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Plants control the seasonal dynamics of microbial N cycling in a beech forest soil by belowground C allocation

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Abstract. Soil microbes in temperate forest ecosystems are able to cycle several hundreds of kilograms of N per hectare per year 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 unraveling 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 and August) and (2) a winter N immobilization phase (November–February). The summer mineralization phase was characterized by a high N mineralization activity, low microbial N uptake, and a subsequent high N availability in the soil. During the autumn/winter N immobilization 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 immobilization 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 and may thus account for almost one-third of the annual plant N demand. Tree girdling strongly affected annual N cycling: the winter N immobilization 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 feed back on plant N availability in the following growing season.

Key words: *beech forest; belowground carbon allocation; ectomycorrhiza; N cycle; N retention; plant–soil interactions; seasons; soil microbial community; tree girdling.*

Introduction

Terrestrial productivity in temperate forests is among the highest globally, necessitating a fast recycling 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 $N \cdot ha^{-1} \cdot yr^{-1}$, which approximately equals annual input of N from aboveground and belowground litterfall (Norby and Iversen 2006, Kreutzer et al. 2009). This high plant N demand is thus met to a high extent (>80%) by ecosystem 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 recycling 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).

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Microbes have been found to process a lot more N than the plant's annual N demand by depolymerization of soil organic matter and repeated turnover of microbial biomass N over the course of a year (Corre et al. 2007, Schmidt et al. 2007, Kreutzer et al. 2009). These processes lead to a continuous supply of short-term available N in the soil, which is subject to competition between microbes and plants (Schimel and Bennett 2004). The combined N uptake of plant and microbes determines the proportion of N that may potentially be recycled in the system vs. the proportion that is lost by leaching (Gundersen et al. 2006). If NH_4^+ released by microbial decomposition together with external inputs exceed the combined uptake of plants and microbes, a higher proportion of NH_4^+ is transformed to nitrate by microbial nitrifiers (Gundersen et al. 2006, Dise et al. 2009), leading to elevated rates of NO_3^- leaching from the soil with well-known consequences such as soil acidification and eutrophication of ground water and downstream water bodies (Vitousek et al. 1997).

The relative strengths of microbial N release on the one hand, and microbial and plant N uptake on the other hand 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 coworkers (2007) presented a theory of seasonal succession of N cycles in alpine systems: in this concept soil microbes immobilize 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 in general, which are able to efficiently utilize 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 coworkers (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 root colonization (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).

These examples demonstrate that plants may pose a strong control on the mechanisms by which N is recycled in the soil. While it is long known that plants may control ecosystem N cycling by producing litter of different substrate qualities, the effect of belowground C allocation on N recycling is less clear. 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 regarding 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 at 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 experiment to elucidate the role of plant-soil interactions in seasonal microbial N cycling (see Plate 1). 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). We propose that the reduced tree root C input to the soil, which resembles a drawback of plant control on soil processes, will have a

significant effect on the seasonal dynamics of microbial nitrogen cycling in the soil.

As a side effect, girdling has often been found to increase levels of inorganic N in the soil, because reduced C supply for roots may lead to decreased plant N uptake (Edwards and Ross-Todd 1979, Guo et al. 2004). In order to distinguish between the effects that may have been caused by increased inorganic N availability and the effect of reduced belowground C allocation, we additionally established a N fertilization treatment in parallel to the girdling treatment, where we fertilized the soil with NH_4NO_3 in monthly time intervals.

Material and Methods

Study site.—This study was carried out in a temperate beech forest (*Fagus sylvatica*) in Austria, approximately 40 km southwest of Vienna (510 m above sea level). Trees were on average 65 years old. The soil at this site is a dystric cambisol (over flysh), with a pH 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 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ from 2002 to 2004 (Kitzler et al. 2006). Sampling plots were chosen according to a randomized plot design (Kaiser et al. 2010). Briefly, three blocks of approximately 1600 m^2, each of which consisted of more or less homogenous vegetation and soil properties, were selected within a total area of ~5400 m^2. Two control plots (5 × 5 m), two fertilization plots (5 × 5 m) and one girdling area (20 × 20 m) were chosen randomly within each block. Two sampling plots (2.5 × 10 m) were installed within the central 10 × 10 m of each girdling area. Each of them was thus surrounded by a buffer zone of at least 5 m of girdled trees. Each of the three blocks thus contained two replicate plots of each treatment, giving a total of six replicate plots per treatment. Control and fertilization plots were at least 10 m apart from the girdling areas. Within the 20 × 20 m girdling areas, all trees (approximately 30 trees per area) were girdled on 9 May 2006 by removing the bark down to the phloem along a 0.2-m section around the trunk at 1.50 m height (see Plate 1). 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_4NO_3 to obtain a final N fertilization of 50 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$. The first fertilization took place in May 2006, one month prior to the first sampling (Kaiser et al. 2010). Fertilization was conducted year-round, except in months when the forest floor exhibited a full snow cover, which happened only in the second sampling year (December 2007–February 2008). In this case, the sum of the suspended fertilization doses was applied as an accumulated N input in the first snow-free month (March 2008).

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 (approximately 250 g soil each) were taken and pooled. In order to avoid sampling of already disturbed soil we used a predetermined sampling scheme. Soil samples were carefully sieved (2 mm), hand-picked from visible roots and kept at 4°C until further processing. All extractions were carried out on fresh soil within 4 days after sampling.

Climatic and abiotic conditions.—Details about the annual course of soil temperature and soil water content in the two sampling years 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 September 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). Fertilization did not affect soil water content.

Tree vitality.—In the first year of girdling (2006), leaf senescence started approximately three weeks earlier in girdled plots compared to control plots. In the subsequent spring (2007), girdled trees still developed a full flush of leaves. Leaf litter traps, which were installed in May 2007, showed that, although there was no difference in the total amount of leaf litter produced by control and by girdled trees in the second year after girdling, litter fall started notably earlier in girdled plots (34% of total leaf litter fall occurred in girdled plots before the end of September compared to only 13% in control plots) (Kaiser et al. 2010). In spring 2008 (two years after girdling) 40% of girdled trees did not develop new leaves. Fine root biomass in girdled plots did not change 4 months after girdling, but decreased by 45% compared to control plots 14 months after girdling (Kaiser et al. 2010).

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 μmol/L; extracts were stored at −20°C) and in KCl extracts (2 g of fresh soil was extracted with 20 mL of 1 mol/L KCl; extracts were stored at −20°C) by a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V, Shimadzu, Vienna, Austria).

Gross and net ammonification and nitrification rates.—Gross nitrogen transformation rates were assessed using

the pool dilution technique (Myrold and Tiedje 1986): 500 μL of $^{15}NH_4Cl$ (ammonification) or $K^{15}NO_3$ (nitrification) (each 0.25 mmol/L, 10 atom% ^{15}N) were applied to subsamples (2 g) of fresh soil, which were then incubated for 4 h and 24 h (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 15 mL of 2 mol/L KCl. For determination of gross ammonification and consumption, NH_3 was diffused from the extracts into acid traps and analyzed for atom-percent excess of ^{15}N by continuous-flow isotope ratio mass spectrometry (IRMS, Delta-PLUS, Thermo Finnigan, Bremen, Germany) using an elemental analyzer as a front-end (EA 1110, CE Instruments, Milano, Italy). 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) 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_2O 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). Net ammonification rates were calculated as the difference between gross ammonification and gross NH_4^+ consumption, i.e., the net change in the NH_4^+ pool over the incubation time (analogue for net nitrification rates, i.e., the net change in the NO_3^- pool over the incubation time),

NH_4^+ transformation rates:

$$\mathrm{gm} = (A_t - A_0)/t \times (\ln[\mathrm{APE}_0/\mathrm{APE}_t]/\ln[A_t/A_0])$$

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

$$\mathrm{nm} = (A_t - A_0)/t$$

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, and APE (atom percent excess) is the at% of ^{15}N in the sample minus at% of ^{15}N of an unlabelled control; and

NO_3^- transformation rates:

$$\mathrm{gn} = (N_t - N_0)/t \times (\ln[\mathrm{APE}_0/\mathrm{APE}_t]/\ln[N_t/N_0])$$

$$\mathrm{gcn} = \mathrm{gn} - (N_t - N_0)/t$$

$$\mathrm{nn} = (N_t - N_0)/t$$

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.

The rate for which we use the term "gross ammonification rate" in this study (in order to distinguish it from gross nitrification) is sometimes referred to as "gross N mineralization rate" in the literature. Both mean in fact the same rate (measured by the $^{15}NH_4^+$-pool-dilution approach) since ammonification is the first step of N mineralization, and nitrification is thought to be a subset of gross ammonification (organic N is first transformed to NH_4^+ and subsequent to NO_3^-). Gross NH_4^+ consumption includes microbial gross NH_4^+ immobilization *and* losses of NH_4^+ by nitrification and volatilization. We did not, however, subtract nitrification rates from gross NH_4^+ consumption rates to get microbial gross NH_4^+ immobilization rates. 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 [Dionex, Vienna, Austria]; Kaiser et al. 2005). Total dissolved N (DN) was analyzed 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 subsample (2 g) of fresh soil was fumigated with ethanol-free chloroform in a desiccator for 48 hours and subsequently extracted with 20 mL of 1 mol/L KCl. Microbial C and N was estimated from the difference of organic carbon and total nitrogen measured by a TOC/TN analyzer 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, Delaware, USA). SYBR-Green assays for the quantification of fungi were performed in an iCycler iQ5 Multicolor Real Time PCR Detection System (BIO-Rad Laboratories, Hercules, California, USA) using primer pair NSI1 (GATTGAATGGCTTAGTGAGG) (Martin and Rygiewicz 2005)/5,8S (CGCTGCGTTCTT CATCG) (Vilgalys and Hester 1990) as described elsewhere (Inselsbacher et al. 2010). Briefly, 25-μL reactions were composed of 12.5 μL 2× IQ SYBR-Green Supermix (BIO-Rad Laboratories), 0.4 μmol/L 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 10 s, 60°C (fungi) for 30 s, 72°C for 30 s 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 replicates were analyzed. Mean values from triplicate measurements of each replicate sample were used to produce quantitative data for fungal and total DNA (see Fig. 4).

Results

The seasonal microbial N cycle

The results of our two-year sampling campaign revealed clear seasonal patterns of soil N pools and microbial N processes in a beech forest soil. In particular, we could identify two time periods, namely from July to August and from November to February with distinct trends for specific N parameters.

The pools of water-extractable N in the control soil (dissolved organic N, ammonium, nitrate) peaked in both years during the summer period (July–August), while exhibiting low levels during the autumn and winter period (Fig. 1). Similarly, gross and net ammonification rates (in control plots) peaked during the summer months, and were lowest during the period between November and February (Fig. 2). Net ammonification, which is the difference between gross ammonification (i.e., gross NH_4^+ production) and NH_4^+ consumption, was positive in both years in summer (July/August) and in early autumn (October). This means, that during these time periods, more N was mineralized to NH_4^+, than NH_4^+ was immobilized from the soil solution into the microbial biomass. However, from November until the next spring (again in both years), this pattern turned to the opposite: net ammonification rates were negative, indicating a higher NH_4^+ uptake than release by microbes during this time. Net nitrification showed similar seasonal trends in both years, i.e., high activities in summer, followed by a decline in September and a second peak in autumn before reaching relatively low levels in winter. Despite seasonal variations, net nitrification was almost always positive.

The trend of microbial biomass N was opposite to the trends of extractable N and N mineralization. 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 (again in both sampling years, Fig. 3). The opposing seasonal trends of microbial N on the one hand, and N mineralization and dissolved N pools on the other hand were also reflected in significant negative correlations between microbial N and net mineralization in the control plots ($R^2 = 0.51$, $P < 0.001$) as well as between microbial N and total dissolved N ($R^2 = 0.29$, $P < 0.01$) in both years (Fig. 5). Furthermore, we observed a strong positive relationship between gross mineralization and total dissolved N measured in KCl extracts ($R^2 = 0.78$, $P < 0.001$) and between gross N mineralization and net mineralization ($R^2 = 0.48$, $P < 0.001$). These correlations confirm the seasonal trends we observed for certain N cycling parameters over the course of the year: In the summer phase, microbial biomass N is low while process rates and N availability are high, whereas during the winter period, the situation is precisely the opposite.

Unlike microbial biomass N, microbial biomass C did not show a clear seasonal trend. Microbial C:N ratios used to be higher in the summer compared to the winter, with strong fluctuations, especially in the second sampling year. In both years, we found a steep increase in the microbial C:N ratio between June and August. In the first year, C:N ratio increased from 5 to 12, whereas in the second year from 7 to 22. The increase in the first year was accompanied by an increase in the relative percentage of fungal DNA (Fig. 4). Unfortunately, no DNA data are available for the second year. Total microbial DNA was, similar to microbial biomass N, higher during the winter period compared to the summer period (Fig. 4). Toward December, both fungal and total DNA steeply increased. Fungal DNA decreased in midwinter (February), but total DNA did not, indicating an increasing ratio of bacteria : fungi at that time.

Effects of girdling and fertilization on the seasonal microbial N cycle

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 (Fig. 1, Table 1). In the last three months of the sampling period, however, girdling strongly enhanced DON and had a less pronounced effect on ammonium and nitrate. The initial increase of ammonium in girdled plots leveled off at around 250 nmol N/g dry soil in midsummer, whereas nitrate continued to increase during the autumn from 20 nmol up to 250 nmol N/g dry soil, which is more than 10 times higher than the control nitrate level. Fertilization also enhanced nitrate levels but to a lower extent than girdling and showed no clear effect on ammonium or DON.

Girdling significantly decreased gross ammonification over the whole year, but had no overall significant effect on gross NH_4^+ consumption rates (as assessed by factorial ANOVA, Table 1). However, when analyzing only the winter period (November–February) of both years, girdling exhibited a weak significant decrease of gross NH_4^+ consumption rates compared to control plots ($P = 0.096$, Table 1). Girdling affected the seasonal pattern of net ammonification (net NH_4^+ production) rates (Table 1). Although overall net ammonification was decreased on average by girdling in each of the two years, it was significantly enhanced toward positive values during both winter periods in girdled plots ($P = 0.014$, Table 1). This led to a "loss" of the phase of net NH_4^+ immobilization during winter in girdled plots.

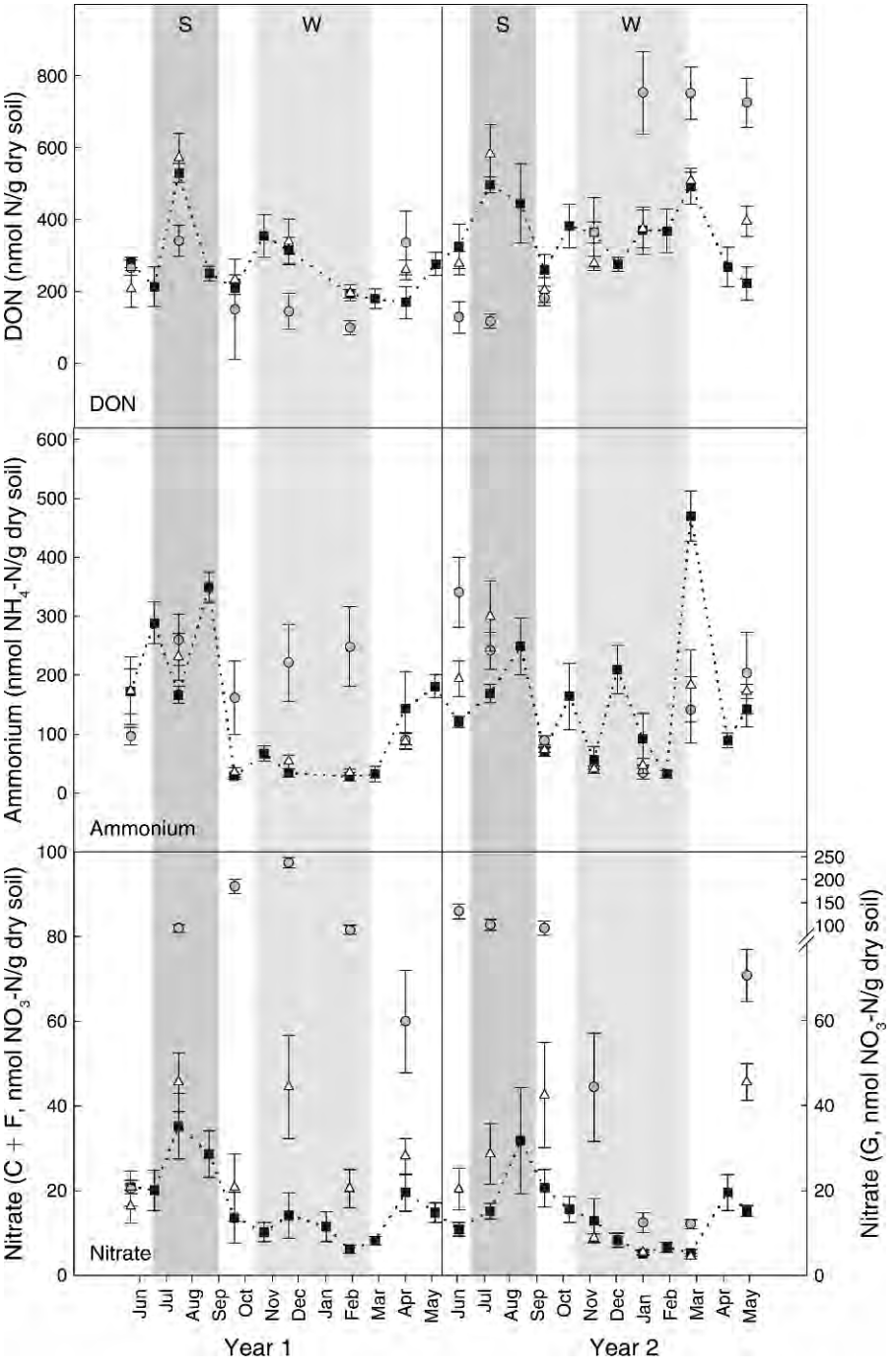

FIG. 1. Dissolved organic nitrogen (DON), ammonium, and nitrate in soil water extracts over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to visualize seasonal trends. Summer (S) and winter (W) periods are highlighted in gray to facilitate identification of seasonal pattern. Note the different scales for nitrate in girdled and control/fertilized plots (C, controls; F, fertilized; G, girdled). All data are plotted on the actual sampling date; ticks indicate the 15th day of each month. Error bars indicate ±SE ($n = 6$).

In contrast to gross ammonification, girdling strongly enhanced gross nitrification rates. It also enhanced gross NO_3^- consumption 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 fertilization on gross N transformation rates were generally smaller compared to the effect of

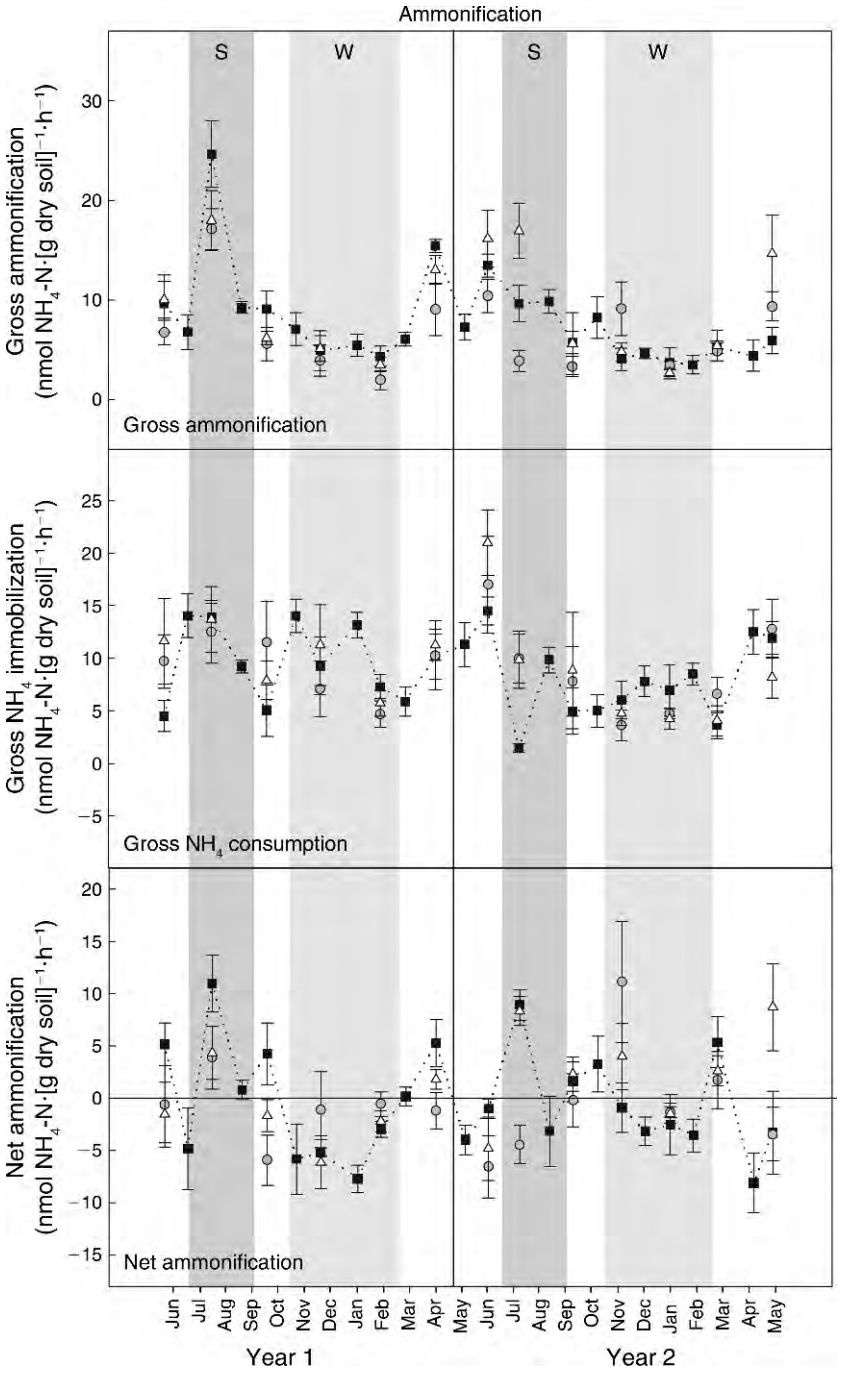

FIG. 2. Nitrogen transformation processes over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to visualize seasonal trends. Summer (S) and winter (W) periods are highlighted in gray. All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate ±SE ($n = 6$).

girdling, and varied with sampling dates. Fertilization had a significantly negative effect on net ammonification rates and a positive effect on gross NO_3^- production in the first year. Overall, fertilization had a weak positive effect on nitrification rates (Fig. 2, Table 1).

Tree girdling significantly diminished the build-up of microbial N during the winter period that had been observed for control plots (Fig. 3, Table 1). Midwinter values for microbial N in girdled plots were decreased by 18% and 44% compared to control plots in the first and

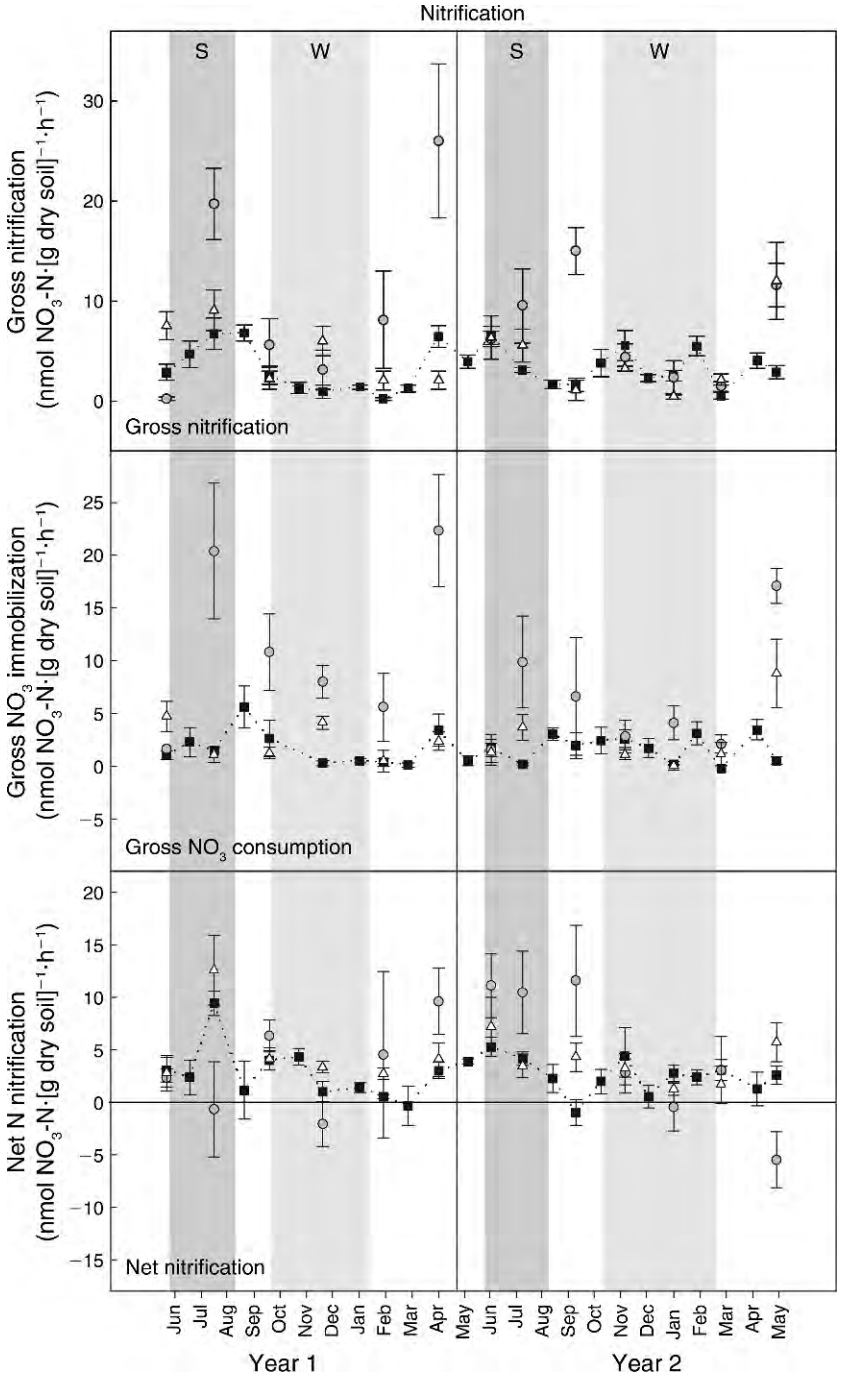

FIG. 2. Continued.

the second sampling year, respectively (Fig. 3). This is consistent with the disappearance of the microbial N immobilization phase (decrease of gross NH_4^+ consumption and increase of net ammonification) during winter in girdled plots (Fig. 2). In contrast, fertilization 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 (−60%), February (−59%) and April (−69%).

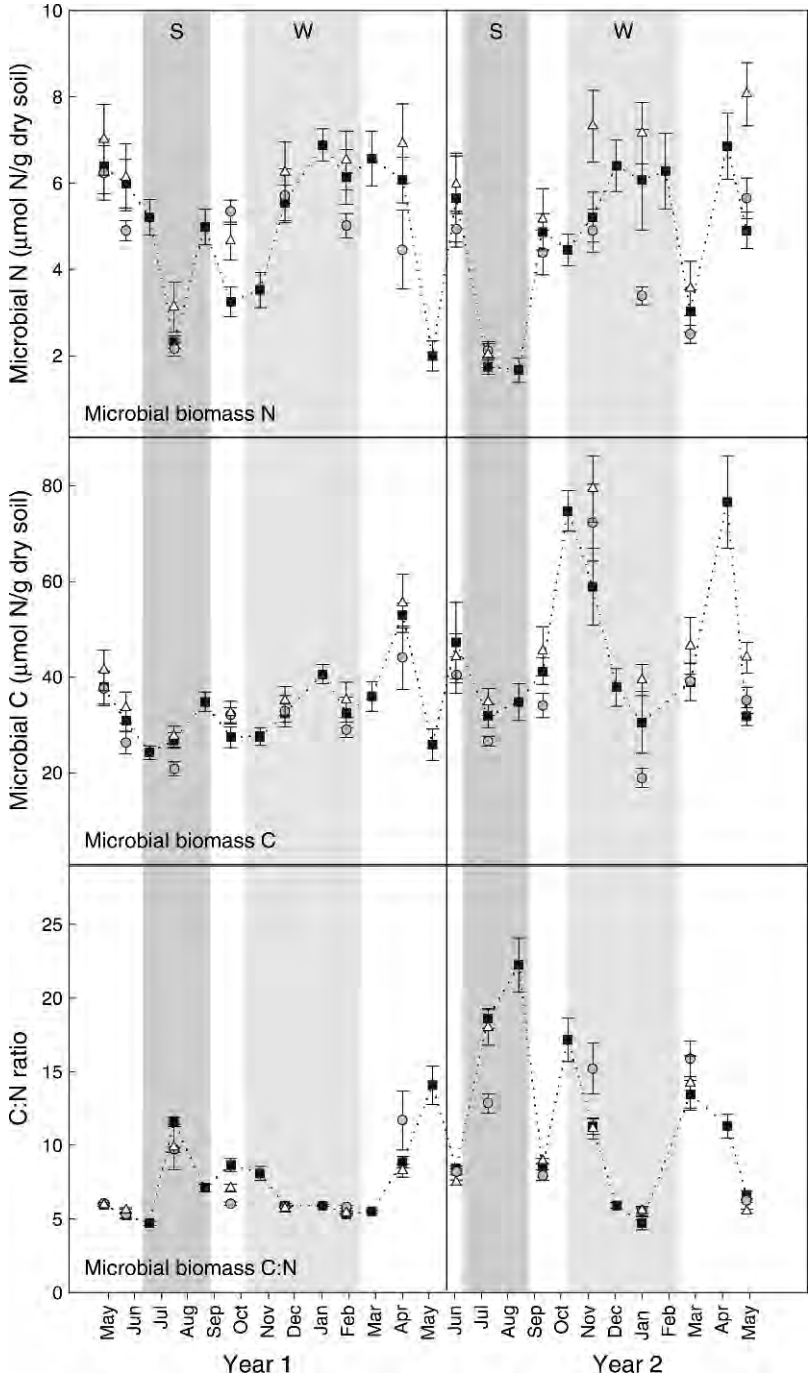

FIG. 3. Microbial carbon and nitrogen over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to indicate the seasonal trend. Summer (S) and winter (W) periods are highlighted in gray. All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate ±SE ($n = 6$).

The seasonal trends of microbial N, dissolved N pools and N cycling processes that were present in the control plots, were significantly affected by girdling. In particular the opposing relation between microbial N on the one hand and N pools/processes on the other hand over a year's course disappeared in girdling plots. This was confirmed by the absence of significant negative correlations between these parameters in girdled plots (Fig. 5). By altering the winter increase of microbial N immobilization and reducing the summer peak of N

mineralization, girdling led to a decoupling of the year-round relations between these parameters that was found in control plots.

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. Similarly, the effect of tree belowground C allocation and mycorrhizal fungi to the soil microbial N cycle is still not well understood. In this study we provide a set of N pool and process data from a girdling experiment in a temperate beech forest for two full years in order to contribute filling this gap. Our results revealed clear seasonal trends of soil 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 immobilization of N, low availability of dissolved N in the soil solution, and high levels of N in the microbial biomass. These results point to a strong temporal partitioning of nitrogen between microbes and plants in a beech forest soil. Furthermore, our results revealed a strong plant control over these seasonal patterns: the reduction of belowground C allocation by tree girdling diminished not only the peak summer N mineralization, but also the winter immobilization of N into the microbial biomass, suggesting that plants may actively control the annual forest N re-cycling.

Seasonal patterns of microbial N cycling

The summer peaks of dissolved N and (gross and net) N mineralization and nitrification rates suggest a high availability of N for plants during that time of the year. In a parallel study we observed that extracellular phenoloxidase and peroxidase activities (measured from the same plots) were also at their maximum levels during July and August, while cellulytic enzyme activities were low (Kaiser et al. 2010). This indicates that humified soil organic matter may be the source for increased N mineralization in summer. Concomitantly, we found a steep increase in the microbial C:N ratio between June and August in both years, which was accompanied by an increase in percent of fungal DNA. The wideness of microbial C:N ratios in the summer was a consequence of extraordinarily low levels of N in the microbial biomass whereas levels of microbial C were average. Fungi are known to exhibit more variable C:N ratios compared to bacteria, depending on the C:N ratio of their substrate (Sterner and Elser 2002). It is thus possible that the increase of microbial C:N ratios

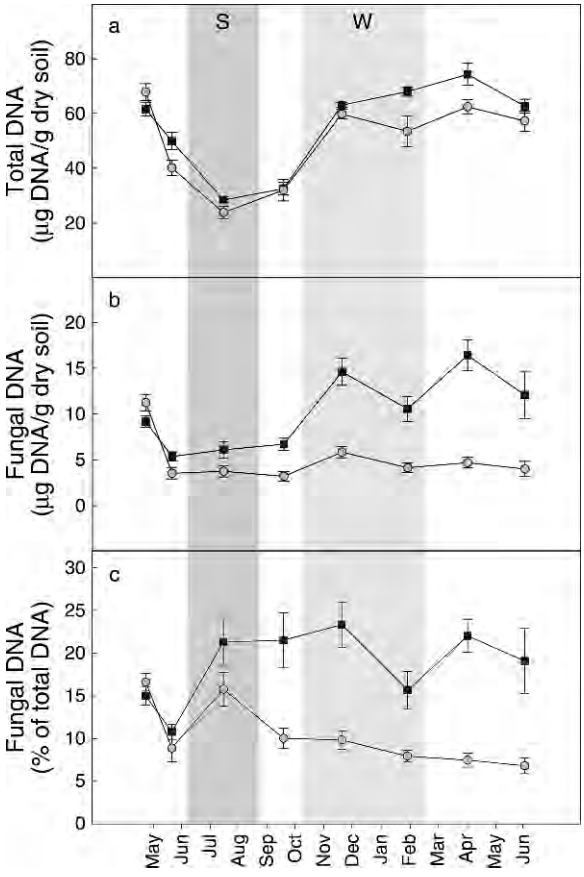

Fig. 4. Fungal DNA and total DNA in soils of a beech forest ecosystem over the course of the first sampling year in girdled and control plots (squares, controls; circles, girdled plots). Summer (S) and winter (W) periods are highlighted in gray. Error bars indicate ±SE ($n = 6$).

reflected the specific situation for microbes in this part of the year (high plant N demand), together with an increase of the fungal population. Although rather high (particularly in year 2), these C:N ratios are still in the range of what was found in other studies on natural communities (Cleveland and Liptzin 2007). Our results of summer soil N trends are consistent with results of a previous study, which reported a peak of available NO_3^- coupled to a substantial decline of microbial biomass N in mid-summer in a beech forest soil (Zechmeister-Boltenstern et al. 2002).

During autumn and winter, when plant N uptake ceased, microbes switched from net N mineralization to net N immobilization which apparently led 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

TABLE 1. Effects of girdling, fertilization, and season on N-transformation processes, N pools, and microbial C and N for each sampling year and the winter period of both sampling years, as assessed by ANOVA.

Parameter	Month of sampling	Girdling			Fertilization		
		Difference, G − C	Treatment	Interaction	Difference, F − C	Treatment	Interaction
A) Year 1							
All seasons							
Nitrate	***	1.56	***	***	0.18	**	†
Ammonium	***	0.08	***	**			
DON	***	−0.09	*				
Mineralization							
Gross NH_4 production	***	−4.39	***		−2.27	†	
Gross NH_4 consumption	**						
Net NH_4 production	***	−3.83	**	*	−3.75	**	
Nitrification							
Gross NO_3 production	***	6.81	***	**	1.44	*	**
Gross NO_3 consumption	*	12.36	***				
Net nitrification	†	−0.06		†	1.43	†	
Microbial C	***	−2.97	†		3.02	†	
Microbial N	***	−0.29		**	0.79	*	
Microbial C:N ratio	***	−0.20		***	−0.69	*	
B) Years 1 and 2							
Winter only (Nov–Feb of both years)							
Gross NH_4 consumption		−2.39	†				
Net NH_4 production		5.08	*				
Microbial N		−0.99	*		1.07	*	

Notes: For "all seasons," the effect of sampling month was analyzed for each year by one-way ANOVA using values of all months and all treatments (controls, C; girdled, G; fertilized, F), including months, where only control plots had been sampled ($n = 144$ for year 1 and $n = 156$ for year 2). For the effect of treatments (girdling or fertilization), only data from those months were used (treatment and control), in which the treatment plots were sampled ($n = 72$ for year 1, $n = 84$ for year 2). The effect of each treatment was assessed by a two-way ANOVA, using the respective treatment and sampling month as factors (effect of sampling month not shown; interaction describes interaction between treatment effect and effect of sampling month). For the "winter only" analysis, sampling data from the period of November to February of both years were used ($n = 49$). Units for mean differences between treatment and control plots are μmol NO_3-N/g dm (nitrate), μmol NH_4-N/g dm (ammonium), μmol N/g dm (dissolved organic nitrogen [DON] and microbial N), nmol NH_4-N·[g soil]$^{-1}$·h^{-1} (NH_4 gross production, gross consumption, and net production), nmol NO_3-N·g^{-1}·h^{-1} (NO_3 gross production, gross consumption, and net production), and μmol C/g dm (microbial C), where dm is dry soil mass.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.1$.

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 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 immobilization. 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 et al. 1999, Schadt et al. 2003, Schmidt et al. 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 et al. 1999, Schadt et al. 2003), thereby leading to increased N availability for plants at the start of the growing season (Jaeger et al. 1999, Bardgett et al. 2005). 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 dry soil) accounts for 38 kg/ha, if calculated on an areal basis, which is as much as one third of the estimated annual N demand of the vegetation of a temperate forest (Kreutzer et al. 2009). This remarkably high amount suggests that N immobilization and storage by soil microbes over the winter may be an essential prerequisite for spring plant growth in a temperate beech forest.

The effect of tree girdling on seasonal patterns of N pools and processes

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 NH_4^+ and NO_3^- levels in the soil.

TABLE 1. Extended.

Month of sampling	Girdling			Fertilization		
	Difference, G − C	Treatment	Interaction	Difference, F − C	Treatment	Interaction
***	0.88	***	***	0.16	***	**
***	−0.07		***	−0.25		***
***	0.00		*			
***	−0.44		**	2.73	*	†
***				1.31		†
***	−1.82		**	1.31		*
***	3.88	***	***	1.12		**
**	5.95	***	**	1.91	*	†
**	1.48		**	0.61		†
***				7.73	**	
***	−0.47		†	1.05	**	
***	−0.03		***			

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. Reduced gross N mineralization rates have also been reported from a girdling experiment in an old-growth spruce forest (Zeller et al. 2008) and gross mineralization 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 mineralization.

In spite of reduced ammonification rates, we observed a strong accumulation of inorganic N (NH_4^+ and NO_3^-) in girdled plots. A possible explanation for this may be that roots, which were cut-off from the C supply by the tree, had to reduce the uptake and assimilation of NH_4^+ and NO_3^- since especially NO_3^- assimilation is an energy-demanding process. The reduced C supply of roots (and thus N uptake capacity) was confirmed by a 45% decline of fine root biomass and a 60% decline of ectomycorrhizal colonization of the remaining vital roots 14 months after girdling (Kaiser et al. 2010). This may have led to the strong accumulation of NH_4^+ in the soil, which, in turn, may have triggered the increase of nitrification and subsequent accumulation of NO_3^- 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 et al. 2010).

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 emphasizing the importance of these parameters for interpretation.

The role of mycorrhizal fungi for ecosystem N recycling

Although ammonium and nitrate availability were strongly enhanced in the girdling treatment there was a significantly lower immobilization of N into the microbial biomass over the winter compared to control and fertilization plots. This raises the question, what

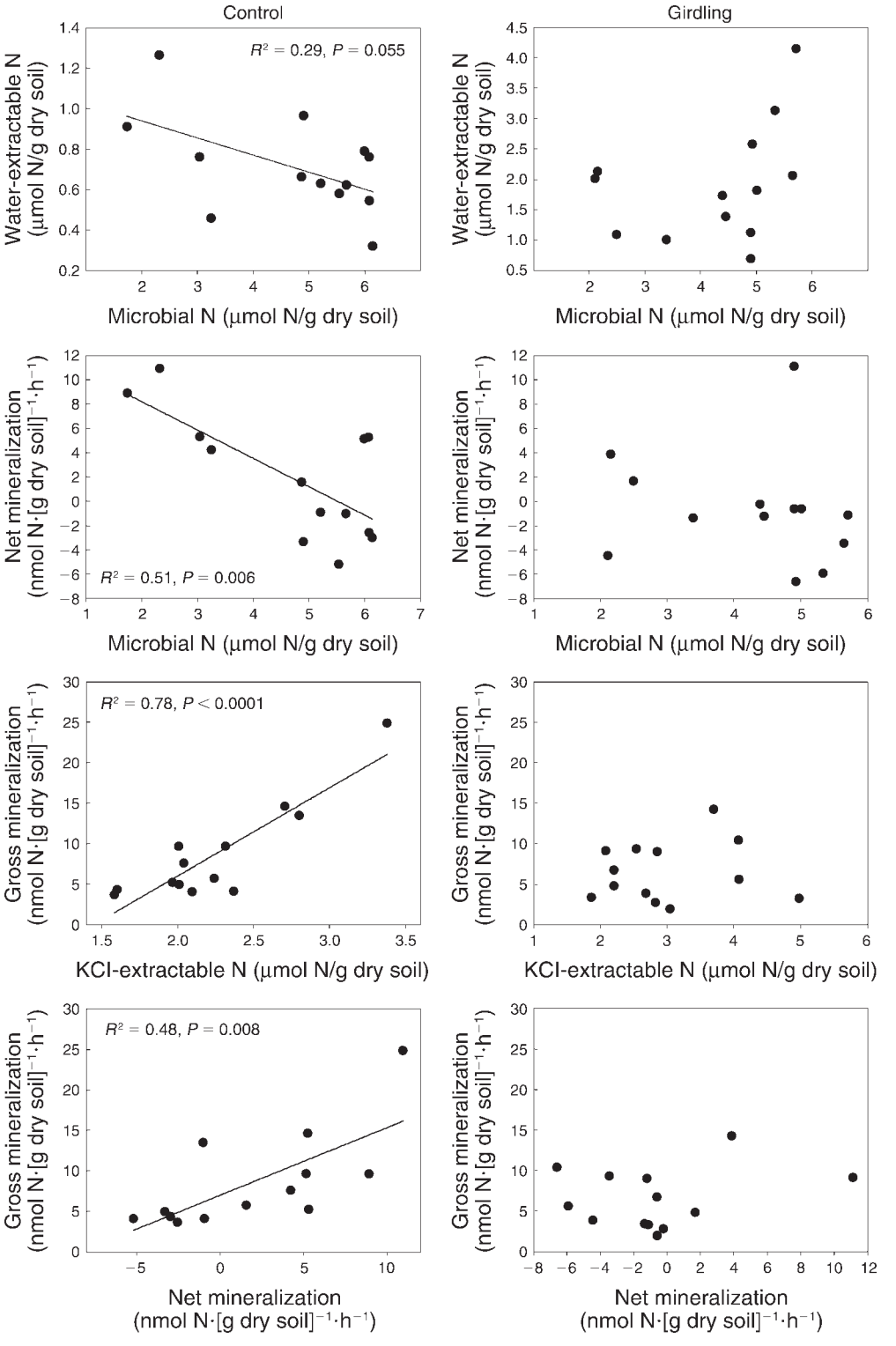

FIG. 5. Correlations between N pools and turnover rates in control and girdled plots. Each point represents the mean value ($n = 6$) of one of 13 sampling dates within two years (bimonthly samplings). The goodness of fit (R^2) and significance of the regression (P value) are given for all cases in which the correlation was significant. Water-extractable N and KCl-extractable N comprise dissolved inorganic nitrogen and dissolved organic nitrogen in water and 1-mol/L KCl extracts, respectively.

PLATE 1. Girdled beech trees (*Fagus sylvatica*) at our study site in Austria in April 2007 (one year after girdling). Photo credit: Franz Hadacek.

actually induced the switch from net mineralization to net immobilization in autumn in control and fertilized 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 immobilization 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 immobilization 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 particular 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). It is noteworthy that, in contrast to girdling, fertilization exhibited a positive effect on microbial winter N storage, especially in the second sampling year. It is thus unlikely that the increased concentrations of inorganic N in the soil of girdled plots may have been responsible for the loss of the wintertime N immobilization phase.

Naturally diminishing root C input towards winter is likely to affect mycorrhizal fungi in the first place. Additionally, girdling strongly decreased mycorrhizal fungi *and* led to the loss of the winter N immobilization phase. It is thus reasonable to consider a possible contribution of mycorrhizal fungi to the increasing microbial N levels towards winter. Mycorrhizal fungi are stronger N sinks than other microorganisms since they are not C limited due to the C supply by their hosts (Högberg et al. 2007, 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 immobilized 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 emphasizes 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 $^{13}CO_2$ labeling experiment in a boreal forest revealed

that belowground C allocation of plant C was about five 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 ^{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, emphasizing 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 specialized 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 recently observed late season peak in tree root exudates may represent an important function for the plant control on belowground N recycling.

Acknowledgments

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ERRATUM

There was an error of omission in Table 1 of Kaiser et al. in the May 2011 issue (pp. 1046–1047). The extended table on p. 1047 should have included some indication that it pertains to year 2 (not to year 1, as in the left-hand page of the table). We apologize to the authors and to our readers for this error, which was apparently introduced by the Publications Office.

Save the Dates!

FUTURE ANNUAL MEETINGS

97th ANNUAL MEETING
Sunday, August 5–Friday, August 10, 2012
Oregon Convention Center
Portland, Oregon

98th ANNUAL MEETING
Sunday, August 4–Friday, August 9, 2013
Minneapolis, Minnesota

99th ANNUAL MEETING
Sunday, August 3–Friday, August 8, 2014
Sacramento, California

100th ANNUAL MEETING
Centenial Celebration
Sunday, August 9–Friday, August 14, 2015
Baltimore Convention Center
Baltimore, Maryland