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Variance components of the respiration rate and chemical characteristics of soil organic layers in Niepołomice Forest, Poland

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Abstract. Respiration rates and chemical characteristics of soil organic layers were measured at 40 experimental plots, 5 sampling sites per plot, in a moderately polluted Niepołomice Forest, S. Poland. The respiration rate was positively related to pH, water content and concentrations of Ca and K, and negatively to N_{tot}, Zn and Pb ($p < 0.001$ for all variables). No significant correlation was found between the respiration rate and Na, Cu or Cd ($p > 0.25$ in all cases). The regression model explained 73% of the total variance. Analysis of variance components revealed that ca. 35% of the total variance in the respiration rate can be explained by the vegetation types covering the area: oak-hornbeam vs. pine-oak forests. The next 40% was explained by the variability between sampling plots and the remaining 25% by within-plot variability among sampling sites. Similar results were obtained for water content. The variance in pH was split 30%:39%:31% between vegetation types:plots:sampling sites. No variance in Ca and Na was explained by the forest type, and approximately half of the variance was due to between-plot and half to within-plot variability. In contrast, potassium concentration differed between forest types (58% variance explained), more than 25% of total variance was due to between-plot variability and only 15% due to within-plot variability. For Zn the results were 1%, 66% and 33%, for Cu 0%, 38% and 62%, for Pb 48%, 18% and 34%, and for Cd 0%, 33% and 67%, respectively. The study clearly shows (1) substantial variance in some soil characteristics between sampling sites and (2) a different split of variance among spatial scales for different soil characteristics.

Introduction

Under natural conditions the rate of forest litter decomposition depends mostly on temperature, soil characteristics (such as pH), water content, concentration of major nutrients (e.g., Ca, K, N) (Meentemeyer 1978; Swift et al. 1979; Berg and Staff 1980; McClagherty and Berg 1987), and substrate chemical composition – e.g., contents of water-soluble compounds, easy-degradable sugars, lignin, etc. (Melillo et al. 1982; Taylor et al. 1991; Berg et al. 1993; Cotrufo et al. 1995). In Central Europe and large areas of the temperate climatic zone the major factors that may affect decomposition rate locally are the soil and vegetation types. In fact, both factors interact with each other, as pure, sandy soils usually support pine or pine-oak forests whose litter cause even further podsolization, while hardwoods, growing mostly on richer brown soils, produce even richer soil conditions through incorporation of dead organic matter into the soil profile. As a result organic matter decomposition tends to be fast in woods such as oak-hornbeam, and slow in needle forests (Swift et al. 1979; Coleman and Crossley 1996). Generally, the higher the soil pH and moisture, the higher the decomposition rate (Bååth et al. 1980; Hågvar 1988). Also, the more enriched in nutrients the soil is, the faster the decomposition (Berg and Staff 1980; Schlesinger 1991). Decomposition rate also increases with an increase in water-soluble materials in litter (Berg et al. 1982; Berg and Lundmark 1987) and decreases with lignin content (Melillo et al. 1982; Berg 1986; Taylor et al. 1989; Couteaux et al. 1995).

In highly industrialized and urbanized areas, litter decomposition may also be affected by pollutants, such as SO_2 (through acidic precipitation) and heavy metals (Zn, Pb, Cd, and others) (Grodziński and Yorks 1981; Berg et al. 1991). Although negative effects of some pollutants on organic matter decomposition has been proven beyond any doubt (e.g., Bååth (1989) and Berg et al. (1991)), most of these data come either from laboratory experiments where the pollutants were used at high concentrations and in an easily bioavailable form (for example, heavy metals commonly used as soluble salts) (e.g., Laskowski et al. (1994)), or from areas extremely polluted by industry (Strojan 1978; Freedman and Hutchinson 1980; Nordgren et al. 1983). Determining an effect of pollution on litter decomposition in moderately and slightly polluted areas appeared more difficult, presumably due to confounding effects of other, natural factors, and the high variability in soil characteristics, uneven spatial distribution of pollutants and other factors difficult to control.

The aim of this study was to detect the most important factors controlling the decomposition rate in a forest ecosystem and to examine the variance of the respiration rate and soil characteristics on different spatial scales. This is a part of a larger project aimed at resolving factors affecting ecosystem function (such as soil organic matter decomposition rates) into natural and anthropogenic effects, based on the long-term ecological research area of Niepołomice Forest, Poland (Weiner 1999).

Methods

Study site

The Niepołomice Forest is a typical lowland forest of continental Europe, situated in the Vistula River Valley, 20 km east of the urban and industrial area of Cracow (50°07′ N, 20°23′ E; Figure 1a). It consists of two major sections: an extensive mixed pine forest of the *Pino-Quercetum* type on the podsolic and seasonally wet soils in the south, and a smaller, deciduous, oak-hornbeam *Tilio-Carpinetum* forest on brown soils in the north (total area of about 11 000 ha (Kleczkowski 1981;

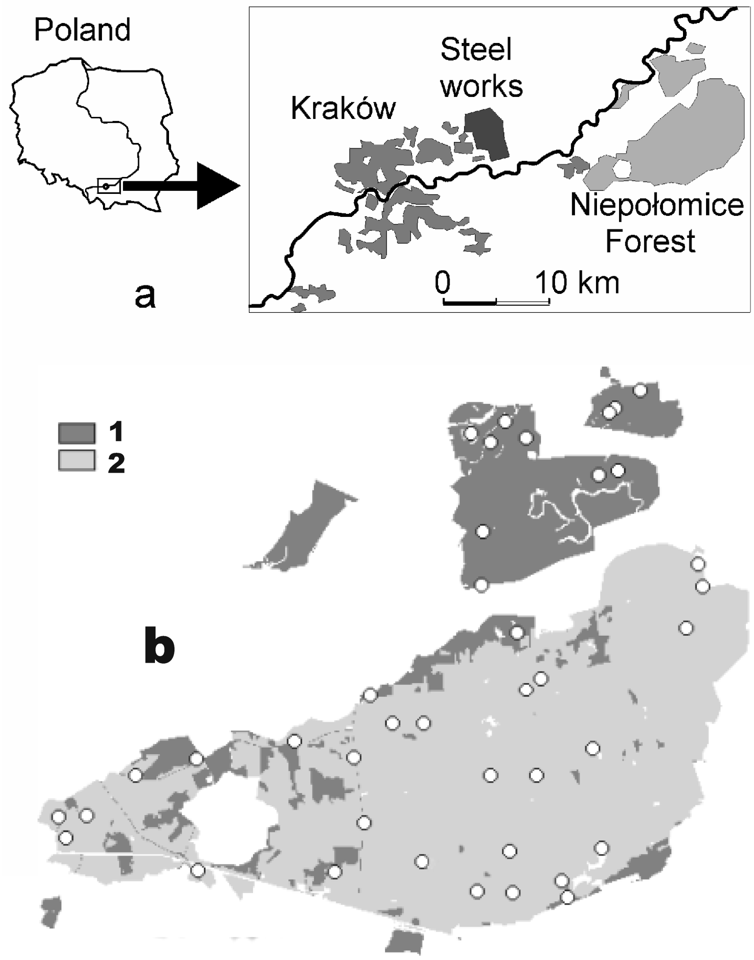

Figure 1. a. Location of the study area; b. Distribution of study plots in the Niepołomice Forest. 1 – oak-hornbeam forests, 2 – mixed forests.

Grodziński et al. 1984; Weiner et al. 1997). The Niepołomice Forest represents a variety of habitats, with two distinct, decoupled gradients: a latitudinal (N-S) gradient of soil moisture/fertility and a longitudinal (W-E) gradient of industrial pollution. Within the area, 40 study plots were established so as to cover maximum expected variance in pollution and to represent both major forest types: pine (22 plots) and oak-hornbeam (18 plots) (Figure 1b).

Sampling and respiration measurements

Samples of ca. 0.07 m^2 of the soil organic layer ($A_{0L} + A_{0F} + A_{0H}$) were collected from the forest floor at the 40 plots, ca. 400 m^2 each ('macro-scale'), in November

1998. The main sampling schedule consisted of five sampling points at each plot, located 5 m from each other ('meso-scale'), along a transect traced from the center of the plot. Additionally, at one oak-hornbeam and one pine-oak plot this schedule was supplemented with additional samples collected at the 'micro-scale', 1 m apart, next to the main sampling points. All samples were collected during two consecutive days.

Each sample was passed through a sieve (1 cm-mesh), transported to the laboratory in a perforated plastic bag and thoroughly mixed. Before starting the incubation, dry weight was measured by drying five subsamples in an oven at 105 °C for 12 hours. Samples containing 5.00 g (on a dry weight basis) of humus were placed in airtight glass jars (ca. 300 cm^3 volume) and incubated at 17 °C and their respective field-moisture conditions. The respiration rates were measured for approximately 12 hours by CO_2 absorption in 0.2 N NaOH. The excess NaOH was titrated with 0.1 N HCl, using a digital Jencons burette with 0.01 ml precision. The incubation time was recorded to the nearest minute.

Chemical analyses

All samples were analyzed for pH_{H2O} (digital pH-meter, Nester Instr.), organic matter content (550 °C, 12 h) and chemical elements. For chemical analysis, the samples were ground to powder in an agate planetary mill (Fritsch, Pulverisette 5) and approximately 1 g of a ground sample was put into a pre-weighed conical flask (100 cm^3). After drying at 105 °C for 12 h and cooling to room temperature, the flasks with the samples were weighed and the exact sample weight was recorded. Twenty ml of super-pure nitric acid (Spectrally Pure HNO_3, POCh, Poland) was added to each sample and left overnight at room temperature. With each batch of samples 3 blank samples (only acid) and 3 samples with reference standard certified material (Promochem GmbH, Germany; Chinese Soil 4, GBW07404) were prepared in the same way. After initial digestion at room temperature the samples were boiled at 130 °C until the fumes turned white. Then, the samples were cooled down to room temperature, filled with deionized water to 100 ml and left overnight to settle. Such digested samples were analyzed for Ca, Na and K by emission flame spectrometry (Jenway Ltd., model PFP 7), for Zn and Cu by flame atomic absorption spectrometry (AAS; Perkin-Elmer AAnalyst 800), and for Cd and Pb by graphite furnace AAS (AAnalyst 800). Analytical precision was checked against certified standard material and most samples fell within ± 10% of the certified value. Total C and total N were determined in five replicate mixed samples per plot, using a Perkin–Elmer CHN–analyzer.

Statistical analyses

The relationship between the respiration rate and chemical characteristics of the humus was studied with multiple regression analysis, using $\log_{10}$ of all independent variables (except for pH which is the $\log_{10}$ itself). The fit of the model was judged from the observed/predicted values plot, and the effects of particular variables were plotted as component + residual plots. The latter plots are constructed so that each graph shows the portion of the fitted model relating the respiration rate to a particular independent variable. The line shows the relative change in the predicted values of the respiration rate, which occurs when changing the independent variable over its observed range in both directions from the average, and all other variables are held constant at their averages. Each point is then plotted by adding its residual to the line. The percentage of the total variance that was explained by the model was reported as the R^2 value adjusted for degrees of freedom. The model was checked for possible multicollinearity, that is correlation between predictor variables, with estimated correlations between the coefficients in the fitted model.

The spatial variability of the respiration rate and humus characteristics for different spatial scales was analyzed with variance components analysis, using the hierarchical (nested) ANOVA with Expected Mean Squares method (Polhemus 2001). Two different data sets were used in two separate analyses. The main data set, consisting of the data for all 40 plots with 5 sampling sites per plot, was used to analyze the split of the total variance between forest type (oak-hornbeam vs. pine-oak), sampling plot (the within-forest variance) and sampling site (the within-plot variance). Thus, sampling sites were nested within the plot, and sampling plots were nested within the forest type. Then, the variance components ascribed to the particular level of this hierarchy were plotted as relative variance bar plots (so that the total variance was expressed as 100%, making different variables visually comparable). The second data set was limited to only two plots, one per forest type. At these plots, however, the samples were collected with one hierarchical level more to make it possible to estimate the ’error’ source originating from the microhabitat diversity (’micro-scale’; see sampling). The statistical method was the same as described for the main data set, however the highest rank was the sampling plot (in this particular case equivalent to the forest type), with the subsamples nested within the sampling site and the sampling sites nested within the plot. The variance due to subsampling within the sampling site (’micro-scale’ variance) was called the ’error’. The results are presented in a tabular form reporting variance components for all variables and as variance-split plots. The plots are constructed so that the average for a particular spatial scale is indicated by a horizontal line with individual data points stretching up and down from this line. Thus, at the higher hierarchy level the horizontal line indicates an average for a particular plot, and the points indicate results for the sampling sites. At the lower hierarchy level, the horizontal lines indicate averages for sampling sites, while points represent individual subsamples. All analyses were performed with the Statgraphics Plus 5.1 software (Statistical Graphics Corporation).

Table 1. Relation between the respiration rate and soil organic matter characteristics – multiple regression results.

Equation parameter	Parameter value	Significance level
Intercept	−50.2	0.0008
pH	13.2	<0.0001
moisture	13.9	0.0005
Ca	9.5	<0.0001
K	10.2	0.0002
N_{tot}	−9.1	0.0001
Zn	−18.7	0.0005
Pb	−14.1	<0.0001
Na	3.2	>0.40
Cu	3.7	>0.28
Cd	0.9	>0.81

Results

Chemical characteristics of the soil organic layer in Niepołomice Forest

The average pH of the humus used in the studies was 4.58 with a range 3.80–6.00. From the point of view of heavy metal concentrations, the area may be considered slightly to moderately contaminated. Average zinc concentration in the humus was 181 mg kg^{-1} dwt, with a range from 49 to 385 mg kg^{-1} dwt. The range of copper concentration (> 0–45 mg kg^{-1} dwt) with an average 23 mg kg^{-1} was typical for unpolluted organic soils, however the average concentration of lead (118 mg kg^{-1} dwt, range 39–684) and Cd (2.2 mg kg^{-1} dwt, range 0.4–9.3) were higher than in unpolluted soils (Kabata-Pendias and Pendias 1999). Concentration ranges of Ca, Na and K were relatively wide: 75–9297 (average 926), 66–695 (average 180), and 470–5636 (average 1651) mg kg^{-1} dwt respectively.

Relation between the respiration rate and humus characteristics

The multiple regression analysis let us distinguish clearly between the factors affecting the respiration rate and non-significant variables. Humus moisture and concentrations of Ca and K promoted the respiration rate, while concentrations of H^+, N_{tot}, Zn and Pb correlated negatively with CO_2 evolution ($p < 0.001$ for all). The concentrations of Na, Cu and Cd appeared non-significant ($p > 0.25$) (Table 1). The model was highly significant ($F = 53.5$, $p < 0.0001$) and explained 73% of the total variance of the respiration rate (R^2 adjusted for degrees of freedom = 0.73) (Figure 2). No significant multicollinearity of the predictor variables was found: only one correlation between the coefficients in the fitted model had the absolute value greater than 0.5 (N_{tot}/humus moisture, − 0.65; see Table 2).

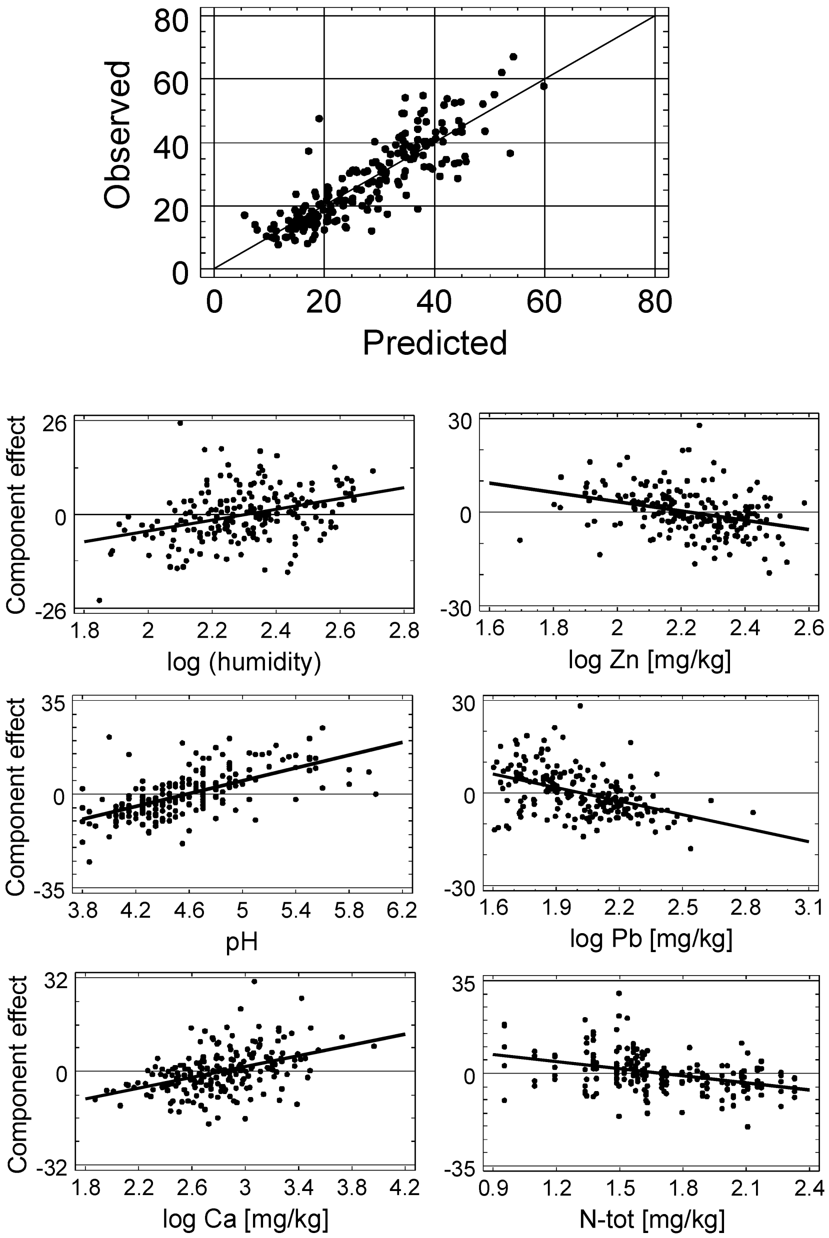

Figure 2. Multiple regression analysis results: the model fit (observed *vs*. predicted plot) and effect of each significant variable on the respiration rate (component effect plots: thick lines show the relative change in the predicted values of the respiration rate, which occur when changing the independent variable over its observed range in both directions from the average and all other variables are held constant at their averages; for more details see Methods).

Table 2. Test for multicollinearity: correlation matrix for coefficient estimates for particular independent variables used in the regression analysis to relate organic matter respiration rate to soil factors (n = 197). Note that in one case only the correlation coefficient is greater than |0.5|.

variable	Na	K	Zn	Cu	Pb	Cd	moisture	pH	N_{tot}
Ca	−.08	.42	−.43	.04	.32	−.02	−.20	−.14	.10
Na		−.26	−.29	−.02	−.25	.18	−.04	.04	−.12
K			−.42	−.09	.45	.01	−.05	−.27	.05
Zn				−.17	−.16	−.35	.04	−.08	.00
Cu					−.01	−.34	−.01	.14	−.15
Pb						−.35	.02	.31	−.09
Cd							−.01	−.17	.01
moisture								.03	−.65
pH									.12

Spatial variability of the respiration rate and humus characteristics

Respiration rates differed significantly between the forest types ($p = 0.00012$) and sampling plots ($p < 0.0001$), and the variance was split almost equally among the forest types (35%) and plots (40%), while the remaining variance was caused by within-plot variability (25%). Because respiration rate is strongly determined by soil/humus pH and moisture, it might be expected that these two characteristics should follow a similar split of variability among the forests and sampling sites. In fact, ca. 30% variance of humus pH was explained by the forest type, 39% by the sampling plot, and 31% was due to local within-plot variability. As for the respiration rates, the effects of both the forest type ($p = 0.0006$) and the plot ($p < 0.0001$) were significant. Humus moisture content followed a similar distribution of variance: 28% was explained by the forest type ($p = 0.002$), 46% by between-plot variability ($p < 0.0001$), and the remaining 26% was the within-plot variance (Figure 3).

The forests differed also in humus concentrations of K ($p < 0.0001$) and Pb ($p < 0.0001$). As much as 58% of the variance in K concentration was explained by the forest type, 27% by between-plot variability and 15% by within-plot variability. Forty eight percent of the lead variance was explained by the forest type, 18% by between-plot variability, and 34% by within-plot variability (Figure 3).

The concentrations of the remaining elements, that is Ca, Na, Zn, Cu and Cd differed only between the sampling plots, but not the forest types. Forty five percent of the variance of Ca concentration was explained by the between-plot variability, and 55% was due to within-plot variability. Similarly, the variance in Na concentration was split between plots (46%) and sampling sites within plots (64%). The variance in Zn was explained by forest type (1%), between-plot variability (66%), and within-plot variability (33%). For Cu concentration the numbers were 0%, 38% and 62% respectively, and for Cd – 0%, 33% and 67% (Figure 3).

Detailed analysis of the variability of the respiration rate and humus chemistry at different spatial scales revealed surprisingly large variance for the lowest scale

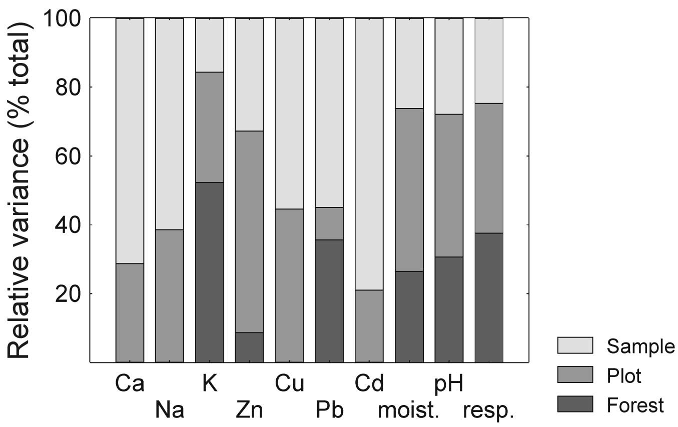

Figure 3. Variance components for humus characteristics measured at the 40 plots distributed among all soil types in the Niepołomice Forest. The variance was split between two major forest types: oak-hornbeam *vs*. pine-beech (macro-scale), study plots (mezo-scale) and sampling sites (micro-scale).

studied – among subsamples taken 1 m apart at each sampling point at the plot (*cf.* Methods) (Figures 4 and 5). Such a detailed study was made on one plot per forest type only. Thus from the point of view of the main data set that part of the variance that was due to variability between subsamples can be regarded as an error. As can be seen from the Table 3 and Figure 4, which show the split of the total variance among the three levels of hierarchy in the nested ANOVA, only 9% of the variance in the respiration rate was explained by between-plot variability (which was equivalent to the differences between forest types in this particular case), 13% was due to within-plot variability, and as much as 78% could be regarded as an error (variability among subsamples). The high variability on the meso- and micro-scales (the within-plot variability and 'error') was even more strongly emphasized for some other humus characteristics. Thus, the variability of Na was almost totally due to error (99.5%), and only 0.5% could be explained by the variability among the main sampling sites (Table 3, Figure 4). Other examples are shown in Table 3, Figures 4 and 5. On the other hand, even with that small sample size (only one plot per forest type), the forest type explained the variance in K concentration in humus (91%), and the remaining 9% only was due to an error (variability among subsamples) (Figure 4). Nevertheless, it may be said that generally the variance in most humus characteristics was dominated by the variability at small spatial scales, such as the 20-m transects in the plots (within-plot variance) and 1-m distances between subsamples at each sampling point (the 'error') (Table 3, figures 4 and 5).

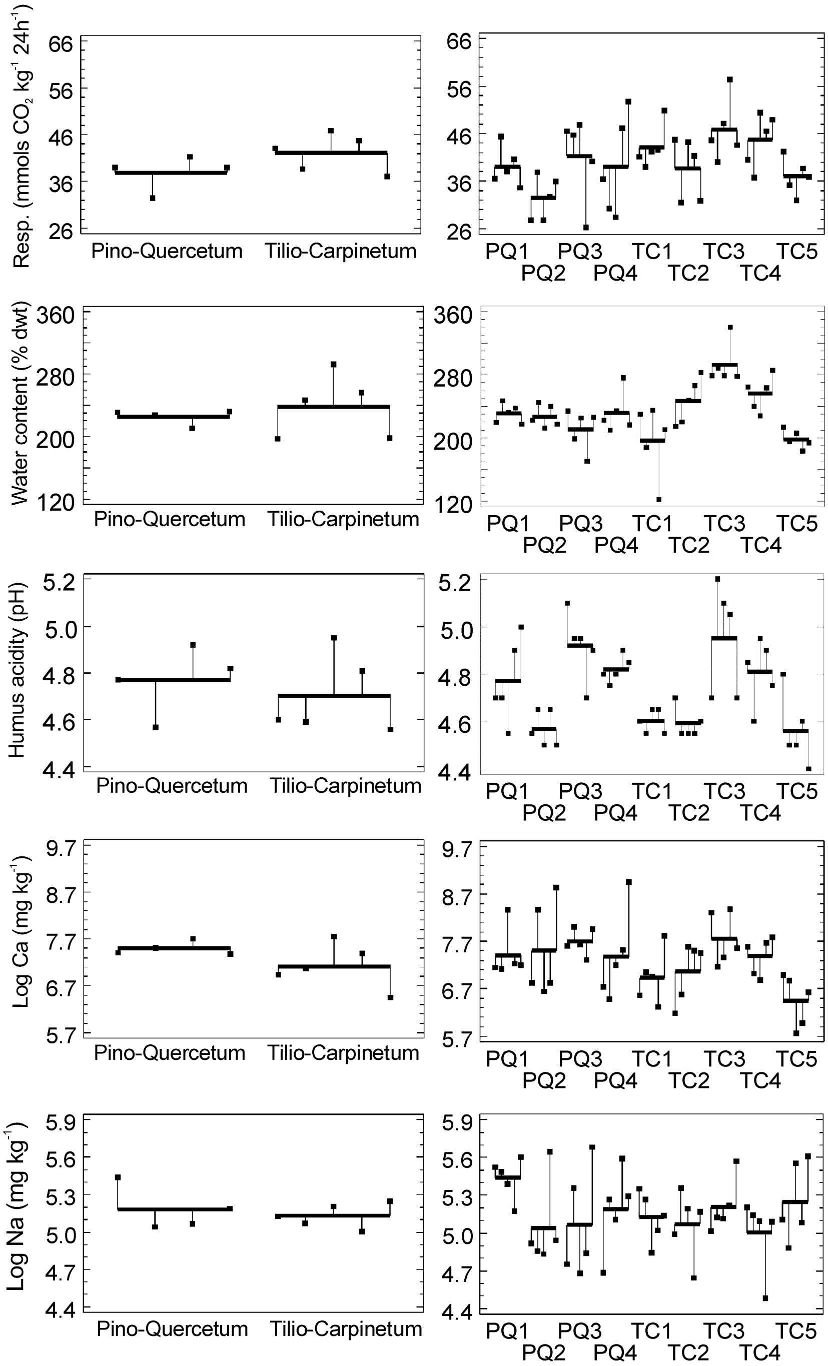

Figure 4. Variance split at the ″macro″ (left-hand column) and ″micro″ (right-hand column) scales for the respiration rate, water content, pH, and concentrations of Ca and Na. Points indicate either samples (″macro″ scale) or individual subsamples (″micro″ scale), and horizontal lines are the respective averages.

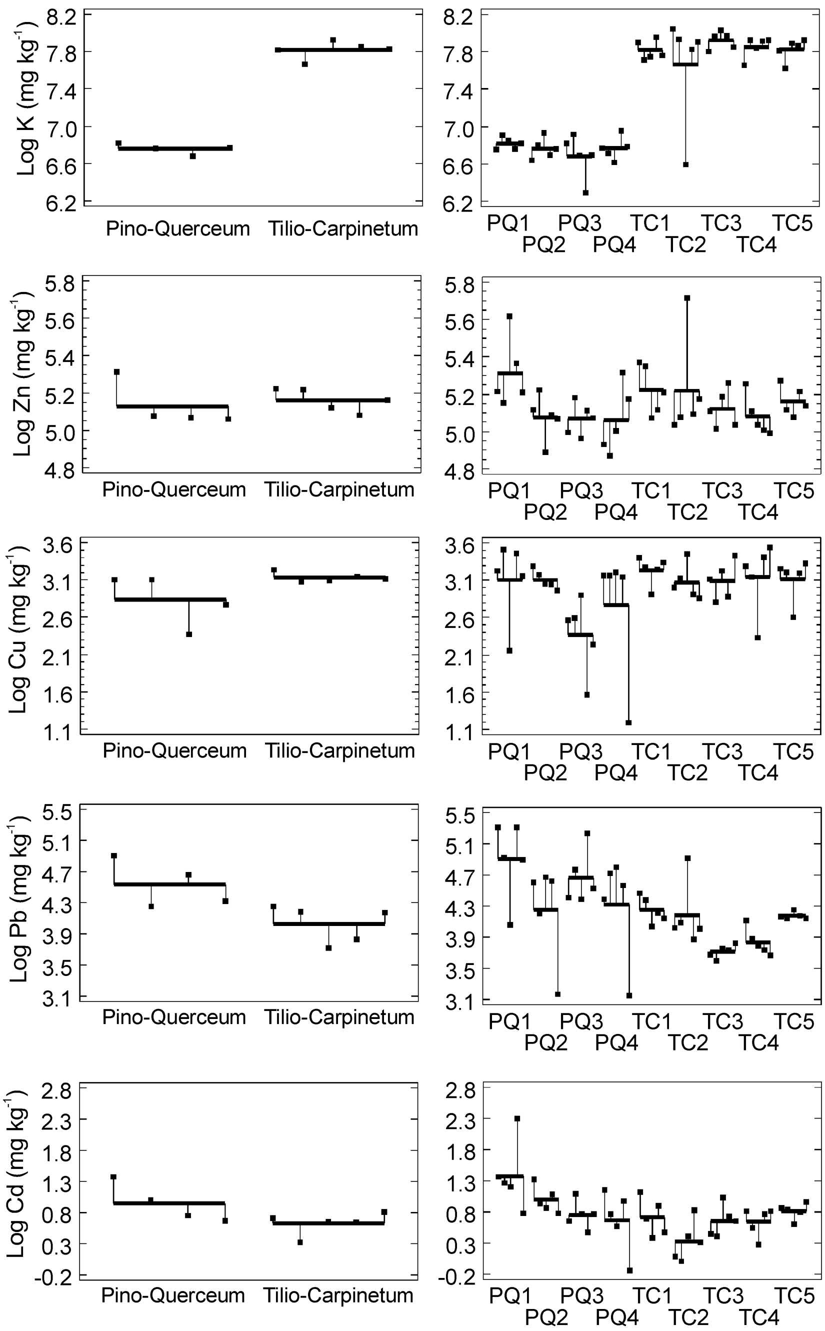

Figure 5. Variance split at the ″macro″ (left-hand column) and ″micro″ (right-hand column) scales for the concentrations of K, Zn, Cu, Pb and Cd. Points indicate either samples (″macro″ scale) or individual subsamples (″micro″ scale), and horizontal lines are the respective averages.

Table 3. Variance components of the humus respiration rate and soil organic layer characteristics studied at three different spatial scales: two plots, each located in a different forest type (Plot), five sampling sites per plot located 5 meters apart (Sampling site), and 5 (oak-hornbeam) or 4 (pine-oak) subsamples taken at each sampling site ('Error').

Variable	Percent total variance		
	Plot	Sampling site	'Error'
respiration rate	9.0	13.2	77.8
PH	0.0	54.7	45.3
moisture	0.0	56.1	43.9
Ca	7.5	11.5	81.0
K	91.0	0.0	9.0
Zn	0.0	11.3	88.7
Pb	35.2	12.2	52.6
Na	0.0	0.5	99.5
Cu	9.2	9.9	80.9
Cd	19.3	21.7	59.0

Discussion

Micro-scale spatial variability

Micro-scale spatial variability of decomposition rate and other soil characteristics has rarely been investigated until now. More often differences between forest types at macro- or regional scales are investigated. Janssens and Ceulemans (1998) mentioned that even within homogenous patches of Scots pine without understory great variability in respiration rate between plots occurred. However, a detailed analysis of spatial variability was not the main purpose of that experiment. Gibson (1986) measured concentrations of ions obtained from soil solutions in grassland soils sampled at 10 cm intervals along 3.2 m transects. The large coefficients of variation (ranging from 36.8% to 73.1%) in the supply of Ca^{2+}, Na^{+}, K^{+}, NO_3^- and NH_4^+ reflected substantial micro-spatial heterogeneity of these soil characteristics. Ion supply was correlated with micro-topography and the occurrence of plant species (Gibson 1986). Dwyer and Merriam (1981) demonstrated a significant effect of micro-topography upon surface litter accumulation in a wood near Ottawa. The distance between sampling points was ca. 5–6 m (n = 66). However, variability of forest floor properties in homogenous forest stands, i.e., soil respiration rate and soil characteristics, at different spatial scales has not been well documented so far. *In situ* measurements of soil respiration rates in Niepołomice Forest (6 plots, 12 sampling sites per plot; P. Nycz-Wasilec, unpubl.) indicated that the main part of the variance in respiration rate (ca. 60%) could be attributed to the 'error', that is within plot variability.

This variability on a small spatial scale indicates that unsuitable sampling design (e.g., too few samples or too large samples) may significantly influence statistical and biological conclusions on meso- or regional scales. Such effects were observed for both forested and non-forested ecosystems (Kuzel et al. 1993; Xu and Qi 2000), where correlations between plant species and soil characteristics or just between soil characteristics (e.g., SOC, pH, Cd content) were different at different spatial scales. However, in both these studies, the soil and ecosystem characteristics were measured at a scale not smaller than 10 m distance between sampling sites.

Distinguishing between natural and anthropogenic factors affecting soil processes

We might assume that the largest part of the variance in anthropogenic factors could be expected at the macro-scale, that is at distances of kilometers between plots. By contrast, the prevailing part of the variance in natural factors may be expected to reflect the major differences between the forest types, as well as spatial heterogeneity at the micro-scale. In the Niepołomice Forest the major source of heavy metals in the humus layer is anthropogenic deposition of industrial dust, since the steelworks near Kraków have emitted these elements with particulate pollutants in large amounts until the early 1990's (Weiner et al. 1997). Our results (Table 1) show that zinc and lead concentrations significantly affect decomposition. Analysis of Variance for the whole Niepołomice Forest shows that most of variance in zinc concentration can be explained at the macro-scale but only minor part can be ascribed to differences between the two major forest types (Figure 3). However, the second data set, with only two sampling plots, demonstrates large variance also at the micro-scale (Table 3, Figure 5). In the case of lead, the variance components analysis has attributed only a small part of the total variance to the macro-scale in both data sets (Figure 2, Table 3), suggesting that the lead contamination is not related to industrial emissions. High maximum local lead concentration was found in the humus layer in *Pino-Quercetum* forests (about 330 mg kg^{-1}), resulting in most of the variance being explained by the forest type (Figure 3). In addition, its uneven spatial distribution can perhaps be explained by a locally high automobile traffic. In fact, the southern, *Pino-Quercetum* part of the Niepołomice forest is crossed by a number of roads and exposed to heavy transit and tourist traffic, while most of the oak-hornbeam forests are either completely closed to traffic, or the traffic is limited to local transportation of low intensity.

Conclusions

1. Although the contamination level of the Niepołomice Forest is not high, the concentrations of zinc and lead appeared sufficient to retard the decomposition rate significantly. Due to the uneven split of variance in the respiration rate and chemical soil/humus characteristics among different spatial scales, such correlations can be detected only with specific sampling programs/designs.
2. The load of zinc and lead in the Niepołomice Forest can certainly be attributed

to human activities. The split in the pattern of variance in zinc concentration between different spatial scales suggests that it can be explained by a persistent load of former deposition with industrial dust. In contrast, lead contamination can be attributed to automobile traffic rather than to the former deposition of industrial dusts.
3. Surprisingly large variance component of the decomposition rate and organic soil layer characteristics can be attributed to the micro-scale (1-m distance). That part of variability can be attributed to natural factors rather than to direct anthropogenic effects.

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