The effect of resource availability, microbial community composition and abiotic conditions on soil organic matter decomposition
Alice laughed. “There’s no use trying,” she said “One can’t believe impossible things”.

“I dare say you haven’t had much practice” said the Queen. “When I was your age, I always did it for half an hour a day. Why, sometimes I’ve believed as many as six impossible things before breakfast.”

— Lewis Carrol
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Introduction

The atmospheric carbon (C) balance is not only threatened by the introduction of new anthropogenic C flows, such as burning of fossil fuels or emissions caused by land use change, but also by the perturbation of natural C flows between terrestrial ecosystems and the atmosphere (IPCC 2007). The terrestrial C cycle is governed by two major processes: net primary production (i.e. photosynthetic fixation of CO₂ minus autotrophic respiration) on the input front and heterotrophic respiration by microbial decomposers and animals from an output perspective (Prentice et al. 2001). The rates of these two processes are roughly balanced for ecosystems near to steady state (Chapin et al. 2009), which lead to a total global flux of approximately 60 Gt C per year between the atmosphere and the terrestrial biosphere in either direction (Prentice et al. 2001). However, both processes respond to changing environmental conditions but not necessarily in the same way and are due to the complexity of natural systems, not always easy to predict (Trumbore 2006, IPCC 2007). Net primary production, for example, has been found to respond to variations in atmospheric CO₂ concentrations, temperature, precipitation, and nitrogen (N) deposition (Schulze 2006, LeBauer & Treseder 2008, Friend 2010). In addition, there are, however, other factors that go far beyond a direct physiological effect on photosynthesis, e.g. changes in plant species composition or in season length, which have the potential to fundamentally alter ecosystem carbon dynamics (De Deyn et al. 2008).

The process that counters net primary production in terms of carbon cycling, i.e. the decomposition of soil organic matter is primarily controlled by microorganisms. Decomposition of organic matter is directly affected by changing temperature and soil moisture and by the availability of nutrients (Mack et al. 2004, Fierer et al. 2006, Meier & Leuschner 2010). Although less obvious than for net primary production, environmental change may affect decomposition of soil organic matter in a similarly complex way that goes beyond simple microbial physiology. Potentially important mechanisms for a C cycle feedback of decomposition, which have increasingly drawn attention in the last years, include the link between microbial community composition and function, plant-soil interactions, and the role of mycorrhizal fungi for decomposition processes (Bardgett et al. 2005, Chapin et al. 2009, Bahn et al. 2010, Courty et al. 2010). There are, however, still considerable knowledge gaps on how these factors may influence decomposition processes (Chapin et al. 2009, Bahn et al. 2010). Exploring those mechanisms may thus be a critical step towards a better understanding of the terrestrial ecosystem-climate feedback.

Decomposition in Arctic soils: Effect of physical and abiotic factors

It is well known, that soil organic matter decomposition is susceptible to changing abiotic conditions. It had been shown, for example, that atmospheric warming and soil water content significantly affect decomposition rates in soil (Thomsen et al. 1999, Miller et al. 2005, Fierer et al. 2006, Allison & Treseder 2008, Biasi et al. 2008, Karhu et al. 2010, Meier & Leuschner 2010). Decomposition in cold biomes may be adapted to low temperatures, e.g. by sustained microbial activity in frozen soils, or by a greater potential of the cold-adapted
community to degrade organic matter at low temperatures, compared to microbial communities of other ecosystems (Santruckova et al. 2003, Schimel et al. 2007). The consensus is that long-term exposure to unfavorable abiotic conditions, such as low temperature and high soil moisture has substantially delayed decomposition processes in soils of arctic regions and therefore has led to the accumulation of large amounts of soil C in the Arctic. Although the northern permafrost region covers only 16% of the global soil area, it has been estimated to store 1672 Gt C, which is approximately 50% of the global soil C pool and more than double the amount of C present in the atmosphere (Tarnocai et al. 2009). It is likely that global climate change will have a larger effect on northern latitudes and thereby lead to a higher regional warming in the Arctic, compared to the rest of the world (Kattsov & Källen 2004). Thus, the large amount of C stored in Arctic soils may be under threat by increasing decomposition rates caused by warmer temperatures (Zimov et al. 2006, Khvorostyanov et al. 2008, Schuur et al. 2008, McGuire et al. 2009). The response of Arctic soils to warming is however difficult to predict (Davidson & Janssens 2006). One reason for that is that the C is unevenly distributed in Arctic soils: a large part of the Arctic is or has been affected by cryoturbations, processes which lead, by repeated freezing and thawing of the soil, to the intermixing of soil layers in the soil profile and thereby to broken, warped and distorted soil horizons. The translocation of C-rich topsoil layers into deeper, colder and wetter soil layers may considerably slow down the decomposition of this C pool (Ping et al. 2008). It has been found that Arctic soils with signs of cryoturbation were those which had the highest C stocks (Michaelson et al. 1996), which emphasizes the importance of the process of cryoturbation for soil organic matter storage in arctic soils and thus for the global C cycle.

The effect of resource availability on microbial processes and microbial community composition

In temperate ecosystems, physical controls such as temperature and moisture are probably less important than in arctic systems, instead the availability of labile C and N may play a key role in determining decomposition rates (Schimel & Weintraub 2003). The stoichiometry of available key elements (predominantly C and N) determines microbial growth and activity, because microbes have to maintain a relatively constraint C:N ratio in their biomass, which is usually lower than that of their substrate (Makino et al. 2003). An increasing number of studies in the last years have explored the effect of enhanced N availability on decomposition rates, with sometimes contrasting results (Saiya-Cork et al. 2002, Mack et al. 2004, Knorr et al. 2005, Janssens et al. 2010). Increased N availability in a long term soil fertilisation experiment in the arctic tundra, for example, had caused a substantial C loss from deep soil layers (Mack et al. 2004) whereas soil decomposition rates of temperate forests have been shown to decrease in response to increased N availability in most of the N-addition experiments conducted in the last years (Janssens et al. 2010). However, under certain conditions the opposite effect occurred, and N addition led to the acceleration of soil organic matter decomposition in temperate forests. The latter happened mostly when forests were strongly N limited or when N addition was followed by enhanced photosynthesis (e.g. in very young forests or in experiments with elevated CO₂). The results of these studies suggest that
N limited microbes are able to increase growth and activity by the addition of N, whereas N addition may have a completely different, even opposing, effect when the microbial community is not N limited.

These effects, however, may possibly be disguised by more complex responses of the soil biosphere, for example when altered C or N availability induces a shift in the microbial community composition. Microbes are structurally very diverse, ranging from small, single-celled, ball-shaped bacteria to fungal hyphae networks and may thus exhibit different C:N ratios as well as different abilities to store excess C and N, and therefore different C and N demands for growth (Elser et al. 2003). Additionally, due to different surface-to-volume-ratios, microbes may exhibit greatly different uptake rates for available nutrients. Varying C and N availability may favour some species, and be detrimental to others, thereby provoking community changes. The addition of easily available N may for example favour more efficient (i.e. faster growing), but N-demanding species which may be able to outcompete slower growing species that are instead specialised on degrading complex substrates and thus may have an advantage in times of low N availability.

**Belowground C allocation**

The largest input of readily assimilable C to the soil comes from belowground C allocation by plants. A considerable part of recent photosynthates is transported from the tree canopy to the root-mycorrhizal system and subsequently excreted to the soil, where it serves as an important energy source for soil microbes (Högberg & Read 2006). This leads to the establishment of a specific rhizospheric community consisting of mycorrhizal fungi and other microorganisms that thrive on the rhizosphere substrates. Half of the microbial activity in a forest soil is thought to be fuelled by recent photosynthates, which were assimilated by the autotrophs only a few days prior to exudation (Högberg et al. 2008). Moreover, a large part of this tree derived C is stored in the mycorrhizal hyphal networks which make up the most dominant C input to soil organic matter pool, exceeding even inputs of leaf and fine root litter (Godbold et al. 2006).

**Rhizosphere priming effect**

Belowground C input by tree roots has been shown to increase the decomposition of recalcitrant soil organic matter (Carney et al. 2007, Dijkstra & Cheng 2007, Ekberg et al. 2007). Experimental doubling of CO₂ concentrations, for example, has led to a long-term net loss of C from the soil in spite of higher plant growth, due to enhanced microbial degradation of soil organic matter (Carney et al. 2007). In a 13 month greenhouse study, soil organic matter decomposition was significantly greater in soils planted with trees compared to unplanted controls (Dijkstra & Cheng 2007). These and other studies indicate that interactions between soil and tree roots accelerate soil organic matter decomposition. The mechanisms underlying this so called ‘rhizosphere priming effect’ are, however, not completely resolved (Fontaine et al. 2003, Kuzyakov et al. 2009, Paterson 2009). The degradation of humified soil organic matter is a highly energy demanding process which does not release as much C as
required for its degradation, and therefore proceeds at slow rates in the soil (Kuzyakov 2002, Kuzyakov et al. 2009). If energy limitation is relieved by the input of easily available C, microbes may be able to enhance soil organic matter degradation in order to gain limiting nutrients (Paterson 2009), which may be one possible explanation for the rhizosphere priming effect. In contrast, some studies suggest that the rhizosphere priming effect does not come from increased breakdown of recalcitrant soil organic matter, but from improved turnover of the microbial biomass and subsequent higher mineralisation rates of microbial endocellular reserves (De Nobili et al. 2001, Weintraub et al. 2007). Thus, the rhizosphere priming effect may be more complex than previously thought. (Blagodatskaya & Kuzyakov 2008) recently suggested the presence of a short-term priming effect driven by the activation of endogenous microbial resources, which in turn facilitates a long term priming effect, involving the activity of specialist soil microbes capable of degrading complex soil C and N resources. However, so far there is little scientific data to back up this theory and the experimental evidence that unravels the priming mechanism in natural systems is still missing, highlighting the need for further research.

The effect of microbial community dynamics on decomposition functions: extracellular enzyme activities

The widely observed phenomenon that varying C and N availabilities affect both, decomposition rates and microbial community composition (Brant et al. 2006, Phillips & Fahey 2006, Carney et al. 2007), indicates that there may be a link between these two factors. A precondition for such a link, is that microbial taxa or groups need to be functionally different from each other. Some microbial functions (e.g., N\textsubscript{2} fixation, nitrification, lignin degradation) are known to be restricted to certain microbial species, although many of these processes may be highly redundant within the microbial community. Due to the vast microbial diversity and the difficulty to link functions to species within natural microbial communities, it may thus be useful to define ‘functional groups’, which comprise microbes with specific ecological functions regardless of their phylogenetic classification (Hodkinson & Wookey 1999, Prosser et al. 2007). One possible way to classify microbes according to their ecological function may be by using their ability to produce specific extracellular enzymes to decompose important substrates in the soil (e.g. lignin, cellulose, humified soil organic matter, proteins). Both, the ability and the capacity to produce certain enzymes may differ widely among the microbial community, although the majority of species contributing to a certain functional group of enzyme producers is probably still unexplored due to the fact that many microbes can not be cultured (van der Heijden et al. 2008). Linking microbial diversity to ecosystem function is one of the most challenging tasks in current microbial ecology that requires the application and combination of new approaches and technologies such as functional metatranscriptomics and meta-proteomics and novel isotope methods, such as PLFA- and protein-SIP and gross process rate measurements (e.g. Urich et al. 2008, Bastida et al. 2009, Wanek et al. 2010).

Extracellular enzyme activities are of great importance for soil decomposition processes, because the depolymerization of complex substrates by such enzymes is thought to be the
rate-limiting step of soil organic matter decomposition (Schimel & Weintraub 2003). It has been shown that varying C and N availability affect extracellular enzyme activities (Gallo et al. 2004, Allison & Vitousek 2005, Sinsabaugh et al. 2008). It is, however, not completely clear, whether this is caused by changes in microbial metabolism or by microbial community dynamics. It has been suggested that the production of specific enzymes to acquire C, N and P would increase when assimilable nutrients are scarce and complex substrates are present (‘economical’ regulation on organism level) (Allison & Vitousek 2005, DeAngelis et al. 2008, Geisseler & Horwath 2009). However, as discussed above, the response of enzyme production to varying nutrients may also be controlled by changes in microbial community composition. For example, N has been found to increase enzyme activities in low lignin litter, whereas it suppressed ligninolytic enzyme activities in high lignin litters (Carreiro et al. 2000). The reduced ligninolytic enzyme activity at high N availability may be caused either by suppressed enzyme production at the organism level or by a community shift, caused by a competitive disadvantage of the microbial group specialised on producing ligninolytic enzymes in high lignin litter. The latter would point to a strong control of the microbial community composition on the functional response to N addition. It is likely, however, that the overall regulation of exoenzyme activity by varying C and N input may be a combination of both, regulation of microbial metabolism and microbial community dynamics. Enzymes produced by a wide range of microorganisms (e.g. protease) may be regulated at a physiological level (Sinsabaugh et al. 2002, DeAngelis et al. 2008, Geisseler & Horwath 2008), whereas enzymes that are produced by specialized microbial taxa (e.g. ligninases, cellulases) may be affected to a larger extent by changes in microbial community composition resulting from different nutrient limitations. A vivid example for the close coupling between microbial community composition, resource availability and extracellular enzyme activities is the microbial succession that occurs during litter degradation. At the beginning of litter decomposition when leachates consisting of low molecular weight compounds (e.g. sugars, amino acids) are still present, the microbial community is dominated by fast growing bacteria. Only with time, when easily available compounds get exhausted, bacteria are replaced by slow-growing fungi which are able to produce enzymes necessary to degrade lignin and other complex compounds of the decomposing leaf and the accumulated dead microbial biomass (Hättenschwiler et al. 2005).

These findings clearly demonstrate that microbial community composition may be a crucial, albeit often overlooked factor that drives the response of decomposition processes to changing environmental conditions. Due to methodological limitations, the effects of microbial community dynamics on ecosystem processes are much more difficult to assess compared to their macro-scale counterparts, i.e. effects of plant or animal community dynamics. This may be the reason why it has taken somewhat longer to recognize their potential importance in ecosystem processes (Wall et al., Fitter et al. 2005, Bardgett et al. 2008). However, the effect of microbial community dynamics in response to changing environmental conditions may be even more significant compared to macro-scale community dynamics, due to the by orders of magnitudes smaller time-scales at which community changes are able to occur.
Plant-soil interactions: Mycorrhiza

It is increasingly recognized that microbial soil organic matter decomposition cannot be analysed independent of the interaction with plants. One crucial component that has not been addressed so far is the function of mycorrhizal fungi in soil organic matter decomposition and hence in terrestrial nutrient cycling. The development of the mycorrhizal symbiosis between plants and fungi dates back to a very early time of evolution of terrestrial plants (Alexander 2006, Bonfante & Selosse 2010), indicating that this may be a highly specialised and optimized system. While for a long time the prevailing opinion was that mycorrhizal fungi mainly act as a kind of root prolongation, increasing the access of plants to nutrients in exchange for C derived from canopy photosynthesis, there is now evidence accumulating that the ecological function of mycorrhizal fungi may be much broader and as such not as easy to classify. It has been suggested recently, for example, that ectomycorrhizal fungi may possess significant saprotrophic capacities, which may not allow their classification as pure biotrophic organisms (Koide et al. 2008, Courty et al. 2010). Moreover, the ectomycorrhizal fungal community has been found to exhibit a high functional diversity with different species (colonizing the same tree) showing different abilities to produce certain extracellular en-
zymes (Buee et al. 2007). Additionally, the ectomycorrhizal community showed strong seasonal variations in temperate deciduous forests, indicating its responsiveness to changing environmental or nutritional conditions (Buee et al. 2005, Courty et al. 2008). Although ectomycorrhizal fungi have been found to be able to secrete a wide range of extracellular enzymes, their ability to degrade complex ligno-cellulosic or humic compounds in the forest floor is still not proven beyond doubt and thus remains a controversial issue (Talbot et al. 2008, Baldrian 2009, Cullings & Courty 2009). Talbot and co-workers (2008) suggested three mechanisms by which mycorrhizal fungi may be involved in the degradation of soil organic matter. First, mycorrhizal fungi may degrade soil organic matter as an alternative C source in times of low photosynthetic activity of the tree. This hypothesis has been supported recently by two studies, one of which found increased catabolic activities of mycorrhizal fungi after defoliation of pine trees (Cullings et al. 2008), whereas the other could demonstrate an increased production of cellolytic and lignolytic enzymes by ectomycorrhizal fungi in times of bud-break of oak trees, i.e. when photoassimilates were not yet available and the carbon demand of the tree was high (Courty et al. 2007). Second, mycorrhizal fungi may decompose soil organic matter as a side-effect when mining for nutrients and third, mycorrhizal fungi may decompose soil organic matter at times of high belowground C allocation, when their growth and activity is ‘primed’ by recent plant photosynthates (Talbot et al. 2008).

There may be an additional mechanism of how mycorrhizal fungi affect decomposition processes. Decomposition of a specific substrate in the soil is thought to be either C or N limited, depending on the C:N ratio of the substrate and the C:N ratio of the microorganism (Schimel & Weintraub 2003). Due to their ability to translocate excess C or N to either C or N limited microsites in the soil through their extramatrical hyphae network, mycorrhizal fungi may easily overcome such elemental limitation during decomposition. The direct access to C from their host trees may enable mycorrhizal fungi to decompose N-rich compounds at C-limited microsites more effectively than any other group of microorganisms. Subsequently, they could then transfer N from these spots to other microsites which are rich in C but low in N, enabling them to degrade C-rich compounds (Boberg et al. 2010). Overall, this would lead to a more efficient degradation of substrates in the heterogenous soil system and to a lower effective N mineralisation (because N in excess is taken up and transported somewhere else instead of being mineralised). In fact, gross mineralisation has been found to be strongly negatively correlated to the fungal to bacteria ratio in boreal forests, which supports this concept (Högberg et al. 2007).

Recent studies have demonstrated, that some mycorrhizal fungi may promote a closed N cycle by short-circuiting the passage of litter-N back to the plant by bypassing the soil organic matter pool and/or the pool of dissolved N in the soil (Chapman et al. 2006, Wurzburger & Hendrick 2009). Instead of litter-N being mineralised by free living microbes and subsequently taken up by plants, N may be mineralised directly from litter by mycorrhizal activity and channelled back into the host-plant (Chapman et al. 2006). This may avoid N-losses from the system and thus could be an advantage for the plants, especially in N-limited ecosystems. It is hence not surprising that the strongest evidence for this kind of short-
circuit has been found in ericoid mycorrhizal symbiosis which is predominantly found in N-limited (e.g., arctic or alpine) ecosystems (Wurzburger & Hendrick 2009).

These findings indicate that mycorrhizal symbiosis may be a key factor for decomposition processes and for ecosystem C and N cycling. Mycorrhizal fungi may however react highly sensitive to environmental change and to resulting variations in C and N availabilities. Rising CO$_2$ concentrations, for example, may increase photosynthesis and thus belowground C allocation, which in turn may increase the abundance of mycorrhizal fungi (Carney et al. 2007). Increased N deposition, on the other hand, has been found to strongly decrease belowground C allocation in boreal forests (Högberg et al. 2010) probably because it lowers the demand for trees to invest C in the root-mycorrhizal system to improve N uptake (Hermans et al. 2006). It is therefore not surprising that mycorrhizal fungi have been shown to rapidly decline in response to rising N deposition, with unknown implications for decomposition of soil organic matter and CO$_2$ release from forest soils (Nilsson & Wallander 2003).

Summary

There is growing evidence that – in addition to abiotic factors - interactions of microbes and their biotic environment strongly influence soil organic matter decomposition. Hence, there is a strong need to account also for these effects when assessing the effect of climate change on C fluxes from soils to the atmosphere. We are only at the beginning of understanding the complex mechanisms behind the regulation of soil organic matter decomposition. According to the latest IPCC report (2007) the residual terrestrial C sink (i.e., the additional uptake of atmospheric CO$_2$ by terrestrial ecosystems, which offsets a part of the anthropogenic emissions) has apparently increased by approximately 1 Gt C yr$^{-1}$ between the 1980s and the 1990s. However, neither the fluxes responsible for the residual terrestrial C sink nor the mechanisms behind its variations have yet been identified. Increasing our knowledge on soil microbial ecology and its complex interactions with environmental conditions and the C cycle will be an important step forward towards a better understanding of the response of terrestrial ecosystems to climate change.
Aim and Outline of the thesis

The aim of my thesis was to elucidate mechanisms of microbial soil organic matter decomposition. In the first part of my work I focused on the effect of cryoturbations (i.e., processes in arctic soils that lead to the long-time exposure of humic-rich top soil layers to unfavourable abiotic conditions) on soil decomposition processes. In the follow up work I shifted the focus from a tundra ecosystem in Siberia to a temperate beech forest ecosystem near the city of Vienna, which enabled me to study the link between resource availability, microbial community composition and function as well as plant-soil interactions in a seasonal context.

This thesis comprises four manuscripts:

Chapter 2: Conservation of soil organic matter through cryoturbation in arctic soils in Siberia

We found strong signs of cryoturbation at our study site at the G’dansky peninsula (N 69°43.0’, E 74°38.8’), showing highly contorted soil horizons in 12 soil profiles within a 4 ha area. In this publication I report on the physical, chemical and microbial characteristics of these soil horizons and discuss how they relate to decomposition processes. Additionally, I present the C distribution within the profiles and the mean residence times of C for each layer. My results provide insight in the effect of cryoturbations on soil organic matter decomposition and their possible consequences for the arctic C cycle.

Chapter 3: Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil

We investigated microbial community composition, enzyme activities and C and N availabilities in monthly to bi-monthly time steps in a two-year field experiment where C and N availability in the soil was altered by tree girdling (which interrupts belowground C allocation) and N-fertilisation, respectively. This chapter provides insight into the coupling between microbial community composition and function in a seasonal context in a temperate beech forest. Furthermore, it shows the effects of belowground C allocation and N availability on community composition and function over the course of the year.
Chapter 4: **Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2\omega6,9 and 18:1\omega9**


The widely used fungal phospholipid fatty acid biomarkers 18:2\omega6,9 and 18:1\omega9 are known to occur also in plant tissue, which has led to doubt over validity of their use as biomarkers for fungi in soil. In this paper I used the results from a girdling experiment together with measurements of PLFAs in beech roots to calculate the possible contamination of these biomarkers in sieved soil samples with PLFAs originating from root remains. This novel approach allowed me to show that it is safe to use the two mentioned biomarkers to quantitatively characterize the fungal communities of soil.

Chapter 5: **Plants control the seasonal dynamic of microbial N cycling in a beech forest soil by belowground allocation of recently fixed photosynthates**


In the last chapter I focused on elucidating the effect of tree girdling and N fertilisation on the control of the microbial N cycle over all seasons. I present seasonal cycles of N mineralisation, N nitrification, N in the microbial biomass, fungal biomass and dissolved organic nitrogen, ammonium and nitrate in the soil solution. The results of this study demonstrate that plants exert a strong control over the seasonal microbial N cycling by recent photosynthates and emphasise the possible role of mycorrhizal fungi for ecosystem N cycling.
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Chapter 2:
Conservation of soil organic matter through cryoturbation in arctic soils in Siberia
Conservation of soil organic matter through cryoturbation in arctic soils in Siberia

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Abstract

Cryoturbation (mixing of soil layers due to repeated freeze-thaw processes) is a major soil forming process in arctic regions, which may contribute to long-term storage of C in soils of northern latitudes. Our goal was to determine the effect of subduction of organic matter by cryoturbation on microbial decomposition processes in tundra soils. Buried layers were situated at 30-60 cm depth, between Bg and B horizons, but exhibited a C and N content highly similar to present-day A horizons. Radiocarbon dating revealed, however, that the mean age of C in the buried layer was three times higher (~1,300 years BP) than in the A horizon (~400 years BP), suggesting that decomposition rates in the buried layer were delayed. The observed microbial processes support this result: gross C and N mineralization rates were substantially lower in the buried layers than in the respective A horizons. The amount of C stored in the buried layer still doubles the amount of C stored in top-soil horizons (O and A). Assuming that the buried layer originates from both, O and A horizons, this indicates that O and A horizon at time of burying (800-1300 years BP) must have been significantly thicker and present-day O and A horizon at this site may still have the capacity to accumulate additional C. Cryoturbation therefore may lead to additional long-term storage of carbon in the system by (1) retarding decomposition processes of buried organic material and (2) enabling the soil to restart C accumulation in top soil layers.

Keywords: cryoturbation, high-latitude soils, carbon cycle, microbial decomposition
Conservation of Soil Organic Matter through Cryoturbation

Introduction

Cryoturbation is a major soil forming process in arctic regions. The movement of water along thermal gradients in the soil combined with repeated freezing and thawing can lead to the mixing of soil layers and thereby to the subduction of humic-rich top-soil horizons into deeper soil layers [Van Vliet-Lanoe, 1998; Van Vliet-Lanoe, 2004]. Abiotic conditions in deep soil are different from top-soil, e.g. deep soil is colder, frozen for longer time periods and exhibits a prolonged wetness during the growing season, which may slow down decomposition of the buried material. Most soils today are in steady state in respect to C storage and therefore exhibit no or only a very limited capacity to sequester additional CO₂ from the atmosphere [Schlesinger, 1990]. The mixing of C-rich top soil material into lower soil layers through cryoturbation in arctic regions may thus, by retarding microbial decomposition, constitute an important mechanism of terrestrial C sequestration [Davidson and Janssens, 2006]. This effect may be even stronger when the cryoturbated organic-rich material becomes finally encased in permafrost [Ping et al., 1998].

Cryoturbation is a widespread phenomenon in arctic regions. Studies from Alaska (e.g. Seward peninsula, Northern Brooks Range, Kaparuk River Basin) constantly found signs of strong cryoturbation in at least 40% of the observed soil profiles [Hofle et al., 1998; Munroe and Bockheim, 2001; Ping et al., 1998]. In arctic Canada, Turbic Cryosols cover 79% of the soil area with considerable amounts of C stored in the cryoturbated horizons (Tarnocai, 2003). Older estimates of the arctic terrestrial carbon pool (e.g. [Post et al., 1982]) could be underestimated by a factor of 2, because C stored (predominately by cryoturbation) in the upper permafrost was largely ignored [Michaelson et al., 1996; Zimov et al., 2006].

The importance of cryoturbation for the arctic C cycle may change in the future: since frost heave strongly depends on soil temperature and soil water content cryoturbation is sensitive to changes in climate. Understanding the fate of cryoturbated organic C therefore becomes a potentially important factor for predicting arctic C fluxes under changing climatic conditions. While many studies have identified the presence of buried C in cryoturbated soils in the Arctic [Bockheim et al., 2003; Bockheim and Tarnocai, 1998; Hofle et al., 1998; Michaelson and Ping, 2003; Michaelson et al., 1996; Munroe and Bockheim, 2001], to our knowledge, none of these studies investigated decomposition processes in the buried organic layers.

Our goal was to determine the effect of organic matter subduction by cryoturbation on microbial decomposition processes and to assess whether cryoturbation effectively acts to inhibit decomposition and preserve C pools in the soil. Our study site, located on the Gydansky peninsula, Siberia, shows strong cryoturbation, with highly contorted soil horizons and frequent organic intrusions within the soil profiles. To assess the relevance of the buried C pool for the C budget of this (and similar) tundra regions, we measured the amount of the buried soil organic carbon and the carbon isotope composition and mean age of organic material in the different horizons. Furthermore, the chemical and microbial properties of the soil layers as well as microbial respiration and gross N mineralization rates were characterized.
Materials and Methods

Study site

Our study site was located at the Gydansky-Peninsula in Siberia (N 69° 43.0’, E 74° 38.8’; 74 m a.s.l.), in the arctic bioclimate subzone D [CAVM Team, 2003], also called typical tundra subzone in the Russian classification [Chernov and Matveyeva, 1997]. The vegetation consisted of dwarf-shrubs (e.g. Betula nana, Salix glauca, Vaccinium vitis-idea, Dryas punctata), graminoides (e.g. Carex arctissibirica, Arctagrostis latifolia, Calamagrostis holmii) and a well developed moss and lichen layer. The study site was located near a frostboil tundra [Biasi et al., 2005; Kaiser et al., 2005], but did not exhibit primary patterned ground.

The soils were Epigleyi-Turbic Cryosols (having gleyic properties within 50 cm from the soil surface) [FAO, 1998] (corresponding to Typic Aquiturbels in the Soil Taxonomy classification system) with a small O and A horizon (< 8 cm together), followed by a Bg horizon (thickness 20-30 cm) and a B horizon (thickness 20-30 cm); active layer thickness was on average 60.3 cm. Buried organic layers were found in all 12 soil profiles of the study area (200 x 200 m). They were located in the soil profile below 20 cm depth within the B horizons and down to the permafrost (at 60 cm depth). The buried horizons (Ajj horizon) formed irregular, broken horizons of a dark colour (colour and structure were similar to the A horizon) within the brighter coloured B horizons (Fig. 1). There were no signs for active cryoturbation processes at this site, as it was entirely covered by vegetation and did not exhibit distorted O and A horizons. Also, these horizons were not connected to the buried A horizon. This implies that the buried horizons were formed during historic cryoturbation processes.

C and N content and microbial biomass-N

O, A, Bg, B and Ajj horizons from 6 soil profiles were sampled. Soil samples were sieved (< 2 mm) and soil moisture and bulk density were determined gravimetrically. Total C and N content and δ¹³C values were determined by elemental analyzer-isotope ratio mass spectrometry (EA-IRMS) of finely-ground soil sub-samples. Soils were extracted with 0.5 M K₂SO₄ (1 g fresh soil per 15 ml, for one hour). The extracts were kept cool (buried in tundra soil) until their transport to the laboratory for analysis.

For determination of microbial biomass N, a sub-sample (2 g) of fresh soil was fumigated with ethanol-free chloroform in a desiccator for 24 hours (at the study site, immediately after soil sampling) and subsequently extracted with 15 ml 0.5 M K₂SO₄ for determination of microbial biomass (chloroform-fumigation-extraction method, [Amato and Ladd, 1988]). Dissolved organic nitrogen (DON) was measured in the extracts of fumigated and non-fumigated soils by an alkaline persulfate digestion [Sollins et al., 1999] and determination of the resulting NO₃⁻ by ion chromatography (HPLC). Microbial biomass N was calculated from the difference of DON before and after fumigation multiplied by the factor 0.54 [Brookes et al., 1985].
Soil respiration

Soil respiration rates were assayed in aerobic incubations: 10 g soil samples were incubated at ambient soil temperatures (buried in the respective soil horizon) over a period of 2-3 days in a closed glass jar along with 5 ml 0.25 M NaOH. The absorbed CO₂ was precipitated by adding 10 ml 1 M BaCl₂ and the remaining NaOH was titrated with 0.1 M HCl and phenolphthalein as indicator.

Gross N mineralization rates

We used the \(^{15}\text{N}\) pool dilution technique to determine gross N mineralization rates [Myrold and Tiedje, 1986; Schimel et al., 1986]. We thoroughly mixed two sub-samples (1 g) of fresh soil with 500 µl of 0.25 mM \((^{15}\text{NH}_4\text{})_2\text{SO}_4\) and incubated them under field conditions for 4 and 24 hours, respectively. The incubation was terminated by extraction with 15 ml 1M KCl. Approximately 10 ml of the extracts was transferred into glass flasks and 100 mg MgO was quickly added along with an acid-trap, to trap released ammonia. Acid-traps were prepared by wrapping two circular ash-free filter papers (diameter 5mm), each containing 15 µl 2.5 M KHSO₄, in Teflon tape. The flasks were sealed immediately and shaken at 100 rpm at room temperature (approximately 22 °C). After five days the acid-traps were removed, dried and the nitrogen isotope ratio of the trapped \(\text{NH}_4^+\) was measured by isotope ratio mass spectrometry (IRMS) using an elemental analyser coupled to a gas IRMS system (DeltaPLUS, Finnigan MAT, Bremen, Germany). Rates of gross N mineralization were calculated using the equation in [Barrett and Burke, 2000].

Radiocarbon data

Radiocarbon measurements of the humic acid fraction (i.e., alkali soluble, acid insoluble; [Kristiansen et al., 2003]) of soil organic matter were made by accelerator mass spectrometry (AMS) at the Vienna Environmental Accelerator (VERA #2495-#2501). The \(^{14}\text{C}\) data (corrected for isotopic fractionation) are presented as pMC (percent Modern Carbon) and, where pMC values were < 100, as radiocarbon age (years BP) [Stuiver and Polach, 1977; Wang et al., 1996], which represents the mean age of C in the regarding pool.

Profile C distribution

Horizon depths of six soil profiles in the study area were measured and drawn to scale. Photographs of these soil profiles were taken and transformed to remove perspective distortions. These corrected images were then used to determine the area of the irregular-shaped buried horizon. The volume of each horizon for the site was then estimated by extrapolation of the mean area of each horizon in the profiles. By using bulk density and C content data we calculated the amount of C stored in each layer on a kg C m\(^{-2}\) basis.
Results

Abiotic soil properties
Gravimetric soil water content generally decreased with depth, with the exception that soil moisture in the buried horizons were higher than in the B horizon (but still lower than in the A horizon) (Table 1). Mean July soil temperatures strongly decreased with depth (from 4.5°C in 5 cm to 0.2°C in 50 cm depth). Bulk densities increased from O to A to B horizons and were slightly higher in the buried horizons compared to the A horizons, but lower compared to the B horizons (Table 1).

C and N content, profile C distribution
The amount of C stored in the whole active layer averaged 13.5 kg m⁻², from which approximately 50% was situated in the B horizon. O and A horizon only held a small part of the total C (0.78 kg and 1.5 kg m⁻², respectively) whereas the buried layer (Ajj horizon) accounted for more than one third of the total C stock (4.7 kg m⁻²). While the C and N content of the buried horizon was similar to the C and N content of the A horizon (5.2 % C; 0.35 % N; Table 1), the amount of carbon in the buried horizon was more than double the amount of C in the O and A horizon together (Fig. 2).

Table 1: Physical properties, Radiocarbon age, C and N content of soil horizons of a cryoturbated tundra site. O, O horizon; A, A horizon; Bg, Bg horizon; Ajj, buried (cryoturbated) A horizon; B, B horizon. n=5, except for radiocarbon dating (one analysis per regular horizon, three for the Ajj horizon).

<table>
<thead>
<tr>
<th>depth (cm)</th>
<th>Layer</th>
<th>Percent Modern Carbon (%)</th>
<th>Radiocarbon age (years BP)</th>
<th>Total C (%)</th>
<th>Total N (%)</th>
<th>C/N ratio</th>
<th>Soil moisture (%)</th>
<th>Bulk density (g m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>O</td>
<td>113.79</td>
<td>&gt;modern</td>
<td>18.85 (2.73)</td>
<td>0.98 (0.12)</td>
<td>19.3 (1.7)</td>
<td>55.9 (4.1)</td>
<td>0.18 (0.05)</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>95.66</td>
<td>355+-40</td>
<td>6.21 (0.71)</td>
<td>0.39 (0.05)</td>
<td>16.1 (0.5)</td>
<td>34.1 (2.1)</td>
<td>0.84 (0.03)</td>
</tr>
<tr>
<td>15</td>
<td>Bg</td>
<td>62.70</td>
<td>3750+-35</td>
<td>1.13 (0.28)</td>
<td>0.08 (0.02)</td>
<td>13.5 (0.6)</td>
<td>16.8 (1.3)</td>
<td>1.57 (0.01)</td>
</tr>
<tr>
<td>25</td>
<td>Ajj</td>
<td>84.01</td>
<td>1400+-35</td>
<td>5.2 (0.46)</td>
<td>0.35 (0.03)</td>
<td>15.0 (0.2)</td>
<td>24.2 (2.7)</td>
<td>1.1 (0.01)</td>
</tr>
<tr>
<td>50</td>
<td>B</td>
<td>46.33</td>
<td>6180+-40</td>
<td>0.6 (0.1)</td>
<td>0.05 (0.01)</td>
<td>11.1 (0.6)</td>
<td>15.5 (0.7)</td>
<td>1.57 (0.01)</td>
</tr>
<tr>
<td>60</td>
<td>Ajj</td>
<td>85.71</td>
<td>1240+-30</td>
<td>8.8</td>
<td>0.56</td>
<td>15.6</td>
<td>59.1</td>
<td></td>
</tr>
</tbody>
</table>

Microbial biomass and processes
Microbial biomass N and microbial gross N mineralization were significantly lower in the buried horizons than in the A horizon (Fig. 3). Soil heterotrophic respiration was also lower in the buried layer compared to the A horizon, although this difference was not significant.

Values of microbial process rates in the buried layer were generally in the range of the values found in the surrounding B horizon, if calculated per g dry soil (Figure 3). Per unit soil C (or N) process rates in the buried Ajj horizons were much lower, even compared to the B
horizons (Figure 3). The buried horizons therefore showed the lowest transformation rates of all horizons.

On a soil dry matter basis the microbial biomass pool in the buried layer was less than one third of that in the chemically similar A horizon, but higher than that of surrounding B horizons. Per unit soil N, however, the buried horizon had about the same amount of microbial biomass than the surrounding B horizon (Figure 3).

**Figure 1**: Selected drawings of 4 soil profiles (active layer) from a cryoturbated site at the Gdansky Peninsula, Siberia. O, O horizon; A, A horizon; Bg, Bg horizon; Ajj, buried humic-rich layer; B, B horizon.

**Radiocarbon measurements**

Since the radiocarbon age and various soil parameters were similar across and within the buried horizons of all soil profiles, we regarded the buried horizons in this area as a single cryoturbated horizon with the same origin and age which has undergone the same development.

For a pool that is in long-time equilibrium and has a continuous input and output, the radiocarbon age (i.e., the mean age of all C atoms in this pool) reflects the mean residence time and therefore the turnover time of this specific pool [Trumbore et al., 1995; Trumbore et al., 1992]. Therefore, the radiocarbon data of the O, A, Bg and B horizons do not represent their
true age, but their mean residence times of <50, 355, 3750 and 6180 years, respectively (Table 1).

For the buried horizon, however, the interpretation of the radiocarbon data is not straightforward. Before the cryoturbation occurred, the buried horizon consisted of a top-soil horizon, which probably had a similar $^{14}$C age than the present-day O and A horizons (as the $^{14}$C age of soil stays constant after reaching steady state, and then is characteristic for the turnover time in a special climate and region, [Wang et al., 1996]). After the top-soil horizons were mixed into the deep soil, however, conditions that affect the composition of radiocarbon age changed: (1) input of new C into this horizon decreased sharply compared to input of C to a top-soil horizon and (2) some mixing with the B horizon may have taken place.

It is beyond the means of this study to clearly identify the point in time when the cryoturbation did happen. However, by making some simple assumptions and calculations we can at least restrict the range of time within which the cryoturbation has taken place. First, the formation of the recent O and A horizon could only have started after the former top-soil horizon has been mixed down by cryoturbation. Since the “new” A horizon has a radiocarbon age of approximately 350 years and the $^{14}$C age of any organic matter fraction can be interpreted as minimal ages for soil formation [Wang et al., 1996], the cryoturbation must have happened more than 350 years ago. Our second assumption is that the input of new C decreased sharply after cryoturbation and would have been in the range of the input of new C into the B horizon. The radiocarbon age of the B horizon of approximately 4000 years corresponds to turnover rate of 0.025% (1/4000). This corresponds to an input of 0.0044 mg C cm$^{-3}$ per year into the B horizon; given the higher C content of the buried horizon this translates to an annual input of only 0.0069% of the C pool in the Ajj horizon. By using equation 1 and the assumptions that the buried horizon had the same $^{14}$C content as the current A horizon and that the input of new carbon has the same $^{14}$C signature as the atmospheric CO$_2$, we are then able to calculate that the buried horizon would have needed 1118 years to reach its measured radiocarbon age of 1400 years.

Eq. 1: \[
\frac{dR_{Ajj}}{dt} = (R_{Ajj} - e^{-\lambda t}) \times (1 - i - b) + 100 \times i + R_B \times b,
\]

where $R_{Ajj}$ is the $^{14}$C content of the buried horizon (as pMC), \(\lambda\) is the decay constant of $^{14}$C (1/8033 per year); $R_B$ is the $^{14}$C content of the surrounding B horizon (54.5 pMC), \(i\) is the input rate of new C per year (0.0069%), and \(b\) is the rate of B horizon mixed into the buried horizon per year (varied from 0.00 to 0.07%). We used a starting pMC of 95.66 % for the Ajj horizon at time of burying (i.e. the pMC of the present day A horizon).

Any mixing of C of the surrounding B horizon into the buried SOM would make the Ajj horizon reach its measured radiocarbon age in less than the calculated 1118 years. Therefore the cryoturbation must have happened between 1118 to 350 years BP, depending on the amount of carbon of the B horizons that was mixed into the buried layer.
Since the burying of soil must have happened at least 350 years BP we can use equation 1 to estimate the amount of C of the B horizon that may have been mixed into the buried SOM to be 22.5% at maximum (0.065% per year, assuming a mean radiocarbon age of 4873 years for B horizon carbon).

**Figure 2**: Active layer carbon distribution in cryoturbated soil profiles (Gdansky peninsula, Siberia). O, O horizon (depth: 0-3cm); A, A horizon (depth: 3-8cm); Ajj, Ajj horizon (depth: 25-60cm); B, B horizon (depth: 8-60 cm). The amount of total C stored in the profile was 13.5 kg C m⁻² (± 0.5 kg C m⁻²). Data represent the mean of 6 soil profiles.

*Isotopic signature - δ¹³C*

We observed an enrichment in ¹³C with time and depth in the non-cryoturbated horizons (O, A, Bg, B; Figure 4). However, the isotopic signature of the buried layer showed a distinct pattern, being statistically significantly more enriched in ¹³C than A horizon and Bg horizon and being in the range of enrichment of the lower B horizon, although both upper and lower B horizon have a much higher radiocarbon age (Figure 4).
Figure 3: Microbial biomass and processes (heterotrophic respiration and gross N mineralization) in different soil horizons of a cryoturbated soil profile, Gdansky Peninsula, Siberia on a soil dry matter basis (left panels) and a per unit C or N basis (right panels).

O, O horizon (depth: 0-3 cm, n=5); A, A horizon (depth: 3-8 cm, n=7); Bg, Bg horizon (situated above the buried Aj horizon, depth: 8-25 cm, n=4); Ajj, Ajj horizon (buried humic-rich horizon, depth: 25-60 cm, n=6); B, B horizon (situated below the buried Aj layer, depth: 30-60 cm, n=4). Different letters indicate statistically significant differences (multiple range test, LSD, p<0.05).
Discussion

In the Russian arctic, cryogenic soil features have been found related to cryogenic activity of both Pleistocene and Holocene periods [Morozova and Nechaev, 1997; Van Vliet-Lanoe, 1998]. Major cryoturbation activity in the holocene occurred predominately in periods of cold climate [Van Vliet-Lanoe, 1998]. Our data suggest that the mixing of the humic layers into the deep soil must have happened some 350 – 1180 years ago (Table 1). Generally, in the Arctic a major cooling period took place before the onset of a relatively mild period starting around 1000 BP (analogous to the medieval warm period known for the north-atlantic region) [Van Vliet-Lanoe, 1998; Velichko et al., 1997]. In Western Siberia a cooling phase was identified between 1400 to 950 BP by dendrochronology (Taymir peninsula;[Naurzbaev et al., 2002]) and between 1050 to 900 BP by reconstruction of the polar tree-line (Yamal peninsula; Hantemirov and Shiyatov, 2002). Therefore, it is likely that the cooler climatic period between 1400 and 900 years BP drove the cryoturbation activities that produced the soil features we observed. These cryoturbations are thus relatively young compared to the buried layers dated along an arctic transect in Alaska, which were on average 7000 years old [Ping et al., 1998].

Humic acids of the buried horizon were on average three times older than in the A horizon, while they exhibited highly similar physical and chemical characteristics (Table 1). Together with the high amount of SOM in the buried layer (still more than present A and O horizons together; Figure 2) this strongly suggests, that decomposition rates in the buried layers have been substantially delayed. The current microbial properties support this result: the buried layer showed significantly lower N mineralization rates, microbial biomass and lower microbial respiration rates compared to the A horizon (Figure 3). Per unit soil C or N the buried horizons showed even lower transformation rates than the surrounding B horizon, although they had about the same amount of microbial biomass (Figure 3). This suggests that deep soil decomposition may not have been limited by substrate availability. From this data we can infer that decomposition rates in the buried layer must have been constrained by other factors than substrate pools and microbial biomass.

One of the most important constraints on microbial process rates in the buried horizon may have been the unfavourable abiotic conditions at deeper soil layers, such as lower summer temperatures, higher soil moisture content and prolonged period, where the soils are frozen. At our study site, the summer temperature at 5 cm depth (were the A horizon is located) averaged 4.5 °C (±0.06, in 2001) whereas at 30 cm depth (the area of the main part of the inclusions) temperature averaged only 2.0 °C (±0.02) (data not shown). Organic material that has been mixed down into the B horizon therefore experienced temperatures during summer that were significantly lower than they would have been at the soil surface. Lower temperatures substantially decrease microbial processes, such as respiration, and affect microbial life-time and turnover [Garzon, 2004; Price and Sowers, 2004]. Soil respiration in cold environments is known to be highly responsive to temperature [Michaelson and Ping, 2003; Mikan et al., 2002]: Q10 values for microbial respiration have been found to be much higher.
for tundra soils than for temperate or tropical soils, ranging from 4.6 - 9.4 for temperatures above 0 °C and from 63 - 237 in frozen soils [Mikan et al., 2002]. Additionally, deeper soil layers stay frozen for a longer time of the year which slows down decomposition processes considerably. Soil moisture regime is also likely to be different in the deeper soil layers compared to the top-soil – the deep soil is nearer to the permafrost table, so it may stay wetter for longer time periods than top-soil layers, again leading to a decrease of microbial processes.

Figure 4: Stable carbon isotope signature of soil horizons (solid circles) and a cryoturbated (buried) soil horizon (Ajj, open circle) of different radicarbon age. O, O horizon (depth 3 cm); A, A horizon (depth 8 cm); Bg, gleyic B horizon located above the buried horizon (depth 15 cm); Ajj, buried humic-rich horizon (depth 25 cm); B, B horizon located below the buried horizon (depth 50 cm). Bars represent standard errors of the mean. Ajj was significantly enriched compared to the A horizon (p < 0.05).

However, during winter, deeper soil layers experience warmer temperatures than lower soil layers [Romanovsky and Osterkamp, 2000]. Since wintertime respiration has been found to account for a substantial part of total CO2-loss from arctic ecosystems [Elberling, 2003; Hobbie and Chapin, 1996] and decomposition below 0 °C is highly sensitive to temperature, deep soil respiration may be an important part of total CO2-loss in winter [Schimel et al., 2006]. In contrast to A horizons, a considerable part of the decomposition in the buried horizons may therefore have taken place during winter.

Additional evidence that decomposition in buried horizons may have been different than in A horizons comes from the stable C isotope signature (δ^{13}C) of SOM (Figure 4). SOM is
known to enrich progressively with age in $^{13}$C, due to discrimination processes associated with microbial SOM turnover [Agren et al., 1996; Boutton, 1996; Ehleringer et al., 2000; Feng, 2002; Poage and Feng, 2004] and this enrichment is thought to be greater, when decay rates are faster [Agren et al., 1996; Feng, 2002; Poage and Feng, 2004]. Since the buried horizons should have had lower decomposition rates, we expected lower $^{13}$C enrichment compared to "regularly" developed soil horizon. However, our results demonstrated a higher enrichment of the buried inclusions, even compared to the B horizons, which had a much higher $^{14}$C age (Figure 4). This may be due to differences in microbial physiology (e.g. number and type of microbial metabolites, aeration status [Blair et al., 1985; Santruckova et al., 2000; Santruckova et al., 2004]), differences in microbial community composition [Poage and Feng, 2004] or different mechanisms of decomposition between frozen or unfrozen soils [Michaels and Ping, 2003; Mikan et al., 2002; Schimel et al., 2006].

The higher $^{13}$C enrichment of the buried layer thus suggests that (a) turnover of C did take place in buried horizons and (b) this turnover must have been substantially different in its rates and processes from the other soil horizons.

Despite some decomposition activity and despite the fact that only negligible input of new carbon occurred, the buried horizons still stored about one third of the total C stock of the active layer after approximately 1000 years. Moreover, the buried horizons contained twice as much C than the present-day O and A horizon together (Figure 2). If we assume that the buried layer originated from a mixture of former O and A horizons we may conclude that O and A horizon at time of subduction must have been substantially thicker than present-day O and A horizon. This strongly indicates that present-day O and A horizon at this site may not have reached steady state and are still accumulating organic matter. This is further supported by the fact that the cryoturbation occurred less than 1500 years ago – a too short time period to reach steady state [Schlesinger, 1990].

Interestingly, the average C amount stored in the whole active layer (13.5 kg m$^{-2}$) was in the same range than the average C amount of a microsite with similar soil moisture at a nearby frostboil tundra (11.5 kg C m$^{-2}$ [Kaiser et al., 2005]). At this site – which had no inclusions in the soil profile - a higher part (approximately one third) of the whole C stock was stored in O and A horizon (4.2 kg m$^{-2}$). In our study site – by contrast – a similar amount of C (4.7 kg m$^{-2}$) was held by the buried layer, while the O and A horizon only contained a much smaller part of the whole C (2.3 kg m$^{-2}$). This underpins that the former O and A horizon may have been at a later stage of development than the present topsoil horizons, similar to the undisturbed O and A horizon of this nearby site, which are probably near to or at steady state.

Cryoturbation buries the humic-rich topsoil horizons (i.e., O and A horizons) into the deep soil. This leaves behind an unvegetated B horizon exposed to the atmosphere. Once the cryoturbation processes stop, the bare soil surface will be re-vegetated, which starts the formation of a new O and A horizon. In contrast to soils in a steady state, the rate of C accumulation is high during early stages of soil development [Amundson, 2001; Schlesinger, 1990]. Therefore, such restart of top-soil formation may have been an important mechanism that contributed to the high carbon storage capacity of arctic soils during the last millennia.
especially in the light that time periods with cryoturbation activities may have occurred repeatedly (i.e., by alternate periods of cooler and warmer climatic periods, which have occurred at least 3-5 times during the Holocene). The importance of cryoturbation for C storage in the Arctic is also emphasized by observations in arctic Alaska, which showed that the active layer of cryoturbated sites averaged 62% higher C stores than non-cryoturbated sites, indicating that cryoturbation may play a key role in the distribution of C stocks [Michaelson et al., 1996].

Taken together this demonstrates that the cryoturbation activity several hundred years ago has led to an additional long-term storage of a substantial amount of C in this system, because it (1) delayed decomposition of buried soil organic matter and (2) enabled the soil to restart accumulation in top soil layers.

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Chapter 3:

Belowground carbon allocation by beech affects seasonal dynamics of soil extracellular enzyme activities and microbial community composition
Belowground carbon allocation by trees drive seasonal pattern of extracellular enzyme activities by altering microbial community composition in a beech forest soil

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Abstract

- Plant seasonal cycles alter C and N availability for soil microbes which may affect microbial community composition and thus feed back on microbial decomposition of soil organic material and plant N availability. The temporal dynamics of these plant-soil interactions are, however, ambiguous.

- We experimentally manipulated the C and N availability in a beech forest by N fertilisation or tree girdling and conducted a detailed analysis of seasonal pattern of microbial community composition and decomposition processes over two years.

- We found a strong relationship between microbial community composition and enzyme activities over the course of seasons. Phenoloxidase and peroxidase activities were highest during late summer, whereas cellulase and protease peaked in late autumn. Girdling, and thus loss of mycorrhiza, resulted in an increase of soil organic matter degrading enzymes and a decrease of cellulase and protease activity.

- Temporal changes of enzyme activities suggest a switch of the main substrate for decomposition between summer (soil organic matter) and autumn (plant litter). Our results indicate that ectomycorrhizal fungi are possibly involved in autumn cellulase and protease activity. Our study shows that, by belowground C allocation, trees significantly alter soil microbial communities which may affect seasonal patterns of decomposition processes.

Key words: microbial community dynamics, seasonal dynamics, plant-soil interactions, extracellular enzyme activities, rhizosphere priming, girdling, ectomycorrhizal fungi, soil organic matter decomposition
Introduction

Seasonal cycles of plants and soil microbes in temperate forests are closely linked by the availability of nutrients and labile carbon. Plants affect C and N availability for soil microbes due to competition for nutrients during active growth, exudation of labile C via roots and substrate input by litterfall (Wardle et al. 2004, Bardgett et al. 2005, Harrison et al. 2008). Microbes, on the other hand, control nutrient availability for plants by carrying out a wide spectrum of decomposition processes (Schimel et al. 2007, Schmidt et al. 2007). Thus, seasonal variations in plant growth phases as well as in microbial community composition and physiology may strongly affect C and N availability, which in turn feed back on plant and microbial processes over the course of a year.

Microbial decomposition processes in soil have been shown to be highly sensitive to the availability of labile C and N (Schimel & Weintraub 2003). For example, the degradation of humified soil organic matter (SOM) is thought to be energy limited, leading to very slow SOM decomposition rates in soils (Kuzyakov et al. 2009). The input of labile C by plant root exudation, however, may provide energy and enable microbes to degrade SOM to gain limiting nutrients (Paterson, 2009). Plant exudates may also fuel decomposition of other complex substrates, such as soil proteins (De Nobili et al. 2001, Weintraub et al. 2007) or litter (Subke et al. 2004). Complementarily, many studies have shown that changes in N availability for soil microbes lead to alteration of decomposition rates of SOM and litter (Waldrop & Zak 2006, Allison et al. 2008, Keeler et al. 2009). Together, these findings indicate that the availability of labile C and N control the decomposition of complex substrates and thus, in turn, of the availability of nutrients for plants.

The mechanism, by which C and N availability may control decomposition rates, however, remains ambiguous. One possible explanation for this phenomenon may be the tight coupling between microbial community structure and function that has been proposed recently (Prosser et al. 2007, Strickland et al. 2009). Due to different nutrient demands and growth characteristics of specific microbial groups, altered C and N availability may favour the growth of certain microbial groups over others, thereby leading to microbial community shifts (Brant et al. 2006). This may, in turn, strongly influence decomposition processes, since different microbial groups may exhibit different capacities to degrade high molecular weight substances, such as lignin, cellulose or humified soil organic matter.

In temperate ecosystems, a strong effect of seasons on plant-soil interactions lead to seasonal variable C and N availabilities for both, plants and microbes. As described above, C and N availability may be – together with abiotic factors – major drivers of microbial decomposition processes in soils. Microbial decomposition of soil organic matter, however, represent a key driver of the forest’s C and N cycle, which highlights the need for a better understanding of how this process is influenced by plant-mediated C and N availability. Unraveling the control of aboveground - belowground relationships on ecosystem processes and function on seasonal time-scales still remains one of the major challenges in ecosystem ecology (Bardgett et al. 2005). To improve our understanding of that relationship, it is, however,
necessary to get a deeper insight into the link between C and N availability, microbial community dynamics and microbial decomposition processes in a seasonal context.

The aim of this study was to explore this link over the course of two years in a temperate forest. We hypothesized that (1) the seasonal changes in microbial community composition and physiology are driven by C and N availability in addition to abiotic factors and that (2) microbial community composition affects specific decomposition processes mainly through the production of distinct sets of extracellular enzymes. We tested these hypotheses by analysing the seasonal patterns of nutrient availability, microbial community structure and decomposition processes in monthly to bi-monthly measurements over a time-period of two years. Additionally, we experimentally altered the C and N availability and the C:N stoichiometry by N fertilization and by tree girdling. Tree girdling has been shown to effectively cut off the translocation of photo-assimilates from the canopy to the roots, thereby prohibiting the exudation of labile C from plant roots into the soil (Högberg et al. 2001, Subke et al. 2004, Scott-Denton et al. 2006). To link microbial community composition to decomposition processes we analysed if seasonal or treatment-specific microbial community changes were related to changes in specific enzyme activities.

Material and Methods

Study site

Our study site was located in a mature beech forest (*Fagus sylvatica*) approximately 40 km south-west of Vienna, Austria (510 m a.s.l.). The age of the trees was on average 65 years. The soil was a dystric cambisol (over flysh) with a pH between 4.5-5.1 (CaCl$_2$). Organic C and total N content comprised 7.45% and 0.48% of dry soil, respectively. Despite the proximity to the city of Vienna, the site received a N input by atmospheric deposition of only 12.6 kg N ha$^{-1}$ year$^{-1}$ (Kitzler et al. 2006).

Experimental set up

The selection of control and treatment plots followed a randomized block design: First, three blocks within an area of approximately 5,400 m$^2$ were selected based on a geobotanic characterization, such that each block had more or less homogenous vegetation and similar soil properties. Within each block, two control plots, two fertilisation plots (5x5 m each) and one girdling area (20x20m) have been chosen randomly. Two girdling plots were installed in the central 10 m x 10 m of each girdling area. Each block thus held 2 replicate plots of each treatment.

The fertilization treatment plots were fertilized once a month (after the soil sampling) with 29.7 g NH$_4$NO$_3$ dissolved in 2 l water to reach a final N fertilization of 50 kg ha$^{-1}$ yr$^{-1}$. The fertilizer was evenly distributed on each plot by spraying. The first fertilizer application was on May 9th 2006, one month prior to the first sampling. At the same day, all trees within each girdling area were girdled by ripping off a 20 cm strip of bark around the stem at approximately 1.50 m height. Understory plants, which consisted mainly of beech seedlings, a few
herbs and sedges, were removed from all plots (girdling, control and fertilization). Understory plants were again removed in the following spring.

*Soil sampling*

Soil samples were taken from the upper 5 cm of mineral soil (A horizon). Soils from control plots were sampled every month between June 2006 and June 2008 (24 samplings). Soils from fertilized and girdled plots were sampled every two months, except June and July 2007 (which were both sampled; 13 samplings in total). Four subsamples were taken from each of the six replicate plots for treatments and controls and pooled to give one replicate. Sampling was based on a pre-determined sampling scheme in order to avoid sampling of already disturbed soil. Soil samples were carefully sieved (2 mm), freed from visible roots by hand-picking and kept at 4°C until further processing. All extractions were carried out on fresh soils within four days after sampling.

*Fine root biomass*

Fine root biomass was determined 4, 14 and 28 months after girdling. The determination of root biomass 4 months after girdling was conducted in a parallel girdling experiment at the same study site, which started in May 2008 (3 new girdling areas, same outline and experimental set-up as the girdling experiment described above). Thus, root biomass was determined in July 2007 (14.5 months after girdling) and September 2008 (4 and 28 months after girdling). Five soil cores (7 cm in diameter and 14.5 cm in height) were taken from the upper mineral soil (A horizon) of each of the six replicate control and girdled plots. For each soil core, fine roots (diameter < 1 mm) were carefully separated from coarse and visibly dead roots, thoroughly washed and weighted to determine fine root biomass. Fine roots were pooled for each replicate plot. Mycorrhizal colonization was determined from aliquots of pooled samples.

*Determination of mycorrhizal root colonization*

Entire root subsamples were evenly dispersed in a 9-cm diameter petri dish, and root length was measured by a line intersect method at 30 x magnification (Newman 1966). The degree of mycorrhizal root colonization was determined by counting all ectomycorrhizal root tips in four randomly selected squares making up 11% of the total area of the petri dish.

*Soil moisture and temperature*

Soil moisture was detected gravimetrically from soil samples. Soil temperature was measured by Pt100 sensors (Kucera Company, Brno, Slovakia) at 5 cm depth and data were collected every 0.5 hours. Presented soil temperature data are means of soil temperature of 6 days preceding the sampling date.
**DOC and total N**

Dissolved organic carbon (DOC) and total dissolved N (dN) were measured in soil water extracts (2 g fresh soil was extracted with 20 ml analytical grade water; extracts were stored at -20°C) by a TOC/TN analyzer (TOC-V CPH E200V / TNM-1 220V, Shimadzu).

**Phospholipid fatty acids (PLFAs)**

PLFAs were analyzed using a modified procedure described in Frostegard et al. (1991). Samples were processed within one day after sampling. Total lipids were extracted with chloroform/methanol/citric acid buffer (0.15 M), pH 4.0 (1:2:0.8, v/v/v). Neutral lipids were separated from phospholipids on silica columns (Supelco, LC-Si SPE) by elution with chloroform, acetone and methanol. After adding methyl-nonadecanoate (19:0) as internal standard, phospholipids were converted to fatty acid methyl esters (FAME) by alkaline methanolysis. Dried FAMEs were redissolved in isoctane and analyzed by gas chromatography (HP G1530A) on a DB23 column (Agilent). A bacterial FAME mix, (Supelco) was used as qualitative standard. Concentrations of FAMEs were calculated using the internal standard (19:0) peak as a reference.

We used i15:0, a15:0, i16:0, i17:0, a17:0 as indicators for Gram-positive bacteria, 18:1ω7, cy17:0, 16:1ω7, 16:1ω9, cy18:0, cy19:0 and 16:1ω5 as estimates for Gram-negative bacteria, the sum of Gram-positive and Gram-negative biomarkers together with 18:1ω5, 17:0, 15:0, 17:1ω6, 17:1ω7 as a measure for total bacteria. The biomarkers 18:2ω6,9, 18:1ω9 and 18:3ω3,6,9 are frequently used as fungal markers (Hill et al. 2000, Leckie 2005, Högberg 2006, Joergensen & Wichern 2008). However, since they also have been found to occur in plants (Zelles 1997, Laczko et al. 2004) we measured the concentrations of these biomarkers in beech roots and calculated that the possible contribution of root-borne PLFAs (based on fine root biomass measurements and the assumption that 95% of roots were removed by sieving) to our soil samples was less than 0.6% for 18:2ω6 and 18:1ω9 (or less than 0.31% in girdled plots) and up to 3.8% for 18:3ω3,6,9 (or 1.2% in girdled plots). Thus, the observed fine root loss in girdled plots would account for 0.78%, 1.25% and 6% of the observed decrease of 18:1ω9, 18:2ω6 and 18:3ω3,6,9, respectively (C. Kaiser et. al., unpublished).

We used the sum of all PLFAs described above together with the PLFAs 20:2ω6,9 (protozoa), 10Me16:0 (actinomyceta) and 14:0, i14:0, 16:0, 19:1ω8, i17:1ω8, 18:0, 16:1ω11, 16:1ω6, 19:1ω7, 20:0, 14Me15:0, 20:1ω9, which are not specific for any microbial group, as a measure of total microbial biomass.

**Extracellular enzymes**

Potential extracellular enzyme activities were measured by microplate fluorometric and photometric assays. All activities were measured within 48 hours after sampling of soils. One g of sieved soil was suspended in 100 ml sodium acetate buffer (100mM, pH 5.5) and ultrasonicated at low-energy (Stemmer et al. 1998, Marx et al. 2001). β-1,4-Cellobiosidase (“cellobiosidase”), β-1,4-N-acetylglucosaminidase, chitinase/lysozyme (“chitinase”) and leucine
Seasonal Enzyme Activities and Microbial Community Dynamics

amino-peptidase ("peptidase") were measured fluorimetrically (Marx et al. 2001, Saiya-Cork et al. 2002). Two hundred µl soil suspension and 50 µl substrate (4-methylumbelliferyl-β-D-cellobioside, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide, 4-methylumbelliferyl-β-O-N,N‘,N‘‘-triacetylchitotrioside and L-leucine-7-amido-4-methyl coumarin, respectively) were pipetted into black microtiter plates in 3 analytical replicates. Methylumbelliferyl was used for calibration of cellobiosidase, N-acetylglucosaminidase and chitinase, whereas aminomethylcoumarin was used for calibration of leucine amino-peptidase. Plates were incubated for 140 min in the dark and fluorescence was measured at 450 nm emission at an excitation at 365 nm (Tecan Infinite M200 fluorimeter).

Different enzymes with different abilities to cope with steric hindrance may be involved in the degradation of polymers, such as chitin. We therefore assayed chitinases with two kinds of substrates: 4-methylumbelliferyl-β-D-N,N‘,N‘‘-triacetylchitotrioside, consisting of three units of N-acetyl-β-D-glucosaminide (component of chitin), and 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide consisting of only one unit.

Phenoloxidase and peroxidase activities were measured photometrically according to standard methods (Sinsabaugh et al. 1999), with small modifications. Subsamples were taken from the soil suspension (see above) and mixed with a 20 mM L-3,4-dihydroxyphenylalanin (DOPA) solution (1:1). Samples were shaken for 10 minutes, centrifuged and aliquotes pipetted into microtiter plates (6 analytical replicates per sample). Half of the wells additionally received 10 µl of a 0.3% H₂O₂ solution for measurement of peroxidase. Absorption was measured at 450 nm at the starting time point and after 20 hours. Enzyme activity was calculated from the difference in absorption between the two time points divided by the molar extinction coefficient, which had been determined in a pre-experiment.

**Actual extracellular enzyme activities**

"Actual" enzyme activities were measured without substrate and buffer additions, but with the addition of toluene to inhibit microbial uptake of enzymatic products, leading to their accumulation in the soil suspension (Boschker et al. 1995, Watanabe & Hayano 1995, Lipson et al. 1999, Weintraub & Schimel 2005). This method has been used before to determine actual protease activities (accumulation of amino acids; Lipson et al. 1999, Weintraub & Schimel 2005) and to determine polysaccharide degradation (accumulation of glucose and xylose; Boschker et al. 1995). We measured both activities in the same assay: 8 g of fresh soil were mixed with 80 ml distilled water and 0.8 ml toluene in glass jar and shaken for 4 hours. Subsamples for the detection of amino acids and glucose were sampled after 0.5 and 4 hours. For measuring amino acid production, subsamples were centrifuged and supernatants were mixed with the same volume of a solution containing 0.11 M trichloroacetic acid, 0.22 M sodium acetate and 0.33 M acetic acid, to stop further enzyme activity (Weintraub & Schimel 2005). Samples were analyzed for amino acids by a photometric assay with ninhydrin (Moore 1968). For measuring glucose production, subsamples were centrifuged twice at 15,000 g to remove soil particles and microbes and were incubated at 100°C for 15 min to stop enzyme activities. Samples were then analyzed for glucose by HPLC (DIONEX ICS-3000;
CarboPac PA20 column, 10mM NaOH, pulsed amperiometric detection). In a pre-
experiment, the accumulation of both amino acids and glucose was shown to be linear up to
6 hours. Glucose accumulation in the soil solution was most likely caused by cellulase and/or
amylase activity (Boschker et al. 1995). We detected small accumulation of cellobiose and
maltose in the soil solution, but to a much lower extent than glucose, indicating that these
intermediate products were rapidly further degraded. Thus, we used glucose production as a
proxy for the combined action of cellulose and amylase. We ascribed accumulation of amino
acids in the soil solution to protease activity (Weintraub & Schimel 2005).

Statistics
We carried out a canonical correspondence analysis (CCA (Anderson & Willis 2003, Gonzalez
et al. 2008)) on a data set of 11 samplings (bimonthly from August 2006 to May 2008; Au-
gust 2007 had to be excluded due to missing data on phenoloxidase and peroxidase activity,
n=195). A total of 37 individual PLFA biomarkers were used as community matrix (nmol
PLFAs g\(^{-1}\) dry soil), whereas different sets of constraining variables (abiotic conditions, C and
N Pools and microbial processes) were used to compare the influence of different groups of
environmental parameters on microbial community composition. Contribution of con-
strained variability to total community variability (= fraction of variability explained by the
parameter) of each analysis were used as measure for the influence of the respective pa-
rameter set on community variation (used in Table 2). Results from a CCA conducted with all
parameters (except glucose production which was not available for the first two samplings)
as constraining matrix are presented graphically in more detail (used in Fig. 6). We con-
ducted a linear regression analysis (ordinary least square regression) between all individual
PLFA biomarkers and all measured soil parameters of the 2-year sampling period. All statisti-
tical analyses were performed in R 2.8.1. (R-package vegan).

Results
Climate and abiotic conditions
Temperature and precipitation patterns were different between the two sampling years
(June 2006 to May 2007 and June 2007 to May 2008). The first autumn and winter period
(Sept 2006 to January 2007) was on average 2.2°C warmer compared to the second au-
tumn/winter, and exceptionally dry with almost no precipitation and no snow cover. By con-
trast, the second sampling year was characterised by a continuous snow cover from Nove-
ember to March. Soil water content ranged from 25% to 30% between October and January of
the first sampling year, and from 30% to 40% in the same period of the second year (Fig. 1b).
In both years, soil water content was lowest in August (23%). Soil temperature was approx-
imately 1.3°C higher between September and May of the first year compared to the second
year (Fig. 1a).
Girdling affected soil water content significantly. The magnitude of this effect, however, de-
depended on season. Soil moisture was significantly higher in girdled plots from September to
December of both years (by 5% - 10%), and in spring and summer of the second year. No difference, however, was observed between January and March of both years (Table 1, Fig. 1b). Fertilisation did not affect soil water content. There was no difference in soil temperature between treatments and controls.

**Figure 1.** (a) Mean soil temperature (at 5 cm depth) during the two sampling years (2006/2007 and 2007/2008). Each data point represents the average of the daily mean soil temperatures of 7 days preceding each sampling date (for all treatments: open symbols, year 1; filled symbols, year 2). (b) Gravimetric soil water content (0 - 5cm depth) for control and girdled plots (squares, controls; circles, girdled plots) during the two years. Error bars indicate 1 SE (n=6).

**Effect of Girdling on tree vitality**

In the first year, leaf senescence started approximately 3 weeks earlier in girdled trees. In the following spring, however, girdled trees still exhibited a full flush of leaves. The amount of leaf litter in the second sampling year was similar in all plots, although litterfall occurred earlier in girdled plots (34% of total leaf litterfall in girdled plots but only 13% in control plots took place already before the end of September 2007, Figure S1). In spring 2008 (2 years after girdling) 40 % of girdled trees did not produce new leaves.
Figure 2. Dry fine root biomass (a) and ectomycorrhizal root tips (b) per dm$^{-3}$ of soil in control and girdled plots at 4, 14.5 and 28 months after girdling. Black bars, controls; grey bars, girdled plots. Error bars indicate 1 SE (n=6). Asterisks indicate statistical significant differences between control and girdled plots for each time point (n.s., not significant; *, p<0.05; **, p<0.01; ***, p<0.001).

**Fine root biomass and ectomycorrhizal colonization**

Fine root biomass in control plots was around 1-1.2 g dry fine roots dm$^{-3}$ soil in the upper 14.5 cm of soil. We observed no difference in fine root biomass or ectomycorrhizal root tip colonization between control and girdled plots 4 months after girdling (Fig. 2). Fine root biomass decreased by 45% and 60% in girdled plots 14 and 28 months after girdling, respectively. Mycorrhizal root colonisation was reduced by 60% 14 months after girdling, but only
by 27% 28 months after girdling. However, due to the lower amount of vital roots at the latter time point, the total amount of mycorrhizal root tips per cm$^3$ soil was decreased by around 70% for both, 14 and 28 months after girdling (Fig. 2).

**DOC and N levels**

In both years, DOC content in soil water extracts was low in early autumn (September to October), increased in late autumn (November to December) and decreased again to low levels between January and April, followed by a sharp increase in May (Fig. 3b). Thus, autumn and winter dynamics were similar between the two years, probably indicating a general seasonal trend. In contrast, we could not observe similar DOC dynamics in early and late summer of the two years. Tree girdling decreased DOC in soil water extracts predominantly in July of both years (-21.7%; p=0.058 and -20%; p=0.044, respectively), but did not affect DOC levels during autumn and winter (Fig. 3b). Total (=organic and inorganic) dissolved N in soil water extracts peaked around mid-summer (August) in both years, followed by a sharp decrease in autumn and low levels in winter (Fig. 3a). Levels of dissolved N were significantly enhanced by fertilisation and girdling, on average (over the whole sampling period) by 28% and 170%, respectively (p<0.001 and p<0.05, respectively, Fig. 3, Table 1).

**Soil enzyme activities**

Soil enzyme activities were high in spring, late summer and autumn, and low in mid-summer and winter (Fig. 4). The spring peak was highly synchronous for all measured enzymes (in 2007), starting with fairly low levels in March increasing to maximum levels in June, followed again by a summer decline. The second (‘autumn’) peak occurred at different times for different enzymes: (potential) phenoloxidase and peroxidase showed maximum activities already in late summer (around September) whereas glucose production (by actual cellulase/amylase activity; Fig. 4g) and actual protease activity (Fig. 4h) had their autumn maximum around November. At this time phenoloxidase and peroxidase activities had already dropped to very low levels (Figure 4e,f).

We found no significant effect of girdling or fertilisation on enzymes measured by fluorescent substrates (chitinase, cellobiosidase, N-acetylglucosaminidase and peptidase) during the first year (Table 1, Fig. 4a-d). In the second year, chitinase was found to be significantly decreased in girdled plots (Table 1, Fig. 4a). ‘Actual’ protease and cellulase activities were markedly reduced in girdled plots, whereas phenoloxidase and peroxidase activities were significantly enhanced (Fig. 4 e-h, Table 1). These shifts in activities occurred already in the first summer after girdling. Fertilisation affected phenoloxidase and peroxidase activity only in the second year (Table 1, Fig. 4e,f).
Table 1. Significance of the effects of girdling, fertilisation and sampling month on various soil parameters derived from an analysis of variance (ANOVA).

<table>
<thead>
<tr>
<th>Soil parameter</th>
<th>Girdling Year 1</th>
<th>Fertilisation Year 1</th>
<th>Girdling Year 2</th>
<th>Fertilisation Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m, df=5</td>
<td>g, df=1</td>
<td>m, df=5</td>
<td>g, df=1</td>
</tr>
<tr>
<td>Soil water content</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Cellobiosidase</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
</tr>
<tr>
<td>Chitinase</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>N-Acetylglycosaminidase</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>Leu-Peptidase</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>Phenoloxidase (^a)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Peroxidase (^a)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Actual protease (^b)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Glucose production (^c)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>DOC</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
</tr>
<tr>
<td>Total dissolved N (dN)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>DOC: dN</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>CCA1</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
</tr>
<tr>
<td>CCA2</td>
<td>*</td>
<td>***</td>
<td>n.s.</td>
<td>***</td>
</tr>
</tbody>
</table>

Presented are levels of significance resulting from factorial ANOVA (with month and treatment as factors) conducted separately for the girdling and fertilisation treatment, and for each year of the experiment. Abbreviations: m, month of sampling; g, girdling; f, fertilisation; g x m and f x m, interaction of month and treatment. Number of replicates for the first and second year was n=72 (6 samplings) and n = 84 (7 samplings), respectively. Degrees of freedom (df) for each factor as indicated in the table headings, with the following exceptions: \(^a\), n=72 and df (m and m x treatment) = 5 for year two; \(^b\), n=60 and df (m and m x treatment) = 4 for year one; \(^c\), n = 48 and df (m and m x treatment) = 3 for year one. CCA1, CCA2: sampling scores of the first two axes of the canonical correspondence analysis (Figure 6, Figure S2). Significance levels: ***, p<0.001; **, p<0.01; *, p<0.05; °, p<0.1; n.s., not significant.

**Microbial biomass**

The annual course of the amount of total PLFAs, as a measure of microbial biomass, was similar in the two sampling years in control plots, besides a small time shift (Fig. 5a). Microbial biomass showed two phases of lower levels during the course of a year: the first in June (July in the second year) and the second in February (March in the second year). In both years we observed a sharp increase of biomass in spring and a period of high values in autumn (Fig. 5a, supporting information, Table S1).

Girdling decreased the total amount of PLFAs by 15% on average between July 2006 and January 2008 (2 to 20 months after girdling). In the same time period, the sum of bacterial biomarkers was decreased by around 14%, the amount of the fungal biomarker 18:1ω9 by 16% and the fungal biomarker 18ω2ω6,9 by as much as 51% (Fig. 5).

Fungi are thought to contain less PLFAs per g biomass compared to bacteria due to a higher ratio of cell volume to cell surface, which makes it difficult to estimate the real fungi to bacteria ratio from PLFA data (Joergensen & Wichern 2008). Thus, the reduction of 51% of the 18:2ω6,9 biomarker may translate into a much higher reduction of the total microbial biomass than the 15% loss of total PLFAs suggests. It is worth noting, that in girdled plots the
seasonal trend was maintained for bacteria and the fungal biomarker 18:1ω9, but not for the fungal biomarker 18:2ω6,9, which started to decrease strongly already two months after girdling (Fig. 5). Fertilisation had no significant effect on the total amount of bacterial or fungal PLFA biomarkers.

**Figure 3.** Total dissolved nitrogen (a) and dissolved organic carbon (b) in soil water extracts over the course of the two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots; dotted lines connect control values to visualize the seasonal trend). All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate 1 SE (n=6).

**Microbial community composition**

We conducted a canonical correspondence analysis (CCA) in order to assess how far the variation observed in the microbial community composition was related to specific environmental parameters. By contrast to unconstrained multivariate analysis, CCA displays only
the part of the variation in the community data that can be explained by the used constraints. Our analysis revealed that soil temperature and soil water content were related to 20% of the PLFA variability, whereas labile C and N (DOC and dissolved N) could only explain 4.3% of the total community variability (Table 2). Enzyme activities, however, were linked to 24% of the variability in the community matrix. From all enzymes, phenoloxidase, peroxidase and peptidase were most strongly related to community structure (19.9%) whereas other enzymes only covered much smaller parts of the variance (e.g., N-acetyl-glucosaminidase, chitinase and cellobiase only 5.4%; Table 2).

Altogether, about 36% of the variability of the microbial community matrix throughout seasons and treatments could be related to a combination of enzymatic activities and environmental parameters (Fig. 6, Table 2). From the constrained variability, CCA axis 1 (CCA 1) accounts for 72.2%, CCA axis 2 (CCA2) for 12.4%. Both CCA1 and CCA2 were significant (p<0.001, permutation test). We found significant effects of seasons and girdling on microbial community composition along these two axes (Fig. 6, Table 1). Different sampling months were mainly separated on CCA1 whereas control and girdled plots could be separated on CCA2 (Fig. 6b). No general trend for specific microbial groups (Gram-, Gram+, bacteria, fungi) was observed along CCA1, indicating that seasonal microbial community shifts were caused by changes within all groups. Community shift along CCA2 (reflecting the effect of girdling) was dominated by the influence of two fungal biomarkers (18:2ω6,9 and 18:3ω3,6,9) and one bacterial biomarker (18:1ω5), which were negatively correlated to that axis (indicating reduced abundance of these markers in samples from the girdling treatment). The influence of the third fungal biomarker (18:1ω9) was comparably low (Figure 6).

From all sampling months February of the first year and November of the second year got the highest scores on CCA1 (supporting information, Fig. S2). Both were the first months in which winter conditions (high soil moisture and low soil temperature) occurred in the respective year. Spring and summer months were characterized by lower scores (supporting information, Fig. S2). Soil temperature and soil water content as well as peroxidase and peptidase activities exhibited a strong effect on CCA1 indicating that they were closely related to seasonal variation of microbial community composition (Fig. 6).

A regression analysis of each individual biomarker with each microbial process revealed interesting links between microbial processes and community structure (Table 3). The results of this regression analysis showed that enzymatic activities can be separated into three groups, each of them relating to a specific set of individual PLFA biomarkers. First, phenoloxidase and peptidase activities were correlated with peroxidase activity and all three enzymes were correlated to the same set of individual PLFAs including the majority of Gram+ biomarkers (i15:0, a15:0, i16:0), certain Gram- (16:1ω7, 16:1ω9) and a few general bacterial biomarkers (14:0, 16:1ω11) (Table 3). Second, cellobioidase, N-acetylglucosaminidase and chitinase were correlated with each other. Overall, these enzymes did not correlate with any specific PLFA biomarker, with the exeption of N-acetylglucosaminidase and chitinase that were correlated to the microbial biomass (sum of all PLFAs). The third group of enzyme activities, comprising “actual” protease and cellulase /
amylase activities, were not correlated to any bacterial biomarker, but – together with chitinase activity - to the fungal biomarkers 18:2ω6,9 and 18:3ω3,6,9 (Table 3). There was no correlation of one of the fungal biomarkers with peroxidase or phenoloxidase activity. However, when examining the regression analysis only for girdled plots, a strong correlation ($r^2=0.54$, $p<0.005$) between 18:2ω6,9 and phenoloxidase activity was found (supporting information, Table S2).

Table 2. Relationship between different soil parameters and soil microbial community composition.

<table>
<thead>
<tr>
<th>Set of constraints</th>
<th>Explained fraction of variability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil temperature and soil water content</td>
<td>20.3</td>
</tr>
<tr>
<td>DOC and dN</td>
<td>4.3</td>
</tr>
<tr>
<td>N-acetyl-β-D-glucosaminidase, chitinase and</td>
<td>5.4</td>
</tr>
<tr>
<td>cellobiosidase</td>
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Presented are the contributions of constrained variability to total community variability by canonical correspondence analysis conducted with different sets of constraining variables. CCA was based on data from bimonthly samplings of control, fertilised and girdled plots between August 2006 and May 2008 (with exclusion of August 2007 due to missing peroxidase and phenoloxidase data; 11 samplings, $n=195$). Additionally, data for July 2006 are missing for cellulose/amylase activity (*; 10 samplings, $n=177$).
Figure 4. Soil extracellular enzyme activities in control, fertilised and girdled plots throughout the two year sampling period (squares, controls; circles, girdled plots; triangles, fertilised plots; dotted lines connect control values to visualize the seasonal trend). Figures a – f show ‘potential’ activities (activities measured with fluorometric and photometric methods after addition of a specific substrate). Figures g and h show ‘actual’ enzyme activities (no substrate added). All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate 1 SE (n=6).
Discussion

Tree roots provide an important source of easily assimilable carbon for soil microbes and allow the establishment of a specific microbial community (Brant et al. 2006). In temperate forests, an important part of this community comprises ectomycorrhizal fungi (Read & Perez-Moreno 2003, Jones et al. 2004), but also other microbial groups benefit from this source of carbon (Butler et al. 2003). Eliminating belowground carbon allocation by tree girdling in our study had a two-fold effect on C and N availability for microbes. First, it led to decreased DOC levels in the soil during the summer. This effect was similar to that of other girdling studies which also found small season-dependent effects of girdling on DOC levels in soil water extracts (Weintraub et al. 2007, Zeller et al. 2008). Since DOC is a labile pool with fast turnover times, its absolute level may provide little information about substrate supply to microbes. We, however, also observed a lowered microbial biomass and a strong decline of soil CO$_2$ efflux (B. Kitzler et al., unpublished) confirming that the availability of easily assimilable C was substantially depleted in the girdling treatments. By contrast, N levels were strongly enhanced in the soil water of girdled plots, presumably due to reduced plant N uptake caused by decreasing mycorrhizal hyphae and fine roots in the soil (Jordan et al. 1998). Increased inorganic N levels in response to girdling have also been found in other studies (Weintraub et al. 2007, Zeller et al. 2008, Dannenmann et al. 2009) and may affect microbial community composition in addition to the loss of root C input.

The effect of girdling on soil C and N availability led to substantial changes in microbial biomass and community composition. Although only a small proportion of the bacterial community (15%) was affected, the fungal biomarker 18:2\omega 6,9 was reduced by 51%. This is consistent with other girdling studies, which showed a strong decline of the fungal biomarker 18:2\omega 6,9, but almost no response of 18:1\omega 9. Compared to other types of fungi, mycorrhizal fungi depend to a much higher degree on belowground C allocation by trees. It is therefore safe to assume that the reduction of fungal biomarkers after girdling was related mainly to the reduction of mycorrhizal fungi, as has also been suggested by others (Högberg 2006, Högberg et al. 2007, Yarwood et al. 2009). Surprisingly, we found a strong decrease of 18:2\omega 6,9 already two months after girdling (Fig. 5), although we did not observe any decrease of mycorrhizal root colonization four months after girdling (Fig. 2). The latter is consistent with the results of another beech girdling experiment (Dannenmann et al. 2009), in which girdling had no effect on mycorrhizal root colonization in the first year. In our study, however, mycorrhizal root colonization decreased by as much as 60% 14 months after girdling (Fig.2). Our results suggest that reducing the input of labile root C initially resulted in a strong decrease of the mycorrhizal extramatrical hyphae network in the soil already within the first two months, followed only later by a decrease of ectomycorrhizal root tips and a loss of fine root biomass.
Potential activities of cellulobiosidase, N-acetylglucosaminidase and peptidase were not significantly affected by girdling, demonstrating that they were not linked to root exudations. These enzymes (measured using relatively simple low-molecular weight substrates) possibly represent a general trait of microbial groups other than mycorrhizal fungi and rhizosphere bacteria, which were not affected by girdling. In contrast to the above mentioned enzyme activities, our results showed a clear decrease of actual cellulase and protease activities in girdled plots already in the first year, especially at times when these rates were at peak levels in control plots, i.e. in spring and autumn (Fig. 4). The diminishing of the spring peak in girdled plots indicates that this peak is linked to root exudates. Spring exudates may have accelerated the turnover of microbes leading to a flush of proteins and carbohydrates (De Nobili et al. 2001, Kuzyakov 2002, Phillips et al. 2008). The autumn peak in enzyme activity, on the other hand, may be explained by the input of fresh litter at that time. Approximately 70% of N-rich low order roots are thought to have turnover times of less than a year (Kielland et al. 2006, Guo et al. 2008, Pritchard & Strand 2008) and a large proportion of fine root mortality is usually found at the end of the growing season (Ruess et al. 2003). Together with
leachates from leaf litter, this peak of fine root mortality in autumn may therefore have led to a large input of cellulose and protein into the soil and subsequently to increased glucose and amino acid production.

Since there was no reduction in overall fine root biomass in the first year of girdling, we assume that this input of fresh substrates did occur in control as well as in girdled plots. Nevertheless, girdling strongly reduced this autumn peak of glucose and amino acid production (by more than 50%) in November of both years (Fig. 4). This reduction was correlated to a strong reduction of the autumn and winter peak of fungal biomarkers in girdled plots (Fig. 5b, Table 3) suggesting that mycorrhizal fungi may be highly involved in protease and cellulase/amylase activity in autumn. At the time when amino acid and glucose production rates exhibited their autumn peak (in November), (potential) phenoloxidase and peroxidase activities, strongly decreased to very low levels (Fig. 4). Thus, we observed a temporal shift in enzyme activities from an early autumn maximum of oxidative enzymes to a late autumn maximum of hydrolytic enzymes. While the maxima of cellulase and protease activities can be readily explained by increased substrate inputs in late autumn (see discussion above), the reasons causing the drop of oxidative enzymes by about 70% from September to December are less clear. According to our data it is not likely that this drop was driven by abiotic factors. For example, October 2006 exhibited warmer soil temperatures than September, although there was already a strong decrease (by 45%) in oxidative enzymes in this period (Fig. 4). Potential measurements of oxidative enzyme activities may however reflect the abundance of the microbes producing them, rather than the actual rates of these enzyme activities. Phenoloxidase and peroxidase catalyze the key reactions in the degradation of lignin and humified SOM and have to be carried out by specialist microbes (Hatakka 2001, Baldrian 2009). However, specialists for SOM degradation will only have an advantage over others when other sources of nutrients (such as fresh organic matter, FOM) are scarce (Fontaine et al. 2003, Kuzyakov et al. 2009, Paterson 2009), which is possibly the case during summer. Plant root exudates may provide a constant energy supply at low N levels during summer, thereby creating optimal conditions for SOM degraders (Fontaine et al. 2003). This situation may change fundamentally, when N-rich substrates, such as dying fine roots and leaf litter leachates appear in autumn. Soil organic matter degrading microbes may then lose their competitive advantage over microbes decomposing FOM, explaining the rapid autumn and winter decrease of phenoloxidase/peroxidase.
Table 3. Linear regressions of phospholipid fatty acid biomarkers and various soil parameters.

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Presented are goodness of fit ($R^2$) of statistical significant (p<0.01) relationships between parameters; empty fields indicate that regression was not significant. Bold values highlight $R^2$ higher than 0.5. Regressions are based on data from control and girdling plots from all samplings over the two year sampling period (mean values of each sampling). *Cellob*, cellobiosidase; *chitin*, Chitinase; *N-acet*, N-acetyl-glucosaminidase; *L-pept*, Leucine-peptidase; *ph.ox.*, phenoloxidase; *perox*, peroxidase; *act prot*, actual protease; *gluc p.*, glucose production; *dN*, total dissolved nitrogen; *actmyc*, actinomycetes; *bact.*, bacteria; *f&p*, fungi and plant.

Competition between microbial groups could also have been responsible for the shift of enzyme activities from hydrolytic enzymes to oxidative enzymes in girdled plots. Mycorrhizal fungi are known to dominate the rooted soil layers due to a competitive advantage by access to root carbon, whereas saprotrophic fungi are thought to be more competitive in the litter.
layer (Hobbie & Horton 2007, Lindahl et al. 2007). Although ectomycorrhizal fungi have been found to be able to produce cellulases, proteases and phenoloxidases (Luis et al. 2005, Cullings & Courty 2009), saprotrophic fungi are thought to possess a much higher capacity to produce peroxidases (Luis et al. 2005, Baldrian 2009, Cullings & Courty 2009). Girdling strongly decreased the abundance of mycorrhizal fungi, thereby possibly rendering a competitive advantage for saprotrophic fungi also in the rooting zone. At the same time, girdling reduced easily assimilable C in soils, which may have led to starvation of microbes, while N availability increased to very high levels (Fig. 3). According to the current understanding of rhizosphere priming, this would not favour SOM degradation, because under such conditions there is no need to mineralize N and SOM degradation to gain C would be too energy demanding (Kuzyakov et al. 2009, Paterson 2009). Nevertheless, potential oxidative enzyme activities rose in girdling plots in our experiment suggesting that, at least in the longer term, the reduced abundance of mycorrhizal fungi may have released the competitive disadvantage of slow growing specialist decomposers (e.g. saprotrophic fungi) which possess the ability to degrade SOM by releasing peroxidases and phenoloxidases (Hobbie & Horton 2007, Baldrian 2009).

Alternatively, reducing C input from the host tree could have led to a shift within the ectomycorrhizal community towards species with a higher ability to produce SOM degrading enzymes or to a physiological switch of ectomycorrhizal fungi towards increased saprotrophic activity (Buee et al. 2007, Talbot et al. 2008, Cullings & Courty 2009). Different mechanisms have recently been suggested, which may lead to the decomposition of SOM by mycorrhizal fungi, e.g. the need for an alternative C source when supplies of photosynthetates from plants are low, or, in contrast, the ‘priming’ with high amounts of photosynthetates, which may accelerate growth and activity of ectomycorrhizal fungi (Courty et al. 2007, Cullings et al. 2008, Talbot et al. 2008). Our results suggests that if mycorrhizal fungi were able to switch to saprotrophy at low C supply (as indicated by increased phenoloxidase and peroxidase activities in girdled plots), it was apparently not very effective, as fungal biomass strongly decreased in girdled plots. Instead, it seems plausible that fungi were primed by tree C in control plots, in which they grew to high levels from summer to autumn and were linked to high levels of cellulase and protease activity, both of which diminished in girdled plots. An increase of phenoloxidase and peroxidase activities after girdling was also found by Weintraub and co-workers (2007), who suggested that this may have been caused by increasing availability of dead fine root biomass due to girdling. However, due to the fast increase of oxidative enzymes in girdled plots already two months after treatment and a lack of decrease of fine root biomass in the first 4 months (Fig. 2), we rule out this explanation for our experiment.

Regression analysis of PLFAs and enzyme activities over all seasons revealed three ‘groups’ of enzymes, each related to a specific set of individual PLFA biomarkers (Table 3). From all measured enzyme activities, phenoloxidase, peroxidase and leucine-peptidase were those linked to the largest part of variation (20%) of the microbial community composition, whereas cellobiosidase, N-acetylglucosaminidase and chitinase showed the weakest relation to community composition (Table 2,3). The high amount of significant correlations between
PLFA biomarkers and enzymes suggest that the observed enzyme pattern was determined to a large extent by microbial community dynamics, rather than by individual physiological response of microbes to varying nutrient and energy availability. Correlations between phenoloxidase, peroxidase and individual PLFAs were stronger and also involved the fungal biomarker 18:2ω6,9 ($R^2=0.54$, $p=0.007$, Table S2) when girdled plots were analysed alone, indicating that more microbial groups were connected to these enzyme activities after girdling (supporting information, Table S2).

**Figure 6.** Relation between microbial community composition and various soil parameters analysed by canonical correspondence analysis (CCA). PLFA data were used as community matrix; environmental and microbial process data as a constraining matrix. Analysis was based on data from all samplings between August 06 and May 08, except July 07. (a) The distribution of individual PLFA biomarkers (weighted scores) is shown on the first two axes. Additionally, the biplot scores of constraining variables depicting the influence of soil parameters are shown on these axes. Biomarkers are coloured according to their allocation to specific microbial groups. Abbreviations: a*, 18:1ω7 and cy17:0; N_ac, N-acetyl-ß-D-glucosaminidase; cell, cellobiosidase; stemp, soil temperature; act_prot, actual protease; pept, leucine-peptidase. (b) The distribution of the bi-monthly samplings for the two years is shown along the first two axes. Grey circles, girdled plots; black squares, control plots; open triangles, fertilized plots. The contribution of constrained variability to total variability was 36%, from which CCA 1 accounts for 74.8% and CCA2 for 12.5%. CCA1 and CCA2 were both significant ($p<0.001$, permutation test). The distribution of bi-monthly samplings in the course of time is shown in Fig. S2 (supporting information).
Tree carbon allocation to the belowground microbial community has been shown to enhance the decomposition of recalcitrant C compounds (Ekberg et al. 2007) and to result in a long-term net C loss from soil (Carney et al. 2007, Dijkstra & Cheng 2007). Carney et al. (2007) showed that elevated root C input altered the microbial community structure towards a higher proportion of fungi, which in turn led to a higher priming of SOM decomposition by leaf litter. This indicates that SOM decomposition may be determined by microbial community composition and an appropriate source of energy. In the present study, we compared a system with a specialised root-C-based microbial community to a system inhabited by a community not supported by tree root C. In the ‘intact’ system, we see clear seasonal trends, suggesting that fresh organic matter degradation, as indicated by actual cellulase/amylase and protease activities, seems to be enhanced in autumn, whereas SOM degradation, as indicated by a higher potential activity of oxidative enzymes, may be enhanced during the summer months. In the girdled system, however, this trend disappeared and lower rates for cellulase and protein degradation, especially in autumn, were found. The possible conclusion that mycorrhizal fungi may be involved in autumn litter degradation, however, still needs to be confirmed by direct measurements.

Overall, our study demonstrated the importance of plant-decomposer interactions for C and N cycling in temperate forests on a seasonal time-scale. Our results showed that extracellular enzymes mediating the decomposition of SOM and litter in a beech forest soil are linked to microbial community composition and exhibit a strong seasonal pattern. Our findings indicate that, by belowground C allocation, trees alter microbial community structure and thus may affect seasonal pattern of microbial decomposition processes.

Acknowledgements

This work was supported by the Austrian Science Fund (FWF, Project number: P18495-B03) and in part by the FWF National Research Network MICDIF (S1001-B07). We are thankful to Ieda Nunes-Cornelio Hämmerle and Birgit Wild for valuable help in the field and to four anonymous reviewers for helpful comments on the manuscript.
References


Supporting material:

**Figure S1.** Leaf litterfall collected by litter traps in the second sampling year (August – December 2007) in control and girdled plots.

**Figure S2.** Microbial community composition of bi-monthly samplings on the first two axes of the canonical correspondence analysis.
### Table S1: Phospholipid fatty acids (nmol g\(^{-1}\) dry soil) of different microbial groups in control, girdled and fertilised soils over a time period of two years.

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#### fungi (18:2ω6,9)

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Bold letters indicate statistical significant differences among treatments (C, Control; G, Girdling; F, Fertilisation) for each month and microbial group; levels of significance are given in the last column (***, p<0.001, **: p<0.01, *: p<0.05, tested by ANOVA). Different superscript letters indicate statistical significant differences between specific treatments (Tukey HSD; p<0.05). The statistical difference between treatments over all samplings (assessed by ANCOVA with sampling month as covariate) is indicated for each microbial group together with the overall mean.
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**Note:** The values represent the relative abundance of each substance in the control and girdling plots, with no significant differences noted.
Chapter 4:

Contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2ω6,9 and 18:1ω9
Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2w6,9 and 18:1w9

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Soil Biology and Biochemistry (2010), accepted for publication
Abstract

The phospholipid fatty acid biomarkers 18:1\textsubscript{ω9}, 18:2\textsubscript{ω6,9} and 18:3\textsubscript{ω3,6,9} are commonly used as fungal biomarkers in soils. They have, however, also been found to occur in plant tissues, such as roots. Thus, the use of these PLFAs as fungal biomarkers in sieved soil, which may still contain small remains of roots, has been questioned. We used data from a recent beech tree girdling experiment to calculate the contribution of roots to these biomarkers and were able to demonstrate that not more than 0.61% of 18:1\textsubscript{ω9} and 18:2\textsubscript{ω6,9} in sieved soil samples originated from roots (but 4% of 18:3\textsubscript{ω3,6,9}). Additionally, the abundance of the biomarker 18:2\textsubscript{ω6,9} in the soil was found to be highly correlated to ectomycorrhizal root colonization, which further corroborates its fungal origin. PLFA biomarkers were substantially reduced in vital roots from girdled trees compared to roots of control trees (by up to 76%), indicating that the major part of PLFAs measured in roots may actually originate from ectomycorrhizal fungi growing inside the roots. We calculated, that even a near to 50% reduction in fine root biomass – as observed in the girdling treatment - accounted for only 0.8% of the measured decrease of 18:2\textsubscript{ω6,9}. Our results demonstrate that both 18:1\textsubscript{ω9} and 18:2\textsubscript{ω6,9} are suitable biomarkers for detecting fungal dynamics in soils and that especially 18:2\textsubscript{ω6,9} is a reliable biomarker to study mycorrhizal dynamics in beech forests.

Keywords: PLFAs, fungal biomarkers, 18:2\textsubscript{ω6,9}, 18:1\textsubscript{ω9}, soil microbial community, ectomycorrhizal fungi, girdling, beech roots
Phospholipid fatty acid (PLFA) analysis is widely used to quantitatively assess microbial community composition of soils. One of the advantages of this approach is that it covers a wide range of microbial groups, including both bacteria and fungi. The PLFAs 18:2ω6,9, 18:1ω9 and to a lesser extent also 18:3ω3,6,9 are commonly used as fungal biomarkers in soil microbial community studies (e.g. Hill et al., 2000; Högb erg, 2006; Joergensen and Wichern, 2008; Leckie, 2005) but have also been found to occur in plant tissues (Laczko et al., 2004; Zelles, 1997). Soil samples used for PLFA analysis are usually sieved and/or hand-picked to remove visible roots prior to analysis. Although this pre-treatment certainly eliminates the majority of all roots, remains of fine roots in the soil samples can not be ruled out. It has therefore been argued that analysis of fungal PLFA biomarkers in soils may be biased by possible contribution of plant remains to soil samples (Joergensen and Wichern, 2008; Leckie, 2005; Olsson, 1999). This possibility of contamination with root-borne PLFAs may thus reduce the reliability of results from studies that use PLFAs as fungal biomarkers in soil. Despite this limitation, there has been, to our knowledge, no attempt to quantify the possible contamination of plant roots to PLFAs measured from soil samples so far.

In a large-scale girdling experiment, we found a strong decrease (about 50% on average between 2 to 20 months after girdling) of the biomarker 18:2ω6,9 compared to untreated controls (Kaiser et al., 2010). A similar effect has also been found before by other girdling studies (Högb erg et al., 2007). The obvious interpretation that the reduction of this biomarker after girdling reflects the decline of ectomycorrhizal fungi has recently been supported by DNA profiling of the soil microbial community (Yarwood et al., 2009). However, since tree girdling reduces fine root biomass in soil, some part of this reduction could also have been caused by decreasing fine root biomass if the methodology used reflects to some extent also PLFAs in roots. To address this problem we measured the PLFA content of fine roots of beech from our experiments and calculated the contribution of fine roots to the total 18:2ω6,9, 18:1ω9 and 18:3ω3,6,9 biomarkers in our soil samples.

**PLFA measurements of forest soil and beech root samples from a girdling experiment**

Soil and root samples were taken from a tree girdling experiment in an approximately 65 year old beech forest (*Fagus sylvatica*) near the city of Vienna (for details see: Kaiser et al., 2010). The soil was a dystric cambisol (over flysh, pH 4.8) and samples were taken from the upper mineral soil (A horizon). Girdling had taken place 14.5 months before the sampling (May 9th, 2006) in three 400m² girdling areas. Two 5 x 5 m sampling plots were installed in the central part of each girdling area; two control plots were installed in the vicinity of each girdling area (i.e., six girdling and six control plots in total). Five soil cores (14.5 cm height, 7 cm diameter) were taken randomly from each of the girdling and control plots and pooled to give one replicate sample. Fine roots (diameter < 1mm) were thoroughly separated from soil and from coarse roots, washed carefully and weighed. Soil samples were sieved to 2 mm and freed from all visible roots by hand picking (n=6). Ectomycorrhizal root colonisation was measured from aliquots of the pooled fine root samples by counting ectomycorrhizal root tips in petri-dishes at 30 x magnification (n=6). For PLFA analysis, roots were pooled to three
final samples from girdling and control plots, respectively (n=3). Fine root samples were kept frozen (-20°C) until PLFA extraction and crushed in liquid N₂ with a mortar and pestle immediately before extraction. Soil samples were processed fresh (within 24 hours of sampling). PLFAs were extracted in soil samples and in fine roots using a modified procedure as described by Frostegård and co-workers (1991). Phospholipids were converted to fatty acid methyl esters (FAME) by alkaline methanolysis. FAMEs were analyzed by gas chromatography on a DB23 column (J&W 60m x 0.25mm, 0.25µm). Mixtures of bacterial FAMEs (Bacterial acid methyl ester mix, Supelco, and 37 Comp. FAME Mix, Supelco) were used as qualitative standards. Concentrations of single FAMEs were calculated using the internal standard (19:0) peak as a reference.

**PLFA fungal biomarkers found in beech roots are predominantly of mycorrhizal origin**

Fine roots of beech contained all of the three PLFAs 18:2\(\omega_{6,9}\), 18:1\(\omega_{9}\) and 18:3\(\omega_{3,6,9}\) commonly used as fungal biomarkers (Högberg et al., 2007; Joergensen and Wichern, 2008): 18:2\(\omega_{6,9}\) showed the highest concentration of all biomarkers, followed by 18:3\(\omega_{3,6,9}\) and 18:1\(\omega_{9}\) (303, 130 and 59 µg g\(^{-1}\) dry fine roots, respectively; Table 1). Interestingly, these biomarkers were substantially reduced in vital roots from girdled trees by as much as 56 to 76%. Since we have no reason to believe that girdling alters the PLFA composition or concentration of the membranes of vital roots, the reduced concentration of these biomarkers in roots of girdled trees most likely reflects the decrease of ectomycorrhizal fungi growing inside the roots. This is supported by a weak correlation of the concentration of these biomarkers in fine roots with the ectomycorrhizal root colonization (\(R^2=0.39, p=0.185\), Figure 1a) and suggests that the predominant part of the PLFAs measured in root tissue may in fact originate from colonization with ectomycorrhizal fungi.
Table 1: Dominant PLFA biomarkers in fine roots of *Fagus sylvatica* and soils of girdled and control plots 14.5 months after girdling and estimation of the contribution of root-borne PLFAs to PLFAs measured in sieved soil samples. Values are means (1 SE). Roots, n=3; soil, n=6.

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<thead>
<tr>
<th>PLFA concentration in soils (µg g⁻¹ dry soil) a</th>
<th>Control</th>
<th>Girdling</th>
<th>Difference G - C (%)</th>
</tr>
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<tbody>
<tr>
<td>18:1ω9</td>
<td>7.46 (1.45)</td>
<td>7.15 (1.05)</td>
<td>-4.1</td>
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<tr>
<td>18:2ω6,9</td>
<td>4.34 (0.44)</td>
<td>1.82 (0.27)</td>
<td>-58.0</td>
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<tr>
<td>18:3ω3,6,9</td>
<td>0.28 (0.05)</td>
<td>0.12 (0.05)</td>
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<td>18:0</td>
<td>1.82 (0.38)</td>
<td>1.98 (0.31)</td>
<td>+9.1</td>
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<td>0.81 (0.05)</td>
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<tr>
<td>17:0</td>
<td>0.51 (0.06)</td>
<td>0.44 (0.07)</td>
<td>-12.5</td>
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<table>
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<tr>
<th>Root-borne PLFAs in soil samples b</th>
<th>Control</th>
<th>Girdling</th>
<th>Contribution of fine root biomass loss to the observed decrease in soil PLFAs (%) d</th>
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<tr>
<td>Root-borne PLFA (ng g⁻¹ dry soil)</td>
<td>Root-borne PLFAs (%) c</td>
<td>Root-borne PLFA (ng g⁻¹ dry soil)</td>
<td>Root-borne PLFAs (%) c</td>
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<td>4.97 (0.43)</td>
<td>0.07 (0.01)</td>
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<td>25.51 (1.68)</td>
<td>0.61 (0.10)</td>
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<td>18:3ω3,6,9</td>
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<td>4.05 (0.55)</td>
<td>1.48 (0.97)</td>
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<td>2.53 (0.58)</td>
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<td>0.14 (0.06)</td>
<td>0.11 (0.10)</td>
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<td>16:0</td>
<td>12.30 (1.83)</td>
<td>0.18 (0.05)</td>
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<td>17:0</td>
<td>0.51 (0.72)</td>
<td>0.11 (0.03)</td>
<td>0.13 (0.12)</td>
</tr>
</tbody>
</table>

a Soil was sieved and visible roots were hand-picked prior to analysis.
b Calculated contribution of root-borne PLFAs to total PLFAs measured in sieved soil samples. Calculations were based on the observed PLFA-concentrations in fine roots and on fine root biomass measurements for each plot (control plots: 1.68± 0.09 mg g⁻¹ soil, girdling plots: 0.914 ±0.27 mg g⁻¹ soil) under the assumption that 5 % of roots remained in soil samples after sieving and hand-picking (PLFA concentration in fine roots x total fine root biomass per g soil x 0.05).

c Estimated contribution of root-borne PLFAs to total PLFAs measured in sieved soil samples in percent (root-borne PLFAs per g soil / total PLFAs per g soil x 100).

d Estimated contribution of the root biomass decrease (~45%) to the total observed decrease of soil-borne PLFAs in girdled plots compared to controls (difference between root-borne PLFAs per g soil in control and in girdled plots / difference between total PLFAs per g soil in control and in girdled plots x 100).
The contribution of roots to $18:2\omega_{6,9}$ and $18:1\omega_{9}$ found in soil samples is negligible

Our data allowed us to calculate the possible contribution of $18:2\omega_{6,9}$, $18:1\omega_{9}$ and $18:3\omega_{3,6,9}$ from root tissue to soil samples. Control plots contained on average 1.68 mg fine roots g$^{-1}$ soil ($\pm$ 0.09 mg g$^{-1}$) in the upper 14.5 cm (Kaiser et al. 2010). Based on a $18:2\omega_{6,9}$ concentration of 303 µg g$^{-1}$ fine roots, the amount of this biomarker in soil originating from fine roots would be 0.51 µg g$^{-1}$ soil if all roots were present in the soil. However, since our soil samples were sieved to 2 mm and freed from all visible roots by hand picking prior to analysis, we assume that we removed at least 95% of all roots by this treatment. Thus, the amount of root-borne $18:2\omega_{6,9}$ in our soil samples (contaminated with 5% of the original fine root biomass) would be 0.025 µg g$^{-1}$ soil, or only 0.6% of of the amount of $18:2\omega_{6,9}$ measured (Table 1). The contribution of roots to the other two biomarkers would also be small, but of different magnitudes (0.07% and 4% for $18:1\omega_{9}$ and $18:3\omega_{3,6,9}$, respectively, Table 1). These differences are caused by the difference of the ratios at which these biomarkers occur in roots (where the ratio of $18:1\omega_{9}$ to $18:2\omega_{6,9}$ to $18:3\omega_{3,6,9}$ is equal to 1:5:2) and soils (27:15:1, respectively). The differences of these ratios on their own, already suggest that there is no significant contribution of root-borne PLFAs to sieved soil samples, which would have required a higher similarity in the relative abundances of these markers in roots and soil.

**Figure 1**: Correlations between ectomycorrhizal root tips and the amount of the PLFA biomarker $18:2\omega_{6,9}$ found in beech fine roots (a) and bulk soil (b). In (a) each point represents roots from two plots (comprising five subsamples each) which were pooled to give three samples from girdled and three samples from control plots. In (b) each point represents one plot (five pooled subsamples). Black circles, control plots; grey circles, girdling plots. Significance of regressions: (a) $p=0.185$ (not significant), (b) $p=0.0038$ (**).
**Effect of a reduction in fine root biomass on fungal biomarkers found in soil**

The observed decrease of fine root biomass by 45% in girdled plots at the time we took these samples would translate into a decrease of the root-borne \(18:2\omega_{6,9}\) biomarker in sieved soil samples by 0.020 µg g\(^{-1}\) soil (i.e. from 0.0255 in girdled to 0.0056 µg g\(^{-1}\) soil in control plots, Table 1). However, both, amount and changes of the fungal biomarker measured in our soil samples were by levels of magnitudes higher than that, i.e. \(18:2\omega_{6,9}\) decreased from 4.34 µg g\(^{-1}\) soil in control plots to 1.82 µg g\(^{-1}\) soil in girdled plots. Thus, a variation of 0.02 µg g\(^{-1}\) soil caused by the loss of fine root biomass in girdled plots would account for less than 0.8% of the observed change of \(18:2\omega_{6,9}\) in soil. The influence would be higher for \(18:1\omega_{9}\) and \(18:3\omega_{3,6,9}\) (1.25% and 6.0%, respectively), due to less change of these biomarkers in response to girdling.

In conclusion our results show that, although the concentrations of \(18:2\omega_{6,9}\) and \(18:1\omega_{9}\) in beech roots are high, their contribution to the total PLFA content of a sieved beech forest soil is negligible, mainly due to the high abundance of these biomarkers in the soil. If there would be a significant contribution of roots to total soil PLFAs we would expect similar ratios of these biomarkers in soils and roots, which clearly is not the case. Rather, we found a significant correlation between fungal PLFA biomarkers in the soil and ectomycorrhizal colonization of root tips, suggesting the fungal origin of these biomarkers in the soil. \((R^2 = 0.58, p<0.005\) Figure 1b\)). PLFAs with relatively low concentrations in soils, but high concentrations in roots, such as \(18:3\omega_{3,6,9}\) may, however, still be biased to some extent by root-borne PLFAs. We conclude that the PLFA biomarkers \(18:2\omega_{6,9}\) and \(18:1\omega_{9}\) are well suited for reliably capturing fungal dynamics in sieved forest soils.

**Acknowledgements**

We thank Peter Schweiger for determination of ectomycorrhizal colonization of roots and Jörg Schnecker for help in fine root biomass determination. This work was supported by the Austrian Science Fund (FWF, Project numbers P18495-B03 and S1001-B07).
References


Chapter 5:

Plant control seasonal microbial nitrogen cycling in a beech forest soil
Plants control the seasonal dynamic of microbial N cycling in a beech forest soil by belowground allocation of recently fixed photosynthates

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Abstract

Soil microbes in temperate forest ecosystems are able to cycle several hundreds of kg of N ha\(^{-1}\) yr\(^{-1}\) and are therefore of paramount importance for N retention. Belowground C allocation by trees is an important driver of seasonal microbial dynamics and may thus directly affect N transformation processes over the course of the year. Our study aimed at unravelling plant controls on soil N cycling in a temperate beech forest at a high temporal resolution over a time period of two years, by investigating the effects of tree girdling on microbial N turnover. In both years of the experiment, we discovered (1) a summer N mineralization phase (between July – August) and (2) a winter N immobilisation phase (November - February). The summer mineralization phase was characterised by a high N mineralization activity, low microbial N uptake and a subsequent high N availability in the soil. During the autumn/winter N immobilisation phase, gross N mineralization rates were low and microbial N uptake exceeded microbial N mineralization, which led to high levels of N in the microbial biomass and low N availability in the soil. The observed immobilisation phase during the winter may play a crucial role for ecosystem functioning, since it could protect dissolved N that is produced by autumn litter degradation from being lost from the ecosystem during the phase when plants are mostly inactive. The difference between microbial biomass N levels in winter and spring equals 38 kg N ha\(^{-1}\), and may thus account for almost one third of the annual plant N demand. Tree girdling strongly affected annual N cycling: the winter N immobilisation phase disappeared in girdled plots (microbial N uptake and microbial biomass N were significantly reduced, while the amount of available N in the soil solution was enhanced). This was correlated to a reduced fungal abundance in autumn in girdled plots. By releasing recently fixed photosynthates to the soil, plants may thus actively control the annual microbial N cycle. Tree belowground C allocation increases N accumulation in microorganisms during the winter which may ultimately feedback on plant N availability in the following growing season.

Keywords: belowground carbon allocation, beech forest, N cycle, seasons, soil microbial community, plant-soil interactions, ectomycorrhiza, N retention, tree girdling.
Introduction

Terrestrial productivity in temperate forest is among the highest globally, necessitating a fast re-cycling of N in these latitudes (Huston and Wolverton 2009). Annual uptake of nitrogen into the plant biomass in temperate forests has been found to range between 80 and 150 kg N ha\(^{-1}\) year\(^{-1}\) which approximately equals annual input of N from aboveground and belowground litter (Norby and Iversen 2006, Kreutzer et al. 2009). This high plant N demand is thus met, to a high extent (>80%) by N recycling, i.e. by microbial mineralization of litter and soil organic matter, whereas external N input by atmospheric N deposition or N\(_2\)-fixation, only represents a small proportion of the total plant N demand (Schlesinger 1991, Kreutzer et al. 2009). For growth and maintenance of temperate forests, an efficient re-cycling of N from dead plant material is thus of pivotal importance, especially since (in spite of increasing anthropogenic deposition) N is still a limiting element for the majority of such ecosystems (LeBauer and Treseder 2008, Finzi 2009).

Microbes have been found to process a multiple of the plant’s annual N demand by repeated turnover of microbial biomass N over the course of a year (Corre et al. 2007, Schmidt et al. 2007, Kreutzer et al. 2009), emphasising the importance of microbial N immobilisation for ecosystem N retention (Corre et al, 2007). Microbial N immobilisation and mineralization processes may occur at the same time in different microsites of the soil, and determine, together with plant N uptake, the proportion of N that may be re-cycled in the system (Schimel and Bennett 2004). The relative strengths of these three processes may, however, vary with seasons leading to different levels of dissolved N that can possibly be lost by leaching at different times of the year. Since leaching of dissolved N from soils occurs presumably at times when both plant and microbial N demand is low (Neff et al. 2003), temporal partitioning of N between plants and soil microbes may be a key mechanism that minimizes N losses from ecosystems. Such a partitioning has been observed in several studies of strongly N-limited ecosystems (Jaeger et al. 1999, Lipson et al. 1999, Schmidt et al. 2007). Schmidt and co-workers (2007) presented a concept of seasonal succession of N cycles in alpine systems: in this concept soil microbes are immobilizing N in the absence of plant N uptake during autumn and winter, and release N at a time of maximum plant N demand. It was further suggested that fungi, which are able to efficiently utilize inorganic and organic nitrogen from complex plant residues, dominate the microbial community during winter, whereas bacteria, fuelled by root exudates, are more active during summer (Bardgett et al. 2005). Such a concept of seasonal succession of N cycles has, to our knowledge, not yet been tested for temperate forests.

It has been the prevailing opinion until recently that decomposition of plant litter and thus ecosystem N cycling is under the predominant control of soil microbes (Knops et al. 2002). There is now, however, increasing evidence that plants may exert much greater influence on soil N cycling than previously thought (Chapman et al. 2006, Högberg and Read 2006). Recent studies have shown that almost half of the soil respiration in forests is derived from belowground C allocation of recent photosynthates (Högberg et al. 2001, Högberg and Read 2006), and it was further estimated (although with a large uncertainty) that up to 20-30% of
the net primary production of temperate and boreal trees is possibly invested to support ectomycorrhizal fungi (Hobbie 2006, Courty et al. 2010). This large input of C to the soil microbial community may in turn strongly affect soil N cycling. A few years ago, Chapman and co-workers (2006) presented a concept of how N cycling may be controlled by different types of plant-decomposer interactions. Fast-growing, N-rich plant species (e.g., some grasses or tropical trees), for example, produce litter which decomposes quickly and exhibit only a loose plant-decomposer coupling. They are mainly associated with arbuscular mycorrhizal fungi, which possess only a low ability to degrade complex compounds (Cornelissen et al. 2001, Read and Perez-Moreno 2003). In this case, litter N is mineralized mainly by free living microbes and N is recycled through the microbial and the labile soil N pool back to plants. More ‘nitrogen-conservative’ plants (such as temperate or boreal trees), on the other hand, produce more recalcitrant, low-nutrient litter and at the same time support high levels of (ecto-)mycorrhizal symbionts (Cornelissen et al. 2001). Ectomycorrhizal fungi are able to degrade complex organic compounds and thus make plant litter N directly accessible to their host-plants (Read and Perez-Moreno 2003). This mechanism short-circuits the mineralization of N through the labile soil N pool and may thereby minimize possible losses of N from the respective ecosystem (Chapman et al. 2006).

Thus, by releasing recently assimilated photosynthates to the soil, trees may pose a strong control on the mechanisms by which N is recycled in the soil. Since temperate forests depend on high N recycling from dead plant biomass, it would be conceivable that the soil microbial community established under the influence of tree C input advances the efficiency of N recycling in the forest soil. While several studies have addressed possible mechanisms how plants may control N cycling in different ecosystems (Chapman et al. 2006) the seasonal aspects of this plant control has received little attention so far. Thus, the aims of our study were (1) to investigate the seasonal microbial N cycle in a temperate beech forest by close monitoring of relevant processes and pools in monthly intervals over a time period of two years, and (2) to elucidate the effect of belowground C allocation and the presence of ectomycorrhizal fungi on the microbial N cycling in a seasonal context.

We used a beech tree girdling and N fertilization experiment to elucidate the role of plant-soil interactions in seasonal microbial N cycling. Girdling, which interrupts the C flow from the tree canopy to the roots, has led to an approximate 50% reduction of ectomycorrhizal fungi in our study site for a time period of 2-20 months after girdling (Kaiser et al. 2010). Here, we focus on the effect of a reduced plant control on the seasonal dynamics of microbial N cycling.
Material and Methods

Study site

This study was carried out in a temperate beech forest (*Fagus sylvatica*) situated in Lower Austria. Trees were on average 65 years old. The soil at this site is a dystric cambisol (over flysh), with a pH value of 4.5-5.1 (CaCl$_2$) and a C to N ratio of 15.5 (N: 0.48% of dry soil). Despite its proximity to Vienna, the atmospheric N deposition was on average only 12.6 kg ha$^{-1}$ yr$^{-1}$ from 2002-2004 (Kitzler et al. 2006).

Six control plots, 6 fertilisation plots (5 x 5m each) and 3 girdling areas (20 x 20 m) were established at the study site following a randomized block design: each of two control plots, two fertilised plots and one girdling area were chosen randomly within one geobotanically homogenous block. Two girdling sampling plots were installed in the central 10 m x 10 m of each of the girdling areas. Each of the three blocks thus held 2 replicate plots of each treatment and of the control. Within the 20 x 20m girdling areas, all trees (approximately 30 trees per area) were girdled on May 9$^{th}$ 2006 by removing the bark down to the phloem along a 0.2 m section around the trunk at 1.50 m height. Understory vegetation was clipped from all plots. Girdling and removal of understory vegetation was repeated in the following spring wherever necessary (some girdled trees produced small amounts of new bark, which did not, however, bypass the girdle). Fertilization plots were fertilized once a month (always after soil sampling) with NH$_4$NO$_3$ to obtain a final N fertilisation of 50 kg ha$^{-1}$ yr$^{-1}$. The first fertilisation took place in May 2006, one month prior to the first sampling (Kaiser et al. 2010).

Soil sampling

Soils from control plots were sampled once a month from June 2006 to June 2008, whereas fertilized and girdled plots were sampled only every two months, except June and July 2007 (which were both sampled), adding up to 24 samplings for controls and 13 samplings for treatments over a time period of two years. Soil was taken from the upper 5 cm of mineral soil (A horizon); from each replicate plot, four subsamples were taken and pooled. In order to avoid sampling of already disturbed soil we used a pre-determined sampling scheme. Soil samples were carefully sieved (2 mm), hand-picked from visible roots and kept at 4°C until further processing.

Climatic and abiotic conditions

Details about the annual course of soil temperature and soil water content in the two sampling years, as well as the progressive effect of girdling on tree vitality have been presented elsewhere (Kaiser et al. 2010). Briefly, temperature and precipitation patterns were different between the two sampling years such that soil experienced warmer and dryer conditions in the first autumn and winter period (i.e, no snow cover from Sept 2006 to January 2007) compared to the second autumn/winter. Soil moisture was significantly higher in girdled
plots from September to December of both years (by 5% - 10%), and in spring and summer of the second year (no difference between January and March of both years). Fertilisation did not affect soil water content.

**DOC and total dissolved N**

Dissolved organic carbon (DOC) and total dissolved N (dN) were measured in water extracts (2 g of fresh soil was extracted with 20 ml laboratory grade Water; HgCl was added to a final concentration of 10 μM; extracts were stored at -20°C) and in KCl extracts (2 g of fresh soil was extracted with 20 ml 1 M KCl; extracts were stored at -20°C) by a TOC/TN analyzer (TOC-V CPH E200V / TNM-1 220V, Shimadzu).

**Gross and net ammonification and nitrification rates**

Gross nitrogen transformation rates were assessed using the pool dilution technique (Myrold and Tiedje 1986): 500µl of $^{15}$NH$_4$Cl (ammonification) or $^{15}$NO$_3$ (nitrification) (each 0.25 mM, 10 atom% $^{15}$N) were applied to subsamples (2g) of fresh soil, which were then incubated for 4h and 24h (incubation temperature depending on the season: 5°C in winter and 10°C and 15°C in spring/autumn and summer, respectively) and finally extracted with 15ml of 2M KCl. For determination of gross ammonification and consumption, NH$_3$ was diffused from the extracts into acid traps and analysed for atom-percent access of $^{15}$N by continuous-flow isotope ratio mass spectrometry (IRMS) using an elemental analyser as a front-end (DeltaPLUS, Finnigan MAT, Bremen, Germany). For determination of gross nitrification and NO$_3^-$ consumption rates the $^{15}$N/$^{14}$N ratio of NO$_3^-$ was determined by the SPINMAS system (Stange et al. 2007): to achieve this soil extracts were mixed in a reaction vial with 2 ml of acidic V(III)Cl$_3$ solution at 85°C to form NO. The produced NO was transported with Helium as a carrier gas (10 ml min$^{-1}$) to the inlet capillary (open split) of a quadrupole mass spectrometer (GAM 400, InProcess Instruments GmbH, Bremen, Germany) where the $\delta^{15}$N values of the NO were analyzed. Prior the transfer in the quadrupole mass spectrometer H$_2$O and CO$_2$ were removed by a cryotrap (-120 °C). Rates of gross N mineralization and gross nitrification were calculated according to the following equations (modified from Bengtson et al. 2006):

$$\text{NH}_4^+ \text{ transformation rates:}$$

$$\text{gm} = \frac{(A_t - A_0)}{t} * (\ln (\text{APE}_t / \text{APE}_0) / \ln (A_t / A_0))$$

$$\text{gcm} = \text{gm} - (A_t - A_0) / t$$

$$\text{nm} = \frac{(A_t - A_0)}{t}$$

where gm is gross ammonification, gcm is gross NH$_4^+$ consumption, nm is net ammonification, $A_t$ is the pool size of NH$_4^+$-N at time t, $A_0$ is the initial NH$_4^+$-N, APE (atom percent excess) is the at% of $^{15}$N in the sample minus at% of $^{15}$N of an unlabelled control.
Plant control of soil microbial N cycling

NO$_3^-$ transformation rates:

\[ gn = \frac{(N_t - N_0)}{t} \times (\ln \left( \frac{APE_0}{APE_t} \right) / \ln \left( \frac{N_t}{N_0} \right)) \]

\[ gcn = gn - \frac{(N_t - N_0)}{t} \]

\[ nn = \frac{(N_t - N_0)}{t} \]

where \( gn \) is gross nitrification, \( gcn \) is gross NO$_3^-$ consumption, \( nn \) is net nitrification, \( N_t \) is the NO$_3^-$ N pool after time \( t \), \( N_0 \) is the initial NO$_3^-$ N pool.

Gross NH$_4^+$ consumption includes gross NH$_4^+$ immobilisation and losses of NH$_4^+$ by nitrification and volatilisation. We did not, however, subtract nitrification rates from gross NH$_4^+$ consumption rates to get gross NH$_4^+$ immobilisation rates, since nitrification rates may also include processes where NO$_3^-$ is formed from DON directly. Rather, we present all N transformation processes separately, which we think gives the most useful information.

**DON, nitrate and ammonium**

NO$_3^-$ and NH$_4^+$ were determined from water extracts by chemically suppressed ion chromatography (HPAEC on a Dionex AS11 column for anions and HPCEC on a Dionex CS16 column for cations) (Kaiser et al. 2005). Total dissolved N (dN) was analysed in water extracts by a TOC/TN analyzer (TOC-V CPH E200V / TNM-1 220V, Shimadzu). Dissolved organic N was calculated by subtracting inorganic from total N.

**Microbial biomass C and N**

Microbial biomass C and N was determined by the chloroform-fumigation-extraction method (Amato and Ladd 1988). A sub-sample (2 g) of fresh soil was fumigated with ethanol-free chloroform in a desiccator for 48 hours and subsequently extracted with 20 ml 1 M KCl. Microbial C and N was estimated from the difference of organic carbon and total nitrogen measured by a TOC/TN analyzer (see above) in KCl extracts of fumigated and unfumigated soils.

**Fungal biomass**

Bulk soil DNA was isolated using the FastDNA® Spin for Soil Kit (MP Biomedicals, Solon, Ohio, USA) and extracts were quantified photometrically (Nanodrop ND-1000, Nanodrop Technologies, Wilmington, DE, USA). SYBR-Green assays for the quantification of fungi were performed in an iCycler iQ5 Multicolor Real Time PCR Detection System (BIO-Rad Laboratories) using primer pair NSI1 (GATTGAATGGCTTAGTGAGG) (Martin and Rygiewicz 2005)/5,8S (CGCTGCGTTCTTCATCG) (Vilgalys and Hester 1990) as described elsewhere (Inselsbacher et al. 2010). Briefly, 25 µl reactions were composed of 12.5 µl 2x IQ™ SYBR®-Green Supermix
(BIO-Rad Laboratories), 0.4 μM of each primer and 0.2 mg/ml BSA. Standards and samples were processed in triplicates. The thermocycler program was set on 95°C for 3 min and 40 cycles of 95°C for 10s, 60°C (fungi) for 30s, 72°C for 30s with data collection at 72°C. Melting curve analysis was done in order to confirm the specificity of the PCR product. As standard pure culture genomic DNA from Cadophora finlandica PRF15 (Gorfer et al. 2007) was used as reference. To produce the quantitative data for fungal and total DNA (for Fig. 4) three technical repetitions of six biological were analyzed.

Results

Dissolved inorganic (DIN) and organic (DON) nitrogen measured from soil water extracts showed remarkable similar seasonal trends between the two sampling years. Ammonium, nitrate and DON pools peaked in both years during the summer period (July-August), and exhibited lowest levels during the autumn and winter period (Figure 1). The period of low N levels in autumn and winter was most pronounced for nitrate, whereas DON and ammonium showed some irregularities during that period.

Girdling strongly enhanced the amount of ammonium and nitrate in the soil but decreased the amount of DON in the first 18 months after girdling (Figure 1, Table 1). In the last three months of the sampling period, however, girdling strongly enhanced DON and had a less clear effect on ammonium and nitrate. The initial increase of ammonium in girdled plots levelled off at around 250 nmol N g⁻¹ dry soil in mid-summer, whereas nitrate continued to increase during the autumn from 20 nmol up to 250 nmol N g⁻¹ dry soil, which is more than ten-fold higher than the control nitrate level. Fertilisation also enhanced nitrate levels but to a lower extent than girdling and showed no clear effect on ammonium or DON.

Similar to DIN and DON pools, gross and net ammonification rates peaked in control plots during the summer months, and were lowest during the period between Nov-Feb (Figure 2). Net ammonification was negative during the winter period, which is consistent with a higher microbial NH₄ immobilization than production rate (gross consumption exceeded gross mineralization during that time period). This pattern was visible in both years. A general trend for gross nitrification and NO₃ immobilisation could not be worked out. Net nitrification, however, showed similar seasonal trends in both years, i.e. high activities in summer, followed by a decline in September and a second peak in late autumn before coming to relatively low levels in winter. Despite seasonal variations, net nitrification was almost always positive.

Girdling significantly decreased gross ammonification over the whole year, but had no overall significant effect on gross NH₄⁺ consumption rates (as assessed by factorial ANOVA, Table 1). When analysing only the winter period (Nov-Feb) of both years, girdling had a weak significant negative effect on gross NH₄ consumption rates (p=0.096, Table 1). In a full-year assessment, girdling significantly affected net ammonification rates, but the effect was inverse in the summer and in the winter season (significant interaction, Table 1). Girdling decreased overall net ammonification rates in the first and in the second year. When analysing only the
winter periods of both years, however, girdling significantly increased net ammonification rates in this season by 5.1 nmol NH$_4$-N g$^{-1}$ h$^{-1}$ on average ($p=0.014$, Table 1).

**In contrast to ammonification, girdling strongly enhanced nitrification rates.** It also enhanced gross NO$_3$ immobilisation rates, which resulted in net nitrification rates that were not significantly different between girdled and control plots except for the time period between 0.5 and 1.5 years after girdling. After 1.5 years the positive effect of the girdling treatment on gross nitrification rates diminished (Fig. 2, Table 1).

The effects of fertilisation on gross mineralisation and consumption were generally small and varied with sampling dates. This treatment had, however, a significantly negative effect on net ammonification rates in the first year, but again only for the sampling dates in the
second year. Overall fertilisation had a weak positive effect on nitrification rates (Fig. 2, Table 1).

Microbial biomass N showed a clear seasonal trend which was opposite to the trends of DIN, DON and to NH₄ mineralization. In both years, microbial N was lowest during the summer period (July-August), increased steeply towards autumn and winter (interrupted only by a short depression between September and October), and then stayed at high levels until spring (Figure 3). The trends of microbial biomass C and the C:N ratio were less clear, but it can be inferred from these data that the C:N ratio was higher in the summer compared to the winter of each year, with some fluctuations in between (e.g., high values around October). In general – and similar to microbial biomass N – microbial DNA was higher during the winter period compared to the summer period (Figure 4). In December, both fungal and total DNA steeply increased. Fungal DNA decreased in mid-winter (Feb), but total DNA did not, indicating an increasing ratio of bacteria:fungi at that time.

Girdling significantly decreased microbial N during the winter period (Figure 3, Table 1). This is consistent with the loss of microbial net N immobilisation during winter in girdled plots (Figure 2). In contrast, fertilisation significantly increased microbial N during autumn and winter (Table 1). Girdling reduced microbial C only at specific sampling times (e.g. in summer of both years, and in the winter of the second year only). Girdling progressively decreased the abundance of fungal DNA in the first 12 months. The loss of fungal DNA was most pronounced in December (-59.5%), February (-58.7%) and April (-68.8%).

Negative correlations between microbial N and net mineralization in the control plots (R²=0.51, p<0.001) as well as between microbial N and total dissolved N (R²=0.29, p<0.01) were observed. Furthermore, we observed a strong positive relationship between gross mineralization and total dissolved N measured in KCl extracts (R²=0.78, p<0.001) and between gross N mineralization and net mineralization (R²=0.48, p<0.001). These correlations basically reflect the opposite trends of process rates and N availability on the one hand and microbial N on the other hand over the course of the seasons. Microbial biomass N is low when process rates and N availability are high (e.g., in summer), whereas during the winter period, the situation is precisely the opposite. This opposite relation between N cycling processes during the different seasons is weakened by fertilisation and even diminished by girdling, as shown by the lower correlations strengths for these treatments (Fig. 5).
Figure 2: Nitrogen transformation processes over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to visualize seasonal trends. Summer (S) and winter (W) periods are highlighted in grey to facilitate identification of seasonal pattern. All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate 1 SE (n=6).
Figure 3: Microbial carbon and nitrogen over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to visualize the seasonal trend. Dotted lines connect control values to visualize seasonal trends. Summer (S) and winter (W) periods are highlighted in grey to facilitate identification of seasonal pattern. All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate 1 SE (n=6).
Discussion

The seasonal dynamics of microbial N cycling may be one of the most important determinants of the ability of ecosystems to retain N (Monson et al. 2006, Schmidt et al. 2007). Although extensive research on the seasonal N cycle has been conducted in alpine and arctic systems, little is known from other ecosystems, in particular about winter processes. In this study we provide a set of N pool and process data from a temperate beech forest for two full years in order to contribute filling this gap.

Our results revealed clear seasonal trends of microbial N cycling over the course of a year. In particular, we observed contrasting pictures in summer and in winter. While summer months (July-August) were generally characterized by high N mineralization rates, high dissolved N in the soil solution and low N levels in the microbial biomass, the winter period (Nov-Feb) exhibited the opposite trend: net microbial immobilisation of N, low availability of dissolved N in the soil solution, and high levels of N in the microbial biomass. Trends for early autumn and spring were less clear, they were characterized by fluctuations in microbial biomass C, microbial biomass N, DOC and N mineralization. These results point to a strong temporal partitioning of nitrogen between microbes and plants.

Together with high (gross and net) N mineralization and nitrification rates during summer, high levels of dissolved N in the soil solution suggest a high plant availability of during that time of the year. Our results support that of others, who reported peaks of N2O emission together with NO3 accumulation and a substantial decline of the microbial biomass N during July in a temperate beech forest (Zechmeister-Boltenstern et al. 2002). In our experimental plots, phenoloxidase and peroxidase activities were at maximum levels during July and August (Kaiser et al. 2010), indicating that humified soil organic matter may be the source for increased N mineralisation. The absence of complex fresh organic matter input at that time of the year may have rendered soil organic matter (SOM) degrading K-strategist microbes a competitive advantage, leading to the enhanced degradation and mineralization of N-rich SOM (Fontaine et al 2003). Additionally, higher N mineralization rates in summer may have been caused by root exudations, which may have accelerated microbial turnover and thereby N release to the soil (Moore et al. 2003). The same mechanism (increased microbial growth and predation triggered by labile C input) may also have kept the microbial N pool low during that time of the year.

During autumn and winter, when plant N uptake ceased, microbes switched from net N mineralisation to net N immobilisation which apparently leads to an accumulation of N in the microbial biomass over the winter. Again, year-round enzyme measurements at the same study site have shown that actual cellulase and protease activities were at maximum levels during autumn, which points to the degradation of fresh litter during that time being the most important process (Kaiser et al 2010). Our results suggest that microbes immobilize the N released by enzymatic break-down of litter (e.g., fine root litter, leaf litter) in autumn and store it at least in part in their biomass over the winter, at a time period when uptake by plants is negligible. This temporal partitioning of N between plants and microbes may be of great importance for ecosystem N retention, especially with regard to the fact, that the
Table 1. ANOVA analysis of treatment and seasonal effects on N transformation processes, N pools and microbial C and N for year 1 and year 2 of the experiment. Part A: seasonal (=sampling month) effects were analysed using values of all months and all treatments (controls, girdled, fertilized), including months, where only control plots had been sampled (n=144 for year 1 and n=156 for year 2, respectively). For the effect of treatment (girdling, fertilisation) only those months were used, in which treatment plots were sampled (n=72 for year 1, n=84 for year 2). Part B: for the ‘Winter only’ analysis, sampling data from the period of November to February of both years were used (n=49). Bold values indicate that either the treatment effect or the effect of interaction between treatment and month of sampling was significant for the respective parameter. Significance codes: ***, P<0.001; **, P<0.01; *, P<0.05, °, P<0.1.

(Part A)

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<th>All seasons</th>
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<th>Year 2</th>
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<td>Girdling</td>
<td>Fertilization</td>
<td>Month of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sampling</td>
<td>Mean diff.</td>
<td>Treatment</td>
<td>Mean diff.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G-C</td>
<td>Interaction</td>
<td>G-C</td>
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<tr>
<td>Nitrat</td>
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<td>Gross NH₄ consumption</td>
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<td>Net NH₄ production</td>
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<td>-0.06</td>
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<td>*</td>
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(Part B)

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<th>Fertilization</th>
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<td>Treatment</td>
<td>Inter-</td>
<td>Mean diff.</td>
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<td>G-C</td>
<td>Interaction</td>
<td>action</td>
<td>G-C</td>
</tr>
<tr>
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<td>-2.39</td>
<td>*</td>
<td>.</td>
<td>.</td>
</tr>
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<td>Net NH₄ production</td>
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<td>Microbial N</td>
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<td>µmol N g⁻¹ dm</td>
<td>-0.99</td>
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major input of fresh organic substrate to the soil in temperate forests occurs in autumn, when plants are thought to be inactive. The most N-rich constituent of plant litter (i.e., proteins) is degraded relatively fast, and the resulting reactive N could thus easily be lost by leaching or volatilization in the absence of microbial immobilisation. By storing this N pool at least in part in the microbial biomass over the winter it could be retained in the system and accessed by plants in the following spring. Such temporal partitioning of the N between plants and microbes has been observed before in arctic and alpine systems (Lipson, 1999, Schadt, 2003, Schmidt 2007). In these studies microbial biomass had been found to peak under late-winter snowpack followed by a significant decline when soils were thawing (Lipson 1999, Schadt 2003), thereby leading to increased N availability for plants at the start of the growing season (Bardgett et al, 2005, Jaeger 1999). Our results strongly suggest that a similar mechanism exists for temperate forests: the spring decline of microbial biomass N (from ~6 to ~2 µmol N g⁻¹ dry soil) accounts for 38 kg ha⁻¹, if calculated on an areal basis, which is as much as one third of the annual N demand of the vegetation of a temperate forest (Kreutzer et al. 2009).

**Figure 4:** Fungal DNA and total DNA in soils of a beech forest ecosystem over the course of the first sampling year in girdled and control plots (squares, controls; circles, girdled plots). Summer (S) and winter (W) periods are highlighted in grey to facilitate identification of seasonal pattern. All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate 1 SE (n=6).
Although dissolved inorganic and organic N showed the same seasonal trends, they were affected in opposite ways by the girdling treatment, which reduced DON, but strongly enhanced NH₄ and NO₃ levels in the soil. Root exudates are thought to promote rapid growth of r-selected microbes, thereby increasing predation, turnover and subsequent DON availability (Moore et al. 2003). Thus, stopping root exudates by girdling may have led to decreased microbial turnover and decreased DON levels in soil. The same mechanism may have led to reduced ammonification rates in girdling plots, which, however, seem to be contradictory to the strong accumulation of inorganic N in girdled plots. Reduced gross N mineralisation rates have also been reported from a girdling experiment in an old-growth spruce forest (Zeller et al. 2008) and gross mineralisation rates have been found to be significantly greater in the presence of Populus and Pinus seedlings compared to non-planted controls (Dijkstra et al. 2009). These findings are consistent with our results, which strongly indicate that belowground C allocation by trees has a positive effect on soil organic matter decomposition and subsequently on gross N mineralisation. A possible explanation for the increased levels of inorganic N in the soil solution may be that roots, which were cut-off from the C supply by the tree, had to reduce the uptake and assimilation of NH₄ and NO₃ since especially NO₃ assimilation is an energy-demanding process. This may have led to the strong accumulation of NH₄ in the soil, which—in turn—may have triggered the increase of nitrification and subsequent accumulation of NO₃ in the soil. The increase of nitrification rates in girdled plots was apparently caused by an increase of bacterial and archaeal nitrifiers in the soil, which had been observed by functional gene analysis of soil samples in the same experiment (Rasche and co-workers, unpublished).

A positive effect of girdling on ammonium and nitrate levels in soils has also been observed before in other girdling experiments conducted on various tree species (Pinus contorta, Weintraub et al. 2007, Picea abies, Zeller et al. 2008, Fagus sylvatica, Dannenmann et al. 2009), although this effect was not always as clear as in our study (Weintraub et al. 2007, Dannenmann et al. 2009). Similarly, reduced DON levels have been observed in some studies (Weintraub et al. 2007), but not in others (Zeller et al. 2008, Dannenmann et al. 2009). These differences may be attributed to different site characteristics but also to different times of girdling and sampling. Our results demonstrate that the response of soil N pools and fluxes to girdling strongly depend on season and on time elapsed since girdling thereby emphasising the importance of these parameters for interpretation.

Although ammonium and nitrate availability were strongly enhanced in the girdling treatment there was a significantly lower immobilisation of N into the microbial biomass over the winter compared to control and fertilisation plots. This raises the question, what actually induced the switch from net mineralization to net immobilisation in autumn in control and fertilised plots. An intuitive explanation would be that there is lowered competition with plant roots for N uptake at the end of the vegetation period, which may have enabled microbes to increase immobilisation of N. However, our measurements of N mineralization rates were always made in absence of plant roots, which rebuts this argument. Furthermore, if reduced plant N uptake had been the cause of increased microbial N uptake, microbial N...
immobilisation rates would have increased in girdled plots where plant N uptake was reduced and N availability was high. This was, however, not the case. Instead, several other reasons may have led to the switch of microbial N cycling processes in autumn, as for example physiological adaptation of the microbial community to winter conditions, diminishing root C input to the soil and/or a change of the microbial community composition. In particul-
lar the stop of root C input to the soil substantially changes the limitation status of the soil microbial community (Fontaine et al. 2003), decreases microbial turnover rates (Moore et al. 2003) and changes microbial community composition (Yarwood et al. 2009, Kaiser et al. 2010). Thus, one reason for the increase of microbial N towards winter could be, that fast-turnover-bacteria, which were fuelled by root exudates and fresh organic matter degradation in autumn, switch – in the absence of labile C input in winter - to dormant stages (physiological adaptation to unfavourable conditions), keeping the N in their biomass. Another possibility may be a contribution of mycorrhizal fungi to that process: they are stronger N sinks than other microorganisms since they are not C limited due to the constant C supply by their hosts (Högberg et al. 2007, Högberg et al. 2008) and due to their ability to translocate C within their mycelia to spots with high N concentration, which enables them to avoid N mineralization even during degradation of N-rich substrate (Boberg et al. 2010). It could thus be, that during summer, N taken up by mycorrhizal fungi is mostly transported to the tree (explaining low N levels in the microbial biomass), whereas during autumn and winter, it could be efficiently immobilised and stored in the fungal hyphae network, which we found to grow to maximum levels during that time (Fig. 4). Our results from the girdling treatment strongly emphasises this explanation: microbial winter N storage was strongly reduced when tree root C input was stopped by girdling. Since tree girdling changes the C supply compared to control plots only during the photosynthetic active period, this can only be explained by being an effect of the strongly decreased fungal (i.e. mycorrhizal) abundance in girdled plots.

The greatest fluxes of root exudations have been found to occur in the late season (Horwath et al. 1994, Kagawa et al. 2006, Högberg et al. 2010). A recent $\text{^{13}CO}_2$ labelling experiment in a boreal forest revealed that belowground C allocation of plant C was about 5-times higher in the late season compared to the early season and that the majority of this C was taken up by fungi, as indicated by $\text{^{13}C}$ PLFA-analysis (Högberg et al. 2010). Autumn, however, is also the time of increased N input by litterfall, which has been found to lead to increased cellulase and protease activities (Kaiser et al. 2010). This high simultaneous C and N availability may thus explain the observed increase of total and fungal biomass in autumn. The large input of recent photosynthetates to the soil by trees in the late season may be a plausible mechanism to provide C for mycorrhizal growth, thereby enabling the fungi to accumulate N released by litter degradation. Without the energy from root exudates in autumn, the ability of soil microbes to grow and incorporate N may be limited, as suggested by our girdling plot results. According to our data this limitation strongly affects fungal (especially mycorrhizal) species, emphasising the important role of mycorrhiza for decomposition and N recycling in temperate forests (Read and Perez-Moreno 2003, Talbot et al. 2008, Wurzburger and Hendrick 2009).

Taken together, our results indicate that temperate (deciduous) trees may actively control the recycling of N originating from the degradation of autumn litter input from one year to the next by supporting a specialised belowground microbial community. Thus, our data expand the recently developed concept that soil N cycling is more controlled by plants and less by microbes than previously thought (Chapman et al. 2006, Högberg and Read 2006, Chapin
et al. 2009) for temperate forest ecosystems. Furthermore, our results suggest that the often observed late season peak in tree root exudates may represent an important function for the plant control on belowground N recycling.

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Chapter 5


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Chapter 6:
Summary
Summary

Microbial decomposition of soil organic matter (SOM) is a key process for the global carbon cycle. Decomposition processes may be limited by abiotic conditions, such as temperature or soil moisture, but also by available resources, such as carbon (C) and nitrogen (N).

Unfavorable abiotic conditions, such as low temperature and high soil moisture substantially delay decomposition processes in soils of Arctic regions, which has led to the accumulation of huge amounts of C in these soils. Assessing the impact of these conditions on arctic C storage is, however, complicated by the fact that soil layers with different C content have frequently been mixed by freeze-thaw processes (‘cryoturbations’) leading to distorted, broken and warped horizons in large parts of the Arctic region. Cryoturbations may lead to the long-time exposure of humic-rich topsoil layers to unfavourable abiotic conditions in deeper soil layers (i.e. nearer to the permafrost).

In the first part of this thesis I report on an investigation of the effect of such cryoturbations on soil decomposition processes at the G’dansky peninsula, Western Siberia. I found that C and N mineralisation rates were substantially delayed in humic-rich, buried O and A-horizons, which were found in approximately 30 -60 cm depth. These layers, which exhibited a mean C age of 1,300 years (measured by radiocarbon dating), still contained more C compared to the newly formed topsoil horizons (with a mean C age of approximately 400 years). This indicates that O and A horizons at the time of burying must have been significantly thicker than present-day O and A horizons, which thus may still posses the capacity to accumulate C. Cryoturbations may therefore be an important, but largely overlooked mechanism for soil C storage in arctic soils, which at the same time retards decomposition processes in buried layers and promotes C accumulation in newly formed topsoil layers.

In addition to abiotic conditions, resource availability and microbial community composition may be of central importance for decomposition of organic matter in soils. Due to a wide range of different cell structures, volume to surface ratios and biomass C:N ratio within the microbial population, certain microbial groups may exhibit different stoichiometric demands of C and N. Additionally, different microbial groups may exhibit different capacities to produce specific enzymes for the degradation of complex compounds, such as lignin, cellulose or humified SOM. Thus, it is likely that resource availability may affect microbial community composition (due to different C and N demands and due to different capacities to degrade specific substrates) whereas microbial community composition in turn will affect decomposition rates (due to different enzyme production capacities). The second and third part of this thesis focused on the link between resource availability, microbial community structure and decomposition of organic matter. The research for this part was conducted in a temperate beech forest in which plant seasonal cycles strongly alter C and N availability for soil microbes, which may affect microbial community composition and thus feed back on decomposition processes and N availability for plants. In order to get a better understanding for these interactions, C and N availabilities were experimentally altered by N fertilisation and tree girdling (which interrupts the transport of photosynthetates from the tree canopy to the roots). Soil processes and microbial community composition were closely monitored over a period of two years revealing a strong relationship between community structure and extracellular enzyme activities over the course of seasons. Enzymes involved in SOM degradation (phenoloxidases and peroxidases) showed highest activities during summer and early autumn, whereas cellulases and proteases peaked in late autumn - probably triggered by the input of fresh litter. Reducing C input by girdling resulted in a loss of mycorrhizal fungi and
some associated bacterial groups. At the same time, it led to an increase of SOM degrading enzymes and to a decrease of cellulase and protease activity, especially during autumn. This indicates that mycorrhizal fungi may be involved in the autumn degradation of root litter. N was immobilized into the microbial biomass predominantly in autumn and winter and released in spring, whereas microbial N mineralisation peaked in summer. These seasonal phases of “summer mineralization” and “winter immobilisation”, however, disappeared in the girdling treatment, indicating that they were driven by tree belowground carbon allocation, and were probably linked to a specific microbial community composition and/or the presence of mycorrhizal fungi. Taken together, these results demonstrate that SOM decomposition is, apart from abiotic factors, also driven by microbial community composition. Seasonal variations of abiotic factors, plant phenology and C and N availability affect microbial community composition and thereby decomposition processes over the course of the year. By releasing C to the soil, plants fundamentally alter microbial community structure and thus strongly affect seasonal patterns of microbial decomposition processes.
Zusammenfassung


Im ersten Teil dieser Dissertation wurde der Effekt von Cryoturbationen auf mikrobielle Abbauprozesse untersucht. Bodenprofile in einem ca. 4 ha großen Untersuchungsgebiet auf der G’dansky Halbinsel in Westsibirien (N 69 °43.0’, E 74°38.8’) wiesen durchwegs einen verzerrten, humusreichen Bodenhorizont in etwa 30-60 cm Tiefe, inmitten des vorhandenen B-Horizonts, auf. In diesem vergrabenen, ehemaligen O- und A-Horizont, dessen Kohlenstoffatome ein mittleres Alter von etwa 1300 Jahren (14C-Datierung) aufwiesen, waren sowohl Kohlenstoff (C) als auch Stickstoffmineralisierungsraten deutlich geringer, als in den aktuellen O- und A-Horizonten (mittleres C Alter etwa 400 Jahre). Darüberhinaus enthielt der vergrabene humusreiche Bodenhorizont im Durchschnitt mehr C pro m² als die beiden Oberbodenhorizonte zusammen, was darauf hindeutet, dass dieser Horizont zum Zeitpunkt seiner Verlagerung wesentlich mächtiger gewesen sein musste als die aktuellen Humushorizonte, und diese daher noch in der Lage sein sollten, weiteren C zu akkumulieren. Cryoturbationen könnten daher einen wichtigen, aber bislang unterschätzten Mechanismus für die C-Speicherung in arktischen Systemen darstellen, einerseits durch die Einschränkung von Abbauprozessen von organischem Material in tieferen Bodenschichten und andererseits dadurch, dass sie die Bildung neuer Humushorizonte auf den neu entstandenen, offenen (Mineral-)bodenflächen ermöglichen.


Der zweite und der dritte Teil dieser Dissertation beschäftigt sich daher mit der Verbindung von Ressourcenverfügbarkeit, mikrobieller Gemeinschaft und dem Abbau von organischem Material im Boden. Die Untersuchungen dazu wurden in einem Buchenwald in Niederöster-
Appendix
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Appendix

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Date of birth: 16. 7.1974
Nationality: Austria
Children: one daughter, Katharina Ranefeld (1997)

Education

1989-1994 Higher technical school (HTL) for Information technology (IT) and Organisation, Vienna, A-level exam
1995-2003 Study of Ecology at the University of Vienna
2003 M.Sc. in Ecology (Department of Chemical Ecology and Ecosystem Research, University of Vienna)
Diploma thesis „Soil carbon and nitrogen cycling in arctic tundra ecosystems in response to global warming“
2003-2010 Ph.D. study (Department of Chemical Ecology and Ecosystem Research, University of Vienna) covering two topics: (1) Decomposition of soil organic matter in cryoturbated arctic ecosystems and (2) Resource limitation of microbial decomposition of soil organic matter in a temperate beech forest.

Study abroad

2003 ERASMUS study abroad semester: Environmental Chemistry, University of Glasgow, UK (6 months)

Further Education

2004 2nd Envisat-Summerschool: Earth System Monitoring and Modelling, European Space Agency, Esrin, Italy (2 weeks)
2007 ALTER-Net Training Course: An Introduction to Ecological-economic Modelling for Designing and Evaluating Biodiversity Conservation Policies and Strategies, Bad Schandau, Germany (1 week)
2009 UFZ Training course: Ecological Modelling, Center for Environmental Research (UFZ), Department of Ecological Modelling, Leipzig, Germany (2 weeks)
Professional experience

Software development


1998-1999 Software development for Inbound Supply Management (Java, Paradine, Inc.)

2000 Database application development (Visual Basic, Access, Analog & Digital Messtechnik Ltd.)

University

2001 Field work in the arctic tundra, Siberia.

2002-2005 Tutor for the course “Carbon and Nitrogen cycles in alpine ecosystems”, University of Vienna

2003–2004 Collaboration in various research projects at the Department of Chemical Ecology and Ecosystem Research, University of Vienna

2004-2005 Collaboration in a research project on canopy nitrogen uptake of montainous beech and conifer forests (Integrated Monitoring Site Zöbelboden, Upper Austria, Umweltbundesamt Austria)

2006-2008 Collaboration in the research project 'LTSER Eisenwurzen: Development of an integrated model of socio-economic and ecological material and substance flows'. Within the project I was responsible for the development of the ecological model part (Institute of Social Ecology, University of Klagenfurt & Dept Chem. Ecology and Ecosystem Research, University of Vienna).

2006-2008 Collaboration in the research project „Resource limitation of microbial decomposition of soil organic matter“ as part of my doctoral thesis (Department of Chemical Ecology and Ecosystem Research, University of Vienna)

2008-2009 Assistant lecturer for the practical course „Interaction of terrestrial and aquatic Ecosystems“ (Bachelor program Ecology, University of Vienna)

Practical experience

HPLC (anions, cations, LMW-carbohydrates), soil extracellular enzyme activities, standard soil methods (CFE, ammonia, amino acids), soil respiration (IRGA), stable isotope approaches (¹³C labelling experiments, ¹⁵N pool dilution method), PLFAs, modelling of ecosystem C and N flows, as well as of spatial explicit microbial community dynamics in the soil (Java, Anylogic)

Interests

Soil microbial ecology (linking community structure and function), ecosystem ecology, biogeochemical cycles, seasonal dynamics of C and N cycling, ecological modelling (individual-based modelling), global change, plant-soil interactions, mycorrhizal fungi
Publications

Peer-reviewed journals:


Appendix


**Contributions to Conferences:**


"He said he would come in", the White Queen went on, "because he was looking for a hippopotamus. Now, as it happened, there wasn't such a thing in the house, that morning."

"Is there generally?" Alice asked in an astonished tone. "Well, only on Thursdays," said the Queen.

— Lewis Carrol