THE ECOLOGICAL INDICATOR ROLE OF PERIPHYTON IN ECOLOGICAL RESTORATION FOR WETLANDS, NORTHEAST OF CHINA

ZHAO, J. Y. – LIU, M. H.* – MING, X. Y. – BAO, C. L. – MENG, X. Z. – YIN, J. H.

College of Wildlife and Protected Area, Northeast Forestry University, Harbin 150040, China

**Corresponding author*
e-mail: manhong@nefu.edu.cn

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Abstract. Currently, aquatic ecosystems are experiencing significant changes due to wetland restoration processes. This study examines the response of periphyton to environmental changes by using wetlands with varying restoration durations within the Naolihe National Nature Reserve, Northeast of China, as research sites. Natural wetlands within the reserve were used as a control to compare biodiversity differences and assess the impact of aquatic environmental factors on community diversity. Additionally, the variation in periphyton attached to different vascular plants was analyzed. The results showed that the species composition was dominated by Bacillariophyta and Chlorophyta. Wetlands restored for two years had the highest periphyton density, while those restored for eight years had the lowest among the three groups. Only the two-year restored wetlands exhibited indicator species, including *Aphanizomenon flos-aquae*, *Euglena viridis*, and *Ulothrix* sp. Seven species were dominant in the two-year restored wetlands, while 10 species dominated the eight-year restored wetlands. Among these, common dominant species in both natural and restored wetlands included *Anabaena circinalis*, *Tribonema affine*, and *Tribonema viride*. The Shannon-Wiener diversity index, Margalef richness index, and Pielou evenness index showed no significant differences among wetlands with varying restoration durations. NMDS and ANOSIM analyses further confirmed no significant difference in the periphyton community structure. Pearson correlation analysis revealed a significant positive correlation between NO_3^--N and *E. viridis*, *Ulothrix* sp., *T. affine*, *T. viride*, and *Tribonema utriculosum*.
Keywords: *periphyton, restored wetlands, biodiversity index, indicator species, Naolihe Nature Reserve*

Introduction

The conflicts between population growth, resource exploitation, and environmental degradation has resulted in severe ecological damage and frequent disasters. Examples include the deterioration of hydrological basin integrity, regional environmental decline, and biodiversity loss, which hinder the potential of wetland resources and their environmental functions. Consequently, there is a growing awareness of the necessity for ecological restoration. Aquatic organisms are widely used to assess and restore wetland ecosystems because of their strong association with environmental factors in aquatic habitats (Giorgio et al., 2016; Voss et al., 2012). As primary producers in aquatic ecosystems, algae serve as key indicator organisms of water quality. Additionally, algae possess the capacity to reduce nutrient levels in wastewater and remove organic pollutants, heavy metals, and antibiotics, offering significant potential for ecological restoration (Yang et al., 2024).

Algae are a ubiquitous feature of various aquatic ecosystems, playing a pivotal role as primary producers in the food chain. Their movement in water can be categorized into two types: phytoplankton, which drift with water currents, and

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periphyton, which adhere to substrates such as stones and plants (Hu et al., 2022). These two communities are closely interconnected and can transition between forms (Wang et al., 2019). Algae are diverse, small organisms with short life cycles and high reproductive rates (Hou et al., 2022). As the primary producers in aquatic ecosystems, they are directly affected by physical and chemical factors, responding rapidly to pollutants entering the water through various pathways (Chao et al., 2024). Periphyton often thrive on the surfaces of stones, plants, and other substrates (Le et al., 2023). Due to their immobility, periphyton provide a reliable means of assessing water quality. Additionally, they contribute to the restoration of eutrophic ecosystems by consuming phosphorus (Sapucaia et al., 2024). While phytoplankton have been widely used as biological monitoring indicators in the Naoli River wetlands (Hou et al., 2022; Dong et al., 2022), fewer studies have focused on periphyton (Xing et al., 2017).

In the 1950s, large-scale reclamation activities were carried out in the wetlands of the Naoli River Basin in China. Continuous development and utilization have since caused significant shrinkage of these wetlands (Wang, 2021). By the end of 1990, large areas of natural wetlands had been lost, leading to a degradation of ecological functions and reduced resilience to natural disasters. Since the establishment of the Naolihe Nature Reserve in 2002, the Reserve Management Bureau has proactively engaged in wetland restoration, collaborating with various agricultural companies. By the end of 2021, all farms had successfully returned cultivated land to wetland, ensuring the effective protection and restoration of the ecological environment.

In this study, periphyton in the Naolihe Nature Reserve were selected as the research subject. The sampling site was located at Hongqiling Farm within the reserve, where periphyton were collected from wetlands restored in 2014 and 2020. The natural wetlands served as a control. After identifying the periphyton, the community structure characteristics were investigated to understand the effects of environmental changes on periphyton following wetland restoration. The study employed diversity indices, significance analysis of differences, and correlation analysis to achieve these aims. The findings provide a scientific basis for the ecological restoration and protection of the Naolihe Nature Reserve.

Materials and methods

Study area overview

The Naolihe Nature Reserve (132°22’29″–134°13’45″E, 46°30’22″–47°24’32″N) is located in the hinterland of the Sanjiang Plain in northeast China, covering an area of 160,601 hm^2. The reserve includes a core area of 37,047 hm^2, a buffer zone of 53,128 hm^2, and an experimental area of 70,426 hm^2. The region has a typical humid continental monsoon climate with four distinct seasons. Precipitation is primarily concentrated between May and September, averaging around 420 mm, accounting for approximately 78% of the annual rainfall (Wang et al., 2023a).

The reserve is characterized by extensive swamp areas and a diverse array of higher vascular plants, including *Phragmites australis*, *Deyeuxia angustifolia*, *Miscanthus sinensis*, *Setaria viridis*, *Zizania latifolia*, *Equisetum arvense*, and other species (Jiang et al., 2021). With a total of 1047 plant species (Wang et al., 2023a) and 539 animal species (Liu et al., 2022), the Naolihe Nature Reserve is regarded as a rare gene pool of natural biological resources.

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Sampling sites

This study set up nine sampling sites in June (spring), 2023, within the Hongqiling Farm in the Naolihe Nature Reserve (*Fig. 1*). Natural wetlands served as controls. These wetlands are located in the flood plain wetland, which is a native wetland and connected with the Naoli River channel. The restored wetlands used to be farmlands and were restored for 2 years (R2) and 8 years (R8) respectively (*Fig. 2*). The detailed information is shown in *Table 1*. The dominant vascular plants observed in the water bodies of the sampling sites were *P. australis*, *Z. latifolia*, *D. angustifolia* and *Trapa manshurica*.

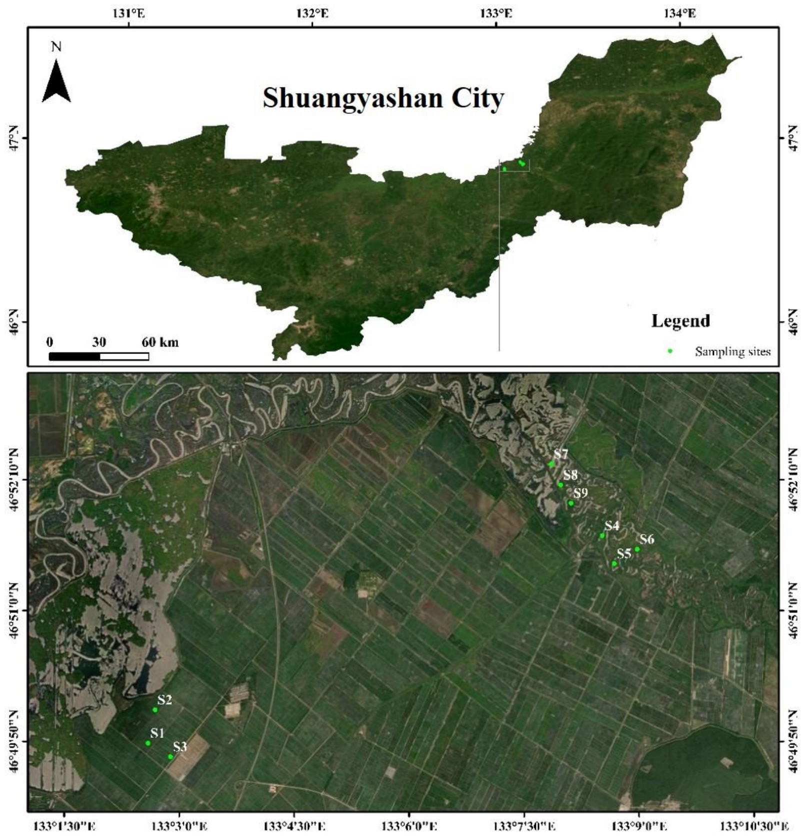

Figure 1. *Sampling sites of the Hongqiling Farm in the Naolihe Nature Reserve*

Figure 2. *Sampling sites and habitats*

Table 1. *Information of sampling sites in wetlands with different restoration years and natural wetlands*

Information of sampling sites	Number	Dominant species of vascular plants	Geographic position
NW	S1	*Phragmites australis* *Zizania latifolia*	N46°49′40.23″, E133°2′16.64″
	S2	*Deyeuxia angustifolia* *Phragmites australis* *Zizania latifolia*	N46°49′41.42″, E133°2′16.58″
	S3	*Deyeuxia angustifolia*	N46°49′39.58″, E133°2′17.45″
R2	S4	*Deyeuxia angustifolia*	N46°51′40.09″, E133°8′52.19″
	S5	*Trapa manshurica*	N46°51′39.44″, E133°8′54.34″
	S6	*Trapa manshurica*	N46°51′41.23″, E133°8′57.45″
R8	S7	*Trapa manshurica*	N46°52′20.51″, E133°8′25.66″
	S8	*Zizania latifolia* *Trapa manshurica*	N46°52′18.46″, E133°8′26.92″
	S9	*Trapa manshurica*	N46°52′15.74″, E133°8′30.42″

R2 represents wetlands restored for 2 years, R8 represents wetlands restored for 8 years and NW represents natural wetlands

Sample collection and measurement

Collection and measurement of periphyton

The sample collection protocol followed the guidelines outlined in the "*Technical Specifications for Aquatic Ecological Monitoring—Fresh Water Periphytic Algae*" (China National Environmental Monitoring Center, 2022). At each sampling site, three clumps of the dominant vascular plant species were randomly selected, and the underwater portions were collected using gentle movements to minimize periphyton detachment. The plants were placed in sealed bags, and the periphyton were scraped off using a toothbrush and spatula in the laboratory. The surface area of the plant samples was then calculated. Periphyton species were identified using references such as *The Freshwater Algae of China—Systematics, Taxonomy and Ecology* (Hu et al., 2006), *Atlas of Common Algae in China's Inland Waters* (Ministry of Water Resources, 2012), and *Atlas of Common Freshwater Planktonic Algae in China* (Weng et al., 2010).

Collection and measurement of environmental factors

WD (water depth, WD), WT (water temperature, WT), DO (dissolved oxygen, DO), Cond (conductivity, Cond), and pH were measured using the YSI-6600 portable water quality analyzer. SD (secchi disk depth, SD) was determined using a Secchi disk. At each sample site, 500 ml water samples were collected in labeled plastic containers and transported to the lab for analysis. The water samples were analyzed for NH_4^+-N (ammonia nitrogen, NH_4^+-N), TP (total phosphorus, TP), TN (total nitrogen, TN), NO_3^--N (nitrate nitrogen, NO_3^--N), and COD_{Mn} (the permanganate index, COD_{Mn}) following the "*Methods for Monitoring and Analysis of Water and Wastewater*" (4th Edition, State Environmental Protection Administration, 2002).

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Data analysis and processing

Dominant species were determined based on a dominance value (*Y*), with $Y \geq 0.02$ signifying dominance (Hou et al., 2022). The dominant species equation was as follows (*Eq. 1*).

$$Y = (n_i / N) f_i \quad \text{(Eq.1)}$$

where f_i is the frequency of occurrence of the specie *i* at each point, n_i is the total number of individuals of species *i*, N is the total number of species.

PAST (version 326b) software was used to calculate the Shannon-Wiener diversity index (*H'*), Pielou evenness index (*J*), and Margalef richness index (*D*) (Dong et al., 2022).

ANOVA (One-way analysis of variance, ANOVA) was used to assess the statistical significance of the observed differences between the restored and natural wetlands with respect to each water environment factor. NMDS (Non-metric multidimensional scaling analysis, NMDS) (https://www.chiplot.online/) and ANOSIM (Analysis of similarities, ANOSIM) (https://www.cloudtutu.com.cn) were used to test the significance of the differences between the restored wetlands and the natural wetlands in terms of periphyton communities. Pearson correlation analysis was also employed to determine the correlation between the water environment factors and the dominant species of the periphyton (https://www.chiplot.online/). Indicator Species Analysis was conducted using PC-ORD 7 to identify indicator species for different wetlands.

Results and analysis

Characteristics of the species composition

In this study, 91 species of periphyton from 51 genera and 5 phyla were identified. These included 21 genera and 44 species of Bacillariophyta, accounting for 47.83%; 17 genera and 24 species of Chlorophyta, accounting for 26.09%; 4 genera and 10 species of Euglenophyta, accounting for 10.87%; 7 genera and 8 species of Cyanophyta, accounting for 8.70%; and 2 genera and 6 species of Xanthophyta, accounting for 6.52%. In terms of restoration years, 46 genera and 81 species were identified in R2, 37 genera and 60 species in R8, and 40 genera and 69 species in NW. There was no significant difference in species richness among wetlands with different restoration years (*Fig. 3*).

Additionally, the periphyton attached to different vascular plants showed variation in species richness. The most abundant periphyton were found on *Trapa manshurica*, with 47 genera and 86 species identified. This was followed by *Deyeuxia angustifolia* with 43 genera and 75 species, *Zizania latifolia* with 32 genera and 53 species, and the lowest number on *Phragmites australis*, with 24 genera and 32 species.

The density of periphyton in wetlands varies significantly depending on the restoration year. The highest density was observed in R2, at 2918×10^4 cells/cm², while the lowest density was found in R8, at 192×10^4 cells/cm². The natural wetland exhibited a density of 532×10^4 cells/cm², falling between the two restored sites. The maximum density, 5060×10^4 cells/cm², occurred in R2 on *Trapa manshurica*, while the minimum value, 27×10^4 cells/cm², was observed in R8 on *Zizania latifolia*.

There were also differences in the density of periphyton attached to different vascular plants. The highest density, 2130×10^4 cells/cm², was recorded on *Deyeuxia*

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angustifolia, followed by 1180×10⁴ cells/cm² on *T. manshurica* and 91×10⁴ cells/cm² on *Z. latifolia*. The lowest density was found on *Phragmites australis*, at 68×10⁴ cells/cm².

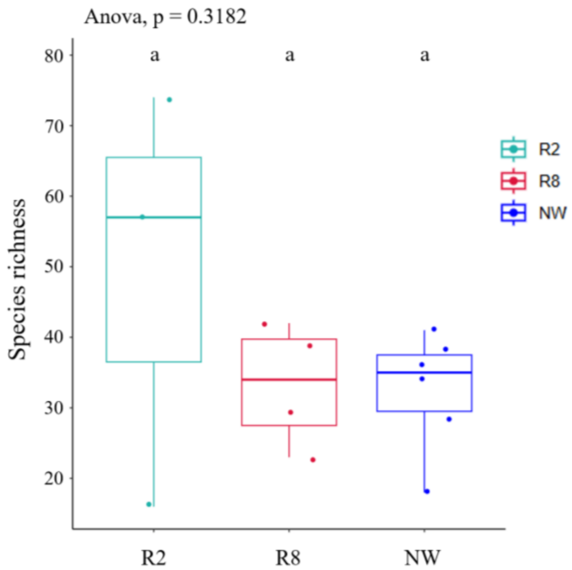

Figure 3. *Species richness in different wetlands. R2: wetlands restored for 2 years, R8: wetlands restored for 8 years, NW: natural wetlands*

Screening of indicator species

A high indicator value means that the average abundance of the species within a quadrat group exceeds that of other quadrat groups, and the species is present in most quadrats within the group (Wang et al., 2023b). In this study, species with an indicator value greater than 50 and $P < 0.05$ were identified as indicator species of periphyton in wetland ecosystems. Three indicator species of periphyton were selected from R2 (*Table 2*): *Aphanizomenon flosaquae*, *Euglena viridis*, and *Ulothrix* sp. However, no indicator species were found in R8 or NW.

Table 2. *Indicator species and value of periphyton of wetlands in R2*

	Indicator species	Indicator value	*P* value
R2	*Aphanizomenon flosaquae*	87.50	0.038
	Euglena viridis	100.00	0.034
	Ulothrix sp.	93.90	0.034

R2 represents wetlands restored for 2 years, R8 represents wetlands restored for 8 years and NW represents natural wetlands

Dominant species

Dominant species, which are significant in terms of abundance, biomass, and community function within wetland ecosystems, hold a key position and serve as indicators of the water environment (Sheng et al., 2024). Analysis revealed seven dominant species in R2 and ten in R8, compared to only four in NW. *Anabaena circinalis*, *Tribonema affine*, and *Tribonema viride* were common dominant species

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across all three types of wetlands. The dominant species in all three types of wetlands mainly belonged to Cyanophyta and Xanthophyta, with Bacillariophyta dominant species present only in R2 and R8. The dominant species *Synedra ulna* was present exclusively in R2, while five other species were found only in R8 (*Table 3*).

Table 3. *Dominant species of periphyton of restored and natural wetlands*

Phyla	Dominant species	Dominant degree		
		R2	R8	NW
Cyanophyta	*Oscillatoria princes*	0.037	0.023	——
	Anabaena circinalis	0.033	0.105	0.037
	Anabaena oscillarioides	——	——	0.057
	Aphanizomenon flosaquae	0.066	0.119	——
Xanthophyta	*Tribonema affine*	0.033	0.165	0.347
	Tribonema viride	0.063	0.026	0.022
	Tribonema utriculosum	0.067	——	——
Bacillariophyta	*Synedra ulna*	0.033	——	——
	Achnanthes lanceolata	——	0.024	——
	Stauroneis anceps	——	0.038	——
	Navicula simples	——	0.038	——
	Nitzschia palea	——	0.022	——
	Nitzschia amphibia	——	0.040	——

R2 represents wetlands restored for 2 years, R8 represents wetlands restored for 8 years and NW represents natural wetlands

Analysis of difference significance for diversity index

Biodiversity within a community is often characterized by the diversity index (Wang et al., 2022), which quantitatively reflects the species succession, richness, and distribution uniformity. In this study, the biological diversity of periphyton was analyzed using the Shannon-Wiener Diversity Index, Margalef Richness Index, and Pielou Evenness Index.

The results indicated that R8 had the highest Shannon-Wiener diversity and Pielou evenness indices, followed by NW, while R2 had the lowest values. Conversely, the Margalef Richness Index showed that R2 had a higher species richness compared to R8, which in turn exceeded NW. However, further analysis revealed no statistically significant difference in biodiversity indices between R2 and R8 when compared to NW ($P \geq 0.05$) (*Table 4; Fig. 4*).

Table 4. *Diversity index of restored and natural wetlands (mean ± standard deviation)*

	R2	R8	NW
Shannon-Wiener diversity index	2.00 ± 0.50	2.78 ± 0.33	2.54 ± 0.58
Margalef richness index	5.68 ± 2.76	5.48 ± 1.10	4.92 ± 0.91
Pielou evenness index	0.55 ± 0.16	0.80 ± 0.11	0.69 ± 0.17

R2 represents wetlands restored for 2 years, R8 represents wetlands restored for 8 years and NW represents natural wetlands. Means in each bar sharing the same superscript letter or absence of superscripts are not significantly different ($P \geq 0.05$), the same applies below

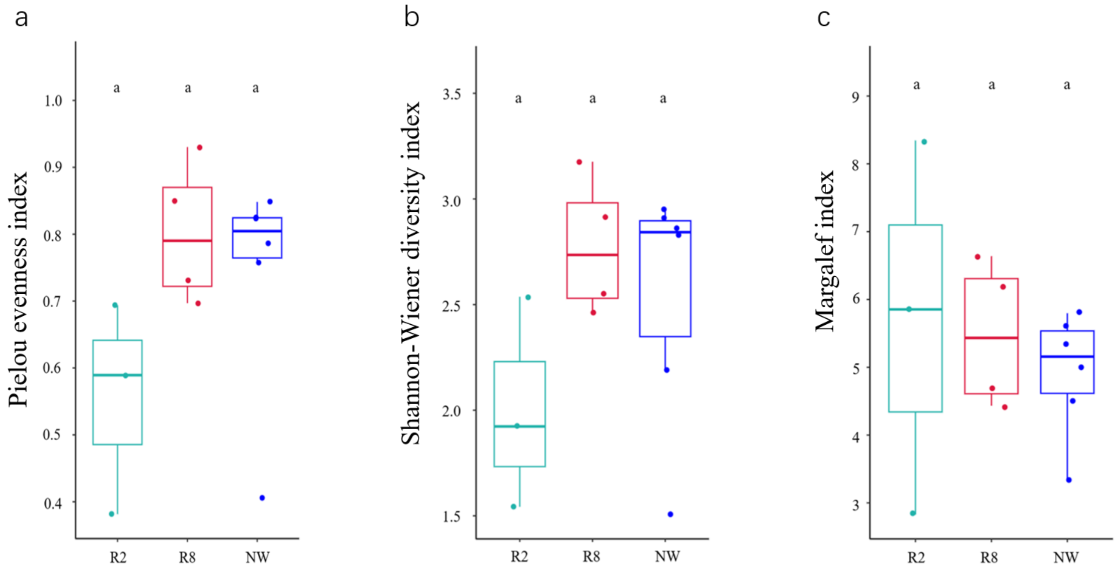

***Figure 4.** Biodiversity index of periphyton. (a) Pielou evenness index. (b) Shannon-Wiener diversity index. (c) Margalef index. R2: wetlands restored for 2 years, R8: wetlands restored for 8 years. NW: natural wetlands*

Analysis for periphyton community structure

Non-metric multidimensional scaling (NMDS) and Analysis of Similarities (ANOSIM) were used to assess the similarity of periphyton community structure between restored and natural wetlands. The NMDS results indicated that the differences were not statistically significant among R2, R8, and NW (*Fig. 5*). Similarly, ANOSIM showed that the dissimilarity between groups was minimal compared to within-group variation, with no significant differences between the groups ($R < 0$, $P \geq 0.05$), consistent with the NMDS findings (*Fig. 6*).

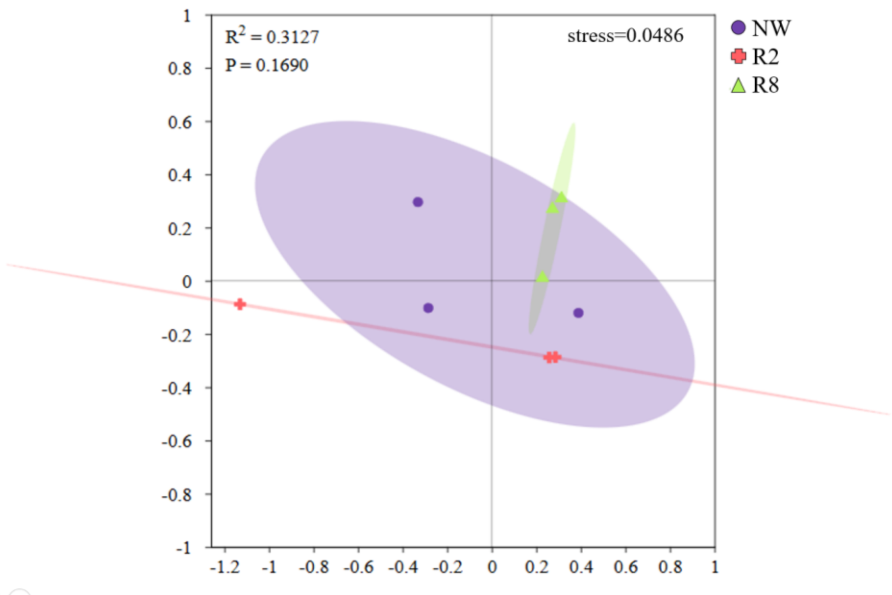

***Figure 5.** NMDS analysis of periphyton community structure. R2: wetlands restored for 2 years, R8: wetlands restored for 8 years. NW: natural wetlands*

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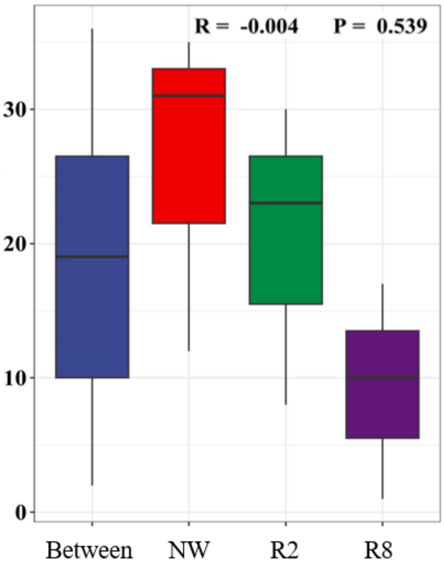

Figure 6. *ANOSIM analysis of periphyton community structure. R2: wetlands restored for 2 years, R8: wetlands restored for 8 years. NW: natural wetlands*

Analysis for aquatic environmental factors

Aquatic environmental factors interact with one another, influencing the periphyton within the ecosystem and exerting direct or indirect effects on their growth and distribution. An analysis of 11 aquatic environmental factors revealed that both WT and Cond in R2 and R8 were significantly different from those in NW (P < 0.05), but there were no significant differences between the restored wetlands. Other aquatic environmental factors showed no significant differences. The values of WD, DO, COD_{Mn}, NH_4^+-N, and TN were higher in NW compared to both R2 and R8. The SD was higher in the restored wetlands than in the natural wetlands. Additionally, TP and NO_3^--N levels were higher in R2 compared to both NW and R8. The pH values indicated that the water bodies in the protected area were weakly alkaline (*Table 5; Fig. 7*).

Table 5. *Aquatic environmental factors of wetlands (mean ± standard deviation)*

	R2	**R8**	**NW**	***P* value**
WD (m)	0.39 ± 0.05	0.39 ± 0.03	0.43 ± 0.16	0.88
SD (m)	0.38 ± 0.02	0.38 ± 0.01	0.35 ± 0.06	0.61
WT (°C)	25.91 ± 0.56^a	26.59 ± 0.73^a	22.99 ± 0.32^b	**<0.001**
DO (mg/L)	7.74 ± 1.05	8.27 ± 0.57	9.30 ± 0.67	0.12
Cond (μS/cm)	75.00 ± 24.64^b	90.00 ± 0^b	222.00 ± 2.00^a	**<0.001**
pH	7.65 ± 0.50	8.26 ± 0.62	7.82 ± 0.18	0.32
COD_{Mn} (mg/L)	2.52 ± 0.64	2.90 ± 1.32	2.95 ± 0.85	0.84
NH_4^+-N (mg/L)	3.07 ± 1.35	2.79 ± 0.80	3.54 ± 0.51	0.63
TN (mg/L)	4.30 ± 1.52	3.74 ± 1.49	4.41 ± 0.63	0.80
TP (mg/L)	0.17 ± 0.04	0.14 ± 0.04	0.08 ± 0.04	0.11
NO_3^--N (mg/L)	0.37 ± 0.48	0.12 ± 0.08	0.15 ± 0.14	0.54

NW represents natural wetlands, R2: wetlands restored for 2 years; R8: wetlands restored for 8 years; WD: water depth; SD: Secchi disk depth; WT: water temperature; DO: dissolved oxygen; Cond: conductivity; COD_{Mn}: permanganate index; NH_4^+-N: ammonia nitrogen; TN: total nitrogen; TP: total phosphorus; NO_3^--N: nitrate nitrogen

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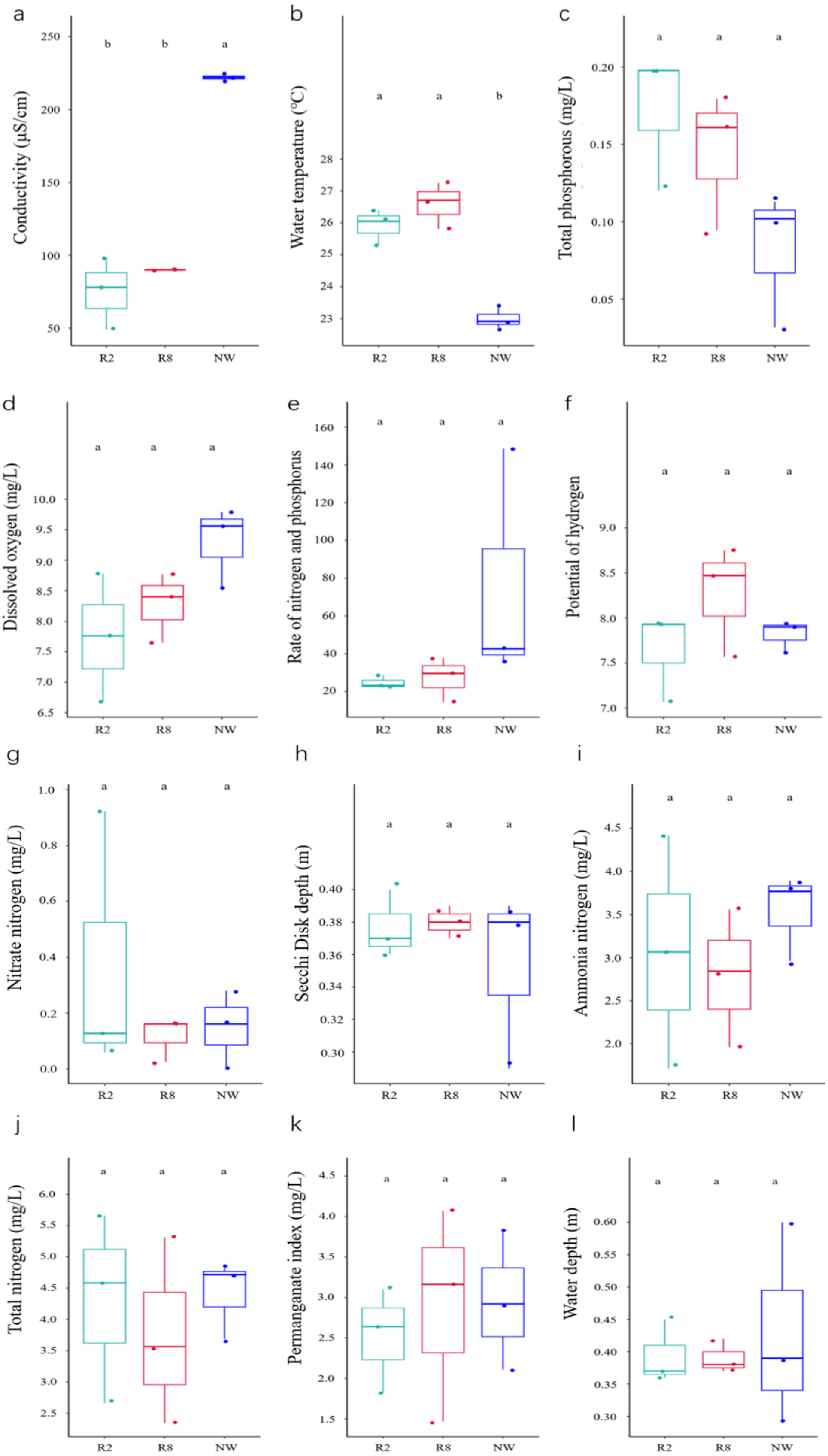

Figure 7. *Difference significance analysis of aquatic environmental factors. R2: wetlands restored for 2 years, R8: wetlands restored for 8 years. NW: natural wetlands*

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Pearson correlation analysis of periphyton and aquatic environmental factors

Pearson correlation analysis was performed to examine the relationship between aquatic environment factors and indicator species as well as dominant species of periphyton (*Fig. 8*). The results revealed significant and extremely significant positive correlations ($P < 0.05$, $P < 0.01$) among NO_3^--N and indicator species like *E. viridis*, *Ulothrix* sp., and dominant species like *T. affine*, *T. viride*, and *Tribonema utriculosum* as well. No significant correlations were found among aquatic environmental factors and other indicator species or dominant species ($P \geq 0.05$).

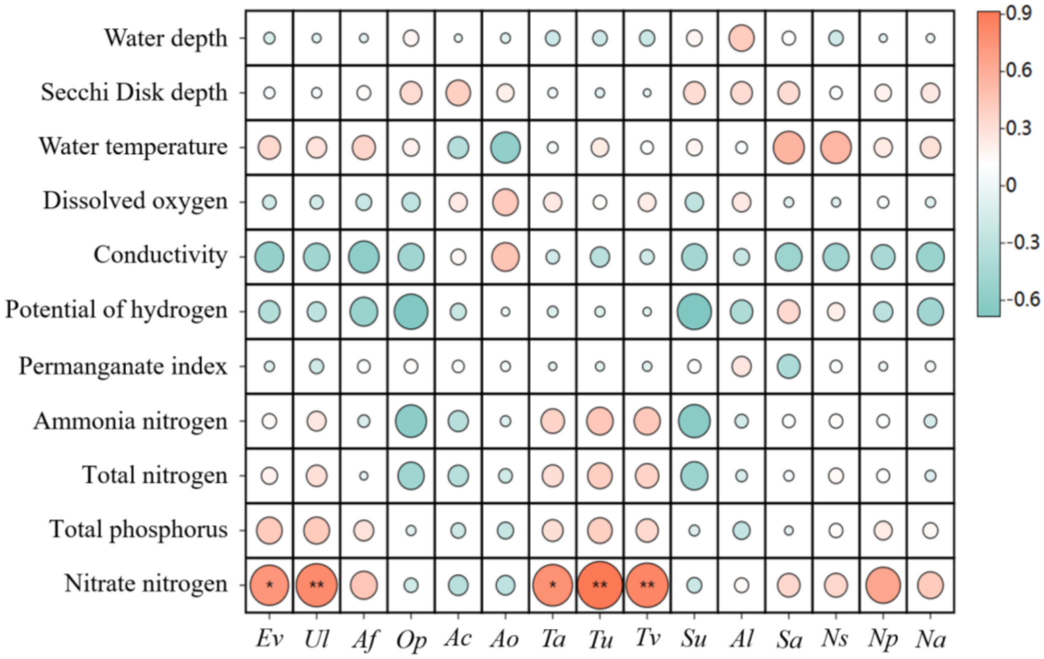

Figure 8. *Pearson correlation analysis between periphyton indicator and dominant species and aquatic environmental factors. R2: wetlands restored for 2 years, R8: wetlands restored for 8 years, NW: natural wetlands. * means significant, P < 0.05; ** means extremely significant, P < 0.01. Ev: E. viridis, Ul: Ulothrix. sp., Af: A. flosaquae, Op: O. princes, Ac: A. circinalis, Ao: A. oscillarioides, Ta: T. affine, Tv: T. viride, Tu: T. utriculosum, Su: S. ulna, Al: A. lanceolate, Sa: S. anceps, Ns: N. simples, Np: N. palea, Na: N. amphibia*

Discussion

Periphyton are considered as suitable organisms for surface water biological monitoring (Gökçe, 2016). Their small size, short life cycle, and heightened sensitivity to environmental changes enable them to promptly respond to fluctuations in their surroundings. Their inability to migrate renders them unable to evade environmental stressors, making them a more accurate reflection of alterations in their aquatic habitat (Hu et al., 2022; Liu et al., 2020; Tulp et al., 2024).

Community structure variability of periphyton

The NMDS analysis results (*Fig. 3*) indicated some differences in the periphyton community structure between R2 and R8, although these differences were not statistically significant. The connectivity of aquatic bodies plays a crucial role in

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wetland restoration (Cui et al., 2016). The reclaimed wetland at Hongqiling Farm in the Naolihe Nature Reserve was previously farmland, and barrages were constructed to control flooding from the Naoli River. Despite micro-topographic transformations following the conversion of farmland to wetland, the sampled areas in this study still exhibited water system separation between wetlands of different restoration years and natural wetlands. We observed poor water flow between different parts of the wetland during the study, which may explain the lack of significant differences in periphyton community structure between R2 and R8. This finding is supported by the ANOSIM analysis (*Fig. 4*), suggesting that the reversion of farmlands to wetlands has had a positive impact on the ecological conditions within the restored wetland area, indicating progress toward better ecological conditions.

Density and diversity of periphyton

In this study, the density of periphyton was highest at R2 and lowest at R8. This may be attributed to the shorter recovery time at R2, which likely retained higher nitrogen and phosphorus nutrients from previously cultivated soil. The restoration initiatives led to these nutrients being continuously released into the water environment, providing a rich source of nitrogen and phosphorus for periphyton (Dong et al., 2022), resulting in higher density in R2 compared to R8.

Different life-type plants in water can affect the attachment of periphyton by influencing water nutrition. Emergent plants such as *Phragmites australis* and *Zizania latifolia* mainly absorb nutrient elements through developed roots in the bottom mud, thus having little influence on the concentration of nitrogen nutrients in the water. Floating plants like *Trapa manshurica* have a strong ability to use nitrogen and can affect the algae community structure by competing for nitrate nitrogen (Li et al., 2024). *Deyeuxia angustifolia* has long thin stems and a larger attachment area, and the plants are closely arranged, which can slow down the flow rate of the water between the plants, facilitating the adhesion of periphyton and making them less likely to be washed off by the water. The stems of *Z. latifolia* and *P. australis* are smooth, tall, and upright, adjusting to protrude out of the water surface, thus providing a smaller attached area compared to *D. angustifolia*. The leaves of *T. manshurica* are spiral-shaped and float on the water surface, exposing the attached algae to sunlight (Anjuman et al., 2023), which aids algae growth. The described growth morphology of different higher vascular plants may explain the results showing densities of periphyton on different plants ordered from high to low: *D. angustifolia, T. manshurica, Z. latifolia,* and *P. australis*.

The Shannon-Wiener Diversity Index, Margalef richness index, and Pielou evenness index were utilized to analyze the periphyton, indicating that species richness showed a pattern of R2 > R8 > NW. Furthermore, species evenness and diversity showed a pattern of R8 > NW > R2. Nutrients in the restored wetland primarily originated from gradual and stable soil release, while the natural wetland received high concentrations of nutrients from agricultural runoff, promoting the growth of nutrient-rich algae. Species diversity serves as a comprehensive indicator of richness and evenness, with low species diversity typically reflecting water eutrophication (Yuan et al., 2024).

Correlation of indicator and dominant species with aquatic environment factors

The indicator species identified in R2 were *Aphanizomenon flosaquae, Euglena viridis,* and *Ulothrix* sp., which are commonly found in waters with high concentrations of

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nutrient (Xue et al., 2024; Lin et al., 2018; Ji et al., 2024). R2 and R8 were farmlands before restoration. R2, with its shorter fallow period and higher nutrient concentrations in soil, provided favorable conditions for the growth of these species through releasing these nutrients into the water. Besides, the data in *Table 5* reveals that the average nitrate nitrogen of R2 is higher than that of the other two types of wetlands. And the correlation analysis showed that there is a significant or strong positive correlation between nitrate nitrogen and indicator species of R2. It means that nitrate nitrogen promotes the growth of indicator species. Indicator species were not identified in R8 or NW, likely due to higher biodiversity and more uniform species distribution in these areas.

The analysis of 11 aquatic environmental factors revealed that water temperature (WT) and conductivity (Cond) in R2 and R8 were significantly different from those in NW. However, no significant differences were identified between R2 and R8. This discrepancy may be due to the natural wetland's location in the floodplain of the Naolihe Nature Reserve, which is a native wetland, whereas the restored wetlands were previously used as agricultural land. The construction of dams has caused separation between the restored and natural wetlands. Additionally, the low precipitation levels in 2023 further diminished the water connection between the restored and natural wetlands. Thus, the restored wetlands have shallow, small, and stagnant water bodies, unlike the natural wetland, which is connected to the Naoli River. This situation may explain the significant differences in WT and Cond between the restored and natural wetlands.

Water environment factors such as temperature, nitrogen, and phosphorus directly affect the composition, density, life cycle, and distribution of algae (Sapucaia et al., 2024; Li et al., 2023b). In both restored and natural wetlands, algae from Bacillariophyta were the most abundant, followed by those from Chlorophyta, Cyanophyta, and Euglenophyta. Xanthophyta species were the least abundant.

Algae from Bacillariophyta tend to proliferate in colder water temperatures (Zhou et al., 2019), while algae from Chlorophyta begin to grow optimally at water temperatures between 20°C and 25°C (Nalewajko and Murphy, 2001; Wang et al., 2021). Cyanophyta and Chlorophyta thrive in static or weakly turbulent waters (Chen et al., 2022), whereas diatoms have a robust ability to adapt to diverse water environments and hydrodynamic conditions (Li et al., 2023a), giving them a proliferative advantage in flowing or turbulent environments. These findings are consistent with those reported by Li et al. (2020) and Shang et al. (2024).

The nutrient content in water, especially nitrogen and phosphorus, is a key factor influencing algae growth and composition (Zhang et al., 2024). In this study, the water body contained low levels of total phosphorus (TP), and the weak correlation between TP and the indicator or dominant species may be due to phosphorus consumption by periphyton during their proliferation. This process reduces phosphorus content, weakening its impact on algae. There was a significant positive correlation between NO_3^--N and indicator species like *E. viridis* and *Ulothrix* sp., as well as dominant species such as *Tribonema affine*, *Tribonema viride*, and *Tribonema utriculosum*. Suitable temperatures can enhance nitrogen utilization by periphyton. Bacteria can convert nitrate nitrogen (NO_3^--N and NO_2^--N) into NH_4^+-N, which algae can directly absorb for growth (Zheng et al., 2023). Common dominant species across the restored and natural wetlands were *Anabaena circinalis* (Cyanophyta), *T. affine*, and *T. viride* (Xanthophyta). Nitrogen has a significant impact on the growth of algae from Cyanophyta and Xanthophyta. Additionally, the water in the study sites was weakly alkaline, which facilitates algae's absorption of CO_2 for photosynthesis (Chao et al.,

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2024). However, the growth of Xanthophyta is subject to various environmental factors, with studies showing that high transparency, low conductivity, low chemical oxygen demand (COD), and low ammonia and nitrogen content are more conducive to Xanthophyta growth (Gui et al., 2007). In this study, only *A. flosaquae* occurred in both indicator and dominant species. It was an indicator species for R2, as well as the dominant species for R2 and R8. This species is capable of rapid proliferation in alkaline eutrophic waters (Xue et al., 2024), and the environmental conditions of R2 are more conducive to competition for resources by this species.

In this study, only *A. flosaquae* appeared in both indicator and dominant species categories. It was identified as an indicator species for R2 and a dominant species in both R2 and R8. This species is capable of rapid proliferation in alkaline eutrophic waters (Xue et al., 2024), and the environmental conditions in R2 were conducive to its competitive advantage.

Dominant species belonging to Bacillariophyta were exclusively found in the restored wetlands, particularly in R8. This could be because diatoms prefer water environments with high dissolved oxygen concentrations (Tian et al., 2022), whereas R2 had lower dissolved oxygen levels, which were not conducive to diatom growth. The nitrogen-phosphorus ratios of R2 and R8 were 25:1 and 26:1, respectively, but it was 55:1 in the natural wetland. The higher nitrogen-phosphorus ratio in NW may have exerted an inhibitory effect on diatoms (Shang et al., 2024), which could explain the absence of dominant diatom species in the natural wetland.

Conclusion

A total of 91 species of periphyton, belonging to 51 genera across 5 phyla, were identified in this study. The species composition was dominated by Bacillariophyta and Chlorophyta. Periphyton density was highest in R2, lowest in R8, and intermediate in NW. Through Indicator Species Analysis, three indicator species were identified in R2: *A. flosaquae*, *E. viridis*, and *Ulothrix* sp. No indicator species were identified in R8 or NW. Common dominant species across restored and natural wetlands were *A. circinalis*, *T. affine*, and *T. viride*. There were no significant differences in biodiversity indices or periphyton community structure among R2, R8, and NW, suggesting that the ecological status of the restored wetlands has improved and is approaching that of the natural wetland. The restoration measures appear to be effective, but further strengthening of the restoration is recommended. In particular, efforts should be made to enhance the connectivity between the restored wetlands and the natural wetland water system to achieve optimal restoration outcomes.

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