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THE ROLE OF EDAPHIC AND VEGETATION FACTORS IN STRUCTURING BETA DIVERSITY OF THE SOIL MACROFAUNA COMMUNITY OF THE DNIPRO RIVER ARENA TERRACE

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Abstract

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The article presents the results of evaluation of the role of edaphic and vegetation factors on beta diversity of soil macrofauna by means of the MDM-approach. The multinomial diversity model (MDM) is a method for relating the Shannon diversity to ecological factors. The research was conducted in the 'Dnipro-Orils'kiy' Nature Reserve (Ukraine). The research polygon was laid in the forest within the Orlova ravine (48°31'13 "N, 34°48"15 "E). The study site comprises 1.0 ha of deciduous woodland bordered by an area of herbaceous cover within the ravine. In the soil of the studied polygon, 38 species of soil invertebrates were identified, which characterizes the gamma diversity. Alpha diversity, or the number of species on average at each sample point is 4.3. Beta diversity is 8.8. The principal component analysis of the edaphic parameters revealed four statistically significant principal components. For vegetation characteristics, six statistically significant principal components were identified. The sequential analysis of the effects shows that edaphic factors accounted for 20.9% (0.81 bit) of the available entropy (1.71–0.91). The largest decrease in the community entropy takes place under the action of the principal components 2 and 3 (0.06 bit and 0.05, respectively). A permutation test showed that these effects are statistically significant. In turn, 28.4% of the community β-diversity is attributable to vegetation factors. The greatest decrease in community entropy is related to the principal vegetation components 1, 3 and 4 (0.07, 0.05 and 0.04 bits, respectively). A permutation test indicated that this effect is statistically reliable. Geostatistical models substantially describe the varying effects on the beta-diversity of edaphic principal components 1 and 2, and the vegetation principal components 1 and 3. It was found that edaphic and plant factors play an important role in structuring the communities of soil macrofauna on the level of beta diversity. Community sensitivity to environmental factors varies in space and is spatially structured. For different environmental factors, specific spatial patterns of community sensitivity are allocated. Beta diversity may be due to the fact that the species of soil macrofauna communities also vary in the degree of sensitivity to various environmental factors. The species of soil microfauna are also divided according to their extent of sensitivity to different ecological factors.

Key words: diversity, ecological factors, spatial patterns, variogram, Mattern model.

Introduction

Soils provide one of the most important ecosystem services such as supporting the most agro-sylvo-pastoral production systems (Lavelle et al., 2006). Soil biota is highly diverse, representing 23% of the described organism diversity (Decaëns et al., 2006). Forest soil biodiversity responds to environmental changes and has been shown to be one of the key drivers of ecosystem function and service delivery (Lukac et al., 2017). An important component of soil biota is represented by soil macrofauna (Lavelle, 1997). Soil macrofauna significantly contributes to the dynamics of the soil properties (Ayuke et al., 2009).

Soil biodiversity demonstrates considerable spatial and temporal heterogeneity at multiple scales (Carpenter et al., 2012; Eggleton et al., 2005; Burton, Eggleton, 2016). Scale-dependent drivers affect the species distributions and community composition at various spatial levels (Berg, 2012). The scale-specific response to habitat heterogeneity may be an essential property of a given taxon or species (Vanbergen et al., 2007). The large-scale determinants of soil macrofauna diversity are climate, soil type, land-use management practices and landscape structure (Dauber et al., 2003). Very little is known about the effect of landscape variables on soil biota (Wolters, 2001; Dauber et al., 2005). Soil animals significantly vary in size, adaptations to movement and, consequently, vary considerably in mobility (Gilarov, 1949; Zhukov, 2015). Differences in local community structure may be affected by ecological processes occurring at larger spatial scales. Species differing in size and mobility can be regulated by different processes on one and the same spatial scale (Olff, Ritchie, 2002). It is highly probable that functional or ecological features of species are the important determinant of which habitat heterogeneity component is relevant and at which spatial scale (Dauber et al., 2005). Differences were found in the spatial scales where the landscape affects species abundances and species richness for Collembola (Chust et al., 2003), Homoptera and Diptera (Chust et al., 2004).

Heterogeneity in soil properties induced by vegetational spatial patterns define the patchy distribution of soil organisms (Berg, Bengtsson, 2007; Berg, 2012). It has been discovered that on a smaller spatial scale, the diversity of tree species influences the earthworm density (Cesarz et al., 2007). Diverse tree cover is important in the conservation of the soil macrofauna communities and in making a significant contribution to their activity in the soil ecological functions (Kamau et al., 2017). The litter quality of a given tree species can significantly contribute to the changes observed in the soil fauna communities (Korboulewsky et al., 2016). The importance of small scale heterogeneity has been shown for plant and soil macrofauna biodiversity (Burton, Eggleton, 2016). The presence of dead wood is positively correlated with soil arthropod abundance and diversity (Jabin et al., 2004). It has been shown that spatial patterns of herbaceous vegetation influence soil macrofauna biodiversity; therefore, full understanding of the soil macrofauna distribution in a grassland ecosystem requires an accurate study of the vegetation cover around the places where the samples of animals were collected (Mathieu et al., 2009). Soil animals, which are classified as ecosystem engineers, can significantly increase the spatial heterogeneity of the soil, and hence, the spatial patchiness of soil fauna (Nuutinen et al., 2017). Ecosystem engineers are able to modify important drivers of the spatial distribution of soil organisms such as soil structure, pore space, porosity and bulk density, water content, and mix organic matter and inorganic matrix (Lavelle, 2002; Berg, 2012).

Diversity is the most important aspect of the community structure. Diversity can be seen in terms of three components: alpha, beta and gamma. Alpha diversity is the species diversity at individual sites. Gamma diversity is that of the whole region of interest of the study. Beta diversity is the variation in species composition among sites within the geographic area of interest (Legendre et al., 2005). Variation of biological communities across space or time (i.e., beta diversity) has attracted increasing attention (Alahuhta et al., 2017; Viana et al., 2016). Beta-diversity partition may provide additional insights into the causes of spatial variability in biotic communities compared to the total beta diversity itself (Soininen et al., 2017). Beta diversity can reflect two different phenomena: nestedness and spatial turnover (Baselga, 2010). The principal current hypotheses about the origin of beta diversity are as follows: 1) species composition is uniform in large areas; 2) species composition fluctuates in a random, autocorrelated way; 3) species distributions are related to environmental conditions (Legendre et al., 2005). Obviously, all these mechanisms may occur in relation to soil macrofauna communities. It is important to directly relate community beta-diversity to multiple environmental factors. This problem may be resolved by means of the multinomial diversity model (MDM). This approach can divide community entropy and diversity within and between sites, species, and models, and changes in entropy or diversity can be attributed to model predictors (De'ath, 2012).

The aim of our work is to define the role of edaphic and vegetation factors in the partitioning of beta diversity of the soil macrofauna community.

Material and methods

Site description

Studies were conducted in the 'Dnipro-Orils'kiy' Nature Reserve (Ukraine). The research polygon was laid in the forest within the Orlova ravine (48°31'13 "N, 34°48" 15 "E). The territory has a temperate-continental climate with an annual mean maximum decade temperature of 25.7 °C, and a minimum of –10.0 °C, and with a mean annual precipitation of approximately 565 mm (20-year average according to the data of the Dnipro meteorological station).

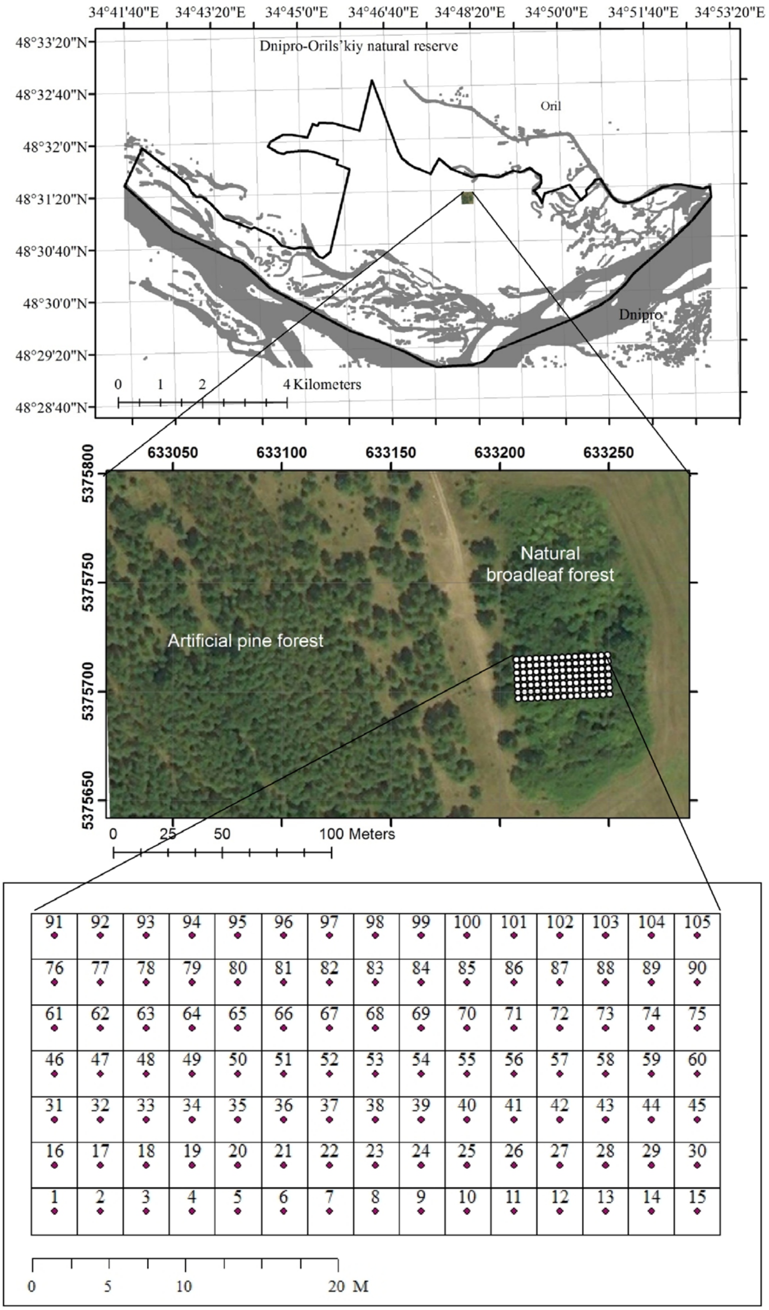
The study site comprises 1.0 ha of deciduous woodland bordered by grassland valley territory (Fig. 1). Forests in the steppe zone of Ukraine have a very restricted distribution and usually have an island status. To the east, the natural forest of the site borders an artificial pine plantation. The soils are fertile sandy loam with the underlying geology comprising quaternary aeolian sandy sediments. The site consists of 7 transects. Each transect is made up of 15 test points. The distance between rows in the site is 3 m.

Sampling methods

Soil macrofauna was defined as invertebrates visible to the naked eye (macroscopic organisms) (Warren, Zou, 2002). Geobionts (large soil invertebrates that permanently inhabit the soil) and geophiles (organisms that live in the soil only for some phase of their life) (Krivolutsky, 1994; Gholami et al., 2016) were assessed. Sampling was carried out during May 2016. Samples consisted of a single block of soil, 25×25×30 cm^3 deep, dug out quickly. A quadrat was fixed on the soil surface prior to taking the soil samples. The litter macrofauna was collected from the soil samples hand. The soil macrofauna were sorted and the animals were stored in 4% formaldehyde (Mathieu et al., 2004).

Vegetation survey

This was carried out in 9 m^2 quadrats, in the centre of which the macrofauna samples were collected. The projective cover of plant species was recorded at ground level, the understory (up to 2 m height) and canopy (above 2 m height). We were able to make species level identification for all the quadrats. Within the studied polygon, 48 species of plants were found. The forest stand was dominated by *Quercus robur* L. and *Pyrus communis* L. *Sambucus nigra* L.,



91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Fig. 1. Placing of experimental polygon and the sampling points.

Acer tataricum L. and *Crataegus fallacina* Klokov predominated among the bushes. The herbaceous layer was dominated by *Urtica dioica* L., *Anthriscus sylvestris* (L.) Hoffm., *Chelidonium majus* L., *Glechoma hederacea* L. and *Vincetoxicum hirundinaria* Medikus. In syntaxonomic aspect, the vegetation can be identified as follows (Sokolova, 2011):
Class *Querco-Fagetea* Br.-Bl. et Vlieger in Vlieger 1937,
Ordo *Quercetalia pubescenti-petraeae* Klika 1933,
Union *Aceri tatarici-Quercion* Zolyomi 1957,
Ass. *Vincetoxico hirundinariae-Quercetum roboris* Sokolova, 2011.

Environmental variables

Based on the geobotanical descriptions, phytoindicative assessment of environmental factors, according to Belgard (1950, 1971), Didukh (2011, 2012) and Ellenberg (1974), was made.

A system of plant ecomorphs was used according to Belgard (1950) and Tarasov (2012). Hygromorphs are represented by xerophytes (humidity level 1), mesoxerophytes (humidity level 2), xeromesophytes (humidity level 3), mesophytes (humidity level 4), hygromesophytes (humidity level 5). The humidity level by hygromorphic structure *(Hygr)* is calculated as:

$$Hygr = \frac{\sum_{i=1}^{i=N}(i \times P_i)}{100},$$

where i is the moisture level; P_i is the projective cover of plants of the corresponding hygromorph (Zhukov, Zadorozhnaya, 2016).

Trophomorphs are represented by oligotrophs (trophy level 1), mesotrophs (level of trophy 2) and megatrophs (trophy level 3). Nutrient status level by trophomorphic structure (*Troph*) is calculated as:

$$Troph = \frac{\sum_{j=1}^{j=N}(j \times P_j)}{100},$$

where j is the level of trophicity; P_j is the projective cover of plants of the corresponding trophomorph.

Heliomorphs are represented by heliosciophytes (level of light 2), scioheliophytes (level of light 3), helophytes (level of light 4). The level of illumination by the heoliomorphic structure (*Hel*) is estimated as:

$$Hel = \frac{\sum_{z=1}^{z=N}(z \times P_z)}{100},$$

where z is level of light; P_z is the projective cover of plants of the corresponding heliomorph.

Didukh phytoindication scales (2011, 2012) include edaphic and climatic scales. The edaphic phytoindication scales include the soil water regime (Hd), the variability of damping (fH), the soil aeration (Ae), the soil acidity (Rc), the total salt regime (Sl), the carbonate content in the soil (Ca) and nitrogen content in the soil (Nt). The climatic scales include the parameters of the thermal climate (thermoregime, Tm), humidity (Om), cryo-climate (Cr) and the continentality of climate (Kn). In addition to these, the lighting scale (Lc) is highlighted, which is characterized as a microclimate scale. Thermal properties of soils are indicated by a scale of the thermal regime, and hydrothermal is the scale of ombro mode. Phytoindicational evaluation of the environmental factors is performed by the ideal indicator method of Buzuk (2017).

Ellenberg indicator values (1974) include: *L*-scale of illumination/shading (9 classes, *Light Regime*), *T*-scale of thermo climate (9 classes, *Temperatures*), *K*-scale of climate continentality (9 classes, *Continentality of Climate*), *F*-scale of soil moisture (9 classes, *Humidity*), *R*-scale of soil acidity (9 classes, *Acidity*), and *N*-scale of soil nitrogen (9 classes, *Nutrients Availability*). Calculation of values of environmental factors was carried out using the method of average weighted values of indicator scales taking into account the projective cover of plants.

Measurement of soil mechanical impedance was carried out in the field using a hand penetrometer Eijkelkamp, to a depth of 100 cm with an interval of 5 cm. The average error of the measurement results of the device is ± 8%. The measurements were made by a cone with a cross-sectional dimension of 2 cm^2. Within each measurement point, the mechanical impedance of the soil was made in a single repeatability.

To measure the electrical conductivity of the soil *in situ*, a sensor HI 76305 was used (Hanna Instruments, Woonsocket, R. I.). This sensor works in conjunction with the portable device HI 993310. The tester estimates the total electrical conductivity of the soil, that is, combined conductivity of soil air, water and particles. The results of measurements of the device are presented in the units of saturation of the soil solution with salts is g/l. Comparison of measurement results of HI 76305 with laboratory data allowed us to estimate the conversion factor of units as 1 dS/m = 155 mg/l (Pennisi, van Iersel, 2002).

The aggregate structure was evaluated by the dry sieving method, according to Savinov (Vadunina, Korchagina, 1986). The percentage content of such fractions is established: < 0.25, 0.25–0.5, 0.5–1, 1–2, 2–3, 3–5, 5–7, 7–10, > 10 mm, and plant roots. The soil bulk density was estimated by Kachinskiy and the soil moisture by weight method (Vadunina, Korchagina, 1986).

Soil macrofauna identification

Adult and larvae specimens were counted and identified to species level. Earthworms were identified using Perel (1978), Vsevolodova-Perel (1997), and Kunah et al. (2010), Lithobiomorpha with Zalesskaya (1978), Geophilomorpha using Bonato et al. (2014), Diplopoda using Cherny and Golovach (1993), imago ground beetles using Kryzhanovsky (1964), larvae of ground beetles using Gilyarov (1964), Dolin (1978), Andreeva (1990), Kabakov (2006), and Krivosheina (2012), woodlice using Schmolzer (1965), molluscs using Gural-Sverlova and Gural (2012).

Statistical analysis

Statistical calculations were performed using the Statistica 7.0 program and the Project R ‘R software shell: A Language and Environment for Statistical Computing’ (http://www.R-project.org/). Estimation of confidence intervals and the standard deviation of the number of soil animals was made using a bootstrap approach and implemented by means of the bootES package (Kirby, Gerlanc, 2013).

The assessment of the soil macrofauna community biodiversity and 95% of their confidence intervals and its partitioning on alpha, beta and gamma diversity was done by using the entropart package (Marcon, Herault, 2015). Environmental variables were studied through the analysis of principal component using the package vegan (Oksanen et al., 2017). The number of significant principal components was calculated on the basis of the Horn procedure (Horn, 1965). The operation was completed using the paran package (Dinno, 2012). The partitioning of beta-diversity in relation to external predictors was conducted using multinomial diversity models with the help of MDM (De’ath, 2012, 2013). Spatial variation of differential entropy was displayed using the ‘Surfer® 12 from Golden Software, LLC (www.goldensoftware.com)’.

Geostatistical analysis

Kriging is an important tool in geostatistics. Kriging is a linear predictor by the method of the least squares (Minasny, McBratney, 2005). The variogram is a key concept in geostatistics. Knowledge of the exact mathematical form of the variogram allows one to quantify spatial variation (McBratney, Pringle, 1999) as well as the prediction of soil properties on a local or regional level (Minasny, McBratney, 2005). A variogram is usually calculated using spatial data using the method of moments, and subsequent fitting to the theoretical model of empirical variogram using a nonlinear least-squares method (Webster, Oliver, 2001). It is customary to refer to the intercept of the variogram model curve as the nugget (τ^2), the difference between the asymptote and the nugget as the sill (σ^2), and the distance at which the theoretical variogram curve reaches its maximum as the range. For models with an infinite range, the value at which the variogram reaches 95% of the asymptote is called the practical range. These names correspond to the parameters τ^2, σ^2 and φ respectively, where the latter is usually multiplied by a constant depending on the model. For instance, the practical range is 3φ for the exponential, $\sqrt{3}\varphi$ for the Gaussian, 4φ and 5φ for the Mattern model with $\kappa = 1$ and 2, respectively, and equals φ for the spherical model (Ribeiro et al., 2003).

However, the method of moments can give erroneous results, as commonly used variogram models (spherical, exponential and gauss) are characterized by lack of flexibility (Stein, 1999). As an alternative, one can consider the Mattern variogram class of models (Matern, 1986). Mattern models have considerable flexibility for modelling the spatial covariance and are able to describe a wide variety of local spatial processes. Based on this, the Mattern model is proposed to be used as a general approach for the simulation of soil properties (Minasny, McBratney, 2005). Mattern isotropic covariance function has the form (Handcock, Stein, 1993; Stein, 1999):

$$F(h) = \frac{1}{2^{\kappa-1}\,\Gamma(\kappa)}\left(\frac{h}{\varphi}\right)^{\kappa} K_{\nu}\left(\frac{h}{\varphi}\right),$$

where h is the separation distance; K_ν is the modified Bessel function of the second kind of order κ (Abramowitz, Stegun, 1972), Γ is the gamma function, φ is the range or distance parameter ($\varphi > 0$), which measures how fast correlation decays with distance; κ is the smoothness parameter. The Mattern model is characterized by high flexibility compared with conventional geostatistical models in view of the smoothing parameter κ. When the κ parameter is small (κ → 0) the model assumes a rough spatial process, if the κ parameter is large (κ → ∞) it assumes a smoothed spatial process (Minasny, McBratney, 2005). When the parameter κ = 0.5 , the Mattern model fully corresponds to an exponential model. When κ → ∞ , the Mattern model corresponds to a Gaussian model. If κ = 1, it corresponds to a Whittle's function (Whittle, 1954; Webster, Oliver, 2001; Minasny, McBratney, 2005). If the range parameter *r* is large (*r* → ∞), then the spatial process is approximated by the power function when κ > 0, and a log function or de Wijs function if κ → 0 (de Wijs, 1951, 1953). Calculations are made using geoR library (Paulo et al., 2016).

The nugget to sill ratio is an indicator of the strength of the spatial autocorrelation. A variable is considered to have a strong spatial dependence if the ratio is less than 25%, and has a moderate spatial dependence if the ratio is between 25 and 75%; otherwise, the variable has a weak spatial dependence (Sun et al., 2003).

Map accuracy, cross-validation, ME, NRMSE, and MSDR

To measure the accuracy of differential entropy maps, we use the cross-validation procedure and consequently, we compute the normalized root mean squared error (NRMSE), mean error (ME) and mean squared deviation ratio (MSDR) (Vašát et al., 2013). Mean squared error (RMSE) was calculated as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(x_{1,i} - x_{2,i})^2}{n}} .$$

Normalized root mean squared error (NRMSE) was calculated as follows:

$$NRMSE = \frac{RMSE}{x_{1,max} - x_{1,min}} .$$

Mean error (ME) was calculated as follows:

$$ME = \frac{\sum_{i=1}^{n}(x_{1,i} - x_{2,i})}{n} .$$

Mean squared deviation ratio (MSDR) was calculated as follows:

$$MSDR = \frac{\sum_{i=1}^{n}\left[\frac{(x_{1,i} - x_{2,i})^2}{var_i}\right]}{n} .$$

Where x_1 is a prediction of the variable X; x_2 is a measure of that variable; *n* is the number of records; *var* is a kriging variance. The smaller the NRMSE and ME values, the more accurate the map. The MSDR indicates whether the variance of measurement data is well reproduced with the kriging interpolation, and ideally, it equals to 1 (Vašát et al., 2013). The *R*-squared of the regression between the observed and predicted after cross validation values was used as they are very intuitive. Cross-validation procedure was performed using the function *xvalid* from the package geoR library (Ribeiro, Diggle, 2016).

Results

In the soil of the studied polygon, 38 species of soil invertebrates were found, which characterises the gamma diversity : (2.5% quantile is 34.2, 97.5% quantile is 41.2) (Fig. 2). Alpha diversity, or the number of species on average in each sampling point is 4.3 (2.5% quantile is 4.2, 97.5% quantile is 4.5). Beta diversity is 8.8 (2.5% quantile is 8.0, 97.5% quantile is 9.6).

The abundance of soil macrofauna was 197.8 ± 27.9 ind./m^2 (Table 1). The endogeic earthworm *Aporrectodea rosea* was the dominant species in the macrofauna community. Endogeic *A. trapezoides*, epigeic *Dendrobaena octaedra* and anecic *Octodrilus transpadanus* were represented in the community. Millipedes were represented by four species, with domination of endogeic Geophilidae compared to epigeic Lithobiidae. There was a variety of soil insects,

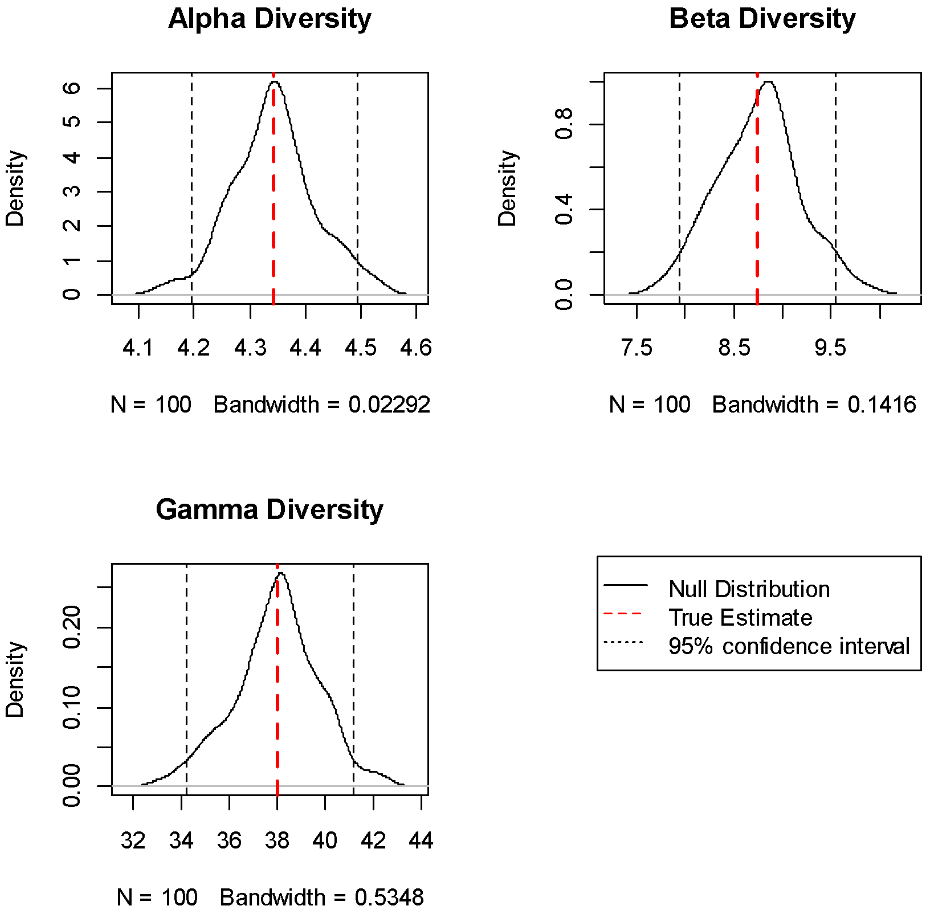


Fig. 2. Alpha, beta and gamma diversity of soil macrofauna.

among which were both imagoes and larvae. Molluscs were represented by five species, but their abundance was not great.

Beta diversity of the community may be affected by edaphic and (or) vegetation factors. Principal component analysis of the edaphic indicators revealed 9 principal components, having eigenvalues greater than 1. Using the Horn procedures, we found that the first four principal components are statistically significant. These components describe 64.2% of the total variation of the edaphic indicators. Principal component 1 is correlated with the soil mechanical impedance at a depth of 35–100 cm, litter depth, density and electric conductivity of the soil and the content of the roots and aggregate fractions of size < 0.25–1 mm and 5–10 mm (Table 2). Principal component 2 is correlated with the soil mechanical impedance

T a b l e 1. Taxonomic composition and abundance of soil macrofauna.

Taxons		**Density, ind./m^2 ± st. error**
Phylum Annelidae Class Oligohaeta Order Haplotaxida		
Family Lumbricidae	*Aporrectodea caliginosa trapezoides* (Duges, 1828)	9.90 ± 1.39
	Aporrectodea rosea rosea (Savigny, 1826)	117.49 ± 8.15
	Dendrobaena octaedra (Savigny, 1826)	15.54 ± 2.27
	Octodrilus transpadanus (Rosa, 1884)	5.64 ± 1.17
Order Tubificida		
Family Enchytraeidae	*Enchytraeus albidus* Henle 1837	7.92 ± 1.56
Phylum Arthropoda Class Arachnida Order Araneae		
Family Lycosidae	*Pardosa lugubris* (Walckenaer 1802)	0.91 ± 0.42
Class Chilopoda Order Geophilomorpha		
Family Geophilidae	*Geophilus proximus* C.L.Koch 1847	3.96 ± 1.00
	Pachymerium ferrugineum (C.L.Koch 1835)	1.83 ± 0.56
Order Lithobiomorpha		
Family Lithobiidae	*Lithobius (Monotarsobius) aeruginosus* L. Koch 1862	0.91 ± 0.42
	Lithobius (Monotarsobius) curtipes C.L. Koch 1847	1.83 ± 0.59
Class Insecta Order Coleoptera		
Family Carabidae	*Amara (Amara) aenea* (De Geer 1774)	0.15 ± 0.15
	Amara similata (Gyllenhal, 1810)	2.29 ± 0.85
	Calathus (Calathus) fuscipes (Goeze, 1777)	0.15 ± 0.15
	Carabus (Cancellocarabus) cancellatus Illiger, 1798	0.30 ± 0.30
Family Cetoniidae	*Cetonia aurata* (Linnaeus 1761) (larvae)	0.46 ± 0.33
Family Chrysomelidae	*Chrysolina (Fastuolina) fastuosa* (Scopoli 1763) (larvae)	0.76 ± 0.32
Family Curculionidae	*Otiorhynchus (Cryphiphorus) ligustici* (Linnaeus 1758) (larvae)	0.30 ± 0.21
Family Elateridae	*Athous (Athous) haemorrhoidalis* (Fabricius 1801) (larvae)	6.70 ± 1.18
	Agriotes (Agriotes) lineatus (Linnaeus 1767) (larvae)	0.15 ± 0.15
	Cardiophorus rufipes (Goeze, 1777) (larvae)	0.46 ± 0.26
Family Staphylinidae	*Othius angustus angustus* Stephens 1833 (larvae)	0.15 ± 0.15
	Othius punctulatus (Goeze 1777) (larvae)	0.15 ± 0.15
Family Tenebrionidae	*Helops coeruleus* (Linnaeus 1758) (larvae)	0.30 ± 0.22
Family Melolonthidae	*Anoxia pilosa* (Fabricius 1792) (larvae)	0.30 ± 0.21
	Melolontha melolontha (Linnaeus 1758) (larvae)	2.29 ± 0.58
	Serica brunnea (Linnaeus 1758) (larvae)	0.46 ± 0.26
Order Dermaptera		
Family Forficulidae	*Forficula auricularia* Linnaeus 1758	0.61 ± 0.31
Order Diptera		
Family Rhagionidae	*Rhagio scolopaceus* (Linnaeus 1758) (larvae)	4.88 ± 1.21
Family Stratiomyidae	*Stratiomys longicornis* (Scopoli 1763) (larvae)	0.15 ± 0.15
Family Tabanidae	*Tabanus bromius* Linnaeus 1758 (larvae)	0.30 ± 0.21
Family Tipulidae	*Tipula (Lunatipula) lunata* Linnaeus 1758 (larvae)	3.66 ± 0.73
Order Lepidoptera		
Family Noctuidae	*Agrotis clavis* (Hufnagel 1766) (larvae)	5.03 ± 1.07
Class Malacostraca Order Isopoda		
Family Trachelipodidae	*Trachelipus rathkii* (Brandt 1833)	0.15 ± 0.15
Phylum Mollusca Class Gastropoda Order Pulmonata		
Family Cochlicopidae	*Cochlicopa lubrica* (O.F. Muller 1774)	0.15 ± 0.15
Family Helicidae	*Cepaea (Austrotachea) vindobonensis* (C. Pfeiffer 1828)	0.15 ± 0.15
Family Succineidae	*Succinella oblonga* (Draparnaud 1801)	0.61 ± 0.30
Family Valloniidae	*Vallonia pulchella* (O.F. Muller 1774)	0.15 ± 0.15
Family Vitrinidae	*Vitrina pellucida* (O.F. Muller 1774)	0.61 ± 0.30

Table 2. Descriptive statistics and principal component analysis of the edaphic parameters (presenting statistically significant correlation coefficients with $p < 0.05$).

Parameters, mean ± st. error		**Principal components**			
		1	**2**	**3**	**4**
Soil mechanical impedance at depth, MPa					
0–5 cm	0.65 ± 0.01	–	–0.55	–	–
5–10 cm	0.76 ± 0.02	–	–0.76	–	–
10–15 cm	0.89 ± 0.04	–	–0.90	–	–
15–20 cm	0.96 ± 0.04	–	–0.91	–	–
20–25 cm	0.98 ± 0.04	–	–0.89	–	–
25–30 cm	0.95 ± 0.04	0.20	–0.84	–	–
30–35 cm	0.94 ± 0.03	–	–0.78	–	–
35–40 cm	1.04 ± 0.03	–0.41	–0.59	–0.26	–
40–45 cm	1.31 ± 0.03	–0.50	–0.42	–0.47	–
45–50 cm	1.56 ± 0.03	–0.66	–0.32	–0.32	–
50–55 cm	1.81 ± 0.04	–0.72	–0.33	–0.23	–
55–60 cm	2.02 ± 0.04	–0.82	–0.24	–	–
60–65 cm	2.28 ± 0.05	–0.79	–0.21	–	–
65–70 cm	2.41 ± 0.05	–0.87	–	–	–
70–75 cm	2.53 ± 0.05	–0.90	–	–	–
75–80 cm	2.59 ± 0.05	–0.89	–	–	–
80–85 cm	2.69 ± 0.06	–0.87	–	–	–
85–90 cm	2.72 ± 0.06	–0.90	–	–	–
90–95 cm	2.74 ± 0.07	–0.91	–	–	–
95–100 cm	2.84 ± 0.08	–0.84	–	–	–
Other edaphic parameters					
Litter depth, cm	3.40 ± 0.10	–0.35	0.55	–	–
Moisture, %	30.96 ± 0.69	–	–0.59	–	–
Bulk density, g/cm^3	0.98 ± 0.01	–0.71	0.58	–	–
Electrical conductivity, dSm/м	0.21 ± 0.01	–0.35	0.55	–	–
Aggregate fractions (mm) and plant roots content, %					
> 10	12.91 ±0.71	–	–	0.86	–
7–10	7.28 ± 0.28	–0.24	–	0.64	–
5–7	9.81 ± 0.35	–0.26	–	–	0.52
3–5	23.13 ± 0.85	–	–	–0.54	0.58
2–3	27.68 ± 0.82	–	–	–0.79	–
1–2	13.96 ± 0.86	–	–	–0.66	–0.41
0.5–1	1.37 ± 0.12	0.27	–	–0.44	–0.60
0.25–0.5	2.29 ± 0.29	0.22	–	–0.35	–0.61
< 0.25	0.61 ± 0.09	0.37	–	–0.27	–0.66
Roots	0.95 ± 0.08	0.25	–	–	0.36
Eigen values		9.42	6.72	3.23	2.47
% total variation		27.71	19.77	9.50	7.27

Notes: Litter – thickness, cm; Moisture – moisture of soil, %; Density – soil density, g/cm3; E – electronic conductivity of the soil, d Sm/M.

Table 3. Descriptive statistics and principal component analysis of the vegetation parameters (presenting statistically significant correlation coefficients with $p < 0.05$).

Parameters, mean ± st. error		**Principal components**					
		1	**2**	**3**	**4**	**5**	**6**
Diduh scales							
Hd	9.20 ± 0.13	−0.21	–	−0.69	–	–	–
fH	5.16 ± 0.10	−0.53	0.50	−0.24	0.37	–	–
Rc	6.11 ± 0.14	−0.39	–	−0.26	0.26	−0.50	−0.47
Sl	7.10 ± 0.10	–	−0.49	–	0.41	−0.65	–
Ca	4.97 ± 0.23	−0.84	–	0.21	–	–	–
Nt	12.21 ± 0.12	0.60	–	0.19	−0.53	−0.33	−0.20
Ae	5.50 ± 0.16	–	0.37	−0.51	−0.36	–	–
Tm	11.18 ± 0.06	0.38	–	0.48	–	–	–
Om	12.56 ± 0.13	0.71	0.21	−0.49	–	–	–
Kn	7.05 ± 0.20	−0.86	–	0.30	–	−0.22	–
Cr	9.93 ± 0.10	0.27	0.56	–	0.43	–	0.30
Lc	5.30 ± 0.15	−0.72	–	−0.21	–	–	−0.26
Indexes based on Belgard’s ecomorphs							
Troph	2.28 ± 0.02	−0.26	0.57	–	−0.46	−0.39	0.32
Hygr	3.23 ± 0.03	0.25	–	0.84	–	–	–
Hel	3.00 ± 0.01	−0.78	−0.31	–	–	0.40	–
Ellenberg scales							
Light Regime	6.79 ± 0.03	−0.52	−0.66	–	–	0.34	–
Temperatures	5.80 ± 0.02	−0.70	–	–	−0.33	−0.45	–
Continentality of Climate	5.31 ± 0.04	−0.81	−0.37	–	–	–	0.24
Humidity	5.15 ± 0.02	0.71	−0.33	–	0.23	–	0.29
Acidity	6.02 ± 0.02	–	−0.51	0.69	0.29	–	−0.23
Nutrients Availability	6.74 ± 0.08	0.89	–	0.23	–	0.20	–
Raunkiaer’s life forms							
Ph	0.34 ± 0.011	0.20	−0.30	−0.78	–	−0.22	0.40
nPh	0.27 ± 0.011	–	−0.84	–	–	–	−0.40
HKr	0.28 ± 0.012	–	0.59	0.50	0.42	–	0.22
T	0.10 ± 0.007	–	0.62	0.31	−0.44	0.14	−0.28
G	0.01 ± 0.002	−0.32	0.44	−0.39	–	0.29	−0.28
Eigen values		7.19	4.21	3.96	2.01	1.77	1.48
% total variation		27.64	16.19	15.22	7.74	6.79	5.6

Notes: Hd – soil water regime; fH – variability of damping; Rc – soil acidity; Sl – total salt regime; Ca – carbonate content in soil; Nt – nitrogen content in soil; Ae – soil aeration; Tm – thermoregime; Om – humidity; Kn – continentality of climate; Cr – cryo-climate; Lc – lighting scale; Hygr – humidity level by hygromorphic structure; Troph – nutrient status level by trophomorphic structure; Hel – level of illumination by the geoliomorphic; Ph – phanerophytes; nPh – nanophanerophytes; HKr – hemicryptophytes; T – therophytes; G – geophytes.

at a depth of 0–65 cm and some other edaphic parameters. Principal component 3 correlated with soil mechanical impedance at a depth of 35–55 cm and a content of aggregate fractions of size < 0.25–5, 7–10 and > 10 mm. Principal component 4 is correlated with the content of roots and aggregate fractions < 0.25–2 and 3.7 mm.

Principal component analysis of plant indicators identified 7 principal components, the eigenvalues of which are greater than 1. Using the Horn procedure, we found that the first six principal components were statistically significant. These components describe 73.6% of the total variation in the vegetation indicators (Table 3). The first principal component is characterized by the greatest correlation with the level of carbonates in the soil, as well as scales of continentality and illumination. This component is meaningful and can be interpreted as the ecotone effect. Principal component 2 is characterized by a positive correlation with phanerophites and nanophanerophites and negative correlation with other life forms according to Raunkiaer's classification. Thus, the principal component 2 represents the ratio between the herbaceous layer, on the one hand, and the shrub layer (to a lesser extent with forest stands), on the other hand. Correlation with other environmental factors reveals the contents of the processes associated with the specified value. The principal component 3 is correlated with phanerophytes; this allows us to interpret it as the forest stand density. The humidity, acidity, ecotope aeration, as well as some other ecological factors are connected with forest stand density. The principal component 4 is characterized by the greatest correlation with soil nitrogen content. The principal component 5 can be interpreted as the mineralization of the soil solution. The principal component 6 most likely represents the level of soil solution acidity.

The principal components are orthogonal, that is, mutually independent. However, vegetation and edaphic principal components can be correlated (Table 4). Only the principal component 6 is independent and reflects plant community properties only, since it is not correlated with the edaphic principal components. The edaphic principal components always correlate with vegetation principal components. These results indicate the complexity of the interaction between soil and vegetation.

On the basis of the MDM-approach, we evaluated the role of edaphic principal components in the partitioning of the macrofauna community β-diversity (Table 5). The results indicate that the γ-diversity is 1.71 bits, and a α-diversity is 0.91 bit. We found that 20.9% (0.81 bit) of β-diversity was caused by the edaphic factors. The largest decrease in community entropy took place under the action of principal components 2 and 3 (0.06 bit and 0.05 bit, respectively). The permutation test reveals that these effects are statistically significant. The influence of the principal component 1 and 4 is lower and is not statistically significant.

In turn, 28.4% of community β-diversity was caused by the plant factors (Table 6). The greatest decrease in community entropy is related to vegetation principal components 1, 3 and 4 (0.07, 0.05, 0.04 bits, respectively). The permutation test reveals that these effects are statistically significant. The effect of principal components 2, 5 and 6 are not statistically significant.

The effect of ecological factors leads to a change in the entropy of the community. This change can be positive, and then the factor information is transferred to the community. Or a change can be negative, and then the community is sent misinformation. Between the

T a b l e 4. Correlation matrix of the edaphic and vegetation principal components (presenting statistically significant correlation coefficients with $p < 0.05$).

		Vegetation principal components					
		Ph_1	**Ph_2**	**Ph_3**	**Ph_4**	**Ph_5**	**Ph_6**
Edaphic principal components	**Ed_1**	–	–0.20	–0.48	–	–	–
	Ed_2	0.37	–0.21	0.37	–	–0.34	–
	Ed_3	–	–	0.20	–	–	–
	Ed_4	–	–	–	0.22	–0.24	–

T a b l e 5. The analysis of deviance, entropy and diversity under edaphic impact on macrofauna on the basis of MDM-approach.

Model	**df**	**Δdf**	**Dev**	**ΔDev**	**Ent**	**ΔEnt**	**p–level**	**Div**	**ΔD**
γ-diversity	3432	–	359.5	–	1.71	–	–	5.54	–
Ed1	3399	33	353.8	5.74	1.69	0.03	0.37	5.39	1.03
Ed1+Ed2	3366	33	340.8	12.96	1.62	0.06	0.00	5.07	1.06
Ed1+Ed2+Ed3	3333	33	330.6	10.27	1.57	0.05	0.00	4.83	1.05
Ed1+Ed2+Ed3+Ed4	3300	33	324.3	6.25	1.54	0.03	0.29	4.69	1.03
α-diversity	0	3300	190.2	134.18	0.91	0.64	0.01	2.47	1.89

Notes: Ed1–4 – edaphic principal components; df – degrees of freedom; Δdf – changes in df; Dev – deviance; ΔDiff – changes in deviance; Ent – entropy; ΔEnt – changes in entropy; *p*–level – the significance level based on sequential permutation tests; Div – diversity; ΔD – proportional change in diversity.

T a b l e 6. The analysis of deviance, entropy and diversity under vegetation impact on macrofauna on the basis of MDM-approach.

Model	**df**	**Δdf**	**Dev**	**ΔDev**	**Ent**	**ΔEnt**	**p–level**	**Div**	**ΔD**
γ- diversity	3432	–	359.5	–	1.71	–	–	5.54	–
Ph1	3399	33	344.1	15.48	1.64	0.07	0.01	5.15	1.08
Ph1+Ph2	3366	33	337.9	6.20	1.61	0.03	0.28	5.00	1.03
Ph1+Ph2+Ph3	3333	33	328.3	9.56	1.56	0.05	0.02	4.78	1.05
Ph1+Ph2+Ph3+Ph4	3300	33	320.1	8.16	1.52	0.04	0.05	4.59	1.04
Ph1+Ph2+Ph3+Ph4+Ph5	3267	33	316.3	3.84	1.51	0.02	0.87	4.51	1.02
Ph1+Ph2+Ph3+Ph4+Ph5+Ph6	3234	33	311.4	4.90	1.48	0.02	0.57	4.41	1.02
α-diversity	0	3234	190.2	121.25	0.91	0.58	0.01	2.47	1.78

Notes: Ph1–Ph6 – edaphic principal components; df – degrees of freedom; Δdf – changes in df; Dev – deviance; ΔDiff – changes in deviance; Ent – entropy; ΔEnt – changes in entropy; *p*–level – the significance level based on sequential permutation tests; Div – diversity; ΔD – proportional change in diversity.

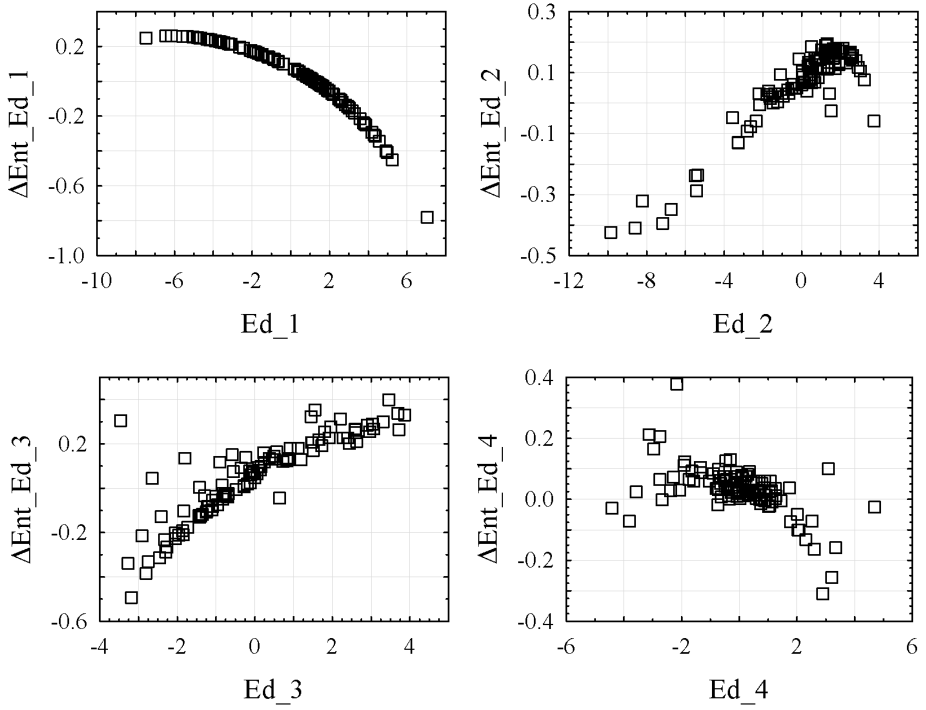


Fig. 3. Dependence of differential entropy on edaphic principal component scores.

values of the edaphic principal components, which reflect the cumulative variation of soil properties , and changes of entropy in each site, there is a dependency (Fig. 3). For example, the positive values of the principal components 1 lead to misinformation of the community. On the other hand, negative values are perceived by the community as an organization factor. Thus, the effects of ecological factors on the macrofauna community do not occur across the full range of values of the ecological factors. All dependencies are nonlinear, indicating that the there is a range of values of the principal components, when the highest amount of information is transmitted. In other words, when the community is most sensitive to the corresponding principal components.

Similar results were obtained for the dependence of entropy change of the vegetation principal component values (Fig. 4). All principal components have a range of values of the principal component, when the community receives misinformation. Also, for certain values of principal components, the transmitted information reaches the maximum values.

The MDM-approach evaluates the components of entropy by sites. This provides a good opportunity to examine how changes in entropy vary in geographic space (Figs 5, 6). Within the study area, for each of the principal components, areas can be distinguished within which

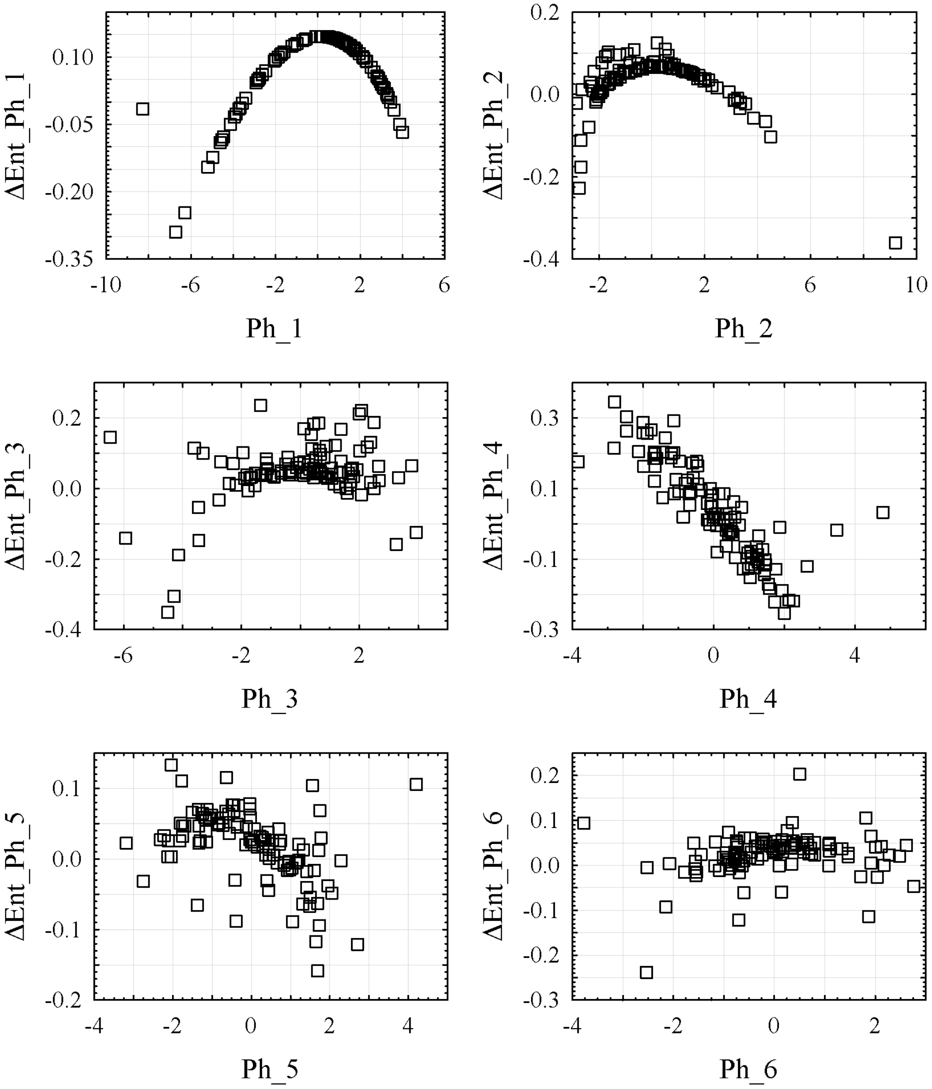


Fig. 4. Dependence of differential entropy on vegetation principal component scores.

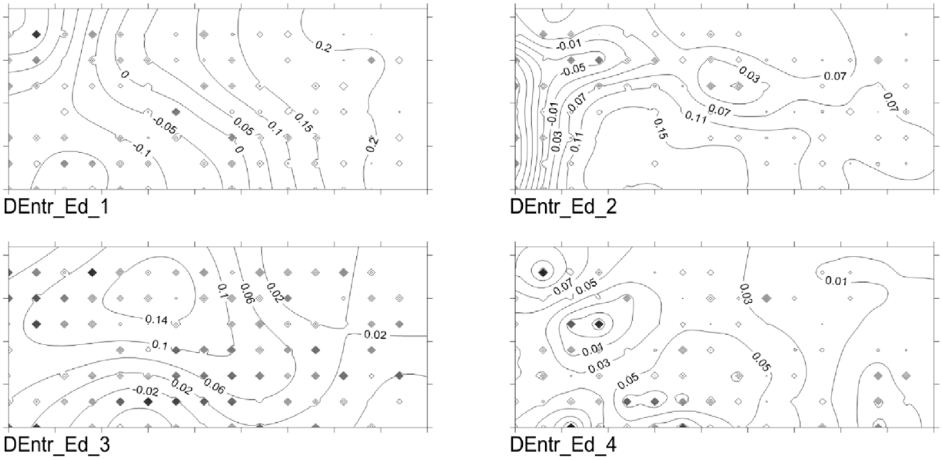


Fig. 5. Spatial variation of differential entropy under influence of the edaphic principal components.

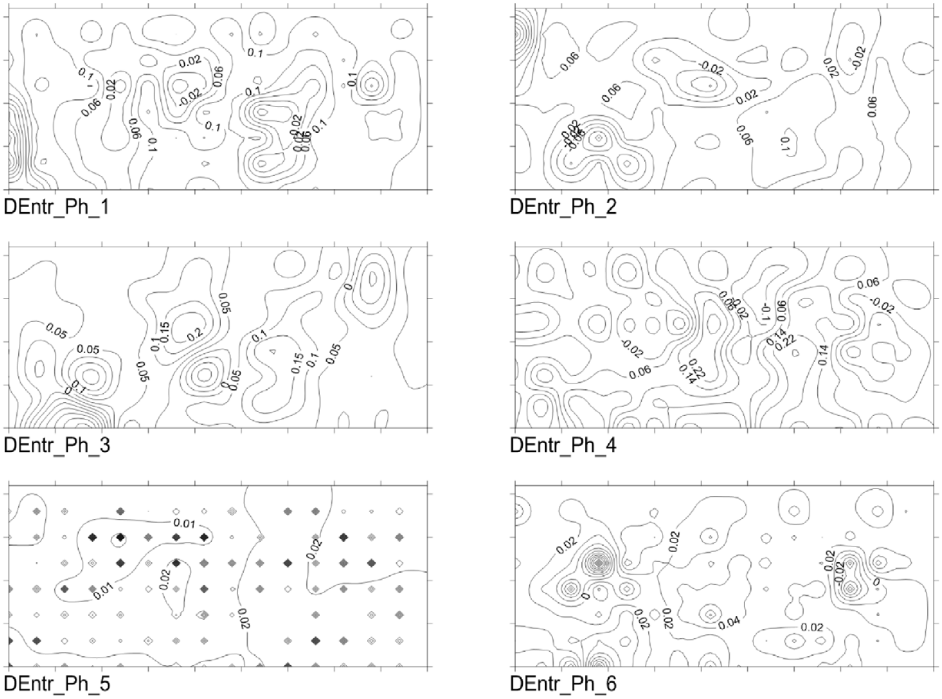


Fig. 6. Spatial variation of differential entropy under influence of the vegetation principal components.

Table 7. Geostatistics of the spatial variation in the entropy change under the influence of edaphic and vegetation factors.

PC	Phi	Pr_Range	Sill	Nugget	SDL	Kappa	NRMSE	ME*10^{-5}	MSDR	R^2
Edaphic principal components										
Ed1	9.86	39.98	0.03	0.0097	23.92	1.03	0.11	5.50	0.38	0.61
Ed2	3.67	10.98	0.01	0.0064	41.41	0.50	0.16	-2.44	0.56	0.49
Ed3	1.55	15.59	0.01	0.0271	70.20	8.00	0.20	-2.53	0.95	0.05
Ed4	23.00	196.15	0.04	0.0056	11.15	5.60	0.12	-0.14	1.01	0.00
Alpha	0.20	0.12	0.13	0.0831	38.64	0.03	0.18	0.00	1.01	0.00
Vegetation principal components										
Ph1	22.69	113.55	2.53	0.0029	0.11	1.70	0.13	4.49	0.79	0.25
Ph2	5.66	30.14	8.07	0.0000	0.00	1.97	0.09	-15.99	1.44	0.09
Ph3	21.62	135.25	71.01	0.0002	0.00	2.83	0.06	4.38	0.55	0.47
Ph4	28.22	150.39	92.87	0.0004	0.00	1.97	0.21	23.24	2.03	0.01
Ph5	19.33	61.03	0.00	0.0025	81.58	0.57	0.17	1.18	0.96	0.03
Ph6	1.00	3.45	0.00	0.0001	3.83	0.70	0.11	0.20	1.01	0.00
Alpha	27.67	45.20	0.09	0.17	66.76	0.13	0.19	6.55	0.99	0.01

Notes: Phi is the range or distance parameter of the Mattern model; Pr_Range is a practical range; Kappa is a smoothing parameter; the nugget is the y-intercept of the graph, the sill is the semivariogram value (y value) where each graph becomes a plateau, the range is the distance (x value) where the plateau begins, and the spatial dependence level SDL ((Sill-Nugget)/Sill) is the ratio of structural to population variance, R^2 of cross-validation.

the relevant ecological factor has a structuring effect on the macrofauna community. Also, there are areas within which the ecological factor transmits misinformation to the community. The spatial patterns of the entropy changes are different under the influence of specific ecological factors.

Geostatistical models best describe the variation of entropy change induced by edaphic principal components 1 and 2 (Table 7). These models respectively describe 61 and 49% of their variation. The impact of principal component 1 on entropy change has a strong spatial dependence (SDL = 23.90%). The impact of principal component 2 has a moderate spatial dependence (SDL = 41.41%). The Mattern model can be regarded as a generalization of a number of theoretical variogram models (Minasny, McBratney, 2005). The geostatistical model of principal component 1 is closest to the Whittle function, as in the general Mattern model kappa = 1.03 (for the Whittle model kappa = 1) (Whittle, 1954). Spatial variation in differential entropy under the influence of the principal components 2 is best modelled by an exponential model for which kappa = 0.5 (Webster, Oliver, 2001; Minasny, McBratney, 2005). The effect on the community of edaphic components 3 and 4 has no significant spatial dependence or generally may be purely spatially modelled.

The spatial model best describes the effect of the vegetation principal components 1 and 3 on the soil macrofauna community (R^2 = 0.25 and 0.47 respectively). The impact of the vegetation principal components 1 and 3 on entropy change has a strong spatial dependence.

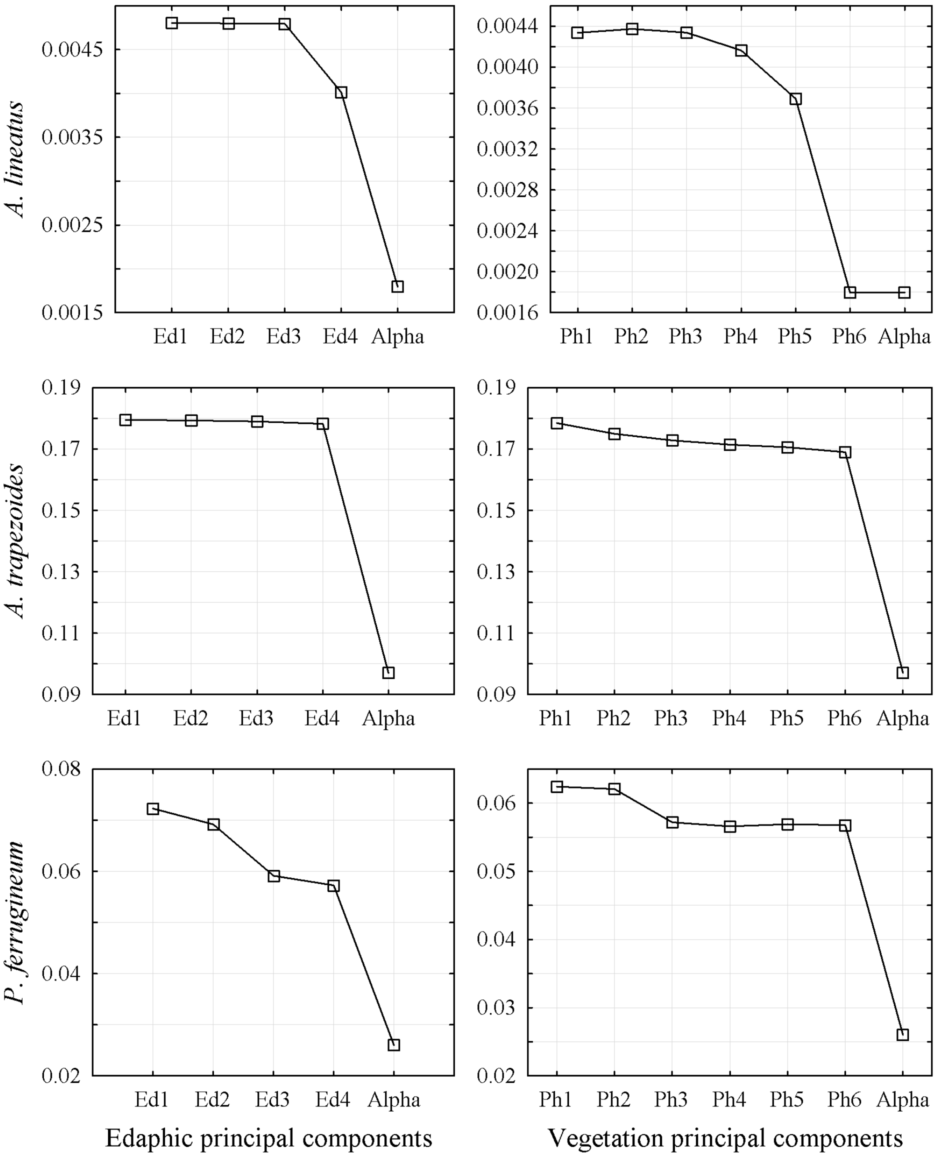


Fig. 7. Differential entropy for some species of soil invertebrates.

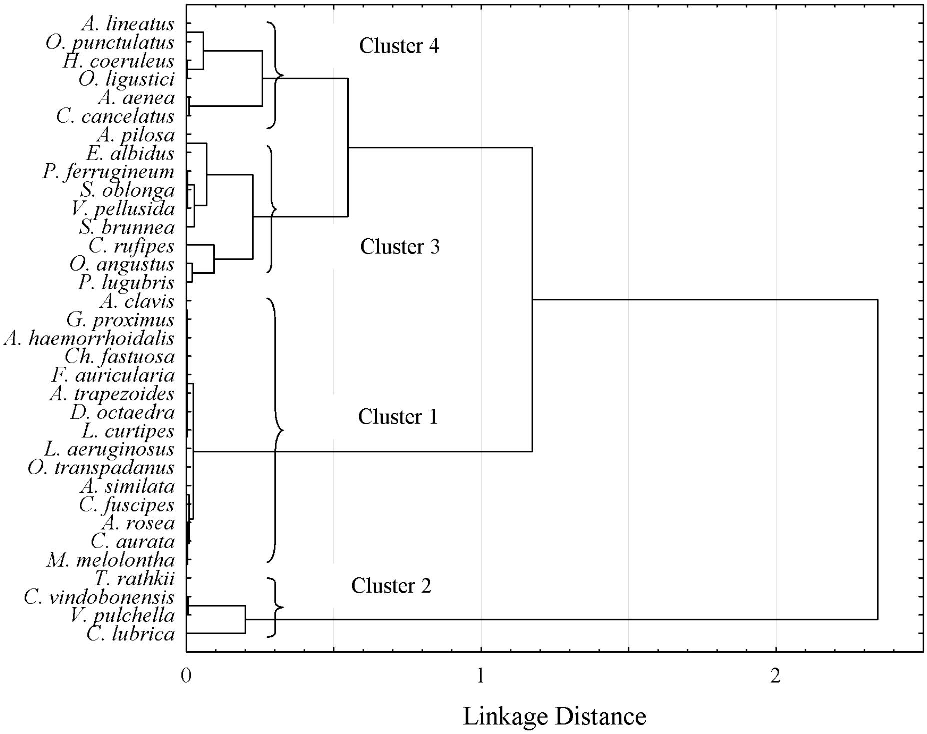


Fig. 8. Classification of macrofauna species on the basis of beta diversity transformation patterns under the influence of edaphic factors.

Parameter kappa indicates that the spatial patterns for the principal components 1 and 3 are smoother than the Whittle pattern.

The MDM-approach estimates the components of entropy by species (Fig. 7). The corresponding curves characterize the ecological features of the species that make up the community. According to the degree of similarity of these curves, one can perform a classification of the community by cluster analysis (Fig. 8). The cluster analysis of the species according to their response to the edaphic factors reveals four clusters. It is possible to build the integral curves of reaction to the environmental factors of the species included in each cluster. Cluster 1 combines the species for which edaphic factors have no effect on contribution to the total β-diversity of the community (Fig. 9). Cluster 2 combines the species sensitive to the action of the principal components 1 and 2. Cluster 3 combines the species in the community that are sensitive to all the principal components. Cluster 4 combines species sensitive to the action of principal components 3 and 4.

With respect to the influence of vegetation factors, the macrofauna species are classified into four clusters (Fig. 10). Cluster 1 combines species not susceptible to the action of

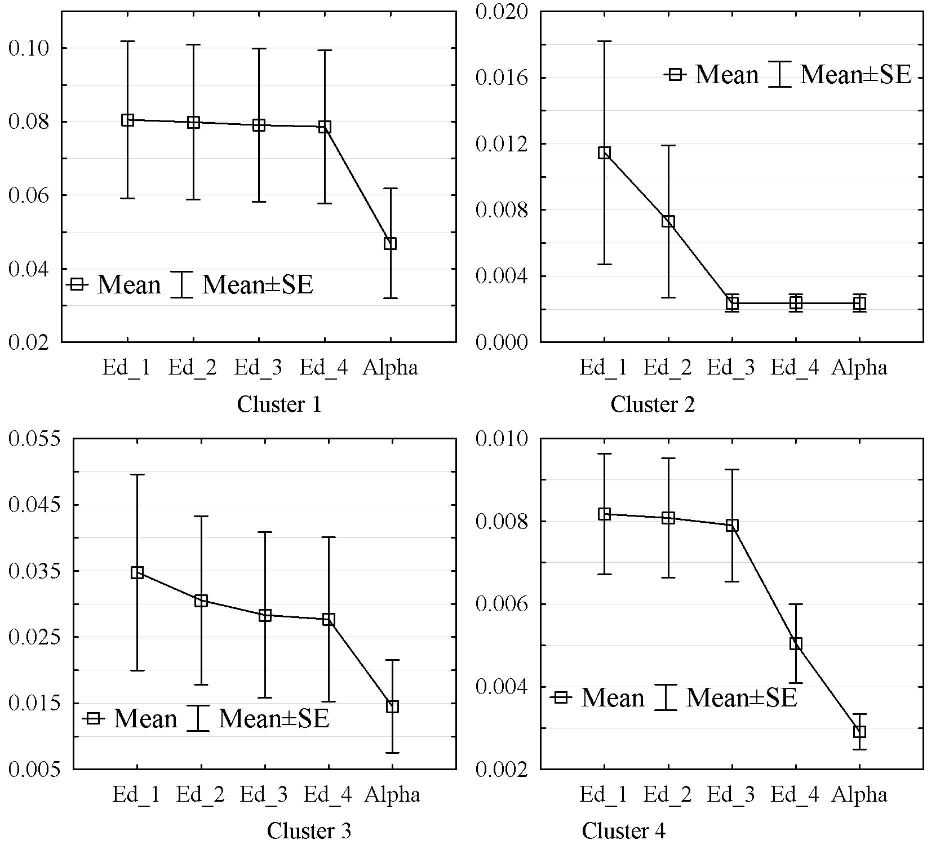


Fig. 9. Differential entropy under the influence of edaphic factors for clusters.

vegetation principal components within the studied site (Fig. 11). Species that are included in cluster 2 are sensitive to all vegetation principal components. Cluster 3 combines species sensitive to the action of vegetation principal components 1 and 2. Species included in cluster 4 are sensitive to principal components 1–5.

Discussion

The overall inertia of the tables of species occurrence has been demonstrated to be capable of corresponding with common diversity indices of species richness, such as the Simpson diversity, or the Shannon information index. This result allows one to examine from a general point of view the ordination techniques such as Correspondence Analysis, Non-Symmetric

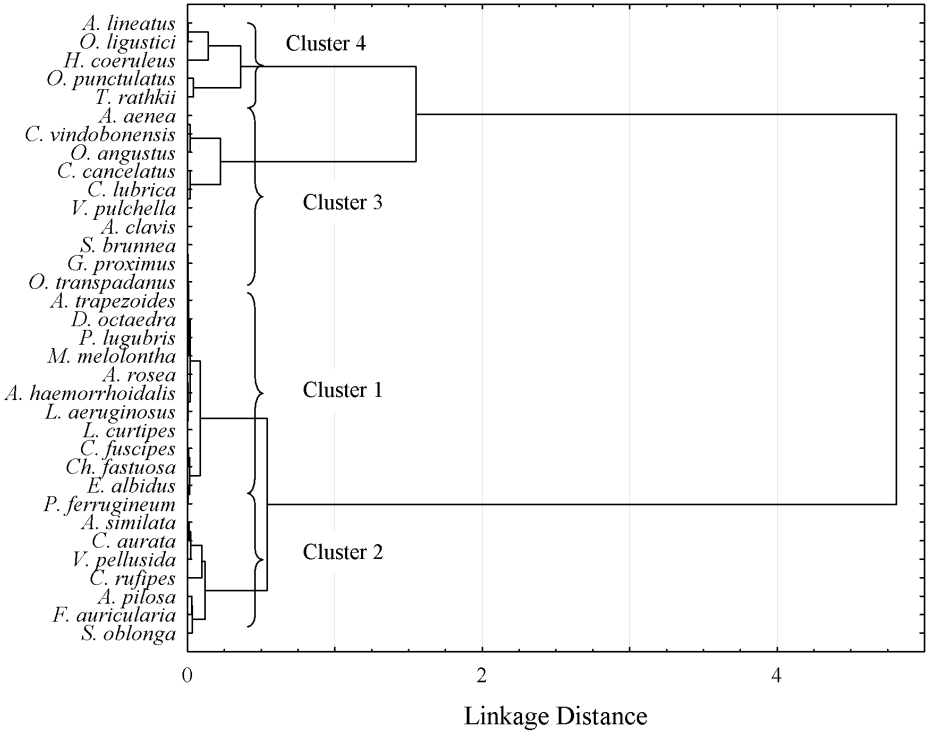
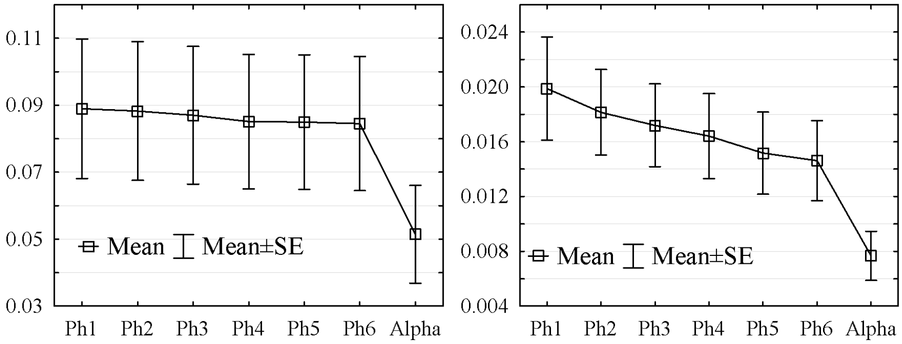


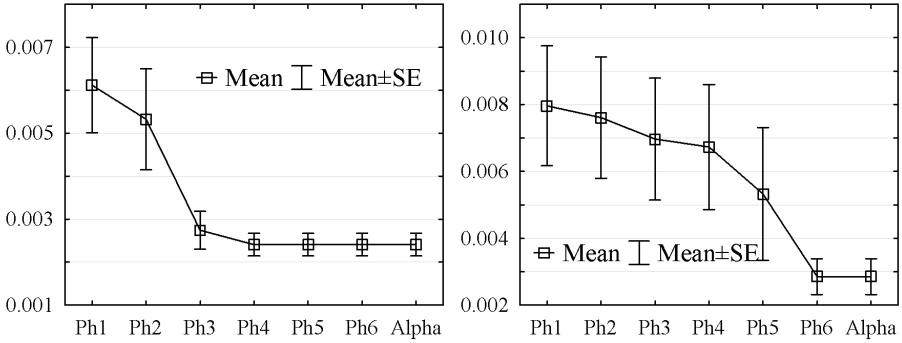
Fig. 10. Classification of macrofauna species on the basis of beta diversity transformation patterns under the influence of vegetation factors.

Correspondence Analysis, Canonical Correspondence Analysis, and Redundancy Analysis, and provides greater insight into interrelations between the ordination methods and diversity indexes (Pélissier et al., 2003). These interrelations explain why ordination techniques are widely used in studying beta diversity (Eggleton et al., 2005; Carpenter et al., 2012; Zbinden, Matthews, 2017). The multinomial diversity model (MDM) is a method for relating Shannon diversity to complex environmental, spatial and temporal predictors (De'ath, 2012).

In our work, we established the influence of plant and edaphic factors on beta diversity of a macrofauna community. Within the relatively restricted area of the studied polygon, beta diversity of the soil macrofauna community is 8.8 (2.5% quantile-8.0, 97.5%-9.6). We have shown that the effect of plant factors on beta diversity is greater than the effect of edaphic factors. It is notable that the edaphic principal component 1, which is dominant on level of variation, has no statistically significant effect on beta diversity of the macrofauna community. Probably, the reason for this is that the variation of relevant properties of edaphic properties is perceived by the community as a source of information and as a source of misinformation. Information increases the negative entropy and organizes community. In turn, the impact



Cluster 1 Cluster 2



Cluster 3 Cluster 4

Fig. 11. Differential entropy under the influence of vegetation factors for clusters.

of misinformation increases entropy and disorder in the community. Only negative values of edaphic principal components 1 pass information to the community, whereas positive values are a source of misinformation. The edaphic principal component 1 is characterized by correlation with indicators of soil mechanical impedance at a depth of 35–100 cm. In the conditions of the floodplain at the specified depth, variation of the soil mechanical impedance can be a consequence of the mobility of the parent deposition. High soil mechanical impedance at relatively great depths may characterize the stable areas of the floodplain. Low soil mechanical impedance can be the result of the fact that the soil pores and cracks within moving parts of the floodplain are filled with loose material. Mechanical instability can lead to different scenarios of soil dynamics, to which soil animals react as to factors of disorganization of the community.

The edaphic principal components 2 and 3 are sensitive to the variations of the soil mechanical impedance at a depth of 0–65 and 35–55 cm. These dynamics of soil properties may be due to spatial variation of the vegetation structure. Plant and edaphic principal components are correlated with each other. Statistics cannot confirm a causal relationship, but vegetation can be assumed to play a leading role in shaping soil ecological regimes at this scale level. This is also in line with the views on the factors of soil formation that ascribe the leading role to vegetation (Dokuchaev, 1883; Jenny, 1941; Bockheim et al., 2014).

The impact of vegetation principal components 1, 3 and 4 on the beta diversity of soil macrofauna community is statistically significant. Plants modify the microclimate in their vicinity by cooling down the soil and air in the shade of their leaves. They also modify humidity by intercepting wind and rain, and by absorbing water in the soil. As a consequence, vegetation creates specific physical conditions for the survival of macrofauna and influences the food availability regime (Jackson, Caldwell, 1993). The principal component 3 reflects the varying ecological regimes associated with an abundance of woody plants. There was a positive correlation between the diversity of the two groups but only at the local scale (single sample data), indicating that tree diversity can increase lumbricid diversity by the mechanism of creating small scale microhabitat diversity (Cesarz et al., 2007; Migge-Kleian et al., 2007).

The effect of vegetation principal component 2 on the soil macrofauna is not statistically significant. This component mainly reflects the ratio between nanophanerophytes and hemicryptophytes. Probably, the nature of the impact of these plant ecological groups on the soil environment is uniform and does not find its specific response in the community structure of soil macrofauna. The character of dependence of differential entropy on principal component 2 confirms this assumption (Fig. 4). The vegetation principal components 5 and 6 do not provide statistically significant effects on beta diversity of the soil macrofauna in view of the low level of the variability.

To determine the causes of community variation, it is necessary to link the scales at which variation is measured to the scales at which the processes potentially affecting diversity actually operate (Huston, 1999). In this regard, an important role is played by the spatial properties of the processes. Geostatistics provides an opportunity to assess the spatial distribution of the variability of environmental properties and soil organisms (Rossi et al., 1996; Rossi, 2003). Geostatistical models are sufficiently good at describing the varying effects on the beta-diversity of edaphic principal components 1 and 2 and the vegetation principal components 1 and 3. Effects of other principal components cannot be well described by geostatistical models in the framework of the chosen procedure. It is likely that to identify the relevant patterns, a detailed large-scale survey of the territory must be made.

It is worth taking into account the indicator differential entropy. This indicator reflects the sensitivity of beta-diversity of the community to the impact of external factors. Most often, attention is paid to the spatial characteristics of the soil properties (Jackson, Caldwell, 1993, 1996; Reza et al., 2016) or separate species (Gongalsky et al., 2009) or the community of living organisms (Gongalsky et al., 2008). Differential entropy characterizes the relationship between environmental factors and the community. Spatial structuring of differential entropy indicates that the community sensitivity to environmental factors is not uniform in space (Figs 5, 6).

In the framework of the MDM approach, the effects of the model can be expressed as changes in entropy. Entropy can be partitioned within and between sites, species and models, and changes in entropy can be attributed to model predictors (De'ath, 2012). We have found that soil macrofauna species vary in sensitivity to action of edaphic and vegetation factors. Similarities in these reactions serves as a basis for the classification of species. A significant group of species is insensitive to the action of both edaphic (16 species) and vegetation factors (16 species). 12 species within the community are not sensitive to the action of both edaphic and vegetation factors. It can be assumed that these species are sensitive to these ecological factors on other spatial levels or that interspecies interaction plays an important role in the organization of the community represented by these species.

Conclusion

We found that edaphic and vegetation factors play an important role in structuring the soil macrofauna community on the level of beta diversity. The sensitivity of the community to environmental factors varies in space and is spatially structured. For edaphic and vegetation factors, specific spatial patterns of community sensitivity are allocated. Beta diversity may be due to the fact that the species of soil macrofauna community also vary in the degree of sensitivity to various environmental factors. A considerable part of the community is represented by the species indifferent to the impact of ecological factors within the studied spatial scale.

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DETERMINATION OF ORGANIC FRACTIONS AND ENZYMATIC ACTIVITY IN FOREST SPRUCE SOIL OF TATRA NATIONAL PARK

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Abstract

Gáfriková J., Hanajík P., Zvarík M.: Determination of organic fractions and enzymatic activity in forest spruce soil of Tatra National Park. Ekológia (Bratislava), Vol. 37, No. 4, p. 328–337, 2018.

The formation and quality of soil organic matter (SOM) highly depends on the input of organic material and microbial enzymatic activities. Soil extractions with specific nonpolar and polar extractives can be used to identify qualitative changes in SOM. The aim of this paper was to understand the correlations among microbial enzymatic activity and specific organic fractions in acidic spruce forest soil. Klason lignin (KL), acid soluble lignin (ASL), holocellulose (HC), SOM content, and potential enzymatic activity (FDA and phosphatase) was measured and analyzed. We sampled Dystric Cambisol of forest spruce stands (*Picea abies*) in Tatra National Park (Slovakia). The SOM fractions were determined gravimetrically based on their extractivity in nonpolar (dichloromethane (DME)) and polar (acetone (AE), ethanol (EE), water (WE)) solvents Total extractives content was 0.079% and nonpolar extractives 0.036%. The mean amount of polar extractives tented to increase in the order EE<AE<WE. The total lignin content was determined to be 1.079% and HC 0.774%. FDA negatively correlated with KL (r=-0.873 p<0.05) and DME (r=-0.913 p<0.05). Phosphatase positively correlated with WE (r=0.972 p<0.01) and KL (r=0.957 p<0.01).

Key words: soil organic matter, organic matter fractions, soil enzymes, Tatra Mts.

Introduction

Soils consist of various organic and inorganic compounds that are mixed at different levels. The organic component of soils, commonly known as soil organic matter (SOM), and processes of its formation were widely studied especially in the last decades (Crawford D.L., Crawford R.L., 1980; von Lützow et al., 2007; Špoljar et al., 2014). In the forests, decaying plant biomass tends to accumulate on the surface of the soil and its size is reduced by soil microbiota (Bardgett, 2005). Litter, dead animal and plant bodies, which are mainly built from organic compounds, are suitable target for soil decomposers (bacteria and microscopic fungi) (Cotrufo et al., 2013). The level of soil organic matter decomposition was shifted at various sites in TANAP although the overall amount of soil organic carbon did not change rapidly (Don et al., 2012).

Cellulose, hemicelluloses, and lignin are the main chemical compounds of plant cell walls (Štursová et al., 2012). Transformations of plant residues are carried out by enzymes produced by soil bacteria and fungi that greatly depend on the source of carbon (C) (Baldrian et al., 2012). For instance, cellulose, proteins, and fats, which easily decompose, are the ready sources of C. Otherwise, compounds such as lignin and other phenolic plant constituents are subjected to slow decomposition (Miranda et al., 2013). Microbial characteristics studied at in TANAP showed significant spatial variability that can be reflected in the transformation of SOM (Gömöryová et al., 2008). The chemical analysis of SOM can serve as a good indicator of its quality. The chemical methods of examining SOM fractions are based on its extraction in aqueous solutions, with or without electrolytes, or in organic solvents (Hanajík et al., 2016b; Kutsch et al., 2009; von Lützow et al., 2007). Generally, about 20% of terrestrial C is stored in forest soils. In Tatra Mts., the forest covers approximately 60,000 ha at both Slovak and Polish sides (Jonášová et al., 2010). Thus it serves as the suitable location for monitoring the soil chemical and biological properties (Hanajík, Fritze, 2009; Gáfriková, Hanajík, 2016; Hanajík et al., 2016a; 2017).

The aim of this paper was to perform a quantitative determination of SOM fractions, determine their potential enzymatic activity, and to understand the correlation among enzymatic activities and SOM fractions of Dystric Cambisol in the spruce forest of Tatra National Park (TANAP), Slovakia.

Material and methods

Soil was sampled in July 2017 in forest spruce stands (F) near the spa hamlet Vyšné Hágy (N49°07′17.5″; E20°06′16.4″). The sampling locality is at an altitude of 1222 m a.s.l. and is a part of TANAP (Fig. 1). The dominant species were *Picea abies* and *Vaccinium myrtillus* and main soil type is clay to sandy-clay Distric Cambisol.

The topsoil samples (F1–F3) were collected from a 15×15 m area by random choice. The soil samples were homogenized by 2.0 mm mesh and refrigerated (at 4 °C). For the analysis of SOM fractions, the soil was dried at 40 °C for 4 consecutive days to constant mass (95–96% dry mass). For enzymatic analyses, the soil was stored at 4 °C for 2 weeks and then analyzed. The SOM fractions referred to as extractable substances were determined gravimetrically based on their solubility in nonpolar and polar extractives. Dichloromethane (DME) was used to remove nonpolar extractives, acetone (AE), ethanol (EE), and water (WE) to remove polar extractives (Wieder, Starr, 1998). The Klason lignin (KL) was determined from extractive-free samples that were hydrolyzed in sulfuric acid. The nonhydrolyzable residue was defined as KL. The acid-soluble lignin (ASL) was determined spectrophotometrically from KL (Ehrman, 1996). Holocellulose (HC) (sum of cellulose and hemicellulose) was determined from extractive-free residue samples by sodium chlorite method (Allen, 1974). Potential soil enzyme activity was estimated using the colorimetric method with a spectrophotometer. The hydrolytic capacity of soil was estimated by fluorescein diacetate (FDA) hydrolysis in potassium phosphate buffer (pH 7.6). The method was described by Schnürer and Rosswall (1982), Shaw and Burns (2005), and Green et al. (2006). The potential phosphatase activity (PH) was determined according to Tabatabai and Bremner (1969) using nonbuffered system by distilled water instead (Eivazi, Tabatabai, 1977; Shaw, Burns, 2005). The dry weight (DW%) was determined by drying soil samples at 105 °C overnight. The SOM content was measured gravimetrically as loss on ignition (LOI) from dried samples at 550 °C for 4 h.

Results and discussion

Soil organic matter fractions

We analyzed SOM fractions of Distric Cambisol originating from forest spruce stands (*Picea abies*) according to their solubility in non-polar and polar chemical substances (Table 1). As re-

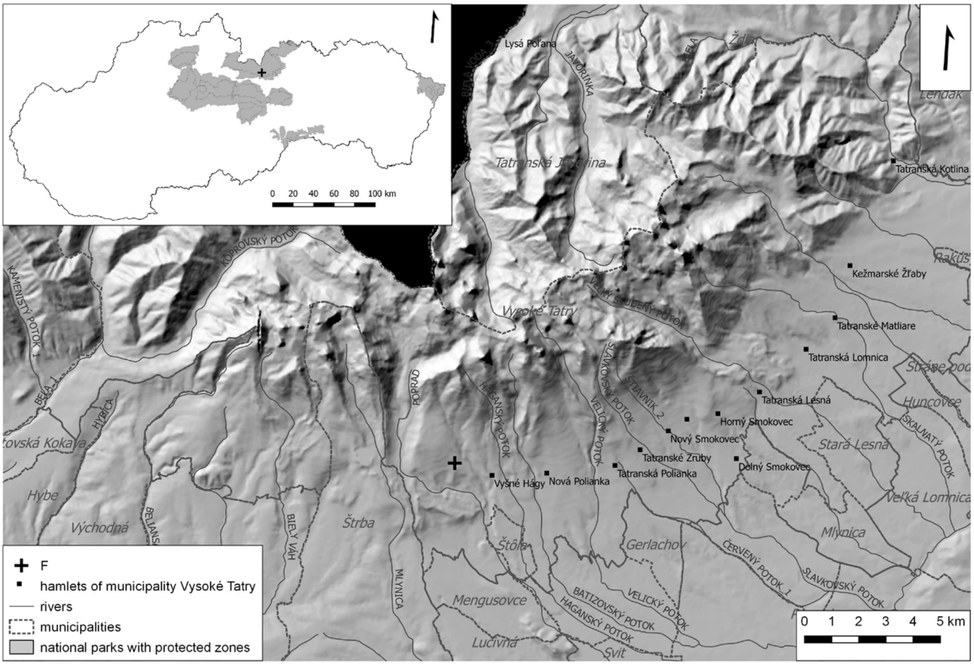

Fig. 1. The position of sampling locality (F).

gards to the total extractives content (non-polar and polar) forest spruce stand soil had 0.079%. During an extraction process using dichlormethane (DME) non-polar extractives (NPE) such as oils, waxes and fatty acid e.g. were extracted. The total amount of NPE reached up to 0.036% (±0.003%), which represents 45.67% of total extractives content. The total number of polar extractives (PE) was to 0.043% (±0.002%). Carbohydrates, ketones, and other nonpolar compounds make up 54.33%. The mean amount of polar extractives tended to increase in the order: EE<AE<WE (Fig. 2).

There are several published articles on the quantitative determination of organic fractions. A majority of them focus on the branch litter (Vávřová et al., 2009), eucalyptus (Yadav et al., 2002) and birch (Miranda et al., 2013) bark, or different ecosystems such as peatlands (Straková et al., 2010; Wieder, Starr, 1998) and tropical pastures (Marín-Spiotta et al., 2008). For the branch litter of Scott pine, Vávřová et al. (2009) reported 31.4–87.6 mg.g^{-1} of dry mass of NPE. The yield of PE increased in the order AE<WE<EE. In regard to birch bark composition, Miranda et al. (2013) reported total extractives of 17.6% (DME=5.1% EE=5.5% WE=5.2%). Wieder and Starr (1998) the amount of soluble fats, oils, and waxes to 5.23% in peat soils.

The total lignin content (Klason+ASL) was determined to be 1.079%, the mean yield of KL reached 0.628%, and the ASL was 0.4510%. The mean yield of HC was 0.774%. The amount of KL and ASL differs within vegetation. For instance, in hardwood of birch (*Betula papyrifera*), KL ranged between 24 and 32% and ASL 2–4% (Nicholson et al., 2014) and fine

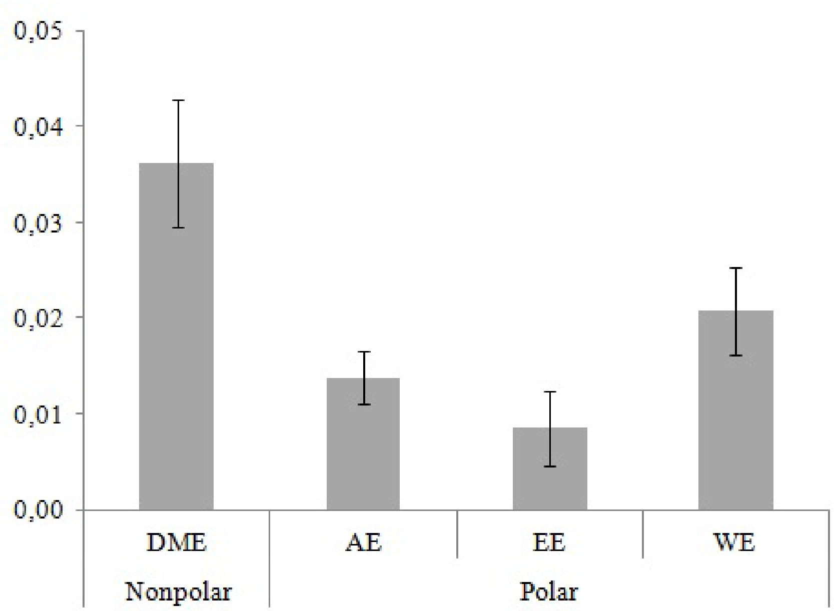

Fig. 2. Nonpolar (DME) and polar (AE, EE, WE) soil organic matter extractives expressed as % g.dw^{-1}.
Notes: DME – dichloromethane; AE – acetone; EE – ethanol; WE – water; ±SD, *n*=6.

branches of Scott pines (*Pinus sylvestris*) included 427–436 mg.g^{-1} of KL and 7–10 mg.g^{-1} of ASL (Vávřová et al., 2009). The total content of lignin in organic peatland soil was 36.74% (Wieder, Starr, 1998). Lignin belongs to the most abundant aromatic plant component. Thus their input via plant litter (aboveground and belowground) to soil represents approximately 20%. According to its abundance, lignin is considered as a major component of SOM (Higuchi, 2006). Lignin decomposes slowly and its degradation is mediated by bacteria (*Streptomyces* sp., *Nocardia* sp.) and basidiomycetes brown and white-rot fungi (Crawford D.L., Crawford R.L., 1980). During the processes of its biodegradation, lignin is transformed into nonlignin products and incorporated into SOM (Thevenot et al., 2010).

The holocellulose (HC) content of Dystric Cambisol of *Picea abies* forest varied between 0.73 and 0.82%. Cellulose and hemicellulose are considered to be the most abundant and moderately labile components of plant litter and are degraded more easily than recalcitrant fractions (Bardgett, 2005; Kutsch et al., 2009). The content of cellulose depends on sampled litter type a soil horizon. Štursová et al. (2012) sampled the litter layer and organic horizon of topsoil of *P. abies* forest. The cellulose content was determined to 36° mg.g^{-1} for litter layer, and 11 mg.g^{-1} for organic horizon. The HC content was determined in twings and branches of *Pinus sylvestris* to 500–560 mg.g^{-1} (twings) and 600–700 mg.g^{-1} (branches) (Vávřová et al., 2009). In highly organic peat soil, HC represented 51.35% (Wieder, Starr, 1998).

In soil, the content of litter and bulk organic matter is influenced by many environmental factors such as pH, soil moisture, and climate but also by vegetation cover and land use (Gruba, Mulder, 2015). These variables might have an impact on lignin degradation as well. The optimal mineralization of lignin and cellulose by *Streptomices viridiosporus* was observed at pH 8.5 (Pometto, Crawford, 1986). However, soil pH of the studied site was found to be strongly acidic (pH_{H2O} 4.0; pH_{KCl} 3.2) (Hanajík et al., 2017), which means suppressed biodegradation and SOM turnover.

Soil moisture content can be reflected in dry weight percentage (DW%). High DW% indicates low moisture content and on the contrary, low DW% shows favorable moisture conditions. SOM serves not only as a source of energy and nutrients for microorganisms but also provides organic compounds for stabilization of organic matter. The mean DW% determined in our study was 63.4%, and the mean content of SOM was 26.6%, which is comparable to previous research (Ďugová et al., 2013; Gáfriková, Hanajík, 2016; Hanajík et al., 2017).

The overall values of every extractive were lower in contrast to different scientific papers that were predominantly focused on litter quality, different soil type, or vegetation. Generally, in topsoil, plant litter is mixed and incorporated into soil and undergoes changes via microbial transformation. These processes can be enhanced or reduced by several environmental factors (mainly soil pH, soil moisture, and substrate availability). In Dystric Cambisol, soil pH indicated unfavorable conditions for organic matter turnover. Thus in this case an accumulation, rather than transformation of SOM, can be observed. Also, the quality of SOM is considered low and SOM is transformed into more stable forms (humus). In TANAP forest spruce stands, based on low pH values and altitude, the major contributors to SOM turnover are microscopic fungi, but the optimal mineralization of lignin and cellulose is completed at around a pH of 8. Higher values of lignin yield can indicate its slower mineralization. Moreover, the yield of organic fractions, lignin, and HC is generally higher in fresh material (e.g., leaves, bark) than in soil, because it contains more organic compounds than soil.

T a b l e 1. Soil organic matter fractions, dry weight, and SOM of forest spruce stands (F1–F3).

	F 1	**F 2**	**F 3**	**mean**	**CV**
DME*	0.029	0.037	0.043	0.036 ±0.003	18.47
AE*	0.014	0.011	0.016	0.014 ±0.001	20.16
EE*	0.007	0.012	0.007	0.009 ±0.002	45.53
WE*	0.018	0.018	0.026	0.005 ±0.019	22.08
KL*	0.545	0.594	0.743	0.628 ±0.038	14.91
ASL*	0.498	0.504	0.351	0.451 ±0.035	18.77
HC*	0.824	0.766	0.731	0.774 ±0.016	5.06
DW**	60.47	66.02	63.83	63.439 ±1.204	4.65
SOM**	21.59	26.38	31.85	26.607±1.877	17.28

Notes: DME – dichloromethane; AE – acetone; EE – ethanol; WE – water; KL – Klason lignin; ASL – acid-soluble lignin; HC – holocellulose; DW – dry weight; SOM – soil organic matter; CV – coefficient of variance (%); ±SE; *n*=6. *DME, AE, EE, WE, KL, AL, HC expressed as % g.dw^{-1}; **DW, SOM expressed as %.

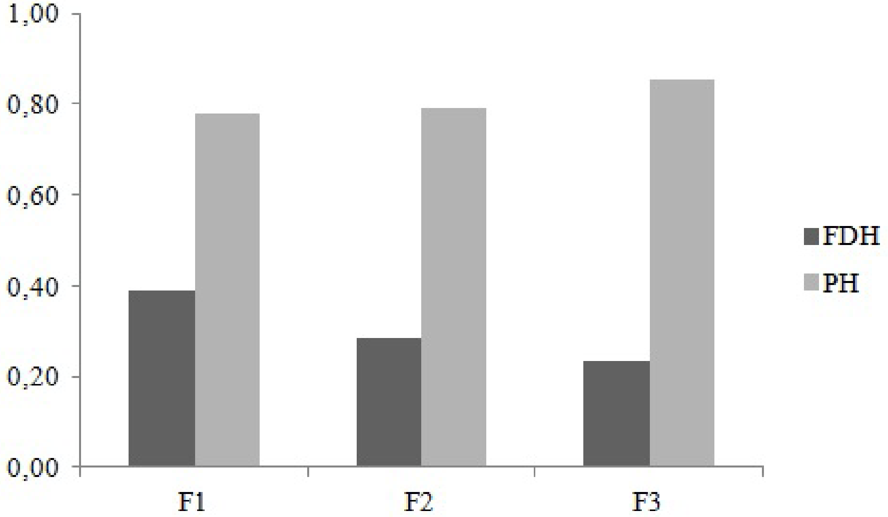

Fig. 3. Potential enzymatic activity of each forest spruce stand sampling plot (F1, F2, F3).
Notes: FDH – fluorescein diacetate (FDA) hydrolysis expressed as μg fluorescein/g dry soil/0.5h; PH – phosphomonoesterase activity expressed as μg *p*-nitrophenol/g dry soil/h.

Potential enzymatic activity

Enzymatic activity in the soil reflects the activity of soil microbiota, especially bacteria and fungi that are crucial for sustaining fluxes of essential chemical elements and supporting SOM turnover. Hence, the analysis of soil enzyme activities can be necessary for the evaluation of soil and SOM quality assessment (Shaw, Burns, 2005; Błońska et al., 2016). The potential FDA activity of the forest spruce stand soil (Fig. 3) varied between 0.24 and 0.39 μg fluorescein/g dry soil/0.5 h, with a mean value of 0.3028 μg fluorescein/g dry soil/0.5h. FDA in soil is hydrolyzed by a variety of enzymes, for example, proteases, lipases, and esterases. Bacteria and fungi are considered to be a major decomposer in the soil ecosystem. In general, they are responsible for more than 90% of the energetic flux in soil. Therefore, this method reflects potential hydrolytic capacity of the soil (Adam, Duncan, 2001; Green et al., 2006). FDA activity depends on several soil properties, such as pH, soil texture, and SOM content. These variables could be taken into account in the interpretation of results (Schnürer, Rosswall, 1982). The FDA activity of Haplic Chernozems was higher than that of Haplic Luvisols and ranged between 0.05 and 0.20 and 0.24 and 0.39 μg fluorescein/g dry soil/0.5h (Javoreková, Hoblik, 2004).

The acid phosphomonoesterase activity of Dystric Cambisol varied between 0.78 and 0.86 μg *p*-nitrophenol/g dry soil/h and the mean value for this variable was 0.81 μg *p*-

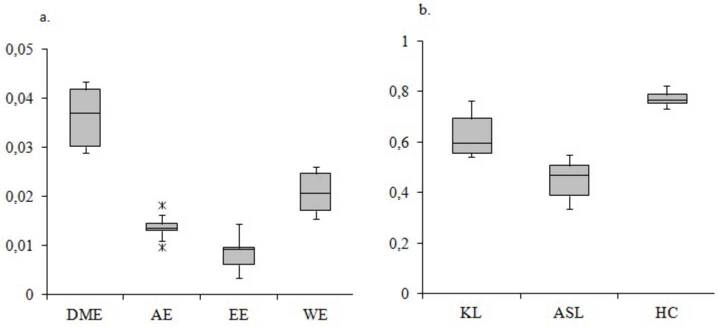

Fig. 4. Box plots showing the data variability of organic fractions.
Notes: DME – dichloromethane; AE – acetone; EE – ethanol; WE – water; KL – Klason lignin; ASL – acid-soluble lignin; HC – holocellulose; *n*=6.

nitrophenol/g dry soil/h (Fig. 3). The activity of soil phosphates is necessary for bioutilization of phosphorus for and its availability for plants. Thus, increased PH activity was observed in soil rhizosphere (Nannipieri et al., 2011). Phosphomonoesterase activity can be influenced by soil pH, C, N and P content (Šantrůčková et al., 2004) and can vary seasonally (Margesin et al., 2014).

Statistical analysis

The data variability is presented in Fig. 4. The highest variability among organic extractives was determined for DME fraction. DME_{max} reached 0.043% and DME_{min} 0.029% . Among samples solubilized in polar extractives, WE showed the highest variability (WE_{max} 0.026%; WE_{min} 0.016%). The less variable data were observed for AE; however, this is the only extractive where outliers, upper and lower, were calculated. Regarding EE, most of the values were below median (EE_{med} 0.0092%).

In the case of KL and ASL, data were not normally distributed. The 75% of values for KL were over the median (KL_{med} 0.6) and about 30% for ASL values were below the median (ASL_{med} 0.47). The values of HC showed low variability in comparison to KL and ASL.

The simple linear regression showed a strong linear relation between enzymatic activity and DME ($r = -0.913$; $p<0.05$ and $r=0.843$; $p<0.05$), KL ($r=-0.873$ $p<0.05$ and $r=0.957$; $p<0.01$) and SOM ($r=-0.95$; $p<0.01$ and $r=0.899$; $p<0.05$). In each case, FDH indicated a negative and PH a positive correlation. Furthermore, PH positively correlated with WE ($r=0.972$; $p<0.01$) and negatively with AL ($r=-0.975$; $p<0.01$).

The number of tested samples provided the first approach information about the studied soils in TANAP though formulation of sufficient conclusions needs to be tested on wider set of samples in order to obtain statistically stronger findings.

T a b l e 2. Correlation coefficients.

	DME	**AE**	**EE**	**WE**	**KL**	**ASL**	**HC**	**DW**	**SOM**
FDH	-0.913*	-0.238	-0.182	-0.633	-0.873*	0.687	0.295	-0.578	-0.95**
PH	0.843*	0.502	-0.314	0.972**	0.957**	-0.975	0.413	0.205	0.899*

Notes: *α=0.05; **α=0.01. DME – dichloromethane; AE – acetone; EE – ethanol; WE – water; KL – Klason lignin; ASL – acid-soluble lignin; HC – holocellulose; DW – dry weight; SOM – soil organic matter; FDH – fluorescein diacetate; PH – phosphomonoesterase.

Conclusion

The quantitative determination of organic fraction of forest spruce stands in Tatra National Park indicate higher amount of polar extractives (e.g., carbohydrates) compared to nonpolar extractives (e.g., waxes, oils). Lignin content and cellulose content were ten times lower than in, for example, litter or bark. The overall FDA capacity of soil was indeed optimal compared to different soil types; here we can claim that it reached slightly higher values. Otherwise, overall PH activity was found to be lower. The data revealed high variability of polar extractives and FDA hydrolysis as well as a strong correlation between FDA and Klason lignin ($r=-0.873$; $p<0.05$) also FDA and DME ($r=-0.913$; $p<0.05$). PH correlated with WE ($r=0.972$; $p<0.01$) and Klason lignin ($r=0.957$; $p<0.01$).

Acknowledgements

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CONTENT OF PLASTID PIGMENTS IN THE NEEDLES OF *Pinus pallasiana* D. DON IN DIFFERENT FOREST GROWTH CONDITIONS OF ANTI-EROSION PLANTING

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Abstract

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The influence of different forest growth conditions on the content of plastid pigments in *Pinus pallasiana* D. Don needles in the anti-erosion planting of the steppe zone of Ukraine was investigated. The reduction in the total chlorophyll *a* and *b* concentration in the pine needles in the area with insufficient water supply (xerophilous and mesoxerophilous hygrotopes) was found, compared with more favourable conditions for water supply (mesophilous hygrotope). The reduction in the content of green pigments in arid conditions is due to chlorophyll *a*. Chlorophyll *b* showed high resistance to moisture deficiency. There is an increase in the concentration of carotenoids in pine needles in forest growth conditions with insufficient water supply (xerophilous and mesoxerophilous), compared with more favourable conditions (mesophilous), which can be explained by the adaptive role of these pigments in the processes of stabilising the functioning of the photosynthetic apparatus in adverse environment, and it is considered as a protective reaction of *P. pallasiana* to the effects of drought.

Key words: anti-erosion planting, forest growth conditions, *Pinus pallasiana*, needles, plastid pigments.

Introduction

Since the second half of the twentieth century, *Pinus nigra ssp. pallasiana* (*P. pallasiana*), has been increasingly used in forestry, for planting of cities (Korshikov et al., 1998; Terlyga et al., 2002) and in anti-erosion plantation (Bessonova et al., 2015) in the steppe zone of Ukraine. However, there is a little research on its physiological features and adaptive responses in different growth conditions.

The vital activity of plants depends largely on how effective photosynthetic reactions are, which are affected by many internal and external factors. An important role is played by the pigment chloroplasts. The pigment system of plants is complex and extremely sensitive; it responds dynamically to stress factors of the environment (Bessonova, 2006a; Usypiva, Samko, 2009; Iusypiva, Drotik, 2015). As the energy state of plant organisms depends entirely on the quantitative parameters of the pigment apparatus, their violation leads to a deterioration of the energy supply of plants (Hryhoriukh et al., 2004).

The stability of the functioning of the photosynthetic apparatus is ensured by changes in the content and the redistribution of green and yellow pigments in the light-harvesting complex (LHC) and the reaction centre (RC) photosystem. Increasing or decreasing the concentration of pigments that take part in photosynthesis shows the level of adaptive reactions of the organism to stressful environmental influences (Ivanova, Suvorova, 2015). Moreover, the study on the content of chlorophyll and carotenoids in the leaves provides important information for analysing physiological adaptation and productivity of plant organisms (Andrianova, Tarchevs'kyj, 2000; Titova, 2010; Halil, 2012; Iusypiva, Vegerich, 2014; Bessonova, Ivanchenko, 2016). These indicators are often used in environmental studies as the most important and easily identifiable indicators of the functional state of plants (Ivanova, Suvorova, 2015).

The purpose of this article is to analyse the influence of different forest growth conditions on the content of plastid pigments in the pine needles of *P. pallasiana* in the anti-erosion planting of the steppe zone of Ukraine.

Material and methods

The study was conducted in *P. pallasiana* trees (*P. pallasiana* D. Don) aged 25–27 years, the artificial stand of which is located on the slope of the southern exposition of wooded ravine Vijs'kovij (coordinates between $48^0$11'08" N $35^0$07'45" E and $48^0$10'41" N $35^0$10'12" E), situated near Vijs'kove village in Solonyansky district of Dnipropetrovsk region. It belongs to the southern geographical variant of ravine forests of the steppe zone of Ukraine (Belgard, 1971). The total length of the ravine is 3.2 km. The ravine has three forks. It features the areas of natural groves as well as anti-erosion artificial plantations on the slopes (Bessonova et al., 2015). The research area is characterised by low rainfall (420-450 mm) and low humidity factor (0.67). The samples were collected from three sites of artificial planting. The first test area (TA1) is located in a talweg on its flat and slightly raised part 1.5–2 m above the level of the stream, which flows along the bottom of the ravine in a deep streambed. Moistening is groundwater and atmospheric. The mechanical composition of the soil is loam (CL). Forest growth conditions (according to Belgard, 1971) are clay-loam soil (CL_2) (mesophilic, fresh soil). The second test area (TA2) is laid in the middle, the steepest part of the slope. Moistening is atmospheric transit. Forest growth conditions are CL_1 (mesoxerophilic, somewhat dry, or semi-arid). The third test area (TA3) is located on the top of the slope. Moistening is atmospheric-transit. Forest growth conditions are CL_{0-1} (xerophilic, arid).

Two-year-old pine needles were selected at an altitude of 2m from the eastern side of trees growing in identical lighting conditions. The pigments were determined after extraction with 100% acetone on SF-2000. Their concentrations were calculated using the Wettstein formulas (Bessonova, 2006b).

Statistical analysis was carried out using Microsoft Office Excel 2007. The data was analysed as mean ± SEM (standard error of the mean). In order to estimate the significant differences between paired data, Student's t-test method was used at the level of significance $p \leq 0.05$.

Results and discussion

The results of our study indicate that the forest-based conditions affect the content of chlorophyll in *P. pallasiana* needles. The lowest concentration of this pigment in the investigated organs is observed in plants of the upper third of the slope (TA3); the highest concentration is observed in mesophilic fresh soil growth conditions (TA1) (Table 1).

Table 1. Influence of forest growth conditions on the content of chlorophylls (*a* + *b*) in pine needles of *Pinus pallasiana*, mg g^{-1} wet weight, M ± m, n = 4.

Test area	**20 May**	**Share of reference data (%)**	**18 July**	**Share of reference data (%)**	**5 September**	**Share of reference data (%)**
TA1	1.17 ± 0.03		1.33 ± 0.04		1.37 ± 0.03	
TA2	1.12 ± 0.04*	95.7	1.23 ± 0.02*	92.5	1.18 ± 0.03	86.1
TA3	1.02 ± 0.02	87.2	1.08 ± 0.03	81.2	1.05 ± 0.04	76.6

Note: * the value differs insignificantly from TA1 ($p \leq 0.05$).

Table 2. Influence of forest plant conditions on the content of chlorophyll *a* and *b* in the pine needles of *Pinus pallasiana*, mg g^{-1} wet weight, M ± m, n = 4.

Test area	**20 May**	**Share of reference data (%)**	**18 July**	**Share of reference data (%)**	**5 September**	**Share of reference data (%)**
Chlorophyll *a*						
TA1	0.82 ± 0.02		0.90 ± 0.03		0.93 ± 0.04	
TA2	0.76 ± 0.02*	92.7	0.78 ± 0.02	86.7	0.76 ± 0.02	81.7
TA3	0.70 ± 0.01	85.4	0.67 ± 0.03	74.4	0.65 ± 0.02	69.9
Chlorophyll *b*						
TA1	0.35 ± 0.02		0.43 ± 0.04		0.42 ± 0.02	
TA2	0.36 ± 0.01*	102.9	0.44 ± 0.03*	102.3	0.46± 0.03*	109.5
TA3	0.32 ± 0.02*	91.4	0.41 ± 0.04*	95.3	0.40± 0.02*	95.2

Note: * the value differs insignificantly from TA1 ($p \leq 0.05$).

The difference between the content of chlorophyll in the needles of plants in TA2 relative to TA1 is minor (insignificant at $p \leq 0.05$) in May and July. However, it becomes reliable in September and makes 13.9%. The difference in this indicator in trees of TA3 compared to TA1 is reliable in all months and makes 12.8% in May, 18.8% in July and 23.4% in September. This is in line with the data of other researchers who found that deterioration of water supply reduces the accumulation of chlorophyll in assimilation organs of plants (Bessonova et al., 1976; Shmat'ko et al., 1989; Kisljuk et al., 1997; Loggini et al., 1999; Hryhoriukh et al., 2004). In their research paper, Korytova et al. (1976) indicate that the pine tree grown in a fresh subor has a higher total chlorophyll content *a* and *b* than that grown in semi-arid wood conditions.

The effect of insufficient water supply to plants on the content of each of the forms of green pigments (*a* and *b*) is uneven. As can be seen from Table 2, the number of chlorophyll *a* in TA2 decreases significantly in July and September by 13.3% and 18.3%, respectively, when compared with TA1 and – even more – by 25.6 and 30.1%, respectively, when compared with TA3.

It was found that the growth conditions do not affect the concentration of chlorophyll *b* in the pine needles (Table 2). Consequently, this form of green pigment shows greater resistance to insufficient soil moisture.

The obtained data are consistent with the results of a number of researchers (Kushnirenko, Medvedeva, 1969), which indicate that under more arid conditions, the reduction in the concentration of green pigments occurs because of chlorophyll *a*, which is a more labile form. The study of the content of plastid pigments in leaves of *Robinia pseudoacacia*, growing in different growth conditions (fresh and arid), also showed that the amount of chlorophyll *b* is almost the same in both variants of the experiment during the vegetation (Bessonova et al., 1976).

Similar results were obtained in experiments with common pine, which grew in a fresh subor and semi-arid wood conditions (Korytova et al., 1976). The fact that chlorophyll *b* is a less mobile component of the pigment system and its content almost does not change during the vegetation was pointed out by Novikov and Vitkovskaja (1959). Stroganov et al. (1970) explained the stability of this form of chlorophyll by a stronger bond of pigment with membranes of chloroplasts.

Chlorophyll *b* is one of the leading factors in the stability of pigment–protein complexes of photosynthetic membranes (Tanakav R., Tanaka A., 2011). As a specific chlorophyll of antenna complexes, chlorophyll *b* increases light gathering in low light, and in bright conditions, it takes part in the dissipation of the excess of absorbed light energy, in the formation of the light energy migration paths within the antenna and from the antenna to the photosystems, and is also a direct component of centres of thermal dissipation (e.g. antenna protein Lhcb 4). The lack of chlorophyll *b* causes changes in the size and composition of the photosynthetic complexes of granal thylakoids, which affects the nature of their stacking in the membrane. This limitation of lateral diffusion of macromolecular protein–pigment complexes complicates the processes of their repair, and the limitation of the diffusion of plastoquinone and plastocyanin can seriously limit the linear transport of electrons (Vojcehovskaja, 2015). According to Bossmann et al. (1999), the lack of chlorophyll *b* destabilises the light-harvesting antenna, first of all, LHC2, because of proteolytic degradation of the Lhcb 1–2 proteins, which leads to a decrease in the intensity of photosynthesis. Consequently, maintaining the content of chlorophyll *b* in *P. pallasiana* needles in conditions of poor water supply ($CL_{0\text{-}1}$) at the same level as in the conditions of better water supply (CL_2) has a significant adaptive value.

Changes in the concentration of green pigments in arid forest growth conditions led to a violation of the ratio of chlorophylls *a* and *b* during the month when the moisture content in the soil in the upper part of the slope was significantly lower than that of talweg. Although the difference in the value of this parameter between the variants of the experiment was not evident in May, it increased and became significant in July and September. As the negative influence of arid conditions of growth affects the content of chlorophyll *a* and almost does not affect the concentration of chlorophyll *b*, then the chlorophyll *a/b* ratio decreases in TA2 and TA3 plants relative to TA1 (Table 3).

The content of carotenoids is higher in the pine needles of *P. pallasiana* in the arid forest growth conditions of the upper ($CL_{0\text{-}1}$) and medium (CL_1) slopes in comparison with

Table 3. Influence of forest growth conditions on Chlorophyll *a/b* Ratio, M ± m, n = 4.

Test area	**20 May**	**18 July**	**5 September**
TA1	2.34 ± 0.05	2.09 ± 0.10	2.11 ± 0.11
TA2	2.21 ± 0.04*	1.77 ± 0.05	1.65 ± 0.05
TA3	2.18 ± 0.06*	1.63 ± 0.11	1.62 ± 0.07

Note: * The value differs insignificantly from TA1 ($p \leq 0.05$).

Table 4. Influence of forest growth conditions on carotenoid content in the needles of *Pinus pallasiana*, mg g^{-1} wet weight, M ± m, n = 4.

Test area	**20 May**	**Share of reference data (%)**	**18 July**	**Share of reference data (%)**	**5 September**	**Share of reference data (%)**
TA1	0.12 ± 0.005		0.19 ± 0.006		0.20± 0.010	
TA2	0.14 ± 0.004	116.7	0.24 ± 0.004	126.3	0.25 ± 0.006	125.0
TA3	0.15 ± 0.009	125.0	0.25 ± 0.008	131.6	0.28 ± 0.007	140.0

this indicator in plants of talweg (CL_2). In the trees of the upper part of the slope (TA3), in comparison with those in the talweg (TA1), the concentration of yellow pigments increases in May by 25.0%, in July by 31.6% and in September by 40.0%. The conditions in September were the most unfavourable because of the lack of rain during August. In the plants in TA2, the number of carotenoids in the needles also increases compared with this indicator in the area with favourable conditions of water supply (TA1), but to a lesser extent (Table 4). In our study, the growth of the concentration of carotenoids in the tissues of *P. pallasiana* needles under water-deficit conditions can be considered as a protective reaction to the effects of drought. A number of research articles also provide data on the increase in the content of carotenoids in the assimilation bodies of plants under the influence of stress factors of moderate strength, mainly in drought-resistant species (Tuba, 1984; Taran, 1999; Bessonova, Ponomar'ova, 2017).

Carotenoids play an important role in the adaptive reactions of plant organisms. They expand the spectral range of photosynthetic radiation, take part in energy migration, neutralise active forms of oxygen, protect chlorophyll from photodegradation, regulate the heat exchange of the lamina and stabilise photosynthetic membranes (Adams et al., 1990; Demmig-Adams, 1990, Demmig-Adams, Adams, 2000; Hryhoriukh et al., 2004; Ladygin, Shirshikova, 2006; Shadchyna et al., 2006; Ralituho et al., 2007).

Conclusion

Thus, chlorophyll *a* + *b* content in the needles of *P. pallasiana* under conditions of insufficient water supply (xerophilous, CL_{1-0} and mesoxerophilous, CL_1) is lower than that in the trees under fresh growth conditions (mesophilous, CL_2). The reduction in the concentration of green pigments in TA2 and TA3 relative to the TA1 indices is due to chlorophyll *a*; chlorophyll *b* showed high resistance to poor water supply. This leads to a decrease in the chlorophyll *a/b* ratio in the needles of plants in more arid areas compared with this indicator in trees of mesophilic growth conditions.

The quantity of carotenoids in needles under insufficient water supply is higher than that in the trees under fresh forest growth conditions, which can be explained by the adaptive role of these pigments in the processes of stabilising the functioning of the photosynthetic apparatus under adverse growth conditions and considered as a protective reaction of *P. pallasiana* on the effect of drought.

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INFLUENCE OF SALINITY ON PHYSIOLOGICAL RESPONSE OF THE BEARDED HORSE MUSSEL *Modiolus barbatus* and NOAH'S ARK SHELL *Arca noae*

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Abstract

Vlašić M., Glavić N., Bolotin J., Hrustić E., Dupčić Radić I.: Influence of salinity on physiological response of the bearded horse mussel *Modiolus barbatus* and Noah's ark shell *Arca noae*. Ekológia (Bratislava), Vol. 37, No. 4, p. 345–357, 2018.

Bearded horse mussel *Modiolus barbatus* and Noah's ark shell *Arca noae* are a species of interest for the diversifying shellfish aquaculture on the south-eastern coast of the Adriatic. In this study, oxygen consumption (OC), total ammonia excretion (TAM) and clearance rate (CR) responses to the changes in seawater salinity (37, 30, 25 and 20) were investigated in the laboratory. There is a statistically significant influence of salinity on oxygen consumption and TAM excretion of Noah's ark shell, while the time of exposure to different salinities is significantly correlated to TAM excretion by the bearded horse mussel. Mean OC of Noah's ark shell ranged from 0.14 ± 0.06 to 0.54 ± 0.27 mg $O_2 g^{-1} h^{-1}$ and that of bearded horse mussel from 0.18 ± 0.17 to 0.26 ± 0.14 mg $O_2 g^{-1} h^{-1}$. Mean values of TAM excretion of Noah's ark shell ranged from 2.14 ± 1.52 to 7.22 ± 6.04 μmol $g^{-1} h^{-1}$ and for bearded horse mussel from 0.98 ± 0.53 to 2.78 ± 2.96 μmol $g^{-1} h^{-1}$. Salinity and exposure time have a significant influence on the CR of Noah's ark shell, whilst salinity has been found to be the determining factor for the bearded horse mussels' CR. Mean values of Noah's ark shell CR ranged from 0.96 ± 0.54 to 4.18 ± 1.15 l $h^{-1} g^{-1}$ and for bearded horse mussel from 2.43 ± 0.99 to 4.23 ± 0.84 l $h^{-1} g^{-1}$. Higher oxygen consumption to total ammonia excretion (O:N) ratios at lower salinities indicated the use of proteins as a metabolic substrate for both species. Noah's ark shell has greater energy expenditure related to respiration and TAM excretion than the bearded horse mussel.

Key words: shellfish, salinity, clearance rate, oxygen consumption rate, ammonium excretion rate, O:N ratio.

Introduction

Bivalves *Arca noae* Linnaeus, 1758 and *Modiolus barbatus* (Linnaeus, 1758) have become species of interest in recent years and significant research has been carried out on them.

Various biological data was documented for bearded horse mussel, such as age, growth rate, reproduction and aquaculture potential (Mladineo et al., 2007; Peharda et al., 2007, 2013). Energy budget and other physiological responses were also investigated for bearded horse mussel (Ezgeta-Balić et al., 2011; Pörtner, 2012). Age, growth rate, reproductive biology and aquaculture potential studies were performed on Noah's ark shell (Peharda et al., 2002, 2006, 2013). Literature shows very little data on the physiological response of Noah's Ark shell (Glavić et al., 2018).

Estuaries and coastal areas are under a strong influence of tides and freshwater from rivers (Levinton et al., 2011). Therefore, salinity is one of the most important abiotic ecological factors in the estuaries and coastal seas (Berger, Kharazova, 1997). Bivalves respond to changes in the ambient salinity by closing shells and decreasing the rate of feeding and breathing; but gradually, they recover their osmotic balance and return to normal physiological conditions (Almada-Villela, 1984). Several studies have been carried out on the effect of changes in salinity on oxygen consumption (OC), total ammonia (TAM) excretion e and clearance rate (CR) (Navarro, 1988; Navarro, Gonzalez, 1998; Tang et al., 2005), but there is no available information, to our knowledge, on the influence of salinity on the metabolism of *Arca noae* and *Modiolus barbatus*. Metabolic responses to changes in the environment are important when selecting the farming area and our research contributes to the estimates of farming opportunities of the investigated species. The aim of this study was to determine OC, TAM excretion, O:N ratio and CR of bearded horse mussel and Noah's ark shell related to abrupt changes in water salinity (37, 30, 25 and 20 psu).

Material and methods

Sampling site and procedures

For the measurements of OC and TAM excretion, individuals of *Arca noae* and *Modiolus barbatus* were collected by autonomous diving in November 2015 and April 2014, respectively in Bistrina Bay (42° 52'11.41"N, 17° 42 '06.73"E) located in Mali Ston Bay (south-eastern Adriatic) . The animals for CR measurements were collected at the same place, *Arca noae* in May 2016 and *Modiolus barbatus* in June 2016. Mali Ston Bay is one of the most important areas for the production of shellfish in Croatia, with annual salinity fluctuations from 17.48 to 36.93 psu (Jasprica et al., 1997).

Bivalves were transferred to the Institute for marine and coastal research, where they spent seven days acclimating to the ambient aquarium conditions (salinity 37±1.0 psu, temperature 20±1 °C). Experimental shellfish were fed daily, in the morning with the green algae *Tetraselmis suecica* (Kylin) Butcher, 1959, at a density of 3000 cells ml^{-1} (0.43 mg l^{-1}dry algal mass, according to Widdows, Staff, 2006). Abiotic water parameters (temperature, salinity) were measured daily by using the WTW Profiline Cond 3110 conductivity and temperature probe, whilst dissolved oxygen (DO) concentration was measured by using the Oxyscan graphic oxygen probe (UMS Gmbh, Germany).

Experimental design

Measurements of OC and TAM excretion were carried out on the organisms under different salinities: 37, 30, 25 and 20 psu. Sample groups of individuals were transferred from the sea water of ambient salinity into the waters of prepared different salinities. Sea water of different salinity was prepared by diluting the filtered (10/5/1 μm) and sterilized (UV-B lamp) aquarium sea water with the calculated portions of distilled water. Physiological response for each salinity was measured after 24 and 120 hours (1 and 5 days) to determine the acclimation to changed salinity. Measurements were performed in triplicate groups with five individuals per group (a total of 15 individuals per species). OC measurement was performed at the end of the period between two feedings to avoid the influence of feeding (Widdows, Staff, 2006). Size of animals selected for the experiment was 40–50 mm for both species (usual market size).

OC, TAM excretion and CR measurements

OC and TAM excretion measurements were performed for each shellfish in a 429.39 ml volume closed respiratory chamber. Prior to introducing individuals in the chamber, they were cleaned from epibionts. After placing the shellfish in the chamber, it was closed and filled with pure sea water. Prior to physiological measurements, the oxygen enriched seawater was circulated through the chamber for an hour to allow the bivalves opening of the shells. Then the chamber inlet and outlet valves were closed, and the oxygen concentration decrease was measured for 40 minutes with the Oxyscan graphic probe (UMS Gmbh, Germany). No significant drop in oxygen was observed in a closed control chamber with pure sea water without shellfish during the measurement period.

After the experiment, the volume of individual shellfish was measured, and the soft tissue was dried in a drying chamber at 60 °C for 24 h to a constant mass and subsequently weighted. The dry weight (DW) of shellfish was taken as the basis for calculating the specific (per gram) physiological rate. Dissolved oxygen spent during one hour, that is, VO2 (mg O_2 h^{-1}) by individual shellfish was calculated according to Widdows, Johnson (1988) using equation:

$$VO_2 = 60\times \left[C\ (t_0)\ - C\ (t_1)\right]\ \times\ (Vr)/(t_1 - t_0)$$

where: t_0 and t_1 - initial and final points (min) of the measurement period, C (t) - oxygen concentration in water at time t, Vr - volume of respirometeric chamber reduced by the volume of shellfish.

TAM excretion was measured simultaneously with the consumption of oxygen. After opening the chamber where OC was measured, 50 ml of seawater sample was taken, preserved with 2 ml of 1 M phenol in 95% ethyl alcohol vol/vol solution and stored in the refrigerator until spectrophotometric measurement conducted within 72 hours. Reference sample for ammonia concentration in the seawater at the beginning of each experiment was sampled the same way. These TAM concentrations were subtracted from the TAM concentrations obtained at the end of each experiment. The concentration of TAM was determined spectrophotometrically at 634 nm from the non-filtered samples using indophenol blue method (Solorzano, 1969) modified by Ivančić, Degobbis (1984). The TAM excretion rate was calculated according to Sobral, Widdows (1997) using equation:

$$U\ =\ (T - C)\times(V/1000)/t$$

where: U - TAM excretion rate (μmol NH_4-N h^{-1}), T - concentration of TAM (μM) in the sample, C - concentration of TAM (μM) in the control sample, V - volume (ml) of chamber, t - time (h).

Using the values of specific OC and TAM excretion, O:N atomic ratio was calculated and expressed according to Hawkins et al. (2002) using the equation:

$$O/N = (mg\,O_2/16)/(mg\,NH_4/14)\ .$$

CR can be determined by measuring the decrease in concentration of suspended algal cells added to the seawater. A closed metering system was used in which CR was measured in 5 l water tank over a period of 2 hours (Widdows, Staff, 2006). Bivalves were cleaned from epibionts, left for 20 minutes to open the shells and then *Tetraselmis suecica* algal cells were introduced at a concentration of 20 000 cell ml^{-1}. Mixing of water was achieved by aeration. Every 30 minutes for a period of 2 hours, a sample of 20 ml was taken to determine the algal concentration, which was performed using the Hach DR2500 spectrophotometer by reading the absorbance of visible light at 750 nm. Separate string of known algal concentrations was analysed for absorbance to produce the regression equation of the dependence of absorbance on algal concentration. Subsequently, polynomial equation of that regression curve was used to calculate the algal concentrations in 20 ml samples from the absorbance data, similar to Rodrigues et al. (2011). No significant drop of algal cell concentration was observed during the experimental period in the control tanks without shellfish. The CR of each bivalve was calculated using the following equation (Coughlan, 1969):

$$CR\ (l\ h^{-1}) = Vol\ (l)\ \ x\ (lnC1\ -\ lnC2)/time\ interval\ (h)$$

where: Vol - volume of water, C1 and C2 - cell concentrations at the beginning and end of time interval (h).

In order to standardize the values of physiological rates and eliminate the influence of different animal weight on OC, CR and TAM excretion, all the physiological rates were converted to a specific physiological rate per gram of dry mass of the animal. Standardized rates were calculated according to Bayne, Newell (1983) using the following equation:

$$Ys = \left(Ws/We\right)^{b} \times \; Ye$$

where: Ys - the physiological rate for the animal of standard mass (1 g), Ws - standard mass (1 g), We - the observed mass of the individual in grams, Ye - the uncorrected (measured) physiological rate, b - the mass exponent for the physiological rate. The mean b exponent mass was 0.67 for the CR and 0.75 for the OC of bivalve (Savina, Pouvreau, 2004), and a value of 0.78 was used for the TAM excretion (Hawkins et al., 2000).

Energetic expenditures were respiratory energy expenditure (R) and energy lost as excreta (U). Calculation of R and U (all in $Jg^{-1}h^{-1}$) was as follows (Widdows, Johnson, 1988):

$$R = VO_2\left(ml\ O_2 g^{-1}h^{-1}\right) \times 20.33\ J\ ml^{-1}O_2\,;$$

$$U = mg\ NH_4 g^{-1}h^{-1} \times 19.4\ J\ mg^{-1}NH_4\,.$$

Statistical analysis

The data collected were tested for variance homogeneity using Levene's test using the Statistica package 12.0. Normality of data was estimated by Kolmogorov-Smirnov and Liliefors test. For the analysis of OC, TAM excretion, O:N ratio and CR with respect to different salinity and exposure time to specific salinity, 'nested' ANOVA and Tukey post-Hoc analysis was used. To compare the metabolic rates between *Arca noae* and *Modiolus barbatus*, a t-test was used. Significant probability for all analyses was given as $p < 0.05$, if not expressed by an exact number.

Results

Respiration rate, that is, OC rate

Significant difference in OC was observed for Noah's ark shell (F = 9.964; $p = 0.000$) exposed to different salinities. Difference was significant between groups kept at 25 and 30 psu (Tukey, $p = 0.001$). Mean OC values ± SD are shown in Table 1.

Bearded horse mussel did not show statistically significant difference in OC at different salinities and at different exposure times. Mean OC values ± SD are shown in Table 2.

Table 1. Mean values (± SD) of *Arca noae* oxygen consumption, respiration energy loss, TAM excretion, excretion energy loss, O:N ratio and clearance rate for different salinities after one and five days of exposure.

Salinity (psu)	**Day**	**Respiration rate mg $O_2g^{-1}h^{-1}$**	**Respiration energy loss J $g^{-1}h^{-1}$**	**Excretion rate µM$NH_4g^{-1}h^{-1}$**	**Excretion energy loss J $g^{-1}h^{-1}$**	**O:N ratio**	**Clearance rate l $h^{-1}g^{-1}$**
37	1	0.38±0.24	5.36±3.46	5.53±4.58	1.94±1.60	35.02±100.38	4.19±1.15
30	1	0.48±0.18	6.85±2.51	7.22±6.04	2.36±2.14	11.21±21.81	1.29±0.58
30	5	0.54±0.28	7.73±3.95	5.46±5.37	1.91±1.88	13.75±20.21	2.04±0.32
25	1	0.14±0.07	2.00±0.95	2.14±1.52	0.75±0.53	7.71±9.92	3.01±0.66
25	5	0.16±0.13	2.29±1.82	5.00±4.42	1.62±1.56	3.44±4.19	3.05±1.47
20	1	0.42±0.41	5.99±5.83	4.11±4.13	1.34±1.44	13.02±22.88	0.96±0.54
20	5	0.38±0.30	5.44±4.21	2.60±2.80	0.91±0.98	22.36±26.23	2.66±1.06

T a b l e 2. Mean values (± SD) of *Modiolus barbatus* oxygen consumption, respiration energy loss, TAM excretion, excretion energy loss, O:N ratio and clearance rate for different salinities after one and five days of exposure.

Salinity (psu)	**Day**	**Oxygen consumption mg $O_2g^{-1}h^{-1}$**	**Respiration energy loss J $g^{-1}h^{-1}$**	**TAM excretion µMNH4$g^{-1}h^{-1}$**	**Excretion energy loss J $g^{-1}h^{-1}$**	**O:N ratio**	**Clearance rate l $h^{-1}g^{-1}$**
37	1	0.21±0.16	2.92 ± 2.30	1.21 ±0.78	0.40±0.28	32.20±61.81	2.43±0.99
30	1	0.23±0.12	3.20 ± 1.67	1.02 ±0.94	0.36±0.33	30.88±38.58	2.86±0.82
30	5	0.25±0.16	3.63 ± 2.22	1.29 ±0.65	0.45±0.23	11.40±10.11	3.22±0.51
25	1	0.26±0.13	3.74 ± 1.85	2.34 ±1.35	0.82±0.47	8.21±8.56	3.72±0.49
25	5	0.26±0.15	3.77 ± 2.09	1.98±1.11	0.60±0.43	15.75±26.32	4.23±0.84
20	1	0.18±0.17	2.63 ± 2.48	0.98 ±0.53	0.34±0.19	11.42±9.21	2.75±0.21
20	5	0.17±008	2.48 ± 1.07	2.78 ±2.96	0.97±1.03	12.51±14.51	2.79±1.00

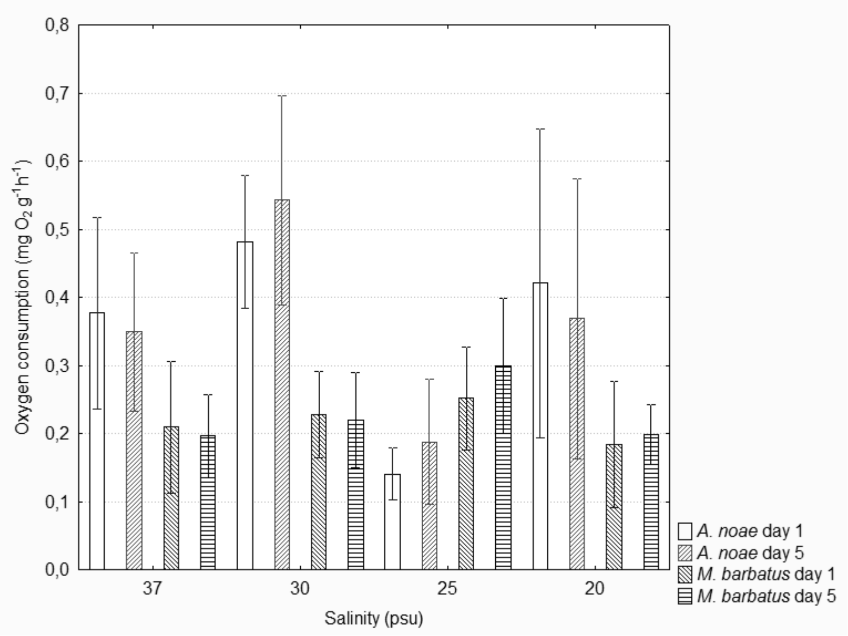

Fig. 1. Comparison of oxygen consumption (mg $O_2 g^{-1}h^{-1}$) between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

A comparison of OC between the investigated species is shown in Table 3. OC were found to be significantly different between the species at all tested salinities, except for the measurements performed after the exposure for 5 days at 25 psu and after 24 h at 20 psu. *M. barbatus* exhibited lower OC than *Arca noae* at all salinities (Fig. 1).

T a b l e 3. Summary of t-test results for oxygen consumption, TAM excretion, O:N ratio and clearance rate between Noah's ark shell and bearded horse mussel.

Salinity (psu)	**Day**	**Oxygen consumption**		**TAM excretion**		**O:N ratio**		**Clearance rate**	
		t	***P***	***t***	***P***	***t***	***P***	***t***	***P***
37	1	-2.247*	0.032	3.481*	0.001	-0.091	0,927	2.591*	0.032
30	1	-4.668*	0.000	3.925*	00.00	1.673	0,105	-3.482*	0.008
30	5	-3.591*	0.001	3.087*	0.004	-0.412	0,682	-4.343*	0.002
25	1	3.104*	0.004	-0.352	0.727	0.140	0,889	-1.866	0.104
25	5	1.980	0.059	2.391*	0.025	1.599	0,123	-1.556	0.158
20	1	-1.991	0.056	2.011	0.054	-0.262	0,795	-6.823*	0.000
20	5	-2.296*	0.031	-0.163	0.871	-1.235	0,226	-0.212	0.836

Note: * significant difference.

TAM excretion rate

There was a statistically significant difference in Noah's ark shell TAM excretion (F = 2.896; $p = 0.039$) due to different salinity and there was the largest difference between the group of bivalves exposed one day (24 hrs of influence) to salinity of 25 psu and a group exposed one day to salinity of 30 psu (Tukey, $p = 0.044$). The mean values of TAM excretion ± SD are shown in Table 1.

There was a statistically significant difference in the influence of the exposure time to TAM excretion of bearded horse mussels (F = 4.304; $p = 0.006$). The mean values of bearded horse mussel TAM excretion are shown in Table 2. The lowest values of TAM excretion rate were recorded for the bivalves exposed to 20 psu for one day (24 hrs of influence) and the highest after five days (120 hrs of influence) of exposure to 20 psu (Fig. 2). The post hoc Tukey test results showed the difference between shellfish exposed one day and five days to salinity of 20 psu (Tukey, $p = 0.018$).

A comparison of TAM excretion between the investigated species is presented in Table 3. There was a statistically significant difference between the two species, except for those exposed to 25 psu for one day and to 20 psu for one and five days.

O: N ratio

The mean values of O:N ratio of Noah's ark shell ± SD are shown in Table 1. There was no statistically significant difference in its O: N ratios due to different salinities.

The mean values of the O:N ratio of bearded horse mussel ± SD are shown in Table 2. The influence of salinity and exposure time on O:N ratio was not statistically significant.

A comparison of the O:N ratios of *A. noae* and *Modiolus barbatus* due to different salinities and exposure time showed no statistically significant differences between the species. The lowest values of O:N ratio for both species were determined at 25 psu (Fig. 3).

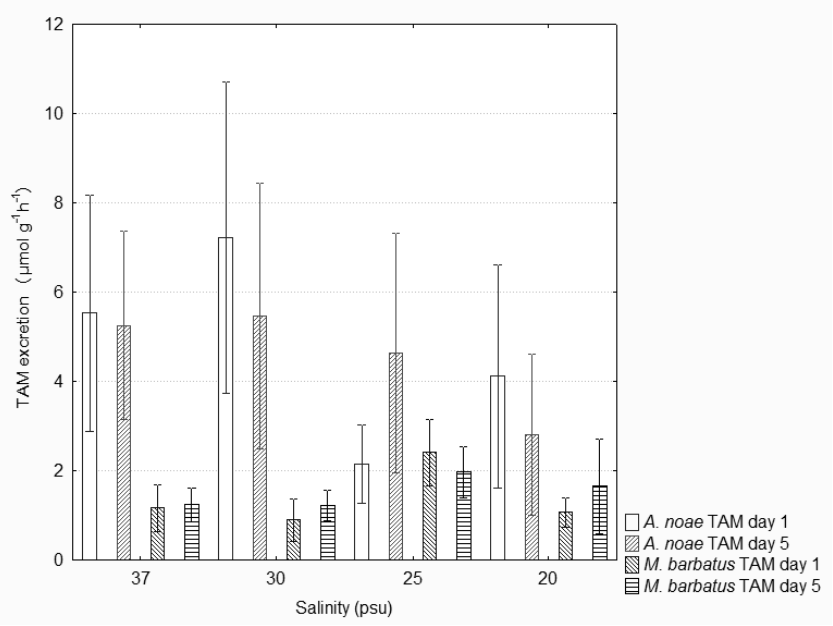

Fig. 2. Comparison of TAM excretion (μmol NH_4 $g^{-1}h^{-1}$) between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

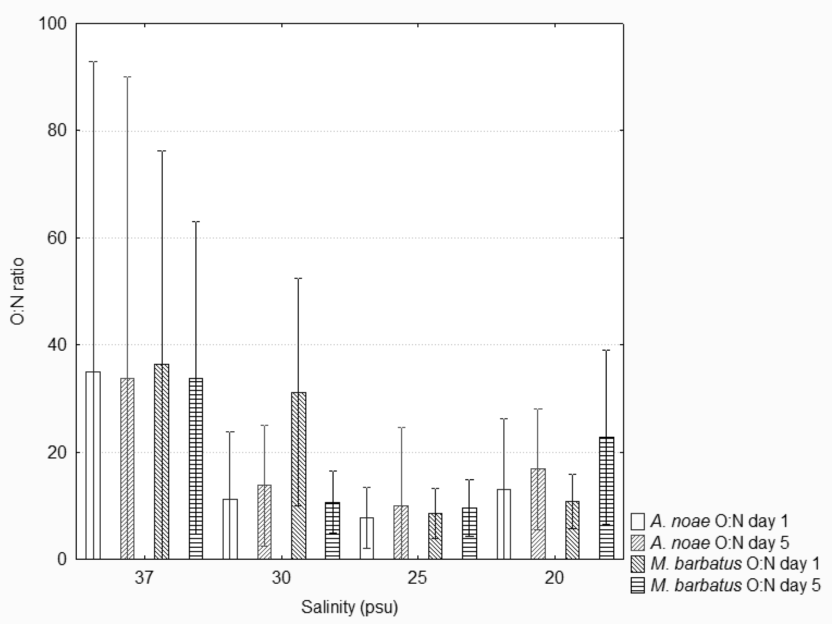

Fig. 3. Comparison of O:N ratio between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

Clearance rate

There was a statistically significant difference in the CR of Noah's ark shell (F = 15.814; *p* = 0.000) due to different salinity. The statistically significant difference (F = 3.255; *p* = 0.034) also occurred due to time of exposure. The mean values of Noah's ark shell CR ± SD are shown in Table 1. The lowest values were observed for groups exposed to salinity of 20 psu for one day, and the highest values were observed at ambient salinity (37 psu). The post hoc Tukey test showed a significant difference between the groups of bivalves exposed to the following conditions: 1. one day at 20 psu and one day at 25 psu (Tukey, *p* = 0.039), 2. one day at 20 psu and five days at 25 psu (Tukey, *p* = 0.019). There was a statistically significant difference in the bearded horse mussel CR (F = 7.456; *p* = 0.000) due to different salinity. The mean CR ± SD at different salinities are shown in Table 2. The lowest values were observed for bivalves exposed to ambient salinity, and the highest at 25 psu after five days of exposure. Values of CR increased to reach a maximum at 25 psu and then decreased. The influence of exposure time was not statistically significant, although the measured values were somewhat larger after five days. The post hoc Tukey test showed a significant difference in the CR between the bivalves exposed to 37 psu for one day and to 25 psu for five days (Tukey, *p* = 0.002).

Comparison of the CR between species is shown in Table 3. Significant differences in CR between the species were observed for all sets of conditions, except for the groups exposed to 25 psu for one and five days and to 20 psu for five days (Fig. 4).

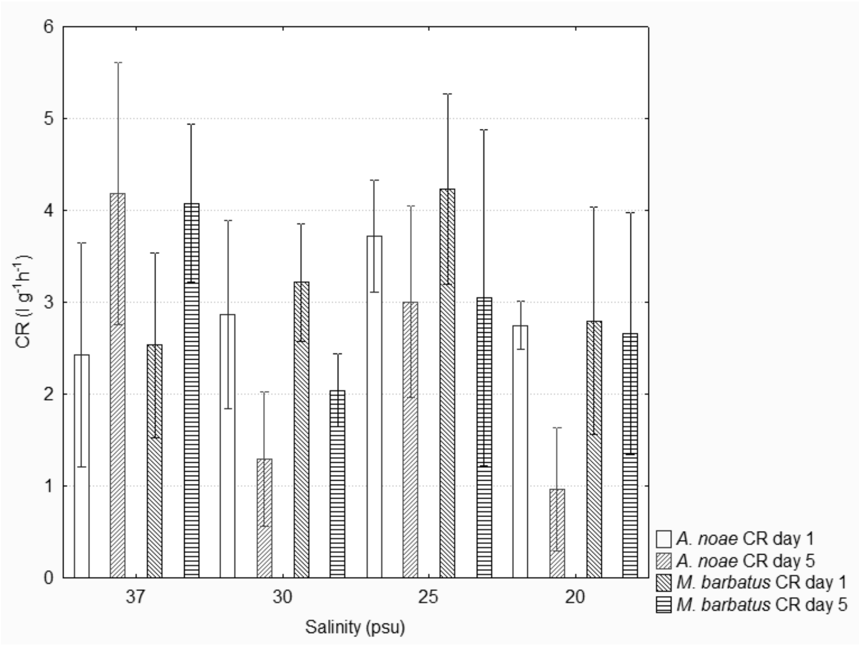

Fig. 4. Comparison of clearance rate (CR) between Noah's ark shell and bearded horse mussel. Values are given as mean ± SD, N = 15.

Discussion

Koehn, Bayne (1989) defined stress as a change in the environment that resulted in decrease in net energy balance. This paper analyses the physiological response of two shellfish species to changes in salinity. The first response of bivalves to a stressful change in salinity is the closure of the shells that separates the animal from the outside environment. This response helps in the prevention of osmotic stress but provides only short-term protection from adverse conditions (Berger, Kharazova, 1997). Pierce, Greenberg (1972) noted that salinity decrease caused the increase of free amino acids degradation and TAM excretion, while increase in salinity had the opposite effect.

OC values for bearded horse mussel and Noah's ark shell partly overlap. Our results are consistent with the results obtained for the mussel *Mytilus edulis* by Hawkins et al. (1985) and for the dog cockle *Glycymeris glycymeris* by Savina, Pouvreau (2004). Ezgeta-Balić et al. (2011) documented OC of 0.31 to 0.67 mg $O_2g^{-1}h^{-1}$ for bearded horse mussels. The results of this study are from 0.14 to 0.54 mg $O_2g^{-1}h^{-1}$, being somewhat lower than those obtained by Ezgeta-Balić et al. (2011). This could be caused by different seasons in which the experiments were conducted as well as by the different phases of the gametogenetic cycle.

Noah's ark shell showed fluctuating OC values with the highest value at 30 psu and the lowest at 25 psu. In some studies, with salinity drop, OC decreased as recorded for the ark shell *Anadara broughtonii* by Shin et al. (2006) and for the gren-lipped mussel *Perna viridis* by Wang et al. (2011). In other studies, the decreasing salinity increased OC in the clam *Meretrix meretrix* (Tang et al., 2005) and the mussel *Mytilus galloprovincialis* (Hamer et al., 2008). OC of the coot clam *Mulinia lateralis* and brown mussel *Perna perna* oscillated with the highest value at 20 psu (Williams, 1984; Resgalla Jr. et al., 2007) and at 16 psu for the clam *Meretrix meretrix* (Tang et al., 2005).

In this experiment, salinity changes did not significantly affect OC of bearded horse mussel, which was consistent with the study of Paganini et al. (2010) for the clam *Potamocorbula amurensis*. Different salinity regimes did not affect OC of *Mytilus edulis* in field research, although the highest OC was recorded at 25 psu (Landes et al., 2015). Williams (1984) noticed a higher OC at 20 psu that could be a referendum of salinity because it was similar to the average salinity of the habitat. In both investigated species in our experiment, an intensive physiological response was observed at 25 psu, and this should be further explored.

The effect of salinity on the TAM excretion was significant for Noah's ark shell, while the influence of exposure time on the TAM excretion was significant for the bearded horse mussel. Noah's ark shell demonstrated higher values of TAM excretion than bearded horse mussel. The highest TAM excretion values of bearded horse mussel overlapped with the lower values of the Noah's ark shell. Our results of TAM excretion are in accordance with the values given for the scallop *Argopecten purpuratus* by Navarro, Gonzalez (1998), and for the mussel *Mytilus edulis* by Bayne, Thompson (1970), Bayne, Scullard (1977b) and Gilek et al. (1992). The value of the TAM excretion rate that Navarro (1988) noticed for the mussel *Choromytilus chorus* kept at 18 psu corresponded to the lowest values for bearded horse mussel exposed to 20 psu for one day in our study. Presumably, there was an influence of type of food (*Tetraselmis suecica*) on TAM excretion due to the dietary conditions and higher content of proteins in the mentioned algal species (Brown, 1991). Feeding with algal species *T. suecica* could increase TAM excretion rate (Bayne, Scullard, 1977a). Bivalves from the brackish environment have higher values of TAM excretion (Gilek et al., 1992). That paper also showed the variability of all the measured physiological responses for shellfish collected in the Vråganskär area that was unpolluted and brackish, similar to Mali Ston Bay.

Ratio of consumed oxygen and excreted nitrogen can help to reveal the nature of the substrate used to maintain metabolism (Corner, Cowey, 1968). Low value of O:N ratio (≤ 10) is connected to significant protein decomposition (Bayne, Newell, 1983) indicating the state of stress (Widdows, 1978). Metabolism relying more on carbohydrates and lipids produces values of O:N ratio higher than 30, while protein decomposition results in values below 30 (Bayne, Thompson, 1970). Similar to our study results, the O:N ratio decreased with reducing salinity in the Chilean scallop *Argopecten purpuratus* (Navarro, Gonzalez, 1998) and in the green-lipped mussel *Perna viridis* (Wang et al., 2011).

In our experiment, Noah's ark shell had O:N ratio over 30 when kept at 37 psu and below 30 at lower salinities. This indicates a stress when the environmental salinity was changed. Values of O:N ratio were above 30 for the bearded horse mussel at 37 psu and for the ones exposed to 30 psu for one day. For the bearded horse mussel, due to the stage of the gametogenetic cycle and the period of the fastest growing, lower value of the O:N ratio was expected at ambient salinity, but it has shown enough energy for both activities as noted by Ezgeta-Balić et al. (2011). Regardless of disadvantageous results for bearded horse mussel there was no statistically significant difference in the O:N ratio between the species.

CR is the indicator of feeding activity. Changes in salinity limit maximum feeding rate, and salinity fluctuations can cause severe disruption to normal diet physiology (Navarro, González, 1998), as also evidenced by the results of our study. For Noah's ark shell , the CR values were reduced by salinity decrease, which was consistent with the results of Wang et al. (2011) for the green-lipped mussel *P. viridis* and Kang et al. (2014) for the clam *Mactra veneriformis*. The values measured after 5 days of exposure to a particular salinity differed significantly from the values achieved after one day. Pleissner et al. (2013) suggested that filtration was strongly influenced by the rate of salinity change, that is, faster the salinity decreased or increased, a more pronounced change was in CR. In this study, an abrupt change of salinity was provoked. The highest observed values of CR were consistent with the results obtained by Widdows et al. (1990) for the species *Arca zebra* at salinity of 36 ± 0.5 psu and Albentosa et al. (2007) for the clam *Ruditapes decussatus* at ambient salinity. The lower values were consistent with the results documented by Navarro (1988) for the mussel *Choromytilus chorus* at 18 psu and Resgalla Jr. et al. (2007) for the brown mussel *Perna perna* at 20 psu. The CR values were not reduced with salinity decline for the mussel *M. odiolusbarbatus*, unlike the ark shell *Arca noae* in our study. Upward trend to one point and then a decrease in CR was found in the brown mussel *Perna perna*, which showed the lowest values at 15 and 40 psu, and the highest value at 20 psu (Resgalla Jr. et al., 2007). In this paper, the values of OC and TAM excretion for *Modiolus barbatus* were the highest at 25 psu, and the O:N ratio was the lowest, suggesting that salinity of 25 psu increased the energy needs due to the osmoregulatory energy expenditure. Increased energy needs could be the reason for greater filtration at this salinity. Furthermore, increased CR caused greater TAM excretion and OC.

Ezgeta-Balić et al. (2011) observed a much lower CR (0.17 to 0.23 $lh^{-1}g^{-1}$) for the mussel *M. barbatus*. Experimental shellfish were fed with somewhat larger amount of different food, *Isochrysis galbana* (Prymnesiophyceae). The individuals used by Ezgeta-Balić et al. (2011) were collected in November; while for this study, the samples were collected at the end of May when the bivalves were in a different phase of the gametogenetic cycle. Since *Modiolus barbatus* grows faster from May to August (Peharda et al., 2007), it would have higher energy needs that could cause a greater need for nutrition. Due to the whole range of differences during research, it was difficult to compare results, especially due to the lack of information on the cumulative effects of different impacts.

Conclusion

For Noah's ark shell, salinity reduction to 30 and 20 psu, respectively, increased OC and TAM excretion, and reduced CR presumably due to protein mobilization as a metabolic substrate during a reduced food intake period. From the results, it was likely that for the Noah's ark shell, salinity of 25 psu was favourable. Strong reaction of both species to salinity of 25 psu may be the result of natural occurring changes in Mali Ston bay salinity caused by river Neretva and submarine groundwater discharges.

Our research has shown that the investigated species had similar physiological responses at lower salinities of 20 and 25 psu. From the aspect of energy loss, Noah's ark shell lost more energy than the bearded horse mussel. Bearded horse mussel was more tolerant to salinity changes, which was indicated by the O:N ratio greater than 30 at ambient salinity and 30 psu, whereas Noah's ark shell achieved O:N ratio greater than 30 only at ambient salinity. Noah's ark shell achieved the highest CR at ambient salinity, and exhibited lower CR at reduced salinity. In contrast to that, the bearded horse mussel showed a higher CR when exposed to lower salinity than the ambient, achieving higher energy gain. Therefore, in an area such as the Mali Ston Bay, which is subject to constant changes in salinity, the bearded horse mussel seems to be more the economical choice for farming. Physiological condition indexes could be used to determine the degree of stress that bivalves experience during farming. In order to determine the influence of stress, there was need for data collecting on normal seasonal variations in the physiological indexes of *M. barbatus* and *Arca noae*.

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IMPORTANCE OF WETLAND REFUGIA IN AGRICULTURAL LANDSCAPE PROVIDED BASED ON THE COMMUNITY CHARACTERISTICS OF SMALL TERRESTRIAL MAMMALS

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Abstract

Kalivodová M., Kanka R., Miklós P., Hulejová Sládkovičová V., Žiak D.: Importance of wetland refugia in agricultural landscape provided based on the community characteristics of small terrestrial mammals. Ekológia (Bratislava), Vol. 37, No. 4, p. 358–368, 2018.

Intensification of agriculture has led, among other negative consequences, also to drying out of wetlands. Nevertheless, some of the wetland biotopes were preserved as small spots. This paper discusses the importance of those areas serving as refugia for small terrestrial mammals. Because small terrestrial mammals in the middle of food webs, they serve as an indicator for the presence of food sources (plants and invertebrates) and suggest the potential of the area as a living space for predators. The experiment took place at lowland agricultural landscape with wetland patches in west and west-east Slovakia (Záhorská and Podunajská nížina lowlands) using catch-mark-release method from 2015 to 2017. The importance was assessed according to abundance, biodiversity, persistence of species during seasons and habitat preference of small terrestrial mammals and equitability of the biotopes. Overall 368 individuals belonging to 12 species were recorded. The lowest abundance and diversity were registered at field biotopes where *Apodemus sylvaticus* was the most abundant. *Microtus arvalis, Clethrionomys glareolus* and *Sorex araneus* dominate at wetland biotopes. The higher biodiversity and abundance of small mammals were recorded at the wetland refugia. The results, together with position of small mammals in food webs, lead to conclusion that the wetland refugia are important habitats for the overall preservation of biodiversity and maintaining them is a part of the strategy for sustainable agriculture.

Key words: small terrestrial mammals, refugia, agricultural landscape, wetlands.

Introduction

The Slovak landscape has been influenced and changed by agricultural intensification, similar to other European countries, mostly by two driving forces – 19th-century agricultural revolution and 20th-century intensive agriculture. Both of them were a result of bigger demand for food and technical crops due to increasing population (Skokanková et al., 2016).

Productive agriculture in post-socialist countries, like Slovakia, was intensively connected with collectivisation as well as by the recent changes in agricultural practices (Stoate et al., 2001). This trend, characterized by the extensive use of heavy mechanization, pesticides and fertilizers (Kuskova et al., 2008), has resulted in the expansion of agricultural land and the rapid reduction of the natural landscape and its pollution. The changes led to decreasing of diversity and abundance of organisms (Baessler, Klotz, 2006; Donald et al., 2001; Stoate et al., 2001; Storkey et al., 2011).

Even though agricultural landscape has enlarged, still some biocorridors were preserved in the Slovak landscape. Those may serve, obviously, as paths during migration or transferring of organisms but for fauna of smaller size, as well as refugia, as a proper and stable biotope.

Research of small terrestrial mammals in Slovakia started more significantly in the 1960s. At first, research was focused mostly on diseases that are spread by micromammals and centres of their expansion. Data about occurrence, abundance and biodiversity as secondary information were the focus of many studies from Ambros and Dudich (for example, Ambros, 1986; Dudich, 1994) but also from other authors (Elischerová, 1989; Gaisler et al., 1967; Kocianová, Kožuch, 1988). Research on the examination of owl pellets to define owl feed composition was also done (Latková, 2008; Obuch, Kürthy, 1995), which is unfortunately not a reliable source for the determination of exact localities where small mammals range. In past 25 years, more and more research aimed at their ecology, population characteristics and influence of landscape changes has been performed (Baláž, Ambros, 2012; Krištofík, 2001).

Marshall and Moonen (2002) presented an extensive summary explaining the functions and relationships between agricultural land and field margins. Most of the experiments were aimed at refugia in an agricultural landscape focusing on plant species (Fried et al., 2009; Smart et al., 2002) and invertebrates (Čejka et al., 2018; Harding et al., 2006; Šustek, 1994).

Jančová et al. (2008) studied the biodiversity of small mammals in ponds and fishponds near the town Nitra and considered them as important oases of life in a monotonous agricultural land. However, the study (Jančová et al., 2008) was focused mainly on species richness in the localities as the community characteristic and a comprehensive comparison was not done. Bryja and Zukal (2000) studied the population characteristics of species in new forestry biocorridors. Malzahn and Fedyk (1982) compared trappability of small mammals at a bog biotope transformed to intensively used meadows, wooded reserves and shelterbelts and proved that unmown meadows, shelterbelts and wooded reserves act like refugia for relict bog species. Atanasov et al. (2012) and Heroldová et al. (2007) claim that even intermediately disturbed habitat shows relatively low small mammal diversity and species evenness. That is why we expect wetland patches at fields to serve as a biotope for micromammals.

Small terrestrial mammals form an important part of an environment. These organisms have been chosen for their designation in the refugia of importance because they not only are herbivores and insectivores, which means that their presence indicates sufficient plant and invertebrate sources of nutrition but also they can serve as food source for secondary consumers (predators).

The purpose of this study is to examine the importance and rate of use of the wetland patches in agricultural landscape as refugia (shown by the difference in biodiversity and abundance of small mammals in the studied areas), point out how sustainable the environment for small mammals is through equitability, to compare results from studied lines at different localities to see the similarities in an identical environment but in different areas and to show the desirable environment for small terrestrial mammals expressed by the results of their habitat preference.

Material and methods

Study area

The experiment was performed at two localities (Fig.1) at Záhorská nížina lowland in the west of Slovakia – Káňa (K, N 48.506° E 16.940°) and Ploština (P, N 48.489° E 16.973°) and at one locality at Podunajská nížina lowland in the south-west of Slovakia – Veľké Kosihy (VK, N 47.769° E 17.859°). All the localities are situated in agricultural land that is arable periodically with some reed wetland patches, which may serve as refugia.

Mammalian sampling

Three trapping lines consisting of 25 „Chmela" type live traps with a 5 m distance between were laid at each locality for two to five nights from November 2015 to February 2017, and overall, four field works were carried out. Trapping lines A (VKA, KA, PA) at each locality were placed at ecotone between a field and wetland, lines B (VKB, KB, PB) at a field that was arable during late spring and summer and nonproductive during fall and winter season and lines C (VKC, KC, PC) at wetland type of biotope to see whether the higher abundance and diversity is at field or wetland type patches. The traps were checked twice a day (after sunrise and after sunset if possible). Oat flakes and larvae of *Tenebrio molitor* were provided as the feed. In colder months also, a piece of cloth in each trap was offered for better survival. Catch-mark-release method was used throughout the whole experiment.

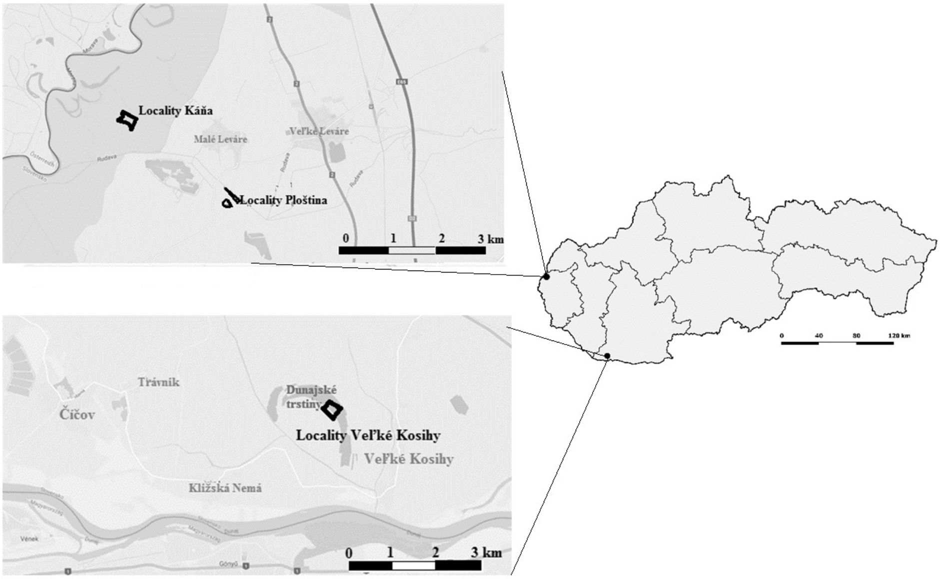


Fig. 1. The map of the studied localities.

Data analysis

Population characteristics

Figures of Shannon diversity index were determined according to the equation

$$H = -\sum_{i=1}^{n} pi, \ln$$

where $pi = \frac{\mathrm{ni}}{\mathrm{N}}$ (*N* is the number of all captured individuals, n_i the number of individuals belonging to one species), figures of Pielou´s evenness index according to

$$E = \frac{\mathrm{H}}{\mathrm{Hmax}}$$

where $H_{\max}$ stands for index of diversity with maximal equitability of all present species, which is determined by the equation $H_{\max} = \ln s$ (*s* is the number of all present species in a community).

We determined the persistence of the species during the seasons by the equation

$$K = \frac{\mathrm{ni}.100}{s} \quad (\%)$$

where *ni* is the number of trapping actions where a species was noticed and *s* is the number of all trapping actions.

Statistical analysis

Nearest-neighbour cluster analysis based on Euclidean space (Lacevic, Amaldi, 2010) showing which lines are the most similar was made using PAST 3.13 (Hammer et al., 2001).

Habitat preference of the species was estimated with CCA after applying DCA on species data (gradient length ≥3,2) using CANOCO 4.56 and CANODRAW for a diagram. The species *Mus* sp. was excluded because just one individual of this species was captured and CCA would not show the real preference for the species. Every single trapping of an individual was included in the analysis because we expect that individuals are trapped multiple times and they stay in the biotope and that is why we consider it is as suitable for them. Excluding those data would underestimate the suitability of the biotope. Environmental factors (variables) for the diagram were tested using Monte-Carlo permutation test under full model using 2999 iteration (at significance level $\alpha = 0.05$) and selected according to how much (≥0.05) of the variability they explain.

Results and discussion

Altogether 368 individuals were noticed, with 121 individuals at the locality Káňa, 131 individuals at the locality Ploština and 116 individuals at the locality Veľké Kosihy. In the west of Slovakia (localities Káňa and Ploština) we observed nine species of small mammals: three species belonging to the order Eulipotyphla – *Sorex araneus* (Soar, 19.4%), *S. minutus* (Somi, 3.2%), *Crocidura leucodon* (Crle, 1.2%) and remaining to the order Rodentia – *Apodemus flavicollis* (Apfl, 7.5%), *A. sylvaticus* (Apsy, 21.4%), *Clethrionomys glareolus* (Clgl, 29%), *Micromys minutus* (Mimi, 3.2%), *Microtus arvalis* (Miar, 15.1%), *Mus* sp. (Musp, 0.4%) and 11 species in the south of Slovakia (locality Veľké Kosihy): two belonging to the order Eulipotyphla – *Sorex araneus* (Soar, 29.3%), *S. minutus* (Somi, 0.9%) and the remaining to the order Rodentia – *Apodemus agrarius* (Apag, 16.4%), *A. flavicollis* (Apfl, 2.6%), *A. microps* (Apmi, 4.3%), *A. sylvaticus* (Apsy, 10.3%), *Clethrionomys glareolus* (Clgl, 0.9%), *Micromys minutus* (Mimi, 8.6%), *Microtus arvalis* (Miar, 21.6%), *M. oeconomus* (Mioe, 5.2%), *Mus* sp. (Musp, 0.9%). The exact numbers of each of the species at each locality are shown Fig. 2.

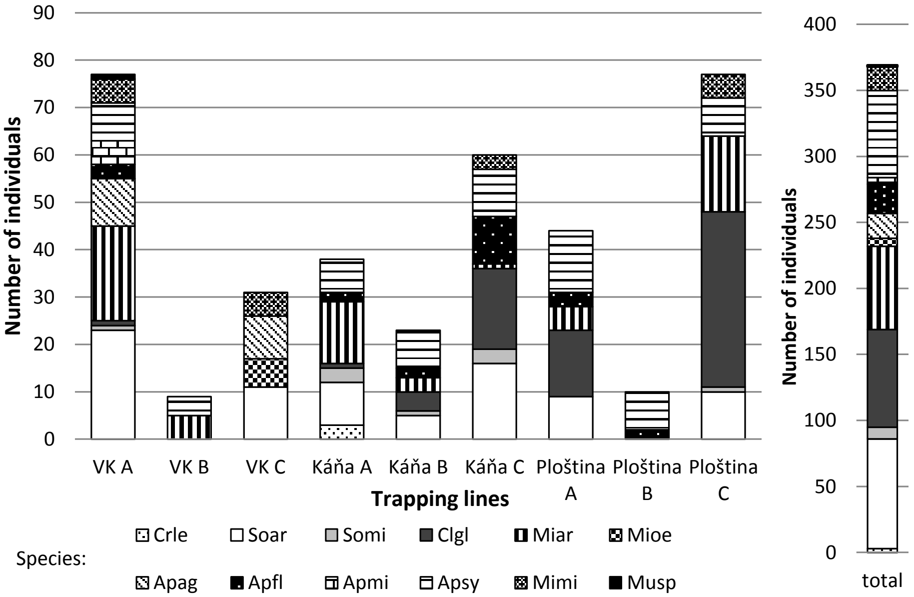


Fig. 2. An overview of caught individuals at each of the trapping lines at the localities.

Order Eulipotyphla at each trapping line is represented mostly by the species *Sorex araneus,* while *S. minutus* and *Crocidura leucodon* comprise just a small part of the communities. At Záhorská nížina lowland, the most abundant species is *Clethrionomys glareolus* and *Microtus arvalis.* On the other hand, in the Podunajská nížina, together with the species *M. arvalis*, a considerable part of the community is formed by the species *Apodemus agrarius* as well as the glacial relict and the endemic subspecies *Microtus oecomomus mehelyi* (Hulejová Sládkovičová et al., 2018). The difference in the structure of the communities between the west and south-west localities could be caused by the expansion of *Apodemus agrarius* that was proven to influence the abundance of the other species at some localities in south-western Slovakia (Tulis et al., 2016).

Even though the abundance of species at the trapping lines is markedly different, significant difference of the lines was confirmed by ANOVA test just at the locality Veľké Kosihy (F=3.77, p=0.0335) but not for the locality Káňa with F=1.79, p=0.1918 nor for the locality Ploština F=2.15, p=0.1409.

At the B lines, *A. sylvaticus* predominates (at Veľké Kosihy and *Microtus arvalis* just slightly). Similar results obtained by Heroldová et al. (2007), except that they observed also *Apodemus microps* in the field habitat, which is really rare at both of the studied regions. At trapping lines situated at wetland biotopes the most abundant species were *Microtus arvalis, Clethrionomys glareolus* and *Sorex araneus*. At forestry biocorridors, *Apodemus sylvaticus* was the most abundant (Bryja, Zukal, 2000).

Abundance and also biodiversity of small mammals at the lines located on a field biotope was lower than on the others, which can be caused by lack of vegetation (mainly causing lack of a shelter as crops provide plant food sources) or partly by using herbicides. Sullivan et al. (1998) found out that using herbicides negatively affects the abundance of small terrestrial mammals; on the other hand, Fisher et al. (2011) claim that organic farming is important mostly at conventional fields and at a type of landscape where our research was done, the diversity of the landscape increases biodiversity and abundance of small terrestrial mammals.

Diversity and evenness of the biotopes

The diversity of small terrestrial mammals shows variety and richness of the biotope, while the equitability (evenness) shows how sustainable the biotope is not only for them but also for species that condition their occurrence (plant and invertebrate food sources). We use both these indices to characterize the quality of biotopes and thus we present Shannon index (H) and Pielou´s evenness index (E) values calculated for each trapping line at the localities from data gathered during the whole experiment (Fig. 3).

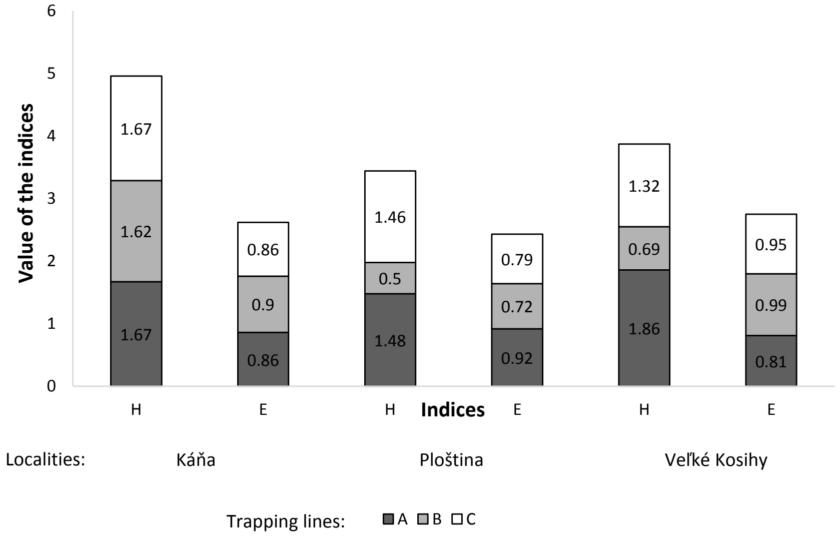


Fig. 3. Indices of diversity (H) and evenness (E) at the trapping lines in total (for all seasons).

Shannon index of diversity is the lowest at the B-lines (the traps laid on a field) at each locality showing that this biotope is suitable only to a less number of species than does the environment with more diverse vegetation. This differs from the results of Bryja and Zukal (2000) who reported lower diversity at forestry biocorridors. Also, the highest biodiversity index they noticed was in a forest habitat but slightly lower (1.8) than at Veľké Kosihy trapping line A (1.86) in ecotone wetland/field. Evenness shows how balanced the trapping lines are. Those numbers vary less at the lines, which is the result of capturing more individuals at lines with higher diversity and their even distribution at those lines.

Indices of diversity and evenness fluctuate a lot during the seasons. The highest diversity was mainly recorded in fall – after summer litters (KA-1.47, KB-1.54, KC-1.67, PA-1.42, VKC-1.31) and summer – after spring litters (PB-1.01, VKB-0.69). On the other hand, the lowest diversity was marked in winter (KA, KB, PB, VKB-0, PA-0.79) and spring (KB, PB, VKB, VKC-0) when conditions (food, temperature or gradation of individuals) are not befitting to such an extent as during rest of the year. Evenness was the lowest at Veľké Kosihy locality in summer 2016 (VKA-0.44, VKC-0.59), excluding spring 2016, because just one species was captured or no individuals were noticed at all. In general, evenness was high in autumn 2015 and summer 2016.

Similarity of the trapping lines

Based on abundance of the species at the lines, the most similar are trapping lines B that were in the field environment. At all of these lines, abundance and diversity were shown to be low. Even though microclimate at the Veľké Kosihy locality is different (slightly warmer because of the position in the south of Slovakia) and the localities are quite distant, the trapping lines at the localities were not compound to one cluster. However, the results are in agreement with the nature of the environment at the lines. Vegetation is one of the most important factors for the presence of small terrestrial mammals. It does not only serve as a food source for herbivore species but also acts as a living space and a shelter for all of the present species. Vegetation at trapping line Káňa A was reduced rapidly during our research, which makes it closer to the B-line type of habitat. The furthest is Ploština C with distinct community characteristic. The remainder of the trapping lines were located at more or less dense reed vegetation and their position in the dendrogram (Fig. 4) corroborates with the uniformity of the trapping lines.

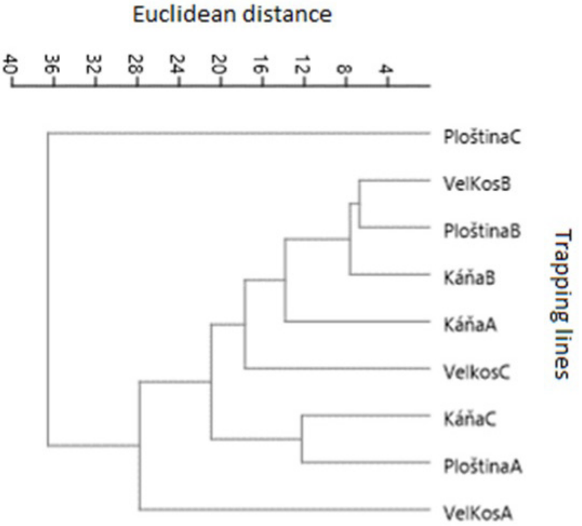


Fig. 4. Cluster analysis of the trapping lines at the surveyed localities.

Persistence of the species during seasons (%)

Persistence of the species (based on presence/absence of a species during a trapping action) is in general the lowest at the lines B at all the localities for every species (Table 1). The only exception is *A. sylvaticus*, which is the most likely caused by significant mobility of the species and ability to move quickly and for longer distances. That is why it might be able to survive also in the long term at field biotopes even when it is not covered by crops. Other species reside at these biotopes just occasionally as the figures of persistence confirm. Even though the B lines present the most of null values (prompted bold), there is no significant difference proved by ANOVA test (localities: Veľké Kosihy - F=1.79, p=0.185, Káňa – F=1.03, p=0.3756, Ploština – F=2.1, p=0.148) between the localities.

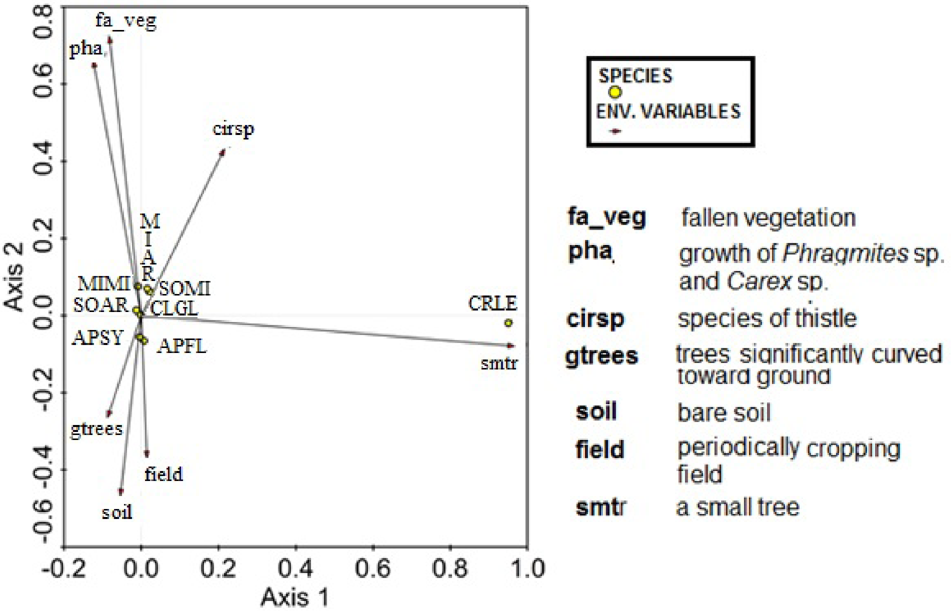
Table 1. Persistence of the species during the research at particular trapping lines

	VK A	**VK B**	**VK C**	**K A**	**K B**	**K C**	**P A**	**P B**	**P C**
Crle	0	0	0	25	**0**	0	0	**0**	0
Soar	75	**0**	75	50	25	100	100	**0**	100
Somi	25	**0**	0	25	25	50	0	**0**	25
Clgl	25	**0**	0	25	50	100	100	**0**	100
Miar	75	75	0	50	50	25	25	**0**	100
Mioe	0	**0**	50	0	0	0	0	0	0
Apag	25	**0**	75	0	0	0	0	0	0
Apfl	50	**0**	0	50	50	75	75	25	0
Apmi	25	**0**	0	0	0	0	0	0	0
Apsy	50	50	0	100	75	75	100	100	75
Mimi	25	**0**	50	0	**0**	25	0	**0**	50

Habitat preference of the species

Sorex araneus and *Clethrionomys glareolus* are, according to the position in the diagram (Fig. 5), considered as generalist species. In accordance with a recent research (Lešo, Kropil, 2017) *Sorex araneus* should not be considered as a forest species as it was present before and its habitat preference recorded during this research affirm the statement. More movable species such as *Apodemus flavicollis* and *A. sylvaticus* prefer an environment with bare soil and less vegetation, concurs with the result of Baláž et al. (2016), which explains their occurrence at the B lines. On the other hand, Klimant et al. (2015) categorized these species as exoanthropic (lesser affinity to humans). That could mean that even the field biotopes changed by human activity are more suitable for those species more than suburban and urban landscape. Other species are more attached to wetland type of biotope with the growth of *Phragmites* sp. and *Carex* sp., fallen vegetation and occurrence of *Cirsium* sp.

Like Heroldová et al. (2007), our research confirms that at non-crop habitats, communities are more diverse and species more abundant. Bryja and Zukal (2000) observed moving of small mammals from fields to newly planted biocorridors during part of the season, as a con-



fa_veg	fallen vegetation
pha	growth of *Phragmites* sp. and *Carex* sp.
cirsp	species of thistle
gtrees	trees significantly curved toward ground
soil	bare soil
field	periodically cropping field
smtr	a small tree

Fig. 5. Habitat preference diagram of the listed species.

sequence of the food scarcity or the threat from agricultural actions. Dennis and Fry (1992) found out that field margins do not serve as the only wintering refugia for arthropods. However, we did not notice this tendency probably because the wetland patches are old and have been serving as refugia for a long time and they serve as stable habitat throughout the year.

Semi-natural habitats like the wetland refugia that we studied are not only important for abundance and diversity of small mammal but also it is proven (even though more research in other segments is needed to be done) that they provide many ecosystem services like soil conservation, support of biological control and much more (Holland et al., 2017).

Conclusion

The higher abundance and biodiversity of small mammals were recorded at the wetland habitats. Most of the observed species show preference for habitats with diverse vegetation, as a source of diverse food and more shelters, and simultaneously do not tend towards field habitats. The presence and numbers of top predators are naturally dependent on the prey abundance and, despite the yet not concluded debate on the low ability of predators to influence numbers of their prey (see review of White, 2013), the small mammals are important part of their food base. That leads us to the conclusion that the presence of small terrestrial mammals in the wetland refugia offers an opportunity as a food source for higher components of food webs, shows sufficiency of plant and invertebrate food resources occurring also in the properly composed agricultural landscape and contributes to higher biodiversity overall. That is why the refugia should be considered as important habitats and a point of interest for future sustainable agriculture and retention of diverse landscape and biodiversity.

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LAND SNAILS IN THE SLOVAK OPEN- AIR GARDEN CENTRES

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Abstract

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In last decades, the number of non-native land snails increased up to 15 percentages; they create more than eight percent of all the Slovakian species. Trend of newly established snail species corresponds with increases in the average temperatures as well as the intensity of foreign trade, suggesting a synergistic effect of both climatic conditions and socioeconomic factors. The research of the open-air garden centres in Slovakia confirmed both factors. We report here some of the newly established populations of sixteen mollusc species. In the old garden centres, the number of species as well as the number of individuals decreased slightly. Area of the garden centre has a very high impact on both abundance and species diversity. The size and age of garden centre proportionally influences the composition of mollusc assemblages. Two new species *Cornu aspersum* and *Cepaea nemoralis* were noticed for the first time in Slovakia. The recent findings of the introduced populations demonstrate the potential of this snail to colonise new areas.

Key words: Garden centres, mollusc, urban area, Slovakia.

Introduction

Central Europe currently faces an influx of expanding terrestrial invertebrates from more southerly situated regions that are often climate restricted in their distribution. Molluscs are sensitive indicators of environmental conditions, and they constitute a group particularly suited to reconstruction of changes of habitats and ecosystems, occurring as the effect of climate or human activity (Alexandrowicz, 2013). Snails are notoriously known for their limited ability for active dispersal; some spectacular range expansions aided by human activities have been recently observed in Europe (Rabitsch, 2006). A majority of socio-economic changes in Eastern Europe were followed by a dramatic increase in imports, and also in transmit traffic in the last 15 years (Peltanová et al., 2012). Langraf et al. (2016) considered these changes as a demonstration of ecological degradation of ecosystems, which are connected to anthropogenic activities in the country. Problems resulting from the introduction of plant and animal species have become increasingly serious. Ignoring their presence might lead to impoverishment of native animal and plant communities; economic losses and the process of their spreading may become impossible to stop (Šteffek, 2007).

The open-air garden centres, as the transfer station of the spreads of invasive organisms, are rarely studied areas. The penetration of non-native species into new areas may lead to biotic homogenization, that is, increase in the similarity of species composition between different areas, which may cause the decline of native species, especially. Number of non-native species grows, and more than half of them seem to come from the Mediterranean area. This trend accelerates; seven new species (six from Mediterranean) have appeared during the last two decades. In Slovakia, some of mollusc species were found only in greenhouses, for example, non-native snail *Hawaiia minuscula* (Binney, 1841) was found in two greenhouses in Slovakia (Čiliak et al., 2016); or in the thermal waters (*Helisoma trivolvis, Menetus dilatatus, Melanoides tuberculata, Planorbella duryi*, *Holandriana holandrii, Gulella io, Opeas goodallii, Zonitoides arboreus, Pseudosuccinea columella*) (Šteffek, 2007). Others expand quickly and efficiently to favourable habitats (*Potamopyrgus antipodarum*, *Physella acuta*, *Arion vulgaris, Sinanodonta woodiana, Corbicula fluminea*, *Dreissena polymorpha*) or form the populations there (*Ferrisia clessiniana, Lucilla singleyana, Boettgerilla pallens*) according to Šteffek (2007). In 2015, thanks to an accidental malacological research of the open-air garden centre in Bratislava, there was discovery of viable populations of non-native species for Slovakia - *Hygromia cinctella* (Draparnaud, 1801) and *Cepaea nemoralis* (Linnaeus, 1758), several empty fresh shells of the green garden snail mentioned Čejka (2015a). A viable population of the brown garden snail *Cornu aspersum* (O. F. Müller, 1774) was found in 2015 for the first time (Čejka, 2015b). Čejka, Čačaný (2014) found that *Helix lucorum* in Slovakia came from south-east Mediterranean. Populations of *H. lucorum* were also identified in Prague (Czech Republic) (Peltanová et al., 2012); all the individuals were able to survive the winter condition in Central Europe. These populations were able to live more than four hundred kilometres out of the area of their origin. Similar results were found by Peltanová et al. (2012) and Holienková, Krumpálová (2016) in the Nitra city (Slovakia). Generally, two major driving forces accelerating the global trend of biological invasion are discussed – global climate change (Walther et al., 2009), and human pressure through various socio-economic activities (Pyšek et al., 2010). All these indicate synergic effect of climate conditions and social-economic facts (Peltanová et al., 2012). Horsák et al. (2016) suggest that recent climate warming may foster geographical expansions of many non-native land snail species, as their distributions are controlled mainly by the January temperature. The range expansion of certain gastropod taxa is often correlated with the loss of retreat of many native species. General habitat degradation, fragmentation and loss of habitat connectivity have endangered some native species (Horsák et al., 2010; Hedblom et al., 2017). Extremely important is the elimination of organisms in the initial stages of invasion; the high effect is successful when the populations are small and the reproduction is low (Horsák et al., 2010).

Two ways that are considered in the study of gardens are: i) To specify and qualify the relationship among the age and proportion of garden centres and snail assemblages; and ii) Identification of habitat preferences, which gardens to species living there offer.

Material and methods

The research was carried out in 2016. For research, all potential types of garden centres on the gradient from western to central parts of Slovakia were recognized and considered. Because the molluscs of different urban habitats cannot be sampled using the standard methods (e.g., square method), we used individual collection per time unit (from 15 min. to 1 hour, depending on the size of garden and microhabitats), which corresponded with similar samplings in the urban environment (e.g., Horsák et al., 2009; Lososová et al., 2011, 2012a,b; Clergeau et al., 2001). Specimens were determined following Ložek (1956) and Horsák et al. (2013a).

According to Horsák et al. (2009), we used a similar method to compare the impact of habitat disturbance and isolation of the site. Each garden centre was categorised, we considered its age and size (Appendix 1, Map). We ranked the species according to Horsák (in verb) to the slightly modified ecological groups sensu Lisický (1991).

Using multidimensional statistic method (Hammer et al., 2001), we evaluated the effect of size and age of gardens on the transfer of new species. We also described similarity, equitability, species diversity and species richness of mollusc assemblages.

Study sites

We sampled garden centres in twenty cities that were chosen in the western part of Slovakia (Appendix 1, Map). The garden centres were categorised according to the age of the building: new - 1 (established among 2010–2016), older - 2 (2000–2010) and old (before the year 2000); and according to the area size: small - 1 (size under 0.5 ha); medium - 2 (up to one ha); and large - 3 (more than one ha).

Results

In the twenty open-air gardens, we found 543 individuals belonging to 16 mollusc species (Table 1); no specimens were found in the four newly established gardens. All the data were tested by Friedman test ($p = 0.006$; DF = 16). Species diversity was low; the highest species diversity (Shannon index) was confirmed in large older garden as the highest value of Margalef's index and equitability (Table 1).

Most of the species were found in the old and large gardens. The highest number of individuals was found in the large gardens with pre-dominance of *Succinea putris* (up to 251 individuals) or with high dominance of *Discus rotundatus* (30 individuals) (Table 1).

Succinea putris was dominant in the seven open-air gardens, with a dominance of nearly 60% and even more (Table 1). Constant occurrence of three species was recorded – *Oxychilus draparnaudi* - 69%, *Cepaea hortensis* - 63% and *Deroceras agreste* - 50% in all the studied gardens; three species had accessorial and ten species occasional occurrence. Frequent eco-elements in gardens were agricolae, sivicolae agricols and praticolae silvicols (Fig. 1).

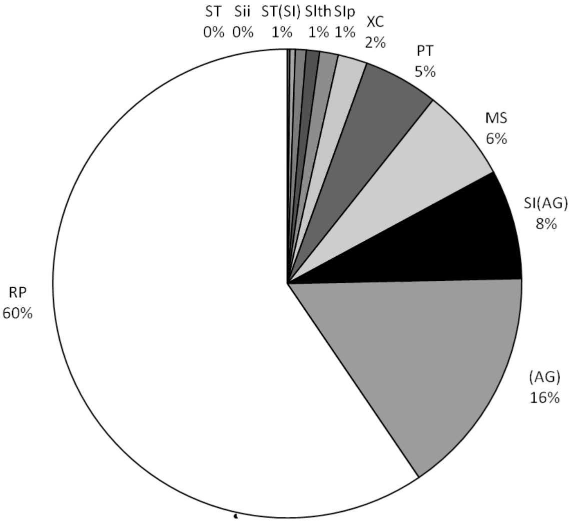

Fig. 1. Percentage of individuals representing eco-elements in open-air gardens.
Notes: AG – agricolae, RP – ripicolae, SI – silvicolae, SI(AG) – silvicolae agricols, MS – euryvalent species, PT – praticolae, XC – xericolae, ST – stepicolae, Sii – silvicols of inundations, ST (SI) – stepicoles of deciduous forests, SIth – silvicols in brushes, SIp – agricols at stones (according to Lisický 1991).

T a b l e 1. Land snails in open-air gardens in Western Slovakia (L1 - L20).

Taxa	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**L8**	**L9**	**L10**	**L11**	**L12**	**L13**	**L14**	**L15**	**L16**	**L17**	**L18**	**L19**	**L20**	**Eco elements**
Succinea putris (Linnaeus, 1758)	53		2							2	14	251	1								RP
Lacinaria plicata (Draparnaud, 181)																				1	SIp
Arion fuscus (O. F. Müller, 1774)			1							1											Sii
Arion vulgaris Moquin-Tandon, 1855	15	5							3	6						6					MS
Discus rotundatus (O. F. Müller, 1774)		15																			SI(AG)
Cepaea hortensis (O. F. Müller, 1774)	4	2	2		2	2	2		4		1		3		2						SI(AG)
Cepaea nemoralis (Linnaeus, 1758)		1											1								SI(AG)
Cepaea vindobonensis (Férussac, 1821)							1					1	3								ST(SI)
Cornu aspersum (O. F. Müller, 1774)	4	2			3																AG
Helix pomatia (Linnaeus, 1758)	1						1		1						1						Slth
Monacha cartusiana (O. F. Müller, 1774)	1					1							9								XC
Xerolenta obvia (Menke, 1828)																				1	ST
Oxychilus draparnaudi (Beck, 1837)	46		4	4	2	6			1	1	2				3	3				3	AG
Limax maximus Linnaeus, 1758	4										1		1								SIp
Deroceras agreste (Linnaeus, 1758)	6	5			3	2	2				6		2	2							PT
Deroceras reticulatum (O. F. Müller, 1774)			1		1																AG
number of individuals	134	30	10	4	11	11	6		9	10	24	252	20	2	6	9				5	
number of species	9	6	5	1	5	4	4		4	4	5	2	7	1	3	2				3	
Shannon H´	1.5	1.4	1.4	0	1.5	1.2	1.3		1.2	1.1	1.1	0.1	1.6		1.0	0.6				0.9	
Margalef	1.6	1.4	1.7	0	1.7	1.3	1.7		1.4	1.3	1.3	0.2	2.0		1.1	0.5				1.2	
Equitability_J	0.7	0.8	0.9	0	1.0	0.8	1.0		0.9	0.8	0.7	0	0.8		0.9	0.9				0.9	

The age of gardens proportionally influences the composition of mollusc assemblages. A decrease of species spectrum and number of individuals was recorded in the oldest and large garden centres (Fig. 2). On the other hand, in the new garden centres, we found sites without individuals, or numerous. In addition, we observed a huge predominance of some species, namely *Succinea putris*, in the new and middle-aged garden centres. In most of the middle-aged and old gardens, the assemblages were more stable with balanced species spectrum (Fig. 2).

The size of area of the open-air garden centre seems to have a huge impact on both abundance and species diversity (Table 1, Fig. 3). In small and medium sized gardens, the number of species and number of specimens were similar; on the contrary, with increase in the size of gardens, the number of species and individuals increased. Large garden centres give them plenty of potential shelters and living conditions.

Based on the qualitative and quantitative similarity (Bray-Curtis single linkage cluster analysis), a set of snails coenoses were divided into branch and four separate lines at a low level of similarity (0.25). Separately, the lines belong to the old and large gardens detached on the basis of a higher number of species and a high number of collected individuals (Fig. 4). The molluscs' assemblages in large gardens were separately allocated based on the predominance of *Succinea putris* and occurrence of *Limax maximus*. Coenoses of large and old garden (L2) was self-allocated due to the dominance of *Discus rotundatus*, which was found only in this garden. High similarity was between the largest gardens due to the same number of individuals and with the dominance of *Oxychilus draparnaudi*, or between the snail assemblages with higher dominance of *Cepaea hortensis*. The highest similarity was between coenoses in large sized gardens based on the occurrence of *Arion vulgaris* at both sites.

Coenoses of gardens, which differed in age and size, have been characterized by the occurrence typically introduced snails to the environment by human, such as *Monarcha cartusiana*, *Cepaea nemoralis* and *C. vindobonensis*. Presence of *Arion vulgaris* in five gardens signalizes the appropriate conditions there for spreading of this non-native species.

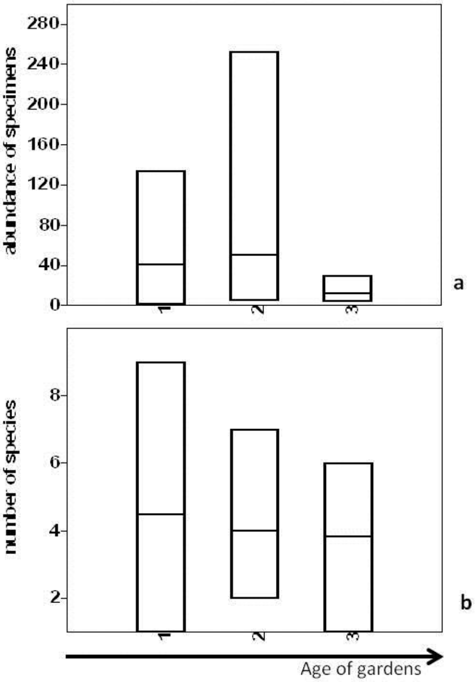

Fig. 2. Box - plot (mean, min-max, 95% confidence limit, p ≤ 0.005), number of individuals (a) and species (b) in open-air gardens of different ages.
Notes: 1 - new (2010 - 2016), 2 - older (2000-2010), 3 - old (before the year 2000).

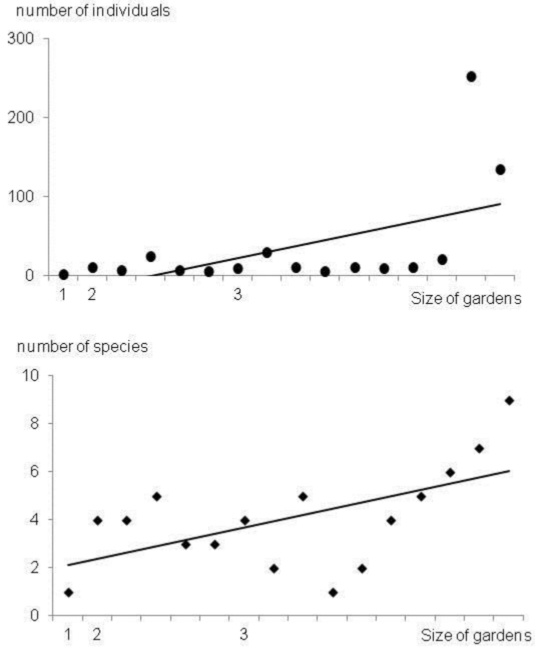

Fig. 3. Trend line of number of individuals and species in different size of gardens.
Notes: 1 - small, 2 - medium, 3 – large garden.

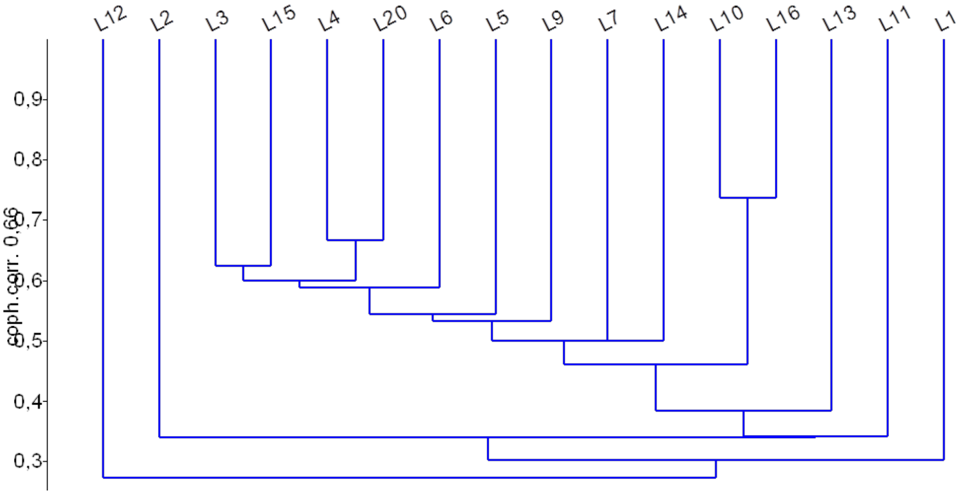

Fig. 4. Bray-Curtis similarity of land snail assemblages in open-air garden centres (Euclidean distance, paired-group; *coph. corr.*: 0.66).

Discussion

In the twenty gardens studied, we recorded two new species for Slovakia – *Cepaea nemoralis* (two gardens) and *Cornu aspersum* (three gardens). *C. nemoralis*, the West European species, was recorded in the Czech Republic for the first time at the end of the 19th century; there was only a slow increase in numbers (Peltanová et al., 2012). It occurs in urban areas, gardens, parks and abandoned urban areas. We found it in two old and large gardens (Bratislava and Zvolen cities); in both we noticed one living exemplar, only. Contrary to the findings of Peltanová et al. (2012), the assumed dispersal mode may result in successful colonization by numerous individuals of even relatively large-shelled in a single event, for example, nearly fifty individuals travelling on the outer surface of a car boot in Prague. The relationship between climbing, traffic intensity, and snail dispersal is now well documented (Aubry et al., 2006). *Cornu aspersum* is a native species common in the Mediterranean region (including Egypt) and Western Europe, from northwest Africa and Iberia, eastwards to Asia Minor, and northwards to the British Isles. In the Czech Republic, *C. aspersum* was for the first time recorded in 2009 (Juřičková, Kapounek, 2009) in two sites in Prague. In Slovakia, it was found in 2015 (Čejka, 2015a). We confirmed its occurrence in three gardens in the Bratislava city; the records were in the old and large open air gardens (Table 1), in which there were found empty shells and living specimens. Based on the number of empty shells and living individuals, we can suppose that its survival in this type of habitat is probable. In agreement with Peltanová et al. (2012), the spreading is supported by an increase of local traffic, the abundance of disturbed sites enriched with nutrients (especially calcium), and microclimatic conditions that facilitate invader survival. Horsák et al. (2013b) found that the number of non-native land snail species in urban habitats was significantly greater in more humid climates. However, for both native and non-native species, the effect of climate on the number of species in the individual habitat types was much less pronounced than the effect of habitat management and disturbance regimes (Horsák et al., 2013b).

The species richness of the garden centres is comparable with the species richness of the urban habitats in Slovakia (Lososová et al., 2011; Čejka 2015a,b; Holienková, Krumpálová, 2016; Mesárošová, Holienková, 2016). Eleven species (from 16) found in the garden centres were confirmed in the cities too. Indeed, there are considerable similarities between the urban biotopes and garden centres. Specific similarity was found between the old and large gardens and city parks or grasslands near apartments. As in open-air gardens and in urban habitat, we found occurrence of typical undemanding synanthropic species such as *Oxychilus draparnadii* or *Deroceras agreste*. Some gastropod species, including ubiquitous species from more southerly European regions, benefit from the ruderalisation of open areas and replace sensitive species. Habitat type explained higher proportions of the total variation in snail species composition than did climate (Lososová et al., 2011). In the near future, we may expect a further increase in the number of non-native species well adapted to human-impacted environments. Newly established urban environments might help alien species to leave their original ranges and to adapt to the new conditions. It is likely that there are general differences in the levels of invasion of cities in different climatic regions, and in the effects of the factors (land-use type and its spatial structure, climatic, edaphic and socio-economic conditions, disturbance and other stochastic processes) on species of different origin (Horsák et al., 2016). Climatic and socio-economic factors separately would not lead to such a high number of successful land snail expansions, but together constitute suitable conditions for spreading (Pyšek et al., 2010; Hedblom et al., 2017; Ružička, Mišovičová, 2013). Assemblages of snails in old garden centres were characterised by a decrease in the number of species and in the number of individuals. On the other hand, the size of the open-air garden centres seems to have a huge impact on both, on the abundance and the species spectrum. The garden conditions are appropriate refugee for the spreading of non-native species.

Conclusion

We report here some of the newly established population of sixteen Land snail species in the Slovak open-air garden centres. In the old garden centres, the number of species as well as the number of individuals decreased slightly. Area of the garden centre has a very high impact on both the abundance and the species diversity. The size and age of garden centre proportionally influences the composition of mollusc assemblages. Coenoses of gardens, which differed in age and size, have been characterized by the occurrence typically introduced snails to the environment by human, such as *Monarcha cartusiana*, *Cepaea nemoralis* and *C. vindobonensis*. Presence of *Arion vulgaris* in five gardens signalizes the appropriate conditions there for spreading of this non-native species. The recent findings of the introduced populations demonstrate the potential of the snail to colonise new surrounding areas.

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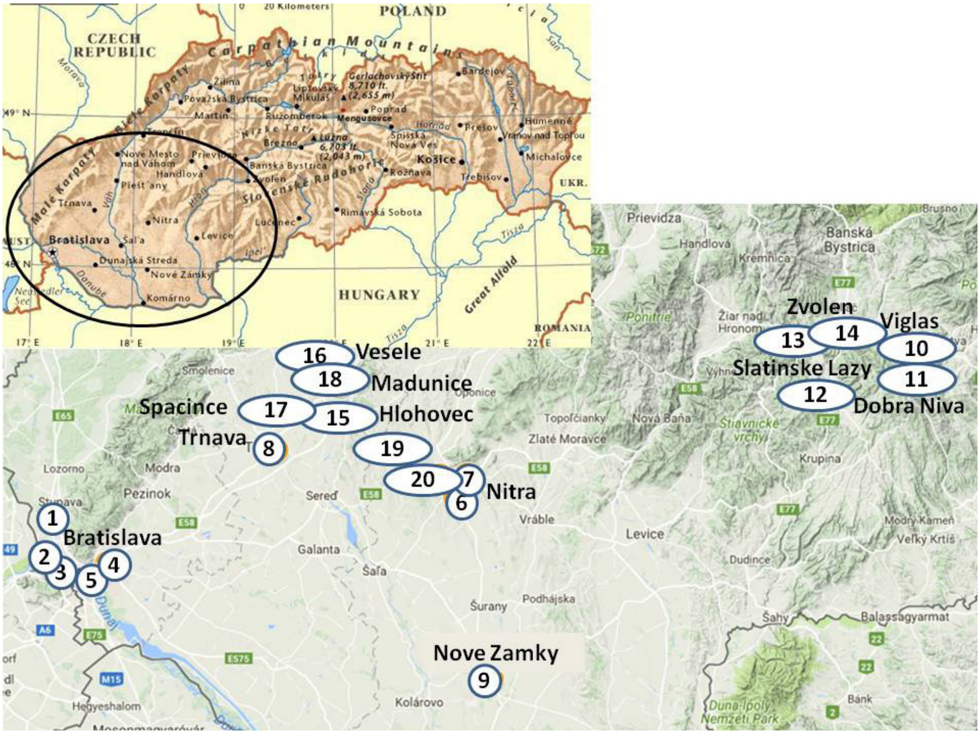
Appendix 1. Map of study sites, the open-air garden centres in western part of the Slovakia.



Notes: L1. Siky Gardens, Bratislava, altitudes: 48.24662, 17.02331, date of establishment: 2004, area 3, age 1. Date of collection: 28 September 2016.

L2. Agapé Bratislava, altitudes: 48.15159, 17.03142, date of establishment: 1996, area 3, age 3. Date of collection: 28 September 2016.

L3. Zreuz, Bratislava, altitudes: 48.15676, 17.01348, date of establishment: 1998, area 3, age 3. Date of collection: 28 September 2016.

L4. Kulla, Bratislava, altitudes: 48.16398, 17.15914, date of establishment: 1992, area 3, age 3. Date of collection: 28 September 2016.

L5. Atelier Duma ltd., Bratislava, altitudes: 48.1425, 17.13391, date of establishment: 1999, area 3, age 3. Date of collection: 28 September 2016

L6. Agapé plus ltd., Nitra, altitudes: 48.28133, 18.09111, date of establishment: 2012, area 2, age 2. Date of collection: 27 September 2016.

L7. Top Garden, Nitra, altitudes: 48.32160, 18.12070, date of establishment: 1998, area 2, age 2. Date of collection: 27 September 2016.

L8. Oazis Lestra & Co ltd., Trnava, altitudes: 48.36601, 17.6101, date of establishment: 2016, area 1, age 1. Date of collection: 27 September 2016.

L9. Grandiflora, Nové Zámky, altitudes: 47.96021, 18.18909, date of establishment: 1991, area 3, age 3. Date of collection: 20 August 2016.

L10. Garden centre, Vígľaš, altitudes: 48.54906, 19.3032, date of establishment: 2010, area 3, age 2. Date of collection: 10 August 2016.

L11. Gardens, Slatinské Lazy, altitudes: 48.50157, 19.3022, date of establishment: 2012, area 2, age 1. Date of collection: 12 August 2016.

L12. Gardens, Dobrá Niva, altitudes: 48.46605, 19.10039, date of establishment: 2000, area 3, age 2. Date of collection: 12 August 2016.
L13. Tília, Zvolen, altitudes: 48.56698, 19.16046, date of establishment: 2000, area 3, age 2. Date of collection: 8 August 2016.
L14. Siki gardens, Zvolen, altitudes: 48.57126, 19.10972, date of establishment: 2012, area 1, age 1. Date of collection: 8 August 2016.
L15. Garden design, Hlohovec, altitudes: 48.42486, 17.8148, date of establishment: 2002, area 2, age 2. Date of collection: 27 September 2016.
L16. Plantex, Veselé, altitudes: 48.55128, 17.73267, date of establishment: 1996, area 3, age 3. Date of collection: 28 September 2016.
L17. Hadzima plant, Špačince, altitudes: 48.43654, 17.60881 39, date of establishment: 2010, area 3, age 1. Date of collection: 28 September 2016.
L18. Smaragd gardens, Madunice, altitudes: 48.48191, 17.78345, date of establishment: 2013, area 1, age 1. Date of collection: 28 September 2016.
L19. Garden centre, Rišňovce, altitudes: 48.36774, 17.896, date of establishment: 2008, area 1, age 1. Date of collection: 28 September 2016.
20. Garden centre Terra design, Nitra, altitudes: 48.31901, 18.05128, date of establishment: 2013, area 2, age 1. Date of collection: 27 September 2016.

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WOODY SPECIES DIVERSITY, REGENERATION AND SOCIOECONOMIC BENEFITS UNDER NATURAL FOREST AND ADJACENT COFFEE AGROFORESTS AT BELETE FOREST, SOUTHWEST ETHIOPIA

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Abstract

Yasin H., Kebebew Z., Hundera K.: Woody species diversity, regeneration and socioeconomic benefits under natural forest and adjacent coffee Agroforests at Belete forest, southwest Ethiopia. Ekológia (Bratislava), Vol. 37, No. 4, p. 380–391, 2018.

The southwest Ethiopian Afromontane moist forests are recognized as one of the biodiversity hotspots as they are the centres of origin and domestication of Arabica coffee. But they are under threat due to deforestation and conversion to coffee farms. Coffee agroforests are believed to buffer the natural forest from these threats. The study was conducted to investigate the importance of coffee agroforest adjacent to Belete forest southwest Ethiopia. Vegetation data were collected from 68 plots (34 each) and socioeconomic data were collected from 136 households (68 each). The results showed that statistically, there were no significant differences between the natural forest and adjacent coffee agroforests in the species composition, species richness and Shannon diversity index of the woody species($P > 0.05$). The socioeconomic benefit result showed a number of forest products that are collected from the coffee agroforest. There were statistically significant differences between the natural forest and coffee agroforest in the forest income and the Simpson Diversification Index of the households ($p < 0.05$). Coffee agroforests contribute to the conservation of woody species through the retention of woody species and reducing pressure on the natural forest. Therefore, the socioeconomic benefits of coffee agroforests must take into account the conservation of woody species in linking the conservation and development arena.

Key words: diversity, forest incomes, conservation, Afromontane, southwest Ethiopia.

Introduction

A large proportion of the planet's land surface has been transformed as a result of conversion of the natural landscape for human use (Foley et al., 2005). The most practiced land-use changes threatening the tropical forests are the clearing of forests for subsistence agriculture, intensification of farmland productivity and expansion of urban centres (DeFries et al., 2004).

The southwest Ethiopian Afromontane moist forests are recognized as one of the biodiversity hotspots and are the centres of origin and domestication of Arabica coffee (Anthony et al., 2002) and supports diverse species of plants (Tadesse et al., 2014). Despite rapid forest destruction in Ethiopia, primarily, these moist forests are still the main natural production assets, since a great proportion of the population living close to the forest patches depends on the forest products (Stellmacher, 2008).

These remaining Afromontane forests have got much attention due to their ecological and economic importance. But they are under continuous change as the results of coffee management intensification (Schmitt et al., 2010; Tadesse et al., 2014) and are now modified to coffee agroforest that are mainly managed by smallholder farmers' (Aerts et al., 2011).

Despite the forest modification to coffee agroforest, many indigenous tree species are retained with it and has attracted much attention for woody species conservation (Tadesse et al., 2014). Recent study by Molla and Kessew (2015) has shown the significant contribution of traditional agroforestry towards addressing the conservation of indigenous tree species. Compared to large coffee plantation, the smallholder farmers' coffee farms have retained many woody species in southwest Ethiopia (Tadesse et al., 2014). Komar (2006) has also stated the greater conservation value of coffee agroforest. Currently, many conservation organizations have given due attention to coffee agroforests for the conservation of biodiversity and the livelihoods of smallholder farmers' (Gordon et al., 2007). Some studies have demonstrated the persistence high pressure on the natural forest (Didita et al., 2010; Gole et al., 2008). Coffee agroforest is a foreseen option among strategies proposed in reducing pressure on the remaining natural forest as a buffer zone.

The natural forest is the natural capital of the forest dependent households. Coffee agroforestry is a means of natural forest exploitation to get more benefits from the forest. Seemingly, coffee intensification results in a change in the woody species composition and regeneration status. As a result, one can notice a variation from the undisturbed natural forests to intensively managed coffee agroforest (Fisher et al., 2009). Study by Hundera et al. (2013) has revealed the vegetation composition, structure and regeneration difference due to coffee intensification in southwest Ethiopia. This has risen the notion of balancing conservation and development in human dominated land use system. Nevertheless, there is still an opinion that believes coffee agroforest is compatible with conservation in many ways. First there are different types of agroforests with different management intensity under different socioeconomic conditions (Wiersum, 2004). Secondly, coffee forests are nearby or adjacent to the natural forest (Moguel, Toledo, 1999).

Although there are many studies focusing on the Afromontane forest in southwest Ethiopia (Aerts et al., 2011; Hundera et al., 2013; Senbeta, Denich, 2006), literature that bring into attention the comparative analysis of woody species conservation and socioeconomic benefit is missing. The way the forest resources are used under coffee agroforests affect the woody species conservation. Hence, understanding the woody species diversity and socioeconomic benefits provides insights into resources use and conservation (Gomez-Baggethun et al., 2010). Therefore, this study was aimed at investigating the woody species diversity, regeneration status and socioeconomic benefits under natural forest and coffee agroforestry. The paper tries to answer the following research questions:

- Is there a major change in the species composition, structure, regeneration and diversity between a natural forest and coffee agroforestry?

- Do the households exploit coffee agroforest in a way that hinder the regeneration and conservation of woody species?

Material and methods

Description of study area

The study was conducted at Belete forest located in Shabe Sombo district, Jimma Zone, southwest Ethiopia. It is found along Jimma-Bonga main road at 50 km from Jimma town. Geographically, it is found between 7°30' and 7°45' N latitudes, 36°15' and 36°45' E longitudes. The altitude of the area ranges between 1300 to 3000 m above sea level (Cheng et al., 1998). The annual precipitation ranges from 1800 to 2300 mm with maximum rainfall between the months of June and September. The mean annual minimum and maximum annual temperature of the area ranges is 15 and 22 °C, respectively (Hundera, Gadissa, 2008).

Belete forest is part of Belete Gera National Forest Priority Areas in Ethiopia. The forest is part of the remnant moist evergreen Afromontane forest of southwest Ethiopia. For effective management, the forest is under participatory forest management since 2003, and currently, it is under the concession of Oromia Forest and Wildlife Enterprises. The total area of the forest is about 25,597.94 ha. The forest is dominated by trees like *Syzigium guineense*, *Olea welwitschii*, *Prunus africana* and *Pouteria adolfi-friederici*. This forest is among the forests that are rich in biodiversity (Schmitt et al., 2010). As a result, it has a great importance for biodiversity conservation and socioeconomic contribution.

Methods of data collection

Both vegetation and socioeconomic data were collected in 2016. Two stage sampling techniques were applied to collect the data. The forest was divided into natural forest and coffee agroforest. Fourteen (seven each) transect lines and a total of 68 plots (34 each) with an area of 20x20 m at a distance of 100 m between transect and within the plots were established to collect the vegetation data. Within the main plot, subplots of 10x10 and 5x5 m were nested for saplings and seedlings assessment, respectively. The starting point of the first transect line was located randomly. To avoid the edges effects, all the sample plots were established at least 50 m from the forest edges or roads inside the forest (Senbeta, Teketay, 2001). Total number of individuals for mature trees, saplings and seedlings were identified and counted within the main and subplots, respectively. Diameter at Breast Height (DBH) was measured for all woody species in the main plot for individuals with height ≥ 2 m and DBH ≥ 10 cm. Woody species with height ≤ 50 cm and DBH ≤ 10cm and height > 50 cm and DBH ≤ 10 cm were counted as seedlings and saplings, respectively (Kelbessa, Soromessa, 2008). Plant identification was carried out at the field with the help of Flora of Ethiopia and Eritrea (Edwards et al., 2000; Hedberg et al., 2006) and Useful Trees and Shrubs for Ethiopia (Bekele-Tesemma, 2007). Plant specimens were collected, pressed, dried and brought to Jimma University Department of Biology for further identification and deposition.

Natural forest and coffee agroforest are bordered by seven kebeles. Four kebeles (two for each) were selected randomly, Atro Gefere and Sombo Daru for the natural forest and Yanga Duguma and Sebeka Debye for coffee agroforestry. Socioeconomic information focusing on household characteristics, forest income in a form of non-timber forest products, forest products and forest utilization pattern was collected on the benefits of natural forest and coffee agroforestry through the household survey. Structured and semi structured questionnaire was prepared to collect the information. The sample size was determined using the formula following Barlett et al. (2001). Accordingly, a total of 136 households were calculated and proportionally allocated to the total number of households (Table 1). The interviewed households were selected randomly using the lottery approach.

Table 1. Total number of households and sample size.

Name of Kebele	Total households	Sample size
Yanga Duguma	499	31
Sebeka Debye	694	43
Atero Gefere	540	33
Sombo Daru	467	29
Total HH	2200	136

Data analysis

Data on vegetation and socioeconomic benefits were analysed descriptively and tested by independent t-test using Microsoft Excel and the IBM SPSS version 20. The sufficiency of the total number of plots was checked by drawing species accumulation curve. Shannon diversity index, Sorensen's similarity index and Important Value Index were used to analyse vegetation data. The indexes were calculated using the formula following Magurran (2004).

Shannon diversity index:

$$H' = -\sum_{i=1}^{s} P_i \, lnP_i$$

where: H' = Shannon diversity index, Pi= proportion of individuals found in the ith species.

Sorensen's similarity index

$$S_s = \frac{2C}{(2C + A + B)}$$

where: Ss = Sorensen's similarity index, A = number of species in sample one, B = number of species in sample two, C = number of species common to both sample.

Important Value Index:

$$\text{IVI} = \text{Relative dominance} + \text{Relative frequency} + \text{Relative Abudance}$$

The regeneration status was decided based on the recruitment of seedlings, saplings and mature trees following Dibaba et al. (2014), as given below:

- good regeneration pattern when the number of Seedling > sapling > trees
- fair regeneration when the number of seedling outnumbers sapling and trees but sapling less than trees
- poor regeneration pattern when no individual in seedling and sapling stages but relatively many standing individual tree

Forest product diversity index, Simpson diversity index and Relative forest income were used to analyse the socioeconomic benefits under coffee agroforest and natural forest. Forest product diversity index was calculated adopting the Shannon diversity index as follow.

where: H' = Shannon's diversity index, S = total number of forest products collected, Pi = ni/N, the number of forest products as a proportion of the total number of forest products used, In = natural logarithm to base e.

$$(\text{H}') = -\sum_{1=i}^{s} \text{piln(pi)}$$

Simpson Diversity Index (SDI) was calculated using the formula following Illukpitiya and Yanagida (2010):

$$\text{SDI} = 1 - \sum_{i=1}^{N} p_i^2$$

where: Pi is the proportion of forest income from source i. Pi value varies from zero to one. If there is just one forest product type, Pi = one and hence, SDI = zero. As the number of sources increase, the shares (Pi) decline so that SDI approaches to one. SDI = zero implies specialization, whereas SDI = 1 implies diversification resource use types. According to Saha and Bahal (2010), the value of SDI always falls between zero and one. Households with most diversified incomes have the largest SID, and the less diversified incomes are associated with the smallest SDI.

Relative forest income was calculated by estimating the total volume of all types of forest products collected by a household and multiplied by the local market price of each of the products per unit volume. Relative Forest Incomes (RFI) is calculated as the proportion of total income originating from forest use and with total household income (Vedeld et al., 2007). Household annual income was estimated using the formula as has been used previously by Belay et al. (2013), Gobeze et al. (2009) and Tieguhong, Nkamgnia (2012).

$$\begin{aligned} &Household\ Annual\ Income \\ &\quad = \sum Forest\ income + Agricultural\ income + Other\ incomes \end{aligned}$$

The total value products from crop production (I1), livestock products (I2), NTFPs (I3), off-farm activity (I4) and remittance (I5) then sums up to total household income (THI) and finally, relative forest income (RFI) that is calculated using the formula:

$$RFI = \frac{TFI}{THI} * 100$$

where: RFI = Relative forest income, TFI = Total forest income, THI = Total household income.

Results and discussion

Woody species composition of coffee agroforest and natural forest

Species accumulation curve levels first for coffee agroforest and then natural forest. It flattens at 25th for agroforestry and 28th for the natural forest (Fig. 1). The result shows the probability of getting new species is less. This implies that the total number of sample plots taken for the study were sufficient.

A total of 67 woody species belonging to 38 families were recorded. The natural forest accounts for 55 species belonging to 35 families, whereas the coffee agroforest accounts for 33 species belonging to 23 families (Appendix 2 and 3). The relative proportion of each families account for less than 10% in the natural forest and 12% in the coffee agroforest. Fabaceae was the family that accounts for the largest proportion, both in natural forest (7%) and coffee agroforest (12%). The Sorensen's similarity index showed that 47% of the total woody species recorded were found both in the natural forest and coffee agroforest. Although there were more species in the natural forest than agroforest, there were 5 woody species recorded only in the coffee agroforest, implying the importance of coffee agroforest for woody species conservation. The presence of 5 woody species in the coffee agroforest is probable due to overexploitation in the natural forest in the past. The finding supports the idea that the coffee

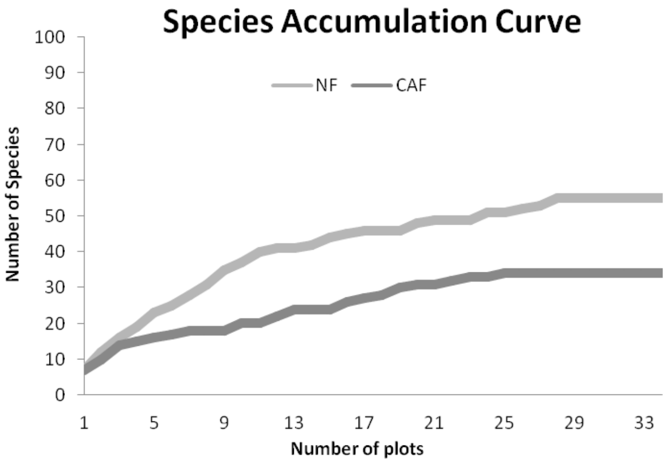

Fig. 1. Species accumulation curve of natural forest (NF) and coffee agroforests (CAF).

agroforest serves as a repository of native tree species as forest loss continues (Tadesse et al., 2014). The dominance of species belonging to the Fabaceae family might be attributed to the nature of species regeneration and coffee shade tree preference by the smallholder farmers.

During the assessment, trees, shrubs and lianas were recorded both in the natural forest and coffee agroforest. The proportion of trees, shrubs and lianas were 53, 31 and 16% in the natural forest and 73, 25 and 2% in the coffee agroforest. The proportion of trees, shrubs and lianas in the coffee agroforest shows the simplification of forest composition towards mature trees. This might be due to coffee management practices that discourage undergrowth (Hundera et al., 2013). The number of trees and lianas were significantly higher in the natural forest than the coffee agroforests ($p < 0.05$). The total number of shrubs recorded in the natural forest and coffee agroforest is not statistically significantly different ($p > 0.305$). Although the magnitude of woody species differ, coffee agroforest hold similar woody species in that trees, shrubs and lianas were recorded in the coffee agroforest.

The Importance Value Index (IVI) shows the top ten important woody species in the natural forest and coffee agroforests. It shows different species in the natural forest and coffee agroforest. Woody species with the highest IVI in the natural forest were *Syzygium guineense* (20%), *Croton macrostachyus* (14%), *Maytenus arbutiolia* (13%) and followed by other species. Whereas, woody species such as *Coffea arabica* (31%), *Millettia ferruginea* (30%) and *Albizia gummifera* (21.7%) and followed by other species were the species with higher IVI in coffee agroforest (Table 2).

Woody species richness and diversity

Species richness and diversity result showed that both the natural forest and coffee agroforest support many species. The Shannon's diversity index of the natural forest and coffee agroforest were H' = 3.79 and H' = 2.82, respectively. The species evenness for the natural forest and coffee agroforests were 0.95 and 0.81, respectively. Although the result shows more woody species richness and Shannon's diversity index under natural forest, the difference is not sta-

Table 2. Importance value index of woody species under coffee agroforest and natural forest.

Natural forest		**Coffee agroforests**	
Scientific name	IVI	Scientific name	IVI
Syzygium guineense	20.03	*Coffea arabica*	30.90
Croton macrostachyus	13.59	*Millettia ferruginea*	29.60
Maytenus arbutiolia	13.42	*Albizia gummifera*	21.07
Olea capensis	13.19	*Ficus sycomorus*	18.71
Celtis africana	12.86	*Ficus vasta*	18.69
Pittosporum viridiflorum	12.29	*Cordia africana*	18.50
Teclea nobilis	11.34	*Bersema abyssinica*	16.06
Pouteria adolfi-friederici	10.05	*Ehretia cymosa*	15.07
Flacourtia indica	9.51	*Sapium ellipticum*	13.86
Ehretia cymosa	9.47	*Syzygium guineense*	12.36

Table 3. Diversity woody species in natural forest and coffee agroforests.

Forest site	Richness	Diversity	
		Shannon index	Evenness
Natural Forest	55	3.79	0.95
Coffee agroforests	33	2.82	0.81
p-value	0.134	0.826	0.50

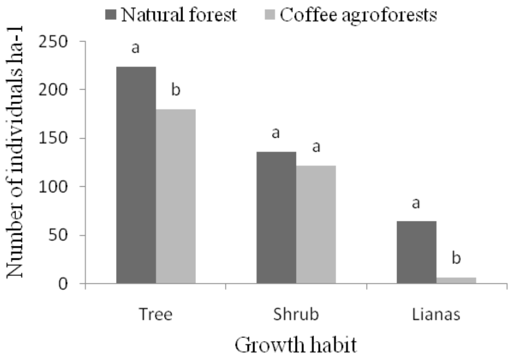

Fig. 2. Growth habit of woody species recorded in the natural forest and coffee agroforests.

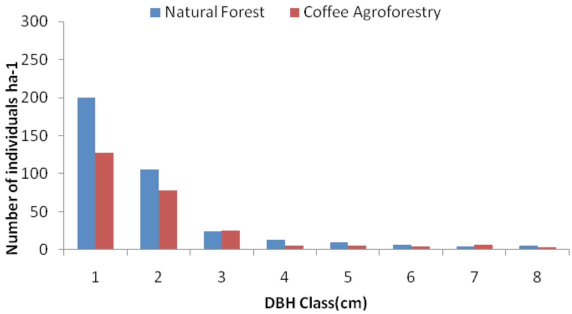

Fig. 3. Diameter class distributions of woody species in natural forest and coffee agroforests.
Notes: DBH class: 1 = 10–20 cm; 2 = 20–30 cm; 3 = 30–40 cm; 4 = 40–50 cm and 5 = 50–60 cm; 6 = 50–60 cm; 7 = 60–70 cm; 8 = > 80 cm.

tistical significant ($p > 0.05$) (Table 3). This might be due to land use history as most of the coffee agroforests are original natural forests. It indicates the importance of conservation of coffee agroforest. This study is in agreement with Tadesse et al. (2014) who reported 27 woody species in semi-forest coffee in south west of Ethiopia. Molla and Asfaw (2014) also reported 32 woody species in the enset based coffee agroforestry in the Midland of Sidama Zone in Ethiopia.

Regeneration under the coffee agroforest and natural forest

The diameter distribution of the individuals in the natural forest and coffee agroforest showed more or less inverted J-shape implying that there are greater number of individuals in the lower diameter class (Fig. 2). The larger diameter classes were dominated by *Pouteria adolfi-friederici, Ficus sycomorus, Prunus africana, Sapium ellipticum* and *Syzygium guineense species* (Fig. 3).

Table 4 shows the number of seedlings, saplings and trees under the natural forest and coffee agroforest. The result showed that overall, there are more number of seedlings than trees both in the natural forest and coffee agroforest. However, it differs from species to species. Regeneration category result showed that the largest proportion of species are under good regeneration under the natural forest and fair regeneration under the coffee agroforest (Fig. 4). No regeneration of *Olea welwitschii, Schefflera abyssinica, Prunus africana, Pouteria adolfi-friederici, Podocarpus falcatus* in the natural forest and *Ficus sycomorus, Prunus africana, Ficus vasta, Fagaropsis angolensis, Ekebergia capensis, Sapium ellipticum, Acacia abyssinica* in the coffee agroforest was recorded. The implication is that these species need attention in the future for conservation.

Table 4. Number of seedlings, saplings and trees in the natural forest and coffee agroforest.

Growth stages	**Natural forest**	**Coffee agroforests**	**P-value**
	Density ha-1	**Density ha-1**	
Seedling	1950	1448	0.038
Sapling	579	424	0.034
Tree	458	424	0.207

To compare species regeneration under the natural forest and coffee agroforest, the species recorded in both were selected. The result showed that a greater number of seedlings and saplings were recorded under the coffee agroforest. No regeneration of certain species were recorded both under the natural forest and coffee agroforest (Table 5). This implies that the absence of regeneration under the coffee agroforest is not always due to the practices, rather it might be attributed to the nature of the species regeneration.

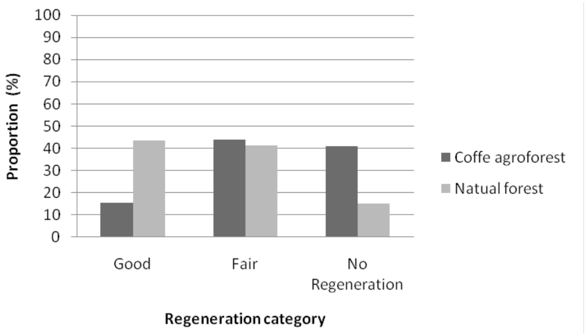

Fig. 4. Regeneration category of species in the natural forest and coffee agroforest.

Socio-economic Benefits

Diversity of forest products

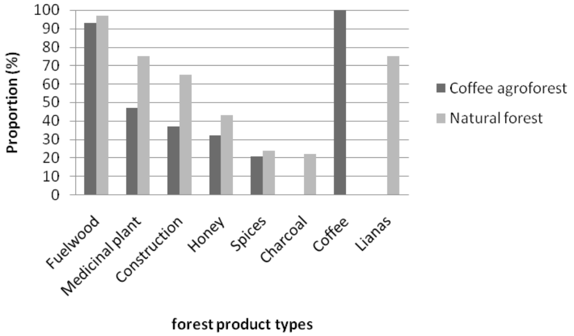

Fig. 5. Different forest products collected from coffee agroforest and natural forest.

Different forest products are collected from the coffee agroforest and natural forest. Figure 5 shows the major forest products that are collected from the forest. The largest proportion of households collect forest products that can be collected from the natural forest. The Shannon Diversity Index shows that there is no statistical significant difference between coffee agroforest and natural forest in the number of forest products that can be collected (Table

Table 5. Regeneration extent of species recorded both in the coffee agroforest and natural forest.

	Coffee agroforest			Natural forest		
	Seedlings	**Saplings**	**Trees**	**Seedlings**	**Saplings**	**Trees**
Albizia gummifera	+++	++	++++	+	+	++
Allophylus abyssinicus	++++++	-	++++	++	+	+++
Bersama abyssinica	+++++	+++	++++	++	++	+
*Clausena anisat*a	-	-	+++	+	++	+
Cordia africana	+++	-	+++	-	+	++
Croton macrostachyus	-	++++	+++++	++	+	+++
Diospyros abyssinica	++++++	+++++	++++	+++	+	++
Dracaena afromontana	-	++++	-	++	+++	+
Ehretia cymosa	++++++	+++++	++++	+	+++	++
Ekebergia capensis	-	-	+++	-	+	++
Fagaropsis angolensis	-	-	++++	+++	+	++
Ficus sycomorus	-	-	+++	-	+	++
Maytenus arbutifolia	-	++++	-	+++	++	+
Millettia ferruginea	+++++	++++	++++	+++	++	+
Olea capensis	+++++	++++	++++	+++	++	+
Phoenix reclinata	-	-	++	-	-	+
Polyscias fulva	++++++	++++	+++++	+	++	+++
Prunus africana	-	-	++	-	-	+
Sapium ellipticum	-	-	+++	-	+	++
Syzygium guineense	-	++++	+++++	++	+	+++

Notes: - sign indicates no regeneration; + sign indicates regeneration and extent of regeneration.

Table 6. Forest product types, relative importance and nature of dependence.

Study area	**Coffee agroforest**	**Natural forest**	**P-value**
Shannon's Diversity Index	4.18	4.16	0.799
Relative Forest Income	49	11	< 0.0001
Simpson Diversity Index	0.45	0.16	0.000

6). Household uses the forest as a source of income. The relative forest income from coffee agroforest account for 49% of the total household income. This is attributed to coffee, as it is highly linked to market to generate cash. The Simpsons Diversification Index shows that there is statistically significant difference between coffee agroforest and natural forest usage. Forest products' usage tends towards diversification and specialization for coffee agroforest and natural forest, respectively. The specialization of forest product use under the natural forest shows a shift from natural forest to other sources implying a reduction of pressure on the natural forest. The local communities do have different feelings towards the natural forest and coffee agroforest in terms of usage. About 90% of the respondents said that the natural forest is a common resource implying all have access to the forest. Whereas there is a restriction imposed to access the coffee agroforest (Fig. 6). The use rights, control rights and authoritative rights are the fundamental issues in forest governance (Sikor et al., 2017). Coffee agroforest has shown a sign of more control in forest resource use than the natural forest.

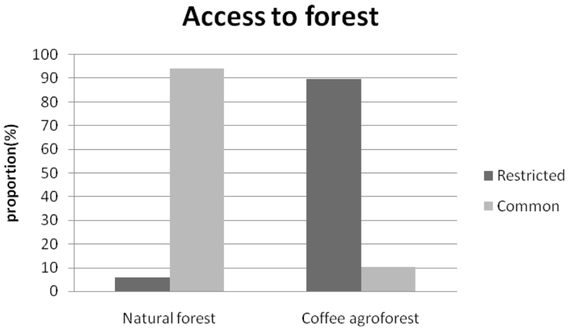

Fig. 6. Household response to access rights to the natural forest and coffee agroforest.

Conclusion

Forest of southwest Ethiopia has a vast ecological and economic importance, but due to human induced factors, there is a persistently high rate of biodiversity loss. There is a need of biodiversity conservation. The results of the present study confirm that the natural forest and coffee agroforests constitute larger proportions of woody species, which may be a reflection of the conservation of biodiversity. Coffee agroforests are conserving woody species through selective management practice in maintaining more species as shade of coffee and economically useful species. This implies that coffee agroforests indirectly contribute to the conservation of biodiversity through reducing pressure that would be exerted on natural forests, so coffee agroforests serve as a buffer zone in natural forest conservation. The study compared a consistent set of description of the characteristics of the forest product and diversification strategy. In the natural forest, household incomes show as specialized because more households are engaged in agricultural practise. However, coffee agroforestry shows that household income is diversified with high value product and is engaged in different activities. Different ways to address dependence on forest products incomes, in case of natural forest, the relationship between diversification and relative forest incomes indicates specialization. Therefore, dependence on agricultural and other incomes simply represents the utilization of the additional income opportunities that the forests provide less. Yet, coffee agroforestry, the relationship between diversification and relative forest incomes implies that it is diversified and is increasing with forest income. Therefore, coffee agroforestry provides different forest products incomes and reduces dependence from natural forest.

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CRITICAL ANALYSIS OF SATELLITE DATA OF NSIDC, NOAA NESDIS IN DETERMINING THE SPATIAL DISTRIBUTION OF ICE ON LAKES

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Abstract

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The process of formation and rotting of ice on lakes is an integral part of the hydrological cycle of many lakes. The conditions of the ice regime significantly influence the ecological system of lakes. The article includes calculation and analysis of errors in the determination of the spatial ice distribution (spatial resolution of 4–6 km) on Lake Onego, Lake Ladoga, Lake Segozero and Lake Vigozero within the period of 2006–2017 according to National Snow and Ice Data Center (NSIDC), National Oceanic and Atmospheric Administration National Environmental Satellite, Data, and Information Service (NOAA NESDIS) data with regard to reliable Moderate Resolution Imaging Spectroradiometer (MODIS) data (spatial resolution of 500 m). It was established that within the monitoring period, NSIDC data have the minimum mean values of errors in determining the spatial distribution of ice on lakes (3–10%) compared to NOAA NESDIS data (11–19%) and are also of more practical interest in estimating the ice coverage of lakes. The dependence of the mean value of errors that occur in the determination of the spatial distribution of ice (according to NSIDC, NOAA and NESDIS data) on the actual value of ice coverage (according to MODIS) was revealed. The results show that the NSIDC data allow estimating adequately the phases of the ice regime; however, the formation of a daily time series of ice coverage during freeze-up and break-up phases is possible only with a significant error (mean value of absolute deviations according to MODIS data is up to 35%).

Key words: NSIDC, NOAA NESDIS, MODIS, spatial distribution of ice, ice coverage, lakes.

Introduction

The formation of the ice cover of lakes depends on a large number of factors both those that influence in the short term (water temperature, air temperature, wind, etc.) and those that affect long before the onset of winter (thermal capacity of the lake). Therefore, ice is a sensitive indicator reacting on changes in these factors (Adrian et al., 2009; Karetnikov, Naumenko, 2008).

In this regard, studies of the annual recurring ice regime of lakes are necessary for solving an important environmental objective that is the establishment of trends in global and regional climate change (Magnuson, 2000).

In addition, the formation and rotting of ice on lakes directly affects the aquatic ecosystem of lakes. In particular, the ice coverage on lake reduces the sunlight penetration, reduces the air–water heat transfer to the atmosphere and stops the kinetic wind to water energy transfer. These factors form ecological system of lakes in winter (Karetnikov, Naumenko, 2008; Filatov, 2012).

Studying the nature of the course of ice regime of lakes comes down to an assessment of the dependence of ice coverage of lakes (parts of the aquatic area covered with ice) on time.

At present, satellite data are widely used to obtain the information on the condition of the earth's surface as well as on the ice coverage of lakes. During the past few years (10–25 years), satellite sensors (MODIS, Visible Infrared Imaging Radiometer Suite (VIIRS), Atmospheric Infrared Sounder (AIRS), Multi-angle Imaging SpectroRadiometer (MISR) and many others) daily perform multi-zone observation of the area in different ranges of electromagnetic wave (visible, infrared and microwave) (Abd Rahman et al., 2016, 2017; Baklagin, 2017).

It is possible to calculate ice coverage of lakes with high accuracy by means of visual expert evaluation based on satellite images of the MODIS sensor made in the visible range (synthesised RGB images (is an additive color model in which Red, Green and Blue light are added together in various ways to reproduce a broad array of colors) or true color images) with high spatial resolution (up to 250 m in open access: https://earthdata.nasa.gov/earth-observation-data).

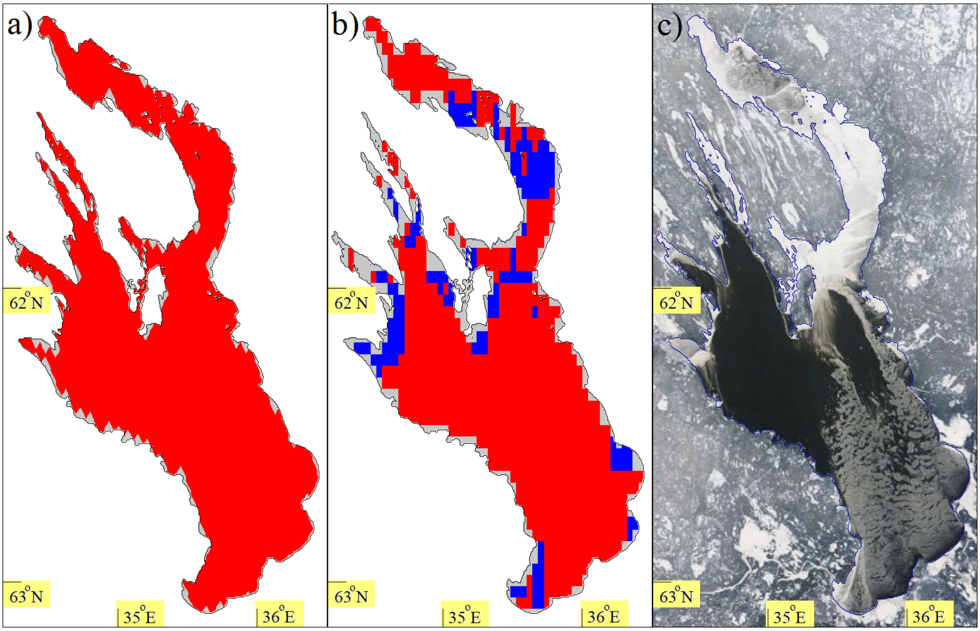

Fig. 1. Visualisation of satellite data on the state of ice coverage of Lake Onego dated 25 January 2012: (a) according to NSIDC data; (b) according to NOAA NESDIS data and (c) according to the MODIS sensor. Red colour represents ice and blue water (a, b).

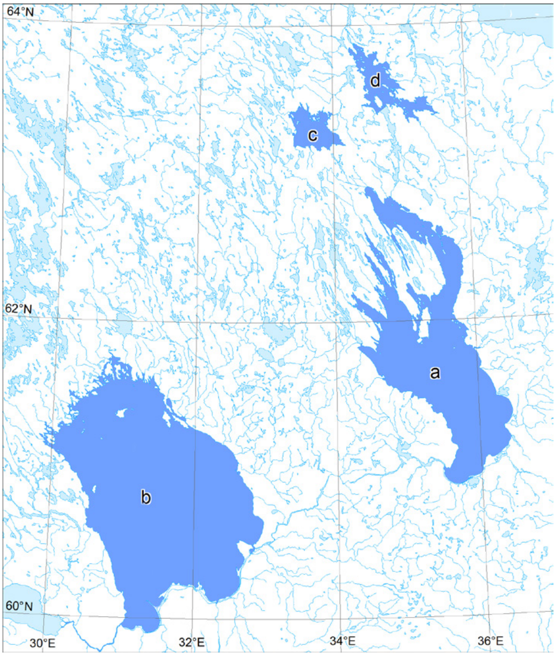

Fig. 2. The lakes in the Mercator projection (the aquatic surface area): (a) Lake Onego (9720 km^2), (b) Lake Ladoga (17 700 km^2), (c) – Segozero (815 km^2) and (d) Vigozero (1250 km^2).

However, in most cases, it is not possible to calculate the ice coverage of lakes according to satellite images of the visible range because of the clouds. For example, during the observation period of 2006–2017, 648 satellite images were taken to estimate the state of the ice cover of Lake Onego and 447 images were taken to estimate the state of the ice coverage of Lake Ladoga because of the greater aquatic area (the larger the aquatic area of the lake, the lesser is the likelihood of aquatic area to be completely free from clouds). Therefore, it is not possible to form a continuous series of values of ice coverage of lakes using only these images.

However, there are multi-sensor microwave radiometers that can perform multi-zone observation in the microwave range (Advanced Microwave Sounding Unit (ASMU-A), Advanced Technology Microwave Sounder (ATMS), Advanced Very-High-Resolution Radiometer (AVHHR), MODIS, VIIRS and others) that allows recording the scene daily regardless of the clouds. Automatic mapping of multi-zonal images of these systems results in obtaining the data on snow and ice coverage of the planet. This data is presented by the National Snow and Ice Data Center (NSIDC) and the NOAA NESDIS Center for Satellite Applications and Research (ftp portal in the public domain: ftp://sidads.colorado.edu/DATASETS/NOAA/G02156/ and ftp://ftp.star.nesdis.noaa.gov/pub/smcd/emb/snow/binary/multisensor/global/). Despite the modest spatial resolution of 4–6 km, the data are suitable for ice coverage calculation of many lakes.

However, it was established that in some cases, automatic interpretation of satellite data generates significant differences in the determination of the spatial distribution of ice on lakes. All this leads to errors in the calculation of the ice coverage of lakes. For example, according to NSIDC and NOAA NESDIS, some aquatic areas of lakes can be covered with ice (Fig. 1a and b), whereas in reality, there is no ice in these areas (Fig. 1c).

In this regard, the purpose of this article is to verify the adequacy of the use of NSIDC and NOAA NESDIS data to determine the spatial distribution of ice on lakes as well as to calculate the ice coverage of lakes. The study includes the following stages:

1. Collection and analysis of satellite data of NSIDC and NOAA NESDIS on the state of the ice coverage of lakes for the period of 2006–2017.

2. Calculation and analysis of satellite data errors of NSIDC and NOAA NESDIS arising while determining the spatial distribution of ice on lakes as well as while calculating the ice coverage of lakes when reliable data is available.

Material and methods

The lakes located in the Republic of Karelia of the Russian Federation were selected for the purposes of conduction critical analysis of NSIDC and NOAA NESDIS satellite data when determining the spatial distribution of ice. Lake Onego and Lake Ladoga are the largest European lakes located in this geographical region; small lakes that are suitable for the analysis of the ice cover with a spatial resolution of 4–6 km can also be found in this region; they are Lake Segozero and Lake Vigozero (Fig. 2). The lakes are covered with ice completely or partially yearly, which is a necessary prerequisite for carrying out this study.

This made it possible to determine the discrepancy between the NSIDC and NOAA NESDIS satellite data in determining the spatial distribution of ice and calculating the ice coverage of lakes that are approximately of the same size and those that are different in the same climatic conditions.

The time interval of satellite data used in the study corresponds to 2006–2017. At this time interval, data from all the sources are available.

MODIS data with a spatial resolution of 500 m were used in this study as reliable data, whilst NSIDC and NOAA NESDIS data have a spatial resolution of 4–6 km. Therefore, a comparative analysis of the spatial distribution of ice in the aquatic area of lakes has features stipulated by the comparison of data of different spatial resolution.

According to MODIS data, homogeneous parts of aquatic area of lakes $A_{small\ 1}, A_{small\ 2}, \dots, A_{small\ i}, \dots, A_{small\ n}$ correspondingly have an area of $S_{small\ 1}, S_{small\ 2}, \dots, S_{small\ i}, \dots, S_{small\ n}$. According to NSIDC and NOAA NESDIS data, homogeneous parts of aquatic area of lakes $A_{large\ 1}, A_{large\ 2}, \dots, A_{large\ j}, \dots, A_{large\ m}$ correspondingly have an area of $S_{large\ 1}, S_{large\ 2}, \dots, S_{large\ j}, \dots, S_{large\ m}$; $A_{large\ j} = \{{}^{water}_{j}A_{small}, {}^{ice}_{j}A_{small}, {}^{land}_{j}A_{small}\}$.
${}^{water}_{j}A_{small} = \{{}^{water}_{j}A_{small\ 1}, {}^{water}_{j}A_{small\ 2}, \dots, {}^{water}_{j}A_{small\ w}, \dots, {}^{water}_{j}A_{small\ w\_count}\}$.

According to the MODIS data, whilst lots of homogeneous parts corresponding to the area $A_{large\ j}$ in geographical coordinates are the aquatic area of the lake, it is not ice.
${}^{ice}_{j}A_{small} = \{{}^{ice}_{j}A_{small\ 1}, {}^{ice}_{j}A_{small\ 2}, \dots, {}^{ice}_{j}A_{small\ ic}, \dots, {}^{ice}_{j}A_{small\ ic\_count}\}$.

Also, lots of homogeneous parts corresponding to the area $A_{large\ j}$ in geographical coordinates are the aquatic area of the lake and it is ice.
${}^{land}_{j}A_{small} = \{{}^{land}_{j}A_{small\ 1}, {}^{land}_{j}A_{small\ 2}, \dots, {}^{land}_{j}A_{small\ l}, \dots, {}^{land}_{j}A_{small\ l\_count}\}$.

In addition, lots of parts corresponding to the area $A_{large\ j}$ in geographical coordinates are not the aquatic area of the lake.

Calculation of the satellite data error (NSIDC and NOAA NESDIS) in determining the spatial distribution of ice on lakes for each time point was carried out according to the formula:

$$\delta = \frac{\sum_{e=1}^{r} {}^{error}S_{large\ e}}{S_{total}} \cdot 100\%$$

where $S_{large\ e}$ is the area of the homogeneous part ${}^{error}A_{large\ e}$ of the set $\{{}^{error}A_{large\ 1}, {}^{error}A_{large\ 2}, \dots, {}^{error}A_{large\ 1}, {}^{error}A_{large\ r}\}$ that are inconsistent with the MODIS data; S_{total} is the number of homogeneous parts that are inconsistent with MODIS data; is the total area of the lake's aquatic area stipulated by the spatial resolution of the satellite data $S_{total} = \sum_{j=1}^{m} S_{large\ j}$.

The discrepancy in data of each part of the aquatic area of lake $A_{large\ j}$ with MODIS data was considered if the following restriction is fulfilled:

$$\left(A_{large\ j} = ice \wedge \sum_{w=1}^{w\_count} {}^{water}_{j}S_{small\ w} > \sum_{ic=1}^{ic\_count} {}^{ice}_{j}S_{small\ ic}\right)$$

$$\vee \left(A_{large\ j} \neq ice \wedge \sum_{w=1}^{w\_count} {}^{water}_{j}S_{small\ w} \leq \sum_{ic=1}^{ic\_count} {}^{ice}_{j}S_{small\ ic}\right)$$

If the restriction is met, the area of lake's aquatic area $A_{large\ j}$ is included in the set ${}^{error}A_{large\ e}$.

Calculations of δ for lakes were performed at time points at which at least one of the data sources (NSIDC, NOAA NESDIS and MODIS) showed ice formations on the lake. Then the mean value of errors $\overline{\delta}$ was calculated for each lake for all the time points.

Results

For the period of 2006–2017, the number of MODIS sensor images (without clouds) showing ice formation of the within the periods of ice phenomena on the lakes amounted to 648 satellite images of Lake Onego, 447 satellite images of Lake Ladoga, 644 satellite images of Lake Segozero and 584 satellite images of Lake Vigozero. These images were used to calculate the data errors of NSIDC and NOAA NESDIS (for each time point at which there is an image).

The mean values of errors in the determination of the spatial distribution of ice on lakes and the mean values of the absolute deviations of satellite data NSIDC and NOAA NESDIS on ice coverage of lakes as regard to the actual ice coverage data (according to MODIS) $\overline{\mathrm{MAD}}_{\mathrm{ice}}$ are presented in Table 1.

T a b l e 1. Mean values of errors of satellite data of NSIDC and NOAA NESDIS in the determination of the spatial distribution of ice on lakes and the mean absolute deviations of ice coverage of lake .

Lakes	(%)		(%)	
	NSIDC	**NOAA NESDIS**	**NSIDC**	**NOAA NESDIS**
Lake Onego	6.1	18.5	4.9	16.1
Lake Ladoga	9.6	10.6	8.2	7.2
Lake Segozero	3.2	12.6	3.1	12.3
Lake Vigozero	5.8	11.5	5.4	11.1

The results show that NSIDC data have lower $\bar{\delta}$ values than NOAA NESDIS data ($\bar{\delta}_{NSIDC}<\bar{\delta}_{NESDIS)}$.

At the same time, the following is true for all lakes except for Lake Ladoga $\bar{\delta}_{NSIDC}<7\%$.

The ice coverage not only characterises the areas of ice formations on lakes but does not characterise their location so the following equation is correct: $\overline{MAD}_{ice}<\bar{\delta}$. Ice coverage on lakes is of great practical importance in determining the timing and duration of the ice regime phases. NSIDC data allow performing calculation of ice coverage of lakes with a smaller value than $\overline{MAD}_{ice}$ NOAA NESDIS data for all lakes other than Lake Ladoga ($\overline{MAD}_{iceNSIDC}=8.2\%$).

According to the satellite data of NSIDC and NOAA NESDIS, the daily series of ice coverage values of lakes are formed: Lake Onego, Lake Ladoga, Lake Segozero and Lake Vigozero for the period of 2006–2017. Graphs of dependence of ice coverage of lakes on time are shown in Figure 3.

The mean absolute deviations of ice coverage values obtained from the NSIDC data from the ice coverage values obtained from NOAA NESDIS data are 18.9% for Lake Onego, 11.7% for Lake Ladoga, 19.3% for Lake Segozero and 12.9% for Lake Vigozero.

According to the NSIDC data, the dates of beginning and ending as well as the duration of ice phenomena on lakes are illustrated in Figure 4 in the form of diagrams.

The determination of the dates of beginning and ending of ice phenomena on lakes according to NOAA NESDIS data is complicated by the fact that in some years, ice phenomena also occur in summer. For example, according to NOAA NESDIS data, in 2013, in August and September, ice formations were observed on Lake Ladoga, whilst in July and October,

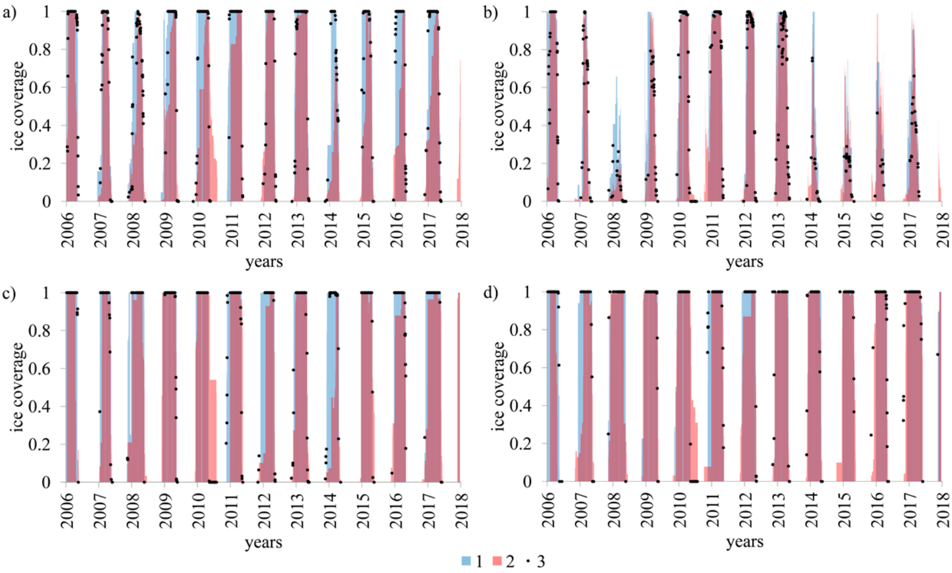

Fig. 3. Graphs of dependence of ice coverage on time: (a) Lake Onego, (b) Lake Ladoga, (c) Lake Segozero and (d) Lake Vigozero. Notation: (1) according to NSIDC, (2) according to NOAA NESDIS and (3) according to MODIS.

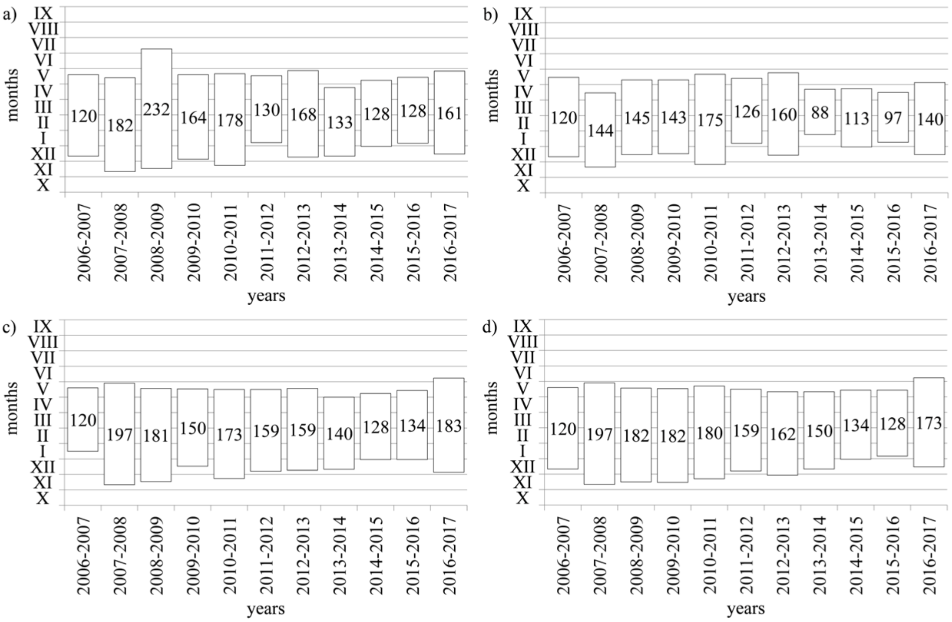

Fig. 4. The dates of beginning and ending of ice phenomena (with an indication of duration) according to NSIDC data for the lakes: (a) Lake Onego, (b) Lake Ladoga, (c) Lake Segozero and (d) Lake Vigozero.

they were not. This is not true for these lakes, which in summer are always completely free of ice. It is impossible to determine the boundaries of beginning and ending of two adjacent periods of ice phenomena. Therefore, it was not possible to compare the dates of beginning and ending of ice phenomena according to NSIDC and NOAA NESDIS data.

According to the NSIDC and NOAA NESDIS data, the number of days the ice phenomena were observed on the lakes during the period of 2006–2017 is correspondingly 1877 and 2294 days for Lake Onego (relative deviation, $\varepsilon = 20.0\%$); 1566 and 2190 days for Lake Ladoga ($\varepsilon = 33.2\%$); 1853 and 2159 days for Lake Segozero ($\varepsilon = 15.3\%$) and 1972 and 2378 days for Lake Vigozero ($\varepsilon = 18.7\%$).

Discussion

Values of $\bar{\delta}_{NSIDC}$ are satisfactory (<7%) when determining the spatial distribution of ice of all lakes, except for Lake Ladoga (9.6%). Therefore, NSIDC data are of greater interest in the determination of the spatial distribution of ice and also in the calculation of ice coverage than NOAA NESDIS data. However, the $\bar{\delta}_{NSIDC}$ value in the determination of the spatial distribution of ice on Lake Ladoga is almost twice as much as that of other lakes. In this regard, a detailed analysis of the errors that occur whilst determining the spatial distribution of ice on lakes in accordance with NSIDC and NOAA NESDIS data was carried out and the dependence of the mean error value in determining the spatial distribution of ice on lakes on the actual ice coverage of the lakes $\bar{\delta}(ice)$ was deduced.

Also the dependence $\overline{MAD}_{ice}(ice)$ characterising the distribution $\overline{MAD}_{ice}$ of actual values of ice coverage (according to MODIS data) was determined.

It was found out that the dependence $\bar{\delta}(ice)_{NSIDC}$ increases steadily whilst actual ice coverage (according to MODIS data) reaching a peak ($\bar{\delta}(0.3\text{-}0.4)_{NSIDC} = 39.2\%$) with ice coverage value of 0.3–0.4, then the error decreases steadily to the minimum value ($\bar{\delta}(1)_{NSIDC} = 0.7\%$) complete freeze-up phase). The graph of the dependence $\bar{\delta}(ice)_{NSIDC}$ is shown in Figure 5.

It should be noted that 70–80% of the satellite images (MODIS) taken for analysis shows lakes that are completely covered with ice, except Lake Ladoga (only 30% of all images). This is explained by the fact that small lakes, Lake Segozero, Lake Vigozero as well as Lake Onego, are completely covered with ice for a considerable time interval of 80–120 days yearly. Lake Ladoga is not always completely covered with ice (Fig. 3b). In 2008–2009 and 2014–2017, there are no MODIS images free from clouds where Lake Ladoga is completely covered with ice. All this explains relatively large value of (9.6%) for Lake Ladoga comparing to other lakes.

It should also be noted that NSIDC data with a small value $\bar{\delta}(0)_{NSIDC}$ (3.9%) make it possible to record the total absence of ice on lake (Fig. 5). Thus thanks to NSIDC data, it is possible to determine sufficiently the dates of beginning and ending of the periods when the lake is completely or partially covered with ice and completely free from ice; at the same time, significant value $\overline{MAD}_{ice\ NSIDC}(0\text{–}1)$ (up to 36%) of freeze-up and break-up phases does not affect the calculation of these dates.

This makes it possible to solve an important hydrological task – to determine the timing and duration of phases of the ice regime of lakes. However, the formation of a daily series of

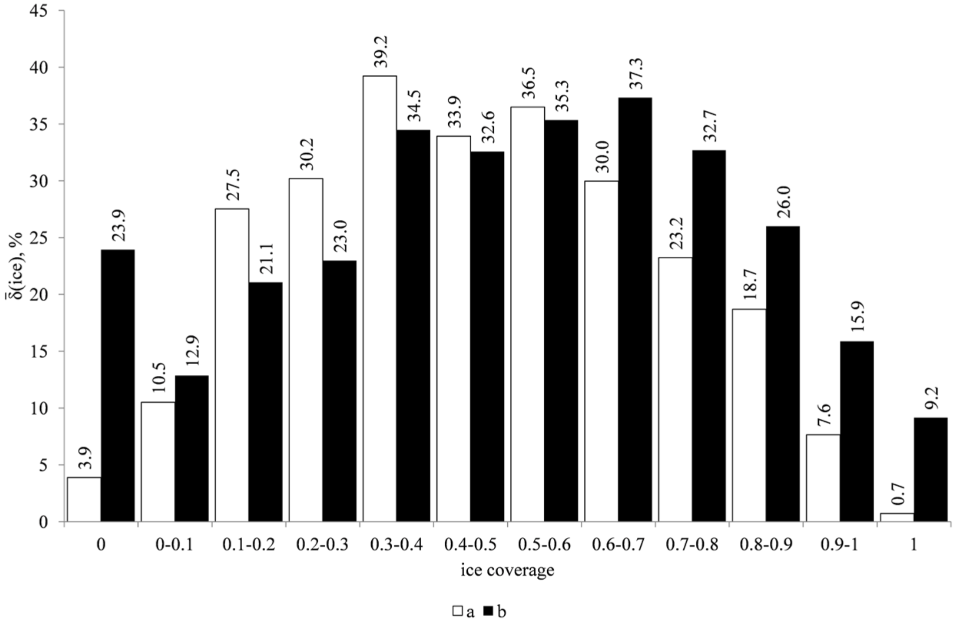

Fig. 5. Graphs of dependence : (a) NSIDC data and (b) NOAA NESDIS data.

values of ice coverage of lakes is impossible with NSIDC data during freeze-up and break-up. To do this, it is necessary to use additional more accurate data.

Dependence $\overline{\delta}(ice)_{NESDIS}$ is similar to dependence $\overline{\delta}(ice)_{NSIDC}$; the maximum value $\overline{\delta}(0.6\text{–}0.7)_{NESDIS}=37.3\%$ is reached when the ice coverage value is 0.6–0.7 and the minimum value is $\overline{\delta}(1)_{NESDIS}=9.2\%$ during the complete freeze-up phase (Fig. 5).

The difference between $\overline{\delta}(ice)_{NESDIS}$ and $\overline{\delta}(ice)_{NSIDC}$ dependences is the presence of a significant value $\overline{\delta}(0)_{NESDIS}=23.9\%$ at the time when lake is completely ice free. Such a value $\overline{\delta}(0)_{NESDIS}$ takes into account the summer periods when according to NOAA NESDIS ice phenomena occur on the lakes, whereas in reality, the lakes are always completely ice free in summer. For the same reason, the number of days within the period of 2006–2017 the ice phenomena were observed on the lakes according to NOAA NESDIS data for all lakes is >15–30% according to NSIDC data.

Conclusion

It was established that comparing to MODIS data, NSIDC data have a smaller value (3–10%) than NOAA NESDIS data (11–19%) for lakes (Lake Onego, Lake Ladoga, Lake Segozero and Lake Vigozero). Therefore, it is advisable to use NSIDC data when determining the spatial distribution of ice on lakes. According to NSIDC (as well as NOAA NESDIS) within the freeze-up and break-up phases, $\overline{\delta}(ice)_{NSIDC}$ can reach an unacceptable value of 40% ($\overline{MAD}_{ice}$

is up to 36%). In this regard, the formation of a detailed time series of ice coverage values within the freeze-up and break-up phases using the NSIDC and NOAA NESDIS satellite data is possible only if it is corrected by more accurate data, for example, MODIS data.

However, the NSIDC data make it possible to calculate the value of the ice coverage of lakes during the complete freeze-up phase and the absence of ice phenomena on the lake with sufficient accuracy ($\overline{MAD}_{ice\ NSIDC}(0)=3.9\%$ and $\overline{MAD}_{ice\ NSIDC}(1)=0.7\%$). This allows us to conclude that the use of NSIDC data is advisable for determining the timing and duration of the ice regime phases.

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