taken on sampling days (Delta-T devices, Cambridge, England).

5.3.7 Data analysis

The initial litter dry mass for each litter bag was corrected for mass loss during transit and installation, using correction factors, obtained from litter bags collected immediately after installations. Litter mass loss was calculated as the percentage mass remaining of the initial litter dry mass:

$$\%\ mass\ remaining = \frac{final\ dry\ mass\ of\ litter\ after\ incubation\ (g)}{initial\ dry\ mass\ of\ litter\ after\ correction\ (g)} \times 100 \qquad (5.1)$$

The decomposition constant (k) was calculated using the equation below:

$$m_1 = m_0 e^{-kt} \qquad (5.2)$$

where m_1 and m_0 are remaining and initial dry mass of litter after installation.

The effect of the presence of roots and/or mycorrhizal fungi on litter mass loss and soil respiration was tested using one-way ANOVA following a mixed effect model with treatment as fixed effect and block and time were assigned as random variable using the *lme*r function in lme4 package. The contributions of root, ectomycorrhizal fungi and soil heterotrophic respiration were calculated as described by (Heinemeyer *et al.* 2007). Where the assumptions of normality and homogeneity of variance were violated, response data were log-transformed. Mass of C and N after 430 days of placements in the field (final harvest) was analysed using one-way ANOVA to test for differences among RMS, MS and S treatments. All analyses were done with R Studio v1.0.143 (R Core Team 2017).

5.4 Results

5.4.1 Litter mass loss

In this beech forest, there was no significant difference in the decomposition of beech litter with or without the presence of roots and/or mycorrhizae hyphae ($P = 0.14$, Fig. 5.1). About 78% of the beech litter remained after 430 days of decomposition in the field, with an average decay constant (k) of 0.2 $year^{-1}$ (Fig. 5.1). There was an initial, rapid mass loss of litter within the first two months of litter installations, which was followed by a gradual loss of litter (Fig. 5.2).

5.4.2 C and N components of litter

The mass of C was significantly lower in the litter, upon harvesting after 430 days of incubation ($P < 0.01$, one-way ANOVA), but did not differ significantly among treatments ($P > 0.05$) (Fig. 5.3). Remaining mass of N in the litter did not differ upon harvesting when compared to the initial mass, and also did not differ significantly among the treatments ($P > 0.05$) (Fig. 5.3). Tukey *post hoc* test showed that final litter N was marginally higher in MS than RMS ($P = 0.1$).

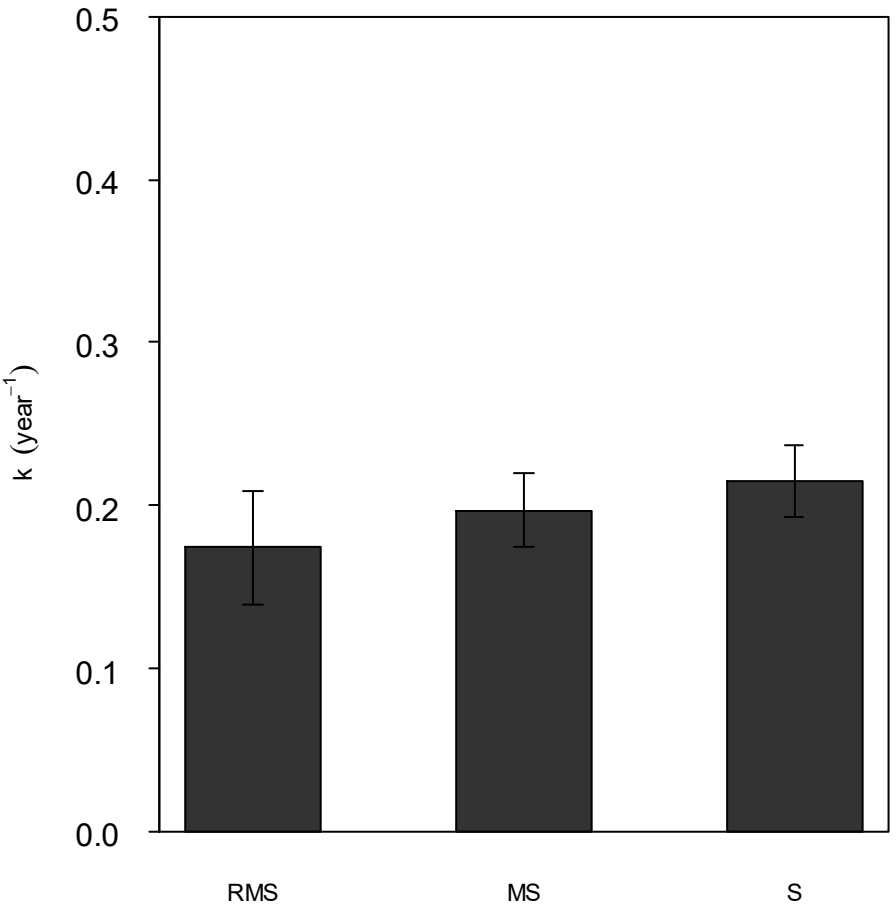

Figure 5.1 Decomposition rate (*k*) of beech litter in RMS, MS and S collars of *Fagus sylvatica* forest collected over five sampling times. Bars are mean ± 1SE (n = 30).

5.4.3 CO_2 efflux

CO_2 efflux in RMS treatments was significantly greater than both MS (Tukey post hoc, $P < 0.05$) and S treatment (Tukey post hoc, $P < 0.001$). MS was also greater than S treatment ($P < 0.001$). Average soil CO_2 efflux measured for the sampling period from RMS collars was 2.31 ± 0.11, μmol m^{-2} s^{-1}, 1.92 ± 0.18 μmol m^{-2} s^{-1} in MS collars and 1.20 ± 0.13 μmol m^{-2} s^{-1} in S collars (Fig. 5.4a). The average proportional contribution of root to soil CO_2 efflux was ~17%, calculated as the difference between RMS and MS treatments, relative to RMS. Ectomycorrhizal hyphae contributed c. 31% to soil CO_2 efflux, whereas heterotrophic respiration contributed c. 52%.

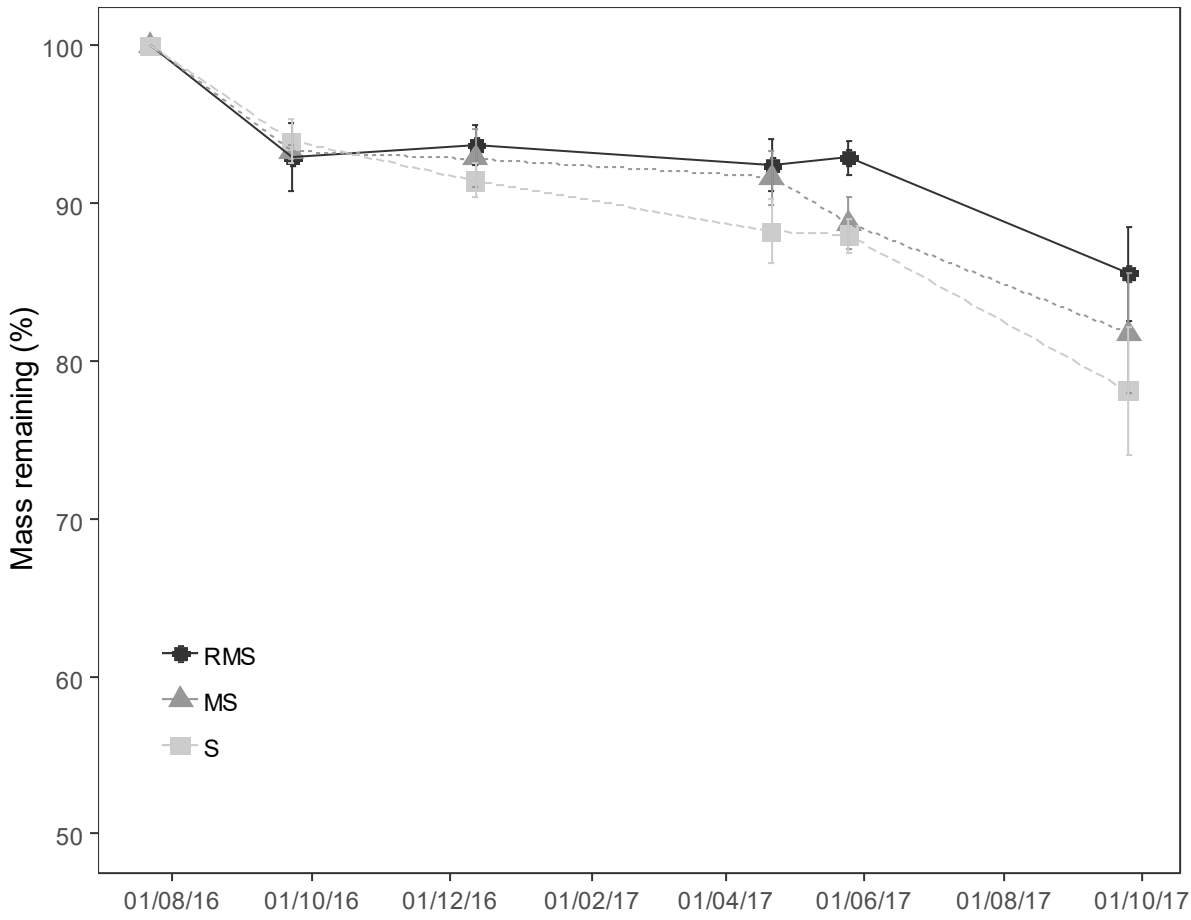

Figure 5.2 Percentage mass remaining of litter over time from 22nd July 2016 to 25th September 2017 when the last litter bags were collected from each collar. Two litter bags were collected on 25th May 2017. Error bars represent ± 1 SE (n = 5).

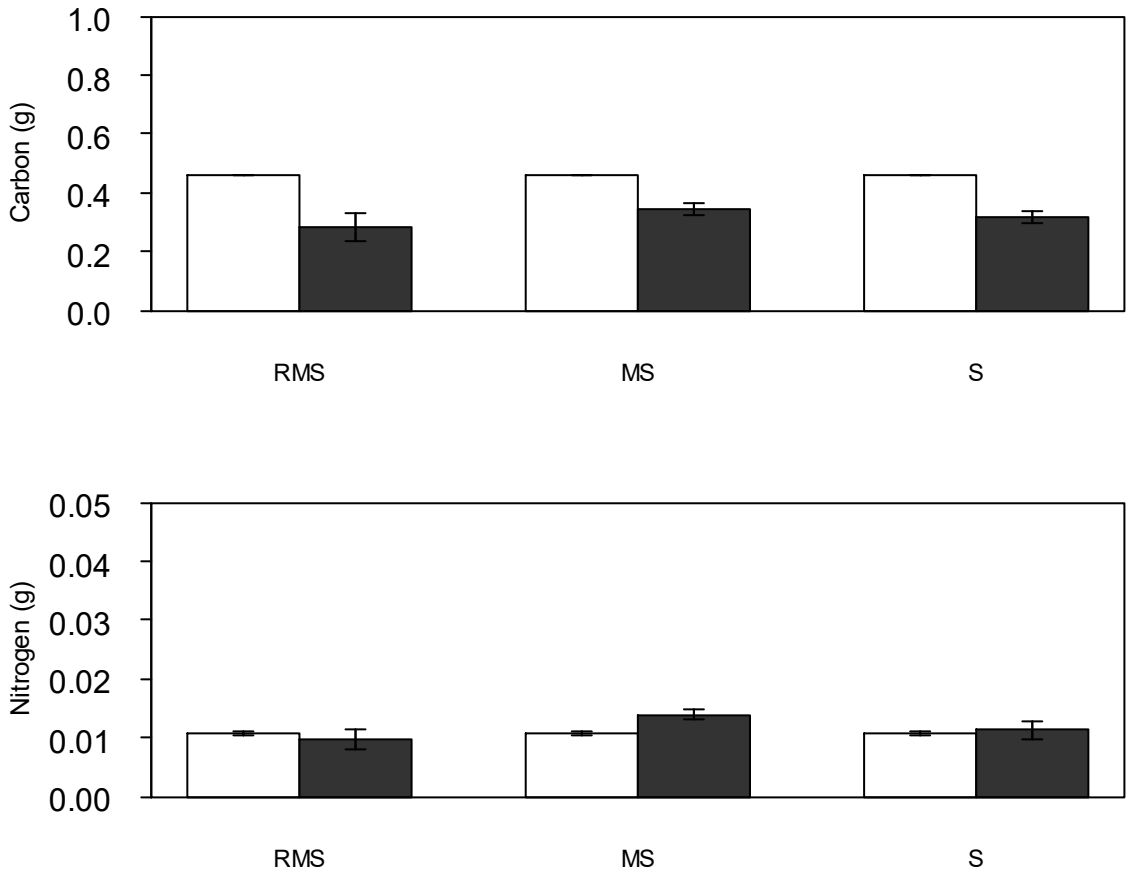

Figure 5.3 Mass of carbon and nitrogen in *Fagus sylvatica* litter after 430 days of decomposition (filled bars) compared with undecomposed control samples (open bars). Error bars are ±1 SE (n = 5). The final mass of C was lower than the initial mass ($P < 0.01$). Final N in litter did not differ from initial N ($P > 0.05$). Remaining C and N in litter were not significantly different among RMS, MS and S treatments ($P > 0.05$). Final N was marginally higher in MS than RMS ($P = 0.1$, Tukey HSD *post-hoc* tests).

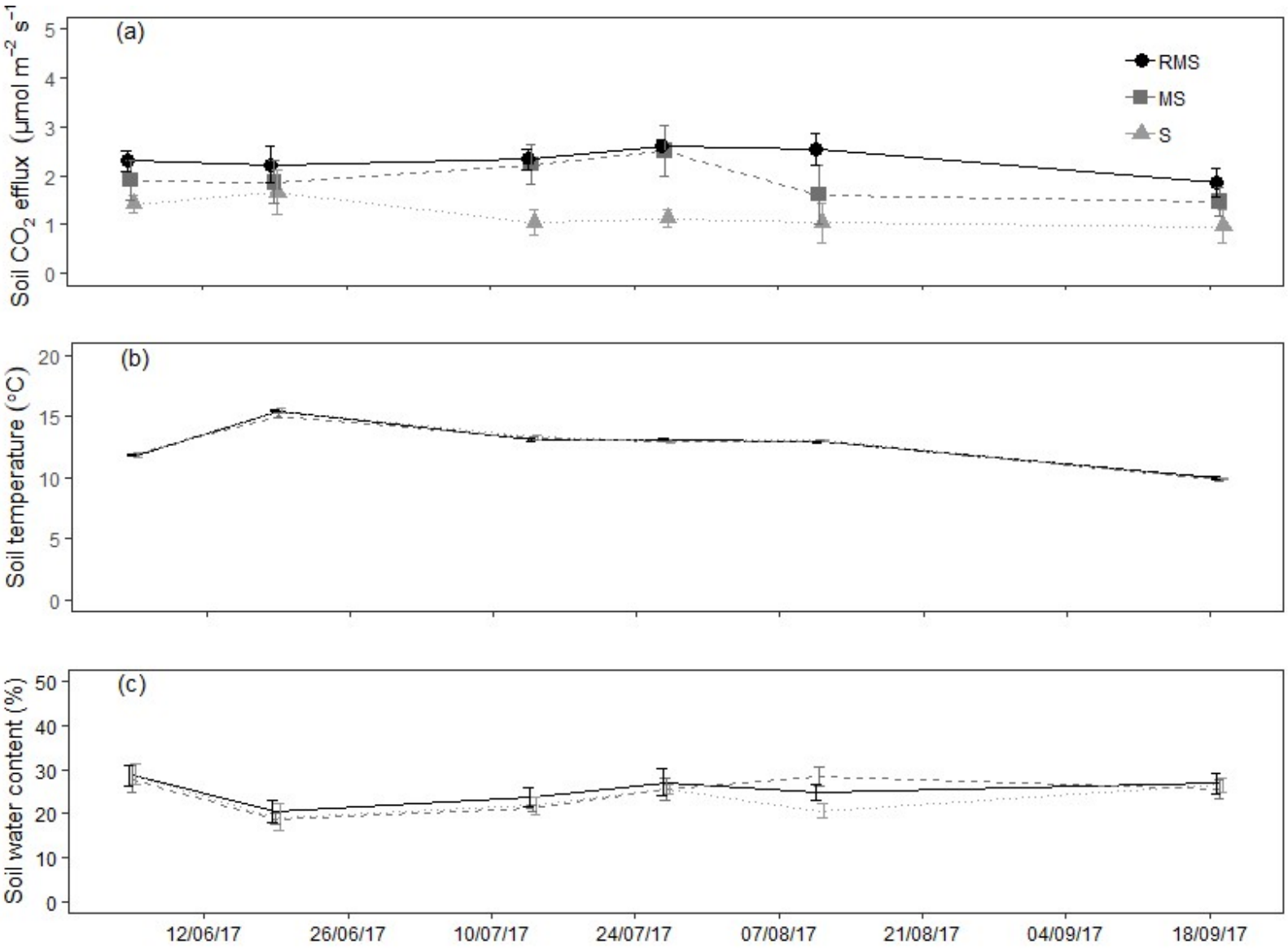
(a)
Soil CO_2 efflux (μmol m^{-2} s^{-1})
RMS
MS
S
(b)
Soil temperature (°C)
(c)
Soil water content (%)
12/06/17
26/06/17
10/07/17
24/07/17
07/08/17
21/08/17
04/09/17
18/09/17

Figure 5.4 Soil CO_2 efflux and environmental conditions over time. Soil CO_2 efflux (a), soil temperature (b) and soil moisture content at 5 cm depth (c) in RMS, MS and S treatments of *Fagus sylvatica* forest. Values are means of five blocks ± 1 SE. CO_2 efflux was significantly higher in RMS than MS ($P < 0.05$) and S ($P < 0.001$) treatments. MS was significantly higher than S ($P < 0.001$). Soil temperature and moisture did not differ among treatments ($P > 0.05$).

5.5 Discussion

Belowground C allocation to the rhizosphere influences soil processes including litter decomposition in forest ecosystems. In contrast to our hypothesis, we did not observe a stimulatory effect of assimilate C supply to the rhizosphere on the mass loss of litter in the beech forest. The rate of decomposition of beech litter was similar in soils with or without roots and ECM hyphae. However, we observed that soil CO_2 efflux was greater in the presence of roots and their associated ECM fungi when compared to soils where roots or hyphae was excluded. This indicates that ECM fungi contribute a significant fraction of the total soil respiration.

The dynamics of litter decomposition has been characterised by an initial rapid decomposition, which is followed by slow decomposition (Berg 2014). The sharp loss of litter observed in all treatments shortly after instalments of litter bags is consistent with the dynamics of litter decomposition. During the early stages of decomposition, rapid mass loss occurs due to leaching of soluble organic components, including simple sugars from the litter, which may be accompanied by increases in the concentration of nutrients, particularly N and P (Berg 2014). The rapid loss of litter was followed by mass loss at a reduced rate, when the degradation of more complex compounds such as hemicellulose and lignin that are not easily degraded by microbes occurs (Swift *et al.* 1979).

In contrast to the Priming effect (Kuzyakov *et al.* 2000), the presence of roots and ECM fungi did not accelerate or retard the decomposition of beech litter. In a recent study, Trap et al. (2017) found that the presence of mature beech trees that were heavily colonized by ECM increased litter mass loss. Likewise, in a girdling study, greater mass loss of hemlock needles was observed in girdled plots, relative to non-girdled plots (Subke *et al.* 2011). In contrast to these findings, (Gadgil & Gadgil 1971, 1975) reported that the competition between ECM fungi and other saprotrophs resulted in the suppression of pine needle decomposition in the presence of plants colonized by ECM fungi. Our findings suggest that ECM fungi had no significant influence on the decomposition of leaf litter in this beech forest as saprotrophic fungi, which are not dependent on assimilate C supply for energy dominated the decomposition of leaf litter. In a recent study, Otsing et al. (2018) used molecular and

bioinformatic techniques to assess the microbial community composition of leaf and root litters, and they reported that saprotrophic fungi dominated the decomposition of both litter types. Spatial separation of litter decomposition between saprotrophic and ECM fungi has been reported, whereby saprotrophic fungi dominate the decomposition of fresh surface litter while ECM fungi and bacteria dominate during the latter stages of decomposition in deeper horizons with low quality substrates (Lindahl *et al.* 2007).

Our findings for C and N dynamics during litter decomposition agree with previous reports of C and N dynamics of litter decomposition in temperate and boreal forests (Moore *et al.* 2005, 2011; Preston *et al.* 2009). Loss of C occurred in all treatments after decomposition, although C in the remaining litter after 430 days of incubation did not differ among RMS, MS and S treatments. This is concurrent with the lack of differences in the remaining mass of beech litter among the treatments. N in litter, on the other hand did not differ after mass loss of beech litter. Long-term studies have shown that litter N increases during the early stages of decomposition and N releases occurs only after c. 40% mass loss (Moore *et al.* 2006; Parton *et al.* 2007). Litter quality is an important factor that influences the decomposition rates of litter (Aerts 1997; Cornwell *et al.* 2008; Zhang *et al.* 2008). Litter recalcitrance of *F. sylvatica* might be responsible for the lack of difference in its decomposition rates between treatments. Beech litter are characterised by low N content, high C:N and lignin:N ratios are generally regarded to be of low quality, and decompose at slow rates (Moore *et al.* 2006; Trap *et al.* 2017). Consistent with our findings, the soils with highly colonized ECM plant roots did not affect beech litter decomposition rates compared to plants with roots with low colonization (Trap *et al.* 2017). It is likely that the large fractions of recalcitrant fractions in the litter reduced the investment of resources by microbes on enzyme production required to degrade the energy poor beech litter. Further study is therefore required to investigate the interaction between the quality of litter and the ECM fungi-root associations.

Our study supports others that indicate that ECM fungi contribute a substantial fraction to forest soil respiration (Heinemeyer *et al.* 2007; Vallack *et al.* 2011). Autotrophic respiration (root + ECM fungi) constituted c. 48% of the total soil respiration that is within the range reported by Subke *et al.* (2006). Recently, Subke *et al.* (2018) estimated that ECM fungi contributed about 15% of the soil respiration in a pine forest. The ECM fungi contribution of ~31% highlights the importance of these symbiotic fungi in forest ecosystems. It is therefore important to understand mycorrhizal-C dynamics and their responses to environmental variations. Though the results of this study showed no difference in the rate of litter mass loss

among the treatments, this may not be consistent for SOM decomposition therefore there is a need for further study.

5.6 Conclusion

This study did not provide evidence for the stimulation or suppression of litter decomposition by roots and ECM. However, this study demonstrates that ECM fungi contribute approximately a third of total soil respiration. Changes in plant allocation to ECM fungi due to global changes such as increased temperature and elevated CO_2 will likely influence the cycling of C and nutrients between the soil and atmosphere, which may result in positive feedback to the global change. This study underlines the need for a mechanistic understanding of the role of ECM fungi in the decomposition of both fresh litter and old SOM. This will aid better predictions of the impacts of changing environmental conditions such as climate and land use change on soil C sequestration in temperate forests.

Chapter 6

General Discussion

Atmospheric CO_2 concentrations have risen at an unprecedented rate since the start of the industrial era, mainly from combustion of fossil fuels and land use change, resulting in climate change (Ciais *et al.* 2013). Forests play a major role in climate change by acting as carbon (C) sinks, removing large amount of CO_2 from the atmosphere and storing them in the vegetation and soils, which could potentially mitigate climate change (Pan *et al.* 2011). Carbon enters soils in different forms such as root exudates, microbial and plant residues to form soil organic matter (SOM) through incomplete decomposition (Cotrufo *et al.* 2015). Roots of temperate and boreal forests form symbiotic associations with ectomycorrhizal (ECM) fungi and allocate a significant fraction of their photo-assimilate C belowground to enhance nutrient uptake. Carbon inputs to the rhizosphere may interact with soil organic matter constituents and have significant impacts on forest soil C stocks. It is therefore important to investigate how plant-soil interactions drive SOM decomposition dynamics in forest soils. Here, we present the priming effect of belowground C allocation on the decomposition of different soil organic C constituents. We also examine the effects of fresh C input on microbial activities and SOM dynamics. The interactions between substrate quality and the rhizosphere were also assessed.

6.1 The influence of belowground assimilate C supply on C cycling

It is crucial to understand the extent to which roots mediate decomposition to predict ecosystem responses to global changes. The potential influence of roots on soil C dynamics in the context of rhizosphere priming effects (RPEs) occurs when the presence of roots retards, accelerates or has no effect on the decomposition of SOM (Cheng *et al.* 2014; Huo *et al.* 2017). A recent synthesis of RPE studies found that roots enhanced soil C decomposition by an average of 59% across all studies (Huo *et al.* 2017), with the direction and magnitude of RPE determined by plant variables such as root exudation, root litter input and mycorrhizal associations.

In this thesis, shading and trenching were used to alter the supply of assimilate C to the rhizosphere. This allowed the investigation of the influence of belowground allocation of

photo-assimilate C on the decomposition of native SOM and that of substrates of ecological significance. Shading and trenching led to reductions in soil CO_2 efflux, in line with the results from previous studies (Sayer & Tanner 2010; Díaz-Pinés *et al.* 2010; Comstedt *et al.* 2011; Hasselquist *et al.* 2016; Savage *et al.* 2018). These reductions were caused by altered or decreased rhizosphere respiration rates following shading or trenching, indicating a strong coupling between aboveground assimilation and respiration (Gavrichkova & Kuzyakov 2017). Soil CO_2 efflux was reduced by about 67% in mesocosms following shading (Chapter 2) but was reduced by only 31% by trenching in the field study (Chapter 3). This discrepancy is likely due to size of the mesocosm and differences in the rooting densities. In a trenching study, Yan et al. (2015) observed that autotrophic respiration reduced significantly with stand age along a poplar chronosequence due to decrease in live fine root biomass observed in older stands. Although trenching may result in an underestimation of autotrophic respiration due to the contribution of decaying dead roots in trenched soils, this was likely not the case in the thesis as the additional CO_2 from decaying roots was accounted for using exponential decay constant. On the other hand, roots use their carbohydrate reserves for maintenance respiration after alteration of assimilate C supply, resulting in underestimation of autotrophic respiration (Aubrey & Teskey 2018). As the availability of root reserves is dependent on the age of the plant (Bahn *et al.* 2006), it is likely that the reserves were rapidly exhausted in the young trees (3 years old) used in the mesocosm study but was used to maintain autotrophic respiration in the older beech forest.

The reduced CO_2 efflux observed after shading or trenching indicates that the supply of assimilate C was effectively severed from roots and their associated rhizosphere microorganisms. However, in contrast to our expectation that the rhizosphere C input will significantly promote C mineralization, the studies presented in this thesis found no significant difference in the decomposition of ^{13}C-labelled glucose, straw, fungal necromass or biochar with or without the rhizosphere C supply (Chapter 2 & 3). Furthermore, litter mass loss did not differ among root, mycorrhizal fungi and soil (RMS), mycorrhizal fungi and soil (MS) and soil only (S) treatments (Chapter 5), and no effect of plant C input to the rhizosphere on microbial activities measured as microbial biomass and enzyme production was observed, with the exception of N-acetyl-β-glucosamindase (NAG) activity that was significantly greater in the absence of plant belowground C supply (Chapter 4). Our findings are contrary to the finding of a girdling study (Subke *et al.* 2011), where litter mass loss was higher in control than girdled plots. Although the acceleration of litter decomposition by

rhizosphere C input has been well documented, other studies have also suggested that the presence of roots retard decomposition due to ECM fungi competing with free living saprotrophs for available nutrients (Orwin *et al.* 2011; Averill & Hawkes 2016).

Plants form symbiotic associations with ectomycorrhizal fungi that produce a wide range of extracellular enzymes to acquire nutrients from soils. The absence of the effect of plant C supply on enzyme activities and thus litter and SOM decomposition suggests that roots do not affect soil C dynamics by simply increasing C availability in general, but instead may affect soil C dynamics through their association with mycorrhizal fungi. Many studies that have reported positive rhizosphere priming effects when comparing decomposition in the presence of plants roots and root-free (un-planted) soils (Zhu *et al.* 2014; Shahzad *et al.* 2015; Kumar *et al.* 2016; Yin *et al.* 2018). In this thesis however, our experiments did not exclude ECM fungi, and hence roots and ECM fungi were present in both treatments where decomposition rates were being compared. Our findings are similar to those from a girdling study where severing belowground C allocation did not significantly alter the decomposition of oak litter in ECM fungi dominated soils, whereas decomposition of sugar maple litter was increased in AM dominated soils (Brzostek *et al.* 2015). Although, ECM fungi derive their C primarily from host roots they may also access C to support their metabolism from the decomposition of SOM in a similar manner to free-living saprotrophs (Rineau *et al.* 2012; Phillips *et al.* 2014). This facultative saprotrophism enables ECM fungi to survive in the absence of C supply from host plant by producing extracellular enzymes involved in the acquisition of C (Buée *et al.* 2005, 2007; Courty *et al.* 2007). It is therefore plausible that in the absence of assimilate C supply in shaded or trenched treatments, ECM fungi decomposed the added substrates to meet their C demands ('Plan B' hypothesis, (Talbot *et al.* 2008)). Given that the availability of C may increase due to global change through CO_2 fertilization, our findings reiterate the need to better understand the role of ECM fungi in the storage and turnover of soil C to predict the C sequestration capacity of ECM dominated forests.

Although plant C supply had no significant effect on the decomposition of added ^{13}C labelled substrates, higher decomposition of SOM was observed with or without root inputs (Chapters 2 & 3). This promoting influence of C input from roots on soil CO_2 efflux was likely a result of microbial nutrient mining. Owing to limited availability of nutrients in these forest soils, the addition of C-rich substrates increased microbial activities that further exacerbated nutrient limitation of microbial communities, hence increased decomposition of SOM to mobilize the required nutrients.

The study presented in Chapter 4 shows that SOM decomposition increased significantly in trenched soils amended with glucose in high doses, however the PE was not significant in the un-trenched soils. Furthermore, the activity of NAG was significantly higher in trenched soils than un-trenched soils following the addition of straw. These results may indicate the dominance of free-living saprotrophic fungi in trenched soils, which were activated by the addition of substrates. Plants and microorganisms require nearly the same nutrients for their maintenance, growth and reproduction (Schimel & Weintraub 2003), and plants therefore compete directly with free-living saprotrophs for N through their mycorrhizal partners (Gadgil & Gadgil 1975; Lindahl *et al.* 2010). ECM fungi derive energy from their host plants to produce extracellular enzymes that can depolymerize complex nutrient-rich substrates to meet their nutrient demands and that of their host root. In so doing, they remove nutrients from the system, exacerbating the nutrient limitations of free-living saprotrophs and reducing decomposition rates (Orwin *et al.* 2011; Averill & Finzi 2011; Averill & Hawkes 2016). Severing C supply to ECM fungi through trenching likely reduced the competitive advantage of ECM fungi for nutrients, thereby increasing the dominance of saprophytic fungi and the production of enzymes that degrade SOM to acquire nutrients.

The results of Chapter 4 contrast with those of Chapters 2 & 3 where there were no differences in SOM decomposition in soils with or without root input. These contrasting results may be due to the short experimental duration of Chapter 4 (eight days) versus thirty days in Chapters 2 & 3. In the presence of an intact rhizosphere, the addition of glucose likely stimulated microorganisms to switch from decomposing complex SOM to utilizing the easily available glucose as their C and energy source (preferential substrate utilization, (Kuzyakov and Bol 2006)). In the absence of assimilate C supply on the other hand, the availability of easily available C from added glucose stimulated microbial activities, which in turn increased nutrient demand, hence the release of enzymes that decompose SOM to release the nutrients contained therein (microbial mining; Chen *et al.* 2014)). It is likely that as the easily available C is gradually exhausted, microbial activities will reduce to the baseline level observed in un-amended soils and the decomposition of SOM will reduce as observed in Chapters 2 and 3. In a recent meta-analysis, experimental duration (i.e. days after planting) was found to be the most influential factor affecting the magnitude and direction of RPEs, accounting for about 23% of the variations in RPE studies (Huo *et al.* 2017). They observed that RPEs significantly increased as the experimental duration increased.

6.2 The effect of substrate quality on decomposition

The rate of decomposition is believed to be primarily controlled by climate and quality of the organic substrate (Zhang *et al.* 2008; Prescott 2010). The quality of the substrate can be classified based on their chemical composition and molecular structure that determines its susceptibility to microbial uptake and enzymatic degradation. The N content, C:N ratio and the concentrations of recalcitrant fractions such as lignin, phenolics and tannins can be used as indicators of substrate quality. In this thesis, the added substrates were classified based on their structural complexities into simple and complex substrates, where glucose that is soluble and readily available for microbial utilization is referred to as a simple substrate whereas straw residues, fungal necromass and biochar that are composed of polymerized C molecules that require enzymatic degradation are regarded as complex substrates.

The results of Chapter 2 and 3 showed that glucose was rapidly utilized by soil microbes, as indicated by the increases in $\delta^{13}C$ of soils in the first seven days of glucose addition, after which glucose-derived CO_2 declined (see Figs 2.2A, B & 3.3). It is likely that the rapid respiration of glucose was a result of the activation of fast-growing r-strategists (Fontaine *et al.* 2003). Complex substrates on the other hand, likely stimulated the activities of slow-growing K-strategists that are better adapted to poorly degradable substrates by producing enzymes of higher substrate affinity (Fontaine *et al.* 2003; Blagodatskaya *et al.* 2009). This was reflected in the slower response, but persistent ^{13}C-labelled efflux observed following the addition of straw, fungal necromass and biochar.

The addition of complex substrates – straw, biochar and fungal necromass – into soils of un-shaded trees resulted in significantly stronger PEs over the 30-day sampling period than the addition of glucose in the mesocosm study in Chapter 2. This contrasts with our expectation that structurally simple, labile substrates will induce greater priming of SOM decomposition than structurally complex substrates. As decomposition of complex substrates requires the actions of extracellular enzymes, it is likely that the input of complex substrates stimulated the activities of K-strategist microorganisms (Fontaine *et al.* 2003; Blagodatskaya & Kuzyakov 2008). K-strategists produce enzymes of greater substrate affinity (Blagodatskaya *et al.* 2009; Loeppmann *et al.* 2016), which may lead to co-metabolism of similar compounds contained in SOM. The C acquired by microorganisms from the decomposition of the complex substrates may also be utilized as energy source to mine for nutrients contained in SOM. This was supported by the results in Chapter 4, where the activities of leucine

aminopeptidase (LAP) and NAG that mobilize N increased, along with β-glucosidase (BG) that hydrolyses the glucosidic bonds in cellulose following the addition of straw and fungal necromass (see Fig. 4.4).

The input of glucose in sufficient amounts significantly increased SOM decomposition by 44% in trenched soils and 28% in un-trenched soils after eight days of its addition, however low glucose input or complex substrates had no significant effect on SOM decomposition, after eight days of addition in the mesocosm experiment in Chapter 4. Again, the difference in the duration of the experiments may be responsible for the discrepancy in the effect of substrate quality between these two mesocosm studies. As complex substrates likely shifted the microbial community towards K-strategists, the lack of significant PE with complex substrates addition in the short-term study was likely due to the slow growth rate of microorganisms (Blagodatskaya *et al.* 2009; Chen *et al.* 2014). These differences in experimental durations may also explain the lack of a significant effect of the quality of the added C (using stoichiometric differences or polymeric structure complexity) on PE in a meta-analytical study of 171 experiments (Luo *et al.* 2016).

Similar to the results from Chapter 4, the field trenching study in Chapter 3 showed the promoting effect of glucose input on SOM decomposition, as significantly higher PEs were observed in the presence of glucose compared to fungal necromass and biochar, whereas straw resulted in a similar PE to glucose. However, results from Chapter 2 did not demonstrate this promoting effect of glucose input on SOM decomposition. The difference in the effects of substrate quality on SOM decomposition between the field and mesocosm studies over the 30-d sampling period suggests an interaction between substrate quality and soil characteristics. In addition to the quality and quantity of substrate added, soil properties (such as C and N contents and microbial communities) and plant characteristics influence the magnitude and direction of PE (Chen *et al.* 2014; Wang *et al.* 2015b; Qiao *et al.* 2016; Xu *et al.* 2018). Since N content were the same between the forest soils, the response of SOM decomposition to substrate addition can be attributed to other factors such as SOC content and microbial biomass and community structure.

It is plausible that the differences between the results of the mesocosm study in Chapter 2 where positive PE was not observed in the presence of glucose compared to the field study in Chapter 3, may be attributed to differences in the soil C and N availabilities. Soils with high C:N ratios could provide more C substrates to microorganisms, thereby increasing the

resources available for enzyme production (Manzoni *et al.* 2012; Sinsabaugh *et al.* 2013). Alternatively, microorganisms could preferentially utilize C-rich substrates to alleviate energy deficiency of enzyme production, when C resources are limited. Therefore, it is plausible that the release of assimilate C supply in the un-shaded treatments and the greater SOC content (5.6%) of the mesocosm soils resulted in an abundance of C resources to produce enzymes to degrade complex substrates, which led to the co-metabolism of SOM. Moreover, the high C:N ratios of the added substrates likely exacerbated nutrient limitation of microbes, leading to the mining of nutrient from SOM (Fontaine *et al.* 2011; Chen *et al.* 2014). On the other hand, the addition of glucose into field soils (Chapter 3) may have alleviated C limitation resulting from the low SOC content (4.5%), thereby resulting in the slight increase in SOM decomposition. Our findings are similar to those of Xu et al. (2018) who investigated the influence of initial SOM content on plant litter turnover, and observed faster decomposition of plant litter soils in soils with higher C:N ratios.

The results of Chapter 4 demonstrate that glucose, when available in sufficient amount, increased the decomposition of SOM by stimulating the synthesis of all five measured hydrolytic enzymes (see Fig 4.4). Likewise, straw and fungal necromass significantly increased the activities of all hydrolytic enzymes. However, biochar addition had no significant effect on any of the hydrolytic or oxidative enzymes. Labile C substrates, such as glucose can be utilized for growth and enzyme production by most microorganisms (Schneckenberger *et al.* 2008). Several studies have reported positive, negative or no priming of SOM in the presence of glucose (Blagodatskaya *et al.* 2007, 2009, 2011b; Garcia-Pausas & Paterson 2011). Preferential utilization of the added glucose rather than SOM may result in negative PE when C and nutrients are abundant in soils. In low-nutrient soils, the addition of labile C may increase the release of nutrients from SOM through decomposition (Fontaine *et al.* 2011). Glucose is the sugar most often released in rhizodeposits (Derrien *et al.* 2004), and the decomposition product of most natural polymers in soils (Kuzyakov 2010); this implies that the release of glucose into forest soils either during decomposition or as rhizodeposits may lead to loss of soil C as demonstrated by increased enzyme activities and SOM decomposition in the studies in this thesis.

Despite their polymeric structural complexity, we found in Chapters 2 and 3 that the input of straw and fungal necromass increased SOM decomposition by stimulating synthesis of extracellular enzymes. Straw is mainly composed of cellulose, which is the most common polysaccharide found in soils (Kuzyakov 2010). This is consistent with the other findings that

observed positive PE following the addition of straw residues or cellulose (Blagodatskaya *et al.* 2009, 2014a; Chen *et al.* 2014; Shahbaz, Kuzyakov & Heitkamp 2017a). Liang et al. (2017) reported stronger increases in fungal PLFAs than bacterial PLFAs in soils amended with maize straw, thereby supporting our suggestion that the decomposition of SOM following the addition of complex substrate was mediated by K-strategists. This group of microorganisms have higher substrate affinity thereby their activity remains stable for a longer period of time (Blagodatskaya *et al.* 2009; Loeppmann *et al.* 2016), as indicated by the persistent emission of substrate-derived CO_2 accompanied by SOM decomposition during the 30-day sampling period. In agroecosystems, straw application has been considered to be beneficial as it been found to promote soil C sequestration, increase N content and improve soil physical properties (Liang *et al.* 2018).

Fungal necromass, obtained in Chapter 2 – 4 of this study from dead ECM fungal extraradical mycelium, is a major component of mycorrhizal fungal symbiosis and a large resource of C that enters into soils, thereby contributing to the formation of SOM (Godbold *et al.* 2006; Drigo *et al.* 2012). Despite their ecological importance, the growth and turnover of ECM fungi is poorly quantified. The greater recalcitrance of chitin in fungal necromass compared to cellulose in plant residues has been used as an explanation for the accumulation of microbial residues and its role in the formation of SOM (Godbold *et al.* 2006). However, another study suggested that fungal chitin is not recalcitrant, as they observed higher decomposiiton of fungal chitin than other fungal tissues (Fernandez *et al.* 2016). The stimulation of microbial activities by the input of fungal necromass in Chapter 2 and 4 of this thesis may be attributed to (I) the rapid utilization of the labile fractions of the substrate by free living saprotrophs (Drigo *et al.* 2012) and (II) microbial mining of the N contained within necromass in order to alleviate N limitation. Fungal necromass may therfore act as a resource of both labile and recalcitrant C that promotes the decomposition of old SOM and also contributes to the formation of new SOM (Drigo *et al.* 2012). Further work is therefore needed to determine the C balance between the loss of old SOM and the formation of new SOM following the addition of fungal necromass.

Biochar was found to induce a positive PE despite little/no decomposition of the added biochar (Chapter 2). However, it had no significant effect on any of the hydrolytic or oxidative enzymes measured in Chapter 4 (see Fig. 4.4). Several studies have reported positive or negative PEs in soils amended with biochar (Cross & Sohi 2011; Zimmerman *et al.* 2011; Whitman, Enders & Lehmann 2014; Luo *et al.* 2017a; DeCiucies *et al.* 2018;

Zimmerman & Ouyang 2019). The extremely low decomposition of biochar observed in Chapters 2 – 4 suggests that biochar was not metabolised by microorganisms. This is inconsistent with other studies that suggested that the positive PE of biochar was triggered by labile C fractions on the surface of the biochar, which activated microorganisms and thus led to co-metabolism of SOM (Zimmerman 2010). In a recent study, Zimmerman and Ouyang (2019) attributed the priming of labile organic matter by biochar to the provision of *habitable surfaces* that encouraged microbial growth and activities. There are many different subsrates and production methods such as the pyrolysis temperature and duration used for biochar production, so different biochar will differ greatly in their chemistry. We suggest that due to its high stability, biochar likely promoted SOM decomposition by changing the physio-chemical characteristics of the soil (e.g. porosity, bulk density and pH) (Sohi *et al.* 2010; Luo *et al.* 2017b).

The studies in this body of work used shading (Chapter 2), trenching (Chapters 3 and 4) and mesh exclusion collars (Chapter 5) to exclude supply of C to roots and ECM fungi. The advantage of shading compared to other root exclusion methods such as trenching and collar insertion is that soil disturbance that may alter soil processes is avoided. Although shading offered a less destructive and effective method of excluding root input into the soil in mesocosms of young trees, it might be technically challenging to use shading for mature forests with closed canopy without altering the soil temperature and site climate. Moreover, root input may not be completely eliminated, and the use of stored root C may contribute to root respiration (Kuzyakov 2006). The dead root biomass in trenched/shaded treatments may also be used as substrate by soil heterotrophs thereby resulting in the overestimation of heterotrophic decomposition (Kuzyakov 2006). In our studies, correction for decaying roots that may contribute to heterotrophic decomposition following trenching and mesh collar insertions, was made to avoid overestimation of heterotrophic decomposition when comparing treatments and control soils. The lack of water uptake by roots in treatments compared to controls may also be a source of bias (Subke *et al.* 2006). Soil moisture did not contribute to the bias in these studies as it did not differ between treatments in the shaded, trenching and mesh collar treatments. However, this suggest the estimates of components in the study should be interpreted with caution as it is possible that the contribution of roots was not completely eliminated in the treatments. This might have contributed to the lack of rhizosphere effects on litter or substrate decomposition between treatments in Chapters 2 – 5.

For further investigations, trenching and collar insertions for root exclusion should be done at depths greater than 30 cm, which will totally eliminate root activities in the treatments.

6.3 Conclusion

Our findings have highlighted the link between aboveground assimilation by trees and belowground processes. They demonstrated that the addition of both simple and complex substrates may accelerate the decomposition of SOM by stimulating microbial production of enzymes that are involved in the degradation of the limiting resources. These findings are critical for the prediction of the response of terrestrial C sink, of which forest soils are a major fraction, to global change. Elevated atmospheric CO_2 and its consequent global warming could influence ecosystem processes such as photosynthesis, belowground C allocation, respiration, and nutrient cycling, all of which may affect soil C storage. There is considerable concern that increases in temperature will result in the release of CO_2 from soils, with a positive feedback for global warming (Yu *et al.* 2015; Wang *et al.* 2015b; Chen *et al.* 2015, 2018; Crowther *et al.* 2016). The increases in soil-derived CO_2 following the input of fresh simple or complex substrates especially when combined with assimilate C supply observed in this body of work supports the conclusion that rapid SOM decomposition observed under elevated CO_2 conditions is caused by greater exploration of microorganisms for limiting N with increased availability of C from rhizodeposition and litter input (Zak *et al.* 2011; van Groenigen *et al.* 2014; Vestergård *et al.* 2016a). Forest ecosystems will likely sustain primary productivity under elevated CO_2 conditions by allocating a significant portion of their assimilate C to their associated ECM fungi partners to enhance N acquisition (Norby *et al.* 2010; Drake *et al.* 2011; Phillips *et al.* 2012; Terrer *et al.* 2016). However, our studies found no evidence to support that positive rhizosphere PEs, whereby the presence of roots and their associated ECM fungi accelerates the decomposition of fresh litter or substrates, mainly by releasing labile C such as root exudates to the soil. Instead, our results indicates that the allocation of C by roots to their ECM fungi partners may likely influence SOM decomposition by competing with free-living saprotrophs (Orwin *et al.* 2011; Averill & Hawkes 2016). This suggests that plants do not influence SOM decomposition by merely supplying labile C to the rhizosphere that stimulates microbial activities. Instead there is a complex plant-soil interaction between the mycorrhizal fungi and free-living decomposers that is poorly understood. There is therefore a need for further work to clearly demonstrate

the role of ECM fungi in the turnover and storage of C in forest soils by excluding ECM fungi from areas of soils, in combination with molecular and other modern techniques to measure changes in microbial communities. This should also be coupled with the measurement of enzyme activities to improve our understanding of the particular decomposer communities involved in the turnover of C, and the mechanisms behind these processes.

References

Aerts, R. (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos*, **79**, 439–449.

Allison, S.D., Chacon, S.S. & German, D.P. (2014) Substrate concentration constraints on microbial decomposition. *Soil Biology and Biochemistry*, **79**, 43–49.

Allison, S.D. & Vitousek, P.M. (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry*, **37**, 937–944.

Aubrey, D.P. & Teskey, R.O. (2018) Stored root carbohydrates can maintain root respiration for extended periods. *New Phytologist*, **218**, 142–152.

Averill, C. & Finzi, A. (2011) Plant regulation of microbial enzyme production in situ. *Soil Biology and Biochemistry*, **43**, 2457–2460.

Averill, C. & Hawkes, C. V. (2016) Ectomycorrhizal fungi slow soil carbon cycling. *Ecology letters*, **19**, 937–947.

Averill, C., Turner, B.L. & Finzi, A.C. (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, **505**, 543–545.

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C. & Wall, D.H. (2009) Home-field advantage accelerates leaf litter decomposition in forests. *Soil Biology and Biochemistry*, **41**, 606–610.

Bahn, M., Knapp, M., Garajova, Z., Pfahringer, N. & Cernusca, A. (2006) Root respiration in temperate mountain grasslands differing in land use. *Global Change Biology*, **12**, 995–1006.

Bahn, M., Lattanzi, F.A., Hasibeder, R., Wild, B., Koranda, M., Danese, V., Brüggemann, N., Schmitt, M., Siegwolf, R. & Richter, A. (2013) Responses of belowground carbon allocation dynamics to extended shading in mountain grassland. *New Phytologist*, **198**, 116–126.

Bahn, M., Schmitt, M., Siegwolf, R., Richter, A. & Brüggemann, N. (2009) Does photosynthesis affect grassland soil-respired CO_2 and its carbon isotope composition on

a diurnal timescale? *New Phytologist*, **182**, 451–460.

Baldrian, P. (2006) Fungal laccases-occurrence and properties. *FEMS Microbiology Reviews*, **30**, 215–242.

Ball, B.A., Carrillo, Y. & Molina, M. (2014) The influence of litter composition across the litter-soil interface on mass loss, nitrogen dynamics and the decomposer community. *Soil Biology and Biochemistry*, **69**, 71–82.

Bengtson, P., Barker, J. & Grayston, S.J. (2012) Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. *Ecology and Evolution*, **2**, 1843–1852.

Berg, B. (2014) Decomposition patterns for foliar litter - A theory for influencing factors. *Soil Biology and Biochemistry*, **78**, 222–232.

Bërg, B. & McClaugherty, C. (2008) *Plant Litter: Decomposition, Humus Formation and Carbon Sequestration*, 2nd Ed. Springer-Verlag Berlin Heidelberg, Berlin, Germany.

Blagodatskaya, E. V., Blagodatsky, S.A., Anderson, T.H. & Kuzyakov, Y. (2007) Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied Soil Ecology*, **37**, 95–105.

Blagodatskaya, E. V., Blagodatsky, S. a., Anderson, T.H. & Kuzyakov, Y. (2009) Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil. *European Journal of Soil Science*, **60**, 186–197.

Blagodatskaya, E., Khomyakov, N., Myachina, O., Bogomolova, I., Blagodatsky, S. & Kuzyakov, Y. (2014a) Microbial interactions affect sources of priming induced by cellulose. *Soil Biology and Biochemistry*, **74**, 39–49.

Blagodatskaya, E. & 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*, **45**, 115–131.

Blagodatskaya, E., Littschwager, J., Lauerer, M. & Kuzyakov, Y. (2014b) Plant traits regulating N capture define microbial competition in the rhizosphere. *European Journal of Soil Biology*, **61**, 41–48.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S. & Kuzyakov, Y. (2011a) Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects. *Soil Biology and Biochemistry*, **43**, 778–786.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S. & Kuzyakov, Y. (2011b) Turnover of soil organic matter and of microbial biomass under C_3 - C_4 vegetation change: Consideration of ^{13}C fractionation and preferential substrate utilization. *Soil Biology and Biochemistry*, **43**, 159–166.

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T. & Kuzyakov, Y. (2010) Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition. *Soil Biology and Biochemistry*, **42**, 1275–1283.

Bonan, G.B. (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science*, **320**, 1444–1449.

Bond-Lamberty, B. & Thomson, A. (2010) Temperature-associated increases in the global soil respiration record. *Nature*, **464**, 579–582.

Bradford, M.A., Berg, B., Maynard, D.S., Wieder, W.R. & Wood, S.A. (2016) Understanding the dominant controls on litter decomposition. *Journal of Ecology*, **104**, 229–238.

Bradford, M.A., Veen, G.F., Bonis, A., Bradford, E.M., Classen, A.T., Cornelissen, J.H.C., Crowther, T.W., De Long, J.R., Freschet, G.T., Kardol, P., Manrubia-Freixa, M., Maynard, D.S., Newman, G.S., Logtestijn, R.S.P., Viketoft, M., Wardle, D.A., Wieder, W.R., Wood, S.A. & van der Putten, W.H. (2017) A test of the hierarchical model of litter decomposition. *Nature Ecology & Evolution*, **1**, 1836–1845.

Bray, S.R., Kitajima, K. & Mack, M.C. (2012) Temporal dynamics of microbial communities on decomposing leaf litter of 10 plant species in relation to decomposition rate. *Soil Biology and Biochemistry*, **49**, 30–37.

Brzostek, E.R., Dragoni, D., Brown, Z.A. & Phillips, R.P. (2015) Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. *New Phytologist*, **206**, 1274–1282.

Brzostek, E.R., Greco, A., Drake, J.E. & Finzi, A.C. (2013) Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in

temperate forest soils. *Biogeochemistry*, **115**, 65–76.

Buée, M., Courty, P.E., Mignot, D. & Garbaye, J. (2007) Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. *Soil Biology and Biochemistry*, **39**, 1947–1955.

Buée, M., Maurice, J.P., Zeller, B., Andrianarisoa, S., Ranger, J., Courtecuisse, R., Marçais, B. & Le Tacon, F. (2011) Influence of tree species on richness and diversity of epigeous fungal communities in a French temperate forest stand. *Fungal Ecology*, **4**, 22–31.

Buée, M., Vairelles, D. & Garbaye, J. (2005) Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. *Mycorrhiza*, **15**, 235–245.

Burke, D.J., Smemo, K.A., Hewins, C.R. & Burke, D.J. (2014) Ectomycorrhizal fungi isolated from old-growth northern hardwood forest display variability in extracellular enzyme activity in the presence of plant litter. *Soil Biology and Biochemistry*, **68**, 219–222.

Chen, J., Luo, Y., García-Palacios, P., Cao, J., Dacal, M., Zhou, X., Li, J., Xia, J., Niu, S., Yang, H., Shelton, S., Guo, W. & van Groenigen, K.J. (2018) Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. *Global Change Biology*, **24**, 4816–4826.

Chen, J., Luo, Y., Xia, J., Jiang, L., Zhou, X., Lu, M., Liang, J., Shi, Z., Shelton, S. & Cao, J. (2015) Stronger warming effects on microbial abundances in colder regions. *Scientific reports*, **5**, 18032.

Chen, J., Luo, Y., Xia, J., Wilcox, K.R., Cao, J., Zhou, X., Jiang, L., Niu, S., Estera, K.Y., Huang, R., Wu, F., Hu, T., Liang, J., Shi, Z., Guo, J. & Wang, R.-W. (2017) Warming Effects on Ecosystem Carbon Fluxes Are Modulated by Plant Functional Types. *Ecosystems*, **20**, 515–526.

Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E. & Kuzyakov, Y. (2014) Soil C and N availability determine the priming effect: Microbial N mining and stoichiometric decomposition theories. *Global Change Biology*, **20**, 2356–2367.

Cheng, W. (1999) Rhizosphere feedbacks in elevated CO_2. *Tree Physiology*, **19**, 313–320.

Cheng, H., Hill, P.W., Bastami, M.S. & Jones, D.L. (2017) Biochar stimulates the decomposition of simple organic matter and suppresses the decomposition of complex organic matter in a sandy loam soil. *GCB Bioenergy*, **9**, 1110–1121.

Cheng, W. & Kuzyakov, Y. (2005) Root Effects on Soil Organic Matter Decomposition. *Agronomy*, pp. 119–144.

Cheng, W., Parton, W.J., Gonzalez-Meler, M. a., Phillips, R., Asao, S., Mcnickle, G.G., Brzostek, E. & Jastrow, J.D. (2014) Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist*, **201**, 31–44.

Churchland, C. & Grayston, S.J. (2014) Specificity of plant-microbe interactions in the tree mycorrhizosphere biome and consequences for soil C cycling. *Frontiers in Microbiology*, **5**, 1–20.

Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J. Galloway, M. Heimann, C. Jones, C. Le Quéré, R.B. Myneni & Thornton, P. (2013) *Climate Change 2013: The Physical Science Basis*. Cambridge University Press, New York, USA.

Cleveland, C.C., Reed, S.C., Keller, A.B., Nemergut, D.R., O'Neill, S.P., Ostertag, R. & Vitousek, P.M. (2014) Litter quality versus soil microbial community controls over decomposition: A quantitative analysis. *Oecologia*, **174**, 283–294.

Comstedt, D., Boström, B. & Ekblad, A. (2011) Autotrophic and heterotrophic soil respiration in a Norway spruce forest: estimating the root decomposition and soil moisture effects in a trenching experiment. *Biogeochemistry*, **104**, 121–132.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., Hobbie, S.E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H.M., Santiago, L.S., Wardle, D. a., Wright, I.J., Aerts, R., Allison, S.D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T. V., Díaz, S., Garnier, E., Gurvich, D.E., Kazakou, E., Klein, J.A., Read, J., Reich, P.B., Soudzilovskaia, N. a., Vaieretti, M.V. & Westoby, M. (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters*, **11**, 1065–1071.

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H. & Parton, W.J. (2015) Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience*, **8**, 776–779.

Courty, P.E., Bréda, N. & Garbaye, J. (2007) Relation between oak tree phenology and the secretion of organic matter degrading enzymes by Lactarius quietus ectomycorrhizas before and during bud break. *Soil Biology and Biochemistry*, **39**, 1655–1663.

Courty, P.-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.-P., Uroz, S. & Garbaye, J. (2010) The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry*, **42**, 679–698.

Courty, P.E., Pritsch, K., Schloter, M., Hartmann, A. & Garbaye, J. (2005) Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New Phytologist*, **167**, 309–319.

Craine, J.M., Morrow, C. & Fierer, N. (2007) Microbial nitrogen limitation increases decomposition. *Ecology*, **88**, 2105–2113.

Cross, A. & Sohi, S.P. (2011) The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biology and Biochemistry*, **43**, 2127–2134.

Crowther, T.W., Todd-Brown, K.E.O., Rowe, C.W., Wieder, W.R., Carey, J.C., Machmuller, M.B., Snoek, B.L., Fang, S., Zhou, G., Allison, S.D., Blair, J.M., Bridgham, S.D., Burton, A.J., Carrillo, Y., Reich, P.B., Clark, J.S., Classen, A.T., Dijkstra, F.A., Elberling, B., Emmett, B.A., Estiarte, M., Frey, S.D., Guo, J., Harte, J., Jiang, L., Johnson, B.R., Kröel-Dulay, G., Larsen, K.S., Laudon, H., Lavallee, J.M., Luo, Y., Lupascu, M., Ma, L.N., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L.L., Schmidt, I.K., Sistla, S., Sokol, N.W., Templer, P.H., Treseder, K.K., Welker, J.M. & Bradford, M.A. (2016) Quantifying global soil carbon losses in response to warming. *Nature*, **540**, 104–108.

Cui, J., Ge, T., Kuzyakov, Y., Nie, M., Fang, C., Tang, B. & Zhou, C. (2017) Interactions between biochar and litter priming: A three-source ^{14}C and ^{13}C partitioning study. *Soil*

Biology and Biochemistry, **104**, 49–58.

DeAngelis, K.M., Pold, G., Topçuoğlu, B.D., van Diepen, L.T.A., Varney, R.M., Blanchard, J.L., Melillo, J. & Frey, S.D. (2015) Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in microbiology*, **6**, 104.

DeCiucies, S., Whitman, T., Woolf, D., Enders, A. & Lehmann, J. (2018) Priming mechanisms with additions of pyrogenic organic matter to soil. *Geochimica et Cosmochimica Acta*, **238**, 329–342.

Derrien, D., Marol, C. & Balesdent, J. (2004) The dynamics of neutral sugars in the rhizosphere of wheat. An approach by 13C pulse-labelling and GC/C/IRMS. *Plant and Soil*, **267**, 243–253.

Derrien, D., Plain, C., Courty, P.E., Gelhaye, L., Moerdijk-Poortvliet, T.C.W., Thomas, F., Versini, A., Zeller, B., Koutika, L.S., Boschker, H.T.S. & Epron, D. (2014) Does the addition of labile substrate destabilise old soil organic matter? *Soil Biology and Biochemistry*, **76**, 149–160.

Díaz-Pinés, E., Schindlbacher, A., Pfeffer, M., Jandl, R., Zechmeister-Boltenstern, S. & Rubio, A. (2010) Root trenching: a useful tool to estimate autotrophic soil respiration? A case study in an Austrian mountain forest. *European Journal of Forest Research*, **129**, 101–109.

Dijkstra, F. a., Carrillo, Y., Pendall, E. & Morgan, J. a. (2013) Rhizosphere priming: A nutrient perspective. *Frontiers in Microbiology*, **4**, 1–8.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A. & Kuzyakov, Y. (2009) Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. *Global Change Biology*, **15**, 1603–1614.

Drake, J.E., Gallet-Budynek, A., Hofmockel, K.S., Bernhardt, E.S., Billings, S.A., Jackson, R.B., Johnsen, K.S., Lichter, J., McCarthy, H.R., McCormack, M.L., Moore, D.J.P., Oren, R., Palmroth, S., Phillips, R.P., Pippen, J.S., Pritchard, S.G., Treseder, K.K., Schlesinger, W.H., DeLucia, E.H. & Finzi, A.C. (2011) Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. *Ecology Letters*, **14**, 349–357.

Drigo, B., Anderson, I.C., Kannangara, G.S.K., Cairney, J.W.G. & Johnson, D. (2012) Rapid incorporation of carbon from ectomycorrhizal mycelial necromass into soil fungal communities. *Soil Biology and Biochemistry*, **49**, 4–10.

Ekblad, A. & Högberg, P. (2000) Analysis of $\delta^{13}C$ of CO_2 distinguishes between microbial respiration of added C_4-sucrose and other soil respiration in a C_3-ecosystem. *Plant and Soil*, **219**, 197–209.

Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, D., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Matzner, E., Neumann, J. & Plassard, C. (2013) The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: Role in carbon cycling. *Plant and Soil*, **366**, 1–27.

Ellsworth, D.S., Anderson, I.C., Crous, K.Y., Cooke, J., Drake, J.E., Gherlenda, A.N., Gimeno, T.E., Macdonald, C.A., Medlyn, B.E., Powell, J.R., Tjoelker, M.G. & Reich, P.B. (2017) Elevated CO_2 does not increase eucalypt forest productivity on a low-phosphorus soil. *Nature Climate Change*, **7**, 279–282.

Epron, D., Le Dantec, V., Dufrene, E. & Granier, A. (2001) Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest. *Tree Physiology*, **21**, 145–152.

Epron, D., Farque, L., Lucot, E. & Badot, P.-M. (1999) Soil CO_2 efflux in a beech forest: the contribution of root respiration. *ANNALS OF FOREST SCIENCE*, **56**, 289–295.

Epron, D., Ngao, J., Dannoura, M., Bakker, M.R., Zeller, B., Bazot, S., Bosc, A., Plain, C., Lata, J.C., Priault, P., Barthes, L. & Loustau, D. (2011) Seasonal variations of belowground carbon transfer assessed by in situ $^{13}CO_2$ pulse labelling of trees. *Biogeosciences*, **8**, 1153–1168.

Fang, Y., Nazaries, L., Singh, B.K. & Singh, B.P. (2018) Microbial mechanisms of carbon priming effects revealed during the interaction of crop residue and nutrient inputs in contrasting soils. *Global Change Biology*, **24**, 2775–2790.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. (1989) Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503–537.

Fernandez, C.W., Langley, J.A., Chapman, S., McCormack, M.L. & Koide, R.T. (2016) The decomposition of ectomycorrhizal fungal necromass. *Soil Biology and Biochemistry*, **93**, 38–49.

Finzi, A.C., Abramoff, R.Z., Spiller, K.S., Brzostek, E.R., Darby, B.A., Kramer, M.A. & Phillips, R.P. (2015) Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology*, **21**, 2082–2094.

Fischer, S., Hanf, S., Frosch, T., Gleixner, G., Popp, J., Trumbore, S. & Hartmann, H. (2015) Pinus sylvestris switches respiration substrates under shading but not during drought. *New Phytologist*, **207**, 542–550.

Flannigan, M., Cantin, A.S., de Groot, W.J., Wotton, M., Newbery, A. & Gowman, L.M. (2013) Global wildland fire season severity in the 21st century. *Forest Ecology and Management*, **294**, 54–61.

Fontaine, S. & Barot, S. (2005) Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecology Letters*, **8**, 1075–1087.

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B. & Rumpel, C. (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, **450**, 277–280.

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S. & Maron, P.A. (2011) Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biology and Biochemistry*, **43**, 86–96.

Fontaine, S., Mariotti, A. & Abbadie, L. (2003) The priming effect of organic matter: A question of microbial competition? *Soil Biology and Biochemistry*, **35**, 837–843.

Freschet, G.T., Aerts, R. & Cornelissen, J.H.C. (2012) Multiple mechanisms for trait effects on litter decomposition: Moving beyond home-field advantage with a new hypothesis. *Journal of Ecology*, **100**, 619–630.

Gadgil, R.L. & Gadgil, P.D. (1971) Mycorrhiza and litter decomposition. *Nature*, **233**, 133–133.

Gadgil, R.L. & Gadgil, P.D. (1975) Suppression of litter decomposition by mycorrhizal roots

of *Pinus radiata*. *New Zealand Journal of Forestry Science*, **5**, 33–41.

García-Palacios, P., Shaw, E.A., Wall, D.H. & Hättenschwiler, S. (2016) Temporal dynamics of biotic and abiotic drivers of litter decomposition. *Ecology Letters*, **19**, 554–563.

Garcia-Pausas, J. & 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*, **43**, 1705–1713.

Gavrichkova, O. & Kuzyakov, Y. (2017) The above-belowground coupling of the C cycle: fast and slow mechanisms of C transfer for root and rhizomicrobial respiration. *Plant and Soil*, **410**, 73–85.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E. & Parton, W.J. (2000) Long-term dynamics of pine and hardwood litter in contrasting environments: Toward a global model of decomposition. *Global Change Biology*, **6**, 751–765.

Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I. a., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., De Angelis, P., Miglietta, F. & Peressotti, A. (2006) Mycorrhizal Hyphal Turnover as a Dominant Process for Carbon Input into Soil Organic Matter. *Plant and Soil*, **281**, 15–24.

Gómez-Guerrero, A. & Doane, T. (2018) The Response of Forest Ecosystems to Climate Change. *Developments in Soil Science*, **35**, 185–206.

van Groenigen, K.J., Qi, X., Osenberg, C.W., Luo, Y. & Hungate, B.A. (2014) Faster decomposition under increased atmospheric CO_2 limits soil carbon storage. *Science*, **344**, 508–509.

Hanson, P.J., Edwards, N.T., Garten, C.T. & Andrews, J.A. (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry*, **48**, 115–146.

Hartley, I.P., Heinemeyer, A. & Ineson, P. (2007) Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biology*, **13**, 1761–1770.

Hasibeder, R., Fuchslueger, L., Richter, A. & Bahn, M. (2015) Summer drought alters carbon allocation to roots and root respiration in mountain grassland. *New Phytologist*, **205**,

1117–1127.

Hasselquist, N.J., Metcalfe, D.B., Inselsbacher, E., Stangl, Z., Oren, R., Näsholm, T. & Högberg, P. (2016) Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. *Ecology*, **97**, 1012–1022.

Heinemeyer, A., Hartley, I.P., Evans, S.P., Carreira De La Fuente, J. a. & Ineson, P. (2007) Forest soil CO2 flux: Uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology*, **13**, 1786–1797.

Heinemeyer, A., Wilkinson, M., Vargas, R., Subke, J.-A., Casella, E., Morison, J.I.L. & Ineson, P. (2012) Exploring the overflow tap theory: Linking forest soil CO_2 fluxes and individual mycorrhizosphere components to photosynthesis. *Biogeosciences*, **9**, 79–95.

Heinrich, S., Dippold, M.A., Werner, C., Wiesenberg, G.L.B., Kuzyakov, Y. & Glaser, B. (2015) Allocation of freshly assimilated carbon into primary and secondary metabolites after in situ ^{13}C pulse labelling of Norway spruce (*Picea abies*). *Tree Physiology*, **35**, 1176–1191.

Hernández, D.L. & Hobbie, S.E. (2010) The effects of substrate composition, quantity, and diversity on microbial activity. *Plant and Soil*, **335**, 397–411.

Hobbie, S.E. (1996) Temperature and Plant Species Control Over Litter Decomposition in Alaskan Tundra. *Ecological Monographs*, **66**, 503–522.

Hobbie, E. (2006) Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*, **87**, 563–569.

Hobbie, S.E. (2015) Plant species effects on nutrient cycling: revisiting litter feedbacks. *Trends in Ecology & Evolution*, **30**, 357–363.

Högberg, M.N. & Högberg, P. (2002) Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, **154**, 791–795.

Högberg, P., Högberg, M.N., Gottlicher, S.., Betson, N.., Keel, S.G., Metcalfe, D.., Campbell, C., Schindlbacher, A., Hurry, V., Lundmark, T., Linder, S. & Nasholm, T. (2008) High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. *New Phytologist*, **177**, 220–228.

Högberg, M.N., Högberg, P. & Myrold, D.D. (2006) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia*, **150**, 590–601.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Löfvenius, M.O. & Read, D.J. (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**, 789–792.

Hopkins, F., Gonzalez-meler, M.A., Flower, C.E., Lynch, D.J., Czimczik, C., Tang, J. & Subke, J. (2013) Ecosystem-level controls on root-rhizosphere respiration. *New Phytologist*, **199**, 339–351.

Hoppe, H.-G. (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Marine Ecology Progress Series*, **11**, 299–308.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Huo, C., Luo, Y. & Cheng, W. (2017) Rhizosphere priming effect: A meta-analysis. *Soil Biology and Biochemistry*, **111**, 78–84.

Jackson, O., Quilliam, R.S., Stott, A., Grant, H. & Subke, J.-A. (2019) Rhizosphere carbon supply accelerates soil organic matter decomposition in the presence of fresh organic substrates. *Plant and Soil*, 1–18.

Janssens, I.A., Lankreijer, H., Matteucci, G., Kowalski, A.S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grünwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, Ü., Morgenstern, K., Oltchev, S., Clement, R., Guomundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R. & Valentini, R. (2001) Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biology*, **7**, 269–278.

Jian, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D. & Luo, Y. (2016) Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biology and Biochemistry*, **101**, 32–43.

Joergensen, R.G. (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EC} value. *Soil Biology and Biochemistry*, **28**, 25–31.

Joergensen, R.G. & Wichern, F. (2018) Alive and kicking: Why dormant soil microorganisms matter. *Soil Biology and Biochemistry*, **116**, 419–430.

Joly, F.-X., Milcu, A., Scherer-Lorenzen, M., Jean, L.-K., Bussotti, F., Dawud, S.M., Müller, S., Pollastrini, M., Raulund-Rasmussen, K., Vesterdal, L. & Hättenschwiler, S. (2017) Tree species diversity affects decomposition through modified micro-environmental conditions across European forests. *New Phytologist*, **214**, 1281–1293.

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

Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A. & Richter, A. (2010) Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. *New Phytologist*, **187**, 843–58.

Keenan, R.J., Reams, G.A., Achard, F., de Freitas, J. V., Grainger, A. & Lindquist, E. (2015) Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. *Forest Ecology and Management*, **352**, 9–20.

Keiser, A.D., Keiser, D.A., Strickland, M.S. & Bradford, M.A. (2014) Disentangling the mechanisms underlying functional differences among decomposer communities. *Journal of Ecology*, **102**, 603–609.

Keiser, A.D., Strickland, M.S., Fierer, N. & Bradford, M.A. (2011) The effect of resource history on the functioning of soil microbial communities is maintained across time. *Biogeosciences*, **8**, 1477–1486.

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

Kumar, A., Kuzyakov, Y. & Pausch, J. (2016) Maize rhizosphere priming: field estimates using ^{13}C natural abundance. *Plant and Soil*, **409**, 87–97.

Kuzyakov, Y. (2002) Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science*, **165**, 382–396.

Kuzyakov, Y. (2006) Sources of CO_2 efflux from soil and review of partitioning methods. *Soil Biology and Biochemistry*, **38**, 425–448.

Kuzyakov, Y. (2010) Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry*, **42**, 1363–1371.

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

Kuzyakov, Y. & Bol, R. (2004) Using natural ^{13}C abundances to differentiate between three CO_2 sources during incubation of a grassland soil amended with slurry and sugar. *Journal of Plant Nutrition and Soil Science*, **167**, 669–677.

Kuzyakov, Y. & Bol, R. (2006) Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biology and Biochemistry*, **38**, 747–758.

Kuzyakov, Y., Friedel, J.. & Stahra, K. (2000) Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry*, **32**, 1485–1498.

Kuzyakov, Y., Horwath, W.R., Dorodnikov, M. & Blagodatskaya, E. (2019) Review and synthesis of the effects of elevated atmospheric CO_2 on soil processes: No changes in pools, but increased fluxes and accelerated cycles. *Soil Biology and Biochemistry*, **128**, 66–78.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I. & Xu, X. (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. *Soil Biology and Biochemistry*, **41**, 210–219.

Lal, R. (2004) Soil carbon sequestration to mitigate climate change. *Geoderma*, **123**, 1–22.

Lang, C., Seven, J. & Polle, A. (2011) Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. *Mycorrhiza*, **21**, 297–308.

Liang, G., Wu, H., Houssou, A.A., Cai, D., Wu, X., Gao, L., Wang, B. & Li, S. (2018) Soil respiration, glomalin content, and enzymatic activity response to straw application in a wheat-maize rotation system. *Journal of Soils and Sediments*, **18**, 697–707.

Liang, X., Yuan, J., Yang, E. & Meng, J. (2017) Responses of soil organic carbon decomposition and microbial community to the addition of plant residues with different C:N ratio. *European Journal of Soil Biology*, **82**, 50–55.

Lindahl, B.D., De Boer, W. & Finlay, R.D. (2010) Disruption of root carbon transport into forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi. *ISME Journal*, **4**, 872–881.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J. & Finlay, R.D. (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist*, **173**, 611–620.

Lindahl, B.D. & Tunlid, A. (2015) Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist*, **205**, 1443–1447.

Liu, S., Ji, C., Wang, C., Chen, J., Jin, Y., Zou, Z., Li, S., Niu, S. & Zou, J. (2018) Climatic role of terrestrial ecosystem under elevated CO_2: a bottom-up greenhouse gases budget (ed J Knops). *Ecology Letters*, **21**, 1108–1118.

Liu, X.-J.A., Sun, J., Mau, R.L., Finley, B.K., Compson, Z.G., van Gestel, N., Brown, J.R., Schwartz, E., Dijkstra, P. & Hungate, B.A. (2017) Labile carbon input determines the direction and magnitude of the priming effect. *Applied Soil Ecology*, **109**, 7–13.

Loeppmann, S., Blagodatskaya, E., Pausch, J. & Kuzyakov, Y. (2016) Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere. *Soil Biology and Biochemistry*, **92**, 111–118.

Di Lonardo, D.P., De Boer, W., Klein Gunnewiek, P.J.A., Hannula, S.E. & Van der Wal, A. (2017) Priming of soil organic matter: Chemical structure of added compounds is more important than the energy content. *Soil Biology and Biochemistry*, **108**, 41–54.

Luo, Y., Lin, Q., Durenkamp, M., Dungait, A.J. & Brookes, P.C. (2017a) Soil priming effects following substrates addition to biochar-treated soils after 431 days of pre-incubation. *Biology and Fertility of Soils*, **53**, 315–326.

Luo, Z., Wang, E. & Sun, O.J. (2016) A meta-analysis of the temporal dynamics of priming soil carbon decomposition by fresh carbon inputs across ecosystems. *Soil Biology and Biochemistry*, **101**, 96–103.

Luo, Y., Zang, H., Yu, Z., Chen, Z., Gunina, A., Kuzyakov, Y., Xu, J., Zhang, K. & Brookes, P.C. (2017b) Priming effects in biochar enriched soils using a three-source-partitioning approach: ^{14}C labelling and ^{13}C natural abundance. *Soil Biology and Biochemistry*, **106**, 28–35.

Maestrini, B., Nannipieri, P. & Abiven, S. (2015) A meta-analysis on pyrogenic organic matter induced priming effect. *GCB Bioenergy*, **7**, 577–590.

Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Ågren, G.I. (2012) Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist*, **196**, 79–91.

Mao, J., Ricciuto, D.M., Thornton, P.E., Warren, J.M., King, A.W., Shi, X., Iversen, C.M. & Norby, R.J. (2016) Evaluating the Community Land Model in a pine stand with shading manipulations and $^{13}CO_2$ labeling. *Biogeosciences*, **13**, 641–657.

Marx, D. (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*, **59**, 153–163.

Matteucci, M., Gruening, C., Ballarin, I.G., Seufert, G. & Cescatti, A. (2015) Components, drivers and temporal dynamics of ecosystem respiration in a Mediterranean pine forest. *Soil Biology and Biochemistry*, **88**, 224–235.

Meentemeyer, V. & Spring, N.L. (1978) Macroclimate the Lignin Control of Litter Decomposition Rates. *Ecology*, **59**, 465–472.

Meier, I.C., Pritchard, S.G., Brzostek, E.R., Mccormack, M.L. & Phillips, R.P. (2015) The rhizosphere and hyphosphere differ in their impacts on carbon and nitrogen cycling in forests exposed to elevated CO_2. *New Phytologist*, **205**, 1164–1174.

Metcalfe, D.B., Williams, M., Aragão, L.E.O.C., da Costa, A.C.L., de Almeida, S.S., Braga, A.P., Gonçalves, P.H.L., de Athaydes, J., Junior, S., Malhi, Y. & Meir, P. (2007) A method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy. *New Phytologist*, **174**, 697–703.

Moinet, G.Y.K., Cieraad, E., Hunt, J.E., Fraser, A., Turnbull, M.H. & Whitehead, D. (2016) Soil heterotrophic respiration is insensitive to changes in soil water content but related to

microbial access to organic matter. *Geoderma*, **274**, 68–78.

Moinet, G.Y.K., Hunt, J.E., Kirschbaum, M.U.F., Morcom, C.P., Midwood, A.J. & Millard, P. (2018) The temperature sensitivity of soil organic matter decomposition is constrained by microbial access to substrates. *Soil Biology and Biochemistry*, **116**, 333–339.

Moore, J.A.M., Jiang, J., Patterson, C.M., Mayes, M.A., Wang, G. & Classen, A.T. (2015a) Interactions among roots, mycorrhizas and free-living microbial communities differentially impact soil carbon processes. *Journal of Ecology*, **103**, 1442–1453.

Moore, J.A., Jiang, J., Post, W.M. & Classen, A.T. (2015b) Decomposition by ectomycorrhizal fungi alters soil carbon storage in a simulation model. *Ecosphere*, **6**, 1–16.

Moore, T.R., Trofymow, J.A., Prescott, C.E., Fyles, J., Titus, B.D. & Cidet Working Group. (2006) Patterns of Carbon , Nitrogen and Phosphorus Dynamics in Decomposing Foliar Litter in Canadian Forests. *Ecosystems*, **9**, 46–62.

Moore, T.R., Trofymow, J.A., Prescott, C.E. & Titus, B.D. (2011) Nature and nurture in the dynamics of C, N and P during litter decomposition in Canadian forests. *Plant and Soil*, **339**, 163–175.

Moore, T.R., Trofymow, J.A., Siltanen, M., Prescott, C. & CIDET Working Group. (2005) Patterns of decomposition and carbon, nitrogen, and phosphorus dynamics of litter in upland forest and peatland sites in central Canada. *Canadian Journal of Forest Research*, **35**, 133–142.

Moorhead, D.L., Currie, W.S., Rastetter, E.B., Parton, W.J. & Harmon, M.E. (1999) Climate and litter quality controls on decomposition: An analysis of modeling approaches. *Global Biogeochemical Cycles*, **13**, 575–589.

Moorhead, D.L. & Sinsabaugh, R.L. (2006) A theoretical model of litter decay and microbial interaction. *Ecological Monographs*, **76**, 151–174.

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S. & Richter, A. (2014) Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources.

Frontiers in Microbiology, **5**, 22.

Moyano, F.E., Vasilyeva, N., Bouckaert, L., Cook, F., Craine, J., Curiel Yuste, J., Don, A., Epron, D., Formanek, P., Franzluebbers, A., Ilstedt, U., Kätterer, T., Orchard, V., Reichstein, M., Rey, A., Ruamps, L., Subke, J.-A., Thomsen, I.K. & Chenu, C. (2012) The moisture response of soil heterotrophic respiration: interaction with soil properties. *Biogeosciences*, **9**, 1173–1182.

Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P. & Paterson, E. (2017) Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. *Plant and Soil*, **417**, 499–510.

Mwafulirwa, L.D., Baggs, E.M., Russell, J., Morley, N., Sim, A. & Paterson, E. (2017) Combined effects of rhizodeposit C and crop residues on SOM priming, residue mineralization and N supply in soil. *Soil Biology and Biochemistry*, **113**, 35–44.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G. & Renella, G. (2003) Microbial diversity and soil functions. *European Journal of Soil Science*, **54**, 655–670.

Ngao, J., Longdoz, B., Granier, A. & Epron, D. (2007) Estimation of autotrophic and heterotrophic components of soil respiration by trenching is sensitive to corrections for root decomposition and changes in soil water content. *Plant and Soil*, **301**, 99–110.

De Nobili, M., Contin, M., Mondini, C. & Brookes, P.C. (2001) Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biology and Biochemistry*, **33**, 1163–1170.

Norby, R.J., Delucia, E.H., Gielen, B., Calfapietra, C., Giardina, C.P., King, J.S., Ledford, J., McCarthy, H.R., Moore, D.J.P., Ceulemans, R., De Angelis, P., Finzi, A.C., Karnosky, D.F., Kubiske, M.E., Lukac, M., Pregitzer, K.S., Scarascia-Mugnozza, G.E., Schlesinger, W.H. & Oren, R. (2005) Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 18052–18056.

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E. & McMurtrie, R.E. (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences*, **107**, 19368–19373.

Nottingham, A.T., Griffiths, H., Chamberlain, P.M., Stott, A.W. & Tanner, E.V.J. (2009) Soil priming by sugar and leaf-litter substrates: A link to microbial groups. *Applied Soil Ecology*, **42**, 183–190.

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

Orwin, K.H., Kirschbaum, M.U.F., St John, M.G. & Dickie, I.A. (2011) Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecology Letters*, **14**, 493–502.

Otsing, E., Barantal, S., Anslan, S., Koricheva, J. & Tedersoo, L. (2018) Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter. *Soil Biology and Biochemistry*, **125**, 328–339.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S. & Hayes, D. (2011) A large and persistent carbon sink in the world's forests. *Science*, **333**, 988–93.

Parker, T.C., Sadowsky, J., Dunleavy, H., Subke, J.A., Frey, S.D. & Wookey, P.A. (2017) Slowed Biogeochemical Cycling in Sub-arctic Birch Forest Linked to Reduced Mycorrhizal Growth and Community Change after a Defoliation Event. *Ecosystems*, **20**, 316–330.

Parker, T.C., Sanderman, J., Holden, R.D., Blume-Werry, G., Sjögersten, S., Large, D., Castro-Díaz, M., Street, L.E., Subke, J.-A. & Wookey, P.A. (2018) Exploring drivers of litter decomposition in a greening Arctic: results from a transplant experiment across a treeline. *Ecology*, **99**, 2284–2294.

Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C. & Fasth, B. (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science*, **315**, 361–4.

Paterson, E., Midwood, A.J. & Millard, P. (2009) Through the eye of the needle: A review of isotope approaches to quantify microbial processes mediating soil carbon balance. *New Phytologist*, **184**, 19–33.

Paterson, E. & Sim, A. (2013) Soil-specific response functions of organic matter mineralization to the availability of labile carbon. *Global Change Biology*, **19**, 1562–1571.

Pausch, J. & Kuzyakov, Y. (2018) Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. *Global Change Biology*, **24**, 1–12.

Pausch, J., Zhu, B., Kuzyakov, Y. & Cheng, W. (2013) Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biology and Biochemistry*, **57**, 91–99.

Peng, F., You, Q.G., Xu, M.H., Zhou, X.H., Wang, T., Guo, J. & Xue, X. (2015) Effects of experimental warming on soil respiration and its components in an alpine meadow in the permafrost region of the Qinghai-Tibet Plateau. *European Journal of Soil Science*, **66**, 145–154.

Phillips, R.P. & Fahey, T.J. (2006) Tree Species and Mycorrhizal Associations Influence the Magnitude of Rhizosphere Effects. *Ecology*, **87**, 1302–1313.

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

Phillips, D.L. & Gregg, J.W. (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**, 171–179.

Phillips, R.P., Meier, I.C., Bernhardt, E.S., Grandy, a S., Wickings, K. & Finzi, A.C. (2012) Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. *Ecology letters*, **15**, 1042–9.

Phillips, L. a, Ward, V. & Jones, M.D. (2014) Ectomycorrhizal fungi contribute to soil organic matter cycling in sub-boreal forests. *The ISME journal*, **8**, 699–713.

Piao, S., Sitch, S., Ciais, P., Friedlingstein, P., Peylin, P., Wang, X., Ahlström, A., Anav, A., Canadell, J.G., Cong, N., Huntingford, C., Jung, M., Levis, S., Levy, P.E., Li, J., Lin, X., Lomas, M.R., Lu, M., Luo, Y., Ma, Y., Myneni, R.B., Poulter, B., Sun, Z., Wang, T., Viovy, N., Zaehle, S. & Zeng, N. (2013) Evaluation of terrestrial carbon cycle models for their response to climate variability and to CO_2 trends. *Global Change Biology*, **19**,

2117–2132.

Pinheiro, J.C. & Bates, D.M. (2000) *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag New York, Inc.

Pold, G., Billings, A.F., Blanchard, J.L., Burkhardt, D.B., Frey, S.D., Melillo, J.M., Schnabel, J., Van Diepen, L.T.A. & Deangelis, K.M. (2016) Long-Term Warming Alters Carbohydrate Degradation Potential in Temperate Forest Soils.

Prescott, C.E. (2010) Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry*, **101**, 133–149.

Prescott, C.E. & Grayston, S.J. (2013) Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. *Forest Ecology and Management*, **309**, 19–27.

Prescott, C.E., Zabek, L.M., Staley, C.L. & Kabzems, R. (2000) Decomposition of broadleaf and needle litter in forests of British Columbia: influences of litter type, forest type, and litter mixtures. *Canadian Journal of Forest Research*, **30**, 1742–1750.

Preston, C.M., Nault, J.R., Trofymow, J.A., Smyth, C. & Group, C.W. (2009) Chemical Changes During 6 Years of Decomposition of 11 Litters in Some Canadian Forest Sites. Part 1. Elemental Composition, Tannins, Phenolics, and Proximate Fractions. *Ecosystems*, **12**, 1053–1077.

Pritsch, K., Courty, P.E., Churin, J.-L., Cloutier-Hurteau, B., Ali, M.A., Damon, C., Duchemin, M., Egli, S., Ernst, J., Fraissinet-Tachet, L., Kuhar, F., Legname, E., Marmeisse, R., Müller, A., Nikolova, P., Peter, M., Plassard, C., Richard, F., Schloter, M., Selosse, M.-A., Franc, A. & Garbaye, J. (2011) Optimized assay and storage conditions for enzyme activity profiling of ectomycorrhizae. *Mycorrhiza*, **21**, 589–600.

Qiao, N., Schaefer, D., Blagodatskaya, E., Zou, X., Xu, X. & Kuzyakov, Y. (2014) Labile carbon retention compensates for CO_2 released by priming in forest soils. *Global Change Biology*, **20**, 1943–1954.

Qiao, N., Xu, X., Hu, Y., Blagodatskaya, E., Liu, Y., Schaefer, D. & Kuzyakov, Y. (2016) Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum. *Scientific Reports*, **6**, 19865.

Le Quéré, C., Moriarty, R., Andrew, R.M., Peters, G.P., Ciais, P., Friedlingstein, P., Jones, S.D., Sitch, S., Tans, P., Arneth, A., Boden, T.A., Bopp, L., Bozec, Y., Canadell, J.G., Chini, L.P., Chevallier, F., Cosca, C.E., Harris, I., Hoppema, M., Houghton, R.A., House, J.I., Jain, A.K., Johannessen, T., Kato, E., Keeling, R.F., Kitidis, V., Klein Goldewijk, K., Koven, C., Landa, C.S., Landschützer, P., Lenton, A., Lima, I.D., Marland, G., Mathis, J.T., Metzl, N., Nojiri, Y., Olsen, A., Ono, T., Peng, S., Peters, W., Pfeil, B., Poulter, B., Raupach, M.R., Regnier, P., Rödenbeck, C., Saito, S., Salisbury, J.E., Schuster, U., Schwinger, J., Séférian, R., Segschneider, J., Steinhoff, T., Stocker, B.D., Sutton, A.J., Takahashi, T., Tilbrook, B., van der Werf, G.R., Viovy, N., Wang, Y.-P., Wanninkhof, R., Wiltshire, A. & Zeng, N. (2015) Global carbon budget 2014. *Earth System Science Data*, **7**, 47–85.

Quilliam, R.S., Marsden, K.A., Gertler, C., Rousk, J., DeLuca, T.H. & Jones, D.L. (2012) Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. *Agriculture, Ecosystems & Environment*, **158**, 192–199.

R Core Team. (2017) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ramirez, K.S., Craine, J.M. & Fierer, N. (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, **18**, 1918–1927.

Read, D.J. & Perez-Moreno, J. (2003) Mycorrhizas and Nutrient Cycling in Ecosystems: Journey Towards Relevance. *New Phytologist*, **157**, 475–492.

Rineau, F., Roth, D., Shah, F., Smits, M., Johansson, T., Canbäck, B., Olsen, P.B., Persson, P., Grell, M.N., Lindquist, E., Grigoriev, I. V., Lange, L. & Tunlid, A. (2012) The ectomycorrhizal fungus Paxillus involutus converts organic matter in plant litter using a trimmed brown-rot mechanism involving Fenton chemistry. *Environmental Microbiology*, **14**, 1477–1487.

Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, K.H., Kuyper, T.W., Taylor, A.F.S. & Finlay, R.D. (2003) Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytologist*, **159**, 775–783.

Saiya-Cork, K.., Sinsabaugh, R.. & Zak, D.. (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biology and Biochemistry*, **34**, 1309–1315.

Savage, K.E., Davidson, E.A., Abramoff, R.Z., Finzi, A.C. & Giasson, M.A. (2018) Partitioning soil respiration: quantifying the artifacts of the trenching method. *Biogeochemistry*, 1–11.

Sayer, E.J. & Tanner, E.V.J. (2010) A new approach to trenching experiments for measuring root-rhizosphere respiration in a lowland tropical forest. *Soil Biology and Biochemistry*, **42**, 347–352.

Schimel, J.P. & Weintraub, M.N. (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model. *Soil Biology and Biochemistry*, **35**, 549–563.

Schlesinger, W.H. & Bernhardt, E.S. (2013) Introduction. *Biogeochemistry*, 3rd ed, pp. 3–14. Elsevier.

Schlesinger, W.H., Bernhardt, E.S., Schlesinger, W.H. & Bernhardt, E.S. (2013) The Biosphere: The Carbon Cycle of Terrestrial Ecosystems. *Biogeochemistry*, 135–172.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S. & Trumbore, S.E. (2011) Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49–56.

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

Schneckenberger, K., Demin, D., Stahr, K. & Kuzyakov, Y. (2008) Microbial utilization and mineralization of [14C]glucose added in six orders of concentration to soil. *Soil Biology and Biochemistry*, **40**, 1981–1988.

Shahbaz, M., Kuzyakov, Y. & Heitkamp, F. (2017a) Decrease of soil organic matter stabilization with increasing inputs: Mechanisms and controls. *Geoderma*, **304**, 76–82.

Shahbaz, M., Kuzyakov, Y., Sanaullah, M., Heitkamp, F., Zelenev, V., Kumar, A. &

Blagodatskaya, E. (2017b) Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds. *Biology and Fertility of Soils*, **53**, 287–301.

Shahzad, T., Chenu, C., Genet, P., Barot, S., Perveen, N., Mougin, C. & 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*, **80**, 146–155.

Simpson, M.J. & Simpson, A.J. (2012) The Chemical Ecology of Soil Organic Matter Molecular Constituents. *Journal of Chemical Ecology*, **38**, 768–784.

Sinsabaugh, R.L. (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry*, **42**, 391–404.

Sinsabaugh, R.L., Hill, B.H. & Follstad Shah, J.J. (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature*, **462**, 795–798.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R. & Zeglin, L.H. (2008) Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, **11**, 1252–1264.

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L. & Richter, A. (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecology Letters*, **16**, 930–939.

Sinsabaugh, R.L. & Shah, J.J.F. (2011) Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: The growth rate hypothesis in reverse. *Biogeochemistry*, **102**, 31–43.

Six, J., Paustian, K., Elliott, E.T. & Combrink, C. (2000) Soil Structure and Organic Matter. *Soil Science Society of America Journal*, **64**, 681.

Smith, S.E. & Read, D.J. (2002) Structure and development of ectomycorrhizal roots. *Mycorrhizal Symbiosis*, Third, p. 163–V. Elsevier.

Sohi, S.P., Krull, E., Lopez-Capel, E. & Bol, R. (2010) *A Review of Biochar and Its Use and*

Function in Soil, 1st ed. Elsevier Inc.

Soil Survey of Scotland Staff. (1981) Soil maps of Scotland at a scale of 1:250 000.

Stewart, C.E., Zheng, J., Botte, J. & Cotrufo, M.F. (2013) Co-generated fast pyrolysis biochar mitigates green-house gas emissions and increases carbon sequestration in temperate soils. *GCB Bioenergy*, **5**, 153–164.

Stone, M.M., DeForest, J.L. & Plante, A.F. (2014) Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biology and Biochemistry*, **75**, 237–247.

Strickland, M.S., Lauber, C., Fierer, N. & Bradford, M.A. (2009a) Testing the functional significance of microbial community composition. *Ecology*, **90**, 441–451.

Strickland, M.S., Osburn, E., Lauber, C., Fierer, N. & Bradford, M.A. (2009b) Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. *Functional Ecology*, **23**, 627–636.

Subke, J.-A., Hahn, V., Battipaglia, G., Linder, S., Buchmann, N. & Cotrufo, M.F. (2004) Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia*, **139**, 551–559.

Subke, J.A., Heinemeyer, A., Vallack, H.W., Leronni, V., Baxter, R. & Ineson, P. (2012) Fast assimilate turnover revealed by in situ $^{13}CO_2$ pulse-labelling in Subarctic tundra. *Polar Biology*, **35**, 1209–1219.

Subke, J.A., Inglima, I. & Cotrufo, M.F. (2006) Trends and methodological impacts in soil CO_2 efflux partitioning: A metaanalytical review. *Global Change Biology*, **12**, 921–943.

Subke, J.A., Moody, C.S., Hill, T.C., Voke, N., Toet, S., Ineson, P. & Teh, Y.A. (2018) Rhizosphere activity and atmospheric methane concentrations drive variations of methane fluxes in a temperate forest soil. *Soil Biology and Biochemistry*, **116**, 323–332.

Subke, J.A., Voke, N.R., Leronni, V., Garnett, M.H. & Ineson, P. (2011) Dynamics and pathways of autotrophic and heterotrophic soil CO_2 efflux revealed by forest girdling. *Journal of Ecology*, **99**, 186–193.

Sulman, B.N., Brzostek, E.R., Medici, C., Shevliakova, E., Menge, D.N.L. & Phillips, R.P.

(2017) Feedbacks between plant N demand and rhizosphere priming depend on type of mycorrhizal association (ed E Cleland). *Ecology Letters*, **20**, 1043–1053.

Sun, T., Hobbie, S.E., Berg, B., Zhang, H., Wang, Q., Wang, Z. & Hättenschwiler, S. (2018) Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proceedings of the National Academy of Sciences*, **115**, 10392–10397.

Sun, J., Xia, Z., He, T., Dai, W., Peng, B., Liu, J., Gao, D., Jiang, P., Han, S. & Bai, E. (2017) Ten years of elevated CO_2 affects soil greenhouse gas fluxes in an open top chamber experiment. *Plant and Soil*, **420**, 435–450.

Swift, M.., Heal, O.. & Anderson, J.. (1979) *Decomposition in Terrestrial Ecosystems*. Blackwell Science, Publications, London.

Talbot, J.M., Allison, S.D. & Treseder, K.K. (2008) Decomposers in disguise: Mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology*, **22**, 955–963.

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R. & Peay, K.G. (2013) Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry*, **57**, 282–291.

Talbot, J.M., Martin, F., Kohler, A., Henrissat, B. & Peay, K.G. (2015) Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry. *Soil Biology and Biochemistry*, **88**, 441–456.

Talbot, J.M. & Treseder, K.K. (2012) Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships. *Ecology*, **93**, 345–354.

Terrer, C., Vicca, S., Hungate, B.A., Phillips, R.P. & Prentice, I.C. (2016) Mycorrhizal association as a primary control of the CO_2 fertilization effect. *Science*, **353**, 72–74.

Terrer, C., Vicca, S., Stocker, B.D., Hungate, B.A., Phillips, R.P., Reich, P.B., Finzi, A.C. & Prentice, I.C. (2018) Ecosystem responses to elevated CO_2 governed by plant-soil interactions and the cost of nitrogen acquisition. *New Phytologist*, **217**, 507–522.

Tian, H., Lu, C., Ciais, P., Michalak, A.M., Canadell, J.G., Saikawa, E., Huntzinger, D.N., Gurney, K.R., Sitch, S., Zhang, B., Yang, J., Bousquet, P., Bruhwiler, L., Chen, G.,

Dlugokencky, E., Friedlingstein, P., Melillo, J., Pan, S., Poulter, B., Prinn, R., Saunois, M., Schwalm, C.R. & Wofsy, S.C. (2016) The terrestrial biosphere as a net source of greenhouse gases to the atmosphere. *Nature*, **531**, 225–228.

Tian, J., Pausch, J., Yu, G., Blagodatskaya, E., Gao, Y. & Kuzyakov, Y. (2015) Aggregate size and their disruption affect ^{14}C-labeled glucose mineralization and priming effect. *Applied Soil Ecology*, **90**, 1–10.

Tian, L. & Shi, W. (2014) Short-term effects of plant litter on the dynamics, amount, and stoichiometry of soil enzyme activity in agroecosystems. *European Journal of Soil Biology*, **65**, 23–29.

Trap, J., Akpa-Vinceslas, M., Margerie, P., Boudsocq, S., Richard, F., Decaëns, T. & Aubert, M. (2017) Slow decomposition of leaf litter from mature *Fagus sylvatica* trees promotes offspring nitrogen acquisition by interacting with ectomycorrhizal fungi. *Journal of Ecology*, **105**, 528–539.

UK Met Office. (2017) No Title. URL https://www.metoffice.gov.uk/public/weather/climate/gcvpnrf34 [accessed 1 February 2017]

Vallack, H.W., Leronni, V., Metcalfe, D.B., Högberg, P., Ineson, P. & Subke, J.-A. (2011) Application of nitrogen fertilizer to a boreal pine forest has a negative impact on the respiration of ectomycorrhizal hyphae. *Plant and Soil*, **352**, 405–417.

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

Vestergård, M., Reinsch, S., Bengtson, P., Ambus, P. & Christensen, S. (2016a) Enhanced priming of old, not new soil carbon at elevated atmospheric CO2. *Soil Biology and Biochemistry*, **100**, 140–148.

Vestergård, M., Reinsch, S., Bengtson, P., Ambus, P. & Christensen, S. (2016b) Enhanced priming of old, not new soil carbon at elevated atmospheric CO_2. *Soil Biology and Biochemistry*, **100**, 140–148.

Vivanco, L. & Austin, A.T. (2008) Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. *Journal of*

Ecology, **96**, 727–736.

Wall, D.H., Bradford, M.A., St. John, M.G., Trofymow, J.A., Behan-Pelletier, V., Bignell, D.E., Dangerfield, J.M., Parton, W.J., Rusek, J., Voigt, W., Wolters, V., Gardel, H.Z., Ayuke, F.O., Bashford, R., Beljakova, O.I., Bohlen, P.J., Brauman, A., Flemming, S., Henschel, J.R., Johnson, D.L., Jones, T.H., Kovarova, M., Kranabetter, J.M., Kutny, L., Lin, K.C., Maryati, M., Masse, D., Pokarzhevskii, A., Rahman, H., Sabará, M.G., Salamon, J.A., Swift, M.J., Varela, A., Vasconcelos, H.L., White, D. & Zou, X. (2008) Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biology*, **14**, 2661–2677.

Wallander, H., Ekblad, a., Godbold, D.L., Johnson, D., Bahr, a., Baldrian, P., Björk, R.G., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Plassard, C. & Rudawska, M. (2013) Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils – A review. *Soil Biology and Biochemistry*, **57**, 1034–1047.

Wallander, H., Nilsson, L.O., Hagersberg, D. & Baath, E. (2001) Estimation of the biomass and seasonal growth of externalmycelium of ectomycorrhizal fungi in the field. *New Phytologist*, **151**, 753–760.

Wang, H., Boutton, T.W., Xu, W., Hu, G., Jiang, P. & Bai, E. (2015a) Quality of fresh organic matter affects priming of soil organic matter and substrate utilization patterns of microbes. *Scientific Reports*, **5**, 10102.

Wang, J., Dokohely, M.E., Xiong, Z. & Kuzyakov, Y. (2016a) Contrasting effects of aged and fresh biochars on glucose-induced priming and microbial activities in paddy soil. *Journal of Soils and Sediments*, **16**, 191–203.

Wang, X., Liu, L., Piao, S., Janssens, I. a, Tang, J., Liu, W., Chi, Y., Wang, J. & Xu, S. (2014) Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. *Global change biology*, **20**, 3229–3237.

Wang, J., Xiong, Z., Yan, X. & Kuzyakov, Y. (2016b) Carbon budget by priming in a biochar-amended soil. *European Journal of Soil Biology*, **76**, 26–34.

Wang, H., Xu, W., Hu, G., Dai, W., Jiang, P. & Bai, E. (2015b) The priming effect of soluble carbon inputs in organic and mineral soils from a temperate forest. *Oecologia*, **178**,

1239–1250.

Wardle, D. a, Nilsson, M.-C. & Zackrisson, O. (2008) Fire-derived charcoal causes loss of forest humus. *Science*, **320**, 629–629.

Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Freitag, T., Guillaumaud, N. & Roux, X. Le. (2006) Maintenance of soil functioning following erosion of microbial diversity. *Environmental Microbiology*, **8**, 2162–2169.

Whitman, T., Enders, A. & Lehmann, J. (2014) Pyrogenic carbon additions to soil counteract positive priming of soil carbon mineralization by plants. *Soil Biology and Biochemistry*, **73**, 33–41.

Wild, B., Alaei, S., Bengtson, P., Bodé, S., Boeckx, P., Schnecker, J., Mayerhofer, W. & Rütting, T. (2017) Short-term carbon input increases microbial nitrogen demand, but not microbial nitrogen mining, in a set of boreal forest soils. *Biogeochemistry*, **136**, 261–278.

Wu, Z., Dijkstra, P., Koch, G.W., Peñuelas, J. & Hungate, B.A. (2011) Responses of terrestrial ecosystems to temperature and precipitation change: A meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927–942.

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R. & Brookes, P.C. (1990) Measurement of soil microbial biomass C by fumigation-extraction: An automated procedure. *Soil Biology and Biochemistry*, **22**, 1167–1169.

Xu, S., Li, P., Sayer, E.J., Zhang, B., Wang, J., Qiao, C., Peng, Z., Diao, L., Chi, Y., Liu, W. & Liu, L. (2018) Initial Soil Organic Matter Content Influences the Storage and Turnover of Litter, Root and Soil Carbon in Grasslands. *Ecosystems*, **21**, 1377–1389.

Yan, M., Guo, N., Ren, H., Zhang, X. & Zhou, G. (2015) Autotrophic and heterotrophic respiration of a poplar plantation chronosequence in northwest China. *Forest Ecology and Management*, **337**, 119–125.

Yin, L., Dijkstra, F.A., Wang, P., Zhu, B. & Cheng, W. (2018) Rhizosphere priming effects on soil carbon and nitrogen dynamics among tree species with and without intraspecific competition. *New Phytologist*, **218**, 1036–1048.

Yin, H., Wheeler, E. & Phillips, R.P. (2014) Root-induced changes in nutrient cycling in

forests depend on exudation rates. *Soil Biology and Biochemistry*, **78**, 213–221.

Yu, L., Wang, Y., Wang, Y., Sun, S. & Liu, L. (2015) Quantifying Components of Soil Respiration and Their Response to Abiotic Factors in Two Typical Subtropical Forest Stands, Southwest China. *Plos One*, **10**, e0117490.

Zak, D.R., Pregitzer, K.S., King, J.S. & Holmes, W.E. (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, **147**, 201–222.

Zak, D.R., Pregitzer, K.S., Kubiske, M.E. & Burton, A.J. (2011) Forest productivity under elevated CO_2 and O_3: positive feedbacks to soil N cycling sustain decade-long net primary productivity enhancement by CO2. *Ecology Letters*, **14**, 1220–1226.

Zhang, D.Q., Hui, D.F., Luo, Y.Q. & Zhou, G.Y. (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology*, **1**, 85–93.

Zhang, W., Wang, X. & Wang, S. (2013) Addition of External Organic Carbon and Native Soil Organic Carbon Decomposition: A Meta-Analysis. *PLoS ONE*, **8**, e54779.

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

Zimmerman, A.R. (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science and Technology*, **44**, 1295–1301.

Zimmerman, A.R., Gao, B. & Ahn, M.Y. (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology and Biochemistry*, **43**, 1169–1179.

Zimmerman, A.R. & Ouyang, L. (2019) Priming of pyrogenic C (biochar) mineralization by dissolved organic matter and vice versa. *Soil Biology and Biochemistry*, **130**, 105–112.

Appendices

Appendix 1

Table S1 Chemical composition of adjusted Modified Merlin Norkans (MMN) growth medium (Marx, 1969) to culture ECM *Hebeloma crustuliniforme* for the production of ^{13}C-labelled fungal necromass

	DJ-MMN: In 1 L distilled H_2O
Malt extract	10 g
D-glucose*	1 g
$(NH_4)_2HPO4$	2 ml
KH_2PO_4	10 ml
$MgSO_4.7H_2O$	2 ml
$CaCl_2.2H_2O$	2 ml
$FeCl_3.6H_2O$ (2%)	1.2 ml
NaCl	1 ml
Thiamin HCl†	100 µl
pH	5.5 – 5.7
Agar (oxoid No. 3)‡	15 g

*^{13}C-labelled medium contained 20% ^{13}C-labelled d-glucose (U-$^{13}C6$, 99 atm%), 80% non-labelled d-glucose

†Added after autoclaving at 121 ^{o}C for 15 minutes.

‡agar excluded from liquid media

Appendix 2

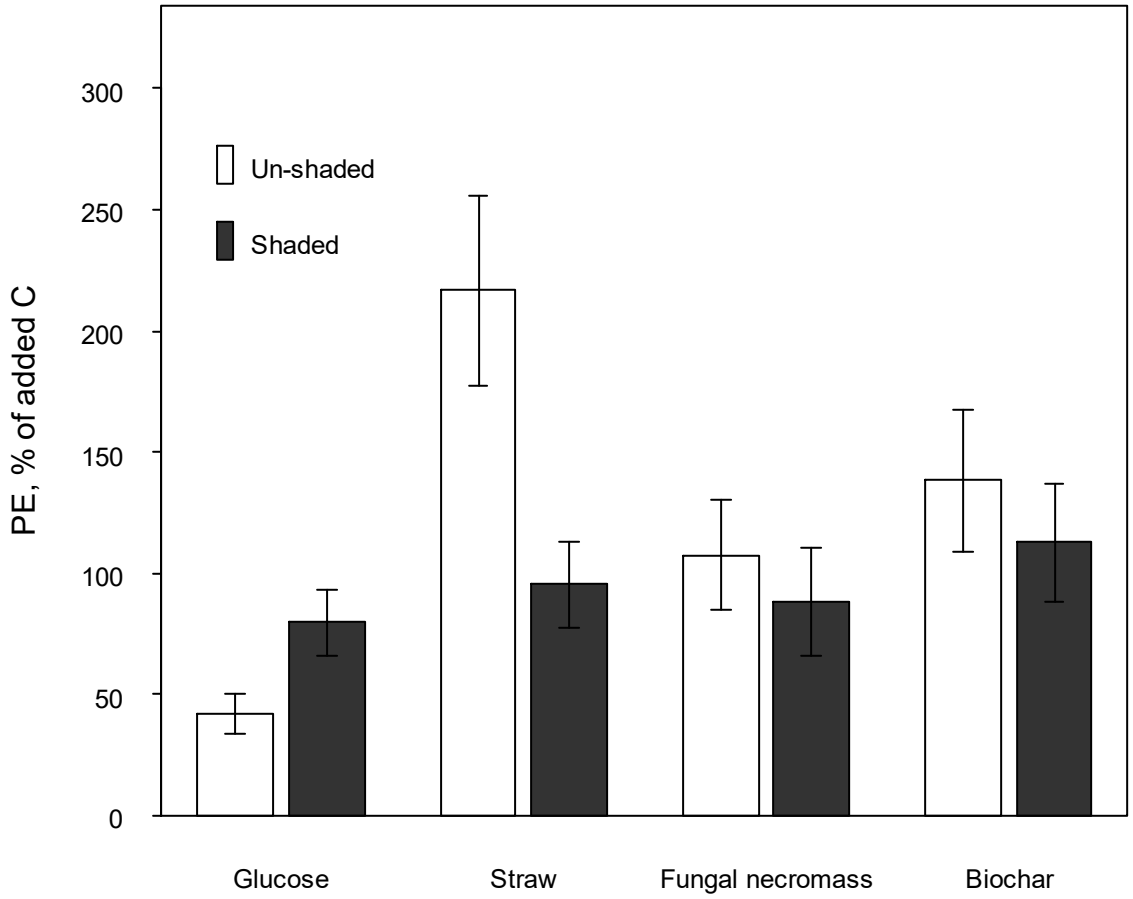

Figure S1 Priming effect in un-shaded and shaded treatments induced by the addition of glucose, straw residues, fungal necromass or biochar presented as percent of respective control. Different letters above bars indicate significant differences among treatments ($P < 0.05$).

Appendix 3

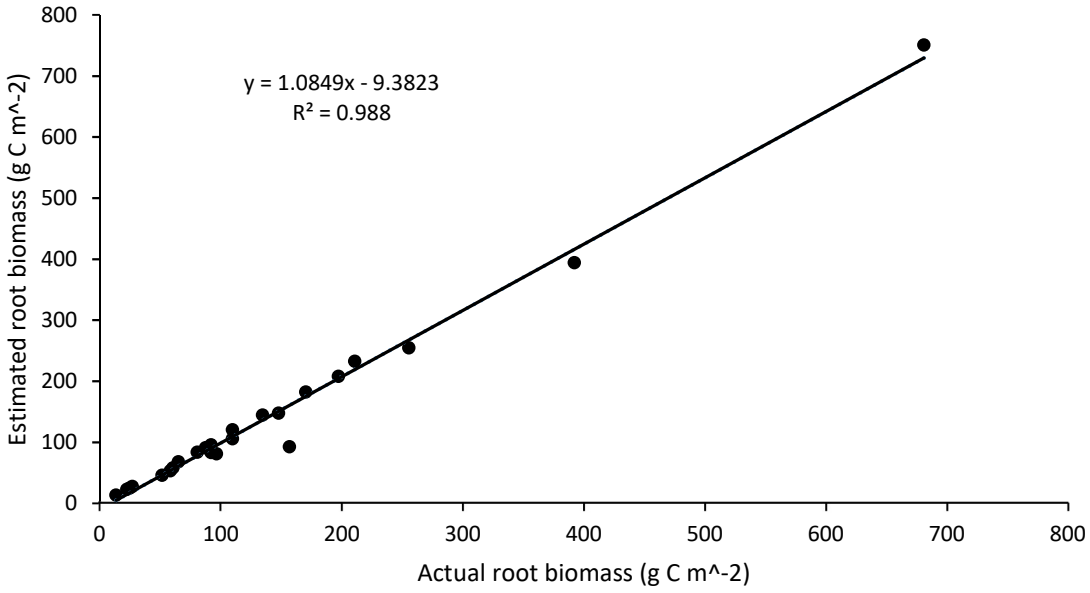

Figure S2. Relationship between actual total and estimated total fine root biomass C obtained from seven core samples where roots were thoroughly picked (see root biomass section in methods).