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Distribution of total and clay-associated organic matter in profiles of arable loamy sand Spodosol

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Abstract

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Stabilisation of soil organic matter (SOM) in clay particles is important when the effect of management practices on organic carbon sequestration is being assessed. The objective of the present study was to quantify the differences in total SOM content in bulk soils and clay particles along four soil profiles under perennial grass-red clover and vetch-oat mixtures with and without farmyard manure (FYM) amendment. The results have shown that the highest accumulation of total SOM in bulk soil was observed in top horizons of soils amended with FYM for both crops. The total SOM content in bulk soil decreased down the soil profile but on average it was higher in the FYM-amended soils. Primary minerals (quartz, feldspar), as compared to phyllosilicates (micas and chlorite), dominated in clay-sized fractions of soils. The highest clay-associated SOM content was also determined in the topsoil horizons. A more pronounced effect of FYM on the content of clay-associated SOM was observed in the topsoil under vetch-oat mixture. The clay-associated SOM content decreased with soil depth and negatively correlated to abundance of micas ($r = -0.50$ to -0.99).

Keywords

crops, farmyard manure, soil profile, total and clay-associated organic matter

Introduction

Maintenance of required levels of soil organic matter (SOM) content is one of the integral aims of soil management practices applied for achieving sustainable soil quality. Labile organic substances are considered to be functionally important agents in carbon biochemical cycling, turnover of nutrients and water-stable aggregates in soils (Cambardella and Elliott, 1992; Balashov and Buchkina, 2011; Tobiašová, 2011). However the efficiency of any management practice in arable soils is mainly quantified in terms of sustainable crop yields and accumulation and protection of inert humic substances (humic and fulvic acids, humins) of SOM. These components of SOM, apart from nonhumic aliphatic and alkyl carbon compounds, are mainly associated with silt- and clay-sized fractions of soils and therefore are stronger protected against the microbial-induced decomposition (Kögel-Knabner et al., 2008) as silt and clay particles show high affinity to adsorption of more oxidized organic substances on mineral surfaces (Von Lützow et al., 2008). A high SOM dynamics in coarse-textured soils can be induced by fast changes in the content of easily decomposable particulate organic matter associated with sand-sized fractions (Cambardella and Elliott, 1992). In the fine-textured soils the dynamics in SOM content is mainly related to the accumulation and loss of stable, humified SOM in silt- and clay-sized fractions (Galantini et al., 2004). Each soil demonstrates a different capacity of saturation by SOM within soil mineral matrix. Hassink (1997) reported that while the total SOM content in sandy grassland soils was higher than that in arable soils, there were no differences in clay- and silt-associated SOM content, i.e. the total SOM content in these particles could have reached its maximum. The results of this research also showed that dominant clay minerals did not affect the relationships between the SOM content in the soil size fractions of <20 µm and the amount of these soil size fractions. Therefore application of different rates of farmyard manure (FYM) can result in different contents of total SOM and its labile components in bulk soils but may not lead to any differences in the SOM content in silt and clay particles if they have a low saturation capacity. According to Baldock and Skjemstad (2000), the mineralogy of clay particles exerts its control over protection of SOM through its effects on the type and density of active sites capable of adsorbing organic materials. The results of Wiseman and Püttmann (2006) noted that the SOM content significantly correlated with kaolinite and illite, but did not demonstrate any significant correlations with smectite content in Antrosol, Vertisol and Gleysol Chernozem.

The objective of the present study was to quantify the differences in total SOM content in bulk soil and clay particles within four soil profiles under mixture of perennial grass and red clover, and vetch-oat mixture with and without application of FYM.

Material and methods

Soil sampling was carried out at the experimental station of the Agrophysical Research Institute in the St. Petersburg region of Russia (59°34’N, 30°08’E) in May of 2011. In the studied region average annual air temperatures reached +5.2, +5.7, +6.2, +5.0, +4.8 and +6.0 °C in 2006, 2007, 2008, 2009, 2010 and 2011, respectively. Average air temperatures during the growing seasons (May–September) of the same years were equal to: +14.8, +14.4, +12.9, +14.1, +16.2 and +15.6 °C, respectively. During the growing season of 2011 average daily air temperature ranged from +1.9 to +26.8 °C.

Annual precipitation was equal to: 959; 914; 1,269; 1,356; 1,026 and 1,263 mm in 2006, 2007, 2008, 2009, 2010 and 2011, while during the growing seasons of these years amount of precipitation reached: 456, 555, 627, 778, 634 and 784 mm, respectively.

The soil was typical for the studied arable area: loamy sand Spodosol. Parent material of the soil was presented by quaternary sand, gravel and clay deposits in ground moraines. A distribution of sand-, loam- and clay-sized fractions in genetic horizons of the loamy sand Spodosol is shown in Fig. 1.

The field experiment was established in 2003 on 1.5 ha plot. The plot was divided into three 0.5 ha (50 × 100 m) sub-plots. The first sub-plot did not receive any FYM. The second sub-plot received, in total, 300 t ha^{-1} of FYM (80 t ha^{-1} in 2003, 80 t ha^{-1} in 2004, 60 t ha^{-1} in 2005 and 80 t ha^{-1} in 2009) and the third sub-plot received, in total, 700 t ha^{-1} of FYM (160 t ha^{-1} in 2003, 320 t ha^{-1} in 2004, 60 t ha^{-1} in 2005 and 160 t ha^{-1} in 2009). In order to establish two crop rotations, each of the three sub-plots was divided into two parts in 2006. Then each part was divided into nine parallel lines representing mineral fertilizer treatments. In the end, each sub-plot part included 3 replicates without mineral fertilizers, 3 replicates with medium rate of mineral fertilizers and 3 replicates with high rate of mineral fertilizers (Olenczenko et al., 2012). There were two crop rotations grown in the experiment. The first one consisted of: white cabbage (*Brassica oleracea* L.), carrot (*Daucus carota* L.), beetroot (*Beta vulgaris* L.), spring barley (*Hordeum vulgare* L.) with undersown mixture of perennial grass and red clover (*Phleum pratense* L. and *Trifolium pratense* L.), mixture of perennial grass and red clover (*Phleum pratense* L. and *Trifolium pratense* L.) of first and second year. The second crop rotation included: spring barley (*Hordeum vulgare* L.) with undersown mixture of perennial grass and red clover (*Phleum pratense* L. and *Trifolium pratense* L.), mixture of perennial grass and red clover (*Phleum pratense* L. and *Trifolium pratense* L.) of first and second year, winter rye (*Secale cereale* L.), potato (*Solanum tuberosum* L.), vetch-oat mixture (*Vicia sativa* L. and *Avena sativa* L.).

Soil sampling was carried out in the last year of the two crop rotations and only on the sub-plots without FYM (“no FYM”) and the sub-plots with the highest FYM rate (700 t ha^{-1}; “FYM”). Four soil profiles were dug in the centers of the lines without mineral fertilizer incorporation. Statistically it would be more reasonable to have more soil profiles but we had to work under very strict conditions not allowing us to destroy significant part of the whole field experiment. Totally, twenty-three composite soil samples were taken from the soil profiles. One composite soil sample consisted of twelve sub-samples collected from a particular soil horizon around a soil profile. All composite soil samples were air-dried and passed through 1-mm or 2-mm sieve. All the analyses were conducted in three replicates and mean values are being used in the discussion.

A pipette method was used to determine amounts of sand (1,000–10 µm), silt (10–1 µm) and clay (<1 µm) particles in the soil samples (Rastvorova, 1983). Besides, clay particles (<1 µm) were subsequently extracted from soil samples by sedimentation and electrophoresis methods. A 24-h sedimentation of soil samples in water was performed in 1-litre glass columns according to the pipette method (Rastvorova, 1983). Before the sedimentation, soil samples were subjected to ultrasonic dispersion in water for 15 min at 315 W using the Branson 450 digital ultrasonic sonifer equipped with a tapped disruptor horn (13 mm in diameter). After the sedimentation, clay particles-water suspension (500 cm^3 in volume) was placed into a work cell of electrophoresis device equipped with a Cu-anode and a Pb-cathode (Moiseev et al., 2012). Electrophoretic extraction of

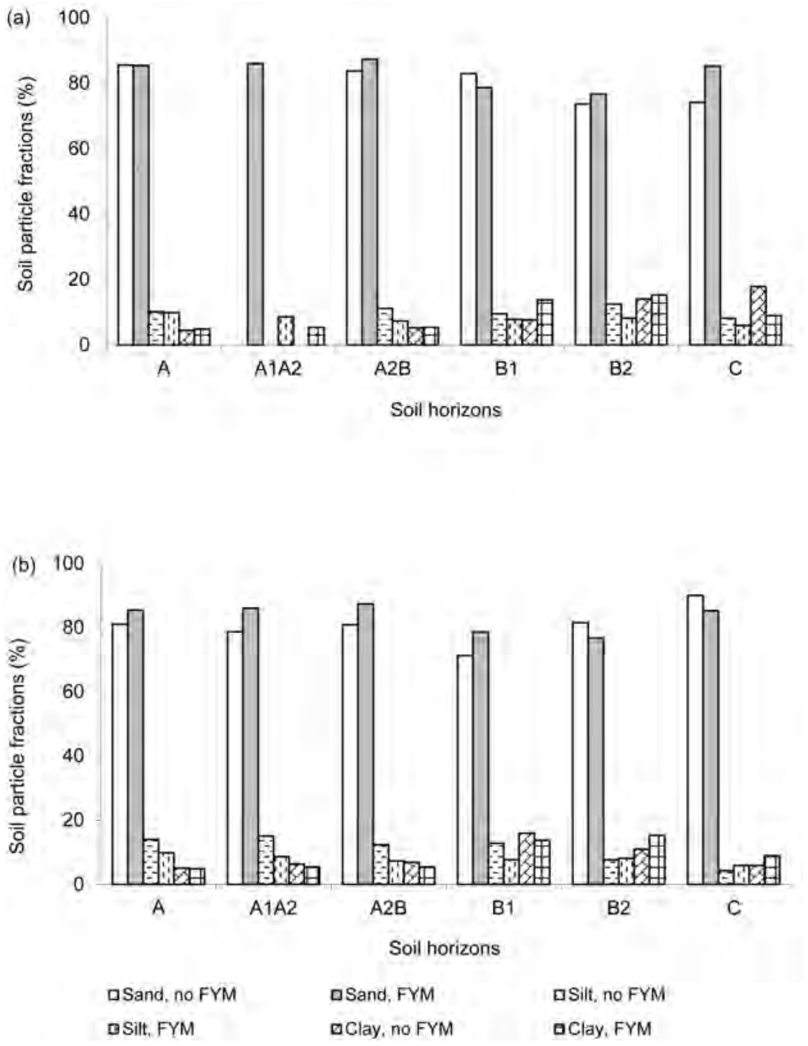

Fig. 1. Distribution of amount of sand, silt and clay size fractions in the profiles of loamy sand Spodosol in the first (a) and second (b) crop rotation on the no FYM and FYM sub-plots.

clay particles was performed at voltage of 320 V, current of 0.25 A and temperature of 20–24 °C and did not exceed 18 min. Then the clay particles were carefully detached from the anode and dried in an oven at temperature of 35–40 °C. The mineral composition of the clay particles was assessed using X-ray diffraction (XRD) analysis of oriented samples using DRON-3 X-ray diffractometer with Cu Kα tube (30 kV, 30 mA). Samples were scanned from 3° to 40°. Quartz, potassium feldspar and plagioclase were identified by the reflections at 0.424, 0.324 and 0.318 nm, respectively. Dioctahedral (muscovite), trioctahedral (biotite) and interlayer deficient (illite) micas were recognized by the reflections at 0.50, 1.00 and 1.08 nm, respectively. Chlorite was identified at the 1.41 nm reflection. Standard reference tables were used for interpretation of the XRD results (Frank-Kamenetzky, 1983).

The total SOM content in the soil samples (later called as bulk soil) and clay-associated SOM content were measured by the Tjurin method of acid dichromate digestion (Rastvorova et al., 1995). Soil pH values were measured by a pH-meter at 1 : 2.5 ratio of soil to 1 N KCl solution (Rastvorova et al., 1995). Amounts of exchangeable P_2O_5 and K_2O in soil samples were determined by a Kirsanov method modified by CINAO using 0.2 M HCl solution for the extraction as well as photoelectric colorimeter and flame photometer for their quantitative determination (GOST SSSR, 1991).

In the first crop rotation, the agrochemical properties of the FYM and no FYM soil under perennial grass and red clover mixture of second year were measured only in the topsoil (A horizon, 0–23 cm). The FYM and no FYM topsoils were respectively, characterized by: pH (KCl) – 6.1 ± 0.1 and 5.3 ± 0.1; exchangeable P_2O_5 – 668 ± 18 and 217 ± 10 mg kg^{-1} soil; exchangeable K_2O – 290 ± 12 and 120 ± 6 mg kg^{-1} soil (Olenczenko et al., 2012).

In the second crop rotation, the agrochemical characteristics of the FYM and no FYM soil under vetch-oat mixture were measured in 2011 not only in the topsoils but also in the lower soil horizons. For the topsoils values of soil pH, contents of exchangeable P_2O_5 and K_2O were equal to: 6.3 ± 0.1 and 5.3 ± 0.1, 515 ± 11 and 237 ± 15 mg kg^{-1} soil, 275 ± 5 and 89 ± 4 mg kg^{-1} soil, respectively, for the FYM and no FYM soil (Olenczenko et al., 2012). According to Vitkovskaya et al. (2014), the agrochemical characteristics in the underlying soil horizons varied in the ranges of: 4.1 ± 0.1 to 6.0 ± 0.1 and 3.9 ± 0.1 to 5.4 ± 0.1 (pH), 108 ± 4 to 553 ± 11 and 122 ± 3 to 295 ± 11 mg kg^{-1} soil (exchangeable P_2O_5), 81 ± 4 to 221 ± 11 and 19 ± 1 to 120 ± 5 mg kg^{-1} soil (exchangeable K_2O), respectively, for the FYM and no FYM soils.

Strength of the relationships between the sets of selected soil parameters in each soil profile was assessed with Spearman's rank correlation coefficients (Table 1) as the studied parameters were related by non-linear, monotonic functions.

A significance of effects of two independent factors (soil horizon, FYM amendment) on the means of the selected soil data sets was estimated by two-way analysis of variance (anova) without repetitions at $p \leq 0.05$ (Table 2).

Results

The high-rate application of FYM resulted in higher contents of exchangeable P_2O_5 and K_2O in the amended topsoil layers under both crop rotations. If the two soil profiles of the second crop rotation were assessed together, the results of two-way analysis of variance showed that FYM amendment significantly ($p < 0.05$) affected only a content of exchangeable K_2O in the whole soil profile (Table 2). Soil horizons showed an insignificant influence only on the profile distributions of exchangeable P_2O_5 and K_2O content.

Our results demonstrated that the FYM incorporation had caused an increase in total SOM content in bulk soil in the topsoil layers (Fig. 2).

For the first crop rotation the highest total SOM content in the bulk soil of the no FYM sub-plot was observed in the topsoil. In the 22–32 cm layer (A2B horizon) it was 15.3 times lower with a slight increase in other underlying layers. In the soil of the FYM sub-plot the total SOM content in bulk soil in the 0–23 cm layer (A horizon) was also high but did not differ from that in the 23–33 cm layer (A1A2 horizon), declined

Table 1. Results of Spearman's rank correlations between parameters: soil organic matter content in bulk soil (SOMbs), clay-associated organic matter content (SOMcl), clay content (Cl), intensities of reflection of primary minerals (Pm) and micas (Mc) in a loamy sand Spodosol in the first and second crop rotation on the no FYM and FYM sub-plots

Crop rotation No., FYM amendment	First rotation, no FYM		First rotation, FYM		Second rotation, no FYM		First rotation, FYM	
Correlations	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
SOMbs vs. SOMcl	0.45	0.19	0.08	0.80	0.66	0.16	0.71	0.11
SOMcl vs. Pm	0.40	0.50	–0.09	0.87	–0.37	0.47	0.14	0.79
Mc vs. Pm	–0.70	0.19	–0.09	0.87	0.37	0.47	–0.14	0.79
Mc vs. Cl	0.90	0.04*	0.58	0.23	0.84	0.04*	0.76	0.08
Pm vs. Cl	–0.90	0.04*	–0.03	0.95	0.06	0.91	–0.58	0.23

Significant differences at $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

Table 2. Changes in soil properties in response to effects of soil horizon layout and FYM amendment

Factor	Parameters							
	Total SOM content in bulk soil	Clay-associated SOM content	Clay content	Primary minerals	Micas	Chlorite	P_2O_5	K_2O
Horizon Layout	0.02*	0.001***	0.01**	0.68	0.04*	0.44	0.11	0.17
Fertilization	0.20	0.24	0.70	0.75	0.52	0.03*	0.20	0.04*

Significant differences at $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, two-way anova.

by 2.8 times only in the 33–50 cm layer (A2B horizon). However the amounts of clay- and silt-sized fractions in these horizons did not differ from those in the A and A1A2 horizon (Fig. 1). Our results also showed a higher total SOM content in bulk soil in the illuvial B1 horizons than in the podzolic A2B ones only in the profile on the no FYM sub-plot. There were higher values of total SOM content in the bulk soil in B2 and C horizons than in the B1 horizon (Fig. 2).

On average, the total SOM content in bulk soil within the soil profile was higher on the FYM sub-plot than on the no FYM sub-plot. In the second crop rotation, the total SOM content in bulk soil showed the similar values in the 0–23 cm (A horizon) layers of the FYM and no FYM sub-plots. In the 22–33 cm layers (A1A2 horizons) the values slightly increased in the no FYM soil and slightly declined in the FYM soil.

There was the substantial decrease in total SOM content in bulk soil in the A2B horizons on both sub-plots. In other three underlying layers the total SOM content in bulk soil, on average, showed slightly higher values in the FYM soil.

The clay-associated SOM content was higher in the upper 50 cm part of the soil profiles (Figs 2 and 3). In contrast to the total SOM content in bulk soil, the FYM amendment has resulted in little differences in the clay-associated SOM contents in the 0–23 cm layers (A horizon) (Fig. 3). The content of clay-associated SOM in the FYM soil in the first crop rotation slightly increased in the 22–33 cm layer (A1A2 horizon). However, the difference was much higher in the FYM soil in the second crop rotation. The clay-associated SOM content in the topsoil layers was on average higher in the second than in the first crop rotation. A drastic decrease in the clay-associated SOM content was observed in the A2B horizons of all the profiles. In the underlying soil horizons (depths of 48–168 cm) the clay-associated SOM content showed much lower val-

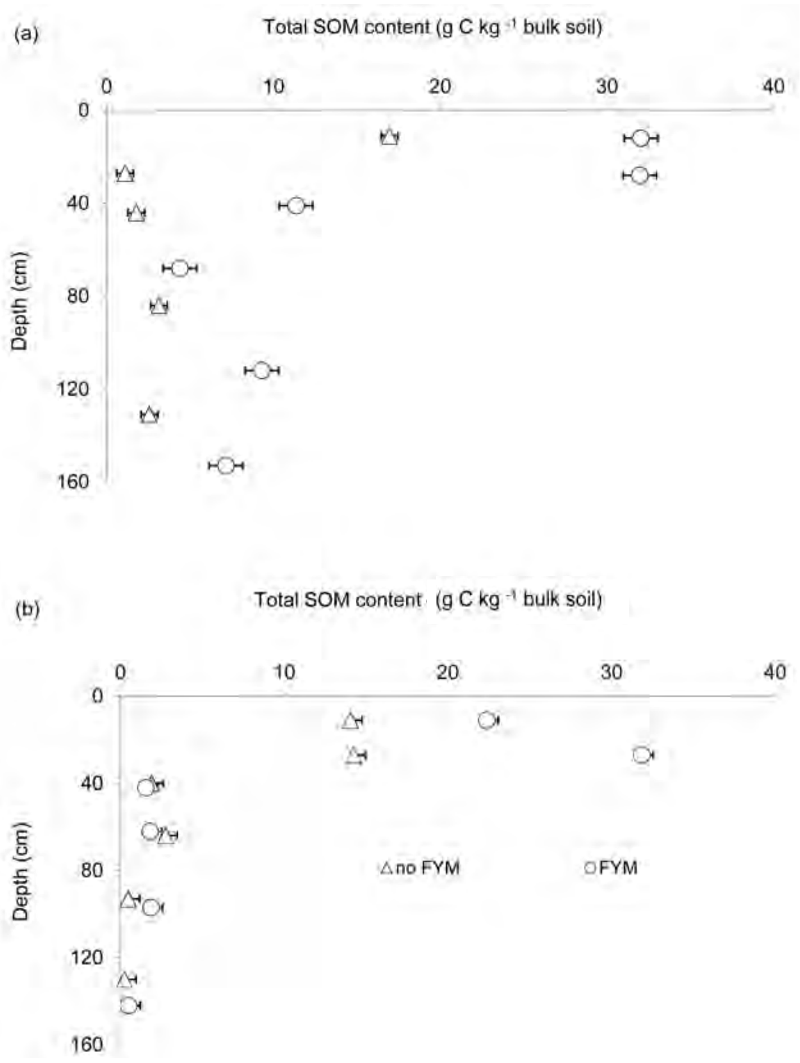

Fig. 2. Distribution of total SOM content in bulk soil in the profiles of loamy sand Spodosol in the first (a) and second (b) crop rotation on the no FYM and FYM sub-plots (horizontal bars are standard deviations at $p \leq 0.05$).

ues except for that in the C horizon on the FYM soil in the first crop rotation. Spearman's rank correlation coefficients between the clay-associated SOM content and total SOM content in bulk soil within the whole soil profiles were positive but insignificant in the first crop rotation on the no FYM and FYM sub-plot, as well as in the second crop rotation on both sub-plots (Table 1). The results of two-way analysis of variance demonstrated that soil horizons significantly and FYM amendment insignificantly affected the clay-associated SOM contents in the second crop rotation (Table 1).

All the soil profiles demonstrated a uniform distribution of sand-sized fraction (Fig. 1). There were only trends in a decrease of silt-sized fraction amounts and in an increase of clay-sized fraction amounts with increasing depth. Among the soil minerals associated with the clay-sized fraction, only the abundance of micas showed positive Spearman's rank correlations with clay content in the soil profiles on the no FYM and FYM sub-plots in the first crop rotation, as well as on the no FYM and FYM sub-plots in the second crop rotation (Table 1). Results of the two-way analysis of variance demonstrated that soil horizons significantly affected the distribution of clay-sized fraction and micas abundance in the second crop rotation (Table 2).

Primary minerals including quartz, potassium feldspar and plagioclase have dominated in the assemblage of soil minerals in the studied soil profiles (Table 3).

There was not any clear distribution of primary minerals within the four soil profiles. Two-way analysis of variance showed insignificant effects of soil horizons and FYM amendment on the abundance of primary minerals in the second crop rotation (Table 2).

In contrast to the profile distribution of primary minerals, the abundance of micas in soil profiles increased with the depth (Table 3). There were negative Spearmen's rank correlations between the intensities' reflection of micas and the clay-associated SOM content in all the soil profiles (Table 1). The results of two-way analysis of variance showed that soil horizons significantly and FYM amendment insignificantly affected the abundance of micas in the soil profiles of the second

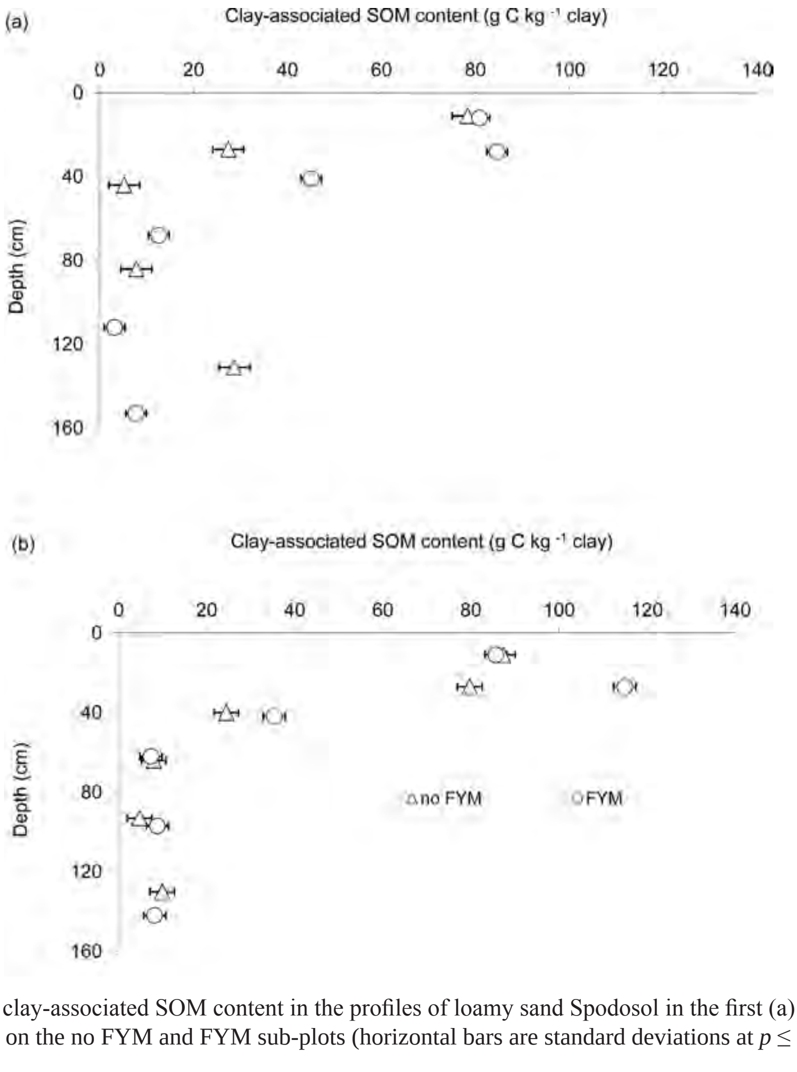

Fig. 3. Distribution of clay-associated SOM content in the profiles of loamy sand Spodosol in the first (a) and second (b) crop rotation on the no FYM and FYM sub-plots (horizontal bars are standard deviations at $p \leq 0.05$).

Table 3. Profile distribution of intensities of reflections of primary and phyllosilicate minerals in clay fraction (<1 µm) of a loamy sand Spodosol in the first and second crop rotation on the no FYM and FYM sub-plots (mean value ± standard deviation)

Treatment, horizon	Depth (cm)	Primary minerals (impulse s^{-1})	Micas	Chlorite
First crop rotation, no FYM				
A	0–22	3,307 ± 166	155 ± 8	152 ± 7
A2B	22–32	3,149 ± 226	133 ± 15	172 ± 12
B1	32–57	1,823 ± 120	185 ± 13	0
B2	57–112	2,865 ± 212	1,009 ± 55	287 ± 22
C	112–150	1,623 ± 107	898 ± 59	0
First crop rotation, FYM				
A	0–23	3,028 ± 241	0	0
A1A2	23–33	2,926 ± 210	665 ± 46	142 ± 12
A2B	33–50	2,945 ± 214	890 ± 79	0
B1	50–85	1,955 ± 152	868 ± 69	158 ± 8
B2	85–138	3,168 ± 255	1,275 ± 92	217 ± 15
C	138–168	2,891 ± 213	1,489 ± 110	135 ± 12
Second crop rotation, no FYM				
A	0–22	1,928 ± 213	143 ± 12	205 ± 15
A1A2	22–31	2,968 ± 233	158 ± 12	182 ± 17
A2B	31–48	2,007 ± 159	1,000 ± 81	192 ± 18
B1	48–79	2,900 ± 234	1,330 ± 105	182 ± 19
B2	79–106	2,920 ± 239	1,593 ± 124	215 ± 16
C	138–168	3,353 ± 261	1,216 ± 95	203 ± 16
Second crop rotation, FYM				
A	0–22	3,143 ± 235	190 ± 14	0
A1A2	22–33	2,915 ± 233	1,013 ± 81	0
A2B	33–50	3,133 ± 235	995 ± 81	138 ± 11
B1	50–75	2,855 ± 235	1,022 ± 83	165 ± 13
B2	75–120	1,545 ± 127	1,424 ± 113	163 ± 12
C	120–165	3,283 ± 242	1,497 ± 177	153 ± 11

crop rotation (Table 2). Chlorite had the lowest abundance and did not show any distinct differences in the profile distribution on all the sub-plots (Table 3).

Discussion

The application of FYM at the total rate of 700 t ha^{-1} expectedly resulted in the improvement of topsoil quality (A horizons) of loamy sand Spodosol in terms of its increased soil pH, contents of exchangeable P_2O_5 and K_2O. The favorable changes in the soil quality indicators contributed to increasing soil productivity. Olenczenko et al. (2012) reported that in 2011 yields of perennial grass and red clover mixture of second year reached 11.6 t ha^{-1} and 13.2 t ha^{-1} on the no FYM and FYM sub-plots. The yield of vetch-oat mixture was also higher on the FYM sub-plot (25.7 t ha^{-1}) than on the no FYM sub-plot (19.8 t ha^{-1}) in 2011.

Maintenance of integrity of the whole soil profiles is also one of the crucial aims of sustainable management practices. Soil inherent mineral and organic properties are key agents in maintaining sustainability of the whole soil profile. Our results showed that the whole soil profile of the no FYM sub-plot in the first crop rotation was shorter than other three soil profiles because of absence of transient A1A2 horizon, probably, due to a disturbance by moldboard ploughing. Therefore we had to perform joint statistical analyses of distributions of selected soil parameters only in two whole soil profiles in the second crop rotation.

The uniform distribution of sand-sized fraction in the soil profile was induced by composition of its parent material, which was presented by quaternary sand, gravel and clay deposits in ground moraines. Therefore the content of clay-associated fraction was higher in a bottom part than in an upper part of the soil profile. In contrast to primary minerals, the abundance of micas increased with increasing soil depth on all the sub-plots (Table 1). These results supported data of scientists showing, firstly, that trioctahedral and dioctahedral micas were more sensitive to anthropogenic disturbing impacts than primary minerals and, secondly, that an increase in amount of micas with increasing soil depth could be induced by their downward migration (CHIZHIKOVA, 2005).

Primary minerals, as compared to phyllosilicates, have lower specific surface area, saturation capacity, and surface reactivity. The application of FYM contributes to an input of aliphatic compounds (polysaccharides, lignin, fatty acids, lipids etc.) into topsoil layers (EUSTERHUES et al., 2003; ŠIMON, 2005). The association of the aliphatic organic compounds with surfaces of primary minerals is induced by H-bonding and ligand exchange (VON LÜTZOW et al., 2008). In the topsoil the highest abundance of primary minerals could induce the accumulation of the greatest amounts of clay-associated aliphatic moieties originated from FYM (KÖGEL-KNABNER et al., 2008). Our results showed that FYM amendment contributed to the increase of total SOM content in bulk soil and did not affect the clay-associated SOM content in the topsoil that supported the data of HASSINK (1997).

The association of phyllosilicates with SOM becomes stronger with increasing degree of SOM humification as a result of dominant ligand exchange reactions producing strong bonds between singly coordinated carboxyl groups at the edges of phyllosilicates and carboxyl and phenolic groups of the organic matter (JONES and SINGH, 2014). The results of our studies demonstrated that the profile distribution of total SOM in bulk soil and clay-associated SOM had positive but insignificant Spearman's rank correlations in all the soil profiles. The micas abundance increased with increasing soil depth, but had negative Spearman's rank correlations with contents of clay-associated SOM in all the soil profiles (Table 1). We assume that micas did not play a key role in the association with the clay-associated organic compounds, which, in the profiles of the studied light-textured soil, could be mainly presented by those with a low degree of humification. Our data supported the results of WISEMAN and PÜTTMANN (2006) that chlorite had played a little role in binding SOM in the soil profiles.

Primary minerals in clay particles were probably main agents in the accumulation of clay-associated SOM in the studied soil profiles. KIEM et al. (2000) reported that unmanured arable soils had higher proportions of aromatic (alkyl) carbon and lower proportions of O/N-alkyl carbon than the FYM-amended soils. Our results demonstrated that the management of loamy sand Spodosol on the FYM sub-plots did not necessarily lead to significant changes in SOM humification in the topsoil horizons and whole soil profiles in two crop rotations. Negative Spearman's rank correlation coefficients (r = –0.50 to –0.99) between the clay-associated SOM content and micas abundance probably supported the opinion that the clay-associated SOM had the low degree of humification and affinity to adsorption on micas surface (JONES and SINGH, 2014). The low degree of SOM humification could be induced by fast turnover of easily decomposable particulate organic matter associated with sand-sized fractions (CAMBARDELLA and ELLIOTT, 1992). The profile distribution of the total SOM in bulk soil and clay-associated SOM reflected inherent processes in the loamy sand Spodosol which had a leaching water regime and were subjected to strong weather and management impacts. Our data showed that (1) the podzolization process could induce a translocation of dissolved low molecular organo-mineral complexes from the A2B to illuvial B horizons, and, (2) there was a difficulty in maintaining a favorable content of SOM with the high degree of its humification in the top horizons at a long-term scale even by application of high rate of FYM.

Conclusions

The results of our study showed that the highest accumulation of total SOM in bulk soil was observed in the top horizons (A and A1A2) of FYM soils in two crop rotations with perennial grass-red clover and vetch-oat mixtures. The total SOM content in bulk soil drastically decreased in subsoil horizons (A2B, B1, B2, C), but on average was also higher in the FYM soil in both crop rotations.

The highest clay-associated SOM content was also determined in the topsoil horizons. A greater effect of FYM on the content of clay-associated SOM was observed in the topsoil under vetch-oat mixture. The clay-associated SOM content decreased down the soil profile and negatively (r = –0.50 to –0.99) correlated to the abundance of micas.

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Influence of the gregarine *Stenophora julipusilli* (Eugregarinorida, Stenophoridae) on the trophic activity of *Rossiulus kessleri* (Diplopoda, Julidae)

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Abstract

Brygadyrenko, V.V., Svyrydchenko, A.O., 2015. Influence of the gregarine *Stenophora julipusilli* (Eugregarinorida, Stenophoridae) on the trophic activity of *Rossiulus kessleri* (Diplopoda, Julidae). *Folia Oecologica,* 42: 10–20.

Analysis of the contents of the intestines of *Rossiulus kessleri* (Lohmander, 1927) revealed presence of gamonts of *Stenophora daulphinia* Watson, 1916, *S. juli* (Frantzius, 1846) Labbe, 1899 and *S. julipusilli* (Leidy, 1853) Crawley, 1903. For the analysis of the consumption of leaves of *Acer negundo* L. in the course of a two week laboratory experiment 122 specimens of *R. kessleri* infested with *S. julipusilli*, and 32 specimens not infested with gregarines were investigated. The initial and final body weight of each millipede was determined as well as the mass of food consumed, the amount of excrement produced and the extent of infestation with gamonts of *S. julipusilli*. No relationship between the amount of food consumed and initial weight of *R. kessleri* specimens was found. Nor was any dependence between the amount of excrement, amount of food consumed and change in body mass established. *S. julipusilli* gamonts and syzygys were found in 79.2% of millipede specimens. The infestation with gamonts of younger *R. kessleri* specimens was significantly lower than with average to large specimens of millipedes. The sex of the host was not found to influence food consumption, change in body mass or in mass of faeces. The extent of infestation with gamonts of *S. julipusilli* did not significantly differ between males and females of *R. kessleri*. In specimens not infested with gregarine gamonts the food consumption during the two-week experiment was at a high level, in those with a slight infestation (1–8 gamonts) it increased by 29%, in those with average and high extent of infestation it decreased by 8% compared with uninfested individuals. During the experiment influence of the number of *S. julipusilli* gamonts upon change in body mass and production of excrement by *R. kessleri* was not observed.

Keywords

change in body mass, gregarine, litter consumption, saprophagous invertebrate, *Stenophora*

Introduction

Up to now no attempt has been made to form a quantitative assessment of the influence of gregarines upon the trophic activity of millipedes. The scattered studies which have been made of gregarines which parasitize insects (Siva-Jothy and Plaistow, 1999; Canales-Lazcano et al., 2005; Rodriguez et al., 2007) show a weakly negative impact of the parasites upon the organisms of their hosts. However, the capacity of many gregarine species to achieve high populations within certain host individuals excludes the possibility of making a clear unambiguous assessment of their impact upon the metabolic activity of a macroorganism.

The ecological peculiarities of diplopod gregarines have been studied in India (Bhamare et al., 2014), Taiwan (Chang et al., 2004), Japan (Hoshide et al., 1970), Poland (Lipa, 1967), Germany, Yugoslavia (Geus, 1969), Slovakia (Valigurová and Matis, 2001) and some other countries. Diplopod gregarines belonging to the Ukrainian fauna have remained unstudied until now.

The species of the *Stenophora* genus are included in a well delineated group – the Stenophoridae family (Geus, 1969). A systematic revision of gregarines (Clopton, 2002, 2009) raised them to the rank of superfamily Stenophoricae. The species of the *Stenophora* genus parasitize centipedes (Myriapoda: Chilopoda) and millipedes (Myriapoda: Diplopoda). The ecological peculiarities of the species of this genus remain unstudied. The literature dedicated to morphology and description of gregarines of this genus (Da Costa and Barth, 1967; Lipa, 1967; Geus, 1969; Hoshide et al., 1970; Valigurová and Matis, 2001) has made only fragmentary references to the peculiarities of the origins of certain development stages of *Stenophora* species.

One of the most common diplopod species of the steppe and forest-steppe zones of Ukraine, Belarus and Russian Federation is *Rossiulus kessleri* (Lohmander, 1927). Scientists have long considered it a convenient object for bioindication and morphological researches (Prishutova, 2001a; Pokhylenko, 2014). Studies have been made of its distribution (Chernyi and Golovach, 1993), development cycle (Striganova, 1996; Prishutova, 1988a, 2001b; Brygadyrenko, 2004), habitat preferences (Pokarzhevskij, 1983; Prishutova, 1988b; Pokarzhevskij et al., 1989), temperature conditions (Striganova, 1972) and range of diet (Striganova and Prishutova, 1990; Kulbachko and Didur, 2012; Svyrydchenko and Brygadyrenko, 2014). The gregarine fauna inhabiting the intestines of this species have been little studied up to now. Geus (1969) reported the discovery in the intestines of this millipede species of *Stenophora juli* (Frantzius, 1846) Labbe, 1899, *S. julipusilli* (Leidy, 1853) Crawley, 1903 and *S. daulphinia* Watson, 1916. Unfortunately, the nomenclature of diplopod species suffers from a confusing range of synonyms, and *R. kessleri* has been described by more than 15 different names (Chernyi and Golovach, 1993). That is why even within a single publication (Geus, 1969) it is designated by a variety of different names.

At the beginning of the research we held a null hypothesis that gregarines in low, average and high numbers have an equally low impact upon their hosts' food consumption. Besides this, the objective of this research is to examine the following hypotheses: (1) maximum increase in body mass will be seen in the millipede specimens that have maximum levels of food consumption, (2) at different development stages diplopods have different levels of gregarine infestation, the level of infestation increasing with the age of the host, (3) the number of gregarines in the intestines will influence changes in body mass and the tempo of faecal formation, the higher the number of gregarines the lower the body mass of the host on account of rapid formation of excrement.

Materials and methods

Objects of the experiment

Specimens of *R. kessleri* were taken manually on 22 May 2014 from the litter and soil surface of a windbreak forest plantation of *Acer negundo* L. near Aleksandrovka village (48°46'N, 34°56'E), Magdalinovka district, Dnipropetrovsk region, Ukraine. 160 *R. kessleri* specimens were used in this study. Green leaves of a single *A. negundo* tree were collected in the middle of May at 2 meters height from the ground. They were collected from the same plantation where the *R. kessleri* were collected. The leaves were air-dried over a week, without exposure to direct sunlight. Then they were sorted (leaf stalks, deformed leaf laminae, leaves damaged by insects, and by bacterial and fungal diseases were removed). The remaining leaves, which were uniform in size and condition were dried over two hours at a temperature of +60 ºC and 20% humidity, weighed to an accuracy of 0.5 mg and placed (1,550 ± 268 mg) in separate containers for the millipedes. The containers were 0.5 l polystyrene cups.

Experimental technique

Before the experiment each millipede was placed in a small separate container without food for no more than 3–4 hours. For preventing infestation of leaves and containers by gregarine gametocytes or oocysts, bacterial spores, microspora, fungal spores, viral polyhedrons, this task was performed with scrupulous care and cleanliness (Tarasevich, 1975). Then each *R. kessleri* individual was weighed and put in a separate experimental container with previously weighed leaves of *A. negundo*. After the experiment the leaves in the 160 experimental and 10 control (leaves without millipedes) containers were moistened from a sprayer, and then covered with clean sheets of standard Xerox paper for preventing moisture evaporation. Each morning the control cups and cups with *R. kessleri* were moistened from a sprayer with equal amounts of distilled water.

Determination of body weight of the millipedes

The weight of the millipedes, their faeces and the litter was determined with the use of a torsion balance (to an accuracy of 0.5 mg). Throughout the study, a consistent temperature of +25 to +27 °C and air humidity of 70–90% was maintained in the laboratory. Food consumption (C_I^*) was determined with a modified formula of David (1998): $C_I^* = (M_0 – M_0D – M_n) / (1 – D)^{1/2}$, where M_0 – initial food mass (dry mass) offered to each millipede for consumption, M_n – food mass (dry mass) not consumed by a millipede by the end of the experiment, *D* – coefficient of reduction of food mass as a result of its microbiological decomposition calculated with the use of control set of experiments ($n = 10$) in identical containers without millipedes ($D = (M'_0 - M'_n) / M'_0$, where M'_0 and M'_n – dry mass of food at the beginning and at the end of control experiment without the presence of millipedes). Microbiological decomposition of leaves, which were moistened in the same way as the containers with millipedes, for two weeks, was 3.5 ± 1.7%.

Identification of gregarines

After the experiment the millipedes were taken out of the containers, transferred into clean containers without food and during two days were analysed to define the extent of infestation of their intestines with gregarines. The observations were made using microscope with ×5, ×10 and ×40 planapochromatic objectives. For controlling the counts of the number of gamonts 10% of specimens were checked a second time. In the control count the coefficient of variation in the number of gamonts was 2–14%. The gregarines were identified using Geus (1969) and Clopton (2002). Additional sources used in identification and morphology of *Stenophora* species were the works of Da Costa and Barth (1967), Lipa (1967), Hoshide et al. (1970), Valigurová and Matis (2001), Bhamare et al. (2014). For identification of the species of the genus *Stenophora* we used total length, length and width of the protomerite, and length and width of the deuteromerite. In addition to this we used the ratios of length of protomerite to total length and width of protomerite to width of deuteromerite. Our morphological data of *S. julipusilli* corresponded very closely to the measurements presented in Geus (1969).

Statistical data processing

Exploratory data analysis (EDA) was performed in the MS Excel software package. The rate of microbial decay, rate of consumption of leaf litter, changes in body weight and daily formation of faeces were calculated for individual specimens of *R. kessleri* for each specific container. Statistical data analysis was performed in Statistica 8.0 software package (Hill and Lewicki, 2007). To characterise particular samples the following characteristics were provided: mean ± SD, minimum and maximum and median. Samples were compared using one-way analysis of variance (anova). Differences between sample means were detected by the Tukey test and considered significant at $P < 0.05$.

Results

Our analysis of the content of the intestines of *R. kessleri* showed gamonts of *Stenophora daulphinia* Watson, 1916, *S. juli* (Frantzius, 1846) Labbe, 1899 and *S. julipusilli* (Leidy, 1853) Crawley, 1903. The first two species comprised no more than 2% of the total number of gregarines in the intestines of the millipede individuals studied in the experiment (taken from Magdalinovka District of Dnipropetrovsk region). The numbers of *S. julipusilli* usually reached 100% of all studied gregarines in the intestine of a single millipede. *S. daulphinia* and *S. juli* were found in the intestines of 6 out of 160 studied specimens. For the sake of the accuracy of the experiment 6 millipede specimens with counts of *S. daulphinia* and *S. juli* under 30% of the total number of gregarines in their intestines were excluded from the subsequent analysis. Thus, 154 millipede specimens infested with *S. julipusilli* were used for analysing trophic activity.

Linear dependence between the amount of food consumed and the body mass of *R. kessleri* was not found (Fig. 1a). There was also no such dependence between the amount of excrement and food consumption (Fig. 1c), and change in body mass (Fig. 1d). An increase in size of millipedes in the experiment also did not bring a statistically significant change in one of the most important ratios, which reflects the efficiency of food processing (the ratio of change in body mass to mass of food consumed, Fig. 1b).

The initial body mass of *R. kessleri* in the experiment was 452 ± 130 (122–692) mg. The males on average weighed 20% less than the females (404 ± 125 and 510 ± 113 mg respectively, $F = 29.84$, $F_{0.05} = 3.90$, df = 1, 152, $P < 0.001$). The asymmetry values (As = –0.19) and excess values (Ex = –0.72) do not exceed critical values for $P = 0.05$ (0.321 and 0.834 respectively). Thus, the distribution of individuals according to weight in the *R. kessleri* population studied may be considered normal. This normal distribution of size classes of *R. kessleri* is typical for three populations of the species which we have studied (in Dnipropetrovsk, Magdalinovka, and Novomoskovsk districts of Dnipropetrovsk region). After the two week experiment the module of asymmetry increased (As = –0.32), and the module of excess decreased (Ex = –0.44): the distribution pattern became less marked by sharp peaks, though larger individuals gained weight more intensively than smaller ones. This led to a shift of distribution peak to the right. Nonetheless the distribution of the animals according to weight remained normal.

The decrease in body mass of *R. kessleri* (mg/individual) during the two-week experiment when fed on leaves of *A. negundo* (Table 1) was on average 15.6 ± 34.2 (from –136 to +81) mg. A statistically significant negative asymmetry (As = –0.36, $P < 0.05$) and positive excess (Ex = 0.89, $P < 0.01$) were registered – i.e. if millipedes gained weight, they significantly increased in size, if they lost weight they only increased in size slightly. No millipedes died in the experiment. During the experiment there were no statistically significant differences in changes in body mass in different size classes of *R. kessleri*. The size classes with minimal and maximum body mass (less than 250 and more than 550 mg) were characterised by minimum variability in body mass.

The millipedes' food consumption during the two-week experiment varied from 1 to 277 mg, on average 113 ± 65 (median – 101) mg. A statistically significant

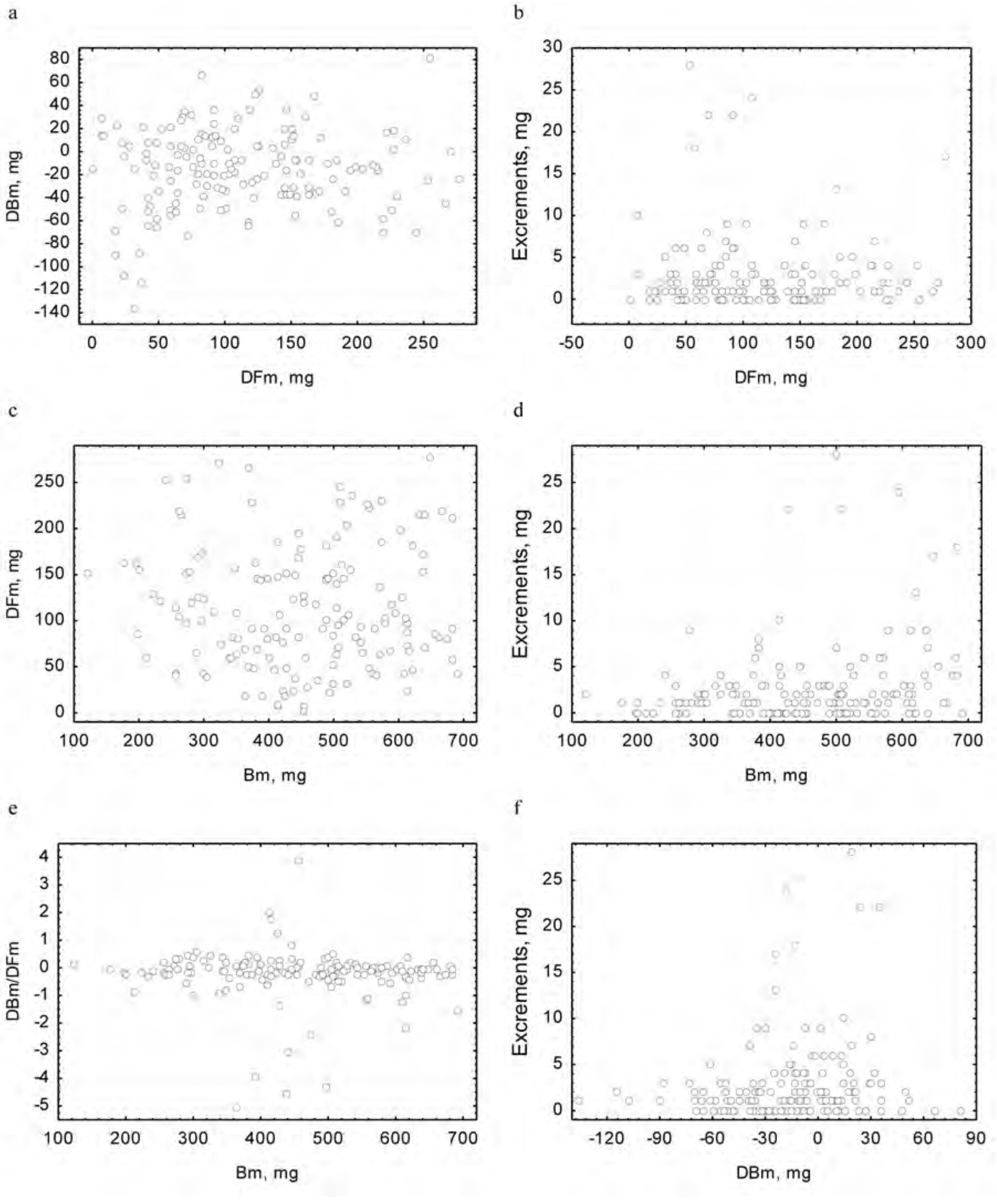

Fig. 1. The characteristics of trophic activity of *R. kessleri* individuals fed on the leaves of *A. negundo* during two weeks: a – relationship between change in body mass (ordinate, DBm, mg) and amount of food consumed (abscissa, DFm, mg); b – relationship between mass of excrements (ordinate, mg) and mass of food consumed (abscissa, DFm, mg); c – relationship between mass of food consumed (abscissa, DFm, mg) and body mass (abscissa, Bm, mg); d – relationship between mass of excrements (ordinate, mg) and the body mass (abscissa, Bm, mg); e – relationship between efficiency of food consumption (ordinate, DBm/DFm) and body mass (abscissa, Bm, mg); f – relationship between mass of excrements (ordinate, F, mg) and the millipedes' body mass (abscissa, Bm, mg).

asymmetry (As = 0.526, $P < 0.01$) and statistically insignificant negative excess (Ex = –0.453, $P > 0.05$) were registered, i.e. food consumption by most of the individuals make up smaller values than the average. No differences in amount of leaves consumed for different size classes of millipedes were registered (Table 1). During two weeks some *R. kessleri* individuals consumed an amount of food that exceeded their body weight (Fig. 1e).

On average during two weeks a single *R. kessleri* individual produced 2.8 ± 4.6 (from 0 to 28, median – 1.0) mg of excrement. Within the distribution of produced excrement (Fig. 1f) a statistically significant positive asymmetry is seen (As = 3.28, $P < 0.001$). The median quantity of excrement increases from 1 to 4 mg, though no statistically significant relationship between increase in the quantity of excrement and increase in an individual's body mass was observed (Table 1).

S. julipusilli gamonts and syzygy were found in 122 of the 154 millipedes individuals used in the experiment (79.2%). On average 17 ± 24 *S. julipusilli* gamonts were found in a single *R. kessleri* individual (median – 8). The distribution of parasites in the selection is asymmetric (2.23, $P < 0.001$), with a notable excess (5.73, $P < 0.001$), which indicates an average and a low rate of infestation of most *R. kessleri* individuals. Although our research concentrates on the weight of the millipedes, it is interesting to note that infestation by gregarine gamonts of second and third year *R. kessleri* individuals (up to 350 mg) is significantly lower than in fourth and fifth year millipedes (Table 1).

No influence of the host's sex was found upon consumption of the food plant (males – 106 ± 69 mg, females – 121 ± 59 mg, F = 1.91, $F_{0.05} = 3.90$, df = 1, 152, $P = 0.169$), changes in body mass (males: –15 ± 38 mg; females: –17 ± 29 mg; F = 0.09, $F_{0.05} = 3.90$, df = 1, 152, P = 0.761) and mass of faeces produced by the millipedes (males: 3.4 ± 5.4 mg; females: 2.1 ± 3.2 mg; F = 3.07, $F_{0.05} = 3.90$, df = 1, 152, P = 0.082). Also no statistically significant differences were found between males and females of the *R. kessleri* specimens in the level of infestation with *S. julipusilli* gamonts (males: 14 ± 22 specimens; females: 21 ± 25 specimens; F = 2.67, $F_{0.05} = 3.90$, df = 1, 152, $P = 0.105$).

According to the results of the experiment it can be stated that food consumption of *R. kessleri* decreased insignificantly when there was an increase in the number of *S. julipusilli* gamonts in their intestines (Table 2). In the absence of gregarine gamonts food consumption was at a quite high level (121 ± 66, median – 109 mg), with slight level of invasion (1–8 gamonts) it increased by 29% (136 ± 69, median – 141 mg), with a high level of invasion (33–133 gamonts) it decreased by 8% (108

Table 1. Change in body mass, food consumption, production of excrement by *R. kessleri* during the two-week experiment and number of *S. julipusilli* gamonts in *R. kessleri* intestines

Characteristics	Body mass, mg	*n*	*x* ± SD	Min	Max	Median	F ($F_{0.05} = 2.28$, df = 5, 148)	*P*
Change in body mass of *R. kessleri*, mg/individual	<250	12	–20.1 ± 20.5	–51	20	–23	1.53	0.183
	250–349	25	0.1 ± 36.2	–58	81	0		
	350–449	37	–14.1 ± 42.1	–114	66	–12		
	450–549	40	–18.2 ± 33.5	–136	35	–15		
	550–649	26	–23.1 ± 27.1	–73	27	–20		
	>650	14	–22.3 ± 25.3	–70	14	–14		
R. kessleri food consumption, mg/individual	<250	12	123.4 ± 60.9	41	253	125	1.33	0.255
	250–349	25	125.5 ± 64.2	39	271	110		
	350–449	37	95.6 ± 64.8	7	267	82		
	450–549	40	110.6 ± 63.1	1	245	103		
	550–649	26	108.6 ± 62.4	23	229	98		
	>650	14	140.7 ± 76.6	42	277	122		
Production of excrement by *R. kessleri*, mg/individuals	<250	12	1.1 ± 1.3	0	4	1	1.93	0.093
	250–349	25	1.7 ± 1.9	0	9	1		
	350–449	37	2.5 ± 4.2	0	22	1		
	450–549	40	2.8 ± 5.5	0	28	1		
	550–649	26	3.6 ± 5.3	0	24	2		
	>650	14	5.6 ± 5.7	0	18	4		
Number of *S. julipusilli* gamonts in *R. kessleri* intestines, mg/individual	<250	12	5.5 ± 9.7a	0	32	1	3.99	0.002
	250–349	25	2.6 ± 4.9a	0	19	0		
	350–449	37	21.2 ± 30.5b	0	133	10		
	450–549	40	24.3 ± 23.1b	0	88	18		
	550–649	26	15.8 ± 21.7b	0	107	9		
	>650	14	24.2 ± 25.3b	0	88	19		

± 67, median – 100 mg) compared to uninfested *R. kessleri* specimens.

No influence was noted of the number of *S. julipusilli* gamonts upon the change in *R. kessleri* body mass and amount of excrement produced when fed on leaves of *A. negundo* (Table 2). Thus, with a low rate gregarine infestation the millipedes, despite an increase in food consumption, produce the same amount of excrement and did not gain weight compared to healthy individuals. An average or a high-rate of gregarine invasion did not lead to a statistically significant increase in the amount of food consumed, body weight and amount of excrement.

If gregarines have a low impact upon the characteristics of trophic and metabolical activities of their hosts, then it is interesting to follow the inverse dependence: how do tempi of food consumption, formation of excrement and changes in body mass affect the numbers of *S. julipusilli*. No statistically significant influence was found of rates of food consumption (Fig. 2), rates of excrement formation (Fig. 2b) and changes in body mass (Fig. 2c) upon the numbers of gregarines (F = 0.85, $F_{0.05}$ = 2.16, df = 6, 147, *P* = 0.533 for food consumption, F = 0.97, $F_{0.05}$ = 2.43, df = 4, 149, *P* = 0.424 for mass of excrement and F = 1.12, $F_{0.05}$ = 2.16, df = 6, 147, P = 0.351 for change in body mass).

Discussion

The consumption of diplopods is not regulated by the regularities general for most invertebrates. The standard ratio for calculating the efficiency of conversion of food consumed into biomass of the body is the ratio of change in body mass (DBm) to mass of food consumed (DFm). If the duration of an experiment is at least 2–3 cycles of filling and voiding the intestine (for most invertebrates that is several days, for a human, in general, – 1.5 days) the DBm/DFm ratio becomes a valuable indicator of an extent of food fixation, its biological significance for an animal. However, for large species of Julidae, for example *R. kessleri*, we have observed rhythmicity in the digestion processes (Svyrydchenko and Brygadyrenko, 2014). Over several days diplopods can intensively consume food, increasing their body mass by 20–80%. After that they do not eat for

Table 2. Influence of number of *S. julipusilli* gamonts in intestines upon food consumption, changes in body mass and mass of excrement of *R. kessleri* during the two-week experiment

Characteristics	Number of gamonts in a single *R. kessleri* individual	*n*	*x* ± SD	Min	Max	Median	F ($F_{0.05}$ = 2.07, df = 7, 146)	*P*
Food consumption of *R. kessleri*, mg	0	32	121.1 ± 65.5a	1.2	270.9	109.2	2.67	0.013
	1–2	22	127.4 ± 73.9a	8.1	266.5	116.6		
	3–4	12	160.6 ± 67.1b	71.1	255.2	172.6		
	5–8	12	126.8 ± 60.5a	45.7	222.2	124.2		
	9–16	22	83.4 ± 44.7a	7.5	181.2	79.6		
	17–32	23	90.2 ± 54.5a	17.6	214.8	91.3		
	33–64	23	115.2 ± 71.2a	7.0	277.3	117.8		
	65–133	8	85.3 ± 50.4a	24.2	180.6	81.2		
Changes in body mass of *R. kessleri*, mg	0	32	–13.1 ± 36.8	–88	66	–16	0.66	0.705
	1–2	22	–16.3 ± 29.9	–70	48	–21		
	3–4	12	–3.4 ± 32.7	–58	81	–13		
	5–8	12	–7.5 ± 29.7	–61	32	–3		
	9–16	22	–13.9 ± 30.2	–66	36	–15		
	17–32	23	–25.1 ± 42.2	–136	36	–14		
	33–64	23	–18.6 ± 35.8	–114	30	–4		
	65–133	8	–22.4 ± 27.2	–53	15	–22		
Mass of *R. kessleri* excrement, mg	0	32	1.1 ± 1.3	0	5	1	1.86	0.080
	1–2	22	3.6 ± 5.3	0	22	2		
	3–4	12	1.8 ± 1.4	0	4	1		
	5–8	12	5.1 ± 5.9	0	22	4		
	9–16	22	3.1 ± 4.6	0	18	2		
	17–32	23	2.4 ± 2.3	0	7	2		
	33–64	23	4.4 ± 7.7	0	28	2		
	65–133	8	1.6 ± 2.4	0	6	0		

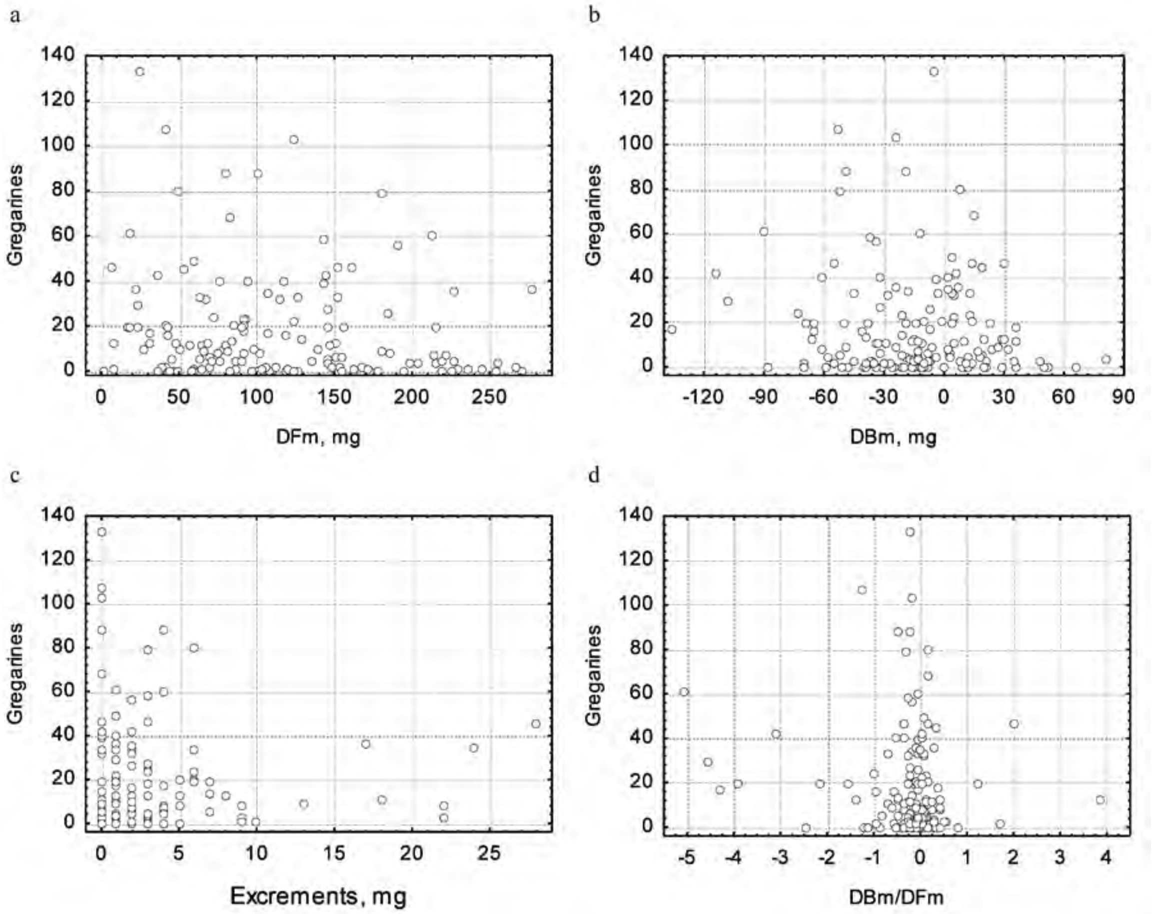

Fig. 2. The relationship between of the number of *S. julipusilli* gamonts in the studied *R. kessleri* specimens on the amount of food consumed (a), change in body mass (b), mass of excrement (c) and the ratio of change in body mass to mass of food consumed (d).

one-two weeks. Intestinal voiding can occur either during the consumption of a new intake of food, and also during the periods between intensive feeding. That is why for conducting laboratory experiments it is important to take into account the rhythmicity of the feeding cycle of *R. kessleri* feeding, its life expectancy, which can exceed 5 years, and the fact that the periods without feeding activities can exceed couple of months (Prishutova, 2001b; Brygadyrenko, 2004).

When analyzing diplopods' trophic activities we have encountered numerous fluctuations in the parameters of trophic activity for certain Julidae individuals (Brygadyrenko and Ivanyshin, 2014; Svyrydchenko and Brygadyrenko, 2014). Experiments with 10 or 12 *R. kessleri* specimens did not provide the desired accuracy in evaluating averages. That is why we set as our objective the evaluation of the overall variability of the diplopods' trophic activity, and assess the influence of gregarines living in the intestines of *R. kessleri*. A necessary stage for such an evaluation is the description of general variability in food consumption, the change in body mass and rates of excrement formation, which is given in the first part of the results of this article. It was discovered that there is no clear relationship between the above-mentioned parameters and the sizes of the animals in the experiment: two to five year old *R. kessleri* specimens might in several weeks only consume food, only void their intestines, do both things at the same time, or do neither. This quite unexpected result makes it more difficult to conduct further experiments on the feeding of *R. kessleri*. The question "Do gregarines influence the basic parameters of the trophic activity of *R. kessleri*?" was the second component of the research.

Data from the literature indicate that gregarines have a pathogenic effect on their hosts during the early stages of their development, as trophozoites attached to the host's epithelium (Valigurová, 2012). Our research, however, did not take into account the relationship between the age of the gregarines and the physiological activities of the hosts.

The influence of gregarines upon their hosts can be classified as negative, positive and neutral. Cases of negative impact can manifest themselves as (1) extension of duration of the entire ontogenesis or a certain developmental stage of the invertebrate host (Harry, 1970; Bouwma et al., 2005; Lord and Omoto, 2012), (2) increase in mortality at certain stages of development (Zuk, 1987b; Bollatti and Ceballos, 2014), (3) increase in mortality through predation, other parasites or diseases of the hosts infested with gregarines, or through damage to their normal intestinal microflora (Byzov et al., 1996; Maraun et al., 2003; Byzov, 2006), (4) the impact upon the population through mechanisms of sexual selection (Zuk, 1987a). It is probable that in the near future other manifestations of the negative impact of gregarines upon multicellular organisms will be identified, though the mechanisms involved in the alteration of the physiological processes of invertebrate-hosts will be studied for a long time to come.

Hypothetically, the positive impact of gregarines upon the carrier (in this case it would not be correct to use such terms as "parasite" or "host") could be realized through of (1) optimization of intestinal microorganism communities, (2) protection against other parasite species entering the intestine and thence other body cavities, (3) extraction by gregarines in the intestine of metabolites, which participate in exchange of matter by the host (4) consumption of chemical compounds present in the intestines unfavorable to the host, etc. However, there are very few examples of research in which these mechanisms have been found. This area of research is only at an early stage.

Many authors have emphasised that a negative impact of certain species of intestinal gregarines only occurs in cases of intensive, "massive" invasion – through mechanical congestion of the intestine (Zuk, 1987). Low and average numbers of intestinal gregarines usually do not affect their hosts. This means that a type of interaction which can be termed a "dose – effect" operates, which appears as a manifestation of the philosophical principle of conversion of quantity into quality. Theoretically a variant is possible, when a low number of gregarines is advantageous for a host (which would have to have mechanisms of optimizing the number of gregarines, for example, by the way of changing the diet), and high number of gregarines is harmful. Most likely, this type of interaction of hosts with high extensiveness and low to medium intensivity of gregarine infestation can be found in infestation of diplopods.

Gregarines, probably, evolved synchronically with the organisms of their hosts (Geus, 1969; Clopton, 2002, 2009). At least, their exceedingly wide-range distribution among invertebrates indicates the possibility that these protists evolved from parasitism to mutualism. In this case there is observed a manifestation of a general rule concerning the parasite's impact upon the host: the earlier the connection "parasite – host" appears within phylogenesis, the weaker becomes the negative impact upon the host's organism (Bulakhov and Pakhmov, 2010). In the process of time phylogenetically young "parasite – host" pairs become stable, mutually advantageous, mutually interacting pairs of species. Diplopoda is one of the oldest, one of the first groups of invertebrate-saprophages to have evolved on land (Hopkin and Read, 1992). Thus, the interaction between representatives of Julidae and Stenophoridae has every chance to become neutralist or even mutual, to lose all the features of parasitism, which is supported by the results of our research. The classification of interaction between species' as "parasitism – neutralism – mutualism" is quite relative and is defined by the intensity of the interaction of a macroorganism with its environment. In unfavorable conditions, which were observed in our laboratory experiment (limitation of space, only a single type of plant food, no possibility of feeding on plant detritus rich in microorganisms) the intensity of the "*S. julipusilli – R. kessleri*" interaction would be expected to sharpen. Perhaps in natural conditions, in order to prevent massive development of gregarines in the intestines, and to speeding-up the movement of food through the intestine *R. kessleri* uses a wide food base (fallen leaves from trees and shrubs, herbaceous plants, excrement of different vertebrates). As with humans the diet of *R. kessleri* should include certain "laxative" and "fixative" types of plant food, whose effect based on secondary metabolites (terpenes, alkaloids, flavonoids, etc.) present in plant remains, and combinations of microorganisms that develop at certain stages of leaf litter decomposition (Svyrydchenko and Brygadyrenko, 2014). Indirect confirmation of this is the increase in *R. kessleri* numbers in ecotones conditions (at the borders of forest and steppe, forest and meadow ecosystems), and also in mixed forests, where within a small territory leaf litter of a maximum number of plant species accumulates and where there are different types of elementary soil processes formation (Pokarzhevskij, 1983; Pokarzhevskij et al., 1989; Brygadyrenko, 2004, 2006).

Gregarine infestation of males and females in some invertebrates can significantly vary (Abro, 1971; Hecker et al., 2002). Perhaps, this is connected with the manifestation of sexual selection: the female chooses the brightest, biggest and strongest males for fertilization. Perhaps resistance to parasites is also a factor in sexual selection (Zuk, 1987b). *R. kessleri* males are smaller than females. The literature contains no information covering the sexual selection of this species of diplopod. This is probably why during our research we did not find any differences in the range of gamont infestation of *S. julipusilli*. This might also indicate that there is little difference in the feeding ecology of males and females of this diplopod species.

The possibility of gregarine infestation increases proportionally with the amount of food consumed by a certain individual (proportional to the life expectancy of a certain host specimen). According to the data of Locklin and Vodopich (2010), heavier specimens of dragonflies (older ones) have statistically significantly higher levels of gregarine infestation. On the other hand, the results of this two-year experiment clearly prove that the intensity of dragonflies' infestation increases from spring to autumn, i.e. with the increase in dragonfly imagos' life expectancy. In our experiment the larger specimens of *R. kessleri* were infested with a higher number of gamonts. Perhaps the results of the experiment were also affected by the high-capacity of the intestines of the larger specimens of *R. kessleri*, which can include a larger of *S. julipusilli* gamonts without causing damage to the host.

Laboratory experiments studying the feeding of diplopods show a significant variability in the consumption of leaf litter depending upon the temperature, moisture, stage of leaves decomposition, species composition of the litter and a number of other factors (Gere, 1956; Kondeva, 1980; Dangerfield and Milner, 1993; Couteaux et al., 2002; Ashwini and Sridhar, 2005; Roy and Joy, 2009). Perhaps a significant contribution to the results of studying trophic activity may be made by study of diplopods' infestation with parasites, and the most common among them – gregarines.

Studying the interaction of diplopods and parasites is a promising research area from an evolutionary perspective, which is important for understanding the processes of regulation of biological populations in natural ecosystems, study of the ways these processes are damaged in anthropogenically affected ecosystems (Köhler and Alberti, 1992; Köhler et al., 1992; Kulbachko and Didur, 2012; Brygadyrenko and Ivanyshin, 2014; Pokhylenko, 2014; Souza et al., 2014) – pollution with heavy metals, radio-nuclides, persistent organic pollutants, pesticides.

The hypotheses formulated in the introduction to this article have partly proved right, partly wrong. Different numbers of *S. julipusilli* gamonts can decrease or increase *R. kessleri*'s food consumption by 25–29%. (1) The hypothesis that maximum increase in the body weight will be observed with specimens which had consumed maximum levels of food proved incorrect. (2) The hypothesis that the extent of infestation with *S. julipusilli* varied at different stages of ontogenesis is proved correct. (3) The hypothesis that the number of gregarines affects changes in *R. kessleri* body weight and rates of faecal formation proved incorrect. The results of this investigation should be useful for better understanding of the relationship between parasites and hosts.

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Assemblages of ground beetles (Carabidae, Coleoptera) in peatland habitat, surrounding dry pine forests and meadows

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Abstract

Igondová, E., Majzlan, O., 2015. Assemblages of ground beetles (Carabidae, Coleoptera) in peatland habitat, surrounding dry pine forests and meadows. *Folia Oecologica*, 42: 21–28.

This research was conducted to study assemblages of ground beetles in peatland, surrounding dry pine forests and meadows in the Šuja peatbog (in northern part of Slovakia) in 2013. The main aim was to classify assemblages of beetles into different habitat types, compare their composition and analyse the relation of species occurrence at study sites. Carabids were sampled between May and October 2013 at 8 study sites using pitfall traps and 1,627 individuals belonging to 55 species were registered in total. All features such as diversity, equitability, species composition, their preference for humidity and vegetation cover, inclusion in the group of their ecological valence and habitat association and community index of the ground beetles were used to characterize ground beetle communities at each study site. In order to evaluate the relationship among the communities of the carabid beetles at the sampling sites we used principal component analysis. The scatter of species formed four groups of species associated with dry open lowland habitats, wetter woodland habitats, hygrofil wetland habitats and dry to semi wet unshaded habitats.

Key words

assemblages, ground beetles, peatland, forests, meadows

Introduction

Peatlands and other wetland ecosystems play an important part in European biodiversity. They provide ideal conditions for a considerable diversity of habitats and species (EC, 2007). Peatlands are very rare, endangered and often relict ecosystems. In general, there is a lack of knowledge about ecosystem functioning and management planning and not all protected areas in Slovakia are protected effectively (Stanová, 2000). Some peatlands have been preserved in a natural state, but most of them have been drained and became highly fragmented, isolated or naturally overgrown by forest. Therefore peatlands and other wetland fragments are mainly surrounded by drier habitats such as forests or meadows.

The environmental sensitivity of peatlands makes insects valuable as bioindicators. With increasing scientific interest in peatland insects and the fact that each relict peatland habitat island is unique, further studies of the diversity of peatland fauna are very important and the conservation of these habitats should be strongly supported (Spitzer and Danks, 2006).

This paper deals with description of ground beetles species composition and characteristics in peatland habitats surrounding dry pine forest and meadows near Šuja peatbog.

According to the range of ecological valence and association with habitat the ground beetles present in Slovakia are divided into three groups: R (relict), A (adaptable), E (eurytop), (Hůrka et al., 1996, Farkač et al., 2007). The fauna of original or little disturbed natural habitats is always composed mostly of A-species as well as some R-species, while E-species are scarcely represented. The number of E-species is increasing with deterioration of the environment (Farkač et al., 2007).

Majority of published research studies refer only marginally to this territory. That applies to invertebrates as well as to other animals. Vertebrate fauna has been researched here by Bitušík and Bitušík (1995). Bitušík (1998) has obtained data of midges fauna (Chironomidae) in the waters of Šujska peatbog. Beetle communities on this site were analyzed by Majzlan et al. (2004).

Study area and sampling sites

The Šuja peatbog nature reserve is subject to 4th level of nature protection (10.8 ha). It is also Special Area of Conservation (SAC), which belongs to the NATURA 2000 network (13.48 ha) located at altitude 470 m. During the 1970s, the territory was destroyed by peat exploitation mainly its northwestern part. Nevertheless, it is still an important wetland area of Slovakia, but there is a threat in increasing succession. *Pinus nigra* is spreading on the northwestern part from the surrounding dry pine forest and more willow shrubs spread to the centre of the peatbog from the east part of Rajčianka riverside. The exploited area is being gradually overgrown by original and ruderal vegetation. In the Šuja peatbog there is an „island“ where *Rubus* sp., *Urtica* sp. appears. This part is influenced by the decline of groundwater level. The edges of neighboring roads are surrounded by trees (*Salix* sp., *Alnus glutinosa*). There is a dense stand of reeds in the northwestern part and eastern part of the territory (*Phragmites* sp.).

Eight sampling sites of peatbog habitats, surrounding dry pine forest and meadows were established (Fig 1):

1 (49°03’40.4”N, 18°36’57.2”E), dry pine forest – forest edge at slope of Strážov hills on the limestone ground with *Pinus* sp.
2 (49°03’37.7”N, 18°36’58.6”E), osier – waterlogged area at mild depression with *Salix repens* and *Salix purpurea*
3 (49°03’37.6”N, 18°37’02.1”E), playground – meadow, mown area, flat surface, without trees, slightly dry, with frequent appearance of molehills
4 (49°03’40.3”N, 18°37’05.2”E), peat – peat bench, unexploited part of peatland, with *Rubus idaeus*
5 (49°03’42.6”N, 18°37’05.8”E), gravel bars – gravel bench in the middle of the reservation with *Betula* sp. and *Pinus* sp.
6 (49°03’44.6”N, 18°37’05.8”E), reed – damp areas around slightly flowing water with *Phragmites australis*
7 (49°03’46.3”N, 18°37’09.8”E), overgrowth – on the peat bench with *Salix* sp. and *Rubus* sp.
8 (49°03’36.9”N, 18°37’18.4”E), field-meadow-edge of the meadow and field by the Rajčianka riverside, ruderal, overgrown with vegetation.

Material and methods

The ground beetles were pitfall-trapped during the year 2013 regularly in bi-weekly intervals (on following dates: 3 May, 17 May, 2 June, 16 June, 1 July, 15 July, 29 July, 12 August, 27 August, 14 September, 27 September, 12 October and 1 November 2013) at eight sampling sites. Pitfall traps were installed on 14 April 2013 and exposed for 201 days. Covered traps (500 ml in size, 10 cm in diameter, half-filled with 4% formalin solution) were used to collect samples. Five traps were placed in lines in each habitat. The carabid beetle individuals were identified using keys of Trautner and Geigenműller (1987) and Hůrka (1996). Characteristics of the environment requirements of species were according to works of Roubal (1930), Nakládal and Hejda (2012) and Šustek (2012, 2010, 2004, 2000). The humidity preference was classified using an eight degree semiquantitative scale (1 – strongly xerophilous, 4–5 mezohygrophilous, 8 – strongly hygrophilous) and preference of the ground beetles for vegetation cover with a five degree semiquantitative scale 1–5 (1 – open landscape species (fields, meadows, ruderals), 2 – indifferent to vegetation cover (eurytopic in largest sense), 3 – herbage cover with disperged group of trees or shrubs, 4 – forest species, 5 – rupicolous species) (Šustek, 2004). As a diversity index, the Shannon-Wiener index (H’) (Shannon and Weaver 1949; Spellerberg and Fedor, 2003), which is a commonly used diversity index considering both abundance and evenness of species present in the community, was chosen. As an equitability index we used Pielou’s Evenness Index or Equitability (E) (Pielou, 1966). All these calculations were carried out using the PAST program. The ground beetles were divided into three groups: R (relict), A (adaptable), E (eurytop) according to the range of their ecological valence and their association with the habitat of Farkač et al., 2007. Based on this species distribution and mathematical model of Boháč (1990), the stage of antropogenetic degradation maybe characterized by a community index of the ground beetles (IKS) (Nenadál, 1998). This index proposes 5 levels of anthropogenic degradation in total: 1 – deeply affected (value 0–15), 2 – strongly affected (value 10–35), 3 – affected (value 30– 50), 4 – slightly affected (value 45–65), 5 – unaffected (value 65–100). Principal component analysis (PCA) was conducted to evaluate the relationship among the communities of the carabid beetles at the sampling sites using the CANOCO software program (TerBraak and Šmilauer, 1998). The longer a single arrow is, the higher the value of single species within the sampling site around them. Though smaller distance between arrow and axis represents a higher correlation between the two values.

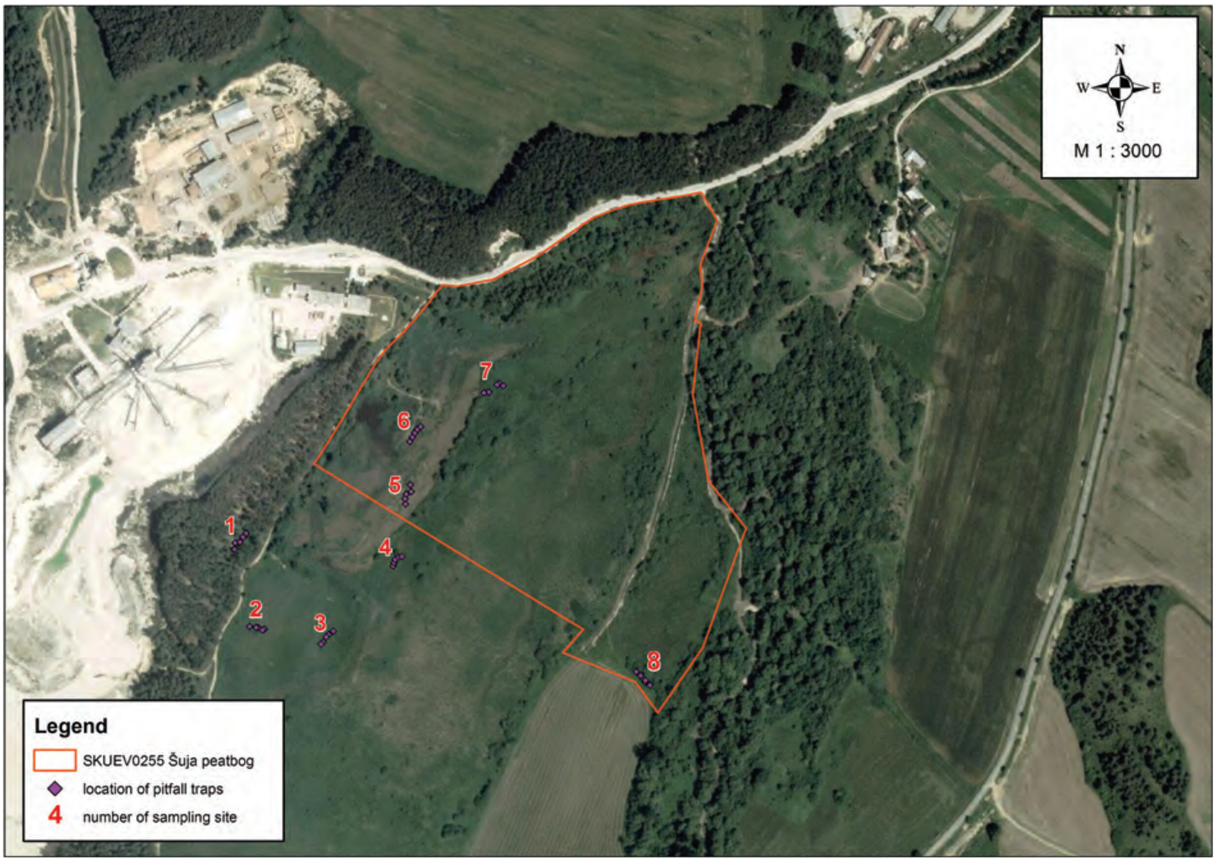

Fig. 1. Distribution of sampling sites in the Šuja peatbog.

Results and discussion

A total of 1,627 individuals belonging to 55 species were registered (Table 1) and all sampling sites demonstrated the high dominance of *Poecilus versicolor* and *Poecilus cupreus*.

Eudominant species (>10%) occur in the forest such as *Abax parallelepipedus* (31.2%), *Carabus violaceus* (23.65%) and *Molops piceus* (12.9%). *Abax parallelepipedus* occurs in wooden habitats and is often a dominant species within forest communities. *Carabus violaceus* and *Molops piceus* prefer shaded habitats and are more or less mezohygrophilous species.

The osier has a balanced dominance of *Pterostichus niger* (27.7%) and *Pterostichus diligens* (17.85%). These species prefer wet and semi-wet places from lowlands to mountains and are also present in other study sites.

The highest number of individuals was recorded at the playground, with three eudominant species *Poecilus versicolor* (22.53%), *Poecilus cupreus* (15.11%) and *Amara communis* (10.25%). *Poecilus versicolor* and *Poecilus cupreus* are eurytopic species, inhabiting unshaded habitats, meadows, fields and ruderal habitats. *Amara communis* prefers open habitats e.g. fields, abandoned fields, managed meadows.

The peat had a balanced representation of two eudominant species *Carabus ullrichi* (24.9%) and *Pterostichus melanarius* (13.83%). These species are mezohygrophilous. However *Pterostichus melanarius* is eurytopic and appears in the fields, meadows, gardens, open lands from lowlands to uplands and *Carabus ullrichi* prefers woodland habitats.

Low number of species following three eudominant species was found in the gravel bars: *Poecilus versicolor* (17.3%), *Amara lunicollis* and *Carabus coriaceus* (13.04%). The first two are heliofil species which prefer unshaded habitats with preference for drier ones. *Carabus coriaceus* is mezohygrophilous forest species.

The reeded area was characterized by eudominant species *Pterostichus diligens* (38.9%), *Pterostichus niger* and *Trechussecalis* (13.8%). *Pterostichus diligens* is eurytopic and *Pterostichus niger* prefers woodland habitats. All these species are hygrofilous. *Trechussecalis* is present in marshes and bogs.

In the overgrowth site *Trechus secalis* (21.25%) and *Patrobus atrorufus* (11.8%) were dominant. In general *Patrobus atrorufus* prefers humid and woodland habitats and occurs only at this site.

Finally, in the field-meadow, the dominant species were *Poecilus cupreus* (19%), *Abax parallelepipedus* (13.63%), *Carabus ullrichi* and *Poecilus versicolor*

Table 1. Abundance of ground beetles (Carabidae) sampled

Species	Abbr	PH	VC	for	osi	plg	pet	gra	ree	ovg	fim
Abax parallelepipedus (Piller et Mitterpacher., 1783)	Abpa	3	4	29	2	1	6	0	0	3	33
Agonum fuliginosum (Panzer, 1809)	Agfu	8	4	0	5	2	2	0	0	1	0
*Agonum sexpunctatu*m (Linnaeus, 1758)	Agse	5	1	0	0	1	0	0	0	1	0
*Amara aulic*a (Panzer, 1797)	Amau	3	1	0	0	0	0	0	0	15	0
Amara communis (Panzer, 1797)	Amco	–	–	0	0	76	8	2	0	1	0
Amara familiaris (Duftschmid, 1812)	Amfa	3	1	0	0	6	0	0	0	1	1
Amara lunicollis (Schiődte,1837)	Amlu	–	–	0	1	55	10	3	3	4	0
Anchomenus dorsalis (Pontoppidan, 1763)	Ando	3	1	0	0	0	0	0	0	0	1
Anisodactylus signatus (Panzer, 1797)	Ansi	5	1	0	0	0	0	0	0	0	2
Badister sodalis (Duftschmid, 1812)	Baso	7	2	0	1	1	0	0	0	0	0
Bembidion mannerheimi (Sahlberg, 1827)	Bema	8	4	0	2	46	5	0	0	2	1
Bembidion properans (Stephens, 1828)	Bepr	3	1	0	1	3	0	0	0	0	1
Bradycellus caucasicus (Chaudoir, 1846)	Brca	3	1	0	1	4	2	0	0	0	0
Calathus fuscipes (Goeze, 1777)	Cafu	4	1	0	0	1	0	0	0	0	0
Calathus micropterus (Duftschmid, 1812)	Cami	–	–	0	0	1	0	0	0	7	0
Carabus convexus (Fabricius, 1775)	Caco	4	4	3	0	33	11	1	0	0	4
Carabus coriaceus (Linnaeus, 1758)	Caco2	5	4	2	2	0	13	3	0	5	6
Carabus granulatus (Linnaeus, 1758)	Cagr	7	2	1	10	3	3	0	2	0	6
Carabus intricatus (Linnaeus, 1761)	Cain	4	4	2	0	0	0	0	0	0	0
Carabus problematicus (Herbst, 1786)	Capr	4	1	4	0	0	0	0	0	0	0
Carabus ullrichi (Germar, 1824)	Caul	4	4	3	7	22	63	0	1	2	28
Carabus violaceus (Linnaeus, 1758)	Cavi	5	4	22	5	16	13	1	0	2	4
Clivina collaris (Herbst, 1784)	Clco	6	2	0	1	5	0	0	0	0	1
Cychrus caraboides (Linnaeus, 1758)	Cyca	5	4	2	0	0	0	0	0	0	0
Dyschirius globosus (Herbst, 1783)	Dygl	8	5	0	0	5	2	0	0	1	1
Harpalus latus (Linnaeus, 1758)	Hala	4	1	0	0	0	0	3	1	1	6
Lebia chlorocephala (Hoffmann, Koch, P. Műller et Linz, 1803)	Lechl	4	4	0	0	1	0	0	0	0	0
Leistus ferrugineus (Linnaeus, 1758)	Lefe	4	3	1	0	18	0	0	0	0	0
Leistus terminatus (Hellwig in Panzer, 1793)	Lete	5	4	0	0	34	1	0	0	3	5
Molops piceus (Panzer, 1793)	Mopi	4	4	12	0	0	0	0	0	0	0
Nebria brevicolis (Fabricius, 1792)	Nebr	6	2	0	0	2	2	0	0	0	5
Notiophilus palustris (Duftschmid, 1812)	Nopa	4	2	1	0	1	1	0	0	2	11
Oodes helopioides (Fabricius, 1792)	Oohe	8	2	0	1	0	1	0	0	0	1
Ophonus azurens (Fabricius, 1775)	Opaz	2	1	0	0	1	0	0	0	0	0
Panagaeus bipustulatus (Fabricius, 1775)	Pabi	4	1	0	0	1	0	0	0	1	0
Paradromius linearis (Olivier, 1795)	Pali	2	1	0	0	0	1	0	0	0	0
Patrobus atrorufus (Stroem, 1768)	Paat	7	4	0	0	0	0	0	0	15	0
Platynus assimilis (Paykull, 1790)	Plas	7	4	0	0	0	1	0	0	0	0
Poecilus cupreus (Linnaeus, 1758)	Pocu	4	1	0	1	112	9	2	0	1	46
Poecilus versicolor (Sturm, 1824)	Pove	2	1	0	1	167	8	4	2	0	28
Pseudoophonus rufipes (De Geer, 1774)	Psru	4	1	0	0	2	0	0	0	1	7
Pterostichus anthracinus (Illiger, 1798)	Ptan	8	4	0	0	0	1	0	0	0	9
Pterostichus burmeisteri (Heer, 1841)	Ptbu	5	4	0	0	0	1	0	0	0	0
Pterostichus diligens (Sturm, 1824)	Ptdi	7	2	2	20	29	13	0	14	6	1

Table 1. Abundance of ground beetles (Carabidae) sampled – continued

Species	Abbr	PH	VC	for	osi	plg	pet	gra	ree	ovg	fim
Pterostichus melanarius (Illiger, 1798)	Ptme	5	2	0	2	52	35	0	0	12	17
Pterostichus minor (Gyllenhal, 1827)	Ptmi	8	5	0	2	0	0	0	0	0	0
Pterostichus niger (Schaller, 1783)	Ptni	6	4	8	31	5	6	3	5	5	5
Pterostichus nigrita (Paykull, 1790)	Ptni2	8	2	0	0	1	1	0	1	2	6
Pterostichus oblongopunctatus (Fabricius, 1787)	Ptob	5	4	1	0	0	0	0	0	0	1
Pterostichus ovoideus (Sturm, 1824)	Ptov	4	2	0	10	8	23	1	2	5	5
Pterostichus strenuus (Panzer, 1797)	Ptst	7	2	0	0	1	0	0	0	0	0
Pterostichus vernalis (Panzer, 1796)	Ptve	8	5	0	0	1	0	0	0	0	0
Stomis pumicatus (Panzer, 1796)	Stpu	6	2	0	0	2	0	0	0	0	0
Syntomus truncatellus (Linnaeus, 1761)	Sytr	4	1	0	0	0	3	0	0	1	0
Trechus secalis (Paykull, 1790)	Trse	–	–	0	6	22	8	0	5	27	0

Abbr, Abbreviations; PH, preference for humidity: scale 1–8 (1 – strongly xerophilous, 4–5 mezohygrophilous, 8 – strongly hygrophilous); VC, vegetation cover preference: scale 1–5 (1 – open landscape species (fields, meadows, ruderals), 2 – indifferent to vegetation cover (eurytopic in largest sense), 3 – herbage cover with dispersed group of trees or shrubs, 4 – forest species, 5 – rupicolous species); for, forest; osi, osier; plg, playground; pet, peat; gra, gravel bars; ree, reed; ovg, overgrow; fim, field-meadow.

(11.6%). The first and last prefer dry open habitats. *Carabus ullrichi* is mezohygrophilous and woodland similarly as *Abax parallelepipedus*.

The results show that the number of species registered in the studied sites was the same in the gravel bars and reed (10). The highest number of individuals (741) refers to the playground, however the equitability reaches its minimum (0.7279). The lowest number of individuals was located in gravel bars (23) and reed (36). The gravel bars show the highest value of equitability (0.9557). The highest diversity index was at the overgrown sampling site (2.737) (Table 2).

During the sampling period no R-species were recorded in all localities. A- and E-species were observed at all localities in different ratios. 22 (59.5%) adaptable and 15 (40.5%) eurytopic species were recorded in the playground. 13 (81.25%) adaptable and 3 (18.75%) eurytopic species were recorded in the forest. 14 (51.8%) adaptable and 13 (48.2%) eurytopic species were recorded in the field-meadow. 7 (70%) adaptable and 3 (30%) eurytopic species were recorded in the reeds. 20 (69%) adaptable and 9 (31%) eurytopic species were recorded in peat. 18 (66.7%) adaptable and 9 (33.4%) eurytopic species were recorded in the overgrown site. 8 (80%) adaptable and 2 (20%) eurytopic were found in the gravel bars. 14 (70%) adaptable and 6 (30%) eurytopic species in the osier. The highest IKS index values were in the forest and gravel bar study sites and the lowest ones at the field-meadow and playground study sites.

The IKS index may be sometimes confusing as E-species occur in all habitats but also in anthropogenetic uninfluenced ones. In contrast R-species are found only in the anthropogenetic uninfluenced habitats.

Principal component analysis (PCA)

The PCA ordination diagram of the carabid beetle communities at 8 sampling sites is represented in Figure 2. Eigenvalues of the two first axes are λ1 = 0.83 and λ2 = 0.078. The first canonical axes account for 83% of the total variance of the species data. The scatter of species forms four groups (Fig. 2).

The scatter of carabid beetle species and sampling sites form four groups (Fig. 2). The first group contains species *Paradromius linearis*, *Syntomus truncatellus*,

Table 2. Diversity and equitability

	for	osi	plg	pet	gra	ree	ovg	fim
Number of species	15	21	37	29	10	10	27	28
Number of individuals	93	112	741	253	23	36	127	242
Diversity (H’)	2,062	2,399	2,628	2,694	2,201	1,903	2,737	2,705
Equitability(E)	0,7613	0,7879	0,7279	0,8	0,9557	0,8265	0,8304	0,8118
Index of ground beetles community (IKS)	40,63	35	29,73	34,49	40	35	33,33	25,93

for, forest; osi, osier; plg, playground; pet, peat; gra, gravel bars; ree, reed; ovg, overgrow; fim, field-meadow.

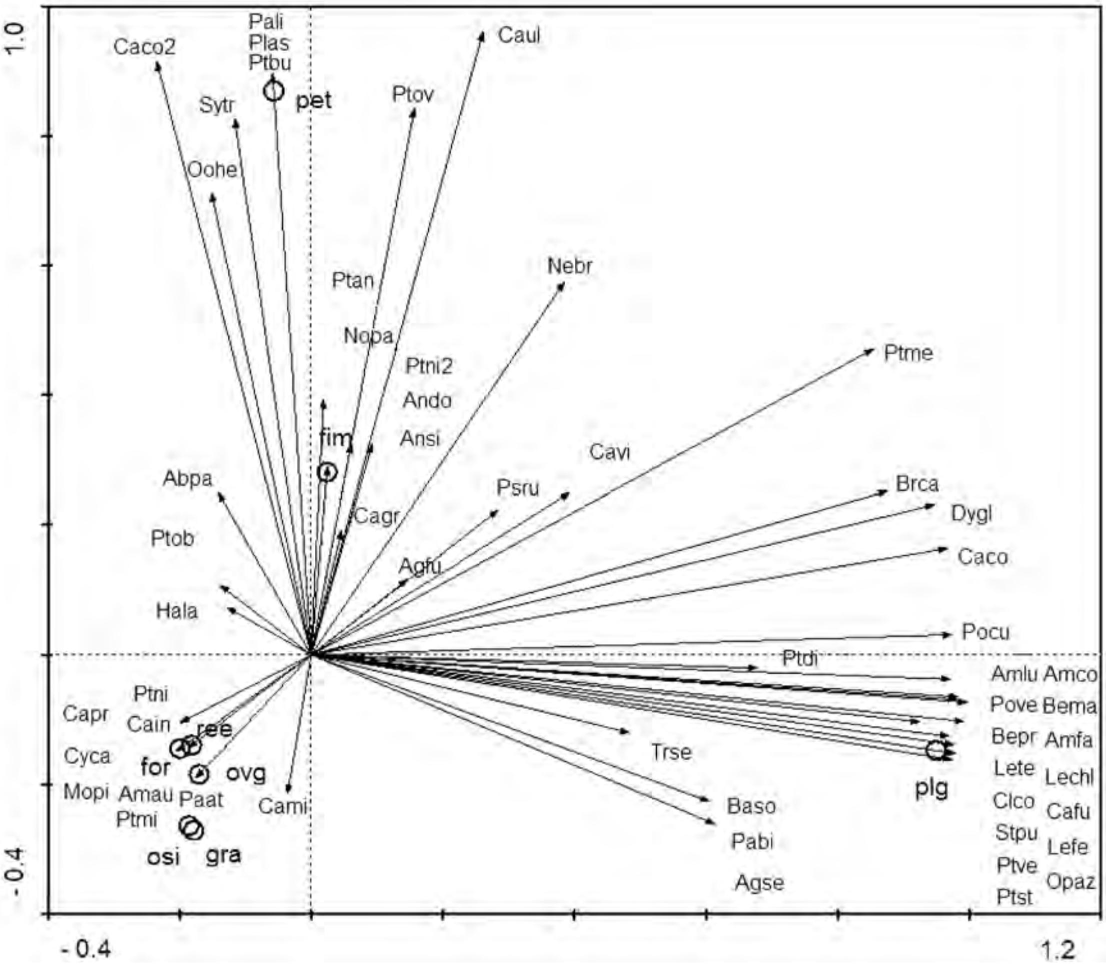

Fig. 2. Ordination diagram based on Principal Component Analysis (PCA) of carabid beetle species and sampling sites. The species are represented by arrows. For abbreviations of species names see the Table 1. Codes of study sites: plg, playground; for, forest; fim, field-meadow; osi, osier; pet, peat; ovg, overgrow; gra, gravel bars; ree, reed.

Carabus coriaceus, *Carabus ullrichi. Paradromius linearis* and *Syntomus truncatellus* are associated with dry, more xerophilous open non-forest habitats in lowlands and ruderal habitats, fields or vineyards. *Carabus coriaceus* and *Carabus ullrichi* are mezohygrophilous and forest species (the upper right quadrat and upper left quadrat of the ordination diagram). Species associated with dry, xerophilous non-forest habitats, but also species associated with more humid woodland habitats had a closer relation with peat sampling site.

The second group contains species *Carabus problematicus, Cychrus caraboides, Molops piceus, Carabus intricatus*, *Abax parallelepipedus*, *Pterostichus oblongopunctatus* associated with mezohygrophilous forest habitats with temporary covers (the lower left quadrat and upper left quadrat of the ordination diagram). Species associated with more or less mesophilic shaded forest habitats had relation with the forest in the peat vicinity.

The third group contains *Trechus secalis*, *Pterostichus diligens* and *Badister sodalist* associated with strongly hygrophilous habitats, near waters, wet habitats, peatbogs and wetlands and *Patrobus atrorufus, Pterostichus minor* associated with strongly hygrophilous habitats (the lower left quadrat and lower right quadrat of the ordination diagram). Species associated with hygrophilic habitats occurred in the gravel bars, osier, reeds and overgrowth, but also in the playground.

The fourth group contains *Poecilus versicolor, Poecilus cupreus*, *Bembidion properans* and *Amara communis* associated with unshaded habitats, more dry to semi wet and open lowland habitats (the upper right quadrat and lower right quadrat of the ordination diagram). Species associated with dry to semi wet and unshaded habitats occurred in the playground and field-meadow. These characteristics of carabid beetles are known; also the result of the analysis demonstrated them.

Carabus intricatus according to IUCN (2013) belongs to lower risk (LR) or near threatened (NT) IUCN threat status. Remaining carabid beetles in the sample had not evaluated (NE) IUCN threat status. *Carabus intricatus* was found in the forest.

Carabus problematicus belongs according to Annex no. 6 to Decree no. 24/2003 Coll. implementing the Law no. 543/2002 Coll. on nature and landscape protection to the list of species of national importance. *Carabus problematicus* was found in the forest. From the nature protection point of view valuable species occurred at the forest sampling site.

Pterostichus diligens is considered to be a tyrphophilous carabid species (Spitzer et al., 1999) and it was dominant at reed and osier sampling sites occurring at gravel bars, osier, reed, overgrowth and also with playground sampling sites.

The most ecosozological value according to the ratio of the adaptable and eurytop species percentage and IKS index values, was found in the forest and gravel bars. In addition, the gravel bars sampling site also indicates the highest value of equitability. The highest diversity index was at the overgrowth sampling site.

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The changes of nutrient and risk elements of top soil layers under canopy of different tree species and grassland in Arboretum Mlyňany, Slovakia

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Abstract

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Litter represents the input-output system of nutrients in forests. Since the aim of study was to extent the knowledge of nutrient and risk elements cycling in the Arboretum Mlyňany (Slovak Republic), we investigated the contents of selected elements in the litter of tree species and grassland, and compared them with the contents of elements in the surface soil layers (0–10 cm). Results showed that the richest on N, P, K macro-elements were sod of grass, rich was litter of maple, oak and surprisingly also yew. The correlation between quantity of exchange calcium, magnesium, total nitrogen, available phosphorus, potassium, copper and iron in the surface soil layers and litters was not significant. On the contrary, statistically significant correlations were found in case of zinc and manganese in litter and their available ions in the surface soil layers (Zn: $r = 0.884$, $P < 0.001$; Mn: $r = 0.501$, $P < 0.05$). Limit values of available cadmium and zinc content were exceeded in the soil for yews, Cd for Himalayan pines and lead for spruces. Higher contents of mentioned heavy metals in soil were attributed to bioaccumulation, as well as to atmospheric deposition.

Keywords

grassland, heavy metals, litter, nutrients, soil, tree species

Introduction

Litter plays a key role in the forest life and forest soil as natural fertilizer. In forests, the amount of litter depends on the tree species composition, age, canopy and quality. At the same stand, the biomass of litter can be year to year different with even double variations (Šály, 1982). For example annual influx of elements with beech litterfall to the soil in Middle Pomerania (Poland) was: 23.2–61.0 kg ha^{-1} of nitrogen, 3.6–7.6 kg ha^{-1} of phosphorus, 8.3–26.2 kg ha^{-1} of potassium and 15.3–22.4 kg ha^{-1} of calcium (Jonczak, 2013).

During decomposition of plant material, part of the nutrient elements are liberated in inorganic dissolved form, for instance as the ions (Ca^{2+}, Mg^{2+}, K^+, NH_4^+, SO_4^{2-}, $H_2PO_4^-$). In soils of humid areas, dissolved nutrients tend to migrate down the soil profile. Under vegetation, the larger part of those nutrients is usually taken up again by plant roots and assimilated by the vegetation, and only a small fraction is lost with the drainage water. An actively growing, closed vegetation may take up several hundreds kg of nutrient elements per ha per year from the root zone, which often extends to several metres below the soil surface. Most nutrients in plants are eventually returned to the soil surface by litter fall. So the net effect of uptake by plants is that nutrients from deeper soil layers are transported to the soil surface. Part of the nutrients in the surface soils is easily available to plants (at the exchange complex or temporarily stored in microbial biomass), but part may be locked up in slowly available mineral form (especially P). This process of "nutrient pumping", which counteracts the leaching of nutrients, is especially important in mature, undisturbed forests (van Breemen and Buurman, 2003).

Up to 70–90% nutrients annually needed for forest growth are released by decomposition of organic detritus. During these processes, nutrients are preserved through their retranslocation and immobilization in order to maintain the productivity of ecosystem (Vogt et al., 1986). The contents of nutrients in soil positively correlate with litter substrate quality, showing that higher contents of soil nutrient are accompanied with good quality of litter substrate, and lower soil nutrients with poor litter quality (Ge et al., 2013).

In order to better know the cycle of nutrients and other risk elements in Arboretum Mlyňany, we investigated the contents of selected elements in litter of tree species and grassland and compared them with contents of elements in surface soil layers.

Material and methods

Study site

Arboretum Mlyňany (48º19'N and 18º21'E) is located in southern Slovakia on the north edge of the Danubian Lowland, in the valley of the Žitava river, on slightly undulated terrain, at an altitude of 165–217 m above sea level. It is situated on a late Tertiary geological formation, represented by Neogene clays, sands and rubble sands. This substratum is almost all covered by wind-deposited loess, mostly without carbonates (Cifra, 1958). Mean temperature in the area is 10.6 °C and mean annual total precipitation is 541 mm (Hrubík et al., 2011). Arboretum in Mlyňany was established in 1892. Recent inventory of the gene pool of trees and shrubs finished in 2012 showed, that the current number of taxa grown in the Arboretum is 1,933 (Hoťka et al., 2013).

Soil sampling

Elemental composition of litter and the contents of elements in soil depth 0–10 cm were investigated under canopy of tree species: natural oak-hornbeam stand (*Quercus robur*, L., *Carpinus betulus*, L.), spruce (*Picea abies*, (L.) Karsten), sugar maple (*Acer saccharinum,* L.), yew (*Taxus baccata*, L.), cherry laurel (*Prunus laurocerasus*, L.), Himalayan pine (*Pinus wallichiana,* Jacks.), Japanese cedar (*Cryptomeria japonica,* D. Don.) and grassland.

Soil and litter samples were taken in autumn 2005.

Soil and litter analyses

- Ca, Mg, Fe, Zn, Mn, Cu in dry litter was analysed by atomic absorption spectrophotometer;
- Total organic carbon content in soil and dry litter – by Tyurin method (Orlov and Grishina, 1981), total nitrogen by Kieldahl method (Fecenko, 1991);
- Soil exchange base cations (Ca^{2+}, Mg^{2+}) by extraction with ammonium acetate (Hrivňáková et al., 2011) available phosphorus and potassium in soil by method of Mehlich III (Mehlich, 1984), potentially available forms of heavy metals in soil (Cu, Zn, Cr, Pb, Cd, Ni, Fe, Mn, Co) (extraction by 2 mol dm^{-3} HNO_3) (Hrivňáková et al., 2011).

Each analysis was done in 3 repeats. Results shown in Tables 1 and 2 represent the average values (mean ± SD). Correlation analysis was used for determination the relationship between elements in the soil and litter. Statistical significance of results was assessed on minimum 95% level.

Results and discussion

Litter represents the input-output system of nutrients in forests. The rate, at which litter accumulates and decomposes, regulates the flow of energy, primary productivity and nutrient cycling in forest ecosystem.

Contents of basic macro-elements (carbon, nitrogen, phosphorus, potassium, calcium, magnesium) and microelements (iron, zinc, manganese, copper) in dry litter are shown in Table 1. The results indicate that the richest on N, P, K macro-elements was sod of grass, whereas other macro- and microelements it contained less. Rich on macro-elements was also litter of maple, oak and surprisingly also yew. Litter of Japanese cedars was characterized by a high content of calcium and that of cherry laurels had high content of magnesium. Compared to other tree species and grassland, the highest content of microelements as iron, zinc and manganese was found in the litter of yew.

Furthermore, in the litter of trees and grasses was evaluated also relative proportion of carbon and nitrogen (Table 1). Generally, C:N ratio significantly affects the rate of organic matter decomposition. The narrower the ratio is, the faster the organic matter decomposes, because contains more N available to microorganisms (Bottner et al., 2006; Gonet and Markiewicz, 2007; Gonet et al., 2008; Ondrišík, 2013) and sup-

Table 1. Content (means ± SD) of selected elements in litter of dry leaves and needles

Stand	C	N	C/N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
	(g kg^{-1})					(g kg^{-1})				(mg kg^{-1})	
1 grassland	507.00	22.59	22.44	2.58	16.49	3.52	3.10	0.32	0.04	7.04	47.34
	±13.29	±0.46		±0.07	±0.11	±0.10	±0.03	±0.00	±0.00	±0.10	±0.49
2 spruces	558.47	12.33	45.31	1.53	5.47	9.58	1.81	0.24	1.13	3.94	40.44
	±17.48	±0.30		±0.11	±0.06	±0.45	±0.01	±0.01	±0.04	±0.08	±0.32
3 oaks-hornbeams	515.10	14.18	36.33	1.66	8.58	12.00	2.94	0.27	2.34	7.30	31.68
	±9.06	±0.13		±0.08	±0.12	±0.32	±0.06	±0.00	±0.01	±0.55	±0.64
4 maples	521.80	13.23	39.43	2.90	7.75	14.41	4.22	0.21	1.12	9.92	129.65
	±20.14	±0.19		±0.14	±0.03	±0.66	±0.10	±0.00	±0.01	±0.37	±2.60
5 yews	548.45	14.44	37.98	1.53	8.20	15.65	3.07	1.40	2.05	5.35	221.86
	±11.02	±0.06		±0.07	±0.05	±0.14	±0.07	±0.01	±0.05	±0.12	±9.76
6 cherry laurels	507.83	9.95	51.05	0.99	4.51	29.71	4.40	0.70	1.57	4.07	70.06
	±7.72	±0.13		±0.02	±0.03	±2.23	±0.12	±0.01	±0.03	±0.11	±2.30
7 pines	602.36	8.54	70.54	0.93	2.22	11.59	1.54	0.62	0.08	3.82	69.66
	±5.46	±0.06		±0.02	±0.03	±0.25	±0.03	±0.01	±0.00	±0.38	±3.80
8 cedars	564.16	5.48	102.92	0.98	1.70	34.94	4.13	0.64	0.27	3.72	47.57
	±5.79	±0.15		±0.07	±0.01	±1.25	±0.11	±0.00	±0.01	±0.09	±0.87

Table 2. Content (means ± SD) of total carbon and nitrogen, available phosphorus and potassium, exchange calcium and magnesium and potentially available forms of selected elements (Fe, Mn Cu, Zn, Cd, Pb, Ni, Co, Cr) in the 0–10 cm soil layer

Stand	C_T	N_T	C_T/N_T	P	K	Ca^{2+}	Mg^{2+}	Fe	Mn	Cu	Zn	Cd	Pb	Ni	Co	Cr
	(g kg^{-1})									(mg kg^{-1})						
1 grassland	22.47	2.49	9.04	10.33	168.17	2,320	1,568	1,653	243.44	3.68	5.34	0.19	12.71	1.30	3.70	1.79
	±0.59	±0.13		±0.36	±3.61	±33.26	±14.00	±50.12	±12.29	±0.11	±0.08	±0.01	±0.15	±0.07	±0.07	±0.06
2 spruces	22.97	2.07	11.10	13.87	173.33	1,678	404	1,591	119.77	2.90	6.22	0.06	37.61	1.93	2.59	1.60
	±1.01	±0.05		±0.84	±2.08	±23.29	±2.08	±18.73	±4.04	±0.24	±0.18	±0.00	±1.25	±0.08	±0.13	±0.05
3 oaks-hornbeams	30.60	2.01	15.20	60.33	193.33	1,814	1,776	1,831	350.96	4.12	8.96	0.09	17.49	2.62	2.78	1.86
	±0.62	±0.04		±1.73	±3.06	±35.30	±18.19	±48.22	±3.61	±0.26	±0.34	±0.00	±0.30	±0.08	±0.12	±0.09
4 maples	28.70	2.99	9.60	12.00	265.17	2,106	848	1,850	245.38	7.47	9.69	0.22	11.90	4.40	2.84	1.61
	±1.23	±0.12		±0.40	±4.36	±54.34	±12.77	±57.09	±6.56	±0.17	±0.42	±0.01	±0.37	±0.16	±0.10	±0.04
5 yews	21.00	1.94	10.85	53.87	402.83	2,214	1,392	1,980	448.16	6.28	157.01	0.48	16.08	4.08	3.32	1.29
	±0.95	±0.08		±1.36	±8.08	±13.11	±11.02	±22.54	±9.17	±0.41	±7.31	±0.01	±0.37	±0.23	±0.08	±0.03
6 cherry laurels	29.70	2.09	14.23	10.00	97.87	2,000	992	1,395	510.33	5.78	8.44	0.24	13.40	5.26	5.88	1.68
	±1.37	±0.11		±0.59	±0.58	±39.89	±9.61	±35.17	±9.50	±0.44	±0.23	±0.01	±0.25	±0.16	±0.10	±0.06
7 pines	26.40	1.66	15.88	14.00	188.00	3,494	1,456	1,810	345.84	7.62	9.34	0.31	12.40	5.60	6.28	3.12
	±1.06	±0.03		±0.87	±2.08	±38.68	±10.54	±56.96	±8.54	±0.13	±0.36	±0.01	±0.14	±0.10	±0.12	±0.14
8 cedars	24.33	2.36	10.32	13.83	314.83	2,906	1,184	1,215	170.15	6.92	9.02	0.16	12.20	2.68	3.50	1.69
	±0.75	±0.12		±0.59	±5.57	±10.58	±17.09	±18.52	±8.72	±0.36	±0.21	±0.01	±0.18	±0.12	±0.11	±0.06

ports the microbial activity (Pengthamkeerati et al., 2011). Faster decomposition of organic matter means faster nutrient cycling in the environment (Sariyildiz et al., 2005). Among studied stands in Arboretum, the narrowest C : N ratio was determined in the sod of grassland (22.4 : 1), then in the litter of oak, yew, maple, spruce, cherry laurels, Himalayan pine and litter from Japanese cedars which overall had the widest C : N ratio (102.9 : 1). The reason of the wide C : N ratio was the fact that Japanese cedar does not throw down individual needles, but gradually, after drying, breaks off small twigs with needles. Therefore, under Japanese cedars the litter contained more twigs than other stands.

Moore et al. (2006) stated that C : N ratio, when there begins the release of nitrogen from litter usually refers to a certain "critical" C : N (25–30), respectively to initial concentration of N in litter 20 g kg^{-1} as threshold for net accumulation or net release of nitrogen. Similar results were reached by Vahdat et al. (2011) who moreover considered also the quality of C and N constituents of litter. They found that the critical levels of N concentration, C : N ratio, lignin and lignin:N ratio of the plant residues at which neither N mineralization nor immobilization would occur were 10.8 g kg^{-1}, 30.7, 253.5 g kg^{-1} and 17.0, respectively. In a multiple model, lignin concentration of the plant residue was the most important factor for predication the net effects of plant residue on soil mineral N dynamics. Generally, lignin physically protects most of the cellulose and hemicellulose from enzymatic hydrolysis. Also Jonczak (2009) confirmed that the different rate of poplar leaves decomposition depended on different chemical composition of the initial material, which depended on age of poplars. Lignin content correlated with the age of poplars, and its proportion decreased with the age of trees.

According to Vogt et al. (1986) about 70–90% of nutrients annually needed for forest growth are provided by decomposition of organic detritus. Therefore, we wanted to determine whether the concentration of total nitrogen, available macro-elements (P, K) and exchange cations (Ca^{2+}, Mg^{2+}) in the surface soil layer in Arboretum Mlyňany was influenced by the contents of these elements in litter. In the past, the soil in the Arboretum was enriched by nutrients released from manure which was regularly supplied to the soil. Whereas since 1965 fertilizers have not been used, it is evident that the majority of mentioned macro-nutrients (N, P, K, Ca, Mg) comes from decomposed litter. Obtained results showed that between the content of available macro-nutrients in the surface soil layer (0–10 cm) and their content in dry litter was not found any statistically significant correlation (N: $r = 0.269$, $P > 0.05$; P: $r = –0.062$, $P > 0.05$; K: r = –0.080, $P > 0.05$; Ca: $r = 0.179$, $P > 0.05$; Mg: $r = –0.046$; $P > 0.05$). The reason could be different rate of nutrients release from the studied litters. For instance, Blair (1988) found that the sequence of release five examined elements was K > Mg > Ca > P > N, whereas Liao et al. (2006) found the sequence: C > Ca > N > K > Mg > Na > P, and stated, that the above sequence of nutrients returned from leaf litter differed from the quantity sequence of these nutrients in the soils indicating that the nutrient status of the forest floor was also impacted by atmospheric deposition, throughfall, stemflow, microbial activities, runoff, erosion, leaching processes and other edaphic factors in the study area. On the base of this statement we conclude that the macro-elements content in the 0–10 cm soil layer of Arboretum Mlyňany in addition to chemical composition of litter was also influenced by other biotic and abiotic conditions, therefore the correlation between macro-elements in the litter and in 0–10 cm soil layer was not found.

Higher quality of grass litter was evident not only from their narrowest C : N ratio, but also from the narrowest ratio of total carbon (C_T) and nitrogen (N_T) in the surface soil layer (0–10 cm) as compared with soil under canopy of tree species (Tables 1–2). Obtained results showed, that differences in $C_T : N_T$ ratios in the soils between studied stands were not so marked as differences in C : N ratios between investigated litters. The same was confirmed by Šimková et al. (2014) who studied the accumulation of C_T and N_T in surface humus and mineral soil layers after the change of tree species composition in nudal beech forests.

Since, in addition to macro-elements, from the decomposed litter are released to the soil also micro-elements we examined, whether the amount of available micro-elements copper, zinc, manganese and iron in the surface soil layer in Arboretum was influenced by the contents of these elements in litter (Tables 1–2). From the results it follows, that statistically significant correlations were found between the amount of zinc, manganese in litter and their available ions in the soil (Zn: $r = 0.884$; $P < 0.001$; Mn: $r = 0.501$, $P < 0.05$), while for copper and iron such correlation was not found (Cu: $r = 0.030$; Fe: $r = 0.160$, $P > 0.05$).

Under studied canopy of tree species and grassland, in the upper soil layer (0–10 cm) was also investigated the content of available heavy metals (Table 2). It was found that the limit values according to *Decree of MA SR 531/1994/540* (1994) for cadmium (0.3 mg kg^{-1}) and zinc (40 mg kg^{-1}) have been exceeded for yews, Cd for Himalayan pines and lead (30 mg kg^{-1}) for spruces. In other stands, the limit values were not exceeded despite the fact that in the soil prevailed acidic pH when heavy metals are mostly in available forms (Kabata-Pendias and Pendias, 1984; Čurlík, 2011).

Actually, the limit values for Cd were exceeded in two stands (Table 2). Forest soils well accumulate cadmium, which in turn negatively affects the growth of trees and reduces their dry matter weight (Tomáš et al., 2007). Cadmium belongs to the most toxic heavy metals and has a high mobility in the soil-plant system (Čurlík, 2011). Zinc is an important microelement for plant physiology and its deficiency causes growth problems. On the other hand, in acidic soils Zn can manifest increased availability and even toxicity (Alloway and Ayres, 1997).

Considering available lead, Novák et al. (2010) stated that the heartwood of spruce had a high permeability, moisture contents and number of rings in sapwood, which could be subjected as geochemical archive of pollution. Also, results of our research in Arboretum confirmed that spruces considerably absorb Pb. The content of available Pb was in 0–10 cm soil layer under spruces on average up to three times higher compared with other tree species (Table 2).

Higher contents of available Cd, Zn and Pb in soil under certain trees in Arboretum Mlyňany were attributed to bioaccumulation (uptake by root system from deeper soil layers and their accumulation from decomposed litter), as well as by atmospheric deposition. It is known that crowns of trees have great filtration capacity and large surface area of their vegetation bodies (10 or more ha per 1 ha of forest area) through which they capture and eliminate considerable quantities of air pollutants (Bublinec, 2000). Thus the trees substantially contribute to air cleaning and recovery, therefore, it is necessary to protect and thoroughly take care of them.

Conclusions

The correlation between the content of macro-elements (N, P, K, Ca, Mg) in the litter and in 0–10 cm soil layer of Arboretum Mlyňany was not found, because the macro-elements content in soil is generally influenced not only by chemical composition of litter but also by other biotic and abiotic conditions.

Contrariwise, the correlations of microelements (Zn, Mn) contents in litter and their available ions in soil were statistically significant.

In the soil under yews, Himalayan pines, and spruces there were exceeded limit contents of Cd, Zn and Pb, which indicates higher bioaccumulation ability as well as filtration of atmospheric deposition by mentioned trees compared to other studied tree species.

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Vegetation dynamics of herb layer in managed submountain beech forest

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Abstract

Schieber, B., Kubov, M., Pavelka, M., Janík, R., 2015. Vegetation dynamics of herb layer in managed submountain beech forest. *Folia Oecologica*, 42: 35–45.

Dynamics of herb layer within two communities in managed submountain beech forest was studied. Sampling was done on three differently managed forest plots over the period of 16 years (1995–2011). The first plot was situated in an original 110-year-old beech stand (control), the second was covered by a thicket (in 2011 – aged ca 23 years) developed after clear-cut. At the third plot, several interventions were performed along with a gradual reduction of the original stand density, and finally the parent stand was completely felled. Results revealed conspicuous changes in community structure, especially in species richness and composition. Also, changes in population dynamics of two dominant herb species were observed, mainly on the managed plots. Using Shannon-Wiener′s index we ranked the communities to the category with low--diversity, however increasing diversity was denoted on the area with complete removal of the tree layer. Bioindication using the Ellenberg’s indicator values for selected ecological factors (temperature, light, soil moisture and nitrogen content) was used to find a reflection of the human-caused environmental changes on herb layer structure in a beech forest. Mean values for the both light and nitrogen content were changed significantly only on the managed plots, while no biologically significant changes in mean values for the both temperature and soil moisture were found.

Keywords

beech stand, bioindication, *Carici pilosae-Fagetum*, density, *Dentario bulbiferae-Fagetum*, species richness

Introduction

Plant communities of beech stands are considered rather poor in the diversity of their herb component. However, also species rich communities of beech forests are reported (Ujházyová and Ujházy, 2007). Hrivnák et al. (2013) described inversely unimodal species richness-altitude relationship in Central European beech forests. The diversity of herb layer depends on multiple factors and has not been explained sufficiently yet. Ground vegetation development depends on light supply (Davis et al., 1998; Härdtle et al., 2003; Diaci et al., 2012). Slowly degradable leaf litter represents barrier for plant germination and growth (Kooijman, 2010). Also water uptake in upper soil layers is another limiting factor which can be affected by strong suction of beech roots (Gálhidy et al., 2006). Typical example of negative interactions among beech and understorey vegetation are “nudal” beech dominated forests classified as *Fagetum pauper* group of forest types in Slovak typological system (Hančinský, 1972) or *Dentario bulbiferae-Fagetum* association (Jarolímek and Šibík, 2008), respectively. They are characterised by poorly developed herbal synusia with both low cover and low number of herb species. Partial or complete removal of the dominant trees from the forest ecosystem results in an increase of litter decomposition and topsoil nitrification rate (Purahong et al., 2014). Increased availability of multiple resources is associated with increasing species diversity and productivity of herb layer in general. As a consequence, seasonal dynamics of the communities are modified (Schieber, 1996, 2007; Graae and Heskjaer, 1997; Pykälä, 2004; Godefroid et al., 2005). Subsequently, restoration of the communities may require a relatively long time (Bossuyt and Hermy, 2000; Łaska, 2000).

The aim was to examine the response of herb understorey to the changed ecological conditions in managed beech forest and to identify the temporal changes in selected ecological factors through the bioindication.

Material and methods

The research was done at the Beech Ecological Experimental Site (BEES) localized in the SE part of the Kremnické vrchy Mts (48°38´N, 19°04´E, 450–520 m asl). Site is located on a west-southwest oriented slope with an inclination of 5–15°. The area belongs to a moderately warm region, moderately warm and humid, in a hilly sub-region (Lapin et al., 2002). The annual mean air temperature was increased from 6.8 °C$_{(1951\text{–}1980)}$ to 7.5 °C$_{(1994\text{–}2013)}$ and the mean annual rainfall is 780 mm. The mean January and July air temperatures are –3.5 °C and +18.0 °C, respectively for the period of 1994–2013 (unpublished). More than 55% of the annual rainfall occurs between April and September. The soils are skeletal cambisols with a moderate acidic reaction and skeletal cover ranging from 10 to 60% (Kukla et al., 1998).

Investigations were carried out in three different plots of the BEES area (Fig. 1):

- K – control plot representing a 115-year-old beech stand with stocking density of 1.0;
- H – formerly a clear-cut area until intervention in 1989. By 2011 this plot had a 23-year-old thicket.
- M – managed; original stocking degree of the parent stand was decreased from 0.9 to 0.7 in 1989. Subsequently stocking degree was reduced to 0.5 in spring 2004 and finally the rest of a parent stand was completely felled in spring 2009.

Dominant tree species in the plot K is *Fagus sylvatica*, and associated species include *Abies alba*, *Quercus dalechampii*, *Carpinus betulus* and *Tilia cordata*. In addition to these, naturally regenerated thicket on the plot H is supplemented with *Salix caprea*, *Populus tremula*, *Betula pendula*, *Acer pseudoplatanus*, *Cerasus avium* and *Picea abies* species. Plant communities of the study area are classified as *Carici pilosae-Fagetum* and *Dentario bulbiferae-Fagetum* associations (Kontriš et al., 1995). More detailed information concerning BEES is in Kontriš et al. (1993a, b), Križová (1993), Kodrík (1997), Kuklová et al. (2005), Kellerová and Janík (2006), Janík (2010), Barna and Schieber (2011) and Janík et al. (2011).

Two subplots of the 400 m^2 area were established on each of three plots. The first subplot represented the association of *Carici pilosae-Fagetum* (*CpF*), the second *Dentario bulbiferae-Fagetum* (*DbF*). Herb species in the understorey were sampled in 1995, and repeatedly in 2008 and 2011 in three pairs of subplots (within K, H, M plots and two associations).

Species richness was evaluated for herb layer excluding tree-species juveniles. Braun-Blanquet combined scale modified by Zlatník (1953) was used to determine the species cover within the herb layer. Shannon-Wiener′s index was applied to evaluate the changes in species diversity. Floristic similarity of the communities was expressed by Sørensen’s index. Communities were considered to be similar if the index was

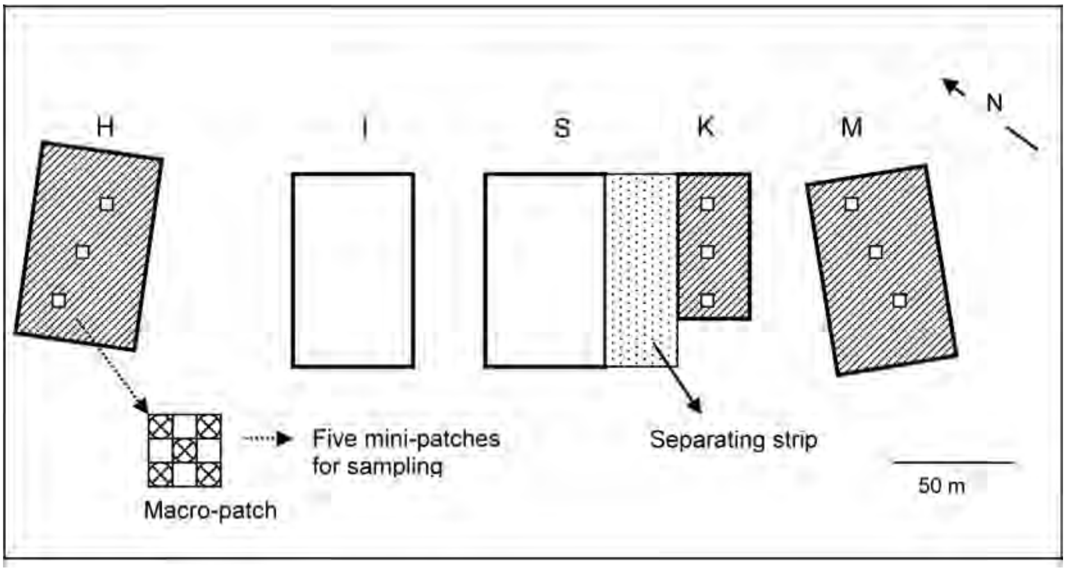

Fig.1. Scheme of sampling in the plots (H, I, S, K, M) of BEES.

equal or higher than 70% (Moravec, 2000). Temporal changes in selected ecological factors (light, temperature, soil humidity and nitrogen content) were analyzed by bioindication method based on eco-analysis using Ellenberg′s indicator values – EIVs (Ellenberg et al., 1992). Ecological analysis was processed manually and calculations were done in regard to percentage cover (dominance) of the species. The difference > 0.5 between two mean values was considered as an ecologically significant change of the factor (Križová and Nič, 2000). Considering that conditions at the whole space on each of study plots were relative homogeneous, the both communities within each plot were merged together as a whole for bioindication analysis. The presence of generative phenophases within all species was also observed to express the vitality of the species (Bottlíková, 1975). The dynamics of population density for two dominant species was analysed at the time of its optimal vegetative development – in May and in July for *Dentaria bulbifera* and *Carex pilosa*, respectively. Density of fertile and sterile shoots (ramets) was sampled on 15 mini-patches with a dimension of 1m^2, which were placed within three macro-patches (9 m^2). Macro-patches were localised along the line transect within each subplot (Fig. 1). Temporal changes in the density within the plots were tested by Fisher’s LSD test (Statistica® program). Measurements of light conditions were done by Luxmeter PU 150 (Metra Blansko) at 7 different points within each studied plot of BEES during anti-cyclonal, sunny conditions in June 1995 and the following measurements were performed repetitively in June 2008 and 2011. Nomenclature for plants and for plant communities followed Marhold and Hindák (1998) and Jarolímek and Šibík (2008), respectively.

Results

Changes in species richness and composition

Changes in species composition of the herb layer within three plots managed by different way during the period of 16 years are summarised in Tables 1–3. Species richness on control plot K with completely closed canopy was relative stable and varied from 12 to 17 taxa. The number of species with cover > 1% was 12 in 1995, but only three species were present in 2011. Conspicuous change on this plot was observed in *Ajuga reptans* and *Veronica officinalis*, within 16 years these taxa had disappeared. Also *Lathyrus vernus* suffered a considerable decrease in its cover. However, some species were absent in at least one association, such as *Fragaria vesca*, *Pulmonaria officinalis* and *Rubus hirtus*. In another plot H with dense young tree stand, 29 taxa were recorded in both associations in 1995, but a 55% decrease to 13 taxa was noted after 16 years. The number of taxa with dominance exceeding 1% on this plot was 16 in 1995, but only 3 in 2011. The largest changes were observed in the *Calamagrostis epigejos*, *Atropa belladona*, *Eupatorium cannabinum* and *Rubus idaeus* species. Their cover in 1995 reached of 5–50%, whereas in 2011 were not present. A considerable drop in dominance or absolute absence was also recorded in the *Ajuga reptans*, *Fragaria vesca*, *Chamerion angustifolium*, *Senecio fuchsii*, *Tussilago farfara*, *Veronica officinalis* and *Viola reichenbachiana* species. On the third plot M, influenced by three cutting operations in 1989, 2005 and in 2009 years, there was found out the opposite trend. The species diversity gradually increased in time. While a total number of taxa was 22 in 1995, increasing to 68 species was detected by 2011. The most remarkable increase in dominance was found in the *Cirsium arvense*, *Carduus nutans* and *Rubus hirtus* species. Spatio-temporal analysis of diversity, expressed by Shannon-Wiener index, showed increasing values on plot M, especially in *DbF*, but the opposite trend was observed on plot H with young thicket. There was found the decrease in values of the index for the both communities. Diversity on the control plot K was relative stable. The values of Shannon-Wiener index were less than 2.5 for all communities on the studied plots, so we can classify them as a low-diversity communities. Only *DbF* community on plot M was the exception with the index of 2.8, which may be ranked to category of medium-diversity (Fig. 2). Floristic similarity of studied communities is expressed by Sørensen’s index (Table 4). The highest values of the index were detected between the communities on control plot K (68.9–72.7%). On the other hand the lowest values were found within the plot M (32.8–41.5). Comparison between the plots showed, that the index of similarity reached the highest values of 77.8% and 63.6% (between K and M) within the both *CpF* and *DbF* communities, respectively. This similarity was found at the beginning of the study period in 1995. Also, high index of similarity (69.6%) was calculated between K and H at the end of the study period in 2011. Contrary to this, the lowest values of index were detected between K and M at the end of the study period (Table 4).

Population dynamics of the dominant species

The presence of generative phases within life cycle of a taxon reflects its good vitality and vice versa. Accordingly, generative phenophases were observed in 81% of the taxa in both associations on plot K in 1995. By 2011, this was noted in 53%, accounting for 9 species including two ferns. Decrease in number of taxa with generative phases from 29 recorded in 1995 to 4 species in 2011 on the second plot H was found. Compared to these plots, the opposite temporal trend in presence of generative phases was observed on the third plot M.

Table 1. Changes in species composition and dominance of herb layer in *Dentario bulbiferae-Fagetum* (*DbF*) and *Carici pilosae-Fagetum* (*CpF*) communities on control plot K; (a) – absence of generative phenophases

Years	1995		2008		2011	
Phytocoenoses	*DbF*	*CpF*	*DbF*	*CpF*	*DbF*	*CpF*
Herb layer						
Ajuga reptans	1 (a)	1 (a)	.	.	.	.
Athyrium filix-femina	.	+	.	r	+	+
Brachypodium sylvaticum	.	.	.	.	.	r
Carex digitata	.	.	.	.	+	.
Carex pilosa	.	4	.	3	.	-2
Cephalanthera longifolia	.	.	r (a)	.	r(a)	+(a)
Dentaria bulbifera	2	2	-2	-2	1	1
Dryopteris filix-mas	+	+	.	r	+	+
Fragaria vesca	1	1	.	+ (a)	r(a)	+(a)
Galium odoratum	2	1	+	1	1	1
Geranium robertianum	r (a)	.	.	.	.	.
Geum urbanum	.	r	.	.	.	.
Lathyrus vernus	.	2	.	r (a)	.	+
Melittis melissophyllum	.	.	.	.	.	r(a)
Oxalis acetosella	.	1	.	+	+	+
Pulmonaria officinalis	1	1	.	+ (a)	+(a)	.
Rubus hirtus	1	2 (a)	.	+(a)	+(a)	+(a)
Sanicula europaea	2	1	.	.	+(a)	.
Symphytum tuberosum	.	.	.	.	.	+(a)
Veronica officinalis	.	1	.	.	.	.
Viola reichenbachiana	1	2	.	+ (a)	+(a)	+(a)
Total number of species	16		12		17	

Table 2. Changes in species composition and dominance of herb layer in *Dentario bulbiferae-Fagetum* (*DbF*) and *Carici pilosae-Fagetum* (*CpF*) communities on plot H – thicket; (a) – absence of generative phenophases

Years	1995		2008		2011	
Phytocoenoses	*DbF*	*CpF*	*DbF*	*CpF*	*DbF*	*CpF*
Herb layer						
Ajuga reptans	1	+	.	.	.	.
Atropa belladona	2	+	.	.	.	.
Calamagrostis epigejos	3	.	.	.	.	.
Campanula persicifolia	.	+	.	.	.	.
Carduus acanthoides	.	+	.	.	.	.
Carex digitata	1	+	+	.	+	+
Carex pilosa	.	4	.	3 (a)	.	-2(a)
Carex sylvatica	+	.	+	.	+	+
Cephalanthera longifolia	.	.	r (a)	.	r(a)	.
Cirsium arvense	.	+	.	.	.	.
Coronilla varia	+	.	.	.	.	.
Dentaria bulbifera	2	2	-2	1	1	1
Epilobium montanum	+	.	.	.	.	.
Eupatorium cannabinum	2	.	.	.	.	.
Fragaria vesca	1	+	.	.	.	.
Galium odoratum	1	+	+	.	+	.
Geranium robertianum	.	.	r (a)	r (a)	r(a)	.
Glechoma hederacea	+	.	.	.	.	.
Hypericum hirsutum	+	+	.	.	.	.
Chamerion angustifolium	1	.	.	.	.	.
Juncus effusus	r	.	.	.	.	.

Table 2. Changes in species composition and dominance of herb layer in *Dentario bulbiferae-Fagetum* (*DbF*) and *Carici pilosae-Fagetum* (*CpF*) communities on plot H – thicket; (a) – absence of generative phenophases – continued

Years	1995		2008		2011	
Phytocoenoses	*DbF*	*CpF*	*DbF*	*CpF*	*DbF*	*CpF*
Herb layer						
Platanthera bifolia	.	.	.	.	.	+(a)
Pulmonaria officinalis	+	+	+ (a)	.	+(a)	.
Rubus hirtus	1	+	1 (a)	+ (a)	+(a)	1(a)
Rubus idaeus	2	.	.	.	.	.
Sanicula europaea	+	.	.	.	.	.
Scrophularia nodosa	+	.	.	.	.	.
Senecio fuchsii	.	1	.	.	.	.
Tithymalus amygdaloides	.	+	+ (a)	.	r(a)	.
Tussilago farfara	.	1	.	.	.	.
Veronica officinalis	1	1	+ (a)	.	r(a)	.
Viola reichenbachiana	1	1	+ (a)	.	+(a)	r(a)
Total number of species	29		12		13	

Table 3. Changes in species composition and dominance of herb layer in *Dentario bulbiferae-Fagetum* (*DbF*) and *Carici pilosae-Fagetum* (*CpF*) communities on plot M; (a) – absence of generative phenophases

Years	1995		2008		2011	
Phytocoenoses	*DbF*	*CpF*	*DbF*	*CpF*	*DbF*	*CpF*
Herb layer						
Ajuga reptans	1 (a)	1 (a)	+	.	.	+
Alliaria petiolata	.	.	.	.	+	.
Asarum europaeum	.	.	.	.	+	.
Astragalus glycyphyllos	.	.	.	.	+	1
Athyrium filix-femina	.	r	+	+	+	.
Atropa belladona	.	.	+	1	+	1
Brachypodium sylvaticum	.	+	.	r	1	.
Calamagrostis epigejos	.	.	.	.	+	.
Campanula trachelium	.	.	.	.	+	.
Cardamine pratensis	.	.	.	.	1	.
Carduus acanthoides	.	.	.	.	1	+
Carex digitata	.	.	+	.	+	.
Carex echinata	.	.	.	.	.	+
Carex pilosa	.	4	.	+4	.	4
Carex sylvatica	.	.	+	.	+	.
Cephalanthera longifolia	.	.	+	.	.	.
Cirsium arvense	.	.	.	r	3	1
Cirsium oleraceum	.	.	.	.	1	.
Cirsium vulgare	.	.	.	.	-2	.
Coronilla varia	.	.	.	.	+	.
Dentaria bulbifera	2	2	+2	2	2	2
Dryopteris filix-mas	.	+	.	+	+	.
Epilobium montanum	.	.	r	r	+	.
Eupatorium cannabinum	.	.	r	.	+	+
Fragaria vesca	1(a)	1(a)	1	.	2	r
Galium aparine	.	.	.	.	.	r
Galium odoratum	2	1	+2	-2	1	2
Geranium robertianum	+(a)	+(a)	1	+	1	1
Geum urbanum	.	r(a)	.	.	.	.

Table 3. Changes in species composition and dominance of herb layer in *Dentario bulbiferae-Fagetum* (*DbF*) and *Carici pilosae-Fagetum* (*CpF*) communities on plot M; (a) – absence of generative phenophases – continued

Years	1995		2008		2011	
Phytocoenoses	*DbF*	*CpF*	*DbF*	*CpF*	*DbF*	*CpF*
Herb layer						
Glechoma hederacea	.	r(a)	.	r	.	.
Hieracium sp.	.	.	.	.	r	.
Hypericum hirsutum	.	.	+	.	+	+
Hypericum perforatum	.	.	.	.	.	+
Chamerion angustifolium	.	.	r	.	.	.
Lactuca serriola	.	.	.	.	+	.
Lathyrus vernus	+	+	.	r	+	.
Leontodon sp.	.	.	.	.	+	r
Lysimachia vulgaris	.	.	.	.	+	.
Lysimachia nummularia	.	.	.	.	+	.
Melica nutans	.	.	.	.	+	.
Melica uniflora	.	.	.	.	+	.
Mycelis muralis	.	r	1	.	+	+
Myosotis sylvatica	+	.	.	.	1	+
Oxalis acetosella	.	+	.	.	.	.
Petasites albus	.	+	.	.	.	.
Plantago sp.	.	.	+	.	.	+
Platanthera bifolia	.	.	.	r	.	r
Prunella vulgaris	.	.	r	.	.	+
Pulmonaria mollis	.	.	.	.	.	+
Pulmonaria officinalis	.	+	.	+	.	+
Ranunculus lanuginosus	.	.	.	.	+	.
Rosa sp.	.	.	r	.	.	.
Rubus hirtus	+	+	+	1	+2	1
Rubus idaeus	.	.	+	+	1	+
Salvia glutinosa	.	.	.	.	.	+
Sanicula europaea	+	+	r	.	.	.
Scrophularia nodosa	.	.	+	+	+	+
Senecio fuchsii	.	.	.	.	+	.
Senecio viscosus	.	.	.	.	+	.
Senecio vulgaris	.	.	.	.	+	.
Stellaria nodosa	.	.	.	.	+	.
Symphytum officinale	.	.	.	.	r	+
Symphytum tuberosum	.	.	.	.	+	.
Stachys sylvatica	.	.	.	.	+	.
Stenactis annua	.	.	.	.	r	.
Taraxacum officinale	.	.	.	.	.	+
Tithymalus amygdaloides	+(a)	+(a)	+	+	+	.
Tithymalus cyparissias	.	.	.	.	1	.
Tragopogon sp.	.	.	.	.	r	.
Urtica dioica	.	.	.	.	+	.
Veronica chamaedrys	.	.	.	.	+	.
Veronica officinalis	1(a)	1(a)	+	.	+	.
Vicia cracca	.	.	+	.	1	+
Viola reichenbachiana	1	2	+	+	r	+
Vignea muricata	.	.	.	.	+	.
Verbascum thapsiforme	.	.	.	.	.	+
Total number of species	22		34		68	

While a relative rate of taxa with generative phases was 68% in 1995, increase to 100% species was detected by 2011.

Figure 3 illustrates variability and differences in density of fertile and sterile shoots in two dominant taxa within the herb layer of the communities during the period of 16 years. No statistically significant changes in density of sterile and fertile shoots of *Carex pilosa* were found on control plot K. On the other hand, significant changes in density were found on the other two plots M

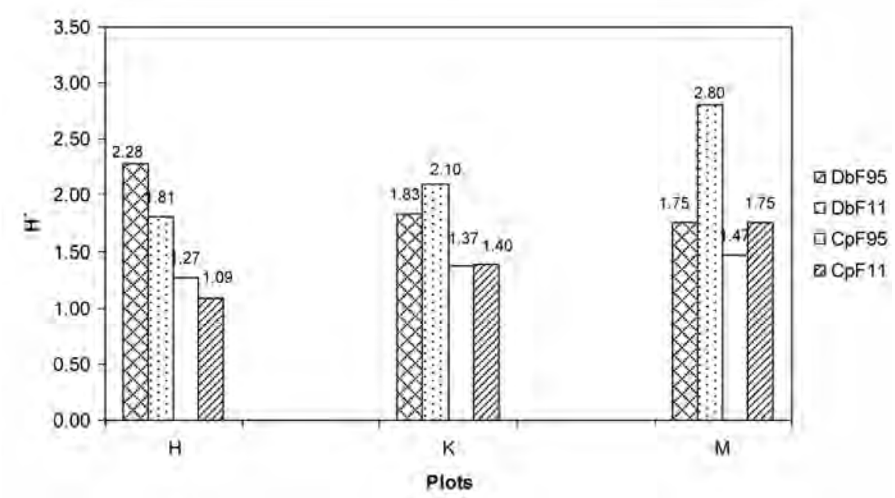

Fig. 2. Shannon-Wiener′s index of diversity within the herb layer of two communities on the studied plots.

Table 4. Sørensen's index of similarity of the communities calculated within the plots and between the pairs of relevés between 1995 and 2011 (values in bold italic denote high similarity > 70 %)

Plots Communities	Sørensen‘s index (%)
Plot H	
DbF_{95}–DbF_{11}	51.6
CpF_{95}–CpF_{11}	40.0
Plot K	
DbF_{95}–DbF_{11}	**72.7**
CpF_{95}–CpF_{11}	68.9
Plot M	
DbF_{95}–DbF_{11}	32.8
CpF_{95} –CpF_{11}	41.5
Plot K–H	
DbF_{95}–DbF_{95}	43.7
DbF_{11}–DbF_{11}	69.5
CpF_{95}–CpF_{95}	54.5
CpF_{11}–CpF_{11}	38.1
Plot K–M	
DbF_{95}–DbF_{95}	63.6
DbF_{11}–DbF_{11}	20.9
CpF_{95}–CpF_{95}	**77.8**
CpF_{11}–CpF_{11}	26.1
Plot M–H	
DbF_{95}–DbF_{95}	47.1
DbF_{11}– DbF_{11}	27.3
CpF_{95}–CpF_{95}	51.3
CpF_{11}–CpF_{11}	30.8

and H for the both sterile and fertile shoots. Similarly as was in the case of *Carex pilosa* species, the density of the both sterile and fertile shoots of *Dentaria bulbifera* species showed no statistically significant temporal changes on plot K. Significant changes in density of sterile shoots were detected on the plots M and H. The density of fertile shoots was changed significantly only on plot M during the study period.

Bioindication analysis

The spatio-temporal changes in selected ecological factors on the plots are shown in Fig. 4. Ellenberg′s indicator values (EIVs) analysis revealed that the both light and nitrogen content were changed significantly on two managed plots (plots H and M), the differences in average values were higher than 0.5. Mean EIVs for light on plot K at the end of the study period changed by 0.45, which was not ecologically significant. The value of 3.64 in 2011 enabled us to conclude that the understory vegetation (herb layer) of the analyzed communities was shade-tolerant or moderately shade-tolerant. Plot H, with the thicket, exhibited more pronounced changes in ecological conditions. In 1995, the communities on this plot were created by hemi-sciophytes and also hemi-heliophytes. However, sixteen years later, the relative presence and dominance of hemi-heliophytes rapidly decreased. Mean EIVs on plot H was reduced by 1.31, and this indicates an ecological change. The character of herb layer on this plot was hemi-sciophytic or hemi-heliophytic in 1995, but only sciophytes or hemi-sciophytes were recorded in 2011. On the other hand, light conditions were gradually improved on the third plot M. Mean EIVs on this plot increased by 1.38 between 1995 and 2011. In 1995 the communities on this plot were created by sciophytes or hemisciophytes, but hemi-sciophytes or hemi-heliophytes were observed in

2011. Nitrogen content was another factor which was changed in time, changes were not as high as it was in the case of light conditions. Nitrogen content mean value, indicated by phytocoenoses, had decreasing trend between 1995 and 2011 on plot H, but it was the opposite on plot M. Mean EIVs vary from 4.95–5.21 on plot K to 5.29–5.96 on plot M. The ecotopes characterised by a moderately rich soils were detected on plots as follows: on plot K during the whole study period, on plot M in 1995 and also on plot H in 2008–2011. Moderately rich soils or rich soils were indicated on plot H in 1995 and also on plot M in 2008–2011. Two other factors soil moisture and temperature were not changed among all studied plots in time, the differences in mean EIVs for these factors were lower than 0.5. Mean values for these two factors showed that the ecotopes of all studied plots can be characterised as a moderately warm and mesic.

Discussion and conclusion

Environmental conditions were changed after human impact. It led to changes in structure of the herb layer of plant communities on the studied plots. The species richness on plot K (control) was relatively stable during the study period. However, moderate changes in communities were also recorded on this plot caused by side-light penetrating through clear-cut strips separating the partial plots, thereby influencing part of the almost fully-shaded plot K (Fig. 1). By 2011, separating strip had grown by natural regeneration of trees and shrubs resulting in considerable decrease in side-light intensity. Evident increase in species richness was observed on managed plot M, where the number of taxa increased more than three times. Improved light conditions and increasing rate of decomposition and nitrification processes after removing of the parent stand on this plot in 2004 and 2009 accelerated the development of natural regeneration of trees. Herb layer was also synchronously supported. Statistically increasing density of sterile and fertile shoots of dominant *Carex pilosa* species was found on this plot. On the other hand, the density of sterile shoots of *Dentaria bulbifera* species decreased in time. It may be caused by increasing cover of fast-growing and high biomass-producing species in *DbF* on this plot, mainly in the representatives of nitratophiles and heliophytes (genus *Carduus*, *Cirsium*, *Rubus* and *Fragaria vesca*), which gradually occupied free niches. These conditions seem to be similar to those, which were reported from the forest gaps (HULL, 2002; RITTER et al., 2005). Similarly KRIŽOVÁ (1993) reported, that the average value of the above-ground biomass of *CpF* association has increased several times after clear-cutting on BEES. Also COUWENBERGHE et al. (2011) found that distribution of 12 common herbal forest species was affected by stand structure (canopy openness) through the light gradient. MCLACHLAN and BAZELY (2001) reported that no significant differences were detected in the diversity of native forest understory species between restored and undisturbed sites, however significant among-site variation was found.

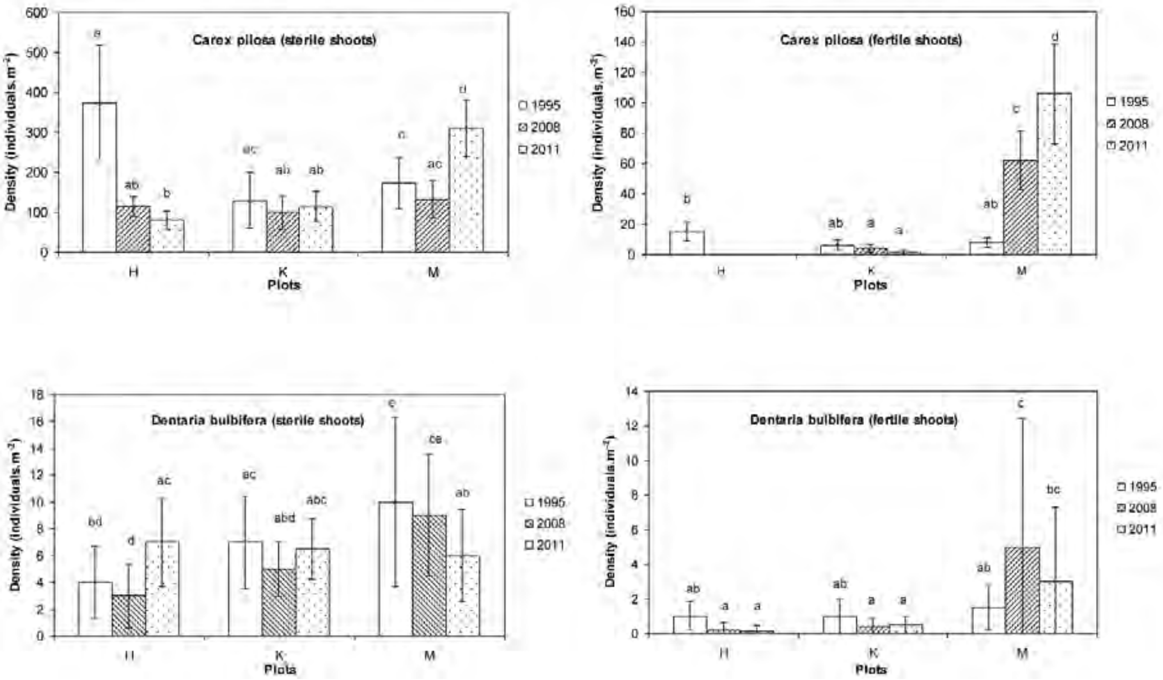

Fig. 3. Changes in density of sterile (ster) and fertile (fert) individuals within two dominant species on differently managed plots (means ± SD and results of Fisher´s LSD test; $n = 15$, $P \leq 0.05$).

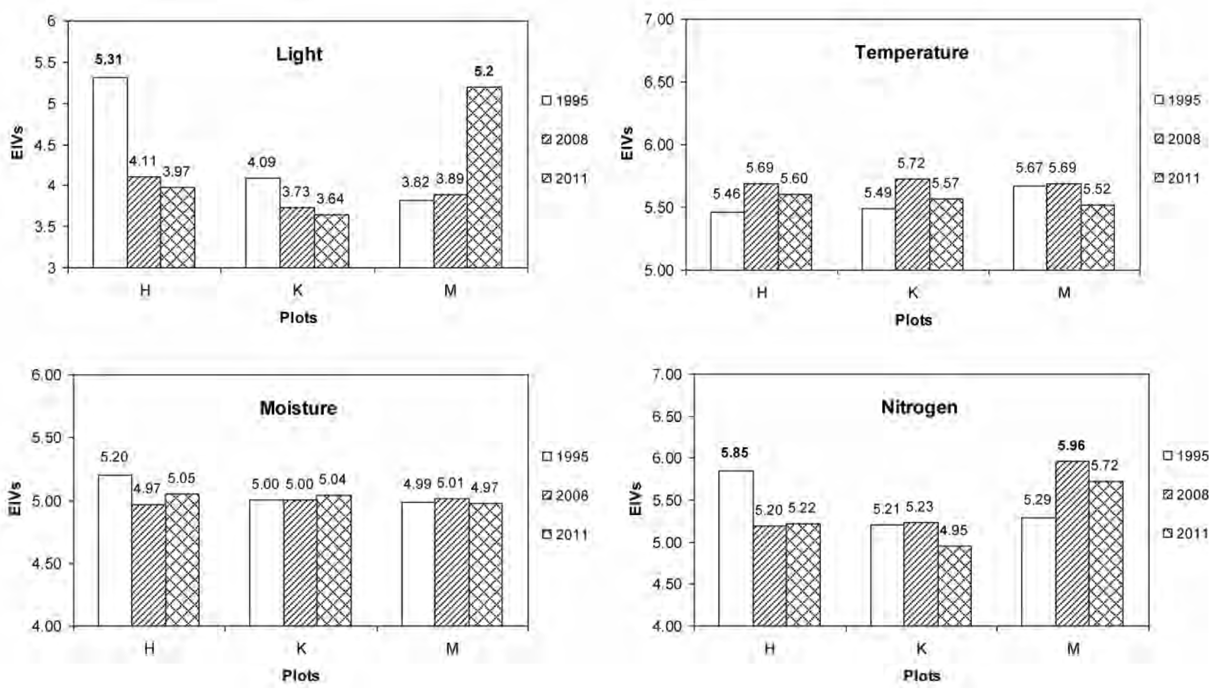

Fig. 4. Temporal changes in mean Ellenberg indicator values (EIVs) for selected ecological factors within the studied plots (values in bold denote significant difference).

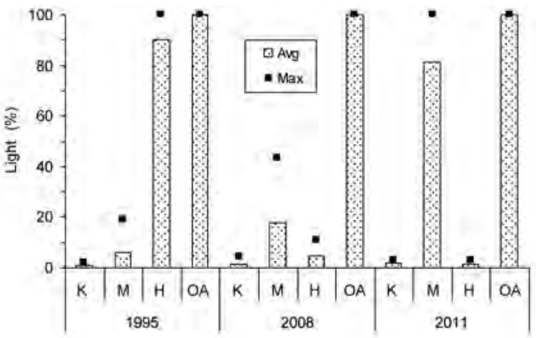

Fig. 5. Average (Avg) and maximum (Max) values of light on the studied plots K, M, and H compared to the open area (OA) measured by luxmeter.

The value of Shannon-Wiener′s index of diversity confirms increasing diversity on this plot for *DbF*, but not for *CpF* community. It seems to be that high density of *Carex pilosa* caused the weakening the competition ability of the other species. Sørensen’s index showed the low similarity between original communities in 1995 and those in 2011 on this plot. While the total number of taxa on the third plot H (thicket) at the beginning of the study period in 1995 was the highest, compared to the other plots K and M, subsequently the species diversity on this plot was rapidly decreased by 2011. The total removal of the tree layer in 1989 opened growth space for vigorously developing natural tree regeneration (Kodrík, 1997). This led to the 23-year old thicket fully covering plot H by 2011 and the light availability to the herb layer became more limited. The values of accessible light measured directly in a space of communities on the plots indicate a conspicuous reduction (Fig. 5). The competition capacity within this natural regeneration of woody plants is considerable, and its continual developing shade lowers vegetation layers. The vastly reduced light supply on plot H was responsible for considerable impoverishment of the species spectrum and a large decrease in species vitality, which was indicated by the absence of the generative phases. Species typical for clearings – *Chamaerion angustifolium*, *Eupatorium cannabinum*, *Atropa belladonna* and *Calamagrostis epigejos* were disappeared (Fajmonová, 1986). Significant decrease in density of sterile and fertile shoots of *Carex pilosa* species was observed. On the other hand, significant increase in density of sterile shoots was detected for *Dentaria bulbifera* species. This fact may be explained by contrasting requirements on light supply during vegetation period. *Carex pilosa* has relative higher demands on stable light conditions compared to *Dentaria bulbifera*, which utilizes more favourable light conditions before full leafing of tree layer. Also sufficiency of free niches supported the development of this species. Sørensen’s index of similarity confirmed, that succession processes on plot H led to development of the communities similar to those recorded on plot K. Bioindication analyses confirmed, that light mean value was changed significantly on managed plots (H and M) but not on control plot K. The light was reported as an important factor in the study presented by Ujházy et al. (2007). They state that disintegration of stand canopy improved the site conditions for some heliophytes, such as *Fragaria vesca, Rubus idaeus, Stellaria nemorum* etc. Our results are comparable to the results of Pykälä (2004), who summarised that the clear-cut areas are characterized by considerable increase in biomass volume and also species richness. However, subsequent succession processes result in a gradual decrease in species diversity, changes in biometric characteristics and changes in phenological traits of individual species, dependent on intensity of disturbance to the original status.

The herb layer in forest understory reflects changes in ecological conditions, especially in changing structure of tree stand. Different intensity of human impact leads to different subsequent changes in ecosystems, whereby destructive management forms (clear-cut) cause more expressive changes (Pilková, 2013) than less disturbed micro-sites which possess more favourable conditions for the survival of sensitive species (Brunet and Oheimb 1998). Also climatic variability may affect forest development, however, stand type-dependent sensitivity is reported by Kardol et al. (2010). Our data and results could be used in the near future for modelling the forest vegetation dynamics following natural or anthropogenic disturbances.

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The assessment of the soil organic matter of different ecosystems according to parameters of carbon

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Abstract

Tobiašová, E., Dębska, B., Drag, M., 2015. The assessment of the soil organic matter of different ecosystems according to parameters of carbon. *Folia Oecologica*, 42: 46–53.

In this study, the differences in the assessment of the soil organic matter (SOM) of 8 soils (Eutric Fluvisol, Mollic Fluvisol, Haplic Chernozem, Haplic Luvisol, Eutric Cambisol, Rendzic Leptosol, Eutric Regosol, and Dystric Planosol) of different ecosystems (forest, meadow, urban, and agro-ecosystem) in Slovakia were compared. The quantity and quality of the SOM was assessed through the parameters of carbon in the total volume of soil and in the fractions of soil aggregates. The significant differences in the parameters of carbon between the ecosystems are more visible in the case of its assessment in the soil aggregates than in the total volume of soil and the results are influenced by the nature of SOM stabilization. The highest contents of total organic carbon (TOC) and labile carbon (C_L) in the total volume of soil were in the forest ecosystem, but more significant differences in the contents of TOC and C_L between the other ecosystems were determined only in the soil aggregates. The urban ecosystem > meadow ecosystem > agro-ecosystem followed then, and the most stabilized carbon was in the urban ecosystem. TOC and C_L had higher portions in larger fractions of aggregates (>2 mm) that had the highest proportion in the meadow ecosystems.

Key words

ecosystem, soil organic matter, water-resistant macro-aggregates

Introduction

Total organic carbon (TOC) is not sufficient parameter in a short-term dynamics of the soil organic matter (SOM) (Hungate et al., 1996), because the changes in this parameter are usually the result of plant growing or used method of its determination. Actual changes in the values of this parameter can be observed at least after two decades, but in the case of labile carbon content (C_L) and other more sensitive parameters (Blair et al., 1995), these changes are possible to be monitored in a shorter period (Tisdall and Oades, 1982). Labile fractions of SOM are important because they regulate ecosystem productivity in a short-term period and are influenced by the land use (Jandl and Sollins, 1997; McLauchlan and Hobbie, 2004; Tobiašová, 2010). Because of the positive correlation between C_L and TOC (Powlson et al., 1987), through the C_L we can also predict the other changes in SOM. They were assessed to this time in the total volume of soil. The SOM is stabilized also through the formation of soil aggregates (Six et al., 2001; Tobiašová, 2011), but the mechanisms of aggregate dynamics (Tisdall and Oades, 1982) are different. They depend not only on the soil, but also on the ecosystem, the stability of which depends mostly on the quantity and quality of SOM (Doran and Parkin, 1994). Therefore, the objectives of this study were as follows: i) to show on the differences in assessment of the quantity and quality of SOM in the total volume of soil and in the fractions of water-resistant aggregates, ii) to assess the differences in SOM of selected ecosystems, and iii) in SOM of the fractions of water-resistant macro-aggregates.

Materials and methods

The experiment includes four types of ecosystems, which present the different land use (forest, meadow, urban, and agro-ecosystem) of eight soil types (Eutric Fluvisol, Mollic Fluvisol, Haplic Chernozem, Haplic Luvisol, Eutric Cambisol, Rendzic Leptosol, Eutric Regosol, and Dystric Planosol) (WRB, 2007).

Characteristics of the territory

Šaľa (Eutric Fluvisol), Horná Kráľová (Mollic Fluvisol, Haplic Chernozem), Veľké Ripňany (Eutric Regosol), and Ludanice (Haplic Luvisol) are located in the Danube Basin with neogene formation, which are covered with younger quaternary rocks (loess and loess loamy) and brackish sediments (gravels, sands, and clays) (Pristaš et al., 2000). The original vegetation consists mostly of oak-ash-elm-alder forests and along the Vah River, willow-poplar forests are preserved. The average annual temperature of the studied area is 9.3 to 9.8 °C and the average rainfall per year is 568 to 607 mm (Korec et al., 1997). Pružina (Eutric Cambisol, Rendzic Leptosol) is located in the north-eastern foothills of the highest mountain Strážov, in the valley of Pružinka River. A substantial part of the mountain consists of nappe-folded mesozoic complexes with highly variable rock resistance (Buday et al., 1967). The original vegetation consists mostly of beech and oak forests blended with pine tree and in a higher altitude, pine forest with fir and with higher portion of conifers. The average annual temperature of studied area is 8.2 °C, and the average rainfall per year is 718 mm (Korec et al., 1997). Hrachovo (Dystric Planosol) is located in Matra – Slaná area with weathered non-carbonate rocks (crystalline volcanic rock) (Buday et al., 1967). The average annual temperature of studied area is 8.8 °C, and the average rainfall per year is 640 mm (Korec et al., 1997).

Soil samples and analytical methods used

The soil samples for determination of the physical and chemical characteristics were collected in the spring in three replicates to a depth of 0.3 m in four ecosystems of eight soil types. The basic chemical and physical properties of the soils are characterised in the Table 1. All samples for the determination of physical properties were dried in a constant-temperature room of 25 ± 2 °C. The soil samples for determination of the composition of soil aggregates were after drying divided by the wet sieve to size fractions of water-resistant aggregates. The particle size distribution was determined after dissolution of $CaCO_3$ with 2 mol dm^{-3} HCl and decomposition of the organic matter with 30% H_2O_2. After repeated washing, samples were dispersed using $Na(PO_3)_6$. Silt, sand and clay fractions were determined according to the pipette method. To determine the chemical properties, the soil samples were dried and ground. The total organic carbon (TOC) was determined by wet combustion (Orlov and Grishina, 1981) and the labile carbon (C_L) by $KMnO_4$ oxidation (Loginov et al., 1987). The non-labile carbon (C_{NL}), lability of carbon (L_C), index of carbon lability (LI_C), carbon pool index (CMI), and carbon management index (CMI) were calculated according to equations 1–5 (Blair et al., 1995).

$$C_{NL} = TOC - C_L \text{ (mg kg}^{-1}\text{)} \tag{1}$$

$$L_C = \frac{C_L}{C_{NL}} \tag{2}$$

$$LI_C = \frac{L_C \text{ in variant}}{L_C \text{ in control}} \tag{3}$$

$$CPI = \frac{TOC \text{ in variant}}{TOC \text{ in control}} \tag{4}$$

$$CMI = LI_C \times CPI \times 100 \tag{5}$$

The soil pH was measured potentiometrically in a supernatant suspension of a 1 : 2.5 soil: liquid mixture. The liquid is 1 mol dm^{-3} KCl (pH/KCl). The cation exchange capacity (CEC) was determined according to Jackson (2005).

The obtained data were analysed using Statgraphic Plus statistical software. A multifactor anova model was used for individual treatment comparisons at $P < 0.05$, with separation of the means by LSD multiple-range test.

Results and discussion

The content of total organic carbon (TOC) in a total volume of soil was significantly higher in the forest ecosystem (Table 2). This is the natural forest and its soil has almost the same properties like the soil of undisturbed area, the soil of which is according to Doran and Parkin (1994) considered the soil of the highest quality. One of the reasons is the fact that in the natural ecosystems all plant residues remain, so that there is not the loss from the soil by this way. The content of the labile carbon (C_L) is more sensitive parameter than TOC. The C_L had also the highest proportion in the forest ecosystems, in which the values of soil pH are usually lower in comparison with other ecosystems, and in the case of a lower soil pH, more carbon is in an active form (Tobiašová, 2010). In the forest ecosystem, the intensity of mixing of the organic and mineral compounds is also limited, which is one of the factors of stabilization of the organic substances in the soil. This fact is confirmed by the values of the lability of carbon (L_C).

Table 1. Chemical and physical properties of the soils in different ecosystems

Soil type	Ecosystem	pH/KCl	TOC	CEC	Sand	Silt	Clay
			(%)	(mmol kg^{-1})		(%)	
EF	FO	7.28	2.955	392.73	46.25	42.46	11.29
	ME	7.28	2.113	303.43	41.20	43.28	15.52
	UR	6.92	1.936	347.18	32.00	43.33	24.67
	AG	7.28	1.546	401.56	30.26	51.59	18.15
MF	FO	6.14	1.496	321.83	54.43	35.13	10.44
	ME	7.39	1.144	400.17	44.64	31.20	24.16
	UR	7.50	1.766	401.23	27.11	52.33	20.56
	AG	6.77	2.095	378.34	46.69	37.08	16.23
HC	FO	6.56	2.165	324.80	53.05	32.34	14.61
	ME	7.98	2.013	399.90	45.49	39.01	15.50
	UR	7.27	1.822	400.17	38.03	49.75	12.22
	AG	7.10	1.762	330.20	32.43	50.13	17.44
HL	FO	5.44	2.662	165.42	26.70	59.46	13.84
	ME	5.91	1.806	229.22	24.75	59.23	16.02
	UR	6.98	2.026	257.11	40.05	46.86	13.09
	AG	6.00	1.592	174.34	18.57	52.55	18.88
EC	FO	6.16	3.076	319.24	28.97	53.49	17.54
	ME	7.01	2.040	326.96	59.83	28.38	11.79
	UR	6.94	2.370	321.64	35.58	48.14	16.28
	AG	6.36	1.222	312.61	30.24	50.88	10.65
RL	FO	7.25	3.168	403.57	43.58	41.11	15.31
	ME	7.36	1.765	403.23	43.41	37.18	19.41
	UR	7.26	1.158	402.57	41.42	40.08	18.50
	AG	7.04	1.312	384.06	33.99	50.48	15.53
ER	FO	4.87	1.136	278.40	28.72	61.63	9.65
	ME	6.06	1.003	244.80	36.84	50.92	12.24
	UR	5.97	1.208	254.00	34.28	54.78	10.94
	AG	6.05	1.141	300.80	43.60	41.22	15.18
DP	FO	4.23	1.725	100.37	25.24	58.01	16.75
	ME	5.13	0.977	140.43	47.88	36.95	15.17
	UR	4.34	0.773	153.38	43.90	26.23	29.87
	AG	4.54	1.162	163.21	30.37	43.64	25.99

EF, Eutric Fluvisol; MF, Mollic Fluvisol; HC, Haplic Chernozem; HL, Haplic Luvisols; EC, Eutric Cambisol; RL, Rendzic Leptosol; ER, Eutric Regosol; DP, Dystric Planosol; FO, forest ecosystem; ME, meadow ecosystem; UR, urban ecosystem; AG, agro-ecosystem; TOC, total organic carbon; CEC, cation exchange capacity.

However, more significant differences in these parameters between the ecosystems take effect in the case, if we consider only the organic matter bound in the soil aggregates, because the losses of soil organic carbon are determined primarily by the destruction of macro-aggregates (Elliott, 1986). In the case of incorporated carbon in the aggregates (Table 3), the significant differences were observed also between the other ecosystems. In the case of evaluation of the TOC and C_L in a total volume of the soil, the values in the meadow and urban ecosystems are nearly the same, but if we evaluate their contents only in the fractions of water-resistant macro-aggregates, the differences are statistically significant. Second highest contents of the TOC and C_L, after forest ecosystem, were in the urban ecosystem. In the case of both ecosystems, meadow and urban, the vegetation cover were grasses, therefore the differences in their contents in a total volume of the soil were not more significant. The source of organic matter in the meadow ecosystem, compared to the for-

Table 2. Statistical evaluation of carbon parameters in soil to a depth of 0.3 m

Factor	TOC (mg kg^{-1})	C_L (mg kg^{-1})	C_{NL} (mg kg^{-1})	L_C	LI_C	CPI	CMI
Ecosystem							
FO	25,267b	3,549b	21,719b	0.181b	–	–	–
ME	17,086a	1,923a	15,163a	0.127a	77.48a	0.686a	51.67a
AG	14,918a	1,939a	12,979a	0.153ab	96.49a	0.655a	60.30a
UR	16,695a	1,869a	14,801a	0.134a	87.22a	0.691a	56.00a
Soil type							
EF	18,648b	2,247b	16,401b	0.137abc	90.45bcd	0.631ab	57.12ab
MF	16,683b	1,862ab	14,821b	0.121ab	61.03ab	1.115c	73.54b
HC	18,658b	1,944ab	16,713b	0.117ab	37.82a	0.862bc	32.50a
HL	18,078b	2,428b	15,651b	0.155bc	117.97de	0.679ab	80.38b
EC	18,772b	1,899ab	16,806b	0.121ab	81.53bc	0.610ab	48.28ab
RL	14,115ab	1,173a	12,943ab	0.094a	68.50ab	0.446a	29.18a
ER	15,201ab	2,312b	12,889ab	0.181c	110.99cde	0.512a	56.56ab
DP	9,707a	1,418ab	8,288a	0.177c	128.20e	0.563a	70.34b

FO, forest ecosystem; ME, meadow ecosystem; AG, agro-ecosystem; UR, urban ecosystem; EF, Eutric Fluvisol; MF, Mollic Fluvisol; HC, Haplic Chernozem; HL, Haplic Luvisols; EC, Eutric Cambisol; RL, Rendzic Leptosol; ER, Eutric Regosol; DP, Dystric Planosol; TOC, total organic carbon; C_L, labile carbon; C_{NL}, non-labile carbon; L_C, lability of carbon; LI_C, index of carbon lability; CPI, carbon pool index; CMI, carbon management index; different letters (a, b, c, d, and e) between the factors show statistically significant differences ($P < 0.05$) – LSD test.

est ecosystem, comes mainly from the underground biomass. This is not only visible rich root system, but the plants during their life produced a huge amount of root exudates. These substances are mainly polysaccharide nature, it means they represent the labile fraction of organic matter, which is an immediate source of the carbon and energy for soil microflora (Huang and Schoenau, 1996) and the secondary can support the growth of microbial population. In these ecosystems, larger aggregates (>3 mm) had significantly higher proportion. The formation of these size fractions is supported mainly by the roots of plants, which have the largest proportion in the grass ecosystems (Table 4). Soils of urban ecosystems are more compact (Puskás and Farsang, 2009), and this causes the inhibition of microbial activity. The root exudates, which are the main source of labile forms of carbon in the grass ecosystems (Hütsch et al., 2002) are therefore in the soil of urban ecosystem less exploited by the microorganisms. This organic matter is stabilized mainly physically through its incorporation into the soil aggregates, in which is protected by inhibiting against carbon oxidation (Six et al., 1998; Hernanz et al., 2002).

The lowest carbon contents in a total volume of the soil as well as in the soil aggregates were in the agro-ecosystem, in which the inputs of organic matter into the soil are the lowest (Tobiašová, 2010; Tobiašová, 2014) and also the intensity of oxidation of the organic matter is the highest. The reason is mainly mechanical destruction of aggregates (Šimanský et al., 2008), because the carbon inside of these aggregates is physically protected (Elliot, 1986; Tobiašová, 2011). The part of the year is a soil in the agro-ecosystem without a vegetation cover, which is a next reason of the low content of carbon (Cheng et al., 2003).

In the larger fractions of water-resistant macro-aggregates (>3 mm), higher amounts of TOC and C_L were included, but the organic matter in them subjected more intensive oxidation (Sohi et al., 2001). These larger macro-aggregates had significantly higher proportion in the grass ecosystems. On the other hand, the content of carbon in smaller aggregates was lower, but better stabilized; however the increased proportion of smaller size fractions of aggregates is characteristic for degraded soils (Whalen and Chang, 2002). Smaller fractions of macro-aggregates had higher proportion in the agro-ecosystem.

However, if we compare the quantity and stability of SOM of the individual ecosystems with the forest ecosystem, we can observe the changes that occur in the studied ecosystems. Suitable parameters for tracking of these changes are parameters such as lability of carbon (LI_C), carbon pool index (CPI), and carbon management index (CMI) that were described by Blair et al. (1995). In the case of these parameters, more significant differences were recorded in the soil aggregates than in a total volume of the soil. The CPI values indicate the highest contents of organic matter in the aggregates of urban ecosystem while the values of LI_C indicate the highest lability of this organic matter. Parameter of

CMI also shows on the highest changes. The lower is the value of CPI, the higher is the loss of organic matter from the soil (Blair et al., 1995; Conteh et al., 1998). This fact also shows on a great importance of the physical stabilization of carbon as the prevention from its loss from the soil. It follows that a higher proportion of labile forms of organic matter does not mean its more rapid loss from the soil. The increase of CMI values is not the result of formation of the organic compounds as a result of the increased annual input of carbon, but as a result of the changes in a quality of SOM, especially C:N ratio, contents of lignin, cellulose, hemicellulose, proteins, and carbohydrates that affect the lability of carbon to oxidation of $KMnO_4$ (Tirol-Padre and Ladha, 2004). The LI_C were significantly higher in the agro-ecosystem than in the meadow ecosystem, but the values of CMI were fairly balanced. In the agro-ecosystem, besides of the primary source of organic matter of the plant residues, the secondary source was farmyard manure. It increased the lability of organic matter (Kalbitz et al., 2003); therefore the values of LI_C were higher in the agro-ecosystem than in the meadow ecosystem. Farmyard manure also stimulates biological fixation of N_2 in the soil (Ladha et al., 1989), which can cause the increase of the values of CMI. The reasons of increased values of CMI in the agro-ecosystem are moreover leguminous plants (Vieira et al., 2007).

In the case of soil type, the highest content of TOC was in the Eutric Cambisol and the lowest in the Dystric Planosol in a total volume of the soil (Table 2) as well as in the soil aggregates (Table 3). The tendency was nearly the same also in the case of other soil types. However, if we compare the other parameters of carbon the values are different in the case of a total volume of the soil, but higher differences are in the case of soil aggregates. More significant differences are observed, if we make the comparisons to the control, it means against to the soil of the forest ecosystem. The LI_C was the lowest in Haplic Chernozem in a total volume of the soil as well as in the soil aggregates. In this soil the labile components of organic matter are chemically and also physically stabilized. On the other hand, the highest values of LI_C were in Dystric Planosol and Haplic Luvisol in a total volume of the soil as well as in the soil

Table 3. Statistical evaluation of carbon parameters in water-resistant aggregates

Factor	TOC (mg kg^{-1})	C_L (mg kg^{-1})	C_{NL} (mg kg^{-1})	L_C	LI_C	CPI	CMI
Ecosystem							
FO	27,889c	3,775c	24,114c	0.159c	–	–	–
ME	18,125ab	2,070a	16,056ab	0.123a	81.14a	0.779a	65.93a
AG	16,956a	2,066a	14,889a	0.142b	91.83ab	0.797a	75.68a
UR	20,607b	2,782b	17,826b	0.152bc	99.45b	1.023b	123.18b
Soil type							
EF	19,435ab	2,596abc	16,839b	0.152bcd	80.47ab	0.790c	64.49ab
MF	20,109b	1,959a	18,151bc	0.105a	83.43b	1.145d	101.08bc
HC	24,967b	3,277c	21,690cd	0.147bc	63.56a	0.689bc	43.28a
HL	19,726b	2,834bc	16,891b	0.163cde	121.81d	1.052d	129.48c
EC	25,636b	3,203bc	22,433d	0.138b	86.57bc	0.504ab	44.32a
RL	21,481ab	2,038a	19,443bcd	0.096a	67.16ab	0.324a	19.99a
ER	20,405b	2,961bc	17,444b	0.173de	105.65cd	0.480ab	49.98a
DP	15,395a	2,516ab	12,880a	0.178e	117.80d	1.946e	253.50d
Fraction of water-resistant macro-aggregates (mm)							
> 5	18,762b	2,462b	16,300b	0.145a	93.24a	0.774a	82.26a
5–3	19,077b	2,454b	16,623b	0.144a	89.18a	0.843a	86.84a
3–2	19,133b	2,374ab	16,759b	0.139a	79.76a	0.871a	83.09a
2-1	19,555b	2,446b	17,108b	0.141a	94.16a	0.910a	102.84a
1–0,5	18,483ab	2,200ab	16,283ab	0.135a	92.19a	0.904a	84.92a
0.5–0.25	16,367a	1,898a	14,469a	0.130a	96.32a	0.895a	89.64a

FO, forest ecosystem; ME, meadow ecosystem; AG, agro-ecosystem; UR, urban ecosystem; EF, Eutric Fluvisol; MF, Mollic Fluvisol; HC, Haplic Chernozem; HL, Haplic Luvisols; EC, Eutric Cambisol; RL, Rendzic Leptosol; ER, Eutric Regosol; DP, Dystric Planosol; TOC, total organic carbon; C_L, labile carbon; C_{NL}, non-labile carbon; L_C, lability of carbon; LI_C, index of carbon lability; CPI, carbon pool index; CMI, carbon management index; different letters (a, b, c, d, and e) between the factors show statistically significant differences ($P < 0.05$) – LSD test.

Table 4. Statistical evaluation of % proportion of size fractions of water-resistant macro-aggregates in soil to a depth of 0.3 m

Factor	>5 mm	3–5 mm	2–3 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm
				(%)		
Ecosystem						
FO	11.01ab	14.43ab	18.13a	18.06a	13.57a	9.49a
ME	16.92b	17.72b	17.92a	17.37a	10.69a	8.81a
AG	3.65a	9.02a	15.13a	19.48a	20.06b	15.37a
UR	14.61b	17.35b	18.47a	19.05a	11.70a	9.33a
Soil type						
EF	29.01b	17.88bc	14.86a	14.29ab	10.90ab	5.55a
MF	5.83a	10.60ab	13.04a	17.35abc	17.33b	18.62c
HC	5.75a	13.36abc	22.26b	21.75c	16.03ab	13.24abc
HL	11.67a	12.02abc	11.32a	12.15a	17.14ab	15.84bc
EC	14.08a	18.45bc	20.61b	20.10bc	10.68a	5.38a
RL	12.45a	18.90c	22.82b	19.21abc	10.82ab	6.52ab
ER	4.34a	8.86a	13.74a	18.54abc	16.18ab	14.28abc
DP	9.26a	16.96abc	20.64b	24.52c	12.94ab	6.56ab

FO, forest ecosystem; ME, meadow ecosystem; AG, agro-ecosystem; UR, urban ecosystem; EF, Eutric Fluvisol; MF, Mollic Fluvisol; HC, Haplic Chernozem; HL, Haplic Luvisols; EC, Eutric Cambisol; RL, Rendzic Leptosol; ER, Eutric Regosol; DP, Dystric Planosol; different letters (a, b, c, d, and e) between the factors show statistically significant differences ($P < 0.05$) – LSD test.

aggregates, and not in the Mollic Fluvisol and Rendzic Leptosol as we would expect, because the contents of C_L in these soils were low. The lowest contents of C_L in a total volume of the soil were in the Rendzic Leptosol, which assumes mainly the chemical stabilization by the effect of carbonates while the lowest contents of C_L were in the soil aggregates of Mollic Fluvisol, which assumes mainly the physical stabilization inside of soil aggregates and the dominance of stabilized humus substances. This fact is confirmed with the highest values of CMI in a total volume of the soil in the case of soils (Dystric Planosol, Haplic Luvisols, Mollic Fluvisol), that are characterized by an alternating of the dry and wet periods, which have positive influence on the formation of soil aggregates (Bravo-Garza et al., 2009; Šimanský et al., 2008) and also the stabilization of humus substances and the formation of stronger links with the mineral proportion of soil (Denef et al., 2002). The next proof can be the lowest CPI values of District Planosol in a total volume of the soil and vice versa the highest values of this parameter in the soil aggregates. It is known that in this soil the majority of organic matter is physically stabilized.

Conclusion

More significant differences in the parameters of carbon between the ecosystems are visible in the case of assessment of the organic matter in soil aggregates than in a total volume of the soil and the results are influenced by the nature of stabilization of the organic matter.

The highest contents of TOC and C_L in a total volume of the soil were in the forest ecosystem, but more significant differences between the other ecosystems were recorded only in their contents in water-resistant macro-aggregates. After the forest ecosystem, the urban > meadow > agro-ecosystem followed, and the most stabilized carbon was in the urban ecosystem.

The TOC and C_L had higher proportions in larger fractions of the macro-aggregates (>2 mm), which had the highest proportion in the meadow ecosystems.

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Vegetation characteristics of species-rich grasslands in the National Park Slovenský raj, Slovakia

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Abstract

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The paper presents phytosociological data of grassland communities in Kopanecké lúky in the National park Slovenský raj. Regarding the species richness, the studied meadows are extraordinary valuable and contain one of the highest number of vascular plant species at small scales in Europe. In 2012, we recorded 100 vascular plant species in the area of 25 m^2. Within the study area, eight plant communities or vegetation types (characterised by specific species combination and species dominance) were ordered into the alliances *Arrhenatherion elatioris*, *Violion caninae*, *Nardo strictae-Agrostion tenuis*, *Polygono bistortae-Trisetion flavescentis*. All types of recorded vegetation represent transitional and successional stages. After 13–17 years the vegetation studied on permanent monitoring plots 5 × 5 m showed some changes. Meadows which have been regularly managed since 1990 have relatively stable species composition and species diversity. Abandoned plots experienced decrease in the total number of species and the emergence of expansive grasses like *Calamagrostis varia* (mesic and subxerophilic sites), *Brachypodium pinnatum* (wetter, mesic and subxerophilic sites) and *Calamagrostis arundinaceae* (mesic oligotrophic and acidic sites). In areas where cutting of trees, restoration or irregular mowing of meadows took place the number of species slightly increased. On the present, the plots are still threatened by expansion of *Calamagrostis arundinacea* species.

Keywords
diversity, grassland, phytosociology, Slovenský raj

Introduction

Semi-natural grasslands belong to the plant communities with the highest species diversity, especially in a small scale (Kull and Zobel, 1991; Klimeš, 2001, Wilson et al., 2012). Meadow and pasture communities occupying only 5% of the area of the National park Slovenský raj are the result of the traditional land-use in the past. Kopanecké lúky (meadows) ranks among the most valuable and most integrated complex of meadows in NP Slovenský raj. These communities represent the richest grasslands in Central Europe. In 2000, seventy-five vascular plants were recorded per 1 m^2 and 109 per 25 m^2, respectively (Šeffer et al., 2010). Many authors Smiešková (1970), Dzubinová (1984), Pitoniak (1978), later Dražil et. al. (1998) studied flora and vegetation of Kopanecké lúky. This paper introduces phytosociological characteristics of grassland communities in the area and the description of their changes on the 11 monitoring plots after 13–17 years. The study area is situated in the south-western part of the NP Slovenský raj in the cadaster of Vernár, district Poprad. The altitude of the aforesaid area ranges from 900 to 1,186 m asl.

Material and methods

Fifty five relevés from 55 monitoring plots (MP) recorded during the growing seasons 2012–2014 were used for the phytosociological description of the area. Between 1996 and 1999 relevés from 11 MPs and one relevé from 2000 (old relevés) (Dražil, 2004) were recorded in order to compare the species composition. Between 2012 and 2013 relevés were again recorded in 11 MPs. Some MPs are documented by more than one relevé in the old or new set of relevés. Nomenclature of vascular plants follows Marhold et al. (1998). Names of syntaxa follow Hegedüšová Vantarová et al. (2014). All relevés have been recorded according to the principles of the Zürich-Montpellier school (Braun-Blanquet, 1964) using the new Braun-Blanqet 9-degree cover scale (Barkman et al., 1964). Mosses and lichens were not determined. The size of each plot was 5 × 5 m. Relevés were stored in a database program Turboveg (Hennekens and Schaminée, 2001) and imported into the program Juice (Tichý, 2002). As the gradient length in DCA analysis was short (2.961 and 2.375 for the first two axes), the PCA (principal component analysis) which is included in the program Canoco 4.5 (Ter Braak, Šmilauer, 2002) was applied. Divisive polythetic classification Twinspan (Hill, 1979) in program Juice (Tichý, 2002) was used for the numerical classification of relevés. Pseudospecies cut levels were set to 0, 5, 15, 25. Diagnostic or differential taxa were determined according to fidelity and for its calculation the phi coefficient and standardization of the number of relevés in the synoptic column were carried out. The statistical significance of fidelity was tested by Fisher exact test ($p < 0.01$) (Chytrý et. al., 2002). Species with phi > 0.30 were considered as diagnostic. Species with a cover > 30% in relevés were considered to be dominant and species with a frequency > 90% in column were considered to be constant. Apart from diagnostic species, constant species were also decisive for differentiation of communities due to relative small dataset. Shannon diversity index and the mean unweighted values of Ellenberg`s ecological indices (Ellenberg et al., 1992) for nutrients, soil reaction, light, temperature, continentality and moisture were calculated for individual relevés in the program Juice (Tichý, 2002). They entered the analysis as supplementary data. To test the correlation of mean values of Ellenberg´s ecological indices (EIIs) calculated for grassland communities with 1. and 2. PCA axis „Modified randomized test with species indicator values“ was used (Zelený and Schaffers, 2012).

Results and discussion

Phytosociological characteristics of grassland communities

Typical plant communities or vegetation types of the studied area in various stages of transition or succession were documented by phytocoenological relevés. The historically developed secondary meadow communities are influenced by the dominance of expansive species (*Arrhenatherum elatius*, *Brachypodium pinnatum*, *Calamagrostis arundinacea*, *C. varia*). Also Norway spruce (*Picea abies*) has a high contribution to the overgrowing of meadows. Irregular and poorly managed parts of the grassland complex Kopanecké lúky

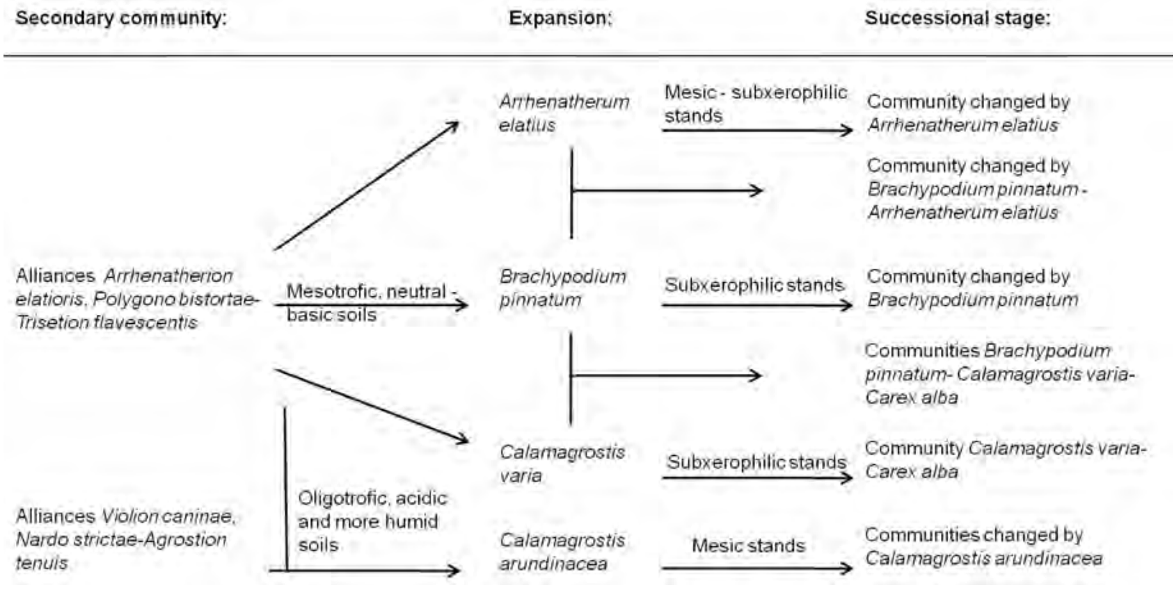

Fig. 1. Successional scheme of the most widespread communities in complex of Kopanecké lúky (authors: Vadel and Dražil, 2014).

are most affected by succession (Fig. 1). Therefore, it was not possible to classify some relevés exactly to syntaxa so we characterized them only as vegetation types.

55 relevés representing grassland communities were divided into 8 groups using a classification program Twinspan (Hill, 1979). In PCA analysis environmental factors as supplementary data were derived from mean values of Ellenberg`s ecological indices (EIIs). The first ordination axis explains 17.3% and the second axis 13.2% of the variability of species data. According to "Modified randomized test with species indicator values" (Zelený and Schaffers, 2012), EIIs for moisture, soil reaction and nutrients had a significant correlations with the first two PCA axes ($p < 0.05$). Moisture had positive correlation, but soil reaction had negative correlation with both PCA axes. Nutrients show negative correlation with 1.PCA axis and positive with 2.PCA axis (Fig. 2).

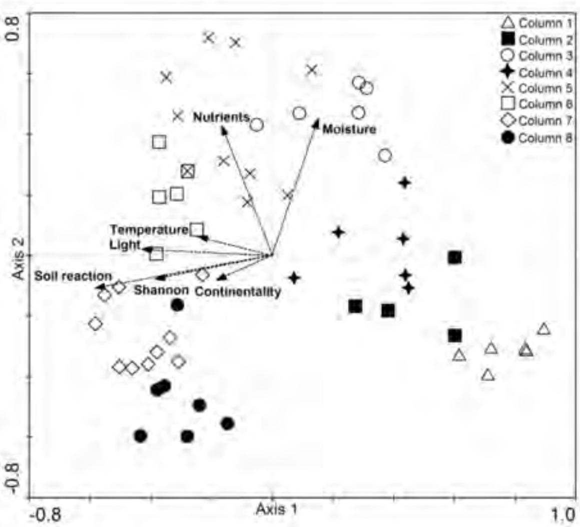

Fig. 2. Ordination graph of principal components analysis (PCA) shows the position of 55 relevés from 55 monitoring plots classified to 8 groups.

The first group of relevés (Table 1) represents transitional stages of communities of the alliance *Nardo strictae-Agrostion tenuis*. These mesic and oligotrophic grasslands are represented by acidophilous diagnostic species as *Avenella flexuosa*, *Nardus stricta*, *Soldanella hungarica, Trommsdorfia uniflora*. Constant species are *Pyrethrum clusii*, *Carlina acaulis* and *Luzula luzuloides*. Two relevés show affinity to the association *Viola sudeticae-Agrostietum capillaris*. The original vegetation is changed by expansion of grass species *Calamagrostis arundinacea* which is dominant and diagnostic for this group. It is the consequence of inadequate management in the particular part of the area. These secondary communities were developed after deforestation of fir-beech and spruce forests (Ujházy and Kliment, 2007). Some relevés contain subalpine elements, such as *Pulsatilla scherfelii*, *Trientalis europaea*, *Homogyne alpina*, *Veratrum album* subsp. *lobelianum*.

Dominant *Calamagrostis arundinacea* affects vegetation in the second group of relevés as well (Table 1). These communities have successional character of alliance *Violion caninae*, which are typical of mixture of species of meadow and mat-grass communities (Kliment and Ujházy, 2014). Diagnostic species are *Hieracium lachenalii*, *Vaccinium myrtillus*, *V. vitis-idaea*. Species *Agrostis capillaris, Avenella flexuosa, Veronica chamaedrys*, *Fragaria vesca*, *Campanula patula*, *Jacea phrygia*, *Lotus corniculatus*, *Luzula luzuloides*, *Hypericum maculatum*, *Pimpinella saxifraga*, *Ranunculus polyanthemos*, *Cruciata glabra* etc. are constant. Several of these species are typical of the *Polygono bistortae-Trisetion flavescentis* and *Arrhenatherion elatioris* alliances. In comparison with the previous group, these communities are species-richer and *Nardus stricta* occurs only with low abundance in one relevé.

Grasslands formed by species *Agrostis capillaris*, *Arrhentherum elatius* and *Festuca rubra* with lower species diversity on more humid and deeper soils are documented in the third column of Table 1. The group is differentiated by species *Deschampsia cespitosa*, *Poa humilis*, *Achillea distans*, which have higher fidelity index. Characteristic species composition is complemented by constant species, such as *Crepis mollis*, *Cardaminopsis halleri*, *Stellaria graminea*, *Acetosa pratensis*, *Festuca rubra*, *Ranunculus acris*, *Hypericum maculatum*, *Trifolium pratense* etc. Swards can be characterised as successional stages of the most widespread grassland association in Slovenský raj – *Antoxantho odorati-Agrostietum tenuis* (Pitoniak, 1978), with close relation to the communities of the *Polygono bistortae-Trisetion flavescentis* alliance. *Deschampsia cespitosa* species occurs on sites of association *Antoxantho odorati-Agrostietum tenuis* at higher altitudes (Uhliarová et al., 2014).

The fourth column of Table 1 documents species-rich communities despite being influenced by *Calamagrostis arundinacea*. Species *Cerastium holosteoides*, *Prunella vulgaris*, *Viola canina* and *Avenella flexuosa* differentiate the group. Constant species of grasses are represented by *Briza media*, *Festuca rubra, F. ovina*. Species *Agrostis capillaris* has the frequency of 83%. Species *Cardaminopsis halleri*, *Trifolium repens* and *T. pratense*, *Alchemilla* sp. div. are typical of lower herb layer. These swards having characteristic species *Luzula luzuloides*, *Ranunculus polyanthemos* and *Thymus pulegioides* occur on nutrient-poorer soil. This group represents successional stages of communities of alliance *Polygono bistortae-Trisetion flavescentis*. According to species composition and ecological conditions, some relevés probably represent a successional stage of association *Crepido*

Table 1. Synoptic table containing only diagnostic species (with fidelity over 30, in gray fields), constant species and species with frequency over 65%

Group number	1	2	3	4	5	6	7	8
Number of relevés	6	4	6	6	10	6	10	7
Alliance/Species				Frequency (%)				
Alliance *Nardo strictae-Agrostion tenuis*								
Nardus stricta	100$^{75.1}$	25^{---}	.$^{---}$	17^{---}	10^{---}	.$^{---}$	10^{---}	.$^{---}$
Soldanella hungarica	50$^{68.3}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$
Trommsdorfia uniflora	100^{62}	50^{---}	33^{---}	33^{---}	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$
Hieracium murorum	50^{51}	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	29^{---}
Gymnadenia conopsea	100$^{49.7}$	50^{---}	17^{---}	17^{---}	10^{---}	17^{---}	40^{---}	43^{---}
Alliance *Violion caninae*								
Hieracium lachenalii	17^{---}	75$^{75.4}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$
Vaccinium vitis-idaea	100^{62}	100^{62}	.$^{---}$	17^{---}	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$
Vaccinium myrtillus	67$^{44.5}$	75$^{52.4}$	.$^{---}$	17^{---}	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$
***Antoxantho odorati-Agrostietum tenuis* – successional stages**								
Deschampsia cespitosa	.$^{---}$	25^{---}	100$^{59.2}$	17^{---}	20^{---}	50^{---}	20^{---}	.$^{---}$
Poa humilis	$^{---}$	25^{---}	83$^{53.3}$	17^{---}	20^{---}	17^{---}	10^{---}	.$^{---}$
Achillea distans	33^{---}	.$^{---}$	67$^{51.4}$	17^{---}	.$^{---}$	.$^{---}$	.$^{---}$	14^{---}
Alliance *Polygono bistortae-Trisetion flavescentis* – successional stages								
Cerastium holosteoides	.$^{---}$	.$^{---}$	.$^{---}$	83$^{53.6}$	40^{---}	33^{---}	30^{---}	.$^{---}$
Viola canina	17^{---}	25^{---}	33^{---}	100$^{53.2}$	40^{---}	33^{---}	20^{---}	.$^{---}$
Calamagrostis arundinacea	100$^{44.3}$	100^{---}	17^{---}	100$^{44.3}$	10^{---}	.$^{---}$	10^{---}	.$^{---}$
Avenella flexuosa	100$^{42.5}$	100^{---}	33^{---}	100$^{42.5}$	10^{---}	.$^{---}$	10^{---}	.$^{---}$
Prunella vulgaris	.$^{---}$	25^{---}	.$^{---}$	100$^{43.1}$	20^{---}	100$^{43.1}$	60^{---}	43^{---}
Poo-Trisetetum flavescentis								
Trisetum flavescens	.$^{---}$	.$^{---}$	.$^{---}$	17^{---}	80$^{60.9}$	33^{---}	.$^{---}$	14^{---}
Crepis biennis	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	30$^{52.2}$	.$^{---}$	.$^{---}$	.$^{---}$
Myosotis nemorosa	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	30$^{52.2}$	.$^{---}$	.$^{---}$	.$^{---}$
Avenula pubescens	17^{---}	.$^{---}$	17^{---}	.$^{---}$	90$^{52.1}$	67^{---}	20^{---}	14^{---}
Trollius altissimus	17^{---}	.$^{---}$	.$^{---}$	.$^{---}$	50$^{45.1}$	17^{---}	10^{---}	.$^{---}$
Taraxacum sp.	.$^{---}$	.$^{---}$	50^{---}	.$^{---}$	80$^{42.5}$	33^{---}	40^{---}	29^{---}
Carex pallescens	.$^{---}$	.$^{---}$	17^{---}	50^{---}	70$^{37.4}$	50^{---}	10^{---}	14^{---}
Primula elatior	.$^{---}$	.$^{---}$	17^{---}	33^{---}	80$^{35.3}$	50^{---}	60^{---}	43^{---}
Rhinanthus serotinus	50^{---}	50^{---}	17^{---}	83^{---}	100$^{31.5}$	83^{---}	60^{---}	29^{---}
***Arrhenatherion elatioris* - successional stages**								
Colymbada scabiosa	.$^{---}$	.$^{---}$	.$^{---}$	17^{---}	10^{---}	100$^{52.4}$	90$^{44.4}$	57^{---}
Carum carvi	.$^{---}$	.$^{---}$	33^{---}	.$^{---}$	50^{---}	83$^{47.8}$	50^{---}	.$^{---}$
Arrhenatherum elatius	33^{---}	.$^{---}$	50^{---}	17^{---}	60^{---}	100^{47}	40^{---}	14^{---}
Alliance *Polygono bistortae-Trisetion flavescentis* – warm type of community								
Carex ornithopoda	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	40$^{60.7}$	.$^{---}$
Carex panicea	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	30^{---}	33^{---}	90$^{57.5}$	43^{---}
Plantago major	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	30$^{52.2}$	.$^{---}$
Arenaria serpyllifolia	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	30$^{52.2}$	.$^{---}$
Sanguisorba minor	.$^{---}$	25^{---}	.$^{---}$	.$^{---}$	.$^{---}$	83^{---}	100$^{51.5}$	71^{---}
Carex alba	.$^{---}$	50^{---}	.$^{---}$	.$^{---}$	.$^{---}$	17^{---}	90$^{51.5}$	71^{---}
Carex caryophyllea	17^{---}	25^{---}	.$^{---}$	17^{---}	50^{---}	33^{---}	100$^{50.9}$	43^{---}
Festuca pallens	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	40$^{42.4}$	29^{---}
Viola hirta	.$^{---}$	.$^{---}$	.$^{---}$	17^{---}	.$^{---}$	.$^{---}$	40$^{41.4}$	14^{---}
Galium mollugo	17^{---}	.$^{---}$	67^{---}	33^{---}	20^{---}	67^{---}	100$^{40.3}$	71^{---}
Trifolium montanum	17^{---}	25^{---}	.$^{---}$	50^{---}	60^{---}	50^{---}	100$^{39.0}$	86^{---}
Plantago media	.$^{---}$	.$^{---}$	.$^{---}$	67^{---}	50^{---}	100^{---}	100$^{38.9}$	71^{---}
Clinopodium vulgare	.$^{---}$	25^{---}	.$^{---}$	83^{---}	20^{---}	50^{---}	80$^{34.8}$	29^{---}
Festuca pratensis	.$^{---}$	.$^{---}$	33^{---}	50^{---}	80^{---}	100^{---}	100$^{34.6}$	71^{---}
Polygonatum verticillatum	33^{---}	50^{---}	.$^{---}$	50^{---}	.$^{---}$	33^{---}	80$^{34.5}$	43^{---}
Plantago lanceolata	17^{---}	.$^{---}$	50^{---}	17^{---}	50^{---}	100^{---}	90$^{33.6}$	43^{---}
Dianthus carthusianorum	33^{---}	75^{---}	.$^{---}$	83^{---}	50^{---}	67^{---}	100$^{30.0}$	86^{---}
***Calamagrostis varia* – (*Sesleria albicans*) – vegetation type**								
Aquilegia vulgaris	.$^{---}$	.$^{---}$	.$^{---}$	33^{---}	.$^{---}$	17^{---}	20^{---}	71$^{53.3}$
Arabis hirsuta	17^{---}	.$^{---}$	.$^{---}$	.$^{---}$	10^{---}	17^{---}	50^{---}	71$^{47.5}$
Galium pumilum	17^{---}	.$^{---}$	.$^{---}$	17^{---}	.$^{---}$	17^{---}	50^{---}	71$^{46.1}$
Acinos alpinus	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	17^{---}	90$^{58.3}$	86$^{54.5}$
Calamagrostis varia	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	10^{---}	17^{---}	90$^{53.5}$	100^{62}
Carduus glaucinus	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	70^{45}	100$^{72.8}$
Constant and other species								
Anthyllis vulneraria	17^{---}	50^{---}	.$^{---}$	83^{---}	30^{---}	100^{---}	90^{---}	86^{---}
Potentilla heptaphylla	.$^{---}$	.$^{---}$	.$^{---}$	.$^{---}$	40^{---}	67^{---}	20^{---}	29^{---}

Table 1. Synoptic table containing only diagnostic species (with fidelity over 30, in gray fields), constant species and species with frequency over 65% – continued

Group number	1	2	3	4	5	6	7	8
Number of relevés	6	4	6	6	10	6	10	7
Alliance/Species				Frequency (%)				
Constant and other species								
Campanula glomerata	. ---	50 ---	. ---	83 ---	. ---	33 ---	70 ---	57 ---
Linum catharticum	17 ---	. ---	. ---	17 ---	50 ---	83 ---	80 ---	86 ---
Brachypodium pinnatum	. ---	25 ---	. ---	17 ---	80 ---	100 ---	60 ---	86 ---
Poa chaixii	50 ---	75 ---	83 ---	83 ---	30 ---	. ---	10 ---	. ---
Lilium bulbiferum	. ---	. ---	. ---	17 ---	. ---	67 ---	40 ---	57 ---
Sesleria albicans	. ---	. ---	. ---	. ---	. ---	. ---	20 ---	29 ---
Phyteuma orbiculare	17 ---	50 ---	17 ---	83 ---	70 ---	33 ---	90 ---	100 ---
Cirsium arvense	. ---	75 ---	50 ---	. ---	. ---	50 ---	. ---	. ---
Thymus pulegioides	67 ---	75 ---	17 ---	100 ---	80 ---	100 ---	100 ---	86 ---
Jacea phrygia	50 ---	100 ---	83 ---	100 ---	100 ---	83 ---	90 ---	100 ---
Helianthemum	17 ---	25 ---	. ---	50 ---	40 ---	83 ---	70 ---	43 ---
Leontodon hispidus	33 ---	75 ---	17 ---	67 ---	60 ---	100 ---	90 ---	86 ---
Heracleum sphondylium	17 ---	50 ---	50 ---	67 ---	40 ---	33 ---	30 ---	29 ---
Cruciata glabra	83 ---	100 ---	100 ---	100 ---	100 ---	100 ---	90 ---	100 ---
Fragaria vesca	17 ---	100 ---	33 ---	83 ---	20 ---	67 ---	80 ---	71 ---
Cardaminopsis halleri	83 ---	75 ---	100 ---	100 ---	100 ---	100 ---	100 ---	100 ---
Ajuga reptans	. ---	50 ---	. ---	67 ---	10 ---	33 ---	60 ---	29 ---
Carlina acaulis	100 ---	100 ---	. ---	100 ---	60 ---	17 ---	90 ---	100 ---
Festuca rubra	67 ---	75 ---	100 ---	100 ---	100 ---	100 ---	70 ---	57 ---
Veronica chamaedrys	67 ---	100 ---	100 ---	100 ---	100 ---	100 ---	70 ---	71 ---
Stellaria graminea	67 ---	75 ---	100 ---	67 ---	90 ---	33 ---	40 ---	29 ---
Alchemilla sp.	50 ---	75 ---	83 ---	100 ---	100 ---	100 ---	90 ---	29 ---
Dactylis glomerata	. ---	75 ---	100 ---	67 ---	90 ---	83 ---	90 ---	29 ---
Ranunculus polyanthemos	83 ---	100 ---	83 ---	100 ---	100 ---	83 ---	100 ---	71 ---
Leucanthemum vulgare	50 ---	75 ---	33 ---	83 ---	80 ---	83 ---	100 ---	86 ---
Rumex acetosa	50 ---	75 ---	100 ---	100 ---	100 ---	50 ---	80 ---	14 ---
Campanula patula	33 ---	100 ---	83 ---	100 ---	70 ---	67 ---	30 ---	14 ---
Hypericum maculatum	67 ---	100 ---	100 ---	83 ---	80 ---	83 ---	60 ---	29 ---
Campanula persicifolia	50 ---	75 ---	67 ---	100 ---	20 ---	83 ---	70 ---	86 ---
Colchicum autumnale	. ---	25 ---	67 ---	17 ---	90 ---	100 ---	100 ---	100 ---
Trifolium pratense	17 ---	75 ---	100 ---	100 ---	100 ---	83 ---	70 ---	43 ---
Lotus corniculatus	83 ---	100 ---	83 ---	83 ---	100 ---	100 ---	100 ---	100 ---
Trifolium flexuosum	33 ---	50 ---	50 ---	67 ---	60 ---	67 ---	50 ---	14 ---
Luzula luzuloides	100 ---	100 ---	67 ---	100 ---	40 ---	50 ---	60 ---	14 ---
Trifolium repens	33 ---	75 ---	83 ---	100 ---	80 ---	83 ---	100 ---	43 ---
Ranunculus acris	17 ---	25 ---	100 ---	83 ---	80 ---	83 ---	50 ---	43 ---
Lathyrus pratensis	. ---	25 ---	50 ---	50 ---	80 ---	100 ---	90 ---	71 ---
Anthoxanthum odoratum	83 ---	50 ---	17 ---	83 ---	60 ---	100 ---	90 ---	57 ---
Pimpinella major	67 ---	50 ---	100 ---	100 ---	90 ---	83 ---	80 ---	71 ---
Vicia cracca	. ---	25 ---	50 ---	33 ---	90 ---	67 ---	90 ---	71 ---
Pyrethrum clusii	100 ---	100 ---	83 ---	100 ---	80 ---	67 ---	100 ---	86 ---
Crepis mollis	17 ---	75 ---	100 ---	50 ---	100 ---	67 ---	90 ---	43 ---
Briza media	50 ---	50 ---	33 ---	100 ---	90 ---	100 ---	100 ---	100 ---
Achillea millefolium agg.	17 ---	75 ---	33 ---	83 ---	90 ---	100 ---	100 ---	86 ---
Festuca ovina	83 ---	100 ---	67 ---	100 ---	80 ---	67 ---	80 ---	71 ---
Astrantia major	50 ---	. ---	17 ---	67 ---	70 ---	33 ---	40 ---	29 ---
Potentilla aurea	83 ---	75 ---	33 ---	50 ---	50 ---	67 ---	50 ---	14 ---
Vicia sepium	. ---	. ---	67 ---	50 ---	50 ---	33 ---	. ---	. ---
Luzula multiflora	50 ---	25 ---	33 ---	50 ---	50 ---	67 ---	30 ---	43 ---
Crepis conyzifolia	33 ---	50 ---	. ---	67 ---	60 ---	17 ---	40 ---	14 ---
Leontodon hispidus	50 ---	50 ---	33 ---	67 ---	80 ---	100 ---	100 ---	57 ---
Potentilla erecta	50 ---	75 ---	83 ---	33 ---	80 ---	50 ---	30 ---	29 ---
Agrostis capillaris	83 ---	100 ---	100 ---	83 ---	90 ---	100 ---	80 ---	29 ---
Polygala amara	83 ---	. ---	. ---	33 ---	60 ---	33 ---	70 ---	71 ---

Gray highlight – the occurrence of diagnostic species with fidelity (coefficient phi Φ) over 30 in particular type of phytocoenoses (local validity). Probability of non-random occurrence was evaluated by Fisher's exact test, $p > 0.01$. The size of each column was standardized to 12.5% of the size of the dataset.

mollis-Agrostietum capillaris. It belongs to species-rich communities of mountain meadows. In one relevé from 2013 seventy-three species were recorded. Such community occurs in colder and more humid places of submontane and montane zones (700–1,200 m asl) in the Slovenský raj, Nízke Tatry, Poľana, Muránska planina Mts. (Hegedüšová Vantarová, 2014). Ružičková (2004) regards association as a Carpathian vikariant of the *Cardaminopsio halleri-Agrostietum* Moravec 1965 which has been described in the Šumava Mts. as an replacement community of acidophilous beech forests. The change of land-use caused the change of species composition and decline of species of mesophilous meadows. Species of poor habitats appear increasingly and succession continues in the communities of alliance *Nardo strictae-Agrostion tenuis* (Hegedüšová Vantarová, 2014).

Mesophilous grasslands on moister and nutrients-rich soils belong to the *Poo-Trisetetum flavescentis* association (Table 1, column 5). Phytocoenoses are characterized by diagnostic species *Trisetum flavescens, Avenula pubescens, Myosotis nemorosa, Crepis biennis, Trollius altissimus, Carex pallescens, Taraxacum* sp. Species *Trisetum flavescens* and *Festuca rubra* are dominant grasses and *Briza media, Agrostis capillaris, Dactylis glomerata* are constant grasses. *Trollius altissimus* and *Myosotis nemorosa* occur in moister stands. Lower herb layer consists of *Trifolium pratense, Lotus corniculatus* and *Cruciata glabra* species. In the lower swards *Rhinanthus serotinus* prevails, during spring *Primula elatior* prevails among the flowering individuals. Species such as *Crepis mollis* and *Cardaminopsis halleri* of the *Polygono bistotrae-Trisetion flavescentis* alliance have high constancy. Community forms transitional successional stages. In comparison with the *Anthoxantho odorati-Agrostietum tenuis* it occurs on deeper soils with higher content of available nutrients (Uhliarová et al., 2014).

Successional stages of mesic communities of the *Arrhenatherion elatioris* alliance with the dominance of *Arrhenatherum elatius* and *Brachypodium pinnatum* are documented in the sixth synoptic column of Table 1. The composition of grasses is complemented by *Briza media*, *Festuca pratensis, F. rubra*, *Anthoxanthum odoratum* and *Agrostis capillaris* species. This group is characterised by the following diagnostic species: *Colymbada scabiosa, Carum carvi, Prunella vulgaris* and *Arrhenatherum elatius*. Lower herb layer is formed by *Alchemilla* sp. div, *Cardaminopsis halleri, Thymus pulegioides, Lotus corniculatus, Cruciata glabra, Leontodon hispidus* and *Veronica chamaedrys*. *Colchicum autumnale* flowers during late summer. The communities are relatively rich in species; the number of species in relevés varied from 52 to 74.

The seventh group of relevés (Table 1) includes species-rich phytocoenoses with subxerophilic character that represent warm type of communities of the *Polygono bistortae-Trisetion flavescentis*. Thermophilous taxa of the *Festuco-Brometea* class bound to the mesotrophic soil derived from calcareous bedrock such as *Carduus glaucinus*, *Carex caryophyllea*, *Colymbada scabiosa*, *Dianthus carthusianorum*, *Plantago media*, *Sanguisorba minor*, *Trifolium montanum* are characteristic for this group. Physiognomy is mostly affected by grass species *Calamagrostis varia* and *Carex alba,* on some plots accompanied by *Brachypodium pinnatum* and *Agrostis capillaris,* less frequently also by *Briza media* and *Festuca pratensis* species. *Sesleria albicans* was recorded on dolomite substrate in plots located around the top of the hill Javorina which is the highest point of the studied area (1,186 m asl). The species of the *Arrhenatherion elatioris* alliance are frequent, e.g. *Leucanthemum vulgare, Trifolium repens, Colchicum autumnale* and *Leontodon hispidus*. In spring *Crocus discolor* and *Primula elatior* species dominate among the flowering individuals. According to the species composition, the group is closely related to the association *Campanulo glomeratae-Geranietum sylvatici*. Swards are influenced by *Calamagrostis varia* and *Brachypodium pinnatum* species to lesser extent. They occur on warmer south-facing slopes with lower and open vegetation. In the MP 10, an extraordinary species-richness was recorded in 2000 (Dražil, 2004) and 2012 with 109 and 100 vascular plant species, respectively.

Relevés in the eighth synoptic column of Table 1 were classified as a vegetation type composed of calcareous grasses *Calamagrostis varia – Sesleria albicans* that follows previous group along a succession gradient. *Calamagrostis varia* as expansive and dominant species indicates successional trend. These unmanaged communities were documented on the southern slopes with favourable thermic conditions and subxerophilous character. Relevés with *Sesleria albicans* were documented on dolomite substrate under the hill Javorina. Thermophilous species are typical of these warm slopes, e.g. *Carduus glaucinus, Aquilegia vulgaris, Galium pumilum, Arabis hirsuta*. Lower herb layer is formed by *Acinos alpinus, Cruciata glabra, Lotus corniculatus, Cardaminopsis halleri*, etc. Smiešková (1970) mentioned a similar example from Kopanecké lúky where *Festuca rubra*, *Carex alba*, *Calamagrostis varia* occur but *Brachypodium pinnatum* has only low abundance. She describes such community as calcareous and more xerophilous occupying small area on a south-facing slope. Soil was characterized as shallow, calcareous rendzic leptosols with higher content of the skeleton. Along a temperature gradient, this vegetation type is the most xerophilous in the study area. In the relevés, thermophilous species of the class *Festuco-Brometea* were recorded (*Arabis hirsuta*, *Festuca pallens*, *Leontodon incanus*, *Colymbada scabiosa*, *Erysimum witmannii*, *Bupleurum falcatum*, *Carduus glaucinus*).

According to the plant communities and vegetation types occurring in transitional and successional stages of development, a direction of succession in the area is indicated where expansive grasses assume dominance throughout time. Smiešková (1970) described stands of phytocoenoses as homogeneous because they had been managed regularly. The current physiognomy of the stands in the meadows of Kopanecké lúky is more heterogeneous and mosaic depending on the frequency of mowing and the length of abandonment in particular parts.

Comparison of monitoring plots (MP)

On 11 monitoring plots relevés were recorded during the years 1996–1999 and one relevé from the MP 10 in 2000. Some relevés have been repeatedly made during this period. Differences in species composition on MP are influenced by the used method and by intensity of management. During the years 1996–2013 these monitoring plots were managed differently. Meadows were restored, mowed on a regular basis or sporadically, or abandoned. It was reflected in the dynamics of plant communities on monitoring plots.

The difference in mean values of Ellenberg`s ecological indices (EII) calculated for new (2012–2013) and old relevés (1996–2000) indicates the changes of particular environmental factors (Table 2). The changes of EII could not be tested by t-test because of very small sample size.

Re-sampling of relevés on monitoring plots (MP) has shown that the trend in the development of phytocoenoses is dependent on their management. Meadows which have been regularly managed since 1990 have relatively stable species composition, species diversity and moderate increase of heliophilic species (MP 1, 2, 10). For MP 1 (column 5, Table 1) is characteristic the dominant occurrence of species *Trisetum flavescens* whose abundance has varied in the past 17 years (Table 3). *Luzula luzuloides* disappeared while during the period 1996–1999 its cover ranged from 3 to 2m; on the other hand, the abundance of *Agrostis capillaris* species increased. The dominant grass *Brachypodium pinnatum* occurring on MP 2 (column 5) is complemented by species *Agrostis capillaris* and *Festuca rubra*. The cover of *Luzula luzuloides* and *Cardaminopsis halleri* species decreased considerably (Table 3). *Dactylis glomerata* and *Trifolium repens* occurred with higher cover in 2013. The extremely species-rich plot MP 10 (column 7, Table 1) is remarkable by very high number of species per 25 m^2 ranged from 93 to 109. New relevés do not contain significant dominant species; therefore, Shanonn diversity index (2012: 4.29; 2013: 4.21) and value of evenness (2012: 0.93; 2013: 0.92) are high (Table 2). Abundance of the expansive grass *Brachypodium pinnatum* decreased from cover value 4 to 2b. This condition results from regular mowing since 1999 and restoration of some parts of meadows which keeps this plot in a favourable condition. MP 4 (column 7) is not mown every year, but on the other side, it is grazed by wild animals just like the abandoned MP 15 (column 7). Such moderate disturbance keeps these plots relatively stable. Community with subxerophilic character on MP 4 is mowed sporadically, every 2nd–3rd year. In the new relevés, the cover of *Brachypodium pinnatum* as a dominant species increased, but *Carex alba*, *C. panicea* and *Calamagrostis varia* species decreased considerably. MP 15 is the highest plot of the area. In new relevés abundance of *Agrostis capillaris* and *Festuca pratensis* species increased. This plot is species-rich; in 2013 eighty-five species were recorded but only 74 species in 1998, although Shannon diversity index was higher.

Abandoned plots recorded a decrease in the number of species and the emergence of expansive grasses which are dominant in the communities (MP 8, 14, 16). Mainly MP 14 (column 7, Table 1) is changed by expansion of *Calamagrostis varia* which replaced the dominant species *Agrostis capillaris*. The contact zone of the plot is affected by natural regeneration of *Picea abies* having the maximum height of 2 meters. Shannon diversity index decreased from values 4.25 or 4.26 to 3.86 in 2012 (Table 2). Ninety-three or ninety-five species occurred on 25 m^2 in 1998 and 1999, but only 76 in 2012. The absence of management indicates an increase in the abundance of *Carlina acaulis* species. In 2012 many rare species were not confirmed at all, e.g. *Ophioglossum vulgatum*, *Listera ovata*, *Lilium bulbiferum*, *Gymnadenia conopsea*, *Coeloglossum viride*. On the unmowed plot MP 16 (column 8) with the dominant species *Calamagrostis varia*, the diversity decreased moderately. The abundance of *Carex alba* species decreased from value 3 (1998) to 2a (2013) (Table 3). The unmanaged MP 8 with wetter soil is affected by expansion of *Brachypodium pinnatum*. *Dactylis glomerata* acted as subdominant species in new relevé whereas in old relevés it had only low abundance (Table 3). Decrease of species diversity is the result of abandonment of the plot throughout the years.

In the areas where cutting of trees, restoration of grassland (especially in the years 2003–2006) or sporadic mowing of meadows took place the number of species increased slightly (MP 5, 13, 11 – oligotrophic stands), although plots are still threatened by expansion of grass *Calamagrostis arundinacea* which displaces competitively weaker and more heliophilic species. Halada et al. (2010) argued that this grass reacts on restoration of grassland rather weakly. MP 5 (column 4) was abandoned, then mown irregularly about every 3rd–5th year, last time in 2011. In comparison with the old relevés *Luzula luzuloides*, *Hypercium maculatum* and *Fragaria vecsa* species have higher abundance. New relevés contain 68 species, which is on 8 or 9 more than in the old relevés (Table 2). Relevés have lower values

Table 2. The Shannon-Wiener index of diversity and values of ecological factors calculated according to ELLENBERG et al. (1992) for old and new phytocoenological relevés

Characteristic	Index*		Light		Temperature		Continentality		Moisture		Soil reaction		Nutrients		Number of species	
Relevés	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New
**Mp1	3.58	3.58	7.08	7.11	4.43	4.5	3.81	3.8	5.04	5.22	5.96	6.06	3.87	4.18	64.5	68
Mp2	3.49	3.62	7.06	7.15	4.56	4.51	3.8	3.79	4.74	4.77	5.99	5.84	3.67	3.72	68	63.5
Mp4	3.53	3.79	7.04	7.12	4.82	5.07	3.9	3.91	4.62	4.53	6.62	7.01	3.32	3.47	67.5	68
Mp5	3.36	3.66	6.94	6.74	4.66	4.84	3.64	3.74	4.78	4.92	5.83	5.79	3.88	4.09	59.5	68
Mp8	3.45	3.28	7.04	7.02	4.94	4.97	3.76	3.85	5.16	5.28	6.71	6.51	4.32	4.59	61.5	57
Mp10	4.08	4.25	7.07	7.13	4.88	4.81	3.89	3.8	4.69	4.69	6.48	6.48	3.68	3.65	98.6	97.5
Mp11	2.8	3.15	6.64	6.95	4.27	4.32	3.88	3.72	4.77	4.72	5.07	5.17	2.87	3.12	34	45.5
Mp13	3.39	3.76	6.61	7	4.67	4.7	3.7	3.84	4.83	4.73	5.5	5.76	3.83	3.52	63	74
Mp14	4.25	3.86	7.07	7.18	4.75	4.81	3.76	3.88	4.51	4.6	6.37	6.49	3.43	3.77	94	76
Mp15	3.99	3.95	7.07	6.97	4.46	4.66	3.83	3.8	4.58	4.66	6.35	6.27	3.42	3.44	74	82.5
Mp16	3.71	3.47	6.94	6.91	5.03	5.01	3.92	3.89	4.4	4.31	7.16	7.06	3.41	3.39	72	65

*Shannon-Wiener index, ** Monitoring plot.

Table 3. Changes of abundance of plant species during the years 1996–2013 (highlighted are significant differences)

Monitoring plot	1				2						4			5				8			10					11			13				14			15			16		
Year	1996	1999	2012	2013	1996	1997	1998	1999	2012	2013	1996	2012	2013	1996	1997	2012	2013	1996	1996	2012	1996	1997	2000	2012	2013	1996	2012	2013	1997	1999	2012	2013	1998	1999	2012	1998	2012	2013	1998	2012	2013
Species	Abundance																																								
Trisetum flavescens	3	a	a	4	1	1	1	+	.	+	.	.	.	.	.	.	.	+	.	.	+	+	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.
Avenula pubescens	a	1	3	a	a	a	1	+	1	a	.	+	+	.	.	.	.	1	m	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.
Agrostis capillaris	+	a	b	3	a	3	a	a	b	m	.	.	.	a	3	a	a	1	1	1	1	b	a	b	a	.	m	+	a	3	1	.	3	3	1	m	3	3	.	.	.
Dactylis glomerata	1	1	a	a	.	+	.	.	.	a	+	+	m	1	a	1	b	a	a	3	1	a	1	1	a	.	.	.	.	.	1	a	1	1	1	.	.	+	.	.	.
Crepis mollis	.	+	+	a	.	.	+	.	+	+	+	+	.	.	.	+	1	.	+	1	.	.	m	+	+	.	1	+	.	.	1	+	+	.	1	1	+	+	.	.	.
Arrhenatherum elatius	.	.	+	a	.	.	.	.	.	m	.	1	m	.	.	.	.	.	.	a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Gladiolus imbricatus	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	m	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium repens	.	.	.	a	.	.	+	+	+	a	.	+	m	+	.	1	m	.	.	.	.	+	a	+	m	.	.	.	+	.	1	m	.	+	1	.	+	+	.	.	.
Myosotis nemorosa	.	.	+	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Cerastium holosteoides	.	.	+	r	.	.	+	.	.	.	.	.	.	.	+	.	.	.	.	.	+	1	r	+	.	.	.	.	.	.	+	+	+	+	.	1	+	.	.	.	.
Pimpinella major	.	.	1	+	.	.	+	.	+	+	.	+	+	+	1	1	1	a	1	a	+	+	1	a	a	.	+	1	+	1	1	+	+	+	.	1	1	1	+	+	a
Crepis biennis	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trifolium flexuosum	.	.	.	a	.	.	.	.	+	m	.	.	m	.	.	.	+	.	.	.	.	1	.	.	.	.	.	.	a	.	.	.	.	.	+	.	.	.	.	.	.
Anthyllis vulneraria	1	+	.	.	+	+	.	+	.	.	+	+	+	.	.	+	.	+	.	.	1	b	1	1	b	+	.	.	.	+	1	+	1	1	1	1	1	+	1	a	1
Polygala vulgaris	+	+	.	.	+	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Luzula luzuloides	3	m	+	.	a	3	a	m	a	+	+	.	.	a	a	b	3	.	.	+	1	a	1	+	.	1	b	m	b	a	b	a	.	.	1	+	a	+	.	.	.
Cardaminopsis halleri	1	m	1	+	a	3	b	m	1	+	1	+	+	a	3	1	m	.	+	+	+	a	b	1	+	+	+	+	a	m	1	m	+	+	1	1	1	+	+	+	m
Orchis ustulata	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dactylorhiza sambucina	.	.	.	.	.	.	.	.	r	r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Clinopodium vulgare	.	.	.	.	.	.	.	.	+	+	.	.	.	+	1	1	+	.	.	.	.	.	.	1	+	.	.	.	.	.	1	+	.	.	+	.	+	+	.	1	+
Ranunculus acris	1	+	a	+	.	.	.	.	+	+	+	+	+	+	a	.	+	+	1	+	+	.	r	+	+	.	.	.	+	.	.	+	+	+	.	1	1	+	.	+	.
Gymnadenia conopsea	r	+	+	.	+	r	+	+	.	.	+	+	r	.	.	.	.	.	+	.	.	.	+	+	.	1	r	+	.	r	+	.	+	+	.	+	.	.	+	.	.
Coeloglossum viride	.	.	.	.	r	r	r	.	.	.	r	.	.	.	.	.	.	.	.	.	+	+	+	.	.	.	.	.	+	.	.	.	+	+	.	+	.	.	.	.	.
Avenella flexuosa	.	.	.	.	+	1	+	.	.	.	.	.	.	+	1	+	a	.	.	.	.	.	.	.	.	a	b	3	1	+	a	b	+	.	.	.	+	.	.	.	.
Listera ovata	.	.	.	.	r	.	+	.	.	.	+	+	+	.	.	.	.	.	.	.	+	+	r	.	.	.	.	.	.	+	.	.	+	r	.	.	.	.	+	.	+
Carex ornithopoda	.	.	.	.	.	+	+	.	.	.	+	.	.	.	.	.	.	.	.	.	+	1	1	1	.	.	.	.	.	.	.	.	1	1	+	+	.	+	1	.	.
Gentianella lutescens	.	.	.	.	+	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	1	+	.	1	.	.	.	.	.
Taraxacum sp.	1	1	+	+	1	+	+	+	.	.	+	.	.	.	.	.	.	1	1	.	+	a	m	+	+	.	.	.	.	.	.	.	+	1	+	+	.	.	+	.	.
Brachypodium pinnatum	1	+	.	m	4	4	4	3	3	3	a	a	3	.	.	.	.	4	4	4	3	3	4	+	b	.	.	.	.	.	.	.	+	+	.	.	.	.	+	1	b
Carex alba	.	.	.	.	.	.	+	.	.	.	3	a	m	.	.	.	.	.	.	+	1	a	m	b	b	.	.	.	.	.	.	.	1	a	b	m	b	a	3	a	b
Carex panicea	.	.	.	+	.	.	.	.	.	.	3	+	+	.	.	.	.	.	+	.	1	3	m	a	+	.	.	.	.	.	.	.	.	.	+	+	+	m	.	.	.
Potentilla erecta	a	m	b	m	1	b	m	m	1	m	+	a	a	+	+	m	+	1	+	1	+	+	+	+	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.

Table 3. Changes of abundance of plant species during the years 1996–2013 (highlighted are significant differences) – continued

Monitoring plot	1				2						4			5				8			10					11			13				14			15			16		
Year	1996	1999	2012	2013	1996	1997	1998	1999	2012	2013	1996	2012	2013	1996	1997	2012	2013	1996	1996	2012	1996	1997	2000	2012	2013	1996	2012	2013	1997	1999	2012	2013	1998	1999	2012	1998	2012	2013	1998	2012	2013
Species	Abundance																																								
Campanula glomerata	.	.	.	.	.	.	.	.	.	.	.	**1**	+	.	.	+	+	.	.	.	+	+	1	1	+	.	.	.	.	.	.	r	+	+	+	.	.	.	1	1	1
Erysimum witmannii	.	.	.	.	.	.	.	.	.	.	.	.	**r**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+
Galium boreale	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Salvia verticillata	.	.	.	.	.	.	.	.	.	.	.	+	**1**	.	.	.	.	.	.	.	+	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex flacca	.	.	+	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	+	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pimpinella saxifraga	1	m	b	m	1	a	a	m	a	m	.	+	**m**	1	1	+	1	.	+	.	+	+	a	a	+	+	1	+	1	1	1	+	+	+	1	m	1	+	+	.	.
Viola hirta	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
Origanum vulgare	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
Potentilla heptaphylla	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	**1**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Bupleurum falcatum	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Trollius altissimus	a	a	a	a	.	.	.	.	.	.	+	.	.	.	.	+	.	a	1	a	.	.	r	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Gentiana cruciata	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.
Salvia pratensis	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Festuca pallens	.	.	.	.	.	+	.	.	.	.	**1**	+	.	.	.	.	.	.	.	.	+	+	+	.	.	.	.	.	.	.	+	.	.	1	1	.	.	.	.	**a**	+
Knautia maxima	r	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	r	.	+	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	+	.	.	.	.
Cardaminopsis arenosa	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	.	.
Hypericum maculatum	1	+	1	+	+	+	+	+	+	+	.	+	.	**1**	**1**	**b**	**3**	+	+	+	+	+	+	1	+	.	.	.	+	+	**b**	**a**	+	**1**	.	.	.	**1**	.	.	.
Fragaria vesca	.	.	.	.	.	.	.	.	.	.	+	+	m	+	+	**b**	**3**	.	.	.	+	1	1	1	m	.	.	.	+	+	1	.	m	m	1	.	+	+	+	+	+
Festuca rubra	4	3	3	3	3	b	3	a	b	b	.	.	.	+	.	**a**	**a**	.	.	.	.	.	m	a	m	.	**1**	+	+	.	**1**	**a**	m	1	1	1	1	.	+	.	.
Pyrethrum clusii	1	.	+	.	a	a	a	1	1	b	+	a	m	**a**	**a**	**1**	+	.	.	**1**	+	+	1	+	1	1	1	1	1	1	+	+	1	1	1	+	+	+	+	+	+
Campanula persicifolia	.	.	.	.	.	.	.	.	.	.	+	.	+	+	+	**1**	**a**	.	+	.	+	1	m	.	+	+	+	+	+	1	+	m	+	+	.	.	.	.	1	1	m
Angelica sylvestris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	**1**	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Carex pallescens	+	+	1	+	+	a	+	+	1	m	.	.	.	.	.	+	+	.	.	**1**	.	1	+	+	+	.	.	.	+	+	.	+	.	.	.	.	.	.	.	.	.
Heracleum sphondylium	.	.	.	.	+	+	r	+	.	+	.	.	.	.	.	**1**	+	+	1	1	+	+	+	+	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.
Briza media	+	1	1	m	1	1	1	+	1	+	1	1	m	.	.	.	**a**	**1**	**1**	.	**1**	**a**	**m**	**1**	**b**	.	**1**	**m**	+	+	**1**	**a**	m	1	1	1	a	+	+	+	.
Convallaria majalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	+	.	+	**1**	.	.	.	.	.	.	.	.	+	+	+
Rubus idaeus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**1**	**1**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rosa canina agg.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**r**	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+
Asarum europaeum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	**1**	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	+	+	+
Viola riviniana	.	.	.	.	.	.	.	+	.	.	.	.	+	.	.	**m**	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
Mercurialis perennis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	**r**	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	+	r	.
Polygonatum verticillatum	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	+	.	.	.	.	+	r	+	+	.	+	+	.	.	+	+	.	r	1	+	r	+	+	+	+
Festuca pratensis	1	m	.	+	+	.	.	.	+	+	.	.	+	.	.	**1**	**a**	+	1	+	**1**	**1**	**m**	**b**	**b**	.	.	.	.	.	+	.	1	1	1	**1**	**b**	**b**	.	.	.
Galium mollugo	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	**1**	+	+	1	+	+	.	+	+	1	.	.	.	.	.	**1**	+	+	+	**3**	.	**1**	**m**	+	**a**	**m**
Lotus corniculatus	**3**	**m**	**b**	+	3	b	3	m	b	m	1	a	m	**1**	**a**	**m**	.	a	+	1	1	3	m	b	m	1	1	m	a	1	1	+	m	m	1	1	+	+	+	1	m
Rhinanthus serotinus	1	m	a	a	a	1	3	3	1	3	a	1	m	+	+	.	.	**1**	+	.	.	+	+	**b**	**m**	.	**1**	**b**	1	+	+	m	.	.	.	.	+	.	.	.	.
Trifolium montanum	1	+	1	.	3	3	a	m	a	b	a	a	m	+	+	.	.	.	.	+	1	a	m	1	m	.	.	.	.	.	+	**a**	1	m	1	m	1	m	+	+	.
Digitalis grandiflora	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	**a**	**b**
Trifolium badium	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Poa pratensis	.	+	.	.	+	.	.	+	.	.	.	.	.	+	**1**	.	.	**a**	**1**	.	.	.	1	.	.	.	.	.	.	+	.	.	.	1	.	.	.	+	.	.	.
Deschampsia cespitosa	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	+	**a**	.	.	.	.	+	.	.	.	.	.	.	+	.	+	.	.	**1**	**a**	.	.	.
Chaerophyllum aromaticum	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	**a**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Acetosa pratensis	1	1	1	+	+	+	+	+	+	+	.	.	.	+	1	+	1	.	.	+	+	1	1	+	1	.	.	.	+	+	1	+	+	+	+	+	+	+	.	.	.
Roegneria canina	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**1**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Geranium pratense	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Ajuga reptans	.	.	.	.	.	.	.	+	+	+	.	.	+	+	1	+	+	.	.	+	+	+	1	1	+	.	.	.	+	.	+	.	+	+	.	.	.	+	.	.	.
Crepis praemorsa	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	+	+	.	+	.	.	.	1	.	.	.	+	.	.	.	.	.	.
Vicia sylvatica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.
Cirsium erisithales	.	.	.	.	.	.	.	.	.	.	+	.	.	1	1	+	+	.	.	**r**	.	r	.	.	.	.	.	.	.	+	+	+	+	+	.	.	+	+	+	1	1
Jacea phrygia	1	1	b	a	1	1	1	1	1	1	1	1	1	1	a	1	1	**1**	**1**	.	**1**	+	**1**	**a**	**a**	.	.	+	+	**1**	**1**	**a**	1	a	1	1	1	1	1	1	1
Ranunculus auricomus	+	+	+	.	.	.	.	.	.	.	.	.	.	+	.	+	.	+	**1**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Phyteuma orbiculare	1	1	+	r	1	b	1	1	+	+	1	+	+	+	+	1	+	+	+	.	**1**	**a**	**b**	**a**	+	.	+	+	1	+	.	+	m	m	1	1	+	+	+	1	+
Trifolium pratense	1	+	a	1	+	1	+	+	1	+	+	.	+	+	1	1	m	+	+	.	1	+	m	a	m	.	.	.	+	+	.	+	m	1	1	1	+	.	+	+	+
Plantago lanceolata	+	+	+	.	.	.	.	.	.	.	+	+	.	.	.	.	.	+	+	.	+	+	m	1	1	.	.	.	.	.	.	.	**m**	**m**	+	**1**	**a**	**a**	.	.	.
Tragopogon orientalis	1	1	1	+	+	1	+	+	+	+	+	.	+	.	.	.	.	**1**	+	.	1	+	1	+	1	.	.	.	.	.	.	.	**1**	+	.	1	+	m	+	.	.
Potentilla inclinata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	**1**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Colymbada scabiosa	.	.	.	.	.	.	.	.	.	.	a	b	b	.	.	.	.	.	.	.	+	+	+	+	**a**	.	.	.	.	.	.	.	+	**1**	**a**	+	.	+	+	+	+
Thymus pulegioides	1	+	+	+	1	+	+	+	+	+	+	1	m	1	+	1	+	+	.	.	+	**b**	**a**	**1**	+	.	**1**	+	1	+	+	m	m	m	1	**m**	**m**	**b**	1	+	m

Table 3. Changes of abundance of plant species during the years 1996–2013 (highlighted are significant differences) – continued

Monitoring plot	1	2	4	5	8	10	11	13	14	15	16
Year	1996 1999 2012 2013	1996 1997 1998 1999 2012 2013	1996 2012 2013	1996 1997 2012 2013	1996 1996 2012	1996 1997 2000 2012 2013	1996 2012 2013	1997 1999 2012 2013	1998 1999 2012	1998 2012 2013	1998 2012 2013
Species	Abundance										
Festuca ovina	. . . m	. 1 . 1 . a	1 1 .	+ + 1 m	. . .	1 3 3 a +	1 3 a	1 a 1 m	m m .	a b m	+ . +
Bromus erectus	. . . .	. . . . . .	. . .	. . . .	. . .	. . . + +	. . .	. . . .	. . .	. . .	. . .
Tussilago farfara	. . . +	. . . . . .	. . .	. . . .	+ + 1	. . . + +	. . .	. . . .	. . .	. . .	. . .
Euphrasia rostkoviana	. . . .	. . . . . .	. . .	. . . .	. . .	+ + 1 . .	. . .	. . . .	+ 1 .	m . .	. . .
Aquilegia vulgaris	. . . .	. . . . . .	. . .	+ + . .	+ . .	+ . + . .	. . .	. . + +	+ + .	+ . +	. . .
Scabiosa lucida	. . . .	. . . + . .	+ + +	+ + . .	+ . .	+ 1 1 . .	. + .	. . . .	+ + +	+ + 1	. + .
Pilosella cymosa	. . . .	. . . . . .	. . .	. . . .	. . .	. + + . .	. + .	. . . .	. . .	. . .	. . .
Ophioglossum vulgatum	. . . .	. . . . . .	1 + +	. . . .	. . .	+ . + . .	. . .	. . . .	1 1 .	. . r	+ . .
Hieracium murorum	. . . .	. . . . . .	. . .	. . . .	. . .	. + r . .	1 . .	+ + . .	. . .	. . .	. + +
Trommsdorfia maculata	. . . .	. . . . . .	. . .	. . . .	. . .	1 + . . .	. . .	. . . .	. . .	. . .	. . .
Antennaria dioica	. . . .	. . . . . .	. . .	. . . .	. . .	. + r . .	+ . .	. . . .	1 + .	r . .	. . .
Carlina acaulis	+ . . +	1 a 1 1 a 1	1 1 1	1 1 1 1	+ + 1	1 1 1 + +	+ 1 3	a 1 a b	+ 1 a	+ a 1	+ + 1
Nardus stricta	. . . .	. + . . + .	. . .	. . . .	. . .	. . . . .	. 1 a	. . . .	1 + .	. . .	. . .
Trientalis europaea	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. m m	. . . .	. . .	. . .	. . .
Crepis conyzifolia	+ + + +	+ + + 1 1 a	. . .	. . . .	. . +	+ + . + +	. a +	+ 1 1 +	+ + 1	+ . .	. . .
Leontodon hispidus ssp. *danubialis*	. 1 1 1	. . 1 m + .	. + 1	. . . .	. . .	. . m 1 1	. 1 1	. . 1 +	1 m a	1 . .	1 + 1
Leontodon hispidus ssp. *hispidus*	1 m a +	a b m 1 a 1	1 + 1	+ + + .	. + .	a 3 a a 1	. 1 1	+ . 1 1	1 m a	m 1 +	+ . 1
Veronica chamaedrys	1 + 1 m	+ 1 1 1 + +	+ . .	a a 1 m	1 1 1	+ 1 1 + +	. + m	+ + 1 +	1 1 1	+ + +	+ + m
Gymnadenia densiflora	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. r r	. . . .	. . .	. . .	. . .
Dianthus carthusianorum	+ . . .	1 + + + + +	+ + +	. . . .	. . .	1 1 1 1 +	. + +	+ + + +	+ + +	1 1 m	1 + m
Ranunculus polyanthemos	1 + + +	1 a + 1 + +	1 + +	1 1 1 +	. . .	1 1 1 1 1	. + +	1 1 1 +	1 m 1	1 + +	+ + +
Potentilla aurea	+ . . .	+ + 1 + + +	. . .	. + . .	. . .	+ + 1 + +	. 1 m	+ + . +	1 1 .	m + m	. . .
Anthoxanthum odoratum	1 + 1 m	a a m + 1 a	+ . .	1 + + +	. . .	1 1 m a b	. . a	+ + + a	1 1 +	r + +	. . .
Astrantia major	. 1 1 a	+ + + + + +	. . .	. + 1 1	+ 1 a	+ + + + +	+ . .	1 + 1 1	+ + .	+ + +	+ 1 1
Homogyne alpina	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	+ . .	. . . .	. . .	. . .	. . .
Gentiana asclepiadea	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	+ . .	. . . .	. . .	. . .	. . .
Vaccinium myrtillus	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	b a m	1 m a b	. . .	. . .	. . .
Poa chaixii	. . . .	. . . . . .	. . .	a 3 b b	. . .	. . . . .	. . .	+ + 1 3	. . .	. . .	. . .
Helianthemum grandiflorum ssp.. *obscurum*	a . . m	a b m m b m	. . .	1 1 1 m	. . .	+	. . .	+ 1 a a	a a 1	. . .	. + +
Stellaria graminea	+ + 1 +	+ + + + . +	. . .	. 1 + .	+ . +	+ 1 + + +	. . +	. . 1 +	+ + .	m + .	. . .
Populus tremula	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. . .	. . + +	. . .	. . .	. . .
Picea abies	. . . .	r + r . + r	r + .	. . . .	. . .	. . . . .	. . .	. . + +	. r +	. + +	. . .
Botrychium lunaria	+ . . .	+ + + + r .	. . .	+ + . .	. . .	. . . . .	+ 1 +	. . + +	+ + r	+ r +	. . .
Lilium bulbiferum	. . . .	. . . . . .	. . .	. . . .	. . .	+ + + + r	. . .	. . r +	+ + .	+ . .	r r +
Prunella vulgaris	. . . .	. . . . . .	. . .	. 1 . +	+ + +	1 1 m a m	. . .	. . + +	m 1 +	+ + +	. . .
Epilobium collinum	. . . .	. . . . . .	. . .	. . . +	. . .	. . . . .	. . .	. . + +	. . .	. . .	. . .
Laserpitium latifolium	. . . .	. . . . . .	. . .	. . + .	. . .	. . . . .	. . .	. . + +	. . .	. . .	+ + +
Trommsdorfia uniflora	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	a + b	a . + .	. . .	. . .	. . .
Soldanella hungarica	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	1 . +	+ + . .	. . .	. . .	. . .
Galium pumilum	. . . .	. . . . . .	+ . +	. . . .	. . .	. . + . +	. . +	+ + . .	+ + .	1 . m	1 . +
Viola reichenbachiana	. . . .	. . . . . .	. . .	. + . .	+ . .	. . . . .	. . .	+ 1 . .	. . .	. . .	. + .
Primula elatior	a m a +	+ + + + + +	+ . .	+ + + +	+ 1 1	+ + + 1 +	. . .	1 + . .	+ + .	1 . .	+ . +
Aconitum firmum	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. . .	r + . .	. . .	. . .	. . .
Calamagrostis varia	+ . . .	1 + + + 1 .	3 3 m	. . . .	. . .	+ 1 1 1 a	. . .	. . . .	1 a 3	1 b m	3 4 4
Thalictrum aquilegiifolium	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. . .	. . . .	. . r	+ . .	. . .
Euphrasia rostkoviana ssp. *montana*	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. . .	. . . .	. . +	. a +	. . .
Campanula patula	+ + . +	+ + + + + +	. . .	1 1 1 m	. + +	. . . . +	. . .	+ 1 + m	. . +	. . .	. . .
Carum carvi	1 + + +	1 . + . + +	. . .	. . . .	+ 1 +	1 + b 1 +	. + .	. . + .	. . 1	. . .	. . .
Crocus discolor	. + + +	. 1 + 1 + .	. . .	. + . .	. . .	. + + + .	. . .	. . . .	+ + .	+ . .	. . .
Thesium alpinum	. . . .	+ r + + + +	. . .	+ . + .	. . .	+ + + 1 +	. . .	+ . + +	+ + .	+ . .	. + +
Polygala amara	. . . +	. + . . . +	. . +	. . . .	. . .	+ + r + +	. . .	. . . +	+ + .	+ + +	+ . .
Pilosella bauhinii	. . . .	+	. . .	. . . .	. . .	1 1 m + +	. . .	. . . .	+ + .	. . +	+ + .
Danthonia decumbens	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. . .	. . . .	+ + .	. . .	. . .
Festuca tatrae	. . . .	. . . . . .	. . .	. . . .	. . .	. . . . .	. . .	. . . .	1 . .	m a b	+ . .

Table 3. Changes of abundance of plant species during the years 1996–2013 (highlighted are significant differences) – continued

Monitoring plot	1				2						4			5				8			10					11			13				14			15			16		
Year	1996	1999	2012	2013	1996	1997	1998	1999	2012	2013	1996	2012	2013	1996	1997	2012	2013	1996	1996	2012	1996	1997	2000	2012	2013	1996	2012	2013	1997	1999	2012	2013	1998	1999	2012	1998	2012	2013	1998	2012	2013
Species	Abundance																																								
Alchemilla sp.	3	m	b	b	1	a	m	m	1	1	.	.	1	a	a	1	a	b	1	1	1	a	a	a	a	.	1	+	**.**	**.**	**1**	**1**	a	m	a	**3**	**a**	**1**	.	.	.
Acinos alpinus	.	.	.	.	.	.	.	.	.	.	+	+	m	.	.	.	.	.	.	.	+	1	+	1	m	.	.	.	.	.	.	.	+	+	+	**+**	**1**	**b**	m	1	+
Sesleria albicans	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**.**	**a**	**a**	.	.	.
Arabis hirsuta	.	.	.	.	.	.	.	.	.	.	+	r	+	.	.	.	.	.	.	.	+	.	.	+	+	.	.	.	.	.	.	.	+	.	.	**.**	**+**	**+**	+	+	+
Calamagrostis arundinacea	.	.	.	.	.	.	.	.	.	.	.	.	.	4	4	3	3	.	.	.	.	.	.	.	.	3	3	3	4	4	4	b	.	.	.	**.**	**1**	**+**	.	.	.
Viola lutea ssp. *sudetica*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**.**	**r**	**r**	.	.	.
Vicia cracca	+	m	+	+	+	1	+	+	+	+	+	+	+	+	+	+	+	1	+	1	1	1	b	1	+	.	.	.	+	+	+	+	.	.	.	**.**	**1**	**+**	+	+	.
Acer pseudoplatanus	.	.	.	.	r	r	r	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**.**	**r**	**r**	.	.	.
Lathyrus pratensis	+	1	1	+	1	+	+	+	+	+	.	.	.	+	1	1	m	a	a	1	1	+	m	+	+	.	.	.	+	.	1	+	.	+	+	**.**	**1**	**+**	1	+	+
Parnassia palustris	.	1	+	+	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**.**	**+**	**+**	.	.	.
Koeleria pyramidata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**.**	**a**	**m**	.	.	.
Larix decidua	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	r	.	.	.	.	.	.	.	.	.	**.**	**r**	**r**	.	.	.
Viola rupestris	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	+	.	.	+	.	.
Carduus glaucinus	.	.	.	.	.	.	+	.	.	.	1	1	a	.	.	.	.	.	.	.	+	+	+	+	+	.	.	.	.	.	.	.	+	+	+	+	.	+	**1**	**1**	**a**
Phyteuma spicatum	.	+	.	.	+	.	.	+	.	.	.	+	.	.	.	.	r	**1**	**+**	**.**	+	.	.	+	+	+	+	.	+	+	.	+	**.**	**.**	**1**	.	+	.	**.**	**+**	**+**
Knautia arvensis	.	.	.	r	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	1	+	.	.	.	.	.	.	.	.	.	.	.	.	+	**.**	**+**	**1**
Erysimum odoratum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**.**	**.**	**+**
Orobanche reticulata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**.**	**r**	**r**
Anacamptis pyramidalis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**+**	**.**	**+**
Hieracium bifidum	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**+**	**.**	**.**
Carex caryophyllea	1	+	+	.	1	+	1	m	1	a	+	.	+	.	.	.	.	.	.	.	1	b	a	a	+	.	.	.	.	.	.	.	m	1	1	1	.	+	**1**	**.**	**.**
Viola collina	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	**+**	**.**	**.**

of Ellenberg`s ecological indices for light, soil reaction, temperature as well as Shannon diversity index, because soil is moderately acidic and the spreading of more termophilic and heliophilic species in the lower layer is inhibited by the expansion of *Calamagrostis arundinacea* species. The oligotrophic sward on MP 13 (column 4) is influenced by the dominance of *Calamagrostis arundinacea* as well although its abundance decreased in 2013. The abundance of *Poa chaixii* increased and along with *Calamagrostis arundinacea* species it forms physiognomy of community (Table 3). Shannon diversity index is higher in new relevés (Table 2). The above mentioned plot is mown irregularly about every 3rd–5th year, last time in July 2011. The species-poorer MP 11 (column 1) with oligotrophic character contains a higher number of acidophilous species. In new relevés dominate species *Avenella flexuosa*, *Calamagrostis arundinacea*, *Carlina acaulis* and *Pulsatilla scherfelii*. In comparison with 1996, species diversity has increased, which may be the result of grassland restoration and irregular mowing in this part of meadows. Relevé in 1996 had lower EII for light. All relevés have low EII for soil reaction, light, temperature and Shannon diversity index (Table 2), which is associated with the occurrence of typical psychrophilous mountain and acidophilous species (e.g. *Trientalis europaea, Nardus stricta, Potentilla aurea*).

Some plots where Dražil (2004) recorded relevés during the years 1996–1999 have been already overgrown by a closed spruce forest with almost no undergrowth. Kopanecké lúky is a remarkable site from the point of view of species diversity resulting from the traditional land-use in the past. Nowadays, in the past formed communities are in various stages of secondary succession, which is reflected by changed character resulting from the expansion of competing grasses and their lower syntaxonomical representativeness. Further development of the meadows is dependent on appropriate management which is crucial for the preservation of their biodiversity.

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Short communication

Sordaria fimicola (Ascomycota, Sordariales) on *Acer palmatum*

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Abstract

Ivanová, H., 2015. *Sordaria fimicola* (Ascomycota, Sordariales) on *Acer palmatum*. *Folia Oecologica*, 42: 67–71.

During an investigation of the mycoflora of Japanese red maple trees growing in an urbanized area of Nitra, Slovakia, *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not) was isolated from affected leaves and branches of *Acer palmatum* ´Atropurpureum´ L. This fungus was associated with symptoms of brown wood discoloration and leaf spottiness of these trees. *Sordaria fimicola* was recorded for the first time on *Acer palmatum* ´Atropurpureum´ in Slovakia. The fungus is characterized by dark brown ascomata clothed with setae, fasciculate, unitunicate, cylindrical asci, and olivaceous to olivaceous-brown, one-celled, ellipsoidal, smooth-walled ascospores with a colourless basal germ pore and surrounded by a gelatinous sheath**.**

Keywords

ascomycetes, Japanese red maple, pathogenic fungus

Introduction

In recent years stems and branches of woody hosts such as fruit and ornamental trees have been shown to share the same range of fungi, which are able to migrate between these different hosts (Mostert et al., 2005, 2006; Essakhi et al., 2008). However, most of these genera are typical inhabitants of wood and bark, occurring on a broad spectrum of trees and shrubs worldwide (Schoch et al., 2009; Zhang et al., 2009).

These fungi associated with symptoms of brown wood discoloration and leaf spottiness include several ascomycetes such as *Sordaria*. Several members of this order have been isolated from remains of plant biomass, live plants, from seeds and from soil (Lundqvist, 1972; Doveri, 2004; Richardson, 2008) and they are important candidates for studies in genetics and biochemistry (Kendrick, 2000). Natural habitat of this species is dung of herbivorous animals (Fields, 1970).

The aim of this work was to isolate and identify the organism occurring on infected Japanese red maple leaves and branches in green areas of Nitra.

Material and methods

The samples of leaves and twigs of *Acer palmatum* ´Atropurpureum´ showing blight symptoms were gathered from plants growing in private gardens of the town Nitra, during summer–autumn 2013 and summer 2014. Altogether 15 trees were studied. The age of evaluated trees was between 5–10 years. The collected material was deposed in herbarium at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plant Biology in Nitra. [*Sordaria fimicola*, *Acer palmatum* ´Atropurpureum´, Slovakia, Nitra, Zobor-hill, 2. July 2013, leg. H. Ivanová (IFE SAS, Nitra, Slovakia, NR 5190)]. Pure cultures were obtained through cultivation on nutritive 3% PDA medium in a test chamber with constant temperature and humidity (24 ± 1 °C and 45% humidity in dark conditions in a versatile environmental test chamber MLR-351H – Sanyo). Leaves and twigs cut from the diseased plants were surface-sterilized for 20 minutes. Study of fungal structures was performed with a light clinical microscope BX41 (Olympus) under 400× and 1,000×

magnification. Measurements were made using QuickPhotomicro 2.2 programme and the morphometric values were compared with previously published data for the taxa (Lundquist, 1972; Alexopoulos et al., 1996; Crous et al., 2009).

Results and discussion

Many fungal diseases cause damage to ornamental tree species in the genus *Acer*, including *Acer palmatum* ´Atropurpureum´. Among pathogenic fungi, microscopic pathogens isolated and identified from the affected leaf and branch tissues include ascomycetous fungi in the genus *Sordaria*. The causal organism – *Sordaria fimicola* (Roberge) Ces. & De Not., (syn. *Sphaeria fimicola* Roberge in Desm.) (Sordariomycetes, Ascomycota) was systematically isolated from the leaf and twig tissues showing rusty to brown coloured blight symptoms. Microscopic examination of fresh material indicated that the ascomycete fungus from Slovakia fits well within the genus *Sordaria*. Thick-walled, obpyriform, densely aggregated ascomata, as well as olivaceous, dark brown ascospores at maturity with a basal germ pore suggest this fungus is *Sordaria fimicola* on *Acer palmatum* ´Atropurpureum´.

White at the beginning homothallic colonies fast growing on PDA (Fig. 1a) formed sparse aerial mycelium pale white colour. Dark, mostly densely aggregated superficial, obpyriform pycnidia were formed after 1 week of inoculation in dark conditions (Fig. 1b). Vegetative hyphae were thin-walled, septate, branched (Fig. 1c), lacking chlamydospores. Macroconidia did not discover. The ascomata were superficial, glabrus or sparsely covered with flexuous, colourless hairs (Fig. 1d), pear-shaped or obpyriform, with central ostiole (Fig. 1e). The ascocarps walls were thick, composed of several layers, on the outer surface with hyaline, straight or bent short setae 80–100 × 6 μm in size, sometimes shorter. Paraphyses absent, periphyses lining the ostiole. Asci (Figs 1f, g) with eight uniseriate ascospores on ascus arranged obliquely and formed rosettes (Figs 1h, i), growing from the bottom of the perithecium. Ascospores were olivaceous to olivaceous-brown, aseptate with a colourless basal germ pore (Fig. 1j), immature were granular (Fig. 1k), mature ascospores (Fig. 1m) were brown and ellipsoidal to obovoid.

According to Alexopoulos et al. (1996) and García et al. (2004) species are characterized by black globose or flask-shaped solitary perithecia, which are ostiolate, usually with stiff setae, forming cylindrical asci with an apical ring. The ascus apex usually has one or several germ pores and a refractive ring through which the ascospores are discharged. *Sordaria* species have smooth-walled, dark brown ascospores, generally aseptate, with the surface smooth, pitted, reticulate or striate, sheathed or unsheathed. Spores surrounded gelatinous sheath which is sometimes thick and conspicuous, or it is difficult to detect. Darkly pigmented ascospores show wide variation in the kinds of appendages or sheaths. (Important aspect of the life cycle of this species is that no macroconidia are formed). Microconidia are produced, functioning as male gametes in sexual reproduction. Germination of microconidia may occur, but it is very poor.

When compared morphologically with the species occurring on different trees reported in the literature (Lundquist, 1972; Doveri, 2004), *Sordaria fimicola* differs from *S. macrospora* in having smaller spores, ellipsoidal rather than broadly ellipsoidal and smaller perithecia and asci (Crous et al., 2009). Comparison of the main morphological characteristics of *S. fimicola* identified on different hosts and examined material is described in Table 1.

Up to now, fungus *Fusarium* sp. has been known as an opportunistic and quite common pathogen associated with affected Japanese red maple leaves and branches. This fungus, which caused *Fusarium* wilt disease, remains in infested soils for up to ten year. Wilts may be contracted through infected seed, plant debris or soil. The fungus begins and multiplies during the cool, moist season, becoming obvious when weather turns warm and dry. Plants wilt because the fungus damages their water conducting mechanisms (Ivanová, 2013). Important finding is that *S. fimicola* was identified for the first time as a new pathogenic fungus associated with infected *Acer palmatum* ´Atropurpureum´ in Slovakia. Further studies are required for determination of pathogenicity and relevance of *Sordaria* infection in connection with Japanese red maple tree damage.

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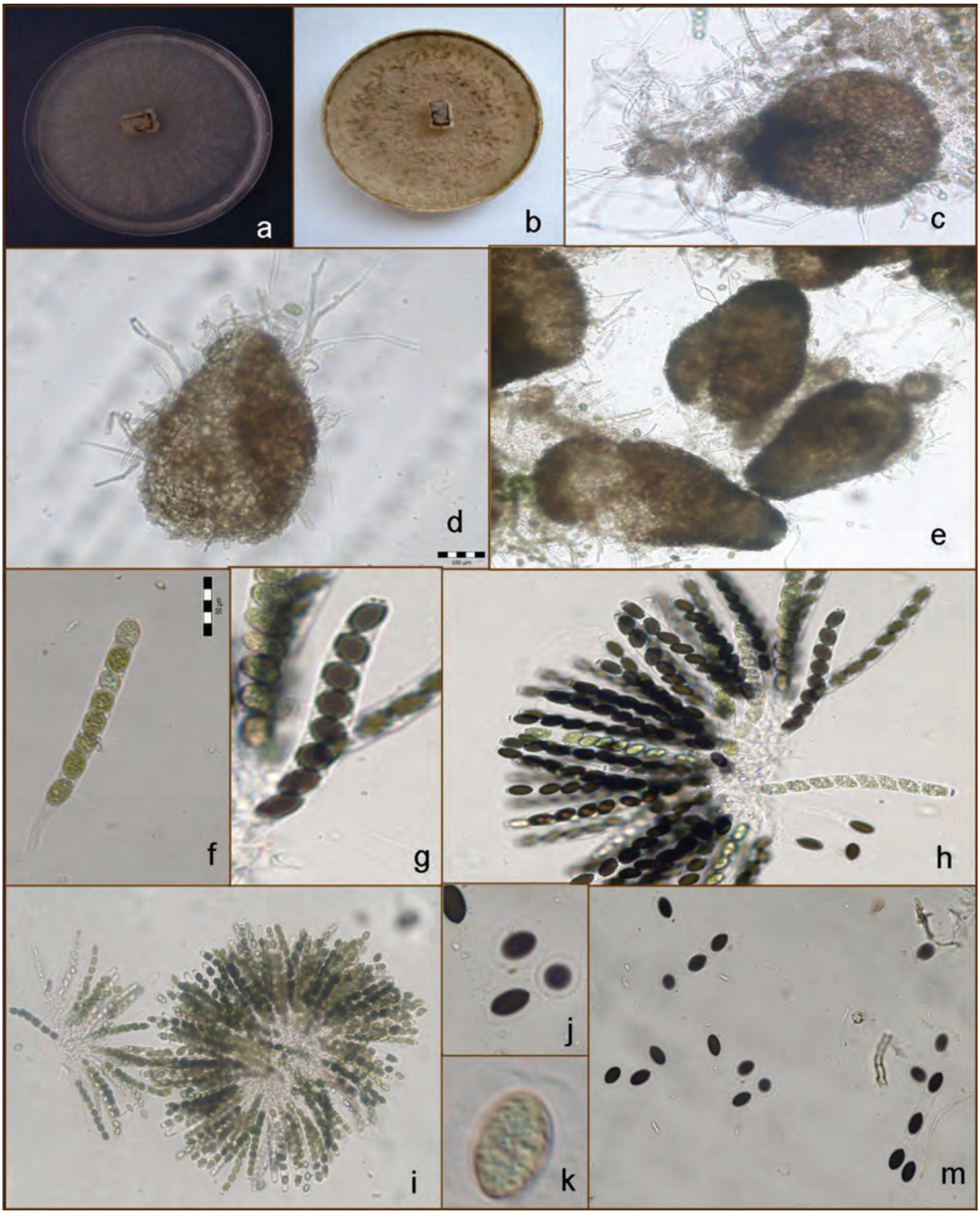

Fig. 1. *Sordaria fimicola* on *Acer palmatum* ´Atropurpureum´ – a, colony of *S. fimicola* on PDA; b, dark pycnidia after 1 week of inoculation; c, hyaline vegetative hyphae and dark pycnidium; d, ascomata of *S. fimicola* with colourless hairs; e, mature pear-shaped ascomata of *S. fimicola;* f, immature ascus; g, mature ascus with small inamyloid apical ring; h, rosettes of unitunicate asci of *S. fimicola;* i, rosettes of asci of *S. fimicola*; j, mature ascospores surrounded by a gelatinous sheath (detail); k, immature ascospore with granular content (detail); m, masses of mature ascospores of *S. fimicola*. Scale bars: i, k = 10 µm, g, j = 20 µm, f, h, m = 50 µm, c, d, e = 100 µm.

Table 1. Comparison of morphological characteristics of *Sordaria fimicola* identified on different hosts and on examined material

Authors	**Sampled plant material under examination**	**Lundquist, 1972**	**Mungai et al., 2012**
Host plant	*Acer palmatum*		
Causal agent	*Sordaria fimicola*	*Sordaria fimicola*	*Sordaria fimicola*
Ascomata	Perithecial, subglobose to pyriform, solitary, 370 × 320 µm, neck 100–160 µm	Mostly densely aggregated, superficial, obpyriform, glabrus 360–420 × 240–325 µm, papilose, neck cylindrical, 120–240 × 100 µm	Perithecium, semi-immersed to superficial, 550–620 µm high., dark brown, ovoid to pyriform, neck conical or subcylindrical 111–120 × 120–150 µm
Setae	Brown or hyaline, straight setae 80–100 × 6 µm	Flexous colourless hairs	Hyphoid hairs sparsely covered ascomata
Paraph.	Absent	Not observed	Moniliform, septate with segments 4.5–12.5 µm broad, abundant, containing hyaline vacuoles
Asci	150 (165) × 15 µm, fasciculate, unitunicate, cylindric, with a truncate apex and small apical rings, 8 ascospores/ascus	Cylindrical, short-stipitate, 8-spored, in a single row, apical ring, (155–) 170–215 × 14–17 µm, with a truncate 9 µm apex	8-spored, 111–163 × 10.5–14 µm, cylindrical, flattened at apex, short stipitate, with a lobate stipe and prominent apical apparatus
Ascospores	Green to brown, one-celled, ellipsoidal, smooth-walled, without guttules, 17–22 × 10–11 µm, with granular contents, surrounded by a gelatinous sheath, germ pore	Aseptate, binucleate, at maturity dark brown, ellipsoidal to obovoid (17–) 18–24 × (9.5–)10–13 µm, often slightly inequilateral, rounded above, somewhat apiculate below with a basal germ pore, gelatinous sheath surrounding the spore except for a basal invagination	15.5–18.5 × 9.5–11.5 µm, obliquely to vertically uniseriate, dark brown, ellipsoidal, occasionally ovoid, smooth, slighty pointed and apiculate base, surrounded by a gelatinous sheath. Germ pore single and basal
Hyphae	Hyaline, 2 µm wide		–

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