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Tree girdling increases soil N mineralisation in two spruce stands

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Abstract

Tree girdling is a common practice in forestry whenever trees are to be killed without felling. The effect of tree girdling on soil nitrogen (N) mineralisation was estimated in both an old and a young spruce forest. The dynamics of mineral N (NO_3^-–N and NH_4^+–N) and soil microbial biomass carbon (MBC) and N (MBN) were determined for different seasons. The in situ net N mineralisation was measured by incubating soil samples in stainless steel cylinders and the gross N mineralisation rates were measured by ^{15}N pool dilution method. Mineral N concentrations increased significantly in the girdled plots in both old and young spruce forests and showed variations between soil horizons and between sampling times. Tree girdling significantly increased net N mineralisation in both spruce forests. Annual net N mineralisation was 64 and 39 kg N ha^{-1} in O horizon of the girdled plots in old and young forest plots, respectively, compared to 25 and 21 kg N ha^{-1} in the control plots. Annual N mineralisation in A horizon was similar between girdled and control plots (31 kg N ha^{-1}) in the old forest whereas in the young forest A horizon N mineralisation was about 2.5 times higher in the girdled plots. As a result, the annual carbon budget was significantly more positive in the girdled plots than in the control plots in both old and young forests. However, we found significantly higher gross N mineralisation rates in both horizons in the control plots than the girdled plots in the old forest, but no differences between the treatments in the young forest. The MBC and MBN contents only showed significant changes during the first three months of the experiment and were similar later on. They first decreased as girdling removed the root carbohydrate, amino and organic acid exudation from the C sources for microorganisms then increased two months after the treatment root dieback acted as a new source of C. Mineralising microorganisms enhanced the mineral N concentrations in girdled plots as a result of greater activity rather than larger population size.

Keywords: Ammonium; Microbial biomass; Nitrate; N mineralisation; Spruce forest; Tree girdling

1. Introduction

In forest soils, N mineralisation of soil organic matter is an important mechanism as it is the main source of mineral N in these ecosystems. N mineralisation is a process, which converts organic N into ammonium N (NH_4^+–N). During this ammonification, organic N is transformed into NH_4^+–N by a variety of bacteria and fungi. Nitrification is the process of oxidising the NH_4^+–N into nitrate N (NO_3^-–N) and it is performed by chemoautotrophic bacteria or heterotrophic microorganisms using, respectively, CO_2 or soil organic matter as C sources (de Boer and Kovalchuk, 2001; Brierley et al., 2001). The respective contribution of ammonification and nitrification depends for a great part on site and stand characteristics. The net N mineralisation, that is the addition of new NH_4^+ and NO_3^- to the soil from organic matter, is calculated as the sum of net ammonification and net nitrification. It may be positive, which corresponds to an increase in ammonium and nitrate pool size over time, or negative, which is due to immobilisation and happens when mineral N is taken up by plants and microorganisms.

Since soil microorganims are the main actors of N mineralisation, factors that affect their activities and/or the

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size of their populations may also exert an indirect positive or negative regulation on soil N mineralisation. Factors that have been consistently reported to affect soil micro-organisms include soil temperature and humidity (Niklaus, 1998; Blumfield and Xu, 2003; Chen et al., 2003; Burton et al., 2007), C availability and the C/N ratio of the soil organic matter (Chen et al., 2004; Burton et al., 2007). Moreover, recent studies suggest that trees may also directly regulate soil N mineralisation. Colin-Belgrand et al. (2003) demonstrated that living tree roots can modify N mineralisation rate and that the rhizosphere seems to be the hotspot where regulation processes take place (Xu and Chen, 2006). Several studies have shown that nitrification is greater under deciduous tree species than under conifers (Augusto and Ranger, 2001). This may be due to a negative regulation of nitrification by the conifers that are well known to take up NH_4^+–N rather than NO_3^-–N (Buchmann et al., 1995). Natural inhibitors of nitrification such as polyphenol compounds or monoterpenes have indeed been identified in pine litter and in spruce forests (Northup et al., 1995, Paavolainen et al., 1998). There also may be a direct root excretion of nitrification inhibitors or activators of specific micro-organisms beneficial for the plant N supply (Erickson et al., 2000).

Tree girdling is a common silvicultural practice used for killing trees without felling them, e.g. for thinning and controlling the stocking of young forest stands while enhancing the wildlife habitat diversity. This operation consists of removing the bark, cambium, and sometimes the sapwood in a ring that extends entirely around the trunk of the tree. If this ring is wide enough and deep enough, it will keep the cambium layer from growing back. After the phloem layer is completely removed, carbohydrates produced in the shoots can no longer be transported to the roots that will die once they exhaust their carbohydrate reserves. This treatment is often done during spring, as the plant sensitivity is greater when its reserves are low. However, little is known about the effect of this method on the soil despite its common use and there is no straightforward way to estimate it. Root dieback should benefit soil moisture and then microbes for N mineralisation, especially if girdling removes the control that tree roots may exert on the soil microbes. But the carbohydrate starvation in roots will also lead them to suppress the root carbohydrate exudation, which is an important source of C for heterotrophic N mineralising microorganisms. Högberg et al. (2001) reported that girdling reduced soil respiration to 37% of the control plots within 5 days in conjunction with a root exudation decrease. Moreover, needle fall will increase following root dieback, in the case of conifers, and this may increase the polyphenol concentration in soil organic matter above thresholds that inhibit microorganisms.

Our study took place in Wetzstein, Germany, and was performed on both a young and a mature spruce forest. We studied soil net and gross N mineralisation, net nitrification, microbial C and N and dissolved organic N (DON) in both organic and mineral soil horizons. Measures were taken from the girdling time for one year in the old stand and two years in the young one. The aims of our work were to: (1) evaluate the effect of tree girdling on soil mineral N contents (NH_4^+, NO_3^-); (2) measure net and gross rates of N mineralisation following girdling; (3) compare the response of N mineralisation to girdling between the lower organic and upper mineral soil on one hand and between young and an old forest on the other hand; and (4) study if the microbial biomass C (MBC) and N (MBN) would respond to girdling and whether the possible response would be positive or negative.

2. Material and methods

2.1. Study site

The experimental sites were 90-year-old and 35-year-old Norway spruce (*Picea abies* (L.) Karst.) stands, respectively, both located in a small mountain range in southeast Germany (Wetzstein, Thüringisches Schiefergebirge, 50°27′N, 11°27′E, about 780 m a.s.l.). There was no under-story vascular plant vegetation. The soil was sandy loam overlying quartzite bedrock. Extensive monoculture of spruce has resulted in low pH (3.75) of the upper soil strata and subsequent podzol formation. In the old spruce stand, the average depth of the organic layer was 10.0 ± 0.7 cm and it stored 4370 ± 332 g C m^{-2} and 202 ± 12 mg N m^{-2}. The top 30 cm of the mineral soil contained 10403 ± 374 g C m^{-2} and 484 ± 21 mg N m^{-2}. In the young spruce stand, the organic layers was on average 8.0 ± 0.5 cm deep and stored 3870 ± 490 g C m^{-2} and 388 ± 49 mg N m^{-2}. The top 30 cm of the mineral soil in the young spruce stand contained 8080 ± 1.050 g C m^{-2} and 166 ± 24 mg N m^{-2}. Annual precipitation averaged 1000 mm and the mean annual temperature was 6 °C.

2.2. Experimental design

In April 2002, four plots of equal areas (400 m^2) were selected in homogeneous parts of each forest stand (no canopy gap, regular tree spacing) for a total of eight plots. Two plots per stand were used as control and the two others were treated by tree girdling. Girdling consisted of removing the bark and phloem of all trees in the plot all around the stems over 50 cm long sections at about 1.5 m above ground. This allowed the transport of water from roots to shoots via the xylem but prohibited phloem transport of photosynthetic products from shoots to roots.

In all plots, a soil sampling area (4 m × 4 m) was defined in the middle of the plot. This allowed a large enough buffer zone to avoid any influence from the neighbouring ungirdled stands on the sampled soil materials. Sampling areas were chosen so as to have their sides roughly equidistant to the neighbour trees.

Net soil N mineralisation was estimated using in situ soil incubation. This method consisted of isolated soil cores in

steel cylinders, thus suppressing root uptake. It enabled the incubation under field conditions, capturing the variability due to diurnal temperature fluctuations and weather conditions (Verchot et al., 2001). It is therefore the best way to estimate annual net N mineralisation (Persson et al., 2000; Jussy et al., 2004). Soil samples were collected every month from April 2002 to October 2002 in all the plots and from July 2003 to August 2003 only in the four plots of the young stand. The old spruce stand had been felled in July 2003 because girdled trees were heavily infected by bark beetles.

On each sampling occasion, six pairs of cores of undisturbed soil were collected along a line in the sampling area of each plot. Cores from a pair were separated by 10 cm and two successive pairs of cores were separated by 50 cm. Every month, cores were sampled on a new line 40 cm away from the previous line. On each sampling occasion, one core from each pair was taken to the laboratory to assess the soil parameters at the beginning of the month/end of the previous month (annotated as T_0 and T_{1S} values later on). The other cores from each pair were separately inserted in stainless steel cylinders (7.6 cm inner diameter, 15 cm long, both ends left open to rain, drainage and evaporation) as described in Raison et al. (1987) and left in place for incubation until the next sampling occasion. They were used to measure the soil net N mineralisation (annotated as T_{1C} values later on). On each sampling occasion, T_0 cores from the two upmost, the two bottommost and the two middle pairs of cores of the same sampling line were combined together which yielded three soil samples per plot and six per stand per treatment. The same was done for T_{1C} cores that had finished their incubation period.

2.3. Sample analysis

In the field, each collected soil core was separated into organic soil (LFH layer, 5–10 cm thick) and mineral soil (A horizon). Each part was then sieved to pass a <4 mm mesh, homogenized, stored in plastic bags and transferred in a cool box to the laboratory where it was analysed the following day. Nitrate and ammonium concentrations were measured in all soil samples by a flow injection analyser (TRAACS 2000, Bran and Luebbe) after extraction by 1 h mechanical shaking of 10 g of organic soil or 20 g of mineral soil in 100 ml of 1 M KCl. In April, July and October 2002 and in July and August 2003, MBC and MBN were measured by fumigation–extraction (Vance et al., 1987). The amounts of 10 g organic soil and 20 g mineral soil were fumigated with alcohol-free chloroform for 24 h at room temperature in darkness. The soluble C and N were then extracted from both fumigated and non-fumigated samples by 1 h mechanical shaking with 100 ml of 0.5 M K_2SO_4 (Chaussod et al., 1987). Dissolved organic C (DOC) and total soluble N concentrations in the fumigated and non-fumigated extracts were determined using a TOC-TN 5050 analyser (Shimadzu Corporation, Kyoto, Japan). DON was calculated by the difference between total soluble N and mineral N. Microbial C and N were calculated from the difference between fumigated and non-fumigated extracts and no correction factor was applied. Indeed, we were only interested in changes of microbial C and N during the time, not in exact values at any given time. These changes were independent from the correction since the same kec factor would have been used to correct all data. Sample moisture was determined on a sub-sample dried at 105 °C.

Gross N mineralisation rates were measured by ^{15}N pool dilution method. Each soil layer was sampled four times (2 g for each replicate). Two samples were incubated with 500 µl of $(^{15}NH_4)_2SO_4$ solution (250 µmol l^{-1}, 98 atom% ^{15}N) on a horizontal shaker for 2 or 24 h. The two other samples were left in the same room conditions with 500 µl of deionised water to evaluate the ^{15}N natural abundance in the soil layers. Thereafter, the 2 h incubation and natural abundance samples were added with 15 ml 2 M KCl to stop the reaction and to extract the ammonium pool and the 24 h incubation and natural abundance samples were treated to isolate ammonium by micro diffusion into acid traps over 5 days after addition of 100 mg MgO. The acid traps consisted of two circular 5-mm diameter ash-free filter papers, each containing 4 µl 2.5 M $KHSO_4$ and wrapped in Teflon tape. Before isotopic analysis acid traps were dried, and the filter discs transferred to tin capsules. The ^{15}N enrichment was measured by continuous flow isotope ratio mass spectrometry (IRMS) using an elemental analyser (EA 1110, CE Instruments, Italy) coupled to a gas isotope ratio mass spectrometer (DeltaPLUS, Finnigan MAT).

2.4. Conceptual equations/calculation of N fluxes

Net N mineralisation, net ammonification and net nitrification were calculated using a set of equations from Raison et al. (1987) based on mineral N budgets outside and inside the cylinders corresponding to situations with and without root uptake. We did not take into account N inputs or losses in the incubation cylinders by deposition or leaching, respectively. Jussy et al. (2004) showed that N loss by leaching from cylinders is limited. Soil mineral N losses including tree uptake and leaching losses were calculated as the differences between final soil mineral N content in the tree uptake area (outside cylinders) and the initial mineral N available between outside and inside the cylinders.

$$\text{Net ammonification} = T_{1C}\,(NH_4^+ - N) - T_0(NH_4^+ - N), \quad (1)$$

$$\text{Net nitrification} = T_{1C}\,(NO_3^- - N) - T_0(NO_3^- - N), \quad (2)$$

$$\text{Net mineralisation} = \text{Net Ammonification} + \text{Net Nitrification}, \quad (3)$$

$$\text{N losses} = \text{final mineral N} - (\text{intial mineral N} + \text{net N mineralisation}) = T_{1S}(NO_3^- - N + NH_4^- - N) - T_{1C}((NO_3^- - N + NH_4^+ - N), \quad (4)$$

where T_0 is the soil mineral N concentrations (NH_4^+–N and NO_3^-–N) at the beginning of the 4-week period; T_{1C} the soil N concentration (NH_4^+–N and NO_3^-–N) at the end of the incubation period inside the cylinder; and T_{1S} the soil N concentration (NH_4^+–N and NO_3^-–N) at the end of the 4-week period outside the cylinder.

The gross N mineralisation (GM) was calculated as

$$GM = \frac{C_{t1} - C_{t0}}{t} \times \frac{\ln(APE_{t0}/APE_{t1})}{\ln(C_{t1}/C_{t0})}, \quad (5)$$

where C_{t1} and C_{t0} are the NH_4^+–N concentrations after 24 and 2 h of incubation with ^{15}N, respectively; t is the duration for the incubations (22 h); APE_{t1} and APE_{t0} are the ^{15}N enrichment values after 24 and 2 h of ^{15}N incubation, respectively; and APE was calculated as

$$APE = \text{atom\% } {}^{15}N_{\text{incubated}} - \text{atom\% } {}^{15}N_{\text{natural abundance}}.$$

2.5. Data analyses

Data analyses were carried out using the SAS software (SAS Institute Inc., Cary, USA). For all statistical tests, we chose the probability level to reject the null hypothesis to be less than or equal to 0.05. Data were analysed using the linear model (I). When model (I) below exhibited significant interactions between the Date effect and the Treatment effect, data were subsequently analysed date by date using model (II) in order to scrutinise more precisely these interactions.

(I) $V = \text{Treat} + \text{Date} + \text{Age} + \text{Horizon} + \text{Plot(Age)} + \text{Treat} \times \text{Date} + \text{Treat} \times \text{Age} + \text{Treat} \times \text{horizon},$

(II) $V = \text{Treat} + \text{Age} + \text{Horizon} + \text{Plot(Age)} + \text{Treat} \times \text{Age} + \text{Treat} \times \text{horizon},$

where V is the explained variable, Treat the effect of the treatment, Date the effect of the sampling time, Age the effect of the stand age, Horizon the effect of the measurement depth and Plot(Age) the effect of the forest plot nested in age effect. Plot(Age) was declared as a random variable. Effects linked by a star are interaction effects. In all analyses, the effect of the forest plot was not significant; so it has been removed from the subsequent result description. When differences between the treatments arose in the models, they were further analysed using Tukey's HSD.

3. Results

3.1. Soil moisture

Soil moisture content was higher in the organic layer than in the mineral soil in both age stands ($P<0.01$). It did not vary significantly between girdled and control plots during the first months after the treatment (Fig. 1). Starting from October 2002, girdled treatments exhibited greater soil moisture in both soil horizons. In October 2002, differences appeared only in the old forest stand ($P<0.01$), while in 2003 girdled plots from the young forest also exhibited greater soil moisture ($P<0.01$). Additionally, the soil moisture reached higher levels in O horizon of the young stand control plots in July 2002 ($P<0.05$).

3.2. Mineral N

The nitrate concentrations depended on the stand age (Table 1) and were greater in the old forest in both O and A horizons (Fig. 2a, $P<0.001$). In both stands, the nitrate concentrations were higher in O horizon ($P<0.001$) and also exhibited an annual variation. Nitrate concentrations increased from April to August/September, depending on the treatment, and then decreased ($P<0.01$). Shortly after girdling, the nitrate concentration increased in the girdled plots and became significantly higher than in the control plot in June for the old stand and in July for the young plot ($P<0.001$). The difference between the treatments increased with the time ($P<0.01$) up to a 10-fold increase in the young plot in July 2003. In contrast, nitrate concentrations decreased slowly in the control treatments to near zero values at the same date. The control treatment exhibited very low NO_3 concentrations for both horizons in the young stand.

The ammonium concentrations in the organic and mineral soil were 2–5 times higher than the nitrate concentrations in both forest types (Fig. 2b, $P<0.001$). There was a significant difference in ammonium concentration between the old and young forest for A horizon ($P<0.05$). There was no difference between the treatments before June 2002. Thereafter, the ammonium concentrations increased significantly in the girdling plots and became greater than in the control plot ($P<0.001$). The difference in ammonium concentrations between the treatments was larger in the young forest. Except in O horizon of the young girdled plot, the ammonium did not show any significant difference linked to the stand age. Both nitrate and ammonium were positively correlated with soil moisture (Table 1).

3.3. Microbial C and N and extractable organic C and N

The MBC and MBN were greater in the surface O horizons for both stand ages. Moreover, the young stand showed a greater biomass than the old stand for both MBC and MBN in O horizon ($P<0.01$) (Fig. 3). The MBC and MBN exhibited an annual variation with a slight decrease from April to October 2002 ($P<0.01$). The same result was observed in both age stands and in both horizons.

The variations of MBC and MBN between the girdled and control plots contrasted strongly with the NO_3 and NH_4 results. In April 2002, MBC and MBN were lower in

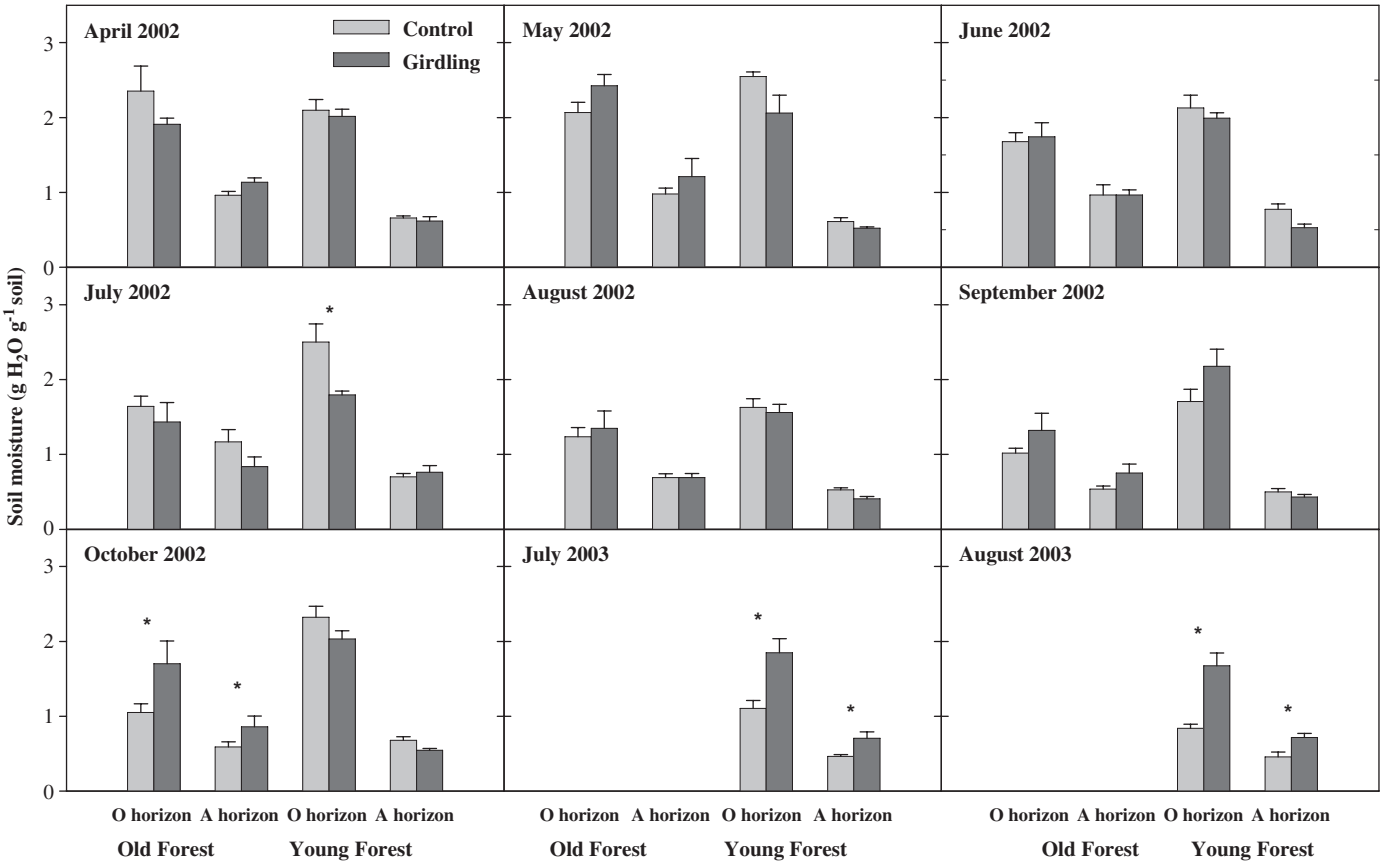

Fig. 1. Variation of the soil moisture in control and girdled plots in an old and a young spruce forest located at Wetzstein. Error bars represent the standard errors for the means ($n = 6$). Asterisks indicate significant differences between the control and the girdling plots ($P<0.01$).

Table 1
Factors affecting the mineral N concentrations, microbial C and N biomass and N mineralization in a spruce forest

Source	Treatment	Sampling date	Stand age	Soil horizon	Treatment × date	Treatment × age	Treatment × horizon	Soil moisture
Nitrate	***	***	***	***	**	n.s.	***	*
Ammonium	***	***		**	*	***	***	***
Microbial N biomass	*	***	*	***	***	n.s.	n.s.	*
Microbial C biomass	*	***	***	*	***	n.s.	n.s.	*
Microbial C/N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N mineralization	***	***	**	***	n.s.	n.s.	*	n.s.

Stars indicate the probability level of rejecting the null hypothesis for given effects (*$P<0.05$, **$P<0.01$, ***$P<0.001$, n.s. = not significant).

the girdled plots than in the controls ($P<0.01$). However, this result reversed in July 2002, when the girdled treatment showed for both stand age and horizons a greater MBC and MBN than the control ($P<0.01$). During the following months, the difference between the treatments decreased. In 2003, MBC and MBN were first greater in the control ($P<0.01$) in July than not different between treatments in August. MBC and MBN were positively related to the soil moisture (Table 1). At all sampling times, the microbial C/N ratios did not vary significantly either between treatments or stands (Table 1).

Extractable DON concentrations were in the range of 40–70% of the total extractable N. During the course of the experiment, N fraction did not show any significant difference between the control and girdled plots (Fig. 4).

3.4. Net and gross N mineralisation rates and N budget

In May 2002, one month after the tree girdling, the net N mineralisation rate in O horizon was greater in the girdled plots compared to the controls but no difference was found for A horizons (Fig. 5, $P<0.01$). From June to September 2002, net N mineralisation became consistently higher in the girdled plots for all horizons in the young forest (Fig. 5, $P<0.001$). However, net N mineralisation in O horizon was higher in the girdled plots than the control plots only

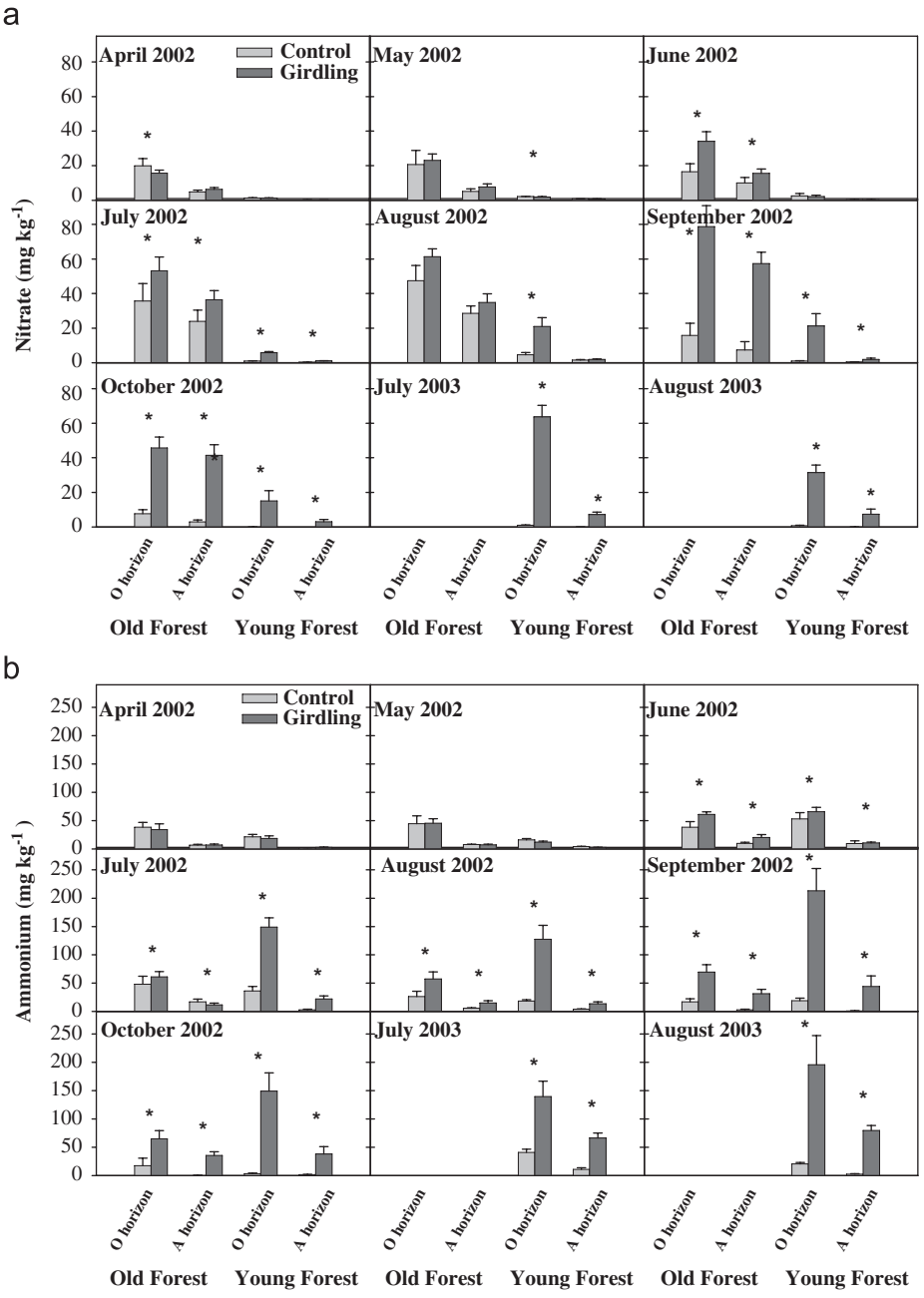

Fig. 2. (a) Variation of the nitrate concentration in control and girdled plots in an old and a young spruce forest located at Wetzstein. Error bars represent the standard errors for the means ($n = 6$). Asterisks indicate significant differences between the control and the girdling plots. (b) Variation of the ammonium concentration in control and girdled plots in an old and a young spruce forest located at Wetzstein. Error bars represent the standard errors for the means ($n = 6$). Asterisks indicate significant differences between the control and the girdling plots.

in July and September (Fig. 5, $P < 0.001$). N mineralisation rate exhibited an annual trend with increasing values from May to July ($P < 0.01$) and decreasing from July to October ($P < 0.01$). In October 2002, the N mineralisation rates were around zero and no differences were found between the treatments. To complement net N mineralisation rates we measured gross N mineralisation rates in August 2002. Interestingly, we found significantly higher gross rates in both horizons in the control plots than the girdled plots in the old forest ($P < 0.05$ and $P < 0.01$ for O and A horizons, respectively), but no differences between the treatments in the young forest (Fig. 6). Gross N mineralisation rates were 3–25 times higher than net N mineralisation rates. The difference between gross and net N mineralisation was significantly greater in the control plots of both horizons in the old forest stand (41.4 vs. 22.5 and 10.3 vs. 4.3 mg N kg^{-1} dry soil per day for O and A horizons, respectively). No such differences were found in the young forest stand.

Annual net N mineralisation was 25 and 64 kg N ha^{-1} in O horizon of the control and the girdled old forest plots, respectively, compared to 21 and 39 kg N ha^{-1} in the control and girdled young forest (Table 2). In A horizon,

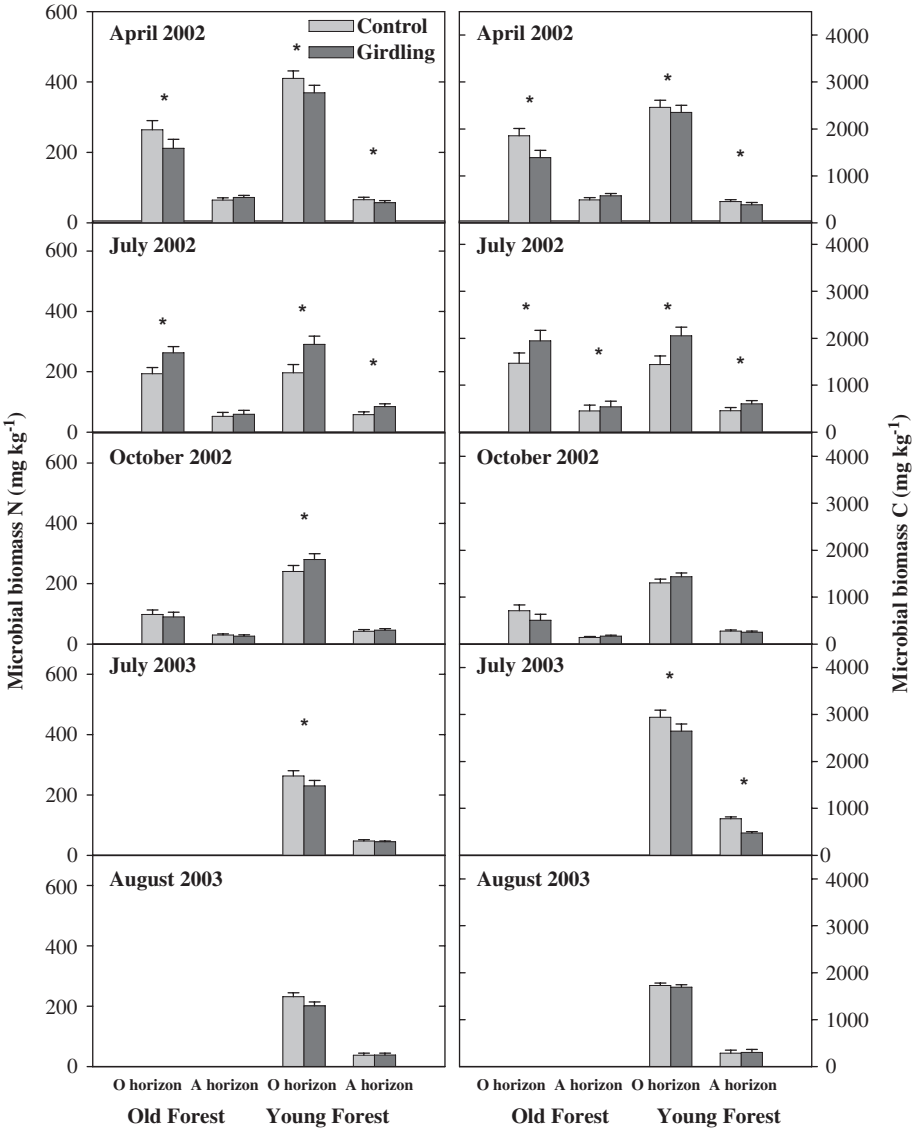

Fig. 3. Variation of microbial biomass C and N in control and girdled plots in an old and a young spruce forest located at Wetzstein. Error bars represent the standard errors for the means ($n = 6$). Asterisks indicate significant differences between the control and the girdling plots.

annual N mineralisation was similar (31 kg N ha^{-1}) in the old spruce, whereas in the young spruce N mineralisation was about 2.5 times higher in the girdled plots. Mineral N loss was similar in the control and girdled plots in the young spruce. In the old spruce, N loss in the girdled plots was higher than in the control plots in O horizon, whereas the opposite was observed in A horizon. As for the annual N budget, there was significantly higher mineral N in the girdled plots than in the control plots in both old and young forests (Table 2).

4. Discussion

4.1. Soil moisture

In our experiment, the soil moisture increased in the girdling treatment compared to the control treatment 6 months after the start of the girdling treatment (October 2002). Such a result has already been observed by Olsson et al. (2005) in a similar experiment and by Ekberg et al. (2007) at the same sites as our study, while Högberg et al. (2001) did not observe any moisture change following tree girdling.

Water uptake by roots is mostly a passive mechanism driven by canopy transpiration and it should not be directly sensible to changes in root metabolic activity. The six month delay in our experiment between the treatment application and first occurrence of increased soil water content suggests that roots had stored enough reserves to pursue normal activity during this period and that root decay became significant only after this.

4.2. Microbial biomass

The microbial biomass showed a decline in the girdled plots in the month that directly followed the treatment. Soil

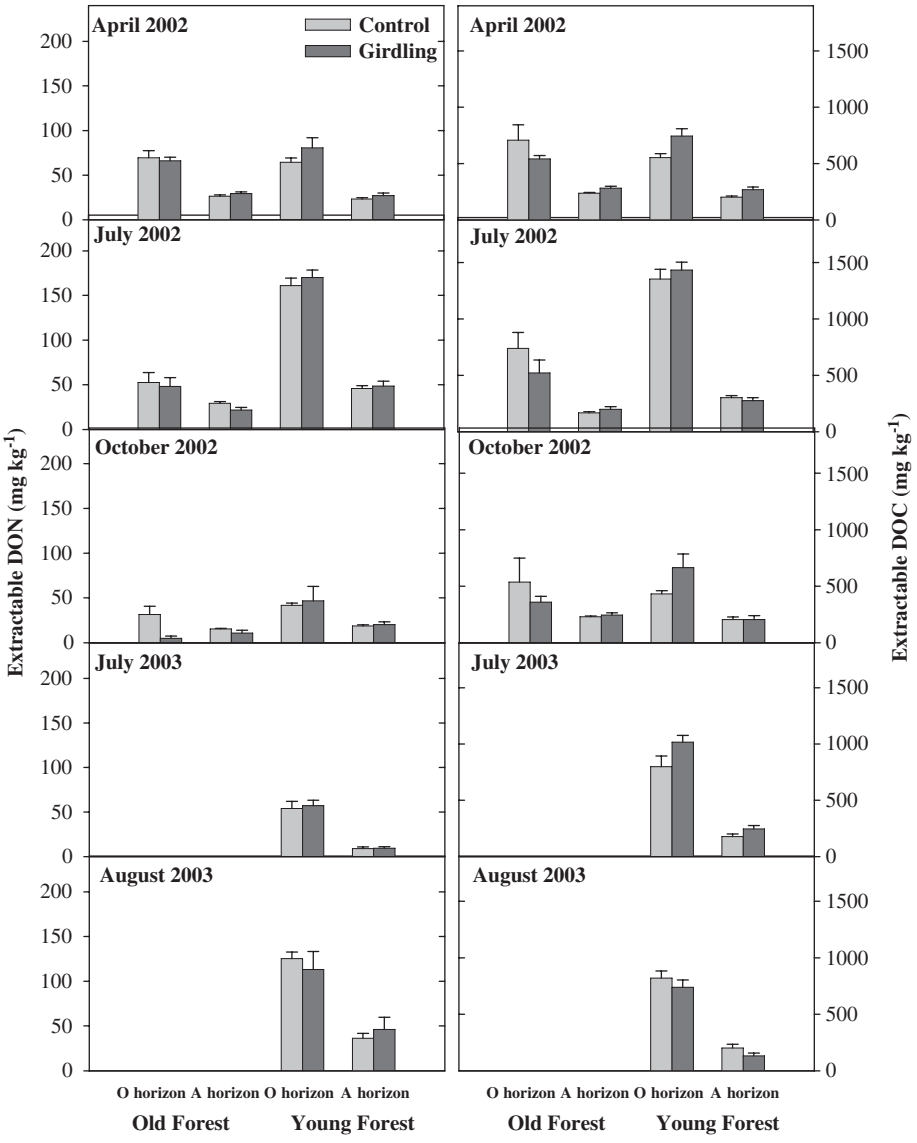

Fig. 4. Variation of extractable organic C (DOC) and N (DON) in control and girdled plots of an old and a young spruce forest located at Wetzstein. Error bars represent the standard errors for the means ($n = 6$). Asterisks indicate significant differences between the control and the girdling plots.

microbes are highly dependent on flux of labile C (Holmes and Zak, 1994). The immediate negative effect of tree girdling on the microbial population was probably due to the cessation of root carbohydrate, amino and organic acid exudation when their transport to roots was stopped, which removed one important C source for microorganisms. However, this trend reversed two months after the treatment in July 2002, with larger microbial populations in the girdled plots. This is probably the time when fine roots with lesser reserves started to decay, causing a new source of C to appear in the soil through the decomposition of decaying root material. This stimulated the growth of microbial populations and then the size of the pool of biomass C and N. Moreover, soil moisture increased in the girdled plots and enhanced soil moisture benefits microbe populations (Niklaus, 1998) as shown by the positive correlations we found between the two parameters. Contrary to Högberg and Högberg (2002), the C/N ratio of the microbial biomass remained stable over the whole observation period and did not exhibit any significant variation between the control and girdled plots.

Interestingly, the girdling treatment affected either positively or negatively the microbial biomass in both O and A horizons in the young stand while only the O horizon was affected in the old stand. Therefore, the effect of girdling on the whole soil microbe biomass depends on the stand age with a greater effect on deeper soil layers in young stands. The effect of girdling on microbial biomass depended on the time, negative at first and then positive.

4.3. Net N mineralisation

The comparison of gross and net N mineralisation rates showed that the difference between gross and net rates, i.e.

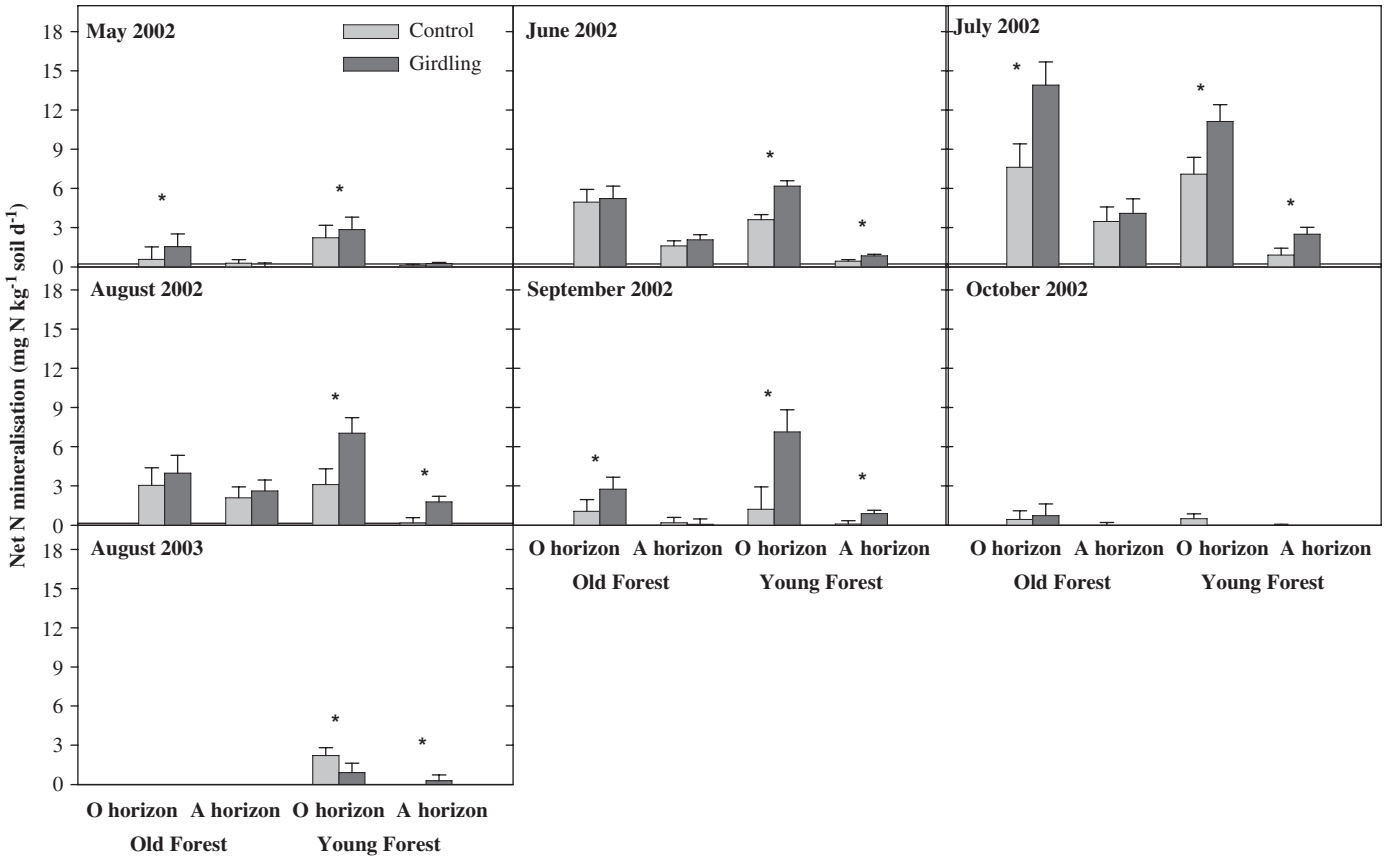

Fig. 5. Variation of net N mineralisation rate in control and girdled plots in an old and a young spruce forest located at Wetzstein. Error bars represent the standard error from the mean ($n = 6$). Asterisks indicate significant differences between the control and the girdling.

the microbial ammonium and nitrate immobilization and/or plant uptake was always higher in the controls compared to girdled plots in the old forest stand, again indicating that plant uptake was considerably lower. In contrast, no significant differences were found in the young forest. This implies that in the young stand the microbial N immobilization was able to compensate for the reduced plant uptake, which in turn implies that microbes exhibited a stronger N demand in the younger stand. The latter was also supported by the generally higher gross N mineralisation rates in the older compared to the younger forest stand.

In both girdled and control plots, net N mineralisation rates were always higher in the organic layers than in the mineral soil at least on a dry matter basis. Similar results were also found by Persson and Wiren (1995) and Persson et al. (2000). With increasing soil depth, the age and the resistance to mineralisation of soil organic matter generally increase together with a decrease in microbial activity, consequently lowering the N mineralisation (Raison et al., 1987). We observed daily net N mineralisation rates in A horizon that were in the range of values reported in the literature for forest soils (Federer, 1983; Vervaet et al., 2002).

Following girdling, the root exudation of carbohydrate and other labile C was stopped and that should have negatively impacted microbe activity. The immediate increase of N mineralising activity suggests then that another mechanism limiting this activity was also stopped by girdling, probably the secretion of a mineralisation inhibition factor by the spruce (Erickson et al., 2000). The difference in net N mineralisation between the control and the girdling treatment increased during the season, probably as a consequence of starting root decay which resulted in a new and potentially bigger source of available C (Smith and Paul, 1990; Hart et al., 1994; Hamer and Marschner, 2005) or by imposing the negative control of spruce.

Girdling had an immediate and very positive effect on net N mineralisation in O horizon that was especially strong in the young stand. The lack of extensive and consistent changes in the microbial biomass C and N suggests that the greater net N mineralisation in girdled plots was probably driven by a higher microbial activity rather than by changes in the size of the microbial pool. As for microbial biomass, girdling affected A horizon only in the young stand. Since the rooting depth was similar in both young and old stands, it seems that younger trees may exert a stronger regulation on lower horizons and thus

girdling would also affect those horizons while modifying root functions.

4.4. Ammonium and nitrate pools

Over the whole observation period, nitrate concentrations remained close to zero in the control plots of the young stand while they were higher in the control plots of the old stand. The absence of net nitrification in the young forest supports the hypothesis that spruce regulates the nitrification step. The regulation may be due to two mechanisms: excretion of nitrification inhibitors such as monoterpenes that would be stronger from young plants (Paavolainen et al., 1998) or competition for the same N resource. Jussy et al. (2004) interpreted low nitrification in a young spruce forest as a result of fast tree growth and low N deposition. This resulted in reduced ammonium concentrations in the soil since spruce prefers ammonium as N source in a variety of soils (Buchmann et al., 1995; Stober et al., 2000) and naturally limited the rate of nitrification.

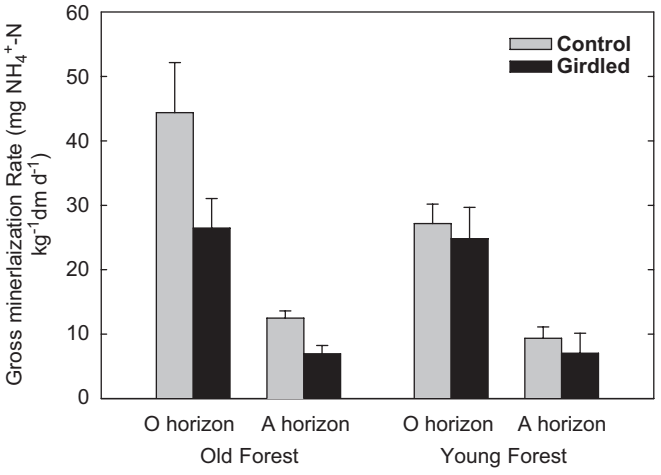

Fig. 6. Gross N mineralisation rates in August 2002 in control and girdled plots in an old and a young spruce forest located at Wetzstein. Error bars represent the standard error from the mean ($n = 6$). Asterisks indicate significant differences between the control and the girdling.

The nitrate concentrations were very low in the young control plots which suggests that young spruce is efficient in inhibiting the nitrification process either by competition for NH_4^+ or by the exudation of nitrification inhibitors.

After girdling which greatly limited root activity, in terms of mineral uptake or root exudation, the ammonium concentration increased more in the young stand while the nitrate increased more in the old stand. Moreover, we obtained similar results from the control plots when comparing soil cores taken outside and inside incubation cylinders. Only root uptake that was prevented inside the cores differed between the outside and the inside cores, all other parameters being the same. Inside cylinders, ammonium concentration greatly increased after 1-month incubation for the young stand plots while nitrate concentration remained very low, roughly equal to the outside values. By comparison, both nitrate and ammonium increased inside cylinders from the old stand plots. These results show that nitrification inhibition in the young stand was mostly performed through the release by roots of inhibitors that can remain effective for a long time after they were released. In the old stand, spruce limited nitrification by a simple competition effect.

The soil N cycle is intimately coupled with the C cycle during the mineralisation and immobilization processes (Mary et al., 1996) and C is a fundamental element driving the interactions between plants and microorganisms in the soils. Knops et al. (2002) showed that plants may control

Table 2
N mineralization, N loss (tree uptake + gaseous and aqueous losses) and N budget (mineralization−N loss) in the organic and mineral soil of girdled and non-girdled forests

Parameters	Soil layers	Control (kg N ha^{-1} yr^{-1})		Girdling (kg N ha^{-1} yr^{-1})	
N mineralization					
Old forest	O horizon	25.44	(4.86)	64.24	(9.00)
	A horizon	30.78	(9.28)	31.09	(8.77)
Young forest	O horizon	21.24	(5.92)	39.00	(5.87)
	A horizon	4.56	(2.05)	13.52	(5.63)
N loss					
Old forest	O horizon	32.69	n.a.	47.25	n.a.
	A horizon	32.01	n.a.	17.44	n.a.
Young forest	O horizon	25.89	n.a.	21.80	n.a.
	A horizon	4.68	n.a.	5.56	n.a.
N budget					
Old forest	O horizon	−7.26	n.a.	17.00	n.a.
	A horizon	−1.23	n.a.	13.64	n.a.
Young forest	O horizon	−4.65	n.a.	17.20	n.a.
	A horizon	−0.12	n.a.	7.96	n.a.

Standard errors are in the brackets, n.a. = not applicable.

the N cycle via the allocation of C compounds to the soil (root exudates and turnover of fine roots and mycorrhizas). Therefore, we expected the girdling to decrease N availability by reducing the flux of C from the roots to the soil.

However, we observed the opposite trend as tree girdling led to increases in both nitrate and ammonium concentrations during the growing season. Greater mineral N concentrations in the soil following girdling were probably the consequence of two mechanisms: increased microbial mineralising activity as discussed previously and significantly reduced N uptake by the roots. Mineral element uptake by the plant is an active process, which requires energy from carbohydrates whether it performed by the roots or by associated myccorhizas fed by the plant. Therefore, terminating the carbohydrate flux from the shoots to the roots by girdling probably led to a quick decrease of the root uptake, resulting in greater concentrations of mineral N in the soil.

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