Application of the bait-lamina method to measure the feeding activity of soil fauna in temperate forests

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ABSTRACT

The aim of the study was to compare the feeding activity of soil fauna in seven temperate forests with the application of the bait-lamina method. Seven types of temperate forests located throughout Poland (East-Central Europe) were tested, ranging from dry pine forest with a typical poor quality soil to eutrophic riparian fresh deciduous forest. Each forest type was represented by five stands and all stands altogether represented natural gradient of soil fertility, texture and vegetation diversity. Despite clear diversification between the studied forest types according to a range of soil physicochemical properties and vegetation characteristics in addition to applying recommended measurement conditions for the method, we determined that the feeding activity of soil fauna did not differ between forest types. The activity of soil fauna did not depend on site botanical characteristics or any soil physical or chemical features, indicating that the bait lamina method was useless in measuring the feeding activity of soil fauna in temperate forest soils. Differences in the feeding activity of soil fauna might result from other environmental factors that influence soil fauna feeding activity in forest stands that were not measured here, i.e., soil temperature and humidity. The differences could also be attributable to the attractiveness of the bait substrate to soil fauna, which may be different in various soil conditions.

INTRODUCTION

Primary production and the decomposition of organic matter are basic ecosystem processes in terrestrial ecosystems. However, the relationships between the below- and aboveground parts of ecosystems remain elusive (Wardle *et al.* 2004). Soil organisms decompose the soil organic matter (SOM), which is composed primarily of plant and animal dead biomass. Soil bacteria and fungi dominate these processes (van der Heijden *et al.* 2008). However, soil fauna, which are animals of different sizes and life history traits, remain an important component of the belowground biological system.

Soil fauna facilitate the microbial attack on SOM, both directly, by the consumption and fragmentation of SOM, and indirectly, by their influence on microbial activity, biomass and community composition (Cortet *et al.* 1999, Bonkowski *et al.* 2000, Sławska 2004, Rożen *et al.* 2010). Soil fauna is influenced by a variety of environmental factors, such as local climatic conditions, edaphic characteristics, vegetation and anthropogenic factors (Rożen *et al.* 2010). These factors may result in diversified activity and taxonomic composition of soil fauna community in different forest ecosystems; thus, soil fauna can be treated as indicators of soil health and fertility.

For example, in temperate forests, a keystone group of soil fauna are enchytraeids (Didden and Fluiter 1998), which are common in the coniferous forests currently dominating in Europe because of former silviculture management (Bobiec *et al.* 2000, Blicharska *et al.* 2012). In soils characterized by a low pH, enchytraeids occur in huge amounts, with up to 200 000 individuals per square meter, and supersede earthworms, which prefer soils with a higher pH (Niklińska

and Klimek 2011). In turn, springtails are common in soils of early successional stages (Petersen and Luxton 1982). Also, organisms traditionally linked with water ecosystems, such as rotifers, can be found in the soil (Devetter 2010). Another group are organisms that do not have an accurately determined taxonomical position, such as slime moulds (Bochynek and Drozdowicz 2012).

Soil fauna can be classified based on taxonomic position, the functions they perform in the soil or food preferences. Notwithstanding, classification according to body size remains a very practical approach, and the division to micro-, meso- and macrofauna is widely practiced (Cortet *et al.* 1999, Bonkowski *et al.* 2000). For certain research applications, arduously counting and identifying numerous small and soft-body animal species that can be found in the soil is not necessary. For some research, a general assessment of field fauna activity is a sufficient indicator. The aim of our study was to compare differences in soil fauna field activity in different temperate forests types, representing of natural gradient of soil fertility, texture and vegetation diversity, which may affect soil biological properties.

The method used in our study to assess soil fauna field activity was a common method of measuring the activity of soil fauna, the bait lamina strips test. This particular method was first described by Von Törne (1990). The method is to use plastic bait-lamina strips that are perforated and fill the openings with a bait substrate; the rate of bait consumption is a measure of the feeding activity of soil invertebrates (Hamel *et al.* 2007, Niklińska and Klimek 2011). The bait-lamina method allows the assessment of the feeding activity of living soil fauna with negligible effects from soil microorganisms and abiotic decay (Helling *et al.* 1998, Hamel *et al.* 2007). Bait can be composed of different ingredients but should be an attractive food for many groups of soil mesofauna. This can be achieved by adding pulverized nettle leaves, which are rich in protein (Helling *et al.* 1998).

The extraordinary simplicity and low cost of the bait-lamina method explain why this approach is widely used to study the activity of soil fauna in various applications. Hamel *et al.* (2007) found differences in the activity of soil fauna on experimental meadow sites, monocultures and multispecies assemblages. Similarly, detecting the effects of soil pollution using the bait lamina has been shown by many authors for heavy metals (André *et al.* 2009) or pesticides (Reinecke *et al.* 2002). Additionally, the effects of agrotechnical (Jacometti *et al.* 2007, Reinecke *et al.* 2008) or recultivation treatments (Hohberg *et al.* 2001) were shown. Rożen *et al.* (2010) used the bait-lamina method to study the vertical stratification of soil fauna in the soil profile. Römbke *et al.* (2006) proposed this method as an standard international test to measure soil quality.

Multiple applications of the method have encouraged us to test its usefulness in measuring the activity of soil fauna in temperate forest stands that differ in humidity and soil fertility, as well as vegetation diversity. Seven types of temperate forest assemblages throughout Poland were investigated, from a dry pine forest to a species-rich eutrophic floodplain forest. We expected to observe differences in the activity of soil fauna between forest types and that soil fauna activity will be higher in deciduous forests than in coniferous forests, which can possibly be driven by site characteristics, such as the soil texture or soil pH.

MATERIALS AND METHODS

Botanical characteristics of research sites

Seven types of temperate forests were investigated throughout Poland, an East-Central Europe country with a range of mean annual temperatures from 6°C in the northeast to 10°C in the southwest and an average annual precipitation for the entire country of 600 mm (Fig. 1). Within each forest type, five sites (replicates) were tested and they were distributed throughout Poland for a total of 35 stands. The plant species were identified in the field during at least three visits to each site from the early spring to the summer. The plant species were determined according to Szafer *et al.* (1986) and Mirek *et al.* (2002). There were two types of coniferous forests and five types of broadleaved forests. Detailed descriptions are given below:

- Dry coniferous forest – associations of *Cladonio-Pinetum* and V*accinio-Pinetum*, with a typical poor forest floor with *Cladonia* lichens (described here as DC),
- Fresh coniferous forest – associations of *Peucedano-Pinetum* and *Leucobryo-Pinetum* (FC),
- Acid beech forest – associations of Luzulo-Fagenion from *Luzulo pilosae-Fagetum* and *Luzulo luzuloides-Fagetum* (AB),
- Fertile beech forest – Dentario glandulosae-Fagetum (FB),
- Fresh mixed forest dominated by hornbeam – *Carpinion betuli* (MH),
- Fresh mixed forest dominated by oak –*Potentillo albae-Quercetum* (MO),
- Eutrophic fresh deciduous forest dominated by ash – *Alno-Ulmion*, mainly *Ficario-Ulmetum minoris* and *Fraxino-Alnetum* (EA).

The data on the vegetation structure and diversity were taken from botanical relevés, carried out on 100 m2 of representative forest patches according to the Brown-Blanquet method, where vascular plant species including trees, shrubs and the herbaceous layer, were identified and the coverage of particular species on the relevé was expressed as the % of the total area. The only total number of tree species was counted over area of 1 ha

(10 000 m^2) and expressed as separate stand botanical parameter; these was done to capture the spatial scale of tree diversification, which is typically larger for trees than for herbaceous plants.

The vegetation diversity at each site was calculated using the H'plant Shannon-Wiener diversity index. Data on plant coverage in 100 m2 relevés were transformed from the Brown-Blanquet scale onto the 0–9 ordinal scale, where 0 means lack of species, 1 means a species coverage <5% of the total 100 m2 area coverage and subsequently, 9 means a species coverage of 75–100% of the relevé area (Piernik 2008). H'plant was calculated according to the equation:

where pi denotes the proportion for i-th species of plant coverage in total coverage area of the plant, and N is the number of species at a particular relevé (Piernik 2008).

Bait lamina test

To perform a bait-lamina test, 525 strips were prepared for a total of 15 pieces per site. Plastic strips 120 mm ´ 6 mm ´ 1 mm were cut down at one end, which facilitates placing them into soil. A homogenous substrate was composed of cellulose (Sigma-Aldrich), agar (Becton, Dickinson Co.) and pulverized, dried nettle leaves (Herbapol, Poland) (1:1:1, w/w/w). Strips were filled with bait of the consistence of paste one day before they were placed into the soil. Openings were filled manually, with controlled the precision of the work under the light.

The experiment was performed in June 2013. Strips were exposed in the soil for at least 12 days (days of the beginning and the end of exposition were noted). Strips were placed in the A soil horizon, below the litter and humus layers, on linear transects at each research site at distances of 1 m. Immediately after collection, the perforated holes in the strips were checked and noted. The feeding activity of soil fauna was expressed as a % of the perforated holes in entire strip set per each site.

Soil sampling

At each research site, the A layer of the soil was collected to determine detailed physicochemical characteristics. One mixed soil sample was collected per research site. Each soil sample consisted of five subsamples; one sample was collected with a steel core sampler at each site in the centre of the 100 m2 plot where plant diversity was assessed, and four were taken from the four corners and joined to obtain one mixed soil sample for each plot. Directly after collection, the samples were sieved (through a 0.2 cm sieve) to remove the green parts of plants, stones and roots, packed into plastic boxes and transported to the laboratory still moist from the field. The soil was stored at 4°C before further analyses.

Soil physicochemical laboratory analysis

The dry weights (DW) of the soil samples were determined by drying them at 105°C for 24 h. Next, the organic matter content (OM) in dry weight was determined as the loss on ignition at 550°C for 24 h. The water holding capacity (WHC) was measured by a standard gravimetric method. The soil pH was measured in air-dried subsamples (1.5 g) shaken in distilled water (1:10 w/v) for 1 h at 200 rpm. The soil texture was assessed by the sieving method as a % of sand, clay and silt in the mineral fraction. The organic C and total N were analysed by dry combustion with an elemental analyser (Vario Micro Cube, Elementar Analysensysteme GmbH). The concentrations of dissolved organic carbon (DOC) were measured in water extracts obtained from 3 g soil dry mass equivalent shaken for 1 h at a 10:1 water-to-soil ratio at 200 rpm (TOC-VCPN, Shimandzu).

The total concentrations of elements (Ca, Mg, K, Na, Mn, Fe) were determined in each soil sample after wet digestion of 0.5 g of dwt in 10 ml of a mixture of suprapure concentrated HNO3 and HClO4 (7:1 v/v) (Sigma-Aldrich). The elements' concentrations were measured by atomic absorption spectrometry with a flame and graphite furnace nebulizer (Perkin-Elmer). To check the accuracy of the method, four blank samples and three replicates of a standard certified material (CRM025-050, Sandy Loam 8, RT Corp.) were analysed with the samples. Each sample was analysed in three to four replicates from every soil sample, and averaged. Together with the soil samples, the bait substrate was analysed in five replicates for the elements' concentrations.

Statistical analysis

A one-way ANOVA was performed to test the differences in the number of mean plant species, as well as in H'plant values between forest types (DC, FC, AB, FB, MH, MO, EA). The normality criterion for the data distribution within groups was checked with the Shapiro-Wilk test and transformed if needed. Significant differences in mean values were compared using the Tukey test. The results were considered significantly different at $P < 0.05$. A one-way ANOVA was run to compare the differences in soil physicochemical characteristics and the granulometric composition of the soil mineral fraction between forest types. A one-way ANOVA was run to test the differences in the mean soil fauna feeding activity (% of holes perforation) between the forest types.

To assess the simultaneous effect of various site characteristics on the feeding activity of soil fauna, a multiple regression was used with independent variables chosen based on a multiple-variable analysis. Next, the physicochemical characteristics of the soil were subjected to a Principal Component Analysis to reduce the number of variables, and a multiple regression was performed to determine the relationship between the activity of soil fauna and the main principal components (PCs). Additionally, simple linear regressions were used to correlate the activity of soil fauna and each single site characteristics, including PCs and H'plant.

Calculations for H'plant for botanical data were performed using the MSVP 3.22 software. All statistical analyses were carried out using Statgraphics Centhurion (StatPoint Technologies Inc., Warrenton VA, U.S.A.).

RESULTS

Differences between forest types

Forest types differed in the mean number of vascular plant species ($P < 0.0001$; Table 1). The dry coniferous forest was characterized by the lowest mean number of species (3.2), whereas the eutrophic fresh deciduous forest dominated by ash (riparian forests) was the richest forest, and the number of species was of order of magnitude higher (35.4).

The H'plant index differed between forest types ($P < 0.0001$), and the means ranged from 0.39 for the dry coniferous forest to 1.51 for the eutrophic fresh deciduous ash-dominated forests (Table 1). However, the vegetation diversity gradient was slightly different than expected, and the acid beech forest was less diverse than the fresh coniferous forest according to both the number of plant species and the H'plant index.

There was no difference in the feeding activity of soil fauna between the seven forest

Table 1. Mean values (n = 5) and standard deviations (in parenthesis) for plant diversity characteristics (total number of vascular plant species, number of tree species and the Shannon-Wiener H'plant index) and soil fauna feeding activity (% of strips perforation per day). One-way ANOVA test results are presented; significant differences (if existed) between forests are indicated by small letters in superscripts (a, b, c, d). Forest types: DC – dry coniferous forest, FC – fresh coniferous forest, AB – acid beech forest, FB – fertile beech forest, MH – mixed hornbeam dominated forest, MO – mixed oak dominated forest, and EA – eutrophic ash dominated forest.

Forest type	Number of plant species	H'_{plant}	Soil fauna activity (% per day)
DC	3.2 (1.1) ^{a}	0.39 (0.13) ^{a}	2.6 (0.6)
FC	18.4 (2.2) ^{b}	1.20 (0.73) ^{c}	2.3 (0.9)
AB	10.0 (1.7) ^{a}	0.91 (0.77) ^{b}	2.8 (1.0)
FB	17.8 (6.6) ^{b}	1.16 (0.20) ^{c}	2.7 (2.0)
MH	21.6 (5.7) ^{b}	1.26 (0.14) ^{c}	2.4 (1.9)
MO	24.0 (1.9) ^{b}	1.32 (0.05) ^{cd}	1.5 (0.6)
EA	35.4 (1.3) ^{c}	1.51 (0.02) ^{d}	2.2 (1.3)

Table 2. Mean values (n = 5) and standard deviations (in parenthesis) for soil physicochemical properties: maximum water holding capacity WHC, dissolved organic carbon content, pH and sand, silt and clay content in the soil mineral fraction. One-way ANOVA test results are presented; significant differences (if existed) between forests are indicated by small letters in superscripts (a, b, c, d). Forest types: DC – dry coniferous forest, FC – fresh coniferous forest, AB – acid beech forest, FB – fertile beech forest, MH – mixed hornbeam dominated forest, MO – mixed oak dominated forest, and EA – eutrophic ash dominated forest.

Forest type	Soil parameter						
	OM	WHC	DOC	pH	sand	slit	clay
	(%)		(mg g^{-1} OM)		(%)		
DC	3.8 (1.2) ^{a}	45.2 (7.7) ^{a}	14.9 (3.5) ^{c}	4.32 (0.18)	93.4 (2.1) ^{b}	5.2 (2.2) ^{a}	1.6 (2.3)
FC	7.0 (2.5) ^{a}	62.4 (13.1) ^{ab}	9.2 (4.0) ^{b}	4.44 (0.83)	87.6 (3.4) ^{b}	9.8 (4.0) ^{ab}	2.6 (1.8)
AB	23.7 (8.8) ^{b}	185.2 (64.2) ^{c}	4.0 (1.2) ^{a}	4.50 (0.59)	54.6 (20.7) ^{ab}	39.2 (21.2) ^{bc}	6.2 (3.4)
FB	15.8 (4.7) ^{ab}	158.4 (30.6) ^{bc}	4.2 (1.9) ^{a}	5.15 (0.70)	39.,6 (21.8) ^{a}	48.4 (19.6) ^{c}	12.2 (5.4)
MH	12.9 (4.6) ^{ab}	128.2 (53.0) ^{abc}	2.6 (1.4) ^{a}	4.91 (0.48)	33.0 (26.2) ^{a}	55.0 (23.6) ^{c}	12.0 (10.6)
MO	12.5 (9.2) ^{ab}	120.2 (71.3) ^{abc}	2.7 (1.6) ^{a}	5.07 (0.58)	65.,4 (30.9) ^{ab}	23.4 (18.8) ^{abc}	11.2 (14.1)
EA	14.8 (9.0) ^{ab}	122.8 (64.6) ^{abc}	2.8 (1.1) ^{a}	5.43 (1.38)	67.0 (23.4) ^{ab}	24.4 (14.0) ^{abc}	8.6 (9.6)

types studied ($P = 0.2345$) (Table 1). The mean activity of soil fauna (% of strips holes perforated per day) seemed to be lower in the oak dominated forest than in the other forest types (Table 1), but the effect was not significant.

The coniferous forests were characterized by a lower OM content ($P = 0.0016$) and thus a lower WHC than the deciduous forests ($P = 0.0015$) (Table 2). The mixed oak forest had the lowest DOC concentration and the acid beech forest had the highest DOC concentration ($P < 0.0001$) (Table 2). The soil mineral fraction granulometric composition differed between forest types for sand ($P = 0.0005$) and silt fractions ($P = 0.0003$). The highest sand concentration was found in coniferous forests and the lowest in the fertile beech forest and in the mixed forest dominated by hornbeam (Table 2). The opposite trend was observed for silt content. The soil pH did not differ between forest types ($P = 0.2091$) (Table 2).

Coniferous forest types differed from the acid beech forest in C and N concentrations ($P = 0.0024$ and $P = 0.0008$, respectively) (Table 3). Low N concentrations in the coniferous forests resulted in a higher C:N ratio than in other forest types ($P < 0.0001$) (Table 3). Additionally, the concentrations of other chemical elements were lower in coniferous than in deciduous forests, including K ($P < 0.0001$), Na ($P < 0.0001$), Ca ($P = 0.0027$), Mg ($P < 0.0001$), Mn ($P < 0.0001$) and Fe ($P < 0.0001$) (Table 3).

The bait substrate biogens composition (mean values) were as follows: C – 37.37%, N – 1.13% and the C:N was of 33.3. The total

Table 3. Mean values (n=5) and standard deviations (in parenthesis) for the total chemical element contents in the soil (C, N, K, Na, Ca, Mg, Mn, Fe) and the C:N ratio. One-way ANOVA test results are presented; significant differences (if existed) between forests are indicated by small letters in superscripts (a, b, c, d). Forest types: DC – dry coniferous forest, FC – fresh coniferous forest, AB – acid beech forest, FB – fertile beech forest, MH – mixed hornbeam dominated forest, MO – mixed oak dominated forest, and EA – eutrophic ash dominated forest.

Forest type	Soil parameter								
	C	N	C:N	K	Na	Ca	Mg	Mn	Fe
	(% DW)			(mg kg^{-1} DW)					
DC	1.91 (0.62) ^{a}	0.09 (0.02) ^{a}	21.8 (2.4) ^{b}	243 (88) ^{a}	17 (4) ^{a}	202 (146) ^{a}	114 (108) ^{a}	24 (14) ^{a}	2440 (1010) ^{a}
FC	3.64 (1.28) ^{a}	0.16 (0.06) ^{ab}	22.8 (3.1) ^{b}	334 (109) ^{ab}	22 (5) ^{ab}	649 (894) ^{ab}	158 (75) ^{ab}	47 (33) ^{ab}	3015 (1120) ^{ab}
AB	12.06 (4.53) ^{b}	0.68 (0.30) ^{c}	18.2 (1.6) ^{ab}	2900 (2755) ^{c}	141 (84) ^{c}	812 (341) ^{ab}	694 (368) ^{bc}	250 (104) ^{bc}	13938 (7042) ^{c}
FB	7.39 (2.63) ^{ab}	0.52 (0.11) ^{bc}	14.2 (3.7) ^{a}	5551 (3377) ^{c}	202 (95) ^{c}	2485 (2399) ^{b}	2207 (1410) ^{c}	878 (501) ^{c}	21472 (8774) ^{c}
MH	6.11 (2.23) ^{ab}	0.44 (0.13) ^{abc}	13.6 (1.8) ^{a}	3138 (1665) ^{c}	115 (55) ^{c}	1592 (738) ^{ab}	1399 (762) ^{c}	1107 (1030) ^{c}	12614 (4427) ^{c}
MO	5.50 (4.99) ^{ab}	0.37 (0.26) ^{abc}	14.0 (2.6) ^{a}	1674 (1951) ^{bc}	83 (62) ^{bc}	6364 (10793) ^{b}	1610 (1731) ^{c}	557 (775) ^{c}	14685 (15876) ^{bc}
EA	6.59 (4.,40) ^{ab}	0.48 (0.29) ^{abc}	13.2 (2.5) ^{a}	2261 (1882) ^{c}	122 (88) ^{c}	6710 (8722) ^{b}	1212 (1037) ^{c}	717 (955) ^{c}	14014 (10296) ^{c}

content of other chemical elements in the bait substrate was: K – 185 mg kg^{-1} DW of soil, Na – 10430 mg kg^{-1} DW of soil, Ca – 9116 mg kg^{-1} DW of soil, Mg – 1051 mg kg^{-1} DW of soil, Mn – 18 mg kg^{-1} DW of soil and Fe – 129 mg kg^{-1} DW of soil.

The effects of site characteristics on fauna feeding activity

A multiple-variable analysis indicated that soil parameters were highly correlated (data not shown). Only three independent variables could be used in the multiple regression: the C:N ratio as a measure of SOM quality, the water holding capacity (%) as a measure of the water holding potential of the soil and the Ca concentration (mg kg–1 DW) as a proxy of the content of macro elements. The model for these three factors was not significant (P = 0.9296) and all tested factors were not significant, even when the backward and forward factors selection was run.

The Principal Component Analysis (PCA) allowed the inclusion of more variability in the soil data and allowed these variables to be tested as to their possible effects on the feeding activity of soil fauna. Three PCs explained subsequently 52, 15 and 10% of the variability in the data (Fig. 2). The first PC was based on the sand component, DOC, N, Mg and Na concentration and was a compilation of information on soil fertility and organic matter content. The second PC was composed of the pH, Ca concentration and C:N ratio, and the third one was based on the soil texture and soil C content. There was not, however, a significant relationship between the PCs and the feeding activity of soil fauna (P = 0.9422).

Finally, the feeding activity of soil fauna was not dependent on any soil physicochemical characteristics when tested with a simple regression analysis.

DISCUSSION

Despite the wide range of soil physicochemical and vegetation characteristics along our temperate forest gradient and the application of the recommended measurement conditions for the bait lamina method, we demonstrated that the feeding activity of soil fauna did not differ between seven types of temperate forests. Moreover, the feeding activity of soil fauna did not depend on the botanical characteristics of the site or any physical or chemical features of the soil that were measured, suggesting that the usefulness of the method is limited.

The potential reason for the lack of diversification in soil fauna in our study could be that we conducted our study on forest stands over a gradient of many environmental features. Earlier studies showed a change in the feeding activity of soil fauna with changes in some soil features, but the research was conducted on experimental plots with strong and clear effects from experimental manipulation. For example, Geissen and Brümmer (1999) conducted their research on the feeding activity of soil fauna along a gradient of soil properties, which was obtained by soil liming and mineral fertilization, in just one type of temperate forest, a mixed oak-birch forest (*Quercus robur/ Carpinusbetulus*) in Germany. They stated that the feeding activity of soil fauna was related to soil pH or the element content (Ca, Mg, K) in the soil. Additionally, Rożen *et al.* (2010) who studied the feeding activity of soil fauna in experimental research plots that were tree monocultures found differences between monocultures. They found higher fauna activity in plots where spruce (*Picea abies*) and larch (*Larix decidua*) dominated, comparing to twelve other monoculture plots. In our study, we measured the feeding activity of soil fauna in natural stands that were not manipulated and were characterized by higher natural variability.

We expected that we would find differences in the feeding activity of soil fauna between tested forest types, at least between coniferous and deciduous broadleaved forests. We expected that pine dominated forest soils would be characterized by lower soil fauna activity. Pine forest soils were characterized by less favourable conditions that drive soil biota activity, which are lower SOM content, coarser soil texture, lower water holding potential, and less soil affluent in macro- and microelements. Nevertheless, we did not confirm our hypothesis. Moreover, none of the measured environmental traits were related to the feeding activity of soil fauna.

The feeding activity of soil fauna can be dependent not only on soil physicochemical properties, but on other environmental features. The significance of temperature as a factor determining the results of the bait lamina test was shown by Gondalsky *et al.* (2008) in a laboratory experiment. They showed that bait lamina perforation was the highest at 24°C and was much less at the lower temperatures tested, which included 14°C, 5°C and –4°C (Gondalsky *et al.* 2008). In turn, Rożen *et al.* (2010), who did not find a direct relationship between the feeding activity of soil fauna in the field and soil physicochemical characteristics, suggested that the activity of soil fauna may be dependent on other environmental factors, i.e., soil temperature or humidity.

In our study, we did not determine these traits. However, it can be expected that the soils of pine forests of a coarser soil texture and lower water holding capacity are more susceptible to drying than the riparian eutrophic forest, which are located at the edges of rivers. On the other hand, thick humus and litter layers in pine forests may keep soil moisture in the upper soil layers and protect deeper soil horizons from drying. Additionally, plant coverage may affect the soil temperature. Less occlusive pine forests may foster increases in the soil temperature, whereas dense tree coverage in riparian forests may reduce it. Despite a wide gradient of soil physicochemical characteristics, the soil temperature and humidity could be the confounding factors in our study. Because we did not control the soil temperature and humidity, such a hypothesis cannot be confirmed or excluded without the repetition of the experiment.

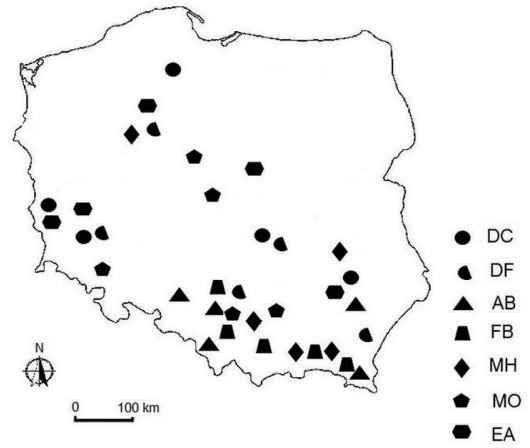

Fig. 1. Location of study sites (Poland, East-Central Europe). Forest types: DC – dry coniferous forest, FC – moist coniferous forest, AB – acid beech forest, FB – fertile beech forest, MH – mixed hornbeam dominated forest, MO – mixed oak dominated forest, and EA – eutrophic ash dominated forest.

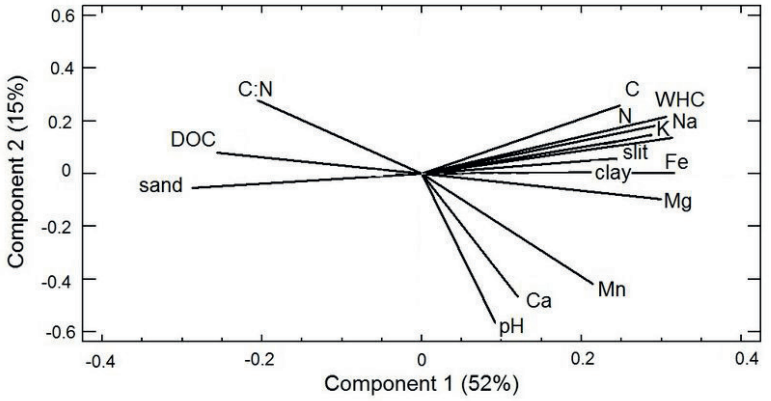

Fig. 2. Interdependencies between the physical and chemical properties of the soil (results of PCA analysis). The first and second components are presented. All soil data used in the PCA analysis are presented in Tables 1–3 (means and their standard deviations).

We did not find an effect from vegetation diversity on the feeding activity of soil fauna. The expected result was that a greater diversity of vegetation, that is, primarily SOM producers, is favourable for higher feeding activity of soil fauna. A wider range of chemical compounds contained in the litter and root exudates creates more ecological niches that are potentially available for soil biota (Klimek *et al.* 2015). However, this seems to be the case for soil microorganisms but not for soil fauna. We defined vegetation diversity as the total vascular plant diversity, including trees, shrubs and vascular plants on the forest floor. Such an approach is more comprehensive because the majority of the biological diversity of vegetation in forests is from the herbaceous layer, not the trees species (Eisenhauer *et al.* 2011). However, litter production from trees overlap the litter production from herbaceous layer in temperate forests. Additionally, the dominant tree species may strongly affect soil properties by producing litter of different chemical compositions (Kiikkilä *et al.* 2006). For example, evergreen needle trees produce more acids and litter that is more difficult to decompose than do broadleaved trees (Adamczyk *et al.* 2008).

Because of the strong diversification in the number of plant species and the H'plant index between the forest types we studied, as well as the diversified physicochemical properties of the soil A horizon, the lack of a difference between forest stands may result from differences in the attractiveness of the bait substrate to soil fauna between forest types. The bait is expected to be attractive to soil fauna thanks to the high nitrogen content (nettle leaves) compared to the soil. However, in dry poor pine forests, the bait could be a more encouraging substrate, and the energetic outlay from soil biota to burrow for food could be more cost-effective than in the eutrophic broadleaved forest. In more nutritive soils in terms of food supply, the soil fauna does not need to translocate for feeding. Such a hypothesis could be tested in a separate experiment by using bait lamina strips of different bait substrate compositions.

Some current research has shown that the bait lamina test did not provide satisfactory results (Domene *et al.* 2014). There are suggestions to use many more than a dozen strips to take into account the spatial variability of the soil (Gondalsky *et al.* 2004, Hamel *et al.* 2007) and the natural aggregative structure of the spatial distribution of some soil fauna groups, such as enchytraeids (Niklińska and Klimek 2011). Undoubtedly, the advantages of the method are the low costs and the fact that the experiment is quick and easy to perform (Kratz 1998). A less obvious advantage that has rarely been raised is the possibility of performing non-destructive research on soil fauna in the field. This allows research to be conducted in valuable ecosystems, especially when there is a need to repeat the studies, for example, to create comparisons between seasons or years. These features state that the bait lamina method, despite its

low sensitivity and lack of measurement precision, can be treated as interesting competition for traditional taxonomic studies.

CONCLUSIONS

Despite clear diversification between the studied forest types according to a range of soil physicochemical properties and vegetation characteristics in addition to applying the recommended measurement conditions for the bait lamina method, we did not observe a difference in soil fauna feeding activity between seven temperate forest types. Soil fauna feeding activity did not depend on the botanical characteristics or any basic soil physical or chemical features of the site, which made the method for measuring the feeding activity of soil fauna useless in temperate forest soils. Differences in the feeding activity of soil fauna might result from the influence of other environmental factors on test results, such as soil temperature and humidity, which can be expected to differ between stands. The other possible reason could be the different levels of attractiveness of the bait substrate for soil fauna in different soil conditions.

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