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Macrophytes as bioindicators of the physicochemical characteristics of wetlands in lowland and mountain regions of the central Balkan Peninsula

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ABSTRACT

The simultaneous monitoring of vegetation, water and sediments was conducted in wetlands of the central Balkan Peninsula due to the lack of available knowledge on the univariate response of macrophytes along environmental gradients. The environmental preferences and bioindicator potential of macrophytes were assessed using Huisman-Olff-Fresco (HOF) models. *Bolboschoenus maritimus* and *Scirpus lacustris* subsp. *tabernaemontani* are valuable bioindicators of slightly saline (electroconductivity of 2000–4000 μS cm^{-1} in the sediment) and alkaline habitats that are rich in SO_4^{2-}. Their ecological niches are partially overlapped. *Bolboschoenus maritimus* prefers saltier and more alkaline habitats for optimal development. The salinity and alkalinity of habitats are decisive factors in the ecological diversification of the *Boloboschoenus* species. *Bolboschoenus glaucus* is adapted to non-saline (400–900 μS cm^{-1}) and slightly alkaline habitats, unlike *Bolboschoenus maritimus*. Relatively deep, slightly acid waters which are poor in SO_4^{2-} (0.30 mg/l), and sediments with low values of electroconductivity and K_2O (6.8 mg/100 g sediment) are preferred by *Typha angustifolia, Sparganium erectum* and *Typha latifolia*. The abundance of *Phalaris arundinacea*, *Scirpus lacustris*, *Carex riparia* and *Eleocharis palustris* increases when there is a decrease in the amount of nutrients (NH_4^+, PO_4^{3-} and SO_4^{2-}) in the water. *Phragmites australis* has low indicative value and regional bioindicator potential. The data obtained in the study may serve as a basis for adjusting the existing indicator values of these species and extending indicator systems by defining the indicator values of species with respect to environmental variables which have not yet been considered.

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1. Introduction

Macrophytes are essential and relevant elements in the biological assessment of habitat quality (Onaindia et al., 2005). They are able to respond to altered environmental conditions through changes in their growth and distribution (Steffen et al., 2014). Aquatic plants are not early warning indicator organisms due to their relatively long life cycles and tolerance of short-term changes in environmental conditions (Brabecz and Szoszkiewicz, 2006). Nevertheless, they are formally recognized under the Water Framework Directive of the European Union (European Commission, 2000) as valuable bioindicators for estimating the ecological status of surface waters.

Numerous ecological studies have been focused on investigating the relationship between aquatic plants and the physicochemical properties of the water and sediment (Onaindia et al., 2005; Kočić et al., 2008; Kłosowski and Jabłońska, 2009; Lukács et al., 2009; Steffen et al., 2014) in order to assess their bioindicator potential. The results of such studies facilitate the correction of the existing indicator values for plant species (Ellenberg et al., 1991; Kojić et al., 1997; Pignatti, 2005) through the collection of information on their habitat affinities. In the past, the indicator values of vascular plants were proposed for certain areas, mostly on the basis of expert judgment, so that they can be considered as surrogates for actual field measurements (Thompson et al., 1993). Presently, ecologists are making efforts to calibrate the existing indicator values of plant species in accordance with the results obtained by measur-

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ing environmental variables (Thompson et al., 1993; Ertsen et al., 1998; Wamelink et al., 2002) and to develop an indicator systems based on these variables (Wamelink et al., 2005). Although the indicator value approach is widely used in bioindication (Godefroid and Dana, 2007), it does not provide important information on the ecological amplitudes of a species (Wamelink et al., 2005).

It has been suggested that the indicator value approach is appropriate for assessing environmental quality only when the whole plant species assemblages are used as indicators of environmental conditions (Wamelink et al., 2005). However, the use of species as bioindicators is more cost-effective and can be accurately estimated by all of the personnel involved in monitoring (Niemi and McDonald, 2004). This gives the use of species a slight advantage over communities. The introduction of more effective approaches in bioindication has become necessary. Species response curves (SRCs) have been recognized as an effective and valid tool (Peppler-Lisbach, 2008) for univariate species response modeling. SRCs provide precise information, not only on the optimum, but also on the other niche parameters, including tolerance and range of the response of species along environmental variables. The shape of SRCs "hides" important data, too. It indicates interspecific interaction (Lawesson and Oksanen, 2002) through determining the density and identity of potential competitors. There are several techniques which can be used to model SRCs, out of which the HOF models are the best option from the ecological point of view (Huisman et al., 1993; Lawesson and Oksanen, 2002; Jansen and Oksanen, 2013). The environmental preferences of species defined on the basis of HOF models are expressed in physical units (Štechová et al., 2008; Uğurlu and Oldeland, 2012), as opposed to indicator systems that use a semiquantitative, arbitrary scale, so they are more meaningful.

The main aims of the present study are: 1) to define the environmental preferences of macrophytes with respect to the physicochemical properties of the water and sediments using HOF models, 2) to assess the bioindicator potential of the species studied, 3) to determine the existence of seasonal variability in the environmental variables, 4) to discuss the impact of seasonal variability in the environmental variables on defining the ecological preferences of the species examined.

2. Material and methods

2.1. Vegetation sampling

In order to determine the environmental preferences of *Bolboschoenus glaucus* (Lam.) S. G. Sm., *Bolboschoenus maritimus* (L.) Palla, *Carex riparia* Curtis, *Eleocharis palustris* (L.) Roemer & Schultes, *Phalaris arundinacea* L., *Stachys palustris* L., *Phragmites australis* (Cav.) Trin. ex Steudel, *Scirpus lacustris* L., *Xanthium strumarium* L. subsp. *italicum* (Moretti) D. Löve, *Scirpus lacustris* L. subsp. *tabernaemontani* (C. C. Gmelin) Syme in Sowerby, *Sparganium erectum* L., *Lemna minor* L., *Typha angustifolia* L., *Potamogeton lucens* L. and *Typha latifolia* L. with respect to the physicochemical characteristics of their habitats, 50 permanent vegetation plots were sampled over five months during one growing season to collect vegetation, water and sediment data. The above species were recognized to be statistically significant indicator species for 11 emergent macrophyte communities developed in the central part of the Balkan Peninsula (Jenačković et al., 2016). The procedures for both vegetation data collection, as well as determining the plant species, are described in detail in an earlier published study (Jenačković et al., 2016). Also, basic data on the geographical positions and climate conditions of the localities investigated are given in the previously mentioned paper.

2.2. Water sampling and analysis

For each vegetation plot where the sediment was covered with water, one 1000 ml water sample was taken by putting a sample bottle 20 cm below the water surface. After collection, the water samples were transported in a hand fridge and kept at 4 °C prior to laboratory analyses. Before chemical analysis, the samples were filtered through 0.45 μm PTFE filters. The concentration of ammonium-ion (NH_4^+), nitrate (NO_3^-) and orthophosphates (PO_4^{3-}) was measured using the Shimatzu UV–vis spectrophotometer according to APHA (1995) standard methodology. The TURB 355 IR turbidity meter (WTW, USA) was used for assessing the quantity of SO_4^{2-} (Radojević and Bashkin, 1999). The concentration of Cl^- was assessed using the argentometric titration method with $AgNO_3$.

Water conductivity (μS cm^{-1}) and pH were measured in situ using a portable WTW multi 340i probe. The water depth was measured from the shallowest to the deepest points in the vegetation plots.

2.3. Sediment sampling and analysis

The sediment samples for each vegetation plot were made by mixing three sub-samples which were collected in the zones of the root system (0–25 cm). The field-moist sediment samples were used to gravimetrically determine the current moisture content by drying them at 105 °C to their constant mass (ISO 11465, 1993).

Chemical analysis was carried out on the sediment samples after they were cleaned of mechanical and organic impurities, air dried, crushed and sieved through 2 mm mesh. These sediment samples were utilized for determining the following properties: sediment reaction, electrical conductivity (EC), concentration of available potassium (K_2O) and phosphorus (P_2O_5), concentration of carbonates and bicarbonates and chloride content. The sediment pH values were determined in 1:2.5 (w/v) suspensions of sediment in water (Van Reeuwijk, 2002) using a pH meter (CyberScan pH 510). The content of available potassium was determined by flame photometry using a Carl Zeiss Jena FLAPHO 4 flame photometer according to the Egner and Riehm (1958) method. The concentration of available phosphorus was determined using a Secomam Anthelie UV-V spectrophotometer (Egner and Riehm, 1958). The saturation extract (1:5 w/v, sediment to distilled water) was used for measuring EC and the concentration of Cl^-, HCO_3^- and CO_3^{2-}. The EC was measured using a SensION5 conductivity meter (HACH, USA) according to the Rayment and Higginson (1992) method. The chloride content was determined by argentometric titration with $AgNO_3$ as the titrant, and with K_2CrO_4 as an indicator, according to Mohr's method (Richards (Ed.), 1954). Titration with 0.01 M HCl was applied to determine the concentration of HCO_3^- and CO_3^{2-} using methyl-orange and phenolphthalein as indicators, respectively (Richards (Ed.), 1954).

2.4. Statistical analysis of the data

Defining the environmental preferences of the species studied was based on species response curves. Modeling of the species response curves for all of the species included in the present study, with respect to the physicochemical characteristics of the water and sediment analyzed, was performed with logistic regression models which are also known as Huisman-Olff-Fresco (HOF) models (Huisman et al., 1993). HOF models are a hierarchical set of five models, namely: I – flat with no response, II – monotone decreasing or increasing, III – monotone increasing to a plateau, IV – symmetric unimodal and V – asymmetric unimodal. This statistical routine was developed by David Zelený and Lubomír Tichý (http://davidzeleny.net/juice-r/

doku.php/scripts:species-response-curves) and it was run externally from the JUICE 7.0 software package (Tichý, 2002). All of the response models were fitted with untransformed data on the species abundances and environmental variables.

The type I HOF model is considered indifferent (Balkovič et al., 2012) because it does not have a specific shape, and thus does not provide valid information on the species optimum (Uğurlu and Oldeland, 2012). In order to fill gaps in the data, the model optima derived from the type I HOF model are replaced with raw optima. The raw optimum is the mean value of all of the environmental variable values recorded for a species (Wamelink et al., 2005; Uğurlu and Oldeland, 2012).

Seasonal variability in the physicochemical properties of the water and sediment was tested by applying an alternative, nonparametric ANOVA technique (the Friedman test) in STATISTICA 8.0 (StatSoft, 2007).

3. Results

3.1. Environmental preferences of macrophytes in regard to the physicochemical properties of the water

Species response curves were modeled using the HOF approach for 13 macrophyte species with respect to eight physicochemical properties of water (Fig. 1). Both the linear decreasing or increasing models and the symmetric unimodal model were the most common. They were fitted to 80 (76.92%) of the 104 species-environmental variable combinations. The type I HOF model occurred in 23.08% of all models while the type III and type V HOF models did not occur in this data set.

The values of the raw and model optima were significantly different for all species-environmental variable combinations that were derived using the type II HOF model (Table 1, Fig. 1). The raw optima values were higher than the model-derived optima for the linear decreasing model while their values were lower than the model-derived optima for the linear increasing model. The values of the raw optima were similar to the optima values derived using the type IV HOF model. Generally, the degree of similarity increases when the tolerance values decrease (Appendix A of Supplementary material).

The species *B. maritimus*, *P. arundinacea* and *S. lacustris* subsp. *tabernaemontani* showed responses to all of the environmental variables while *C. riparia*, *P. lucens*, *S. lacustris* and *T. angustifolia* showed the fewest responses (Fig. 1). It is characteristic that *C. riparia* and *E. palustris* were either unresponsive to the environmental gradients or they showed a monotone response which peaked at the start of the gradients.

The results of the HOF analysis indicate a high level of niche diversification of the species (Fig. 1). Deviation from this phenomenon was recorded for *S. erectum* and *T. latifolia.* These species had similar preferences in regard to all of the physicochemical characteristics of the water except the amount of Cl^- (Table 2). The probability of finding these species was highest in relatively deep, slightly acid waters with low EC values that were poor in $SO_4{}^{2-}$ and rich in $NH_4{}^+$ (Fig. 1, Tables 1 and 2). *Typha angustifolia* showed similar environmental requirements with respect to water depth, acidity, electroconductivity and the concentration of $SO_4{}^{2-}$ in the water.

The habitat requirements of *B. maritimus* were seen to be different compared to the other macrophyte species. *Bolboschoenus maritimus* showed a preference for saline and alkaline waters, rich in nutrients, especially with $PO_4{}^{3-}$, $SO_4{}^{2-}$ and Cl^-. The niche of *S. lacustris* subsp. *tabernaemontani* was seen to be in an intermediate position (between *B. maritimus* and the other species) with respect to EC, and the concentrations of $SO_4{}^{2-}$ and Cl^-.

The broadest niche widths (presented in Appendix A of Supplementary material as tolerance) were for *B. maritimus* ($NO_3{}^-$, $NH_4{}^+$, $SO_4{}^{2-}$), *L. minor* (pH, EC, $NO_3{}^-$), *P. australis* (EC, $PO_4{}^{3-}$) and *S. lacustris* subsp. *tabernaemontani* (EC, $NH_4{}^+$, Cl^-) while *P. arundinacea* and *S. palustris* possessed the narrowest niches (only the unimodal HOF model was taken into account).

3.2. Environmental preferences of macrophytes in regard to the physicochemical properties of the sediment

The five HOF models were successfully used to infer the response of the macrophyte species along the environmental gradients which correspond to the physicochemical properties of sediments (Fig. 2). The HOF analysis suggests that the response of the macrophytes along the environmental gradients analyzed is most approximated by models type II (34.82%) and type IV (28.57%) while model type III (5.36%) had the lowest frequency. The species *B. glaucus*, *B. maritimus*, *S. lacustris* subsp. *tabernaemontani* and *S. palustris* showed responses along all environmental gradients while *E. palustris* and *P. australis* showed the fewest responses.

The largest differences between the model and raw optima were found for the species-environmental gradient combinations that were derived with type II and III models (Table 3, Fig. 2). It was observed that the differences in values between the raw and model-derived optima depend on the types of HOF model (Fig. 2) and values of tolerance (for type IV and V HOF models) (Appendix B of Supplementary material).

A high level of similarity between *S. erectum*, *T. angustifolia* and *T. latifolia* in terms of their environmental preferences was recorded (Fig. 2, Tables 3 and 4, Appendix B of Supplementary material). These species not only show similar preferences with respect to some water properties, but also with respect to sediment properties. They prefer acidic sediment with low EC values and low levels of available potassium. *Phalaris arundinacea*, *Stachys palustris* and *Scirpus lacustris* have a similar response to alkalinity, EC and nutrient content of the sediment. The highest probability of finding *B. maritimus* and *S. lacustris* subsp. *tabernaemontani* was in habitats with alkaline sediments and high EC values. The niches of *B. maritimus* ($CO_3{}^{2-}$), *P. australis* ($HCO_3{}^-$, K_2O and P_2O_5) and *S. lacustris* (Cl^-) were seen to overlap almost all of the ecological niches of the other species.

Detailed information about the environmental preferences of the macrophytes analyzed, with respect to the physicochemical properties of the water and sediment, is given in Figs. 1 and 2, Tables 1 and 2, and Appendix A and B of Supplementary material.

3.3. Seasonal variability of the physicochemical environmental properties

The existence of seasonal variability for almost all of the physicochemical properties of the water analyzed was established according to the results of the Friedman test. The exceptions from this are the pH and amount of $NO_3{}^-$ (Fig. 1). The Friedman test also confirmed seasonal variability for the following sediment properties: water content, pH values, amount of bicarbonates, carbonates, available phosphorous and chloride (Fig. 2).

4. Discussion

The environmental preferences established using the HOF models and data published in relevant literature sources could be used as a basis for assessing the bioindicator potential of the macrophytes studied here. However, a comparison between these results and those already published would be difficult for the following reasons: 1) literature sources rarely contain information about the ecological optimum values and ecological tolerances of particular

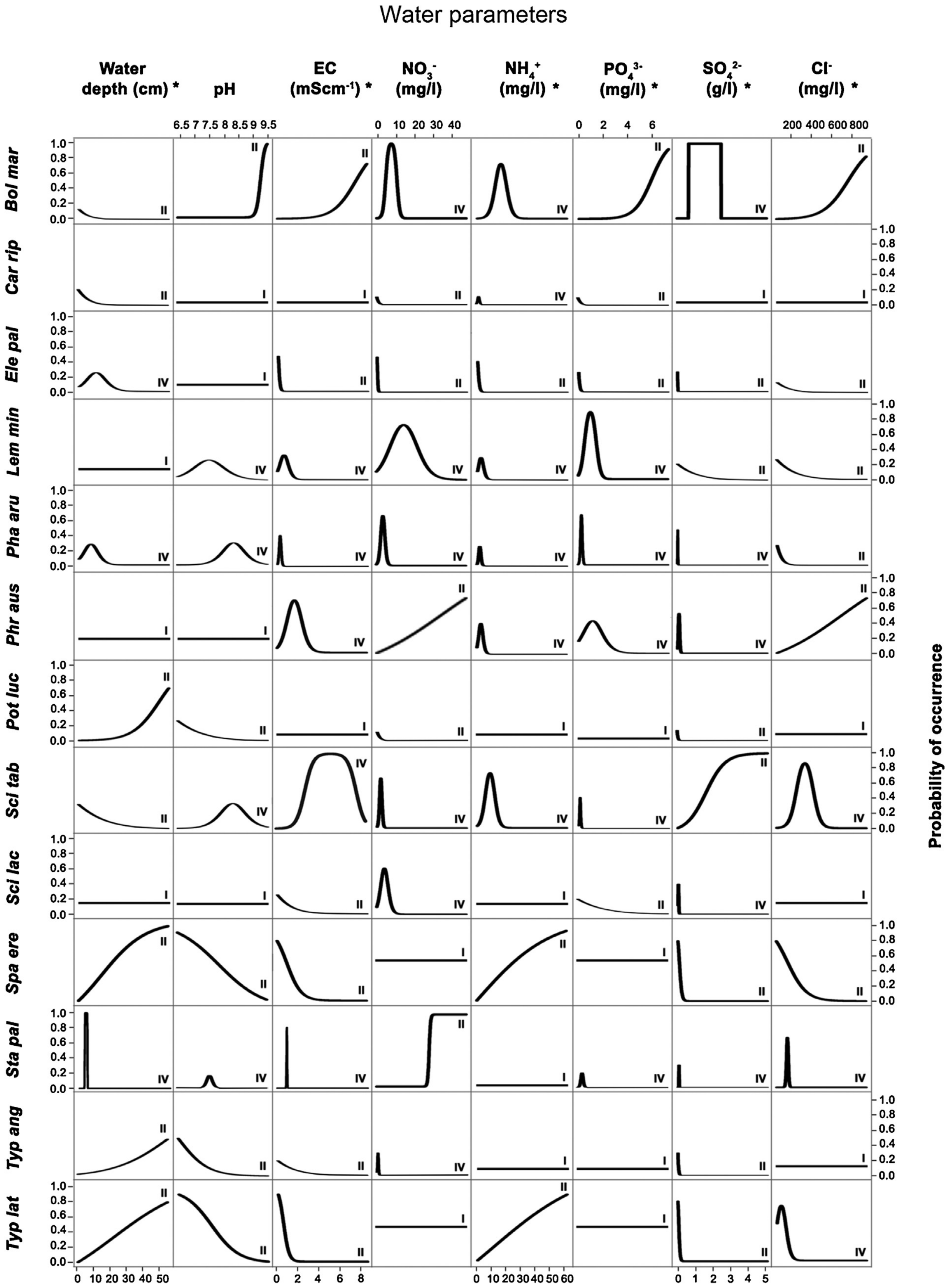

Fig. 1. The species response curves for 13 macrophyte species (Bol mar – *Bolboschoenus maritimus*, Car rip – *Carex riparia*, Ele pal – *Eleocharis palustris*, Lem min – *Lemna minor*, Pha aru – *Phalaris arundinacea*, Phr aus – *Phragmites australis*, Pot luc – *Potamogeton lucens*, Sci tab – *Scirpus lacustris* subsp. *tabernaemontani*, Sci lac – *Scirpus lacustris*, Spa ere – *Sparganium erectum*, Sta pal – *Stachys palustris*, Typ ang – *Typha angustifolia*, Typ lat – *Typha latifolia*) along 8 environmental variables (physicochemical properties of water) were obtained using logistic regression and five hierarchical Huisman-Olff-Fresco (HOF) models (I – flat with no response, II – monotone decreasing or increasing, III – monotone increasing to a plateau, IV – symmetric unimodal and V – asymmetric unimodal). Detailed data (minimum values, maximum values and tolerance) on species response along the environmental variables analyzed are given in Appendix A of Supplementary material. The values on the x-axis are shown alternately on the bottom

Table 1
The raw and HOF modeled optimum of the macrophyte responses along environmental gradients (physicochemical properties of water). The optimal values obtained using HOF model type I are not applicable (NA). The modeled optimal values were untransformed for all parameters.

Species	Water depth (cm)		pH		EC ($\mu S\,cm^{-1}$)		NO_3^- (mg/l)		NH_4^+ (mg/l)		PO_4^{3-} (mg/l)		SO_4^{2-} (mg/l)		Cl^- (mg/l)	
	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model
Bolboschoenus maritimus	6.00	2.00	9.29	9.44	6430.00	8430.00	5.18	7.77	10.47	16.59	6.04	7.14	1357.07	1545.16	617.70	926.55
Carex riparia	6.25	2.00	7.42	NA	505.80	NA	0.51	0.03	1.79	2.02	0.10	0.01	56.36	NA	133.13	NA
Eleocharis palustris	12.13	12.12	7.51	NA	240.28	182.20	0.29	0.03	1.26	0.43	0.06	0.01	6.95	0.30	124.86	74.55
Lemna minor	22.50	NA	7.50	7.52	685.86	792.54	5.53	14.47	2.29	2.94	0.57	0.98	63.66	0.30	131.53	74.55
Phalaris arundinacea	9.29	9.05	8.05	8.32	441.57	429.63	2.18	3.52	2.07	2.39	0.25	0.29	30.59	34.83	104.98	74.55
Phragmites australis	22.06	NA	7.73	NA	1068.38	1782.27	5.68	47.21	2.41	3.18	0.35	1.13	59.76	84.88	227.35	926.55
Potamogeton lucens	39.17	55.00	7.32	6.46	796.83	NA	0.60	0.03	3.83	NA	0.35	NA	18.62	0.30	191.76	NA
Scirpus lacustris subsp. *tabernaemontani*	10.91	2.00	8.03	8.28	4453.09	5188.61	1.39	1.97	5.38	9.25	0.10	0.13	1961.48	4975.70	269.15	342.93
Scirpus lacustris	16.15	NA	7.75	NA	557.17	182.20	1.40	4.09	2.69	NA	0.25	0.01	22.74	30.15	144.21	NA
Sparganium erectum	24.42	55.00	7.49	6.46	561.78	182.20	2.68	NA	7.05	61.66	0.72	NA	34.20	0.30	132.81	74.55
Stachys palustris	5.00	5.82	7.53	7.53	1092.50	1056.47	41.58	44.50	2.50	NA	0.34	0.38	58.00	64.98	170.40	176.79
Typha angustifolia	29.40	55.00	7.25	6.46	608.22	182.20	0.55	0.60	2.87	NA	0.23	NA	15.38	0.30	167.24	NA
Typha latifolia	23.43	55.00	7.38	6.46	404.01	182.20	2.17	NA	7.23	61.66	0.63	NA	24.50	0.30	118.28	114.59

Table 2
Summary review of the environmental preferences of 13 macrophyte species with respect to eight physicochemical properties of the water. The species were classified in categories based on their values for the HOF modeled optimum for each environmental variable included in the analysis. The ranges of the environmental variable values for each category were established using percentiles and are presented in Appendix C of Supplementary material. The species that showed no response along the environmental gradients are included in the special category — Ïndifferent.

	Water parameters							
	Water depth	pH	EC	NO_3^-	NH_4^+	PO_4^{3-}	SO_4^{2-}	Cl^-
Bolboschoenus maritimus	shallow	alkaline	high	rich	rich	rich	rich	rich
Carex riparia	shallow	indifferent	indifferent	poor	moderate	poor	indifferent	indifferent
Eleocharis palustris	moderate	indifferent	low	poor	poor	poor	poor	poor
Lemna minor	indifferent	slightly alkaline	moderate	rich	moderate	rich	poor	poor
Phalaris arundinacea	shallow	alkaline	moderate	rich	moderate	moderate	moderate	poor
Phragmites australis	indifferent	indifferent	high	rich	moderate	rich	moderate	rich
Potamogeton lucens	deep	acid	indifferent	poor	indifferent	indifferent	poor	indifferent
Scirpus lacustris subsp. *tabernaemontani*	shallow	alkaline	high	rich	rich	moderate	rich	rich
Scirpus lacustris	indifferent	indifferent	low	rich	indifferent	poor	moderate	indifferent
Sparganium erectum	deep	acid	low	indifferent	rich	indifferent	poor	poor
Stachys palustris	shallow	slightly alkaline	moderate	rich	indifferent	moderate	moderate	moderate
Typha angustifolia	deep	acid	low	moderate	indifferent	indifferent	poor	indifferent
Typha latifolia	deep	acid	low	indifferent	rich	indifferent	poor	moderate

Table 3
The raw and HOF modeled optimum of the macrophyte responses along environmental gradients (physicochemical properties of sediment). The optimal values established using the type I HOF model are not applicable (NA). The modeled optimal values were untransformed for all parameters.

Species	Water content (%)		pH in water		HCO_3^- (mg/l)		CO_3^{2-} (mg/l)		EC ($\mu S\,cm^{-1}$)		mg K_2O/100 g sediment		mg P_2O_5/100 g sediment		mg Cl^-/100 g sediment	
	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model	Raw	Model
Bolboschoenus glaucus	25.50	8.33	7.93	8.03	131.35	134.59	0.00	0.00	493.40	655.02	28.32	30.58	15.98	13.91	18.24	90.04
Bolboschoenus maritimus	28.03	8.33	8.83	9.28	253.08	318.98	19.86	15.85	945.76	2393.38	55.19	62.29	18.96	23.76	12.47	4.62
Carex riparia	33.60	8.33	7.33	5.08	101.87	67.76	0.00	0.00	408.65	54.10	36.88	NA	16.41	0.06	13.98	20.25
Eleocharis palustris	27.71	8.33	7.40	5.08	131.43	NA	3.00	14.82	365.36	54.10	39.01	NA	14.19	0.06	14.85	NA
Phalaris arundinacea	32.01	23.40	7.68	7.72	108.96	112.85	0.00	0.00	415.14	471.06	42.76	NA	22.16	17.70	15.01	21.45
Phragmites australis	40.46	NA	7.80	NA	150.27	265.03	3.82	9.01	553.71	NA	48.70	70.22	23.22	39.86	14.62	NA
Potamogeton lucens	55.11	68.52	6.98	5.08	107.77	NA	0.00	0.00	250.50	222.72	15.38	14.62	8.02	7.55	12.89	NA
Scirpus lacustris subsp. *tabernaemontani*	51.65	59.12	8.50	8.24	230.28	817.40	11.38	114.00	1272.62	3120.00	38.40	31.89	14.27	13.91	12.70	13.33
Scirpus lacustris	37.58	NA	7.58	7.44	107.65	107.21	0.00	0.00	398.79	452.67	42.32	NA	20.16	24.06	15.81	34.52
Sparganium erectum	46.37	83.41	6.87	5.08	86.92	12.20	0.19	0.00	265.40	54.10	31.29	6.80	18.21	NA	17.02	90.04
Stachys palustris	28.50	8.33	7.74	7.87	112.85	119.29	0.00	0.00	410.23	489.46	52.24	115.40	25.56	21.81	15.76	26.66
Typha angustifolia	43.28	NA	6.81	5.08	93.03	12.20	0.00	0.00	249.94	54.10	29.30	6.80	10.75	0.06	15.41	NA
Typha latifolia	40.99	33.42	7.01	5.08	88.77	12.20	0.00	0.00	430.48	54.10	27.67	6.80	19.38	NA	14.78	NA
Xanthium strumarium subsp. *italicum*	35.64	NA	7.87	7.97	114.88	NA	0.00	0.00	502.50	563.04	26.16	6.80	22.92	22.22	20.59	27.85

species with respect to local or regional estimated environmental variables (Štechová et al., 2008; Uğurlu and Oldeland, 2012), 2) the values of measurement for environmental variables are expressed as their ranges or mean/median values (Onaindia et al., 2005), 3) the assessment of bioindicator potential is often given for the communities (Kłosowski and Jabłońska, 2009; Lukács et al., 2009) and

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and top of figure for each of eight environmental variables. The asterisks show that the seasonal variability of particular physicochemical water properties is statistically significant ($p < 0.05$) according to the Friedman test.

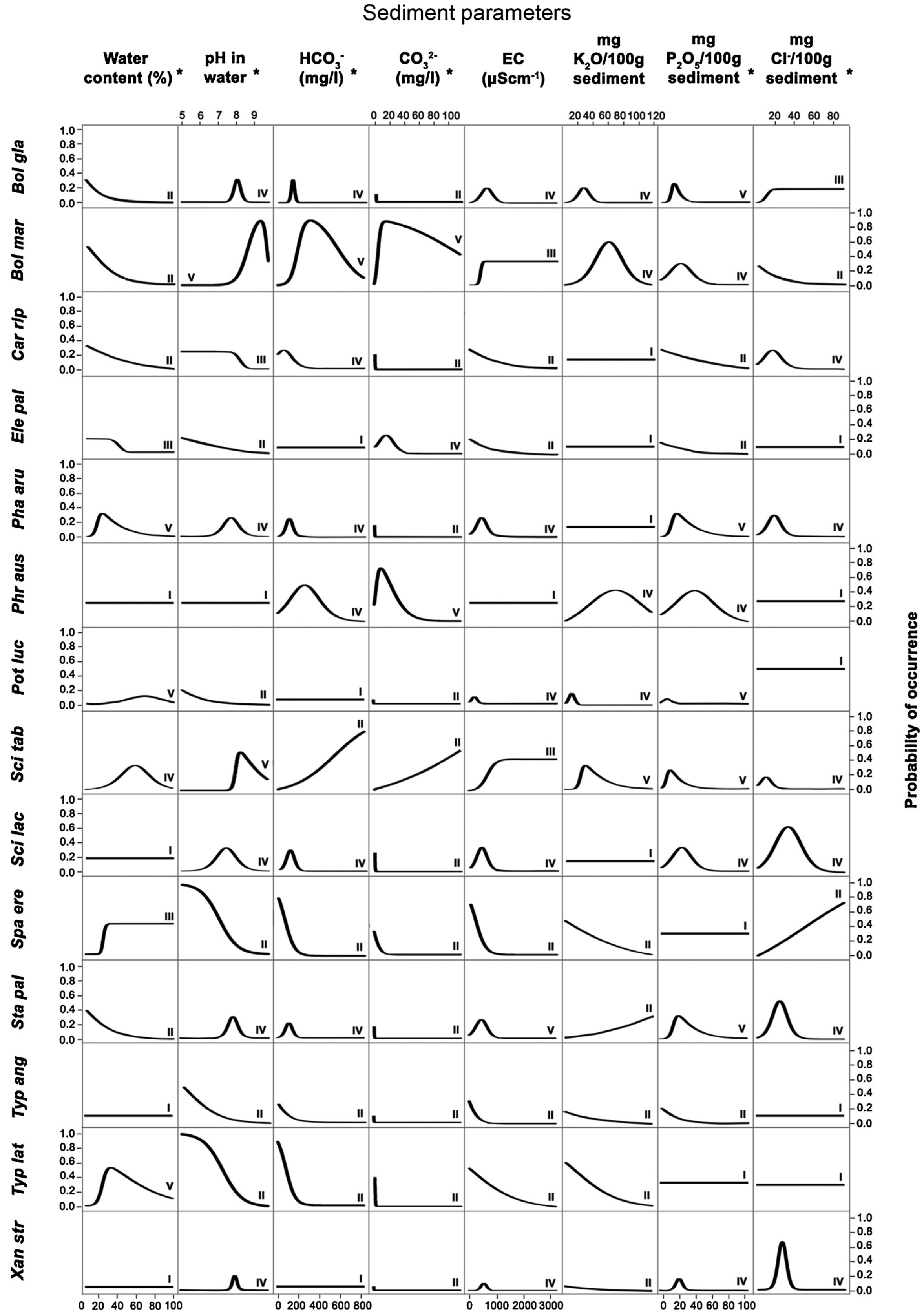

Fig. 2. The species response curves for 8 physicochemical properties of sediments obtained using logistic regression and five hierarchical HOF models (I – flat with no response, II – monotone decreasing or increasing, III – monotone increasing to a plateau, IV – symmetric unimodal and V – asymmetric unimodal) for 14 macrophyte species (Bol gla – *Bolboschoenus glaucus*, Bol mar – *Bolboschoenus maritimus*, Car rip – *Carex riparia*, Ele pal – *Eleocharis palustris*, Pha aru – *Phalaris arundinacea*, Phr aus – *Phragmites australis*, Pot luc – *Potamogeton lucens*, Sci tab – *Scirpus lacustris* subsp. *tabernaemontani*, Sci lac – *Scirpus lacustris*, Spa ere – *Sparganium erectum*, Sta pal – *Stachys palustris*, Typ ang – *Typha angustifolia*, Typ lat – *Typha latifolia*, Xan str – *Xanthium strumarium* subsp. *italicum*). See Appendix B of Supplementary material for detailed data (minimum values, maximum values and tolerance) on species response along the environmental variables studied (physicochemical properties of the sediment). The values

Table 4
Overview of the environmental preferences of 14 macrophytes with respect to the physicochemical characteristics of the sediments. The macrophyte species were classified in categories based on their values for the HOF modeled optimum for each of 8 environmental variables. The range of environmental variable values for each category was established using percentiles and they are presented in Appendix D of Supplementary material. The species that showed no response along the environmental gradients are classified in special category – "Indifferent".

	Sediment parameters							
	Water content	pH in water	HCO_3^-	CO_3^{2-}	EC	K_2O	P_2O_5	Cl^-
Bolboschoenus glaucus	low	slightly alkaline	moderate	absent	moderate	moderate	moderate	rich
Bolboschoenus maritimus	low	alkaline	rich	present	high	rich	moderate	poor
Carex riparia	low	acid	poor	absent	low	indifferent	poor	rich
Eleocharis palustris	low	acid	indifferent	present	low	indifferent	poor	indifferent
Phalaris arundinacea	low	slightly alkaline	moderate	absent	moderate	indifferent	moderate	rich
Phragmites australis	indifferent	indifferent	rich	present	indifferent	rich	rich	indifferent
Potamogeton lucens	high	acid	indifferent	absent	low	poor	moderate	indifferent
Scirpus lacustris subsp. *tabernaemontani*	high	alkaline	rich	present	high	moderate	moderate	moderate
Scirpus lacustris	indifferent	slightly alkaline	moderate	absent	moderate	indifferent	moderate	rich
Sparganium erectum	high	acid	poor	absent	low	poor	indifferent	rich
Stachys palustris	low	slightly alkaline	moderate	absent	moderate	rich	moderate	rich
Typha angustifolia	indifferent	acid	poor	absent	low	poor	poor	indifferent
Typha latifolia	moderate	acid	poor	absent	low	poor	indifferent	indifferent
Xanthium strumarium subsp. *italicum*	indifferent	slightly alkaline	indifferent	absent	moderate	poor	moderate	rich

not for their indicator species. Although the ecological amplitudes of communities are not the same as the ecological amplitudes of their indicator species (Pełechaty, 1999), such data are significant. They provide information about the regional differences between habitats in terms of their physicochemical properties and possible stochastic occurrences of the species in suboptimal conditions (Štechová et al., 2008).

The high frequency of *B. maritimus* and *S. lacustris* subsp. *tabernaemontani* found in habitats with high EC and pH values for both sediments and water suggests that these species could be potentially significant ecological indicators of saline (Piernik, 2003) and alkaline habitats. The peak of their abundance is recorded in a range between 2000 and 4000 $\mu S\,cm^{-1}$ for the EC of the sediment (Fig. 2, Table 3). According to Jackson's scale (Jackson, 1958) of salinity such a range of EC corresponds to slightly saline sediments. The ecological niches of *B. maritimus* and *S. lacustris* subsp. *tabernaemontani* only partially overlap along the pH and EC gradients (particularly in terms of the pH and EC of water). *Bolboschoenus maritimus* requires saltier (Piernik, 2012) and more alkaline habitats for optimal development as opposed to *S. lacustris* subsp. *tabernaemontani*.

The results of a study conducted by Hroudová et al. (2014) indicate that salinity and nutrient gradients are decisive factors in the ecological diversification of the *Boloboschoenus* species (Table 4). Their results suggest that *B. maritimus* is adapted to saline habitats and is sensitive to increased amounts of nutrients, which is similar to the results obtained in our study (Fig. 2, Tables 3 and 4). The environmental tolerances of *B. maritimus* and *B. glaucus* are different, since the occurrence of *B. glaucus* increases with decreased salinity and alkalinity in its habitat. Hence, the high frequency of *B. glaucus* could be an indicator of non-saline, slightly alkaline and relatively nutrient poor habitats.

It has already been mentioned that *T. angustifolia, S. erectum* and *T. latifolia* have similar environmental preferences with respect to some physicochemical properties of water (water depth, pH, EC and SO_4^{2-}) and sediment (pH, HCO_3^-, CO_3^{2-}, EC and K_2O). They can be considered as useful bioindicators of habitats characterized by the following properties: relatively deep water, slightly acid water and sediments with low EC values, poor in SO_4^{2-} and K_2O (Tables 2 and 4). The assessment of their requirements with respect to nutrient content (NO_3^-, NH_4^+, PO_4^{3-} and P_2O_5) can only be made on the basis of data from relevant literature and raw optima values (Tables 1 and 3) because these species are mostly unresponsive (Figs. 1 and 2) to the mentioned environmental variables. *Typha angustifolia* is most likely to be found in nutrient poor habitats (low in NO_3^- and P_2O_5) (Figs. 1 and 2, Tables 1 and 2), which is partly confirmed by the results for the *T. angustifolia* community (Lukács et al., 2009). Other studies state that *S. erectum* can be found in areas with higher amount of nitrates (Kočić et al., 2008), and that there is a rapid increase in the total shoot density of *T. latifolia* after adding nutrients (especially nitrogen) (Grace, 1988). These characteristics show that *S. erectum* and *T. latifolia* are better potentially significant bioindicators for nutrient enrichment than *T. angustifolia*. Numerous studies (Lukács et al., 2011; Steffen et al., 2014) relate these species with slightly alkaline habitats while the results of our statistical procedures show that they have acidophilous behavior.

Phragmites australis is often described as a species with low indicative value (Piernik, 2012; Steffen et al., 2014). It has broad environmental tolerance of many physicochemical properties, including EC and concentration of PO_4^{3-} in the water (Fig. 1), and the amount of HCO_3^-, available potassium and phosphorus in sediments (Fig. 2). The wide ecological amplitudes of *P. australis* make it capable of growing in a wide range of habitats, including salt marshes, brackish and fresh swamps, riversides, lakesides and sand dunes (Nada et al., 2015). Consequently, *P. australis* may have only a regional bioindicative value (Pełechaty, 1999).

Habitats with a low or moderate concentration of NH_4^+, PO_4^{3-} and SO_4^{2-} in the water are suitable for the development of *C. riparia*, *E. palustris*, *P. arundinacea*, *S. lacustris* and *S. palustris* (Fig. 1, Tables 1 and 2). Their abundances increase when the concentration of nutrients in the water decreases (Lukács et al., 2009), so they can be considered as potential bioindicators of nutrient poor habitats. *Stachys palustris* partially deviates from the aforementioned trend because it shows preferences towards a high amount of particular nutrients (NO_3^- and K_2O).

The authors consider that the data obtained on the habitat affinities of some species with respect to some environmental variables are insufficient for making any conclusions about their bioindicator potential. These species are either indifferent to environmental variables or their response curves show only one segment of the environmental gradients which are correlated with the low probability of their occurrence. It therefore seems necessary to carry out further investigations because a high proportion of the linear HOF models indicates truncated environmental gradients (Uğurlu and Oldeland, 2012).

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on the x-axis are presented alternately on the bottom and top of figure for each environmental variable included in the analysis. Statistically significant seasonal variability of the physicochemical properties of the sediment, established using the non-parametric ANOVA technique (the Friedman test), is marked with asterisks.

Defining the environmental preferences of the species studied is based on the results of repeated measurements during one growing season. This can be significant because the habitat requirements were mainly assessed for perennial macrophytes. Perennial macrophytes are able to be metabolically active throughout the entire range of conditions encountered during a growing season by means of changes in their metabolic pathways and shoot morphologies as the season progresses (Keddy and Reznicek, 1986). Their life cycles are not usually interrupted even with ecophase alternation, in contrast to free floating (*L. minor*) and submerged (*P. lucens*) macrophytes that are sensitive to the withdrawal of surface water from their habitats (Hejný et al., 1998). The statistical analyses demonstrate that the physicochemical properties of water (Rothwell et al., 2010) and sediment (Kwon et al., 2007) show significant seasonal variability (Figs. 1 and 2). Accordingly, it can be concluded that only simultaneous monitoring of species and habitats several times during their growing seasons can provide precise information about the environmental preferences of a perennial species and increase the usefulness and validity of the data obtained. In this way the importance of their use as bioindicators will be increased, especially if they have narrow environmental tolerance and prefer discrete habitat types.

This study represents the first step in creating a national or even regional database on the univariate response of species along environmental gradients that is based on the results of continuously monitoring wetlands. Collecting information on the univariate response of species has significant scientific value. These data can be used in assessing the bioindicator potential of species (Peppler-Lisbach, 2008), and for adjusting the existing indicator values of plant species with respect to measured environmental variables (Wamelink et al., 2005; Balkovič et al., 2012). Even the creators (Kojić et al., 1997) of the indicator systems indicate the significance of the data obtained in a gradient analysis because they can be used to adjust the indicator values due to a possible inadequacy or omission in their definition. Further, data on the univariate response of species are an appropriate basis for designing new local or regional indicator systems (Godefroid and Dana, 2007) and extending existing systems through defining indicator values for species in regard to environmental variables which have not yet been considered.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2016.06.003.

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