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Dynamics and stratification of soil biota activity along an altitudinal climatic gradient in West Carpathians

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We employed field methods to investigate the factors affecting soil biota activity in forest stands at four altitudes (600, 800, 1000, 1200 m a.s.l.) on three different mountains (as replicates) in the Beskidy Mts in Poland. Soil biota activity was estimated in two different soil organic layers (Olf and Ofh) representing two stages of litter decomposition. Nylon bags with cellulose filters and the bait-lamina method were used to study the likely effect of climatic parameters on soil microbial and fauna activity in natural field conditions. Also, the density of the enchytraeids (*Oligochaeta*), key mesofauna group in boreal forests, was measured. The results showed significant differences of soil biological activity by altitude, although the altitudinal gradient studied can be considered short (only 600 m altitudinal difference), with slight differences in climatic conditions. The activity of soil microorganisms (especially cellulytic bacteria and fungi) and the mesofauna density (enchytraeids) were found to depend on altitude for both the Olf and Ofh soil organic layers.

Key words: mountains, mesofauna, cellulose filters, bait-lamina test, enchytraeids density.

INTRODUCTION

Organic matter decomposition depends on the integrated activities of microorganisms and soil animals (meso- and macrofauna) (Gestel *et al*., 2003). The majority of forest soil activity is due to soil microbes (fungi and bacteria) able to decompose various organic compounds with a wide range of physical and chemical properties (Berg & Laskowski, 2006). Soil meso- and macrofauna biomass may be up to three orders of magnitude lower than microbial biomass, but the role of soil animals in soil functioning cannot be neglected (Paul & Clark, 1996). Soil animals contribute to litter decomposition, directly by consumption and fragmentation, or indirectly by their influence on microbial activity (Gestel *et al*., 2003). Soil animals translocate soil particles, increasing their susceptibility to microbial attack. Decomposition therefore is not simply due to the sum of the activities of microflora and soil fauna, but is largely the result of multitrophic interactions between the two as well as environmental conditions and carbon resource quality (Gestel *et al*., 2003; Bardgett *et al*., 2005). Thus, the effects of microbial-feeding microfauna on microbial activity and nutrient mineralization are generally positive (Mamilov *et al*., 2001), but the final composition and activity of soil microorganisms and microfauna is a result of its relationships with environment factors, like quality of soil carbon (Rovira & Vallejo, 1997). Environmental factors as temperature and soil moisture directly influence the activity of soil decomposers and the rate of organic matter decomposition (Berg *et al*., 1993; Howard & Howard, 1993). Soil properties and the quality and quantity of organic substrates strongly influence this process (Kirschbaum, 2000). However, there is still substantial uncertainty about common effects of environmental factors, and their interactions with the different soil biological and chemical compartments of soil on soil biota activity (Kirschbaum, 2006).

The enchytraeids (*Oligochaeta*) are the key mesofauna species in northern and coniferous temperate forest. Species diversity is generally low in these forests, and the enchytraeid community is usually strong-

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ly dominated by *Cognettia sphagnetorum* (Didden & de Fluiter, 1998). Enchytraeids can comprise up to ~70% of the total animal biomass in some ecosystems (Cragg, 1961). The contribution of enchytraeids respiration to total soil CO_2 emissions has been reported to vary from 1% to 40% (Lundkvist, 1982; Heck & Römbke, 1990; Briones *et al*., 2004). It is already known that feeding activities of enchytraeids increase soil microbial activity (total and fungal) by 35% in blanket peat (Cole *et al*., 2002). Thus, the interactions between soil microorganisms and enchytraeids are very important for soil biological processes. The majority of soil invertebrates occur in the surface horizons where they are the most likely to be exposed to environmental changes (Briones *et al*., 1997). Enchytraeids do not survive a soil water content of less than 10% of field capacity and are mainly restricted to moist habitats (Abrahamsen, 1971), but they are able to move deeper into the soil in response to moisture changes (Springett *et al*., 1970). Generally, populations of microorganisms and soil fauna tend to decrease with soil depth as a result of depletion of easily degradable compounds (Agnelli *et al*., 2004; Gongalsky *et al*., 2004).

The aim of this study was to compare soil biological activity *in situ* along a climatic gradient, represented by 600 m mountain elevation gradient in the Beskidy Mts in Western Carpathians. Mountain areas with vertical gradients of temperature and precipitation provide an opportunity to observe climate changes similar to those observed at various latitudes. The novelty of our study was the mountain elevation gradient, which allowed us to study the soil biological activity in soil along a climatic gradient, but without basic differences in soil bedrock, as occurs with parallel climatic gradients. Moreover, we applied true replication on 3 different mountains that helps to isolate pure elevation effects from confounded effects of vegetation or other, which may be dissimilar at different mountains. Two forest soil organic layers were investigated, Olf and Ofh. The soil biological activity was measured using nylon mesh bags with cellulose filters and also by the bait-lamina method. The enchytraeids (*Oligochaeta*) density was also measured. We related each of the measured biological activities to soil physicochemical factors potentially affecting it (pH and the concentrations of the biogens Ca, Mg, Mn, N, Na and K) in order to find the relationships with climatic conditions.

MATERIALS AND METHODS

Study plots

Twelve study plots of 30 m^2 were selected along an altitudinal gradient in the Beskidy Mts, Western Carpathians, southern Poland. Three mountain peaks at similar latitudes and of similar geological structure were chosen: Polica (1369 m a.s.l., 19°37′E and 49°37′N) and Romanka (1366 m a.s.l., 19°14′E and 49°33′N), both in the Beskid Żywiecki range and Radziejowa (1262 m a.s.l., 20°36′E and 49°26′N) in the Beskid Sądecki range (for more details see Niklińska & Klimek, 2007). In each mountain, four study plots at 600, 800, 1000 and 1200 m a.s.l. were established. The study was conducted in summer 2005. Since the temperature and moisture content of mountain soils depend highly on the slope and aspect, all plots were selected to face north with similar slope. All the mountains are on approximately the same E–W parallel and they were treated as experimental replicates. At each plot, two distinguished organic layers (Olf and Ofh) were investigated. The thickness of the two soil layers was different at different sites (2-15 cm depth). The plots at the three lowest altitudes are covered by 30-40-year-old spruce forests mixed with beech, alder and fir while 50-year-old spruce forests predominate on the plots at 1200 m. The plant species composition of the forests at the lower altitudes has been affected by human activities such as nearby agriculture, pasturing, and collection of litter from the forest floor (Grodzińska & Szarek-Łukaszewska, 1997). The soils at the sampling plots were generally podzol and brown earth.

Estimation of Enchytraeids density

The density of the enchytraeids (*Oligochaeta*) was measured for each studied plot and in both organic layers. Ten samples per plot (30 m^2 area) were taken randomly by a steel soil sampler (5.2 cm diameter). In the field, the samples were divided into Olf and Ofh layers, and intact soil cores were transported to the laboratory. Enchytraeids were extracted from each core separately. The enchytraeids were collected after 5 hrs of extraction by the Tullgren funnel method, fixed in formalin solution and counted under magnification. Mean enchytaeids density was expressed as number per m^2. Standard deviation and the coefficient of variation were calculated for each plot and, separately, for elevation and soil organic layer (with mountains as replicates).

Bait-lamina test

Bait-lamina test is a relatively new screening method to estimate soil faunal activity in soil (von Törne, 1990). Plastic bait-lamina strips are perforated and the openings are filled with a bait substrate; the rate of bait consumption is a measure of soil invertebrates activity (Hamel *et al*., 2007). The bait-lamina method allows one to assess the feeding activity of living soil invertebrates with negligible effect of soil microorganisms activity (Helling *et al*., 1998). Bait-lamina strips were used to study effects of climatic conditions (Gongalsky *et al*., 2004), effects of recultivation of post-mining landscapes (Dunger *et al*., 2001) and effects of toxic substances like pesticides (Burrows & Edwards, 2002) on soil invertebrates. In our study, standard plastic bait-lamina strips (120 mm long, 6 mm wide, 1 mm thick) perforated at 5 mm intervals by 16 apertures (Terra Protecta Gmbh) were used. The openings (1 mm diameter) were filled with a bait substrate consisting of cellulose, starch and ground nettle leaves (2:1:1) mixed with water to form a paste. Sets of 25 filled strips were inserted horizontally into the middle of the Olf and Ofh organic layers at each studied plot (since the thickness of the layers varied between plots). After 32 days all the bait-lamina strips were gently pulled from the soil and transported to the laboratory. Biological activity was assessed as the percentage of perforated apertures in all sticks used per plot and organic matter layer. Standard deviation and variance coefficient was calculated elevation and soil organic layer (with mountains as replicates).

Cellulose filters decomposition

Cellulose filters (7 cm diameter) were oven-dried at 105˚C, weighted to the nearest mg accuracy and placed in 10×10 cm nylon mesh bags (1×1 mm mesh) with numbered labels. The use of standard material eliminated the influence of substrate morphology and chemical composition. In June 2005, the bags were inserted horizontally into both studied soil layers, 20 bags for each layer in each plot (total 480 bags). The bags were collected at four equal intervals up to 12 weeks. At each interval, 5 bags as replicates were taken randomly from each organic layer at every sampling plot and transported to the laboratory, cleaned of soil particles and dried before weighting. The cellulose decomposition rates were calculated as k parameters in Olson's model:

$$Wt = W_0 \times e^{-kt}$$

where W_0 is initial cellulose filter mass (g), W_t is mass (g) after incubation time t (days) and k is the decomposition constant (rate) (Berg & Laskowski, 2006).

Chemical analyses

Analyses of soil samples included pH in water and pH in KCl in mixed soil from each plot and organic layer. Soil pH was measured in 2 g subsamples shaken for 1 hr at 20 cm^3 deionized water (pH_{H_2O}) or 1 M KCl (pH_{KCl}). Also the concentrations of some nutrients (Ca, Mg, Mn, K, Na, C and N) in mixed soil were determined. Total Ca, Mg, Mn, K and Na were measured after wet digestion of 0.5 g subsamples in 10 ml concentrated HNO_3. As a check on the method, three blank samples and three replicates of standard certified material (Rye grass, PROMOCHEM, GmbH) were analyzed with each batch of samples. Ca, K and Na were measured by emission flame spectrometry (JENWAY, model PFP 7) and Mg and Mn by flame atomic absorption spectrometry (PERKIN-ELMER, model AAnalyst 800). Total C and N content were analyzed with a CHNS analyser (Vario EL III, Elemental Analyser GmbH). Organic matter (OM) content was determined as loss on ignition at 550˚C for 24 hrs.

Statistical analysis

Two-way ANOVA was used to test for differences in the physicochemical parameters between organic matter layers (Olf, Ofh) and elevations (600, 800, 1000, 1200 m a.s.l.), and the interactions between them. These included OM content, pH_{H2O} and pH_{KCl}, concentrations of Ca, Mg, Mn, Na, K, N and C:N ratios. To satisfy ANOVA model assumptions, we log-transformed some of the variables to normalize the data. When significant differences were found, the means were compared using Tukey's test and the results were considered significantly at $p < 0.05$. Non-significant interactions were removed from the models.

Two-way ANOVA was used to test differences in the log number of enchytraeids between elevations and soil organic layers, and the interactions between them.

Multiple regression analysis was used to test which factors (organic layer, elevation, pH, nutrient concentrations, enchytraeids density) affect field biological activity, measured as invertebrate feeding activity (percentage of perforated apertures in bait-lamina

strips) and cellulose filters decomposition rate (absolute k values of Olson's model). Correlation analysis was used to test which soil characteristics could be treated as independent variables. The right-skewed data of bait lamina test results, Ca and K concentrations and enchytraeids density were log-transformed prior to statistical analyses to fulfil the criteria for normality. Forward and backward selections were employed to estimate the best-fitted model. In the next step, the independent variables were standardized prior to statistical analysis by subtracting the average value and dividing by the standard deviation. After standardization, it was possible to directly assess the effects of significant variables and to evaluate their relative importance with the calculated regression coefficients. All statistical analyses employed Statgraphics v. 5.1.

RESULTS

Soil properties

Table 1 shows data on organic matter content (%), pH_{H2O} and pH_{KCl}, organic matter content (%) and the concentrations of some nutrients (Ca, Mg, Mn, K, Na; mg kg^{-1} dry weight), total N (%) and the C:N ratio in samples from Olf and Ofh forest organic layers along the studied elevation gradient.

Two-way ANOVA for organic matter content in soil showed a highly significant effect for organic layer ($F = 45.80$, $p < 0.0001$) and organic matter content was higher in the upper Olf layer than in bottom Ofh layer. The effects of elevation and the interaction between elevation and organic layer were not significant.

Both elevation and organic layer significantly affected soil pH_{H2O} ($F = 5.37$, $p < 0.001$ and $F = 4.81$, $p < 0.05$) and the interaction term was nonsignificant. The soil pH_{H2O} was higher in Olf than in Ofh layer. The soil pH_{H2O} was the highest in soil at elevation 600 m a.s.l. and decreased with elevation.

Two-way ANOVA for soil pH_{KCl} demonstrated significant effect of elevation only ($F = 4.26$, $p < 0.02$), while the organic layer and the interaction between elevation and organic layer had no significant effect.

Nutrient concentrations like Ca, Mg, Mn, K and Na varied widely within groups; there were no differences between organic layers ($p > 0.1$) or between elevations ($p > 0.2$), and the interaction between them were nonsignificant ($p > 0.5$).

The organic layer had a significant effect on N concentration ($F = 20.82$, $p < 0.0003$), but the effect of elevation and the interactive effect were not significant. Also, the C:N ratio varied between organic layers ($F = 4.83$, $p < 0.05$) but not between elevations, and the effect of interaction between them was not significant.

The correlation analysis showed some soil properties to be highly correlated, for example Mg concentration was correlated with K, Na, N and C:N (Table 2).

Estimation of enchytraeid density

Mean enchytraeid density ranged from ~33000 (Ofh layer at 1000 m a.s.l.) up to 200000 (Olf layer at 1200 m a.s.l.) individuals m^{-2} (Table 3). The mean enchytreaids density was the most variable on elevation 1000 m a.s.l., where difference between mountains ranged an order of magnitude for both soil organic layers. The aggregated distribution of enchytraeids in soil resulted in a non-normal distribution in many groups, so the data were log-transformed prior to analysis. Two-way ANOVA showed a significant effect of elevation only on log enchytraeid number ($F = 32.63$, $p < 0.0001$) (Fig. 1). The enchytraeid number in soil on particular elevations increased in order: 1000 < 600 – 800 < 1200 m a.s.l.

Bait-lamina test

Invertebrate feeding activity in soil (Table 3) was calculated as the percentage of perforated apertures to their total number in 23 sticks per organic layer per plot (23 was the smallest number of strips recovered). Mean invertebrate feeding activity ranged from 9.3% (Ofh layer at 1000 m a.s.l.) to 51.9% (Olf layer at 600 m a.s.l.). Invertebrate feeding activity was the most variable on elevation 800 m a.s.l. with variance coefficient equal of 0.66 for Olf soil organic layer and 0.81 for Ofh layer (mountains as replicates).

Since some soil properties were highly correlated, only organic matter content, pH in water, and Ca, K and Mn concentrations were chosen as independent variables, besides elevation, organic layer and enchytraeid density in multiple regressions to test which factors affected field biological activity. After removal of non-significant factors (backward and forward selection), the whole model for the invertebrate feeding activity (percent of perforated apertures in bait-lamina strips) was statistically significant at $p < 0.001$ ($F = 8.80$) and explained 56.9% of the total variability ($R^2_{adj} = 50.4$). Invertebrate feeding activity was correlated negatively with elevation ($p < 0.0006$) and organic layer ($p < 0.03$), and positively with enchytraeid

TABLE 1. Main properties of Olf and Ofh forest organic layers along the studied elevation gradient

humus organic layer	elevation (m a.s.l.)	organic matter[a]	pH_{H_2O}	pH_{KCl}	Ca[b]	Mg[b]	Mn[b]	K[b]	Na[b]	N[a]	C:N
Olf	600	54.1 ± 2.2	4.3 ± 0.3	3.3 ± 0.4	3877 ± 2031	1343 ± 198	687 ± 324	1353 ± 276	41 ± 11	1.60 ± 0.44	19 ± 3
	800	46.7 ± 16.9	4.1 ± 0.0	3.1 ± 0.1	2378 ± 1270	1028 ± 515	530 ± 217	967 ± 56	30 ± 25	1.67 ± 0.41	19 ± 3
	1000	56.2 ± 10.6	4.0 ± 0.3	3.0 ± 0.3	2136 ± 299	519 ± 146	304 ± 20	856 ± 17	25 ± 24	2.02 ± 0.03	22 ± 2
	1200	58.4 ± 6.2	3.9 ± 0.1	2.8 ± 0.3	1442 ± 763	1340 ± 1024	285 ± 141	1780 ± 942	44 ± 22	2.04 ± 0.10	21 ± 2
Ofh	600	27.0 ± 4.6	4.1 ± 0.2	3.1 ± 0.2	2052 ± 1840	1460 ± 977	451 ± 365	1332 ± 862	39 ± 28	1.12 ± 0.33	19 ± 2
	800	23.7 ± 4.6	4.0 ± 0.0	3.1 ± 0.1	1293 ± 999	1458 ± 938	382 ± 104	1349 ± 480	51 ± 26	1.12 ± 0.33	18 ± 3
	1000	32.2 ± 12.5	3.9 ± 0.2	2.9 ± 0.3	1588 ± 747	1599 ± 1478	252 ± 161	1978 ± 1750	47 ± 31	1.44 ± 0.57	18 ± 3
	1200	40.0 ± 0.8	3.7 ± 0.1	2.7 ± 0.1	1661 ± 434	477 ± 86	321 ± 35	858 ± 52	29 ± 9	1.61 ± 0.13	19 ± 3

Values are means ± SD (n = 3)

[a] As percent of dw
[b] As parts per million dw

TABLE 2. Correlations between soil parameters. Pearson's correlation coefficients (range –1 to +1) are followed in parenthesis by the *p* value for the statistical significance of the estimated correlations. Highest correlations ($p < 0.01$) are in bold

	OM	pH_{H_2O}	pH_{KCl}	Ca	Mg	Mn	K	Na	N	C:N
OM										
pH_{H2O}	–0.0368 (0.8644)									
pH_{KCl}	–0.1563 (0.4658)	**0.9480 (< 10^{-4})**								
Ca	0.3901 (0.0595)	0.3964 (0.0552)	0.3720 (0.0734)							
Mg	–0.3400 (0.1040)	0.4372 (0.0326)	0.4870 (0.0158)	–0.2449 (0.2488)						
Mn	0.1109 (0.6060)	0.3108 (0.1394)	0.2388 (0.2612)	0.0898 (0.6763)	0.2253 (0.2898)					
K	–0.2525 (0.2340)	0.3630 (0.0812)	0.4123 (0.0453)	–0.2895 (0.1701)	**0.8147 (< 10^{-4})**	0.1414 (0.5097)				
Na	–0.1615 (0.4509)	0.2142 (0.3148)	0.2107 (0.3230)	–0.2852 (0.1768)	**0.6575 (0.0005)**	–0.0284 (0.8951)	**0.5661 (0.0039)**			
N	**0.8183 (< 10^{-4})**	–0.1864 (0.3832)	–0.2892 (0.1705)	0.4139 (0.0444)	**–0.5514 (0.0052)**	–0.0102 (0.9621)	–0.4028 (0.0510)	**–0.5246 (0.0085)**		
C:N	**0.5803 (0.0029)**	–0.0921 (0.6686)	–0.2563 (0.2267)	0.3256 (0.1205)	**–0.5794 (0.0030)**	0.1382 (0.5195)	–0.4857 (0.0161)	–0.4872 (0.0157)	**0.7450 (< 10^{-4})**	

TABLE 3. Enchytraeid density and field biological activity (as invertebrate feeding activity and cellulose filter decomposition rate) in Olf and Ofh forest soil organic layers along the studied elevation gradient

soil organic layer	elevation (m a.s.l.)	enchytraeid density[a]	invertebrate feeding activity[b]	cellulose filter decomposition rate[c]
Olf	600	41225 ± 13468	51.9 ± 25.8	14.89 ± 3.92
	800	47249 ± 27410	39.9 ± 26.3	9.37 ± 4.38
	1000	39217 ± 49719	16.5 ± 6.3	4.99 ± 2.45
	1200	200241 ± 150617	22.0 ± 6.2	9.53 ± 4.48
Ofh	600	49759 ± 13 662	43.5 ± 21.9	12.18 ± 4.95
	800	47952 ± 23 664	14.8 ± 11.9	7.64 ± 2.11
	1000	32671 ± 42 509	9.3 ± 5.1	2.73 ± 1.11
	1200	94920 ± 46 388	13.9 ± 7.5	5.43 ± 1.77

Values are means ± SD (n = 3)

[a] as number of individuals m^{-2}
[b] as percent of perforation of holes in 23 bait lamina sticks
[c] as absolute values of k parameters × 10^3

density ($p < 0.02$), according to the equation:

$$\log(\text{invertebrate feeding activity}) = 2.31 - 0.00211 \times \text{elevation} - 0.0534 \times \text{layer} + 0.316 \times \log(\text{enchytraeid density})$$

where the invertebrate feeding activity was expressed as the percentage of perforation of bait-lamina strips, elevation in meters a.s.l., layer as organic layer Olf and Ohf, and enchytraeid density as the mean number of individuals per square meter (Fig. 2). There were no significant and crucial interactions, and all of them were removed from the model. The model based

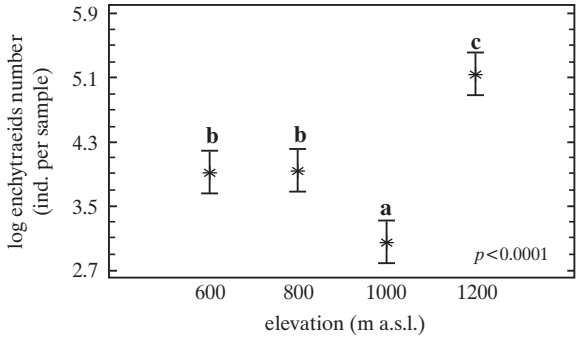

FIG. 1. Number of enchytraeids (individuals per sample) along the elevation gradient ($p < 0.0001$). Central points indicate the sample means, and error bars indicate 95% Tukey HSD intervals (n = 60). Different letters above bars indicate significant differences.

on the standardized values of the variables gave the following equation:

$$\log(\text{invertebrate feeding activity}) = 3.00 - 0.481 \times \text{elevation} - 0.273 \times \text{layer} + 0.302 \times \log(\text{enchytraeid density})$$

This equation allowed the effects and relative importance of significant variables to be assessed by direct comparison of the calculated regression coefficients. In this case, elevation was the most important factor affecting invertebrate feeding activity.

Cellulose filters decomposition rate

The cellulose filters decomposition rate is a measure of mainly the activity of cellulytic bacteria and fungi (Berg & Laskowski, 2006). However, the soil fauna abundance and activity affect the filters decomposition rate. The mean rate of cellulose filters decomposition rate, given as values of the k parameter of Olson's model, ranged from –0.0027350 (Olf layer at 1000 m a.s.l.) to –0.0148895 (Ohf layer at 600 m a.s.l.) (Table 3).

After removal of non-significant factors (backward and forward selection), the whole model for the cellulose filter decomposition rate (k parameter) was statistically significant at $p < 0.001$ ($F = 8.71$) and explained 56.6% of the total variability ($R^2_{adj} = 50.1$). The cellulose filters decomposition rate was correlated negatively with elevation ($p < 0.001$) and positively with enchytraeids density ($p < 0.02$) and organic matter content ($p < 0.055$). The p value for organic matter content was at the limit of significance, but it was decided to include this factor in the model, because it markedly enhanced the R^2 coefficient. The

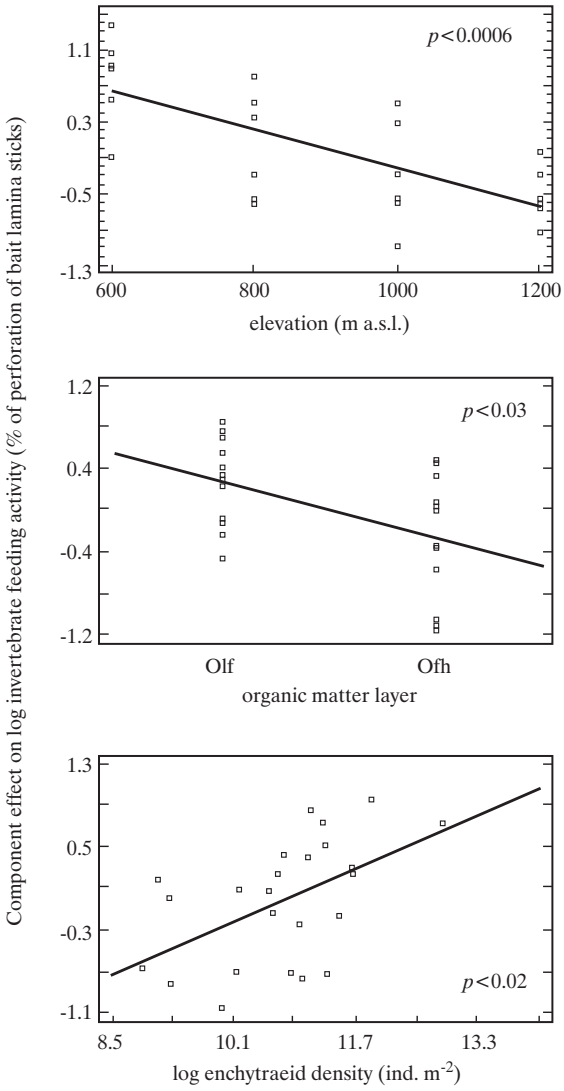

FIG. 2. Component effect on invertebrate feeding activity on elevation, organic matter layer and enchytraeid density. The whole model was significant at $p < 0.001$; the significance of each independent variable is given on the graphs. The line shows the relative change in the predicted values of log respiration rate when changing of parameters over their observed range. Each point is then plotted by adding its residual to the line.

equation of the fitted model was as follows:

$$\text{cellulose filter decomposition rate} = -0.00462 - 0.0000156 \times \text{elevation} + 0.0000994 \times \text{OM} + 0.00212 \times \log(\text{enchytraeid density})$$

where the cellulose filter decomposition rate was expressed as the absolute value of the k parameter of Olson's model, elevation in m a.s.l., organic matter (OM) content as the dry weight percentage, and enchytraeid density as the mean number of individuals

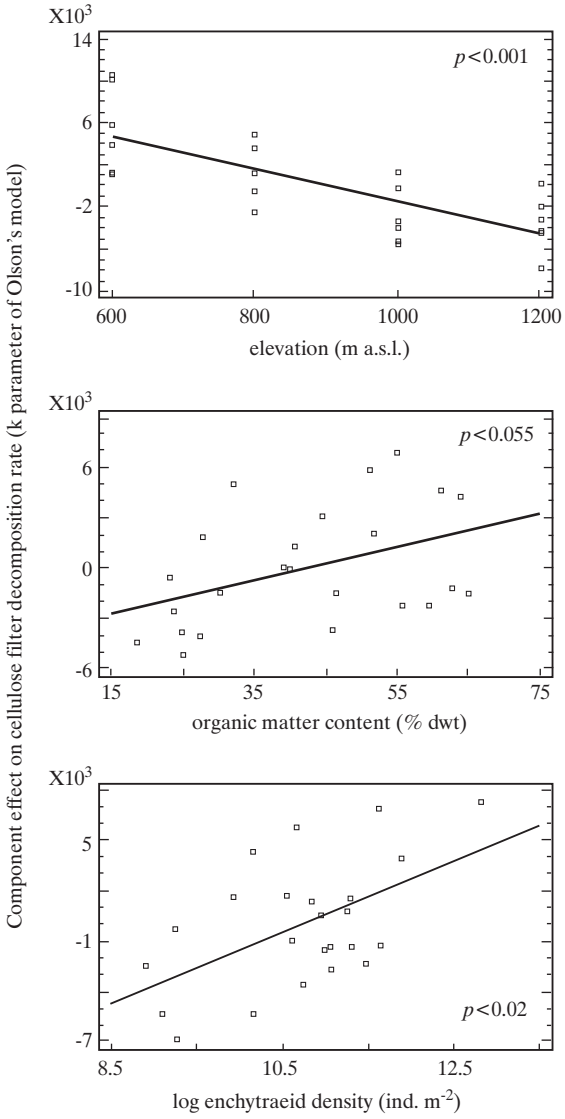

FIG. 3. Component effect on cellulose filter decomposition rate on elevation, organic matter content and enchytraeid density. The whole model was significant at $p < 0.001$; the significance of each independent variable is given on the graphs. The line shows the relative change in the predicted values of log respiration rate when changing of parameters over their observed range. Each point is then plotted by adding its residual to the line.

per square meter (Fig. 3). There were no significant and crucial interactions, and all of them were removed from the model. The model based on the standardized values of the variables indicated that elevation was the most important factor affecting the cellulose filter decomposition rate:

$$\text{cellulose filters decomposition rate} = 0.000835 - 0.00356 \times \text{elevation} + 0.00149 \times \text{OM} + + 0.00203^{*}\log(\text{enchytraeid density})$$

DISCUSSION

Soil chemical properties along the elevation gradient and in organic matter layers

There were no significant differences in the concentrations of Ca, Mg, Mn, K and Na between elevations, although the studied elevation gradient differed in terms of plant species compositions. Thus, the measured differences in soil biological activity cannot be a result of biogens availability. Only the soil pH values (measured in water and in KCl) differed significantly between elevations, but not between the Olf and Ofh layers.

The Olf and Ofh layers differed significantly only in organic matter content and in nitrogen concentration and thus the C:N ratio. However, we did not find any significant differences in organic matter content and C:N ratio along the elevation gradient. Theoretically, if these trends existed, they might indicate a shift in the balance between productivity and decomposition along the elevation gradient as a result of slower decomposition at higher elevations, because decomposition rate is known to be more climate-dependent than ecosystem productivity is (Kirschbaum, 1995; Hyvönen *et al*., 2002). The absence of these trends might be due to changes in plant composition along the mountain elevation gradient, which represents a climatic gradient with different average annual temperatures, and also a floristic gradient with different forest plant species. The increase of spruce admixture with elevation may produce differences in the chemistry of soil organic matter, in particular by increasing the recalcitrant litter fraction, as waxes, resins and lignin, and by reducing pH as observed in this study.

Soil biological activity along elevation gradient and in organic matter layers

By using field techniques, we were able to describe the relationships between soil environmental conditions, soil physicochemical properties and soil biological activity precisely. The novelty of our study was the mountain elevation gradient, which allowed us to study soil biological activity and its vertical stratification in soil along a climatic gradient, but without basic differences in soil bedrock, as occurs with parallel climatic gradients. True replications on 3 different mountains helped to isolate pure elevation effects from the possible confounded effects of other factors (like vegetation), which may be dissimilar at different mountains. In fact, we found some differences in soil bio-

logical activity among mountains. Despite the relatively large variation in each of these measures, we found some general relationships between them and climatic changes along elevation gradient.

Enchytraeids are especially important mesofaunal group for soil functioning, especially in acidic soils of coniferous forests (Laakso & Setälä, 1999; Briones *et al*., 2004). Enchytraeid abudance in soil may range from a few thousand up to 100 thousands of individuals m^{-2} and more (Jänsch *et al*., 2005). We found that mean number of enchytraeids in coniferous mountain forest was up to 200 thousands of individuals m^{-2}. There were significant differences between the numbers of enchytraeids on different altitudes but there was no clear trend. The number of enchytraeids was highest at 1200 m a.s.l. and the lowest at 1000 m a.s.l. in both organic layers, Olf and Ofh. Between measured soil properties, only soil pH changed significantly with elevation. The soil pH_{H_2O} and pH_{KCl} was the lowest in soil at elevation of 1200 m a.s.l. and decreased with elevation. Sites at 1200 m a.s.l. had the lowest soil pH, and the highest number of enchytraeid was found there. Enchytraeids are generally through as acidophilic (Jänsch *et al*., 2005). There is an overall preference for slightly acid to neutral conditions under which enchytraeids species diversity is greatest. However, there is no strong correlation between pH and enchytraeid abundance (Didden, 1993). In our study, the number of enchytraeids was lowest at 1000 m a.s.l., where soil pH was only slightly higher than at 1200 m a.s.l. We did not find significant relations with other soil parameters measured in this study; for example, organic matter content and N concentration apparently had no effect on enchytraeids number, and there was no difference in enchytraeids number between organic layers. Perhaps, other environmental factors are important for enchytraeids biology.

The bait-lamina test was applied to estimate invertebrate (and thereby enchytraeid) feeding activity. Invertebrate feeding activity correlated positively with enchytraeid density, and negatively with elevation and soil depth. Bait-lamina perforation cannot be attributed entirely to a particular group of invertebrates, but the statistically significant correlation with enchytraeid density indicates the important role of this faunal group in the decomposition process. Elevation was the most important factor fitted to this model. The decrease of invertebrate feeding activity with soil depth probably was related to poorer substrate quality and lower microbial biomass in the bottom (Ofh) layer. The more decomposed Ofh organic layers probably means lower food availability for invertebrates as well as microbes; it has been frequently suggested that nutrients from organic matter are less available to soil fauna than those from microbial biomass (Scheu & Schaffer, 1998). Enchytraeids are known to feed on microorganisms (bacteria and fungi) but during grazing they also ingest small particles of organic matter; 80% of their diet consist of microorganisms and 20% of dead organic matter (Didden, 1993). The significantly lower organic matter content in the Ofh layer might indicate lower microbial biomass, because these features are usually correlated (Demoling *et al*., 2007). Because there was no difference in enchytraeids density between organic layers, however, the difference in invertebrate feeding activity between the Olf and Ofh layers should be taken to reflect the activity of the complex mesofauna community. Reduced invertebrate feeding activity with depth may also be related to higher density of organic matter, allowing less migration of invertebrates and less access to oxygen to all heterotrophic organisms.

The significant reduction of bait consumption with elevation may be due mainly to colder climatic conditions, which affect the number and/or activity of soil invertebrates. Gongalsky *et al*. (2004) showed that climatic conditions along north-south gradient in Russia were important for the invertebrate feeding activity. In that study, large differences in soil invertebrate abundance between sites did not correlate with their activity. That work examined different soil fauna groups, which grazed the organic medium from bait-lamina strips at different intensities, and macrofauna rather than mesofauna density was measured; this might account for the lack of correlation. In our study, the observed decrease of invertebrate feeding activity with elevation may be connected with lower invertebrate abundance and with changes in invertebrate species composition. Support for this suggestion is the finding that enchytraeids density did not change gradually with elevation.

The cellulose filter decomposition test was applied to estimate field microbial activity, mainly by bacteria and fungi, which are able to degrade cellulose, although mesofauna may also participate in cellulose filter decomposition by breaking the filters' structure and enlarging the surface area available for microbial attack. We eliminated the influence of substrate morphology and chemical composition by using

standard material. Cellulose filter decomposition (k parameter of Olson's model) correlated positively with enchytraeids density and organic matter content, but negatively with elevation. Elevation was the most important factor in this model. The observed decrease in cellulose filter decomposition rates with elevation was caused by colder climatic conditions, which reduced the rate of cellulose filter colonisation by microfauna. Drewnik (2006) had similar results in studies of cellulose filter decomposition rates in the Polish Carpathians. Coûteaux *et al*. (2002) found decreased decomposition rates with altitude in tropical Andes, using straw as standard material. The increase of the cellulose filters decomposition rate with organic matter content was due to the well-known relation between organic matter and microbial activity. However, we did not find significant differences in the k parameter between the organic layers, which differed significantly in organic matter content. The lower organic matter content and probably lower microbial biomass in the deeper layer may have been compensated by higher moisture during the incubation season, as it would have been better protected physically by the upper layers against drying. Neither soil pH nor any nutrient concentration affected the cellulose filter decomposition rate, probably because of the very high variability of these soil properties found in the analysed samples.

Carbon substrate quality is not the sole factor influencing the temperature sensitivity of organic matter decomposition. In natural systems the relationship between the temperature sensitivity of decomposition and carbon availability may be obscured by complex interactions between soil biota activity and a range of other factors influencing the rate of decomposition (Fierer *et al.*, 2005). There is a continuing need for careful investigations of the dependence of soil biological reactions on temperature and soil moisture content and on changes in meso- and microfauna activity and their community structure; these need to be studied particularly in terms of the different fractions of soil organic matter. Variance coefficients for elevation and soil organic layer (with mountains as replicates) cellulose filters decomposition rate did not exceeded 0.5 and were the lowest when compared to the other measures of soil biological activity, used in our study. This means that the cellulose filters decomposition rate may be especially useful as a measure of environmental factors effect on soil biological activity.

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