SCOTS PINE ROOTS MODIFY THE SHORT-TERM EFFECTS OF TEMPERATURE AND MOISTURE ON SOIL BACTERIA AND FUNGI

KLIMEK, B.*

Institute of Environmental Sciences, Jagiellonian University
Gronostajowa 7, 30-387 Kraków, Poland
(phone: + 4812664519; fax: + 48126646912)

**Corresponding author*
e-mail: beata.klimek@uj.edu.pl

(Received 2nd February 2011; accepted 7th February 2013)

Abstract. Root-delivered exudates affect soil microbial communities and may modify effects of environmental factors such as temperature and moisture on soil microbial communities functioning. There were tested the effects of 1-week incubation under different combinations of temperature (5, 15, 30°C) and moisture (15, 50, 100% Water Holding Capacity) on the soil planted with Scots pine (*Pinus sylvestris* L.) and of unplanted soil. After incubation, microbial activity and metabolic (functional) diversity using Biolog® ECO plates for bacteria and Biolog® SF-N2 plates for fungi were compared. Temperature and moisture affected microbial activity and metabolic diversity of the communities. In suboptimal combinations of temperature and moisture, the cultivable bacteria of planted soil exhibited higher activity and metabolic diversity than those of unplanted soil, and the cultivable fungi of planted soil exhibited higher metabolic diversity than those of unplanted soil.
Keywords: *rhizosphere, Biolog® plates, ECO, SF-N2, metabolic (functional) diversity*

Introduction

Soil microbial metabolism and, in turn, the rate of decomposition of soil organic matter is regulated the most by temperature and moisture as well as soil quality (Buchmann, 2000). The main relationships are well known, but interactions between environmental factors effects on soil microbial communities functioning are still problematic. Especially, studies on the thermal sensitivity of soil rarely comprise the interactions with other abiotic factors such as soil moisture or plant root presence in soil. The temperature effect on the soil microbial communities is strongly modified by soil moisture, as liquid water is necessary for virtually all metabolic reactions of living organisms. Both below and above optimal moisture, soil microbial activity is limited (Howard & Howard, 1993; Bouma & Bryla, 2000; Yuste et al., 2003). Also, some authors suggested that the thermal sensitivity of total soil respiration may depend on the relative contribution of roots and associated rhizosphere microbiota to the total soil CO_2 efflux (Boone at al., 1998). The rhizosphere is defined as few mm zone of influence of plant roots on soil (Kuzyakov, 2002). Plant roots presence considerably alter local soil properties such as nutrient concentration, pH and redox potential, as well as gases such as O_2 and CO_2 exchange (Hinsinger et al., 2006), but the most important phenomenon in the vicinity of roots is stimulation of microorganisms by various organic exudates (Kuzyakov, 2002; Hernesmaa et al., 2005). Antibiotics and hormone-like substances strongly affect the microorganisms of rhizosphere soil; organic acids excretion causes changes in pH and nutrient availability (Baudoin et al., 2001; Hinsinger et al., 2006).

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

Studies on the thermal sensitivity of soil have concentrated mostly on soil respiration (Bouma et al., 1997; Boone at al., 1998; Drewitt et al., 2002). However, the heterotrophic soil respiration rate is a result of the whole microbial community's metabolism as well as extracellular enzyme activity (Insam, 2001). Only a few studies describe the effects of temperature and moisture changes exclusively on soil bacteria, fungi or other soil microorganisms (Ranneklev & Bååth, 2001; Pietikäinen et al., 2005). Not a single study has examined and compared both temperature and moisture effects on microorganisms of the rhizosphere and root-free soil.

Some authors suggest different thermal optima for bacterial and fungal soil communities. However, this might be result of different carbon substrates use efficiency. Higher activation energy for a (bio)chemical reaction is needed to break chemical bounds in recalcitrant carbon compounds (Thornley & Cannel, 2001) and thus elevated temperature may affect the size of the carbon pool available to microorganisms (Ranneklev & Bååth, 2001; Waldrop & Firestone, 2004). Moreover, an increase of the soil respiration rate with a temperature increase above 25-30ºC may be caused by the functioning of extracellular enzymes, and not actual microbial activity (Ranneklev & Bååth, 2001; Pietikäinen et al., 2005).

For such type of studies, microbial techniques are needed which allow separating the actual effects of temperature and moisture on soil microbial communities from possible indirect effects connected with the soil environment. Measuring of ^{14}C-labelled leucine and thymidine incorporation rate to bacterial cells was used previous (Ranneklev & Bååth, 2001; Pietikäinen et al., 2005). The novel idea of my study was to apply the Biolog® microplates to test interactive temperature and moisture effects on bacteria and fungi of the rhizosphere and root-free soil. Both microbial community's activity and the functional (metabolic) diversity can be assessed with Biolog® microplates (Garland & Mills, 1991; Garland, 1996; Verschuere et al., 1997). These multiwell plates contain different carbon substrates; some of them are various soil compounds and plant root exudates such as l-asparginine, d-xylose or γ-hydroxy butyric acid (Campbell et al., 1997). The pattern of utilization of these substrates by soil microbes is specific for the soil microbial community (Glimm et al., 1997; Preston-Mafham et al., 2002), because breaking down of various organic compounds and rate of their usage is not only the sum of community members metabolism, but is also influenced by the interactions between microorganisms (Haack et al., 1995; Loreau & Hector, 2001). Since the method has been invented, it was used in a number of studies, for example on temperature effect on microbial communities of arctic soils (Tam et al., 2001), effects of toxic substances on soil microorganisms (Yao et al., 2003; Wang et al., 2004; Smith et al., 2005) or effects of different management intensity across a range of grasslands (Grayston et al., 2004).

The most common plates used in ecological studies are GN and ECO plates, which have been developed for the bacterial part of a microbial community, although new types known as SF and FF plates are especially recommended for fungi profile analyses (Preston-Mafham et al., 2002; Classen et al., 2003; Klimek & Niklińska, 2007; Stefanowicz et al., 2008). Although Biolog® plates are now commonly used in soil microbial ecology, it has to be stressed that the method met also some criticism (Konopka et al., 1998; Garland, 2001). One of the most important problems with the method is that it is not known how large fraction of soil microbial community is cultivable on Biolog® plates and, thus, to what extent results obtained with this method can be accepted as representative for the whole community. Notwithstanding the

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

weaknesses, the Biolog® method can be used at least as an indicator of (potential) changes in microbial community performance.

Materials and methods

Soil sampling and incubation

Nursery-garden was founded in autumn 2004 in the field station of the State Forests in Jodłówka, southern Poland. The forest soil organic layer with plant green residuals was used as the standard material for tree seedling cultivation. Soil chemical analyses included soil organic matter content, soil pH in water and in KCl, concentrations of major nutrients (Ca, Mg, Mn, K, Na, total C, N and S) and the C:N ratios (see Klimek et al., 2009). After mixing and sieving, the soil was put into 30 standard nursery containers (foil pots with holes in the bottom, volume ca 3 dm^3). Half of them were planted with year-old Scots pine (*Pinus sylvestris* L.) seedlings (5 seedlings per container), and the rest were left without seedlings. In autumn 2005, when the root system was spread through the entire soil volume, all containers were brought to the laboratory for soil sampling.

Bulk soil was sampled from the unplanted pots with a steel sampler from five points of the container. Sampling of planted soil required gentle cutting of the foil pot, careful removal of the plants and separation of soil from the roots. Soil samples were then sieved (2mm) and mixed to obtain two soil kind: planted/rhizosphere (R) and unplanted/bulk soil (B). Before further processing and analyses, the R and B soils were kept for two weeks in the laboratory at 4 °C to minimize the possible effects of sampling (Bloem et al., 2006). Afterwards, the R and B soil samples were incubated in climate chambers at three temperatures (5 °C, 15 °C, 30 °C) and three soil moisture levels (15%, 50%, 100% water holding capacity, WHC), giving altogether eighteen treatments in a full-factorial design. There were three replicates for each combination of soil type, temperature and moisture treatment, for a total of 54 samples. Water holding capacity was measured by a standard gravimetric method using 50 g subsamples. Sample moisture was adjusted with deionised water daily. The samples were incubated for a week; the short incubation time was intended to minimise changes in microbial communities caused by prolonged soil storage, especially at higher temperatures (Pietikäinen et al., 2005). Moreover, rhizosphere soil is known to contain easy degradable carbon sources such as root exudates, dead microbial cells and fine-roots. These resources can be quickly degraded and exhausted under favourable conditions; during prolonged soil incubation the microorganisms of planted soil may be subjected to additional, unplanned stress.

Biolog® plates

The effects of temperature and moisture on the activity and the functional (metabolic) diversity of the microbial communities were investigated using two types of Biolog® plates: ECO and SF-N2 (http://www.biolog.com). The bacterial ECO plates were designed especially for ecological applications and contain three replicate sets of 31 carbon substrates (with tetrazolium dye as the substrate utilization indicator), degradable by different soil bacteria. SF-N2 fungal plates, with 95 sole carbon substrates, have no redox indicator; substrate utilisation is measured as turbidity development.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

The samples of each soil (equivalents of 5 g dwt) were shaken for 1h in 50 ml of 10 mM Bis Tris buffer (pH 7) and allowed to settle for half an hour to decant soil particles. The supernatants containing microbes were frozen immediately at -70 ºC in liquid nitrogen (Van Beelen et al., 2004). The supernatant freezing was applied because experiment described here was one of the series of experiments and it was indispensable to laboratory work organisation. During inoculum preparation the supernatants were defrosted at room temperature and diluted (10^{-2}) in 10 mM Bis Tris solution. The extracts for fungal plates were diluted in Bis Tris buffer with added antibiotics: 1 µg streptomycin sulphate and 0.5 µg chlortetracycline per microtiter plate well (Classen et al., 2003). Addition of the antibiotics is recommended to prevent bacterial growth and development. The solutions were inoculated into the Biolog® plates (125 µl per well in ECO plates or 100 µl per well in SF-N2 plates). All plates were incubated in the dark at 22 °C. All chemical solutions, transfer equipment and glassware were sterilised prior to use, and inoculations were completed under a laminar-flow chamber to minimize the risk of biological contamination.

Substrate utilisation was measured as light absorbance (µQuant spectrometer; BIO-TEK Instruments) at 590 nm for the ECO plates as colour development, and at 750 nm for the SF-N2 plates as turbidity development (mycelium growth). The first measurement was carried out 12 hours after inoculation, and the subsequent readings were taken at 12 h intervals for 168 h for ECO and for 240 h for SF-N2 plates. The absorbance and turbidity measurements for individual wells were corrected against the control well containing only microbial solution. Absorbance values below 0.06 (spectrometer detection limit) were taken as 0.

The following microbial indices were calculated for each sample for bacterial and fungal plates: average well c*olour* development (AWCD) for the ECO plates, average well *turbidity* development (AWTD) for the SF-N2 plates, area under the curve (AUC), functional diversity index H', substrate richness r_s and index of evenness I' (see below). AWCD/AWTD and AUC are used mostly as indicators of general microbial activity (Hackett & Griffiths, 1997; Kong et al., 2006), while H', r_s and I' are indicators of functional (metabolic) diversity based on the structure of substrate use (Dauber & Wolters, 2000; Tam et al., 2001; Bradley et al., 2006).

Because both the density and activity of microbial cells affect the rate of colour/turbidity development, community level physiological profiles were compared at the same plate AWCD or AWTD to compensate for differences in initial inoculum density (Garland, 1997; Preston-Mafham et al., 2002). AWCD or AWTD were calculated for the ECO and SF-N2 plates as:

$$AWCD, AWTD = \sum_{i=1}^{n} \frac{A_n}{n} \qquad \text{(Eq.1)}$$

where A_n is the absorbance of each individual well and n represents the number of substrates on the plate (31 for ECO or 95 for SF-N2). Community-level physiological profiles were compared at AWCD = 0.2 or AWTD = 0.2, irrespectively of incubation time, to compensate for possible differences in initial inoculum density.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

The bacterial and fungal activity was calculated as area under the curve (AUC):

$$AUC = \sum_{i=1}^{n} \frac{(A_n + A_{n+1})}{2} \times (t_{n+1} - t_n) \quad \text{(Eq.2)}$$

where A_n and A_{n+1} are the absorbances of each individual well at two consecutive measurements at times t_n and t_{n+1} (Hackett & Griffiths, 1997). The microbial functional diversity index, based on the Shannon-Wiener biodiversity index H', was derived from the number of substrates used on a plate (s) and the proportion of utilisation of an individual substrate (p_s), calculated as absorbance for a given well divided by the sum of absorbance for all wells (Zak et al., 1994; Kong et al., 2006):

$$H' = -\sum_{i=1}^{s} p_s (\log_{10} p_s) \quad \text{(Eq.3)}$$

Substrate richness r_s is represented by the number of degraded substrates (up to 31 for ECO or up to 95 for SF-N2) and the I' index of evenness (Zak et al., 1994; Kong et al., 2006) was calculated as:

$$I' = \frac{H'}{\log_{10} r_s} \quad \text{(Eq.4)}$$

Each index describes the different aspect of a soil microbial community functioning. The indices may be correlated, but these correlations are not automatic. For example, similar AUC or AWCD/AWTD can be reached when many substrates with low microbial activity are used, or when small number of substrates are utilised intensively (Duelli & Obrist, 2003).

Statistical analyses

For each parameter describing the activity and functional (metabolic) diversity of bacterial and fungal soil microbial communities, three-way ANOVA was used to test the significance of the effects of the soil kind (R, B), temperature (5 °C, 15 °C, 30 °C), moisture (15%, 50%, 100% WHC) and their interactions. Right- or left-skewed data were transformed to fulfil the normality criterion. When differences were significant, the means were compared using Tukey's HSD test. Nonsignificant interactions were removed from the models. When significant interactions were detected, the data were analyzed with one- or two-way ANOVA separately for each soil type to assess the effects of temperature and moisture on the measured microbial parameter. The sequential Bonferroni procedure was used to test for statistical significance within microbial metabolic diversity indices (H', r_s, I') calculated for data obtained with types of Biolog® plates (ECO or SF-N2). To assign corrected alpha levels, the ANOVA results for each tested effect were ranked from smallest to largest; then the smallest p value was tested at α/n, the next one at $\alpha/(n-1)$ and the highest one at $\alpha/(n-2)$, where n is number of tests (n=3 for metabolic diversity indices). Testing stopped when a nonsignificant result occurred. All statistical analyses employed Statgraphics ver. 5.1.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

Results

Effects of temperature and moisture on bacteria activity

The metabolic activity of bacteria was assessed with Biolog® ECO plates and metabolic activity was calculated as area under the curve (AUC). Mean AUC (n=3) was the lowest for B soil incubated at 5 ºC / 15% WHC (AUC=21.43) and the highest for R soil incubated at 30 ºC / 100% WHC (AUC=120.34). Multifactor ANOVA showed a significant effect of soil type ($F=16.15$, $p<0.0003$), temperature ($F=8.55$, $p<0.0008$) and moisture ($F=12.15$, $p<0.0002$). The significantly lower bacterial AUC was measured after soil incubation at 5ºC than at 15ºC and 30ºC. However, there was also a significant interaction between soil type and moisture ($F=4.89$, $p<0.02$), indicating that moisture affected the bacterial activity of R and B soils differently (*Fig. 1A*).

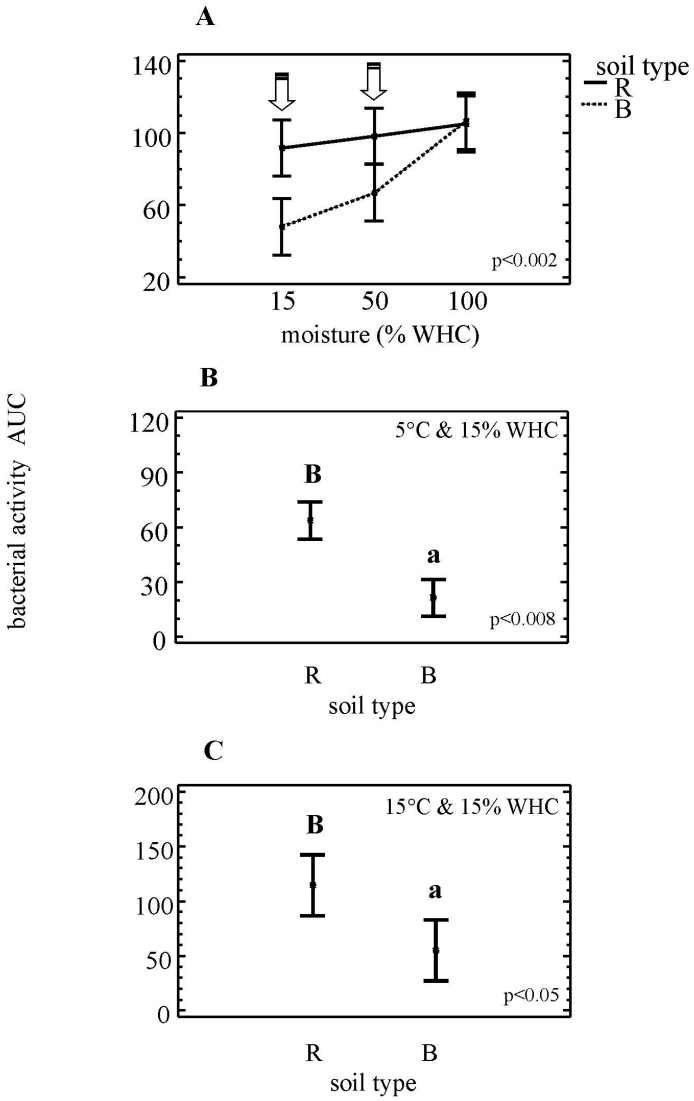

Figure 1. *Effect of soil incubation conditions on bacterial metabolic activity AUC in rhizosphere (R) and bulk (B) soil; (A) interaction between soil type and moisture (differences between soils are marked with arrows) and differences between soils after incubation in combinations of temperature and moisture (B) 5°C and 15% WHC and (C) 15°C and 15% WHC. Central points indicate the sample means, and error bars indicate 95% Tukey HSD intervals. Different letters above bars indicate significant differences*

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

When the data were analysed separately for R and B soil, only temperature effect was found for R soil ($F = 4.23$, $p < 0.03$), while both temperature ($F = 4.17$, $p < 0.03$) and moisture ($F = 19.61$, $p < 0.0001$) were significant for B soil. Bacteria activity in both soils increased with temperature: $AUC_{5°C} < AUC_{30°C} < AUC_{15°C}$. In B soil it also increased with moisture.

One-way ANOVA showed that bacterial AUC in R and B soil differed in some combinations of temperature and moisture. There were statistically significant differences between tested soils at 5 ºC / 15%WHC ($F = 33.14$, $p < 0.005$) and at 15 ºC / 15%WHC ($F = 8.79$, $p < 0.05$), and in both cases bacterial AUC was higher in planted soil (*Fig. 1B, C*).

Table 1. *Metabolic activity of bacteria (H', r_s, I') after incubation of rhizosphere (R) and bulk (B) soil at different temperatures and moisture levels; results of 3-way ANOVA, F and p values for main factors and interactions. Bonferroni-corrected alpha levels for microbial activity indices are given in italics after ANOVA results for tested effect. Within groups, values with different boldface letters differ significantly at p≤0.05 (Tukey test); ns – nonsignificant interaction (removed from a model)*

effect	**bacterial metabolic diversity (mean values)**		
	H'	**r_s**	**I'**
soil type (n=27)	F=2.90, p=0.0952 (*p<0.025*)	F=9.30, p<0.004 (*p<0.01667*)	F=0.02, p=0.8911
R	1.15	24.8 **a**	0.81
B	1.12	27.2 **b**	0.81
temperature (°C) (n=18)	F=2.78, p=0.0723 (*p<0.025*)	F=4.13, p=0.0224 (*p<0.01667*)	F=0.30, p=0.7403
5	1.14	25.7	0.81
15	1.10	24.6	0.80
30	1.16	27.7	0.81
moisture (%WHC) (n=18)	F=5.08, p<0.011 (*p<0.025*)	F=0.08, p<0.93	F=9.20, p<0.0005 (*p<0.01667*)
15	1.17 **b**	25.7	0.84 **b**
50	1.14 **ab**	25.9	0.82 **b**
100	1.09 **a**	26.3	0.77 **a**
soil x temp	ns	ns	ns
soil x moist	ns	F=4.07, p<0.03 (*p<0.05*)	ns
temp x moist	ns	ns	ns

Effects of temperature and moisture on fungi activity

The metabolic activity of fungi was assessed with Biolog® SF-N2 plates and calculated as AUC. Mean fungal activity (n = 3) was the lowest in B soil incubated at 5ºC / 50% WHC (AUC = 105.22), and the highest in R soil incubated at 5 ºC / 50% WHC (AUC=177.74). Multifactor ANOVA showed a significant effect of soil type ($F=39.14$, $p < 0.0001$) with no significant effects of temperature ($F = 0.98$, $p < 0.39$) or moisture ($F = 1.65$, $p < 0.21$). The interactions were nonsignificant. Fungi from R soil exhibited higher activity than fungi from B soil, but soil incubation conditions did not affect fungal activity as measured on Biolog® plates.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

Effects of temperature and moisture on bacteria metabolic diversity

Bacterial metabolic diversity was measured with Biolog® ECO plates and calculated as the functional diversity index (H'), number of substrates used (substrate richness r_s) and index of evenness (I'). The results of the three-way ANOVA for the effects of soil type, temperature and moisture and their interactions for the bacterial metabolic diversity indices are presented in *Table 1*.

Mean bacterial H' (n=3) ranged from 1.05 (R soil incubated at 5 ºC / 100% WHC) to 1.27 (R soil incubated at 5 ºC / 15% WHC). Only moisture affected bacterial H', which was highest in dry conditions (15% WHC) and decreased with the increase of moisture (*Tab. 1*).

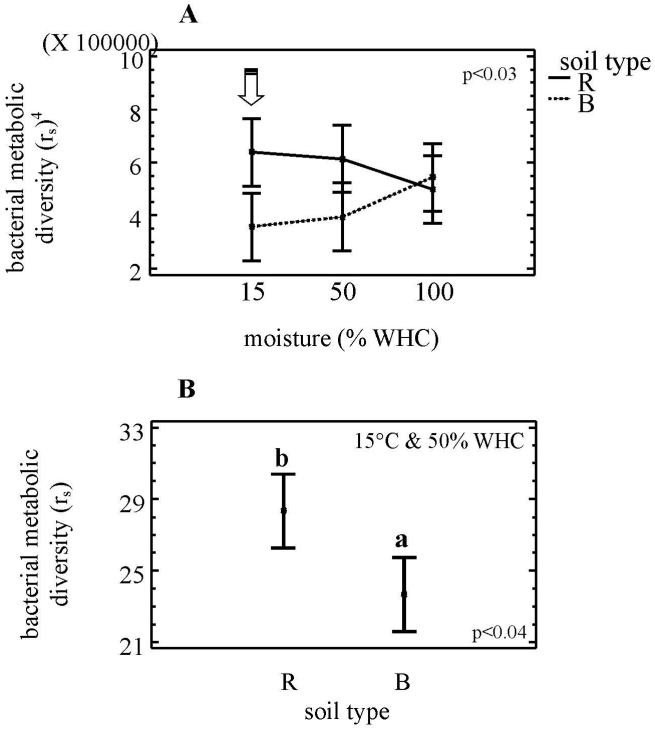

Figure 2. *Effect of soil incubation conditions on bacterial metabolic diversity r_s in rhizosphere (R) and bulk (B) soil; (A) interaction between soil type and moisture (difference between soils is marked with arrow) and (B) difference between soils after incubation in combinations of temperature 5°C and moisture 15% WHC. Central points indicate the sample means, and error bars indicate 95% Tukey HSD intervals. Different letters above bars indicate significant differences*

The mean number (n=3) of substrates used by bacteria on ECO plates (substrate richness, r_s) ranged from 13 (B soil incubated at 15 ºC / 15% WHC) to 29 (R soil incubated at 5 ºC / 15% WHC). There was a significant effect of soil type (*Tab. 1*), and a significant interaction between soil type and moisture (*Fig. 2A*). The data were then analysed separately for R and B soils by two-way ANOVA to check for effects of temperature and moisture on bacterial r_s. There was no significant effect of temperature or moisture for R soil, but the interaction between temperature and moisture was significant at $p < 0.03$ ($F = 3.45$). Consequently, the effect of moisture on bacterial r_s in R soil was analysed separately for each temperature; it appeared significant only at 15ºC ($F = 42.25$, $p < 0.0004$) with r_s increasing in the order: 100% WHC < 15% WHC < 50%

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

WHC. The effect of temperature on R soil tested for a given moisture level was nonsignificant. For B soil the interaction term was nonsignificant, and bacterial r_s was affected by temperature only ($F = 4.52$, $p < 0.03$). Bacterial r_s was the highest at 30 ºC and successively lower at 5 ºC and 15 ºC.

The differences between bacterial r_s in R and in B soils were analysed for particular combinations of temperature and moisture. Bacterial r_s was higher in R than in B soil at 5 ºC / 50% WHC ($F = 9.80$, $p < 0.04$); in those rather cold and moderately wet conditions, bacterial communities of R soil used significantly more substrates (*Fig. 2B*).

The index of evenness (I') for bacterial metabolic diversity ranged from 0.67

(B soil at 30 ºC / 100% WHC) to 0.91 (B soil at 5 ºC / 15% WHC). There was only a significant effect of soil moisture, and bacterial I' decreased with increasing moisture (*Tab. 1*).

The metabolic diversity of bacteria was the highest in dry conditions and decreased with increased moisture (H', I'); r_s differed most between R and B soil in cold and moderately wet conditions.

Table 2. *Metabolic activity of fungi (H', r_s, I') after incubation of rhizosphere (R) and bulk (B) soil at different temperatures and moisture levels; results of 3-way ANOVA, F and p values for main factors and interactions. Bonferroni-corrected alpha levels for microbial activity indices are given in italics after ANOVA results for tested effect. Within groups, values with different boldface letters differ significantly at p≤0.05 (Tukey test); ns – nonsignificant interaction (removed from a model).*

effect	**fungal metabolic diversity (mean values)**		
	H'	**r_s**	**I'**
soil type (n=27)	F=13.29, p<0.0008 (*p<0.01667*)	F=10.15, p<0.003 (*p<0.025*)	F=7.49, p<0.009 (*p<0.05*)
R	1.76 **b**	85.8 **b**	0.912 **b**
B	1.71 **a**	81.0 **a**	0.898 **a**
temperature (°C) (n=18)	F=1.49, p=0.2348 (*p<0.025*)	F=10.77, p<0.0002 (*p<0.01667*)	F=1.16, p=0.3234
5	1.73	84.6 **b**	0.900
15	1.73	78.7 **a**	0.911
30	1.75	86.9 **b**	0.904
moisture (%WHC) (n=18)	F=0.95, p=0.3920 (*p<0.01667*)	F=0.68, p=0.5110	F=0.65, p=0.5283
15	1.73	82.3	0.904
50	1.73	83.6	0.903
100	1.75	84.4	0.909
soil x temp	ns	ns	ns
soil x moist	ns	ns	F=3.21, p<0.05 (*p<0.05*)
temp x moist	ns	ns	ns

Effects of temperature and moisture on fungi metabolic diversity

As in the case of bacteria, fungal metabolic diversity, measured with Biolog® SF-N2 plates, was expressed as H', r_s and I'. The results of three-way ANOVA for the effects of the soil type, temperature and moisture and their interactions on the fungal metabolic diversity indices are presented in *Table 2*.

Mean fungal H' (n = 3) ranged from 1.61 (B soil at 5 ºC / 100% WHC) to 1.85 (R soil at 30 ºC / 100% WHC). Only soil type affected fungal H' significantly (*Fig. 3A*), and it was higher in R soil (*Tab. 2*).

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

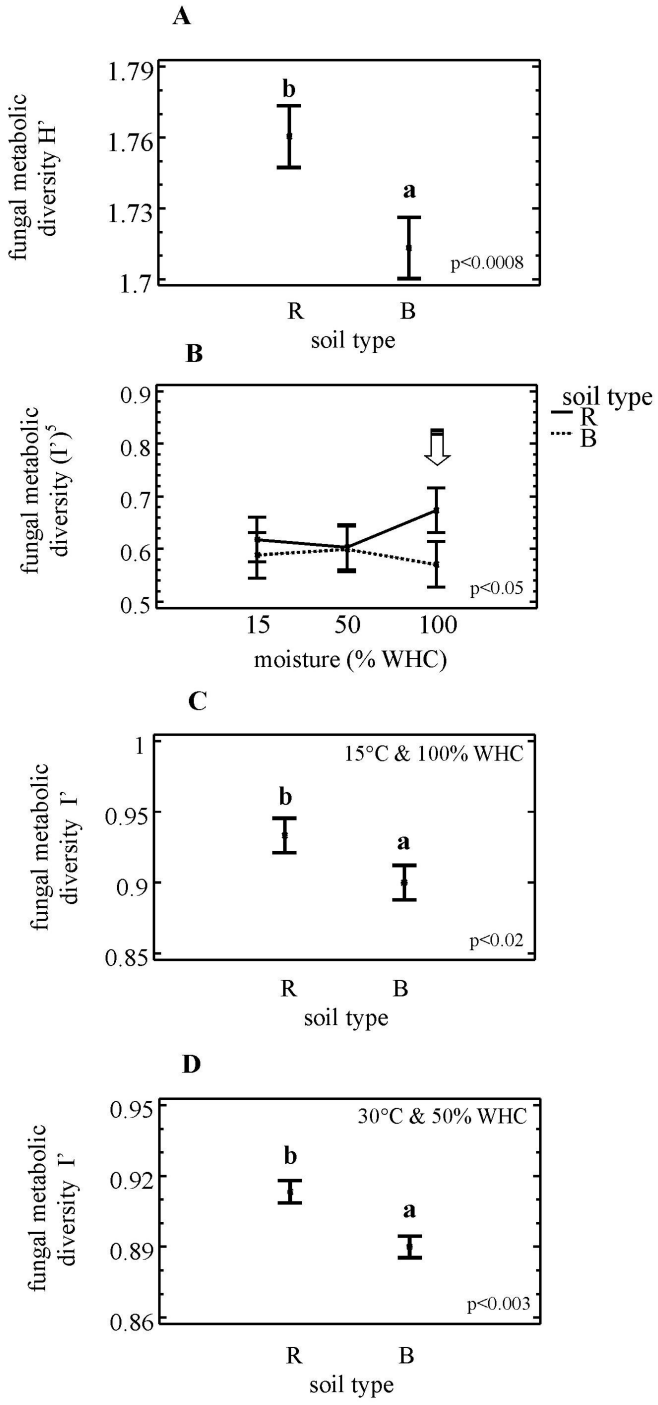

Figure 3. *Effect of soil incubation conditions on fungal metabolic diversity in rhizosphere (R) and bulk (B) soil; (A) difference between soils in fungal metabolic diversity H' (B) interaction between soil type and moisture effect on fungal metabolic evenness I' (difference between soils is marked with arrow) and differences between soils in fungal I' after incubation in combinations of temperature and moisture (C) 15 °C and 100% WHC and (D) 30 °C and 50% WHC. Central points indicate the sample means, and error bars indicate 95% Tukey HSD intervals. Different letters above bars indicate significant differences*

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

The mean number (n = 3) of SF-N2 plate substrates used (r_s) ranged from 75 (B soil at 15 ºC / 50% WHC) to 91 (R soil at 30 ºC / 50% WHC). Interactions and the effect of moisture were nonsignificant; significantly more substrates were used by fungi in R soil, and at 5 ºC and 30 ºC (*Tab. 2*).

Mean (n = 3) fungal metabolic evenness (I') ranged from 0.87 (B soil at 5 ºC / 100% WHC) to 0.94 (R soil at 15 ºC / 100% WHC). Multifactor ANOVA for I' showed a significant effect of soil type (*Tab. 2*), but due to the interaction between soil type and moisture (*Fig. 3B*) the data had to be analysed separately for each soil with two-way ANOVA. It revealed a significant effect of moisture in R soil ($F = 3.45$, $p < 0.05$); neither temperature nor moisture affected fungal I' in B soil. For both soils there was no interaction between temperature and moisture. Metabolic evenness was the highest in fungal communities from R soil after soil incubation at 100% WHC and successively lower at 15% WHC and 50% WHC.

Fungal I' was analysed by one-way ANOVA also to find the combinations of temperature and moisture under which R and B soils differed most. It was also higher in R than in B soil at 15 ºC and 100%WHC ($F = 14.19$, $p < 0.02$) and at 30 ºC and 50%WHC ($F = 43.79$, $p < 0.003$) (Fig. 3C, D).

Summing up, fungi from R soil exhibited higher metabolic diversity than fungi from B soil. Soil incubation temperature affected fungal metabolic diversity in both soils, but moisture affected only R soil. Fungal I' in R soil was especially higher in warm and wet incubation conditions.

Discussion

This study tested the effects of different temperature and moisture incubation regimes on the main soil microbial groups: bacteria and fungi from rhizosphere and bulk soil. The general effects of Scots pine roots presence on soil microorganisms were related mostly to the fungal communities. Fungi from rhizosphere soil cultivated on Biolog® SF-N2 plates were characterized by higher activity (AUC) and higher metabolic diversity (H', r_s) than those from bulk soil under all soil incubation conditions. The higher fungal activity and functional diversity were probably due to the contribution of ectomycorrhizal fungi to the microbial communities in soil planted with Scots pine seedlings, which were supplied from a field culture near a forest and were cultivated in organic soil containing the original forest soil organic layer (humus) supplemented with green manure. Forest humus contains a high concentration of mycorrhizal propagules (Priha et al., 1999). Some symbiotic ectomycorrhizal fungi are obligate symbionts in natural conditions, but can still grow slowly on a synthetic medium containing essential nutrients that can be absorbed by their mycelia (Morton, 1999). The effects of Scots pine roots on the remaining microbial community indices was not so obvious. Because of significant interactions with soil incubation conditions, differences between R and B soil were apparent only for certain combinations of temperature and moisture.

Using Biolog® plates allow separating the actual effects of temperature and moisture on soil microbial communities from possible indirect effects connected with the soil environment. Bacterial and fungal growth rates on Biolog® plates were measured at one standard temperature, irrespective of the earlier soil incubation temperature, so any differences found can be attributed to actual changes in the communities due to the

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

experimental treatments. Higher soil incubation temperature caused bacteria subsequently cultivated on Biolog® ECO plates to exhibit higher activity (AUC). However, difference was significant between 5ºC and both higher tested temperatures and there were no difference in bacterial AUC after soil incubation at 15ºC and 30ºC. That effect could be due to faster decomposition of some substrates and/or expansion of the ability of bacteria to metabolise more compounds.

Different indicators of functional (metabolic) diversity are useful for assessing how the structure of substrate utilisation on Biolog® plates is affected. In this study, no general temperature effect on bacterial functional diversity (H', I') was found. Only bacterial communities of bulk soil exhibited some differences; the number of substrates used (r_s) was the lowest after incubation at 15ºC, ascending in the order 15 ºC < 5 ºC < 30 ºC. The indices of functional metabolic diversity were calculated for AWCD reference values, so the observed differences cannot be the result of higher inoculum density caused by extraction of higher bacterial biomass from soil samples incubated at 15 ºC than at 5 ºC and 30 ºC.

Moisture affected both bacterial AUC and functional diversity indices. The bacterial AUC of bulk soil increased with soil incubation moisture, and for both soils bacterial functional diversity expressed as H' and I' decreased with moisture increase. The number of substrates (r_s) used by bacteria from rhizosphere soil was highest under moderate moisture (50% WHC). At optimal temperature and carbon compounds abundance in soil, the moisture was the limiting factor. Incubation conditions thus must have exerted selection pressure altering the bacterial communities. Bacterial species more adapted to new conditions grow faster, and those not well adapted are outcompeted (Pettersson & Bååth, 2003). However, dominant bacterial species competing for a limited pool of easy degradable carbon compounds can have lower potential metabolic diversity (Fontaine et al., 2003). Generally, the higher the bacterial AUC, the lower the functional diversity in the bacterial communities studied. Possibly the high activity is caused mainly by a few bacterial groups characterized by high efficiency of competition for certain resources present on Biolog® ECO plates.

The effect of incubation conditions on fungal communities was not as pronounced as for bacterial communities; only temperature had some effect on fungal metabolic diversity in both R and B soil. In general, soil incubation temperature was more pronounced as a factor than moisture. The number of substrates used by fungi (r_s) on SF-N2 plates was the lowest after previous soil incubation at 15 ºC, ascending in the order: 15 ºC < 5 ºC < 30 ºC, similarly to bacteria. Apart from that, temperature and moisture had no effect on fungal activity and functional diversity. This result is consistent with the common opinion that fungi are less sensitive than bacteria to such short-term changes in environmental conditions (Vishnevetsky & Steinberger, 1997).

The effects of particular soil incubation conditions on microbial community parameters were compared between R and B soil. In each case where microbial community parameters were found to differ between soil types, the microbial communities of R soil exhibited higher activity or functional diversity as measured with Biolog® plates than B soil, and these differences were seen especially in combinations of suboptimal temperature and moisture. In other words, the results from Biolog® plates may confirm the higher resistance of more diverse communities to stressful environmental conditions (MacArthur, 1955; Pimm, 1984; McCann, 2000; Steiner et al., 2006). Resilience is a component of stability and is defined as the ability of a

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

community to recover over time after a stress, whilst resistance means its ability to withstand immediate stress effects (Seybold et al., 1999).

After incubation of soils at 5 °C / 15% WHC and at 15 °C / 15% WHC, bacterial communities from soil planted with Scots pine exhibited higher activity (AUC) than bacteria from unplanted soil when cultivated on ECO plates, and they are more drought-tolerant. The presence of high amounts of substrates (root exudates) may make a soil microbial community less sensitive to lower soil moisture for example by allowing the microbes to produce a greater amount of protective slime (Chen et al., 2007).

Bacterial functional diversity (r_s) was significantly higher in R than in B soil after incubation at 5 ºC / 50% WHC. Fungal metabolic diversity on Biolog® SF-N2 plates, calculated as the index of evenness (I'), was higher for fungal communities of R than of B soil at two combinations of soil incubation conditions: 15 ºC / 100% WHC and 30ºC / 50% WHC. These results suggest different optima for bacterial and fungal communities. Pietikäinen et al. (2005) found that fungi and bacteria had optimum temperatures around 25-30 ºC. At temperatures higher than that, the decrease in growth rate was more drastic for fungi than for bacteria; at low temperatures the trend was the reverse, indicating that fungi were more adapted to low temperature than bacteria. Those results are consistent with mine: bacteria seemed more sensitive to cold, whereas for fungi warmer conditions were more stressful. Despite bacteria are generally more sensitive to cold than fungi, in temperature 5 ºC the bacterial communities of R soil were characterized by higher functional diversity (r_s) than these of B soil.

It remains an open question whether the differences in substrate utilisation observed on Biolog® plates reflect actual functional differences in soil microbial communities (Classen et al., 2003). The Biolog® technique measures the potential rather than actual carbon sources utilisation pattern, and caution is needed in extrapolating the data to actual carbon source availability in soil (Garland, 1996). The Biolog® technique, like other culture-dependent methods, tends to select fast-growing microorganisms capable of living in high concentrations of substrates, so higher microbial activity and metabolic diversity in rhizosphere microorganisms is often observed (Verschuere et al., 1997; Tam et al., 2001). Rhizobacteria are adapted to environments rich in easily degradable C compounds; better growth and development of rhizosphere bacteria on artificial media has been noted by Grayston et al. (1998). Some of the substrates on Biolog® plates are such root exudates such as l-asparginine, d-xylose or γ-hydroxy butyric acid (Campbell et al., 1997). Benizri et al. (2002) showed that adding root exudates to soil, with the effects of other chemical factors eliminated, can stimulate soil bacteria. Further work should examine soil biological responses to temperature and soil moisture changes in more detail, especially in terms of the different fractions of soil organic matter, which are suspected to differ in their sensitivity to climate.

Acknowledgements. This study was supported by a grant from the Polish State Committee for Scientific Research (4650/P04/2005/29) and Institute of Environmental Sciences, Jagiellonian University (DS-758).

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

REFERENCES

[1] Baudoin, E., Beizri, E., Guckert, A. (2001): Metabolic fingerprint of microbial communities from distinct maize rhizosphere compartments. – European Journal of Soil Biology 37: 85-93.

[2] Benizri, E., Dedourge, O., Dibattista-Leboeuf, C., Piutti, S., Nguyen, C., Guckert, A. (2002): Effect of maize rhizodeposits on soil microbial community structure. – Applied Soil Ecology 21: 261-265.

[3] Bloem, J., Hopkins, D.W., Benedetiti, A. (2006): Microbiological Methods for Assessing Soil Quality. – CABI Publishing, 875 Massachusetts Avenue, Cambridge MA 02139 USA CAB International, Wallingford, Oxfordshire OX10 8DE, UK.

[4] Boone, R., Nadelhoffer, K., Canary, J., Kayne, J. (1998): Root exert a strong influence on the temperature sensitivity of soil respiration. – Nature 396: 570-572.

[5] Bouma, T.J., Nielsen, K.L., Eissenstat, D.M., Lynch, J.P. (1997): Estimating respiration of roots in soil: Interactions with soil CO_2, soil temperature and soil water content. – Plant and Soil 195: 221-232.

[6] Bouma, T.J., Bryla, D.R. (2000): On the assessment of root and soil respiration for soils of different textures: interactions with soil moisture contents and soil CO_2 concentrations. – Plant and Soil 227: 215-221.

[7] Bradley, R.L., Shipley, B., Beaulieu, C. (2006): Refining numerical approaches for analyzing soil microbial community catabolic profiles based on carbon source utilization patterns. – Soil Biology and Biochemistry 38: 629-632.

[8] Buchmann, N. (2000): Biotic and abiotic factors controlling soil respiration rates in *Picea albies* stands. – Soil Biology and Biochemistry 32: 1625-1635.

[9] Campbell, C.D., Grayston, S.J., Hirst, D.J. (1997): Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. – Journal of Microbial Methods 30: 33-41.

[10] Chen, M.M., Zhu, Y.G., Su, Y.H., Chen, B.D., Fu, B.J., Marschner, P. (2007): Effect of soil moisture and plant interactions on the soil microbial community structure. – European Journal of Soil Biology 43: 31-38.

[11] Classen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T., Hart, S.C. (2003): Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences contrasting soils. – FEMS Microbiology Ecology 44: 319-328.

[12] Dauber, J., Wolters, V. (2000): Microbial activity and functional diversity in the mounds of three different ant species. *Soil Biology and Biochemistry* 32: 93-99.

[13] Drewitt, G.B., Black, T.A., Nesic, Z., Humphreys, E.R., Jork, E.M., Swanson, R., Ethier, G.J., Griffis, T., Morgenstern, K. (2002): Measuring forest floor CO_2 fluxes in a Douglas-fir forest. – Agricultural and Forest Meteorology 110: 299-317.

[14] Duelli, P., Obrist, M.K. (2003): Biodiversity indicators: the choice of values and measures. – Agriculture Ecosystem Environment 98: 87-98.

[15] Fontaine, S., Mariotti, A., Abbadie, L. (2003): The priming effect of organic matter: a question of microbial competition? – Soil Biology and Biochemistry 35: 837-843.

[16] Garland, J.L., Mills, A.L. (1991): Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. – Applied and Environmental Microbiology 57: 2351-2359.

[17] Garland, J.L. (1996): Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. – Soil Biology and Biochemistry 28: 213-221.

[18] Garland, J.L. (1997): Analysis and interpretation of community-level physiological profiles in microbial ecology. – FEMS Microbiology Ecology 24: 289-300.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

[19] Garland, J.L., Mills, A.L., Young, J.S. (2001): Relative effectiveness of kinetic analysis vs single point readings for classifying environmental samples based on community-level physiological profiles (CLPP). – Soil Biology and Biochemistry 33: 1059-1066.
[20] Glimm, E., Heuer, H., Engelen, B., Smalla, K., Backhaus, H. (1997): Statistical comparisons of community catabolic profiles. – Journal of Microbial Methods 30: 71-80.
[21] Grayston, S., Shenquiang, W., Campbell, C., Edwards, A. (1998): Selective influence of plant species on microbial diversity in the rhizosphere. – Soil Biology and Biochemistry 30: 369-378.
[22] Grayston, S.J., Campbell, C.D., Bardgett, R.D., Mawdsley, J.L., Clegg, S.D., Ritz, K., Griffiths, B.S., Rodwell, J.S., Edwards, S.J., Davies, W.J., Elston, D.J., Millard, P. (2004): Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. – Applied Soil Ecology 25: 63-84.
[23] Hackl, E., Pfeffer, M., Donat, C., Bachmann, G., Zeichmeister-Boltenstern, S. (2005): Composition of the microbial communities in the mineral soil under different types of natural forest. – Soil Biology and Biochemistry 37: 661-671.
[24] Hackett, C.A., Griffiths, B.S. (1997): Statistical analysis of the time-course of Biolog substrate utilization. – Journal of Microbial Methods 30: 67-69.
[25] Hernesmaa, A., Björklöf, K., Kiikkilä, O., Fritze, H., Haahtela, K., Romantschuk, M. (2005): Structure and function of microbial communities in the rhizosphere of Scots pine after tree-felling. – Soil Biology and Biochemistry 37: 777-785.
[26] Hinsinger, P., Plassard, C., Jaillard, B. (2006): Rhizosphere, A new frontier for soil biogeochemistry. – Journal of Geochemical Exploration 88: 210-213.
[27] Howard, D.M., Howard, P.J.A. (1993): Relationships between CO_2 evolution, moisture content and temperature for a range of soil types. – Soil Biology and Biochemistry 25: 1537-1547.
[28] Insam, H. (2001): Developments in soil microbiology since the mid 1960s. – Geoderma 100: 389-402.
[29] Klimek, B., Niklińska, M. (2007): Zinc and copper toxicity to soil bacteria and fungi from zinc polluted and unpolluted Soils – a comparative study with different types of Biolog plates. – Bulletin of Environmental Contamination and Toxicology 78: 102-107.
[30] Klimek, B., Choczyński, M., Juszkiewicz, A. (2009): Scots pine (*Pinus sylvestris* L.) roots and soil moisture did not affect soil thermal sensitivity. – European Journal of Soil Biology 45: 442-447.
[31] Kong, W.D., Zhu, Y.G., Fu, B.J., Marschner, P., He, J.Z. (2006): The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. – Enironmental Pollution 143: 129-137.
[32] Konopka, A., Oliver, L., Turco, R.F. (1998): The use of carbon substrate utilization patterns in environmental and ecological microbiology. – Microbiology Ecology 35: 103-115.
[33] Kuzyakov, Y. (2002): Review: factors affecting rhizosphere priming effects. – Journal of Plant Nutrition and Soil Sciences 165: 382-396.
[34] Loreau, M., Hector, A. (2001): Partitioning selection and complementarily in biodiversity experiments & Erratum. – Nature 412: 72-76 & 413: 548.
[35] MacArthur, R.H. (1955): Fluctuations of animal populations and a measure of community stability. – Ecology 36: 533-536.
[36] McCann, K.S. (2000): The diversity-stability debate. *Nature* 405: 228-233.
[37] Morton, J.B. (1999): Fungi. – In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A. (eds.): Principles and Applications of Soil Microbiology, pp. 72-93, Prentice Hall Inc., New York, USA.
[38] Pettersson, M., Bååth, E. (2003): Temperature-dependent changes in soil bacterial community in limed and unlimed soil. – FEMS Microiology Ecology 45: 13-21.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

[39] Pietikäinen, J., Pettersson, M., Bååth, E. (2005): Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. – FEMS Microbiology Ecology 52: 49-58.
[40] Pimm, S.L. (1984): The complexity and stability of ecosystems. – Nature 307: 321-326.
[41] Preston-Mafham, J., Boddy, L., Randerson, P.F. (2002): Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles – a critique. – FEMS Microbiology Ecology 42: 1-14.
[42] Priha, O., Grayston, S., Pennanen, T., Smolander, A. (1999): Microbial activities related to C and N cycling and microbial community structure in rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in a organic and mineral soil. – FEMS Microbiology Ecology 30: 187-199.
[43] Ranneklev, S.B., Bååth, E. (2001): Temperature-driven adaptation of the bacterial community in peat measured by using thymidine and leucine incorporation. – Applied Environmental Microbiology 67: 1116-1122.
[44] Seybold, C.A., Herrick, J.E., Brejda, J.J. (1999): Soil resilience: a fundamental component of soil quality. – Soil Science 164: 224-234.
[45] Smith, H., Haapakangas, H., van Beelen, P. (2005): Effects of antibiotics on soil microorganisms: time and nutrients influence pollution-induced community tolerance. – Soil Biology and Biochemistry 37: 1882-1892.
[46] Stefanowicz, A.M., Niklińska, M., Laskowski, R. (2008): Metal affect soil bacterial and fungal functional diversity differently. – Environmental Toxicology and Chemistry 27: 591-598.
[47] Steiner, C.F., Long, Z.T., Krumins, J.A., Morin, P.J. (2006): Population and community resilience in multitrophic communities. – Ecology 87: 996-1007.
[48] Tam, L., Derry, A.M., Kevan, P.G., Trevors, J.T. (2001): Functional diversity and community structure of microorganisms in rhizosphere and non-rhizosphere Canadian arctic soils. – Biodiversity and Conservation 10: 1933-1947.
[49] Thornley, J.M., Cannel, M.G.R. (2001): Soil carbon storage response to temperature: an hypothesis. – Annals of Botany 87: 591-598.
[50] van Beelen, P., Wouterse, M., Posthuma, L., Rutgers, M. (2004): Location-specific ecotoxicological risk assessment of metal-polluted soils. – Environmental Toxicology and Chemistry 23: 2769-2779.
[51] Verschuere, L., Fievez, V., Van Vooren, L., Verstraete, W. (1997): The contribution of individual populations to the Biolog pattern of model microbial communities. – FEMS Microbiology Ecology 24: 353-362.
[52] Vishnevetsky, S., Steinberger, Y. (1997): Bacterial and fungal dynamics and their contribution to microbial biomass in desert soil. – Journal of Arid Environments 37: 83-90.
[53] Waldrop, M.P., Firestone, M.K. (2004): Altered utilization patterns of young and old soil C by microorganisms caused by temperature shifts and N additions. – Biogeochemistry 67: 235-248.
[54] Wang, A., Chen, J., Crowley, D.E. (2004): Changes in metabolic and structural diversity of a soil bacterial community in response to cadmium toxicity. – Biology and Fertility of Soils 39: 452-456.
[55] Yao, H., He, Z., Wilson, M.J., Campbell, C.D. (2000): Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. – Microbiology Ecology 40: 223-237.
[56] Yuste, J.C., Yuste, I.A., Janssens, A., Carrara, L., Meiresonne, L., Ceulemans, R. (2003): Interactive effects of temperature and precipitation on soil respiration in a temperate maritime pine forest. – Tree Physiology 23: 1263-1270.
[57] Zak, J.C., Willig, M.R., Moorhead, D.L., Wildmand, H.G. (1994): Functional diversity of microbial communities: a quantitative approach. – Soil Biology and Biochemistry 26: 1101-1108.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)