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Nitrogen availability in Norway spruce forest floor — the effect of forest defoliation induced by bark beetle infestation

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The objective was to evaluate whether lower nitrogen (N) immobilization by spruce trees (*Picea abies*) or higher microbial N mineralization in the soil is the main mechanism changing the soil N balance after forest defoliation caused by bark beetle. We measured *in situ* mineral N availability using ion exchangers, net N mineralization (N_{miner}, ammonification and nitrification) and N content in microbial biomass (N_{microb}) in the forest floor of infested and control plots in an unmanaged area of The Bohemian Forest National Park. *In situ* N availability already increased before the defoliation culminated, which affirms the primary effect of reduced N immobilization by vegetation. N mineralization was enhanced after maximum forest defoliation (2 *vs*. 30 μg N g^{-1} d^{-1}). The contribution of N_{miner} to *in situ* N availability was supported by the correlations found between *in situ* mineral N availability and N_{miner}/N_{microb} and N_{miner}/C_{miner} ratios. The influence of litter input with low C/N ratio on N mineralization is discussed.

Introduction

Spruce forests in the Bohemian Forest Mountains, Czech Republic, have been endangered by bark beetle attack (*Ips typographus*) since the 1990s and, at present, a large area of the forest has already been affected. It can be expected that nitrogen (N) leaching from soils will distinctively increase in the invaded area. Many deforestation studies showed that N leaching from soil increased after forest decline (e.g. Miller 1979, Frazer *et al*. 1990, Stevens and Hornung 1990, Walley *et al*. 1996, Carmosini *et al*. 2003, Grenon *et al*. 2004, Rothe and Mellert 2004, Huber *et al*. 2004b, Gundersen *et al*. 2006).

Nitrogen leaching from soils increases when the balance between N mineralization (ammonification and nitrification) and N immobilization is disturbed and the rate of mineral N production exceeds that of mineral N immobilization, either by vegetation or soil microbial biomass. As nitrates ($N\text{-}NO_3$) make up the bulk of the leached mineral N, N leaching is mainly enhanced in conditions of high nitrate production and low nitrate immobilization or reduction. Nitrate production increases with increased ammonium ($N\text{-}NH_4$) availability (e.g. Stark and Hart 1997), which can result from both an increase in organic matter mineralization and/or restriction of N immobilization by vegetation and soil microbial

biomass. Even though higher nitrate leaching from the forest floor after forest decline has been frequently observed, it is still unclear whether lower N immobilization or higher N mineralization is the main mechanism affecting the change in N balance (Grenon *et al*. 2004).

Forest decline is undoubtedly accompanied by reduction of tree N demand. Nitrogen immobilization by vegetation decreases until N demand of the regenerating vegetation is equal to N demand of the original forest. Moreover, forest decline is accompanied by massive needle fall prior to their natural senescence. Higher N content, and a more favourable carbon to nitrogen ratio (C/N) of this litter (Morehouse *et al*. 2008), can accelerate N mineralization processes in the soil. The most active part of the forest soil profile is the forest floor with its litter horizon (Schimel and Firestone 1989, Binkley and Hart 1989, Tietema 1998), which can react to changes in litter input very quickly due to its fast turnover. Šantrůčková *et al*. (2009) showed that the floor (litter + humus horizons) of spruce forests in the Bohemian Forest Mountains contains up to 40% of total available N. They also demonstrated that any small shift in the balance between microbial N mineralization and N immobilization in the forest floor can be reflected by important changes in N release, which can end in enhanced N terrestrial export.

Many techniques have been used to derive N availability ($N\text{-}NH_4$ and $N\text{-}NO_3$) in the forest floor. Measurement of *in situ* mineral N availability using resin bags (Binkley and Matson 1983) represents the amount of N percolating through soil horizons with soil water and shows whether mineral N availability exceeds N immobilization by vegetation and soil microbial biomass. On the other hand, laboratory measurements of microbial N mineralization (ammonification and nitrification) and N content in microbial biomass represent potential values. An approximation of the role of N immobilization by plants is possible when using both approaches.

We report on *in situ* mineral N availability ($N\text{-}NH_4$ and $N\text{-}NO_3$), and potential microbial N mineralization (ammonification and nitrification) and N in microbial biomass, in the forest floor (litter + humus horizon) of a mountain spruce forest in the Bohemian Forest Mountains. Bark beetle has attacked this forest and nearly 100% of the trees in the study plot were defoliated in 2004. Forest dieback was followed by a substantial increase of $N\text{-}NO_3$ terrestrial export from the watershed, which exceeded total mineral N deposition by more than 100% in 2005 and 2006 (Kopáček *et al*. 2006). The aim of the study was to determine whether reduced N uptake by vegetation or higher microbial N mineralization plays a crucial role in the enhanced N leaching observed after forest decline. Our starting hypotheses were: (1) enhanced terrestrial N export is accompanied by increased *in situ* N availability in the forest floor, and (2) higher *in situ* N availability is caused primarily by a contemporary decrease of N immobilization by vegetation, but also by an increase of potential microbial N mineralization.

Material and methods

Site description

The Plešné Lake (PL) watershed (48°47´N, 13°52´E, average elevation 1200 m a.s.l.) is situated in the Bohemian Forest (the Hercynian crystalline mountain massive). The Bohemian Forest Mountains have been exposed to increasing sulphur and N depositions over more than one century and the forests were, similarly to other European ecosystems, acidified (Veselý 1994, Kopáček *et al*. 2002, Kopáček and Veselý 2005). Although these depositions decreased in the second part of the last century, the forest soils still show an acidification effect, which is stimulated by (i) acidification due to spruce plantations grown since the 19th century (Oulehle *et al*. 2006), (ii) low base saturation of soils developed on crystalline rocks (e.g. Kennedy 1992), and (iii) global warming (Kettle *et al*. 2003).

The bedrock is predominantly composed of granite. Soils are ~0.5 m deep dystric cambisols; podzols and shallow (< 0.2 m) undeveloped soils rich in organic matter (occurring on steep slopes of the watershed). Generally, the respective mean effective cation exchange capacity of the PL soils is 129 meq kg^{-1}, with low base saturation (15%), but high exchangeable aluminium (57%) and proton concentrations (28%)

Table 1. Mean needle litter input to infested and control study plots and the C/N ratio of needle litter over the study period 2003–2007 (J. Kopáček unpubl. data). Weight is that of samples dried at 50 °C for 12 hours. Mean ± SD (n = 5) is given for litter input. Prefixes 's' and 'w' mean summer and winter, respectively.

Plot	s2003	w2003/2004	s2004	w2004/2005	s2005	w2005/2006	s2006	w2006/2007
Litter input (g m^{-2})								
Infested	180 ± 244	111 ± 33	596 ± 490	110 ± 39	24 ± 25	25 ± 27	343 ± 437	17 ± 14
Control	47 ± 6	128 ± 18	64 ± 12	141 ± 14	167 ± 166	191 ± 96	776 ± 424	118 ± 106
Litter C/N								
Infested	41	41	32	42	38	40	42	36
Control	51	54	52	60	50	52	51	45

(Kopáček *et al*. 2002). Soil pH is low, with the lowest pH_{CaCl2} values in the humus (A) horizon (2.5–3.3) and the highest in the mineral (C) horizon (3.2–4.5) (Kopáček *et al*. 2002). The watershed was almost completely (> 90%) covered by unmanaged 90–160 year-old Norway spruce forest (~99%, *Picea abies*), with sparse beech (*Fagus sylvatica*), fir (*Abies alba*), and mountain ash (*Sorbus aucuparia*) untill 2002. Dominant types of understorey vegetation in the watershed are *Calamagrostis villosa, Vaccinium myrtillus*, *Avenella flexuosa*, *and Athyrium alpestre* (Svoboda *et al*. 2006). Bark beetle attack culminated in 2004 when 40% of the forest in the watershed was defoliated (Hájek and Svoboda 2007). Forest dieback was followed by a substantial increase in $N\text{-}NO_3$ terrestrial export from the watershed, which exceeded total mineral N deposition by more than 100% in 2005 and 2006 (Kopáček *et al*. 2006). The study was conducted at two research plots. One of them was the bark-beetle infested plot where trees were almost completely defoliated in summer 2004 (Table 1: infested plot). The second plot with vigorous trees that started to slightly defoliate in summer 2006 because of incoming bark beetle attack (Table 1: control plot). Needle fall in the infested plot had a lower C/N ratio over the whole study period than the needle fall below the vigorous trees in the control plot (Table 1). Accordingly, C/N ratios of litter and humus horizons were lower in the infested as compared with that in the control plot (Table 2).

Measurement of *in situ* N availability

We used a mixed-bed cation–anion resin (IER) for the *in situ* N availability determination (Binkley and Matson 1983). The cation resin (Purolite C100E) was strongly acid-sulfonated DVB-styrene, with Na^+ as the ionic form. The anion resin (Purolite A520E; highly selective for nitrate) was composed of strongly alkaline DVB-styrene with quaternary amine functional groups and Cl^- as the ionic form. Moist resins were mixed (1:1 by volume) and the resulting IER was pre-treated by subsequent shaking with 0.5 l of NaCl solution (100 g l^{-1}) per 300 g of the resin in 32 steps as follows: (i) the first step with NaCl solution, (ii) five steps with demineralised water, (iii) one step with NaCl solution, and finally (iv) 25 steps with demineralised water. This procedure was shown to be sufficient for decreasing the N background level of this type of mixed resin (blanks under the limits of measurement: $N\text{-}NO_3$ 6 μg l^{-1}; $N\text{-}NH_4$ 1 μg l^{-1}).

We prepared bags for *in situ* exposition by placing pre-treated resin into plastic rings (1 cm in height, 6 cm in diameter, 27.3 cm^2 in area) covered with a polyamide net (Uhelon 160T, Silk & Progress). In the field, we placed two bags horizontally, one in the litter horizon (upper 1–5 cm of soil profile depending on the depth of litter horizon) and the other one at 10 cm in the humus horizon; both bags were in one pit, but

Table 2. C/N ratio in soil from litter and humus horizons in the study period 2003–2007.

Plot	2003	2004	2005	2006	2007
Infested					
Litter	25	22	22	27	25
Humus	24	22	23	26	23
Control					
Litter	27	28	27	30	26
Humus	28	31	30	29	27

not below each other. In total, there were 10 pits in each of the two research plots in the watershed with a 3-m distance between the pits. The bags were exposed for approximately 6-month periods, from November to May (henceforth winter) and then from May to October (henceforth summer) in each year from 2003 to 2008 reflecting the persistence of snow cover (usually from November to May). We calculated and tested the capacity of the bags for a 6-month exposition period to be sufficient before we conducted the study (Skopcová 2005).

After the assay period, we removed the resin from the bags, washed it with demineralised water and extracted it by the repeated elution procedure in a glass column (19.5 cm long, 2 cm in diameter, inbuilt sintered glass and stopcock) with eight elution steps (100 g NaCl l^{-1} as the elution solution), which were finally combined to obtain one sample (400 ml). The $N\text{-}NO_3$ and $N\text{-}NH_4$ concentrations in the samples were determined using a flow injection analyzer (FIAstar 5012, Foss Tecator, Sweden). The absolute amounts of $N\text{-}NO_3$ and $N\text{-}NH_4$ accumulated in the bags (mg) were then calculated using regression equations below, taking into account the elution efficiencies of both ions (98% ± 1.89% $N\text{-}NH_4$; 87% ± 9.14% $N\text{-}NO_3$, $n = 26$, Skopcová 2005):

$$N\text{-}NO_3 = (E_{N\text{-}NO3} + 0.21)/0.92 \quad (1)$$
$$N\text{-}NH_4 = (E_{N\text{-}NH4} - 0.08)/0.96 \quad (2)$$

where E is the amount (mg) of ions after elution. For each study season, absolute available $N\text{-}NO_3$, $N\text{-}NH_4$ and mineral N content in each bag were expressed in mg m^{-2} day^{-1}. In total, there were 10 values for each plot, layer and/or assay used for statistical comparison. Mean annual mineral N availability was calculated from the winter and summer values for comparison of *in situ* mineral N availability and potential microbial N mineralization rate.

Laboratory measurements

Soil sampling

We collected soil samples in late May (after snow melt) from both research plots: in years 2001–2008 (except 2003) from the infested plot and in years 2004–2008 from the control plot. We took them from 16 random locations in each plot, separately from the litter and humus horizons. We prepared four samples representative for a plot by mixing aliquot parts of soil samples taken from the same horizon of four locations in each plot. Sieved (5 mm) and homogenized soil samples were stored in polyethylene bags (PE) at 4 °C for 3 weeks until analysed.

Soil analyses

We used three-week incubations (100 ml flasks, 10 g of soil, 60% water holding capacity (WHC), covered with perforated parafilm, 15 °C) without substrate addition for measuring N mineralization (ammonification and nitrification, Ste-Marie and Paré 1999, Zhu and Carreiro 1999). Mineral N, ammonium and nitrates, concentration in KCl extracts [40 ml 2 M KCl; shaken 40 min, 10 min centrifuged at 4000 g, supernatant filtered through glass-fibre filter (GF/F)] was analysed using a flow injection analyser (FIAstar 5012, Foss Tecator, Sweden). Net N mineralization rate (N_{miner}) was calculated as the difference between final (21 days) and beginning (7 days) concentrations of mineral N ($N\text{-}NH_4$ and $N\text{-}NO_3$) divided by the number of days (Šantrůčková *et al*. 2001). We measured nitrogen in soil microbial biomass (N_{microb}) using the chloroform fumigation–extraction method (Vance *et al*. 1987). Briefly, extraction from 10 g of soil was carried out either immediately [shaken with 40 ml of 2 M KCl for 40 min, centrifuged for 10 min at 4000 g, and supernatant filtered through a glass-fibre filter (GF/F)] or the soil was fumigated first (24 h). Total N in the soil extracts was determined after alkaline persulfate digestion (Cabrera and Beare 1993). N_{microb} was calculated as the difference between N content in fumigated and non-fumigated extracts using a conversion factor of 0.54 to convert chloroform flush N to N_{microb} (Brookes *et al*. 1985). We measured carbon mineralization rate (C_{miner}) after 7 days of incubation (300 ml sealed flasks, 10 g of soil, 60% WHC, 15 °C). Evolved CO_2 was trapped in 0.5 M NaOH and determined by volumetric titration (0.05 M HCl) after addition of $BaCl_2$. Quanti-

Table 3. Total *in situ* mineral available N fluxes (N-NO_3 and N-NH_4, mg m^{-2} d^{-1}) in the forest floor (litter + humus horizon) of the Plešné Lake watershed over the study period 2003–2008. Asterisks mark significant differences between infested and control plots (Chi-square: $p < 0.01$, df = 1). Prefixes 's' and 'w' mean summer and winter, respectively.

Year	w2003/2004	s2004	w2004/2005	s2005	w2005/2006	s2006	w2006/2007	s2007	w2007/2008	s2008
Infested	47*	18*	67*	103*	165*	91*	57	38*	98*	31*
Control	17	4	37	12	57	33	68	78	54	124

ties were expressed per gram of soil dry weight (105 °C). Total C and N contents were measured in dried (60 °C) and finely milled soil samples using a NC elemental analyser (ThermoQuest, Germany). All measurements were performed in three laboratory replications for each soil sample.

Statistics

We tested *in situ* and laboratory data for normality (Shapiro-Wilk's test and histogram plots). *In situ* data were not normally distributed and therefore they were log-transformed for statistical comparison. For data evaluation we used factorial analysis of variance [horizon, year, season (only for *in situ* data), infestation (yes/no) as factors] followed by mean separation test (Tukey's HSD test). We checked homogeneity of variances using the Hartley-Cochran-Bartlett's tests. Statistical analyses were performed using Statistica 7 for Windows (Statsoft, Inc.). All data are presented without transformation.

Results

In situ N availability

Total *in situ* mineral N availability (N-NH_4 + N-NO_3) was the highest in the first and second years (2005 and 2006) after maximum tree defoliation in the infested plot. N availability was higher in the infested as compared with that in the control plot until summer 2006, when the control plot was also invaded (Table 3). Evaluating N-NH_4 availability data across the entire study period did not reveal any significant effect of infestation in the infested plot (Table 4), because N-NH_4 availability was higher in the infested than in the control plot only untill winter 2005/2006 and it was even lower from summer 2007 onwards (Fig. 1a). Ammonium availability was significantly lower in summer than winter, but the effect of soil horizon was insignificant (Table 4). Infestation was followed also by an increase of N-NO_3 availability. In the infested plot, increased N-NO_3 availability persisted untill winter 2007/2008 and in the control plot untill the end of the study (Fig. 1b). Generally, the difference in *in situ* N-NO_3 availability was significantly affected by all measured factors (infestation, season, year and horizon; Table 4). The effect of infestation was more apparent in the humus than in the litter horizon, which was reflected by a significant difference between the horizons (Fig. 1b and Table 4). Lower N-NO_3 availability in summer than winter was detected before main tree defoliation: in 2004 in the infested plot and from 2004 to 2006 in the control plot (Fig. 1b).

Potential microbial activity

All measured microbial characteristics were significantly higher in the litter than the humus

Table 4. Effects of horizon, year, season and infestation on *in situ* available N-NO_3 and N-NH_4 (factorial analysis of variance). ns = not significant.

		N-NO_3		N-NH_4	
	df	*F*	*p* <	*F*	*p* <
Horizon	1	17.8	0.001	0.1	ns
Year	4	2.6	0.08	6.3	0.01
Season	1	7.5	0.01	20.4	0.001
Infestation	1	76.3	0.001	1.5	ns
Year × infestation	4	5.2	0.01	34.8	0.001
Season × infestation	1	13.9	0.001	16.9	0.001

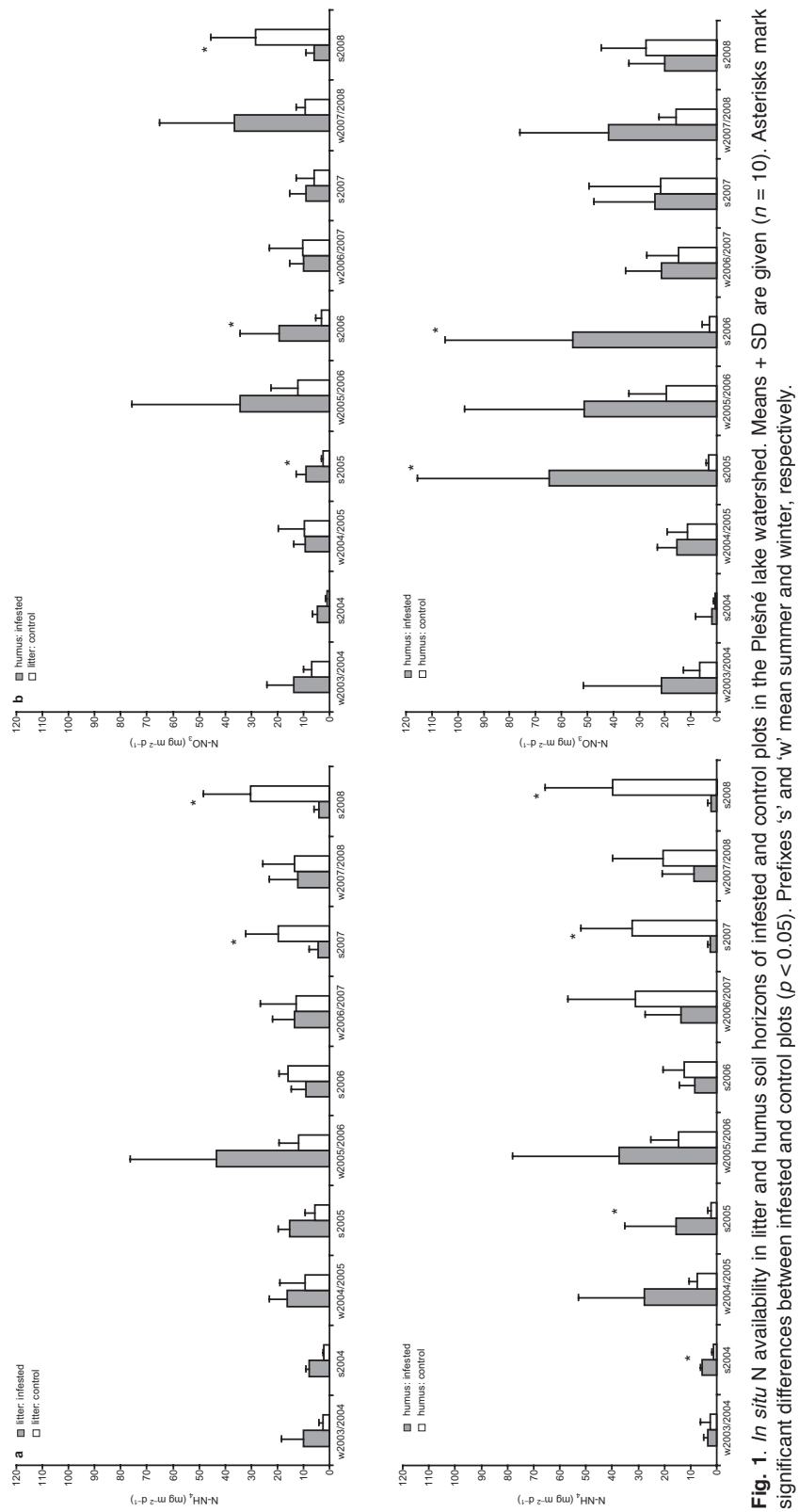

Fig. 1. *In situ* N availability in litter and humus soil horizons of infested and control plots in the Plešné lake watershed. Means + SD are given ($n = 10$). Asterisks mark significant differences between infested and control plots ($p < 0.05$). Prefixes 's' and 'w' mean summer and winter, respectively.

horizon and the between plot differences were significantly affected by infestation and year (Table 5).

Net N mineralization was generally higher in the infested than in the control plot. On average, N_{miner} was 10.2 g^{-1} d^{-1} and 4.9 μg g^{-1} d^{-1} in the litter horizons of the infested and control plots, respectively, and 2.9 g^{-1} d^{-1} and 2.0 μg g^{-1} d^{-1} in the humus horizons of the infested and control plots, respectively. In the infested plot, N_{miner} significantly increased in both horizons in 2005–2006, one year after maximum defoliation (Table 5). In the control plot, N_{miner} increased from 2007 in the litter and 2008 in the humus horizon; again one year after maximum defoliation in this plot. Nitrogen content of microbial biomass (N_{microb}) was generally lower in the infested than in the control plot in both soil horizons (Table 5; 495 *vs*. 818 μg g^{-1} in the litter and 337 *vs*. 471 μg g^{-1} in the humus, on average for the infested and control plots, respectively). Carbon mineralization was slightly, but significantly, lower in the infested than in the control plot (Table 5); the difference was higher in the humus than in the litter horizon. The average C_{miner} for the period from 2004 to 2008 were 174 and 181 μg g^{-1} d^{-1} in the litter, and 58 and 77 μg g^{-1} d^{-1} in the humus, for the infested and control plots, respectively.

Potential microbial net N mineralization rate increased with increased *in situ* annual mineral N availability and, if an outlier (N mineralization in the litter horizon of the infested plot in 2005) was excluded from the statistical evaluation, the correlation was significant ($r = 0.45$, $p < 0.05$, Fig. 2). *In situ* annual mineral N availability was even more tightly correlated with the N_{miner}/N_{microb} ratio ($r = 0.69$, $p < 0.001$), which

Table 5. Potential microbial activity characteristics (N_{miner}, N_{microb}, C_{miner}) in soil from litter and humus horizons of infested and control plots in the Plešné Lake watershed and the effects of horizon, year and infestation on potential microbial activity (factorial analysis of variance). Means ± SD for all representative samples are given ($n = 4$). Different letters indicate significant differences among years (factorial analysis of variance, $p < 0.05$). Asterisks mark differences between infested and control plots in the year (factorial analysis of variance, $p < 0.05$). nd = not determined. ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

		Infested			Control		
	Year	N_{miner} (μg g^{-1} d^{-1})	N_{microb} (μg g^{-1})	C_{miner} (μg g^{-1} d^{-1})	N_{miner} (μg g^{-1} d^{-1})	N_{microb} (μg g^{-1})	C_{miner} (μg g^{-1} d^{-1})
Litter	2001	3.7 ± 0.9^{a}	528 ± 69^{a}	338 ± 72^{c}	nd	nd	nd
	2002	3.6 ± 0.8^{a}	620 ± 45^{a}	211 ± 7^{b}	nd	nd	nd
	2004	3.0 ± 1.6^{a}	527 ± 33^{a}	149 ± 10^{a}	2.2 ± 0.7^{a}	812 ± 202^{ab}	139 ± 19^{a}
	2005	30.2 ± 3.3^{c*}	526 ± 375^{a*}	228 ± 26^{b}	5.1 ± 2.8^{a}	1103 ± 81^{b}	170 ± 17^{b}
	2006	16.7 ± 1.5^{b*}	592 ± 26^{a}	156 ± 60^{a}	3.3 ± 0.6^{a}	724 ± 38^{ab}	120 ± 17^{a}
	2007	3.5 ± 0.4^{a}	413 ± 53^{a}	163 ± 35^{a*}	7.1 ± 0.6^{a}	620 ± 32^{a}	240 ± 22^{b}
	2008	10.4 ± 2.3^{b}	756 ± 130^{a}	175 ± 41^{ab*}	7.0 ± 1.4^{a}	832 ± 89^{b}	235 ± 29^{b}
Humus	2001	2.7 ± 1.3^{a}	342 ± 111^{a}	108 ± 17^{a}	nd	nd	nd
	2002	1.1 ± 0.2^{a}	342 ± 31^{a}	67 ± 5^{a}	nd	nd	nd
	2004	2.6 ± 3.9^{a}	351 ± 46^{a}	60 ± 12^{a}	1.2 ± 1.2^{a}	646 ± 466^{a}	72 ± 19^{a}
	2005	3.5 ± 0.3^{a}	349 ± 98^{a}	43 ± 7^{a}	1.5 ± 4.3^{a}	475 ± 179^{a}	62 ± 19^{a}
	2006	7.0 ± 1.2^{b}	309 ± 49^{a}	46 ± 7^{a}	1.3 ± 0.5^{a}	417 ± 34^{a}	79 ± 24^{a}
	2007	1.9 ± 0.5^{a}	252 ± 2^{a}	96 ± 14^{a}	1.7 ± 0.2^{a}	208 ± 16^{b}	86 ± 5^{a}
	2008	1.2 ± 0.7^{a}	416 ± 38^{a}	46 ± 7^{a}	4.7 ± 2.4^{a}	607 ± 114^{a}	86 ± 19^{a}
Effect source	df	*F*	*F*	*F*			
Horizon	1	36.7***	28.6***	472.0***			
Year	4	8.7***	6.9***	13.8***			
Infestation	1	16.8***	11.1**	9.3**			
Horizon × infestation	1	12.7***	5.0*	0.3^{ns}			
Horizon × year	4	3.6**	0.3^{ns}	7.9***			
Year × infestation	4	7.3***	0.6^{ns}	6.2***			

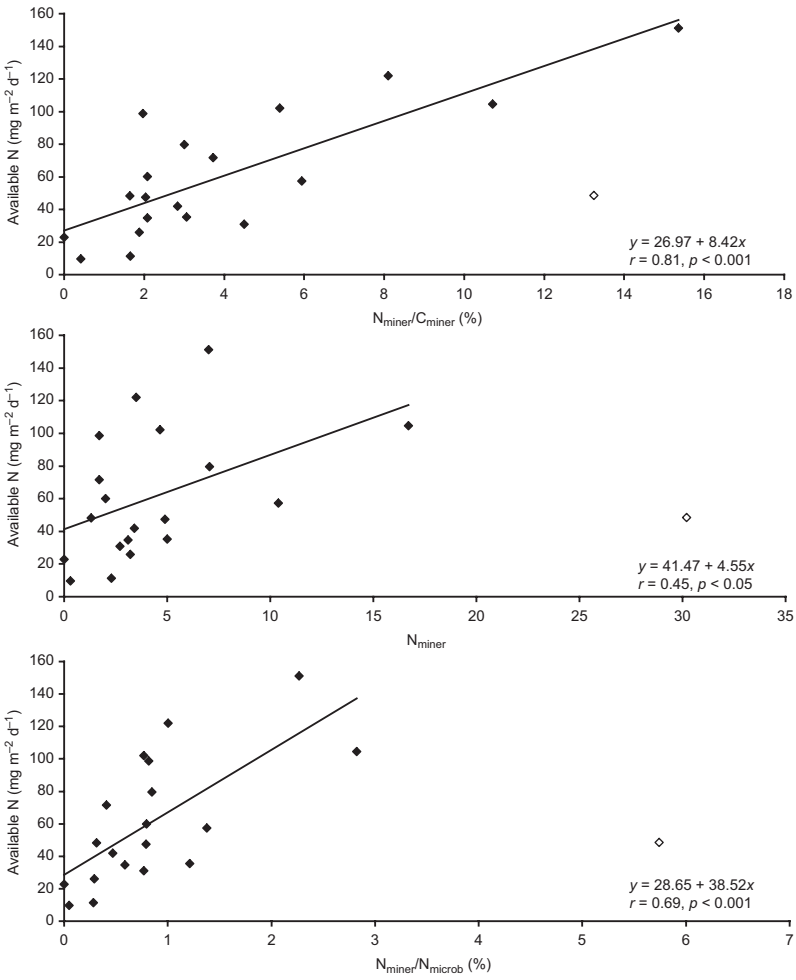

Fig. 2. Correlations of N_{miner} (ammonification and nitrification), N_{miner}/N_{microb} and N_{miner}/C_{miner} ratios with mean annual *in situ* N availability ($N\text{-}NH_4$ + $N\text{-}NO_3$). The outlier year 2005 is displayed as an empty point and was excluded from all correlations.

characterizes prevalence of N mineralization over N immobilization, and to the N_{miner}/C_{miner} ratio (r = 0.81, p < 0.001), an increase of which expresses excess of mineral N release (Fig. 2).

Discussion

In total, *in situ* mineral N availability was significantly enhanced in the forest floor of the infested plot already before maximum forest defoliation (Table 3), while enhancement of potential microbial N mineralization occurred thereafter (Table 5). This indicates that reduced N uptake by trees was the primary cause of increased *in situ* mineral N availability in the forest floor at the infested plot.

In situ $N\text{-}NH_4$ availability had already significantly increased in the year of main litter fall (Fig. 1a, summer 2004), while that of $N\text{-}NO_3$ increased significantly one year later (Fig. 1b, summer 2005). Conifers have been reported to prefer $N\text{-}NH_4$ over $N\text{-}NO_3$ with tree uptake of $N\text{-}NH_4$ being 20 times higher than uptake of $N\text{-}NO_3$ (Kronzucker *et al*. 1997, Ohlund *et al*. 2001). As a result, $N\text{-}NH_4$ should have been mainly enhanced when N uptake of invaded trees was restricted. Similarly to our study, Morehouse *et al*. (2008) compared *in situ* mineral N availability using resin bags in soils of a bark-beetle infested and uninfested pine forest (*Pinus ponderosa*). They found a significant increase of *in situ* $N\text{-}NH_4$ availability in infested plots, but only a small increase of *in situ* $N\text{-}NO_3$ availabil-

ity. In our case, *in situ* availability of both ions was enhanced, which is in agreement with their findings, except that the increase of $N\text{-}NO_3$ was significant in our study.

In situ $N\text{-}NH_4$ availability was enhanced in the litter and humus horizons, whereas $N\text{-}NO_3$ availability was enhanced mostly in the humus horizon. This corresponds to the high mobility of nitrate ions (Jones *et al*. 2005) with seepage water and reported increase of $N\text{-}NO_3$ leaching from the soil after defoliation (from 1.1 to 2.1 g m^{-2} yr^{-1}; Kopáček *et al*. 2006). Such a typical enhancement of $N\text{-}NO_3$ leaching was observed also in many other deforestation studies (e.g. Vitousek *et al*. 1979, Weis *et al*. 2001, Rothe and Mellert 2004, Huber *et al*. 2004a, 2004b, Huber 2005). According to Huber (2005), who studied bark-beetle attacked spruce forests (*Picea abies*) in the Bavarian Forest National Park, $N\text{-}NO_3$ concentration in seepage water was enhanced for seven years after forest dieback with the maximum occurring five years after dieback. Nitrate availability in our infested plot remained enhanced till the end of our study in both horizons (four years after forest defoliation) with the peak during the first year after defoliation. To the best of our knowledge, there has not been another long-term study regarding natural forest dieback. However, forest clear-cuts, which are more frequent, also show nitrate peaks shortly (one-two years) after harvesting (e.g. Dahlgren and Driscoll 1994, Carmosini *et al*. 2003). Huber (2005) explained the five-year delay in $N\text{-}NO_3$ leaching by a temporary increase of microbial N immobilization due to decomposition of new organic material with high C/N ratio from dead trees (breaking down of the stems, Borman and Likens 1979). In this case, N_{microb} should increase and, at the same time, the ratios characterizing N excess (N_{miner}/N_{microb} and N_{miner}/C_{miner}) should decrease. However, we did not detect increasing N_{microb} nor decreasing N_{miner}/N_{microb} and N_{miner}/C_{miner} ratios. In addition, needles, which formed the main part of litter input after tree dieback, had lower C/N ratio than litter fall from healthy trees. Low litter fall C/N ratio was also found by Morehouse *et al*. (2008).

In our study, decreased *in situ* $N\text{-}NO_3$ availability in the infested plot was apparent in 2006 in summer and in the 2006/2007 winter (Table 3). While N availability had a descending trend untill the end of the study, winter N availability increased again in 2007/2008. The decrease of mineral N availability could be caused by mineral N immobilization by developing vegetation cover. As light can reach the forest floor, grassy patches rapidly start to develop in open areas decreasing N availability in the soil solution (Stevens and Hornung 1990, Emmett *et al*. 1991, De Keersmaeker *et al*. 2000, Weis *et al*. 2001, Parfitt *et al*. 2002, Fiala *et al*. 2005). *Calamagrostis villosa* spread very quickly after 2004 in the infested plot and dominated the vegetation in 2006 (M. Svoboda unpubl. data). The dominating grass biomass is rich in N and its litter has a low C/N ratio. During litter decomposition, which starts in late autumn, N mineralization prevails over microbial N immobilization due to the low C/N ratio in decomposing material. The C/N ratio of the grasses at our study plots ranged from 24 to 30 (Svoboda *et al*. 2006) with release of mineral N during decomposition rising inversely to the C/N ratio, with an exponential shape for C/N below 32 (Šantrůčková *et al*. 2006, Bárta *et al*. 2010). This may be the reason why the nitrate concentration in soil water could be independent of ground vegetation cover in the later years after forest dieback and why $N\text{-}NO_3$ availability in our study increased again in winter 2007/2008 (Fig. 1b). This is in agreement with an observation by Huber (2005) who found a positive correlation between ground vegetation cover and $N\text{-}NO_3$ concentration in soil leachate from the second to the fifth year after forest dieback; after that the correlation disappeared.

The results indicate that increased *in situ* mineral N availability is caused by both decreased vegetation N uptake and excess microbial N release. Decreased spruce tree N uptake contributed to increased mineral N availability in summer while enhanced microbial N release took place over the whole year. It is widely accepted that microbial N mineralization, even when decreased at low temperature, is of importance during wintertime (e.g. Nadelhoffer *et al*. 1991, Fitzhugh *et al*. 2001, Groffman *et al*. 2001); this was ascertained by Šantrůčková *et al*. (2009) in the studied soils. The contribution of microbial N mineralization to *in situ* mineral N availability is supported by the correlation

between *in situ* mineral N availability and the N_{miner}/N_{microb} and N_{miner}/C_{miner} ratios. An outlier was excluded from the correlation values of the ratios from the litter layer of the infested plot in 2005. The decision was based on the rate of N mineralization (ammonification and nitrification), which was much higher in the litter horizon than in the humus horizon in 2005 in the infested plot, while total *in situ* available N was, on the contrary, higher in the humus than in the litter horizon. There was high input of new organic material to the litter horizon in 2004, which caused a maximum increase of potential N mineralization in 2005. However, *in situ* available N contains nitrates, which are very mobile and are easily washed down to the humus horizon. This can be the reason for the disproportion found between the rate of N mineralization and the amount of *in situ* available N in 2005 in the litter horizon.

Net N mineralization results from a balance between sources of mineral N (gross ammonification and nitrification) and microbial N immobilization. When N production is in equilibrium with N immobilization, the rates of net N mineralization are low. A shift towards N mineralization brings an increase in the net rate of the processes. In our study, the increase in net N mineralization has to be caused by an increase in N mineralization rate as neither N_{microb} nor C_{miner}, which are often used as estimates of N immobilization rate (Hart *et al*. 1994, Aber 1998, Micks *et al*. 2004), were enhanced. The increase in net N mineralization rate was caused, most likely, by decomposition of a high amount of needle litter with low C/N ratio after tree dieback (in 2004 in the infested plot and 2006 in the control plot) and decomposition of grass litter after 2006 in the infested plot. The increased input of plant debris with low C/N ratio was always followed by decreased C/N ratio of the soil (Table 2).

The results affirm that, in the studied plots, *in situ* N availability was influenced by a combined effect of N consumption by vegetation and microbial N transformation in soil. In the first stage of bark beetle attack, N availability in soil was increased mainly due to reduced N consumption by dying spruce trees. It then decreased again during regeneration of new vegetation cover, which was represented mainly by grasses. Microbial N release contributed to the change of *in situ* N availability after input of a high amount of plant material with favourable C/N ratio.

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