List of papers

I. Li, J., Bengtson, P. Quantification of the rhizosphere priming effect differs widely depending on the choice of control

II. Li, J., Zhou, M., Alaei, S., Bengtson, P. Rhizosphere priming effects differ between Norway spruce (Picea abies) and Scotch pine (Pinus sylvestris) seedlings cultivated under two levels of light intensity.

III. Li, J., Alaei, S., Zhou, M., Bengtson, P. Root influence on soil nitrogen availability and microbial community dynamics results in contrasting rhizosphere priming effect in pine and spruce soil.

IV. Alaei, S., Li, J., Jackowica-Korczynski, M., Bengtson, P. The influence of elevated CO2 and N fertilization on rhizosphere priming, C sequestration and N cycling in soil planted with Picea abies seedlings.

Mechanisms and regulations of priming effects in soil

Jian Li

DOCTORAL DISSERTATION

By due permission of the Faculty of Science, Lund University, Sweden.
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Faculty opponent
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The University of Sydney, Sydney, Australia
Abstract

Soil organic matter (SOM) plays a significant role in the global carbon (C) and nutrients cycles, not only since SOM is a major storage reservoir of C and plant nutrients, but also since microbial decomposition of SOM releases large quantities of CO₂ to the atmosphere. The accelerated or reduced turnover rate of SOM after the input of labile C compounds is a phenomenon known as the priming effect (PE). When the labile C originates from root exudate, the effect is referred to as the rhizosphere PE. The rhizosphere PE has been reported to increase SOM decomposition by up to 380%, but also to reduce SOM decomposition by up to 50% compared to rootless soil. Although PE is an important regulator of SOM decomposition and nutrient cycling, little is known about the mechanisms and regulations of PEs.

In this thesis, I performed several greenhouse experiments with living tree seedlings to investigate how different tree species, light intensity, root exudation rate, elevated CO₂ and nitrogen (N) fertilization influence the rhizosphere PE on SOM decomposition and gross N mineralization. I also performed an incubation experiment to compare the effect of increased C and N availability on the depolymerization of native SOM. I further measured the microbial growth rate, microbial community composition and potential enzyme activities to investigate the underlying mechanisms and role of different microbial groups in regulating the PE.

My results showed that the magnitude and direction of the rhizosphere PE differed between plant species. Light intensity also played an important role in regulating the rhizosphere PE, depending on the plant species present. My results further showed that the effect of N-fertilization and elevated CO₂ on the partitioning of N between plants and microbes was of importance for SOM decomposition and PEs. Moreover, rhizosphere PE was not dependent on the root exudation rate. Instead, my studies suggest that variation in soil N availability is a key regulator of the PEs. My studies further revealed that fungi are responsible for the PEs, while potential enzyme activities are poor indicators of the PEs.

In conclusion, my studies suggest that soil C and N cycling are strongly affected by the PEs caused by living roots. Therefore, the regulation of PEs on SOM decomposition and N mineralization could play an important role in the terrestrial ecosystem’s feedback to global change in response to e.g. elevated CO₂ and N deposition. My results also demonstrated that the PE is regulated by multiple environmental factors, e.g. plant species, light intensity, atmospheric CO₂ concentration, and soil N availability. In order to accurately predict how the PE on soil C and N cycling responds to environmental change, these factors need to be considered more in future studies.

Keywords: Soil organic matter decomposition, gross N mineralization, root exudate, plant species, soil C:N, microbial growth rate, microbial community composition, enzyme activity

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Mechanisms and regulations of priming effects in soil

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Table of Contents

List of Papers........................................................................................................................................... 8
Author Contributions ................................................................................................................................. 9
Popular science summary......................................................................................................................... 10

Introduction ......................................................................................................................................................... 11
  Definition of the priming effect ................................................................................................................. 11
  Mechanisms ................................................................................................................................................. 13
    Positive priming effects .......................................................................................................................... 13
    Negative priming effects .......................................................................................................................... 14
  Regulating factors ....................................................................................................................................... 17
    Microbial activity, biomass and community composition ................................................................. 17
  Microbial community composition ........................................................................................................... 17
    Plant species and physiology .................................................................................................................. 18
    Soil properties .......................................................................................................................................... 19
    Amount of the ‘primer’ .............................................................................................................................. 22

Aim and approaches of studies ...................................................................................................................... 23
  Paper I ......................................................................................................................................................... 23
  Paper II ....................................................................................................................................................... 24
  Paper III ...................................................................................................................................................... 25
  Paper IV ...................................................................................................................................................... 26
  Paper V ....................................................................................................................................................... 27

Methodology ...................................................................................................................................................... 29
  Priming of N mineralization ....................................................................................................................... 29
  Priming of SOM decomposition ............................................................................................................... 30
  Root exudation rate ..................................................................................................................................... 31
  Microbial growth rate ................................................................................................................................. 32
  Microbial community composition .......................................................................................................... 33
  Potential enzyme activities ......................................................................................................................... 33
Main findings ........................................................................................................ 35
  Evaluation of different controls................................................................. 35
  Plant species effects on rhizosphere PEs................................................. 36
  Light intensity effect on rhizosphere PEs............................................... 38
  Elevated CO₂ and N fertilization effects on rhizosphere PEs ............... 39
  Root exudate effects on the rhizosphere PE ...................................... 39
  Soil C and N availability effect on the depolymerization ..................... 41
  The role of the different microbial groups in the PEs ......................... 41
  The role of the extracellular enzyme in the PEs................................. 42

Conclusion and future perspectives .......................................................... 43

Acknowledgments ......................................................................................... 45

References ..................................................................................................... 47
List of Papers

In this thesis, the papers are referred to by the following roman numerals:

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Author Contributions

I. JL, and PB designed the experiment. JL performed the experiment with help from MZ and SA. JL and PB wrote the manuscript.

II. JL, PB, SA and MZ designed the experiment. JL, MZ and SA performed the experiment. JL and MZ analyzed the data. JL and MZ wrote the manuscript with assistance from PB and SA.

III. JL, PB and SA designed the experiment. JL, MZ and SA performed the experiment. JL analyzed the data and wrote the manuscript with assistance from PB. All co-authors provided comments on manuscript drafts.

IV. JL, PB and SA designed the experiment. JL, MJ and SA performed the experiment. SA analyzed the data and wrote the first draft of the manuscript, with assistance from JL and PB. All co-authors provided comments on manuscript drafts.

V. BW, TR and PB designed the experiment. JL, BW and JP performed the experiment, JL and BW analyzed the data. BW wrote the manuscript, with the assistance of the co-authors.

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Popular science summary

Soils contain more organic carbon (C) than the total terrestrial biomass combined. A large part of this C is stored in complex soil organic matter (SOM) with long turnover times. SOM is also the dominant pool of nutrients required for plant and microbial growth, but the availability of these nutrients is dependent on microbial decomposition of SOM into bioavailable forms. Labile C compounds exuded by plant roots can regulate the decomposition rate of SOM. This belongs to the phenomena of priming effects (PEs). Altered rates of native SOM turnover in the presence of labile C compound is increasingly considered as a mechanism central for the coupling of plant and soil biological productivity. The development of high precision isotopic techniques now enables us to directly quantify PEs, and ample evidence suggests that the PEs are large enough to influence the C balance of terrestrial ecosystems, with implications for the global C cycle and atmospheric CO₂ concentration.

Plant's roots can exude labile C compounds into the soil. Accordingly, living roots can increase SOM decomposition by up to 380% or reduce it by up to 50% compared to rootless soil, through the PEs. This large variation can be caused by many controlling factors. For instance, plant species, root exudates, soil N availability, microbial activity, and community composition have been shown to significantly affect PE. Accordingly, several potential mechanisms have been proposed to explain why PE occurs. For instance, positive PE can be caused by microbial activation in response to the input of labile C compounds. In addition, when N is not sufficient for microbial growth, microorganisms will use labile C to mineralize N out of SOM, resulting in stimulation of SOM decomposition and positive PEs. Preferential utilization of the labile C compounds by soil microorganisms, meaning that they do not have to decompose SOM to gain C, have been suggested as a potential reason for negative PEs. Another proposed explanation to negative PEs is that low N availability results in competition for N between plants and microorganisms, leading to a reduction of microbial ability to decompose SOM. Each of these mechanisms may act alone or together, further complicating our understanding of why both positive and negative PEs can occur.

In this thesis, I aimed to investigate the regulation of PEs in soil, with a particular focus on how the input of labile C (e.g. root exudates and sugar) influence SOM decomposition and N mineralization. In summary, my thesis suggested that soil C and N cycling are strongly affected by PE. The PEs were interactively controlled by multiple regulators, including the plant species, light intensity, CO₂ concentration, and N availability. My finding also suggested that variation in soil N availability was of particular importance for regulating the PEs, and different subsets of the microbial community are responsible for inducing positive or negative PEs. Taken together, my results suggest that PEs on SOM decomposition lay an important role in the ecosystem's feedback to global change in response to e.g. elevated CO₂ and N deposition.
Introduction

The largest terrestrial carbon (C) pool is stored in soils as complex soil organic matter (SOM) (Yiqi and Zhou, 2010; Lal, 2018), and at the global scale, nearly half of total soil respiration originates from the decomposition of SOM (Davidson and Janssens, 2006). As a result, even a small change in the SOM decomposition rate can have a strong influence on the atmospheric CO$_2$ concentrations, and hence the global climate (Davidson and Janssens, 2006; Heimann and Reichstein, 2008). The decomposition of SOM is dependent on transformations mediated by soil microorganisms that decompose SOM to gain energy and nutrients. The decomposition activities of these microorganisms are in turn strongly affected by the presence of living roots, since their biomass and activity are promoted by labile C compound supplied in root exudates (Van Hees et al., 2005). In fact, changes in decomposition of native SOM in response to input of labile C compounds, referred to as priming effects (PEs), is increasingly considered a central mechanism for soil C and nutrient turnover, which potentially plays a key role in regulating the global C cycle (Cheng et al., 2014; Guenet et al., 2018).

Definition of the priming effect

The PE was first identified by Lohnis in 1926. It is a phenomenon where the SOM decomposition rate is changed after the addition of exogenous labile C substrates (e.g. root exudates, fresh plant residues, manure, dead microorganisms). The SOM decomposition rate can be increased or decreased after the input of the labile C, referred to as positive PE and negative PE, respectively (Kuzyakov, 2002; Cheng et al., 2014) (Fig. 1). Positive PEs can strongly influence SOM decomposition rates, and large amounts of C and N could be mobilized in a very short time as a result (Kuzyakov et al., 2000; Zhu et al., 2014).
Fig. 1. The priming effect (PE) - interactions between added labile C compound and soil organic matter (SOM) decomposition: (a) acceleration of SOM decomposition - positive PE; (b) reduction of SOM decomposition - negative PE. (Adapted from Kuzyakov 2002).

PEs are divided into two classes: real PE and apparent PE. Jenkinson et al. (1985) suggested that real PE is an increase in the decomposition of recalcitrant SOM, whereas apparent PE is an increase of microbial C turnover, which is not linked to changes in SOM decomposition. In other words, real PEs are a change in the SOM decomposition rate by input of labile C compound, while apparent PE results from an acceleration of the CO₂ evolution in response to the activation of microbial metabolism and higher microbial biomass turnover, which is not related to SOM turnover (Blagodatskaya and Kuzyakov, 2008).

In this thesis, I determined PEs induced by the input of labile C compounds such as sugar, and also the rhizosphere PEs, which is the change in SOM decomposition and gross N mineralization caused by labile C exuded by living roots.
Mechanisms

Several possible mechanisms of the PEs have been proposed in recent years. In the following section, I summarized the most commonly proposed mechanisms.

**Positive priming effects**

*Microbial activation*

Soil microbes have a starving-survival lifestyle of dormancy or low activities (Hobbie and Hobbie, 2013). The microbial activation theory suggests that the input of glucose or other labile C compounds can trigger microorganisms to an active stage, leading to an increase of microbial activity, biomass and SOM decomposition (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Accordingly, several studies have demonstrated that stimulated SOM decomposition in response to labile C input is accompanied by higher microbial activity and biomass (Xiao et al., 2015; Kumar et al., 2016; Li et al., 2018). Even a very low amount of labile C can activate microorganisms and increase the SOM decomposition rate (Kuzyakov et al., 2000; Cheng et al., 2014), which might explain why PEs often result in a disproportional increase in SOM decomposition relative to the amount of labile C that is added or exuded by living roots. For instance, Bengtson et al. (2012) found each mg C exuded by roots C released 6 mg bioavailable C from SOM.

*Increased enzyme production and activity*

SOM decomposition is mediated by extracellular enzymes secreted by soil microorganisms (Marx et al., 2001; Sinsabaugh et al., 2008). The input of labile C substrates can serve as an energy source for the synthesis of such enzymes, resulting in an increase of SOM decomposition (Blagodatskaya and Kuzyakov, 2008). Accordingly, changes in extracellular enzyme production, promoted by the addition of labile C, have been suggested as a possible explanation for PEs. Labile C input might not only increase the microbial production of enzymes, but also stimulate the activity of enzymes already present in the soil. Oxidative enzymes that depolymerize macromolecules and produce soluble substrates for microbial assimilation appear to be particular importance for the initial stages of SOM decomposition (Hofrichter et al., 2010). Root exudates can stimulate the activity of these enzymes, by providing the substrate for accessory H$_2$O$_2$-generating oxidases (Ander and Marzullo, 1997). Accordingly, living roots have been found to stimulate the potential activity of both hydrolytic and oxidative enzymes (Zhu et al., 2014; Kumar et al., 2016). However, there are also examples of positive PE that did not co-occur with an increase in potential enzyme activity (Wild et al., 2017). These ambiguous results demonstrate that there is no straightforward relationship between
enzyme activity and PEs, possibly since the methods used measure the concentration of enzymes, and not the \textit{in situ} enzyme activity (Tiedje, 1982).

\textbf{Microbial N-mining}

The N-mining hypothesis suggests that when inorganic N is limiting microbial growth and activity, microbes in the rhizosphere utilize C-rich rhizodeposits to ‘mine’ N out of SOM (i.e. mineralize organic N or depolymerize proteins and other organic N sources), which results in an accelerated SOM decomposition and positive PEs (Kuzyakov et al., 2000). This can explain why the input of easily available C often increases the CO\textsubscript{2} efflux from native soil if microorganisms become N-limited when supplied with an abundant source of C and start to actively degrade organic materials to gain N (Garcia-Pausas and Paterson, 2011). In contrast, when inorganic N is sufficient, microbes in the rhizosphere assimilate the C exuded by roots instead of decomposing SOM to release available C, which results in lower SOM decomposition and a negative PEs (Preferential substrate utilization, see below) (Cheng, 1999). The N mining hypothesis is supported by several studies that have found a close connection between soil N content and the PE (Chen et al., 2014; Qiao et al., 2016; Xu et al., 2018).

\textbf{Negative priming effects}

\textit{Preferential substrate utilization}

The input of labile C into the soil does not always result in increased SOM decomposition. A recent review paper showed that the PE caused by the labile C input can also reduce the decomposition of SOM, with as much as 50\% (Cheng et al., 2014). This can be explained by the preferential substrate utilization hypothesis, which suggests that microorganisms use the most energy-rich and easily available substrates first (Cheng, 1999). Therefore, under conditions where mineral nutrients are sufficient, the microorganisms are more likely to use labile root-derived C than more complex SOM-derived C, leading to decreased decomposition of SOM. Accordingly, some studies have found that the SOM decomposition rate decreased in N fertilization treatments relative to unfertilized controls (He et al., 2016; Aye et al., 2018; Hicks et al., 2018).

\textit{Plant-microbial competition}

Competition between roots and soil microorganisms for available N may suppress microbial growth and activity when N availability is limited (Månsson et al., 2009) and subsequently the SOM decomposition rate (Kuzyakov, 2002; Kuzyakov and Xu, 2013). This mechanism has been suggested as a possible cause of the negative PEs since the input of labile C in root exudates might aggravate the competition (Kuzyakov and Xu, 2013). Accordingly, it has been observed that the PEs change
from negative to positive with N fertilization (Chen et al., 2014; Lu et al., 2018), but there are also observations on the contrary (Aye et al., 2018; Hicks et al., 2018). It seems like the competition mechanism dominates under extremely N limiting conditions, while the N-mining mechanism will be dominant when N is at a moderately limited level, and the preferential substrate use mechanism will dominate when mineral N is abundant (Kuzyakov et al., 2000).

In reality, these mechanisms may individually or in combination regulate the PEs. Which mechanism that dominates could depend on the specific circumstances since many abiotic and biotic factors (e.g. soil physicochemical properties, soil microbial activity and community composition, plant community structure, etc.) can influence the direction and magnitude of PEs.
Regulating factors

As mentioned above the magnitude and direction of PEs vary considerably among studies (Cheng et al., 2014). The large variation can be caused by many factors. Variations in microbial community composition and activity can have a profound effect on SOM decomposition rates (Strickland et al., 2009; Blagodatsky et al., 2010; Garcia-Pausas and Paterson, 2011), and could potentially explain the large variation in PEs among different studies. Differences in environmental factors (e.g. soil moisture, pH) and SOM quality (e.g. chemical recalcitrance and C:N ratio) are also of importance for SOM decomposition rates, meaning variations in one or several of these biotic and abiotic factors are likely to have a strong effect on the magnitude and direction of PEs. Finally, plant species differences in quality and quantity of root exudates are thought to be key plant traits controlling the PEs (Wang et al., 2016b). In this section, I summarized the current knowledge about how some of these factors influence PE.

Microbial activity, biomass and community composition

Microbial activity and biomass

The first step in the microbial utilization of SOM is its decomposition into compounds that can be taken up and metabolized by microorganisms. Variations in the biomass and activity of microorganisms will, therefore, result in corresponding variations in the turnover rate of SOM (Blagodatsky et al., 2010; Thiessen et al., 2013). In other words, a change in microbial activity and/or biomass, caused by the input of labile C that can serve as the primary energy source, can potentially be responsible for the PE (Kuzyakov, 2002; Cheng and Kuzyakov, 2005). Accordingly, many studies have found a significant relationship between PEs and microbial activity and biomass (Xiao et al., 2015; Wang et al., 2016a; Fang et al., 2018; Li et al., 2018), and a review by Kuzyakov et al. (2010) concluded that the most important mechanism for regulating PEs is the acceleration or retardation of SOM turnover due to increased activity or amount of microbial biomass. This effect might be linked to increased microbial enzyme production since enzyme production is strongly correlated to the microbial biomass and activity (Albiach et al., 2000).

Microbial community composition

Variations in the PEs might be caused by changes/variations in the microbial community composition (Blagodatskaya and Kuzyakov, 2008; Su et al., 2017). It is commonly proposed that PE involves a succession of processes that are partly connected to the succession of microbial communities and their functions (Blagodatskaya and Kuzyakov, 2008). The addition of labile C may first stimulate
the growth and metabolism of microbial r-strategists, followed by a gradual increase in the abundance of K-strategists (Fontaine et al., 2003). Bacteria are generally considered to be r-strategists, while fungi are considered as K-strategists. According to this theory, the initial microbial community response to the addition of easily available substrates is the activation of bacterial metabolism, which results in apparent PE, while fungal activity increases over time and is responsible for the later phase when real PEs occur (Blagodatskaya and Kuzyakov, 2008). In addition, fungi can grow better than bacteria in low nutrient zones since they can access and degrade substrates that are not available to most bacteria, and acquire nutrients from distantly-located substrates by their hyphae (Chalot and Brun, 1998; Otten et al., 2001). Thus, the dynamic of long-term PEs can possibly be explained by successional changes in microbial community composition, manifested as a change in the fungal/bacterial ratio (Fontaine et al., 2003). Accordingly, several studies have demonstrated that the PE is accompanied by such changes in the composition of the soil microbial community (Fontaine et al., 2011; Bastida et al., 2013; Zhao et al., 2018).

Plant species and physiology

Processes in the rhizosphere are primarily controlled by interactions between plant roots and the microbiological community (Cheng et al., 2014). Plant roots influence the soil's physical, hydrological, and chemical environment (e.g. SOM content, water content, pH, etc.) and associated biological processes, all of which are important for the turnover of C and other nutrients (Gregory, 2006). Part of the plants recently photosynthesized C is allocated belowground to root exudates, thereby influencing the microbial metabolism. Therefore, the SOM decomposition rate is strongly influenced by plants and their rhizodeposits (Cheng and Kuzyakov, 2005). In the following section, I summarized the current knowledge about how plant species, plant phenology and plant age influence PEs.

Plant species

Many studies have shown that decomposition and nutrient release from SOM varies among plant species, and different plant species induce different rhizosphere PEs (Zhu et al., 2014; Wang et al., 2016b; Xu et al., 2017; Yin et al., 2018). For example, soybean induced higher PEs than that of wheat when the two species were grown under the same environmental conditions (Cheng et al., 2013). A meta-analysis found that woody species induce the highest PEs followed by grasses, while crops induce the lowest level of PEs (Huo et al., 2017). The response of the PE to environmental changes also seems to differ among plant species. For instance, Xu et al. (2017) found that elevated CO₂ increased the PE induced by white lupin by 47%, but decreased the PEs induced by wheat by 22%. They suggested that changes
in the quality and quantity of root exudate in response to elevated CO₂ might account for the species-specific responses in PEs.

In addition, a study with different tree species showed that up to 60% of plant photosynthates C are transferred to the soil by mycorrhizal hyphae (Godbold et al., 2006). Mycorrhizal partners, which vary in their associations with different plant species (Phillips and Fahey, 2006), might, therefore, be an important factor that influences the PE.

Plant phenology and plant age
Several studies have found that the magnitude of the PE differs among different plant phenological stages (Fu and Cheng, 2002; Cheng et al., 2003). For example, a study with spring wheat found that the PE of wheat reached 287% at the flowering stage and declined significantly afterward (Cheng et al., 2003). Furthermore, although young roots exude organic substances more intensively than old roots (Warembourg and Estelrich, 2001), the fully developed plant roots can occupy more soil space than the younger ones. This might be the reason why older plants cause stronger PEs, at lease when expressed on soil weight or soil volume basis. In line with this proposal, Kuzyakov et al. (2001) found PEs induced by Lolium to increase during a 3.5 months cultivation period. In the first half of the observation period, a small non-significant negative PEs was observed, but the PEs became positive after 2.5 months of vegetative growth. Other studies have found similar results.

Soil properties
As mentioned above, SOM decomposition and PE are mediated by microorganisms, whose activity, in turn, is influenced by various soil properties.

In this section, I reviewed the current knowledge about how some of the most influential soil properties influence microbial SOM decomposition and PEs.

Soil type
SOM is made up of a heterogeneous mixture of compounds of varying chemical complexity that contain nutrients essential for microorganisms and for plant growth (Lal, 2009; Schmidt et al., 2011). Accordingly, the rhizosphere PE has been found to differ between different soil types. For example, soybean and sunflower caused a positive PEs when grown in agricultural soil, but no PEs when grown in annual grassland soil under the same environmental conditions (Dijkstra et al., 2006). The authors suggested that rhizosphere PE of SOM decomposition is more likely to occur in soils of relatively high fertility that can sustain high plant productivity, and possibly in soils where the native SOM contains less labile C compounds. Zhang et
al. (2017) found distinctly different PEs in a Mollisol and an Alfisol. Positive PEs occurred in the Mollisol, while negative PE was found in the Alfisol. They suggested that the differences in the PE between two soils largely stem from differences in the soil clay content.

Soil C is generally the driving force of most microbially mediated processes (Demoling et al., 2007). Accordingly, a laboratory experiment showed that a fine loamy Gleyic Cambisol containing 4.7 % organic C induced 30- to 100-times higher PE when compared to a sandy Gleyic Cambisol with 0.7 % organic C (Kuzyakov et al., 2001). In contrast, the relative PE was 1.1-3.3 times stronger in mineral soil than in the organic layer in a study by Wang et al. (2015). These contrasting results demonstrate that the importance of organic C for regulating the PEs not straightforward, and that other soil and/or plant properties are at least equally important to consider. For example, grasses allocate a larger portion of net assimilated C belowground and exude more C to the rhizosphere in soils poor in organic C, than in soils rich in organic C (Warembourg and Estelrich, 2001). A probable reason is that plants stimulate microorganisms to degrade SOM and mobilize nutrients by increasing their root exudation rate in poor soils. Therefore, plants and microorganisms in soils that are rich in organic C are less dependent on increasing SOM decomposition to obtain nutrients.

Soil pH

Soil pH can affect almost all chemical, physical and biological soil properties (Kemmitt et al., 2006), and is often regarded as one of the most important factors regulating the microbial biomass, activity and community composition (Bååth and Anderson, 2003; Aciego Pietri and Brookes, 2009). Soil pH has a particularly strong influence on the relative abundance and growth of fungi and bacteria (Rousk et al., 2009), meaning that soil pH could also potentially be an important regulator of the PE. Accordingly, an analysis of six previously published studies revealed a linear increase in the priming efficiency (the PE expressed as a percent of added C) in the pH range 2.7 to 7.8 (Blagodatskaya and Kuzyakov, 2008). Likewise, Aye et al. (2017) observed that the PE caused by the addition of plant residues increased as a result of long-term liming of acid soils. However, another recent study by Aye et al. (2018) showed that PE was not related to soil pH during a 30-day incubation period. In this study, the highest PE occurred at pH 4.1, the second-highest at pH 6.6, and the lowest at pH 4.7. The authors suggested that the high PE in pH 4.1 soil could be ascribed to the greater net increase in microbial biomass carbon in response to labile C input, which was because the net increase of microbial biomass carbon in response to C-substrate treatment69% at pH 4.1 was, 29% at pH 4.7 and 48% at pH 6.6.
Soil nitrogen

Nitrogen is the most commonly limiting nutrient of primary production and is thus of crucial importance for regulating the C balance of terrestrial ecosystems. Nitrogen also influences belowground processes and PE. As described above, positive PE may occur if the soil is rich in available C but low in available N, due to microbial N mining (Kuzyakov et al., 2000). On the other hand, if N is abundant this can lead to negative PEs due to preferential substrate utilization (Cheng, 1999). At severely N limiting conditions, competition for N between microorganisms and plants might also result in negative PEs (Kuzyakov, 2002). However, the effect of N fertilization on the PE is contentious, and different studies have found decreased (He et al., 2016; Aye et al., 2018), enhanced (Chen et al., 2014; Lu et al., 2018) or no effect (Qiao et al., 2016; Di Lonardo et al., 2019) of N fertilization on the PE. These discrepant results might be caused by variations in C substrate quality, the amount of N applied relative to the indigenous soil N content, and other physical, chemical and biological soil properties. Hence, the role of N in regulating the PE still warrants further investigations.

Differences in the soil C:N ratio might be one factor that could explain why N fertilization has such contrasting effects on the PE. Kuzyakov (2002) have proposed four scenarios that determine how the SOM decomposition rate can change depending on the amount of decomposable organic C and N in soil: (1) The competition for N between plants and microorganisms will become dominant when both C and N are simultaneously limited in the soil. This would inhibit microbial activity and biomass, leading to decreased SOM decomposition. (2) If available C is limiting microbial activities, this would result in preferential substrate utilization where microorganisms preferentially use labile C (rhizodeposits) instead of decomposing SOM to gain C, resulting in decreased SOM decomposition. (3) In cases where labile C is added to soils with low C:N ratio, microorganisms are activated by the input of labile C and use it to decompose SOM for acquiring additional N (4) When both available C and N are sufficient in the soil, no changes in SOM decomposition rates will be observed, since microorganisms have the optimal nutritional conditions and do not need to acquire additional nutrients from SOM when labile C is added.

Soil water content

Soil moisture can significantly influence the PE through its impact on microbial activity, plant growth, and root exudation rates (Dijkstra and Cheng, 2007; Dijkstra et al., 2010). Root exudates may be more effective in stimulating microbial activity and decomposition in wet than in dry soils, because of more rapid diffusion of exudates away from the root and reduced chances of exudates being actively re-adsorbed by roots (Jones and Darrah, 1993). For example, Dijkstra et al. (2010) suggested that when water availability is low, plant species complementarity and
selection effects on water and N use can decrease soil C decomposition and the rhizosphere PE, based on results from a greenhouse experiment with five semi-arid grassland species and two water levels. In contrast, Lu et al. (2018) found that high soil moisture reduced the rhizosphere PEs. They suggested that this was caused by increased microbial nutrient limitation in the high soil moisture treatment. High moisture increased the plant biomass and plant N uptake, which might have resulted in the plant-microbe competition for mineral N and decreased the microbial activity, and hence depressed SOM decomposition. It is not clear why this would be the case, but the contrasting results demonstrate that there are no straightforward relations between water content and PEs.

**Amount of the ‘primer’**

The amount of the “primer”, i.e. the labile C that induces the PE, is considered an important regulator of PEs. Higher amounts of labile C input will result in a more pronounced stimulation of microbial activity, resulting in stronger PEs (Guenet et al., 2010; Bengtson et al., 2012). Therefore, the rate of available C input to the soil will regulate the magnitude of the PE. However, it appears as if there is an upper limit for this effect. For example, Bengtson et al. (2012) found that each mg of exuded C resulted in decomposition and release of 6 mg bioavailable C from SOM, but there was no or little additional increase in the relative PE when the root exudation rates exceeded 16 mg C per kg soil per day. This suggests that other factors also need to be taken into consideration. For example, Blagodatskaya et al. (2008) suggested that the amount of added substrate relative to the amount of microbial C is a key factor regulating PEs, rather than the amount of substrate on its own. Furthermore, a combined addition of different substrates can induce a higher positive PE than single substrate additions (Hamer and Marschner, 2005). The authors further suggested that the substrate concentrations, as well as the adaptation of microorganisms to the added substrate, are important factors controlling PEs.
Aim and approaches of studies

This section of my thesis is focussed on describing the aims and approaches of Paper I-V, while the methodology is described in greater detail in the next section.

Paper I

Most experiments aimed at investigating the rhizosphere PE in the presence of living roots have been performed using unplanted soil as the control, which could potentially complicate quantification of the rhizosphere PE and mislead our understanding of the underlying mechanisms. In order to evaluate the best choice of control to achieve the aim of this thesis, I performed an experiment in with Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) seedlings, and two different controls, 1. a never planted control (unplanted control), and 2. A control where planted seedlings were cut at the soil surface five days before the measurement (planted control). The aim was to determine how the choice of control will influence the calculated rhizosphere PEs, as well as to test if the variation of the calculated rhizosphere PE can be linked to biological and environmental differences between the two controls.

The rhizosphere PE of SOM decomposition and N mineralization, microbial growth rate, and community composition, as well as the potential enzyme activity, were measured to achieve the aim of the study. The $^{15}$N pool-dilution technique was used to determine the gross N assimilation and mineralization rate (Davidson et al., 1991), and the rhizosphere PEs of SOM decomposition and gross N mineralization were calculated as in Bengtson *et al.* (2012). Microbial community composition was determined by phospholipid fatty acid (PLFA) analysis (Frostegård and Bååth, 1996), and the fungal and bacterial growth rate was determined using isotope incorporation methods (Bååth, 1994; Bååth, 2001). The potential enzyme activity was determined by using a combination of fluorometric and photometric methods (Marx et al., 2001; Saiya-Cork *et al.*, 2002).
Paper II

The objective in Paper II was to investigate if the rhizosphere PE differs between two tree species (Norway spruce and Scots pine) and if their rhizosphere PE responds differently to different light intensities. Plant photosynthetic intensity, which is a main controlling factor of belowground plant C allocation and root exudation rate (Kuzyakov and Cheng, 2001), is a possible reason why the rhizosphere PE varies widely among plant species. I, therefore, hypothesized that the rhizosphere PEs would differ between the two tree species under the same environmental conditions since they have different light saturation and compensation points and different rhizosphere environments. I further hypothesized that light intensity would influence the rhizosphere PEs through its effect on the photosynthetic intensity and root exudation rates. The hypothesis was tested in a greenhouse experiment with pine and spruce seedlings that were cultivated for two months before the measurements.

The rhizosphere PEs of SOM decomposition and gross N mineralization was determined as described in the paper I above. A $^{13}$CO$_2$ pulse-chase method was used to determine the root exudation rate, as in Bengtson et al. (2012). I also measured the microbial growth rate and microbial community composition as in paper I, to investigate their role in rhizosphere PEs.
Paper III

In my third experiment, I cultivated the same tree species (Norway spruce and Scots pine) as in Paper II, but this time for 6 months. Five days prior to measurement of the root exudation rate and the PE, some of the seedlings were cut at the surface of the soil, leaving 0, 1, 3 or 5 intact seedlings in each pot (Fig. 3). The amount of the primer is considered as an important regulator of PEs (Bengtson et al., 2012), and I hypothesized that higher root exudation rates in pots with higher numbers of intact seedlings would result in stronger stimulation of microbial SOM decomposition and gross N mineralization rates, and consequently a stronger rhizosphere PE. I further hypothesized that the rhizosphere PE is caused by increased microbial enzyme production and that this would be reflected in higher potential enzyme activity in treatments with high rhizosphere PEs. A stable isotope probing (SIP) experiment measuring the incorporation of deuterium (D) (derived from D₂O) and ¹³C (derived from root exudates) into microbial biomarker PLFAs was also performed to identify which microbial groups that grew on root exudates and/or on resources released as a result of the rhizosphere PE. SOM decomposition, gross N mineralization, root exudation rate, fungal and bacterial growth rates, and the rhizosphere PE was determined in the same way as in Paper I and II.
Belowground C allocation by plants can be affected by the CO₂ concentration (Ainsworth and Long, 2005), which might thus be an important regulator of the PE. As stated above, soil N availability has also been proposed as a key role in regulating the PEs (Dijkstra et al., 2013), and it is possible that plant-mediated belowground responses to elevated CO₂ are dependent on the amount of plant available N (Kuzyakov and Blagodatskaya, 2015). In this experiment, I, therefore, quantified the combined effect of N fertilization and elevated CO₂ on the root exudation rate and rhizosphere PEs. I hypothesized that elevated CO₂ would increase the root exudation rate and result in higher rhizosphere PEs. This hypothesis was tested by exposing spruce seedlings to ambient (400 ppm) and elevated CO₂ (600 ppm) environment for 7 days (Fig. 4). The CO₂ treatments were combined with three levels of N fertilization, to test if any changes in the root exudation rate and rhizosphere PEs at elevated CO₂ are moderated by the N availability. The relationship between rhizosphere PEs and plant N uptake was also tested. SOM decomposition, gross N mineralization, root exudation rate and the rhizosphere PEs were determined in the same way as above.
Plant available N and microbial available N is produced by the breakdown of soil polymers (e.g. chitin and protein) that represent a major fraction of soil N. Soil microorganisms may adjust the production of enzymes that target different polymers, resulting in a balanced supply of available C and N (Burns et al., 2013; Bell et al., 2014). In this experiment, I first hypothesized labile C addition would increase microbial N demand and hence stimulate the depolymerization of lignin, chitin, and protein. Although lignin does not contain N, lignin depolymerization can still increase soil N availability (Carreiro et al., 2000; Schimel and Bennett, 2004; Knicker, 2011). Secondly, I hypothesized that the opposite effect would occur after the addition of available N.

The hypotheses were tested by using a combination of two complementary ten-day incubation experiments. In experiment 1, the soil was amended with $^{13}$C enriched glucose and available N (ammonium). In experiment 2, the same soil was mixed with lignin, chitin, or protein before the addition of available C or N as above. In the second experiment, glucose had natural $^{13}$C content, while lignin, chitin, and protein were enriched $^{13}$C. Microbial community composition and potential enzyme activity were also measured to the hypotheses.
Methodology

This chapter provides a short overview of the methods used to achieve the aims and test the hypotheses in paper I-V.

Priming of N mineralization

In paper I, II, III and IV, gross N mineralization was determined using the $^{15}$N-pool dilution method (Davidson et al., 1991). Briefly, $^{15}$N enriched NH$_4$Cl was injected into the soil at different positions. Half of the replicates were harvested within 2 hours after the $^{15}$NH$_4$Cl addition ($T_0$) and the rest 24 hours after the $^{15}$NH$_4$Cl addition ($T_1$). The concentration of $^{15}$NH$_4^+$-N and $^{14}$NH$_4^+$-N at $T_0$ and $T_1$ was determined by isotope ratio mass spectroscopy (IRMS) after extraction and diffusion according to standard procedures (IAEA, 2001). Then the gross N mineralization and assimilation rates were calculated using the FLAUZ model (Mary et al., 1998), from the differences in the concentration of N and $^{15}$N of ammonium between $T_0$ and $T_1$.

The PE of N mineralization was calculated as:

$$N_{\text{primed}} = N \text{ mineralization}_{\text{treatment}} - N \text{ mineralization}_{\text{control}}$$

$$N_{\text{primed}} (\%) = \frac{N \text{ mineralization}_{\text{treatment}} - N \text{ mineralization}_{\text{control}}}{N \text{ mineralization}_{\text{control}}} \times 100 \quad (1)$$

Where $N$ mineralization$_{treatment}$ is the gross N mineralization in treatment samples, and $N$ mineralization$_{control}$ is the gross N mineralization in control samples.
**Priming of SOM decomposition**

In **Paper I, II, III** and **IV**, SOM decomposition was calculated from N assimilation measured as above, using the following equations:

\[
C_{\text{assimilated}} = N_{\text{assimilated}} \times f^{15}\text{N}_{\text{mic}} \times C: N_{\text{microorganisms}}
\]  

(2)

Where \(C_{\text{assimilated}}\) is the microbial C assimilation, \(N_{\text{assimilated}}\) is the total N assimilation by plants and microorganisms calculated from the \(^{15}\text{N}\)-pool dilution method above, \(f^{15}\text{N}_{\text{mic}}\) is the fraction of the total assimilated \(^{15}\text{N}\) that was assimilated by the microorganism, and \(C: N_{\text{microorganisms}}\) is the soil microorganism C: N ratio.

\[
\text{SOM}_{\text{decomposed}} = \frac{C_{\text{assimilated}}}{\text{CUE}}
\]  

(3)

Where \(\text{SOM}_{\text{decomposed}}\) is the SOM decomposition and CUE is microbial C-use efficiency.

Then the PE of SOM decomposition was calculated:

\[
\text{SOM}_{\text{primed}} = \text{SOM decomposition}_{\text{treatment}} - \text{SOM decomposition}_{\text{control}}
\]

\[
\text{SOM}_{\text{primed}}(\%) = \frac{\text{SOM decomposition}_{\text{treatment}} - \text{SOM decomposition}_{\text{control}}}{\text{SOM decomposition}_{\text{control}}} \times 100
\]

(4)

Where \(\text{SOM decomposition}_{\text{treatment}}\) is the SOM decomposition in the treatment with intact plants and \(\text{SOM decomposition}_{\text{control}}\) is the SOM decomposition in the control treatment with cut plants.

In order to correctly represent the uncertainty of the assumed variables (C: N_{microorganisms} and CUE), I performed the calculations in @RISK 7.6 (Palisade Corporation, Ithaca, NY, USA) using a probabilistic Monte Carlo analysis. Significant differences among treatments were assessed by testing if the 85% confidence intervals of these calculations overlapped (Payton et al., 2000; Payton et al., 2003).

In **Paper V**, the respiration and isotopic composition of respired CO\(_2\) were measured immediately and then during 10 days after the start of the experiment. Briefly, gastight flasks equipped with a septum was evacuated four times and refilled with pressurized air of known concentration and isotopic composition of CO\(_2\). Empty flasks were processed in the same way and used as blanks. The flasks were incubated for between 4-8 hours before analysis of the concentration and isotopic composition of accumulated CO\(_2\). The analyses were performed using a Delta Ray Isotope Ratio Infrared Spectrometer (Thermo scientific), calibrated against two CO\(_2\)
standards of different isotopic composition. The CO₂ concentration and isotopic composition were corrected for the initially present CO₂ using the values derived from the blanks. We then distinguished CO₂ from different sources using the following equations:

\[ C_1 = C_{\text{Total}} \times \frac{\text{atom}\%_{\text{Total}} - \text{atom}\%_2}{\text{atom}\%_1 - \text{atom}\%_2} \]  
\[ C_2 = C_{\text{Total}} - C_1 \]

Where \( C_{\text{Total}} \) was the concentrations of total CO₂, \( C_1 \) and \( C_2 \) were the concentrations of CO₂ from source 1 and from source 2, respectively. The \( \text{atom}\%_{\text{Total}}, \text{atom}\%_1 \) and \( \text{atom}\%_2 \) were the corresponding \(^{13}\text{C}\) contents (in atom\% \(^{13}\text{C}\)) of respired CO₂.

In experiment 1, source 1 was the added \(^{13}\text{C}\) enriched glucose and source 2 was the natural abundance of \(^{13}\text{C}\) in soil organic C. In Experiment 2, source 1 was the added \(^{13}\text{C}\) enriched polymers (lignin, chitin, or protein), while source 2 included both natural abundance of \(^{13}\text{C}\) in soil organic C and the added glucose.

The concentration and \(^{13}\text{C}\) content of microbial C was measured in 0.5 M K₂SO₄ extracts (after chloroform fumigation) and \(^{13}\text{C}\) in dissolved organic C in 0.5 M K₂SO₄ extracts of fresh soil, using an LC Isolink IRMS system. To assess the effect of C and N addition on the mobilization of C from SOM in experiment 1, and from polymers (lignin, chitin and protein) in experiment 2, the sum of SOM-derived C and polymer-derived C in dissolved organic C, microbial C and cumulative respiration from SOM and polymers, was calculated (as shown in Fig. 1 in paper V).

Root exudation rate

In Paper II, III and IV a \(^{13}\text{CO}_2\) pulse-chase method was used to estimate the root exudation rate, as in Bengtson et al. (2012). Seedlings were labelled with \(^{13}\text{CO}_2\) by placing them in a plexiglass chamber (Fig.2A). 10 mL (in paper II) or 20 mL (in paper III and IV) \(^{13}\text{CO}_2\) (99 atom % \(^{13}\text{C}\)) was injected into the chambers and the concentrations of \(^{12}\text{CO}_2\) and \(^{13}\text{CO}_2\) were measured during the next 1.85 hour, using a Picarro G2201-i analyzer (Picarro Inc., Santa Clara, CA, USA). The seedlings were then destructively harvested five days after the \(^{13}\text{CO}_2\) labelling and soil samples were freeze-dried for analysis of the soil \(^{13}\text{C}\) content in a Flash 2000 elemental analyzer (Thermo Scientific Inc., Bremen Germany).

The root exudation rate was calculated based on the soil \(^{13}\text{C}\) content:
Root exudation rate = $\frac{13C_{\text{soil}} \times (t_1/t_2) / (1 - f^{13C_{\text{lost}}})}{13C-CO_2/100}$ (9)

Where $13C_{\text{soil}}$ is the amount of $\text{CO}_2$ derived $13\text{C}$ that recovered in soil, $f^{13C_{\text{lost}}}$ is the fraction of $13\text{C}$ lost from the soil due to microbial respiration, $t_1$ is the duration of the light period (12 hours), $t_2$ the length of $13\text{CO}_2$ labelling (1.85 hours), and $13C-CO_2$ the atom percent of $13\text{C}$ excess in $\text{CO}_2$. The short duration of the $13\text{CO}_2$ pulse-labelling was to ensure that there was no significant dilution of the $13\text{CO}_2$ label caused by plant or microbial respiration and that no recycling of fixed $13\text{C}$ that occurred during the pulse-labelling time. The calculation in equation 9 was performed in @RISK 7.6 as mentioned above. I used 0.71±0.09 as the value of the 1- $f^{13C_{\text{lost}}}$, based on Bengtson et al. (2012).

Microbial growth rate

I measured bacterial and fungal growth rates in Paper I, II, III and IV, to investigate how the fungal and bacterial activity influences the PEs.

The bacterial growth rate was estimated by the $3\text{H}$-leucine incorporation method. The method is based on the fact that bacteria will take up and incorporate amino acids (e.g. leucine) into their proteins at a rate corresponding to the growth rate. Therefore, the bacterial growth rate can be indicated by the amount of incorporated leucine before and after incubation with isotopically labelled leucine (Bååth, 1994). Briefly, a diluted $3\text{H}$-leucine solution is added after the extraction of bacteria from soil. Washing and subsequent measurement of radioactivity are performed after incubation for 2 hours, and the amounts of leucine derived $3\text{H}$ measured using a liquid scintillation counter (Perkin-Elmer Life Sciences). The amount of $3\text{H}$-leucine incorporated into bacteria per hour per gram of soil was then used as a measurement of bacterial growth, after conversion to bacterial biomass C production.

The fungal growth rate was estimated by the $14\text{C}$-acetate incorporation method, as in Bååth (2001). Quantifying fungal biomass can be performed by estimating a substance present in fungal structures. Ergosterol is a membrane lipid (a sterol), which does not occur in considerable amounts except in fungi. Therefore, the rate of synthesis of ergosterol can be used as an indicator of fungal growth rate. In order to estimate ergosterol synthesis, I used $14\text{C}$-acetate, which is a precursor of lipids. Briefly, fresh soil was transferred to test tubes a $14\text{C}$-acetate solution added. Formalin was added to terminate the fungal growth after incubation for 4 hours. Ergosterol was then extracted, separated, and the amount of incorporated radioactivity determined. The amount of $14\text{C}$-acetate incorporated into fungal
ergosterol per hour per gram of soil was used as a measurement of fungal growth, after conversion to fungal biomass C production.

**Microbial community composition**

In **Paper I, II, III, IV** and **V**, microbial community composition was determined by measuring microbial biomarker phospholipid fatty acids (PLFAs) (Frostegård and Bååth, 1996; Bååth and Anderson, 2003). Phospholipids occur in all living microorganisms and degrade fast when the organism dies. The total amount of microbial phospholipids can, therefore, be used as a measure of the living microbial biomass (Frostegård and Bååth, 1996). The fatty acid part in phospholipids differs among microorganisms, and fatty acids can, therefore, be used to distinguish different groups of microorganisms, like fungi and bacteria. I also used stable isotope (13C) probing (SIP) to investigate the incorporation of isotopes into different microbial biomarker PLFAs. In **paper III**, a combination of 13C and D SIP was performed, which enabled me to determine which microbial group that was growing directly on the root exudates, and on resources released from SOM by the PEs. I assumed that microorganisms that assimilated D, but not 13C, was responsible for the observed PEs (Hungate et al., 2015). Microbial incorporation of 13C and D into PLFAs were calculated from the atom% 13C and atom% D excess in individual PLFAs.

**Potential enzyme activities**

In **Paper I, III** and **V**, I investigated if increased enzyme production and/or activity is the mechanism underlying PEs. The potential activity of six extracellular hydrolytic enzymes and two oxidative enzymes (Summarized in Table 1) were measured by using a combination of fluorometric and photometric methods (Marx et al., 2001; Saiya-Cork et al., 2002).

Fresh soil was amended with fluorescence-labeled substrates to analyze hydrolytic enzyme activities (Table 1). Fluorescence was then measured after incubation to determine the concentrations of released 4-methylumbelliferone (MUF) or 7-amido-4-methylcoumarine (AMC), respectively. Potential hydrolytic enzyme activities were expressed in nmol MUF or AMC per gram dry soil per hour.

Potential phenoloxidase and peroxidase enzyme activity were mixed with L-3,4-dihydroxyphenylalanin as the substrate. Samples for measurements of peroxidase activity also receive 10 µl 0.3% H2O2 (Table 1). The absorbance was then measured.
before and after 20 hours incubation. Potential oxidative enzyme activity is expressed in nmol DOPA per gram dry soil per hour.

**Table 1. Extracellular enzymes and their abbreviations, substrate, and their function for the degradation of macromolecules.**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Degradation of macromolecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-1,4-glucosidase</td>
<td>4-MUB-α-D-glucosidase</td>
<td>Maltose</td>
</tr>
<tr>
<td>β-1,4-xylosidase</td>
<td>4-MUB-β-D-xylosidase</td>
<td>Hemicellulose</td>
</tr>
<tr>
<td>β-1,4-glucosidase</td>
<td>4-MUB-β-D-glucosidase</td>
<td>Cellulose and cellobiose</td>
</tr>
<tr>
<td>L-leucine aminopeptidase</td>
<td>L-leucine-7-amino-4-methylcoumarin</td>
<td>Protein and peptides</td>
</tr>
<tr>
<td>β-1,4-N-acetylglucosaminidase</td>
<td>4-MUB-N-acetyl-β-D-glucosaminide</td>
<td>Chitin and bacterial peptidoglycan</td>
</tr>
<tr>
<td>Phenoloxidase</td>
<td>L-3,4-dihydroxyphenylalanine</td>
<td>Phenolic compounds</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>L-3,4-dihydroxyphenylalanine and H₂O₂</td>
<td>Peroxides (e.g. Hydro peroxides and lipid peroxides).</td>
</tr>
</tbody>
</table>
Main findings

Evaluation of different controls

In paper I, I compared two kinds of controls (unplanted and planted) to investigate how the choice of control influences the calculated rhizosphere PE. The unplanted control consisted of soil that had never been planted and the planted control of pots with soil where the seedlings had been cut at the soil surface five days before the measurements.

My results showed that the rhizosphere PE varied widely depending on the control. In the pine treatment, the rhizosphere PE of SOM decomposition did not differ significantly when calculated using the planted control and unplanted control, respectively, while rhizosphere PE of gross N mineralization was much higher when calculated from the unplanted control. In the spruce treatment, the rhizosphere PE of SOM decomposition was negative when calculated using the planted control, and positive when calculated from the unplanted control. As in the pine treatment, rhizosphere PE of gross N mineralization was much higher when calculated from the unplanted control. (Fig. 1 in paper I). The variation in the rhizosphere PE was caused by lower SOM decomposition and gross N mineralization rates in unplanted than planted control. The reduction seemed to be caused by lower microbial biomass, lower bacterial and fungal growth, and higher bacterial dominance in unplanted control, but possibly also by lower inorganic N concentrations in the planted control compared to the unplanted control (Fig. 1 and Table 1 in paper I).

I also performed an experiment with $^{13}$C labelled plants, which showed that a 5-day time frame that cut seedlings above-ground parts was sufficient to let root activity cease and short enough to prevent any PEs induced by decomposed dead roots (Fig. 2 in paper I). Based on these experiments, I found that the planted control, where above-ground plant parts removed only a few days before measurement the rhizosphere PE, was a better choice for achieving the aims of my thesis and investigate the direct effect of living roots on belowground SOM decomposition.
Plant species effects on rhizosphere PEs

In paper II, my results demonstrated that spruce induced positive rhizosphere PEs, while pine induced a negative rhizosphere PEs (Fig. 2 and 4 in paper II). I suggested that the negative rhizosphere PE in the pine treatment was caused by the competition for available N between plant and microorganisms (Kuzyakov and Xu, 2013). Accordingly, we found that both the inorganic N concentration and the gross N mineralization rate were lower in soil with pine seedlings than in soil with spruce seedlings (Table 2 and Fig. 4 in paper II).

I concluded that higher N availability in combination with input of labile C by root exudates might have increase microbial decomposition of SOM as well as gross N mineralization rates in the spruce treatment, while available N was not enough for this occur in the pine treatment, resulting in a competition for N between plants and microorganisms and a negative rhizosphere PE. Accordingly, my results showed that there was a significant positive relationship between inorganic N concentration and rhizosphere PEs (Fig. 3 in paper II).

In paper III the presence of living plants did not influence the gross N mineralization rate, meaning that no rhizosphere PE of N mineralization occurred (Table 2 in paper III). Possible reasons include more efficient microbial use of already available N (Wild et al., 2017), due to decreasing inorganic N concentrations as the experiment progressed (Table 1 in paper III). Accordingly, more efficient microbial recycling of N occurs in response to low N availability (Bengtson and Bengtsson, 2005).

Even if no rhizosphere PE of gross N mineralization occurred in paper III, pine seedlings induced a positive rhizosphere PE of SOM decomposition while spruce seedlings induced a negative rhizosphere PE of SOM decomposition (Table 2 in paper III). My results suggested that the negative rhizosphere PE that occurred in the spruce treatment was caused by an opportunistic subset of the microbial community that grew on root-derived labile C compounds, while at the same reducing the activity of SOM decomposers by depleting available N. For some reason, this did not appear to occur in the pine treatment, where available N was sufficient for root exudate to stimulate fungal decomposers and induce positive rhizosphere PEs, despite low inorganic N concentrations. I discuss the possible reasons below.

Interestingly, my results in paper II and III showed opposite rhizosphere PEs of the two tree species. The contradictory findings might have been caused by changes in soil N availability and microbial community composition during the cultivation of the seedlings. The seedlings were cultivated for two months in paper II and for six months in paper III, and as previously discussed plant age is a possible factor regulating PEs (Cheng et al., 2003). Furthermore, due to the longer cultivation time
in paper III, the soil inorganic N content decreased from 1.19 µg N g⁻¹ dw soil in paper II, to 0.47 µg N g⁻¹ dw soil in paper III in the spruce treatment, (Fig. 5A). This decrease might have induced competition for N in paper III, resulting in suppressed activity of microbial decomposers and thus negative PEs. Accordingly, I found that addition of N fertilizer changed the rhizosphere PEs from negative to positive in paper IV (Table 1 in paper IV), where the seedlings had been cultivated for 4 months. It is likely that the N deficiency was even more pronounced after 6 months of cultivation, as in paper III.

The soil inorganic N content decreased also in the pine treatment, but not to the same low level as in the spruce treatment (from 0.98 to 0.68 µg N g⁻¹ dw soil). For some reason, the decrease in inorganic N resulted in the opposite effect compared to the spruce treatment. My analyses of the microbial community composition might provide a clue to why this was the case. There was a much stronger fungal dominance in the pine treatment in paper III than in paper II (Fig. 5B). This microbial community shift from bacterial dominance to fungal dominance occurred due to the difference in cultivation time between the two experiments. Fungi have higher C:N ratios than bacteria and are better adapted to low N conditions (Sterner and Elser, 2002). The shift in microbial community composition may, therefore, have relieved the N limitation experienced by the microbial decomposer community in paper II. The increased abundance of fungal biomarker PLFAs might also be a result of increased ectomycorrhizal colonization of the pine seedlings as time progressed since it has been suggested that ectomycorrhiza might be an important player in the regulation of rhizosphere PEs (Zak et al., 2019). However, my data does not allow me to evaluate if this was the case. Finally, pine is better adapted to low nutrient concentrations than spruce (Merilä and Derome, 2008), and it can be speculated that pine roots selected for a microbial decomposer community that is equally well adapted to low nutrient concentrations.
In conclusion, paper II and III showed that the magnitude and direction of the rhizosphere PE depend on plant species and is also affected by the interaction between N availability, plant roots, and microbial community composition.

**Light intensity effect on rhizosphere PEs**

As stated above, pine induced no or negative rhizosphere PEs on both SOM decomposition and gross N mineralization in paper II. The negative rhizosphere PE of SOM decomposition tended to be more pronounced and was only significantly different from zero under low light (Fig. 2 in paper II). The seedlings also allocated more C belowground to root exudates in the low light treatment, which might have induced stronger competition for N between microbial decomposers and plant roots and their associated ectomycorrhiza. This is consistent with my observation that the soil inorganic N increased with 19% after cutting the pine seedlings in the low light treatment, while it only increased with 12% in the high light treatment (Table 2 in paper II). Likewise, cutting the seedlings resulted in a more pronounced increase in microbial biomass in the low light than in the high light treatment, as indicated by the change in total abundance of microbial PLFAs (Table 3 in paper II).

In the spruce treatment, the positive rhizosphere PEs on SOM decomposition and gross N mineralization were both more pronounced in the low than in the high light treatment (Fig. 2 and 4 in paper II). This was possibly caused by the higher

Fig. 5. (A) The relationship between priming of SOM decomposition and soil inorganic N content, (B) Principal component analysis (PCA) of relative individual PLFAs into total microbial biomarker PLFAs in all treatments in paper II and III.
concentration of available N in low light treatment. Sufficient inorganic N to sustain enzyme production, possibly in combination with root exudates that stimulate the extracellular oxidative enzyme activity (Bengtson et al., 2012; Rousk et al., 2015), might be the reason.

In brief, my results showed that the light intensity is an important regulator of the rhizosphere PEs, but also that its effect is plant species dependent.

Elevated CO₂ and N fertilization effects on rhizosphere PEs

In paper IV, rhizosphere PEs on SOM decomposition was positive in the high N fertilization treatment, and negative in the no N and low N fertilization treatments, irrespectively of the CO₂ concentration (Table 1 in paper IV). In the elevated CO₂ treatment, there was no or very low rhizosphere PE on gross N mineralization, regardless of the N fertilization level, while N fertilization resulted in positive rhizosphere PE on N mineralization in the ambient CO₂ treatment (Table 2 in paper IV). The results could not be linked to an effect of elevated CO₂ and N fertilization on the root exudation rate. The findings instead suggest that the effect of N-fertilization and elevated CO₂ on the partitioning of N between plants and microorganisms are more important for SOM decomposition and rhizosphere PEs than the root exudation rate itself. The results also showed that N-fertilization in combination with elevated CO₂ stimulated microbial decomposition of SOM to a greater extent than photosynthesis, meaning that N fertilization in combination with elevated CO₂ can shift the C balance and result in a reduction of soil C stocks in N limited forest ecosystems.

Root exudate effects on the rhizosphere PE

In paper II, my results showed that the light intensity can regulate the root exudation rate, depending on the plant species. Pine seedlings kept under low light intensity exuded more C rate than those kept at a high light intensity, while no difference was found in the root exudation rate of spruce seedlings kept under low and high light intensity (Fig. 1 in paper II). I can only speculate why pine seedlings allocated more C to belowground at low light intensity, but a possible reason is that shaded pine seedlings will invest more in belowground symbionts, so that it can benefit from the transfer of water, C, and N from larger trees through a common mycorrhizal network (Simard et al., 1997; Beiler et al., 2010; Teste et al., 2010). This did not occur in spruce, possibly since spruce achieves optimal photosynthesis
in a lower range of light intensities than pine (Kurets et al., 1994). Regardless, the variation in root exudation rate among treatments could not explain the different rhizosphere PEs in this experiment. As discussed above, it instead appears as if variations in soil N availability was the reason for different rhizosphere PEs between the two species and light intensities.

In paper III, I estimated the root exudation rate in pots with a varying number of pine and spruce seedlings. My results showed that the root exudation rate increased with the number of intact seedlings. It was 213% higher in pots with five seedlings compared to pots with one seedling in the pine treatment, and 108% higher in pots with five seedlings compared to pots with one seedling in the spruce treatment (Fig.1 in paper III). However, higher root exudation rates did not result in a corresponding increase in the rhizosphere PEs. Conversely, the rhizosphere PE of SOM decomposition was lower in the pine treatment with 5 intact seedlings compared to the treatments with 1 or 3 intact seedlings (Table 2 in paper III). A possible reason for the lower rhizosphere PE of SOM decomposition in the treatment with five pine seedlings is that the root exudation rate expressed as per gram root was reduced with almost 50% compared to pots with one intact pine seedling (Fig. 1 in paper III). However, the results again suggested the variation in the rhizosphere PE on SOM decomposition was more strongly regulated by soil N availability than the root exudation rate.

In paper IV, elevated CO2 increased the root exudation rate per gram root, but only when the elevated CO2 treatment was not combined with N fertilization (Fig. 2 in paper IV). The effect of elevated CO2 on root exudation rates could not explain the detected variation of rhizosphere PEs among treatments. As in paper II and paper III, the results rather suggested that the highly variable rhizosphere PEs was induced by CO2 and N fertilization effects on the partitioning of N between plants and microorganisms, which in turn affected the microbial decomposition of SOM.

In summary, my thesis showed that the root exudation rate is not a decisive factor in regulating the rhizosphere PEs. However, it is also possible that measurements of root exudation rates per gram or cm\(^3\) soil poorly represent the exudate concentration at scales relevant for the microbial community. Soil microbial decomposers respond to conditions in their immediate vicinity (Kuzyakov and Blagodatskaya, 2015), meaning that root exudates expressed on weight or volume basis might not be relevant at the scale experienced by microbial decomposers.
Soil C and N availability effect on the depolymerization

In Paper V, labile N addition significantly reduced the chitin depolymerization, while did not affect the depolymerization of protein and lignin (Fig. 1 in paper V). Degradation of chitin appears to have provided the microbial community with N under N limited condition, but chitin was replaced by the added N as the microbial N source when N availability increased. Glucose addition stimulated the depolymerization of SOM and the microbial N demand but reduced the depolymerization of lignin, chitin, and protein (Fig. 1 in paper V). I suggested that this was because the labile C input reduced the mineral or physical protection of native SOM compounds, but this protection was not present in the added lignin, chitin, and protein. Hence, labile C input increased the SOM mobilization, which replaced the lignin, chitin, and protein as the microbial nutrient source. Protein and chitin likely account for a large part of the microbial N sources in soil. Therefore, these results contrast the hypothesis that positive PEs are caused by the microbial decomposition of such N rich polymers under N low conditions (Kuzyakov et al., 2000; Dijkstra et al., 2013).

The role of the different microbial groups in the PEs

In paper III, I used a combination of $^{13}$C and D stable isotope probing methods to investigate the role of different microbial groups in the PE. By quantifying the incorporation of $^{13}$C derived from root exudates and D derived from water into microbial biomarker PLFAs, I attempted to separate microbial groups that grew on root exudates or on resources released by SOM decomposition. Microorganisms that increase their growth on SOM derived compounds mobilized by the PE, as indicated by the incorporation of water derived D into their biomass, without relying on $^{13}$C from root exudates were assumed to be responsible for the PEs (Hungate et al., 2015). Taken together, the microbial $^{13}$C and D incorporation and microbial growth rates detected in the different treatments (Fig. 2 and 3 in paper III), suggested that fungi were responsible for the positive PE in the pine treatment. However, an opportunistic subset of the fungal community that relied on root-derived $^{13}$C while depleting available N, resulted in a reduction of microbial SOM decomposer activity in the spruce treatment, resulting in negative PEs.

These results show that different functional groups in the fungal community can induce both positive and negative rhizosphere PE, meaning that measurements of the total fungal community biomass and activity are not sufficient to elucidate their role in SOM decomposition, nutrient cycling, and PEs.
The role of the extracellular enzyme in the PEs

In paper III, my results showed that the potential activity of extracellular enzymes were all significantly different between the pine and spruce treatments (Fig. 2 in paper III). However, this variation of extracellular enzyme activity alone cannot explain the difference in rhizosphere PEs between the two treatments. My results also suggested that the concentration of C- and N-targeting enzymes were not a limiting factor for SOM decomposition, which might rather have been limited by the activity of oxidative enzymes, such as peroxidases and phenol oxidases. Accordingly, oxidative enzymes that depolymerize macromolecules and make them available for microbial assimilation are of particular importance for the initial stages of SOM decomposition (Hofrichter et al., 2010; Lindahl and Tunlid, 2015). However, since the enzyme activity appears to be more important than the enzyme concentration, which is what the assays I have used measure, the oxidative enzyme concentration could not explain the observed patterns in rhizosphere PEs. In any case, the weak correlation between the potential enzyme activity and the observed rhizosphere PEs suggest that PEs are not a result of increased extracellular enzyme production.

In paper V, the potential N-targeting enzyme activities were not affected by the C addition and partly affected by the N addition. The potential C-targeting enzyme activities showed different responses to the C addition and generally increased by N addition. The potential oxidative enzyme activity response to C and N addition depended on polymers amendment (Table 2 in paper V). Furthermore, the decomposition of lignin-, chitin-, and protein-derived C was only partly connected to the corresponding potential enzyme activities, and the chitin- and protein-derived C recovered did not correlate with the corresponding hydrolytic enzymes. However, the lignin depolymerization was partly matched by the variation in oxidative enzymes (Fig. 2 in paper V). These results together suggested that oxidative enzyme activities might be more representative of depolymerization reactions than hydrolytic enzyme activities.

In summary, these results again showed that the link between extracellular enzyme activity and SOM decomposition is complicated to elucidate, and suggests that the potential activity of hydrolytic and oxidative enzymes are poor indicators of SOM decomposition and the rhizosphere PE.
Conclusion and future perspectives

Rhizosphere interactions are of importance for the release of fixed plant C back to the atmosphere and regulate all aspects of nutrient cycling (Lambers et al., 2008; Stuart et al., 2011; Hopkins et al., 2013). The rhizosphere PE, which is defined as the stimulation or suppression of soil organic matter decomposition in response to labile C input, is increasingly considered as a central role in regulating soil C and N cycling, as well as the atmospheric CO$_2$ concentration (Cheng et al., 2014). However, the underlying mechanisms and controlling factors of rhizosphere PEs are still unclear. In this thesis, I performed a series of experiments to investigate the mechanisms and regulations of the PE on SOM decomposition and gross N mineralization.

In summary, the main findings of my thesis are:

- The magnitude and direction of the rhizosphere PE vary strongly between different tree species.
- Light intensity is an important regulator of the rhizosphere PE, but the effect is plant species dependent.
- The effect of N-fertilization and elevated CO$_2$ on the partitioning of N between plants and microbes is of importance for SOM decomposition and rhizosphere PEs.
- SOM decomposition and the PE are strongly regulated by N availability.
- Fungi are more important than bacteria in regulating the PEs.

Taken together, my findings suggest that soil C and N cycling is strongly affected by the PE, which is interactively controlled by multiple regulators. Soil N availability seems to have a particularly strong influence on the PE, due to its concentration-dependent effect on plant and microbial community interactions, which needs to be considered in order to accurately predict PEs. My results also showed that root exudation rate measured on soil weight or volume basis might not be a relevant measure of the conditions at the scale experienced by microorganisms and that this needs to be considered when we investigating how factors that influence the root exudation rate will also affect the PE. My results further highlight the importance of considering the metabolic diversity of particularly the fungal community when assessing or modeling their role in regulating the PE.
In conclusion, my studies suggest that PEs can strongly influence SOM decomposition and nutrients cycling. Therefore, the regulation of the PE on SOM decomposition and nutrients cycling could play an important role in the terrestrial ecosystem's C and N budget, and their feedback to global change in response to e.g. elevated CO₂ and N deposition.
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List of papers

I. Li, J., Bengtson, P. Quantification of the rhizosphere priming effect differs widely depending on the choice of control

II. Li, J., Zhou, M., Alaei, S., Bengtson, P. Rhizosphere priming effects differ between Norway spruce (Picea abies) and Scots pine (Pinus sylvestris) seedlings cultivated under two levels of light intensity.

III. Li, J., Alaei, S., Zhou, M., Bengtson, P. Root influence on soil nitrogen availability and microbial community dynamics results in contrasting rhizosphere priming effect in pine and spruce soil.

IV. Alaei, S., Li, J., Jackowica-Korczynski, M., Bengtson, P. The influence of elevated CO2 and N fertilization on rhizosphere priming, C sequestration and N cycling in soil planted with Picea abies seedlings.