



Spatial-temporal characteristics of epilithic algae succession on artificial substrata in relation to water quality in Erhai Lake, Yunnan Province, China

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Abstract

In this study, two sites with different nutritional levels at Erhai Lake were selected to perform an epilithic succession experiment in order to better understand the successional dynamic process and associated controlling factors, providing important insights into the role of epilithon in ecological functions and facilitating eutrophic lake recovery planning. The environmental parameters, epilithic biomass and epilithic diversity were qualitatively and quantitatively assessed. Our results showed that faster biomass peaks (40–50 days colonization) were achieved at Gusheng where nutrient level was higher, comparing with that of Xiahe. A 10-day incubation period is a dividing line from an initial successional stage to the following stage during epilithon succession. The epilithic biomass and biodiversity exhibited distinctly spatial-temporal characteristics, showing higher values at the site being affected by human activities than that being under the natural condition. The spatial characteristic was associated with nitrate enrichment (the most important), and the positively biological responses of the epilithon (higher biomass and diversity) to nitrate enrichment highlight the occurrence of non-point-source pollution on the west bank of Erhai Lake. The temporal characteristic was associated with water temperature (the second important), exhibiting a high biomass accumulation in winter compared with that in summer. This may be caused by other biotic factors not measured in this study, therefore, a detailed investigation regarding the interactions among biotic components in Erhai Lake is essentially needed.

Keywords Epilithon · Erhai Lake · Spatial-temporal succession · Biomass · Diversity

Bo Lan and Hailan Li contributed equally to this work.

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Introduction

Epilithon, a kind of rock-attached algae, plays an important role in the functioning of shallow aquatic ecosystems via its impacts on primary production, nutritional cycling and food web (Sand-Jensen and Borum 1991; APHA 1998; McCollum et al. 1998; Vadeboncoeur et al. 2003; Gaiser et al. 2006). Detailed studies on the dynamics of the epilithic community will provide important insights into the role of epilithon in the functioning of ecosystems for biomonitoring, ecological status assessments and eutrophic lake recovery planning (Jöbgen et al. 2004; Roubex et al. 2011; DeNicola and Kelly 2014; Yang et al. 2016). Epilithic dynamics can be assessed by studying succession, i.e., the process of species replacements. Succession reflects a series of interactions among habitat characteristics, allogenic and autogenic factors, and species compositions (Borduqui and Ferragut 2012).

In this study, we selected Erhai Lake to conduct the succession of epilithon. Erhai Lake is the second largest freshwater lake in Yunnan Province, southwestern China, and it has reached a meso-eutrophication level ten years ago (Peng et al. 2005). Moreover, the local water quality has deteriorated to a critical state because of increases in non-point-source pollution introduced by human activities (Zheng et al. 2004; Ni et al. 2016). This deterioration has threatened the biodiversity in the littoral zone of Erhai Lake, for instance, it increased the number of monotonous biotic communities and reduced the biotic community types (Hu et al. 2005; Tang et al. 2013). Thus, ecological assessments of the littoral ecosystem disturbed by human disturbance and a series of corresponding measures of ecological restoration must be implemented for Erhai Lake immediately. Epilithon, as one of the most important biotic components in the littoral zone of Erhai Lake, is critical to perform its function in littoral ecosystem, showing a great potential for use in bioassessment and ecological restoration. As the drastic vegetation degradation of submerged plants of Erhai Lake in recent decades (Wang et al. 2015), epilithon plays an increasingly important role in the littoral ecosystem to sustain primary production and ecosystem stability of Erhai Lake. So far, only a few studies focus on the ecology of epilithon communities in Erhai Lake (Pei et al. 2008; Lan et al. 2011; Yang et al. 2013), including spatial distributions of epilithon, and similarity among different substrata. However, there is still much ecological knowledge unknown at present, for instance, the characteristics of epilithon succession in Erhai Lake. The ecological knowledge gap may lead to an unfavorable situation in which little information is available on the functional attributes of the epilithon in Erhai Lake ecosystem, limiting the applications of epilithon on bioassessment and ecological restoration.

This study aim to describe the ecological characteristics of epilithon community by virtue of studying epilithon succession process on artificial substrata at two sites of Erhai Lake subjected to different pollution levels. It will be helpful to identify the relationships between epilithon succession and its controlling factors, and ultimately increase our understanding of the functional attributes of the epilithon in this lake.

Methods

Study site

Erhai Lake (i.e., our study area) is a large freshwater body situated in Dali City of Yunnan Province of southwestern China (Fig. 1a). According to the meteorological records from Dali station, the mean annual temperature is 15.1 °C, the mean annual precipitation is 1060 mm, with more than 85% falling between May and October in the catchment (Shen et al. 2005). Erhai Lake has a length of 42 km from north to south and a

maximum width of 8.4 km from east to west, and it covers an area of ~250 km². The average depth is 10.5 m, and the maximum depth is 20.9 m (Peng et al. 2005). Erhai Lake is mainly fed by three rivers (i.e., Miju, Luoshi, and Boluo) and eighteen creeks, and it drains southwards via the natural Xi'er River. To the west of Erhai Lake, a large number of local residents are settled along the foot of the Cangshan Mountains, and a vast expanse of farmland is situated between the residential belt and Erhai Lake. Sewage and agricultural wastewater is discharged into Erhai Lake, leading to algal blooms (Yan et al. 2005; Ni et al. 2016). On the eastern side of Erhai Lake is a sparsely populated shore with a few towns, thus limited disturbances occur along most parts of the eastern shore. Two sites at Erhai aquatic-terrestrial ecotone in the west and the east are selected to perform the epilithon succession, respectively. The west one is Gusheng site (25°48'35.6"N, 100°08'39.5"E) which is identified to be obviously affected by human activities, and the east one is Xiahe site (25°39'25.4"N, 100°17'09.1"E) which is rarely affected by human activities (Fig. 1b). Both sites recorded a water depth of ~2.5 m and found widely distributed submersed macrophytes (e.g., *Potamogeton maackianus*, *Ceratophyllum* sp. and *Vallisneria* sp.).

Sampling methods

The experiments were performed in winter (2009.12–2010.2) and late summer (2010.8–2010.10). Epilithic colonization was conducted on artificial substrata composed of rectangular marbles (8 cm × 5 cm × 1 cm, see Fig. 1c). Thirty marble substrata were vertically immersed in water at a depth of ~0.5 m at Gusheng and Xiahe sites. Each sample was consisted of the epilithon which was randomly collected from a marble substratum using a razor blade and a toothbrush at each site every 10 days after the succession began. And four parallel epilithic samples at each site were conducted every sampling time. In addition, water samples were collected to determine the environmental parameters. Subsequently, all epilithic and water samples were stored in insulation can with ice packs and transported to the laboratory for further analysis. Each epilithic sample was divided into three parts. The first part was used to determine the chlorophyll a (Chl a) concentration, the second part was used to determine the dry weight (DW) and the ash-free dry weight (AFDW), and the third part was preserved in 4% formalin and used for epilithic enumeration.

Analytical procedures

Physical and chemical parameters of lake water were measured at each sample day. Water temperature (WT), pH and dissolved oxygen (DO) were measured in situ at a water depth of ~0.5 m. Water transparency (SD) were

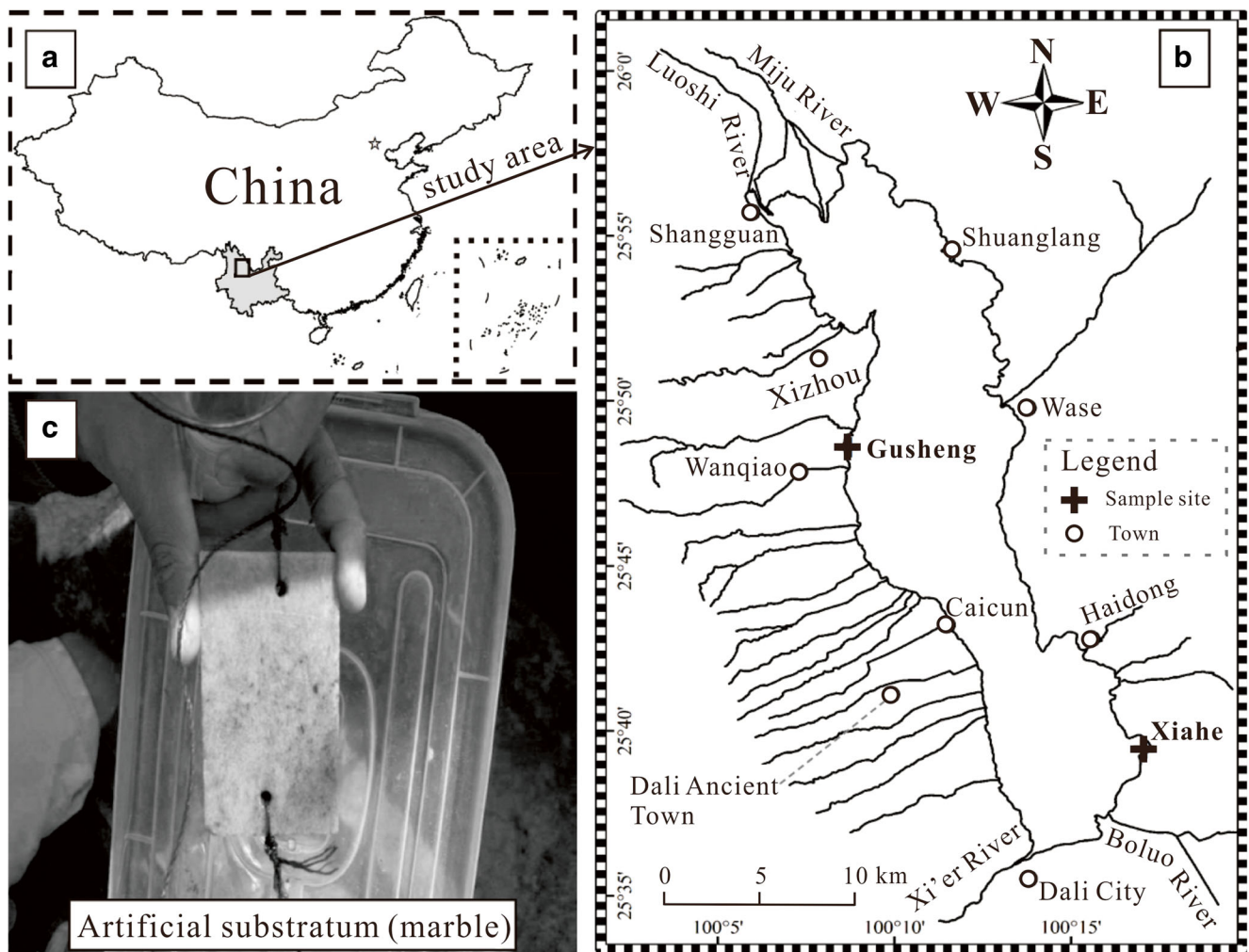


Fig. 1 a Study area, locates in Yunnan Province, southwest of China; b Hydrological regime of Erhai Lake and the sample sites (the cross symbols); c A photo of artificial substratum, made of marble

measured using a black and white quadrant Secchi disk of 20-cm diameter in situ. The total nitrogen (TN), total phosphorus (TP), permanganate index (COD_{Mn}), ammonia nitrogen (NH_4^+-N), nitrate ($NO_3^- -N$), and soluble reactive phosphate (SRP) concentrations were determined according to standard methods used to examine water and wastewater (APHA 1998).

The comprehensive trophic state index (TSI) provides insights into the nutritional status of lake water, specifically, when $30 < TSI \leq 50$, the water quality is still good, indicating a mesotrophic state; and when $50 < TSI \leq 60$, the water quality is polluted, indicating a eutrophic state (Li et al. 2016). The TSI was calculated based on the weighted sum of the TSI_j , which was calculated using five indicators (C_j), including Chl a_{water} , TN, TP, COD_{Mn} , and SD. More details can be found in Huang et al. (2016).

Chl a was extracted using 90% acetone for 12 h after filtering through Whatman GF/F filters. Then, the Chl a concentration was calculated according to the absorbance at 630, 647, 664 and 750 nm (Arar 1997). The DW was

obtained after the epilithic sample was oven-dried at 105 °C for at least 24 h. For the AFDW analysis, the aforementioned oven-dried sample was combusted at 550 °C for 4 h to obtain the ash weight (AW), and the AFDW was calculated by subtracting the AW from the DW (APHA 1998). The DW, AW and AFDW were expressed in $mg\ cm^{-2}$. The autotrophic index (AI) was expressed as the ratio of the AFDW to the Chl a (i.e., $AFDW/Chl\ a$), reflecting the relative importance of autotrophs and heterotrophs in the community (APHA 1998).

Epilithic enumeration of each sample was performed with a microscope at 400-fold and 1000-fold magnification. Morphotypes were identified by referencing Krammer and Lange-Bertalot (1986-1991), Zhu and Chen (2000) and Hu and Wei (2006). The epilithic cell size was estimated using an ocular micrometer. The Shannon-Wiener index (H'), the Margalef index (D), the Simpson diversity index (P), and the Pielou evenness index (J) were employed to depict variations in species biodiversity (e.g., H' and D) and evenness (e.g., P and J) (Pandya et al. 2014).

The temporally and spatially significant differences in the water parameters, biomasses (DW, AFDW, Chl a) and biodiversities (H' , D , P , J) were analyzed by a one-way analysis of variance (ANOVA) in SPSS 19.0, followed by the Student-Neumann-Keuls posthoc (SNK) multiple comparison test to determine significance between groups. A principal component analysis (PCA) and a redundancy analysis (RDA) were conducted using Canoco 5.0 for Windows after species data were square-root transformed to satisfy the assumption of normality and the homogeneity of variance. Only the environmental variables with significant contributions to the axes were chosen for the RDA using forward selection ($P < 0.05$). The level of variance in the biotic data (biomass, diversity and AI) explained by the environmental variables according to the RDA was tested using Monte Carlo simulations with 999 unrestricted permutations.

Results

Water quality

The physicochemical variables at both sampling sites (i.e., Gusheng and Xiahe) during the study period are summarized in Table 1. According to the significance analysis, the WT, SD and SRP exhibited significant temporal differences, with higher values in summer. TN, NO_3^- -N, and $\text{Chl a}_{\text{water}}$ exhibited significant spatial differences, with higher values at Gusheng ($P < 0.05$). AI values at Xiahe were significantly higher than that at Gusheng ($P < 0.01$), indicating that

heterotrophic organisms were more prevalent at Xiahe. The SD, TP, SRP and the comprehensive trophic state index (TSI) exhibited significant spatial-temporal differences ($P < 0.01$). The TSI value was higher than 50 at the Gusheng in summer, indicating a eutrophic state, whereas the others varied between 40 and 50, indicating a mesotrophic state.

Variations in the epilithic biomass

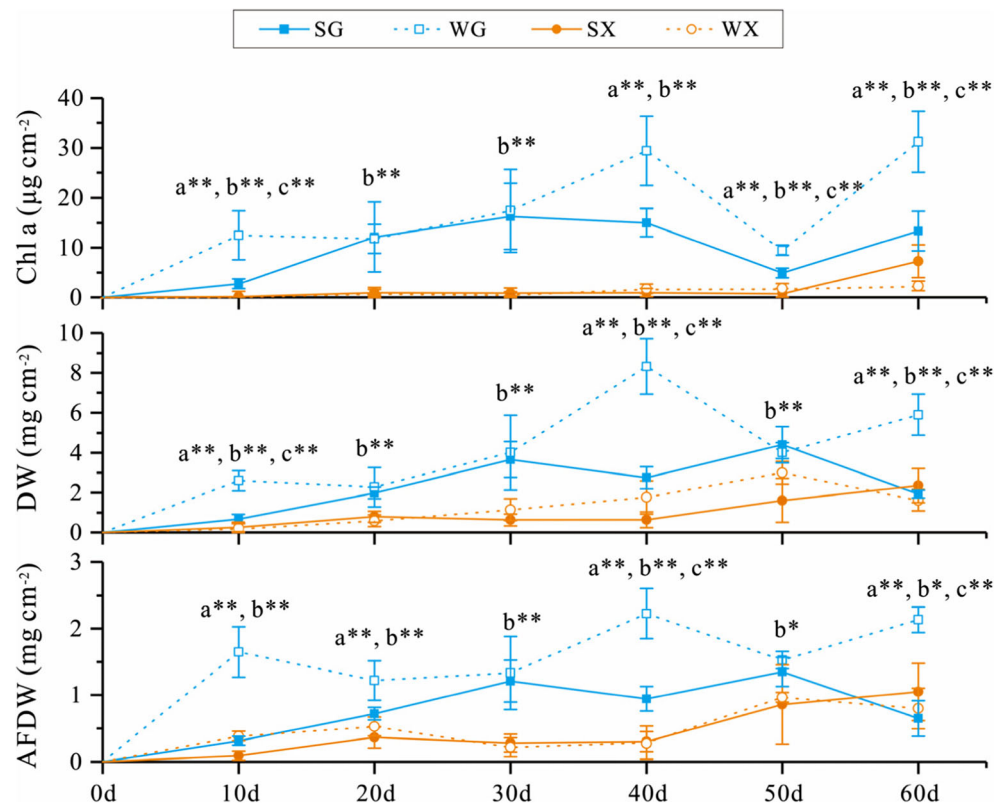
The temporal and spatial changes in epilithic biomass were assessed using the Chl a, DW and AFDW (Fig. 2). All samples were abbreviated, referring to the annotation of Fig. 2, e.g., the sample colonized for 40 days in winter at Gusheng was abbreviated as WG40d, and so on. At the Gusheng site, although faster colonization was observed in summer, the highest epilithic biomass was achieved in winter. At the Xiahe site, the epilithon biomass tended to increase with colonization time in winter and summer. The biomass at Gusheng was always higher than that at Xiahe. Relative high epilithic biomasses (Chl a, DW and AFDW) were observed in the WG40d and WG60d samples with no significant difference between each other ($P > 0.05$), however, both samples were significantly higher than the other samples ($P < 0.05$). Specifically, the WG40d biomasses were as follows: Chl a, 22.5–36.3 $\mu\text{g cm}^{-2}$; DW, 6.9–9.7 mg cm^{-2} ; and AFDW, 1.8–2.6 mg cm^{-2} , respectively, higher than the other biomasses colonized for the same days (i.e., WX40d, SG40d, SX40d). In general, the biomass exhibited significant spatial-temporal variations, i.e., $\text{Biomass}_G > \text{Biomass}_X$ in both seasons, $\text{Biomass}_W \geq \text{Biomass}_S$ at both sites.

Table 1 Environmental parameters (means \pm standard deviation) at both sites in summer and winter

No	Parameters	Gusheng		Xiahe		Significance
		summer	winter	summer	winter	
1	WT ($^{\circ}\text{C}$)	21.70 \pm 0.71	12.58 \pm 1.39	21.20 \pm 0.78	10.92 \pm 1.14	a**
2	pH	8.29 \pm 0.31	8.46 \pm 0.22	8.30 \pm 0.22	8.30 \pm 0.16	–
3	DO (mg L^{-1})	8.77 \pm 0.62	8.97 \pm 0.48	8.10 \pm 1.10	8.29 \pm 0.73	–
4	SD (m)	1.21 \pm 0.08	1.16 \pm 0.15	1.39 \pm 0.12	0.98 \pm 0.12	a**, c**
5	TN (mg L^{-1})	1.16 \pm 0.33	0.89 \pm 0.30	0.72 \pm 0.09	0.60 \pm 0.30	b*
6	TP (mg L^{-1})	0.070 \pm 0.021	0.018 \pm 0.002	0.02 \pm 0.00	0.035 \pm 0.019	c**
7	COD_{Mn} (mg L^{-1})	5.26 \pm 0.99	4.19 \pm 0.46	5.00 \pm 0.99	5.21 \pm 0.99	–
8	NH_4^+ -N (mg L^{-1})	0.12 \pm 0.071	0.18 \pm 0.09	0.13 \pm 0.02	0.23 \pm 0.07	–
9	NO_3^- -N (mg L^{-1})	0.36 \pm 0.34	0.21 \pm 0.05	0.06 \pm 0.02	0.04 \pm 0.01	b*
10	SRP ($\mu\text{g L}^{-1}$)	13.60 \pm 6.08	2.01 \pm 0.42	2.25 \pm 0.14	3.76 \pm 0.75	a*, c**
11	$\text{Chl a}_{\text{water}}$ ($\mu\text{g L}^{-1}$)	35.94 \pm 8.00	19.81 \pm 6.73	16.59 \pm 5.12	19.51 \pm 1.41	b*
12	AI	105 \pm 78	103 \pm 34	389 \pm 193	417 \pm 171	b**
13	TSI	53.4 \pm 1.3	45.3 \pm 0.2	46.2 \pm 1.3	48.6 \pm 2.5	b*, c**

a represent significant seasonal difference (late summer vs. winter); b represent significant spatial difference (Gusheng vs. Xiahe location); c represent significant spatial and temporal difference ($c = a \times b$). significance levels: * $P < 0.05$, ** $P < 0.01$

Fig. 2 Variations of periphytic biomasses at both sampling sites in summer and winter, including chlorophyll a concentration (Chl a), dry weight (DW) and ash-free dry weight (AFDW)



Variations of epilithic species

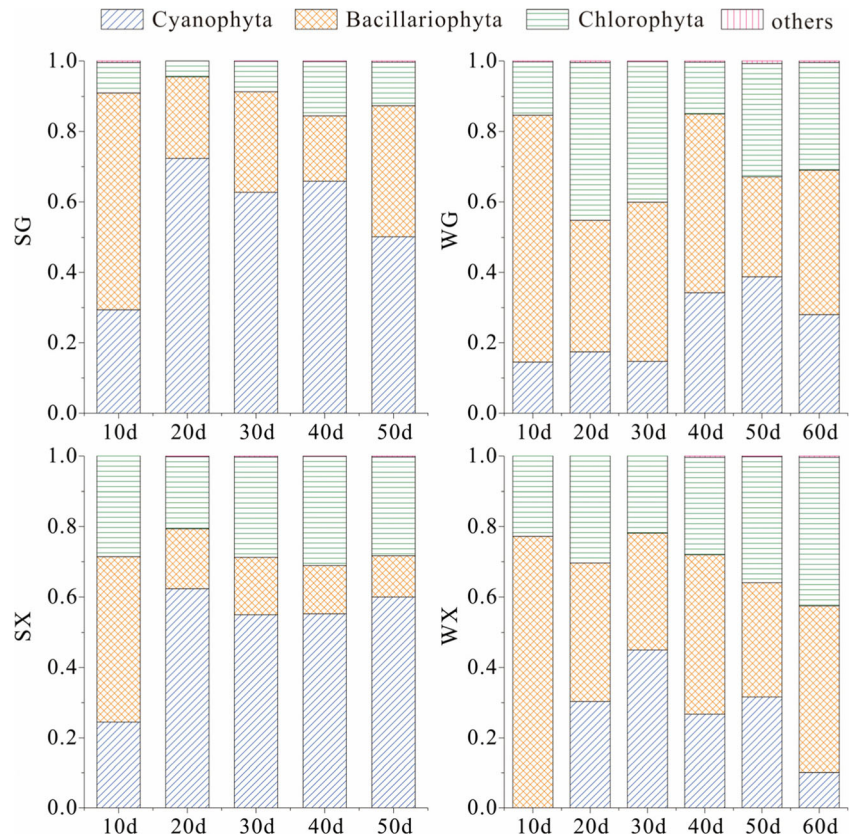
A total of 118 epilithic taxa were identified at the two sampling sites during the study period. The epilithic taxa were dominated by Bacillariophyta, Chlorophyta and Cyanophyta, and a few species of Cryptophyta, Pyrrophyta and Euglenophyta (not shown) were also observed (Fig. 3). Single-celled algae (e.g., *Placoneis amphibola*, *Navicula* sp2., *Stauroneis anceps*, *Tetraedron* sp. and *Cosmarium* sp.) and non-filamentous multicellular algae (e.g., *Fragilaria virescens*, *Fragilaria lyngbye* and *Desmodesmus quadricauda*) occurred at the beginning of succession. Some filamentous algae, such as *Phormidium faveolarum* and *Aphanizomenon flosaquae*, were also observed at the initial stage. Although a large number of cells of the filamentous algae were identified, these organisms are so tiny (~0.5 to ~2 µm) that they only accounted for a small proportion of the biovolume.

To explore the spatial-temporal variations of the epilithic communities, we adopted PCA ordination to visually characterize the epilithic successions (Fig. 4). Based on the results of the PCA, three sample groups were differentiated. The first group was composed of the initial phase samples (i.e., SG10d, SX10d, WG10d, WX10d), which were characterized by the abundant species of Bacillariophyta (e.g., *F. lyngbye*, *P. amphibola*, *Rhoicosphenia abbreviata* and *S. anceps*), as well as species of Cyanophyta and Chlorophyta (e.g., *P. faveolarum*, *A. flosaquae*, *D. quadricauda*, *Cosmarium* sp. and *Tetraedron* sp.). The second group (SX20d, SX30d,

SX50d, WX20d, WX40d, WX50d and WX60d) was dominated by *Anabaena azotica*, *Psephonema aenigmaticum* and *Calothrix* sp. The third group was composed of samples colonized for 20–60 days at the Gusheng site in both seasons and was dominated by *Pseudanabaena* sp., *Cyclotella meneghiniana*, *P. aenigmaticum* and *Cymbella ventricosa*. Species of Bacillariophyta, such as *Nitzschia palea*, *Hantzschia amphioxys*, *Fragilaria capucina* and *Navicula* sp1., were frequently found in this group.

The PCA biplot can be used to identify the potential factors influencing the species variations. The first two axes of the PCA accounted for 42.5% of the total species variance, with the first axis (PCA 1) explaining 23.4% and the second axis (PCA 2) explaining 19.1%. Considering that all samples at the initial stage of succession (i.e., 10 d) were situated on the positive side of PCA 1 and the samples of the following successional stages (i.e., 20–60 d) were situated on the negative side of PCA 1, the succession trajectory was distinctly dependent on incubation time (Fig. 4). Thus, the first axis may be associated with the temporal effect of incubation time on the community structure of epilithon. Most samples collected from Xiahe site were located on the positive side of PCA 2, whereas samples collected from Gusheng site were located entirely on the negative side of PCA 2, indicating that PCA 2 may be associated with the effect of spatially related factors on the community structure of epilithic algae.

Fig. 3 Relative abundance of epilithic aglae



Variations of epilithic biodiversity

Several indices are employed to evaluate the diversity, richness and equitability of species during succession

(Fig. 5). The results showed that the highest Shannon-Wiener diversity index (H') and Margalef richness index (D) were obtained from SG samples, whereas the lowest aforementioned indices were obtained from SX samples. In general, the epilithon reached the first diversity peak within 30 days in summer, whereas the first diversity peak was reached within 50 days in winter. The Simpson evenness index (P) and Pielou evenness index (J) exhibited higher values at the initial colonization stage, followed by a sharp decline on the 20th day and a slightly increasing trend thereafter.

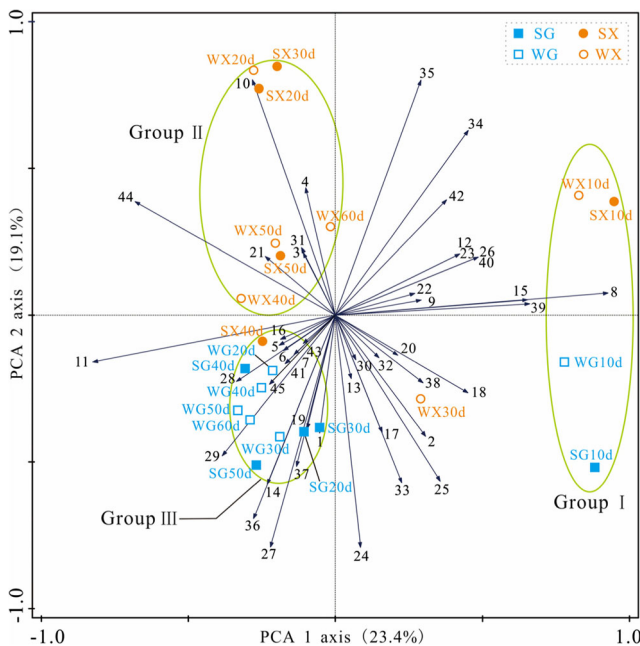
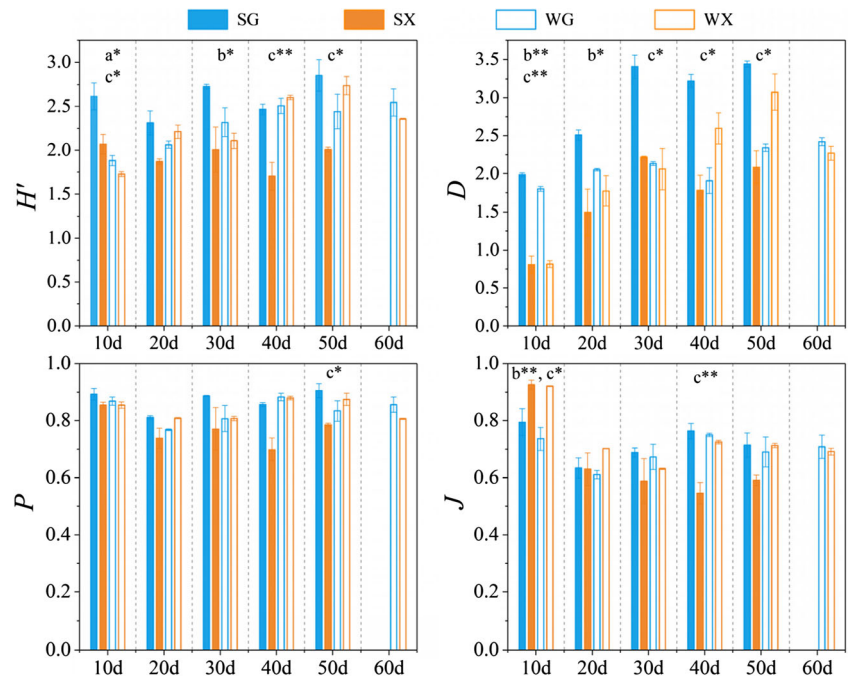


Fig. 4 The principal component analysis (PCA) based on species data (relative abundance >2%). The numbers denote for species, corresponding to the ID number of algae in Supplementary Table S1

The biotic variables (biomass, diversity and AI) and environmental parameters (only shown the significant ones with the first two axes, $P < 0.01$) were subjected to a RDA to determine the relationships among the aforementioned data. According to the RDA results, the first two axes explained 52.0% of the observed variations in biotic data (Fig. 6), with the first axis (RDA 1) explaining 50.6% of the variations and second axis (RDA 2) only explaining 1.4% (Table 2). The biotic data, including the epilithic biomass (Chl a, DW and AFDW) and diversity (H' and D), were positively correlated with RDA 1. Considering that Gusheng site were situated on the positive side of RDA 1, while Xiahe site were situated on the negative side of RDA 1, thus the first axis reflected the effects of the spatial factor on the biotic data of the epilithon. Moreover, nitrate concentration, as one of the

Fig. 5 Spatial-temporal variations of biodiversity, including Shannon-Wiener index (H'), the Margalef index (D), the Simpson diversity index (P) and the Pielou evenness index (J)



most important environmental variables affecting the epilithon succession, was highly significant correlated with PCA 1 ($r = 0.654$, $P < 0.01$; Table 2). It implied that the spatial variability of nitrate was the spatial factor and had a striking function on epilithon succession, resulting in the spatial characteristic of epilithon communities. Similarly, the positive side of RDA 2 mainly contained

biotic data in summer, and the negative side of RDA 2 contained biotic data in winter, implying that RDA 2 reflected the seasonal factor. The seasonal factor was associated with variations of WT based on the relationship between WT and RDA 2 ($r = 0.415$, $P < 0.05$; Table 2).

Discussion

Features of epilithic succession

Faster biomasses peaks were achieved at Gusheng than that at Xiahe, implying that the period of ecological restoration using epilithon colonized on artificial substrata are needed to be differentiated at different nutrient levels. For instance, 40–50 days colonization is recommended at Gusheng and more than 50 days colonization is needed at Xiahe.

The evenness of the epilithic community was initially high, lower in the intermediate phase and again slightly higher later on, being analogous with the result of others (Szabó et al. 2008). Community composition after 10 days colonization was different from following stages whereas there were only minor changes subsequently. That is, the epilithic community tended to stabilize after 20 days colonization. After 20 days, a sharply decreasing evenness index was observed, because certain successional species accounted for relatively high proportions. From 30 to 60 days, filamentous algae (e.g., *Oscillatoria*, *Lyngbya*, *Phormidium*, *Anabaena*, *A. flosaquae*, and *P. aenigmat*) increased proportionally to higher levels, implying that habitat space or light availability may be limiting resources that forced the algal mat

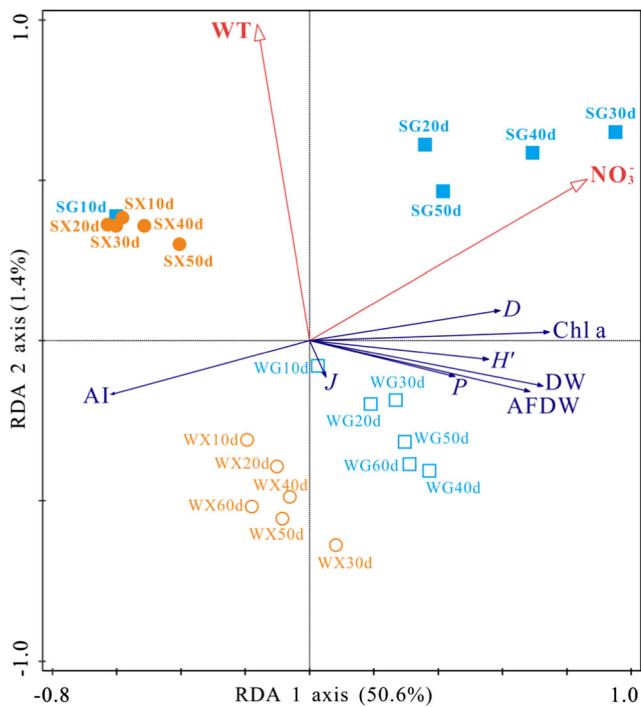


Fig. 6 RDA biplot of biotic data (biomass, diversity and AI) and environmental parameters in Erhai Lake

Table 2 Summary of RDA on biotic data (biomass, diversity and AI) and environmental parameters during the succession

	Axis 1	Axis 2
Eigenvalue	0.506	0.014
Variance explained (%)	50.6	1.4
<i>Pseudo-canonical correlations</i>	0.759	0.440
<i>Cumulative percentage variance</i>		
Of response data	50.6	52.0
Of fitted response data	96.8	99.5
Sum of all eigenvalues	1.000	
Sum of all canonical eigenvalues	0.523	
<i>Canonical coefficients</i>		
NO ₃ ⁻ -N	0.654	0.218
WT	-0.125	0.415

to develop into a three-dimensional stereoscopic structure in following successional stages (Johnson et al. 1997; Jackson 2003). Nevertheless, the dispersed distribution of the samples colonized for 10 days may be associated with the randomly homogenized attachment of epilithic algae; and the samples colonized for 20–60 days were clustered on the negative side of the PCA biplot, suggesting relatively high similarities after 20 days colonization. Thus, a 10-day incubation time is a key criterion for the succession of epilithon and represents a dividing line from the initial to the following successional stage.

E. sorex and *Epithemia gibba* almost solely occurred at Xiahe site during the succession. These taxa are regarded as common epiphytic species (Zalat and Vildary 2005; Sivaci et al. 2008; Malkin et al. 2009), usually accompanied by macrophytes in water environments. This situation is basically in accord with the actual distribution of macrophytes which has been found averagely wider around Xiahe site than Gusheng site by field investigations (Hu et al. 2005; Chu et al. 2014).

Factors influencing the biotic data during succession

Among the environmental parameters, the most important one influencing the biotic data (i.e., biomass, diversity and AI) is nitrate concentration, showing a significant spatial variation. The significant spatial nitrate difference may be associated with anthropogenic effects on the west lakeshore (e.g., Gusheng). In details, spring water originated from Cangshan Mountain flows through residential areas and vast areas of farmland, discharges into Erhai Lake ultimately, leading to nutrient accumulations along the west bank of the lake. Thus, Erhai Lake likely suffers from non-point-source pollution, especially in summer. Excluding nitrate, other nutrients were also highly enriched, and the highest average TSI value was also observed at Gusheng site in summer. The higher nutrients and TSI values at Gusheng in summer than in winter

further highlighted the seasonal characteristics of non-point-source pollution. It is likely to be associated with high quantities of fertilizer and excrement inputs and the low use efficiency of fertilizer and living waste (Yang and Song 2006). Thus, the high nutritional load (e.g., nitrate) leads to higher biomass at Gusheng than at Xiahe, showing an obvious spatial characteristic. The diversity indices were also positively associated with the concentration of nitrate during the epilithic succession. Reports have indicated that a moderately increased nitrate concentration may improve biodiversity (Peterson and Grimm 1992; Baker et al. 2009; Shylla and Ramanujam 2013), resulting in a higher species diversity in meso-eutrophic lakes comparing with that in oligotrophic or eutrophic lakes (Jeppesen et al. 2000). Therefore, the meso-eutrophic waterbody at Gusheng may contribute to the higher biodiversity relative to that at Xiahe.

Among the environmental parameters, the second most important factor influencing the biotic data was WT, although it only accounted for 1.4% of the variations in the biotic data. The epilithic biomass was weakly negatively correlated with WT in this study, with generally higher biomass in winter than in summer. It should be pointed out that the WT is significantly higher in summer than in winter in our study area ($P < 0.01$). This negative correlation between epilithic biomass and WT is different from most cases because biomass accrual rates are positively correlated with WT normally (Francoeur et al. 1999). Epilithic biomass is related to many factors, such as nutrient, grazing, allelopathy and so on (Feminella and Hawkins 1995; McCollum et al. 1998; Hillebrand and Kahlert 2001; Winkelmann et al. 2014). In our study (e.g., Gusheng site), the epilithic biomass was supposed to be higher in summer than in winter because both of the WT and nutritional loads are significantly higher in summer than in winter and both factors usually lead to a higher biomass, however, a reverse result was observed. It can be inferred that certain seasonal variables that were not measured in this study, such as the effects of grazing or interactions with the macrophytes, have exerted more influences on epilithic biomass than WT and nutrients (e.g., nitrate). These undetermined factors (such as grazing intensity and interaction effects with macrophytes) should be included in future studies to further explore their roles on epilithon.

Conclusion

In this study, an epilithic successional experiment was performed at two sites with different nutritional levels in Erhai Lake. The result revealed that faster biomasses peaks (40–50 days colonization) were achieved at Gusheng where nutrient level was higher, comparing with that of Xiahe. A 10-day incubation time is a key criterion for the succession of epilithon in Erhai Lake and represents a dividing line from

the initial to the following successional stage. The biomass and biodiversity of epilithic community were higher at the site being affected by human activities than that being under the natural condition. Positively biological responses of the epilithon (higher biomass and diversity) to nitrate enrichment highlight the occurrence of non-point-source pollution on the west bank of Erhai Lake. And epilithic biomass responses to water temperature call for a detailed investigation regarding the interactions among biotic components in aquatic ecosystem.

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Author contributions B.L. and H.L. are co-first authors with equal contribution to this work. B.L. and H.L. conducted the experiments and analyzed the data. X.X. conducted field work and offered assistance to identify the algae. S.Y. and B.L. designed the project. All authors co-wrote and co-edited the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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