

# **Terrestrial mosses as living environment for invertebrates**

**BSc. Bojana Božanić**

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Supervisor: RNDr. et Mgr. Ivan H. Tuf, Ph.D.  
Consultant: RNDr. Zbyněk Hradílek, Ph.D.

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## **Proclamation**

I, Bojana Božanić hereby proclaim that I have made this study by myself, under the supervision of Dr. Ivan H. Tuf and Dr. Zbyněk Hradílek and using only cited literature.

May 5th, 2011 in Olomouc

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## **Abstract**

Mosses could be playing important role in the life of terrestrial macro-invertebrates. They are a microhabitat used by invertebrates while hiding from predators or unfavourable weather conditions, feeding, hunting or simply laying eggs. The existence of mutual use is shown on several examples of invertebrates dispersing moss propagules.

In our study we examined how moss features and factors affecting mosses influence invertebrates within. Measured parameters were type of substrate, species of bryophyte, height above ground, tree diameter, moss cushion thickness and percentage of shade above place from which samples was taken. Obtained in two seasons, 120 samples were heat-extracted and later on sorted.

Explored were all together 20 species of bryophytes and 13 taxa. Identified to species level were 7 taxa (Aranea, Chilopoda, Diplopoda, Formicidae, Isopoda, Opilionidea and Pseudoscorpionida), counting 51 species in total. *Atrichum undulatum* was the species with the highest diversity of invertebrates, mainly growing on ground. The most abundant taxa were Acarina (2946 individuals in samples, 222 in control samples) and Collembola (1341 individuals in samples, 137 in control samples). Difference in age of the two distinct parts of the forest samples were taken from, showed no influence on abundances. On the other hand, comparison of abundances in samples and control samples showed that samples were richer in terms of invertebrates. The factors, that turned to be significant, were sample size and thickness (on taxa), and thickness and tree diameter (on species) in samples. On control samples, significant influence had season (spring/autumn) and substrate (dead tree/ground).

Božanič B.: Suchozemský mechy jako životní prostředí pro bezobratlé živočichy. Katedra ekologie a životního prostředí, Přírodovědecká fakulta, Univerzita Palackého v Olomouci, 42 s.

## **Abstrakt**

Mechy hrají důležitou roli v životě suchozemských bezobratlých živočichů. Představují mikro-stanoviště, které využívají bezobratlí jako úkryt od predátorů a nepříznivých podmínek, potravu, loviště anebo jen pro kladení vajec. Na existence vzájemného použití ukazují několika příkladů roznášení propagulí mechů bezobratlým.

V naší studii jsme prozkoumali jak rysy mechů a faktory působící na mechy ovlivňují bezobratlé žijící uvnitř. Měřené parametry byly typ substrátu (živý strom, mrtvý strom, povrch půdy), druh mechu, výška nad zemí, průměr stromů, tloušťka a velikost mechového polštáře a procento oslunění místa, z kterého jsme vzali vzorek. Celkově jsme, v dvou sezonách získali 120 vzorků, z kterých jsme posléze tepelně extrahovali bezobratlé, a setřídily je.

Vyzkoumáno bylo dohromady 20 druhů mechů z 13 taxonů. Do druhů byl určen 51 druh ze 7 taxonů (Aranea, Chilopoda, Diplopoda, Formicidae, Isopoda, Opilionidea and Pseudoscorpionida). *Atrichum undulatum*, který převážně rostl na zemi, byl druh s největší diverzitou bezobratlých. Nejhojnější taxony byly Acarina (2946 exemplářů ze vzorků, 222 z kontrolních vzorků) a Collembola (1341 exemplářů ze vzorků, 137 z kontrolních vzorků). Věkový rozdíl dvou části lesu, z kterého jsme vzaly vzorky, neukázal žádný rozdíl v abundancích. Na rozdíl od toho, porovnání abundancí ve vzorcích a kontrolních vzorcích ukázalo, že vzorky byly bohatší na bezobratlé. Faktory, mající signifikantní vliv byly velikost vzorků a tloušťka mechu (na taxony), tj. tloušťka mechu a průměr stromu (na druhy). U kontrolních vzorků, signifikantní vliv mělo roční období (jaro/podzim) a typ substrátu (mrtvý strom/povrch půdy).

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## **Introduction**

Bryophytes are widely spread in variety of habitats and play irreplaceable role in many ecosystems such as peat bogs, forests, tundra, alpine ecosystems, spring areas etc. They are often inhabited by spectrum of invertebrates, from microscopic aquatic ones to larger predatory beetles. Even though both mosses and invertebrates are being rather common, their association has been poorly studied and not yet completely comprehended. Most of the available literature about relationship of invertebrates and mosses deals with aquatic environment (e.g. Suren, 1993; Englund, 1991; Henrikson, 1993; Glime et Clemons 1972; etc.).

Mosses attract invertebrates, primarily by their physical characteristics of water absorption and retention, as water is being one of the most important conditions for survival in terrestrial environments. During the unsuitable weather conditions (drought, temperature extremes etc.), they can completely desiccate and stay in that state for quite long period of time (Kinchin, 1990). The revival of mosses and life within begins as the missing moisture is retrieved, upon which mosses restart the photosynthesis and proceed with growth (During et van Tooren, 1986). Similar to mosses, some invertebrates are capable of surviving water deficits by entering different stadia or using different mechanisms. For instance, members of aquatic bryofauna (tardigrades, nematodes and rotifers) are overpassing inhospitable environmental conditions using anhydrobiosis (Kinchin, 1990). Other invertebrates, incapable of some sort of quiescent state, are often using mosses as shelters. The ability of mosses to provide a humid terrestrial microenvironment could have had a role in the evolution of Dipterans (Gerson, 1969) and may as well explain why bryobionts prefer moss' microclimatic factors over the others (Drozd et al., 2007). Now-a-days mosses are used as indicators of diverse environmental pollution, mostly of air and water. They are used in bio monitoring of environmental pollution, since unable to avoid absorption and retention of heavy metals (Basile et al., 1995) leading to their accumulation in considerable quanta (Basile et al., 2001).

The ecological role of mosses lies also in their abilities to protect invertebrates from climate oscillations. They provide insulation against rapid temperature and humidity changes by creating spaces filled with air inside their tissue structure (Gerson, 1969),

thereby buffering the bryofauna (Kinchin, 1990; Merrifield et Ingham, 1998). Knowing that in the milder regions mosses serve as refugia (Smith, 1982), it is no wonder that under extreme conditions survival and abundance of some invertebrates fully depend on their presence (Gerson, 1969).

Generally speaking, mosses are one of the pioneers in colonization of devastated and newly formed habitats, making it suitable for further colonization of almost any possible substrate, stabilizing it, restraining erosion, producing litter, and retaining water, as it was mentioned above. Spectrum of invertebrates living in mosses is wide, from microscopic to macroscopic ones, from permanent dwellers and to others, which only occasionally appear. Kinchin (1990) classifies bryofauna as bryobionts, bryophiluses, bryoxenuses and occasionals. Subclass of Acarina is one of the most common taxa living in mosses. One order of Acarina, Oribatida, is known as moss-mites (Gerson, 1969).

Whether invertebrates feed on mosses or not, yet remains an open question in moss-invertebrate relations. Invertebrates can feed on both dead and live mosses, but also on algae, detritus and bacteria situated within growths of mosses (Hodkinson et al., 1994). Gerson (1969) finds that some species of beetles, orthopterans, springtails, caterpillars or aphids feed on mosses. For example, it is proven that ground hopper *Tetrix ceperoi*, feeds on *Bryum argenteum* (Kočárek et al., 2008). In case of *Onychiurus arcticus* laboratory examinations showed that it feeds on a whole range of bryophytes (Hodkinson et al., 1994). Several detritophagous species (as millipedes, woodlice, earthworms) are also finding food inside moss growths, especially those using dead wood as substrate.

As it is used as a hideout for some invertebrates, mosses are a perfect hunting ground for larger predators. Invertebrates do not only use it as a hiding place, but often mime it or cover in moss. Gerson (1969) mentions that larvae of Tipulid *Trigoma trisulcata*, that lives attached to moss *Fontinalis antipyretica*, resemble moss. Another invertebrate, stick insect *Trychopeplus laciniatus* has also ability to mime moss (Kinchin, 1990). Some New Guinean curculionid beetles had been found with bryophytes covering them (Geressitt et al., 1968).

Examples of invertebrates using mosses for placing their eggs, such as crane fly *Dolichoheza* and mite *Eustigmaeus* (Smith, 1982) are well-known. Cricket species

*Pteronemobius palustris* and *Pteronemobius fasciatus* are laying their eggs on *Sphagnum* (Gerson, 1969). Some insect, temporarily using bryophytes, might also pupate therein.

This particular relationship between mosses and its inhabitants certainly is two-sided. One of the most important benefits for mosses is probably the distribution of their propagules by invertebrates. Moss species *Dicranum flagellare* uses forest slugs for transport of their asexual branches in order to colonize disturbance gaps on decaying logs, as it has been mentioned in Kimmerer and Young (1995). Another author, Gerson (1969), mentions routine of coprophilous species of flies that can be enticed by color or secretion of also coprophilous *Splachnaceae*, unintentionally helping the dissemination. The important factor here is also the morphology of moss spores, which are sticky and usually attach to bodies of flies. Rudolphi (2009) showed another example of passive dispersal of propagules on ants *Lasius platythorax* and moss species *Aulacomnium androgynum*.

This paper examines invertebrates extracted from forest mosses and next-to-moss surrounding (control samples). The aims of this research were:

1. To describe the invertebrate communities inhabiting mosses in Vrapač NNR.
2. Make a comparison among such communities, and with control samples.
3. To compare them by environmental factors: type of substrate (dead wood, living tree or ground) and other characteristics (insulation, tree diameter, thickness of moss growth, decay level of dead tree etc.)

## Materials and Methods

### *Study site*

Research was conducted in the Vrapač National Nature Reserve ( $17^{\circ}02' E$ ,  $49^{\circ}42' N$ ), east of the town Litovel, The Czech Republic, belonging to vast complex of Litovelské Pomoraví Protected Landscape Area. Vrapač NNR has total area of 80.69ha, with average altitude of 235m above sea level. NNR consists of complex of flood-plain forests situated in the alluvial valley of Morava River (Machar, 2009). Dominant unit is hard wood *Quercus-Ulmetum* flood-plain forest (Šafář et al., 2003) but older robust specimens of *Quercus robur*, *Fraxinus excelsior* and *Ulmus laevis* can be also found. Spring flora consists of *Galanthus nivalis*, *Leucojum vernum*, *Pulmonaria obscura*, *Primula elatior*, *Corydalis cava* and *Corydalis solida*. *Allium ursinum* and *Urtica dioica* represent typical summer flora. In the growths, there could be found mountain and submontane plants such as *Anthriscus nitida*, *Geranium phaeum* and *Isopyrum thalictroides*. More precisely, the study area was categorized into two selected sections, which were compared, later on. The first section is represented by 110 years old growths, whereas the second section is consisting of 150 years old growths of oak and ash trees with the lower floor consisting of 80 years old growths of maple and lime tree.

### *Sampling and extraction*

Altogether, 180 bryophyte samples were collected (120 samples and 60 control samples), first half of them in spring (May 5<sup>th</sup> and June 1<sup>st</sup>) and the rest in autumn (October 18<sup>th</sup> and 27<sup>th</sup>) of the year 2010. The moss samples that were taken from the dead tree or ground were taken together with a thin layer or substrate (bark or layer of soil). Samples, taken from the living tree were usually scratched off the tree into a net. Control samples were taken from the nearest possible place to the moss sample, meaning that control samples are bark, dead wood or soil samples. Invertebrates were heat-extracted from the mosses later on in the laboratory using Tullgren funnels (Tuf et Tvardík, 2005). The

samples were placed in apparatuses for approximately seven days. Extracted invertebrates were eventually sorted, counted, and identified at species level.

### ***Measured parameters***

Each sample was characterised by evaluation of several environmental parameters. **Moss species** – taken samples consisted from the one moss species, mixed growths were ignored; **size of sample** – size was chosen by the size of the moss pillow (i.e. 20 × 20cm, 5 × 20cm or 5 × 15cm , respectively); **substrate** – taken samples of moss were growing on either decomposing fallen tree, living tree or ground; **tree diameter** – if the samples were taken from decomposing fallen tree or living tree, the tree diameter was measured at the height of 130cm above ground; **height above ground** (further on HAG)– was measured on all the samples except for the ones living on the ground; **shading** of the sampled moss – was evaluated as a proportion of canopy closure in photographs taken towards the sky perpendicularly to the ground at the sampling point, and scaled in percents (e.g. 80% means that biggest part of the view above moss was covered with tree trunks, branches and leaves, only 20% was clear sky) using Adobe Photoshop CS4; **pillow size** - was scaled from 1-4 (1=100%, 2=50%, 3=20-50%, 4=0-10% of the whole moss growth); **decay level** - was scaled from 1-4 (1=wood is hard, bark everywhere, 2=wood is softish, bark on more than 50%, 3=wood is pretty soft, bark on less than 50%, 4=wood is completely soft, without bark ); **thickness** of the moss growth – was measured on all the samples.

### ***Statistical analysis and data analysis***

Quantitative data from the sample collection were analysed in CANOCO4.5© programme for Windows (Ter Braak et Šmilauer 1998) and Excel 2007 spreadsheet from Microsoft Office pack.

We used Excel's Data Analysis module; *F-test* was used to find out the equality/inequality of data variances between samples and control samples, and further on *t-test* to see weather abundances differ or not.

First we used DCA - *detrended correspondence analysis* to find out lengths of gradients in species data. Effects of environmental factors on distribution of invertebrate

taxa in samples were evaluated by linear RDA- *redundancy analysis*. For test of relation between species of invertebrates from samples, and both taxa and species relation from control samples with environmental parameters, we used unimodal *canonical correspondence analysis* – CCA.

For testing of relation between species data and factors of environment we used Monte-Carlo permutation tests. To see relation (dependence) between species and environmental factors from CCA analysis, we used GAM – *generalised additive models*.

For graphic representation we used programme CanoDraw for Windows 4.0© (part of CANOCO software).

Visualization of sampling points and the study area was done in ArcGIS/GoogleEarth environment.

## Results

### *Sampled invertebrates communities*

In total 180 samples, we had 120 samples containing 20 species of bryophyte (Table 4), and 60 control samples. *Hypnum cupressiforme* was the most abundant species, appearing in 18 samples.

All together, 12 groups of invertebrates extracted from moss samples were evaluated. Acarina (2946 individuals), Collembola (1341 individuals) and Isopoda (320 individuals) were the most numerous groups, whereas Gastropoda (33 individuals), Opilionida (22 individuals) and Pseudoscorpionida (12 individuals) were the rarest groups. As far as control samples are concerned, smaller counts apply, as predicted. The most numerous were Acarina (222 individuals), Collembola (137 individuals) and Formicidae (135 individuals). Corresponding to moss samples, least abundant were Gastropoda (10 individuals), Opilionida (8 individuals) and Pseudoscorpionida (only 2 individuals). Final count of identified species is 51, consisting of: 15 species of Araneae, 11 species of Isopoda, 9 species of Chilopoda, 8 species of Diplopoda, 4 species of Formicidae, 3 species of Opilionida, and only single species of Pseudoscorpionida. Not all of the groups were identified to species level for different reasons, e.g. Enchytreidae could be determined only when alive. Determined species found only in samples are presented in table 3.

Simpson's diversity indexes were calculated (Tables 1 and 2), for both samples and control samples. The highest diversity rate was found in sample of *Atrichum undulatum* growing on the ground, with shade of 84% ( $D=93$ ). Next highest diversity ( $D=0.9$ ) was within the sample of *Plagiomnium undulatum* also growing on ground, with shade of 88%. In control samples situation is slightly different. There were only 5 samples with diversity higher than 0. The highest diversity was in control sample 1, 2, 3 from the date of 5.5.2010. Sample was taken from the living ash tree with the shading of 84%.



Table 1. Diversity indexes in samples

5.5.		1.6.		18.10.		27.10.	
sample	D	sample	D	sample	D	sample	D
1	0.76	1	0.00	1	0.67	1	0.67
2	0.80	2	0.00	2	0.00	2	0.67
3	0.00	3	0.00	3	0.00	3	0.87
4	0.00	4	0.00	4	0.00	4	0.00
5	0.83	5	0.74	5	0.00	5	0.00
6	0.00	6	0.86	6	0.00	6	0.82
7	0.00	7	0.00	7	0.67	7	0.00
8	0.67	8	0.00	8	0.00	8	0.67
9	0.00	9	0.00	9	0.00	9	0.00
10	0.00	10	0.87	10	0.00	10	0.00
11	0.76	11	0.87	11	0.00	11	0.00
12	0.00	12	0.00	12	0.00	12	0.00
13	0.00	13	0.00	13	0.00	13	0.00
14	0.00	14	0.00	14	0.00	14	0.00
15	0.00	15	0.00	15	0.67	15	0.84
16	0.00	16	0.90	16	0.00	16	0.00
17	0.00	17	0.00	17	0.00	17	0.67
18	0.00	18	0.00	18	0.84	18	0.00
19	0.00	19	0.00	19	0.00	19	0.00
20	0.00	20	0.67	20	0.00	20	0.80
21	0.67	21	0.60	21	0.00	21	0.00
22	0.00	22	0.00	22	0.00	22	0.00
23	0.00	23	0.00	23	0.67	23	0.00
24	0.00	24	0.00	24	0.00	24	0.00
25	0.93	25	0.00	25	0.00	25	0.00
26	0.84	26	0.00	26	0.33	26	0.00
27	0.00	27	0.00	27	0.84	27	0.87
28	0.00	28	0.00	28	0.74	28	0.00
29	0.00	29	0.67	29	0.00	29	0.00
30	0.00	30	0.84	30	0.00	30	0.00

Table 2. Diversity indexes in control samples

5.5.		1.6.		18.10.		27.10.	
controls	D	controls	D	controls	D	controls	D
1,2,3	0.84	1,2	0.75	1	0.00	1,2	0.00
4	0.67	3	0.00	2,3,4,5	0.00	3,4	0.00
5	0.00	4	0.00	6	0.00	5,6,7	0.00
6	0.00	5,6,7	0.00	7,8	0.00	8,9	0.00
7	0.00	8,9	0.00	9,10	0.00	10,11	0.00
8,9,10	0.00	10,11	0.00	11,12	0.00	12,13	0.00
11	0.00	12,13,14	0.00	13,14	0.00	14	0.00
12	0.00	15	0.00	15	0.00	15,16,17,18	0.00
13,14	0.00	16,17,18	0.00	16,17,18	0.00	19,20	0.00
15,16	0.00	19,20,21	0.00	19	0.00	21,22,23,24	0.00
17,18	0.00	22,23,24	0.00	20,21	0.00	25,26,27	0.00
19,20	0.00	25,26	0.00	22,23	0.70	28,29,30	0.00
21	0.00	27,28,29,30	0.00	24,25,26	0.00		
22	0.00			27,28	0.00		
23	0.00			29,30	0.00		
24	0.00						
25,26	0.70						
27	0.00						
28,29	0.00						
30	0.00						

Table 3. List of invertebrate species extracted from moss samples with basic ecological characteristics.

	<i>A. serpens</i>	<i>A. attenuatus</i>	<i>A. undulatum</i>	<i>B. rutabulum</i>	<i>B. moravicum</i>	<i>D. montanum</i>	<i>E. hians</i>	<i>F. taxifolius</i>	<i>H. seligeri</i>	<i>H. trichomanoides</i>	<i>H. cupressiforme</i>	<i>L. heterophylla</i>	<i>M. furcata</i>	<i>P. cuspidatum</i>	<i>P. rostratum</i>	<i>P. undulatum</i>	<i>P. cavifolium</i>	<i>P. succulentum</i>	<i>P. repens</i>	<i>R. punctatum</i>
Isopoda: Oniscidae	0	2	1	4	0	1	4	0	0	8	5	0	1	5	0	6	0	5	2	4
<i>Androniscus roseus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Hyloniscus riparius</i>	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	+	-	-
<i>Hyloniscus spp.</i>	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
<i>Lepidoniscus minutus</i>	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Ligidium hypnorum</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Porcellium collicolla</i>	-	+	+	+	-	-	+	-	-	+	+	-	+	-	-	-	-	+	+	+
<i>Porcellium conspersum</i>	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	+	-	+	+	+
<i>Trachelipus rathkei</i>	-	-	-	+	-	+	-	-	-	+	+	-	-	+	-	+	-	-	-	+
<i>Trachelipus ratzeburgii</i>	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+
<i>Trachelipus spp.</i>	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-
<i>Trichoniscus pusillus</i>	-	+	-	-	-	-	+	-	-	+	+	-	-	+	-	+	-	+	-	-
Aranea	1	1	3	0	0	0	4	0	0	3	1	0	0	5	0	5	0	0	0	0
<i>Ballus chalybeius</i>	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Centromerus sylvaticus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>Diplocephalus latifrons</i>	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Entelecara acuminata</i>	-	-	+	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-
<i>Linyphiidae spp.</i>	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-
<i>Macrargus rufus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>Mangora acalypha</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Neottiura bimaculata</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ozyptila sp.</i>	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-
<i>Pirata sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Porhomma sp.</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tenuiphantes sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Walckenaeria acuminata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-

Table 3. (continued)

	<i>A. serpens</i>	<i>A. attenuatus</i>	<i>A. undulatum</i>	<i>B. rutabulum</i>	<i>B. moravicum</i>	<i>D. montanum</i>	<i>E. hians</i>	<i>F. taxifolius</i>	<i>H. seligeri</i>	<i>H. trichomanoides</i>	<i>H. cupressiforme</i>	<i>L. heterophylla</i>	<i>M. furcata</i>	<i>P. cuspidatum</i>	<i>P. rostratum</i>	<i>P. undulatum</i>	<i>P. cavifolium</i>	<i>P. succulentum</i>	<i>P. repens</i>	<i>R. punctatum</i>
Opiliona	1	1	1	0	0	1	2	1	0	2	1	0	0	0	0	1	0	2	0	1
<i>Lopophilio palpinalis</i>	-	-	-	-	-	+	+	+	-	+	+	-	-	-	-	+	-	+	-	-
<i>Mitostoma chrysomelas</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trogulus tricarinatus</i>	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	+
Chilopoda	0	3	4	6	0	1	4	1	1	4	5	1	0	5	0	4	0	4	0	2
<i>Geophilus flavus</i>	-	-	+	+	-	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-
<i>Lithobius agilis</i>	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Lithobius erythrocephalus</i>	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Lithobius forficatus</i>	-	-	-	+	-	-	+	-	-	+	+	-	-	+	-	-	-	+	-	+
<i>Lithobius mutabilis</i>	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
<i>Lithobius piceus</i>	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
<i>Lithobius spp.</i>	-	+	+	+	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	-
<i>Schendyla nemorensis</i>	-	+	+	+	-	+	+	-	+	+	+	+	-	+	-	+	-	+	-	+
Pseudoscorpionida	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	1
<i>Neobisium carcinoides</i>	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+
Formicidae	0	0	2	0	0	0	0	1	0	2	3	0	0	0	0	1	0	1	2	0
<i>Lasius brunneus</i>	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-
<i>Leptothorax sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>Myrmica rubra</i>	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Myrmica ruginodis</i>	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-	-	+	-
Diplopoda	2	0	2	2	0	0	4	0	0	1	1	1	0	5	0	2	0	4	0	0
<i>Chordeumatida sp.</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
<i>Glomeris tetrasticha</i>	-	-	+	-	-	-	+	-	-	+	-	+	-	+	-	+	-	+	-	-
<i>Julida sp.</i>	+	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
<i>Melogona voigti</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Nemasoma varicorne</i>	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
<i>Strongylosoma stigmatosum</i>	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
number of species/sample	4	7	13	12	0	3	18	3	1	21	16	3	1	19	0	20	0	17	4	8
Simpson's index of diversity	0.17	0.25	0.14	0.33	1.00	1.00	0.18	1.00	1.00	0.19	0.19	1.00	1.00	0.19	1.00	0.17	1.00	0.17	0.33	0.25
1-D	0.83	0.75	0.86	0.67	0.00	0.00	0.82	0.00	0.00	0.81	0.81	0.00	0.00	0.81	0.00	0.83	0.00	0.83	0.67	0.75

Table 4. Moss species found in samples. Abbreviations: D – dead tree, G – ground, L – live tree

	number of samples	number of species/sample	average number of species/sample	substrate
<i>Amblystegium serrepens</i>	1	4	0.6	D
<i>Anomodon attenuatus</i>	3	7	1.0	L
<i>Atrichum undulatum</i>	5	13	1.9	G,D
<i>Brachythecium rutabulum</i>	12	12	1.7	D,G,L
<i>Bryum moravicum</i>	1	0	0.0	L
<i>Dicranum montanum</i>	3	3	0.4	L,D
<i>Eurhynchium hians</i>	13	18	2.6	G
<i>Fissidens taxifolius</i>	5	3	0.4	G
<i>Herzogiella seligeri</i>	1	1	0.1	D
<i>Homalia trichomanoides</i>	12	21	3.0	L
<i>Hypnum cupressiforme</i>	18	16	2.3	L,D
<i>Lophocolea heterophylla</i>	5	3	0.4	D
<i>Metzgeria furcata</i>	1	1	0.1	L
<i>Plagiomnium cuspidatum</i>	10	19	3.0	G,D,L
<i>Plagiomnium rostratum</i>	2	0	0.0	G
<i>Plagiomnium undulatum</i>	10	20	2.9	G,D,L
<i>Plagiothecium cavifolium</i>	1	0	0.0	G
<i>Plagiothecium succulentum</i>	9	17	2.4	L,D
<i>Platygyrium repens</i>	5	4	0.6	D,L
<i>Rhizomnium punctatum</i>	3	8	1.1	D

### ***Older forest vs. Younger forest samples***

Species abundance per samples from mosses vs. control samples outside mosses was compared. Sampling from two parts of forest of distinct age was done in order to see if there is any difference between them (in terms of invertebrate communities). For spatial position of samples inside the area, see map (Appendix 2). Firstly, total abundances of older/younger samples were counted. The results of t-test ( $t_{26}=-0.035$ ,  $p=0.48$ ) had shown us that there is no particular difference between invertebrates abundance in two parts of the forest. Complete results are visible in Table 5.

Table 5. T-Test: Paired Two Sample test for Means

	<b>older</b>	<b>younger</b>
Mean	231.8571429	234.2857143
Variance	241458.4396	164296.3736
Observations	14	14
Pearson Correlation	0.855651774	
Hypothesized Mean Difference	0	
df	13	
t Stat	-0.03566758	
P(T<=t) one-tail	0.486044644	
t Critical one-tail	1.770933383	
P(T<=t) two-tail	0.972089288	
t Critical two-tail	2.160368652	

### ***Samples vs. control samples***

Species abundance per samples from mosses vs. control samples outside mosses was compared. The results show that variances are the same ( $F_{119, 59}=1.41$ ,  $p=0.07$ ) but abundances of given samples were significantly higher in mosses contrary control samples outside mosses ( $t_{178}=3.52$ ,  $p=0.0005$ ).

### ***Environmental analysis of taxa – samples***

Lengths of gradients were from 1.840 and 2.207, indicating that  $\beta$  diversity is probably low (Zuur et al., 2007) and that data have linear distribution. Because of that, RDA analysis was used (Figure 1, Table 6). According to the RDA analysis, significant environmental factors were Sample size and Thickness.

Table 6. Description of RDA model for taxa distribution with conditional effects of environmental variables

<b>Axes</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>Total variance</b>
Eigenvalues:	0.116	0.071	0.003	0.001	1.000
Species-environment correlations:	0.539	0.360	0.467	0.325	
Cumulative percentage variance					
of species data:	11.6	18.7	19.0	19.1	
of species-environment relation:	60.6	97.7	99.1	99.8	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.191

<b>Variable</b>	<b>Lambda</b>	<b>P</b>	<b>F</b>
Sample size	0.08	0.002	10.10
Thickness	0.06	0.006	7.99
Pillow size	0.01	0.192	1.80
Diameter	0.02	0.138	1.96
Shading	0.00	0.230	1.35
HAG	0.01	0.258	0.99
ground	0.01	0.558	0.69
spring	0.00	0.710	0.32
dead	0.00	0.890	0.17
Decay level	0.00	0.886	0.11

\*\* -  $p < 0.01$ ; \* -  $p < 0.05$ ; n.s. – not significant

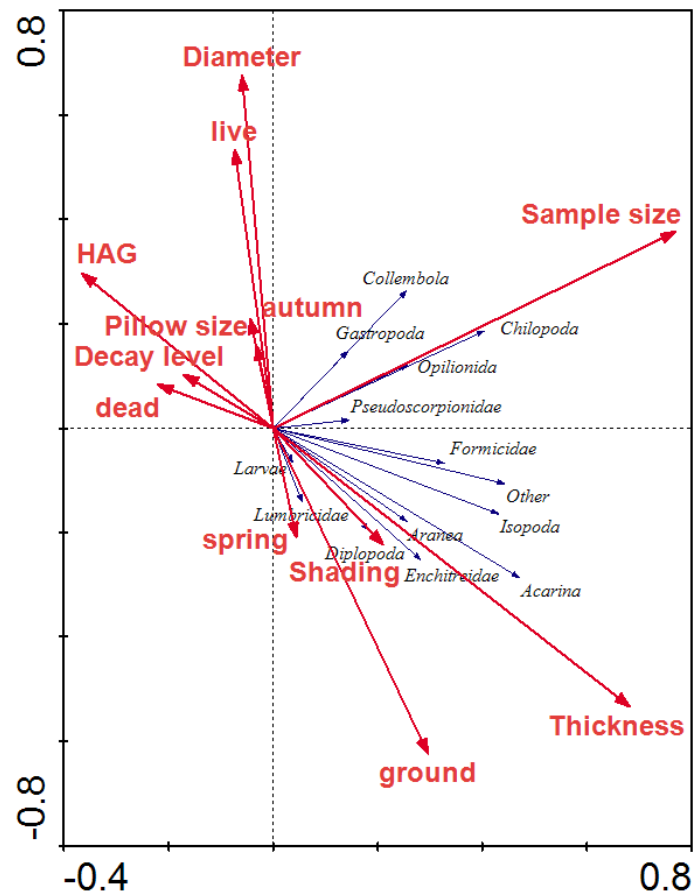


Figure 1. Ordination plot of influence of environmental factors on invertebrate taxa in samples

Figure above depicts correlation of the attributes' canonical components (red axes) and specimens' components (blue axes) of the symmetric plot. Due to obtuse angles between the axis (Yelland, 2006) attributes of Diameter, HAG (Height above ground), Pillow size, autumn, Decay level, and dead stand uncorrelated with both, the majority of remaining attributes (some correlation is achieved in relation to Sample size) and specimen components (blue axes). High levels of error probabilities (Table 6, column p) go in favor of the latter. On contrary, animal specimens are generally correlated and all fall to the 1<sup>st</sup> and the 4<sup>th</sup> quadrant of the plot. Further, the taxa which fall to the 4<sup>th</sup> quadrant are well correlated with the following attributes: Thickness, ground, Shading, spring, while Sample size is very well correlated with some taxa. In such context it could be speculated that Sample size is particularly influencing Chilopoda class, as Chilopoda frequency is higher

than average for the Sample size attribute. Some similar speculations hold true for Thickness to Enchytreidae, Araneae and Acarina, spring to Lumbricidae and so forth.

In this case we used GAM for further analysis of significant parameters, considering that we could not count on linear response. Sample size had significant impact on 8 taxa (excluding mixed group Other, consisting of other samples not included in these bigger groups) – Lumbricidae, Araneae, Isopoda and Gastropoda (Figure 2, left) and Acarina, Collembola, Formicidae and Chilopoda (Figure 2, right). Collembola and Acarina had the strongest responses, being abundant in bigger samples around 400cm<sup>2</sup>. Formicidae, Chilopoda and Isopoda were also most abundant in biggest samples of 400cm<sup>2</sup>, as well as Gastropoda, but with weaker response. Araneae preferred samples around 200cm<sup>2</sup> (15cm x15cm). Lumbricidae were equally abundant in all sample sizes.

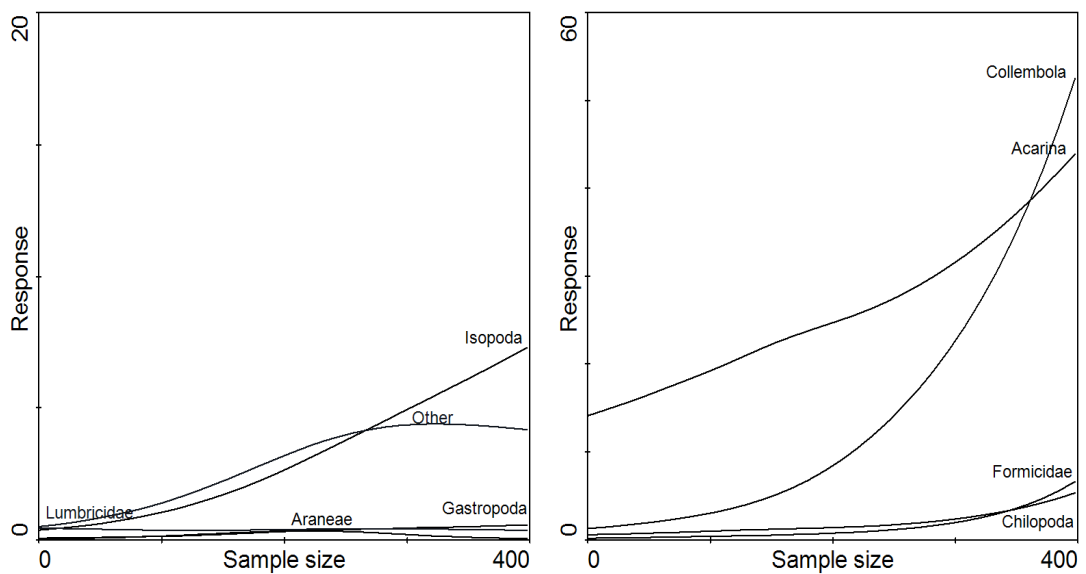


Figure 2. Dependence of taxa distribution on Sample size

Thickness had significant influence on 7 taxa (Figure 3). The strongest response had Acarina, preferring thicker moss samples, consisting mostly of *Plagiomnium undulatum* and *P. cuspidatum*. Thicker moss was also chosen by Isopoda, Enchytreidae, Formicidae, Araneae and Pseudoscorpionida. Chilopoda were the only group showing the preference towards middle depth of the moss (around 2cm).



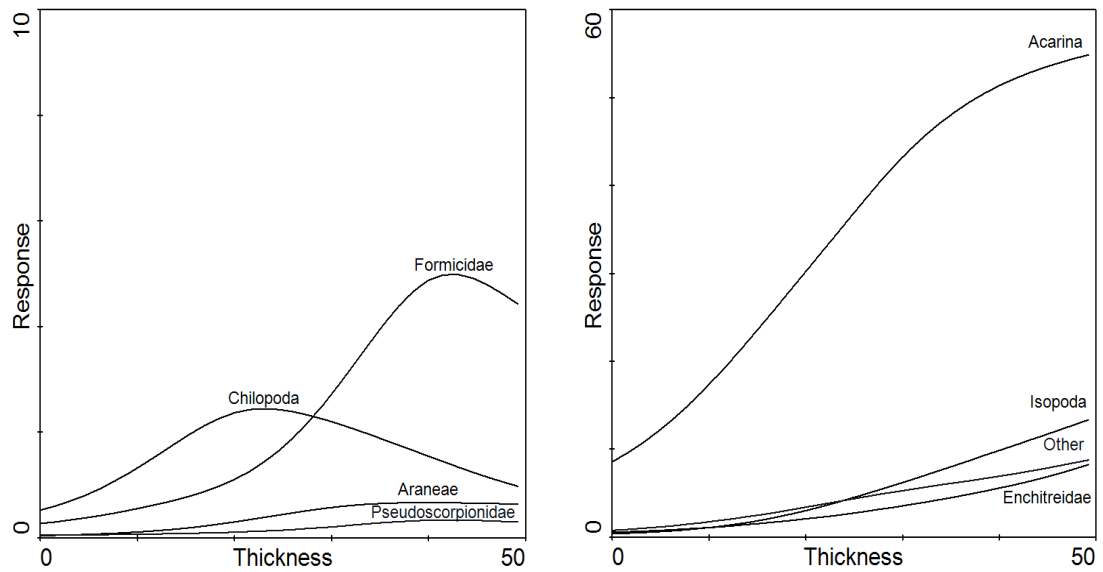


Figure 3. Dependence of taxa distribution on moss thickness

### *Environmental analysis of species – samples*

For this analysis we used CCA (Figure 4). Lengths of gradients were high, from 3.400 till 6.385, indicating high  $\beta$  diversity. The whole model was significant ( $F=1.402$ ,  $p=0.0020$ ). First canonical axis showed 52.8% of variability, second 32.3% (Table 7). According to the results, significant factors were diameter, thickness and spring (Table 7, column p).

Table 7. Description of CCA model for species distribution with conditional effects of environmental variables

<b>Axes</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>Total inertia</b>
Eigenvalues :	0.528	0.323	0.309	0.230	12.471
Species-environment correlations :	0.846	0.756	0.696	0.655	
Cumulative percentage variance					
of species data :	4.2	6.8	9.3	11.1	
of species-environment relation:	26.5	42.6	58.2	69.7	
Sum of all eigenvalues					12.471
Sum of all canonical eigenvalues					1.995

Table 7. (continued)

Variable	Lambda	P	F
Diameter	0.45	0.002	3.32
spring	0.29	0.002	2.21
Thickness	0.25	0.004	1.86
Pillow size	0.19	0.108	1.42
ground	0.18	0.056	1.43
Sample size	0.17	0.118	1.31
Decay level	0.13	0.410	1.01
Shading	0.14	0.476	1.02
HAG	0.10	0.658	0.80
dead	0.09	0.844	0.71

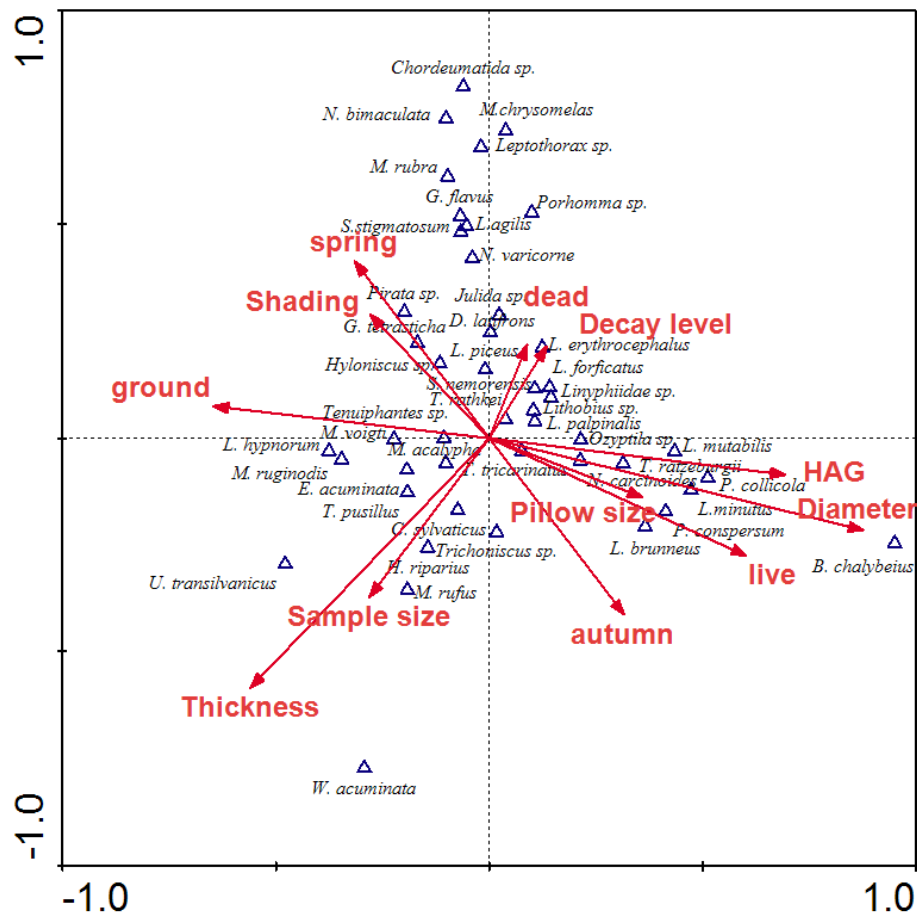


Figure 4. Ordination plot of influence of environmental factors on invertebrate species in samples

For further analysis, species, whose sum of individuals was exceeding 10, were chosen. Diameter of trees, from which samples were taken, had an influence on 6 species.

The strongest effect (Table 8) was on ant species *L. brunneus* (F=27.51) and woodlouse *P. collicola* (F=17.70).

Woodlice *P. collicola* and *P. conspersum* both preferred robust trees with tree diameter around 110cm, which was as well the largest diameter we had in our samples (Figure 5, right). *L. brunneus* preferred trees with diameter approximately around 90cm, unlike centipede *G. flavus* which was mostly found in the samples taken from the ground.

Thickness of the moss sample had a significant influence on 6 species. The thickest moss was *Plagiomnium undulatum* with 50mm. This moss thickness was preferred by woodlice *T. pusillus* (with F=14.20 representing highest influence) and *H. riparius* (Figure 5, left). Ant species *M. ruginodis* was found mainly in moss species with thickness between 40 and 50mm. Preference of the lowest thickness was found in species *N. varicorne*.

Table 8. Influence of diameter and thickness on abundance of invertebrate species

Species	Diameter	Thickness
<i>G. tetrasticha</i>	n.s.	n.s.
<i>G. flavus</i>	3.79*	n.s.
<i>H. riparius</i>	n.s.	9.70**
<i>L. brunneus</i>	27.51**	n.s.
<i>L. forficatus</i>	n.s.	n.s.
<i>L. mutabilis</i>	9.11**	4.71**
<i>L. palpinalis</i>	n.s.	n.s.
<i>Lithobius</i> sp.	n.s.	n.s.
<i>M. ruginodis</i>	n.s.	3.31*
<i>N. varicorne</i>	n.s.	4.74*
<i>P. collicola</i>	17.70**	7.23**
<i>P. conspersum</i>	10.30**	n.s.
<i>N. carcinoides</i>	n.s.	n.s.
<i>S. nemorensis</i>	n.s.	n.s.
<i>T. pusillus</i>	n.s.	14.20**
<i>T. rathkei</i>	4.32*	n.s.
<i>Trichoniscus</i> sp.	n.s.	n.s.

\*\*- p < 0.01; \*- p < 0.05; n.s. – not significant

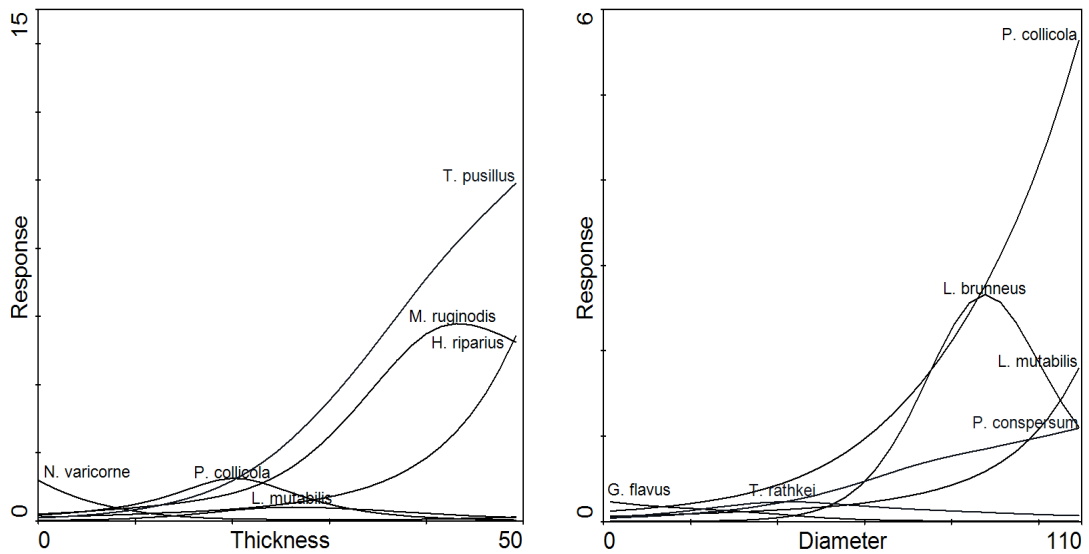


Figure 5. Dependence of species distribution on moss thickness (left), and tree diameter (right)

### ***Environmental analysis of taxa – control samples***

Lengths of gradients were from 2.741 to 3.993, indicating low  $\alpha$  diversity, but, unlike in previous analysis,  $\beta$  diversity is probably high (Zuur et al., 2007). In this case data are having unimodal distribution, hence CCA analysis took place (Figure 6). Whole model was significant ( $F=2.618$ ,  $p=0.002$ ), with the first axis showing 41.1% of variability and the second one showing 28.5% (Table 9). CCA showed that only 3 factors were significant – autumn, ground and dead. Having in mind that these factors were nominal, no further analysis was done.

In the Figure 6 we can see that Enchytreidae reacted on Sample size, which corresponds to our previous investigation (Božanić, 2008). Lumbricidae were only found in control samples taken from ground, as no earthworms live in trees. Both Chilopoda and Larvae showed preference to shade percentage. Group consisting of Gastropoda, Diplopoda, Opilionidae and Acarina were more abundant in samples taken in spring time. On contrarily, Pseudoscorpionida and Collembola were more abundant in autumn.

Table 9. Description of CCA model for distribution of taxa in control samples with conditional effects of environmental variables

Axes	1	2	3	4	Total inertia
Eigenvalues:	0.411	0.285	0.135	0.069	3.385
Species-environment correlations:	0.824	0.731	0.606	0.498	
Cumulative percentage variance					
of species data:	12.1	20.6	24.6	26.6	
of species-environment relation:	41.1	69.7	83.2	90.1	
Sum of all eigenvalues					3.385
Sum of all canonical eigenvalues					0.999

Variable	Lambda	P	F
autumn	0.38	0.002	7.27
ground	0.25	0.004	5.04
dead	0.13	0.006	2.60
Sample size	0.06	0.172	1.45
HAG	0.07	0.188	1.35
Shading	0.06	0.216	1.36
Decay level	0.03	0.746	0.59
Diameter	0.02	0.932	0.42

\*\* -  $p < 0.01$ ; \* -  $p < 0.05$ ; n.s. – not significant

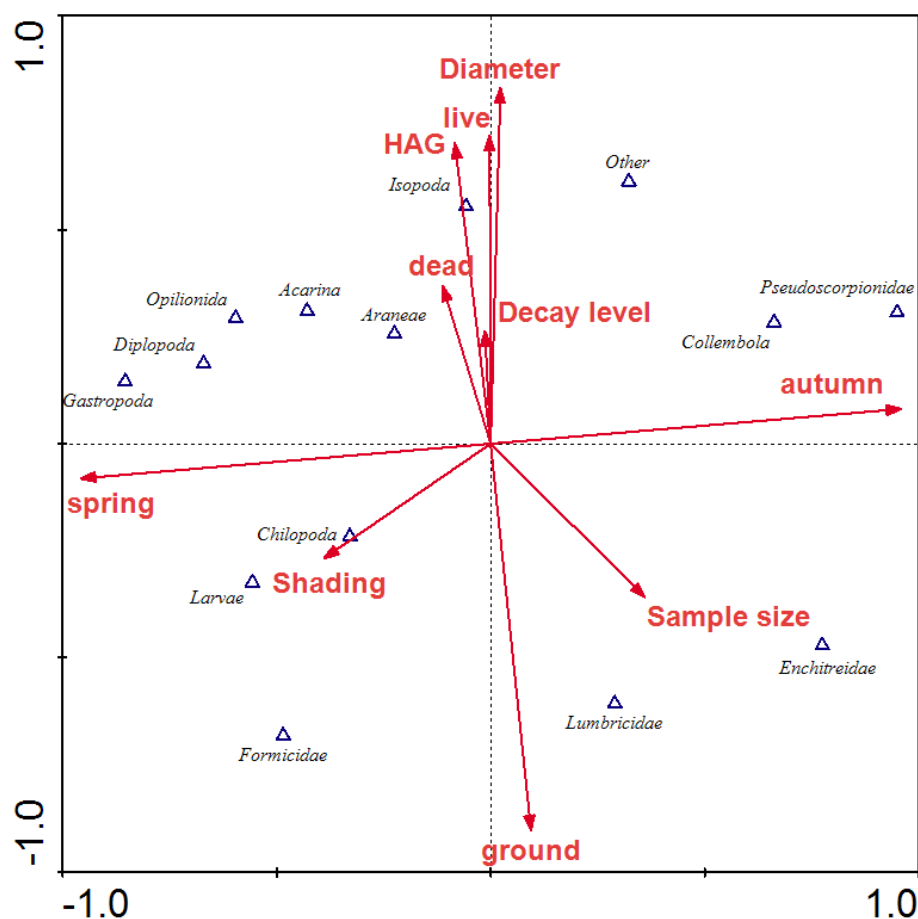


Figure 6. Ordination plot of influence of environmental factors on invertebrate taxa in control samples

### ***Environmental analysis of species – control samples***

Lengths of gradients were from 0 to 37.694. Having all of the gradients except for one over 7, we used CCA. Tested significance of all canonical axes, using Monte Carlo test, turned out significant ( $F=1.362$ ,  $p=0.006$ ). Results showed 80.7% of variability of first axis and 67.8% of the second (Table 10). Only 3 factors showed significant influence, ground, dead and autumn.

*Table 10.* Description of CCA model for distribution of species in control samples with conditional effects of environmental variables

<b>Axes</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>Total inertia</b>
Eigenvalues:	0.807	0.678	0.611	0.456	11.861
Species-environment correlations:	0.962	0.935	0.877	0.845	
Cumulative percentage variance					
of species data:	6.8	12.5	17.7	21.5	
of species-environment relation:	21.8	40.1	56.6	68.9	
Sum of all eigenvalues					11.861
Sum of all canonical eigenvalues					3.704

<b>Variable</b>	<b>Lambda</b>	<b>P</b>	<b>F</b>
ground	0.75	0.002	2.09
autumn	0.63	0.002	1.81
HAG	0.52	0.102	1.53
dead	0.55	0.020	1.64
Shading	0.46	0.192	1.37
Decay level	0.31	0.482	0.93
Sample size	0.30	0.490	0.92
Diameter	0.18	0.938	0.53

In the ordination plot Figure 7. we can see some distinct groups of parameters. One group is represented by tree diameter, HAG and live tree, and is positively correlated with the first axis. Also correlated with first axis but negatively, is a parameter ground. Another smaller group consists of parameters shading and spring. The last group is made of sample size, decay level, dead and autumn. It is interesting that parameters of decay level and dead completely overlap.

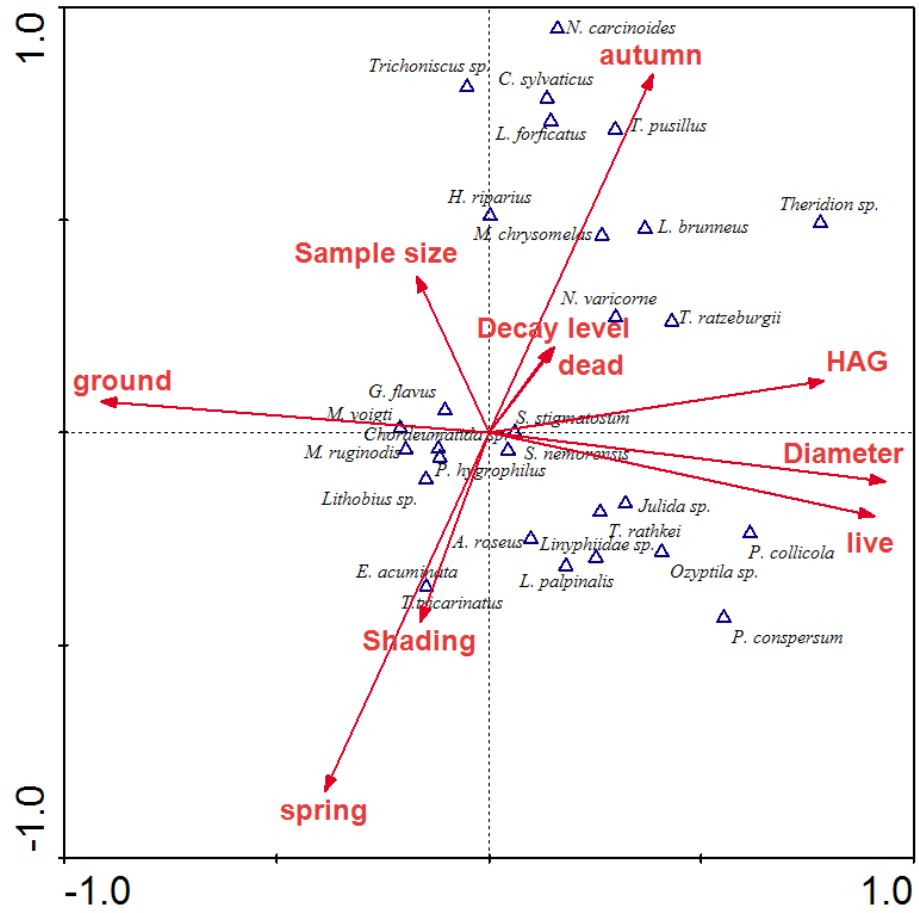


Figure 7. Ordination plot of influence of environmental factors on invertebrate species in control samples

## Discussion

### *Mosses*

In samples all together we had 20 moss species out of total 89 species found in recent years in Vrapač (Hradilek, 2009). The most abundant species was *Hypnum cupressiforme*. It is a very frequent opportunistic species (Odor et al., 2005), common in ecosystems. Its opportunism is obvious in this work, as well. This species was found on 5 out of 5 tree species present in the samples, and had different shading rates. It was approximately equally represented in both sampling seasons, and also in two of the substrates, live and dead wood.

Two other moss species, that had highest abundance and diversity rates, were *Atrichum undulatum* and *Plagiomnium undulatum*. High percentages of shade are not unusual, considering the fact that mosses are adapted to low light rates in forests (Glime, 2007) and generally have negative correlation with leaf litter cover (Marialigeti et al., 2009). Vodka et al. (2007) mention that a few edaphic invertebrate generalists were associated with closed canopy.

Two live specimens of *Acer pseudoplatanus* and one dead *Quercus robur* were the biggest trees (in diameter) samples were taken from. It is interesting that both Sycamores were situated in the younger part, forest 110 years old. Lacina (2009) states that Sycamore is a regular and sporadically, even sub-dominant species in Vrapač, but missing in other South Moravian flood-plains. Considering the age difference, we assumed that abundances of invertebrates will differ accordingly, but it seems that there is no significant difference between abundances of two parts of the forest. Both growths (older and younger) were developing for a quite a long time next to each other; they even visually look alike.

Simultaneously, fauna was able to develop freely without any barriers. These 40 years of age difference between them certainly had no influence on the development of the forest. The fact that sub-growth of the older forest also consists of 80 years old trees may have influenced the age average and decreased the abundance.



## *Samples*

When comparing abundances of samples and control samples, by using t-test, we came to the conclusion that abundances were not the same. Abundances of samples, consisting mostly of moss (and thin layer of substrate) were generally higher. This may be explained by invertebrates seeking food (e.g. Chawn, 1993; Smith et al., 2001; etc.), shelter (e.g. Parker et al., 2007) or humidity (e.g. Drozd et al., 2007) inside the moss cushion, or all of the above.

Taxa found in samples had significant response to two factors – sample size and moss cushion thickness. Moss cushion thickness is species-specific and it well predicts temperature regime of substrate under moss mats (Soudzilovskaia et al., 2011). Collembola and Acarina had the strongest response to sample size. Isopoda's appearance in thicker and bigger samples may be explained by their sensitivity to humidity, leading to their aggregation in more humid areas (Waloff, 1941), making the mosses appealing. The thicker the cushion is, more water it preserves. Enchitreidea are also sensitive to water content, having wide tolerance towards it, but are little adapted to drought (Glime, 2007). Members of Chilopoda need to live in moist habitats, due to lack of waxy cuticle, preferring high humidity and low sun intensity (Mitić et Tomić, 2002). We came to the same results, where Chilopoda were most abundant in the middle range of thickness, where they have optimal conditions needed.

In the case of species found in samples, three of the parameters were significant – tree diameter, moss cushion thickness and spring. Thickness was significant for three species of Isopoda – *T. pusillus*, *H. riparius* and *P. collicola*. All three species are generalists, appearing in variety of habitats (Hornung et al., 2008). Overall, *H. riparius* and *T. pusillus* are known to be hygrophilous (Tajovský, 2000), thus probably appearing in thicker mosses, that preserve more water. On the other hand, *N. varicorne* was mostly in lower moss cushions, probably emerging from the soil. It is a species known for being extremely tolerant to high temperature and desiccation (Haacker, 1968; Enghoff, 1976), so it does not really need to use all the conveniences moss has to offer. Ant species *M. ruginodis* seemed to prefer samples around 4cm of thickness. If we take a look at the samples, the most of these ants (49 pieces) were found in a single 4cm thick moss, coming

from the ground. We assume that underneath the moss mat, there could have been an ant nest, which would explain high abundance. Tree diameter was significant on three Isopoda species (*P. collicola*, *P. conspersum* and *T. rathkei*) known for being rather common (Farkas, 2007). Ant species *L. brunneus* was abundant in mosses growing on trees that had diameter from 60 to 110cm. This species is tree inhabitant which nests in older oak trees, but also some other trees (Collingwood, 1957). In our research, all the specimens came from two trees – *Fraxinus excelsior* and *Acer pseudoplatanus*. On the other hand, some species of *Lasius* are known to disperse moss propagules (Rudolphi, 2009), which may be case here as well. Unlike *G. flavus*, which was found mostly in mosses growing on ground level, *L. mutabilis* preferred higher positions. *L. mutabilis* is important wood predator (Voigtlander, 2006), dominant in flood-plain forests of Litovelské Pomoraví (Tuf et al., 2006).

## Conclusion

This work aims to analyse invertebrate species living in related moss species, and to analyse which measured environmental factors have an influence on them. Control samples were taken so that we could prove that invertebrates are in fact seeking shelter inside mosses. This was proven through comparison of abundances, which turned to be higher in samples than in control samples. Moss species with the highest abundances were *Plagiomnium undulatum* and *Homalia trichomanoides*, the highest diversity was found in *Atrichum undulatum*. Results of testing, in which we compared abundances of two forest parts (younger and older) showed no particular difference between them.

Redundancy analysis model showed that on taxa found in samples influence had two measured factors, thickness of moss pillow and sample size. Through further testing we discovered that thickness had specific influence on Acarina, Isopoda, Enchytreidae, Formicidae, Araneae, Pseudoscorpionida and Chilopoda; strong influence of sample size was on all the taxa except for Opiliones and Pseudoscorpionida. Tree diameter and moss thickness were influential environmental factors for distribution of individual species. At bigger tree specimens were found woodlice *P. collicola*, *P. consperum* and *T. rathkei*, then ant species *L. brunneus* and two centipedes *G. flavus* and *L. mutabilis*. In the thickest mosses there were woodlice *T. pusillus* and *H. riparius* and one ant species *M. ruginodis*. In medium moss thickness there could have been found *P. collicola* and *L. mutabilis*, whereas millipede *N. varicorne* favoured shoal mosses.

Regarding control samples, we have learned that influential were season, either spring or autumn, and substrate. Except for live trees, the substrate had significant number of taxa and adjacent species.

Similar investigation could be done in future in, for example, in different types of forests that endorse mosses to grow. Individual species or taxa, of both mosses and invertebrates could be explored in regard to significant factors and during all of the seasons.

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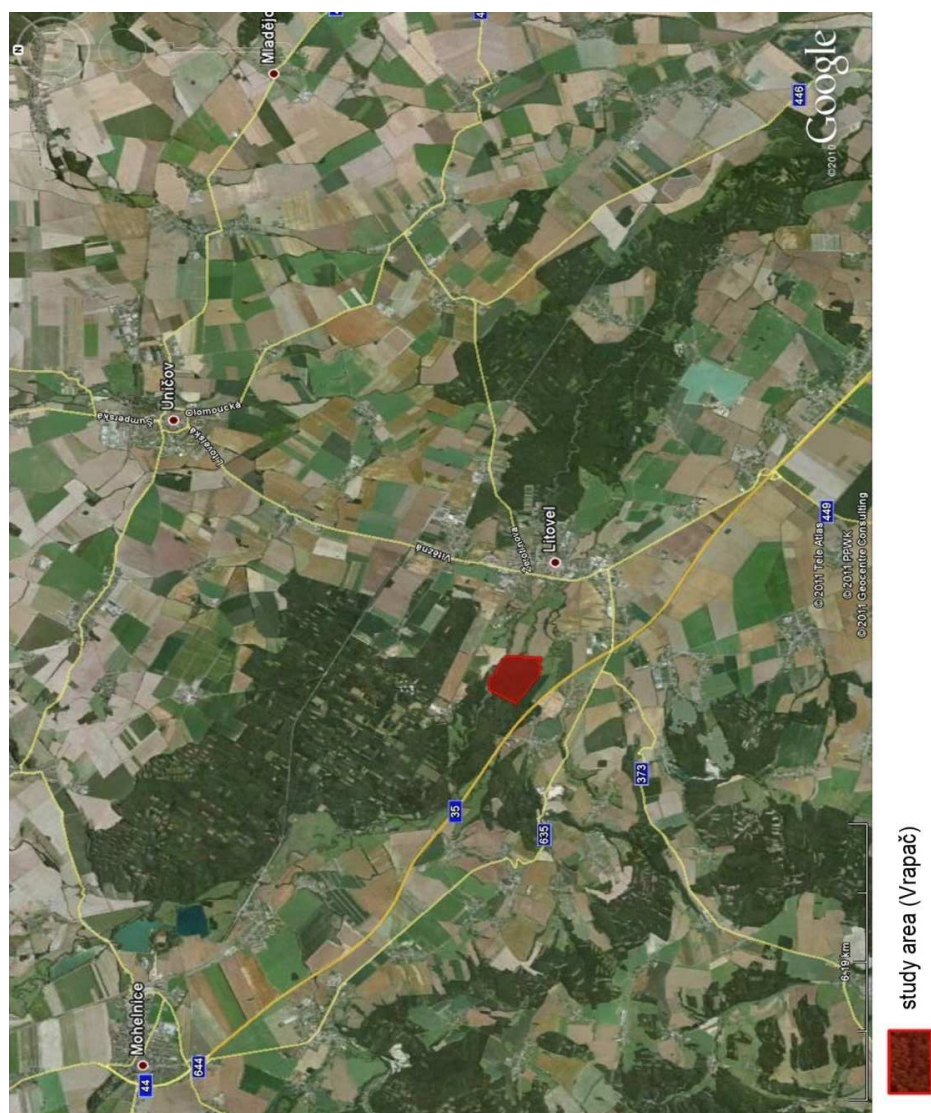
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Appendix 1. Ortho-photo map of Vrapač



 study area (Vrapač)

Appendix 2. Sampling points inside the study area

