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Diversity and Ecology of Charophytes from Vojvodina (Serbia) in Relation to Physico-Chemical and Bioclimatic Habitat Properties

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Abstract: In Serbia, almost all charophyte species belong to one of the IUCN categories. This study aimed to gather more knowledge about their floristic richness, distribution and ecology. During the survey, 262 localities were investigated in the Vojvodina province, northern Serbia. Seventeen charophyte species were found, out of which 16 were included in the analyses. The environmental matrix included 38 localities, each characterized by habitat type and 35 environmental parameters. The canonical correspondence analysis (CCA) showed six parameters as being statistically significant: substrate, altitude, depth, water temperature, temperature annual range and precipitation of the driest month. Together, these explain the 32.34% variability in the species composition and abundance data. Altitude and substrate contributed the most to this. Two main types of habitats were outlined by the analysis. Small and shallow temporary habitats in the river floodplains, at lower altitudes, with muddy and clayish substrate, belong to one type. These areas are inhabited by either very tolerant species, such as *Chara vulgaris* and *C. globularis*, or the so-called "spring" species of the genera *Tolypella* and *Nitella*. Habitats which are located at higher altitudes, when deeper and more permanent with a sandy substrate, such as sandpits and river habitats, belong to the second one. These areas are inhabited by species like *Chara papillosa*, *C. hispida*, and *Nitellopsis obtusa*.

Keywords: charophytes; diversity; ecology; CCA; substrate; altitude

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1. Introduction

Charophyte algae (Charophyceae, Charales) are macroscopic multicellular algae that can thrive in a wide variety of standing and slow-running aquatic habitats. These are mostly freshwater but can sometimes be slightly saline and brackish as well [1–3]. Often, they are related to "hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp." (EU Habitat Directive, Annex I, habitat type No. 3140). However, small, shallow, and even ephemeral ponds and puddles can be very important habitats for charophytes [4–6]. Considering their ability to colonize newly created habitats, they can very often be found as a pioneer species, either in new or in restored habitats [3,5,7,8]. Charophyte species differ in their relation to habitat eutrophication and pollution, ranging from being very tolerant and widespread to very sensitive and rare [8–11]. They can play key roles in the ecosystems they inhabit and influence many aspects of ecosystem functioning, acting as shelter and food for other organism groups, being a "sink for nutrients" and improving water clarity, etc. [10,12,13].

Order Charales comprises one recent family Characeae with six genera. In Serbia, charophytes are represented by four genera and 27 species (personal data), almost all classified in one of the IUCN categories [14]. In Vojvodina, charophyte flora includes

22 species [15]. During our investigations, 17 species were found, while three species, *Chara canescens*, *C. virgata* and *Nitella brachyteles*, historically found on the territory of Vojvodina, were not confirmed. Floristic analysis related to all species of charophytes found during our survey was summarized in [16]. However, during later investigations, *C. canescens* was rediscovered [17–19], and two new species were discovered for the first time for the territory of Vojvodina, *C. baueri* [20] and *C. connivens* [21,22].

The history of the charophyte study and findings on the territory of Vojvodina was given in detail by [16] and recently by [15]. In recent years, charophyte research in Serbia and especially in Vojvodina intensified and new studies have emerged [17–26]. Even though they seemed to be numerous, by reviewing them more carefully one could infer that there were no comprehensive ecological studies among them. Such studies are rare, even in Serbia [27] and the Balkans [28]. Studies summarizing knowledge on charophytes are known from different regions of the world. They are most often related to their diversity and distribution, compiling checklists [29–34], or taxonomic overviews [35–37], with some details on ecology. However, comprehensive and systematic ecological studies on charophytes are rather rare [2,13,38,39]. Hence, this study makes a valuable contribution to enriching the knowledge of charophyte ecology.

This study was the first systematic survey on charophytes in Serbia and was performed with the aim of gathering more knowledge on their floristic richness, distribution, and ecology. A more detailed description of the history of charophyte research, their floristic richness, habitat preferences, and distribution on the territory of Vojvodina is given by [16]. Conversely, in this paper the main aim was to address charophyte algae ecology in the Vojvodina region, i.e., we focus on the ecological preferences of charophyte species in relation to physico-chemical and bioclimatic habitat properties.

2. Materials and Methods

2.1. The Study Area

Vojvodina is a region located in northern Serbia, occupying one-third of its territory. Considering it is situated in the southern part of the Pannonian plain, most of Vojvodina's territory is lowland, with an elevation between 70 and 130 m [40]. The climate is temperate continental, with a mean annual precipitation of around 600 mm [41]. Having numerous streams and rivers, a great number of standing water bodies and an almost 15 thousand kilometer-long network of artificial channels, Vojvodina is hydrologically the richest area in Serbia. The rivers and streams of Vojvodina are naturally characterized by low inclination and slow flow. During their evolution, they have formed wide floodplains and many curves and oxbows. However, these lowland rivers have been an object of intensive regulation works for the last two hundred years. Most streams and rivers are now regulated and controlled, having their branches separated from the main flow [42]. Moreover, as a result of gravel, sand, or other kinds of excavation activities, this region has seen more than one thousand small ponds form in abandoned excavation pits [43]. Many oxbows, as well as excavation pits, are nowadays converted into fishponds. Lakes, natural and artificial, are rare in Vojvodina and usually rather shallow (usually less than 5 m deep).

2.2. Data Collection

The pilot survey was carried out at the territory of the SNR "Zasavica". The results obtained [44] have laid the ground for the intensive field investigations conducted in the period between 2012 and 2014 on the territory of the entire Vojvodina [16]. Altogether, 262 localities have been investigated, 19 in the territory of SNR "Zasavica" and 243 more in the remaining territory of Vojvodina. The investigated waterbodies were diverse in regard to their origin, the quality of water, utilization manner, the status of protection, durability (temporary versus permanent), type (lentic versus lotic), etc. The intent was not to cover only localities with historical charophyte findings but instead to accomplish balanced geographical and ecological coverage of the study area. The habitats were selected in accordance with the habitat diversity of Vojvodina [45] and classified into eleven types: rut, puddle, ephemeral pond, permanent pond, oxbow, fishpond, excavation pit, sandpit, lake, canal, and river. In most cases, the localities were visited only once, or on rare occasions twice, in the period between May and October. Only sampling in SNR "Zasavica" was conducted in all seasons and most of the localities were revisited several times.

The samples were collected from the shore or by boat using a spiny rake. In the case of larger waterbodies, the methodology of transects or profiles, perpendicular to the shore, was used. The distance between transects was about 100 m or transects were done in accordance with habitat transition. All depth zones were covered up until the vegetation limit. If charophyte algae were present, releves were taken using the Braun-Blanquet method [46]. In the case of smaller waterbodies, usually, one releve was used for the entire waterbody.

Data regarding GPS coordinates, altitude, habitat type, and 14 ecological parameters (substrate type, depth and Secchi depth, water and air temperature, pH, oxygen concentration and saturation, conductivity, concentration of ammonium, nitrites, nitrates, total phosphorus, and orthophosphates) were gathered. The findings were mapped with the aid of a GPS positioning method using the eTrex Vista C GPS receiver (Garmin, Olathe, Kansas, USA). The distribution maps of the individual species were given in [16]. Habitat type and substrate type were noted as qualitative parameters. Depth was measured using a graduated rope connected to the sampling rake. The values given represent the depth in the center (or average depth) of the releve taken. Water transparency was measured using a Secchi disk. Water temperature, as well as oxygen concentration and saturation, were measured in the water using a Eutech Cyberscan DO 300 device (Eutech Instruments, Singapore), pH value was measured using a HANNA HI98127 device (Hanna Instruments, Woonsocket, Rhode Island. USA) and conductivity using a HANNA HI8733 device (Hanna Instruments, Woonsocket, Rhode Island. USA). The parameters of the water were measured 20–30 cm below the water surface or less if the waterbody was less than 20 cm deep.

Water samples were collected in the field in 0.5 l plastic bottles (filled to the top) at 20–30 cm below the water surface. Samples were stored at 4 °C and taken to a laboratory to be analyzed. The analysis of water was conducted within the first 24 h. Five chemical parameters were determined: ammonium, nitrites, nitrates, total phosphorus, and orthophosphates. The analyses were conducted in the laboratory of the Institute of Public Health of Belgrade using the following methods [47–50]. All methods used are accredited in accordance with SRPS ISO/IEC 17025:2006 standard.

Charophyte species were identified using the keys proposed by [3,51–53], while vascular plants were determined using the keys by [54–58]. The nomenclature used is in accordance with [59,60]. The algae samples were preserved in an alcohol solution (50% ethanol: glycerin, 1:1), while vascular plants were preserved in a dry herbarium. All samples were deposited in the collection of the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac" (BEOU) at the University of Belgrade.

2.3. Data Analysis

Aiming to give an ecological characterization of habitats inhabited by charophyte algae in the territory of Vojvodina, we gathered all available ecological data, including ecological parameters measured on the site, as well as bioclimatic predictors additionally used to characterize each site. Each location was characterized with a set of 19 bioclimatic parameters, extracted from the WorldClim v.2.1 set of global climate layers [61], as well as with PET (Potential Evapo-Transpiration) extracted from the Global Aridity Index and Potential Evapotranspiration Climate Database v2 [62]. The extraction was performed with QGIS v. 3.24.1 [63]. Finally, each locality was characterized by habitat type, 15 physicochemical parameters, and 20 bioclimatic parameters. Altogether, 36 environmental parameters were used in the analyses. The analyzed data set contains ecological data gathered for the entire territory of Vojvodina. Altogether, there were 38 waterbodies containing charophyte that were used in the analysis. Seven of them belonged to the territory of SNR "Zasavica" and the rest to the remaining territory of Vojvodina. For the rest, there were no ecological data or they were otherwise incomplete. A total of 93 macrophyte taxa were found during our survey. However, rare species were downweighted and 58 remained in the analysis, out of which 17 were determined to belong to charophyte algae. For the purpose of data analysis, Braun-Blanquet values [46] were transformed to the van der Maarel scale [64]. See Table S1.

First, the method of detrended correspondence analysis (DCA) was used to determine species response to the environmental parameters, i.e., the length of the gradients for the first two axes. Given that the relationship between species in relation to environmental variables was unimodal, as the gradient lengths were higher than 4.0 standard deviations [65], the data were analyzed by canonical correspondence analysis (CCA). CCA was conducted in Canoco 5.12 to reveal whether variance in the plant abundance data could be explained by environmental parameters. Prior to CCA, parameters variance inflation factors were calculated, and ones with VIF >10 were omitted from the further analysis. For CCA, the identification of significant environmental parameters was performed using forward selection procedures, with Monte Carlo tests of significance (499 runs). The relative contribution of physico-chemical in relation to bioclimatic parameters to the variability of the presence and abundance of analyzed charophyte species was estimated by variation partitioning [66].

3. Results

During our investigations, representatives of 17 species of charophytes were found. These included seven species of the genus *Chara*, *C. braunii*, *C. contraria*, *C. globularis*, *C. hispida*, *C. papillosa*, *C. tenuispina*, *C. vulgaris*, one species of genus *Nitellopsis*, *N. obtusa*, six species of genus *Nitella*, *N. capillaris*, *N. confervacea*, *N. gracilis*, *N. mucronata*, *N. opaca*, *N. syncarpa*, and three species of genus *Tolypella*, *T. glomerata*, *T. intricata* and *T. prolifera*. On the territory of SNR "Zasavica", they were found at 19 localities and, in the remaining territory of Vojvodina, at 35 out of 243 investigated localities (14.4%). In total, sixteen species were included in the analysis. *Chara globularis* was the most common species, being found at 17 localities, followed by the discovery of *N. mucronata* at 10, *T. prolifera* at 8, and *C. vulgaris* found at 7 localities. On the other side, the most rarely found species, found only in one locality, were *N. capillaris*, *N. confervacea*, and *N. opaca*. *T. glomerata* was also found only once but was not included in the analysis due to a lack of ecological data.

The canonical correspondence analysis (CCA) singled out six environmental parameters as statistically significant: substrate, altitude, depth, water temperature, temperature annual range (BIO7), and precipitation of the driest month (BIO14). Temperature annual range (BIO7) is a parameter given as BIO5-BIO6, where BIO5 is the "Max Temperature of Warmest Month" and BIO6 is the "Min Temperature of Coldest Month". These parameters altogether explained 32.34% variability in the species composition and abundance data (Table 1, Figure 1). Altitude and substrate contributed the most. The set of physicochemical parameters (depth, substrate, water temperature,) explained 21.4% of the variance, while the bioclimatic parameters (altitude, temperature annual range (BIO7), and precipitation of the driest month (BIO14)) explained 15.8% of the variance (Table 2). The independent effect of physicochemical parameters, excluding covariance with bioclimatic parameters, explains 16.5% of the variance, while the covariance of this groups of parameters explains 4.8% of the total variance. In total, 21.4% is explained variance by the set of physicochemical parameters. The same calculation can be performed for the set of bioclimatic parameters. The explained variance is 15.8%, out of which the independent effect is 11% and the covariance 4.8%.

Table 1. Results of canonical correspondence analysis of charophyte presence in relation to environmental variables.

Variables	Axes 1	Axes 2	Axes 3	Axes 4
Eigenvalues	0.65	0.45	0.42	0.28
Species–environment correlations	0.96	0.93	0.89	0.91
Cumulative percentage variance				
of species data	8.83	14.89	20.63	24.41
of species–environment relation	27.29	46.05	63.80	75.48
Weighted correlation coefficients				
Substrate: Clay	−0.775	−0.199	−0.25	−0.182
Substrate: Sand	0.921	0.106	−0.094	−0.011
Substrate: Mud	−0.126	0.198	0.654	0.375
Depth (m)	0.525	0.583	−0.186	−0.029
Water temperature (°C)	−0.003	−0.280	−0.447	0.230
Altitude (m)	0.797	−0.318	0.341	−0.149
BIO7 [1] (°C)	−0.125	−0.029	0.449	0.208
BIO14 [2] (mm)	−0.707	0.228	0.019	−0.478

[1] Temperature Annual Range, [2] Precipitation of Driest Month (BIO14).

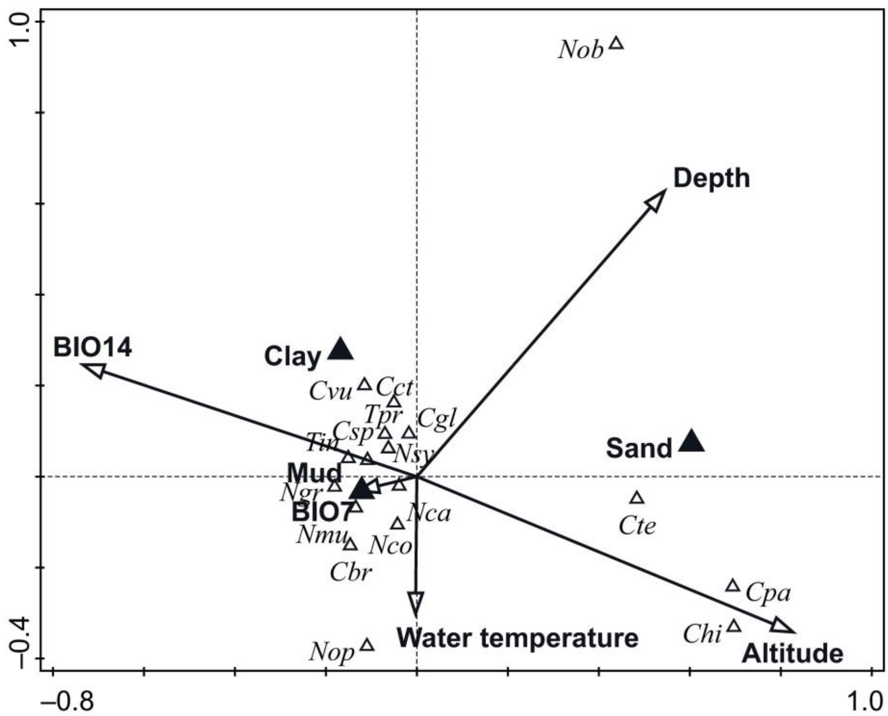

Figure 1. CCA ordination of charophytes and environmental variables in analyzed water bodies in Vojvodina (Serbia). Abbreviations of significant variables ($p < 0.05$) besides Altitude, Depth, Substrate and Water Temperature: BIO7—Temperature Annual Range, BIO14—Precipitation of Driest Month. Abbreviations of Charophyte species: *Cbr—Chara braunii*, *Cct—Chara contraria*, *Cgl—Chara globularis*, *Chi—Chara hispida*, *Cpa—Chara papillosa*, *Cte—Chara tenuispina*, *Cvu—Chara vulgaris*, *Nca—Nitella capillaris*, *Nco -Nitella confervacea*, *Ngr—Nitella gracilis*, *Nmu—Nitella mucronata*, *Nop—Nitella opaca*, *Nsy—Nitella syncarpa*, *Nob—Nitellopsis obtusa*, *Tin—Tolypella intricata*, *Tpr—Tolypella prolifera*.

Table 2. Variation partitioning for environmental variables.

Fraction	Variation	% of Explained	% of All
a—First group [1]	1.22	51.10	16.50
b—Second Group [2]	0.81	34.00	11.00
c—Shared Effect	0.36	15.00	4.80
Total Explained	2.39	100.00	32.30
All Variation	7.38	–	100.00

[1] (a) Substrate, Depth, Water Temperature; [2] (b) Altitude, Temperature Annual Range (BIO7), Precipitation of Driest Month (BIO14)

The substate (sand) and altitude show the strongest and an overall positive correlation with the first axis, while parameters like the precipitation of the driest month (BIO14) and substrate (mud) show a slightly weaker and generally negative correlation. Depth is almost equally correlated with both first and the second axes, while parameters such as temperature of the water (T), temperature annual range (BIO7), and substrate (clay) do not show significant correlations with the first two axes (Table 1). Figure 1 also shows the relations of charophyte species and significant parameters.

The isolated position of the species *Nitellopsis obtusa* is clearly visible. It showed a preference for somewhat deeper water ecosystems with a sandy substrate. *Chara papillosa, C. hispida* and *C. tenuispina* were associated with sand as a substrate and with slightly higher altitudes. *Chara hispida* and *C. papillosa* also showed a preference for deeper waters, as well as for smaller amounts of precipitation during the driest month (BIO14). Other species are mostly related with altitude, having their habitats mostly located at slightly lower altitudes, along with a greater amount of precipitation during the driest month (BIO14).

The majority of *Nitella* species are typically found in shallower waters, except for the species *Nitella syncarpa*, which was found in somewhat deeper waters reflected by its position on the graph. Additionally, the *Nitella* species, except for *N. opaca*, did not show pronounced preferences for altitudes, nor for the precipitation of the driest month (BIO14). *Nitella gracilis* is distinguished from other species of this genus only by a somewhat weaker preference for lower altitudes and higher values of the BIO14 parameter. In contrast, they are all associated with muddy substrates, that is, no *Nitella* species were found on sandy substrates. Some species, such as *Chara vulgaris, C. contraria, C. globularis* and *Tolypella prolifera*, showed a moderate association with deeper water, as well as with increased precipitation of the driest month (BIO14). *Chara braunii* is associated with mud substrates, lower altitudes and shallow waters. It is moderately related with precipitation of the driest month (BIO14).

Although the chemical parameters have not been singled out by CCA as statistically significant, it is worth drawing attention to the values of TP and nitrates in this study. In this survey, waterbodies in which charophytes were found were mostly highly productive and contained increased concentrations of phosphorus in the water. *Nitellopsis obtusa* was the only species found only in waterbodies with low TP values. On the other side, nitrate concentrations were rather low (Table S1), below 1.3 mg/l in all but one waterbody.

What is worth mentioning is that the protected areas of Vojvodina stand out as the richest areas in terms of the number of charophyte findings and the number of charophyte species. More than two-thirds of charophyte findings were located within the protected areas of Vojvodina (see Table S1). SNR "Zasavica" and SNR "Gornje Podunavlje" particularly distinguished during the investigation, with nine species of charophytes found on their territories.

4. Discussion

The percentage of explained variance by significant factors of 32.34% can seem low. However, if we take into consideration that our survey was not primarily focused on the investigation of charophyte historical localities but instead on a variety of habitats in geographic and ecological terms, it can be stated that factors selected by CCA explain charophyte variance rather well. Out of six significant parameters, altitude and substrate contributed the most. Even though the altitude range in this data set is rather small, spanning from 67 to 126 m (see Table S1), altitude was singled out as one of the most significant factors. The present study confirmed that lower altitudes support charophyte species diversity. Many studies have shown this tendency of species number reduction with higher altitude [27,67–69] and that of altitude generally being a significant parameter influencing charophyte diversity [70]. Charophytes in this study were most frequently found in muddy substrates, followed by sand and clay as substrates, which is in accordance with their general ecology [1,3,68]. Together with altitude, substrate was the most significant variable explaining charophyte distribution in Vojvodina.

As shown in Figure 1, CCA analysis separated charophyte species along the first axis, distinguishing two main types of charophyte habitats in Vojvodina. One type was separated in relation to altitude and depth as being small and shallow, located at lower altitudes, and having a muddy or clayish substrate. These habitats were associated with higher values of the "Precipitation of the Driest Month", meaning they were very dependent on precipitation and susceptible to drying out. Such habitats were the most represented in our study, exemplified by ephemeral ruts and puddles in the river floodplains. Two groups of charophyte species were generally found in these habitats.

One group was made of very tolerant species, such as *Chara globularis, C. vulgaris* and *Nitella mucronata*. They have wide ecological amplitude for most environmental parameters and are highly tolerant to the increased trophy of the water [11], making them the most common Serbian [14,27] and European charophyte species [1,3,9,29,53]. *Chara vulgaris* is especially known as a pioneer species, being resistant to drought and frequently found in shallow and often eutrophic ephemeral habitats [3,53,71]. In spite of its wide ecological amplitude, *C. contraria* was found less frequently during our survey, probably owing to its preference for somewhat deeper waterbodies with lower production, in comparison to *C. vulgaris* [53,71]. *Chara globularis, C. vulgaris* and *C. contraria* survival success can be explained by their high fertility, that is, their ability to produce a high number of oospores [71], further influencing their distribution.

The second group of species, inhabiting shallow ephemeral habitats, is made of so-called "spring" species, such as species of the genus *Tolypella* and *Nitella capillaris*. Other *Nitella* species, such as *Nitella opaca*, *N. sycarpa* and *N. gracilis*, that are not typically "spring" species [3,72–74] showed this ability in the environment of Vojvodina. In these ephemeral habitats, they show the ability to develop very early during the spring and finish their life cycle quickly before their habitat dries up during summer. Their oospores are very abundant and resistant and can stay viable for a long period of time, survive unfavorable periods, and germinate when suitable conditions are met. Periods of drought or air exposure can even be favorable for their survival, triggering the germination of oospores [74].

An environmental parameter important to mention here is temperature. The temperature of the water, as well as the bioclimatic parameter BIO7 (temperature annual range), were both significant in this study. This can be explained by their role in oospores germination. Higher temperatures enable faster warming up of the water and hence provide conditions for earlier oospore germination [72,75,76]. In such conditions, even species that usually germinate later during the season start their life cycle sooner. The BIO7 parameter is related to the ability of these species to tolerate higher temperature variation during the year as well as lower temperatures, making them able to start their life cycle already in the spring season. *C. braunii* should be mentioned in relation to these factors as this species showed preferences towards higher water temperature and smaller annual temperature range (BIO7), meaning that it prefers warm waters. In Vojvodina and generally, it typically thrives in shallow and often temporary eutrophic waters [1,53].

One of the significant inferences and challenges in studying these ephemeral habitats is finding the appropriate timing for their investigation. The appearance and subsequent drying out of these habitats depend on differences in bioclimatic parameters from year to year and most often these waterbodies and the vegetation present last only a few months. There remain gaps in the knowledge [8] to be filled in terms of studying the life histories and ecology of individual species [4,74,76], we well as environmental factors' interactive effect on charophytes [77,78]. Our investigations also confirm the predictions of some authors [71,79], in accordance with the predicted climate scenario, that these species successfully thriving in habitats susceptible to desiccation, usually at low altitudes, will in the future have an adaptive advantage compared to the species adapted to more stable environments.

These more stable aquatic habitats made the other habitat type, on the other side of the gradient of the environmental parameters, associated with a higher altitude, depth and sand substrate, less affected by the precipitation parameter and more permanent. Sandpits and river habitats belong to this habitat type. The species inhabiting this type of habitat were *Nitellopsis obtusa*, *Chara tenuispina*, *C. papillosa* and *C. hispida*. *N. obtusa* was found in a sandpit and Danube River Bay, several meters deep, had low TP values, was associated with sandy substrates and made meadows together with other macrophytes, all of which is in accordance with its known ecology [1,3,73]. *C. tenuispina*, one of the rarest European charophytes, was found during this survey twice, both in a sandpit and a slow-running river with high EC and sand substrate, being deeper and at higher altitudes compared to other habitats. During later investigations, this species has been found several times more in Vojvodina at three new localities, all of them being slightly saline. These habitats are not optimal for this species, but it is known to tolerate such conditions [26] and references therein. *C. hispida* and *C. papillosa* were found only in sandpits. *C. hispida* is known to be an ecologically tolerant species inhabiting a wide range of habitats, with a preference for deeper waterbodies with sandy and sandy–muddy substrates [1,31,53]. *C. papillosa* has similar ecology tolerating different ecological conditions but favors highly mineralized and strongly alkaline habitats with sand or sandy–muddy substrates [1,2,73].

Although chemical parameters in this study were not selected by CCA as significant, and samples for chemical analyses were taken only ones during the season, and also taking into consideration the fact that physico-chemical factors in small waterbodies vary greatly [80], there are a few remarks we consider important to give here. Even though charophyte algae are very often mentioned in the literature as indicators of oligotrophic and clear water [10], this study has shown that many species are tolerant to eutrophic or even hypertrophic conditions of the water, judging by high TP values. Similar results are given by [5,38,81,82]. Furthermore, during our investigations, we have found even five species of charophytes thriving together in only a puddle or a rut, which is a remarkable number even for a pond or a lake. It should be emphasized though that light was not a limiting factor in charophyte habitats in Vojvodina, where most of them were transparent to the bottom or very transparent if deeper. *N. obtusa* was the only charophyte species in this study found only in waters with low TP values. According to the literature, this species optimally inhabits meso-eutrophic waters, but it can tolerate eutrophic conditions as well [11,53], unlike hypertrophic conditions [9]. Nitrates were low in the majority of their habitats in Vojvodina, which is in accordance with the position of [38] that nitrates are more likely to be a limiting factor for charophytes than phosphorus. In their study, the limiting concentration for charophytes was 2.5 mg/l. All but one waterbody in our study fits this criterion. Nitrates as detrimental factors for charophyte algae growth are also confirmed by [83], although their influence is dependent on other environmental factors, such as temperature, local adaptations, etc., and is still a subject of investigation [78].

The ability of charophyte algae to colonize newly created habitats, such as ruts, puddles, and sandpits, and act as "pioneer species" [8] is directly related to a high number of their findings in these habitats. On the contrary, in waterbodies already dominated by other submerged macrophytes, filamentous algae, or phytoplankton communities, charophytes are found noticeably less frequently. The main reason for this lies in the reduction of light in such habitats and is due to charophytes being less competitive in such conditions [12,13,81].

Finally, we would like to draw attention to the significance of protected areas of Vojvodina that can be considered real shelters for charophyte species. Unfortunately, the condition of the waters of Vojvodina is far from good, considering that more than 80% of its territory is used for agricultural purposes [84]. Waterbodies are under heavy anthropogenic influence in various possible ways [85]. The newest investigations confirm that protected areas, such as SNR "Zasavica" or SNR "Gornje Podunavlje", are real hotspots of charophyte diversity [20,23,24,44], while other areas should be protected before their habitats and the biodiversity is lost [19,26]. It is also crucial to emphasize the importance of small water waterbodies [86,87] as these habitats are real gems of charophyte and other species' biodiversity.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d15030342/s1, Table S1: The species and environmental matrix used in the CCA analysis.

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References

1. Urbaniak, J.; Gąbka, M. *Polish Charophytes: An Illustrated Guide to Identification*; Uniwersytet Przyrodniczy we Wrocławiu: Wroclaw, Poland, 2014; pp. 1–122.
2. Rey-Boissezon, A.; Auderset Joye, D. Habitat Requirements of Charophytes—Evidence of Species Discrimination through Distribution Analysis. *Aquat. Bot.* **2015**, *120*, 84–91. [CrossRef]
3. Mouronval, J.B.; Baoudouin, S.; Borel, N.; Soulié-Märche, I.; Grillas, P. *Guide des Characees de France Mediterraneenne*; Office National de la Chasse et de la Faune Sauvage: Paris, France, 2015; p. 211.
4. Casanova, M.T.; Brock, M.A. Life Histories of Charophytes from Permanent and Temporary Wetlands in Eastern Australia. *Aust. J. Bot.* **1999**, *47*, 383–397. [CrossRef]
5. Lambert-Servien, E.; Clemenceau, G.; Gabory, O.; Douillard, E.; Haury, J. Stoneworts (Characeae) and Associated Macrophyte Species as Indicators of Water Quality and Human Activities in the Pays-de-La-Loire Region, France. *Hydrobiologia* **2006**, *570*, 107–115. [CrossRef]
6. Rey-Boissezon, A.; Auderset Joye, D. A Temporary Gravel Pit as a Biodiversity Hotspot for Aquatic Plants in the Alps. *Arch. Sci.* **2012**, *65*, 177–190. Available online: http://archive-ouverte.unige.ch/unige:27718 (accessed on 22 March 2022).
7. Azzella, M.M.; Rosati, L.; Iberite, M.; Bolpagni, R.; Blasi, C. Changes in Aquatic Plants in the Italian Volcanic-Lake System Detected Using Current Data and Historical Records. *Aquat. Bot.* **2014**, *112*, 41–47. [CrossRef]
8. Schubert, H.; Blindow, I.; Bueno, N.C.; Casanova, M.T.; Pełechaty, M.; Pukacz, A. Ecology of Charophytes—Permanent Pioneers and Ecosystem Engineers. *Perspect. Phycol.* **2018**, *5*, 61–74. [CrossRef]
9. Penning, W.E.; Mjelde, M.; Dudley, B.; Hellsten, S.; Hanganu, J.; Kolada, A.; Van Den Berg, M.S.; Poikane, S.; Phillips, G.; Willby, N.J.; et al. Classifying Aquatic Macrophytes as Indicators of Eutrophication in European Lakes. *Aquat. Ecol.* **2008**, *42*, 237–251. [CrossRef]
10. Schneider, S.C.; García, A.; Martín-Closas, C.; Chivas, A.R. The Role of Charophytes (Charales) in Past and Present Environments: An Overview. *Aquat. Bot.* **2015**, *120*, 2–6. [CrossRef]
11. Kolada, A. Charophyte Variation in Sensitivity to Eutrophication Affects Their Potential for the Trophic and Ecological Status Indication. *Knowl. Manag. Aquat. Ecosyst.* **2021**, *422*, 30. [CrossRef]
12. Kufel, L.; Kufel, I. Chara Beds Acting as Nutrient Sinks in Shallow Lakes—A Review. *Aquat. Bot.* **2002**, *72*, 249–260. [CrossRef]

13. Pukacz, A.; Pełechaty, M.; Pełechata, A. The Relation between Charophytes and Habitat Differentiation in Temperate Lowland Lakes. *Pol. J. Ecol.* **2013**, *61*, 105–118.
14. Blaženčić, J. Overview of the Stoneworts (Charales) of Serbia with the Estimation of the Threat Status. *Bot. Serbica* **2014**, *38*, 121–130.
15. Trbojević, I.; Predojević, D. Algae in shallow and small water bodies of Serbia: A frame for species and habitat protection. In *Small Water Bodies of the Western Balkans*; Springer Water: Cham, Switzerland, 2022; pp. 147–188. [CrossRef]
16. Vesić, A.; Blaženčić, J.; Šinžar-Sekulić, J. Contribution to Knowledge of the Charophytes (Charales) of Vojvodina (Serbia)—20 Years after the First Review. *Bot. Serbica* **2016**, *40*, 237–247. [CrossRef]
17. Trbojević, I.; Simić, G.S.; Blaženčić, J.; Predojević, D. Rediscovery of *Chara canescens* Loiseleur in Serbia. *Bot. Serbica* **2019**, *43*, 97–102. [CrossRef]
18. Sabovljević, M.S.; Tomović, G.; Boycheva, P.; Ivanov, D.; Denchev, T.T.; Denchev, C.M.; Stevanoski, I.; Marković, A.; Djurović, S.Z.; Buzurović, U.; et al. New Records and Noteworthy Data of Plants, Algae and Fungi in SE Europe and Adjacent Regions, 3. *Bot. Serbica* **2021**, *45*, 119–127. [CrossRef]
19. Sabovljević, M.S.; Tomović, G.; Pantović, J.P.; Djurović, S.Z.; Buzurović, U.; Denchev, T.T.; Denchev, C.M.; Boycheva, P.; Dimitrova, T.; Marković, A.; et al. New Records and Noteworthy Data of Plants, Algae and Fungi in SE Europe and Adjacent Regions, 9. *Bot. Serbica* **2022**, *46*, 311–320. [CrossRef]
20. Trbojević, I.; Milovanović, V.; Simić, G.S. The Discovery of the Rare *Chara baueri* (Charales, Charophyceae) in Serbia. *Plants* **2020**, *9*, 1606. [CrossRef]
21. Trbojević, I.; Marković, A.; Blaženčić, J.; Subakov Simić, G.; Nowak, P.; Ballot, A.; Schneider, S.C. Genetic and Morphological Variation in Chara Contraria and a Taxon Morphologically Resembling *Chara connivens*. *Bot. Lett.* **2020**, *167*, 187–200. [CrossRef]
22. Tomović, G.; Sabovljević, M.S.; Mašić, E.; Popović, S.S.; Marković, A.; Trbojević, I.; Pantović, J.; Sutorý, K.; Niketić, M.; Boycheva, P.; et al. New Records and Noteworthy Data of Plants, Algae and Fungi in SE Europe and Adjacent Regions, 6. *Bot. Serbica* **2021**, *45*, 361–368. [CrossRef]
23. Trbojević, I.S.; Predojević, D.D.; Šinžar-Sekulić, J.B.; Nikolić, N.V.; Jovanović, I.M.; Subakov-Simić, G.V. Charophytes of Gornje Podunavlje Ponds: Revitalization Process Aspect. *Zb. Matice Srp. Prir. Nauk./Matica Srp. J. Nat. Sci.* **2019**, *136*, 123–131. [CrossRef]
24. Trbojević, I.; Marković, A.; Stanković, M. Istraživanja pršljenčica u SRP Zasavica od 2014 do 2020. godine (Charophytes research in the SNR Zasavica 2014–2020). In Proceedings of the "Naučno-Stručni Skup o Biodiverzitetu i Drugim Vrednostima Rezervata Zasavica—Zasavica 2022", Sremska Mitrovica, Serbia, 25 November 2022; pp. 49–58.
25. Damnjanović, B.; Novković, M.; Vesić, A.; Živković, M.; Radulović, S.; Vukov, D.; Anđelković, A.; Cvijanović, D. Biodiversity-Friendly Designs for Gravel Pit Lakes along the Drina River Floodplain (the Middle Danube Basin, Serbia). *Wetl. Ecol. Manag.* **2019**, *27*, 1–22. [CrossRef]
26. Tomović, G.; Sabovljević, M.S.; Irimia, I.; Taşkın, H.; Zupan, E.; Boycheva, P.; Ivanov, D.; Papp, B.; Pantović, J.; Marković, A.; et al. New Records and Noteworthy Data of Plants, Algae and Fungi in SE Europe and Adjacent Regions, 10. *Bot. Serbica* **2022**, *46*, 321–330. [CrossRef]
27. Vesić, A.; Blaženčić, J.; Šinžar-Sekulić, J. Ecological Preferences of Charophytes in Serbia in Relation to Habitat Type and Other Aquatic Macrophytes. *Plant Biosyst.—Int. J. Deal. All Asp. Plant Biol.* **2016**, *150*, 490–500. [CrossRef]
28. Schneider, S.C.; Biberdžić, V.; Braho, V.; Gjoreska, B.; Cara, M.; Dana, Z.; Eriksen, T.E.; Hjermann, D.; Imeri, A.; Jovanović, K.; et al. Littoral Eutrophication Indicators Are More Closely Related to Nearshore Land Use than to Water Nutrient Concentrations: A Critical Evaluation of Stressor-Response Relationships. *Sci. Total Environ.* **2020**, *748*, 141193. [CrossRef]
29. Caisova, L.; Gąbka, M. Charophytes (Characeae, Charophyta) in the Czech Republic: Taxonomy, autecology and distribution. *Fottea* **2009**, *9*, 1–43. [CrossRef]
30. Urbaniak, J.; Sugier, P.; Gąbka, M. Charophytes of The Lubelszczyzna Region (Eastern Poland). *Acta Soc. Bot. Pol.* **2011**, *80*, 159–168. [CrossRef]
31. Barinova, S.S.; Romanov, R.E.; Solak, C.N. New Record of *Chara hispida* (L.) Hartm. (Streptophyta: Charophyceae, Charales) from the Işıklı Lake (Turkey) and Critical Checklist of Turkish Charophytes. *Nat. Resour. Conserv.* **2014**, *2*, 33–42. [CrossRef]
32. Muller, S.D.; Rhazi, L.; Soulie-Märsche, I.; Benslama, M.; Bottollier-Curtet, M.; Daoud-Bouattour, A.; De Belair, G.; Ghrabi-Gammar, Z.; Grillas, P.; Paradis, L.; et al. Diversity and Distribution of Characeae in the Maghreb (Algeria, Morocco, Tunisia). *Cryptogam. Algol.* **2017**, *38*, 201–251. [CrossRef]
33. Romanov, R.E.; Zhakova, L.V.; Bazarova, B.B.; Kipriyanova, L.M. The Charophytes (Charales, Charophyceae) of Mongolia: A Checklist and Synopsis of Localities, Including New Records. *Nov. Hedwig.* **2014**, *98*, 127–150. [CrossRef]
34. Romanov, R.E.; Chemeris, E.V.; Zhakova, L.V.; Ivanova, A.V.; Palagushkina, O.V. The Charophytes (Charales, Charophyceae) from the Middle Volga Region (Russia) Synopsis of Localities and Species Protection. *Nat. Conserv. Res.* **2018**, *3*, 1–20. [CrossRef]
35. Casanova, M.T. An Overview of *Chara* L. in Australia (Characeae, Charophyta). *Aust. Syst. Bot.* **2005**, *18*, 25–39. [CrossRef]
36. Casanova, M.T. An Overview of *Nitella* (Characeae, Charophyceae) in Australia. *Aust. Syst. Bot.* **2009**, *22*, 193–218. [CrossRef]
37. Ahmadi, A.; Riahi, H.; Sheidai, M.; Van Raam, J. Some Charophytes (Characeae, Charophyta) from Central and Western of Iran Including *Chara kohrangiana* Species Nova. *Cryptogam. Algol.* **2012**, *33*, 359–390. [CrossRef]
38. Lambert, S.J.; Davy, A.J. Water Quality as a Threat to Aquatic Plants: Discriminating between the Effects of Nitrate, Phosphate, Boron and Heavy Metals on Charophytes. *New Phytol.* **2011**, *189*, 1051–1059. [CrossRef]

39. Baastrup-Spohr, L.; Iversen, L.L.; Dahl-Nielsen, J.; Sand-Jensen, K. Seventy Years of Changes in the Abundance of Danish Charophytes. *Freshw. Biol.* **2013**, *58*, 1682–1693. [CrossRef]
40. EU-DEM v1.1. Available online: https://land.copernicus.eu/imagery-in-situ/eu-dem/eu-dem-v1.1?tab=metadata (accessed on 5 September 2020).
41. Katić, P.; Đukanović, D.; Đaković, P. *Klima SAP Vojvodine*; OOUR Institut za Ratarstvo i Povrtarstvo, Poljoprivredni Fakultet, Univerzitet u Novom Sadu: Novi Sad, Serbia, 1979; pp. 1–237.
42. Živković, B.; Nejgebauer, V.; Tanasijević, Đ.; Miljković, N.; Stojković, L.; Drezgić, P. *Zemljišta Vojvodine*; Institut za Poljoprivredna Istraživanja Novi Sad: Novi Sad, Serbia, 1972; pp. 1–669.
43. Mesaroš, G.; Dožai, J. Inventarizacija i klasifikacija malih stajaćih voda u Vojvodini. In *Vojvođanske Bare—Vrednosti i mogućnosti*; Mesaroš, G., Ed.; Udruženje Protego: Subotica, Serbia, 2011; pp. 5–14.
44. Vesić, A.; Blaženčić, J.; Stanković, M. Charophytes in the Zasavica Special Nature Reserve. *Arch. Biol. Sci.* **2011**, *63*, 883–888. [CrossRef]
45. Lakušić, D.; Blaženčić, J.; Ranđelović, V.; Butorac, B.; Vukojičić, S.; Zlatković, B.; Jovanović, S.; Šinžar-Sekulić, J.; Žukovec, D.; Ćalić, I.; et al. Staništa Srbije—Priručnik sa opisima i osnovnim podacima. In *Staništa Srbije*; Lakušić, D., Ed.; Institut za Botaniku i Botanička Bašta "Jevremovac", Biološki Fakultet, Univerzitet u Beogradu: Beograd, Serbia, 2005.
46. Braun-Blanquet, J. *Pflanzensoziologie. Grundzüge der Vegetationskunde, 3 ed.*; Springer: New York, NY, USA, 1964.
47. Savezni zavod za Zdravstvenu Zaštitu, NIP "Privredni Pregled". *P-V-2/B Metoda B: Spektrofotometrijski sa Nesslerovim reagensom (bez destilacije) In Voda za Piće: Standardne Metode za Ispitivanje Higijenske Ispravnosti*; Savezni Zavod za Zdravstvenu Zaštitu, NIP "Privredni Pregled": Belgrade, Serbia, 1990.
48. *SRPS EN 26777:2009*; Water Quality—Determination of Nitrite—Molecular Absorption Spectrometric Method (ISO 6777:1984). Institute for Standardization of Serbia: Belgrade, Serbia, 2009.
49. American Public Health Association; American Water Works Association; Water Environment Federation. 4500-NO3-B Nitrogen (Nitrate) Ultraviolet Spectrophotometric Screening Method. In *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; American Public Health Association: Washington, DC, USA, 1995.
50. *SRPS EN ISO 6878:2008*; Water Quality—Determination of Phosphorus—Ammonium Molybdate Spectrometric Method (ISO 6878:2004). Institute for Standardization of Serbia: Belgrade, Serbia, 2008.
51. Krause, W. Charales (Charophyceae). In *Süsswasserflora von Mitteleuropa 18*; Ettl, H., Gärtner, G., Heynig, H., Mollenhauer, D., Eds.; Gustav Fischer Verlag: Jena, Germany, 1997; pp. 1–102.
52. Schubert, H.; Blindow, I. *Charophytes of the Baltic Sea*; The Baltic Marine Biologists Publication No.19; A.R.G. Gantner Verlag: Ruggell, Liechtenstein, 2003; pp. 1–326.
53. Bailly, G.; Schaefer, O. *Guide illustré des Characées du Nord-Est de la France*; Conservatoire Botanique National de Franche: Comté, France, 2010; pp. 1–96.
54. Josifović, M. *Flora SR Srbije I–IX*; Srpska Akademija Nauka i Umetnosti: Beograd, Serbia, 1970–1977.
55. Jávorka, S.; Csapody, V. *Iconographia Florae Partis Austro-Orientalis Europae Centralis = Iconography of the Flora from the South-Eastern Part of Central EUROPE*; Adademiai Kiado: Budapest, Hungary, 1975.
56. Sarić, M. *Flora SR Srbije X*; Srpska Akademija Nauka i umetnosti: Beograd, Serbia, 1986.
57. Sarić, M.R. *Flora Srbije 1, II Izdanje*; Srpska Akademija Nauka i Umetnosti: Beograd, Serbia, 1992.
58. Preston, C.D. *Pondweeds of Great Britain and Ireland*; Botanical Society of the British Isles: London, UK, 1995.
59. Arbeitsgruppe Characeen Deutschlands Hrsg. *Armleuchteralgen. Die Characeen Deutschlands*; Springer Spektrum: Rostock, Germany, 2016; pp. 1–618.
60. IPNI. International Plant Name Index. 2023. Available online: http://www.ipni.org (accessed on 26 January 2023).
61. Fick, S.E.; Hijmans, R.J. WorldClim 2: New 1km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **2017**, *37*, 4302–4315. [CrossRef]
62. Trabucco, A.; Zomer, R. Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v3. figshare. *Dataset* **2022**, *10*, m9. [CrossRef]
63. QGIS Development Team. QGIS Geographic Information System. *Open Source Geospatial Foundation Project.* 2022. Available online: http://qgis.osgeo.org (accessed on 24 March 2022).
64. Van Der Maarel, E. Transformation of cover-abundance values in phytosociology and its effects on community similarity. *Vegetatio* **1979**, *39*, 97–114.
65. Ter Braak, C.J.F.; Šmilauer, P. *CANOCO, Version 4.5*; Biometris-Plant Research International: Wageningen, The Netherlands, 2002.
66. Borcard, D.; Legendre, P.; Drapeau, P. Partialling out the spatial component of ecological variation. *Ecology* **1992**, *73*, 1045–1055. [CrossRef]
67. Auderset Joye, D.; Castella, E.; Lachavanne, J.B. Occurrence of Characeae in Switzerland over the Last Two Centuries (1800–2000). *Aquat. Bot.* **2002**, *72*, 369–385. [CrossRef]
68. Blaženčić, J.; Stevanović, B.; Blaženčić, Ž.; Stevanović, V. Red data list of Charophytes in the Balkans. *Biodivers. Conserv.* **2006**, *15*, 3445–3457. [CrossRef]
69. Kochjarová, J.; Novikmec, M.; Oťaheľová, H.; Hamerlík, L.; Svitok, M.; Hrivnák, M.; Senko, D.; Bubíková, K.; Matúšová, Z.; Paľove-Balang, P.; et al. Vegetation-Environmental Variable Relationships in Ponds of Various Origins along an Altitudinal Gradient. *Pol. J. Environ. Stud.* **2017**, *26*, 1575–1583. [CrossRef] [PubMed]

70. Sametova, E.; Jumakhanova, G.; Nurashov, S.; Barinova, S.; Jiyenbekov, A.; Smith, T. Microalgae Indicators of Charophyte Habitats of South and Southeast Kazakhstan. *Diversity* **2022**, *14*, 530. [CrossRef]
71. Auderset Joye, D.; Rey-Boissezon, A. Will Charophyte Species Increase or Decrease Their Distribution in a Changing Climate? *Aquat. Bot.* **2015**, *120*, 73–83. [CrossRef]
72. Gąbka, M.; Owsianny, P.M. The Occurrence and Habitat Requirements of *Nitella syncarpa* (Thuillier) Chevallier and *Nitelletum syncarpae* (Corillion 1957) Dąmbska 1996 in the Wielkopolska Region. *Rocz. Akad. Rol. w Pozn.* **2005**, *373*, 59–68.
73. Auderset Joye, D.; Schwarcer, A. *Liste Rouge Characées—Espèces menacées en Suisse, État 2010*; Ofce Fédéral de L'environnement: Berne, Switzerland; Laboratoire D'écologie et de Biologie Aquatique de l'Université de Genève: Genève, Switzerland, 2012; pp. 1–72.
74. Auderset Joye, D.; Boissezon, A. New Insights into the Ecology and Phenology of Two Characeae: *N. opaca* (Bruzelius) C. Agardh and *N. gracilis* (Sm.) C. Agardh. *Bot. Lett.* **2018**, *165*, 91–102. [CrossRef]
75. Bonis, A.; Grillas, P. Deposition, Germination and Spatio-Temporal Patterns of Charophyte Propagule Banks: A Review. *Aquat. Bot.* **2002**, *72*, 235–248. [CrossRef]
76. Calero, S.; Auderset Joye, D.; Rey-Boissezon, A.; Rodrigo, M.A. Time and Heat for Sexual Reproduction: Comparing the Phenology of *Chara hispida* of Two Populations at Different Latitudes. *Aquat. Bot.* **2017**, *136*, 71–81. [CrossRef]
77. Rojo, C.; Carramiñana, M.; Cócera, D.; Roberts, G.P.; Puche, E.; Calero, S.; Rodrigo, M.A. Different Responses of Coexisting Chara Species to Foreseeable Mediterranean Temperature and Salinity Increases. *Aquat. Bot.* **2017**, *138*, 53–63. [CrossRef]
78. Puche, E.; Sánchez-Carrillo, S.; Álvarez-Cobelas, M.; Pukacz, A.; Rodrigo, M.A.; Rojo, C. Effects of Overabundant Nitrate and Warmer Temperatures on Charophytes: The Roles of Plasticity and Local Adaptation. *Aquat. Bot.* **2018**, *146*, 15–22. [CrossRef]
79. Rojo, C.; Martínez-Ruiz, C.; Carramiñana, M.; Rodrigo, M.A. Foreseeable Global Warming Will Differentially Affect *Chara vulgaris* Populations from Different Altitudes. *Aquat. Bot.* **2015**, *122*, 20–26. [CrossRef]
80. Szpakowska, B.; Świerk, D.; Pajchrowska, M.; Gołdyn, R. Verifying the Usefulness of Macrophytes as an Indicator of the Status of Small Waterbodies. *Sci. Total Environ.* **2021**, *798*, 149279. [CrossRef]
81. Kłosowski, S.; Tomaszewicz, G.H.; Tomaszewicz, H. The Expansion and Decline of Charophyte Communities in Lakes within the Sejny Lake District (North-Eastern Poland) and Changes in Water Chemistry. *Limnol.—Ecol. Manag. Inl. Waters* **2006**, *36*, 234–240. [CrossRef]
82. Søndergaard, M.; Johansson, L.S.; Lauridsen, T.L.; Jørgensen, T.B.; Liboriussen, L.; Jeppesen, E. Submerged Macrophytes as Indicators of the Ecological Quality of Lakes. *Freshw. Biol.* **2010**, *55*, 893–908. [CrossRef]
83. Rodrigo, M.A.; Puche, E.; Rojo, C. On the Tolerance of Charophytes to High-Nitrate Concentrations. *Chem. Ecol.* **2018**, *34*, 22–42. [CrossRef]
84. Sekulić, P.; Ninkov, J.; Zeremski-Škorić, T.; Vasin, J.; Milić, S. Zemljište. In *Životna Sredina u Autonomnoj Pokrajini Vojvodini: Stanje-Izazovi-Perspektive*; Puzović, S., Radovanović-Jovin, H., Eds.; Pokrajinski Sekretarijat za Urbanizam, Graditeljstvo i Zaštitu Životne Sredine: Novi Sad, Serbia, 2011; pp. 94–133.
85. Dalmacija, B.; Bečelić Tomin, M.; Krčmar, D.; Lazić, N. Vode. In *Životna Sredina u Autonomnoj Pokrajini Vojvodini: Stanje-Izazovi-Perspektive*; Puzović, S., Radovanović-Jovin, H., Eds.; Pokrajinski Sekretarijat za Urbanizam, Graditeljstvo i Zaštitu Životne Sredine: Novi Sad, Serbia, 2011; pp. 94–133.
86. Brock, M.A. Social Awareness of Temporary Wetlands: A Southern Hemisphere Perspective on The Past, Present and Future. In Proceedings of the International Conference on Temporary ponds Menorca 2009, Menorca, Spain, 5–8 May 2009; pp. 363–375.
87. Biggs, J.; von Fumetti, S.; Kelly-Quinn, M. The Importance of Small Waterbodies for Biodiversity and Ecosystem Services: Implications for Policy Makers. *Hydrobiologia* **2017**, *793*, 3–39. [CrossRef]