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The effect of single tree species on soil microbial activities related to C and N cycling in the Siberian artificial afforestation experiment

Tree species and soil microbial activities

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Abstract

The effects of grassland conversion to forest vegetation and of individual tree species on microbial activity in Siberia are largely unstudied. Here, we examined the effects of the six most commonly dominant tree species in Siberian forests (Scots pine, spruce, Arolla pine, larch, aspen and birch) on soil C and N mineralization, N_2O-reduction and N_2O production during denitrification 30 years after planting. We also documented the effect of grassland conversion to different tree species on microbial activities at different soil depths and their relationships to soil chemical properties. The effects of tree species and grassland conversion were more pronounced on N than on C transformations. Tree species and grassland conversion did significantly alter substrate-induced respiration (SIR) and basal respiration, but the differences were not as large as those observed for N transformations. Variances in SIR and basal respiration within species were markedly lower than those in N transformations. Net N mineralization, net nitrification, and denitrification potential were highest under Arolla pine and larch, intermediate under deciduous aspen and birch, and lowest beneath spruce and Scots pine. Tree species caused similar effects on denitrification potential, net N mineralization, and net nitrification, but effects on N_2O reduction rate were idiosyncratic, indicating a decoupling of N_2O production and reduction. We predict that deciduous species should produce more N_2O in the field than conifers, and that Siberian forests will produce more N_2O if global climate change alters tree species composition. Basal respiration and SIR showed inverse responses to tree species: when basal respiration increased in response to a given tree species, SIR declined. SIR may have been controlled by NH_4^+ availability and related therefore to N mineralization, which was negatively affected by grassland conversion. Basal respiration appeared to be less limited by NH_4^+ and controlled mostly by readily available organic C (DOC), which was higher in concentration under forests than in grassland and therefore basal respiration was higher in forested soils. We conclude that in the Siberian artificial afforestation experiment, soil C mineralization was not limited by N.

Introduction

There is increasing interest in linking forest dynamics to ecosystem processes because human-induced environmental changes are likely to change forest composition (Bolker et al., 1995), net primary production (Schulze et al., 1999) and patterns of C and N cycling (Finzi et al., 1998). To understand how changes in vegetation composition will affect ecosystem processes, for example emission of greenhouse gases, it is crucial to clarify the effect of individual tree species on soil microbial processes that mediate soil C and N transformations.

Artificial afforestation experiments provide an opportunity to explicitly identify the influences of individual tree species on soil C and N transforma-

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tions mediated by soil microorganisms, because plots are initially homogenous in microbial, chemical, and physical properties, and trees are of the same age (Binkley, 1994; Menyailo et al., 2002a; Wedin and Tilman, 1990). There are few artificial afforestation experiments where microbiological processes have been studied, and little and contradictory information is available. In some cases, no distinct species effect was found after 23–24 years of stand growth (Priha and Smolander, 1997). By contrast, in another experiment tree seedlings caused changes in C transformation activities in soil after only 4 months of growth (Priha et al., 1999b). The effect of grassland conversion to forest vegetation is much less understood compared to the conversion to cultivated fields or forest conversion to grasslands. Yet, the effects of converting grassland to forest is of special importance as countries pursue efforts to reduce net greenhouse gas emissions.

Little is known about how Siberian tree species influence microbiological processes. To our knowledge, only one study has investigated such effects, showing that individual tree species influence denitrifying enzyme kinetics and their *de novo* synthesis in monodominant stands with different species (Menyailo and Huwe, 1999), where soil samples were taken in canopy 'windows' (inter-space). Whether effects are more pronounced in soils directly beneath trees, and what effects tree species have on other microbial processes in this system remain unknown. The aim of our work was to analyze the effects of the six most commonly dominant tree species in Siberian forests on soil microbial activities related to C and N mineralization and also on two steps of denitrification, which are potentially important for net N_2O fluxes. In the present work, we show the effect of grassland conversion to different tree species on microbial activities at different soil depths as well as their relationships to soil chemical properties. The same microbial parameters were studied in a tropical tree-based agro-forestry system with different tree species and in natural forests in Brazil (Menyailo et al., 2002b). Here, we compare the parameters, their inter-relationships and controlling factors between these two biomes. The comparisons of temperate forests in Siberia and tropical forests in the Brazilian Amazon should elucidate mechanisms regulating microbial C and N transformations.

Materials and methods

Research sites and experimental setup

The same soil samples were used as described in the accompanying paper (Menyailo et al., 2002a). The region is characterized by continental climatic conditions with average rainfall 500 mm $year^{-1}$, average daily summer temperature of 20 °C (at 12:00 h), depth to permafrost 70–170 cm and soil temperature to 20 cm depth in winter −4°to −14°, in summer 10° to 12°. The soil is of gray forest type according to the Russian soil taxonomy and Greyzem according to FAO (1990).

Experimental treatments were established in the early 1970s by the Laboratory of Soil Science of the Institute of Forest, Siberian Branch of the Russian Academy of Sciences. The upper soil horizons (0–50 cm) of the soil were mechanically homogenized to minimize spatial heterogeneity of chemical, physical and biological properties over approximately 1.5 ha. In 1971–1972, 2–3-year-old tree-seedlings of spruce (*Picea abies*), birch (*Betula pendula*), Scots pine (*Pinus sylvestris*), aspen (*Populus tremula*), larch (*Larix sibirica*) and Arolla pine (*Pinus cembra*) were planted. Each plot with one species occupies 2400 m^2. An area of 9600 m^2 was left for grassland as a control; the soil under grass was not mechanically cultivated. Each plot was sub-divided into three parts. In each sub-plot, two trees were chosen and four soil samples were taken at 50 cm from the bole of each tree. Soil samples were taken at three depths (0–10, 10–20 and 20–30 cm) and samples from one depth were bulked. One species resulted in nine soil samples: three sub-plots and three soil depths.

Study of soil chemical properties

Chemical analyses are conducted as described in accompanying paper (Menyailo et al., 2002a). In short, all samples were analyzed for pH in water solution (1:2.5). NH_4^+ and NO_3^- were determined in 1 *M* KCl solution (1:5) by a flow injection analyzer (Lachat). Dissolved organic carbon (DOC) was determined in water extracts (1:5, soil:water) as CO_2 by infrared detection after persulfate oxidation. Total dissolved nitrogen was determined in the same water extract using a Total Nitrogen Analyzer (TN-05, Mitsubishi Kasei Corp.). Dissolved organic nitrogen (DON) was determined as the difference between total dissolved N and inorganic N (measured in water extract). Total C, N and C/N were determined by

Dumas combustion and gas chromatography (Heraeus elemental analyzer). DOC, DON, NH_4^+ and NO_3^- were determined in the central analytical laboratory of BITÖK (Bayreuth Institute of Terrestrial Ecosystem Research). Total C, N and pH were analyzed at the Department of Soil Science of the University of Bayreuth.

Denitrification potential

Five grams of soil were placed in glass flasks (25 mL) and pre-incubated at 28 °C for 3 days to initiate microbial activity. Thereafter, 5 mL of distilled water with KNO_3 and glucose were added to each sample. The resulting concentrations of nitrate and glucose were 100 μg NO_3-N g^{-1} soil and 100 μg glucose-C g^{-1} soil. The flasks were closed with air-tight rubber stoppers and fixed with clamps. Anaerobic conditions were induced by exchanging the gas phase with He for 15 min; 2.5 mL of C_2H_2 (10% v/v) were then added as an inhibitor of the N_2O-reductase, which catalyzes the conversion of N_2O to N_2. All samples were than incubated and after 24 h the headspace of each flask was sampled and analyzed for N_2O using gas chromatography (Shimadzu 14A, N_2 carrier gas, equipped with an electron capture detector (ECD ^{63}Ni), Menyailo and Huwe, 1999). The sample volume was 1 mL. The results were expressed as mg N_2O-N kg^{-1} day^{-1}.

Potential N_2O reduction

From each of the 63 soil samples, four sub-samples were used to measure potential N_2O reduction. As above, 5 g of soil were placed in glass flasks (25 mL) and pre-incubated at 28 °C for 3 days to initiate microbial activity. Thereafter, 5 mL of distilled water with glucose were added to each sub-sample. The resulting concentration of glucose was 100 mg C kg^{-1} soil. The flasks were closed with air-tight rubber stoppers and fixed with clamps. Anaerobic conditions were induced by exchanging the gas phase with He for 15 min. After removing O_2 from the flasks, 1 mL of N_2O was added to two sub-samples as a final electron acceptor. To another two sub-samples, 1 mL of N_2O and 2.5 mL of C_2H_2(10% v/v) were added. The last series of sub-samples were necessary to estimate abiotic N_2O consumption by soil (dissolution in water). One mL of the headspace from each flask was sampled at 0, 24 and 48 h, in order to analyze for N_2O concentration as described above. Biotic N_2O consumption rate were calculated as the difference between changes in N_2O concentration between C_2H_2 treated and untreated samples. For the first incubation day, the following formula was used: N_2O reduction rate = $(B_0 - B_{24}) - (A_0 - A_{24})$, where B_0 is the initial concentration of N_2O in flask without C_2H_2, B_{24} is the concentration of N_2O in this flask after 24 h incubation, A_0 is the initial concentration of N_2O in flask with C_2H_2 and A_{24} is the concentration of N_2O in this flask after 24 h of incubation. For the second incubation day, the analogous formula was used substituting concentrations of N_2O at 24 h as initial and at 48 h instead of 24 h. As the rate of N_2O consumption was linear during 2 days of incubation, mean values were calculated and expressed as mg N_2O-N $kg^{-1}day^{-1}$.

Net N-mineralization and net nitrification

Approximately 15 g were extracted with 1 *M* KCl (1:5) to determine the initial concentration of NH_4^+-N and NO_3^--N with a flow injection analyzer (Lachat). Additionally, 15 g from each soil sample were placed in plastic flasks (150 mL), moistened to 60% of water-holding capacity (WHC), sealed with stoppers and incubated at 28 °C for 30 days. To avoid anaerobic conditions, the flasks were opened every 3 days for 5 min. On day 30, soil samples were analyzed for NH_4^+-N and NO_3^--N as described above.

Net mineralization rate was calculated as the difference in $[NO_3^- + NH_4^+]$ before and after the incubation and was expressed as mg $(NO_3^- + NH_4^+)$-N $kg^{-1}30$ $days^{-1}$. Net nitrification was calculated as the difference in $[NO_3^-]$ before and after the incubation and was expressed as mg NO_3^--N kg^{-1} 30 $days^{-1}$.

Basal and substrate-induced respiration

To study basal respiration, 5 g of soil were placed in a 25-mL flask. Water was added to achieve 60% of water-holding capacity (WHC). The flasks were closed with rubber stoppers, fixed with clamps and incubated at 28 °C for 3 days. Afterwards, flasks were open for 30 min to allow aeration, sealed and incubated at the same temperature for 24 h. A gas sample of the headspace (1 mL) from each flask was taken for analysis of CO_2 concentrations using gas chromatography as described above. For substrate-induced respiration, 5 g of soil were placed in 25-mL flasks and moistured with distilled water. The flasks were closed with rubber stoppers, fixed with clamps and pre-incubated at 28 °C for 3 days. Thereafter, flasks were open a water solution with glucose as a C-source was added to obtain 60% water holding capacity (WHC) and 100 mg glucose-C kg^{-1} soil. Soil

Table 1. Effect of tree species and soil depth on soil microbiological activities in forested soil (n=54)

	Depth (df 2)		Tree species (df 5)	
	F	*P*-level	*F*	*P*-level
Net N Mineralization	7.25	0.002	11.44	<0.001
Net nitrification	NS	NS	12.98	<0.001
SIR	68.47	<0.001	13.11	<0.001
Basal respiration	16.77	<0.001	3.65	0.009
Denitrif. potential	9.01	<0.001	15.27	<0.001
N_2O reduction	NS	NS	15.56	<0.001
DEN/RED ratio	4.38	0.019	9.07	<0.001

NS, not significant.

samples were then incubated for 24 h and 1 mL of the headspace air of each flask was sampled and analyzed for CO_2 as described above. The results were expressed as g CO_2-C kg^{-1} day^{-1}.

Statistical data analysis

All measurements of microbiological activities were done in duplicate for each soil sample. Mean values for one soil sample were taken. All parameters were tested for normality of distribution and were log-transformed where necessary. The main effect of tree species and soil depth was determined by two-way analysis of variance (ANOVA) with three replicates (three soil samples). We considered the effect significant at $P<0.05$. Afterwards, post hoc comparisons with the Tukey honest significant difference (HSD) test was performed to discern under which species the effect of soil depth is significant and at which depth species caused differences. Additionally, we tested for species effects on processes averaged across all depths, thereby assessing the overall effects of species on soil processes (0–30 cm). To study relationships between microbial activities and soil chemical parameters, and within microbiological activities themselves, Spearman rank order correlation coefficients were used. When computing correlations, only soil samples under tree species (not in grassland) and from the same depth were considered (n=18). All statistics were carried out with the statistical package STATISTICA (5.0 for Windows, StatSoft, 1997).

Results

Denitrification potential

Denitrification potential showed high variation and ranged from 2.5 to 20 mg N_2O-N kg^{-1} day^{-1} (Figure 1a). The main effects of tree species and soil depth were significant (Table 1, $P<0.001$). Denitrification declined with depth with significant difference between the deepest and the upper two horizons ($P<0.001$). Within individual species, the effect of soil depth was significant under larch (P=0.009) and birch (P=0.012). The highest denitrification potential was found beneath Arolla pine and larch. Denitrification potential under Arolla pine and larch was significantly higher than beneath spruce, Scots pine and birch, where the lowest denitrification potential was measured. Aspen had a lower denitrification potential than larch (P=0.025) and higher than spruce (P=0.017), and in the same range as under Scots pine, Arolla pine and birch.

Potential activity of N_2O reduction

Potential N_2O reduction varied from 3.8 to 15.3 mg N_2O-N kg^{-1} day^{-1} and values were comparable to those observed for denitrification potential (Figure 1b), indicating that neither NO_3^- nor N_2O was a limiting substrate in our incubation experiments. N_2O reduction was affected only by species ($P<0.001$) and not by soil depth (Table 1). N_2O reduction was thus less modified with soil depth than was denitrification potential. As for denitrification potential, the high rate of N_2O reduction was found under Arolla pine and larch, significantly higher than under all other species ($P<0.035$). In contrast to denitrification potential, low values of N_2O reduction were found under deciduous species aspen and birch, significantly lower than beneath spruce, Arolla pine and larch.

Denitrification potential to N_2O reduction ratio

Soil emissions of N_2O depend on the rates of both N_2O production (due mainly to denitrification) and consumption (N_2O-reduction), so the ratio of these processes (DEN/RED, Figure 1c) shows the potential ratio of N_2O/N_2 the denitrification end products. DEN/RED ranged from 0.3 to 3, depending on species and soil depth, indicating that these factors can substantially influence the balance of the end products (N_2O versus N_2) of denitrification under standard experimental conditions. Both tree species ($P<0.001$)

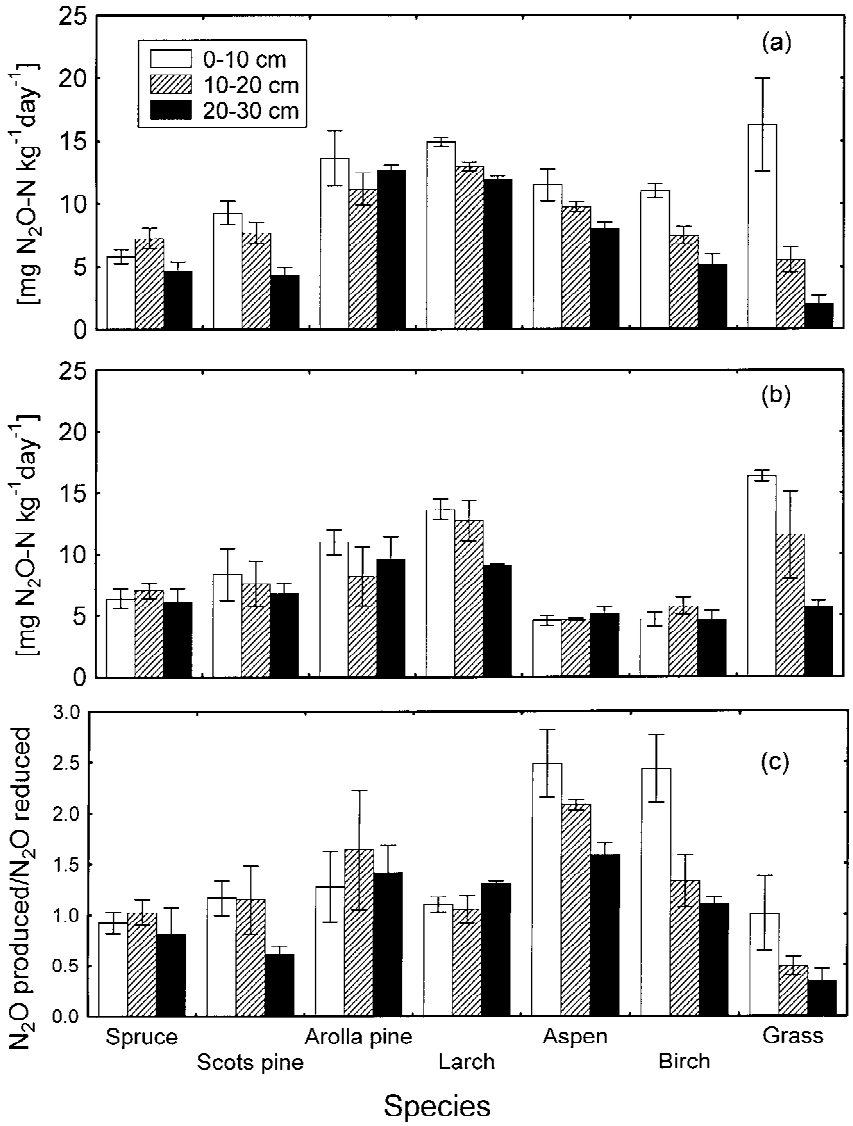

Figure 1. (a) Potential denitrification activity, measured in anaerobic conditions with addition of nitrate, glucose and acetylene in soils beneath six Siberian tree species and grassland at three soil depths (n=3). (b) Potential rate of N_2O reduction, measured under anaerobic conditions with addition of glucose and N_2O in the same soils. (c) Calculated ratios of potential denitrification activity and activity of N_2O reduction.

and soil depth (P=0.012) affected DEN/RED ratio (Table 1). Soil at 20–30 cm depth had significantly lower DEN/RED ratio the two upper horizons. Within species, a significant decline with depth was observed only under the deciduous species aspen (P=0.052) and birch (P=0.011), species under which the highest DEN/RED ratios were found, significantly higher than under spruce and Scots pine. Aspen had also higher DEN/RED ratio than larch (P=0.001).

Net N mineralization

Net N mineralization ranged from 12 to 87.8 mg $(NO_3^- + NH_4^+)$-N kg^{-1} 30 $days^{-1}$ (Figure 2a). Both tree species (P<0.001) and soil depth (P=0.002) affected net N mineralization without interactions between these two factors (Table 1). Net N mineralization rate was significantly higher in the 0–10-cm layer than in the 10–20-cm (P=0.010) and 20–30-cm (P=0.006) layers, but the two deeper layers did not differ significantly (P=0.195). Within species, the ef-

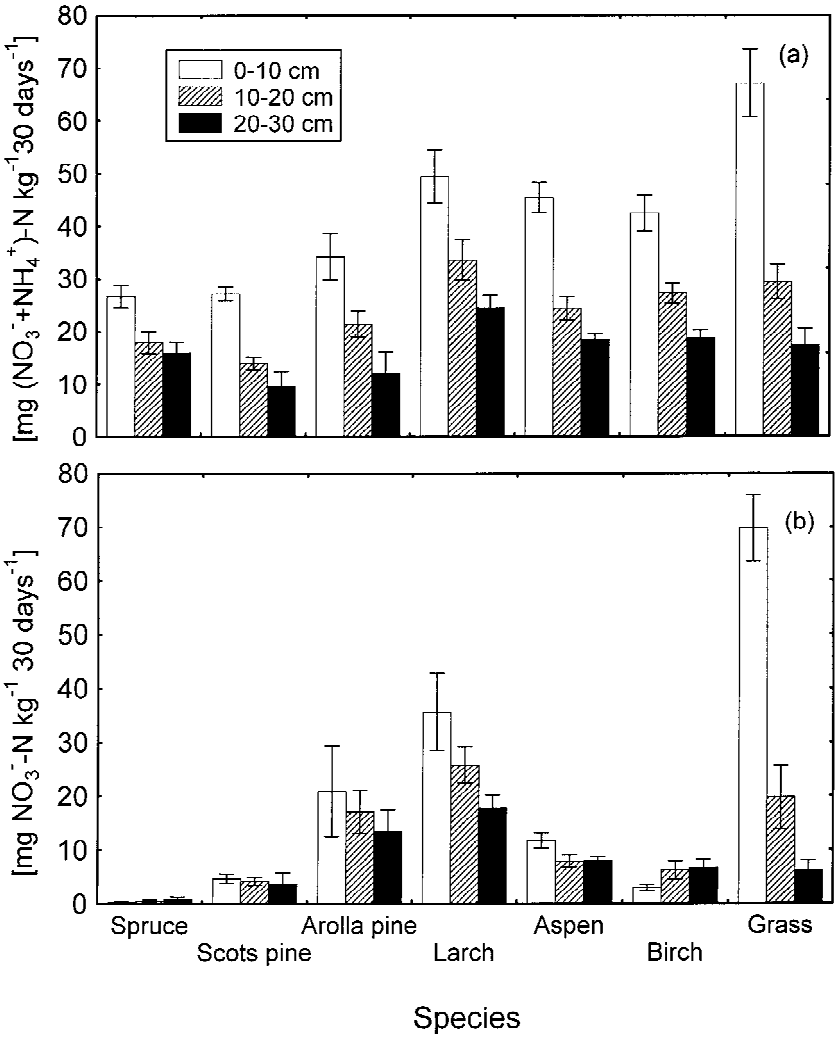

Figure 2. (a) Net N mineralization at three soil depths and under six tree species and grassland (n=3) measured as production of NO_3^- and NH_4^+ in 30 days aerobic incubation at constant soil moisture (60% WHC) and temperature regime (28 °C). (b) Net nitrification rate measured as production of NO_3^- in the same experiment (n=3).

fect of soil depth was significant only under aspen (P=0.004). The highest rate of net N mineralization was found under larch. Larch and Arolla pine increased N mineralization significantly compared to spruce (P<0.001) and Scots pine (P=0.004), where the lowest rate was found. N mineralization under larch was also significantly higher than that under birch (P<0.001) and aspen (P=0.003).

Among all of the measured activities, net nitrification was most strongly affected by tree species (P<0.001) ranging from 0.1 to 54 mg NO_3^--N kg^{-1} 30 $days^{-1}$ (Figure 2b). In contrast to net N mineralization, soil depth did not affect net nitrification (P=0.280). Net nitrification tended to vary among species in the same order as net N mineralization: low activities of both processes occurred under spruce and Scots pine, intermediate values were found under aspen and birch, and the highest rates occurred under larch and Arolla pine. Net nitrification constituted the smallest part of net N mineralization under spruce: 5% in 0–10 cm and 20% in 20–30 cm. Net nitrification was a small proportion of net N mineralization in the upper horizon under birch (around 20%) and increased with depth to 65%. By contrast, net nitrification contributed to 90–100% of net N mineralization under larch and Arolla pine, indicating that almost all of the net increase in inorganic N was due to NO_3^- accumulation.

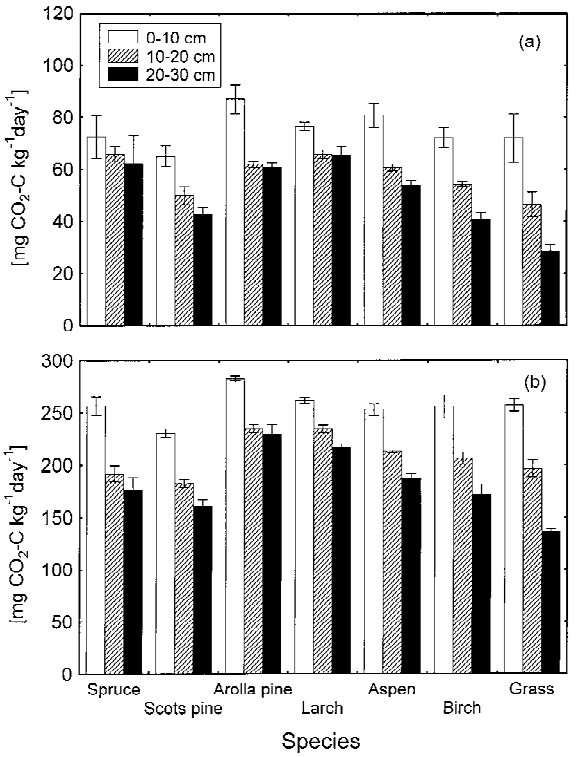

Figure 3. (a) Activity of basal respiration in soil samples at three depths beneath six tree species and grassland (n=3) measured as CO_2 production during 24 h incubation at 60% of WHC and 28 °C. (b) Substrate-induced respiration in the same soil samples measured as described above but with addition of glucose.

Basal respiration and substrate-induced respiration

Basal respiration varied within a very narrow range: 22–99 g CO_2-C kg^{-1} day^{-1} (Figure 3a). Both soil depth (P<0.001) and tree species (P=0.009) influenced basal respiration rate. Basal respiration decreased with soil depth significantly between the 0–10- and 10–20-cm depths (P=0.001), but with no significant difference between the 10–20- and 20–30-cm depths. Within different species, soil depth significantly influenced basal respiration under Arolla pine (P=0.020), aspen (P=0.010) and birch (P=0.006). Arolla pine, larch and aspen increased basal respiration rates in all soil layers compared to Scots pine (P<0.020) and birch (P=0.010). Basal respiration under birch was significantly lower than under spruce (P=0.041). Even though the species effects were significant, the differences among species were not as large as for N transformations.

Substrate-induced respiration through glucose addition caused a 3-fold increase in respiration (Figure 3b). SIR was influenced by both main factors — soil depth and tree species (for both P<0.001). SIR declined with soil depth with significant difference between all horizons and under all species. Arolla pine and larch increased SIR compared to other species (P<0.050). Intermediate values of SIR were found beneath spruce, aspen and birch. The lowest value was found under Scots pine, where SIR was significantly lower than under all other species (P<0.050). Overall, the effects of species on SIR were similar to effects on basal respiration rate.

Overall effect of species (0–30 cm)

Considering all soil depths together (0–30 cm), species significantly affected denitrification potential (P<0.001), N_2O reduction (P<0.001), net N mineralization (P<0.001), net nitrification (P<0.001), basal respiration (P=0.029) and SIR (P=0.017). Arolla pine and larch had higher denitrification potential than spruce, Scots pine, birch and grass (for all, P<0.050). Aspen had higher denitrification potential than spruce (P=0.043). N_2O reduction was also higher under Arolla pine and larch than under spruce (P<0.050), aspen and birch (for both, P<0.010). Larch also differed from Scots pine (P=0.002), and aspen had lower N_2O reduction than Scots pine (P=0.043). Grass had higher N_2O reduction than spruce and Scots pine (for both P=0.009) and aspen and birch (for both P<0.001). Larch had higher net N mineralization than spruce (P<0.001), Scots pine (P=0.001), aspen (P=0.028) and birch (P=0.009). The highest net N mineralization was under grass; the values were significantly higher than under all tree species (P<0.050) except larch. The same differences were found for net nitrification, except that, additionally, Arolla pine differed from spruce (P=0.013). Cumulative basal respiration was lower under Scots pine than under Arolla pine (P=0.025) and larch (P=0.031). Grass had lower basal respiration than spruce (P=0.022), Arolla pine (P=0.008), larch (P=0.010) and aspen (P=0.038).

Interrelationships among microbial parameters

At all three depths, denitrification potential, net N mineralization and net nitrification were intercorrelated (Table 2). The relationship between denitrification and net N mineralization was weaker with increasing depth. In contrast, the relationship between denitrification and net nitrification increased with depth.

Table 2. Spearman correlation coefficients between microbiological parameters measured under different tree species at three soil depths (for each group n=18)

	SIR	Basal respirat.	Net Mineralization	Net Nitrification	Denitrif. potential	N_2O reduction
Depth 0–10 cm						
Basal respiration	0.82***					
Net N mineralization	NS	NS				
Net nitrification	NS	NS	0.95***			
Denitrification potential	NS	NS	0.79***	0.72***		
N_2O reduction	NS	NS	NS	NS	NS	
DEN/RED ratio	NS	NS	NS	NS	NS	−0.70**
Depth 10–20 cm						
Basal respiration	0.53*					
Net N mineralization	0.87***	NS				
Net nitrification	0.85***	NS	0.97***			
Denitrification potential	0.79***	0.47*	0.73***	0.77***		
N_2O reduction	NS	NS	NS	NS	NS	
DEN/RED ratio	NS	NS	NS	NS	NS	−0.76***
Depth 20–30 cm						
Basal respiration	0.82***					
Net N mineralization	0.46*	NS				
Net nitrification	0.61**	NS	0.92***			
Denitrification potential	0.90***	0.71**	0.61**	0.79**		
N_2O reduction	0.57*	0.48*	NS	0.52*	0.61**	
DEN/RED ratio	0.64**	0.56*	0.58*	0.60**	0.74***	NS

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS, not significant.

SIR and basal respiration were correlated at all soil depths. However, the strength of the relationship was not constant with depth, being the weakest at 10–20 cm. While SIR was not related to any other activities in the upper soil (0–10 cm), SIR was positively correlated to net N mineralization and net nitrification in the deeper soil layers; the strongest relation occurred at the 10–20-cm depth and was two times weaker at the 20–30-cm depth. Probably, SIR was limited by either NH_4^+ or NO_3^- availability and these dependences were most pronounced at the 10–20-cm depth, explaining the weak correlation between SIR and basal respiration at this depth. In contrast to SIR, basal respiration was correlated neither to net nitrification nor to net N mineralization, indicating that basal respiration was less limited by inorganic N and controlled, mostly, by easily available organic carbon and/or microbial biomass.

The DEN/RED ratio was only correlated to N_2O reduction rate at 0–10 and 10–20 cm depth. This suggests that, at least in upper soils, the balance between N_2O production and consumption and, largely, the net emission of N_2O from soil to atmosphere, will depend on N_2O-reductase activity and to a lesser extent on the first steps of denitrification. At the 20–30-cm depth, the DEN/RED ratio was correlated to denitrification potential and not to N_2O reduction rate. Also at this deepest soil layer, denitrification potential was correlated to N_2O reduction rate, indicating that different factors control N_2O production and N_2O-reductase activities in the upper horizons.

The strength of relationship between respiration rates and denitrification increased with soil depth. In the two deeper horizons, denitrification potential was most strongly correlated to SIR and to a lesser extent to basal respiration, suggesting that either soil heterotrophic microorganisms were competing with denitrifying bacteria for NO_3^- and/or they competed for easily available organic C. Another possible explanation for this correlation between SIR and denitrification is that SIR was controlled by NH_4^+ and therefore correlated to N mineralization, which, in turn, was correlated to denitrification potential.

The relationships between the microbial activities studied changed markedly with increasing soil depth. C and N transformations were uncorrelated in the

upper 0–10-cm horizon, but SIR was correlated to N mineralization in the deeper depths. N_2O reduction rate and DEN/RED ratio were not related to any microbial activities in the 0–20-cm depth, but were correlated to denitrification potential and, to a lesser extent, to all other processes in the 20–30-cm depth.

Relationship between microbiological activities and soil chemical properties

At the 0–10 and 10–20 cm depths, net N mineralization and net nitrification were correlated to the same soil variables: pH, NH_4^+, total C and total N (Table 3). With increasing depth, net N mineralization and net nitrification were correlated with DON, but not to NH_4^+ and C. Denitrification was correlated to almost the same soil variables due to close interrelationships with N mineralization.

At the 0–10-cm depth, N_2O reduction was correlated to pH and C/N ratio and DEN/RED ratio was correlated to C/N. At the 10–20-cm depth, neither N_2O-reduction nor DEN/RED ratio showed any significant correlations with soil chemical variables. At the deepest layer (20–30 cm), N_2O-reduction was correlated to pH and NH_4^+, and the DEN/RED was correlated to pH, total soil N, and DOC.

In the 0–10-cm depth, SIR was closely correlated to NH_4^+ and only weakly correlated to DOC. In contrast, basal respiration was more strongly correlated to DOC and less to NH_4^+. This is easily explained by the relative availability of C and N (C/N ratio): addition of glucose for the SIR measurement increased the C/N ratio, increasing microbial requirement for inorganic N (Burke et al., 1989; Côté et al., 2000). Close correlation with NH_4^+ rather than with NO_3^- indicates that NH_4^+ is the more preferred form of inorganic N for microbial assimilation in these soils. At 10–20 cm, SIR was related to the same soil properties as N mineralization (pH, NH_4^+, C and N), because at this depth the processes were inter-related. At this depth, initial soil NH_4^+ concentration was not as high as in the surface layer (0–10 cm) and may not have been sufficient to support SIR. Basal respiration, at the same depth, was limited neither by NH_4^+ nor by DOC. However, in the deepest layer, both basal respiration and SIR were limited by DOC concentration and SIR was also linked to the same soil variables as net N mineralization (pH, N, DON), indicating limitation by NH_4^+ availability.

The effect of grassland conversion

To study the effect of conversion, all tree species were combined into one group and the difference between this group and grassland will be called the conversion effect. Considering the main effects of conversion and soil depth, the effect of soil depth was significant for all of the measured activities, except the DEN/RED ratio. The effect of grassland conversion to forest vegetation significantly affected all microbial parameters except denitrification potential. No interaction between the main factors soil depth versus conversion was observed for DEN/RED ratio and basal respiration. Both DEN/RED ratio and basal respiration were higher in all soil depths under tree species than in grassland. For all other activities, significant interactions between depth and conversion were found, indicating that the effect of conversion is different at different depths. This was due to specificity of vertical distribution of microbiological activities in grassland.

Microbial N transformations and SIR showed larger vertical differentiation in grassland than under tree species (Figures 1–3). Denitrification potential, N_2O-reduction, net N mineralization and net nitrification were around two times higher in grassland than under forest in 0–10-cm depth, but activities of these processes were lower in grassland in 10–20- and 20–30-cm depths, providing additional evidence of larger vertical differentiation of microbial activities in grassland than in forest.

Comparisons of the tree species effect in temperate versus tropical forests

The same microbial parameters (except N_2O-reduction) were studied in a tropical tree-based agro-forestry system near Manaus (Central Amazon, Brazil) (Menyailo et al., 2002b). All activities were measured under the same laboratory conditions, though for net N mineralization and net nitrification measurements, soil samples were incubated during 2 weeks instead of 4 weeks as for Siberian soils. Here, we compare the interrelationships among microbial parameters in temperate and tropical climate zones and their relationships to soil chemical variables as well as their mean values (Figure 4). Because in Brazil the soil samples were studied only at 0–10 cm depth (n=21), the same soil depth will be taken for comparisons in Siberian artificial afforestation experiment (n=18) and in Siberian grassland (n=3).

Most microbial parameters measured in the Amazon were inter-related; the only exception was the

Table 3. Spearman correlation coefficients between soil microbial parameters and chemical properties under forest species at three soil depths (for each depth, n=18). The correlations with NO_3^- are not shown because no one was significant

	pH	NH_4^+	N	C	C/N	DON	DOC
Depth 0–10 cm							
Net N mineralization	0.68**	0.63**	0.80***	0.53*	NS	NS	NS
Net nitrification	0.77***	0.59**	0.79***	0.57*	NS	NS	NS
SIR	NS	0.77***	NS	NS	NS	NS	0.51*
Basal respiration	NS	0.53*	NS	NS	NS	NS	0.74***
Denitrif. potential	0.66**	0.57*	0.59**	NS	NS	NS	NS
N_2O reduction	0.65**	NS	NS	NS	0.48*	NS	NS
DEN/RED ratio	NS	NS	NS	NS	−0.67**	NS	NS
Depth 10–20 cm							
Net N mineralization	0.73***	0.82***	0.80***	0.76***	NS	NS	NS
Net nitrification	0.80***0.82***	0.76***	0.76***	NS	NS	NS	
SIR	0.65**	0.88***	0.78***	0.83***	NS	NS	NS
Basal respiration	NS	NS	NS	0.60**	0.60**	NS	NS
Denitrif. potential	0.82***	0.65**	0.71***	0.88***	NS	NS	NS
N_2O reduction	NS	NS	NS	NS	NS	NS	NS
DEN/RED ratio	NS	NS	NS	NS	NS	NS	NS
Depth 20–30 cm							
Net N mineralization	0.72***	NS	0.55*	NS	NS	0.48*	NS
Net nitrification	0.89***	NS	0.59*	NS	NS	0.53*	NS
SIR	0.67**	NS	0.56*	NS	NS	0.53*	0.50*
Basal respiration	NS	NS	NS	NS	NS	NS	0.68**
Denitrif. potential	0.87***	NS	0.57*	NS	NS	0.57*	NS
N_2O reduction	0.53*	0.61**	NS	NS	NS	NS	NS
DEN/RED ratio	0.66**	NS	0.56*	NS	NS	NS	0.58*

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS, not significant.

lack of correlation between net N mineralization and net nitrification ($r = -0.37$, not significant). In Siberia, in contrast, net N mineralization and net nitrification were strongly correlated and they, together with denitrification potential, were not correlated to respiration activities. In Siberia, the lack of such correlation may be explained by lower respiration rates and relatively higher availability of inorganic N. In Brazil, both basal respiration and SIR were correlated only to NO_3^-, whereas in Siberia, NH_4^+ was the more favored N source for heterotrophic microorganisms.

Potential denitrification was higher in Siberian forests and grassland than in the Amazonian forest soils (Figure 4). The difference was, however, not large. In contrast, net N mineralization was higher in the tropics than in Siberian forests and grassland. Net nitrification was higher in tropical than in Siberian forests but lower than in Siberian grassland. In the tropical forest soils, net nitrification was five times lower than net N mineralization, whereas in Siberia, net nitrification was only 25% lower than net N mineralization under forests and was equal in grassland. This indicates that net nitrification contributes far less to total N mineralization in the tropics, possibly owing to higher NO_3^- immobilization in tropics than in Siberia due to microbial preference of NO_3^- rather than NH_4^+ and to higher C mineralization rates in the tropics. The higher contribution of net NO_3^- production to N mineralization in Siberian forests and grassland may also explain the higher denitrification rate in this soil. Potential denitrification in Brazil could be lower if NO_3^- immobilization was a substantial sink for the applied NO_3^-.

Discussion

The Siberian afforestation experiment shows that tree species affect soil microbiological processes. The effects of species and conversion of grassland on SIR

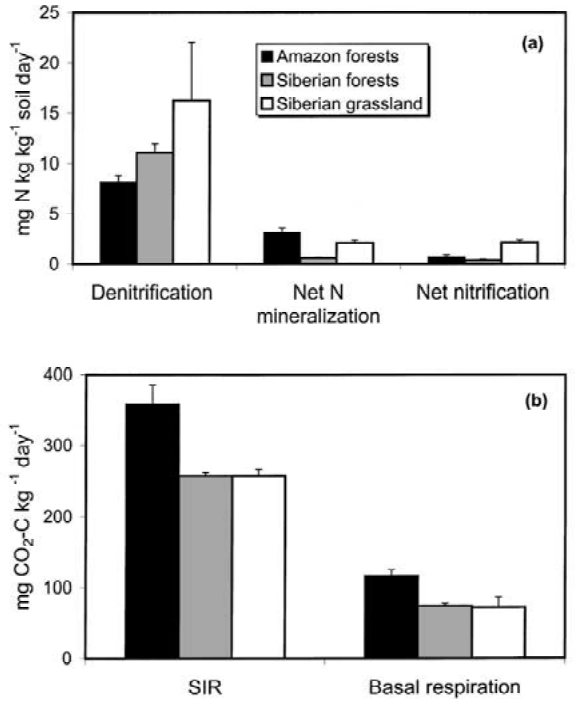

Figure 4. Microbial activities of N transformations (a) and C-respirations (b), measured with the same methods in soil samples (0–10 cm) from artificial afforestation experiments in the Central Amazon, Brazil (EMBRAPA agro-forestry plantations, n=21), in Siberia (n=18) and in Siberian grassland (n=3). Units for N cycling processes are the same as presented in Figures 1–3 (for corresponding processes).

and basal respiration were significant, but the differences among species as well as between grassland and forest were not as large as for N transformations. Our results are in accord with field measurements of CO_2 evolution (Paré, cited by Côté et al., 2000), which indicated no difference between coniferous and deciduous stands. Mikola (1985) studied 30- to 50-year-old spruce and birch forests growing on originally approximately similar sites and found no difference in soil respiration. Also no differences in CO_2 flux were found between an agricultural and forest soil, while much larger N_2O was produced in the agricultural field (van Bachove et al., 2000). These results support our conclusion about stronger effects of species and conversion on N than on C transformation processes. However, this contrasts to other study where tree seedlings of three species (pine, spruce and birch) were growing under greenhouse conditions for 4 months and no differences between species were found on net N mineralization, nitrification and denitrification, while species did affect C mineralization (Priha et al., 1999b).

Overall, the six tree species studied can be separated into three groups according to their effects on net N mineralization, net nitrification and denitrification. All these activities were highest under Arolla pine and larch, intermediate under deciduous aspen and birch, and lowest beneath spruce and Scots pine. These groups correspond to those based on the chemical properties (pH, C, C/N): soils under Arolla pine and larch had the highest pH and C content and intermediate C/N ratios, birch and aspen have intermediate values of pH and C and the lowest C/N, and spruce and Scots pine have the lowest pH and C and the highest C/N (Menyailo et al., 2002a). Thus, tree species caused significant changes in soil N transformations by modifying the amount and quality of soil organic matter and soil pH. Many authors reported that C and N cycling are mostly controlled by organic matter quality (C/N ratio, lignin/N, etc.) and quantity (Aber and Melillo, 1991; Aber et al., 1990; Attiwill and Adams, 1993; Vitousek et al., 1982). In our sites, most measured activities were strongly correlated to soil C and N contents, providing evidence that the amount rather than the quality of SOM was the main regulating factor in these soils.

SIR and basal respiration were differently affected by conversion; if a given tree species caused basal respiration to increase, it caused SIR to decrease. Possibly, SIR was controlled by NH_4^+ availability and related therefore to N mineralization, which was negatively affected by grassland conversion. Basal respiration was likely less limited by NH_4^+ and was thus independent of N mineralization and controlled mostly by easily available organic C (DOC), which was higher in concentration under forests than in grassland (Menyailo et al., 2001a), perhaps explaining why basal respiration was higher in forested soils. It seems that in the Siberian artificial afforestation experiment, C mineralization is not limited by N. This contrasts with the Brazilian forest soils, where SIR as well as basal respiration were closely correlated to NO_3^-. It is known that tropical forest soils have high rates of C mineralization and nitrification (Vitousek and Sanford, 1986), whereas N supply is usually more limited in most temperate as well as boreal forest ecosystems. The possible explanation of our results would be that net nitrification in Brazil was only a small part of gross nitrification due to high rate of NO_3^- immobilization by heterotrophic microorganisms. This would also explain the small contribution of

net nitrification to net N mineralization (around 25%) and the lack of inter-relationships between these processes in Brazil. In contrast, in Siberian soils, most of the net N mineralized was nitrified (up to 100%) and these processes were closely inter-related. Finzi et al. (1998) also found a strong correlation between net nitrification and net N mineralization in temperate forest soils in the USA. In Siberia, the higher soil C content does not stimulate microorganisms to consume all of inorganic N available and produced by mineralization and nitrification because only a small part of the total C is available to microorganisms. Thus, higher inputs to (and higher decomposability of) organic C in Brazilian soils may enhance microbial immobilization of NO_3^-, while the smaller input and lesser decomposability of organic matter in Siberia resulted in a discrepancy between C and N mineralization. It was suggested that soil microorganisms prefer NH_4^+ rather than NO_3^- as their N source (Walley et al., 1996). Stark and Hart (1997) contested this opinion, reporting that soil microorganisms preferred NO_3^- for immobilization in undisturbed coniferous forests of the USA. We demonstrated that microorganisms in different climatic zones differ in preference for forms of inorganic N: NO_3^- was a preferred form in the tropical forest soils, NH_4^+ in the temperate soils.

N_2O reduction was not correlated to the other microbial C and N transformations studied. Probably, some other factors, not measured in this study, could explain the variation in N_2O-reductase activity. It is important that DEN/RED ratio was correlated in upper horizons only with N_2O-reductase activity. This is in agreement with the results of Henault et al. (1998) who reported that actual emission of N_2O from the agricultural field in France was mostly determined by N_2O-reductase activity. As DEN/RED ratio increased with soil depth, the higher O_2 concentration in soil air in the upper soil layers could explain the relatively lower N_2O-reductase activity, because N_2O-reductase is the enzyme most sensitive to O_2 in the denitrification chain (Dendooven and Anderson, 1995). Another possible regulating factor may be the soil C/N ratio, which was the only soil chemical variable correlated to N_2O-reductase and therefore to DEN/RED ratio in the upper soil. In the study of Menyailo and Huwe (1999), C/N was the main factor controlling net N_2O production in inter-space of forest stands at 0–10 cm depth. We demonstrated in this work that soil C/N predicts the N_2O/N ratio only at the upper soil layers but not in the deeper depths. Thus, the lower DEN/RED ratio under deciduous species may be explained, in part, by the lower C/N and, probably, by more anaerobic conditions.

The DEN/RED ratios may indicate under which species, under the same environmental conditions, higher net rates of N_2O production may be observed. We found higher DEN/RED ratio under deciduous than under conifers and propose that, in the field, higher N_2O fluxes should be observed under birch and aspen. This is in accord with the findings of Bütterbach-Bahl et al. (1997); they reported higher field N_2O fluxes in deciduous than under coniferous temperate forests in Germany. Pastor and Post (1988) found that changes in temperature and precipitation resulting from an increase of atmospheric CO_2 concentrations caused a northward migration of the hardwood-conifer forest border in North America. Such migration of the hardwood forests is likely to also occur in Russian Siberia. Based on our results, replacing conifers by deciduous species would approximately double the ratio of N_2O/N_2 as end products of denitrification. Potential denitrification in Siberia was even higher than in the tropical forest soils, the main terrestrial N_2O source (Matson et al., 1990), indicating that the microbial community in temperate forest zone can potentially produce higher amounts of N_2O. By changing environmental conditions due to global changes, atmospheric N deposition or forest fires, which increase soil inorganic N, temperate forests will likely play an increasingly important role in the global N_2O budget.

We found much larger vertical differentiation for most microbiological activities measured in grassland than in forest. One possible explanation would be that grassland, in contrast to forest soils, were not mechanically cultivated (mixed) before afforestation procedures and, thus, the vertical profile in grassland is more naturally differentiated. However, soil chemical properties studied in these soils (Menyailo et al., 2002a) do not support this explanation, as no significant effect of soil depth (no vertical differentiation) was found under grassland, while under tree species, some chemical variables significantly declined with depth. This was because grassland deposits organic matter not only on the soil surface, as forests, but to all depths, making the soil profile more homogeneous. Some other environmental factors, not measured in this study, were likely affecting microbiological activities at different depths. One possible factor could be a large difference in soil organic matter quality and its decomposability in the upper and underlying horizons in grassland. The second possible reason may be

that in grassland there is larger vertical differentiation in O_2 availability through soil profile. However, we have no direct data to support these suppositions. One indirect fact that supports the second idea is that in grassland also the lowest DEN/RED ratio was found in the two deepest layers. If the low DEN/RED ratio was caused by low O_2 content (high level of anaerobiosis), O_2 deficiency in the deeper depth may be responsible for a rapid decline in aerobic microbial processes.

It is widely believed that deciduous species increase nutrient cycling and microbial activities compared to conifers. This is based on the studies of only a few species (spruce, pine and birch). Birch often increases soil microbial biomass C and N, C mineralization, pH, base saturation compared to spruce and pine (Bradley and Fyles, 1995; Mikola, 1985; Priha and Smolander, 1997). We examined more species in this study and found that deciduous aspen and birch have lower microbiological activities than beneath coniferous Arolla pine and larch, probably because these conifers increased soil pH and C compared to aspen and birch. Thus the variation in the litter quality and in the effect on soil is larger among coniferous species and covers the range of such effects of deciduous trees.

We have shown strong effects of grassland conversion and tree species on soil microbiological processes. The species effects are much larger than was previously reported (Priha et al., 1999a,b). Basal respiration was higher in the forests than in grassland, but all other microbial activities were lower. Both species and depth had larger effects on N transformations than on C transformations. Denitrification potential varied under different species in the same way as did net N mineralization and net nitrification, while N_2O reduction rate did not. This caused a large discrepancy between N_2O production and reduction rates under deciduous species. Thus, we predict that deciduous species will produce more N_2O in the field than conifers and that Siberian forests will produce more N_2O if global climate change results in changes in species composition. In the Siberian artificial afforestation experiment, C mineralization did not appear to be limited by N. This contrasts with the Brazilian forest soils, where SIR and basal respiration were closely correlated to NO_3^- availability. We also found correlative evidence that microorganisms in different climatic zones have a different preferences for inorganic N forms: NO_3^- was a preferred form in the tropical forest soils, whereas NH_4^+ was preferred in temperate forest soils.

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