

1 **Algae community structure and relation to** 2 **environmental factors in the Hulanhe Wetland,** 3 **Northeast China**

4
5

6 Hongkuan Hui ^{1,2*}, Min Wang¹, Yunlong Li¹, Yubin Liu¹

7 ¹School of Geography and Tourism, Qilu Normal University, Jinan, Shandong,
8 China

9 ² College of Geography and Environment, Shandong Normal University, Jinan,
10 Shandong, China

11

12

13 ***Corresponding author**

14 Email: hkh8508@163.com (HHK)

Abstract

The distribution characteristic of algae community was evaluated in eight sampling sites based on a monthly survey during May to October 2020 and 2021 in the Hulanhe Wetland, Northeast China. Algae and water environmental factors including water temperature (T), pH, Conductivity (Cond.), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbons (TOC), total phosphorus (TP) and nitrogen (TN) were investigated, and a total of 216 taxa were identified by microscope. The algae community was dominated by Bacillariophyta, Chlorophyta and Euglenophyta. Species such as *Melosira granulate*, *Cyclotella meneghiniana*, *Navicula cryptocephala* and *Pandorina morum* were the most common species. Significant difference of the algae composition and abundance were found in the different sampling sites. Species composition in H8 were different variously with other sites, provide a good chance for us to study the tolerance of the species, compared with other sites, several pollution indicator species were observed. According to canonical correspondence analysis (CCA), TN and TP were the main environmental factors affecting the distribution of algae. Furthermore, temperature and dissolved oxygen were also important to the algae community. It is suggested that the relationship between algae community and environmental factors can point to new directions in future studies on the water quality and habitat conditions in a wetland.

Keywords: Algae community; Environmental factors; CCA; Hulanhe Wetland

45 **Introduction**

46 Wetlands, because of their valuable ecological functions and economic-social
47 value, are recognized as important natural resources for humans and the
48 sustainable development of the ecosystem. Hence, studies of wetlands were given
49 more and more attention and have become the focus of environmental science
50 research [1].

51 Algae are the primary producer in aquatic food webs, and it is of great
52 importance to evaluate the physical, chemical and biological processes of aquatic
53 ecosystems by investigating algae characteristics [2-3]. Algae diversity was
54 influenced by the water environmental conditions [4-5]. Several studies have
55 shown that environment factors such as water temperature, pH, water chemistry
56 and nutrient loading can affect the algae species composition in certain algae
57 community [6-8]. In addition, response of algae to the changes in water quality,
58 such as increased nutrient loading, can be rapid. For their sensitive to
59 environmental conditions, algae can provide information on habitats and reflect
60 changes in different environmental gradients [7,9]. Therefore, algae were widely
61 used as an indicator of the nutritional status of the aquatic environment and
62 increasingly used to assess water quality and, in particular, to monitor
63 eutrophication of lakes in agricultural and urban areas [10-11]. High diversity of
64 species and environmental preference for algae in wetland can also give us a good
65 opportunity to find out the relationship between algae and environmental factors,
66 and assess ecosystem status in wetlands [12-13]. It is essential to investigate the
67 close relationship between the distribution of algae and aquatic environment in
68 lakes and rivers [14-16]. However, algae are poorly described or studied in
69 wetlands in some regions, especially in China.

70 As one of the key restoration wetlands in China, Hulanhe Wetland located in
71 the center of the Songnen Plain, which is in the northern shore of the Songhua
72 River, Northeast China, has an excellent natural wetland ecosystem with abundant
73 fauna and flora. However, there is no research on the algae in Hulanhe Wetland,
74 and also on the assessment of the relationship between algae communities and

water quality. On the current study, the seasonal characteristics of algae from eight sampling sites in Hulanhe Wetland during May to October in 2020 and 2021 were mainly investigated. The specific objectives were to: (1) describe spatial and seasonal distribution of algae assemblages in Hulanhe Wetland; (2) evaluate the linkage between algae communities and environmental factors, and (3) find out the main environmental factors which affect algae communities most. We will be able to provide guidance on using algae as indicator to environmental change and basic ecological information for the future research in Hulanhe Wetland.

Materials and Methods

Study sites and period

Hulanhe Wetland is located in the south of Hulan District, Harbin City, Heilongjiang Province, on the north bank of Songhua River. The study area is in the center of wetlands between latitude $45^{\circ} 5' - 45^{\circ} 51' N$ and longitude $126^{\circ} 38' - 127^{\circ} 14'$ (Fig.1), covering an area of 192.62 km². It belongs to the continental climate of north temperate zone, with four distinct seasons, long winter and short summer, spring and autumn temperature rise and fall rapidly.

Eight sampling sites (H1-H8) were selected across the Hulanhe Wetland from May 2020 to October 2021 (Fig 1). Site H1 is at the 1st of hydrological monitoring station, water belonging to the semi-culture zone. Site H2 is near the pontoon. Site H3 and H4 are in the open water surfaces, not subject to the impact of sewage discharge. Site H5 is located near the outfall of the village, which is disturbed by people. Site H6 is near a village with no sewage into the water, and a large number of sands was mined. Site H7 is located at the 2nd of hydrological monitoring stations, which is far away from villages, and water flow there is much faster. Site H8 is in a closed pool, and there is a large area of farmland surrounded the pool.

Fig 1. Location of the study area and sites at Hulanhe Wetland

105 **Algae**

106 During May to October 2020 and 2021, algae samples were collected from each
107 site monthly at a water depth of 0.5 m with plankton net (mesh size 50 μ m) and
108 were preserved using Lugol's solution. For quantitative analyses of algae
109 communities, 1000 mL mixed water was collected at each site and precipitated for
110 24-36 h, the finally volume set to 30 mL of concentrated sediments. A 0.1mL
111 subsample of this finally concentrate was placed in a perspex counting chamber
112 (Uwitec, Austrian) and enumerated under a light microscope at a magnification of
113 400 $\times$ (Olympus BH $\times$ 2, Japan). Mean figure of statistically values as its biological
114 density and 500 valves were counted from each sample. Identification of taxa
115 followed the volumes by Hu and Lange-Bertalot [17-18].

117 **Water chemistry**

118 Conductivity, pH and water temperature at each site were measured in filed
119 using portable multi-parameter analyzer (DZB-718-A, China). Approximately 500
120 mL of water was collected and placed in polyethylene bottles for chemical
121 analysis in each sampling site monthly. All water chemistry measurements
122 followed standard methods and were completed within 24 h of sample collections
123 [19]. Dissolved oxygen (DO) determinations were carried out by iodometric
124 method. The total phosphorus (TP) and nitrogen (TN) determinations were carried
125 out by UV-Vis spectrophotometer (UV-2500, Shimadzu Corporation, Japan), total
126 phosphorus (TP) using the molybdenum blue method (measure the absorbance at
127 wavelength 700 nm) and total nitrogen (TN) were analyzed with potassium
128 peroxide sulphate (measure the absorbance at wavelength 220 and 275 nm,
129 respectively). Total organic carbons (TOC) were determinated by means of the
130 combustion oxidation-non-dispersive infrared absorption method.

132 **Data analysis**

133 The diversity of each sample was estimated using Shannon and Wiener's

134 diversity index (H), which was calculated with the following:

$$135 \quad H = - \sum (n_i/N) * \log_2(n_i/N)$$

136 Where n_i = the individual number of specie i ; N = the total number of species

137 Margalef's index (D) was calculated in order to indicate richness of the algae
138 assemblage and was calculated as follows:

$$139 \quad D = (S - 1)/\ln N$$

140 Where S = total number of species; and N = total abundance

141 Analysis of variance (ANOVA, SPSS v16.0) was used to test differences
142 between sites with respect to water chemistry, species richness and diversity. To
143 investigate the relationships between algae and water chemistry, a canonical
144 correspondence analysis (CCA) was performed [20]. In this paper, the calculation
145 process and drawing were conducted using Canoco for Windows 5.0 [21].

146

147 **Results and Discussion**

148 **Water chemistry**

149 The ranges of physical-chemical parameters at the eight sampling sites for a
150 twelve-month baseline survey were summarized in Table 1. During two years,
151 water temperature seasonal changed significantly and ranged from 4.3 to 27 °C, it
152 is higher in 2021 than in 2020, but temperature showed minor differences at all
153 sampling sites during our study period. DO ranged from 3.6 to 16.8 mg/L, the
154 concentrations showed significantly differences in different seasons, spring and
155 autumn were higher than summer and higher than 3 mg/L at all eight sites, with
156 the minimum average value at H1 and the maximum at H3. High concentrations
157 of both COD and BOD occurred at H1 and H5, with the maximum in May and
158 minimum in October. The concentrations of TP and TN showed the same trends at
159 all sampling sites in different seasons between 2020 and 2021, higher values at
160 H8, H1 and H5 compared to others. The TOC average concentration value of 4.4
161 mg/L in 2021, was slightly lower than in 2020, with the average value of 4.5
162 mg/L, whereas the concentrations in H5, H8 and H1 were higher than other sites (p

163 < 0.05). The concentration of Conductivity ranged from 175 to 509 $\mu\text{S}/\text{CM}$, and the
164 conductivity at H8 was obviously higher than other sites in the two years.

165

166 **Dynamic characteristics of the algae community structure**

167 During the study period, a total of 216 species, representing 50 genera, 23
168 families, 14 orders, 8 classes, 7 phyla, were identified and quantified in the eight
169 sampling stations. The most species-rich taxa were Bacillariophyta and
170 Chlorophyta, the genera such as *Navicula*, *Nitzschia*, *Surirella*, *Closterium* and
171 *Scenedesmus* were observed in the eight sampling sites, along the 12 sampled
172 months. The number of species varied between 26 (at H8 in May, 2021) and 73, at
173 site H2, in July 2020. In general, the number of species was lower at site H7, and
174 the highest values were registered at H2. Significant differences of species
175 diversity were found among different sites during two years (Fig 2). The mean
176 value from 4.51×10^6 ind./L to 8.47×10^6 ind./L, the highest value was found at H5,
177 the lowest value was found at H7. Monthly mean value of species diversity was
178 various in the two years. The highest mean value was found in May, 2020, the
179 lowest mean value was 0.82×10^6 ind./L in October, 2021. Diversity (H) and
180 species richness (D) showed significant differences among the sampling sites (Fig
181 3). Diversity, values ranged from 1.48 (in September, 2021, at H8) to 3.58 (in
182 June, 2020, at H7). The highest mean value was registered at H7, the lowest at
183 H8. Species richness was found the highest value in May, 2020, at H5 and the
184 highest in October, 2021, at H7.

185

186 **Fig 2. Assemblage composition and relative abundance of the most common**
187 **species in eight sites (a. abundance of the species in 2020; b. abundance of the**
188 **species in 2021)**

189

190 **Fig 3. Species diversity (H) and richness (D) in eight sites between 2020 and**
191 **2021 (a. Shannon-Wiener's diversity index; b. Margalef's index)**

192

193 In spatial scale, the dominant species were presented difference in the eight
194 sampling sites by detrended correspondence analysis (Fig 4). Species such as
195 *Melosira granulate*, *Cyclotella meneghiniana*, *Navicula cryptocephala* and
196 *Pandorina morum* were more abundant in all sites during the study period. The
197 dominant species in H1 were *C. meneghiniana* in 2020 and in spring 2021, but *N.*
198 *cryptocephala* was more abundant in autumn 2021. In H2, the dominant species
199 changed from *M. granulate* in spring to *N. cryptocephala* in summer and autumn
200 2020, the species *C. meneghiniana* and *Euglena acus* were more abundant in
201 2021. H3 was dominated by some diatoms such as *Synedra acus*, *M. granulate*,
202 *Gomphonema acuminatum* and *N. radiosa*. *M. granulate*, *C. meneghiniana* and
203 *Cosmarium granatum* were the dominant species at H4. Eutrophic species such as
204 *Asterionella formosa*, *M. varians*, *N. cryptocephala* and *E. oxyuris* were the
205 common species at H5. H6 was dominated by *Nitzschia palea*, *M. granulate* and
206 *C. meneghiniana*, and *C. granatum*. *C. meneghiniana* were more abundant at H7.
207 Pollution indicator species such as *Trachelomonas oblonga*, *Oscillatoria tenuis*
208 and *Ceratium hiundinella* were the dominant species at H8. Biological
209 characteristics, structure and function of algae showed obvious difference between
210 with other plants in wetland aquatic ecosystems [22]. Species Diversity is the
211 unique biological characteristics of community organizations, which reflects the
212 community-specific species composition and individual density characteristics.
213 Nutrients can affect the phytoplankton number because of dynamic change of
214 wetland water bodies, and phytoplankton as aquatic ecosystems producers, can
215 absorb nutrients in water consumption [23].

216

217 **Fig.4. Detrended correspondence analysis (DCA) of algae assemblages in**
218 **different sites during two years**

219

220 Community structure of algae showed significant spatial differences, mainly
221 diatom and green algae, followed by *Euglena* and *Cyanophyta*. Such as H5, due to
222 the low-lying and water flow was slow (water flow velocity from 0.5 to 0.8 m/s),

the accumulation of nutrients may much and more residential area garbage discharged into water bodies, coupled with sustained wind direction, so resulting in some nutritional indicator species blooms. Algae are the primary producers of aquatic ecosystems, its changes can quickly respond to the nutritional status of water body, dominated by nutritional species such as *E. oxyuris* in H5. Site H8 was surrounded by farmland, some organic pollutants of agricultural production process were discharged into water bodies, the COD concentration increased, and a large number of pollution-resistant species such as *Scenedesmus quadricauda* and *A. formosa* were found. Thus, some organic pollutants in the process of agricultural production through runoff, infiltration into the water [24], the reason for the changes of algae composition maybe human activities, larger changes in nutrient content in water, caused the algae distribution of individual plants differ and breached the community structure [25]. Seasonal variations of the algae community were also significantly affected by the changes of hydrological. Hulanhe Wetland was located in typical cold temperate monsoon climate zone, due to the affection by the monsoon, the precipitation, runoff and water level in spring and autumn. Nutrients of algae growth were easy to accumulate in water bodies, whilst the precipitation increased in summer, large water stir, erosion and dilution effect can affect the algae cell density.

Relationship between algae community and environmental factors

Species composition and structure of the algae community were affected by the environmental factors such as physical, chemical and biological in lakes, reservoirs and other water bodies [26-28]. In general, nutrients and water temperature were the key factors in affecting the growth of algae, but the two factors also were affected by other physical, chemical, and biological factors through the certain way. Community composition and quantity showed significantly different affected by environmental factors in different water bodies [29-30].

Cluster analysis based on species diversity showed that there were significant differences among the eight sites (Fig 5). H2 grouped with H3 and 4, whilst H1, H5 and 6 formed one cluster with two sub-clusters. The latter showed that H5 and 6 were more similar to each other than to the sites including H3 and 4 further away from village. H7 was least similar to all other sites, whilst close to the edge of the wetland, H8 was most distinct.

Fig 5. Cluster analysis of diatom assemblages in based on grouped results for both years

CCA ordination based on algae diversity showed that algae assemblages varied between the sampling sites with different environmental variables (Fig 6). 51.8% of total cumulative percentage variance of species data was explained and the first two axes were 20.2%. The eigenvalues of axis1 was higher than axis2, whilst species- environment correlations in axis 2 were highest among the four axes. Gradients in environmental variables which were significantly related to changes in species assemblage composition included concentrations of the nine factors.

Fi 6. Canonical correspondence analysis (CCA) of algae assemblages in Hulanhe Wetland

Most of environmental factors such like WT, TN, TP, PH, BOD and COD correlated negatively with the first axis, eigenvalues were -0.0265, -0.0466, -0.0975, -0.2876, -0.3026 and -0.3509, respectively. Axis 2 was correlated positively with most of environmental factors except pH and DO. The species were separated into four groups by CCA ordination. Assemblages at H8, the sites with the highest COD and BOD concentrations, differed most from the other sites and were characterized by *M. varians*, *O. tenuis* and *C. hiundinella*. *C. meneghiniana*, *N. cryptocephala* and *E. oxyuris* were most typical at sites H1 and 5 with higher nutrient concentrations included TP and TN. The dissolved oxygen

showed downward trend as long as the water temperature rose, algae consumed most of the nutrients. Then the algae assemblage in sampling sites H8 and 5 showed differ to other sites. Most algae which belong to common freshwater species at other sites were correlated positively with DO concentrations during two years. The difference of seasonal variations of the phytoplankton community structure may were affected by water temperature in Hulanhe Wetland [31-32]. Species composition and structure of the algae community were affected by the environmental factors such as physical, chemical and biological in lakes, reservoirs and other water bodies [26]. Bio-indicators cannot replace physical and chemical analyses, but they provide valuable information of aquatic ecosystems, community composition and quantity showed significantly different affected by environmental factors in different water bodies [33-34]. CCA showed the differences to reflect variations in environmental variables conditions. Some environmental variables such as TN, TP, DO, all of which correlated with assemblage change, were markedly different among the sampling sites. Significantly correlation was represented by the distribution of algae and nutrients in Hulanhe Wetland. *S. quadricauda*、*G. acuminatum*、*C. meneghiniana*、*Oscillatoria tenuis* are good indicator of TP, these species mainly occurred in site H8、H5 and H1. *Oscillatoria tenuis* was reported as potential indicator for the algae bloom; while *C. meneghiniana* is a eutrophic species, also have wide tolerance of temperature, and corresponding to the higher TP values. *M. granulate*、*E. oxyuris* are good indicator of TN, mainly distributed in the sites with high concentration of TN. The growth of algae is closely related to the content of nutrients in water [35-36]. Due to the changes of nutrients, the number of trophic levels in the food chain would change. The phytoplankton community structure would affect the physical and chemical factors of the water body, such as transparency, suspended solids, and pH value [37]. Using relationship between algae and environmental factors shall be an advantage in studying different area with algae diversity, and increases from mesotrophic to meso-eutrophic lakes and wetland [14-15], which was also observed in our study, and higher species

diversity was reported from sites with higher nutrient concentrations. In summary, the algae assemblage are valuable indicators of water quality, and more eutrophic taxa were found showed that most areas in Hulanhe Wetland is heavily impacted by human activities and might be influenced by organic fertilizer during the agricultural production process.

Conclusion

Algae assemblages were investigated at eight sampling sites in Hulanhe Wetland from May to October of 2020 and 2021. During the survey period, a total of 216 algae were found in the study area, in comparison with other algae, Bacillariophyta was the dominant, followed by Chlorophyta and then Euglenophyta. The dominant species in the study area are *C. meneghiniana*, *M. granulate*, *N. cryptotenella* and *E. acus*. *E. viridis*, *O. tenuis* were also found to be responsible for eutrophication. The changes of algae community structure were consistent with the water environment characteristics, so the change of the individual, population or community of algae can reflect the change rule of the water environment in Hulanhe Wetland. Among sampling stations different physical and chemical indicators impact the algal community structure. The distributions were discussed in different seasons. The result of CCA indicated that TN and TP were important environmental variables affecting algal community structure of the Hulanhe Wetland. So it is suggested that further studies are needed to focus on the change of algae community with the wetland restoration. In this study, the results also showed that CCA ordination can explain the response between algae community and environmental factors, and these can point to new directions in future studies on the assessment of water quality of similar wetlands.

Acknowledgements

This study was financially supported by A Project of Shandong Province Higher

342 Educational Science and Technology Program (No. J18KA199).

343 **References**

- 344 1. Amanda A, Richard RH. Wetlands and Urbanization: Implications for the
345 Future. Boca Raton: CRC Press, 2000.
- 346 2. Hulyal SB, Kaliwal BB. Dynamics of phytoplankton in relation to physico-
347 chemical factors of Almatti reservoir of Bijapur District, Karnataka State.
348 Environmental Monitoring & Assessment. 2009; 153(1-4): 45-59.
- 349 3. Reynolds CS. Phytoplankton designer – or how to predict compositional
350 responses to trophic-state change. Hydrobiologia. 2000; 424(1-3): 123-132.
- 351 4. Karydis M, Tsirtsis G. Ecological indices: A biometric approach for assessing
352 eutrophication levels in the marine environment. Science of The Total
353 Environment. 1996; 186(3): 209-219.
- 354 5. Bradshaw EG, Anderson NJ. Validation of a diatom-phosphorus calibration
355 set for Sweden. Freshwater Biol. 2001; 46: 1035-1048.
- 356 6. Round FE. The Ecology of Algae. London: Cambridge University Press;
357 1981. p.1-653.
- 358 7. Kovaács CS, Buczkó K, Hajnal E, Padisák J. Epiphytic, littoral diatoms as
359 bio-indicators of shallow lake trophic status: Trophic Diatom Index for Lakes
360 (TDIL) developed in Hungary. Hydrobiologia. 2007; 589: 141-154.
- 361 8. Quartino ML, Deregibus D, Campana GL, Latorre GEJ, Momo FR. Evidence
362 of Macroalgal Colonization on Newly Ice-Free Areas following Glacial
363 Retreat in Potter Cove (South Shetland Islands), Antarctica. PLoS ONE. 2013;
364 8(3): e58223.
- 365 9. Paver SF, Youngblut ND, Whitaker RJ, Kent AD, Phytoplankton succession
366 affects the composition of Polynucleobacter subtypes in humic lakes,
367 Environmental Microbiology. 2015; 17(3): 816-828
- 368 10. King L, Barker P, Jones RI. Epilithic algal communities and their relationship
369 to environmental variables in lakes of the English Lake District. Freshwater
370 Biol. 2000; 45(4): 425-442.
- 371 11. Bilous OP, Barinova SS, Ivanova NO, Huliaieva OA. The use of

- 372 phytoplankton as an indicator of internal hydrodynamics of a large seaside
373 reservoir – case of the Sasyk Reservoir, Ukraine. *Ecohydrology &*
374 *Hydrobiology*. 2016; 16(3): 160-174.
- 375 12. Mayer PM, Galatowitsch SM. Diatom communities as ecological indicators of
376 recovery in restored Prairie wetlands. *Wetlands*. 1999; 19(4): 765-774.
- 377 13. Dybiec JM, Danz NP, Learman D R, Uzarski DG. Evaluating relationships
378 between plants, water chemistry, and denitrification potential in palustrine
379 freshwater marshes. *Ecological Indicators*. 2021; 131:108-163.
- 380 14. Yang XD, Dong XH, Gao G, Pan HX, Wu JL. Relationship between surface
381 sediment diatoms and summer water quality in shallow lakes of the middle
382 and lower reaches of the Yangtze River. *Journal of Integrative Plant Biology*.
383 2005; 47(2): 153-164.
- 384 15. Fan YW, Li J, Men XY, Liu Y. Preliminary description of diatom community
385 and its relationship with water physicochemical variables in Qixinghe
386 Wetland. *Chinese Journal of Oceanology and Limnology*. 2012; 30(2): 379-
387 387.
- 388 16. Tonetta D, Petrucio MM. Seasonal changes in primary production and
389 respiration in a subtropical lake undergoing eutrophication. *Environmental*
390 *Monitoring and Assessment*. 2020; 192(9): 1-13.
- 391 17. Hu HJ, Wei YX. *The Freshwater Algae of China: Systematic, Taxonomy and*
392 *Ecology*. Science Press, Beijing: Science Press; 2006. (In Chinese)
- 393 18. Lange-Bertalot H, Metzelin D. Indicators of oligotrophy. *Iconogr Diatomol*
394 2:1-390. Los Angeles: Koeltz Scientific Books;1996.
- 395 19. State Environmental Protection Administration. *Water and wastewater*
396 *monitoring analysis method*. 4rd ed. Beijing: China Environmental Science
397 Press; 2002. (In Chinese)
- 398 20. Jongman RHG, Ter Braak CJF, Van Tongeren, OFR. *Data analysis in*
399 *community and landscape ecology*. New York: Cambridge University
400 Press;1995.
- 401 21. Ter Braak CJF, Similauer P. *CANOCO Reference Manual and CanoDraw for*

- 402 Windows User's Guide: Software for Canonical Community Ordination
- 403 (Version 5.0). New York: Microcomputer Power Ithaca; 2013.
- 404 22. Henry CP, Amoros C, Roset N. Restoration ecology of riverine wetlands: A 5-
- 405 year post-operation survey on the Rhône River, France. *Ecological*
- 406 *Engineering*. 2002;18(5): 543-554.
- 407 23. Fore LS, Cygrafe C. Using diatoms to assess the biological condition of large
- 408 rivers in Idaho (U.S.A.). *Freshwater Biology*. 2002; 47(10): 2015-2037.
- 409 24. Wang ZL, Chen DW, Wang SL. Research on agricultural non-point source
- 410 pollution trapping and removing effect of Songbei Wetland in Harbin. *Journal*
- 411 *of Anhui Agri Sci*. 2014; 42: 844-46. (In Chinese)
- 412 25. Figueiras CC, Blanco IÁ, Bécares E, Blanco S. Epiphytic diatoms and water
- 413 quality in shallow lakes: the neutral substrate hypothesis revisited. *Marine and*
- 414 *Freshwater Research*. 2010; 61(12): 1457-1467.
- 415 26. Blanco IÁ, Figueiras CC, Bécares E, Blanco S. Spatiotemporal changes in
- 416 diatom ecological profiles: implications for biomonitoring. *Limnology*.
- 417 2011; 12(2): 157-168.
- 418 27. Kahlert M, Rühland KM, Lavoie I, Keck F, Smol JP. Biodiversity patterns of
- 419 Arctic diatom assemblages in lakes and streams: Current reference conditions
- 420 and historical context for biomonitoring. *Freshwater Biology*. 2020; 67(1):
- 421 116-140.
- 422 28. Patoucheas P, Koukousioura O, Psarra S, Aligizaki K, Dimiza MD, Skampa E.
- 423 et al. Phytoplankton community structure changes during autumn and spring
- 424 in response to environmental variables in Methana, Saronikos Gulf, Greece.
- 425 *Environmental Science and Pollution Research*. 2021; 28(1): 33854–33865.
- 426 29. Liu Q, Yang XD, Anderson NJ, Liu EF, Dong XH. Diatom ecological
- 427 response to altered hydrological forcing of a shallow lake on the Yangtze
- 428 floodplain, SE China. *Ecohydrology*. 2012; 5(3): 316-25.
- 429 30. Bortolini JC, Silva PRLD, Baumgartner G, Bueno NC. Response to
- 430 environmental, spatial, and temporal mechanisms of the phytoplankton
- 431 metacommunity: comparing ecological approaches in subtropical reservoirs.

- 432 Hydrobiologia. 2019; 830: 45-61.
- 433 31. Arrieira RL, Schwind LTF, Bonecker CC, Lansac T, Fábio A. Use of
- 434 functional diversity to assess determinant assembly processes of testate
- 435 amoebae community. Aquatic Ecology. 2015; 49(4): 561-571.
- 436 32. Caroppo C, Roselli L, Di Leo A. Hydrological conditions and phytoplankton
- 437 community in the Lesina lagoon (southern Adriatic Sea, Mediterranean).
- 438 Environmental Science & Pollution Research. 2017; 25(2): 1-16.
- 439 33. Kitner M, Pouličková A. Littoral diatoms as indicators for the eutrophication
- 440 of shallow lakes. Hydrobiologia. 2003; 506-509(1-3): 519-24.
- 441 34. Gaiser E, Rühland K. Diatoms as indicators of environmental change in
- 442 wetlands and peatlands. In: Smol J. P. (Eds). The diatoms: Applications for
- 443 the Environmental and Earth Sciences, 2nd Edition. New York: Cambridge
- 444 University Press; 2010.
- 445 35. Biggs BJF, Smith RA. Taxonomic richness of stream benthic algae: Effects of
- 446 flood disturbance and nutrients. Limnology & Oceanography. 2002; 47(4):
- 447 1175-1186.
- 448 36. Dalu T, Wassermanb RJ. Cyanobacteria dynamics in a small tropical
- 449 reservoir: understanding spatio-temporal variability and influence of
- 450 environmental variables. Science of the Total Environment. 2018; 643: 835-84
- 451 37. Wood RJ, Mitrovic SM, Lim RP, Warne MSJ, Dunlop J, Kefford BJ. Benthic
- 452 diatoms as indicators of herbicide toxicity in rivers – A new SPEcies At Risk
- 453 (SPEARherbicides) index. Ecological Indicators. 2019; 99(APR.): 203-213.

Supporting information

S1 Fig 1 Location of the study area and sites at Hulanhe Wetland

S2 Table 1 Water chemistry at eight sampling sites in Hulanhe Wetland

S3 Fig 2 Assemblage composition and relative abundance of the most common species in eight sites (a. abundance of the species in 2020; b. abundance of the species in 2021)

S4 Fig 3 Species diversity (H) and richness (D) in eight sites between 2020 and 2021 (a. Shannon-Wiener's diversity index; b. Margalef's index)

S5 Fig 4 Detrended correspondence analysis (DCA) of algae assemblages in different sites during two years (a. DCA in 2020; b. DCA in 2021)

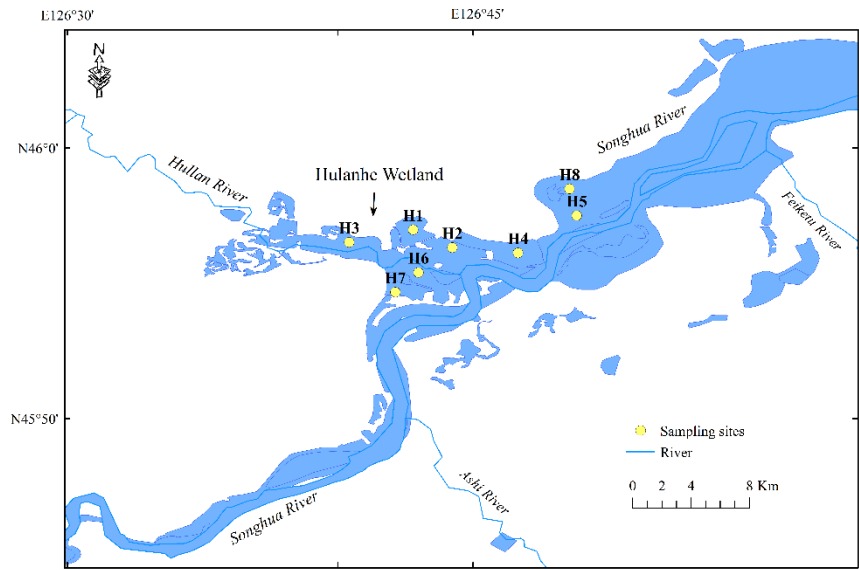
S6 Fig 5 Cluster analysis of diatom assemblages in based on grouped results for both years

S7 Fig 6 Canonical correspondence analysis (CCA) of algae assemblages in Hulanhe Wetland

	Year	H1		H2		H3		H4		H5		H6		H7		H8	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
T	2020	6-26	19 ^a	5-25	18 ^a	6-25	19 ^a	7-26	19 ^a	7-25	19 ^a	7-25	19 ^a	6-26	19 ^a	8-26	20 ^a
(°C)	2021	4-27	21 ^a	4-26	21 ^a	4-26	20 ^a	5.-26	20 ^a	4-26	20 ^a	4-26	20 ^a	4-26	20 ^a	5-26	20 ^a
DO	2020	3.6-14.9	7.97 ^{ab}	4.5-16.6	9.30 ^a	4.3-16.6	9.3 ^a	4.5-16.4	9.3 ^a	3.9-14	7.7 ^b	4.3-16.6	9.3 ^a	4.3-16.6	9.4 ^a	3.8-15.1	7.9 ^{ab}
(mg l ⁻¹)	2021	6.4-16.1	8.35 ^a	4.7-17	9.53 ^{ab}	4.6-16.8	9.2 ^{ab}	4.5-19	9.8 ^b	4-16	8.1 ^a	4.2-16.7	9.3 ^{ab}	4.2-18	9.7 ^b	3.8-14.9	7.9 ^a
BOD	2020	1.8-2.9	2.4 ^{ab}	1.4-2.4	1.93 ^a	1.5-2.4	2 ^a	1.4-2.5	1.98 ^a	2.1-3.2	2.6 ^b	1.5-2.4	2 ^a	1.5-2.5	2 ^a	1.9-2.9	2.45 ^{ab}
(mg l ⁻¹)	2021	1.8-2.6	2.5 ^{ab}	1.5-2.5	2 ^a	1.4-2.4	1.9 ^a	1.4-2.5	1.9 ^a	2.2-2.9	2.5 ^b	1.4-2.4	1.9 ^a	1.3-2.4	2 ^a	2-2.8	2.4 ^{ab}
COD	2020	17-22	21 ^a	16-19	18 ^b	16-19	18 ^b	16-21	18 ^b	17-23	21 ^a	16-19	18 ^b	17-20	18 ^b	18-23	21 ^a
(mg l ⁻¹)	2021	17-23	20 ^a	15-18	17 ^b	16-19	17 ^b	17-20	19 ^{ab}	17-22	20 ^a	15-20	17 ^b	16-20	18 ^b	18-22	20 ^a
TN	2020	1.6-6	3.9 ^a	1.2-5.4	3.3 ^a	1.3-5.4	3.3 ^a	1.4-5.4	3.4 ^a	1.8-6.3	4.2 ^b	1.2-5.4	3.3 ^a	1.2-5.4	3.4 ^a	1.6-6	4 ^b
(mg l ⁻¹)	2021	2.7-5	3.6 ^{ab}	1.7-4.7	2.8 ^b	1.6-4.7	2.8 ^b	2.3-4	2.8 ^b	3.5-5.3	4 ^b	2.3-4.6	2.8 ^a	2.4-4.1	3.1 ^a	2.6-5.1	3.6 ^{ab}
TP	2020	0.09-0.2	0.13 ^a	0.04-0.1	0.07 ^b	0.04-0.11	0.07 ^b	0.03-0.13	0.07 ^b	0.08-0.21	0.13 ^a	0.04-0.13	0.08 ^a	0.04-0.12	0.07 ^a	0.09-0.2	0.14 ^b
(mg l ⁻¹)	2021	0.08-0.17	0.12 ^a	0.02-0.1	0.06 ^b	0.03-0.09	0.06 ^b	0.02-0.1	0.07 ^b	0.08-0.2	0.12 ^a	0.04-0.11	0.06 ^b	0.04-0.1	0.06 ^b	0.1-0.19	0.13 ^a
TOC	2020	4.4-4.8	4.6 ^a	4.3-4.7	4.5 ^a	4.3-4.8	4.5 ^a	4.3-4.8	4.5 ^a	4.5-4.9	4.6 ^a	4.3-4.8	4.5 ^a	4.3-4.7	4.5 ^a	4.4-4.8	4.6 ^a
(mg l ⁻¹)	2021	4.2-4.7	4.4 ^a	4.2-4.5	4.3 ^a	4.2-4.4	4.3 ^a	4.2-4.4	4.3 ^a	4.3-4.7	4.5 ^a	4.2-4.5	4.4 ^a	4.2-4.6	4.4 ^a	4.3-4.7	4.5 ^a
Cond.	2020	144-227	188 ^a	159-223	183 ^a	146-211	175 ^a	139-211	175 ^a	153-308	200 ^a	159-210	183 ^a	149-226	184 ^a	442-498	472 ^b
U	2021	146-227	190 ^a	162-274	202 ^a	148-232	191 ^a	145-218	187 ^a	158-217	188 ^a	159-227	194 ^a	153-416	222 ^a	465-678	508 ^b
pH	2020	6.0-6.5	6.0	6.2-6.5	6.3	6.0-6.4	6.3	5.9-6.5	6.2	6.2-6.6	6.4	5.9-6.4	6.3	6.0-6.8	6.4	6.4-6.9	6.7
	2021	5.9-6.7	6.4	6.3-6.8	6.7	6.3-7.0	6.5	6.1-6.7	6.4	5.8-7.2	6.6	6.2-6.7	6.4	6.5-6.7	6.6	6.8-7.1	6.9

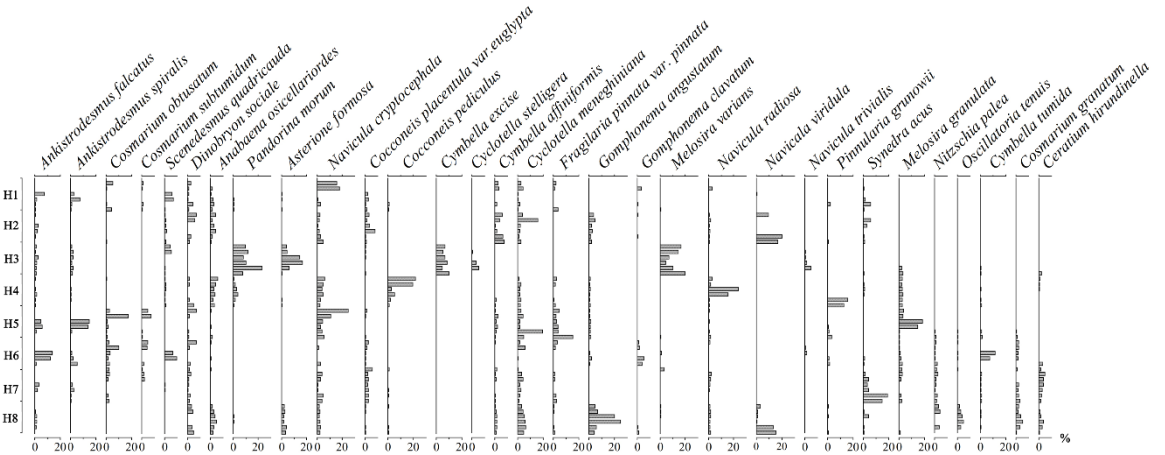
485 Concentration means followed by different uppercase letters (a and b) are significantly different (ANOVA, P < 0.05)

S1 Fig 1

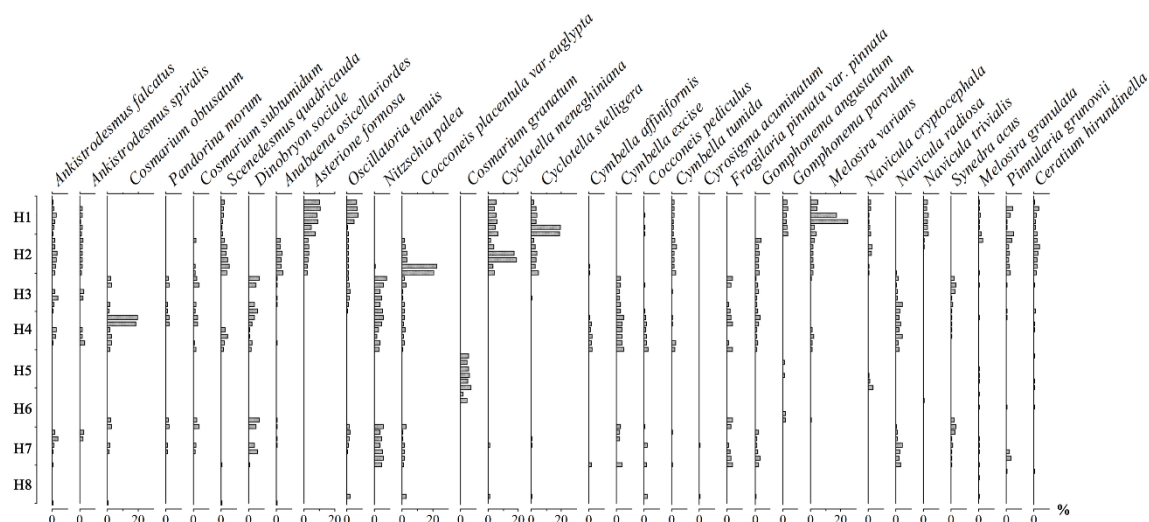


S2 Fig 2

a)

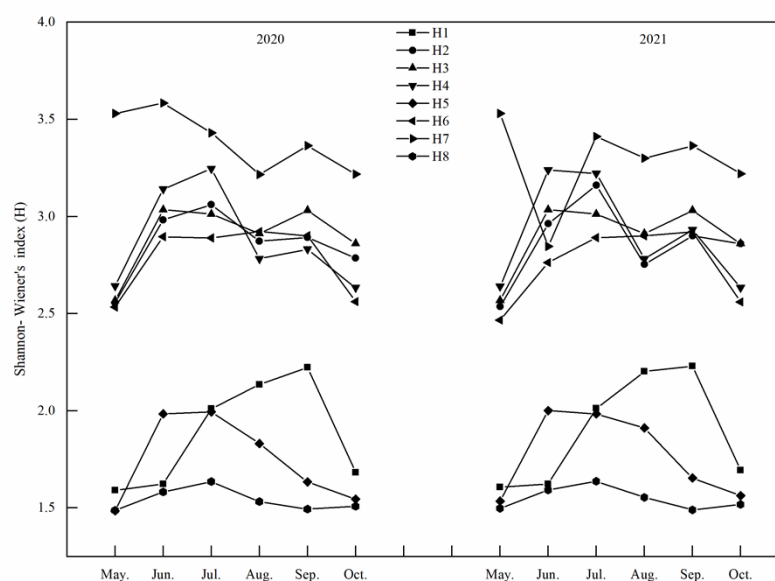


b)

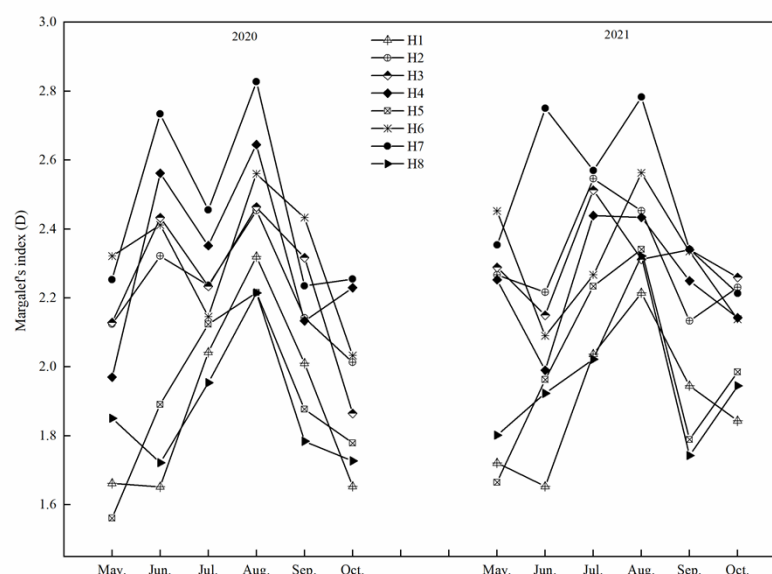


S3 Fig

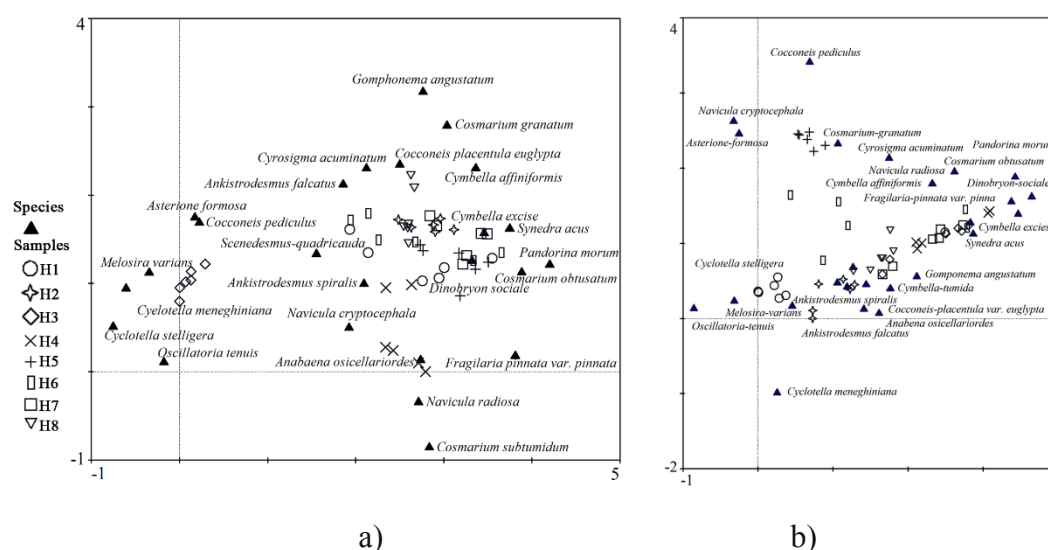
a)



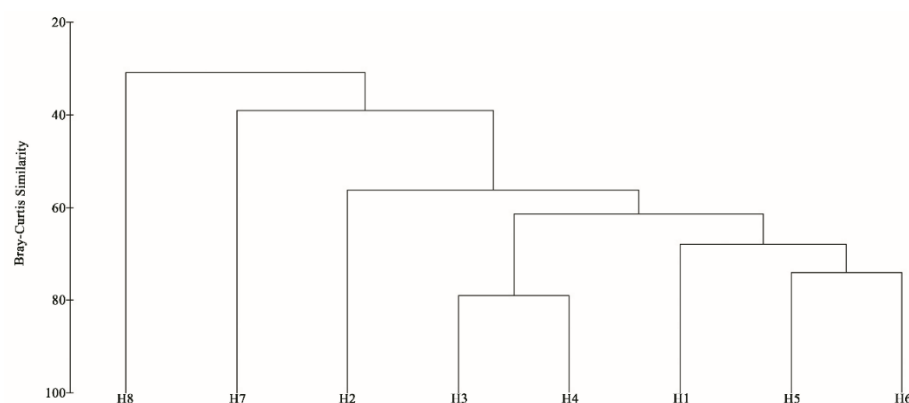
b)



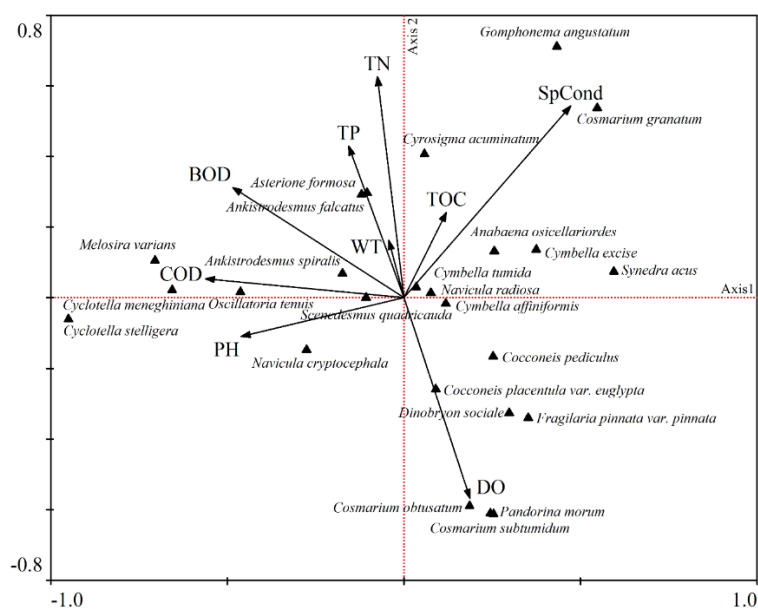
S4 Fig



S5 Fig



505 **S6 Fig**



506