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Effects of Diversity, Coverage and Biomass of Submerged Macrophytes on Nutrient Concentrations, Water Clarity and Phytoplankton Biomass in Two Restored Shallow Lakes

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Abstract: Transplantation of submerged macrophytes to restore shallow lakes has been used as an effective measure to maintain a clear water state. Water quality is highly correlated with submerged macrophytes community, however, the relationships between water quality and the diversity, coverage and biomass of submerged macrophytes are, so far, not yet well studied. We analyzed the correlations of nutrient concentrations, water clarity and phytoplankton biomass with the metrics of submerged macrophytes community in two Chinese restored shallow subtropical lakes, Lake Wuli (Wuli-E, 5 ha) and Lake Qinhu (Qin-E, 8 ha). A similar biomass of submerged macrophytes was transplanted into each lake, while both the species richness and coverage of macrophytes in Qin-E were lower than Wuli-E. After a 1–2-year restoration, the diversity almost had no change, but the biomass density and coverage decreased in Wuli-E. As for Qin-E, the coverage of submerged macrophytes increased but biomass density and diversity decreased. The dominance of canopy-forming submerged macrophyte species *Myriophyllum spicatum* was observed in Qin-E and less meadow-forming biomass and species was observed than that in Wuli-E. Moreover, it was also observed that Wuli-E had a better water quality than that of Qin-E after transplantation. Path analysis results showed that macrophyte coverage and the diversity related to meadow-forming species (e.g., *Vallisneria spinulosa*) had strong effects on enhancing clarity and reducing nutrient concentrations. But the high biomass density accompanied by the canopy-forming species like *M. spicatum* was unfavorable for controlling nutrients. Our results provide important insight into the different roles that macrophyte diversity, biomass and coverage play in improving water clarity and controlling nutrient concentrations. This new knowledge will be instrumental in implementing more effective lake restoration, especially using macrophyte transplantation as a restoration tool in warm shallow lakes.

Keywords: water quality; lake restoration; Shannon–Wiener index; path analysis; canopy-forming; meadow-forming

1. Introduction

Recovery of submerged macrophytes has been considered as an effective measure in restoration of shallow eutrophic lakes. In temperate lakes, macrophyte recovery was mainly achieved naturally through improvements in water quality and transparency, but this approach requires a capacious reservoir of plant propagules and low internal nutrient loadings [1–5]. However, in some eutrophic lakes, submerged macrophytes disappeared long ago; meanwhile the internal nutrient loadings were usually high, the recovery of the diversity of submerged macrophytes community may be delayed due to the lack of diverse propagules in the upper layer of the sediments where the propagules were dominated by eutrophic species (e.g., *Myriophyllum spicatum*) [6–9], most of them are canopy-forming species. The propagule of these canopy-forming macrophytes in the top layer of the sediments will first resume growth after restoration, thereby inhibiting the germination and growth of other species (mainly meadow-forming species) via shading effects [10]. Therefore, natural recovery of submerged macrophytes diversity may be delayed after nutrients control, such as the diversity of submerged macrophytes reached less than 80% of the previous macrophyte diversity in Lake Fure after 48 years [9]. However, transplantation of submerged macrophytes to the eutrophic lakes may favor the quick recovery of macrophyte diversity [4,7,11], especially in lakes with rich sediments and lack of propagules which will limit the natural recovery of submerged macrophytes that may occur, even if water quality and light conditions are improved [12]. In these lakes and the restoration goal including the re-introduction of those species typically dominating in nutrient-poor lakes, transplantation is often deemed relevant after sediment removal and fish community manipulation [4,7,11,13,14].

Submerged macrophytes can improve water quality, but this may require sufficient coverage and biomass of submerged macrophytes [7]. High submerged macrophytes coverage helps much to water quality via covering sediments to inhibit resuspension and nutrients release [15,16]. Similarly, high biomass density helps much via absorbing nutrients from water column [7,10]. In addition, studies reported that diverse macrophytes with different root architectures can inhibit sediment release as well as absorb nutrients from water column [9,17–22]. Although there was no direct evidence that high diversity of submerged macrophytes was conducive to improving water quality, high macrophyte diversity is generally associated with better water quality and long-term declines in macrophyte richness were observed along with eutrophication in many shallow lakes [9,15,20,21].

Submerged macrophyte diversity, biomass density and coverage have been reported to be linked to water quality in both artificial cultivation experiments and field investigations, but the relationship has yet to be fully understood. There are no specific guidelines or management options of macrophyte diversity, biomass density and coverage in macrophyte recovery by transplantation. Although intensive managements of macrophytes were assigned to the transplanted communities, it is still impossible to avoid the collapse of some recovered macrophyte communities. To explore the impact of macrophyte biomass density, coverage and diversity on water clarity in restored subtropical shallow lakes, we conducted a relatively large-scale comparative experiment in two small lakes in the lower reaches of the Yangtze River, China. Submerged macrophyte communities that differed in coverage and diversity, but similar in biomass density were transplanted in two small water bodies no bigger than 8 ha. We predicted that the difference of macrophyte communities would lead to water quality differences in the maintenance of a clear-water state.

2. Methods

2.1. Study Area

Each of the study lakes is part of a large lake in subtropical China, Lake Wuli at 31°32′05.82″ N; 120°14′12.16″ E and Lake Qinhu at 32°37′38.76″ N; 120°05′43.49″ E, and the distance between the two lakes is approximately 120 km. The manmade soil dams were used to isolate the studied area from the main lake (Figure 1). We used abbreviation Wuli-E (experimental) and Qin-E to replace the restored areas in Lake Wuli and Lake Qinhu, respectively, Wuli-C (control) and Qin-C and for the unrestored areas of the two lakes. The Wuli-E covers approximately 5 ha and has a mean depth of 2.0 m. It was used for fish-farming and had a water transparency of 30 cm, an annual TP concentration about 0.06 mg L^{-1} and TN concentration about 1.7 mg L^{-1}. The Qin-E has a mean depth of 1.5 m and a surface area of 8 ha (Table 1). Prior to ecological restoration, the area was also used for fish-farming and had a water transparency of only about 20 cm (Secchi depth), annual TP concentration about 0.06 mg L^{-1} and TN concentration about 1.3 mg L^{-1}.

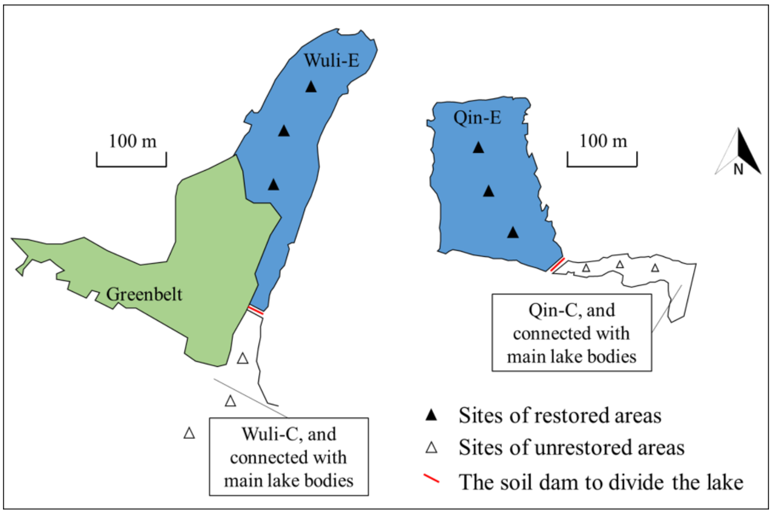

Figure 1. Location of the studied lakes restored (Wuli-E and Qin-E) and the unrestored lake area (Wuli-C and Qin-C) in Lake Wuli and Lake Qinhu with sample sites in this study.

Table 1. Summary of study area information, including the overlying water parameters acquired before restoration and sediment parameters from the restored area. Standard deviations are given. SD refers to Secchi depth, TP refers to concentrations of total phosphorus, TN refers to total nitrogen and TOC refers to total organic carbon. These indicators were monitored one month before the restoration starting.

Study Area	Main Area	Restored Area	Depth of Restored Area (m)	TN (mg L^{-1})	TP (mg L^{-1})	SD (cm)	Sediment Parameters (mg/g)		
							TN	TP	TOC
Lake Wuli	Wuli-C 860 ha	Wuli-E 5 ha	2	1.7 ± 0.2	0.06 ± 0.01	30	1.64 ± 0.33	0.716 ± 0.30	14.7 ± 4.5
Lake Qinhu	Qin-C 130 ha	Qin-E 8 ha	1.5	1.3 ± 0.2	0.06 ± 0.01	20	1.55 ± 0.07	0.768 ± 0.22	13.3 ± 3.2

2.2. Restoration Measures

The restoration measures including lowering down the water level to 0.5–1 m (by pumping water to unrestored areas), dredging of sediment, removal of fish and finally, transplantation of submerged macrophytes were applied to each of the study areas (Wuli-E and Qin-E). Thereafter, water levels were manipulated to the pre-restoration level and keeping a level of 1.5–2 m through pumping water from the unrestored area after macrophyte transplantation.

For both Wuli-E and Qin-E, dredging of sediments was finished by excavator, fish removal was conducted using both electric fishing and gillnets fishing methods to remove most fish in the restored areas and submerged macrophytes were transplanted at a density of 10–30 plants m^{-2} (differed for different species with different size), but similar biomass density (1.7 kg WW m^{-2} in Wuli-E and 1.6 kg WW m^{-2} in Qin-E). Due to different species transplanted and different growth situations, the initial coverage was 40% in Wuli-E and 30% in Qin-E.

In Wuli-E, preliminary works were finished in Jun 2010; submerged macrophytes were planted in July 2010, being comprised of *Vallisneria spinulosa*, *Elodea nuttallii*, *Potamogeton maackianus*, *Hydrilla verticillata*, *M. spicatum*, *Ceratophyllum demersum*, *P. malaianus* and *P. petinatus* and *Najas marina*. Among all the species, 35% of the total biomass was *V. spinulosa*, 20% was *E. nuttallii*, 20% was *P. maackianus*, 5% was *H. verticillata* in Wuli-E. The following year, we negotiated with proprietor and started to restore Qin-E. In Qin-E, the dam was constructed in May 2011 and lowered down the water level in July and submerged macrophytes were planted in August 2011, being comprised of *V. spinulosa*, *H. verticillata*, *M. spicatum* and *C. demersum*, among which *C. demersum* accounted for 40% of the total biomass, followed by 30% *V. spinulosa*. Floating-leaved macrophytes *Nymphoides peltatum* and *Trapa quadrispinosa* emerged spontaneously in both Wuli-E and Qin-E. Hence, there were 11 species (9 submerged and 2 floating-leaved) macrophytes in Wuli-E and 6 species (4 submerged and 2 floating-leaved) macrophytes in Qin-E. It established a diverse macrophyte community and ran the experiments for almost two years in Wuli-E, but one year in Qin-E.

2.3. Sampling and Laboratory Analyses

Before restoration, no submerged macrophytes were found in both restored areas (Wuli-E and Qin-E) and unrestored areas (Wuli-C and Qin-C). After transplantation of submerged macrophytes to the restored lakes, plants were investigated monthly at random using a grab sampler made of two pieces of heavy steel and a net covering a sampling area of 0.25 m^2. The heavy sampler sinks up into the sediment and collects uprooted plants in the net. Three sites in each restored area were set as a transect. Submerged macrophytes were sampled along the transect, 2–3 grabs were conducted at each sampling site to obtain adequate macrophyte species. Macrophyte biomass density was expressed in fresh weight per square meter with belowground biomass included. Percentage coverage was estimated by observing macrophyte canopies under the water surface for the whole restored area. The macrophytes were categorized according to morphology and spatial position as meadow-forming (including *V. spinulosa* and *P. maackianus*, growing at the bottom of the water column and of limited height) and canopy-forming (including *M. spicatum*, *H. verticillata*, *C. demersum*, *E. nuttallii*, *P. malaianus*, *N. marina* and *P. petinatus*, all of which develop long shoots and form a distinct canopy near the water surface) [18,23]. Floating-leaved macrophytes emerged spontaneously also included. Sampling was conducted from August 2010 to May 2012 for almost two years in Wuli-E. Qin-E started following year after Wuli-E from September 2011 to December 2012 for almost one year. The discrepancy in time is due to the divarication with the proprietors. Unfortunately, the sampling of submerged macrophyte had to be discontinued because the proprietors reclaimed the study areas for recreational use.

Although heavy fish removal was implemented, the fish community recovered quickly in both Qin-E and Wuli-E after restoration [24]. In Wuli-E, seven fish species were caught by an 80 × 1.5 m gill net with multiple mesh sizes: 10, 15, 25 and 40 mm one year later after restoration, sharpbelly, crucian carp and bitterling *Acheilognathus macropterus* dominated the fish community. The CPUE of fish in Wuli-E was 35 ind. net^{-1} h^{-1} in numbers and 0.9 kg net^{-1} h^{-1} in biomass. In Qin-E, a total of six fish species were caught following the same method, and silver carp *Hypophthalmichthys molitrix*, bighead carp *Hypophthalmichthys nobilis*, sharpbelly *Hemicculter leuciclus* dominated the fish assemblages. The catch per unit effort (CPUE) of fish in Qin-E reached 120 ind. net^{-1} h^{-1} in numbers and 1.75 kg net^{-1} h^{-1} in biomass.

The monitoring of physicochemical parameters started prior to restoration and continued at least once a month at three sampling sites in each restored area (Wuli-E and Qin-E) and unrestored area (Wuli-C and Qin-C). These parameters prior to restoration started to be monitored in January to April 2011 in Wuli-E and in November 2010 in Qin-E. The monitoring of physicochemical parameters for both lakes lasted until March of 2012. Water depth (WD) was ascertained by a depth-sounder and transparency was recorded as Secchi depth (SD) and SD/WD ratios (Secchi depth-to-water depth ratio) were calculated to evaluate transparency at different water depths. Water samples were collected with a column sampler and used to measure total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS) and chlorophyll *a* (Chl *a*) concentrations. TN was determined using an alkaline potassium persulfate digestion-UV spectrophotometric method, and TP was determined spectrophotometrically according to the molybdenum blue method after digestion with $K_2S_2O_8$ solution. TSS was measured in 100–200-mL water samples filtered through pre-combusted (450 °C for 2 h) and pre-weighed GF/C filters that were subsequently oven-dried to constant weight at 60 °C for 48 h [25]. Phytoplankton was retained on the GF/C filter and extracted in a 90% (*v/v*) acetone/water solution for 24 h. The extracts were subject to spectrophotometric measurement of Chl *a* concentrations, and the results were corrected for pheophytin interference [26].

2.4. Data Analyses

Macrophyte diversity was analyzed by using the Shannon–Wiener index

$$H' = -\sum_{i=1}^{s} \frac{b_i}{b} ln \frac{b_i}{b} \quad (1)$$

where b_i is the biomass density of the i th species, b is the total biomass of all species and s is the total number of macrophyte species. Biomass was used instead of macrophyte number to calculate the index following Jeppesen et al. [15], because the branching and clonal nature of the plants made it difficult to count individuals. We calculated SD/WD (Secchi depth/water depth) to evaluate transparency at different water depths.

Differences in water quality parameters in restoration areas between the two restored areas (Wuli-E and Qin-E) were determined by repeated-measure ANOVA. Correspondingly, the differences between Wuli-C and Qin-C were detected on simultaneous data. The time-series data after restoration (from August 2011 to February 2012, the interval is two months) which covered the entire monitoring period of Wuli-E and the most monitoring period of Qin-E after transplantation was selected to implement the repeated-measure ANOVA. We focused on the differences between the two restored areas of Wuli-E and Qin-E and the difference between the unrestored areas Wuli-C and Qin-C. The previous studies showed significantly lower concentrations of physicochemical parameters in the restored lakes than the unrestored lake areas of Lake Wuli and Lake Qinhu, respectively [27]. The differences between the restored and unrestored areas of each lake (Wuli-E vs Wuli-C; Qin-E vs Qin-C) would not be discussed in our study.

The post hoc pairwise comparisons between the two lakes were performed by estimating marginal means adjusted by Bonferroni's method. These comparisons were performed with the statistical package SPSS version 22.0 (IBM Corporation, Somers, NY, USA).

A hierarchical mixed model estimated by path analysis revealed a quantitative relationship among biomass, biodiversity and water quality [28] and was thus used to isolate the main determinant of water quality parameters and identify direct or indirect effects of drivers based on the log-transformed data. In the path analysis, R-square was given for endogenous variables that indicated explained variance. The path analysis and plotting were done in R software [29] mainly using the packages *sem* [30], *ggplot2* [31] and *vegan* [32].

3. Results

3.1. Changes in the Transplanted Macrophyte Communities

Our results showed that the biomass density, diversity index (Shannon–Wiener index) and coverage of the macrophyte community decreased in both restoration areas from summer to winter and then increased again in the following year. In Wuli-E, recovering biomass density and diversity both almost recovered to their initial levels in the next year, but overall coverage decreased from 75% to approximately 65%, fluctuating around this value, except for a second decrease from the last September to the following March (Figure 2a). At the end of the study, coverage exceeded the original transplanted area (75%) in Qin-E, whereas biomass density reduced to 50% of the initial transplanted levels, diversity index decreased a little (Figure 2b). As a whole, the coverage and species diversity of submerged macrophytes in the two lakes seemed to be stable, while the biomass density changed dramatically from the second year.

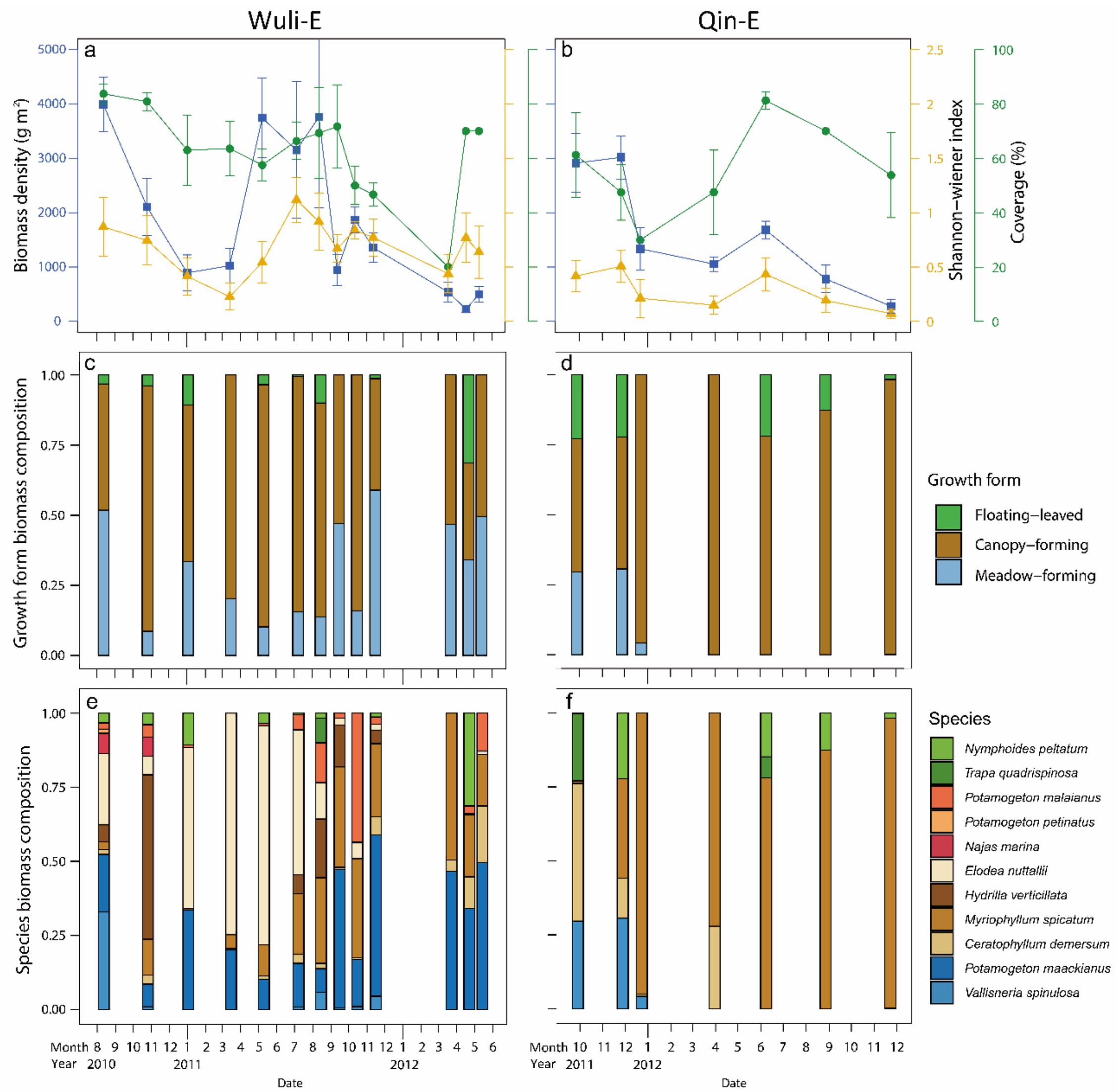

Figure 2. Dynamics of macrophyte biomass density (blue solid squares corresponding to blue vertical axis in (**a**) and (**b**)), Shannon–Wiener diversity index (orange solid triangles corresponding to orange vertical axis in (**a**) and (**b**)), coverage (green solid cycles corresponding to green vertical axis in (**a**) and (**b**)) and growth form biomass composition (**c**) and (**d**) and species composition (**e**) and (**f**) of the restored areas in Lake Wuli (Wuli-E, left panel) and Lake Qinhu (Qin-E, right panel) after macrophyte transplantation (mean values represented the average data for 3 sampling sites in restored lake area in each sampling time). Error bars represent the SE (standard error) of different sampling sites in each sampling time.

In Wuli-E, meadow-forming species occupied a larger percentage at the first sampling event and maintained a presence to the end of the study (Figure 2c). In Qin-E, with the increase of canopy-forming macrophytes and the decline of meadow-forming species, almost all the macrophytes found in Qin-E the year after restoration were canopy-formers, the exception being a few floating-leaved macrophytes that appeared in summer (Figure 2d). The percentage of meadow-forming species declined to a minimum in May of the year and increased in the second year. Although the occurrence of floating-leaved macrophytes was frequent, their proportion of total biomass was low. Notably, due to the dominance of canopy-forming macrophytes, the decline of community diversity was driven by the decline in meadow-forming species.

After transplantation of submerged macrophytes, Wuli-E was dominated variably by the canopy-forming and meadow-forming species in different seasons, while Qin-E was dominated by the canopy-forming species. The dominant species is *M. spicatum* in Qin-E. This dominance of *M. spicatum* was weaker in Wuli-E than in Qin-E, but the presence of other canopy-forming species such as pondweeds *P. malaianus* and *E. nuttallii* which accounted for more than 40% of total biomass during summer supplemented the effect (Figure 2f). The main meadow-forming species in Wuli-E were *V. spinulosa* and *P. maackianus*, but only the former was present in Qin-E. Floating-leaved macrophytes such as *N. peltatum* and *T. potanini* were not part of the initial introduction, but grown from the sediment reservoir naturally.

3.2. Water Quality after Macrophyte Transplantation

Our results suggested that the presence of macrophytes improved water quality in both Wuli-E and Qin-E (Figure 3). Repeated-measure of ANOVA results showed that SD/WD and concentrations of TN, TP and Chl *a* varied significantly with time, not for TSS (Table 2). Significant interaction effects of month and restored lakes on TN were only found between the Wuli-E and Qin-E. The differences in SD/WD and the concentrations of TN, TP and TSS between Wuli-E and Qin-E, were significantly different (Tables 2 and 3). However, the difference in unrestored lake areas between the two lakes was not significant. The SD/WD ratios in Wuli-E were significantly higher, while TN, TP and TSS levels were significantly lower than those in Qin-E (Table 3).

Table 2. Summary of repeated-measure ANOVA results on effects of different month and lake areas (restored and unrestored areas) on the concentrations from three sites in each water quality monitoring of SD/WD (Secchi depth/water depth ratio), total phosphorus (TP), total nitrogen (TN−), total suspended solids (TSS) and chlorophyll *a* (Chl *a*) in restored lake areas (Wuli-E and Qin-E) and the unrestored areas (Wuli-C and Qin-C) of Lake Wuli and Lake Qinhu.

Source of Variation	TN		TP		TSS		Chl *a*		SD/WD	
	F	p	*F*	p	*F*	p	*F*	p	*F*	p
Between subjects										
Restored area	14.94	**0.018**	9.90	**0.035**	21.99	**0.009**	0.34	0.591	17.719	**0.014**
Unrestored area	6.52	0.063	2.64	0.179	0.44	0.542	6.50	0.063	2.08	0.190
Within subjects										
Month	34.51	**0.001**	9.54	**0.012**	1.80	0.240	14.40	**0.012**	7.02	0.012
Month × Restored area	9.06	**0.019**	0.95	0.417	0.93	0.414	0.73	0.461	1.39	0.302
Month × Unrestored area	9.36	**0.009**	11.13	**0.016**	0.74	0.477	7.23	**0.043**	0.41	0.555

Note: values indicate probability levels; bold values are below significance level (0.05).

Table 3. Pairwise comparisons of the concentrations of total phosphorus (TP), total nitrogen (TN), total suspended solids (TSS), chlorophyll *a* (Chl *a*) and SD/WD (Secchi depth: water depth ratio) in restored lake areas (Wuli-E and Qin-E) and the unrestored areas (Wuli-C and Qin-C) after restoration through estimating marginal means (adjusted by Bonferroni's method).

Differences	TN	TP	TSS	Chl *a*	SD/WD
Restored Wuli-E—Qin-E	−0.091 *	−0.016 *	−2.705 **	−1.316	−0.126 *
Unrestored Wuli-C—Qin-C	0.18	−0.011	0.493	5.167	0.013

** $p < 0.01$, * $p < 0.05$.

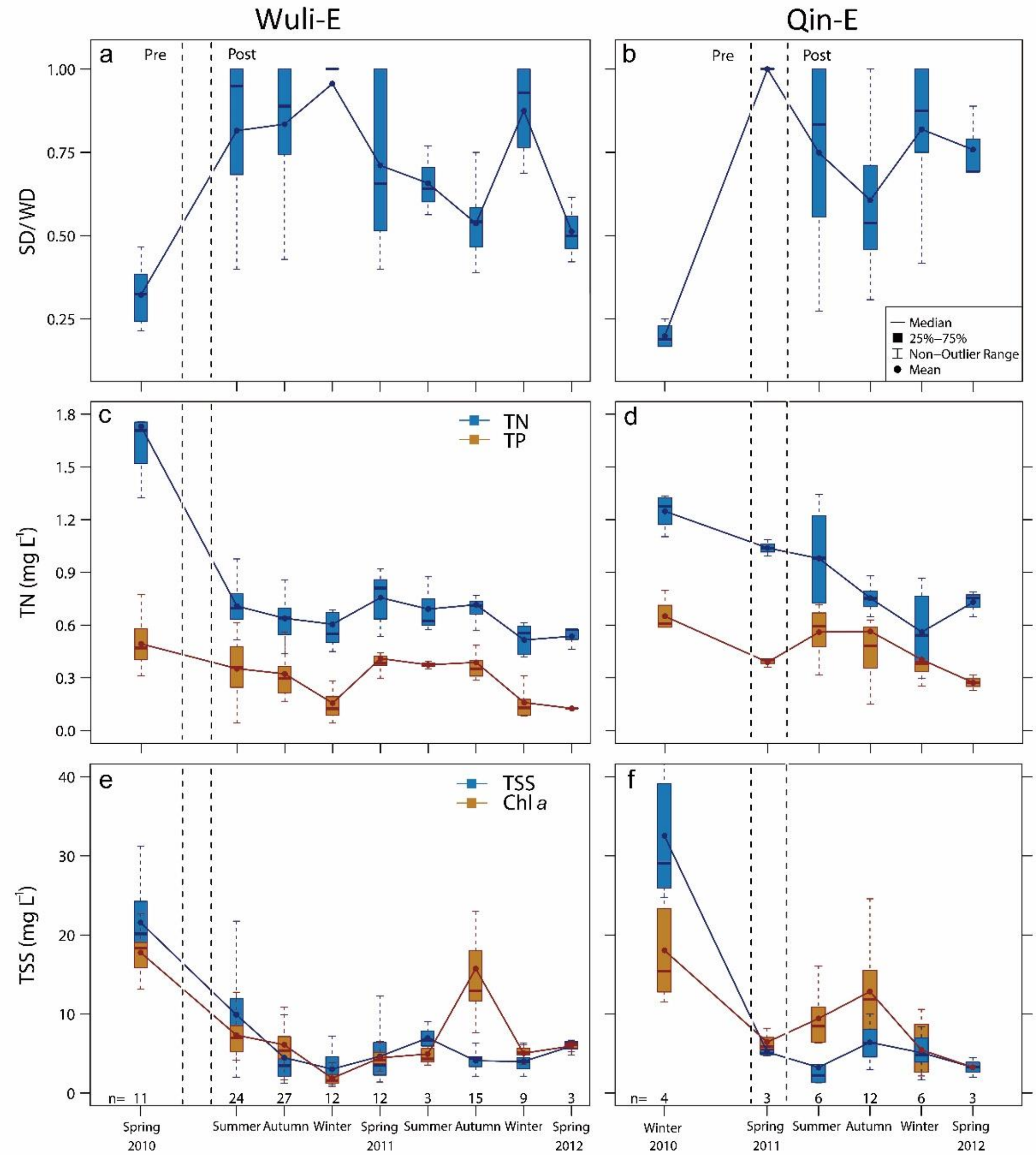

Figure 3. Seasonal variation in SD/WD (Secchi depth/water depth ratio) (**a**) and (**b**) and concentrations of total phosphorus (TP), total nitrogen (TN) (**c**) and (**d**), total suspended solids (TSS) and chlorophyll *a* (Chl *a*) (**e**) and (**f**) in the restored areas of Lake Wuli (Wuli-E, left panel) and Lake Qinhu (Qin-E, right panel). 'Pre' refers to the period before restoration and 'Post' refers to the period of post-restoration. The date of execution of the restoration was shown between the two dashed lines. Spring was considered to be March, April and May. All monitoring data collected from three sites of every lake in each season were used in the boxplot and the numbers of samples (n) were shown above the x-axis.

3.3. Path Analysis between Macrophytes Community and Water Quality Parameters

Taken together, the data from the two study areas demonstrated that biomass density, diversity index depend and coverage of macrophyte showed significant direct and indirect effects on water quality (chi-squared = 27.97, df = 14, p = 0.014). Macrophyte biomass density was positively correlated with TN, TP and TSS (Figure 4). However, diversity index of macrophytes was negatively correlated with TN, TP and SD/WD, while positively correlated with Chl *a*. Moreover, negative relations were found between macrophyte coverage and TSS and Chl *a*, while positive relationships were detected between coverage and SD/WD. As expected, TP was the strongest predictor of Chl *a*, which markedly reduced SD/WD. TN also appeared to have a positive influence on Chl *a*. The R-square values indicate that macrophyte diversity index, biomass density and coverage explain about 60% of the variance in TP, TN and TSS and 87% and 90% of the variance in Chl *a* and SD/WD. Apparently, macrophyte diversity and coverage had a more important positive effect on enhancing the water quality than the biomass density in these two restored lakes.

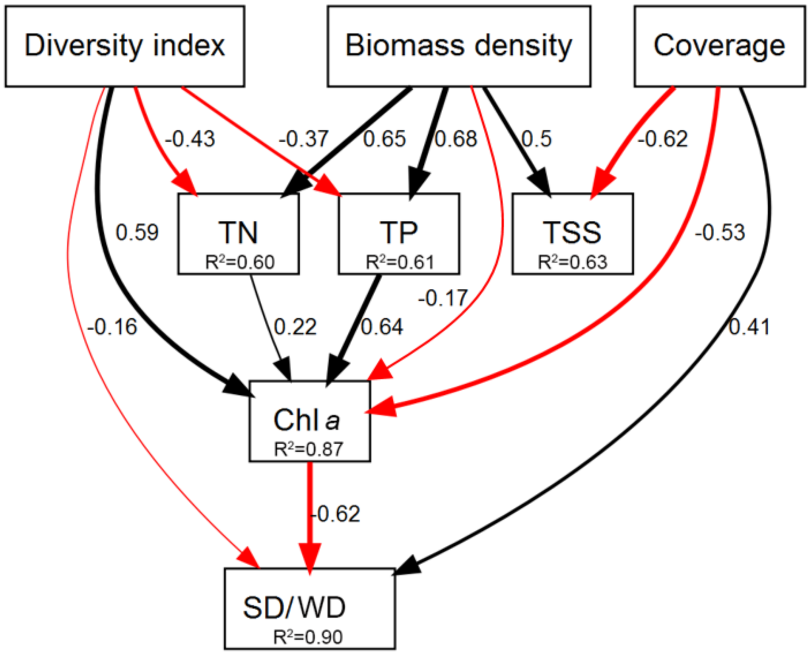

Figure 4. Path diagram between the macrophyte community metrics and the water quality parameters in restored areas (Qin-E and Wuli-E) of Lake Qinhu and Lake Wuli. Factors influencing water quality parameters mainly included SD/WD (Secchi depth/water depth ratio) and concentrations of total phosphorus (TP), total nitrogen (TN), total suspended solids (TSS) and chlorophyll *a* (Chl *a*), averaged for three sites to correspond with mean macrophyte data. All data were log-transformed. Path thickness was proportional to the standardized regression coefficient. Black and red paths represented positive and negative effects, respectively. Paths $p > 0.05$ were not shown, except for two paths from TN to Chl *a* ($p = 0.07$) and the diversity index to SD/WD ($p = 0.08$).

4. Discussion

Our results suggested the restoration by transplantation of the macrophyte communities in two subtropical Chinese lakes, the restored areas of Lake Qinhu (Qin-E) and Lake Wuli (Wuli-E) was successful in improving water transparency and controlling concentrations of TP, TN, Chl*a* and TSS over two years. The transplantation of submerged macrophytes is a popular measure in Chinese lake restoration, combined with other biomanipulation such as dredging and fish removal and has been used in many temperate eutrophic lakes [7]. In warmer shallow lakes in China, the re-establishment of macrophytes may also contribute to improving water state by reducing eutrophication [11,33,34]. However, after the transplantation of submerged macrophytes for a period of time (even only 1 to 3 years), the water quality may deteriorate again [35]. During our investigation period, the submerged macrophyte community stabilized the water quality of restored lake areas.

After transplantation, macrophytes coverages showed seasonal fluctuation and could return to the initial level in the same months with last year in both lakes. Though the diversity index decreased slightly in a fluctuation way in both lakes, remained always higher in Wuli-E than in Qin-E. Biomass densities declined in both two restored lake areas, with more fluctuated biomass density in Wuli-E. Moreover, a loss of meadow-forming macrophytes and the gradual dominance by the canopy-forming *M. spicatum* were found in Qin-E, while the meadow-forming macrophytes persisted in Wuli-E. The submerged macrophyte community stabilized the water quality of restored lake areas after implementing previous restoration measures.

In aquatic ecosystems, biomass, diversity and coverage of macrophytes have been previously shown to affect water quality [7,10,36], it is not clear which of these exerts the most potent effect. This lack of knowledge hampers the effectiveness of restoration by macrophyte transplantation. In our study, results of path diagram showed that the 3 metrics of macrophyte community had different effects on different water parameters. Biomass density of macrophytes had negative effects on water quality due to increase TP, TN and TSS, but could improve transparency (SD/WD) due to inhibiting phytoplankton (Chl *a*). By contrast, macrophyte diversity appeared more beneficial to improvements in water quality due to the decrease in TN and TP but could not inhibit phytoplankton effectively to improve transparency. While coverage inhibited TSS and Chl *a*, with consequent improvements in transparency. Totally, it seemed that diversity and coverage of submerged macrophytes were better drivers of good water quality than biomass. However, this inference was the result of data from just two small restored lake areas. Further case studies are required to reach general conclusions on the influence of submerged macrophyte biomass density, diversity and coverage on lake water quality.

Bottom-up stabilizing mechanisms of macrophytes on water transparency such as increased sedimentation and reduced sediment resuspension depend largely on macrophytes coverage [36]. In Qin-E, macrophyte coverage remained at a high level, which may be the reason for keeping a clear-water state, but was not as good as Wuli-E. The higher diversity of macrophytes created a greater chance that species with specific functions could occur and react, which is important in enhancing ecosystem functioning [37]. Spatial and temporal niche complementarity was reported to help maintain a stable clear-water state [7]. Compared with significant coverage and diversity of submerged macrophytes, high biomass may be a nuisance in warm lakes and generate adverse effects such as oxygen consumption in decomposition, entertainment function reduction [38]. Moreover, the high biomass density is closely related to canopy-forming macrophytes or tropic species such as *M. spicatum*.

Canopy-forming macrophytes can improve transparency rapidly due to their rapid growth, but the concentration of biomass in the upper part of the water column has no good to control the release of nutrients and resuspension from sediment [39]. Though meadow-forming macrophytes grow slowly, but their biomass is mainly close to the sediments which can reduce releasing nutrients and resuspension from sediment [40] and accelerate the nutrients sinks [41], thereby maintaining good water quality for a longer time [18]. In our study, more meadow-forming, but less canopy-forming species were transplanted into Wuli-E than Qin-E. Obviously, the long-term presence of meadow-forming species in Wuli-E contributed to better water quality. However, the canopy-forming species would exert an inhibitory effect on the meadow-forming ones through light competition and potentially result in far less improvement of water quality. In both restored lakes, *M. spicatum* was initially transplanted in alike tiny proportions, while the proportion of *V. spinulosa* was similarly high. However, *M. spicatum* subsequently became the dominant species quickly in Qin-E, but not increased much in Wuli-E. Meanwhile, the advantage of *V. spinulosa* declined over time in both lakes and lost more quickly in Qin-E. It resulted to develop rapidly into a canopy-forming community in Qin-E. Fortunately, there was another meadow-forming species, *P. malaianus*, which dominated the community in Wuli-E replacing*V. spinulosa* to maintain a meadow-forming community at least in summer. The above results indicated that the high species richness was beneficial to inhibiting the development of canopy-forming *M. spicatum* in Wuli-E. However, *V. spinulosa* was easy to disappear because of its lowest height and be shaded to grow. We conclude that growth form, species-specific and species richness should be a serious consideration in the selection of submerged macrophytes for lake restoration.

Our study focuses on the effects of different submerged macrophyte community characteristics on concentrations of nutrient, TSS and Chl *a*, however, other factors may also impact the water quality. For instance, in Qin-E the number and biomass of grass carp (*Ctenopharyngodon idella*), a grazing species who prefers *V. spinulosa* and *C. demersum*, both increased markedly after restoration which may shift to

M. spicatum dominance [42]. In Wuli-E, the fish community was dominated by small omnivores [23]. While pressure on macrophytes from adult fish may prevent the establishment of a stable clear-water state [23], however, it does not appear to affect the composition of the macrophyte community in Wuli-E (Mantel test based on Bray-Curtis distance, $p > 0.05$). Thus, differences in the fish community may partly explain the different results in the two restored areas. However, when the relationships between fish biomass or number and water quality were analyzed, we found no significant impact on either of the two lakes (Mantel test, $p > 0.05$), suggesting that direct effects of fish on water quality were weak in our study. Though the effect of fish appears to be relatively weak and stable in our study area, future plans for strong bottom-up control via submerged macrophytes transplantation should monitor and/or control the fish density [11].

Overall, macrophyte re-establishment was successful in improving water quality in two subtropical Chinese lakes over initial diversity, coverage and biomass density. It seemed diversity and coverage helped much more than biomass density. That may be due to high macrophyte diversity and coverage implied greater opportunities for meadow-forming macrophytes to boost ecosystem functioning and that these were more important than the increased biomass of dense canopy-forming macrophytes. Establishing a less diverse community at the beginning when transplanting potentially allows for aggressive macrophytes such as *M. spicatum* to dominate. Our work confirms macrophyte transplantation as an effective bio-manipulation method in the restoration of eutrophic lakes, but suggests that growth form, diversity and coverage are important considerations in the selection of species, particularly in tropic and subtropical lakes where excessive macrophyte biomass may be problematic.

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