Ecological Indicators xxx (xxxx) xxx-xxx

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: http://ees.elsevier.com

Different but valuable: Anthropogenic habitats as genetic diversity reservoirs for endangered dry grassland species – A case study of *Stipa pennata*

Ewelina Klichowska [a,b], Monika Szczecińska [c], Monika Ślipiko [c], Marcin Nobis [a,d,*]

[a] Institute of Botany, Faculty of Biology, Jagiellonian University, Gronostajowa 3, 30-387 Kraków, Poland
[b] Botanical Garden, Center for Biological Diversity Conservation, Polish Academy of Sciences, Prawdziwka 2, 02-976 Warszawa, Poland
[c] Department of Botany and Nature Protection, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Plac Łódzki 1, 10-728 Olsztyn, Poland
[d] Research Laboratory 'Herbarium', National Research Tomsk State University, Tomsk, Russia

ARTICLE INFO

Keywords
Refuges and substitution habitats
Anthropogenic habitats
Xerothermic species
Genetic diversity
Feather grasses

ABSTRACT

Increasing urbanisation has led to the fragmentation of natural habitats and biodiversity decline. In Central Europe, this fate has befallen semi-natural xerothermic grasslands. However, in the case of species well adapted to open habitats, urbanisation may positively affect dispersal. In many places specialised xerothermic species start to spread in secondary habitats such as roadsides or railway embankments. However, little is known about the genetic variability of such newly-established populations. In an era of growing anthropogenic pressure, understanding the role of secondary, anthropogenic habitats in the maintenance biodiversity should be considered a crucial issue in the conservation of species associated with Central European dry grasslands. Therefore, on the basis of a combined analysis of xerothermic species abundance, bioclimatic and soil data, and genetic diversity, we wish to examine the suitability of secondary habitats (as substitutes for original) and to demonstrate their role in the conservation of xerothermic species. Our results suggest that smaller competition for light and greater habitat connectivity indicate suitability of habitats for xerothermic species conservation. On one hand, the occurrence of these species in secondary habitats may be limited by the pressure of expansive grasses, on the other, the negative impact of the latter is buffered by the reduced presence of shrubs, and thus the greater availability of light. Plant communities from secondary habitats are less richness in xerothermic species, and particular species are less abundant, for this reason secondary habitats should be treated more as refuges (with suboptimal conditions) than as substitution habitats. But yet, *Stipa pennata* populations from secondary habitats do not suffer from genetic depauperation. Populations from secondary habitats are characterised by comparable share of two genotypes, indicating existence of gene flow between populations from south and north-west part of studied area. Through the maintenance of meta-population processes by means of dynamic connectivity between habitats on a landscape scale, secondary habitats possess an undoubtedly high conservation value for xerothermic species.

1. Introduction

In agricultural landscapes, habitats formed or altered directly or indirectly by human activity give shelter to a different groups of organisms (Kütt et al., 2016; Moroń et al., 2014; Rouco et al., 2019), hence they are considered as one of the main source of biodiversity. Among the most species-rich and diversified semi-natural habitats in Central Europe are dry (xerothermic) grasslands, which constitute a key habitat for plants, grazing animals, arthropods, reptiles, and birds, including a large number of rare and endangered species (Comino et al., 2008; Korneck et al., 1996; Sádlo et al., 2007; Tälle, 2018). Because of their high value, semi-natural grasslands located on calcareous substrates (representing the *Festuco-Brometea* class) were included in the EU NATURA 2000 network (Comino et al., 2008). The existence of seminatural grasslands in Central Europe is closely related to long-term human activity such as mowing or grazing, and dates back to agriculture beginning during the Mesolithic–Neolithic transition (Hejcman et al., 2012). Because of their origin, they are unable to survive without further human activity (Barańska, 2014; Tälle, 2018).

Over the past few decades, intensification of urbanisation, by interfering with the surrounding nature, negatively affect all levels of biodiversity through their destructive impact, leading to the fragmentation

* Corresponding author at: Institute of Botany, Faculty of Biology, Jagiellonian University, Gronostajowa 3, 30-387 Kraków, Poland.
E-mail address: m.nobis@uj.edu.pl (M. Nobis)

https://doi.org/10.1016/j.ecolind.2019.105998
Received 8 June 2019; Received in revised form 3 December 2019; Accepted 5 December 2019
Available online xxx
1470-160/© 2019.

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and consequent disappearance of natural habitats (Chytrý et al., 2019; Lin et al., 2019; Liu et al., 2016; Saunders et al., 1991). According to Janssen et al. (2016), over 30% of freshwater and terrestrial habitats in the European Union are considered threatened. The highest percentage of threatened types (categories: critically endangered, endangered, vulnerable) was found among mires and bogs (85%), grasslands (53%), and freshwater habitats (46%). Changes in land use in Europe connected with the abandonment of extensively cultivated grasslands and afforestation, have resulted in the loss and fragmentation of dry grasslands (Schrautzer et al., 2009; Hegedüšová and Senko, 2011; Janišová et al., 2011). Between 1975 and 1998 alone, the total area of grassland in the EU fell by an average of 12% (Comino et al., 2008). Overgrowing of grasslands displaces photophilous species and thus reduces α, β, and γ diversity, as well as affecting diversity at the genetic level as a result of restrictions in gene flow and of genetic bottlenecks (Butaye et al., 2005; Honnay et al., 2007; Durka et al., 2013; Plenk et al., 2019). On the other hand, many grassland species occur also in secondary, anthropogenic habitats such as margins of arable fields, fallows, roadsides, or railway embankments (Jantunen et al., 2006; Tikka et al., 2001). Among such species are also some considered rare, endangered, and/or protected, either in particular countries or throughout the European Union, e.g. *Arabis recta*, *Cardaminopsis petraea*, *Crepis praemorsa*, *Dorycnium herbaceum*, *Draba nemorosa*, *Gentiana cruciata*, *Linum austriacum*, *Rosa gallica*, *Veronica praecox* (Černý et al., 2006; Kaźmierczakowa et al., 2014; Nobis and Nobis, 2012; Nowak, 2005). However, little is known about the genetic variability of populations of these species occurring in such secondary habitats (Helsen et al., 2013; Reisch, 2007). It should be taken into account that the road and rail infrastructure in Central Europe may provide over 1.5 mln km of potential habitats (Eurostat, 2019a, 2019b), affording hope for surveys of photophilous species. However, according to Martínez-Abraín and Jiménez (2016), species may differ in their level of adaptation to occupied anthropogenic, secondary habitats. Most commonly, habitat alteration forces species to relocate to suboptimal habitats, called refuges, where species achieve smaller population sizes than they did in their original habitats. Less often, human activity may lead to the generation of habitats that functionally resemble the original habitats to a great extent and thus provide a species with the same or sometimes even better conditions for growth than in the originals; these are called substitution habitats (Martínez-Abraín and Jiménez, 2016). In the case of grassland species whose original, primary habitats are rapidly disappearing, the degree to which they are able to adapt to anthropogenic, secondary habitats may be crucial for their survival.

In an era of increasing anthropogenic pressure, understanding the role of anthropogenic, secondary habitats in the maintenance of diversity (on both the species and genetic levels) is a crucial issue in the conservation of species associated with central European dry grasslands. Therefore, we decided to examined different levels of diversity on both, primary and secondary habitats, and our research questions are as follows: (1) In terms of what factors, primary and secondary habitats are similar? (2) Is there a difference in genetic diversity and structure between population from primary and secondary habitats (3) What environmental factors affect the abundance and genetic diversity of xerothermic species? (4) What environmental factors indicate habitat suitability for xerothermic species? (5) Substitution or refuges, how should we treat secondary habitats for xerothermic grassland species? As primary habitats we understand semi-natural grasslands, closely related to long-term but extensive human activity, whereas as secondary – habitats under strong anthropogenic pressure such as roadsides or railway embankments. To measure the suitability of secondary habitats as substitution or refuges for xerothermic species, we compare these two types of habitats in terms of environmental (abiotic and biotic) conditions, xerothermic species richness and abundance as well genetic diversity of one selected species. For our study, we chose patches with differing shares of *Stipa pennata* L. (=*S. joannis* Čelak.), treating this flagship steppe species as an indicator of favourable conditions for highly xerothermic specialised plants. It is not without significance that, over the past few decades, due to the loss of original habitats, this perennial grass has decreased in terms of numbers of both populations and individuals and is currently protected and red-listed in many European countries (Poland, Germany, Austria, Czech Republic, Ukraine, Russia) (Didukh, 2009; Grulich, 2012; Kaźmierczakowa et al., 2014, 2016; Korneck et al., 1996; Niklfeld and Schratt-Ehrendorfer, 1999; Trutnev, 2008). *Stipa pennata* is also one of the species that has begun to spread into secondary habitats (Kaźmierczakowa et al., 2014); thus it can serve as a perfect example for examining the suitability of secondary habitats as substitutes for original ones as well as to identify factors indicating this suitability.

2. Materials and methods

In total, 50 plots of vegetation from 32 different localities (including 21 from primary and 11 from secondary habitats) in the north-western range of general distribution were examined (Fig. 1). Plant species composition was documented in plots measuring 4 × 4 m, representing homogenous patches of vegetation. A six-grade cover-abundance scale was used: +: < 1% cover; 1: < 5%; 2: 5–25%; 3: 25–50%; 4: 50–75%; and 5: 75–100% (Braun-Blanquet, 1964). In the case of *Stipa pennata* we additionally estimated the population size. Identification of specimens was carried out using appropriate keys (Jäger, 2009; Pawlus, 1983; Rutkowski, 1998). The plant material is preserved at the Herbarium of the Institute of Botany, Jagiellonian University (KRA). The data was used to calculate the coverage of tree layer per plot (A; %), coverage of shrub layer per plot (B; %), dominance index (DI), ranging from 0 (in plots where all taxa were equally present) to 1 (where one taxon dominated the community completely); species richness (SR, the number of taxa in the plot); and expansive grass cover (egcover, the sum of cover of *Calamagrostis epigejos* and *Arrhenatherum elatius*, grasses which are undesirable in xerothermic grasslands). We also calculated xerothermic species richness (XSR) and xerothermic species abundance (XSA) per plot. As xerothermic we treat species characteristic and distinguishing for the *Festuco-Brometea* and *Trifolio-Geranietea sanguinei* classes (Matuszkiewicz, 2006).

Soil variables including: bulk density in tonnes per cubic-meter (bld), weight percentage of clay particles (<0.0002 mm; cly, weight percentage of silt particles (0.0002–0.05 mm; slt), weight percentage of sand particles (0.05–2 mm; snd), soil organic carbon content in permilles (orc), volume percentage of coarse fragments (>2 mm; crf), Cation Exchange Capacity in cmol+/kg (cec), soil water-holding capacities (AWCtS), were derived from the ISRIC data base (https://www.isric.org/) at a spatial resolution of 250 m^2 (Hengl et al., 2017). Bioclimatic variables: annual mean temperature (bio1), mean diurnal range of temperature (bio2), isothermality (bio3), temperature seasonality (standard deviation × 100) (bio4), maximum temperature of warmest month (bio5), minimum temperature of coldest month (bio6), temperature annual range (bio7), mean temperature of wettest quarter (bio8), mean temperature of driest quarter (bio9), mean tem- perature of warmest quarter (bio10), mean temperature of coldest quarter (bio11), annual precipitation (bio12), precipitation of wettest month (bio13), precipitation of driest month (bio14), precipitation seasonality (coefficient of variation) (bio15), precipitation of wettest quarter (bio16), precipitation of driest quarter (bio17), precipitation of warmest quarter (bio18), precipitation of coldest quarter (bio19) and pH (soil pH × 10 in H_2O), were derived from the WorldClim 1.4 database (http://www.worldclim.org/) at a spatial resolution of 30 arc-seconds (Hijmans et al., 2005). Temperature variables were measured in degrees Celsius × 10; precipitation was measured in whole millimetres

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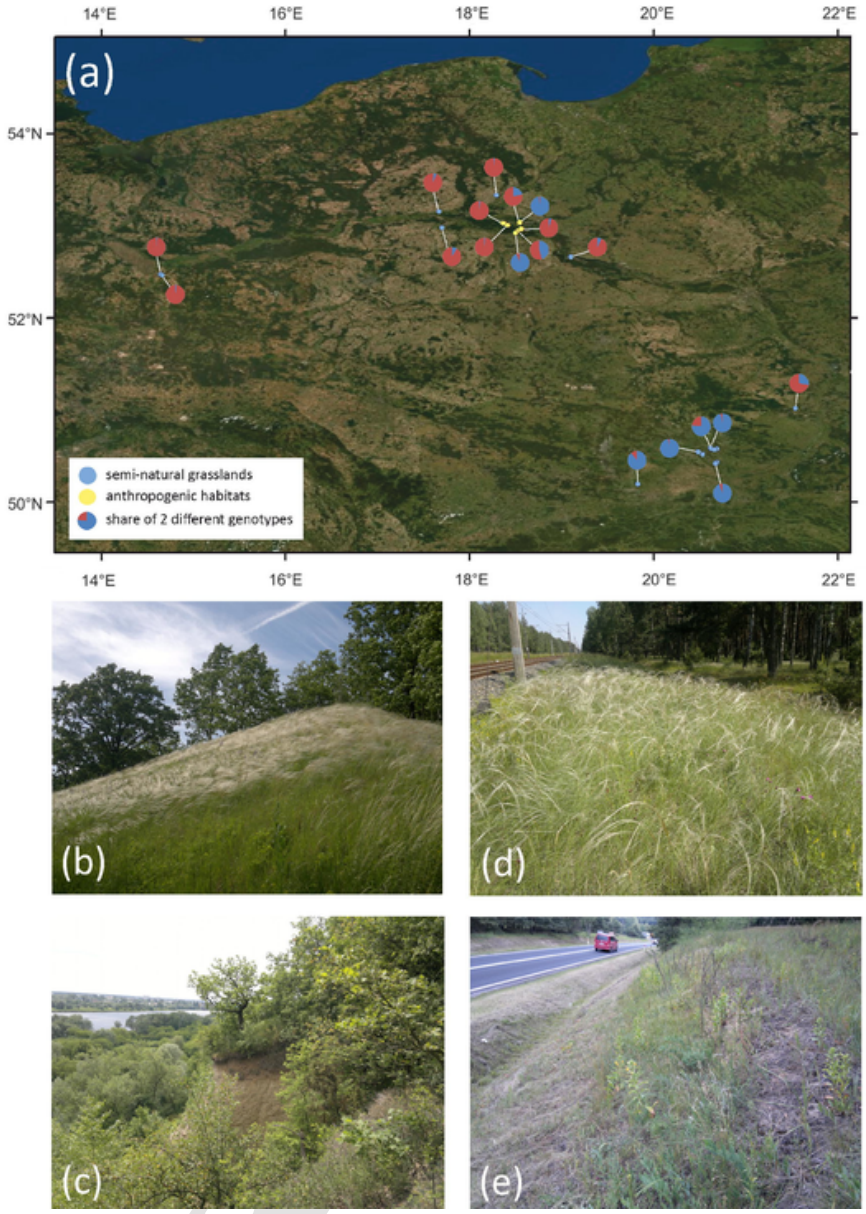

Fig. 1. Localities and habitats of *Stipa pennata* at the north edge of general distribution (a) Geographic distribution of studied populations with proportion of genotype (K = 2) based on STRUCTURE analysis, map generated in ArcMap 10.5 software, map background is the raster layer included in ArcGIS Online (ESRI Inc. 1999–2016); (b) and (c) examples of original habitats (semi-natural dry calcareous grassland with high share of *Stipa pennata* – b, and grassland overgrowing by shrubs with small share of *S. pennata* – c); (d) and (e) examples of anthropogenic habitats (dry grassland on a railway embankment with high share of *S. pennata* – d, extensively mowing grassland on the roadside with small share of *S. pennata* – e).

(Hijmans et al., 2005). We also assessed declination of slope (measured in degrees), during field studies.

For the SSR analysis, leaves from 240 individuals and 19 populations of *Stipa pennata* were sampled (Fig. 1). We chose populations varying in numbers of individuals (from small populations with few up to few thousands of individuals). A total of 4 to 20 samples were obtained from each population (the number of samples depended on the size of *S. pennata* populations). A population was defined as a group of plants separated from their nearest conspecifics by more than 1 km.

Total genomic DNA was extracted from 20 mg of silica-dried leaf material using Genomic Mini AX Plant Spin (A&A Biotechnology Poland). Five microsatellite loci (SP10, SP17, SP15, SP08, and SP23) were used to analyse the genetic variation of the sampled populations. The microsatellite loci, PCR, and electrophoresis conditions used to generate data for the current study were described in our previous paper (Klichowska et al., 2018).

To estimate the level of genetic variation within a population, we calculated the following genetic diversity parameters: mean number of alleles per locus (Na), effective number of alleles (Ne), average observed heterozygosity (Ho), average expected heterozygosity (He), sample-size weighed expected heterozygosity (uHe), fixation index (F_{IS}), and private alleles. To estimate and partition the total variances at three hierarchy levels, within populations, between populations and between groups of populations (two studied types of habitats), Analysis of Molecular Variance (AMOVA) was used. AMOVA was based on genetic distance matrix of pairwise F_{ST} values. All calculations were performed using GenAIEx 6.41 (Peakall and Smouse, 2012).

To explore population genetic substructure, we identified the number of genetic clusters (K) using STRUCTURE v. 2.3.4 (Pritchard et al., 2010). We performed 10,000 Markov chain Monte Carlo (MCMC) repetitions and a burn-in of 20,000 repetitions with 10 independent runs each for K = 1–10. The most likely number of genetic clusters was

determined using STRUCTURE Harvester web version 0.6.94 (Earl and von Holdt, 2012).

To test for an association of genetic distance and geographical distances as well as genetic distance and environmental factors we used a Mantel test (Mantel, 1967), performed in R v. 3.5.1 (R Development Core Team, 2018), package vegan v2.5–6 (Oksanen et al., 2019) with 999 permutations. The Pearson correlation coefficient was calculated to check the relationship between population size and genetic variation, using Statistica software, version 13 (TIBCO, 2017).

In order to reveal significant differences between the mean values of environmental variables and between genetic diversity parameters of secondary habitats vs. primary grasslands, the Mann-Whitney *U* test was carried out. Calculations were performed using Statistica software, version 13 (TIBCO, 2017).

To explore whether environmental factors and population size influence the level of genetic diversity of *Stipa pennata* populations, we run Redundancy Analysis (RDA). In order to linearise relationships and normalise distributions, dependent variables were normalised (margin sum of squares equal to one). To permit detection of straightforward relationships between independent variables, they were standardised (scale × to zero mean and unit variance). To reduce big numbers of variables and avoid multicolinearity we calculate the variance inflation factor (VIF) and Pearson correlation coefficient for both dependent and independent variables. We removed variables strongly correlated (>0.8) with each other. We chose 13 predictor variables including five biotic: A, B, DI, egcover, *Stipa pennata* population size as well as eight abiotic: declination of slopes, bio10, bio18, bio19, bld, crf, pH, and distance to nearest population. As dependent variables we used uHe, Ho and Na. By using automatic backward stepwise model building function with permutation tests, for final model we chose seven variables: bio19, declination, crf, pH, B, egcover and distance to nearest population. The statistical significance of the explanatory variables and axes were tested using a 999 permutation test. Analysis was performed using the computing environment R v. 3.5.1 (R Development Core Team, 2018), with the additional software package vegan v2.5–6 (Oksanen et al., 2019) and car v3.0–3 (Fox et al., 2019).

To analyze the effects that different environmental factors may have on the abundance (population size) of *Stipa pennata* we used a Generelized Linear Mixed Model implemented in lme4 package (Bates et al., 2019) in R v. 3.5.1 (R Development Core Team, 2018). To reduce the high number of factors as well as to solve the problem of multicollinearity we conducted Principal Component Analysis (PCA), however because of large diversity within the factors, and problems with interpreting their impact after a simple combination of variables, we conducted three independent analyzes, grouping the variables into thematic groups: 1) climatic conditions, 2) soil and topography conditions, 3) species competition. We included those three variables as fixed whereas blocking factors (habitat type and geographic region) as random effect. We computed GLMM with Poisson error distribution and link function, which is recommended for count data (Bolker et al., 2009). For checking of violation of assumptions (including overall deviations from the expected distribution, outlier identification and heteroscedasticity, spatial autocorellation) we used 'DHARMa' package, which created readily interpretable residuals (standardised to values between 0 and 1) (Hartig, 2019).

Similarity percentage (SIMPER) analysis was used to identify the patterns of vascular plant species distribution throughout the studied habitats. The Bray-Curtis dissimilarity was used to quantify compositional dissimilarity between groups. To test the statistical significance of differences in plant species composition between secondary habitats and primary grasslands, analysis of similarities (ANOSIM) was calculated (Clarke, 1993) using PAST (Hammer et al., 2001).

3. Results

3.1. Niche comparison

The results of the Mann-Whitney *U* test showed statistically significant differences between secondary and primary habitats in 30 of 39 analysed variables (Table 1). In the context of vegetation cover, secondary habitats were characterised by lower values of species richness (SR), xerothermic species richness (XSR), and abundance (XSA), a lower percentage of moss cover (D), a higher percentage of cover of expansive grasses (egcover), a slightly higher dominance index (DI), and higher percentages of tree cover (tcover) in the immediate area. Moreover, ANOSIM showed that the two types of habitats differed significantly in plant species composition (with $p = 0.001$), whereas SIMPER indicated 17 species primarily responsible for over 30% of the observed difference between the habitats. The greatest impact on the difference between the two types of habitats was exerted by the following grasses: *Calamagrostis epigejos*, *Poa pratensis*, *Brachypodium pinnatum*, *Festuca rupicola*. The communities in the primary habitats are characterised by a greater share of calciphilous species, whereas in secondary habitats species connected with neutral soils (Table 2) are dominant. In secondary habitats, the richness and abundance of xerothermic species are lower than in primary habitats; however, the share of xerothermic species in secondary habitats is over 40% of all species found there (as opposed to ca 60% in primary habitats) (Table 1). Primary dry grasslands are usually located on slopes with greater declination and soil characterised by greater bulk density (bld), a greater percentage of clay particles (cly), a greater cation exchange capacity (cec), a higher pH, and lower soil organic carbon content (orc), a greater percentage of silt particles (slt) and lower percentage of sand particles (snd). Due to geographical location, localities from secondary habitats differ by lower temperatures values and higher precipitation (Table 1). Population size (measured as numbers of generative individuals) is much greater for primary dry grasslands (an average of 1064 vs. 84 individuals); however, the average distance to the nearest population is much less in the case of secondary habitats (1.7 vs 12.6 km).

3.2. Genetic diversity and differentiation

A total of 91 alleles were identified across all loci in 240 individuals. The greatest number of alleles (26) were amplified by S17 primers, the least (14) by S23. Overall genetic diversity in all analysed populations was moderate and varied across the studied locations. Average Ho was 0.30 ± 0.08 for secondary habitats and 0.28 ± 0.07 for primary habitats, and was lower than expected (average He: 0.66 ± 0.07 for secondary habitats and 0.67 ± 0.09 for primary habitats). The average number of alleles per locus (Na) was 4.83 ± 1.17 for secondary habitats and 4.90 ± 0.99 for primary habitats. The fixation index (F_{ST}) was also moderate, 0.53 ± 0.11 for secondary habitats and 0.52 ± 0.20 for primary habitats. The Mann-Whitney *U* test showed that the two types of studied habitats did not differ significantly in terms of any genetic diversity parameters (Na, Ne, Ho, He, I, F_{ST}, private alleles). The genetic diversity indices for five microsatellite loci, calculated for each population, are given in Table 3. Based on the Evanno's method, STRUCTURE Harvester suggested $K = 2$ as the most likely numbers of genetic cluster that best fit the analysed data. Those clusters correspond to geographical distribution of populations (Fig. 1). All populations were characterised by admixture of both genotypes, what suggests a gene flow between populations from particular geographical regions. Populations from secondary habitats, which are located in the core part of studied area, are characterised by more or less equal participation of both genotypes, while populations from primary habitats, both in the northwest and the south, are characterised by the predominance of one of the genotypes. The results of AMOVA shows higher levels of varia-

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Table 1
Ecological factors differentiating two studied types of habitats; Minimum, Maximum, Mean and Standard deviation values as well as results of U Mann-Whitney Test. For abbreviations and description of response and explanatory variables see Materials and Methods.

	Primary habitats (n = 32)				Secondary habitats (n = 18)				U Mann-Whitney Test
	Mean	Min	Max	Std. Dev.	Mean	Min	Max	Std. Dev.	Z
declination	14.2	0.0	40.0	11.6	3.9	0.0	10.0	3.7	**3.4****
bld	1372.4	1270.0	1431.0	44.6	1325.0	1255.0	1427.0	59.9	**2.4***
cec	14.9	9.0	21.0	2.8	10.2	8.0	13.0	1.6	**5.0*****
cly	17.3	9.0	25.0	4.5	7.1	4.0	10.0	2.1	**5.7*****
crf	7.2	4.0	13.0	2.0	6.5	4.0	8.0	1.3	1.0
orc	23.1	15.0	61.0	8.5	31.2	22.0	39.0	6.8	**−4.0*****
ph	61.3	55.0	65.0	2.7	51.3	47.0	60.0	4.5	**5.4*****
slt	34.6	17.0	45.0	7.2	12.6	8.0	20.0	3.9	**5.7*****
snd	48.7	36.0	74.0	10.4	81.1	71.0	88.0	5.8	**−5.7*****
awcts	43.0	41.0	47.0	1.7	43.0	40.0	45.0	1.9	−0.4
bio1	78.7	74.0	91.0	4.9	79.4	79.0	80.0	0.5	**−2.3***
bio2	84.2	78.0	88.0	3.4	83.7	83.0	84.0	0.5	1.9
bio3	26.9	26.0	29.0	0.8	28.0	28.0	28.0	0.0	**−5.1*****
bio4	7834.1	7426.0	8089.0	202.3	7527.4	7474.0	7574.0	30.3	**4.1*****
bio5	234.7	225.0	247.0	5.6	236.1	235.0	237.0	0.9	−1.9
bio6	−72.3	−84.0	−51.0	11.8	−57.8	−59.0	−56.0	1.3	**−3.9*****
bio7	307.0	289.0	317.0	10.0	293.9	293.0	296.0	0.9	**4.7*****
bio8	173.1	165.0	186.0	5.5	173.2	172.0	174.0	0.7	−0.7
bio9	−15.6	−23.0	−2.0	6.4	−9.6	−11.0	−8.0	1.3	**−3.8*****
bio10	173.1	165.0	186.0	5.5	173.2	172.0	174.0	0.7	−0.7
bio11	−29.3	−37.0	−14.0	7.3	−20.7	−22.0	−19.0	1.2	**−3.9*****
bio12	606.6	524.0	715.0	59.5	528.1	522.0	532.0	4.2	**4.6*****
bio13	86.4	69.0	101.0	10.3	76.3	76.0	77.0	0.5	**2.7****
bio14	27.3	22.0	32.0	2.4	22.6	22.0	23.0	0.5	**5.2*****
bio15	38.4	27.0	42.0	5.0	38.0	37.0	39.0	0.9	**2.1***
bio16	240.8	184.0	290.0	33.8	208.1	207.0	210.0	1.0	**2.5***
bio17	90.4	74.0	104.0	7.3	75.9	74.0	77.0	1.4	**5.2*****
bio18	240.8	184.0	290.0	33.8	208.1	207.0	210.0	1.0	**2.5***
bio19	98.5	87.0	109.0	5.7	88.9	87.0	90.0	1.4	**4.7*****
A	0.9	0.0	30.0	5.3	1.7	0.0	30.0	7.1	−0.4
B	2.7	0.0	40.0	8.3	0.0	0.0	0.0	0.0	1.7
C	73.2	40.0	98.0	14.5	70.0	60.0	90.0	8.9	1.3
D	9.6	0.0	40.0	11.8	14.2	0.0	40.0	10.7	**−2.0***
tcover	7.8	0.0	35.0	7.5	69.2	14.0	100.0	26.5	**−5.7*****
egcover	1.4	0.0	8.0	2.4	3.3	0.0	10.0	3.9	**−2.1***
DI	0.1	0.0	0.1	0.0	0.1	0.0	0.2	0.0	**−3.2****
SR	23.5	15.0	36.0	5.7	16.8	6.0	23.0	4.6	**3.7*****
XSR	13.1	0.0	24.0	5.3	6.9	3.0	13.0	2.5	**4.4*****
XSA	81.9	0.0	229.0	61.6	16.8	5.0	43.5	12.2	**4.1*****

Codes of statistically significant effects: <0.001 '***', <0.01'**', <0.05'*'

tion within than between populations, and no variation between two types of habitats (81%, 19% and 0% respectively), with F_{ST} value 0.186.

3.3. Factors affecting S. pennata genetic diversity and differentiation

RDA reveals that majority of variation in genetic diversity could be explained by predictor environmental factors. Constrained axes explain 72.86% of the total variance. First constrained axis explains 61.3% and second 11.5% of the total variance while all unconstrained axes explain together 27.14% of total variance. Permutation test for RDA under reduced model confirm statistical significance of model, however among constrained axis only the first one is statistically significant. Ho and uHe are negatively correlated with first axis while Na positively (Fig. 2). Predictors that significantly affected the level of genetic variation were: distance to nearest population, pH, crf, declination and egcover (Table 4). However, analysis revealed no distinct groups among populations and large overlap between primary and secondary habitats. Also the results of the Mantel test did not support a significant correlation between genetic distance and geographical distance as well as genetic distance and environmental factors.

3.4. Factors affecting S. pennata abundance

After computing PCA for blocks of collinear variables, we received three combined variables corresponding to: 1) climatic conditions, 2) soil and topography conditions, 3) species competition. The first of them is combination of temperature and rainfall factors correlated with first axis, and it correspond to the increase of rainfall and decrease of temperature, towards more humid bioclimatic conditions. The second one is correlation between soil physical and chemical properties, and declination of slopes with loadings of first component. This variable corresponds to decrease in soil compactness and pH, along with increase this factor, soil conditions become less extreme. The third one

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Table 2
Results from the similarity percentages analysis (SIMPER) of communities with share of *Stipa pennata* in two types of examined habitats.

Taxon	Average dissimilarity	Contribution %	Cumulative contribution %	Mean abundance; primary habitats	Mean abundance; secondary habitats
Calamagrostis epigejos	2.56	2.88	2.88	0.37	2.83
Brachypodium pinnatum	1.89	2.13	5.02	2.20	0.00
Poa pratensis	1.89	2.13	7.15	0.47	2.44
Festuca rupicola	1.66	1.88	9.03	1.97	0.00
Medicago falcata	1.64	1.85	10.87	2.00	0.17
Brachythecium albicans	1.61	1.82	12.69	0.37	1.78
Peucedanum oreoselinum	1.54	1.73	14.43	1.47	0.94
Galium verum	1.52	1.71	16.14	2.00	1.56
Achillea pannonica	1.48	1.67	17.81	1.17	1.61
Phleum phleoides	1.45	1.63	19.44	1.50	0.61
Vicia hirsuta	1.44	1.62	21.06	0.07	1.50
Euphorbia cyparissias	1.39	1.57	22.63	1.67	1.28
Potentilla arenaria	1.39	1.56	24.20	1.70	1.22
Salvia pratensis	1.29	1.46	25.65	1.40	0.00
Festuca duvalii	1.26	1.42	27.07	0.00	1.39
Potentilla argentea	1.20	1.35	28.42	0.07	1.39
Rumex acetosella	1.17	1.32	29.75	0.07	1.28
Arrhenatherum elatius	1.16	1.31	31.05	1.10	0.50
Overall average dissimilarity 88.66%					

Table 3
Genetic variability within 19 populations of *S. pennata*. Abbreviations: Na – number of alleles, Ne – number of effective alleles, Ho – observed heterozygosity, He – expected heterozygosity, I – Shannon diversity index, F_{ST} – fixation index, Pa – private alleles. Habitat type: p – primary, s – secondary.

Location (long/lat)	habitat type	uHe	Ho	He	Na	Ne	F	I	Pa
18.521047E/52.944679N	s	0.77	0.42	0.75	6.4	4.38	0.46	1.57	0.6
18.561291E/52.968095N	s	0.58	0.24	0.52	2.8	2.3	0.55	0.86	0
18.359976E/53.033198N	s	0.72	0.28	0.7	5.4	4.26	0.5	1.41	0.4
18.369725E/53.028499N	s	0.67	0.23	0.63	3.8	3.1	0.57	1.15	0
18.499682E/52.924002N	s	0.7	0.27	0.68	5.2	3.45	0.64	1.35	0.4
18.54093E/53.035469N	s	0.71	0.26	0.68	5.2	3.44	0.66	1.35	0
18.54817E/53.039342N	s	0.72	0.42	0.69	5	3.4	0.33	1.33	0.2
Mean		0.70	0.30	0.66	4.83	3.48	0.53	1.29	0.23
SD		0.06	0.08	0.07	1.17	0.70	0.11	0.23	0.24
20.689444E/50.580556N	p	0.74	0.24	0.70	5.80	3.81	0.67	1.46	1.00
20.672307E/50.420727N	p	0.67	0.25	0.64	4.20	3.16	0.58	1.19	0.20
20.616098E/50.593906N	p	0.69	0.25	0.66	4.20	3.08	0.57	1.22	0.40
17.704709E/52.978472N	p	0.46	0.48	0.42	3.00	2.18	−0.08	0.76	0.20
18.296565E/53.330514N	p	0.74	0.27	0.72	6.20	4.66	0.51	1.53	0.60
19.102677E/52.662133N	p	0.79	0.25	0.77	5.80	4.41	0.67	1.59	0.00
14.649526E/52.472001N	p	0.72	0.28	0.70	5.40	3.68	0.50	1.37	0.00
14.666387E/52.465616N	p	0.72	0.29	0.69	5.00	3.50	0.49	1.33	0.00
17.671428E/53.155291N	p	0.77	0.24	0.75	6.00	4.43	0.61	1.56	0.80
21.541111E/51.018889N	p	0.66	0.24	0.63	3.80	2.88	0.54	1.12	0.00
20.486481E/50.548244N	p	0.68	0.21	0.66	4.80	3.16	0.68	1.27	0.20
19.830045E/50.197651N	p	0.71	0.36	0.66	4.60	3.27	0.44	1.28	0.80
Mean		0.70	0.28	0.67	4.90	3.52	0.52	1.31	0.35
SD		0.08	0.07	0.09	0.99	0.72	0.20	0.23	0.36

correspond to competition with other plant species, along with increase of this factor, increase competition (with shrubs, trees and other expansive on grassland species) and decrease availability of open habitats (Table 5). The generalised linear mixed model determined that all three examined, environmental factors significantly affect *S. pennata* population size. Intensification of competition with more expansive species, as well as reducing the availability of open habitats exerts a strong, significant negative effect on the *S. pennata* population size. Also, increasing the humidity of bioclimatic conditions has a negative impact on response variable. While reducing the extremity of soil and topographic conditions had a slight but significant positive effect on the population size of *S. pennata.* Random effects were not statistically significant (Table 6).

4. Discussion

On one hand, urbanisation causes natural fragmentation of habitats and reduces their connectivity; on the other, in the case of species well adapted to open habitats, it may positively affect dispersal and even range expansion (Martínez-Abraín and Jiménez, 2016). Many endangered species are capable to colonising and inhabiting new anthro-

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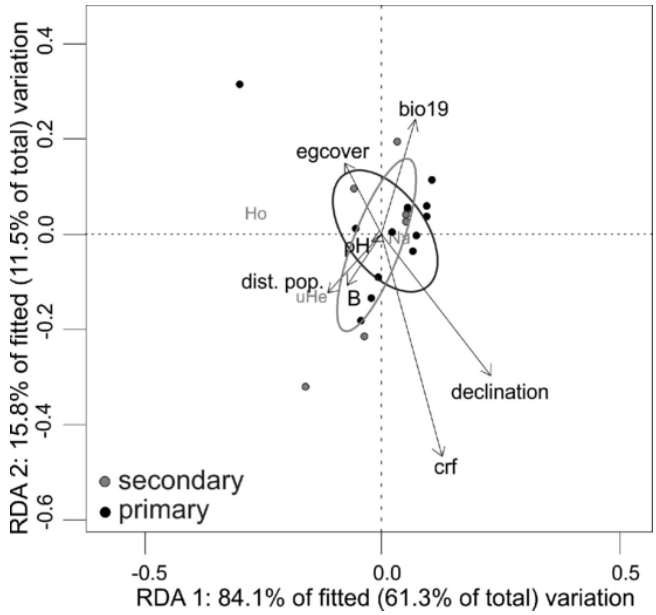

Fig. 2. Results of Redundancy Analysis (RDA) testing the effects of environmental factors on the level of genetic diversity of *Stipa pennata* populations.

Table 4
Results of Redundancy Analysis (RDA) testing the effects of environmental factors on the level of genetic diversity of *Stipa pennata* populations. For abbreviations and description of response and explanatory variables see Materials and Methods.

Factor	Variance	F statistic	P value
B	1.7949e−05	0.6309	0.385
declination	1.2313e−04	4.3275	0.024*
bio19	2.9649e−05	1.0420	0.328
crf	1.4561e−04	5.1177	0.015*
ph	1.9740e−04	6.9377	0.005**
egcover	9.4515e−05	3.3218	0.047*
dist. pop.	2.3204e−04	8.1552	0.003**
Residual	3.1298e−04		

Codes of statistically significant effects: <0.001 '***', <0.01'**', <0.05'*'.

Table 5
Results of Principal Component Analysis (PCA) grouping the variables into tree thematic groups. For each group of variables, factor loadings of PC 1 were presented. For abbreviations and description of response and explanatory variables see Materials and Methods.

Climatic conditions		Soil and topography conditions		Species competition	
Factor	PC1	Factor	PC1	Factor	PC1
bio1	−0.728	declination	−0,467	B	−0.175
bio2	0.765	AWCtS	0,095	A	0.630
bio3	−0.038	bld	−0,553	tcover	0.715
bio4	0.660	cec	−0,712	DI	0.897
bio5	−0.660	cly	−0,922	egcover	0.195
bio6	−0.967	crf	−0,266		
bio7	0.817	orc	0,679		
bio8	−0.667	ph	−0.910		
bio9	−0.914	slt	−0.959		
bio10	−0.667	snd	0.975		
bio11	−0.944				
bio12	0.948				
bio13	0.978				
bio14	0.761				
bio15	0.771				
bio16	0.977				
bio17	0.730				
bio18	0.977				
bio19	0.449				

Table 6
Results of generalized linear mixed models (GLMM) testing the effects of fixed factors on *Stipa pennata* population size. For description of response and explanatory variables see Materials and Methods.

Fixed effects				
	Estimate	*SE*	*Z* value	*P* value
Intercept	4.548	0.997	4.561	5.08e−06***
Species competition	−1.786	0.023	−76.575	<2.00e−16***
Soil and topography condition	0.469	0.017	27.783	<2.00e−16***
Bioclimatic condition	−0.701	0.013	−55.863	<2.00e−16***
Random effects				
	Variance	*SD*		
Geographic region	2.233	1.494		
Habitat type	0.872	0.934		

Codes of statistically significant effects: <0.001 '***', <0.01'**', <0.05'*'.

pogenic habitats, although the secondary habitats may differ from the primary in a number of characteristics (Rewicz et al., 2016; Zielinska et al., 2016). In Central Europe, such habitats have also become a convenient place for *Stipa pennata*, a relict steppe species which, over recent decades, has begun to vanish from Central Europe (Didukh, 2011; Iliashenko and Iliashenko, 2000; Kaźmierczakowa et al., 2014; Korneck et al., 1996; Procházka, 2001). Secondary habitats occupied by *S. pennata* differ from the originals, especially in terms of vegetation cover connected with light availability, type of soil, topography, and climate conditions. Within such habitats, various numbers of xerothermic species may co-occur with *S. pennata*; however, their general species composition definitely differs from that found in primary habitats. Despite the undoubtedly high share of xerothermic species in secondary habitats, their richness and abundance is lower than in primary ones. In this aspect, our observations are consistent with those reported from Finland (Jantunen et al., 2006). Moreover, populations of *S. pennata* in secondary habitats are less abundant than in primary habitats, possibly due to various factors including lower soil fertility, relative younger age of the population, overpowering anthropogenic pressure (e.g. mowing prior to the maturation of seeds, partial destruction of populations through the modernisation of road or railway infrastructures) (Kaźmierczakowa et al., 2014), or even a lower level of mycorrhizal colonisation of roots (Klichowska et al., 2019). Such reduced numbers of individuals in populations may indicate that xerothermic species are not fully adapted to secondary habitats, which are only partially suitable for their development. Hence, such habitats fit the definition of refuges rather than substitute habitats (Martínez-Abraín and Jiménez, 2016).

Europe is characterised by a high level of urbanisation (Nabielek et al., 2016); as a consequence, the natural habitats of many species have been destroyed. On the other hand, species began to colonize new, secondary habitats, Although levels of genetic diversity are often lower in such populations than in established ones (Amsellem et al., 2000; Jacquemyn et al., 2009; Kaňuch et al., 2014; Shirk et al., 2014), the case of *S. pennata* is rather unusual, as similar levels of genetic diversity have been observed in secondary and primary habitats. We also did not observe difference in genetic variation between studied habitat types. In both cases the most of genetic diversity (81%) was observed within populations, whereas 19% of genetic variation existed between the evaluated populations, which is typical for cross-pollinat-

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ing plants (Huang et al., 2016; Szczecińska et al., 2016) including *Stipa* species (Wagner et al., 2012, 2011). The relative small F_{ST} value (0.186), is comparable with the values reported for other cross-pollinating and long-lived perennial species (Huang et al., 2016; Szczecińska et al., 2016). Both, population from primary and secondary habitats are characterised by admixture of two different genotypes. Populations from secondary habitats are located in core of studied area, hence the share of both genotypes is within them comparable. In both, southern and north-western populations from primary habitats, usually only one of genotype predominates. All those results suggests existence of gene flow between populations from studied area and confirm that, in comparison with population from primary habitats, *S. pennata* populations from secondary habitats do not suffer from reduced genetic diversity. Similar situations have been observed in the cases of primary and secondary central European populations of other calcareous species, e.g. *Origanum vulgare* (Helsen et al., 2013) and *Saxifraga tridactylites* (Reisch, 2007), as well as in that of the saline species *Aster tripolium* and *Salicornia ramosissima* (Krüger et al., 2002). Krüger et al. (2002) hypothesised that the loss of genetic diversity in anthropogenic habitats caused by founder effects might be balanced by multiple colonisation events, gene flow between populations, or rapid growth of populations. Reisch (2007), however, explained the absence of genetic depauperation in *Saxifraga tridactylites* in terms of high rates of immigration and population growth after founding. Similar levels of genetic diversity within populations from primary and secondary habitats cannot be explained by population size, since populations from secondary habitats are smaller than those from primary ones. Moreover, rapid population growth is impossible, since *S. pennata* is a multiannual species. Similar levels of genetic diversity may also result from multiple introduction events and/or the long duration of the colonisation event; however, we do not know neither the exact date of the first incident of colonisation nor the demographic history of particular populations; we know only the dates of discovery of some populations (Ceynowa-Giełdon, 1976; Kaźmierczakowa et al., 2014). Nevertheless, xerothermic species in fragmented landscapes can persist only if both suitable habitats and dispersal pathways are maintained. Research confirms that secondary habitats may serve as dispersal corridors for grassland species (Tikka et al., 2001). This is not surprising, since, in comparison to sharply fragmented grasslands, linear landscape elements provide almost continuous strips of available habitats. Such linear elements of the landscape, related to human activity, were repeatedly mentioned as significant for nature conservation (Moroń et al., 2017, 2014). Our results confirm that genetic diversity of *S. pennata* is significantly affected by distance to nearest population. Taking into account that average distances between neighbouring populations at secondary sites are smaller than in primary grasslands, exchanges of genes may be more efficient in the case of former one. We noticed that observed levels of heterozygosity are lower than expected in both primary and secondary habitats, which may suggest the adverse effect of such processes as inbreeding (Freeland et al., 2011) on the conservation of populations from the edges of the general range of distribution.

Specialist species are the winners of competition only under strictly defined conditions, when conditions deteriorate, they give way to generalists (Chmolowska et al., 2019; Hülber et al., 2017; Ramiadantsoa et al., 2018). According to Ramiadantsoa et al. (2018), specialists dominate in landscapes of high quality, whereas generalists thrive in those of intermediate quality, including disrupted and fragmented habitats. Thus, competition, (including: cover of trees, shrubs and more expansive herbaceous plants) appear to be the most important factor affecting not only population size of *S. pennata*, but also directly affecting the genetic diversity of this species. Hence, probably in the long term, the survival of its populations as well. The strong negative impact of competition is rather obvious, since photophilous species such as *S. pennata*, as a result of shading (less light availability, higher humidity), disappear from grasslands overgrown by shrubs (Barańska, 2014; Comino et al., 2008; Tälle, 2018). Plots in secondary habitats with shares of *S. pennata* were often dominated by the expansive *Calamagrostis epigejos* (or this species occurred in the neighbouring plots of vegetation), whereas original habitats were often occupied by another expansive grass, *Arrhenatherum elatius* or *Brachypodium pinnatum*, indicating disturbance of the habitat. Intensive mowing, as it disrupts the growth of shrubs and trees, is an undeniable advantage of secondary habitats; however, in some situations, it can also constitute a disadvantage. According to Barańska (2014), intensive mowing can support the development of clonal species (such as *B. pinnatum*, *C. epigejos*, or *A. elatius*) and reduce the occurrence of the clump ones (such as *S. pennata*, *Festuca duvalii*, *F. rupicola*, *Avenastrum pretense*, and *Phleum phleoides*). The presence of expansive grass species is attributed to changes in soil nutrient availability, humidity of the habitat, and decreased light availability (Barańska, 2014; Butaye et al., 2005; Comino et al., 2008). The increased humidity of secondary habitats associated with the expansion of *C. epigejos* can be indirectly concluded from the greater abundance of mosses. A specialised xerothermic species can survive in extremely dry conditions and occupy niches unsuitable for most species; however, in more mesophilic conditions, it will lose in competition with other species (Barańska, 2014; Butaye et al., 2005; Comino et al., 2008). Most likely, for the same reasons, we noticed a decline in the population of *S. pennata* towards more humid bioclimatic conditions. Yet, *S. pennata*, as a species distributed widely in Eurasian steppes (Kaźmierczakowa et al., 2014), is adapted to relatively dry climatic conditions prevailing there (Werger et al., 2012). Our results are consistent with previous research (Heinicke et al., 2016), which shows that southern Siberian *S. pennata* populations (in the case of size, density and genetic diversity), are negatively influenced by precipitation.

5. Conclusions

In this paper we underline the role of secondary habitats for conservation of xerothermic species. Our results suggest that for xerothermic species secondary habitats should be treated more like refuges (with suboptimal conditions) than substitution habitats. Biotic factors connected with concurrency and habitat connectivity predominantly affect the abundance and genetic diversity of xerothermic species. On one hand, the occurrence of these species in secondary habitats may be limited by the pressure of expansive grasses; on the other, the negative impact of the latter is buffered by the reduced presence of shrubs, and thus the greater availability of light. Despite the differences in ecological conditions between primary and secondary habitats, *S. pennata* populations from the latter one, do not suffer from genetic depauperation. These results indicate the compensating effect of habitat connectivity on small numbers of individuals in populations at secondary sites. Because mowing, which inhibits natural succession, is performed on secondary habitats for practical reasons (to ensure safety on the road), no additional cash outlays are needed for active protection of these habitats. It is possible that slight adjustments in mowing schedules, as well as the application of a restoration method to suppress expansion of *Calamagrostis epigejos* (at least in some of the more valuable patches), may optimise chances of survival for xerothermic species. By maintaining meta-population processes through dynamic connectivity between habitats on the landscape scale, secondary habitats play an undoubtedly important role in the conservation of xerothermic species. In face of negative changes in surrounding landscape, they may become the last refuge of these species in Central Europe.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by National Science Center (Poland), grant numbers: 2014/15/N/NZ8/00340 and 2013/09/B/NZ8/03287. We thank to the Regional Directorates for Environmental Protection in Bydgoszcz, Gorzów Wielkopolski, Kielce and Lublin as well as Ministry of the Environment and Ojców National Park for authorisation for collection of protected plants and conducting the research in the protected areas [decisions numbers WPN.6400.16.2013.JC.1, WPN.6400.26.2015.JC, WPN. 6400.25.2015.JC, WPN.6400.41.2015.NG, WPN.I.6400.6.2015.AC, WPN.I.6400.17.2015.BD, WPN.I.6400.9.2013.BD, WPN-I.6400.61.2014.AT, WPN-I.6205.25.2015.AI, WPN.6400.8.2015.MPR, DNE-0620/1/2015, NB-0620/23/2015, NB-0604.33/2016, DLP-III-4102-227/3/409/15/MD, DLP-III-4102-227/3/4/2/15/MD].

Author contributions

MN and EK planned and designed the research; MN and EK identified plant species and collected samples; EK, MŚ and MSz conducted laboratory analysis; EK performed statistical analysis; EK and MN interpreted the results and wrote the paper with the input and final approval of other co-authors.

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