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Effect of available P and phenolics on mineral N release in acidified spruce forest: connection with lignin-degrading enzymes and bacterial and fungal communities

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Abstract We conducted over four months a short-term laboratory incubation experiment to find the best prediction parameters (i.e. initial chemical characteristics) to explain differences in microbial respiration rates and mineral N (DIN) release in different litter in an acidified spruce forest. In addition, we wanted to find the link between the activity of key extracellular ligninolytic enzymes, phenoloxidases (PhOx) and peroxidases (Perox), microbial respiration and composition of fungal and bacterial communities. Samples of spruce needles (*Picea abies*) and litter of four dominant understorey vegetation; lady fern *(Athyrium alpestre*), blueberry (*Vaccinium myrtillus*), reedgrass (*Calamagrostis villosa*) and hair grass (*Avenella flexuosa*), were collected in 2005, 2006 and 2007 from six sites located in watersheds of two glacial lakes (Plesne Lake and Certovo Lake) in the Bohemian Forest, Czech Republic. Litter samples were incubated at 0 and 10 °C in laboratory controlled conditions for 90 days. Activities of PhOx and PerOx, and C mineralization rate were measured regularly each 14 days. Litter quality characteristics and endophytic microbial community structure, based on 16SrDNA-DGGE fingerprint of bacteria and ITS-DGGE of fungi, were determined at the beginning and end of litter incubation. Our results showed a close correlation of phenolics/P_{OX} with DIN release ($r > 0.74$, $p < 0.001$). Using multivariate analyses, P_{OX} seems to play an important role in the change of litter fungal and bacterial community composition. At 0 °C the fungal and bacterial communities of reedgrass and blueberry litter changed in relation to P_{OX} and Perox activity, while at 10 °C the fungal communities after the incubation were additionally affected by the phenolics/N_{TOT} and phenolics/P_{TOT} ratios.

Keywords Available phosphorus · DGGE · Extracellular enzyme activities · Litter decomposition · N leaching · Phenolics

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Introduction

Forest plant litter decomposition is an important process in C, N and P cycles and soil formation. This process is controlled by abiotic factors, such as temperature, moisture, and chemical composition of the litter, and microbial communities (Aber et al. 1990; Couteaux et al. 1995; Fassnacht and Gower 1999; Park and Matzner 2003; Pregitzer et al. 2004).

Chemical composition of litter influences the composition of fungal and bacterial colonizers, and their enzymatic apparatus (Cox et al. 2001; Lucas et al. 2007). The change in enzymatic activity influences the quantity of nutrients, which are released during litter decomposition, and vice versa, the release of different nutrients influences the activity of specific enzymes (Sinsabaugh et al. 2002, 2005).

Many studies have focused on trying to predict plant litter decomposition from initial litter chemical composition during short-term incubation experiments (Aber et al. 1990; Aber et al. 1998). The carbon/nitrogen (C/N) ratio was widely used for this prediction (Muller et al. 1988; Carlyle et al. 1990). However, the C/N ratio was found to be a poor predictor when trying to estimate the amount of released mineral N (DIN, NH_4^+ and NO_3^-) from highly lignified litter (Prescott 2005). A stronger correlation was found between lignin and the lignin/N ratio and mineral N release (Berg 1986; Berg and Ekbohm 1991).

Some recent studies have demonstrated that, when phenolics are included into the statistical analyses, C mineralization and DIN release rates are more closely correlated with phenolics content and phenolics/N_{TOT} ratio, than they are with C_{TOT}/N_{TOT} ratio, lignin concentration, or lignin/N_{TOT} ratio (Fox et al. 1990; Northup et al. 1995, 1998). One of the first studies focusing on the influence of phenolics was done by Dyck et al. (1987). The authors attributed most of the variation in DIN release from *Pinus radiata* litter to differences in phenolics content. These findings showed that a high content of phenolics and low N availability can be limiting factors of DIN release from decomposing litter in acid forest soils (Kopáček et al. 2002a, b). However, acid forest soils can also be poor in P and it is very likely that low bioavailability of P is another limiting factor of litter decomposition which can affect DIN release. Even if P bioavailability and the amount of phenolics in litter could be the "driving force" in litter decomposition and DIN release in acidified spruce forests, there are still only a few studies on this topic (Šantrůčková et al. 2004, 2006).

Phenolics are the structural unit of lignin and are released during depolymerization by the activity of extracellular lignin-degrading enzymes. Fungal phenoloxidases and peroxidases are the key enzymes involved in this process (Weintraub et al. 2007; Fackler et al. 2006; Söderström et al. 1983; Fog 1988; Kirk and Farrell 1987; Eriksson et al. 1990; Hammel 1997; Waldrop et al. 2004; Sinsabaugh et al. 2002). Depolymerization of lignin is a crucial process because it increases the accessibility of C, N, and P for both fungal and bacterial decomposing microorganisms. Therefore, there should be a tight link between ligninolytic activity, C mineralization and fungal community composition. The depolymerization process also creates different phenolic products with high reactive hydroxyl groups, which can then play various roles in the soil. Released phenolics can react with each other forming humic substances, they can precipitate proteins, reducing the leaching of DIN (Northup et al. 1995, 1998), or predominantly precipitate toxic forms of Al^{3+} thereby increasing P bioavailability (Northup et al. 1998; Tomlinson 2003).

Temperature affects the rate of litter decomposition; its effect is closely interconnected with initial chemical composition of the litter (Meentemeyer 1978). In nutrient rich and phenolic poor litter, temperature sensitivity of litter decomposition is higher than in nutrient poor and lignin rich litter (Domisch et al. 2006). The response to temperature is stronger especially in the early stage of litter decomposition than in later phases (Jansson and Berg 1985). In addition, differential temperature sensitivity of decomposition processes can lead to an imbalance in C and N mineralization.

High DIN leaching is one of the major problems in acidified spruce forests in Central Europe. These forests have undergone great changes during the past few decades, with acid anthropogenic N depositions during the last century and bark beetle infestations being the main disturbances. The high acidity of soils led to the reduction of soil acid neutralizing capacity, increased mobility of toxic Al^{3+} ions and leaching of essential plant cations (Ca^{2+}, Mg^{2+} and K^+) and DIN from these ecosystems (Dauer et al. 2007; Tomlinson 2003; Pawłowski 1997; Kopáček et al. 2002a, b, 2006a, b; Šantrůčková et al. 2006). Soil acidity and Al mobility have been related to the physiological stress suffered by spruce trees since the 1960 s (Šantrůčková et al. 2007), there is currently a high degree of tree disturbance and defoliation. In open patches, understorey vegetation dominated by grasses, blueberries and ferns develop rapidly. Their effect on DIN leaching is still unclear (Svoboda et al. 2006; Šantrůčková et al. 2006).

The purpose of this study was to:

1. evaluate the effect of P bioavailability on litter mineralization and DIN release.
2. find the connection between phenolics/P_{OX} ratio and litter mineralization and N release.
3. determine C mineralization, ligninolytic activity and N release at close to zero temperatures.
4. determine whether there are links between ligninolytic activity, C mineralization and dynamics of litter microbial colonizers during incubation.

Spruce needles (*Picea abies*) and the litter of four dominant understorey species (*Athyrium alpestre* (lady fern), *Vaccinium myrtillus* (blueberry), *Calamagrostis villosa* (reedgrass), and *Avenella flexulosa* (hair-grass) were incubated at 0 (average winter temperature) and 10 °C (average summer temperature) for four months. The three representative litters with different amounts of phenolics (spruce needles, blueberry and reedgrass) were analyzed for lignin-degrading enzyme (PhOx and Perox) activities and composition of fungal and bacterial communities.

Materials and methods

Study site description

Litter was collected from six different sampling sites in the watersheds of two glacial lakes: Plešné and Čertovo, which are situated at 48°47′ and 49°10′N, and 13°52′, and 13°11′E, respectively, at altitudes from 1030 to 1090 m a. s. l. Watershed of Plešné Lake is covered with a 160 year-old Norway spruce (*Picea abies*) forest with small areas of ash. The bedrock is composed of granites. Čertovo Lake is covered with 90–150 year-old Norway spruce forests, with sparse white fir (*Abies concolor*) and European beech (*Fagus sylvatica*); the bedrock is predominantly composed of mica-schist (muscovite gneiss) with quartzite intrusions. The understorey of both watersheds is dominated by hair grass (*Avenella flexuosa*), reedgrass (*Calamagrostis villosa*), blueberry (*Vaccinium myrtillus*), and lady fern (*Athyrium alpestre*, Svoboda et al. 2006). Soil types are mostly cambisols, podzols and litosols on steep slopes in both watersheds. Soil temperatures measured from 2001 to 2002 were close to 0 °C in winter and 10–12 °C in the summer months. Basic physico-chemical and biochemical properties of the soils are described by Kopáček et al. (2002a, b) and Veselý (1994).

Litter collection and experimental set-up

Senescent leaves from four dominant understorey species (hair grass, reedgrass, blueberry and lady fern) and spruce needles were collected in October 2005, 2006 and 2007. Mean values of initial litter quality from the three sampling years are presented in Table 1. Fresh litter was stored in a cold room for a maximum of 24 hours. Then litter moisture was adjusted to 60% and litter was weighed (20 g) into 1,000 ml flasks in three replicates. Samples were incubated in closed flasks at 0 and 10 °C for four months; moisture was adjusted every 14 days. All results further reported in this paper are expressed on a dry weight basis (105 °C) and mean values from three replicates were used for statistical evaluations. C mineralization was determined by measuring the respiration rate, and decomposition by measuring water extractable compounds (dissolved organic C (DOC), dissolved organic N (DON) and dissolved mineral N, DIN (sum of NH_4^+ and NO_3^-) and oxalate extractable P (P_{OX}) after incubation. Oxalate extractable P (P_{OX}) is supposed to characterize biological bioavailability of P in soil (Koopmans et al. 2004; Pote et al. 1996; van der Zee et al. 1987; Schwertmann 1964). Activity of ligninolytic enzymes and composition of the microbial communities were determined on spruce needles, reedgrass and blueberry in 2007. Respired CO_2 was trapped into 1 M NaOH placed in a beaker on the surface of the litter and measurements were performed each 14 days.

Litter quality measurements

C_{TOT}, N_{TOT}, P_{OX} and phenolics were analyzed in air-dried, finely ground litter. C_{TOT} and N_{TOT} were measured using an elemental analyzer (NC Thermo-Quest, Germany). P_{OX} was determined by extraction of 0.5 g of litter with 50 ml of acid ammonium oxalate solution (0.2 M $H_2C_2O_4$ + 0.2 M $(NH_4)_2C_2O_4$ at pH 3) according to Cappo et al. (1987), but with a three-step instead of continuous extraction (Kopácek et al. 2002a). Dissolved organic carbon (DOC) and dissolved nitrogen (DN) were extracted step by step in both cold water (CW) and hot water

Table 1 Selected initial chemical properties of the litter of spruce needles and leaves of four dominant species of understorey vegetation

Litter	Initial chemical characteristics							
	C_{TOT} (mmol g^{-1})	C_{TOT}/N_{TOT} (molar)	C_{TOT}/P_{TOT} (molar)	P_{OX} (μmol g^{-1})	Phenolics (mg g^{-1})	Phenolics/N_{TOT} (w/w)	Phenolics/P_{TOT} (w/w)	Phenolics/P_{OX} (w/w)
Spruce needles	40.4^{B} (1.8)	49.2^{B} (9.9)	1179.2^{AB} (18.8)	1.3^{A} (0.3)	178.6^{D} (31.8)	16.0^{C} (4.3)	157.9^{B} (27.1)	9715.8^{C} (5352.1)
Lady fern	38.5^{AB} (1.3)	38.3^{AB} (12.1)	713.7^{A} (122.4)	9.5^{C} (0.31)	74.1^{BC} (34.9)	5.3^{AB} (3.8)	49.8^{A} (28.3)	244.7^{AB} (109.1)
Blueberry	39.0^{AB} (1.3)	47.8^{AB} (7.4)	1617.3^{BC} (281.7)	7.9^{BC} (1.2)	116.0^{C} (21.4)	10.1^{B} (1.9)	141.5^{B} (48.6)	477.8^{B} (124.1)
Reedgrass	39.3^{AB} (1.8)	44.2^{AB} (9.7)	2149.7^{C} (896.2)	5.1^{B} (2.9)	27.9^{A} (6.0)	2.7^{A} (0.8)	47.4^{A} (30.7)	236.8^{AB} (165.8)
Hair grass	37.1^{A} (0.4)	29.3^{A} (4.2)	784.2^{A} (95.2)	7.4^{BC} (1.8)	32.8^{AB} (5.9)	1.9^{A} (0.2)	20.3^{A} (3.2)	143.6^{A} (18.1)

The data represent averages and standard deviations shown in parenthesis ($n = 30$)

Different letters show significant differences between values within columns ($p < 0.05$)

(HW): water:litter, 10:1, v/w, 30 min at 20 °C and 16 h at 80 °C, respectively . DOC and DN in the cold and hot water extracts were determined on a TOC/TN analyzer (SKALAR FORMACS HT), and NH_4^+ and NO_3^- using a flow injection analyzer (Foss Tecator 5042, Sweden). Total phenolics were determined using the method of Bärlocher and Graça (2005). Dissolved organic nitrogen (DON) was calculated by subtracting the sum of NH_4^+ and NO_3^- from DN.

CO_2 trapped in 1 M NaOH was determined by volumetric titration. Values were used for calculation of cumulative respiration (mg C-CO_2 per g litter).

Analysis of Fe and Al in litter samples was done in accredited laboratory of Czech Geological Survey. In general 1 g of sample was carefully incinerated at 550 °C, than a mixture of HF, $HClO_4$, and H_3BO_3 was added for disruption of litter samples, and finally extracted by HCl. The resulting solution loaded to the FAAS instrument (Perkin–Elmer 3100). Acetylene-air and acetylene-N_2O for Fe and Al was used, respectively. Detection limit for Fe and Al is 0.01 mg.kg^{-1}. Oxalate extractable Al (Al_{OX}) and Fe (Fe_{OX}) were calculated by equations based on a close correlation between Al_{TOT}/Fe_{TOT} and Al_{OX}/Fe_{OX} (Kaňa, unpublished data). 289 litter samples from Plešné and Čertovo Lake watershed were analyzed for Al_{TOT}, AL_{OX}, Fe_{TOT}, Fe_{OX} amount and correlated (Eq. 1):

$$\begin{aligned} Al_{OX} &= 0.1447 \cdot Al_{TOT} + 0.6773,\ r = 0.61,\ p \leq 0.01 \\ Fe_{OX} &= 0.3409 \cdot Fe_{TOT} + 0.1357,\ r = 0.83,\ p \leq 0.001 \end{aligned} \quad (1)$$

The sorption data were fitted to the linear form of the Langmuir equation (also called Lineweaver–Burk regression) and the sorption maximum (X_{MAX}) was calculated for each litter type (Yuan and Lavkulich 1994). Calculations are based on a close correlation between the sum of $Al_{OX} + Fe_{OX}$ and X_{MAX} (Kaňa and Kopáček 2006).

Enzyme assays

Peroxidase (Perox) and phenol oxidase (PhOx) activities were determined in three representative litters with different amounts of phenolics (spruce needles, blueberry and reedgrass) during the 2007 experiment. Activity was measured every 14 days using the methodology of Hendel et al. (2005). In general, three

replicate microtubes were used for each assay; three microtubes were used as negative substrate controls and another three microtubes served as negative sample controls. The assay tubes received 1 ml aliquots of sample suspension and 1 ml of 5 mM L-DOPA substrate in 50 mM acetate buffer. The negative sample control wells contained 1 ml aliquots of sample suspension and 1 ml of acetate buffer. The negative substrate control wells received 1 ml aliquots of acetate buffer and 1 ml substrate. For Perox assays, each well also received 100 µl of H_2O_2 (0.3%). The microtubes were covered with aluminium foil and placed on an orbital shaker for 1 h at 20 °C. Activity was measured spectrophotometrically at 460 nm. Enzyme activity was expressed in International Enzyme Units (IEU). One unit of enzyme activity was defined as the amount of enzyme forming 1 µmol of reaction product per hour per g of organic matter (litter). Overall ligninolytic enzyme activities were calculated as the sum of the average activities of PhOx and Perox during the incubation period.

Extraction of DNA from litter samples and PCR amplification

Composition of the microbial community (bacterial community by studying 16S rDNA and fungal community by studying the ITS regions of their genomic DNA) was determined at the beginning and then after each month of incubation during the 2007 experiment.

A portion of each litter sample (0.15 g) was taken for DNA extraction. Litters were homogenised in liquid nitrogen using a sterile mortar and pestle. Homogenised samples were transported into 1.5 ml Eppendorf microtubes and kept frozen (−70 °C) until the DNA isolation took place. For the isolation of genomic DNA from litter and soil, the DNasy® Plant Mini Kit (Qiagen, USA) was used according to manufacture instructions with some modifications: Bead-Beating was performed after adding the extraction buffer using a Mini Bead-Beater (BioSpec Products, Inc.) for efficient disruption of fungal spores and G^+ bacterial cell membranes. Isolated DNA was stored in 1.5 ml Eppendorf microtubes in a freezer (−20 °C).

Fungal communities were analysed using ITS1F and ITS2 primer pairs to amplify the 300 bp fragment of the fungal ITS rDNA by PCR. The primer ITS1F (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) is higher fungi ITS specific, while ITS2 (5′-GCT GCG TTC TTC ATC GAT GC-3′) is a universal primer amplifying the ITS region from Eucaryotes, including both Ascomycetes and Basidiomycetes (Heuer and Smalla 1997). A 40 bp GC-clamp (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3′) was attached to the 5′ end of the ITS2 primer to avoid a complete separation of DNA strands during denaturing electrophoresis. The reaction medium consisted of 5 µl of PCR buffer (Sigma, 100 mM Tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 1 µl of dNTP (10 mM), 1 µl of each primer (20 µM), 1 µl of GC-rich solution (Sigma), 2 µl of Bovine Serum Albumin (20 mg ml^{-1}), 0.5 µl of Taq-polymerase (5 units/µl, Sigma) and 2 µl of genomic DNA brought to a final volume of 50 µl. The amplification programme consisted of an initial cycle of denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C for 1 min 15 s. The amplification concluded with a final elongation step at 72 °C for 8 min.

Bacterial communities were analysed using the eubacterial primer set, 968f (5′- AAC GCG AAG AAC CTT AC -3′) and 1401r (5′- CGG TGT GTA CAA GAC CC -3′), to amplify by PCR a 475-bp fragment of 16S rDNA from the same DNA extract as above (Heuer and Smalla 1997). A 40-bp GC clamp was attached to the 5′ end of the 968f primer. A 50 µl PCR mixture containing 5 µl of PCR buffer (Sigma, 100 mM Tris–HCl, pH 8.3, 500 mM KCl), 1 µl of dNTP (10 mM), 1 µl of each primer (100 µM), 0.5 µl of Taq-polymerase (Fastart, Roche Diagnostic), 2 µl of $MgCl_2$, 1 µl of GC-rich solution (Sigma), 2 µl of BSA (3%) and 2 µl of genomic DNA was used. The amplification regime consisted of an initial cycle of denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 56 °C for 30 s and extension at 72 °C for 1 min, concluding with an elongation step at 72 °C for 5 min.

Size of PCR amplicons was verified using 1.5% (w/v) agarose and electrophoresed with specific markers.

DGGE analyses

For DGGE analyses, 10 µl of PCR products (approximately 150 ng) were loaded into a polyacrylamide

gel (8%) with a 40% to 70% denaturing gradient for bacteria and 38 to 50% for fungi and electrophoresed for 16 h at 60 V and 60 °C using the DCode™ Universal Mutation Detection System (BioRad, USA). The 100% denaturing stock solution consisted of 105 g urea; 100 ml deionised formamide/250 ml. Gels were stained with SYBR®Green (1:10000) and visualised under UV light.

Bands in acrylamide gels were identified and analysed using the Biorad Quantity One software v. 4.5.2. The position tolerance was set at 1% and background subtraction was applied.

Within-gel comparisons, taking into account both the presence/absence, and intensity (relative abundance of species) of bands, were performed using multivariate analyses.

Statistical evaluation

Basic statistical analyses were performed using Statistica 8.0 (StatSoft). One-way ANOVA (litter as independent variables), followed by the Tukey HSD test wase used for testing differences between initial litter quality and cumulative microbial respiration of the four understorey species and spruce needles. Linear regression was used to test the correlation of NO_3^-, NH_4^+, DOC, DN, DON, DIN, P_{OX} and cumulative mineralization rate on initial litter quality characteristics. Correlation coefficients (r) with p values were evaluated.

A unimodal type of constrained ordination, canonical analysis (CCA), was used to evaluate the relation between the litter chemical properties and extracellular enzymatic activities (explanatory variables, at the beginning and end of incubation) vs. initial and final composition of bacterial and fungal communities (response variables). The contribution of each explanatory variable was tested using forward selection and the Monte-Carlo permutation test within the CCA framework ($p < 0.05$). Only those explanatory variables that showed significant marginal and conditional effects on the microbial communities were included in the diagrams. The results were summarized using biplot diagrams. In each diagram, the percentage of explained variation between samples is shown on first and second canonical axis. The distance between the initial and final bacterial and fungal communities illustrates the magnitude of differences between the litter samples. The relative length and position of arrows show the extent and direction of response of bacterial and fungal community structures to the litter chemical properties and extracellular enzymatic activities. The analysis was performed using CANOCO ver. 4.5 (ter Braak and Šmilauer 1998).

Results

The role of phenolics, N and P content on litter decomposition and nutrient release

Initial litter chemical characteristics differed significantly in the amounts of phenolics, and also in DOC, DON and P_{OX}, and the phenolics/N_{TOT}, phenolics/P_{TOT} ratios. The largest differences were between spruce needles and hair grass litter (Table 1).

There was exponential relationship between the initial amount of phenolics and P_{OX} (Fig. 1). When the amount of phenolics dropped below 0.05 mmol g^{-1}, the amount of P_{OX} dramatically increased. The amount of N_{TOT} and P_{TOT} did not correlate with the amount of phenolics (data not shown).

Initial phenolics/P_{TOT}, phenolics/P_{OX} and phenolics/N_{TOT} ratios were negatively related to NO_3^- amount and positively correlated to the DOC/DN and DON/DIN ratios in water extracts after incubation (Table 2). The amount of NH_4^+ was negatively affected only by initial phenolics/P_{TOT} and phenolics content. The phenolics/P_{TOT} and phenolics/P_{OX} showed in most cases higher correlation coefficients than the phenolics/N_{TOT} ratio (Table 2).

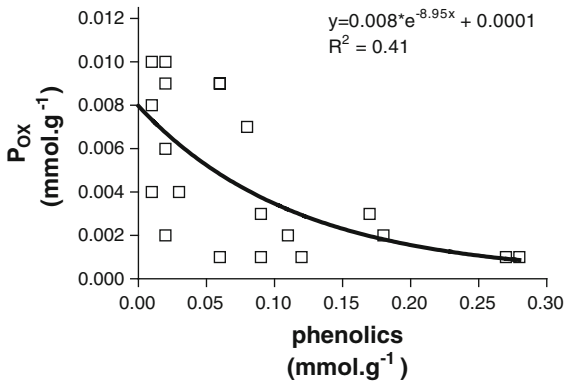


Fig. 1 Relationship between initial amount of phenolics and bioavailable P (P_{OX}). Each point represents a mean value from three replicates

Table 2 Correlation coefficients (r) of selected initial litter quality characteristics (phenolics, phenolics/N_{TOT}, phenolics/P_{TOT}, phenolics/P_{OX}, P_{OX}) versus the amount of NO_3^-, NH_4^+, DOC/DN, DON/DIN in water extracts and P_{OX} after 4 months of litter incubation at 0 and 10 °C (the first column: the sum of HW and CW extracts; the second column: CW extracts)

Initial litter quality	Versus	0 °C		10 °C	
		r (HW + CW)	r (CW)	r (HW + CW)	r (CW)
Phenolics/P_{TOT}	NO_3^-	-0.43^{*}	-0.41^{*}	-0.62^{***}	-0.70^{***}
	NH_4^+	-0.45^{*}	-0.46^{*}	-0.55^{**}	-0.50^{*}
	DOC/DN	0.79^{***}	0.87^{***}	0.68^{***}	0.60^{**}
	DON/DIN	0.73^{***}	0.67^{***}	0.45^{*}	0.51^{*}
	P_{OX}	-0.63^{**}		-0.58^{**}	
Phenolics/P_{OX}	NO_3^-	-0.61^{***}	-0.50^{*}	-0.75^{***}	-0.75^{***}
	NH_4^+	ns	ns	0.40	ns
	DOC/DN	0.67^{***}	0.76^{***}	0.68^{***}	0.42^{*}
	DON/DIN	0.75^{***}	0.77^{***}	0.89^{***}	0.74^{***}
	P_{OX}	-0.61^{**}		-0.60^{**}	
P_{OX}	NO_3^-	0.81^{***}	0.66^{***}	0.62^{***}	0.65^{***}
	NH_4^+	ns	ns	ns	ns
	DOC/DN	ns	ns	ns	ns
	DON/DIN	ns	ns	ns	ns
	P_{OX}	0.88^{***}		0.96^{***}	
Phenolics	NO_3^-	-0.48^{*}	-0.44^{*}	-0.65^{***}	-0.72^{***}
	NH_4^+	-0.40^{*}	-0.42^{*}	-0.52^{**}	-0.48^{*}
	DOC/DN	0.72^{***}	0.80^{***}	0.61^{***}	0.49^{*}
	DON/DIN	0.68^{***}	0.62^{***}	0.50^{*}	0.54^{**}
	P_{OX}	-0.61^{**}		-0.58^{**}	
Phenolics/N_{TOT}	NO_3^-	-0.48^{*}	-0.43^{*}	-0.63^{***}	-0.66^{***}
	NH_4^+	ns	ns	-0.49^{*}	-0.43^{*}
	DOC/DN	0.78^{***}	0.84^{***}	0.67^{***}	0.53^{**}
	DON/DIN	0.47^{*}	0.70^{***}	0.53^{**}	0.59^{**}
	P_{OX}	-0.58^{**}		-0.56^{*}	

ns not significant

Correlation coefficients are given and their significances are marked by asterisks as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Cumulative microbial respiration at the end of incubation was significantly highest in hair grass litter (Tukey HSD test, $p < 0.05$) at both incubation temperatures compared to the other litter (Fig. 2a, b), which then decreased in the order: blueberry > reedgrass > lady fern > spruce needles and reedgrass > lady fern > blueberry > spruce needles at 0 and 10 °C, respectively. However, the differences in cumulative respiration between these four litters were not statistically significant (Tukey HSD test, Fig. 2a). Cumulative microbial respiration at the end of incubation was higher at 10 °C than at 0 °C.

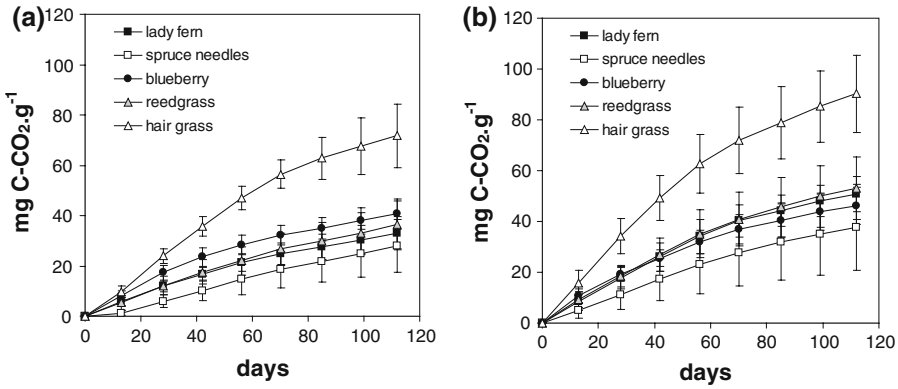


Fig. 2 Cumulative respiration of lady fern, spruce needles, blueberry, reedgrass and hair grass litter incubated at 0 (**a**) and 10 °C (**b**).Mean values from three independent experiments (2005, 2006, 2007) and standard deviation (*error bars*) are given

Between temperature differences were 56.6 ± 20.8% in lady fern litter, 49.0 ± 20.2% in reedgrass litter, 37.4 ± 18.1% in spruce needle litter, 27.2 ± 17.0% in hair grass litter and 24.4 ± 10.4% in blueberry litter (Fig. 2a, b).

Total cumulative respiration was negatively correlated with initial amount of phenolics, and the C_{TOT}/N_{TOT}, phenolics/P_{TOT}, phenolics/P_{OX} and phenolics/N_{TOT} ratios, and positively correlated with P_{OX} (Table 3). The correlations were slightly stronger at 10 °C than at 0 °C.

Relative P saturation was the lowest for spruce needles (6.7%) and the highest for hair grass litter (22.4%, Table 4) at the beginning of litter incubation. Relative P saturation of the five incubated litters after incubation ranged from 44 to 81% at both temperatures. Relative P saturation reached approximately 44% in spruce needle litter, while it was almost 81% in hair grass litter (Table 4).

Table 3 Correlation coefficients (r) of selected initial litter quality characteristics (C_{TOT}/N_{TOT}, phenolics, phenolics/N_{TOT}, phenolics/P_{TOT} and P_{OX}) versus cumulative respiration after the months of litter incubation at 0 and 10 °C

Initial litter quality	Versus cumulative respiration	
	r (0 °C)	r (10 °C)
C_{TOT}/N_{TOT}	-0.45^{*}	-0.41^{*}
phenolics/P_{TOT}	-0.57^{**}	-0.63^{***}
phenolics/P_{OX}	-0.48^{*}	-0.55^{**}
phenolics/N_{TOT}	-0.55^{**}	-0.59^{**}
P_{OX}	0.43^{*}	0.46^{*}
Phenolics	-0.57^{**}	-0.64^{***}

Correlation coefficients are given and their significances are marked by asterisks as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Final DOC, DN, DON, NO_3^-, NH_4^+ amounts and DOC/DN at 0 and 10 °C were closely correlated (Table 5). Temperature affected final DOC, DN and NO_3^- amounts only slightly. This was indicated by the slope of the linear correlation line being close to 1. Final NH_4^+ content in water extracts and DOC/DN were lower at 10 °C than at 0 °C, while DON was higher at 10 °C.

Perox and PhOx dynamics

The activity of PhOx had similar dynamics for spruce needles and blueberry litter at 0 and 10 °C (Fig. 3a). In reedgrass litter, the activity of PhOx was higher at 0 °C than at 10 °C; it peaked at the end of the first month at 12.8 μmol h^{-1} g^{-1} (Fig. 3a). The activity of Perox showed different dynamics than PhOx at both incubation temperatures (Fig. 3b). Perox in reedgrass litter was significantly higher than in the spruce needles and blueberry litter at both temperatures. It fluctuated during incubation at 0 °C and grew at 10 °C from the beginning until the end of the second month, when it peaked at 56.5 μmol h^{-1} g^{-1} (Fig. 3b).

Average activity of PhOx over the entire incubation period was highest in reedgrass litter at 0 °C and in spruce needles and reedgrass at 10 °C (Table 6). However, the differences between spruce needles and reedgrass were not significant (Tukey HSD test, Table 6). The highest average Perox activity and overall ligninolytic activity occurred in the microbial community on reedgrass litter at both temperatures (Table 6). The differences in PhOx, Perox and overall ligninolytic activities between 0 and 10 °C were not statistically significant for all litter.

Table 4 Litter total P sorption capacity ($P_{OX} + X_{MAX}$) and relative P saturation ($P_{OX}/X_{MAX} + P_{OX}$) observed in five major understorey litter before incubation (initial) and after the four month of incubation at 0 and 10 °C

Litter	Initial		After 4th month at 0 °C		After 4th month at 10 °C	
	$X_{MAX} + P_{OX}$ (μmol g^{-1})	$P_{OX}/X_{MAX} + P_{OX}$ (%)	$X_{MAX} + P_{OX}$ (μmol g^{-1})	$P_{OX}/X_{MAX} + P_{OX}$ (%)	$X_{MAX} + P_{OX}$ (μmol g^{-1})	$P_{OX}/X_{MAX} + P_{OX}$ (%)
Spruce needles	14.7	6.7	24.6	44.3	24.4	43.9
Lady fern	16.4	16.4	39.8	65.4	46.8	70.5
Blueberry	16.4	15.5	30.5	54.5	32.0	56.5
Reedgrass	16.2	15.1	44.5	69.3	39.5	65.3
Hair grass	17.7	22.4	73.7	81.4	65.8	79.1

Table 5 Effect of different incubation temperatures on nutrient release, and DOC/DN and DON/DIN ratios

0 °C versus 10 °C	CW		HW + CW	
	r	Slope	*r*	Slope
DOC	0.88	1.02	0.84	0.83
DN	0.89	0.86	0.91	0.92
DON	0.94	1.57	0.92	1.45
NO_3^-	0.77	1.29	0.75	1.15
NH_4^+	0.70	0.50	0.72	0.48
DOC/DN	0.84	0.52	0.87	0.64
DON/DIN	ns	–	ns	–

Correlation coefficients (*r*) and slopes between released nutrients at 0 and 10 °C are shown

ns not significant

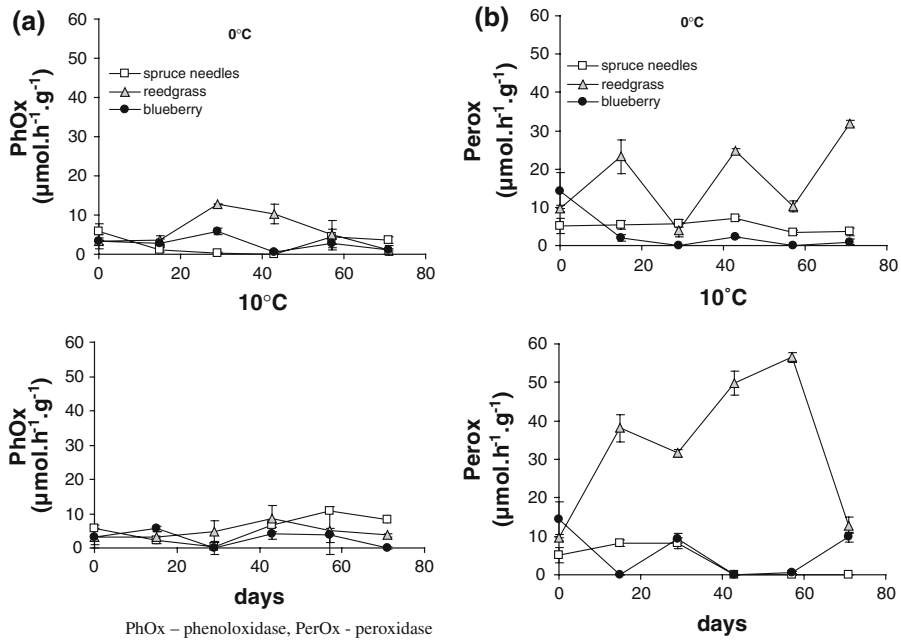
There was a close correlation between overall ligninolytic activity and respiration rate ($r = 0.77$, $p < 0.01$ and $r = 0.82$, $p < 0.01$ at 0 and 10 °C, respectively; Fig. 4). The relationship between ligninolytic enzymes and microbial respiration was similar at both temperatures, which was indicated by the similar slope of the linear regressions. The y intercept (*y* axis = respiration rate) was 2 times higher for 10 °C as compared to 0 °C indicating that microbial respiration was more sensitive to temperature than the activity of ligninolytic enzymes (Fig. 4).

Change of fungal and bacterial communities in relation to phenolics, N and P content

Fungal communities

The first and second canonical axes explained 50.1% of the variability of the fungal communities on spruce needles, blueberry and reedgrass at 0 °C (Fig. 5a). Variance explained by the variables selected by forward selection was 89% and decreased in order: DOC/DN (explained 26%) > P_{OX} (explained 22%) > Perox (explained 21%) > phenolics (explained 20%). During decomposition, all three communities changed their composition with relation to P_{OX}. P_{OX} influenced strongly the blueberry and reedgrass fungal communities and slightly the spruce needle community (Fig. 5a). Additionally, the fungal community of spruce needles showed a slight positive correlation with Perox activity.

At 10 °C, the first and second canonical axes explained 56.8% of the variability of the fungal communities (Fig. 5b). Variance explained by the variables selected by forward selection was 90% and decreased in order: phenolics/N_{TOT} (explained 27%) > phenolics/P_{TOT} (explained 23%) > P_{OX} (explained 21%) > PhOx (explained 19%).The fungal community of reedgrass changed composition again with relation to P_{OX}. Contrary to 0 °C, the



PhOx – phenoloxidase, PerOx - peroxidase

Fig. 3 PhOx (**a**) and Perox (**b**) activity during the decomposition of spruce needles, reedgrass and blueberry litter incubated at 0 and 10 °C. PhOx—phenoloxidase, PerOx—peroxidase

Table 6 Average activity of PhOx, Perox and overall ligninolytic activity (sum of PhOx and Perox activity) during the incubation period at 0 and 10 °C

Litter	Average enzyme activity					
	PhOx (μmol h^{-1} g^{-1})		Perox (μmol h^{-1} g^{-1})		Overall ligninolytic activity (μmol h^{-1} g^{-1})	
	0 °C	10 °C	0 °C	10 °C	0 °C	10 °C
Spruce needles	2.5^{A} (2.4)	5.7^{A} (3.9)	5.0^{A} (1.4)	3.6^{A} (4.1)	7.6^{AB} (1.7)	9.3^{AB} (1.7)
Blueberry	2.7^{A} (1.9)	2.8^{A} (2.3)	3.2^{A} (5.5)	5.7^{A} (6.3)	6.0^{A} (5.9)	8.5^{AB} (5.1)
Reedgrass	6.0^{A} (4.6)	4.7^{A} (2.0)	17.3^{AB} (10.8)	33.1^{B} (19.0)	23.3^{C} (9.6)	37.8^{C} (20.3)

Standard deviations are shown in parenthesis ($n = 18$)

Different letters show significant differences between values within columns at 0 and 10 °C ($p < 0.05$)

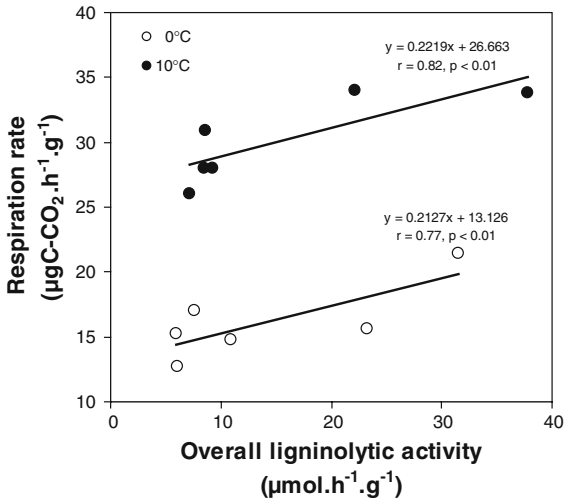


Fig. 4 Respiration rates and overall ligninolytic activities of spruce needles, blueberry and reedgrass litter at 0 and 10 °C

fungal communities of spruce needles and blueberry litter changed composition with relation to PhOx activity (Fig. 5b).

Bacterial communities

At 0 °C, the first and second canonical axes explained 50% of the variability in the bacterial communities of spruce needles, blueberry and reedgrass (Fig. 6a). Variance explained by the variables selected by forward selection was 85% and decreased in order: P_{OX} (explained 35%) > Perox (explained 22%) > phenolics (explained 17%) > phenolics/N (explained 11%). All three communities changed their composition greatly during the incubation. While the bacterial community of spruce needles changed composition under the strong influence of phenolics, P_{OX} strongly influenced the bacterial communities of

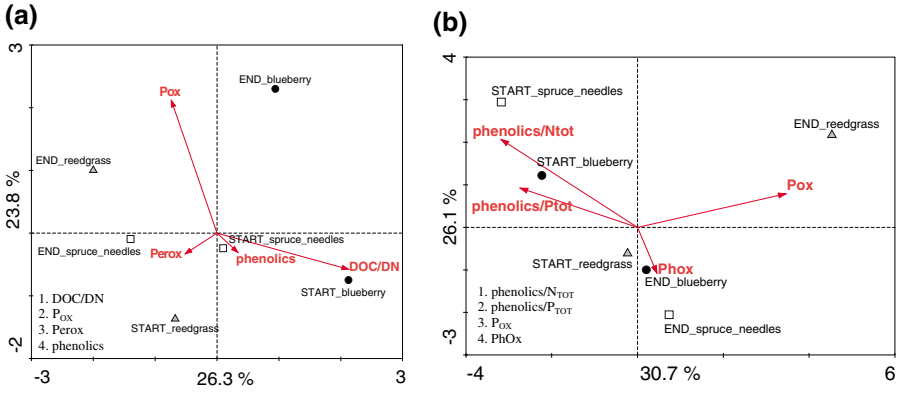


Fig. 5 Ordination biplot of CCA displaying the effect of environmental variables (full line arrows) explaining the variance in fungal community composition (**a**—0 °C, **b**—10 °C) at the beginning and end of the incubation of spruce needle, blueberry and reedgrass litters. Monte–Carlo permutation test was calculated and four environmental variables explaining the most variability in fungal community composition are shown. The order of explained variation by each environmental variable is displayed in *downer left corner* of ordination diagram

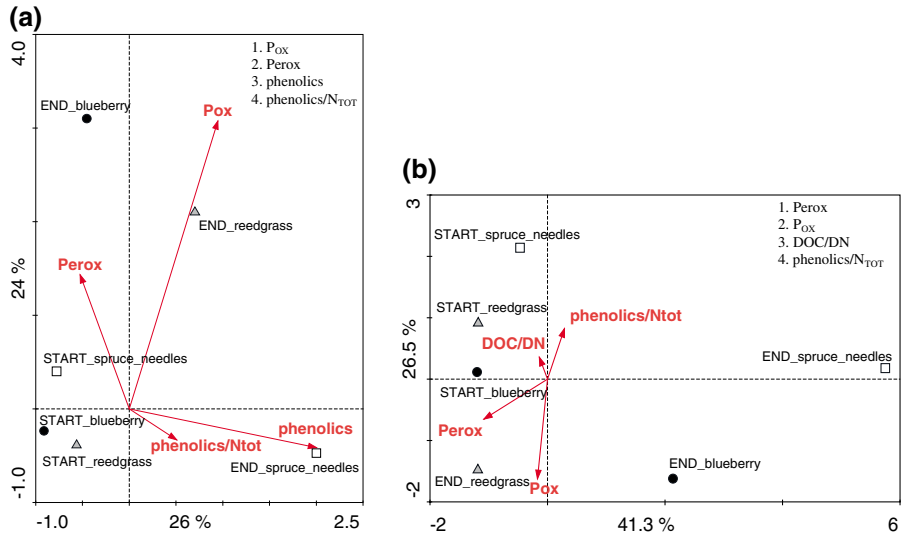


Fig. 6 Ordination biplot of CCA displaying the effect of environmental variables (*full line arrows*) explaining the variance in bacterial community composition (**a**—0 °C, **b**—10 °C) at the beginning and end of the incubation of spruce needle, blueberry and reedgrass litters. Monte–Carlo permutation test was calculated and four environmental variables explaining the most variability of bacterial community composition are shown. The order of explained variation by each environmental variable is displayed in *upper right corner* of ordination diagram

reedgrass and blueberry litter. The bacterial community of blueberry litter was additionally affected by Perox activity.

The first and second canonical axes explained 67.8% of the variability of bacterial communities at 10 °C (Fig. 6b). Variance explained by the variables selected by forward selection was 91% and decreased in order: Perox (explained 37%) > P_{OX} (explained 25%) > DOC/DN (explained 15%) > phenolics/N_{TOT} (explained 14%). All three bacterial communities changed differently during the incubation. P_{OX} and Perox activity had similar effects on the bacterial reedgrass litter community. The blueberry litter and spruce needle communities developed according to unknown environmental variables (Fig. 6b).

Discussion

The role of phenolics, N and P in litter decomposition and nutrient release

The present study confirmed that a low amount of phenolics and low phenolics/N_{TOT} ratio in plant litter is closely related to higher DIN release during the first four mounts of litter decomposition (Northup et al. 1995). In addition, we found that the phenolics/P_{OX} and phenolics/P_{TOT} ratios have similar correlation coefficients for DIN release as the phenolics/N_{TOT} ratio (Table 2).

For a better understanding of how P can influence decomposition processes, we need more information about P bioavailability (Pote et al. 1996). Greater amounts of P_{OX}, as an indicator of bioavailable P, were connected with increased NO_3^- release during litter decomposition in low lignified litter (i.e. reedgrass and hair grass litter, Table 2). It was also indicated by higher N mineralization and nitrification rates in reedgrass and hair grass litter (Tahovská, unpublished data). Similar results were found also *in-situ* for the litter soil horizon in the Bohemian Forest (Kaňa and Kopáček 2006; Šantrůčková et al. 2004, 2006).

Our results additionally revealed that, when phenolics were included into the statistical calculations with relation to P_{OX}, we found a close correlation with the DOC/DN and DON/DIN ratios after four months of litter incubation (Table 2). The strong relationship between phenolics and the DON/DIN ratio was first described by Northup et al. (1995).

Phenolics comprise up to 60% of plant dry weight and are well known for their protective and high reactive properties (Gallet and Lebreton 1995). In the decomposition process phenolics need to be first depolymerized from the lignin matrix by lignin degrading enzymes. The rate of carbon mineralization is a very slow in spruce needle litter as was confirmed by it having the lowest cumulative respiration of the five litters tested, as well as low ligninolytic activity (Figs. 2, 3). The amount of phenolics and P availability could be one of the reasons. Phenolics differ in their structure and complexity among plant species, which influeces the quality of depolymerization products. Northup et al. (1995), explained the close relationship of DON/DIN with phenolics predominantly by the fact that high molecular weight phenolics can precipitate proteins (the major part of DON) and can therefore shift the microbial N metabolism to decrease the loss of DIN; vice versa, low molecular weight, water soluble phenolics can accelerate respiration rate and increase the release of DIN. Also, close connection between phenolics and P_{OX} can be caused by the natural properties of phenolics. Most of the low molecular weight phenolics released by depolymerization have highly reactive ortho-phenolic groups (Comerford and Skinner 1989). When released, they are able to precipitate Al and Fe ions (Northup et al. 1995, 1998; Comerford and Skinner 1989). Precipitation of Al and Fe by low molecular weight phenolics leads to higher amounts of P_{OX} and greater mineral N release from grasses. On the contrary, precipitation of proteins by high molecular weight phenolics leads to lower mineral N release and lower P_{OX} from spruce needle litter. Therefore, not only the amount of phenolics, but also the phenolics/P_{OX} ratio seem to be important indicator of DON and DIN release (Northup et al. 1995; Gallet and Lebreton 1995; Fierer et al. 2001; Kanerva et al. 2006).

In general, there was a negative correlation between the initial amount of phenolics and P_{OX} before and after incubation in our study (Fig. 1; Table 2), which indicates that a low content of phenolics was connected with high P bioavailability. In high phenolic litter this could be caused by reduced depolymerization rates of lignin, and low molecular weight phenolics were not sufficient for precipitation of Al and Fe ions. It resulted in P being predominantly bound to recalcitrant Al and Fe complexes (Northup et al. 1995; Comerford and Skinner 1989). In low phenolic litter (i.e. reedgrass and hair grass), the amount of P_{OX} increased during decomposition. After four months, the amount of P_{OX} almost reached the total P sorption capacity in both grasses (Table 4). Therefore, in areas dominated by reedgrass and hair grass, P_{OX} can leach to litter soil horizon from decomposing litter, because of the P saturation. Similar behavior was previously found for DIN (Šantrůčková et al. 2006; Svoboda et al. 2006; Kopáček et al. 2002a, b; 2006a, b).

Dynamics of Perox and PhOx activities and low temperature effect

The main enzymes which are responsible for lignin degradation and depolymerization are fungal and bacterial ligninolytic enzymes (Baldrian 2004; Snajdr et al. 2008), namely phenoloxidases (PhOx) and peroxidases (Perox). However, PhOx and Perox do not have the same biochemical functions. PhOx uses O_2 molecules for oxidation and is mainly responsible for degradation of more labile low molecular weight phenolics. Meanwhile, Perox uses H_2O_2 for creating unstable and highly reactive phenolic radicals and is predominantly associated with lignin depolymerization (Weintraub et al. 2007). Perox is produced by all white rot basidiomycetes, but PhOx may not be, suggesting that PhOx is not absolutely required in lignin degradation (Srinivasan et al. 1995; Dittmer et al. 1997; Rodriguez et al. 1999; Podgornik et al. 2001; Larrondo et al. 2003; Valaskova et al. 2007).

The activity of Perox increased the most on reedgrass litter at both incubation temperatures, suggesting that depolymerization in low phenolic litter is rapid from the very beginning of decomposition (Fig. 3). Therefore, there can be a sufficient amount of reactive phenolics during reedgrass litter decomposition to precipitate Al and Fe ions, which increases P bioavailability and DIN release. On the contrary, high NO_3^- concentrations are thought to inhibit ligninolytic enzymes unless they are used by the decomposing community as N source or in denitrification processes. In our experiment, NO_3^- concentrations did not reach concentrations to inhibit ligninolytic enzymes as was found by Wardlop and Zak (2006), and Waldrop et al. (2004). These authors did not find any significant decrease in PhOx and Perox activities below 10 μg g^{-1} of NO_3^- which was

the typical concentration in our experiment. However, they found significant decrease in the activity of PhOx (about 30%) and Perox (about 50%) at NO_3^- concentrations of 20 μg g^{-1}.

Additionally, our experiment revealed that microbial processes were also active close to zero and that the activity was not negligible. Sometimes the activity was even higher at 0 °C than at 10 °C (Table 5). The activities of PhOx and Perox at 0 °C were also relatively high (Di Nardo et al 2004; Uchida et al. 2005). Our results confirmed the latest findings that microbial respiration and the activities of lignin-degrading enzymes during the winter period under snow cover are comparable to those during the summer period (Fig. 3; Table 3; Uchida et al. 2005). High Perox activity in low phenolic litter during the winter period can produce more reactive phenolics, increasing the amount of bioavailable P, which reached 69% and 82% of total P litter saturation after the four months in reedgrass and hair grass litter, respectively (Table 4).

Overall ligninolytic activity showed a close positive correlation with microbial respiration. The effect was almost identical at both incubation temperatures (Fig. 4). The differences in the factors measured at the of incubation temperature of 0–10 °C were significant ($p < 0.05$) only for respiration rate, which showed positive effect of temperature. The overall activity of ligninolytic enzymes in all three litter samples was not affected significantly by temperature (Davidson and Janssens 2006).

Dynamics of bacterial and fungal communities

There has been increasing interest in the last few years in using cultivation independent, DNA-based methods for studying bacterial and fungal communities in soil and litter (Coleman and Whitman 2005; Lynch et al. 2004; Fitter et al. 2005; Nannipieri et al. 2003; Kulhankova et al. 2006). However, many of these studies have focused on estimating the bacterial or fungal communities at a specific time of litter incubation or in a particular season and did not study changes in microbial community composition over a time period. Few of them included environmental variables, such as the chemical composition of litter, into the statistical calculations to determine the link between changes in microbial composition and biovailability of key nutrients during litter decomposition (Fromin et al. 2002; Kulhankova et al. 2006).

Because we eliminated the direct contact of litter with soil and the long-term decomposer biota were absent, we assumed that only primary colonizers and those microorganisms already present on plant leaves (endophytes) will be responsible for litter decomposition. The ecological role of microbial primary colonizers, their enzymatic activity and their succession during the first months of litter decomposition are still not clear, but they are often found in the litter soil horizons (Lindahl et al. 2007; Livsey and Barklund 1992). Our results showed that, at a microcosm scale different nutrient availability can be closely related to composition of both bacterial and fungal communities. In general, bacterial and fungal communities compete for available nutrients when litter decomposition starts (Hoshino and Matsumoto 2007; Hoshino and Matsumoto 2004; Hurt et al. 2001). Each group has its advantages over the other. Bacteria can grow faster, but are less tolerant of acidic conditions (Fioretto et al. 2007; Killham 1994). In spruce forest ecosystems, where a lot of lignified litter is decomposed, fungi have an enzymatic advantage over bacteria. Actinomycetes are the only known representatives which are able to compete with fungi in lignin degradation (Waldrop et al. 2004; Waldrop and Zak 2006). New studies also show that Actinomycetes are leaf endophytes (Izumi et al. 2008; Li et al. 2008).

Our results revealed that composition of bacterial and fungal communities changed greatly during litter incubation (Figs. 5, 6). This change was mainly in the number and type of different bands in the DGGE gel, indicating some successional development of primary colonizers and endophytes in the microcosm during the four months (data not shown). However, only the composition of microorganisms at the beginning and end of incubation were compared together with available data on environmental variables (Figs. 5, 6).

At 0 °C, the amount of phenolics was closely connected to initial composition of fungal communities in high lignified litter (i.e. spruce needles), indicating that these may be controlled by phenolics. O the contrary, phenolics were not related to the initial bacterial community on spruce needles (Fig. 6a). This seems to be in agreement with Frankland (1992), who hypothesized that the bacterial primary decomposers first utilize simple and easily available compounds and after that the more

recalcitrant ligno-cellulose complexes. Fast growing bacteria can over dominate fungi in competition for easily available nutrients in this stage of litter decomposition. Fungi, which are then in a nutrient limiting environment are obliged to depolymerize lignin and can be influenced by the amount of available phenolics (Fig. 6).

At 10 °C, fungal and bacterial communities of reedgrass at the end of the incubation responded strongly to P_{OX} (Figs. 5, 6). This is with agreement with the highest respiration rate in reedgrass litter which means that the microbial decomposers would have higher demand on P availability.

The connection of extracellular enzymes (i.e. PhOx and Perox) with the composition of microbial communities may be difficult to interpret. Is the activity of enzymes affecting microbial composition or is it the other way around? The production of extracellular enzymes is C, N and energy intensive, therefore microbes produce enzymes only when there is nutrient limitation (Koch 1985). Microbes that produce PhOx and Perox have to face several important challenges. Because these enzymes are produced and function outside the cell, products of depolymerization process (i.e. low molecular phenolics) may then diffuse away from the enzyme-producing microbes (Allison 2005). Other microbes in the system could take up these products without secreting their own enzymes. This strategy of "cheating" should arise whenever multiple organisms can benefit from the resource investment of a single organism (Velicer 2003), and could represent a competitive threat to enzyme-producing microbes. How they will succeed in "fighting" with "cheater" microbes depends also on the initial population size/density of extracellular enzyme producers (Allison 2005). Therefore, PhOx and Perox activities may affect not only the composition of ligninolytic enzymes producers, but also the composition of other microbes (i.e. fast growing bacterial communities), which lack PhOx and Perox activities.

Only Perox activity had a significant effect on bacterial community, indicating a strong dependence of bacteria on depolymerization processes. However these changes were dependent on litter type and incubation temperature (Fig. 6a, b). At 0 °C, the bacterial communities of blueberry and reedgrass litter at the end of incubation were strongly positively related to Perox activity. We did not observe a similar pattern at 10 °C (Fig. 6b). On the contrary, the bacterial community of spruce needles changed from a positive relation to Perox at the beginning of incubation to a strongly positive relation to the amount of phenolics at the end of incubation. This could be an indication of change in bacterial community from Actinomycetes, which produced Perox at the beginning, to the "cheater" microbes, which used depolymerized phenolics at the end of the incubation. It also confirms previous findings that the competition for available nutrients play crucial role in the composition of the decomposers at the later stages of litter decomposition (Berg et al. 1993). Our results showed that this shift in microbial composition at a microcosm scale can be relatively fast, i.e. during the first four months of litter decomposition.

From the results we conclude:

- The phenolics/P_{TOT} and phenolics/Pox ratios are good predictors of the release of DON and DIN from acid spruce forest litter.
- The higher activity of ligninolytic enzymes (i.e. Perox) in low phenolic reedgrass litter can produce highly reactive phenolics, which can either be used by the microbial community or precipitate Al and Fe ions, thereby increasing the bioavailability of P (P_{OX}) and release of DIN. This can, therefore, lead to higher DIN leaching from the areas dominated by grasses.
- Activity of lignin-degrading enzymes at 0 °C was comparable to those at 10 °C.
- There is a potential risk of bioavailable P (P_{OX}) leaching from grass dominated sites to the soil litter horizon.
- Bacterial and fungal communities of primary colonizers and endophytes change greatly during the early-stages of litter decomposition. While the change in fungal community composition of low lignified litter (i.e. reedgrass) correlates mostly with bioavailable P (P_{OX}), the change in higher lignified litter (i.e. spruce needles) correlates mostly with phenolics/N_{TOT}, phenolics/P_{TOT} ratios and PhOx activity (i.e. fungal community of spruce needles at 10 °C). Perox activity was responsible for the change in bacterial community composition of blueberry and reedgrass litter, while the bacterial community of spruce needle litter changed with relation to phenolics (i.e. bacterial community of spruce needles at 0 °C).

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