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Priming of carbon and nitrogen mineralization in forest soils

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Priming of carbon and nitrogen mineralization in forest soils

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LUND UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Decomposition of soil organic matter (SOM) contributes significantly to the global carbon (C) cycle and climate feedbacks. SOM decomposition depends on soil microbial activities, and these activities are driven by the availability of C and other nutrients. Plant root exudates are known to alter decomposition of SOM, a phenomenon referred to as rhizosphere priming effects (RPE). In order to predict the effect of environmental changes such as elevated CO_2 and increased N deposition on microbial SOM decomposition and release of CO_2 to the atmosphere, we need a better understanding of the factors that regulate RPE.

In this thesis, I present my results from priming experiments with and without plants. I studied the effect of root exudates on SOM decomposition by adding glucose to soil to simulate root exudation. I also performed experiments with living plants. The aim was to investigate how variations in C and N availability influence priming. I further aimed to determine how elevated CO_2, N fertilization, and light intensity influence root exudation rates and priming. I also tested how priming influence gross N mineralization and protein depolymerization, and if this could be linked to the abundance of different microbial functional groups and extracellular enzyme activity.

I found that the soil C:N ratio is a poor predictor of priming. Instead, my findings suggest that the C:N imbalance (soil C:N divided by microbial biomass C:N) could better predict priming. My findings suggest that C:N imbalances could induce priming by increasing the abundance of microbes able to decompose complex substrates such as lignin. My results further suggest that priming is a result of enhanced activity of extracellular oxidative enzymes, rather than a change in the concentration of enzymes. I also found that in addition to increasing N cycling rates, soil microbes could meet their increased N demand caused by C input through using the available N more efficiently. This suggests that plant C input might aggravate N limitation by promoting microbial N sequestration.

My findings highlight that elevated CO_2 and N deposition enhance plant C uptake, but they also increase the microbial respiration of SOM to an even greater extent. These findings suggest that in order to evaluate if elevated CO_2 and N deposition increases terrestrial C sequestration, changes in the microbial decomposition of SOM also needs to be accounted for. Finally, my results demonstrated that physiological traits of different plant species, e.g. response to altered light intensity, also have important effects on RPE.

In summary, my findings suggest that priming is of major importance not only for C cycling in forest soils, but also for N cycling. Stoichiometric imbalances in C and N, plant and microbial nutrient demands, and the microbial response to nutrient deficiency, are important factors regulating RPE. I also conclude that priming a result of stimulated activity of extracellular oxidative enzymes, rather than of increased concentration of such enzymes.

Key words: Soil organic matter, C:N imbalance, root exudates, priming, extracellular enzymes

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To my dad - No one has ever given more loving and unconditional support than I have been given by you. I love you, too.

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List of Papers

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

I. Alaei, S., Wild, B., Knorr, W., Rütting, T., Bengtson, P. Priming induced CO_2 and N fluxes are governed by C and N imbalances and microbial nutrient deficiency. *Manuscript.*

II. Wild, B., Alaei, S., Bengtson, P., Bode´, 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.*

III. Alaei, S., Karhu, K., Li, J., Rütting, T., Bengtson, P. Priming of soil organic matter mineralization by glucose additions in boreal forest soils with different C:N ratios. *Manuscript.*

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

V. Alaei, S., Li, J., Jackowicz-Korczynski, M., Bengtson, P. The influence of elevated CO_2 and N fertilization on rhizosphere priming, C sequestration and N cycling in soil planted with *Picea abies* seedlings. *Manuscript.*

Paper II is reprinted with the permission from the publisher.

Author contributions

I. SA, PB, TR, and WK designed the experiment. SA and BW performed the experiment. SA analyzed the data, wrote the manuscript, and interpreted results with the assistance of PB. All co-authors provided comments on manuscript drafts.

II. SA, BW, PB, TR, and designed the experiment. BW and SA performed the experiment. AR, JS, PBX, SB, and WM performed the enzyme and isotopic analyses. BW analyzed the data and wrote the manuscript with input from SA. All co-authors provided comments on manuscript drafts.

III. SA, KK, PB, JL designed the experiment. SA, JL, KK, and performed the experiment. JL performed enzyme analyses and SA performed the rest of the analyses with assistance from JL and KK. SA wrote the manuscript with input from PB, JL, and KK.

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

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

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Popular Science summary

Carbon dioxide (CO_2) is one of the main gases responsible for greenhouse effect, and human CO_2 emissions have a significant effect on the global climate. After the industrial revolution, human activities such as burning of fossil fuels, deforestation, and increased conventional agricultural production have resulted in a substantial increase in the atmospheric C pool from 285 ppm in 1850 to more than 400 ppm. In spite of global efforts to decrease the atmospheric CO_2 concentration, it reached a new record of 408 ppm in 2018. Since recent efforts to reduce human release of CO_2 to the atmosphere have not been successful, we also need to invest more into gaining knowledge about possible approaches to increase sequestration of atmospheric CO_2 into naturally occurring stable C pools. The main path for C sequestration in terrestrial ecosystems is absorption of CO_2 by plants through photosynthesis. A major part of the photosynthetic C ends up in plant biomass as slowly decomposing compounds like lignin and cellulose, but plants also release a proportion of the recently photosynthesized C from their roots to the soil as fast decomposing root exudates. The increasing atmospheric CO_2 concentration is predicted to enhance photosynthesis, but also root exudation rates.

Root exudates contain easily available organic compounds such as soluble sugars. These compounds are able to alter decomposition of soil organic matter (SOM), a phenomenon termed rhizosphere priming effects (RPE). Even though RPE is one of the fundamental processes that govern SOM decomposition it is currently not accounted for in most global C cycling models. A reason for that could be that the level and even direction of RPE (negative or positive) is different in different soils, and that the cause for this is not known. There are two possible reasons for this ambiguity 1. The controlling factors, underlying molecular mechanisms and microorganisms involved in RPE are still not fully understood. 2. The intricate nature of the plant-microbial interactions is hard to investigate. For instance, increased SOM decomposition can also release plant nutrients that stimulate plant CO_2 uptake and growth and. Thus, the total net outcome of processes such as decomposition and photosynthesis determine future changes in soil C sequestration.

Increased atmospheric CO_2 concentration and anthropogenic N deposition are two of well-known environmental changes that are likely to influence RPE. In my studies, I aimed to address some of the unknowns about RPE, with a particular focus on the effect of C and N availability. Since forest ecosystems are important reservoir of terrestrial C, the studies were carried out in forest soils. By using a combination of laboratory and greenhouse experiments, I investigated the effect of variations in C and N availability on priming of C and N mineralization, as well as on measures of microbial decomposition activities, such as potential activities of extracellular enzymes that decompose SOM.

My findings suggest that the soil C:N ratio is not a good predictor for priming. I suggest that C:N imbalance, which considers both microbial nutrient demand and nutrient provision, could be a better predictor of microbial SOM decomposition and RPE. My findings also suggest that C to N imbalances induce priming because soil microorganisms degrade SOM through enhanced oxidative enzyme activity to acquire their limiting nutrients. Finally, my results highlight that elevated CO_2 and N deposition could enhance plant C uptake, but they also could increase the soil CO_2 efflux. These findings suggest that in order to evaluate if elevated CO_2 and N deposition increase terrestrial C sequestration, gross changes in the soil respiration needs to be accounted for.

Introduction

The global C cycle

The global biogeochemical C cycle is defined as the exchange of C between the atmosphere and terrestrial and aquatic ecosystems (Ciais *et al*., 2013). Movement of C between different C pools is the result of various biogeochemical processes such as photosynthesis and respiration (Fig.1).

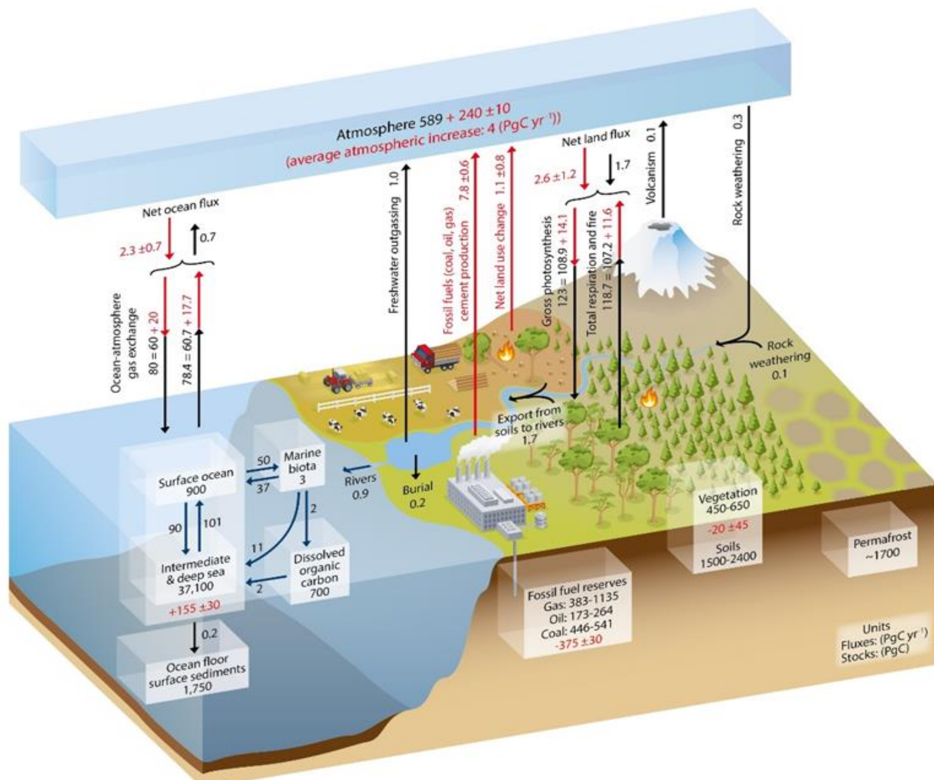

Figure 1. Global C cycle. Numbers in boxes represent reservoir mass (stocks), inventories, or storage in Petra gram of C; PgC (1 Gt) and 2.1 PgC is equivalent to 1 ppm atmospheric CO_2 (Socolow and Lam, 2007). Numbers next to arrows indicate fluxes in PgC/yr. Numbers and arrows in black represent estimates of the pre-industrial so-called natural carbon cycle (ca 1750) and numbers and arrows in red represent estimates of average annual anthropogenic C fluxes (2000 - 2009). Uncertainties are reported as 90% confidence intervals. The image is from Ciais *et al.* (2013).

Atmospheric CO_2 one of the main greenhouse gases (GHGs) responsible for climate change (IPCC, 2013). A part of the sun's infrared radiation, which is reflected from surface of the planet, is trapped by GHGs. Even if trapping the heat in the lower atmosphere is of fundamental importance for preservation of life on earth, the recent increase in the concentration of CO_2 have resulted in a concurrent increase in mean global temperature (IPCC, 2013).

After the industrial revolution, human activities such as burning of fossil fuels, deforestation, and increased agricultural production have raised the concentration of CO_2 in the atmosphere from 285 ppm in 1850 (McCarroll and Loader, 2004) to more than 400 ppm in the current century (Houghton, 2007). Today, human activities are responsible for approximately 9 Gt C emissions to the atmosphere annually, of which approximately 5 Gt C is reabsorbed due to enhanced C uptake by photosynthesis in terrestrial and aquatic ecosystems. The net result is an annual increase of four Gt C in the atmospheric C reservoir (Fig. 1).

The soil C stock (2300 Gt) is approximately three times greater than the current atmospheric C reservoir (Figure. 1). An effective remedy to mitigate the increased atmospheric CO_2 concentration could be to enhance C sequestration in stable C pools in the soil through landscape management and agro-ecological practices (Lal, 2000; Post and Kwon, 2000; Zomer *et al*., 2017). Two of the main fluxes between the soil C reservoir and the atmospheric C pool are the input of plant-derived organic C into the soil, and microbial decomposition of soil organic matter (SOM). The latter results in release of soil C to the atmosphere as CO_2. Plant-derived C enters the soil either as above ground or belowground plant litter, or as root exudates. While a substantial part of the plant's litter is in recalcitrant forms such as lignin, root exudates contain easily available C sources like sugars.

Waksman and Starkey (1931) introduced the role of microbial decomposition in the global C cycle. Since then many studies have aimed to reveal the factors that influence the soil microbial activity and elucidate the mechanisms involved in the microbial decomposition of SOM. Special attention has been given to forest ecosystems, since forests are quantitatively important reservoirs of soil C. For instance, the boreal forests soils alone contain approximately 30% of global terrestrial C (Bradshaw *et al*., 2009; Bradshaw and Warkentin, 2015).

The soil CO_2 flux to the atmosphere is approximately ten times higher than anthropogenic CO_2 emissions (Schlesinger and Andrews, 2000; Raich *et al*., 2002). Root respiration and microbial respiration of SOM are the two main processes contributing to the soil CO_2 efflux (Kuzyakov, 2006). Therefore, even a small change in SOM decomposition can profoundly influence the soil CO_2 emissions to the atmospheric C pool (Todd-Brown *et al*, 2014), which could potentially have a profound impact on climate change (Schlesinger and Andrews, 2000). In other

word, changes in the soil C balance can turn the soil from a sink to a source of C, or vice versa, depending on how the activity and composition of the soil microbial community responds to future climate conditions (Carney *et al*. 2007; Garcia-Pausas & Paterson 2011).

Plant roots contribute to the soil CO_2 efflux through their own respiration and by exuding labile C compounds through their roots. These labile C compounds can have a profound impact on the microbial decomposition and respiration of SOM (Kuzyakov, 2002; Hütsch *et al*. 2002; Blagodatskaya and Kuzyakov, 2008). The effect of labile C compounds on decomposition of SOM is termed "priming effects" (PE), and when the effect is caused by root exudates it is further specified as "rhizosphere priming effects" (RPE) (Helal and Sauerbeck, 1984; Cheng *et al*., 2003; Cheng *et al*., 2014). The magnitude of RPE varies widely among different studies (Cheng *et al*., 2014), ranging from negative effects (Kuzyakov and Cheng, 2001) to four-fold increases in SOM decomposition (Cheng *et al*., 2003). These results suggests that RPE is one of the quantitatively most important processes in the decomposition of SOM, and that RPE is of major importance for how soil C stocks and nutrient cycling will respond to a future climate (Cheng *et al*., 2014; Finzi *et al*., 2015). The increased atmospheric CO_2 concentration is likely to increase plant derived C input to soil (Kuzyakov and Cheng, 2001; Pendall *et al*., 2004; Phillips *et al*, 2009), and increased root exudation could lead to stronger RPE (Bengtson *et al*., 2012). Therefore, in order to estimate how future environmental changes will affect soil C stocks in terrestrial ecosystems, a better understanding of RPE is crucial. Even so, RPE is currently not accounted for in most global C cycling models.

The main aim of this thesis was to investigate the effect of C and N availability on priming of C and N mineralization, and if this could be linked to the potential activities of extracellular enzymes, the abundance of different microbial functional groups, and fungal and bacterial growth rates. I have focused on the priming effects of recently photosynthesized C inputs to the soil, and the term "labile C" refers to the organic C content of the root exudates or compounds found in root exudates, e.g. glucose. Priming effects of more complex substrates, e.g. plant litter, are not included in this thesis.

Factors influencing the priming effects

C and other nutrients

Since RPE is believed to be the result of root exudation, it is reasonable to assume that the magnitude of RPE is related to the root exudation rate. Accordingly, Bengtson *et al*. (2012) found that Sitka spruce (*Picea sitchensis*); Ponderosa pine (*Picea sitchensis*) and Western hemlock (*Tsuga heterophylla*) caused RPE that increased SOM decomposition by between 152-244% in a growth chamber experiment. The variation in priming was linearly related to root exudation rates, with higher priming found at high root exudation rates. Likewise, Yin *et al*, (2014) quantified root exudation rates *in situ* and found that white oak (*Quercus alba*) and American beech (*Fagus grandifolia*) exuded almost twice as much C than sugar maple (*Acer saccharum Marsh*) and tulip poplar (*Liriodendron tulipifera*). There was a positive linear relationship between the RPE and root exudation rates in this study as well.

The findings above have recently been reproduced in a comprehensive laboratory study. Liu *et al*. (2017), estimated the priming effects in a seven week incubation experiment by adding different levels of ^{13}C labelled-glucose once a week (8 to 1606 mg kg^{-1} $week^{-1}$) to different soil types (cool desert grassland, Pinion-juniper woodland, ponderosa pine forest, and mix conifer forest). They found a positive and linear correlation between priming and glucose addition in all soils. Moreover, the results did not show any saturation stage for added glucose. A possible cause for these observations is that bioavailable C restricts the growth and activity of soil microorganisms in many ecosystems (Bååth, 2001; Demoling *et al*., 2007; Kamble *et al*., 2013). Inputs of labile organic C could relieve this limitation and enhance microbial decomposition of SOM to obtain other nutrients necessary for a balanced growth (Paterson and Sim, 2013). Furthermore, soil microbes require extra-cellular enzymes to decompose SOM (Schimel and Weintraub, 2003) and labile organic C in root exudates can provide the required energy needed for enzyme production (Blagodatskaya and Kuzyakov, 2008; Brzostek *et al*., 2013)

However, several lab studies have produced results that contradict the view that priming increases with increasing input of labile C. For example, in a study by Cheng (1996), root-derived C from wheat (*Triticum aestivum*) grown in agricultural soil resulted in a 37.3% decrease in SOM decomposition. Similarly, in a study by Rousk *et al*. (2016) a one-time addition of glucose resulted in decreased respiration of SOM the week after the addition. A possible reason is that when microbes have

access to other mineral nutrients, microbial utilization of easily available labile C tends to be preferred over SOM decomposition (Blagodatskaya *et al*., 2007). This results in decreased decomposition rates, and suggests that nutrients other than C, e.g. N, might have a strong modifying effect on RPE (Paterson and Sim, 2013). Accordingly, in a study by Murphy *et al*. (2017) addition of nutrients (N, P, and K) to soil planted with *Lolium perenne* reduced SOM-C mineralization. Their results suggest that plant-microbial interactions mediate RPE, and that the magnitudes of these effects are moderated by availability of nutrients, e.g. N.

Nitrogen availability can also influence priming indirectly by influencing root exudation rates. Under N limited conditions plants increase root exudation and input of labile C to the soil (Phillips *et al*., 2009), which can result in increased priming. In soils where N availability limits primary production, priming-induced decomposition of SOM could benefit the plant by enhancing the release of bioavailable N (Nie and Pendall, 2016). In contrast, at high N availability plants are likely to reduce belowground C allocation, even if primary production is high (Warembourg and Esterlich, 2001).

In addition to N availability, microbial requirement for phosphorous (P) could also influence RPE (Dijkstra *et al*., 2013). The ratio between N and P in the microbial biomass is lower than this ratio in SOM and plant biomass (Cleveland and Liptzin, 2007). Under conditions where microbial activity is limited by N rather than by P, root exudates could enhance microbial N-miming decomposition activities (Dijkstra *et al*., 2013). On the contrary, when microbial activity is limited by P rather than by N, root exudates could be used for producing hydrolytic enzymes, which releases bioavailable P (Dakora and Phillips, 2002; Dijkstra *et al*., 2013).

In conclusion, the studies cited above demonstrate that the concentration of C as well as other nutrients can have an influence on priming effects. Variations in the concentration of these nutrients relative to each other can also have an influence on plant-microbial interactions and be of importance for regulating of priming effects, which will be explored in the next section.

C:N stoichiometry

Living cells contain specific amounts of different elements, e.g. C and N. Growth and maintenance of soil microorganisms depends on the availability of C and other nutrients in the required proportion. It is likely that microbial communities will be faced with N limitation under conditions where the C:N ratio of microbially available nutrient sources is much higher than the C:N of the microbial biomass,. In contrast, when the soil C:N is much lower than C:N of microbial biomass, the availability of C could limit growth and activity of the soil microbial communities. It is therefore reasonable to suspect that the priming effect is dependent both on the

C:N ratio of the "primer", e.g. root exudates, and the SOM being decomposed. To test this hypothesis, Qiao *et al*. (2016) investigated how the C:N ratio of labile substrates and organic matter (leaf litter, wood, organic matter from organic and mineral horizons) influenced priming effects. They found that labile input with a C:N less than 55, induced minor changes in SOM decomposition with only slight differences among the four OM forms. In contrast, labile input with a C:N higher than 55 induced strong negative priming for the organic matter with high C:N ratios and strong positive priming for organic matter with lower C:N ratios. Their results suggest that priming could be predicted from the C:N ratio of the "primer" and the C:N ratio of decomposing SOM.

Environmental changes in the form of higher atmospheric CO_2 concentration could increase labile C input to the soil via enhanced root exudation (Kuzyakov and Cheng, 2001), and increased N deposition (Vitousek *et al*., 1997; Galloway *et al*., 2008) could enhance the soil N content. This can influence the balance between the soil C:N ratio, and plant-microbial interactions such as competition for nutrients (Bardgett *et al*., 2003; Kuzyakov and Xu, 2013), and as mentioned above, SOM decomposition and priming (Qiao *et al*. 2016). Therefore, in order to understand how environmental changes influence soil C stocks and the net flux of C between soil and the atmosphere, we need a better understanding of the role of C:N stoichiometry in regulating RPE.

Temperature and moisture

Only a few studies that have investigated temperature effects on RPE. Zhu and Cheng (2011) investigated how increased temperature influence RPE by sunflower (*Helianthus annuus*) and soybean (*Glycine max*) in a sandy loam soil. They used continuous ^{13}C-depleted CO_2 labelling in growth chambers and found that Q_{10} of soil respiration ranged between 1.2–1.8 in unplanted soil, and between 2–2.7 in planted soil, implying that the presence of roots increased the temperature sensitivity of SOM decomposition. They further found that RPE caused a 17–163% increase in soil respiration. A possible explanation for higher RPE at higher temperature could be that warming increases root exudation rates. For example, in a study by Yin *et al*. (2013) a 1.8 – 3.6 °C temperature increase enhanced root exudation rate in Chinese spruce (*Picea asperata)* by 78.1% on the basis of root biomass, 68.6% on the basis of root length and 55.0% on the basis of root area. Another possible reason to why priming can increase with temperature is that older SOM is likely to be more recalcitrant, and it has been demonstrated that priming mainly enhances the decomposition of SOM that is several years old (Blagodatskaya *et al*., 2011; Vestergård *et al*., 2016). Decomposition of recalcitrant SOM requires more energy than decomposition of labile organic compounds (Thornley and Cannell, 2001), and reactions with a higher activation energy respond more strongly

to increased temperature (Arrhenius, 1889). As a result, the sensitivity of more recalcitrant SOM pool to increased temperature is stronger than that of labile organic compounds (Bosatta and Agren 1999).

There are also studies that have found RPE to be insensitive to temperature. For instance, Ghee *et al*. (2013) investigated the effect of warming on RPE by applying ^{13}C-enriched glucose daily, and incubating the samples at 15, 20, 25, or 30 °C. They found that basal respiration increased with temperature (Q_{10} =1.6), and that respiration of SOM older than four years was especially sensitive to temperature increases (Q_{10} =2.7). They observed increased SOM-C mineralization in all glucose treatments, but it was that was not sensitive to temperature. Similarly, in a laboratory warming experiment by Hopkins *et al*. (2014), addition of ^{13}C-labelled sucrose resulted in positive priming at all investigated temperatures (5, 15, and 25°C), but the extent of priming was not sensitive to temperature. They suggested that substrate availability restricts priming and not the temperature. Likewise, in a study on the effect of seasonal temperature change (12.8- 22.8 °C) on SOM-C decomposition in unplanted pots and pots planted with cottonwood (*Populus fermontii*), it was found that while SOM-C mineralization was dependent on temperature in unplanted pots, the same was not the case in planted pots (Bader and Cheng, 2007). The authors suggested that the lack of temperature effect on SOM mineralization in planted pots was due to a strong RPE (102%) that masked the temperature sensitivity of SOM respiration. Taken together, the inconsistent results presented above demonstrate the need for more studies on the temperature effect on RPE. Of particular interest is to separate the effect of temperature on root exudation rates from direct effects of temperature on priming rates.

Soil moisture content as well as drying-rewetting cycles can also have an influence on RPE. Dijkstra and Cheng (2007a) studied the effect of soil moisture (45 and 85 % of field capacity) on SOM decomposition and RPE by performing a greenhouse experiment with sunflower (*Helianthus annuus*) and soybean (*Glycine max*). The higher moisture content on average induced higher positive RPE (up to 76% increase in SOM-C mineralization) than in the lower moisture (up to 52% increase in SOM-C mineralization). The highest priming was for soybean at higher moisture content (110% increase in SOM-C mineralization). The authors suggested that the higher RPE at higher moisture content was linked to increased diffusion efficiency of exudates. However, the effect could also be due to higher extent of root exudation at the higher moisture (Gorissen *et al*., 2004).

Drying and rewetting of the soil could also influence RPE. In a study by Zhu and Cheng (2013), repeated drying-rewetting reduced RPE (priming of SOM-C mineralization) to 33%, in comparison with 69% under constantly moist conditions, in a soil planted with sunflower (*Helianthus annuus*). Their results suggest that this effect could be due to lower plant biomass and rhizodeposition in the drying-

rewetting treatment. In contrast, in soil planted with soybean (*Glycine max*) drying-rewetting cycles did not change the plant biomass and rhizodeposition, and the magnitude of RPE (priming of SOM-C mineralization) did not differ between constantly moist and after drying-rewetting cycles (82% vs. 85% respectively) (Zhu and Cheng 2013).

Global warming is only one of the ongoing environmental changes that can influence future SOM decomposition rates and soil C stocks. We therefore need to evaluate the combined effects of other environmental factors besides warming, since the combined interactive effect of different environmental factors on SOM decomposition could be different from the effect of each of the single factors alone. For instance, Reinsch *et al* (2013) investigated the effects of elevated CO_2, higher temperature, and drought on priming, as well as combined effect of these factors. They found positive priming (<15% increase in SOM mineralization) in both ambient and elevated CO_2 concentrations but not under the combination of assessed climatic factors. This could suggest that the combined effects of such environmental factors could counterbalance each other and maintain ecosystem stability.

Plant species and physiology

As discussed above, the root exudation rate and soil N concentration appears to be two of the main factors determining the magnitude of priming. It is therefore reasonable to suspect that different plant species induce different priming responses due to variations in e.g. root exudation rates and nutrient uptake from soil. Accordingly, several studies that have investigated RPE using different plant species, grown in the same soil under the same environmental condition, indicate that RPE varies among species (Fu and Cheng, 2002; Dijkstra & Cheng, 2007b; Bengtson *et al.*, 2012; Xu *et al.*, 2018; Yin *et al.*, 2018). For example, the study by Fu and Cheng (2002) demonstrated that soybean (*Glycine max*) (69.9%) induced higher RPE than sunflower (*Triticum aestivum*) (69.9% vs. 38.5% increase). Likewise, roots of ponderosa pine (*Pinus ponderosa*) induced higher RPE than Fremont cottonwood (*Populus fremontii*) (Dijkstra & Cheng, 2007b). The study by Bengtson *et al*. (2012) suggested that RPE induced by Sitka spruce (*Picea sitchensis,* 244%) is consistently higher than the effect induced by ponderosa pine (*Picea sitchensis*, 156%) or Western hemlock (*Tsuga heterophylla*, 152%). One reason for these variations could be the effect of physiological differences among plant species, such as plant biomass (Dijkstra *et al*, 2006), photosynthetic rate (Kuzyakov and Cheng, 2001) and root exudation rates (Bengtson *et al.*, 2012). Since high photosynthesis generally coincides with high root exudation rates, woody plant species cause higher RPE than herbaceous species due to higher primary production and root exudation rates (Cheng *et al*, 2003; Dijkstra *et al.*, 2006; Bader and Cheng, 2007; Phillips *et al.*, 2011).

Microbial communities involved in priming

The role of different microbial functional groups in priming is not yet clear. It is commonly suggested that priming is the result of uptake and immobilization of labile C by fast-growing microbial r-strategists, which is followed by the emergence of a population of slow-growing oligotrophic decomposers that are responsible for the priming (Fontaine *et al*, 2003; Fontaine *et al*., 2011; Perveen *et al*., 2014). It is usually presumed that the r-strategists consist of bacteria, while K-strategists consist of saprotrophic fungi. However, direct experimental evidence supporting this hypothesis is scarce. Garcia-Pausas and Paterson (2011) found positive priming concurrent with an increase in the uptake of ^{13}C-glucose in the microbial specific biomarkers of fungi and actinomycetes, suggesting an important role of these microbial groups in priming. However, there are also studies that have suggested that bacteria are responsible for RPE (Nottingham *et al*., 2009; Bird *et al*, 2011), while Rousk *et al*. (2015) found that addition of glucose (0-4000 g^{-1} C g^{-1}) resulted in a priming effect of up to 300-350% even if there was no clear response of microbial growth associated with the observed priming.

Another microbial functional group that could possibly contribute to priming is mycorrhizal fungi, but the role of mycorrhizal fungi in RPE is not yet clear. Some studies have demonstrated that mycorrhizal fungi may at least play an indirect role in RPE. For example, Kaiser et al. (2014) used $^{13}CO_2$ labelling to trace the flow of C from wheat (*Triticum aestivum*) to the soil microbial community. Their results suggest that arbuscular mycorrhizal fungi (AMF) associations enhanced the transfer of recently photo-assimilated C to other soil microorganisms. Since the extent of priming commonly is related to the input rate of labile (see discussion above), AMF associations might therefore result in enhanced priming. Accordingly, a study by Paterson *et al*. (2016) demonstrated that AMF associated with *Lolium perenne* strongly enhanced mineralization of SOM.

Ectomycorrhizal fungi (EMF) might have an even stronger influence on RPE. In a field experiment by Yin *et al*. (2014) it was demonstrated that mass-specific exudation rates from white oak (*Quercus alba*) and American beech (*Fagus grandifolia*), which are associated with EMF, were approximately two times greater than sugar maple (*Acer saccharum Marsh*) and tulip poplar (*Liriodendron tulipifera*), which are associated with AMF. The difference resulted in 67% higher RPE in the soil with EMF-associated plants compared to in soil with AMF-associated plants. However, a possible alternative explanation to the observation that EMF caused higher RPE due to higher exudation rates is that EMF themselves are known to decompose SOM (Brzostek *et al*., 2013). In contrast, AMF fungi only have limited means to decompose SOM (Veresoglou *et al*., 2012).

Why priming occurs?

One of the first theories trying to explain priming was the microbial succession theory (Fontaine *et al.*, 2003; Fontaine *et al.*, 2011). As described above this hypothesis suggests that priming is caused by a shift in microbial community composition, such that labile C is first taken up by fast-growing microorganisms (termed as r-strategists). As these microbes turn over, the abundance of slow growing K-strategists microorganisms increases which results in increased SOM decomposition. The theory has been questioned based on evidence that bacteria play a major role in RPE (Nottingham *et al.*, 2009; Bird *et al*, 2011). Furthermore, as mentioned above priming seems to occur even if there is no evidence of a shift in fungal and bacterial growth (Rousk *et al*. 2015). Rousk *et al*. (2015) suggested that priming might rather be the result of increased activity of extracellular enzymes triggered by the input of labile C. This had previously also been suggested by Bengtson *et al*. (2012), who proposed that labile C input could increase in the activity of SOM degrading oxidative enzymes. These oxidative enzymes, e.g. peroxidases, decompose recalcitrant SOM (Drake *et al*., 2011), but their activity depends on the supply and regeneration of hydrogen peroxide (H_2O_2). Labile C compounds (e.g. sugars in root exudates) can serve as a substrate for production of H_2O_2 by oxidases (Ander and Marzullo, 1997; Halliwell and Gutteridge, 1999). However, direct evidence in support of this theory is currently lacking.

A more common view is that priming is a result of increased enzyme production (e.g. Hamer and Marschner 2005b), rather than of stimulated enzyme activity. This theory is based in the fact that degradation of SOM relies on extracellular enzymes (Marxsen and Witzel 1991; Allison and Vitousek, 2005), and labile C could supply the energy needed for enzymes synthesis (Schimel and Weintraub, 2003). Accordingly, Asmar *et al*. (1994) have demonstrated that input of labile C (glucose) accelerates production of dehydrogenase and extracellular proteases, but other studies have not found this to be the case (Geisseler and Horwath, 2008; Wild *et al*, 2017). A possible reason to the contradictory results might be that priming caused by increased enzyme production can only occur if there is a balanced input of C and N, which is also known as the stoichiometric decomposition hypothesis. This theory predicts that C and N stoichiometry regulates the SOM decomposition, such that provision of C and N in proportions matching the C:N ratio of microbial biomass triggers microbial decomposition activity (Hessen *et al*., 2004) and priming effects (Drake *et al*., 2013). In other words, the stoichiometric decomposition hypothesis implies that a balanced availability of C and N induces priming, while microbial nutrient limitation (e.g. N deficiency) decreases microbial decomposition of SOM. However, this is not what is generally observed. In fact, as noted above it seems as if low N availability results in high RPE, which is in line with a theory referred to as the ‘microbial N mining’ hypothesis (Craine *et al*., 2007; Fontaine *et al*., 2011).

This theory suggests that microbial decomposer communities use the labile C input to decompose recalcitrant SOM in order to release N required for growth and activity, while excess C contained in SOM is respired. On the other hand, at high availability of C as well as N, microorganisms switch from SOM decomposition to direct uptake of the available C and N, which might result in negative priming, i.e. reduced SOM decomposition (Cheng, 1999; Blagodatskaya *et al*. 2007). The latter phenomena is referred to as the preferential substrate utilization hypothesis, which implies that soil microbial communities adjust between decomposition of SOM and utilization of more labile C based on the energy cost and nutrients gain of SOM decomposition (Cheng, 1999).

Methods commonly used to study priming

Most priming studies have quantified the priming effect by measuring how soil respiration responds to labile C input (Kuzyakov *et al*. 2000; Cheng *et al*, 2014). Since the measured respiration rate comprises of both SOM-derived CO_2 and CO_2 originating from direct respiration of the labile C input, we need to determine the contribution of the two to sources to the total soil respiration. This is commonly achieved using isotopic techniques (Paterson *et al*, 2009). Below, I have reviewed the most frequently used methods in priming studies.

Methods with plants

Priming studies that includes plants provide a more realistic assessment of priming than methods where labile C compounds are added to unplanted soil. For example, the presence of plant roots accounts for the physical functions of the roots, takes into account the possibility for competition between plants and soil microorganisms for nutrients, as well as the concurrent effects of all different exudates constituents. However, it is also more challenging to control experiments that include plants and separate the multitude of possible effects that roots can have on decomposition rates, besides the effect of root exudation. Furthermore, in studies that include plants priming is usually calculated by subtracting the SOM respiration in unplanted control soil from that in soil containing plant roots. Since the unplanted soil not only lacks input of root exudates, but also is likely to accumulate nutrients and water to a different extent than the planted soil, it can be challenging to separate these effects on soil respiration rates.

Continuous labelling

A common method to quantify priming in the presence of living plant roots is the continuous exposure of the plant shoots to ^{13}C-enriched or ^{13}C-depleted CO_2 over a period of time (Dijkstra *et al*., 2006; Dijkstra and Cheng, 2007; Zhu and Cheng, 2011; Pausch *et al*, 2013; Carrillo *et al*, 2014; Zhu *et al*., 2014). The method allows for calculations of the relative contribution of heterotrophic respiration of SOM and root respiration to the total soil respiration rate, since the two CO_2 sources have a different isotopic ($^{13}C/^{12}C$) signature (Eq.1-2):

$$C_{soil} = C_{total} (\delta^{13}C_{root} - \delta^{13}C_{total}) / (\delta^{13}C_{root} - \delta^{13}C_{soil}) \quad \text{(Eq. 1)}$$

$$C_{root} = C_{total} - C_{soil} \quad \text{(Eq. 2)}$$

Where C_{total}, C_{soil} and C_{root} are the amounts of total respired C, soil-derived C and root-derived C, respectively. Accordingly, $\delta^{13}C_{total}$, $\delta^{13}C_{soil}$, and $\delta^{13}C_{root}$ are $\delta^{13}C$ values of C from total respiration, soil-derived respiration, and root-derived respiration, respectively. Priming is calculated by subtracting the SOM respiration of unplanted soil (control treatment) from SOM respiration in planted soil (Eq. 3).

$$C_{primed} = C\ soil_{planted} - C\ soil_{unplanted} \quad \text{(Eq. 3)}$$

Where $soil_{planted}$, $C\ soil_{unplanted}$, and C_{primed} represent soil derived-C from the planted treatment, respired C from unplanted soil (control treatment), and primed soil-derived C due to the RPE, respectively.

Pulse labelling

In the pulse labelling technique, plants are pulse-labelled with ^{14}C-enriched CO_2 (Kuzyakov *et al*, 1999) or ^{13}C-enriched CO_2 (Bengtson *et al*, 2012). A few days after labelling, the amount of ^{13}C recovered in soil is measured and used to calculate the root exudation rate. Priming is calculated as the difference in SOM decomposition in the treatments with intact plants and the control treatments, where SOM decomposition is assessed by a ^{15}N pool dilution experiment (Bengtson *et al*. 2012):

$$C_{exuded} = {}^{13}C_{soil} \times (t_1/t_2) / (1\text{-}f\ {}^{13}C_{lost}) \quad \text{(Eq. 4)}$$

Where C_{exuded} is root C exudation per day, $^{13}C_{soil}$ is ^{13}C content recovered in the soil, and $f\,^{13}C_{lost}$ is the fraction of ^{13}C lost from the soil i.e. by respiration. Duration of daily light period is shown as t_2 (h), and t_1 is 24 (h).

$$C_{assimilated} = N_{assimilated} \times C{:}N_{microorganisms} \quad \text{(Eq. 5)}$$

Where $C{:}N_{microorganisms}$ is C:N ratio of microbial biomass and assimilated C and N are shown as $C_{assimilated}$ and $N_{assimilated}$, respectively (Eq. 5).

$$SOM_{decomposed} = C_{assimilated} / CUE \quad \text{(Eq. 6)}$$

$$SOM_{primed} = SOM_{decomposed} - SOM_{decomposed\ in\ control} \quad \text{(Eq. 7)}$$

$SOM_{decomposed}$ is decomposed SOM and CUE is carbon use efficiency of microorganisms (Eq. 6) and (Eq. 7). Pulse labelling is challenging because it may result in non-uniform labelling of plant C input, and dilution of labelled input with unlabelled plant C could underestimate input of root-derived C to the soil (Kuzyakov, 2002). Moreover, the method relies on several assumptions that can be challenging to verify (Bengtson *et al*., 2012).

^{13}C natural abundance

C_3 and C_4 plants discriminate against the stable isotope ^{13}C to a different extent during photosynthesis. Consequently, ^{13}C values in C_3 plants (approximately $\delta^{13}C$ -27‰) is dissimilar to C_4 plants ($\delta^{13}C$ -12‰) (Smith and Epstein, 1971). Therefore, C_3 plants that grow in a soil with C_4 cultivation background would respire with a different ^{13}C signature compared to respiration that originates from heterotrophic mineralization of SOM. This means that root respiration can be separated from SOM respiration (Eq. 8 & 9).

$$C_3 = C_{total}\,(\delta^{13}C_{total} - \delta^{13}C_4) / (\delta^{13}C_3 - \delta^{13}C_4) \quad \text{(Eq. 8)}$$

$$C_4 = C_{total} - C_3 \quad \text{(Eq. 9)}$$

Where C_{total}, C_3, and C_4 are the total amount of respired C, respired C from C_3 plant and respired C from C_4 soil, respectively, and $\delta^{13}C_{total}$, $\delta^{13}C_3$, and $\delta^{13}C_4$ are $\delta^{13}C$ values of C from total respiration, root-derived respiration, and soil-derived

respiration, respectively. Priming is then calculated by subtracting the SOM respiration of unplanted soil (control treatment) from SOM respiration in planted soil (Eq. 10).

$$C_{primed} = C_4 - C_{4\ (unplanted)} \qquad \text{(Eq. 10)}$$

Where C_4, $C_{4\ (unplanted)}$, and C_{primed} are respired C from C_4 soil, respired C from unplanted soil (control treatment), and primed soil-derived C due to the RPE, respectively.

Many priming studies have used this method (Cheng, 1996; Fu and Cheng, 2002; Cheng *et al.*, 2003). The method is non-invasive and is applicable for partitioning decomposition of recent vs. old SOM C pools during priming, since periodic changes in C_3-C_4 plants cultivation add distinguishable SOM to the soil. For example. Blagodatskaya *et al.* (2011) used this technique in parallel with application of ^{14}C-labelled substrate to determine the priming effects in old and new SOM pools.

Methods without plants

Priming studies in the absence of plants relies on the addition of ^{13}C or ^{14}C labelled organic compounds representative of compounds in root exudates (Hamer and Marschner, 2005a, 2005b; Nottingham *et al.*, 2009). Calculation of SOM-derived CO_2 compared to respiration of the added substrates is distinguished using the same isotopic mixing models described above, and priming calculated as the difference in SOM respiration in treatments with and without substrate addition. Experiments using these methods are easy to control and convenient for testing new hypotheses. However, they are less realistic than methods with living plant roots.

Objectives of thesis

The objectives of my thesis were to:

- Assess how variations in C and N availability influence priming
- Determine how priming influences depolymerization of nitrogenous organic compounds, gross N mineralization, and plant N availability
- Determine if priming mainly stimulates the oxidative decomposition of complex SOM in order to release bioavailable C and N compounds, or alternatively, if priming is the result of increased production of hydrolytic enzymes that decompose bioavailable C and N compounds
- Determine how elevated CO_2 concentration and N fertilization influence root exudation rates and priming of C and N mineralization
- Test the effect of different light intensity on root exudation and RPE to in different plant species

Hypotheses and approaches of studies

In my first study (**Paper I**), I applied a semi-continuous addition of different concentrations of C (^{13}C-labelled glucose) and inorganic N (ammonium) to the soil via artificial roots (**Fig. 2**).

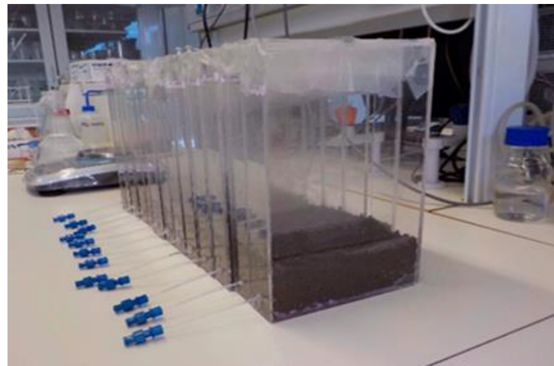

Figure 2. Microcosms consisted of plexiglass boxes containing 250 g soil. Solution containing C and N substrate was transferred to the soil through artificial roots (Paper I).

I investigated how variations in C and N input to the soil influences the extent to which priming is manifested as increased gross protein depolymerization, gross N mineralization, and SOM respiration. I hypothesized that priming is the result of microbial N mining, which enhances the release and uptake of N by soil microorganisms. The hypothesis was tested by measuring gross protein depolymerization, gross amino acid consumption, and gross N mineralization and immobilization. I also investigated how C and N availability influences the abundance of microbial decomposer groups and “cheaters” that do not decompose SOM. For that, I used 96-well MT2 microplates (Biolog, Inc., Hayward, CA, USA) prepared with C substrates of varying recalcitrance. Finally, I aimed at examining if priming mainly stimulates the oxidative decomposition of complex SOM in order to release bioavailable C and N compounds. This was tested by adding a mixture of bioavailable C and N substrates (cellulose, starch, and protein: CSP) to the soil. If

priming mainly enhances oxidative decomposition of recalcitrant SOM to release less recalcitrant compounds, e.g. proteins and carbohydrates, then we would expect priming to be less pronounced under conditions where the concentration of such compounds is high. Conversely, if priming occurs as the result of increased production of hydrolytic enzymes that decompose proteins and carbohydrates, then we would expect priming to be more pronounced where the concentration of such compounds is high.

In my second study (**Paper II**), we studied the effect of soil C and N availability on C and N mineralization and gross depolymerization in a set of boreal forest soils. We hypothesized that an increase in C availability would facilitate the microbial investment in extracellular enzymes that target N-rich SOM polymers and thus promote protein depolymerization, overall leading to higher soil N availability, but also a higher mineralization of SOM-C. I expected that an increase in N availability would have the opposite effects. I further hypothesized that the magnitude of the response to C or N addition would depend on the initial balance between C and N availability in the soil, with strongest C effects where C availability was low, and strongest N effects where N availability was low. I altered soil C or N availability by adding C (^{13}C-labelled glucose) and inorganic N (ammonium) and monitored the release of CO_2 derived from the soil and added substrate over the course of one week. At the end of the incubation, I determined gross rates of protein depolymerization and ammonification using ^{15}N pool dilution assays. I also measured extractable soil C and N pools, microbial biomass, and potential activities of a range of extracellular enzymes involved in the breakdown of different C- and N-rich SOM polymers.

In my third study (**Paper III**), I carried out a one-week incubation experiment on six forest soils with different C:N ratios to investigate the effect of soil C:N ratio on the priming of C and N caused by labile C addition (semi-continuous ^{13}C-labelled glucose addition). I aimed to test if (1) priming of SOM respiration is a result of microbial N mining, and if priming is higher in soils with low N availability (high soil C:N ratio). (2) Priming is caused by an increase in the concentration of C-targeting extracellular enzymes in C-poor soils, and an increase in the concentration of N targeting extracellular enzymes in N-poor soils. (3) The labile C input in soils with unbalanced C:N ratio (dissimilar to microbial C and N requirements) increases the abundance of microbes that decompose more complex SOM.

In this study, the respiration rate was continuously measured using a Picarro analyzer (**Fig. 3**). I also measured the potential activity of a range of hydrolytic and oxidative extracellular enzymes at the end of the incubation, to test the links between priming and potential activity of C and N acquiring extracellular enzymes. Finally, I used a MT2 microplate assay to study the abundance of microbes decomposing substrates of different recalcitrance. As a complementary method, the ^{13}C-labelled

glucose substrate was dissolved in deuterium-labelled deionized water. This enabled us to carry out a novel double labelling approach for microbial community analysis (^{13}C-D PLFA) to assess which microbial groups relied on the labile C input (^{13}C-labelled) and SOM (deuterium-labelled).

Figure 3. PVC microcosms containing soil were connected to Picarro analyzer (G2131-i) through PTFE tubes (Paper III).

In my first priming study with intact plants (**Paper IV**), I aimed to test the effect of light intensity on RPE induced by Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*), which are known to have dissimilar light saturation point (LSP) and light compensation point (LCP). I used a $^{13}CO_2$ pulse-chase experiment to estimate plant photosynthesis as well as exudation rate. In addition, I used ^{15}N pool-dilution method to measure priming of SOM decomposition and N mineralization. I also measured bacterial and fungal growth rates to investigate possible links between RPE and soil microbial growth.

In my last study (**Paper V**), I aimed to quantify the influence of elevated CO_2 and N fertilization on the root exudation rate, microbial growth and priming of C and N mineralization, in a soil planted with Norway spruce (*Picea abies)*. I hypothesized that elevated CO_2 increases root exudation rates. To test the hypothesis I exposed the seedlings to ambient (400 ppm) and elevated CO_2 concentration (600 ppm) for one week (**Fig. 4a**). I also aimed to tease out how the presumed increase in root exudation rate at elevated CO_2 concentration influence priming at different N concentrations, which was achieved by adding two different levels of N fertilizer. I used the ^{13}C pulse-labelling method to determine the photosynthesis and soil respiration (using a Picarro analyzer **Fig. 4b**). Gross N mineralization was measured using the ^{15}N pool dilution method. The root exudation, SOM decomposition, and priming were calculated as in Bengtson *et al*. (2012), using Eq. 4-7. Plant material

was harvested and analyzed to investigate how RPE is linked to plant N uptake, and vice versa.

Figure 4. a) Seedlings were kept under controlled moisture, CO_2 concentration, and light intensity. b) ^{13}C pulse-labelling using plexiglass boxes connected to Picarro analyzer (Paper V).

Methodology

This section provides a short overview of the methods that I used to address the hypotheses in the different studies.

Soil respiration measurement

The effect of labile C input on C mineralization was estimated by measuring soil respiration. Since the measured respiration rate comprise of both SOM-derived CO_2 and CO_2 originating from direct respiration of the labile C input, I used isotope techniques to determine the contribution of each of the two sources in the total soil respiration. In my first three studies (**Paper I-III**), ^{13}C-labelled glucose was added to soil to simulating root exudation. In **Paper I** & **II,** I periodically collected gas samples from mesocosms to estimate the respiration rate. In **Paper III**, I performed continuous respiration measurement using a Picarro system connected to the microcosms. In my greenhouse experiments (**Paper IV** &**Paper V**), I used $^{13}CO_2$-pulse labelling technique to measure photosynthetic C assimilation by seedlings and soil respiration using Picarro system.

Gross N transformation rates

One of the objectives in my priming studies was to test the effect of labile C input on gross N transformation rates. Gross N mineralization was measured using the ^{15}N-pool dilution technique as described in Davidson *et al.* (1991). This method is based on addition of a small amount of ^{15}N (usually ammonium) to the soil, followed by extraction of mineral N immediately (T0) and after a specific time (T1, usually one day). Gross N mineralization is then calculated based on the concentration and the isotopic signature of mineral N in the extracted mineral N pool between the two time points using the FLUAZ model (Mary *et al.*, 1998) (**Paper I, IV** & **V**) or the method proposed by Kirkham and Bartholomew (1954) (**Paper II**).

A large proportion of soil N is contained within nitrogenous organic compounds of varying recalcitrance (i.e. proteins), which only become available for plants and

microbial uptake after depolymerization. Consequently, depolymerization of nitrogenous organic compounds to monomers is considered the rate-limiting step in soil N turnover (Schimel and Bennett, 2004; Jan *et al.*, 2009). In **Paper I** & **II**, I estimated protein depolymerization rate using the ^{15}N pool dilution technique as described by Wanek *et al*. (2010). The method is based on the same principle as the gross N mineralization assay described above, except that the ^{15}N labelled-N source consists of a mixture of twenty ^{15}N-labelled amino acids.

Fungal and bacterial growth rate

The ^{3}H-leucine incorporation technique was used to estimate the bacterial growth rate (**Paper IV** & **Paper V**). This technique is based on the incorporation of the radioactive precursor amino acid (^{3}H-leucine) in bacterial macromolecules produced during a specific time (Bååth, 1994; Bååth *et al*, 2001). A bacterial suspension is produced by homogenization and centrifugation. After addition of the radioactive precursor molecules, the suspension is incubated (2h). Thereafter, the unincorporated leucine is removed by a series of washing steps, and the radioactivity of the samples is measured via a scintillation counter. The amount of ^{3}H-leucine incorporation indicates bacterial growth rate.

I used ^{14}C-acetate incorporation into ergosterol (Bååth, 2001) to estimate fungal growth rate in the soil (**Paper III & Paper V**).Since ergosterol is a fungal specific lipid sterol; ergosterol production rate can be used to estimate the fungal growth rate. Briefly, after addition of the precursor molecules to the soil suspension, samples were incubated (4h) and then ergosterol was extracted. The radioactivity of the extracts was measured in a scintillation counter to estimate ^{14}C-acetate incorporation into the fungal biomass as an index for fungal growth.

Microbial community analyses

Phospholipid fatty acids

Phospholipids exist in all living cells but different subsets of the soil microbial community contain different fatty acids in their phospholipids. This fact is frequently used to estimate the abundance of different groups of microorganisms in soil. Stable isotope probing can be used to investigate the incorporation of a labelled marker into different microbial phospholipids fatty acids (PLFAs) (Nottingham *et al*., 2009; Yao *et al*., 2015). In **Paper III**, I used ^{13}C-labelled glucose and deuterium-labelled deionized water as substrates, which enabled me to estimated incorporation

of ^{13}C and deuterium (D) into microbial PLFAs. The aim was assessing to which extend different microbial groups rely on labile C and SOM for growth. I expected microbes that assimilate D without assimilating ^{13}C in their PLFA to benefit and be responsible for priming

MT2 microplate

In **paper I** & **Paper III**, I determined the abundance of microbes growing on substrates of varying recalcitrance using MT2 microplates (Biolog, Inc., Hayward, CA, USA), where colorimetrical changes of a tetrazolium violet dye contained in the plate-wells indicate microbial utilization of the provided substrate. Briefly, I prepared the wells with C substrates according to the instruction by the manufacturer (Biolog Inc., 1993). Following repeated measurements of the well color development, parameters of the microbial growth curve were then used to estimate the abundance of microbes growing on each substrate. A more detailed description of the calculations is provided in **Paper I** & **Paper III**.

Potential activity of extracellular enzymes

Microbial decomposition activities in the soil are catalyzed by extracellular enzymes. In **Paper II** & **Paper III**, the potential activity of hydrolytic extracellular enzymes including cellobiosidase (CB, targeting cellulose), β-glucosidase (BG, targeting cellulose), N-acetyl-β-D-glucosaminidase (NAG, targeting chitin and peptidoglycan), and leucine-aminopeptidase (LAP, targeting proteins and peptides) was measured using fluorometric and photometric methods modified after Kaiser *et al*. (2010). The potential activity of hydrolytic enzymes was measured fluorometrically by adding fluorometrically labelled substrates to one set of aliquots in four replicates for each soil and enzyme. We used 4- methylumbelliferyl- β -D-cellobiosidase for cellobiosidase, 4-methylumbelliferyl- β -D-glucopyranoside for β -glucosidase, 4-methylumbelliferyl-N-acetyl- β -D-glucosaminide for N-acetyl β -D-glucosaminidase, and L-leucine-7-amido-4-methylcoumarin for leucine-aminopeptidase). Dihydroxyphenylalanine (DOPA) was used to measure the potential activity of phenoloxidase, and, DOPA in combination with H_2O_2 to measure the potential peroxidase activity. The potential C-acquiring enzymes activity were calculated as the sum of the activities of CB and BG and the potential N-acquiring enzymes activities were calculated as the sum of the activities of NAG and LAP. We also calculated the ratio between C and N acquiring enzymes as the ratio between natural logarithm of C and N acquiring enzymes as in Sinsabaugh *et al*. (2008).

Main findings

The effects of C and N availability on priming

If RPE is the result of labile C input to soil, it is reasonable to assume that the extent of priming is related to the extent of labile C input. Accordingly, in **Paper I,** I observed that glucose additions resulted in increased respiration of native SOM, but a strong positive response of SOM respiration to glucose addition (up to 10 times increase) only occurred at the highest glucose addition rate. This is in line with other studies that found a positive link between SOM decomposition and labile C input (Qiao *et al*., 2014, Liu *et al*., 2017, Bengtson *et al*., 2012, Paterson and Sim, 2013; Rousk *et al*., 2015). In **Paper II,** glucose addition did not increase SOM respiration. Likewise, changes in SOM respiration was not related to the root exudation rate in **Paper IV** & **Paper V**.

In **Paper I**, I hypothesized that priming is the result of microbial N mining (Craine *et al*., 2007; Fontaine *et al*., 2011), which enhances the release and uptake of N by soil microorganisms. My results to some extent supported this hypothesis. For instance, when the soil received high concentrations of glucose in combination with inorganic N priming of SOM respiration was less pronounced. However, the results further suggested that priming is not always an N mining response, but rather a result of microbial decomposition of SOM to release the currently deficient nutrient, irrespective of whether the deficient nutrient is C or N. If this is the case and microbial decomposition activities are regulated by the availability of C and N, priming and microbial decomposition of SOM could potentially be predicted by the soil C:N ratio. To test this we measured priming and SOM decomposition in soils with different C:N ratios (**Paper III**). The results showed that glucose additions induced positive priming (12-52% increase in SOM respiration) in all soil types (C:N =11.5-22), but there was no linear relationship between priming and soil C:N ratio.

Contrary to other studies that have suggested that the soil C:N ratio could be an important regulator of the priming effect (Wang *et al*., 2015; Qiao *et al*., 2016), my results suggest that the soil C:N ratio is not a good measure for predicting priming. I instead found that priming of SOM decomposition was negatively correlated to the microbial C:N ratio (**Fig 5a**) and positively linked to the C:N imbalance, where a higher C:N imbalance implies stronger microbial N limitation (**Fig**. **5b**). The C:N

imbalance, which considers both microbial nutrient demand and nutrient provision, is therefore a better predictor of SOM decomposition than the soil C:N ratio. It has also been suggested that the soil C:N ratio is a poor predictor of N-fluxes associated with SOM decomposition (Murphy *et al.*, 2015). A possible reason is that soils with similar C:N ratio could have dissimilar active microbial communities, which induces different microbial demand for nutrients. For instance, higher C:N ratio could favors fungi over bacteria, since fungi are known to have higher biomass C:N ratio (Wallenstein *et al.*, 2006) and higher C use efficiency (Six *et al.*, 2006) than bacteria. Therefore, the fungal C:N imbalance could be expected to be lower than the bacterial C:N imbalance in a soil with high C:N ratio.

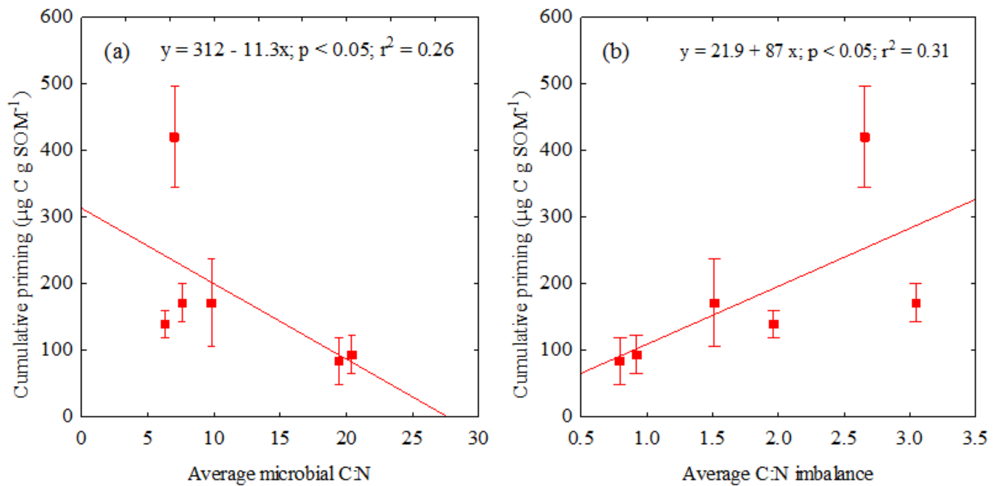

Figure. 5. The relationship between the microbial C:N ratio and cumulative priming and (a) and the relationship between the C:N imbalance and cumulative priming (b). Figure from Paper III.

The role of protein depolymerization in priming

I further tested if priming of SOM decomposition is a result of microbial N mining by measuring the protein depolymerization rate, which I expected to increase if that was the case (**Paper I** & **Paper II**). In **Paper I**, glucose additions increased the gross protein depolymerization rate, the microbial amino acid uptake, and the microbial retention of amino acid N in the biomass, but different indicators of microbial decomposition activities, i.e. SOM respiration, gross protein depolymerization and gross N mineralization, were poorly correlated. In **Paper II,**

the protein depolymerization did not increase although glucose addition promoted microbial growth and N demand. The latter suggests that the soil microorganisms immobilized the already available N more efficiently, as suggested by the observations that gross N mineralization rates and inorganic N concentrations decreased. Taken together the results from **Paper I** and **Paper II** suggest that can cope with N deficiency both by increasing the production of bioavailable N, e.g. ammonium and amino acids, and by using the available N more efficiently.

The role of oxidative and hydrolytic enzymes and different functional microbial groups in priming

Decomposition of SOM depends on extracellular enzymes produced by microorganisms (Marxsen and Witzel 1991; Allison and Vitousek, 2005). Therefore, it is reasonable to assume that priming of SOM decomposition is facilitated by microbial investment of labile C input in extracellular enzymes that target SOM compounds. An emerging question is what role hydrolytic and oxidative enzymes play in priming. I tried to answer this question using two approaches. In the first approach, I measured the potential activity (concentration) of hydrolytic and oxidative extracellular enzymes. In contrast to previous studies (Brzostek *et al.*, 2013), glucose addition to the soil did not influenced the potential extracellular enzyme activities in **Paper II**, even though the glucose input promoted microbial growth and N demand. In **Paper III**, there was no difference in the concentration of oxidative enzymes in the glucose and control treatment, but there was a positive relationship between the potential oxidative enzyme activity and SOM respiration in both the glucose and control treatment. While the slope of the linear relationship between the potential oxidative enzyme activity and SOM respiration was the same in both treatments, respiration of SOM was consistently higher in glucose-amended samples (**Fig. 6).** These results suggest that glucose addition resulted in priming by stimulating the activity rather than the concentration of oxidative enzymes, as previously proposed (Bengtson *et al.*, 2012, Rousk *et al.*, 2015).

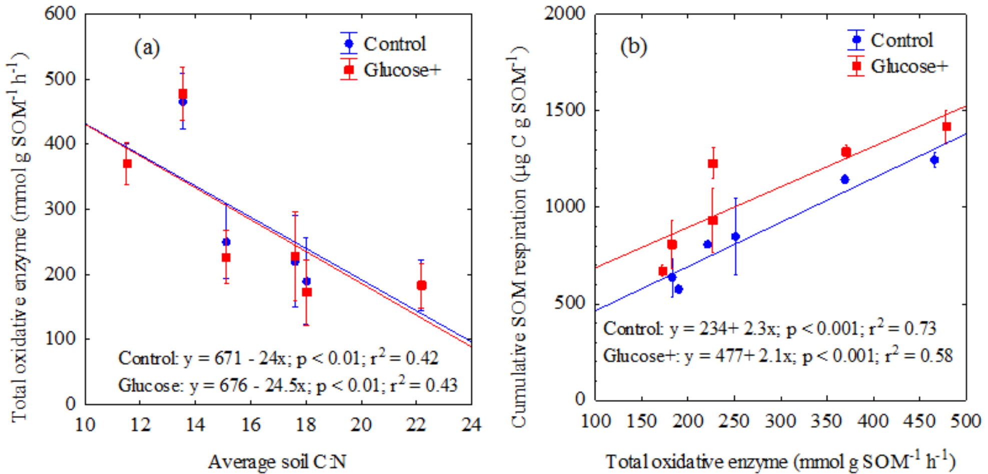

Figure. 6. The relationship between total potential activity oxidative enzymes (Peroxidase+ phenoloxidase) and soil C:N ratio (a) and the relationship between SOM respiration and total potential activity oxidative enzymes (b) at the end of the one-week incubation period. Error bars represent standard error of the mean. Figure from Paper III.

In **Paper I**, I tested if priming mainly stimulates the oxidative decomposition of complex SOM using another approach, where I added a mixture of bioavailable C and N substrates (cellulose, starch, and protein: CSP) to the soil. If priming mainly enhances oxidative decomposition of recalcitrant SOM to release less recalcitrant compounds, e.g. proteins and carbohydrates, then I expected priming to be less pronounced under conditions where the concentration of such compounds is high. Conversely, if priming occurs as the result of increased production of hydrolytic enzymes that decompose proteins and carbohydrates, then I expected priming to be more pronounced where the concentration of such compounds is high.

Glucose addition induced a strong priming effect (up to 10 times increase) on SOM respiration in non-amended soil. At the same time I only observed a small increase in SOM respiration by glucose addition in the CSP treatment, which support our hypothesis that oxidative decomposition play an important role in priming. However, different indicators of microbial decomposition activities, i.e. SOM respiration, gross protein depolymerization and gross N mineralization, responded differently to the glucose additions. While I only observed a weak response in SOM respiration by glucose addition in CSP treatment, gross N mineralization rates responded more strongly to glucose additions in this treatment compared to the non-amended soil.

Glucose addition increased the abundance of microbes decomposing lignin, while the opposite effect was observed when glucose was added in combination with inorganic N (**Fig. 7**). These results imply that priming at least in part is related to the abundance of lignin decomposers, and suggest that an imbalanced C to N supply selects for the trait of being able to release the deficient nutrient by decomposing complex SOM, regardless if the deficient nutrient is C or N. The importance of C:N imbalances for regulating decomposer abundance and priming effects are also in line with my findings in **Paper III**.

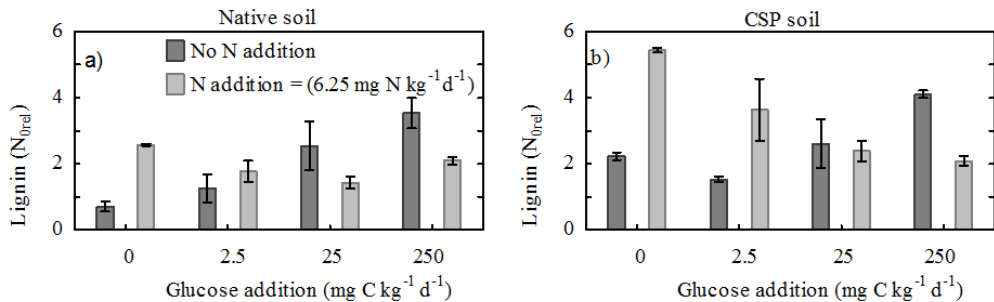

Figure. 7. Abundance of the soil microbes (*N0rel*) growing on lignin, after 12 day of incubation period with C (glucose) and inorganic N addition in native soil treatment (a) and in the soil pre-incubated with CSP (b). Error bars represent standard errors of the mean. Figure from Paper I.

The effect of elevated CO_2 and N fertilization on RPE

Another important factor influencing photosynthesis and plant belowground C allocation is the concentration of CO_2. Increased input of labile C into the soil under elevated CO_2 concentration can increase SOM decomposition and reduce the soil C stocks (Van Groenigen *et al*., 2014). In **Paper V**, I tested the effects of CO_2 and N fertilization on priming. Elevated CO_2 or N fertilization did not induce any significant change in the root exudation rate expressed per gram soil, but elevated CO_2 increased the root exudation rate per gram dry root. When plants were exposed to elevated CO2 in combination with N fertilization this increase was suppressed to the initial level. These findings are in line with previous observation that elevated CO_2 enhances the root exudation rate, while N addition has the opposite effect (Phillips *et al*., 2009).

In **Paper V**, positive priming of SOM decomposition only occurred at the highest N fertilization (**Fig. 8a**, while priming of N mineralization occurred also at the lowest N-fertilization level, but only at ambient CO_2 (**Fig. 8b**). Priming of N mineralization was absent or slightly negative in the elevated CO_2 treatment,

regardless of the N-fertilization level. That is, contrary to what I expected the priming effect was higher in the ambient CO_2 treatment than in the elevated CO_2 treatment. I suggest that these patterns are caused by plant-microbial competition for N, since I could not link the observed priming to the root exudation rate.

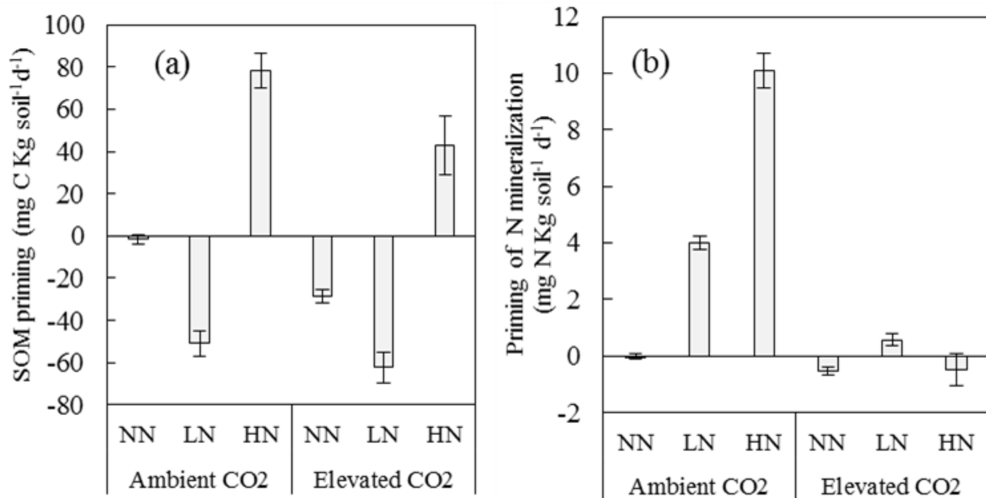

Figure. 8. SOM-C priming (a) and priming of N mineralization. No, low, and high level of N addition is abbreviated to NN, LN, and HN, respectively. Error bars represent 85% CI (n=8). Figure from Paper V.

I further observed that elevated CO_2 in combination with N addition increased photosynthesis (**Fig. 9a**), but also the gross respiration rate to an even greater extent (plant and microbial respiration combined; **Fig. 9b**). The increase in gross respiration could to a large extent be linked to increased microbial decomposition of SOM. These findings suggest that it is not always the case that elevated CO_2 and higher N availability result in higher C sequestration in the soil (Yue *et al.*, 2016), and that the combined effect of elevated CO_2 and N fertilization on the microbial decomposition of stable SOM pools needs to be considered before such forest management practices are proposed.

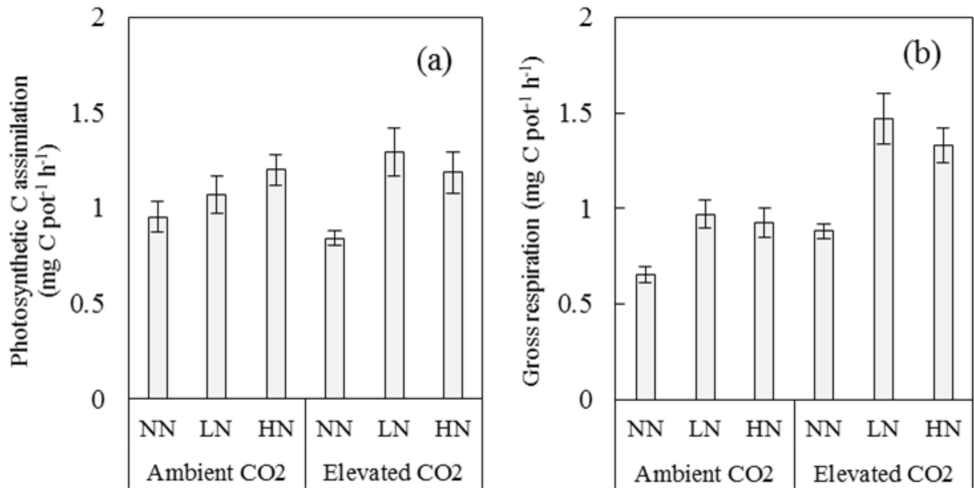

Figure. 9. Photosynthetic C assimilation (a), and gross respiration (b), in treatments incubated at different concertation of CO_2 and different levels of N fertilizer addition. No, low, and high level of N addition is abbreviated to NN, LN, and HN, respectively. Error bars represent standard errors (n=8). Figure from Paper V.

The effect of light intensity on root exudation and RPE

Plant photosynthetic intensity is one of the main factors controlling belowground C allocation and root exudation (Högberg *et al*., 2001; Kuzyakov and Cheng, 2001). In **Paper IV**, I estimated the root exudation rate in seedlings of two plant species (pine and spruce). Spruce is known to have lower light saturation point (LSP) and lower light compensation point (LCP). I expected that spruce seedlings, due their wider range of photosynthetic adaptability, could have higher root exudation rate than pine seedlings, especially at low light intensity. When the seedlings were exposed to low and high light intensities (60 vs. 230 µmol･m-2･s-1±10), the results showed the opposite. Pine seedlings kept at low light intensity had the highest root exudation rate. Even so, priming of C and N mineralization was higher in spruce-planted soil than in pine planted soil. Moreover, low light intensity induced stronger positive priming effects in spruce-planted soil, and stronger negative priming in pine planted soil (Fig. 10a). A possible reason for the negative priming in the pine-planted soil could be plant-microbial competition for N (Yin *et al*., 2018). Accordingly, both the gross N mineralization and inorganic N concentration was lower in the pine-planted soil than in the spruce-planted soil (Fig. 10b). A possible reason to why stronger competition could occur under low light conditions is that shaded seedlings invest more in belowground plant parts and symbionts, in order to benefit from transfer of C and other nutrients from larger trees (Simard *et al*., 1997;

Teste *et al*., 2010; Beiler *et al*., 2010). I therefore suggest that competition for N between roots and microbes (possibly mediated by ectomycorrhizal fungi) could be the reason for the differences between plant species and for the stronger RPE found in the low light treatment.

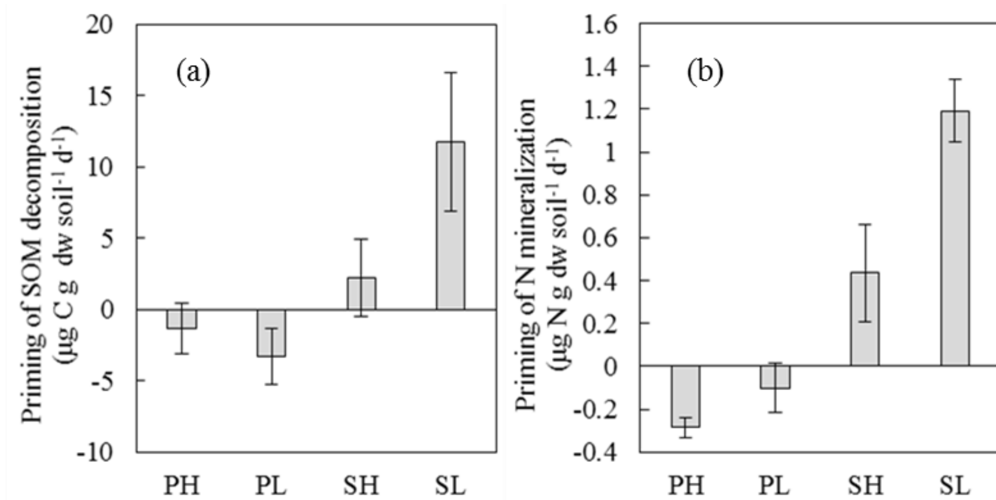

Figure. 10. Priming of SOM decomposition (a) and priming of N mineralization (b) in the soil with pine or spruce seedlings under two levels of light intensities. P represents the pine and S represents the spruce. H represents the high level of light intensity (230 $\mu mol \cdot m^{-2} \cdot s^{-1} \pm 10$) and L represents the low level of light intensity (60 $\mu mol \cdot m^{-2} \cdot s^{-1} \pm 10$). Error bars represents the 85% CI. Figure from Paper IV

Conclusion

One of the main challenges for predicting soil and vegetation feedbacks to changing climate is uncertainties regarding the extent to which SOM decomposition responds to environmental changes (Davidson and Janssens, 2006; Schuur *et al*, 2015). RPE is one of the fundamental processes that controls SOM decomposition and has could provide an important feedback to the global C cycle. However, the controlling factors, underlying molecular mechanisms and microorganisms involved in RPE are still not fully understood. In my studies, I studied some of the factors regulating RPE, with the focus on the effect of C:N stoichiometry on priming of soil C and N mineralization.

In this thesis, I performed experiments without plants (**Paper I-III**) and experiments with plants (**Paper IV** & **Paper V**) to investigate the effects of variations in C and N availability on potential activities of extracellular enzymes, the abundance of different microbial decomposer groups, and priming of SOM decomposition, gross N mineralization, and gross protein depolymerization. The main findings of my thesis are:

- The soil C:N ratio is a poor predictor of priming. Instead, my findings suggest that the C:N imbalance (soil C:N divided by microbial biomass C:N) could better predict microbial SOM decomposition and priming
- An imbalanced C to N supply could select for the microbial trait of being able to release the deficient nutrient (C or N) by decomposing SOM
- Priming is stimulated by the activity rather than the concentration of extracellular enzymes
- C to N imbalances induces priming by enhancing the abundance of microbes that are able to decompose complex SOM such as lignin
- Soil microbes could also meet the increased N demand caused by C input by using the available N more efficiently, suggesting that plant C input might aggravate N limitation by promoting microbial N sequestration

Outlook

My studies provided insights into N transformation rates and the regulation of protein depolymerization (**Paper I** & **Paper II**). I observed that SOM respiration responded in a different way to the C inputs than microbial protein depolymerization, amino acid uptake, and N mineralization. These findings suggest that to estimate priming-induced SOM decomposition and nutrient release in terrestrial environments, we need to move beyond respiration measurements when quantifying priming. Thus, I suggest that indicators of microbial decomposition activities such as gross N mineralization and protein depolymerization should be taken into consideration in future priming studies.

My findings further suggest that the activity of oxidative enzymes limits the decomposition of SOM, and that labile C input could cause priming by increasing the activity of these enzymes (**Paper III**). A possible reason for this could be that the activity of oxidative enzymes is limited by regeneration of H_2O_2 (Bengtson *et al*, 2009), and a labile C substrates like glucose could function as a substrate for H_2O_2 production (Ander and Marzullo, 1997; Halliwell and Gutteridge, 1999). Since the activity of oxidative enzyme systems is considered to be the regulatory step in the decomposition of recalcitrant SOM (Perez *et al.*, 2002), stimulation of H_2O_2 production in response to labile C input might be the main mechanism that results in priming. There is still a need for further studies that could test if this is the case.

I observed that elevated CO_2 concertation increased root exudation rate (per gram dry root), while the combined effect of N addition and elevated CO_2 suppressed this rise back to initial level (**Paper V**). I also found that the same root exudation could induce markedly different priming responses. These findings, together with the intricate effect of light intensity on RPE in different plant species (**Paper IV**), calls for further research on the effect CO_2, N deposition and light intensity on plant-microbial interactions and RPE.

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List of Papers

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

I. Alaei, S., Wild, B., Knorr, W., Rütting, T., Bengtson, P. Priming induced CO_2 and N fluxes are governed by C and N imbalances and microbial nutrient deficiency. *Manuscript.*

II. Wild, B., Alaei, S., Bengtson, P., Bode´, 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.*

III. Alaei, S., Karhu, K., Li, J., Rütting, T., Bengtson, P. Priming of soil organic matter mineralization by glucose additions in boreal forest soils with different C:N ratios. *Manuscript.*

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

V. Alaei, S., Li, J., Jackowicz-Korczynski, M., Bengtson, P. The influence of elevated CO_2 and N fertilization on rhizosphere priming, C sequestration and N cycling in soil planted with *Picea abies* seedlings. *Manuscript.*

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