# Changes of the functional diversity of soil microbial community during the colonization of abandoned grassland by a forest

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## ARTICLE INFO

## ABSTRACT

The impact of secondary succession of grassland communities towards a Norway spruce forest on soil microbial community was studied on a belt transect established in the Pol'ana Mts., Central Europe. Data on understory vegetation, light availability, soil properties and microbial activity were collected on 147 plots distributed over regular grid. Moreover, distributions of functional groups of microorganisms were assessed using BIOLOG analysis on a subset of 27 plots. Mantel partial correlations between microbial community indicators and environmental variables showed that microbial activity generally decreased with increasing tree density and size, whereas it increased with increasing radiation at the soil surface, soil temperature, and cover and diversity of understory vegetation. Functional richness and diversity of microorganisms were positively correlated with solar radiation, but also with plant species richness and diversity. Abundance of several functional groups correlated closely with succession-related variables. Redundance analysis of microbial data provided slightly different outcomes. Forward selection yielded only two environmental variables significantly influencing the composition of the microbial community: tree influence potential and organic carbon content. Abundances of several functional microbial groups correlated with tree influence, documenting that microbial community changes are at least partially driven by the colonization of grassland by trees. Nevertheless, the relative importance of abiotic environment change and plant community succession on microbial community dynamics remains unresolved.

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## 1. Introduction

With the advent of intensive agriculture, large areas of fields, meadows and pastures have been abandoned in Europe and North America. The conversion of abandoned agricultural land into forests either by reforestation or through natural colonization by trees has become an increasingly important ecological phenomenon (FAO, 2001), and various aspects of this process have intensively been studied (Magee and Antos, 1992; Miller and Halpern, 1998; Prévosto et al., 2003; Doležal et al., 2004; Dovčiak et al., 2005, 2008; League and Veblen, 2006; Julien et al., 2006). The main driver of this scientific interest is the fact that this process may play an important role in global geochemical cycles, such as carbon sequestration (Post and Kwon, 2000; Thuille et al., 2000; Thuille and Shulze, 2006), nitrogen cycle (Asner et al., 2001; Zavaletta and Kettley, 2006) and hydrological cycle (Chalita and Letreut, 1994; Kergoat, 1998; Misson et al., 2002; Tett et al., 2007).

In Central Europe, gradual changes in the management of agricultural land have occurred since the early 1950s after the collectivization of agriculture. In the Carpathian range, they have affected mainly mountain grasslands used as meadows and pastures. These areas resulting from deforestation mainly in the 16th and 17th centuries were mostly small, fragmented, and surrounded by forests. Therefore, after their management ceased, they have become gradually recolonized by trees and shrubs (Križová, 1995; Ujházy, 2003).

In spite of a general interest in the conversion of grasslands to forests, several aspects of this process are still poorly understood, including the effects on soil, and in particular on soil microbial communities. The tree layer is supposed to affect the soil through several mechanisms. Trees provide an important part of aboveground and belowground litter which is decomposed by soil microorganisms, modify the access of radiation and precipitation water to the soil, and draw water and nutrients from the rhizosphere. Their roots directly interact with soil microorganisms (Stoyan et al., 2000; Wilkinson and Anderson, 2001). Moreover, trees affect the composition of understory vegetation; changes of density, age, diameter and height structure of trees

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and shrubs during the succession cause that the formerly rather homogeneous grassland vegetation is replaced by a hetero-geneous mosaic within few decades (Grime, 1979; Glenn-Lewin and van der Maarel, 1992; van der Valk, 1992; Lett and Knapp, 2003). As soil microbial communities are frequently limited by carbon and nitrogen (Zak et al., 1994, 2003), a patchy distribution of vegetation types producing litter of different quantity and quality may ultimately lead to spatial aggregation of soil microorganisms (Saetre and Bååth, 2000; Chabrerie et al., 2003; White et al., 2005).

The variety of tree stand properties across the tree invasion front is thus expected to be reflected in the functional diversity of soil microbial communities and should produce a spatial pattern of microbial activity indicators such as soil respiration and enzyme activities. Our goal was thus to assess the responses of the soil microbial community to abiotic and biotic environment changes associated with the progress of the colonization of a pasture by forest trees.

## 2. Materials and methods

### 2.1. Study site

The studied area is a grassland of approximately 100 ha located in Central Slovakia (Western Carpathians) at the Príslopy Pass (48°38′10″N, 19°25′11″E, approximately 900 m a.s.l.). The grassland is surrounded by natural and seminatural forests dominated by silver fir (Abies alba Mill.) and European beech (Fagus sylvatica L.). Mean temperatures in the area reach ca. 14 and −6 °C in July and January, respectively, the annual precipitation is approximately 900–1200 mm, and snow cover lasts more than 100 days (Ministry of Environment of the Slovak Republic, 2002). The soils are mostly deep and fertile cambisols overlaying tuffic sediments and andesite lava flows (Hraško et al., 1980).

The grassland originated around 1800 after forest clearance, and it was utilized as a hay meadow. In the 1890s, an approximately 20 m wide strip of Picea abies was planted along the north-facing forest margin. Mowing was replaced by cattle grazing in the early 1950s, and the grassland was completely abandoned in the 1980s (Ujházy, 2003). The planted Norway spruce began to colonize the grassland in the 1950s, and the invasion was well under way by 1975 (Gömöry et al., 2006). A minor part of the area was restored by manual removal of invading trees and shrubs to preserve biodiversity and commu-nities associated with grasslands, but the remaining area contains a well-developed invasion gradient ranging from the original open grassland to areas covered by a dense spruce thicket.

In 2003, a 170 m × 40 m permanent belt transect was established perpendicularly to contour lines and to the forest edge. The transect crossed all stages of stand development from the strip of planted trees (now over 120 years old and starting to break up), across a dense closed young spruce stand (now approximately 50 years old), to a relatively open grassland with scattered junipers and young spruce trees overgrown by grassland communities with the predominance of Avenula adsurgens, Avenella flexuosa, Carex pilulifera and Nardus stricta. The transition from a relatively open grassland to a closed forest is rather smooth (Fig. 1), and the species composition of the understory vegetation changes accordingly.

We established a 10 m × 10 m grid within the transect and mapped it using an electronic tachymeter (ELTA-4, Carl-Zeiss Jena, Germany). We recorded the position (x, y, z coordinates), breast-height diameter (dbh, at 1.3 m height) and height of all trees >1.3 m tall.
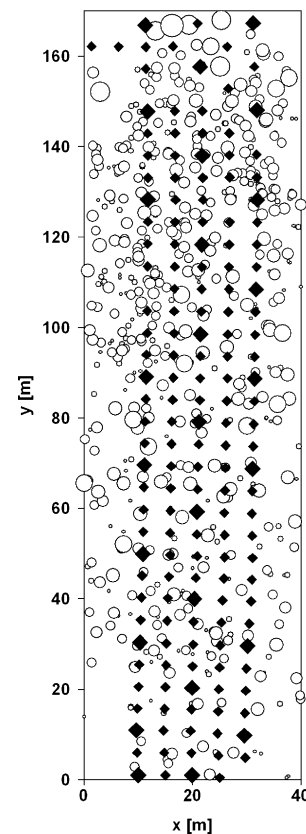


Fig. 1. Distribution of trees and sampling points along the belt transect. Circles, trees (symbol size corresponds to tree diameter at breast height); small diamonds, vegetation plots; large diamonds, vegetation plots used for the Biolog assay.

### 2.2. Vegetation and site condition assessment

Field-layer vegetation was recorded on a more detailed grid of 5 m × 5 m on 147 circular plots of 0.5 m² established within the 20-m-wide central strip of the transect using belt measurement. For each plot, we estimated the percentage cover of vascular plants ($E_1$) and bryophytes ($E_0$), percentage cover of dead herb biomass and percentage cover of fallen needles.

To assess the effects of the colonizing tree species on sampling plots, we calculated an index based on sizes of trees within a 5-m-neighbourhood of the sampling point (centre of the vegetation sampling plots) weighted by their distances from the point:

$$IP_{BA} = \sum_i BA_i e^{-r_i}$$

where $BA_i$ is the basal area (area of the cross-section of the stem at breast height) of the $i$th tree within the 5-m-neighbourhood of the sampling point, and $r_i$ is the distance between the sampling point and the $i$th tree (Kuuluvainen and Pukkala, 1989). The 5 m neighbourhood size corresponds to the crown size of an average adult tree in this study. The index was calculated as a single plot-level statistic for each vegetation plot.

At each vegetation plot, canopy light transmission was characterized using vertical hemispherical photographs taken 20 cm above the soil surface with a Nikon Coolpix 5400 digital camera equipped with a fisheye FC E9 objective. Canopy openness (CO, the percentage of open sky seen from beneath the forest canopy), and the amount of direct ($R_{DIR}$) and diffuse ($R_{DIF}$) solar radiation transmitted by the canopy were estimated from the photographs using Gap Light Analyser 2.0 (Frazer et al., 1999).

To get at least a general idea of the spatial distribution of daily temperature course on the transect, soil temperatures at the depth

of 2 cm were measured using Hg soil thermometers distributed over a subset of 90 points on October 1, 2004 between 08:00 and 17:00. Temperatures were recorded once per hour, whereby the average temperature ($T_{AVG}$) and the amplitude ($T_{AMPL}$) for each point were calculated.

### 2.3. Soil properties and microbial activity analysis

Because vegetation cover varied among points, affecting the character and composition of organic soil horizons, soil samples were taken only from the uppermost mineral horizon (A-horizon, Šály et al., 2000) from the center of each vegetation plot from the depth of 5–10 cm. This is the zone most densely rooted by herb species, where the interaction between the vegetation and the soil microbiota is most intensive. Moreover, it constitutes a direct interface between belowground and aboveground components of the ecosystem. Four subsamples were taken for each sampling point and bulked together to form composite samples. Soil moisture was determined gravimetrically by oven-drying fresh soil at 105 °C for 24 h. Half of each sample was air-dried and used for measurement of soil acidity in water and 1 M KCl suspension (20 g soil plus 50 ml water and KCl, respectively), soil organic carbon content (standard Tyurin titrimetric method), total nitrogen (standard Kjeldahl wet oxidation), phosphorus and potassium (Mehlich 3 extract, K – flame emission spectrometry, P – colorimetry).

Another half of the composite sample was stored in field-moist conditions at 4 °C. To preserve natural conditions for microbiota, samples were not sieved prior to the analysis, but hand-picked to remove macroscopic rock fragments and plant remains. Soil respiration was measured as the amount of carbon dioxide released from 50 g of fresh soil without (24 h incubation; basal respiration) and with glucose amendment (10 mg of D-glucose per 1 g of soil, 4.5 h; substrate-induced respiration, SIR) at 22 °C, absorbed in 25 ml 0.05 N NaOH, whereby the amount of carbonate was determined by the titration with 0.05 N HCl after the precipitation of carbonates by 5 ml 0.5 M $BaCl_2$. Catalase (EC 1.11.1.6) activity was measured 10 min after 20 ml 3% $H_2O_2$ was added to 10 g fresh soil sample based on the volume of discharged oxygen, compared to the respective sample where microbial activity was suppressed by overheating at 180 °C (to account for $H_2O_2$ dissociation caused by soil Fe and Mn oxides; Khaziev, 1976). The rate of cellulose decomposition (as a rough semiquantitative indicator of cellulase activity) was measured using three 5 cm × 1 cm filter-paper strips on samples placed in Petri dishes after 30 days of exposure at 21 °C. Strips were digitally photographed and the decomposition rate was measured as the decrease of the white-area percentage. Microbial biomass was assessed using the microwave irradiation procedure following Islam and Weil (1998): 10 g oven-dried equivalent (ODE) of field-moist soil adjusted to 80% water-filled porosity was irradiated twice by microwave (MW) energy at 400 J g$^{-1}$ ODE soil to kill the microorganisms. The time settings and MW oven power depended on the total amount of soil in the MW oven. After cooling, soil samples were extracted with 0.5 M $K_2SO_4$ and C content in the extract was quantified by the oxidation with $K_2Cr_2O_7/H_2SO_4$, measuring the absorption at 590 nm using a spectrophotometer. The same procedure was done with a non-irradiated sample. The microbial biomass carbon was then determined as $C_{mic} = (C_{irradiated} - C_{non-irradiated})/K_{ME}$, whereby Islam and Weil (1998) recommended the extraction efficiency factor $K_{ME} = 0.213$.

Community-level metabolic profiles of microbial communities were determined in soil samples collected on a subset of 27 points regularly distributed over the transect on a diagonally rotated grid of 14.1 m × 14.1 m within 2 h of a single day, and handled identically as samples for microbial activity assessment, using the Biolog® Eco Plates (31 organic substrates, Insam, 1997). Sample preparation followed essentially Gomez et al. (2004). Inocula were produced by resuspending fresh soil (equivalent of 5 g dry weight) in 45 ml 0.9% NaCl, centrifuged (1000 rpm for 5 min), the supernatant was diluted 1:10,000, and 150 μl of extract were incubated in microtitration plates at 37 °C during 7 days. Absorbance at 590 nm was recorded every 12 h using the Sunrise Microplate Reader (Tecan, Salzburg, Austria). Absorbance values were blanked against the control well. The metabolic activity was calculated as the area below the time–absorbance curve, and was used as a measure of the abundance of the respective functional group.

### 2.4. Data analysis

Species richness of the understory vegetation was assessed as the number of vascular plant and bryophyte species present on the 0.5 m$^2$ sampling plot. Diversity was assessed using the Hill's index: $N_2 = 1/\sum p_i^2$; where $p_i$ is the frequency (calculated on the basis of cover) of the $i$th plant species (Hill, 1973).

The richness of the soil microbial community was assessed as the number of substrates with a non-zero response. The functional diversity of the microbial community was also assessed using Hill's $N_2$ index.

To assess the relationships between the soil microbial community, plant diversity, and environment, two approaches were used.

#### 2.4.1. Univariate approach

We calculated correlations between microbial activity indicators, plant species richness and diversity and environmental factors. Since the data were not spatially independent, we used partial Mantel tests yielding partial correlation coefficients between the matrix of differences in response and predictor variables while controlling the effect of the matrix of spatial distances among plots (Smouse et al., 1986). The significance of the partial correlation coefficients was tested using 100,000 random permutations, and subsequently corrected using sequential Bonferroni procedure (Quinn and Keough, 2002). The calculations were performed using the program zt (Bonnet and Van de Peer, Ghent University, Belgium).

#### 2.4.2. Multivariate approach

Since plant and/or microbial richness and diversity do not completely explain how the structure of communities changes along the successional gradient and how the composition of functional groups of microorganisms is related to the ground-layer vegetation, a multivariate analysis was performed. We chose a direct gradient analysis (redundancy analysis; RDA), allowing to determine environmental variables which best explain the changes of the frequency distributions of microbial functional groups along the colonization gradient. RDA is based on a linear approximation of the species' response to environmental gradients (appropriate when environmental conditions are relatively homogeneous so that the gradient length is small) and yields constrained ordination axes reflecting the direction of the maximum variability within the dataset, that can be explained by the assessed environmental factors (ter Braak and Smilauer, 2002). The significance of environmental variables and RDA axes was tested using Monte Carlo permutation test (9999 runs). Unrestricted permutations were employed, since the abundance of microorganisms and the composition of microbial communities generally change at very short distances, so that we did not expect spatial dependence at the scale of several meters. Significant environmental variables were identified by forward selection.

**Table 1**
Means, standard deviations and ranges of microbial community indicators and environmental variables.

| Variable | Mean ± S.D. | Range |
|---|---|---|
| **Microbial community indicators** | | |
| Basal respiration ($\mu g\,CO_2\,g^{-1}\,h^{-1}$) | 6.11 ± 3.13 | 0.52–14.76 |
| SIR ($\mu g\,CO_2\,g^{-1}\,h^{-1}$) | 28.68 ± 10.13 | 4.73–64.44 |
| Catalase activity ($ml\,O_2\,g^{-1}\,min^{-1}$) | 1.41 ± 0.39 | 0.40–2.70 |
| Cellulase activity (%) | 40.13 ± 10.82 | 19.85–84.77 |
| Microbial biomass ($\mu g\,C\,g^{-1}$) | 361.9 ± 190.6 | 10.2–1270.9 |
| Microbial richness | 18.23 ± 4.48 | 11–28 |
| Microbial diversity | 8.13 ± 2.75 | 3.99–12.85 |
| **Soil properties** | | |
| Organic matter (%) | 8.51 ± 2.84 | 0.34–16.90 |
| Soil moisture (%) | 40.59 ± 6.00 | 25.97–65.95 |
| pH/$H_2O$ | 4.46 ± 0.22 | 3.87–5.03 |
| pH/KCl | 3.38 ± 0.21 | 2.90–3.95 |
| Nitrogen ($mg\,g^{-1}$) | 5.45 ± 1.19 | 3.53–12.35 |
| Phosphorus ($mg\,kg^{-1}$) | 3.67 ± 2.07 | 1.05–15.26 |
| Potassium ($mg\,kg^{-1}$) | 177.4 ± 83.8 | 86.8–582.6 |
| **Colonization stage** | | |
| $IP_{BA}$ ($cm^2\,m^{-1}$) | 1385 ± 825 | 50–4729 |
| **Light and temperatures** | | |
| Canopy openness (%) | 13.09 ± 7.26 | 4.22–41.31 |
| Direct radiation ($mol\,m^{-2}\,day^{-1}$) | 2.86 ± 2.68 | 0.37–13.83 |
| Diffuse radiation ($mol\,m^{-2}\,day^{-1}$) | 4.14 ± 2.24 | 1.09–12.70 |
| Mean temperature (°C) | 7.65 ± 0.51 | 6.3–10.2 |
| Temperature amplitude (°C) | 1.48 ± 0.60 | 0.6–3.4 |
| **Vegetation** | | |
| Plant species richness | 10.18 ± 8.13 | 0–32 |
| Plant species diversity | 2.90 ± 2.20 | 0.00–8.03 |
| $E_1$ cover (%) | 25.27 ± 31.26 | 0–95 |
| $E_0$ cover (%) | 33.06 ± 35.70 | 0–100 |
| Dead herb biomass cover (%) | 5.96 ± 11.95 | 0–70 |
| Needle cover (%) | 49.44 ± 46.64 | 0–100 |

$IP_{BA}$ – tree influence potential, $E_1$ – vascular plants, $E_0$ – bryophytes.

# 3. Results

A basic overview of the investigated microbial and environmental variables is given in Table 1. Microbial activity indicators were mostly inter-correlated, except cellulose decomposition rate. However, their correlations with the richness and diversity of functional groups of microorganisms were generally non-significant (Table 2). Richness and diversity themselves were closely correlated ($r = 0.821^{***}$), indicating that diversity was determined mainly by richness rather than evenness, i.e. the frequency distributions of microbial groups were similar among samples, but the numbers of absent groups were different.

Increasing density of the spruce stand reduced light availability at the ground level, soil temperatures and their fluctuation, and even soil moisture. The losses of water through increased solar radiation in open patches are apparently less important than interception of vertical and horizontal precipitation in tree crowns and water desuction from soil by tree roots. The cover, species richness and species diversity of understory vegetation (both vascular plants and bryophytes) as well as dead herb biomass decreased with the colonization, in contrast to the cover of needles. On the other hand, soil chemical properties remained unaffected by the colonization (Table 2).

As environmental variables largely co-varied along the successional gradient, microbial community indicators exhibit similar responses to the variables related to the same or similar environmental factors (Table 2). Microbial activity and biomass generally decreased with increasing tree density and size, whereas they increased with increasing radiation at the soil surface, soil temperature as well as cover and diversity of understory vegetation. Soil chemical variables were mostly independent from the colonization stage (correlations were non-significant, data not shown), but they also affected microbial activity. Interestingly, the effects of organic carbon and nitrogen supply on microbial activity indicators were mostly non-significant, sometimes even negative. Microbial community generally reacted positively to the supply of herb and moss litter, as indicated by the fact that all significant correlations of microbial activity indicators with ground-layer vegetation cover or biomass were consistently positive. On the other hand, the presence of a thick layer of needles decreased microbial activity and all significant correlations of needle cover with the abundances of functional microbial groups were negative (Tables 2 and 3).

Richness and diversity of functional groups of microorganisms were most strongly correlated with solar radiation. There was also a significant positive correlation with plant species richness and diversity, indicating a direct relationship between microbial and plant communities. Interestingly, microbial diversity and richness were apparently not correlated with most chemical soil properties, including organic matter content. The only exception was nitrogen content, exhibiting positive association with microbial diversity and richness.

Almost all functional groups of microorganisms exhibited some significant correlations with environmental variables, but only in few cases such correlations showed a consistent and interpretable pattern (Table 3). The use of D-glucosaminic acid (s22) and glycyl-L-glutamic acid (s24) was consistently positively correlated with microbial activity indicators. Response to the latter substrate seems to be also dependent on soil chemistry ($C_{org}$, pH, K content). On the other hand, the use of four substrates (α-cyclodextrin (s17), L-asparagine (s8), D-xylose (s6) and DL-α-glycerol phosphate (s30)) increased in insolated places with high soil temperatures. It is, however, questionable whether this response is fostered by the energy input or by the composition and cover of understory vegetation. The use of α-cyclodextrin exhibited high positive correlation with the plant species richness and diversity, whereas the response to the remaining three substrates was found to depend on the amount of herbs and dead herb biomass. None of the substrates exhibited consistently negative correlation with light and temperatures.

Multivariate approach yielded slightly different outcomes. The first RDA axis accounted for 17.4% of variance in the species data and 82.5% of the species–environment relationship. The forward selection of environmental variables in the RDA indicated that the frequency distributions of functional microbial groups along the colonization gradient were significantly affected by tree influence ($IP_{BA}$). The variance explained by this environmental variable represented 12% of the total variance in microbial data (Table 4). Organic carbon content also showed a significant effect (9.1% of the total inertia). Moreover, plant species richness had a significant marginal effect.

The variation in microbial functional groups in relation to environmental variables which passed the forward selection is shown in Fig. 2. Tree influence was strongly positively correlated with the use of phenylethylamine (s28). The other substrates exhibiting higher response in the proximity of trees are D-glucosaminic acid (s22), D-galacturonic acid (s7), 2-hydroxybenzoic acid (s12) and L-asparagine (s8), on the contrary, the use of glucose-1-phosphate (s26), L-threonine (s21), DL-α-glycerol phosphate (s30) and D-xylose (s6) seems to be lower in the neighbourhood of trees. Response to i-erythritol (s10), glycyl-L-glutamic acid (s24), 4-hydroxybenzoic acid (s15), 2-hydroxybenzoic acid (s11) and γ-hydroxybutyric acid (s19) is associated with humus-rich sites, on the other hand, the use of D-malic acid-dependent microbes (s31) decreases with higher organic carbon content. However, most functional groups seem to be indifferent to both environmental factors which proved to be significant.

**Table 2**
Mantel partial correlations (Bonferroni-corrected) between microbial activity indicators and environmental variables.

| Variable | $IP_{BA}$ | Basal respiration | SIR | Catalase activity | Cellulase activity | Microbial biomass | Microbial richness | Microbial diversity |
|---|---|---|---|---|---|---|---|---|
| **Microbial community indicators** | | | | | | | | |
| Basal respiration | ns | | | | | | | |
| SIR | −0.160[***] | 0.135[***] | | | | | | |
| Catalase activity | −0.248[***] | 0.394[***] | 0.404[***] | | | | | |
| Cellulase activity | −0.216[***] | ns | ns | 0.203[***] | | | | |
| Microbial biomass | ns | ns | ns | ns | ns | | | |
| Microbial richness | ns | ns | ns | ns | ns | ns | | |
| Microbial diversity | ns | ns | −0.218[*] | ns | ns | ns | 0.821[***] | |
| **Soil properties** | | | | | | | | |
| Organic carbon | ns | ns | ns | −0.133[***] | ns | 0.114[**] | ns | ns |
| Soil moisture | −0.199[***] | 0.129[**] | 0.414[***] | 0.125[**] | ns | ns | ns | ns |
| pH/H$_2$O | ns | 0.096[*] | 0.127[**] | ns | ns | 0.087[*] | ns | ns |
| pH/KCl | ns | 0.151[***] | 0.120[*] | 0.217[***] | ns | 0.117[**] | ns | ns |
| Nitrogen | ns | −0.114[**] | ns | ns | 0.132[**] | ns | 0.285[*] | 0.190[*] |
| Phosphorus | ns | 0.168[***] | 0.179[***] | 0.256[***] | ns | ns | ns | ns |
| Potassium | ns | 0.083[*] | 0.159[**] | 0.212[***] | 0.087[*] | ns | 0.197[*] | ns |
| **Light and temperatures** | | | | | | | | |
| Canopy openness | −0.577[***] | ns | 0.113[*] | 0.205[***] | 0.275[***] | 0.102[*] | 0.339[**] | ns |
| Direct radiation | −0.397[***] | 0.197[***] | ns | 0.296[***] | 0.243[***] | 0.148[**] | 0.522[***] | 0.325[**] |
| Diffuse radiation | −0.571[***] | ns | 0.130[*] | 0.209[***] | 0.253[***] | 0.101[*] | 0.349[**] | 0.198[*] |
| Mean temperature | −0.473[***] | 0.181[*] | 0.252[*] | 0.306[***] | ns | 0.219[*] | ns | ns |
| Temperature amplitude | −0.514[***] | ns | ns | 0.231[**] | 0.115[*] | 0.204[**] | ns | ns |
| **Vegetation** | | | | | | | | |
| Plant richness | −0.089[*] | ns | 0.097[*] | 0.129[***] | ns | ns | 0.256[*] | 0.281[*] |
| Plant diversity | −0.427[***] | 0.124[**] | 0.084[*] | 0.159[***] | 0.194[***] | ns | 0.393[**] | 0.251[*] |
| $E_1$ cover | −0.650[***] | 0.104[**] | 0.127[**] | 0.333[***] | ns | 0.247[***] | ns | ns |
| $E_0$ cover | −0.590[***] | ns | 0.177[***] | 0.186[***] | 0.075[*] | ns | ns | ns |
| Dead herb biomass | −0.314[***] | 0.180[***] | ns | 0.242[***] | ns | 0.183[***] | ns | ns |
| Needle cover | 0.659[***] | ns | −0.147[**] | −0.270[***] | ns | −0.142[**] | ns | ns |

Significance labels: ns – non-significant. $IP_{BA}$ – tree influence potential, $E_1$ – vascular plants and $E_0$ – bryophytes.
[*] $P < 0.05$.
[**] $P < 0.01$.
[***] $P < 0.001$.

## 4. Discussion

The axis of the transect follows the direction of the colonization of the former pasture by spruce. The density of the newly formed forest stand and the size of trees decrease rather continuously along the transect (Fig. 1). Therefore, we did not try to divide the transect area into parts or patches corresponding to different colonization stages, since such division would necessarily be arbitrary. Instead, we used the tree influence potential ($IP_{BA}$) as a proxy for the colonization stage, as it quantifies the composite effect of tree density and tree size in the neighbourhood of a soil sampling site.

Plant species richness and diversity in our experimental object were only partly correlated with the position within the colonization gradient, tree influence or light availability on the ground level. In fact, plant communities most resembling the original species-rich pasture vegetation have been best preserved in small patches surrounded by trees. Larger open patches in the bottom half of the transect have become rapidly colonized by clonally spreading grasses (mainly *A. adsurgens*, less *Brachypodium pinnatum* and *Calamagrostis arundinacea*), forming species-poor monodominant stands (Janišová et al., 2007). With the progress of the colonization by spruce and increasing shade, clonal grasses are suppressed, and more diverse patches are formed again around the crown perimeters of trees. Consequently, the transect became covered by a mosaic of different vegetation types, out of which several exhibit similar levels of plant species diversity and richness, although having very different species composition (Hrivnák and Ujházy, 2005).

Although the aboveground and belowground components of a terrestrial ecosystem are generally considered to be dependent on each other, the relationship between plant and soil microbial communities is not straightforward. Generally, residues and roots of different plant species are colonized by different decomposer communities (Wardle et al., 1999, 2006; Miethling et al., 2000; Kowalchuk et al., 2002), microbial community composition and biomass may even be different for different genotypes within a species (Schweitzer et al., 2008). A mechanistic view may lead to the expectation that increased plant species diversity should be reflected in a high diversity as well as activity of soil organisms. Actually, empirical studies (mainly those based on manipulative experiments) do not always confirm this expectation. For instance, Rodriguez-Loinaz et al. (2008) or Liu et al. (2008) observed a strong positive correlation between soil functional diversity and plant diversity; on the other hand, non-significant or even negative relationship was reported by Keith et al. (2008) or Kielak et al. (2008). The same applies to microbial activity. In some studies, a positive response of biomass, respiration, N mineralization and enzyme activities on increased plant species richness was observed, but mostly was attributed to a higher production under high diversity treatments (Stephan et al., 2000; Zak et al., 2003; Liu et al., 2008). However, Rodriguez-Loinaz et al. (2008) observed both positive and negative correlations of several enzyme activities with plant diversity in native forest soils. Similarly, Hedlund et al. (2003) did not find an effect of plant diversity on soil decomposer communities including bacteria and saprophytic fungi.

In our case, ground-layer vegetation seems to affect the soil microbial community. Microbial activity generally responded to the variety and amount of plant residues entering the soil, as indicated by consistently positive correlations between microbial activity and biomass indicators and characteristics of understory vegetation, contrasting with negative correlations with spruce

**Table 3**
Mantel partial correlations (Bonferroni-corrected) between selected microbial functional groups, microbial activity indicators, and environmental variables.

| Variable | Metabolized substrate | | | | | | |
|---|---|---|---|---|---|---|---|
| | s22 | s24 | s9 | s17 | s8 | s6 | s30 |
| **Microbial community indicators** | | | | | | | |
| Basal respiration | 0.429*** | 0.500*** | ** | −0.307*** | ns | 0.423*** | ns |
| SIR | ns | 0.308* | ns | ns | ns | ns | ns |
| Catalase activity | 0.315** | 0.458** | ns | ns | ns | ns | ns |
| Cellulase activity | 0.426*** | 0.289** | ns | ns | ns | ns | ns |
| Microbial biomass | ns | 0.419*** | 0.328* | ns | ns | ns | ns |
| Microbial richness | ns | ns | ns | 0.262* | ns | ns | 0.234* |
| Microbial diversity | ns | ns | ns | 0.254* | ns | ns | ns |
| **Soil properties** | | | | | | | |
| Organic carbon | ns | 0.479*** | ns | −0.267* | ns | ns | ns |
| Soil moisture | 0.346** | 0.249* | ns | ns | ns | ns | ns |
| pH/$H_2O$ | ns | 0.442* | 0.208*** | ns | ns | ns | ns |
| pH/KCl | ns | 0.435* | 0.290*** | ns | ns | ns | ns |
| Nitrogen | ns | ns | ns | ns | ns | 0.240* | ns |
| Phosphorus | ns | ns | ns | ns | ns | ns | −0.246* |
| Potassium | 0.565*** | 0.325** | 0.177** | ns | ns | ns | ns |
| **Colonization stage** | | | | | | | |
| $IP_{BA}$ | 0.352*** | ns | ns | ns | −0.189* | ns | ns |
| **Light and temperatures** | | | | | | | |
| Canopy openness | ns | ns | ns | 0.362** | 0.290* | 0.543*** | 0.359** |
| Direct radiation | ns | 0.240* | ns | 0.265* | ns | 0.558*** | 0.313** |
| Diffuse radiation | ns | ns | ns | 0.372** | 0.294** | 0.543*** | 0.373*** |
| Mean temperature | ns | ns | ns | 0.241* | 0.197* | 0.439*** | ns |
| Temperature amplitude | ns | ns | ns | 0.450*** | 0.277* | 0.267* | ns |
| **Vegetation** | | | | | | | |
| Plant species richness | ns | ns | ns | 0.541*** | ns | ns | ns |
| Plant species diversity | ns | 0.317* | ns | 0.391*** | ns | ns | ns |
| $E_1$ cover | ns | ns | ns | ns | 0.281* | 0.508*** | 0.442*** |
| $E_0$ cover | ns | ns | ns | ns | ns | ns | ns |
| Dead herb biomass | ns | ns | ns | ns | 0.288** | 0.720*** | 0.437** |
| Needle cover | ns | ns | ns | ns | ns | −0.357*** | −0.316*** |

Metabolized substrates (Biolog®): s22 – D-glucosaminic acid; s24 – glycyl-L-glutamic acid; s9 – Tween 40; s17 – α-cyclodextrin; s8 – L-asparagine; s6 – D-xylose; s30 – DL-α-glycerol phosphate.
Significance labels: ns – non-significant. $IP_{BA}$ – tree influence potential, $E_1$ – vascular plants and $E_0$ – bryophytes.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

**Table 4**
Redundancy analysis of soil microbial data (percentages of variance in microbial data explained by environmental variables).

| Variable | Marginal effects[a] | Conditional effects[b] |
|---|---|---|
| $IP_{BA}$ | 12.0* | 12.0* |
| Plant species richness | 10.7* | |
| Organic carbon | 7.6* | 9.1* |
| pH/KCl | 7.2ns | |
| pH/$H_2O$ | 6.0ns | |
| Temperature amplitude | 5.4ns | |
| Diffuse radiation | 5.1ns | |
| Canopy openness | 4.7ns | |
| K | 4.6ns | |
| $E_1$ | 3.6ns | |
| Dead herb biomass | 3.5ns | |
| Soil moisture | 2.8ns | |
| Direct radiation | 2.8ns | |
| P | 2.6ns | |
| Plant species diversity | 2.2ns | |
| N | 2.1ns | |
| $E_0$ | 2.1ns | |
| Needle cover | 1.9ns | |
| Mean temperature | 1.5ns | |

Significance labels: ns – non-significant.
[a] Marginal effect: percentage of variance explained by an individual environmental variable while used as the only constraining variable.
[b] Conditional effect: additional variance explained by the variable at the time it was included in the forward selection.
* $P < 0.05$.

needle cover. The lack of correlation between organic carbon and nitrogen content and microbial activity also indicates that it is quality rather than quantity of plant litter as a source of organic matter, which plays an essential role in microbial community development. What we measured was the total amount of organic carbon and total (organic and inorganic) nitrogen, contained in various compounds including relatively stable macromolecules. The proportion of labile compounds which are usually easily degradable and more recalcitrant components protected from decomposition may differ depending from the litter type. Remains of herbs are generally more easily decomposed than conifer needles and twigs. Conifer litter is generally more lignified, contains more epicuticular waxes and more compounds hampering enzyme activities, such as polyphenols and tannins (Kögel-Knabner, 2002). Moreover, patches with a high needle cover are poor in herb species. Consequently, soil under such sites receives less organic compounds in the form of root exudates or other root-borne substances, which may represent a substantial portion of carbon entering the soil (Kuzyakov and Domanski, 2000). Richness and functional diversity of the microbial community are positively correlated with plant species richness and diversity, but not with the amount of plant biomass as measured by the cover of living plants or dead plant residues. Apparently, these correlations are not associated with a higher productivity of plant communities exhibiting higher diversity, as suspected by Zak et al. (2003).

Community-level physiological profiling using the Biolog system has been criticized because it is actually a culture-based
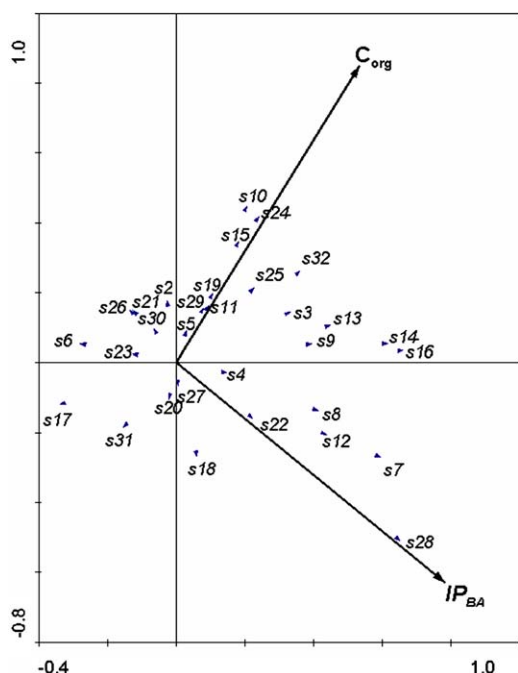
**Fig. 2.** Redundancy analysis of soil microbial data: functional groups positions and significant environmental variables which passed the forward selection. The diagram explains 21% of the total variance. Arrow tips show the positions of functional microbial groups (metabolizing specific substrates): s2, β-methyl-D-glucoside; s3, D-galactonic acid γ-lactone; s4, L-arginine; s5, pyruvic acid methyl ester; s6, D-xylose; s7, D-galacturonic acid; s8, L-asparagine; s9, Tween 40; s10, i-erythritol; s11, 2-hydroxybenzoic acid; s12, L-phenylalanine; s13, Tween 80; s14, D-mannitol; s15, 4-hydroxybenzoic acid; s16, L-serine; s17, α-cyclodextrin; s18, N-acetyl-D-glucosamine; s19, γ-hydroxybutyric acid; s20, L-threonine; s21, glycogen; s22, D-glucosaminic acid; s23, itaconic acid; s24, glycyl-L-glutamic acid; s25, D-cellobiose; s26, glucose-1-phosphate; s27, α-ketobutyric acid; s28, phenylethylamine; s29, α-D-lactose; s30, DL-α-glycerol phosphate; s31, D-malic acid; s32, putrescine.

method, as extracts with low microbial cell densities are inoculated, so that the outcomes depend on the initial physiological state of the microbes (Konopka et al., 1998). This is also one of the reasons why we focused on the mineral soil instead of organic layers, where the abundance and physiology of the decomposers is strongly affected by weather extremes and highly variable both in space and time at very short scales. Moreover, the method provides information only about the culturable fraction of the microbial community (with bias towards quite few taxonomical groups of bacteria) (Konopka et al., 1998; Ros et al., 2008).

Biolog® Eco Plates, although designed just for the analysis of the soil microbial community (Insam, 1997), contain quite few substrates specifically related to plant residues. D-xylose and D-galacturonic acid are components of the xylan-rich portion of hemicelluloses from plant cell walls and fibre. α-Cyclodextrin (cycloamylose) is a cyclic oligosaccharide, composed of six α-D-glucopyranoside units, and arises through enzymatic conversion of starch. D-cellobiose, a disaccharide consisting of two glucose units, is a product of a partial hydrolysis of cellulose. D-mannitol and i-erythritol are polyols (sugar alcohols) contained in plant tissues or produced during their fermentation. Most other substrates (carboxylic acids, aminoacids, saccharide-phosphates, etc.) are produced by or contained in most organisms or arise during their decomposition. However, considering the biomass distribution in terrestrial ecosystems, plant litter or root exudates are probably the main sources of these compounds in the soil as well. The use of only two of the plant-related substrates exhibited consistent response to light availability and soil temperatures as indicated by Mantel correlations: D-xylose and α-cyclodextrin. In both cases,

there seems to be a link to understory vegetation: the processing of D-xylose reacted positively to herb cover and the thickness of herb litter layer, and negatively to a high needle cover, whereas the use of α-cyclodextrin was positively associated with plant species richness and diversity.

Abiotic factors also affected the microbial community. In the case of the relationship between microbial activity and soil chemical properties, causality is not clear. Microbial community may have benefited from higher supplies of available phosphorus and potassium, but a higher content of these nutrients may also result from increased turnover rates due to microbial activity, functioning as a sort of positive feedback (Bever, 1994). Nevertheless, we did not find any association of soil chemistry with the process of secondary succession of plant communities. On the other hand, light and temperature regimes are clearly associated with the changes of the tree layer during the colonization and result in shifts of the functional composition of microbial communities (Sariyildiz et al., 2005; O'Neill et al., 2006). Soils under patches receiving higher amounts of solar energy contain more abundant microbial populations in a better physiological state, which is reflected in a higher activity even under standardized laboratory conditions.

Reports about microbial responses to environmental changes during primary or secondary succession are diverging. Directional changes of microbial biomass, respiration, nutrient turnover rates and other microbial activity indicators were observed mainly when the successional series resulted in a forest (Aikio et al., 2000; Pennanen et al., 2001; O'Neill et al., 2006). On the other hand, no patterns that could be interpreted in terms of succession were found, e.g., by Merilä et al. (2002) and Tscherko et al. (2003). Taxonomical composition of bacterial and fungal communities seems to be independent of the successional stage (Pennanen et al., 1999; Chabrerie et al., 2003), although unidirectional development was also observed (Li et al., 2006). However, many soil processes are covered by groups of taxonomically different microorganisms, which results in a functional redundancy of most microbial species (O'Donnell et al., 2005). Functional diversity of microbiota is thus more likely to be associated with environmental changes than microbial species diversity.

When tracing the causes of microbial community changes during the succession, it is mostly difficult to separate the effects of the successive changes of plant communities as a source of organic matter from the effects of the altered abiotic environment. Manipulative experiments can hardly mimic the course of succession with a sufficient precision. In fact, it is difficult to control the environment even within such experiments: e.g., the interception of radiation and precipitations or water losses from soil through evapotranspiration may strongly depend on the chosen plant species composition determining average height and density of understory vegetation within individual treatments. Nonetheless, distinguishing between these effects and assessment of their relative importance are essential for our understanding of the processes underlying the microbial community response to vegetation succession and possible feedback mechanisms between aboveground and belowground ecosystem components. This issue deserves further detailed fine-scale studies with a careful assessment of both plant chemistry and energy fluxes.

## 5. Conclusion

The response of the soil microbial community to changes of vegetation is not straightforward. The dynamics of soil microbiota is partly driven by intrinsic factors. However, the presented results demonstrated the relationship between the aboveground and belowground components of an ecosystem. Increasing density of the colonizing tree species lead to a decrease of soil microbial

activity, which is associated both with decreasing solar energy influx into the soil and changes of plant litter composition (spruce needles instead of more easily decomposed herb litter). At the same time, colonization by spruce induced directional changes in the representation of functional bacterial groups. Diversity and richness of functional microbial groups is positively associated with the species richness and diversity of the ground vegetation.

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