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GS and ZK designed the study; GS collected vegetation and soil data and together with ZBD carried out statistical analyses; DP and LZ conducted chemical analysis of soil data and interpreted soil–vegetation correlations; GS wrote the manuscript; all authors contributed to the final version by revising the manuscript

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ZK is an associate editor of the *Acta Societatis Botanicorum Poloniae*; other authors: no competing interests have been declared

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ORIGINAL RESEARCH PAPER

A performance comparison of sampling methods in the assessment of species composition patterns and environment–vegetation relationships in species-rich grasslands

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Abstract

The influence that different sampling methods have on the results and the interpretation of vegetation analysis has been much debated, but little is yet known about how the spatial arrangement of samples affect patterns of species composition and environment–vegetation relationships within the same vegetation type. We compared three data sets of the same sample size obtained by three standard sampling methods: preferential, random, and systematic. These different sampling methods were applied to a study area comprising of 36 ha of intermittently wet *Molinia* meadows. We compared the performance of the three methods under two management categories: managed (extensively mown) and unmanaged (abandoned for 10 years). A total of 285 vegetation-plots were sampled, with 95 plots recorded per sampling method. In preferential sampling, we sampled only patches of vegetation with an abundance of indicator species of the habitat type, while random and systematic plots were positioned independently from the researcher by using GIS. The effect of each sampling method on the patterns of species composition and species–environment relationships was explored by redundancy analysis and the significance of effects was tested by the randomization test. Preferential sampling revealed different patterns of species composition than random and systematic sampling methods. Random and systematic sampling methods have resulted in broader vegetation variability than with preferential sampling method. Preferential sampling revealed different relationship between soil parameters and species composition in contrast to random and systematic sampling methods. Although we have not found significant differences in vegetation–environment relationships between random and systematic sampling methods, random sampling revealed a more robust correlation of species data to soil factors than preferential and systematic sampling methods. Intentional restriction of vegetation variation sampled preferentially may be detrimental to statistical inference in studies of species composition patterns and vegetation–environment relationships.

Keywords

preferential; random; systematic; soil properties; *Molinion* meadows

Introduction

One of the most important steps in conducting ecological research is to choose an appropriate sampling method that will ensure the collection of reliable data for statistical processing and ecological inference. The use of different sampling strategies and their effects on the results and their ecological interpretation has been under intensive debate since the early 1950s [1]. It is a well-established fact that sampling strategy is one of the most important factors influencing the observed patterns and processes in ecological studies [2,3]. Sampling methods can be grouped into one of two categories: non-probabilistic (or preferential sampling) and probabilistic sampling. Non-probabilistic sampling involves the choosing of sampling sites based on the subjective decision of the researcher, while the selection of sampling sites in probabilistic sampling is completely independent from the researcher.

Preferential sampling is an indispensable part of Central European phytosociology [4], which since its very beginning has aimed to describe and classify plant communities [5,6]. With this approach, only homogenous and well-developed patches of vegetation are sampled, while those considered “atypical” are simply omitted by the researcher [7,8]. Accordingly, discrete vegetation types are recognized and the researcher aims to explore the environmental differences among these types [9–12]. In preferential sampling, the researcher’s perception of the vegetation and the scale of the study can influence the way in which the environment–vegetation relationships are analyzed [13]. Preferential selection of sampling sites is often applied for ecologically-sound goals [14–18]. Undoubtedly, data collected preferentially are a very valuable source of ecological information [19], however, some results from analyses based on such data should be interpreted with caution because preferentially sampled data may produce biased estimates on some vegetation parameters such as species richness and the representation of some predefined plant groups [20–23].

Lájer [24] has questioned the usability of data that have been collected preferentially in ecological studies and argued that random sampling coupled with the explicit definition of the statistical population is the only appropriate method for obtaining valid data, at least from a strict statistical point of view. Some authors, however, suggested that such preferential data can be applied for testing ecological hypotheses if appropriate statistical tools are used and the results are carefully interpreted considering the preferences of data collectors [25–27]. Generally, ecologists follow the principles of the probability theory and apply random sampling to study interactions between vegetation and environment [28–30]. Systematic sampling is also often applied in ecological investigations, particularly in analyses of spatial vegetation changes along environmental gradients [31,32], although this method does not guarantee the total independence of observations [33].

As of yet, a little attention has been paid to the consequences of different sampling methods on the assessment of species composition patterns within a single vegetation type [25]. The consequences of using distinct sampling methods to assess the environment–vegetation relationships has been poorly studied. The research studies that have addressed this issue were concerned with environmental gradients in a heterogeneous landscape [21,34]. The majority of studies that compared different sampling methods focused on forest vegetation on a broad scale with a high degree of variability [8,22,35–37] or dealt with a variety of habitats occurring across the landscape [21,23,34], while in other studies, sampling methods have been investigated using simulated data [38–40]. The present study is of a different nature in that it compares three sampling methods applied to a well-defined vegetation type in a small spatial scale. Seemingly homogeneous grassland areas are often highly differentiated in a small spatial extent [41], thus it is worth comparing the performance of different sampling methods within a single but internally heterogeneous vegetation type.

Despite the extensive knowledge regarding sampling in ecology, there has been no previous study that would investigate species composition patterns and interactions between species and environmental factors under different sampling methods in species-rich meadows. We compared three different sampling methods that are commonly used in ecological studies: preferential (P), random (R), and systematic (S). For the three approaches, we sampled within a well-defined habitat with high internal heterogeneity, that is intermittently wet meadows (the alliance *Molinion* in phytosociological terms).

Another reason why this study stands out from other research is that we compared different sampling methods within two land use categories of the same habitat: (i) regularly mown and (ii) abandoned for 10 years. We used large data sets of the same sample size and compared them using univariate and multivariate statistics to examine the effects that the three sampling methods might have on our results. Our main research question posed is to what extent do the relationships between patterns of species composition and environment–vegetation differ between the three sampling methods applied to a single vegetation type?

Material and methods

Study area

The study was carried out in a Natura 2000 site located in the southwestern Poland (N 51°15'24.8", E 16°33'24.9") (Fig. 1). The area is situated in lowlands with an altitude ranging from 105 to 125 m above sea level. The average annual precipitation is 600 mm, with a mean annual temperature of 8.5°C [42]. Predominant types of soils are acidic brown soils and alluvial soils. The study area is covered with semi-natural grassland plant communities belonging to the alliance *Molinion caeruleae* Koch 1926 (in phytosociological terms). The entire meadow complex covers about 36 ha. Two land use categories were differentiated: managed (covering 60% of study area) and unmanaged sites (covering 40% of study area). Managed meadows are regularly mown in the late summer, while unmanaged sites had been abandoned about 10 years prior to the study.

Vegetation sampling

Vegetation data were collected from 5 × 5 m plots that were distributed according to each sampling method, P (preferential), R (random), and S (systematic). Vegetation data were sampled from June to late July to eliminate the effect of the phenological differences in the vegetation. The vegetation data were collected solely by the first author

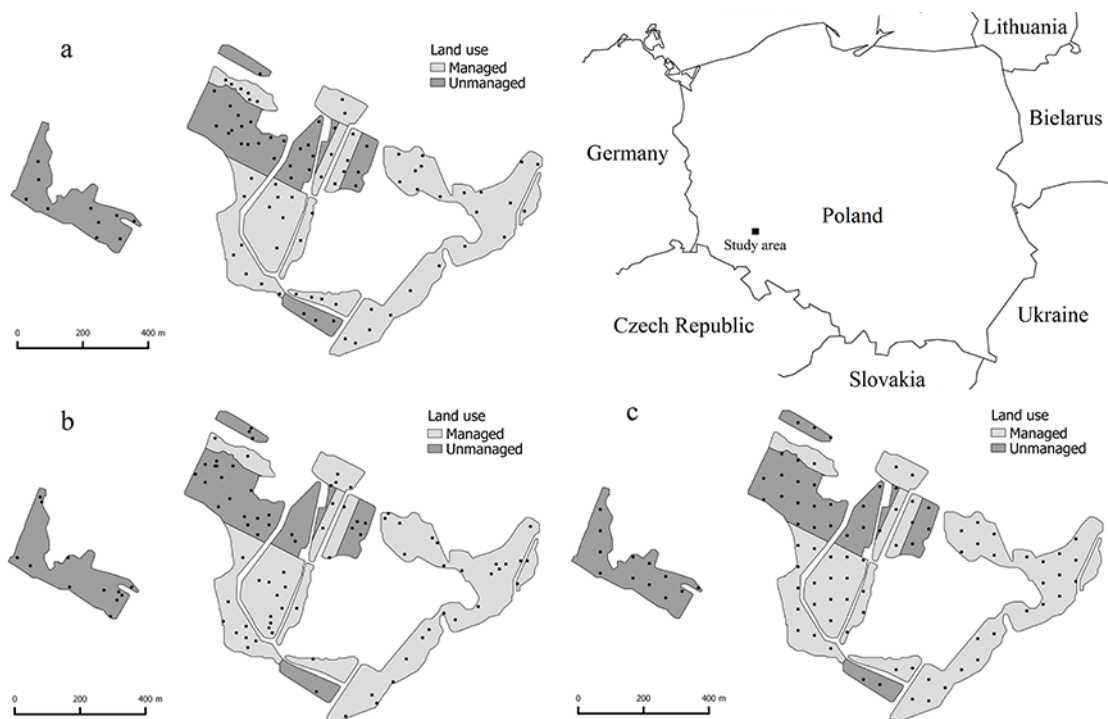


Fig. 1 Location of the study area and spatial distribution of sampling plots in (a) P sampling, (b) R sampling, and (c) S sampling.

of this study to eliminate inconsistency in plant cover estimation between different observers [43,44]. Species cover in all plots was visually assessed using Braun-Blanquet 7-point scale [45].

The selection of stands under the P sampling method aimed at obtaining sites that represent the recognized plant communities of the alliance *Molinion*. This approach required a selection of only so-called “typical” and homogeneous stands with an abundance of indicator species of the vegetation type [46,47]. R and S plots were positioned prior to field sampling using a grid of squares generated in ArcGIS (version 9.3.1, ESRI). Using ArcGIS we created a grid of cells 5 × 5 m for the entire study area and each cell was given a unique number. Each cell was a potential sampling position. R plots were selected by simple randomization among these numbered cells. The S plots were positioned at fixed distances (65 m in length) along a series of transects, orientated in north–south direction. Each transect was separated by a distance of 50 m in an east–west direction. The position of the first plot in a transect was selected randomly. The entire data set consisted of 285 plots and was divided as evenly as possible between the three sampling methods and two land-use management types. We sampled a total of 165 plots from managed sites (P – 55, R – 55, S – 55) and 120 plots from unmanaged sites (P – 40, R – 40, S – 40).

Soil sampling and chemical analyses

Topsoil samples at 10-cm depth were collected from each of the 285 plots. The non-decomposed litter was removed from the top section of soil cores. Five 50-cm³ soil cores were sampled from each plot and merged into a bulk sample for chemical analysis. Prior to the chemical analysis, soil samples were air-dried and sifted through a 2-mm-mesh sieve. Physicochemical analyses were performed in accordance with the methods proposed by Allen [48] and Radojević and Bashkin [49]. Loss-on-ignition as a rough measure of the total organic matter was determined by igniting 2 g of soil in a muffle furnace at 600°C for 6 hours and then cooled overnight. Soil pH was determined potentiometrically in distilled water. Total nitrogen content was determined by the Kjeldahl method. Soluble phosphorus was determined colorimetrically after extracting the soil with 0.5 M sodium bicarbonate solution (pH 8.5). Exchangeable forms of potassium, calcium, and magnesium were extracted with 1 M ammonium acetate (pH 7.0) and determined using the Varian SpectrAA 200 spectrometer operating in the emission mode for potassium and calcium and atomic absorption mode for magnesium.

Statistics

A formalized plot-based classification using the expert system for *Molinion* meadows [50] was applied to assess whether all plots represented the same vegetation type (the alliance *Molinion*). Application of an expert system revealed that 86% of all vegetation-plots matched the formal definition. Only a small fraction of the whole data set did not comply to this formal definition, but it does not mean that these unclassified plots do not represent plant communities of *Molinia* meadows. It has been well recognized that not every vegetation plot can be classified by the formalized classification approach [51,52]. Indicator species of the alliance *Molinion* [50] were well represented in both groups that matched and that did not match the formal definition of the alliance *Molinion* (Fig. S1). Based on the frequency distribution of indicator species it is apparent that transitional plots that have not been assigned to the alliance by formal definition can still be considered the target vegetation type.

The pooled number of species was calculated for each data set obtained by different sampling methods. These data sets were compared in terms of species uniqueness in a pairwise comparison: P versus R, P versus S, R versus S. Then, we calculated number of species for each plot within the three data sets by sampling method. The significance of differences between the three data sets was analyzed using the Kruskal–Wallis test and subsequent multiple comparisons of mean ranks for all groups. For a comparison of species abundance distribution among different sampling methods we delimited five frequency classes: (i) 1–10%, (ii) 11–20%, (iii) 21–30%, (iv) 31–40%, (v) 41–50%.

Species with the frequency of less than 10% were considered rare species, whereas the species with highest frequency values (41–50%) were common species. The values for soil variables deviated from the normal distribution in most cases, therefore non-parametric methods were used. For a comparison of medians, the Kruskal–Wallis test was applied. Dispersion around the mean was explored by a permutation test of mean Euclidean distances [53].

For further analyses, scores of the Braun–Blanquet 7-point scale were transformed to percentage cover values by using the midpoint of each category. To illustrate a distribution of plots from each sampling method in ordination space we used principal coordinate analysis (PCA). Additionally, we used Whittaker's beta diversity index, i.e., the ratio of the total number of species over all sites in the data set to the average number of species per site [54], as the measure of internal heterogeneity of P, R, and S data sets. The effect of the sampling design and soil parameters on species composition patterns was explored by redundancy analysis (RDA) after Hellinger transformation of cover values [55]. Only species with the frequency higher than 5% in the whole data set were included in the ordination-based analyses because rare species may have distorted the results [56]. In RDA model, the vegetation data were related to the type of sampling (a factor with three categories: P, R, S) and soil variables (pH, organic matter, nitrogen, phosphorus, potassium, calcium, and magnesium). Species distribution in the ordination space was visualized as supplementary data on a biplot diagram. Then, separate models were built to show whether sampling methods affected patterns of species composition and vegetation–environment relationships. The strength of the effect of sampling methods and soil variables was quantified by the percentage variance explained, and the significance of the effects was assessed by a randomization test. The results of randomization tests are valid even with observations that are not a random sample [57]. In the analysis of vegetation–environment relationships, the influence that each soil parameter has on species composition patterns was assessed (main effect). Sampling method and its interaction with the studied variable was included in the analysis as an explanatory variable, and then data from different sampling methods were analyzed separately in a pairwise comparison. For showing which species were responsible for significant interaction between a sampling method and soil properties, species were ranked in decreasing order of variance explained by the interaction after partialling out the main effect in partial RDA. Then, species were omitted step-by-step from the model until the interaction became non-significant. Parallel analyses comparing sampling methods were carried out for managed sites and unmanaged sites.

All statistical computations were performed in the R software environment (RStudio, version 0.98.1091, R Foundation for Statistical Computing, Vienna, AT) using the vegan package [58] and the coin package [59]. Ordination diagrams of PCA and RDA were generated in Canoco 5 [60]. The nomenclature of taxa follows Mirek et al. [61].

Results

A total of 272 species was recorded by all three sampling methods. The pooled number of species captured by the P method was smaller (203) than in R (231) and S (212) data sets. The lists of unique species for each sampling method in three sets comparisons: (i) P versus R, (ii) P versus S, (iii) R versus S are presented in Tab. S1.

The sampling method affected the number of species per plot within the two land use categories (Fig. 2a,b). In managed sites, number of species was estimated to be higher in P than in R data set, but S did not differ from either. In unmanaged sites, number of species was significantly higher in P than in R and S data sets, the latter two data sets did not differ in this respect.

The proportion of rare species (species with the frequency of less than 10%) was lower in the P data set than in the R and S data sets (Fig. 3a,b). The proportion of the most common species (frequency class: 41–50%) was highest in the P data set in managed sites. In unmanaged sites, there were no species with the frequency of more than 40%, however, the proportion of species with the frequency 31–40% was highest in the P data set. There was no constant pattern of distribution of species within intermediate frequency classes, neither in managed nor in unmanaged sites.

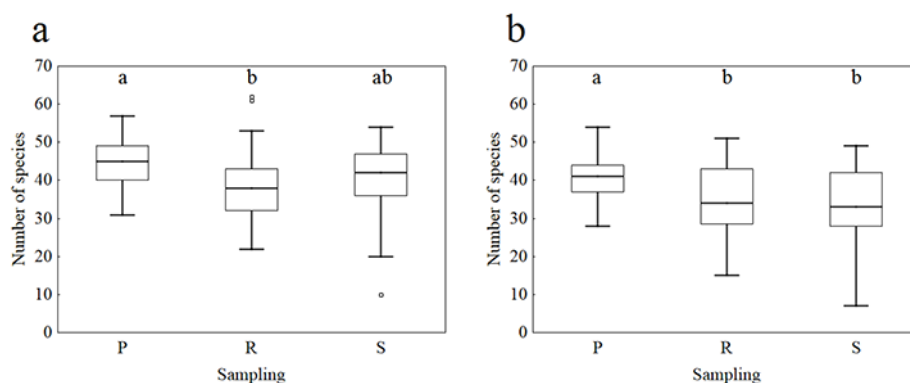


Fig. 2 Number of species in the three sampling data sets for (a) managed and (b) unmanaged meadows. Differences were tested using the Kruskal–Wallis test; data sets with the same letter are not significantly different at $p < 0.05$. Bars represent medians, boxes the interquartile range (25–75 percentiles), whiskers represent non-outlier range, open circles outliers.

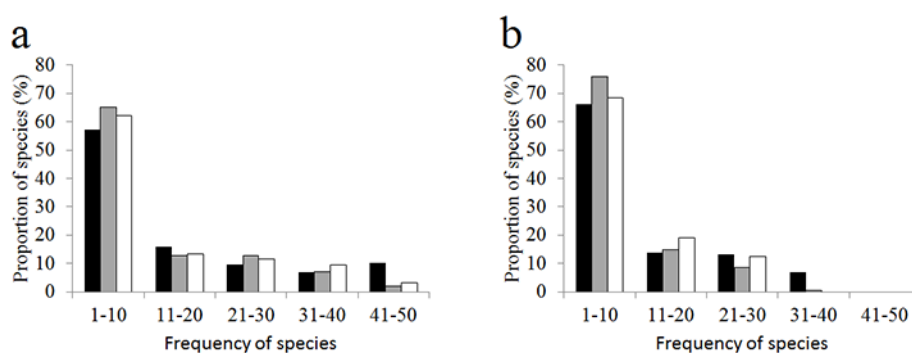


Fig. 3 The proportion of species in relation to their abundance (number of occurrences) for (a) managed meadows and (b) unmanaged meadows. Black columns – P, grey columns – R, white columns – S.

All sampling methods revealed a similar gradient of the soil conditions (Tab. 1). The Kruskal–Wallis test showed that soil parameters were not significantly different between sampling methods. There were also no differences between the sampling methods in a comparison of dispersion around the mean according to the permutation test of mean Euclidean distances.

PCA diagrams showed that P plots were more clumped in the ordination space in contrast to R and S in managed and unmanaged sites (Fig. 4a,b). R and S plots were more scattered than P, reaching the margins of point scatters. Whittaker's beta diversity index revealed low internal heterogeneity of P data set in comparison to R and S data sets in managed sites ($P = 2.53$, $R = 3.68$, $S = 3.30$), as well as in unmanaged sites ($P = 2.60$, $R = 4.15$, $S = 3.72$).

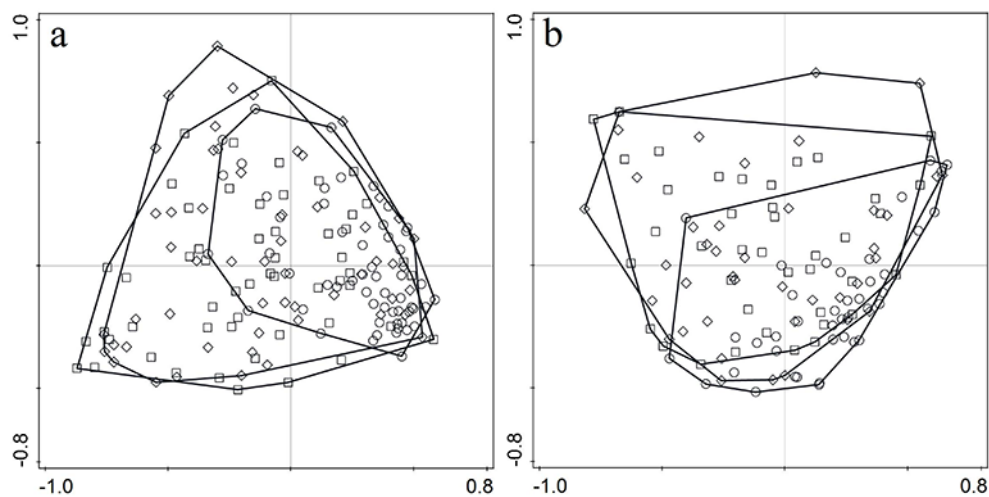
In RDA diagrams, the first axis is strongly correlated with the sampling methods, while the second axis corresponds more to the soil variables (Fig. 5a,b). Centroids representing R and S data sets are situated close to each other whereas P data set is distinctly isolated. The clear differences are visible in the distribution of species in ordination space. Species such as *Calamagrostis epigejos*, *Galium palustre*, *Lythrum salicaria*, *Cnidium dubium*, and *Galeopsis tetrahit* exhibit a high affinity to the R and S sampling methods in managed meadows. In unmanaged meadows, species with high affinity to the R and S sampling methods are *Solidago gigantea*, *Crataegus monogyna*, *Lysimachia vulgaris*, and *Galium aparine*.

In general, species composition differed to a large extent between all sampling methods according to the RDA model used for a comparison of species composition patterns (Tab. 2). A pairwise comparison of sampling methods revealed that these differences refer to P versus R and P versus S. This is true for the analysis comparing sampling methods within managed and unmanaged sites. Most striking differences between sampling methods were revealed for managed sites, where the sampling method accounted for the highest proportion of variance explained.

Tab. 1 General description of soil conditions obtained by different sampling methods within managed and unmanaged sites. The median for soil variables are presented with minimum and maximum values (in parentheses).

	Land use	Sampling method		
		P	R	S
pH	M	5.5 (4.7–6.8)	5.3 (4.5–6.6)	5.5 (3.9–6.4)
	U	5.5 (4.6–6.6)	5.5 (4.5–6.5)	5.5 (4.5–6.4)
Organic matter (%)	M	10.3 (6.1–17.9)	10.1 (5.2–19.2)	8.7 (4.5–18.8)
	U	7.6 (4.6–13.1)	6.7 (2.8–13.2)	7.0 (4.1–13.8)
Nitrogen (%)	M	0.4 (0.1–1.1)	0.4 (0.2–1.0)	0.3 (0.2–1.1)
	U	0.3 (0.1–1.0)	0.3 (0.1–1.0)	0.3 (0.1–0.7)
Phosphorus (mg/100 g soil)	M	1.3 (0.6–2.6)	1.3 (0.6–2.3)	1.3 (0.6–2.5)
	U	1.2 (0.5–3.4)	1.4 (0.6–3.3)	1.3 (0.1–3.3)
Potassium (mg/100 g soil)	M	7.5 (3.1–15.0)	7.3 (3.6–36.1)	7.5 (3.6–22.2)
	U	5.6 (5.6–14.2)	5.3 (2.0–15.1)	5.5 (2.3–14.2)
Calcium (mg/100 g soil)	M	167.9 (54.0–316.7)	143.4 (21.1–337.6)	143.0 (12.1–322.8)
	U	118.9 (39.2–303.7)	102.7 (7.3–251.6)	101.7 (25.6–197.9)
Magnesium (mg/100 g soil)	M	51.9 (22.3–109.1)	50.8 (5.7–152.1)	45.4 (8.8–146.0)
	U	34.7 (15.0–102.6)	31.9 (10.0–94.0)	31.5 (14.1–76.7)

Land use management categories: M – managed, U – unmanaged.

**Fig. 4** Principal coordinates analysis for P (circles), R (squares), and S (diamonds) data sets collected from (a) managed and (b) unmanaged sites.

Although most of the soil parameters affected species composition (main effect), only pH and potassium showed significant interaction between sampling methods (Tab. 3). Statistically significant interactions were revealed for managed, but not for unmanaged sites. The pH–species composition interaction was shown in the comparison of P versus R ($p = 0.008$) and P versus S ($p = 0.006$), while pairwise comparison of R versus S showed no significant differences. Following the exclusion of species with the strongest response to pH (*Betonica officinalis*) from the analysis, the species composition–pH interaction between P versus R became statistically non-significant. Significant interaction between

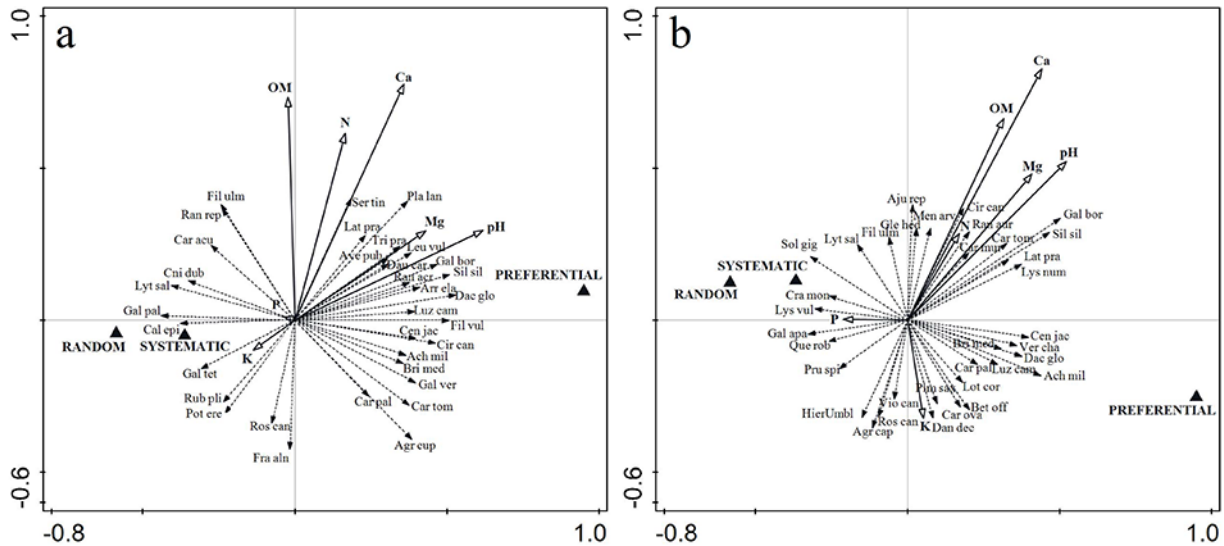


Fig. 5 Ordination diagram of redundancy analysis for (a) managed and (b) unmanaged sites. Species data was related to the sampling methods (triangles) and soil properties (arrows). Thirty-five best fitted species are plotted. For managed sites, percentage variance explained by the first and second axes are 7.23% and 3.63%, respectively. For unmanaged sites, percentage variance explained by the first and second axes are 4.59% and 3.38%, respectively. Abbreviations: Ca – calcium; Mg – magnesium; N – nitrogen; OM – organic matter; P – phosphorous; pH – pH content. Species labels: Agr cap – *Agrostis capillaris*; Agr eup – *Agrimonia eupatoria*; Aju rep – *Ajuga reptans*; Ach mil – *Achillea millefolium*; Arr ela – *Arrhenatherum elatius*; Ave pub – *Avena pubescens*; Bet off – *Betonica officinalis*; Bri med – *Briza media*; Cal epi – *Calamagrostis epigejos*; Car acu – *Carex acutiformis*; Car mur – *Carex muricata*; Car ova – *Carex ovalis*; Car pal – *Carex pallescens*; Car tom – *Carex tomentosa*; Cen jac – *Centaurea jacea*; Cir can – *Cirsium canum*; Cni dub – *Cnidium dubium*; Cra mon – *Crataegus monogyna*; Dac glo – *Dactylis glomerata*; Dan dec – *Danthonia decumbens*; Dau car – *Daucus carota*; Fil ulm – *Filipendula ulmaria*; Fil vul – *Filipendula vulgaris*; Fra aln – *Frangula alnus*; Gal apa – *Galium aparine*; Gal bor – *Galium boreale*; Gal pal – *Galium palustre*; Gal tet – *Galeopsis tetrahit*; Gal ver – *Galium verum*; Gle hed – *Glechoma hederacea*; Hie umb – *Hieracium umbellatum*; Lat pra – *Lathyrus pratensis*; Leu vul – *Leucanthemum vulgare*; Lot cor – *Lotus corniculatus*; Luz cam – *Luzula campestris*; Lys num – *Lysimachia nummularia*; Lys vul – *Lysimachia vulgaris*; Lys sal – *Lythrum salicaria*; Men arv – *Mentha arvensis*; Que rob – *Quercus robur*; Pim sax – *Pimpinella saxifraga*; Pla lan – *Plantago lanceolata*; Pot ere – *Potentilla erecta*; Pru spi – *Prunus spinosa*; Ran acr – *Ranunculus acris*; Ran aur – *Ranunculus auricomus*; Ran rep – *Ranunculus repens*; Ros can – *Rosa canina*; Rub pli – *Rubus plicatus*; Ser tin – *Serratula tinctoria*; Sil sil – *Silva silaus*; Sol gig – *Solidago gigantea*; Tri pra – *Trifolium pratense*; Ver cha – *Veronica chamaedrys*; Vio can – *Viola canina*.

P versus S was not seen following the exclusion of two species (*Carex pallescens* and *Betonica officinalis*) from the analysis. These species proved to be species whose response to pH content differed under different sampling methods.

The correlation between potassium and species composition differed between P versus R ($p = 0.003$). Pairwise comparison of P versus S and R versus S resulted in non-significant differences. Significant interaction between P versus R no longer existed for potassium after removing eight species with the strongest response [*Avena pubescens*, *Arrhenatherum elatius*, *Trifolium pratense*, *Plantago lanceolata*, *Pyrus communis* (seedling), *Carex pallescens*, *Filipendula vulgaris*, and *Anthoxanthum odoratum*] from the analysis. These species turned out be species whose response to potassium content differed under different sampling methods.

A significant finding was the difference in the importance of particular soil factors within each sampling method. The percentage variation explained by pH in managed meadows was similar in all three sampling methods. Potassium had the highest explanatory power of all the soil variables within the P sampling method, however, contradictory results were obtained for R and S where potassium had no significant impact on species composition. In general, the strength of the effect of soil factors was highest for R, but two exceptions were potassium in managed sites and calcium in unmanaged sites.

Tab. 2 The impact of sampling on species composition patterns explored using RDA. The significance of the effects was assessed by a randomization test.

Sampling	M	U
	variance explained (%) in	
All samplings	5.02***	4.43***
P vs. R	5.89***	4.96***
P vs. S	4.84***	4.16***
R vs. S	0.84 ^{NS}	1.10 ^{NS}

Significance codes: *** 0.001; ** 0.01; * 0.05. NS – not significant. Asterisks show significant departure of variation explained by the compared sampling data sets from zero. Land use categories: M – managed; U – unmanaged.

Tab. 3 The effect of environmental factors on species composition explored using RDA. The significance of the effects was assessed by a randomization test.

	Land use	<i>p</i> values for		Variance explained (%) in		
		main effect	interaction with sampling methods	P	R	S
pH	M	0.001	0.008	5.51 ^{***}	5.77 ^{***}	5.05 ^{***}
	U	0.001	0.895	3.67 ^{NS}	4.50 [*]	3.54 ^{NS}
Organic matter	M	0.001	0.142	2.42 ^{NS}	6.07 ^{***}	3.90 ^{**}
	U	0.001	0.544	4.71 ^{**}	5.03 ^{**}	3.12 ^{NS}
Nitrogen	M	0.001	0.454	3.55 ^{NS}	4.24 ^{**}	2.39 ^{NS}
	U	0.168	0.146	3.47 ^{NS}	3.42 ^{NS}	2.20 ^{NS}
Phosphorus	M	0.052	0.631	2.44 ^{NS}	1.83 ^{NS}	1.93 ^{NS}
	U	0.025	0.984	2.34 ^{NS}	2.61 ^{NS}	2.33 ^{NS}
Potassium	M	0.001	0.018	5.78 ^{***}	2.45 ^{NS}	2.41 ^{NS}
	U	0.001	0.969	2.91 ^{NS}	4.16 ^{**}	4.10 [*]
Calcium	M	0.001	0.115	3.33 [*]	5.90 ^{***}	5.17 ^{***}
	U	0.001	0.283	6.59 ^{**}	4.46 ^{**}	4.78 [*]
Magnesium	M	0.001	0.254	3.08 [*]	4.37 ^{**}	4.37 ^{***}
	U	0.002	0.710	3.81 [*]	3.82 [*]	3.34 ^{NS}

Asterisks show significance of the effect of the environmental factor on species composition. Significance codes: *** 0.001; ** 0.01; * 0.05. NS – not significant. Land use categories: M – managed; U – unmanaged.

Discussion

Patterns of species composition

Our study reveals that different sampling methods produce divergent results regarding species composition patterns. The pooled number of species captured in the P data set was smaller than in R and S data sets. A notable finding is that the P sampling resulted in higher number of species per sampling unit, although the pooled species richness of P data set was the lowest. The issue of overestimation of species richness by P sampling method has been frequently raised in the literature [20–23]. Species-poor stands can, however, be preferred in P sampling in the surveys of vegetation types where an increasing number of species indicates disturbance [25].

The P sampling method resulted in a higher number of common species and a lower number of rare species. In the light of these results, common species were typical grassland species that were widely distributed in the study area, while rare species were species typical for other habitats such as forests, tall-sedge vegetation, or wet meadow communities that sporadically occur in *Molinia* meadows (Tab. S1). The common components of the vegetation can be considered a core species, while rare species are satellites species [62]. The study area is surrounded by the habitats that are source of species which can inhabit vegetation of *Molinia* meadows, despite that these species are not constant component of this habitat (spatial mass effect) [63]. It can be concluded that the P sampling method overestimated the number of common species (core species) and underestimated the number of scarce species (satellites species). Our results support the notion that data from P sampling should not be uncritically used in the analyses of species-abundance models [21].

P plots are evidently more clumped in ordination space than the R and S plots, which included plots from the extremities of the existing environmental gradients (Fig. 3). A cloud of points from P sampling overlap only partially with R and S data sets, meaning that only part of the vegetation variability was explored by the P sampling method, while R and S data sets represent a broader variation in vegetation. The intentional sampling of patches with abundance of indicator species of *Molinia* meadows has resulted in a restricted population sample (sample universe) of P data set. This can also be seen from Whittaker's beta diversity index calculated for P, R, and S data sets. From a statistical point of view, this means that P and both R and S samples represent overlapping, but not the same statistical population [25]. As a result, a certain number of species has not been included in the P data set simply because these species occurred in communities omitted by the surveyor. Based on the comprehensive description of plant communities of the alliance *Molinion*, we were able to create an explicit algorithm for the selection of the most characteristic stands to be sampled preferentially [46,47]. In the case of well-described vegetation units, a surveyor can become very familiar with the species composition of a particular vegetation type and subsequently use this knowledge to find the most representative stands [25]. For the poorly studied vegetation types a priori definitions of sampling units based on expert knowledge is rather difficult, thus formal criteria for the selection of sampling sites, preferably using GIS, should be applied [23].

Our study suggests that the P sampling method may produce a biased conclusion about vegetation variability due to the intentional restriction of vegetation variation. These results are in line with the findings of Holeksa and Woźniak [7], who have also shown that selecting the most characteristic stands of vegetation may result in omitting considerable variability of vegetation in the altitudinal gradient analysis of forest communities. Other comparative studies on the performance of sampling methods in a heterogeneous landscape indicated a higher vegetation variability in preferentially collected data sets [8,22]. From these latter studies it can be concluded that the observed vegetation variability increases with the increasing ability to locate rare community types in the landscape. Indeed, P sampling is an efficient method for finding rare vegetation types in the landscape, while probabilistic sampling predominantly records dominant community types [64]. It has previously been emphasized that probabilistic sampling methods result in the under-representation of rare vegetation types if the chosen sample size is not extensive enough in the study [36,65]. On the other hand, very high sampling intensity is impractical, especially in studies at large spatial scales [65].

The most pronounced differences in species composition between sampling methods were found in managed sites. When sampling managed vegetation preferentially, it was possible to find undisturbed and well-developed patches of *Molinia* meadows and ignore those considered atypical or transitional. Sampling method explained less variation in species composition in the unmanaged sites because secondary succession enhances homogenization of species composition [66,67].

Environment–vegetation relationships

Soil conditions within managed and unmanaged sites studied by different sampling techniques were homogenous. This means that none of the data sets consisted of plots placed at a narrow-range gradient of soil chemical conditions. Ordination diagrams of RDA showed that sampling methods coincide with the first ordination axis (i.e., the main gradient of vegetation changes) (Fig. 5). Soil variables included in the analysis corresponded substantially to the second axis. So, it can be interpreted that soil variables were not directly responsible for the separation of sampling data sets in the ordination diagrams. Judging from the positive correlation of moisture-demanding species to the R and S data sets and their negative correlation to the P data set, we assumed that moisture content in the soil was most likely the factor differentiating the data sets. It has to be noted that both *Calamagrostis epigejos* and *Solidago gigantea* were located close to moisture-demanding species in ordination space. The growth of *Calamagrostis epigejos* is enhanced under low-intensity management regime [68], and under moist and nutrient-rich conditions [69]. *Solidago gigantea* also shows tendency to occur in relatively moist sites [70] and it commonly thrives in abandoned or sporadically mown

grasslands [71]. More frequent occurrence of *Calamagrostis epigejos* and *Solidago gigantea* in more wet sites may also be the effect of irregular mowing of these sites that might be omitted by farmers due to the unfavorable ground conditions. With P sampling method, stands with these species were intentionally omitted because the abundance of these species indicates disturbance of habitat [72].

The results showed that most of soil parameters were significantly correlated with species composition (main effect), however, the differences in soil–vegetation relationships between sampling methods were recorded for pH and potassium, and only within managed sites. It needs to be emphasized that pH is important determinant of species composition in grasslands [11] while potassium is an important soil variable limiting or colimiting vegetation growing on soils that can dry out [73]. It can be interpreted that P sampling overestimated the role of potassium due to the constant avoidance of sites invaded by *Calamagrostis epigejos* or *Solidago gigantea*. Species may respond differently simply because a different range of environmental factors was sampled. The lack of interactions between sampling methods in the vegetation–environment relationships in the unmanaged sites can be explained by the increased homogeneity of species composition caused by the abandonment of agricultural management.

In a comparative study on the performance of different sampling methods Diekmann et al. [21] showed that P sampling resulted in larger number of significant correlations of species data to soil variables than R sampling. Bhatta et al. [34] concluded that S sampling produced better correlations between species and environmental data than stratified R sampling. Although we have found no significant differences in vegetation relationships between R and S data sets, R sampling resulted not only in the largest number of significant interactions between species and soil data, but also produced the most robust response of species data to the underlying environmental factors. A drawback of the simple randomization process is that it does not ensure a spatially balanced data set [35,64]. In our study, R sampling oversampled some parts of study area and undersampled others (Fig. 1). It is a well-established phenomenon that neighboring sites are more similar than more distant sites, therefore the robust interactions between vegetation and environment can be explained by the spatial autocorrelation in R data set. As with almost each probabilistic data, spatial autocorrelation tends to be high [40]. To overcome problem of spatial autocorrelation, stratification of the study area by some criteria is often carried out and probabilistic sampling is applied within delimited strata [74–76]. In our study, we focused on a single vegetation type in a small spatial scale, and as a result, we did not consider the stratified sampling method relevant to our case study. The magnitude of differences in vegetation–environment interactions between sampling methods could be even more emphasized if a larger gradient was considered because the magnitude of explanatory power of environmental factors depends on the gradient lengths undertaken in a study [77,78].

Conclusions

In this study, we have attempted to investigate species composition patterns and vegetation–environment relationships in a small spatial scale using different sampling methods. Our study highlights that preferential sampling may result in narrowing the environmental gradient due to intentional restriction of vegetation variation. The differences between samplings, however, diminish with the increasing disturbance of habitat. Preferential sampling may be detrimental to statistical inference in studies of species composition patterns and vegetation–environment relationships. Note that conclusions from this study are valid for that type of preferential sampling where the aim is representing variation in species composition in plant communities that usually correspond to the so-called typical stands. The results of studies where the positioning of samples was influenced by the subjective decisions of a surveyor should therefore be interpreted with caution.

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Supplementary material

The following supplementary material for this article is available at <http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3561/0>:

Fig. S1 The number of indicator species per plot that matched and that did not match the formal definition of the alliance *Molinion* for two management categories (managed and unmanaged).

Tab. S1 List of the unique species for each data set regardless of the management type.

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