diversity

MDPI

Article

Assessment of Anthropogenic Impacts on the Genetic Diversity of *Phragmites australis* in Small-River Habitats

Jolanta Patamsytė [1], Carla Lambertini [2], Jurgita Butkuvienė [1], Donatas Naugžemys [3] and Donatas Žvingila [1,*]

[1] Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio Av. 7, 10257 Vilnius, Lithuania; jolanta.patamsyte@gf.vu.lt (J.P.); jurgita.butkuviene@gmc.vu.lt (J.B.)
[2] Department of Bioscience, University of Milan, Via Celoria 26, 20133 Milan, Italy; carla.lambertini@unimi.it
[3] Botanical Garden, Vilnius University, Kairėnų Str. 43, 10239 Vilnius, Lithuania; donatas.naugzemys@gf.vu.lt
* Correspondence: donatas.zvingila@gf.vu.lt

Abstract: Common reed is often used as a model plant to study the anthropogenic impacts on ecosystems at local and global scales. As a community-forming species, it is directly exposed to the impacts of human activities on the ecosystem. The aim of our study was to evaluate the patterns of genetic diversity in common reed stands located in habitats that are differently affected by anthropogenic factors. We studied whether riverbed modifications, land cover in the neighborhood of the stand and the chemical and physical parameters of the river water affect the genetic diversity of *P. australis* at the studied sites. Using DNA fingerprinting, we genotyped 747 plants from 42 sites located in 16 small Lithuanian rivers. Bayesian clustering and principal coordinate analysis revealed two main gene pools at the population (river) level. At the site level (i.e., considering all sites independently of their rivers), polymorphism was high even between sites in the same river. Our study revealed a negative relationship between the concentration of nitrogen compounds and the genotypic richness of *P. australis* populations. We did not find any correlations between the other chemical parameters of the water and the parameters of the genetic diversity. Additionally, there were no genetic differences between sites in modified and unmodified river sections or between sites that differed in land cover type in the neighborhood of the stand.

Keywords: DNA polymorphism; ISSR; genetic structure; habitat fragmentation; eutrophication; nutrients; clonal diversity

Citation: Patamsytė, J.; Lambertini, C.; Butkuvienė, J.; Naugžemys, D.; Žvingila, D. Assessment of Anthropogenic Impacts on the Genetic Diversity of *Phragmites australis* in Small-River Habitats. *Diversity* **2023**, *15*, 1116. https://doi.org/10.3390/d15111116

Academic Editor: Petras Prakas

Received: 9 October 2023
Revised: 23 October 2023
Accepted: 25 October 2023
Published: 26 October 2023

1. Introduction

Genetic diversity is an essential component of biodiversity. It is an objective measure of species dynamics and is generally regarded as an indicator of environmental conditions on the basis of all processes on which biodiversity (species diversity, ecosystem diversity) depends [1]. The extent of genetic diversity controls the ability of populations to adapt to environmental changes and is therefore the core of their long-term survival [1,2]. Together with species diversity, it can play a very important role in ecosystem responses to stressors. High genetic diversity can, in fact, increase the likelihood in the community of genotypes being resistant to one or multiple biotic or abiotic factors in the community and, at the same time, enhance the ability of the ecosystem to function under adverse conditions [3]. The loss of genetic diversity puts species at risk of extinction [4].

Anthropogenic stressors cause changes in gene flow and selection and can trigger genetic drift and mutations in populations and increase population differentiation [5–7]. It has been demonstrated that fragmentation of natural habitats results in a decrease in genetic diversity within populations [8]. In many cases, anthropogenic pressure interacts with natural factors [9,10]. Therefore, it is important to understand how genetic diversity changes with global environmental changes, especially in habitat-forming species, and predict patterns of ecosystem functioning.

Common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) is a habitat-forming species in aquatic ecosystems that serves important functions such as stabilization and protection of the shore, removing significant amounts of pollutants from agricultural runoffs and providing food and a home to animals and other organisms [11,12]. Common reed is widespread all over the planet along riverbanks and lakeshores on all continents except Antarctica [13–15]. This genetically diverse plant can grow under very different climatic and ecological conditions. Some lineages of the common reed are invasive and colonize various disturbed ecosystems in North America [16–18]. *P. australis*, like many other macrophytes, can reproduce sexually through seeds and asexually through rhizomes or broken rhizome fragments [19,20]. Pollen and seeds are carried by the wind [20]. Dispersal also occurs through water, as rhizome fragments and seeds can float on water [21], or can be carried by birds [13,22]. The role of rhizome fragments in the dispersal of species over large distances appears to be rather limited compared to that of seed dispersal [23–25]. Seed germination usually results in genetically distinct individuals, while spread via rhizome fragments or vegetative growth inside stands determines clonal propagation. Kettenring et al. [17,26] reported that the proportion of viable seeds and seed viability were positively associated with stand genetic diversity; therefore, the greater the genetic diversity of a stand is, the more seeds are produced. It has been recognized that genetic diversity, either from sexual reproduction or from genome structure rearrangements and changes in gene expression, can be an important factor for invasion in new ranges [27–30]. River ecosystems and especially macrophyte communities are strongly impacted by natural and anthropogenic environmental changes. Humankind disturbs river ecosystems via pollution, damming, hydrological alterations and alien species dispersal [10,31–35]. Anthropogenic and natural factors frequently leave detectable footprints on affected populations genetic diversity patterns and structure [8,34,36–38]. For example, Koppitz and Kühl [39], based on DNA polymorphisms in common reed stands, revealed that polymorphic multiclonal populations established by seeds can become dominated by one or a few locally adapted genotypes over time, probably due to competition. In accordance with this, Engloner and Major [40] showed that the genotype number in a population decreases with increasing water depth as a result of competition among clones toward the open water. McCormick et al. [37] revealed that common reed populations were more genetically diverse and had more genotypes as habitat disturbance increased. Hazelton et al. [41] observed that older common reed stands exhibit lower clonal richness and shorter interclonal genetic distances than young stands. Liu et al. [34] highlighted the potentially negative impact of dams on rhizome dispersal and local adaptation of *P. australis*. Kuprina et al. [25] analyzed the genetic diversity of populations from northeastern Germany under different levels of disturbance caused by water salinity and mowing and concluded that disturbance is important for the accumulation of genetic diversity. Guo et al. [18] found that even on a continental scale, the human footprint has a stronger effect than climate on the genetic structure of *P. australis* populations. In contrast, Paul et al. [42] did not find any correlation between the impact of management and/or habitat disturbance and the genetic diversity pattern of common reed stands in the United Kingdom.

The genetic diversity of *P. australis* populations can also be influenced by environmental pollution caused by agriculture, sewage and industry. Reeds thrive in environments enriched with nutrients (eutrophication) and industrial pollution [43–45] and are successfully used for contamination control and phytoremediation [46–48]. For example, it has been shown that reeds can accumulate heavy metals [43,49,50]. On the other hand, heavy metals and other toxic chemicals are environmental stressors and can cause genetic effects in plants [31,51]. Competition between common reed genotypes may also take place in such stressful environments. Coppi et al. [31] found a significant correlation between genetic diversity and environmental concentrations of certain heavy metals and explained this phenomenon as the possible genotoxic effect of heavy metals (Cr, Cu and Zn). Excess nutrients in the environment can also impact the genetic structure of clonal plants that use

both vegetative and sexual reproduction, leading to shifts to prevalent clonal growth and changes in reproductive modes [13,52,53].

Common reed is abundant in all the territory of Lithuania, mainly around water bodies and occasionally in roadside ditches and sandy soils [35]. These plants establish themselves in new sites quite quickly, whenever suitable moisture and nutrient conditions occur. This indicates that there is a constant circulation of seeds of this species in the environment. River ecosystems shape suitable conditions for the establishment of reedbeds and have an impact on their genetic diversity [34]. Lithuania's rivers are suffering eutrophication due to intensive agriculture and urbanization. According to the Lithuanian Environmental Protection Agency (EPA) [54], the concentration of nitrogen nitrate in river catchments in agricultural districts is significantly higher (at least 3–4 times higher) than that in the rivers in forest areas. Nitrate levels in some rivers significantly exceed the values of good water quality by 2–3 times (in 60% of cases), and this excess is 4–9 times higher in one third of the cases. High concentrations of N were detected in common reed leaves collected in many Lithuanian rivers [33]. The reedbeds in Lithuanian rivers were also influenced by river regulation works, which were very intense in 1950–1990 [35]. The excavation works associated with the straightening and modification of the riverbeds and the removal of the upper soil layer could have contributed to the establishment of new reed populations from the soil seed bank or from seed [25,36,55]. In a previous study [35], we used simple sequence repeat (SSR) markers to assess how river modifications impact the genetic diversity patterns of Lithuanian *P. australis* populations. Our results showed that differences in genetic diversity between the populations in modified and natural river sections cannot be explained solely by the hydraulic works carried out in the past century. In this study, we analyzed a larger sample set and other regions of the *Phragmites* genome to assess the anthropogenic factors that can impact the genetic diversity of the populations of this species. Here, we employed intersimple sequence repeats (ISSRs), dominant molecular markers that are highly polymorphic, sufficiently reliable, inexpensive and, as some other dominant markers, equally informative as SSRs in the analysis of polyploid genotypes [56]. The application of the ISSR technique enabled the investigation of DNA polymorphisms between adjacent, inverted microsatellite loci, located at suitable distances for PCR amplification, and produced reproducible multilocus band patterns [38,57–63]. In addition, these markers allowed us to assess a larger number of loci than the 10 SSR loci commonly used for *P. australis* [64].

Our aim was to quantify the genetic diversity of Lithuanian common reed populations using ISSRs and to assess how disturbance, land cover in the neighborhood of the stand and the chemical and physical parameters of the river water affect the genetic diversity of *P. australis* populations. Our study should provide new insights into the adaptation and dynamics of common reed populations to anthropogenic disturbance.

2. Materials and Methods

2.1. Sampling Sites and Common Reed Samples

A total of 747 common reed samples were collected in the summer periods of 2015–2018 from 42 sites located at 16 Lithuanian rivers (Table 1, Figure 1).

Table 1. *Phragmites australis* 42 sampling sites name, code, number of analyzed samples and identified genets, location and some habitat characteristics.

No.	River Site	Code	Samples		Coordinates		L	M	H
			N	G	Latitude	Longitude			
1.	Varėnė 1	VR1	24	9	54.391611	24.407747	A	+	–
2.	Varėnė 2	VR2	16	16	54.329047	24.511211	N	–	+
3.	Varėnė 3	VR3	28	13	54.250614	24.554161	U	–	–
4.	Merkys 1	MR1	24	24	54.436978	24.982144	A	+	+

Table 1. *Cont.*

No.	River Site	Code	Samples		Coordinates		L	M	H
			N	G	Latitude	Longitude			
5.	Merkys 2	MR2	8	7	54.409117	24.910669	A	+	+
6.	Merkys 3	MR3	8	4	54.388197	24.893189	N	−	+
7.	Merkys 4	MR4	7	3	54.337967	24.822267	N	−	+
8.	Merkys 5	MR5	12	6	54.336086	24.808022	N	−	+
9.	Merkys 6	MR6	11	5	54.118106	24.302706	N	−	+
10.	Skroblus	SK	12	1	54.105942	24.279528	N	−	+
11.	Grūda 1	GR1	24	4	54.022956	24.333617	A	+	+
12.	Grūda 2	GR2	14	14	54.110042	24.352975	N	−	+
13.	Verseka 1	VS1	24	4	54.180353	24.949422	A	+	+
14.	Verseka 2	VS2	28	13	54.311475	24.815764	A	−	+
15.	Šalčykščia 1	SC1	10	3	54.255969	25.214581	N	−	−
16.	Šalčykščia 2	SC2	18	8	54.266883	25.178094	N	+	−
17.	Šalčia 1	SL1	18	2	54.319219	25.403611	U	+	+
18.	Šalčia 2	SL2	12	3	54.291917	25.209867	N	−	+
19.	Šalčia 3	SL3	7	4	54.299950	25.202411	N	−	+
20.	Šalčia 4	SL4	3	3	54.304858	25.141147	N	−	+
21.	Beržė	BR	41	4	54.298883	25.204058	N	+	+
22.	Visinčia 1	VN1	24	3	54.323150	25.507589	A	+	−
23.	Visinčia 2	VN2	12	2	54.386056	25.374642	A	−	+
24.	Visinčia 3	VN3	6	1	54.370264	25.271892	N	−	−
25.	Taurupis 1	TR1	16	7	54.284014	24.850161	A	+	−
26.	Taurupis 2	TR2	20	4	54.300647	24.838781	A	+	−
27.	Nevėžis 1	NV1	10	3	55.511533	24.768736	A	+	+
28.	Nevėžis 2	NV2	17	8	55.533814	24.682608	A	+	+
29.	Nevėžis 3	NV3	24	8	55.527264	24.698569	A	+	+
30.	Nevėžis 4	NV4	24	2	55.700283	24.433556	U	−	+
31.	Pienia 1	PN1	24	23	55.511019	24.771975	A	+	+
32.	Pienia 2	PN2	16	1	55.434881	24.928181	U	−	+
33.	Širvinta 1	SR1	18	1	55.062150	25.198025	A	+	+
34.	Širvinta 2	SR2	24	3	55.028725	25.008336	N	−	+
35.	Siesartis 1	ST1	24	8	55.227114	25.270822	A	+	−
36.	Siesartis 2	ST2	18	4	55.291158	24.893558	N	−	+
37.	Siesartis 3	ST3	6	3	55.226875	25.248489	A	+	−
38.	Šešupė 1	SP1	20	11	54.356864	23.063047	A	+	+
39	Šešupė 2	SP2	24	18	54.409133	23.225194	U	−	+
40.	Šešupė 3	SP3	23	17	54.417664	23.250158	N	−	+
41.	Kiauna 1	KN1	24	24	55.306550	25.88635	N	−	+
42.	Kiauna 2	KN2	24	24	55.294283	25.898083	N	−	+

N—number of analyzed individuals per site; G—number of genets (genotypes); L—land cover type in the neighborhood of the stand (N—seminatural, A—agricultural, U—urbanized); M—riverbed modification status; H—availability of hydro-chemical and physical data.

The number of collection sites per river varied from 1 (Skroblus River, Beržė River) to 6 (Merkys River) and was generally proportional to river size. Plants were sampled on transects along the coastline. The distance between samples was at least 5 meters if the stand was small and 10 or more meters if the stand at the sampling site was larger. The tips of the plants with the youngest leaves were separated from the plant with secateurs, placed in a plastic bag with an attached label indicating the location of the plant collection and placed in a car refrigerator on ice. Samples were transported to the laboratory, where leaves were washed and used for DNA extraction.

The prevailing land cover in the neighborhood of the sampled stand was recorded using Google Maps and by directly visiting the sites. The land cover type was assessed as seminatural (N)—undisturbed wetland or forest; urbanized (U)—in the vicinity of artificial surfaces, in most cases in urbanized territories; and agricultural (A)—near areas of intensive agricultural land use. Some hydro-chemical characteristics (pH, dissolved

oxygen, BOD_7 (biochemical oxygen consumption over 7 days), ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, mineral nitrogen, total nitrogen (N_T), phosphate phosphorus, total phosphorus, specific electrical conductivity), during the period of 9 years (2010–2018) were obtained from the Lithuanian EPA database [54]. Averages of the nine-year values of these indicators were used. River modification data were obtained from the Lithuanian EPA and Lithuanian Energy Institute [35].

Figure 1. The location of 42 common reed (*Phragmites australis*) sampling sites. Codes of the sites are explained in Table 1.

2.2. DNA Extraction

Approximately 100 mg of cleaned tissue from young healthy leaves was weighed and used for DNA isolation. DNA was extracted from leaf tissue using a modified version of the CTAB method adapted to extract DNA from small amounts of plant material [65]. The DNA quality was assessed using 0.8% agarose gel electrophoresis and a spectrophotometer. The DNA concentration was determined using a BioPhotometer (Eppendorf, Hamburg, Germany). The DNA samples were stored at −20 °C until use.

2.3. ISSR Marker Analysis

ISSR-PCR conditions were as described previously by Patamsytė et al. [65]. Amplification was performed in a 10 µL volume containing 1× PCR buffer, 10 ng of genomic DNA, 3 mM $MgCl_2$, 5 µM primers, 200 µM dNTPs and 0.4 U of *Taq* DNA polymerase (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). Amplification reactions were run on a Mastercycler Pro (Eppendorf, Hamburg, Germany) under the following protocol: starting denaturation cycle at 94 °C for 7 min; 32 cycles of 30 s at 94 °C, 45 s at the primer-specific annealing temperature (from 45 °C to 51 °C, Table S1) and 2 min at 72 °C, and a final step of 7 min at 72 °C. A total of 7 ISSR primers (CCA$(GTC)_4$, $(AG)_8$CG, GTGC$(TC)_7$, $(CCA)_5$, $(GGGT)_3$GTG, $(CA)_8$A and $(AC)_8$T) suitable for common reed genome analysis were selected from the 20 tested. These primers amplified numerous clear and reproducible DNA bands. The amplification products were analyzed in large 1.5% agarose gels using 0.5×

TBE buffer (4 h, 4 V/cm) and stained with ethidium bromide. ISSR-PCR banding patterns were recorded using a UV transilluminator and BioDocAnalyze software (Biometra, Göttingen, Germany). GeneRuler^{TM} DNA Ladder Mix (100–10,000 bp) (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) was used for DNA fragment size assessment. DNA bands in the size range of 310–2000 bp were scored. Each DNA sample was examined in independent amplification reactions at least twice to ensure reproducibility. The genotyping error rate was 1.6%. Problematic loci were not included in the data analysis.

2.4. Data Analysis

Reproducible DNA bands generated by ISSR-PCR were scored and used to construct a binary data matrix that was used for further analyses. The presence of a DNA band was indicated as 1 and the absence as 0. Monomorphic bands were not included in the analysis.

Genetic diversity parameters and differentiation were assessed on different genotypes (genets). Identical genotypes (ramets) within sites were excluded from these calculations. Clones were defined using the multilocus matches function in GenAlEx v. 6.5 [66]. We calculated the genotypic richness (R) using the formula $R = (G - 1)/(n - 1)$, as suggested by [67], where G is the number of different genotypes (genets) at a studied site and n is the total number of plants collected from a given site. The total gene diversity at the species level (Ht) and average gene diversity at the site and population levels (Hw) were estimated using AFLP-SURV [68]. The number of alleles (Na), the number of effective alleles (Ne) and Shannon's information index (I) were calculated using POPGENE 1.31 software [69]. The percentage of polymorphic loci (P) and expected heterozygosity (He) were estimated using GenAlEx v 6.5 [66].

The analysis of molecular variance (AMOVA; [70]) was used to define genetic variability among *P. australis* populations and within populations. AMOVA was performed using GenAlEx v. 6.5 [66]. AMOVA was also used to assess how genetic diversity is partitioned between rivers (populations), between sampling sites within rivers and within sites based on Euclidean pairwise distances among individuals. Only the populations that had two or more sampling sites were included in this calculation. Significance testing was carried out using 999 permutations. Four sites were not included in the analysis because they contained only one unique genotype (see Table 1). Hierarchical AMOVA was also used to assess genetic differences between sites that varied according to the type of land cover (seminatural—N; agricultural—A; and urbanized—U) in the neighborhood and groups of sites from modified and natural river stretches. Genetic distances among sites and populations were determined using principal coordinate analysis (PCoA) based on the Φ_{PT} matrix. The PCoA was performed using GenAlEx v. 6.5. The correlation between genetic (Φ_{PT}) and geographic distances was estimated and the effect of isolation by distance (IBD) among sites was assessed using the Mantel test (999 permutations) in GenAlEx v.6.5.

The genetic structure and the extent of admixture between genotypes, sites and populations were assessed using the Bayesian clustering method implemented in the software STRUCTURE v. 2.3.4 [71,72] using an admixture model. The likelihood L(K) and the values of K (ΔK) from K = 1–42 were computed for the sites and from 1 to 16 for populations, and the most likely number of clusters (K) was identified according to Evanno et al. [73] with STRUCTURE HARVESTER. The study used an initial burn-in period of 20,000 steps and 40,000 MCMC (Markov chain Monte Carlo) iterations [73] with 10 independent replicates each.

For the examination of differences between groups of sites subdivided according to land-use type in the neighborhood of the stand, we used TIBCO Statistica^{TM} v13.3.0 (TIBCO Software Inc., Palo Alto, CA, USA, 2017) and performed the Kruskal-Wallis H test. We also assessed the relationships between the *P. australis* genetic diversity parameters (R, PL, polymorphism, Na, Ne, I and He) of 32 sites and the water chemical and physical parameters (pH, dissolved oxygen, BOD_7, NH_4-N, NO_2-N, NO_3-N, mineral N, total N, PO_4-P, total P and specific electrical conductivity) using Spearman's rank correlations.

3. Results

3.1. Genetic Diversity Based on ISSR Markers

The 747 DNA samples were successfully amplified and yielded reproducible banding patterns (ISSR phenotypes). Further analysis of these samples with 7 preselected primers identified 182 loci (bands) (Table S1). The number of polymorphic bands per primer ranged from 20 to 31. A total of 339 distinguishable multilocus genotypes (genets) were identified and used for data analysis. Of the 42 sampling sites scored in 16 Lithuanian rivers, 38 showed genotypic polymorphism (Table 1). The number of genets per multiclonal site varied from 24 (MR1, KN1, KN2) to 2 (SL1, VN2, NV4). Only 1 genotype per site was identified at 4 monoclonal sites (SK, VS3, PN2, SR1). Genotypes from these four sites showed site-specific banding patterns with all tested primers. The mean number of genets per site was 8.368 ± 1.130. The percentage of polymorphic loci per multiclonal site ranged from 1.1% (VN2) to 51.1% (KN2), with an average of 26.62 ± 2.36% (Table 2).

Table 2. Sampling site genetic diversity parameters of *Phragmites australis* revealed using ISSR markers.

Site	*PL*	*P*, %	*R*	*Na*	*Ne*	*I*	*He*
VR1	81	44.51	0.348	1.445	1.309	0.256	0.174
VR2	71	39.01	1.000	1.390	1.241	0.210	0.141
VR3	95	52.20	0.444	1.522	1.331	0.284	0.191
MR1	58	31.87	1.000	1.319	1.204	0.171	0.116
MR2	55	30.22	0.857	1.302	1.208	0.171	0.117
MR3	31	17.03	0.429	1.170	1.109	0.092	0.062
MR4	3	1.65	0.333	1.017	1.013	0.010	0.007
MR5	6	3.30	0.455	1.033	1.020	0.018	0.012
MR6	4	2.20	0.400	1.022	1.018	0.014	0.010
SK	0	0	0.000	1	1.000	0.000	0.000
GR1	3	1.65	0.130	1.017	1.009	0.008	0.005
GR2	67	36.81	1.000	1.368	1.262	0.215	0.148
VS1	57	31.32	0.130	1.313	1.221	0.189	0.130
VS2	76	41.76	0.444	1.418	1.285	0.235	0.160
SC1	48	26.37	0.222	1.263	1.182	0.153	0.104
SC2	68	37.36	0.412	1.374	1.266	0.217	0.149
SL1	51	28.02	0.059	1.280	1.184	0.153	0.103
SL2	6	3.30	0.182	1.033	1.017	0.017	0.011
SL3	33	18.13	0.500	1.181	1.128	0.110	0.075
SL4	35	19.23	1.000	1.192	1.140	0.114	0.078
BR	59	32.42	0.075	1.324	1.213	0.180	0.122
VN1	50	27.47	0.087	1.275	1.198	0.162	0.111
VN2	2	1.10	0.091	1.011	1.008	0.007	0.005
VN3	0	0	0.000	0.000	1.000	0.000	0.000
TR1	42	23.08	0.400	1.231	1.154	0.129	0.088
TR2	38	20.88	0.053	1.209	1.148	0.126	0.086
NV1	21	11.54	0.222	1.115	1.081	0.067	0.046
NV2	76	41.76	0.438	1.418	1.304	0.248	0.171
NV3	61	33.52	0.304	1.335	1.225	0.193	0.131
NV4	43	23.63	0.043	1.236	1.167	0.143	0.098
PN1	60	32.97	0.957	1.330	1.206	0.177	0.119
PN2	0	0	0.000	0.000	1.000	0.000	0.000
SR1	0	0	0.000	0.000	1.000	0.000	0.000
SR2	40	21.98	0.087	1.220	1.155	0.128	0.088
ST1	57	31.32	0.304	1.313	1.210	0.178	0.121
ST2	33	18.13	0.176	1.181	1.126	0.107	0.073
ST3	25	13.74	0.200	1.137	1.105	0.083	0.058
SP1	54	29.67	0.526	1.297	1.201	0.168	0.115
SP2	80	43.96	0.739	1.440	1.276	0.236	0.160

Table 2. *Cont.*

Site	*PL*	*P*, %	*R*	*Na*	*Ne*	*I*	*He*
SP3	80	43.96	0.727	1.440	1.291	0.243	0.165
KN1	79	43.41	1.000	1.434	1.261	0.228	0.153
KN2	93	51.10	1.000	1.511	1.302	0.268	0.178
Average [1]	43.83	24.09	0.399	1.169	1.161	0.136	0.092
SE [1]	4.48	2.46	0.052	0.055	0.016	0.014	0.009
Average [2]	48.447	26.62	0.441	1.266	1.178	0.150	0.102
SE [2]	4.300	2.36	0.053	0.024	0.015	0.013	0.009

PL—the number of polymorphic loci; *P*—the percentage of polymorphic loci; *R*—genotypic richness; *Na*—observed number of alleles; *Ne*—effective number of alleles'; *I*—Shannon's information index; *He*—expected heterozygosity; SE—standard error; [1]—all sites; [2]—only multiclonal sites.

The genotypic richness (*R*) at 42 sampling sites ranged from 1 to 0. The highest *R* values were detected at the VR2, MR1, GR2, SL4, KN1 and KN2 sites, and the lowest at the SK, VN3, PN2 and SR1 sites. The mean number of observed alleles (*Na*) at 38 studied polymorphic sites was 1.266 ± 0.024 and the mean number of effective alleles (*Ne*) was 1.178 ± 0.015. The Shannon information index (*I*) was highest at VR3 (0.284) and lowest at the VN2 site (0.007). The average value of *I* was 0.150 ± 0.013. Expected heterozygosity (*He*) ranged from 0.191 (VR3) to 0.005 (GR1, VN2).

When genetic diversity parameters were also calculated for the 16 rivers, the following average values were generated: *R*—0.377 ± 0.068; *Na*—1.482 ± 0.105; *Ne*—1.355 ± 0.033;, *I*—0.299 ± 0.028; and *He*—0.203 ± 0.019 (Table 3).

Table 3. Genetic diversity of *Phragmites australis* populations at ISSR loci.

Population	*PL*	*P*, %	Samples		*R*	*Na*	*Ne*	*I*	*He*
			Total	*G*					
VR	147	80.77	68	38	0.552	1.808	1.494	0.430	0.288
MR	132	72.53	70	49	0.696	1.725	1.458	0.389	0.263
SK	0	0	12	1	0.000	0.000	1.000	0.000	0.000
GR	90	49.45	38	18	0.459	1.495	1.358	0.289	0.199
VS	113	62.09	52	17	0.314	1.621	1.409	0.340	0.231
SC	110	60.44	28	11	0.370	1.604	1.412	0.337	0.230
SL	119	65.38	40	13	0.308	1.654	1.457	0.372	0.254
BR	59	32.42	41	4	0.075	1.324	1.214	0.180	0.122
VN	94	51.65	42	6	0.122	1.517	1.312	0.277	0.185
TR	78	42.86	36	9	0.229	1.429	1.275	0.235	0.158
NV	136	74.73	75	22	0.284	1.747	1.500	0.419	0.285
PN	83	45.60	40	24	0.590	1.456	1.283	0.241	0.162
SR	62	34.07	42	4	0.073	1.341	1.241	0.199	0.136
ST	113	62.09	48	14	0.277	1.621	1.420	0.349	0.238
SP	129	70.88	67	46	0.682	1.709	1.460	0.389	0.263
KN	119	65.38	48	48	1.000	1.654	1.393	0.342	0.229
Average	99.00	54.40	46.69	20.25	0.377	1.482	1.355	0.299	0.203
SE	9.25	5.08	4.16	4.09	0.068	0.105	0.033	0.028	0.019

PL—the number of polymorphic loci; *P*—the percentage of polymorphic loci; *G*—number of genets; *R*—genotypic richness; *Na*—observed number of alleles; *Ne*—effective number of alleles; *I*—Shannon's information index; *He*—expected heterozygosity; SE—standard error.

The highest number of genets was found in the Merkys River (MR)—49; the lowest number of genotypes was detected in samples from the Skroblus River (SK)—1. The mean number of genets per population was 20.25 ± 4.09. The genetic diversity at the species level was calculated with AFLPSURV, *Ht* = 0.3503, and that calculated at the population (river) level was *Hw* = 0.2201.

The correlation between genetic and geographical distances of sites according to the Mantel test was nonsignificant (R^2 = 0.0033, p = 0.255).

3.2. Genetic Differentiation of Sites and Populations

The hierarchical AMOVA partitioned the total genetic diversity of Lithuanian common reed into the following components: among populations (rivers), among sites and within sites. The percentages of these components of genetic diversity were 14, 33 and 53, respectively. The Φ_{PT} value calculated on the basis of ISSR markers was 0.322 (p = 0.001).

We carried out principal coordinate analysis (PCoA) for the comparison of genetic relationships among polymorphic study sites. The first axis of the PCoA explained 22.3% of the genetic variance among sites. Three main axes explained 59.18%. The resulting diagram of the first two principal coordinates shown in Figure 2 illustrates the pattern of site grouping.

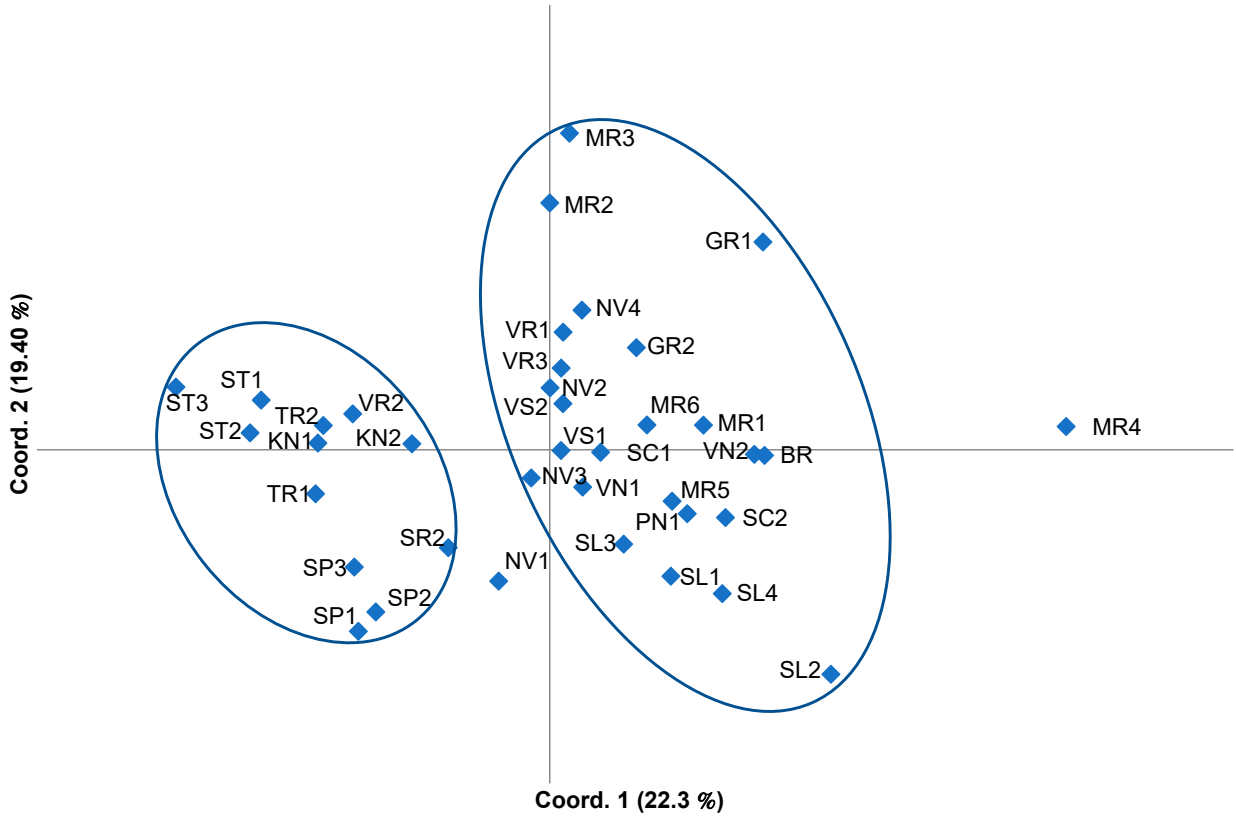

Figure 2. The grouping of 38 polymorphic sampling sites in the PCoA plot. The site codes correspond to the codes explained in Table 1.

The sites were divided into two subgroups. Twenty-four sites formed the larger subgroup on the right side of the PCoA plot. A smaller number of sites (12), including all sites in Siesartis (ST1–ST3), Kiauna (KN1, KN2), Šešupė (SP1–SP3), Taurupis (TR1, TR2) and some other sites, formed a smaller subgroup on the left side of the plot. Some sites from the same river tended to cluster according to their origin. For example, this was typical for sites SP1–SP3, ST1–ST3 and SL1–SL4. The sites from other rivers were scattered on the plot. Sites in the Merkys River (MR2, MR3 and MR4) were especially genetically divergent. Although the sampling sites of some rivers tended to cluster together, their grouping was not related to the geographical location of the rivers.

The analysis of the genetic structure of 16 *P. australis* populations was performed using the Bayesian clustering method using STRUCTURE. The highest Delta K value was observed at K = 2 (144.83) (Figure 3a), which means that the studied reed populations could be divided into two genetic clusters. Additional peaks were detected at K = 9 (17.07) and

K = 12 (7.66) but were very weak and uncertain to be able to recognize a secondary population structure. STRUCTURE analysis revealed two gene pools and divided the populations into green and red clusters (Figure 3b). Two large populations (SP and KN) were attributed to the red cluster, and five populations (MR, GR, VS, VN and PN) were attributed to the green cluster. The remaining populations were admixed. The highest Delta K for sites (Figure 4a) was K = 14 (331.52), with a few small secondary peaks at K = 12 (44.76), 22 (20.02) and 33 (21.83).

Bayesian analysis of the genetic structure of the sites revealed the genetic specificity of separate sites and a high level of admixture (Figure 4b).

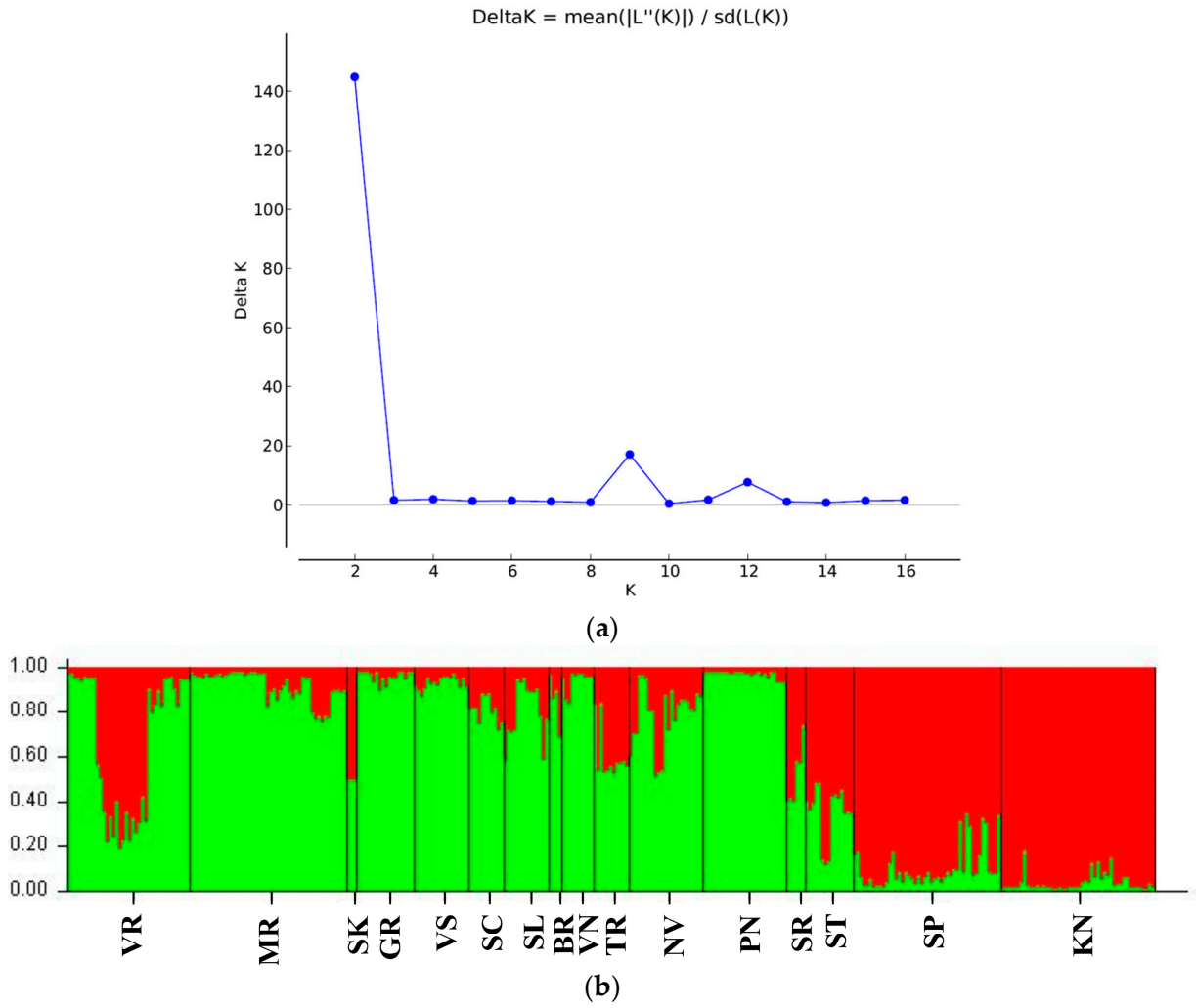

Figure 3. Analysis of the genetic structure of common reed at the population level: (**a**) Most likely number (K = 2) of genetic pools among 16 populations of *P. australis* inferred by Evanno's Delta K method. (**b**) Genetic structure of *P. australis* populations (K = 2) revealed using the STRUCTURE program. Codes below the bar indicate populations according to Tables 1 and 3. Each individual is represented by a thin vertical line and its color depends on its partitioning into the K clusters. Black lines separate plants from different populations.

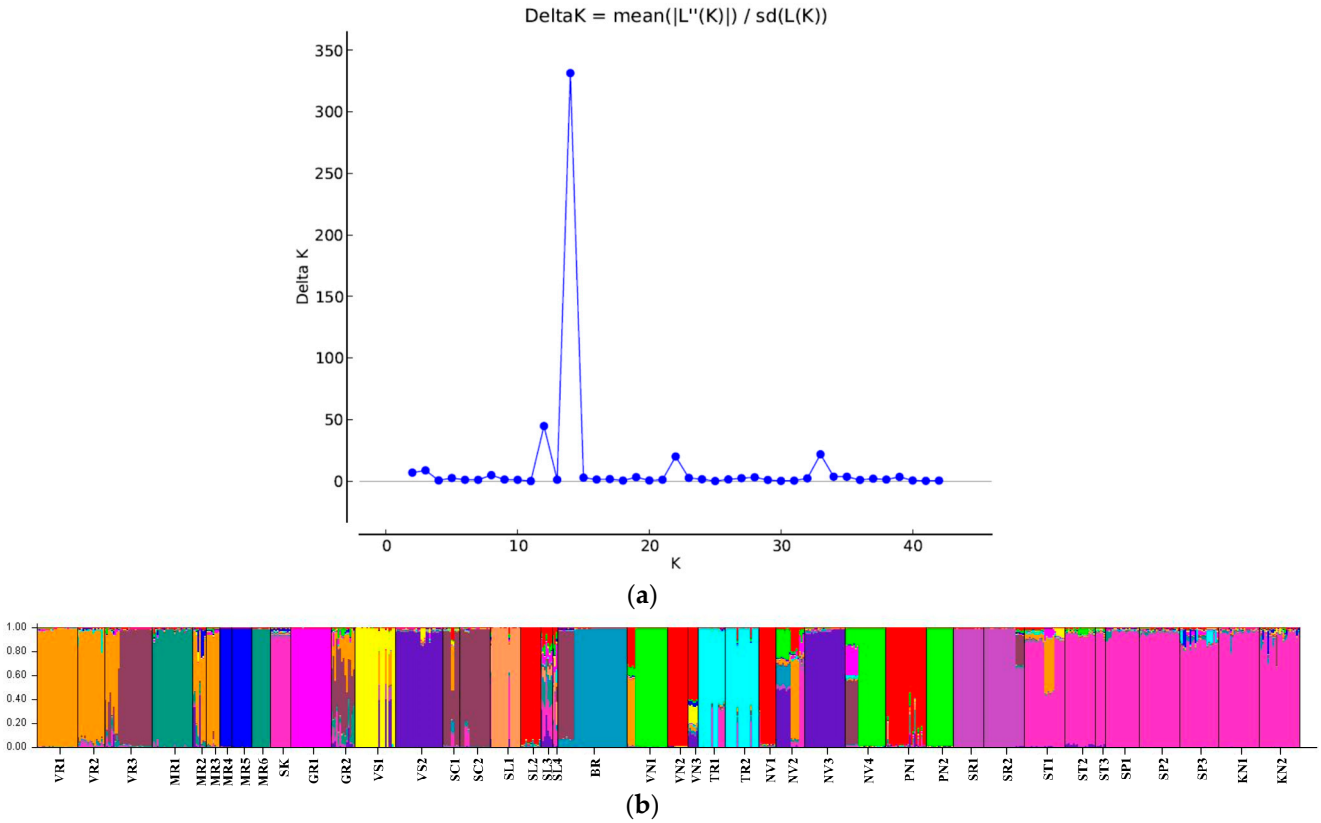

Figure 4. Analysis of the genetic structure of common reed at the level of sites: (**a**) Most likely number (K = 14) of genetic clusters of 42 sites of *P. australis* inferred by Evanno's Delta K method. (**b**) Genetic structure of *P. australis* sites (K = 14) revealed using the STRUCTURE program. Codes below the bar indicate sites according to Table 1. Each individual is represented by a thin line and its color depends on its partitioning into the K clusters. The plants of the same site are separated by black lines.

3.3. Assessment of Environmental and Anthropogenic Impacts on Genetic Diversity

We compared site genetic diversity parameters with water chemical characteristics at 32 sites (Table S2) and found a weak negative correlation between the genetic richness (*R*) and the concentrations of nitrogen forms (R:NO_2—$r = -0.3485$, $p = 0.05$; R:Mineral N:—$r = -0.3458$, $p = 0.05$; R:N_T—$r = -0.3446$, $p = 0.05$). For nitrates, the significance of the correlation with genetic richness was very close to significance (R:NO_3—$r = -0.3401$, $p = 0.0569$). There were no significant relationships between the other genetic diversity parameters of *P. australis* and the river water chemical and physical characteristics at these sites ($p > 0.05$).

Hierarchical AMOVA and the Kruskal–Wallis test did not detect any genetic differentiation among the groups of sites based on similar neighboring land cover; i.e., among seminatural areas, urbanized areas or agricultural land as well as between sites from natural and modified river stretches.

4. Discussion

4.1. Genetic Structure of Populations and Sites

The polymorphism of *P. australis* populations based on ISSR markers was high (*Hw* = 0.2201 ± 0.0127; *Ht* = 0.3503), which corroborates our previous findings based on SSR markers [35] and with the studies of other authors who have shown that high genetic diversity in populations of the common reed is due to gene flow between populations and the relatively frequent establishment by seeds [20,25,36,37,55]. Nevertheless, 4 of our sites (SK, VS3, PN2, SR1) and 1 population (Skroblus River) were monoclonal. This latter population was small and we could sample only 12 plants from it (Table 1). Mono-

clonal patches, stands or populations were found also in several studies [13,20,23,74]. The population genetic structure that was inferred by the Bayesian clustering method showed that there are two allele pools that determine the genetic makeup of studied populations (Figure 3). The Šešupė and Kiauna populations were grouped into the smaller red cluster. The green cluster included populations from the Merkys, Visinčia, Pienia, Verseka and Grūda rivers. The remaining populations were combinations of these two allele pools. Despite their genetic similarity, the Šešupė and Kiauna populations are geographically distant, confirming that there is no correlation between geographic and genetic distances, as shown by the Mantel test. Additionally, STRUCTURE analysis carried out at the site level identified high variation among sites within populations (Figure 4). The sampling sites can be divided into two groups according to their polymorphism patterns. Some sites of the same population were genetically similar and were grouped in the same cluster, for example, sites SP1–SP3 from the Šešupė River and sites SR1 and SR2 from the Širvinta River. Such grouping can indicate gene flow between adjacent sites of *P. australis* [75]. However, some sites that are geographically close to each other were genetically different. For example, sites MR3, MR4 and MR6 from the Merkys River and NV1–NV4 from the Nevėžis River had rather different genetic structures. The changes in the genetic diversity of the reed stands of these two rivers could have been influenced by the modification of the river bed. The part of the Nevėžis River, including riverbank habitats, where our sampling sites (NV1–NV3) are located was strongly modified in the previous century during melioration and riverbed straightening works [35]. The changes in the genetic structure of these sites could therefore be determined by the genetic drift because the disturbance caused by the works, like, for instance, the removal of the upper soil layer, could have created the conditions for the recruitment of propagules coming from different stands or from local seed bank. However, we were unable to identify distinct genetic clusters that could distinguish modified sites from those located in unmodified riverbeds. No correlation of genetic diversity parameters with site disturbance level was found even in several *P. australis* studies [25,42]. The results of the Bayesian clustering generally corroborated the site grouping in the PCoA plot (Figure 2), where sites from the Merkys River and the Nevėžis River were, in fact, very scattered.

4.2. Eutrophication Impact

We analyzed the impact of anthropogenic factors that can have a strong effect on the structure of the genetic diversity of the common reed stands. Our study found a weak negative relationship between the concentration of nitrogen compounds and genotypic richness. Previous studies analyzed the impact of nutrients, particularly of N, on the clonal growth and sexual reproduction of plants [52,53] and concluded that clonal plants can respond to environmental stress by switching reproduction mode [53,76,77]. It was proven experimentally that clonal growth is stimulated by nutrients. Nitrogen fertilization increased, for instance, the growth of *Leymus chinensis* ramets and significantly reduced sexual reproduction [53]. Therefore, the negative relationships that we found in our study between genotypic richness and the concentrations of nitrogen compounds could indicate that an increase in these nutrients in water could stimulate clonal spread. In support of this, Kirk et al. [13] reported a significant negative relationship between soil potassium concentration and genotypic richness. The authors suggested that low environmental stress (consistent with abundant nutrient availability) is beneficial for the clonal spread of *P. australis*. Our study also revealed a new potential relationship of genotypic richness with nitrate (NO_3-N) ($p = 0.0569$). Such a result can be explained by the fact that different forms of nitrogen can have different effects on plant physiology, including a trade-off between the two modes of reproduction [13,78,79]. In contrast, McCormick et al. [37] explained the high genotypic diversity at *P. australis* sites in Chesapeake Bay by disturbance and increased amounts of nutrients caused by eutrophication. This contradiction could be due to the fact that the balance between reproduction modes may be rather plastic and be impacted also by many other factors than nutrients, like stand age, successional status, ramet density and

others [53]. We did not find any relationship between the chemical and physical parameters of the water and the other genetic diversity parameters (*Na*, *Ne*, *I* and *He*) that we measured.

5. Conclusions

The analysis of a large number of common reed samples provided a more detailed picture of the genetic diversity of *P. australis* and its dynamics in Lithuanian rivers. The study showed that of all the anthropogenic factors that were tested, the concentration of nitrogen compounds is the only one that could influence the reed clonal diversity in the studied sites. As corroborated by other studies, increased nutrient availability may have promoted clonal growth and reduced seed recruitments at the studied sites. We did not find any relationship between allelic frequencies and river stretch modifications as well as between allelic frequencies and land cover type in the neighborhood of the stand. Further studies are needed to assess population dynamics and adaptation in response to the human impact.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15111116/s1, Table S1. Oligonucleotide ISSR primers used for diversity analysis in common reed sites and populations, primer annealing temperature, number of scored and polymorphic bands, percentage of polymorphic loci identified per primer and scored DNA fragments size range. Table S2. Water hydro-chemical and physical characteristics of common reed sampling sites.

Author Contributions: Conceptualization, C.L. and D.Ž.; Methodology, J.P., D.N. and D.Ž.; Software, J.P., J.B. and D.N.; Validation, J.P. and J.B.; Formal analysis, J.B., D.N. and D.Ž.; Investigation, J.P.; Resources, D.Ž.; Data curation, J.P.; Writing–original draft, D.Ž.; Writing–review & editing, J.P., C.L. and J.B.; Visualization, J.P.; Supervision, C.L. and D.Ž.; Project administration, D.Ž.; Funding acquisition, D.Ž. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Research Council of Lithuania (SIT-2/2015).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hoban, S.; Archer, F.I.; Bertola, L.D.; Bragg, J.G.; Breed, M.F.; Bruford, M.W.; Coleman, M.A.; Ekblom, R.; Funk, W.C.; Grueber, C.E.; et al. Global genetic diversity status and trends: Towards a suite of Essential Biodiversity Variables (EBVs) for genetic composition. *Biol. Rev.* **2022**, *97*, 1511–1538. [CrossRef] [PubMed]
2. Reed, D.H.; Frankham, R. Correlation between fitness and genetic diversity. *Conserv. Biol.* **2003**, *17*, 230–237. [CrossRef]
3. Roger, F.; Godhe, A.; Gamfeldt, L. Genetic diversity and ecosystem functioning in the face of multiple stressors. *PLoS ONE* **2012**, *7*, e45007. [CrossRef] [PubMed]
4. Hautier, Y.; Tilman, D.; Isbell, F.; Seabloom, E.W.; Borer, E.T.; Reich, P.B. Anthropogenic environmental changes impact ecosystem stability via biodiversity. *Science* **2015**, *348*, 336–340. [CrossRef] [PubMed]
5. Leimu, R.; Mutikainen, P.; Koricheva, J.; Fisher, M. How general are positive relationship between plant population size, fitness, and genetic variation? *J. Ecol.* **2006**, *94*, 942–952. [CrossRef]
6. Sebbenn, A.M.; Carvalho, A.C.M.; Freitas, M.L.M.; Moraes, S.M.B.; Gaino, A.P.S.C.; daSilva, J.M.; Jolivet, C.; Moraes, M.L.T. Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. *Heredity* **2011**, *106*, 134–145. [CrossRef]
7. Beatty, G.E.; Reid, N.; Provan, J. Retrospective genetic monitoring of the threatened yellow marsh saxifrage (*Saxifraga hirculus*) reveals genetic erosion but provides valuable insights for conservation strategies. *Divers. Distrib.* **2014**, *20*, 529–537. [CrossRef]
8. Sampson, J.F.; Hankinson, M.; McArthur, S.; Tapper, S.; Langley, M.; Gibson, N.; Yates, C.; Byrne, M. Long-term 'islands' in the landscape: Low gene flow, effective population size and genetic divergence in the shrub *Hakea oldfieldii* (Proteaceae). *Bot. J. Linn. Soc.* **2015**, *179*, 319–334. [CrossRef]
9. Banks, S.C.; Cary, G.J.; Smith, A.L.; Davies, I.D.; Driscoll, D.A.; Gill, A.M.; Lindenmayer, D.B.; Peakall, R. How does ecological disturbance influence genetic diversity? *Trends Ecol. Evol.* **2013**, *28*, 670–679. [CrossRef]
10. Fuller, M.R.; Doyle, M.W.; Strayer, D.L. Causes and consequences of habitat fragmentation in river networks. *Ann. N. Y. Acad. Sci.* **2015**, *1355*, 31–51. [CrossRef]

11. Kiviat, E. Ecosystem services of *Phragmites* in North America with emphasis on habitat functions. *AoB Plants* **2013**, *5*, plt008. [CrossRef]
12. Engloner, A.I.; Szegő, D. Genetic diversity of riverine reed stands indicating the water regime of the habitat. *Ecol. Indic.* **2016**, *61*, 846–849. [CrossRef]
13. Kirk, H.; Paul, J.; Straka, J.; Freeland, J.R. Long distance dispersal and genetic diversity are implicated in the invasive spread of the common reed *Phragmites australis* (Poaceae) in Eastern North America. *Am. J. Bot.* **2011**, *98*, 1180–1190. [CrossRef] [PubMed]
14. Lambertini, C.; Sorrell, B.K.; Riis, T.; Olesen, B.; Brix, H. Exploring the borders of European *Phragmites* within a cosmopolitan genus. *AoB Plants* **2012**, *2012*, pls020. [CrossRef] [PubMed]
15. Eller, F.; Skálová, H.; Caplan, J.S.; Bhattarai, G.P.; Burger, M.K.; Cronin, J.T.; Guo, W.-Y.; Guo, X.E.; Hazelton, L.G.; Kettenring, K.M.; et al. Cosmopolitan species as models for ecophysiological responses to global change: The common reed *Phragmites australis*. *Front. Plant Sci.* **2017**, *8*, 1833. [CrossRef] [PubMed]
16. Saltonstall, K. Cryptic invasion by a non-native genotype of *Phragmites australis* into North America. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2445–2449. [CrossRef] [PubMed]
17. Kettenring, K.M.; McCormick, M.K.; Baron, H.M.; Whigham, D.F. Mechanisms of *Phragmites australis* invasion: Feedbacks among genetic diversity, nutrients, and sexual reproduction. *J. Appl. Ecol.* **2011**, *48*, 1305–1313. [CrossRef]
18. Guo, W.Y.; Lambertini, C.; Pyšek, P.; Meyerson, L.A.; Brix, H. Living in two worlds: Evolutionary mechanisms act differently in the native and introduced ranges of an invasive plant. *Ecol. Evol.* **2018**, *8*, 2440–2452. [CrossRef] [PubMed]
19. Cronk, J.K.; Fennessy, M.S. *Wetland Plants: Biology and Ecology*; CRC Press/Lewis Publishers: Boca Raton, FL, USA, 2001; pp. 147–188.
20. McCormick, M.K.; Kettenring, K.M.; Baron, H.M.; Whigham, D.F. Extent and reproductive mechanisms of *Phragmites australis* spread in brackish wetlands in Chesapeake Bay, Maryland (USA). *Wetlands* **2010**, *30*, 67–74. [CrossRef]
21. Ishii, J.; Kadono, Y. Factors influencing seed production of *Phragmites australis*. *Aquat. Bot.* **2002**, *72*, 129–141. [CrossRef]
22. Brochet, A.L.; Guillemain, M.; Fritz, H.; Gauthier-Clerc, M.; Green, A.J. The role of migratory ducks in the long-distance dispersal of native plants and the spread of exotic plants in Europe. *Ecography* **2009**, *32*, 919–928. [CrossRef]
23. Fér, T.; Hroudová, Z. Genetic diversity and dispersal of *Phragmites australis* in a small river system. *Aquat. Bot.* **2009**, *90*, 165–171. [CrossRef]
24. Stabile, J.; Lipus, D.; Maceda, L.; Maltz, M.; Roy, N.; Wirgin, I. Microsatellite DNA analysis of spatial and temporal population structuring of *Phragmites australis* along the Hudson River Estuary. *Biol. Invasions* **2016**, *18*, 2517–2529. [CrossRef]
25. Kuprina, K.; Seeber, E.; Schnittler, M.; Landeau, R.; Lambertini, C.; Bog, M. Genetic diversity of common reed in the Southern Baltic Sea region—Is there an influence of disturbance? *Aquat. Bot.* **2022**, *177*, 103471. [CrossRef]
26. Kettenring, K.M.; McCormick, M.K.; Baron, H.M.; Whigham, D.F. *Phragmites australis* (common reed) invasion in the Rhode River subestuary of the Chesapeake Bay: Disentangling the effects of foliar nutrients, genetic diversity, patch size, and seed viability. *Estuaries Coast.* **2010**, *33*, 118–126. [CrossRef]
27. Belzile, F.; Labbé, J.; LeBlanc, M.C.; Lavoie, C. Seeds contribute strongly to the spread of the invasive genotype of the common reed (*Phragmites australis*). *Biol. Invasions* **2010**, *12*, 2243–2250. [CrossRef]
28. Pyšek, P.; Skálová, H.; Čuda, J.; Guo, W.-Y.; Suda, J.; Doležal, J.; Kauzál, O.; Lambertini, C.; Lučanová, M.; Mandáková, T.; et al. Small genome separates native and invasive populations in an ecologically important cosmopolitan grass. *Ecology* **2018**, *99*, 79–90. [CrossRef]
29. Oh, D.-H.; Kowalski, K.P.; Quach, Q.N.; Wijesinghege, C.; Tanford, P.; Dassanayake, M.; Clay, K. Novel genome characteristics contribute to the invasiveness of *Phragmites australis* (common reed). *Mol. Ecol.* **2022**, *31*, 1142–1159. [CrossRef]
30. Tao, F.; Fan, C.; Liu, Y.; Sivakumar, S.; Kowalski, K.P.; Golenberg, E.M. Optimization and application of non-native *Phragmites australis* transcriptome assemblies. *PLoS ONE* **2023**, *18*, e0280354. [CrossRef]
31. Coppi, A.; Lastrucci, L.; Cappelletti, D.; Cerri, M.; Ferranti, F.; Ferri, V.; Foggi, B.; Gigante, D.; Venanzoni, R.; Viciani, D.; et al. AFLP approach reveals variability in *Phragmites australis*: Implications for its dieback and evidence for genotoxic effects. *Front. Plant Sci.* **2018**, *9*, 386. [CrossRef]
32. Erős, T.; Kuehne, L.; Dolezsai, A.; Sommerwerk, N.; Wolter, C. A systematic review of assessment and conservation management in large floodplain rivers—Actions postponed. *Ecol. Indic.* **2019**, *98*, 453–461. [CrossRef]
33. Krokaitė, E.; Shakeneva, D.; Juškaitytė, E.; Rekašius, T.; Nemaniūtė-Gužienė, J.; Butkuvienė, J.; Patamsytė, J.; Rančelienė, V.; Vyšniauskienė, R.; Duchovskienė, L.; et al. Nitrogen concentration of the aquatic plant species in relation to land cover type and other variables of the environment. *Zemdirb. Agric.* **2019**, *106*, 203–212. [CrossRef]
34. Liu, L.; Wang, J.; Ma, X.; Li, M.; Guo, X.; Yin, M.; Cai, Y.; Yu, X.; Du, N.; Wang, R.; et al. Impacts of the Yellow River and Qingtongxia dams on genetic diversity of *Phragmites australis* in Ningxia Plain, China. *Aquat. Bot.* **2021**, *169*, 103341. [CrossRef]
35. Naugžemys, D.; Lambertini, C.; Patamsytė, J.; Butkuvienė, J.; Khasdan, V.; Žvingila, D. Genetic diversity patterns in *Phragmites australis* populations in straightened and in natural river sites in Lithuania. *Hydrobiologia* **2021**, *848*, 3317–3330. [CrossRef]
36. Lambertini, C.; Gustafsson, M.H.G.; Frydenberg, J.; Speranza, M.; Brix, H. Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales. *Aquat. Bot.* **2008**, *88*, 160–170. [CrossRef]
37. McCormick, M.K.; Kettenring, K.M.; Baron, H.M.; Whigham, D.F. Spread of invasive *Phragmites australis* in estuaries with differing degrees of development: Genetic patterns, Allee effects and interpretation. *J. Ecol.* **2010**, *98*, 1369–1378. [CrossRef]

38. Anderson, N.O.; Jocienė, L.; Krokaitė, E.; Rekašius, T.; Paulauskas, A.; Kupčinskienė, E. Genetic diversity of *Phalaris arundinacea* populations in relation to river regulation in the Merkys basin, Lithuania. *River Res. Appl.* **2018**, *34*, 300–309. [CrossRef]
39. Koppitz, H.; Kühl, H. To the importance of genetic diversity of *Phragmites australis* in the development of reed stands. *Wetl. Ecol. Manag.* **2000**, *8*, 403–414. [CrossRef]
40. Engloner, A.I.; Major, Á. Clonal diversity of *Phragmites australis* propagating along water depth gradient. *Aquat. Bot.* **2011**, *94*, 172–176. [CrossRef]
41. Hazelton, E.L.G.; McCormick, M.K.; Sievers, M.; Kettenring, K.M.; Whigham, D.F. Stand age is associated with clonal diversity, but not vigor, community structure, or insect herbivory in Chesapeake Bay *Phragmites australis*. *Wetlands* **2015**, *35*, 877–888. [CrossRef]
42. Paul, J.; Kirk, H.; Freeland, J.R. Genetic diversity and differentiation of fragmented reedbeds (*Phragmites australis*) in the United Kingdom. *Hydrobiologia* **2011**, *665*, 107–115. [CrossRef]
43. Vymazal, J.; Březinová, T. Accumulation of heavy metals in aboveground biomass of *Phragmites australis* in horizontal flow constructed wetlands for wastewater treatment: A review. *Chem. Eng. J.* **2016**, *290*, 232–242. [CrossRef]
44. Uddin, M.N.; Robinson, R.W. Can nutrient enrichment influence the invasion of *Phragmites australis*? *Sci. Total. Environ.* **2018**, *613–614*, 1449–1459. [CrossRef] [PubMed]
45. Prica, M.; Andrejić, G.; Šinžar-Sekulić, J.; Rakić, T.; Dželetović, Ž. Bioaccumulation of heavy metals in common reed (*Phragmites australis*) growing spontaneously on highly contaminated mine tailing ponds in Serbia and potential use of this species in phytoremediation. *Bot. Serbica* **2019**, *43*, 85–95. [CrossRef]
46. Ahmad, S.S.; Reshi, Z.A.; Shah, M.A.; Rashid, I.; Ara, R.; Andrabi, S.M.A. Phytoremediation potential of *Phragmites australis* in Hokersar wetland—A Ramsar Site of Kashmir Himalaya. *Int. J. Phytoremediation* **2014**, *16*, 1183–1191. [CrossRef]
47. Rodrigo, M.A.; Valentin, A.; Claros, J.; Moreno, L.; Segura, M.; Lassalle, M.; Vera, P. Assessing the effect of emergent vegetation in a surface-flow constructed wetland on eutrophication reversion and biodiversity enhancement. *Ecol. Eng.* **2018**, *113*, 74–87. [CrossRef]
48. Carricondo, J.M.; Oliver-Villanueva, J.V.; Turégano, J.V.; González, J.A.; Mengual, J. Use of *Phragmites australis* for controlling phosphorus contamination in anthropogenic wetland ecosystems. *Environ. Technol.* **2021**, *42*, 3055–3064. [CrossRef]
49. Bonanno, G.; Lo Giudice, R. Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. *Ecol. Indic.* **2010**, *10*, 639–645. [CrossRef]
50. Chandra, R.; Yadav, S. Phytoremediation of CD, CR, CU, MN, FE, NI, PB and ZN from aqueous solution using *Phragmites cummunis*, *Typha angustifolia* and *Cyperus esculentus*. *Int. J. Phytoremediation* **2011**, *13*, 580–591. [CrossRef]
51. Labra, M.; Di Fabio, T.; Grassi, F.; Regondi, S.M.G.; Bracale, M.; Vannini, C.; Agradi, E. AFLP analysis as biomarker of exposure to organic and inorganic genotoxic substances in plants. *Chemosphere* **2003**, *52*, 1183–1188. [CrossRef]
52. Piqueras, J.; Klimes, L.; Redbo-Torstensson, P. Modelling the morphological response to nutrient availability in the clonal plant *Trientalis europaea* L. *Plant Ecol.* **1999**, *141*, 117–127. [CrossRef]
53. Bai, W.M.; Sun, X.Q.; Wang, Z.W.; Li, L.H. Nitrogen addition and rhizome severing modify clonal growth and reproductive modes of *Leymus chinensis* population. *Plant Ecol.* **2009**, *205*, 13–21. [CrossRef]
54. EPA (Environmental Protection Agency). Agriculture and Lithuanian Waters. Impact of Agricultural Activity on the Status of Lithuanian Rivers and Pollution Loads in the Baltic Sea. Vilnius. 41 (in Lithuanian). 2018. Available online: https://aaa.lrv.lt/lt/veiklos-sritys/vanduo/upes-ezerai-ir-tvenkiniai/valstybinis-upiu-ezeru-ir-tvenkiniu-monitoringas/upiu-monitoringo-rezultatai (accessed on 28 August 2023).
55. Lambertini, C.; Guo, W.Y.; Ye, S.; Eller, F.; Guo, X.; Li, X.Z.; Sorrell, B.K.; Speranza, M.; Brix, H. Phylogenetic diversity shapes salt tolerance in *Phragmites australis* estuarine populations in East China. *Sci. Rep.* **2020**, *10*, 17645. [CrossRef] [PubMed]
56. Tanhuanpää, P.; Erkkilä, M.; Kalendar, R.; Schulman, A.H.; Manninen, O. Assessment of genetic diversity in Nordic timothy (*Phleum pratense* L.). *Hereditas* **2016**, *153*, 5. [CrossRef] [PubMed]
57. Zietkiewicz, E.; Rafalski, A.; Labuda, D. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics* **1994**, *20*, 176–183. [CrossRef] [PubMed]
58. Gentili, R.; Fenu, G.; Mattana, E.; Citterio, S.; De Mattia, F.; Bacchetta, G. Conservation genetics of two island endemic *Ribes* spp. (Grossulariaceae) of Sardinia: Survival or extinction? *Plant Biol.* **2015**, *17*, 1085–1094. [CrossRef] [PubMed]
59. Lopes, M.S.; Mendonça, D.; Bettencourt, S.X.; Borba, A.R.; Melo, C.; Baptista, C.; da Câmara Machado, A. Genetic diversity of an Azorean endemic and endangered plant species inferred from inter-simple sequence repeat markers. *AoB Plants* **2014**, *6*, plu034. [CrossRef]
60. Cavanzón-Medrano, L.E.; Machkour-M'Rabet, S.; Chablé-Iuit, L.R.; Pozo, C.; Hénaut, Y.; Legal, L. Effect of climatic conditions and land cover on genetic structure and diversity of *Eunica tatila* (Lepidoptera) in the Yucatan Peninsula, Mexico. *Diversity* **2018**, *10*, 79. [CrossRef]
61. Krokaitė, E.; Janulionienė, R.; Jocienė, L.; Rekašius, T.; Rajackaitė, G.; Paulauskas, A.; Marozas, V.; Kupčinskienė, E. Relating invasibility and invasiveness: Case study of *Impatiens parviflora*. *Front. Ecol. Evol.* **2022**, *10*, 845947. [CrossRef]
62. Naugžemys, D.; Patamsytė, J.; Žilinskaitė, S.; Hoshino, Y.; Skridaila, A.; Žvingila, D. Genetic structure of native blue honeysuckle populations in the Western and Eastern Eurasian Ranges. *Plants* **2022**, *11*, 1480. [CrossRef]

63. Lal, A.; Pant, M.; Pant, G.; Palni, L.M.S.; Kumar, A.; Kumar, G. ISSR marker assisted genetic diversity assessment in natural populations of two endemic orchids *Aerides multiflora* and *Rhynchostylis retusa* from Uttarakhand, India. *S. Afr. J. Bot.* **2023**, *157*, 151–158. [CrossRef]
64. Saltonstall, K. Microsatellite variation within and among North American lineages of *Phragmites australis*. *Mol. Ecol.* **2003**, *12*, 1689–1702. [CrossRef] [PubMed]
65. Patamsytė, J.; Naugžemys, D.; Čėsnienė, T.; Kleizaitė, V.; Demina, O.N.; Mikhailova, S.I.; Agafonov, V.A.; Žvingila, D. Evaluation and comparison of the genetic structure of *Bunias orientalis* populations in their native range and two non-native ranges. *Plant Ecol.* **2018**, *219*, 101–114. [CrossRef]
66. Peakall, R.; Smouse, P.E. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* **2012**, *28*, 2537–2539. [CrossRef] [PubMed]
67. Dorken, M.E.; Eckert, C.G. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *J. Ecol.* **2001**, *89*, 339–350. [CrossRef]
68. Vekemans, X.; Beauwens, T.; Lemaire, M.; Roldán-Ruiz, I. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Mol. Ecol.* **2002**, *11*, 139–151. [CrossRef] [PubMed]
69. Yeh, F.C.; Yang, R.; Boyle, T. *POPGENE: Microsoft Windows-Based Freeware for Population Genetic Analysis: Release 1.31*; University of Alberta: Edmonton, AB, Canada, 1999.
70. Excoffier, L.; Lischer, H.E.L. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **2010**, *10*, 564–567. [CrossRef]
71. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef]
72. Falush, D.; Stephens, M.; Pritchard, J.K. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Mol. Ecol. Notes* **2007**, *7*, 574–578. [CrossRef]
73. Evanno, G.; Regnault, S.; Goudet, J. Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]
74. Koppitz, H. Analysis of genetic diversity among selected populations of *Phragmites australis* world-wide. *Aquat. Bot.* **1999**, *64*, 209–221. [CrossRef]
75. Jacquemyn, H.; Honnay, O.; Looy, K.V.; Breyne, P. Spatiotemporal structure of genetic variation of a spreading plant metapopulation on dynamic riverbanks along the Meuse River. *Heredity* **2006**, *96*, 471–478. [CrossRef] [PubMed]
76. Gardner, S.N.; Mangel, M. Modeling investments in seed, clonal offspring, and translocation in a clonal plant. *Ecology* **1999**, *80*, 1202–1220. [CrossRef]
77. Weppler, T.; Stocklin, J. Variation of sexual and clonal reproduction in the alpine *Geum reptans* in contrasting altitudes and successional stages. *Basic Appl. Ecol.* **2005**, *6*, 305–306. [CrossRef]
78. Jampeetong, A.; Brix, H.; Kantawanichkul, S. Effects of inorganic nitrogen forms on growth, morphology, nitrogen uptake capacity and nutrient allocation of four tropical aquatic macrophytes (*Salvinia cucullata*, *Ipomoea aquatica*, *Cyperus involucratus* and *Vetiveria zizanioides*). *Aquat. Bot.* **2012**, *97*, 10–16. [CrossRef]
79. Tho, B.T.; Lambertini, C.; Eller, F.; Brix, H.; Sorrell, B.K. Ammonium and nitrate are both suitable inorganic nitrogen forms for the highly productive wetland grass *Arundo donax*, a candidate species for wetland paludiculture. *Ecol. Eng.* **2017**, *105*, 379–386. [CrossRef]