Microarthropods and vegetation as biological indicators of soil quality studied in the poor sandy stand at former military sites

Iwona Gruss[1], Jacek Twardowski[1], Diana Nebeská[2], Josef Trögl[2], Tatyana Stefanovska[3], Valentina Pidlisnyuk[2], Iva Machová[4]

[1] Wrocław University of Environmental and Life Sciences, Department of Plant Protection, Grunwaldzki Sq.24a, 50-363 Wrocław, Poland

[2] Jan Evangelista Purkyně University, Faculty of Environment, Department of Environmental Chemistry and Technology, Králova výšina 3132/7, Ústí nad Labem, Czech Republic

[3] National University of Life and Environmental Sciences of Ukraine, Faculty of Plant Protection, Biotechnology and Ecology, Heroiv Oborony Str.15, 03041, Kyiv, Ukraine

[d] Jan Evangelista Purkyně University, Faculty of Environment, Department of Environment, Králova výšina 3132/7, Ústí nad Labem, Czech Republic

Correspondence: Jacek Twardowski, jacek.twardowski@upwr.edu.pl (ORCID: 0000-0003-2412-8992)

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Abstract

Biota play major roles in soil function and are highly sensitive to any disturbances including land degradations. The objective of research was to evaluate the effectiveness of different methods used to determine soil quality in sandy soil, in particular to compare the microarthropod and vegetation indices. The following soil fauna indices were used: Collembola and Acari abundance, QBS-ar index, decomposition rate, feeding activity. The Ellenberg index was used as a vegetation indicator, in which the response to pH, nutrients, and moisture was analysed. We based on an experiment conducted at a former military site in the Czech Republic. Soil quality was determined at two sites which differ slightly in nutrient content. Collembola abundance, feeding activity, and QBS-ar index were highly sensitive to minor differences in nutrients. In the group of vegetation indices, only the response to pH was significant. All analysed indices showed better biological quality in soils with higher nutrient content. Collembola were positively correlated with all vegetation indicators, which may indicate a close relation of springtails to certain plant species or similar habitat requirements. Finally, we indicate the usefulness of biological indicators for monitoring the quality of soil, which can be adopted when making various decisions concerning land use.

KEYWORDS

biological indicators, Ellenberg index, soil microarthropods, Collembola, military areas

1 INTRODUCTION

Soil quality is defined as the ability to function within ecosystems which reflects the soil's complexity and its ecosystem services (Bünemann et al., 2018). It is important in suitable soil management, particularly when combined with land use management (Pullemann et al., 2012). Soil quality can be measured by different indicators, which primarily include physical, chemical, biological, and biochemical processes. Among these, biological and biochemical indicators are marginalised (Bünemann et al., 2018). The sensitivity of the soil fauna to environmental variations can be a potential indicator of soil quality, but the interactions between them are relatively unknown (Cunha Neto et al., 2012). Soil is an environment in which many groups of macro- and microorganisms undergo certain stages of development or their entire life cycle (Hättenschwiler et al., 2005; Gessner et al,. 2010). Biota play a crucial role in many soil processes and functions (Pulleman et al., 2012). Any changes in properties of the soil can influence the abundance, species composition, and activity of the biota (Kladivko, 2001; Yi et al., 2021). There are several methods to access soil quality based on fauna. The most common is general evaluation of microarthropod occurrence in the field (Twardowski et al., 2016; Gruss et al., 2018), with Collembola and Acari accounting for about 95% of the total number of arthropods which live in the soil (Neher, & Barbercheck, 1998). However, Collembola and Acari represent different ecological traits and can occupy different soil levels (González-Macé, & Scheu, 2018). The differences include trophic preferences, reproduction type, and dispersal ability (Siepel, 1994; Yin et al., 2019). The ecological traits of soil microarthropods are related to their morphological adaptation to life in the soil (Parisi et al., 2005). Based on this assumption, the QBS-ar index was developed by Parisi (2001) and verified in different ecosystems (Aspetti et al., 2010; Menta et al., 2018; Galli, 2020).

An alternative is to measure the soil fauna activity, expressed through its contribution to soil processes (Briones, 2014). The bait-lamina test allows assessment of the feeding activity of soil organisms. This method, first used by Törne (1990), is proposed as a simple tool for measuring soil fauna activity, the effectiveness of which has been confirmed by many authors (Filzek et al., 2004; André et al., 2009; Klimek et al., 2015). Moreover, litter decomposition, which is one of the most important biogeochemical processes, closely corresponds to the activity of soil biota (Hättenschwiler et al., 2005; Gessner et al., 2010). The direct contribution of soil fauna to decomposition is the fragmenting and burrowing of the surface of litter material, while an indirect contribution relies on stimulating microbial decomposition (Yin et al., 2019).

Plants belong to the soil biota and also have a significant role in many soil functions. For example, it is recognised that plant root exudates and residues form the major source of carbon and energy for heterotrophic soil biota (Pulleman et al., 2012). The occurrence of different plant species enables access to environmental conditions (Shaffers, & Sýkora, 2000). One of the proposed indicators is the Ellenberg index, which assigns indicator values to vascular plant species (Ellenberg et al., 1992). The efficacy of the Ellenberg index was validated in relation to soil properties (Pitman et al., 2014; Chytrý et al., 2018). Considering soil quality, the most useful are the indices with respect to soil moisture, soil nitrogen, and reaction to pH (Schaffers, & Sýkora, 2000). Its value can be useful in choosing ecologically appropriate crops and types of woodland (Hawkes et al., 1997).

To assess soil processes and functions comprehensively and well, it is essential to consider the complex of biotic interactions in the soil in conjunction with the abiotic environment. Most authors assess soil quality using just one independent indicator, but it is preferable to sensibly combine some of these to create a better understanding of soil processes and functioning (Van Leeuwen et al., 2015). In this study, all analysed indices are closely related to soil function. We suppose that the response of all indices will be similar to minor differences in soil nutrient content. We also hypothesise that there is a link between fauna and vegetation indicators.

2 MATERIALS AND METHODS

2.1 Site description

The research site is located in the area of a former military airport, Hradcany, Czech Republic. Since the 1990s, this locality has undergone reclamation after massive contamination by fuels. Currently, the top layers of soil are free of contamination. Residual hydrocarbons can be detected mainly in the saturated zone (at a depth of more than 2.5 m) (Machackova et al., 2012), but it was claimed that the contamination of the site no longer presents health or environment risks (Ministry of the Environment of the Czech Republic 2020). Nevertheless, the area is still affected by past military activity by the presence of building ruins and illegal dumps which are continuously removed, and the area is undergoing restoration.

Two research plots were established to test the possibility of growing an energy crop, *Miscanthus x giganteus* (Greef et Deu), in such conditions as one of the options for future use of the locality. Site 1 (50°37’31″N, 14°43′23″E) was, for most of the time since the 1950s, fully or partly covered by trees, which were felled after 2010, and the surface has remained unforested to this day. Site 2 (50°37′26″N, 14°44′49″E) is located near a jet fuel storage bunker, and in the 1950s–1990s it was intersected by an access road. Compared to site 1, it has been without tree cover since at least the 1950s. Both sites were established in May 2017 with the same management. The soil was ploughed and undesirable materials, e.g., pieces of concrete and large stones, were removed. The sites were both divided into eight subplots (3 × 4 m) which were each planted with 12 *M. x giganteus* rhizomes. There was no following maintenance applied except watering the rhizomes in the first two weeks after planting.

2.2 Soil characteristics

The soil in this area is characterised by its low quality with a high sand content. It is not homogenous, and some differences between sites 1 and 2 can be found. The physicochemical characteristics of the soils are given in Table 1. The soil texture was determined by a combination of sieving and the hydrometer method, as recommended by the ISO (2016). The maximum water holding capacity (WHC) was determined gravimetrically after total saturation of the samples with water and draining for 2 hours on filter paper according to the ISO (2012).

The soil reaction was measured by a pH meter in a suspension of soil with deionised water (pH-H_2O) and 1M KCl (pH-KCl) with a 1:2.5 ratio. The content of total C, N, and S was determined by an elemental analyser, Vario MAX CNS/CN (Elementar Analysensysteme). Humus content was calculated as the C content multiplied by Welte's coefficient 1.724. The available nutrients P, K, Mg and Ca were extracted with Mehlich 3 solution and determined by ICP-OES (Integra XL, GBC Scientific Equipment). Mineral nitrogen (N-NO_3 and N-NH_4) was determined spectrophotometrically according to the standard methodology by Zbíral (2002).

2.3 Soil fauna abundance

Soil samples for microarthropod abundance were taken twice a season in the years 2017–2018 (in June and October). Five soil samples were taken diagonally across each subplot from a depth of 10 cm using a core sampler with a diameter of 5 cm. The number of soil samples in one season was as follows: 5 samples from each subplot × 8 subplots × 2 sites × 2 sampling dates = 160. Soil organisms were extracted in Berlese-Tüllgren funnels for 48 hours using sieves with a mesh size of 0.2 cm. The extracted organisms were kept in 75 % alcohol. All invertebrates were determined to the taxonomic level, which allowed the determination of the

QBS-ar index as proposed by Parisi et al. (2005). The principle of this index is that the presence of higher numbers of taxa with better adaptation to life in soil indicates better soil quality (Parisi, 2001). For each individual invertebrate group, an EMI (Ecomorphological Index) score was assigned, which ranges between 1 (no adaptation to soil – for instance, adult Diptera) and 20 (maximum adaptation to life in the soil – for instance, soil mites). The EMI scores were calculated from five samples from each plot, the result of which is the QBS-ar index. Summing up the scores from the five samples, we obtain the sample size (100 cm^2), which is required in Parisi et al.'s protocol (2005). The scores assigned to each taxon are presented in Appendix 1. Additionally, the total numbers of Collembola and Acari were analysed. The observations were performed under a stereomicroscope (13 to 56x magnification).

2.4 Soil fauna feeding activity and decomposition rates

The feeding activity of soil organisms was determined in June 2018 by using a bait-lamina test (Törne, 1990). The test allows access to the feeding of the bait of soil fauna. Bait-lamina strips were made from PCV and were 120 mm long, 6 mm wide and 1 mm thick. Each strip had a series of 16 holes (2 mm diameter) pierced at 5 mm intervals from each other. The pointed tip at the lower end was inserted into the soil. Holes were filled with bait containing a mixture of microcrystalline cellulose, powdered oatmeal, activated carbon, and agar, in proportions of 35 : 13 : 2 : 50. The strips were inserted vertically in the soil with the uppermost hole under the soil surface and exposed for ten days. One experimental unit (for subplots) was arranged as a group of 16 strips in a 4 × 4 grid within an area of about 30 × 30 cm. The number of strips used in the experiment was as follows: 16 strips for subplots × 8 subplots × 2 sites = 164 strips. At the end of the exposure period, the strips were carefully removed from the soil and each hole was visually assessed. If the bait was fully eaten, it was assessed as 1, partially – 0.5, and uneaten – 0.

The decomposition rates were determined using litter bags (Seastedt, 1984) to estimate the soil organisms' contribution to litter mass loss. We used nylon bags (15 × 10 cm) with a mesh size of 5 mm filled with 7.5 grams of dried plant material consisting of a mixture of grasses (*Festuca rubra* L., *Lolium perenne* L., *Poa pratensis* L., *Festuca ovina*, *Festuca arundinacea* Schreb., *Agrostis capillaris* L.). One litter bag was placed in the middle of each subplot (16 bags in total) and covered with a thin layer of soil. The incubation period was 60 days during the summer of 2018. After retrieval of the litter bags, the remaining litter was separated from soil particles, roots, and non-target soil material. The material from each litter bag was dried at a temperature of 50°C and weighed.

The cleaned litter residues were dried at 70°C for at least three days to a constant weight. Finally, the weight of the remaining litter was recorded to quantify the decomposition rates and soil fauna contribution. The decomposition rates (*k*) were calculated using the model $M_t/M_0=e^{-kt}$, where M_0 is the initial dry mass of the litter (g) and M_t is the dry mass remaining after the incubation period (*t*).

2.5 Vegetation indicator

A botanical survey of the localities was carried out in October 2019 and April 2020, after two years' recovery following establishment of the fields. All plant species which were present inside the fence around the plot and in an undisturbed area up to 5 m from the fence were included in the list.

Ellenberg indicator values (EIV) modified for the Czech Republic by Chytrý et al. (2018) were then assigned to the species present. The mean values for moisture, nutrients, and soil reaction pH were then calculated for each site and used to estimate the site conditions.

Additionally, the most typical vegetation was documented by phytosociological plots of 2 × 5 m in the area in October 2019. The seven-point Braun-Blanquet scale was used to express species abundance (Braun-Blanquet, 1932) (Appendix 3).

2.6 Statistical analyses

The soil fauna abundance data and QBS-ar index were analysed using a mixed model (proc mixed) with years as the repeated factor (type arh1). Decomposition rates, feeding activity, and Ellenberg indicators for both sites were compared using a mixed model (proc mixed) without repeated factors. The fauna and Ellenberg indicators were correlated using the Pearson correlation. The analyses were performed by SAS University Edition, version 9.0. Furthermore, the fauna taxa were presented using DCA (Detrended Correspondence Analysis) with the sites as the supplementary variables. The analysis was performed by Canoco, version 5.0.

3 RESULTS

3.1 Soil fauna indicators

We analysed the effect of the site on five indicators, which are based on different purposes (Table 2). For Collembola and Acari, their abundance was analysed, while the QBS-ar was based on the presence and trait analysis of organisms. Feeding activity and decomposition are related to the functioning of organisms in the soil. Three indicators: Collembola abundance, QBS-ar, and feeding activity, showed a significant response to the site (Table 2).

The mean number of Collembola was significantly higher for site 2 than for site 1 ($p = 0.5$) (Table 2, Fig. 1). The difference was more distinct in the first year of the study (Figure 1). A similar trend was found for Acari, however, there was no significant difference ($p = 0.48$) (Table 2, Figure 2). The value of the QBS-ar index was significantly higher for site 2 than for site 1 ($p = 0.04$), indicating major differences in soil quality (Table 2, Figure 3).

The soil fauna feeding activity was significantly lower for site 1 compared to site 2 ($p = 0.04$) (Table 2, Figure 4). The decomposition rates did not differ significantly between the sites ($p = 0.07$) (Table 2, Figure 5). However, there was a similar trend as observed by feeding activity.

The DCA biplot (Figure 6) presents the most abundant taxa in sites 1 and 2. The proportion of the variance explained was 0.75 according to the first DCA axis and 0.55 according to the second axis (Appendix 2). The highest species scores of DCA 1 was assigned to different Collembola taxa: *Hypogastrura* spp., *Friesea mirabilis,* and Isotomidae, while DCA 2 to Thysanoptera (Appendix 2). Generally, Collembola (*Hypogastrura* spp., *Mesophorura* sp., Isotomidae) were more abundant in site 2, while other arthropods (Diptera larvae, Lepidoptera larvae) or Symphypleona (globular Collembola) were more abundant in site 1. Taxa like Thysanoptera or Hymenoptera did not show any site preferences. Certain Collembola morphotypes (*Mesaphorura* spp.) show the highest degree of specialisation to edaphic life, therefore their presence may indicate better soil quality (Appendix 1).

3.2 Vegetation indicators

The number of plant species was considerably higher for site 2 (52) than for site 1 (37) (Appendix 3). In this case, the purpose of the Ellenberg index is that the higher value of the studied indices (moisture, reaction to pH, and nutrients) indicates better soil quality. According to the Ellenberg index value, only the reaction to pH showed significant differences between the sites, with the higher pH for site 2 (around neutral) ($p = 0.02$). Considering two other indices, moisture and nutrients, there was only a slight, non-significant reaction (both sites are rather nutrient-poor) ($p = 0.5$ and 0.09, respectively) (Table 3). Generally, based on the Ellenberg index, better soil quality was found in site 2. These trends are consistent with the soil's physicochemical characteristics (Table 1) and soil fauna indices (Table 2).

3.3 Link between fauna and vegetation indicators

When comparing the fauna and vegetation indicators, only Collembola was positively correlated with all three vegetation indicators (Figure 7). The highest correlation was found between moisture ($r = 0.32$, $p = 0.02$) after reaction to pH and nutrients (in both cases $r = 0.29$, $p = 0.05$). The correlation between other fauna indicators and vegetation indicators was insignificant. However, the correlation coefficients (r) between decomposition and moisture ($r = 0.49$), decomposition and nutrients ($r = 0.34$), as well as feeding activity and moisture ($r = 0.45$) were considerably high.

4 DISCUSSION

In this study, we found distinct responses of almost all analysed fauna and plant indicators in relation to minor changes in soil properties on degraded military land. Generally, all analysed indices showed better soil quality for site 2, which was characterised by a slightly higher contribution of loam in the soil texture and slightly higher nutrient content (C, N, P, K, Ca, Mg). Some of these indicators: Collembola, QBS-ar, and feeding activity of soil organisms, are highly sensitive to any changes in soil properties and could be widely used in sandy soils. Generally, soil fauna benefits from higher nutrient content in the soil. In the study by Rzeszowski et al. (2017), the gradient of potassium and phosphorus positively affected the Collembola community, although the trends were species specific. In another study (Wang et al., 2016), the soil fauna occurrence was positively associated with carbon, nitrogen, phosphorus, and potassium content.

The QBS-ar index is based on the assumption that the representation of arthropods better adapted to life in the soil (e.g., mites) indicates better soil quality. The indicator is based on the presence/absence of certain taxa and not on its abundance (Parisi, 2011). At the global scale, QBS-ar values are negatively affected by land use intensification (Menta et al., 2018).

The value of the QBS-ar index was relatively low in comparison to studies by other authors (maximal value 90 for site 2). For instance, the maximal QBS-ar index calculated for post-mining was 140 and increased with the succession stage (Madej et al., 2011). In Mediterranean areas, the value of this index reached values of up to 240. As shown by the DCA analysis, the differences in the QBS-ar value between the sites resulted mainly from the more frequent occurrence of soil-dwelling Collembola (with higher EMI scores) in site 2 and the more frequent occurrence of insects' larvae and surface-dwelling Collembola (with lower EMI scores in site 1). Soil-dwelling Collembola are better adapted to living in the soil than other groups, e.g., adult insects.

To determine soil fauna feeding activity, bait-lamina bioassays were performed. In this method, mainly soil macrofauna (e.g., earthworms) and mesofauna (e.g., springtails) contribute to the feeding (Förster et al., 2004; Rombke et al., 2006). The disadvantage of this method could be the inability to distinguish between the different feeding activities of organisms (Musso et al., 2014). However, the short duration of feeding activity bioassays (typically 7–10 days) does not allow microbial activity in perforation (Gongalsky et al., 2004).

A method which includes microbial activity is the use of litter bags to determine the decomposition rate (Gestel et al., 2003). The rate of litter decomposition depends mainly on the origin of the litter, its composition (content of easily degradable simple organic compounds, biodegradable cellulose and hemicelluloses, and poorly biodegradable lignin), abiotic factors (soil moisture and temperature), as well the occurrence of soil organisms (Gessner et al., 2010; García-Palacios et al., 2016). Furthermore, the procedure used could affect the decomposition rates to a large extent (Xie, 2020). For instance, the mesh size could include or exclude particular groups of organisms (Bradford et al., 2002). In our study, the slight decrease in the decomposition rate for site 2 was not significant, and therefore, in our opinion, this method is less effective for soil quality assessment than the bait-lamina bioassay. The 0.5 mm mesh size used in our study allows all groups of organisms to contribute to the decomposition rate. Perhaps the method would be more precise if a procedure allowing indication of the contribution of particular groups of organisms to the overall decomposition was used.

The Ellenberg indexes enable the assessment of environmental variables without direct measurements, and make the method very cheap and quick, which is a significant benefit (Diekman, 2003). Plant-trait-based approaches have been also developed to predict the distribution of biodiversity (Diaz et al., 2016), and to better understand the functioning of soil biota (Laliberté, 2016). In our study, three measures were tested: nutrients, reaction to pH, and moisture. In the study of Hawkes et al. (1997), the index for nutrients and reaction to pH was the most reliable in relation to soil properties. In oak forest, the Ellenberg index was not reliable in the case of nutrients and moisture, while sufficient results were obtained for Ca content (Szymura et al., 2014). Generally, the results of the Ellenberg index are consistent with the fauna indices and confirm the differences in soil nutrient content between both sites.

A significant correlation between Collembola and the three vegetation indicators was also found. There are a few possible explanations for this result:

1. Both Collembola and plants are closely related to soil function. Therefore, their response to changes in the soil may be similar.
2. Changes in soil quality are mediated by plants. Thus, plants indirectly influenced the occurrence of springtails. Plants influence the below-ground ecosystem directly by the kind of leaf litter and root inputs in the soil (Gill, & Jackson, 2000). This means that manipulating plant traits may change the soil functions (Orwin et al., 2020).
3. Certain plant species and Collembola may have similar requirements for soil conditions.

4. The presence of certain plant species and functional groups may be more important for Collembola (Salamon et al., 2004).

5 CONCLUSIONS

The data presented support the following conclusions: Collembola abundance, QBS-ar index, and feeding activity are the most effective soil fauna indicators in relation to differences in nutrient content in sandy soils. These indices are based on different purposes: soil fauna abundance, functional traits, and soil fauna feeding activity, respectively. For the plant indicator (Ellenberg index), only the response to pH showed significant results. Generally, all indicators showed better soil quality for site 2, with a higher nutrient content. We also found that there was a significant correlation between Collembola abundance and the three plant indicators. Collembola and plants are both closely related to soil function, and therefore their responses can be similar. This may also indicate that Collembola and certain plant species have similar environmental requirements or the plants are mediators of changes in the soil and therefore indirectly affect soil fauna. It is also possible that Collembola prefers certain plant species.

In our opinion, the use of soil biological indicators is necessary and useful in soil quality as well land degradation assessment. The response of living organisms may indicate changes within soil functionality, which is important for various agricultural and environmental applications.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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TABLE 1 Physicochemical characteristics of soils

Parameter	Site 1*	Site 2
Soil type	Loamy sand	Sandy loam
Soil texture		
Skeleton (> 2 mm) [%]	3	6
Sand (0.05-2 mm) [%]	71	65
Silt (0.002-0.05 mm) [%]	19	14

Clay (<0.002 mm) [%]	7	15
WHC** [%]	13	9
pH-H_2O	6.50	7.11
pH-KCl	5.57	7.06
C [%]	0.372	0.616
N [%]	0.003	0.031
S [%]	0.126	0.113
Humus [%]	0.642	1.063
N-NO_3 [mg/kg]	0.38	3.85
N-NH_4 [mg/kg]	7.56	9.89
Available P [mg/kg]	20.4	66.2
Available K [mg/kg]	37.8	68.2
Available Ca [mg/kg]	170	1 121
Available Mg [mg/kg]	26	53

* see Material and Methods.

*WHC = maximum water holding capacity.

TABLE 2 The results of mixed model (proc mixed) of Acari and Collembola abundance as well QBS-ar in study sites and years

	Site		
	DF	F	P
Collembola	1	2.86	**0.05***
Acari	1	0.50	0.48
QBS-ar	1	4.62	**0.04**
Feeding activity (%)	1	4.97	**0.04**
Decomposition rate	1	3.98	0.07

*significant differences.

TABLE 3 Values of Ellenberg index in relation to moisture, response to pH and nutrients

Site	1	2	Df	F*	p
Moisture	4.1	4.27	1	0.46	0.5
Response to pH	4.52	5.5	1	5.64	**0.02**
Nutrients	4.16	4.91	1	2.97	0.09

*F, p – results of mixed model.

APPENDIX 1 EMI scores accessed to the taxa occurred in soil samples

Taxa	EMI scores
Acari	20
Collembola	
Mesaphorura sp.	20
Symphypleona	2
Isotomidae	4
Hypogastrura spp.	6
Friesea mirabilis	6
Insecta	
Thysanoptera	1
Hymenoptera (ants)	5
Diptera larvae	10
Diptera adults	1
Coleoptera larvae	10
Lepidoptera larvae	10

APPENDIX 2 Scores for variables and eigenvalues for the first 3 DCA axes

	DCA 1	DCA 2	DCA 3
	Scores for the variables		
Mesaphorura	3.6683	2.4996	2.4396
Thysanoptera	2.0343	4.6946	1.2663
Lepidoptera	1.8837	2.3560	1.0271
Hymenoptera	2.0519	0.0000	2.6022
Diptera larvae	1.4879	2.1585	2.7370
Coleoptera	0.0000	1.7571	2.4139
Hypogastrura spp.	5.6739	2.6284	-0.3850

F_mirabilis	4.3395	3.6379	3.7553
Diptera adults	0.2602	3.5910	-0.1941
Isotomidae	4.2175	1.2400	1.2035
Symphympleona	3.0091	3.0823	2.1218
Acari	3.1136	2.9472	1.0746
Site 1	-0.3447	0.0545	0.0000
Site 2	0.3447	-0.0545	0.000
Eigenvalues			
	0.7513	0.5485	0.3293

Species	Site	Moisture	Reaction to pH	Nutrients
Achillea millefolium	1, 2	5	x	5
Agrostis capillaris	1, 2	x*	4	4
Armeria elongata	2	3	5	3
Artemisia absinthium	2	4	7	7
Artemisia vulgaris	2	5	x	8
Betula pendula	1, 2	x	x	x
Calamagrostis epigejos	1, 2	x	x	x
Calluna vulgaris	1	x	1	1
Carex hirta	1, 2	6	x	5
Cerastium semidecandrum	1	3	6	3
Chenopodium album agg.	1, 2	5	x	7
Cirsium vulgare	2	5	7	8
Conyza canadensis	1	4	x	6
Daucus carota	2	4	x	5
Dianthus deltoides	2	4	4	3
Echinochloa crus-galli	1	5	x	8
Eragrostis minor	2	3	x	6
Erigeron annuus	2	5	x	x
Erysimum durum	2	3	6	4
Euphorbia cyparissias	1	3	x	3
Festuca brevipila	1	3	x	2
Festuca ovina	2	x	3	2
Festuca rubra	2	5	x	x
Filago arvensis	1, 2	3	4	3
Filago minima	1, 2	2	4	2
Fragaria vesca	2	5	x	5
Galeopsis tetrahit agg.	1	5	x	7
Galium album	1	4	6	6
Galium aparine	2	x	6	8
Geranium columbinum	2	4	7	6
Gypsophila muralis	2	6	3	4
Herniaria glabra	2	3	4	4
Hieracium sabaudum	1	5	4	4
Holcus mollis	1	5	3	4
Hylotelephium telephium agg.	2	4	5	x
Hypericum perforatum	1, 2	4	6	4
Hypochaeris radicata	2	5	4	3
Jasione montana	1	2	3	2
Juncus tenuis	1	6	5	5
Knautia arvensis	1	4	x	4
Lepidium densiflorum	2	4	7	6
Luzula multiflora	1	4	4	3
Oenothera biennis agg.	2	4	x	4
Oxalis stricta	2	5	7	7
Pilosella officinarum	1, 2	4	x	2
Pinus sylvestris	1, 2	x	x	x
Plantago lanceolata	2	x	x	x
Poa compressa	2	3	7	3
Polygonum aviculare	2	5	x	7
Populus tremula	1, 2	5	x	x
Potentilla argentea	1, 2	2	5	3
Quercus robur	1	x	x	x
Rumex acetosella	1,2	3	4	2
Salix caprea	1, 2	6	6	6
Scorzoneroides autumnalis	2	5	6	6

APPENDIX 3 Plant species occurred in study sites with their characteristic in Ellenberg scale

*not evaluated.