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Applying trait-based method to investigate the relationship between macrophyte communities and environmental conditions in a eutrophic freshwater lake, China

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A B S T R A C T

Trait-based community assembly analysis, which links the plant traits to the variation of community composition along environmental gradients, has potential to improve the understanding of the impacts of eutrophication on macrophytes and responding process. In this article, we pioneered the use of RLQ and Fourth-Corner methods that establish in a quantitative way the link between macrophyte traits, macrophyte community and water quality in a eutrophic shallow lake, eastern China. A total of 9 environmental variables and 11 macrophyte traits were included in the analysis. We found that total phosphorus in water (25.7%, the contribution to total inertia in RLQ analysis), suspended solids (20.7%), water transparency (17.1%) and Chlorophyll a (14.0%) were the main environmental variables that were associated the macrophyte traits matrix. Among macrophyte traits, life form (15.7%), responded to the environmental conditions the most, followed by leaf nitrogen (13.8%) and specific leaf area (13.6%). The correlation between macrophyte traits and environmental gradients showed that macrophytes with higher leaf nitrogen and higher specific leaf area grew at locations with clear water condition, better water transparency and with less total phosphorus, Chlorophyll a and suspended solids in this shallow eutrophic lake. The results suggested that the leaf nitrogen and specific leaf area of macrophytes had a trend to decrease with the increasing environmental stresses caused by eutrophication. Moreover, we highlighted that total phosphorus in water should be prior considered for the future macrophyte conservation and restoration in this lake.

1. Introduction

A major goal for community ecology is to understand the factors and processes that determine the species composition for any region on earth. Assembly and response rules provide a good perspective for understanding community assembly and allow further prediction of community dynamics under environment changes (Keddy, 1992; Fu et al., 2014). Based on the ubiquitous links between plant traits and environmental factors that have been observed at several spatio-temporal and organizational scales (Violle et al., 2007), from the individual to community level for terrestrial plants (Messier et al., 2010). Recently, an increasing interest has focused on the trait-based approaches to identify the interactions between species fundamental niches and functional traits under the context of abiotic environmental gradients (McGill et al., 2006), for terrestrial vegetation including forests, prairies and agricultural lands (Messier et al., 2010; Garnier and Navas, 2012; Dray et al., 2014). So far these trait-based studies have used numerical and statistical methods, such as redundancy analysis (RDA), a combination of RDA and regression trees, Cluster Regression and RLQ analysis (see details in Kleyer et al., 2012) and generalized linear mixed model (Jamil et al., 2013), to identify determinant traits of species presence-absence or abundance.

Macrophytes, as an important part of freshwater functioning (e.g. Jeppesen et al., 1998), are sensitive to environment variations in freshwater. It was shown that macrophytes change gradually in different zonation of aquatic vegetation or different successional stages of hydrosere, i.e., rooted species occupy the edges of water body, submerged species occur in the littoral zone and floating species occupy the open water zone. Macrophyte community may decline when water quality problems occurred, and their species component (Søndergaard et al., 2010; Alahuhta, 2015), functional group composition (Schneider 2007) and community characteristics (e.g. cover) (Søndergaard et al.,

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2010) would change in the different ecological status of the water body. For example, bottom-dwelling macrophytes are characteristic of infertile sediments and clear waters, whereas erect and rosette macrophytes are at an advantage in regions with intermediate fertility and irradiance (Cao et al., 2011). The trait-based community assembly rules under macrophyte community-environment relationships were most explained at the level of functional groups by description or semi-quantitative methods. Nevertheless, studies that link specific functional traits to the macrophytes abundance variation along environmental gradients (but see Fu et al., 2014) and quantify their correlations are still lacking.

Previous studies have shown, to some extent, the general patterns that macrophyte traits variation perform along aquatic environmental gradients (Bornette and Puijalon, 2011; Fu et al., 2014). For example, from shallower waters to deeper waters, the dominant life form changes from emergent macrophytes to submerged macrophytes (Keddy 2010). Under low light condition, submerged macrophytes tend to have bigger leaf area, longer petiole length and elongated shoot height (Strand and Weisner, 2001; Richards et al., 2011), and specific leaf area tend to increase in order to improve light absorption efficiency (Enriquez and Sand-Jensen, 2003). Other cases can also be found, such as the leaf size may reduce when constantly exposed to waves and currents (Bornette and Puijalon, 2011). Along a gradient of increasing fertility in sediment and decreasing light availability, a decreased root/shoot ratio and divided/feather-like leaves were observed in submerged macrophytes (Barko et al., 1991; Art 2002). Knowledge on the response of macrophytes traits to its living conditions have therefore provided a solid foundation for linking macrophyte traits to the relationship between macrophyte community composition and aquatic environmental conditions.

From 1978–1987, the proportion of eutrophic lake areas in China increased dramatically from 5.0% to 55.0% (Jin, 1995). By 2006, more than 80% of the total lake areas among the 76 main lakes across the country suffered from grade IV level (unhealthy for human contact) or lower water quality (Li, 2006). Studies of lake water quality along the middle to lower reaches of the Yangtze River showed a degraded gradient of turbidity, chlorophyll a and dissolved oxygen (Xu et al., 2005; Wu et al., 2012). Since the early 1990s, a noticeable changing dynamics of macrophyte communities has occurred in one of these lakes-Dianshan Lake (You, 1994; Shi et al., 2011). Pollutants from point and non-point sources caused a heterogeneous environment of water transparency, chlorophyll a, total nitrogen, total phosphorus and dissolved oxygen (Kung and Ying, 1991; Cheng and Li, 2008). Applying trait-based method in this eutrophic lake will help to improve our understanding of the macrophyte community assembly mechanism by determining the trait-sensitive limiting factors, which could be a guidance for the macrophytes restoration in degraded freshwater lakes caused by intense nutrient input.

In this study, our goal is to unravel the driving factors of macrophyte community assembly in a shallow eutrophic lake and identify the key macrophyte traits that respond to the heterogeneous environment. We surveyed macrophyte community data and environmental variables across Dianshan Lake and collected macrophyte traits based on filed measurements and publication review. With the help of RLQ and Fourth-Corner statistic method, the strength of interactions between environment and traits in the underlying macrophytes distribution in this eutrophic shallow lake were assessed to identify the key traits through which the eutrophic water, as environmental filtering, determined the species composition of macrophytes communities. Despite the variances within each species, the functional traits at inter-species level and the abundance of macrophytes in connection with their environmental variables were studied.

2. Methods

2.1. Study area

Dianshan Lake (31°07′N, 120°58′E) is the largest freshwater body in the Shanghai area, bounded by Jiangsu and Zhejiang provinces. The lake is approximately 2 m deep and covers an area of 62.5 square kilometers with a volume of about 80 million cubic meters. The average flow velocity is only 0.03 m/s. The inflows originate from Lake Tai with two main inlets, Jishui Port and Dazhushe. The main outlet Lanlu Port is connected to the Huangpu River. Dianshan Lake is one of the most important drinking water sources for the Shanghai metropolitan area (Kung and Ying, 1991; Cheng and Li, 2010).

2.2. Macrophyte abundance

Macrophytes communities were surveyed during the growing season in 2009 by boat along 22 east-west oriented parallel transects. The distance between each transect was about 600 m. On each transect, the sampling sites were set up with an interval of around 700 m. Three to five 1 m*1 m plots were set up within a radius of 12.5 m (approximately 500 m^2) at each sampling site for the submerged, emergent and floating–leaved macrophytes communities respectively. Moreover, additional sample sites at the border of the lake where macrophytes occurred were surveyed (As shown in Fig. 1)

Relative abundances of macrophyte were assessed using the Braun-Blanquet cover abundance scale. Emergent and floating–leaved macrophyte communities were investigated based on visual inspection, and submerged macrophyte communities were collected randomly 3–5 times using a steel grapple (0.4 m*0.5 m).

A total of 25 species, including 5 emergent, 7 free-floating, 3 floating-leaved and 10 submerged macrophytes, were recorded in 316 plots. Plots with only free-floating macrophytes were removed from further analysis, considering that their occurrences were highly driven by wind or water currency but not by local water quality. Moreover, rare species were also excluded. Rarity was determined by using 2 criteria: 1) species with maximum relative abundance less than 5% in each plot; 2) species with frequency less than 5% for overall plots. Finally, 241 plots with 11 species (3 emergent, 1 floating-leaved and 7

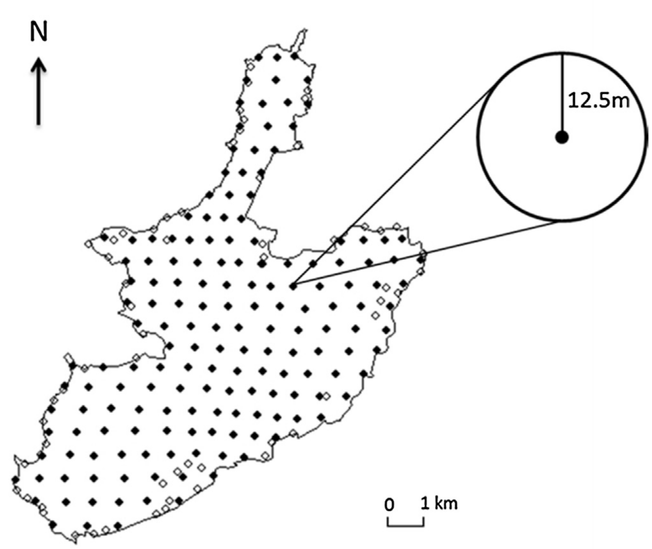

Fig. 1. The lay out of the sampling plots across Dianshan Lake. Black dots in the map for regular sampling sites and open circles for additional sampling sites.

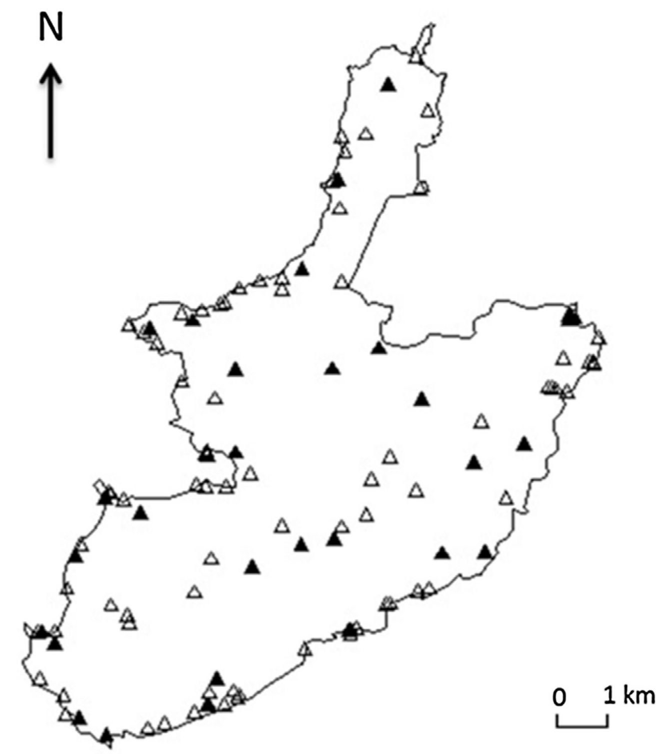

Fig. 2. The layout of sampling sites for environmental variables. The red dots solid triangles for sites that 9 environmental variables were measured and open triangles for sites that only water depth and transparency were measured.

submerged macrophytes) were selected for further analysis.

2.3. Environmental variables

We measured 9 environmental variables 3 times during the growing season from May to September in 2009. Water depth (m) and transparency (m) were measured in 113 monitoring sites. Chlorophyll a (Chla, μg/L), suspended solids (mg/L), water pH, dissolved oxygen (DO, mg/L), total water nitrogen (TN, mg/L), ammonium nitrogen in water (NH_4^+-N, mg/L) and total water phosphorus (TP, mg/L) were measured at 30 sites (as shown in Fig. 2). We determined water depth with a calibrated stick and transparency with Secchi Disc. Water pH was measured in situ with pHep®5 pH/Temperature Tester, HI 98128 (HANNA Instruments USA). DO was determined by Iodometric method. TP was digested with alkaline $K_2S_2O_8$ and then determined by the ammonium molybdate spectrophotometric method. TN was digested with alkaline $K_2S_2O_8$ and then determined by the ultraviolet spectrophotometric method. We determined NH_4^+-N, Chla and suspended solids following environmental quality standards for surface water of the People's Republic of China (GB 3838-2002).

Inverse distance weighted (IDW) method was applied to interpolate the value of environmental variables for the all 241 plots based on measured environment data with its coordination (gstat package, R software). See the interpolation maps of the 9 environmental variables in Appendix.

2.4. Source and selection of plant traits

11 Functional traits were included in the analysis: life form, height (cm), stem diameter (mm), leaf length (mm), leaf width (mm), fruit length (mm), specific leaf area (SLA, cm^2/g), leaf dry matter content (LDMC, mg/g), leaf nitrogen/phosphorus content per dry mass (mg/g) and leaf thickness (mm).

5 Most dominant submerged macrophytes (*Potamogeton malaianus*,

Table 1
The traits of macrophytes used in RLQ analysis and Fourth-corner method.

Species	Life form	Height (cm)	Stem diameter (mm)	Leaf length (cm)	Leaf width (cm)	Fruit length (mm)	Leaf nitrogen (mg/g)	Leaf phosphorus (mg/g)	Leaf thickness (mm)	Specific leaf area (m^2/kg)	leaf dry matter content (g/kg)
Phragmites australis	E	300	40.0	30.0	2.0	1.5	6.7[1]	1.6[1]	3.6[2]	13.1[3]	436.6[4]
Typha orientalis	E	200	*11.6*	70.0	0.9	1.0	15.9[1]	4.1[1]	9.8[2]	11.5[2]	182.0[2]
Zizania latifolia	E	200	10.0	90.0	30.0	12.0	14.6[1]	2.8[1]	*2.5*	*54.4*	*217.6*
Trapa incisa	FL	148	2.5	5.0	7.0	20.0	26.3[5]	1.7[5]	0.4[6*]	30.8[6*]	52.9[6*]
Potamogeton malaianus	S	151[6]	2.0[6]	19.0	2.5	3.0	**29.5**	**4.8**	0.1[6]	89.4[6]	**227.2**
Myriophyllum spicatum	S	250	2.0[6]	3.5	2.5	3.0[7]	**24.6**	**1.4**	0.3[6]	109.6[6]	**115.8**
Ceratophyllum demersum	S	150	1.1[6]	2.0	0.1	5.0	**33.3**	**6.0**	0.5[6]	83.9[6]	**81.7**
Cabomba caroliniana	S	200	*10.1*	2.0	0.3	7.0	37.5[10]	5.4[10]	*2.0*	*61.3*	*188.0*
Potamogeton crispus	S	60	*2.8*	8.0	1.0	3.5	24.9[1]	2.9[1]	0.4[8]	52.5[4]	145.0[4]
Hydrilla verticillata	S	107	1.2[6]	1.7	0.2	25.0[9]	**29.5**	**4.2**	0.1[6]	119.0[6]	**120.5**
Vallisneria natans	S	92[6]	5.3[6]	200.0	2.0	300.0	**32.0**	**5.5**	0.6[6]	73.2[6]	**86.4**

Note: The values in bold were measured in this study. The values without superscript were cited from Flora of China (http://www.floraofchina.org/) and the others with superscripts were cited from different references (Details in reference list), in which three values assigned from the same genus is indicated by asterisk. The values in italic were interpolated by Bayesian Principal Component analysis method (BPCA). E: Emergent, FL: Floating-leaves, S: Submerged.

[1] Lu et al. (2011).
[2] Liu (2008).
[3] Zhong (2014).
[4] Knevel et al. (2003).
[5] Wang (2011).
[6] Fu et al., (2014).
[7] Editing Group of Flora of Zhejiang Province (1993).
[8] Chen et al. (2008).
[9] Ru (2013).
[10] Riemer and Toth (1968).

Myriophyllum spicatum, *Ceratophyllum demersum*, *Hydrilla verticillata* and *Vallisneria natans*) were measured for leaf dry matter content, leaf nitrogen and leaf phosphorus during the macrophytes community investigation (As shown in Table 1.). For each species, 3–6 robust and healthy individuals were sampled. The leaf fresh weight was determined in situ. Samples were stored in sealed plastic bags and taken back to laboratory in 24 h, then oven-dried at 70 °C to constant weight recorded as the leaf dry weight. The leaf dry matter content was calculated as the proportion of leaf fresh weight accounted for by leaf dry matter. Dried leaf samples were then ground and digested with H_2SO_4-$HClO_4$. Leaf nitrogen and leaf phosphorus were quantified using a flow-injection auto-analyzer (Skalar, NL).

The unmeasured traits were compiled from the literature review, and finally Bayesian Principal Component Analysis method (BPCA) was used to interpolate a few missing values (as detailed in Table 1). BPCA uses an expectation maximization approach combined with a Bayesian estimation method to calculate the likelihood of an estimated value (Stacklies et al., 2007).

2.5. Statistical analysis

To quantify the traits-environment-abundance relationships, RLQ analysis and the Fourth-Corner approach, which are considered as the most integrated methods to analyze the relationship between functional traits and environmental factors, were used (Kleyer et al., 2012). RLQ analysis was used to summarize the joint structure among environment, abundance and traits matrix with a multivariate method, and the Fourth-Corner method was used to determine the bivariate associations between traits and environmental variables with permutation procedures to evaluate the significance of these relationships (Dray et al., 2014).

For both methods, three data tables should be constructed: (1) species abundances (columns) in each plot (rows) formed table L; (2) the values of the environmental variables (columns) in each plot (rows) formed table R; (3) the traits (columns) of each species (rows) formed table Q.

For RLQ analysis, three tables described above were separately ordinated. Correspondence analysis (CA) was performed on data in table L. Principal Component analysis (PCA) was computed on table R. Because table Q was a mixture of both quantitative and categorical variables, a Hill and Smith ordination was performed (Hill and Smith, 1976). Then RLQ analysis was applied (Dolédec et al., 1996). Site scores from the CA of the table L were used as row weights in the PCA of the table R, and species scores from the CA of the table L were used as row weights in the Hill and Smith ordination of traits. In RLQ analysis, site scores and species scores of the table L functioned as links between the table R and the table Q. Therefore, RLQ analysis was an extension of co-inertia analysis, which simultaneously took into account the information contained in the tables R, L and Q (Wesuls et al., 2012). The contribution to total inertia were used, as a measure of relative importance of each environmental factor/trait in the RLQ analysis, to identify the most important determinant traits and environmental factors (Wesuls et al., 2012).

To quantify the multivariate associations between macrophyte traits and environmental variables, we applied Fourth-Corner methods. The significance of correlations between macrophytes traits and environmental variables were tested by sequential test (Ter Braak et al., 2012) to control the type I error, and false discovery rate method was used to adjust P values for multiple testing with 49,999 permutations as suggested by Dray et al. (2014).

Because RLQ does not provide significance test, however the Fourth-corner only tests the significance of bivariate associations and it does not consider the covariations among traits or among environmental variables (Dray et al., 2014), we combined both approaches by 1) firstly, a multivariate test was applied to evaluate the global significance of the traits-environment relationships. This test was based on the total inertia of the RLQ analysis (Dray and Legendre 2008). 2) the relationships between traits and environmental gradients, which corresponded to the first two RLQ axes for environmental gradients, were tested (Dray et al., 2014). In this case, we used the site scores of the RLQ axes as synoptic environmental vectors representing linear combinations of environmental variables. The significance of correlations between traits and the RLQ axes for environmental gradients, was tested by using model 2 and model 4 to obtain a global combined test with a significance level at 0.05 with 49999 permutations (Dray and Legendre, 2008; Dray et al., 2014). In the same way, the relationships between environmental variables and traits syndromes corresponding to the first two RLQ axes of traits were tested.

The RLQ and Fourth-Corner analysis were processed by using ade4 library (Dray and Dufour, 2007) in the open source R environment version 3.1.2 (R Development Core and Team, 2014, R Foundation for Statistical Computing, Vienna, Austria).

3. Results

For all environmental variables, the water depth was 1.9 ± 0.7 m (Mean ± SD, n = 113) with the average transparency to 0.5 ± 0.2 m (Mean ± SD, n = 113). The content of Cholorophyll a was 33.9 ± 47.2 μg/L (Mean ± SD, n = 30), and suspended solids was 14.2 ± 10.4 mg/L (Mean ± SD, n = 30). The total nitrogen and total phosphorus content were 3.3 ± 1.4 mg/L and 0.4 ± 0.2 mg/L (Mean ± SD, n = 30) respectively.

The RLQ analysis explained well of the cross-covariance between plant species traits and environmental variables. The first axis of the RLQ only already explained 98.2% total co-inertia. The right (positive) part of the first axis was correlated with all emergent macrophyte species. These species normally have thicker leaves, stem diameter and a higher leaf dry matter content, and they live in more turbid conditions with higher TN, TP, Chla, suspended solids, and with lower water transparency and DO. The left (negative) part of the first RLQ axis identified all submerged macrophyte species with higher SLA, leaf nitrogen and leaf phosphorus. These species require high water quality with higher transparency. (As shown in Fig. 3)

Among all environmental variables, TP contributed the most to 25.7% of the total inertia. Followed by suspended solids (20.7%), transparency (17.1%) and Chla (14.0%). The largest contribution of macrophyte traits to total inertia was life form which accounted for 15.7%, and followed by leaf nitrogen (13.8%) and SLA (13.6%). (As shown in Table 2)

The Fourth-Corner test detected bivariate associations between individual traits and environmental variables. In this case, 24 significant associations ($p < 0.05$) among 99 possible associations were found. The life form responded to most of environmental variables. Higher leaf nitrogen corresponded to habits characterized as clearer water conditions with a lower Chla, suspended solids content, water pH and TP content. Leaf nitrogen and SLA showed similar trend to water quality, i.e. both positively correlated to transparency and negatively correlated to suspended solids and TP significantly. SLA and leaf thickness showed almost an opposite trend. Higher leaf thickness appeared in turbid water condition with higher TP, suspended solids content, TN and lower water transparency. (as shown in Fig. 4)

A significant global relationship between species trait and environmental variables was detected ($p < 0.01$) via the global testing procedure of trait-environment relationship. Direct testing of the associations between RLQ axes and traits/environmental variables showed that the first RLQ axis was significantly negatively correlated with water transparency (clearer water condition) but positively with Chlorophyll a, suspended solids and water pH (turbid water condition with higher alkalinity). Associated traits were higher leaf nitrogen, leaf phosphorus and SLA for clearer water condition, and higher plant height, stem diameter, leaf thickness and leaf dry matter content for turbid water condition with higher alkalinity (Fig. 5).

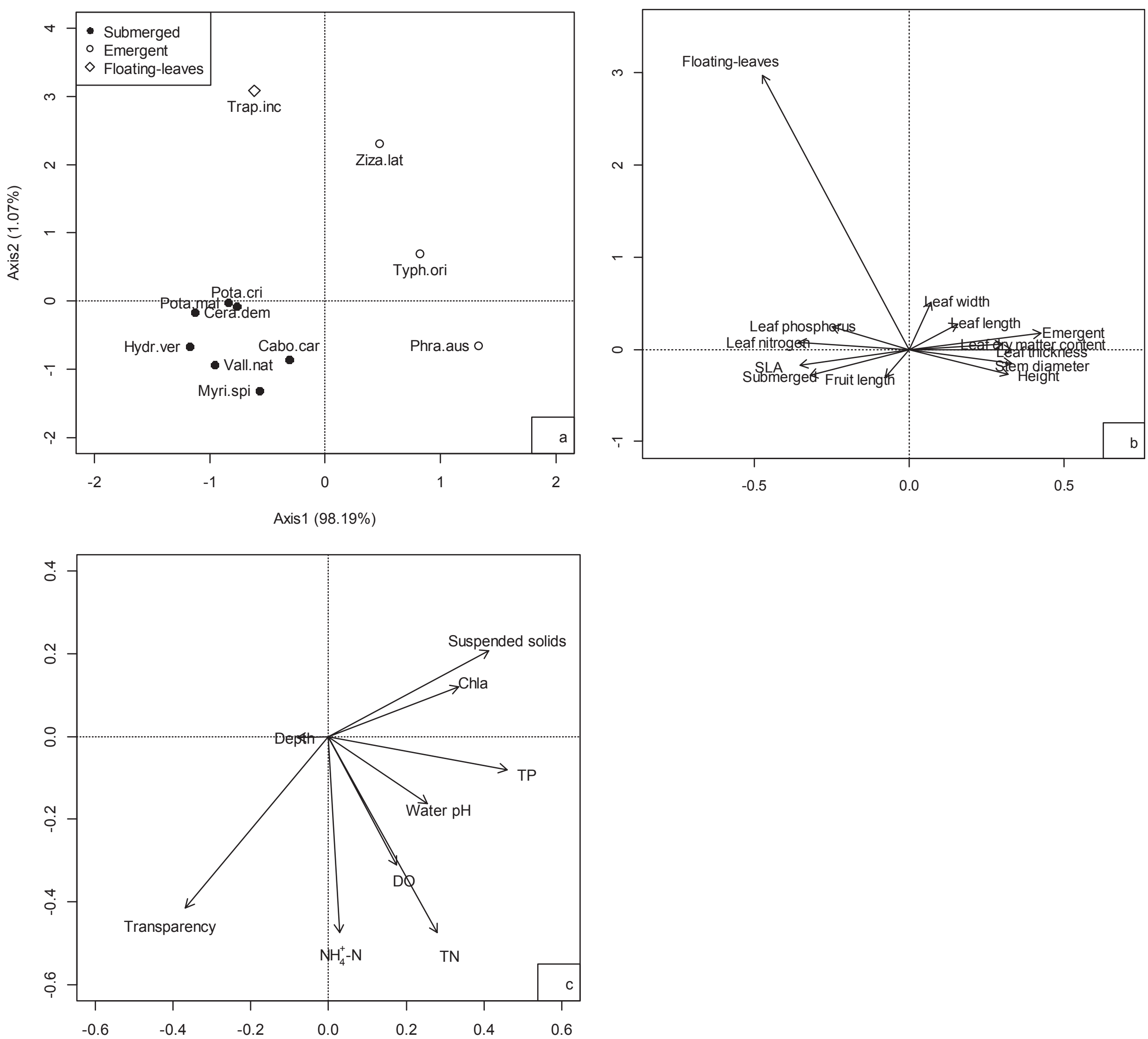

Fig. 3. The results of the first aixs (explained 98.2% total co-inertia) of RLQ analysis: (a) scores of species (*Phragmites australis* as Phra.aus, *Typha orientalis* as Typh.ori, *Zizania latifolia* as Ziza.lat, *Trapa incisa* as Trap.inc, *Potamogeton malaianus* as Pota.mal, *Myriophyllum spicatum* as Myri.spi, *Ceratophyllum demersum* as Cera.dem, *Cabomba caroliniana* as Cabo.car, *Potamogeton crispus* as Pota.cri, *Hydrilla verticillata* as Hydr.ver, *Vallisneria natans* as Vall.nat), (b) traits, (c) environmental variables. The codes for traits and environmental variables in (b) and (c) are following Table 2 (Life form including emergent, floating-leaved and submerged).

Table 2
Contribution of each component to total inertia of environmental variables and macrophyte traits in the RLQ analysis.

Environmental Variable	Contribution to total inertia (%)	Macrophyte trait	Contribution to total inertia (%)
Total phosphorus (TP)	25.7	Life form	15.7
Suspended solid	20.7	Leaf nitrogen	13.8
Transparency	17.1	SLA	13.6
Chlorophyll a (Chla)	14.0	Leaf thickness	12.2
Total nitrogen (TN)	9.3	Stem diameter	11.9
Water pH	8.1	Height	11.3
Dissolved oxygen (DO)	3.8	Leaf dry matter content	10.0
Depth	1.0	Leaf phosphorus	7.1
NH_4^+-N	0.4	Leaf length	2.8
		Leaf width	1.0
		Fruit length	0.8

4. Discussion

In this study, the application of RLQ and Fourth-corner method to investigate the trait-environment associations is novel to aquatic system. The relationships between environmental variables and traits detected in this study could help to identify the key macrophyte functional traits that associate with the environment filters in similar eutrophic shallow lakes. The findings will also give instruction about the impact of eutrophication on macrophytes community in the aspect of functional traits and as a reference for further ecological recovery projects, such as the selection of species for ecological engineering.

Among all environmental variables measured, TP is the most important one (contributed 25.7% to total inertia in RLQ analysis) that impact on macrophytes through traits. This result is in accordance with the previous studies in nearby Tai Lake, which considered TP as the key control factors for both algal biomass and Chla (Chen et al., 2003). The following 3 environmental variables (suspended solids, transparency and Chla) that contribute most to total inertia are all related to the water quality and light availability. This demonstrates that light limitation could be the main driving factor for macrophyte community assembly in Dianshan Lake. The light has been considered as important

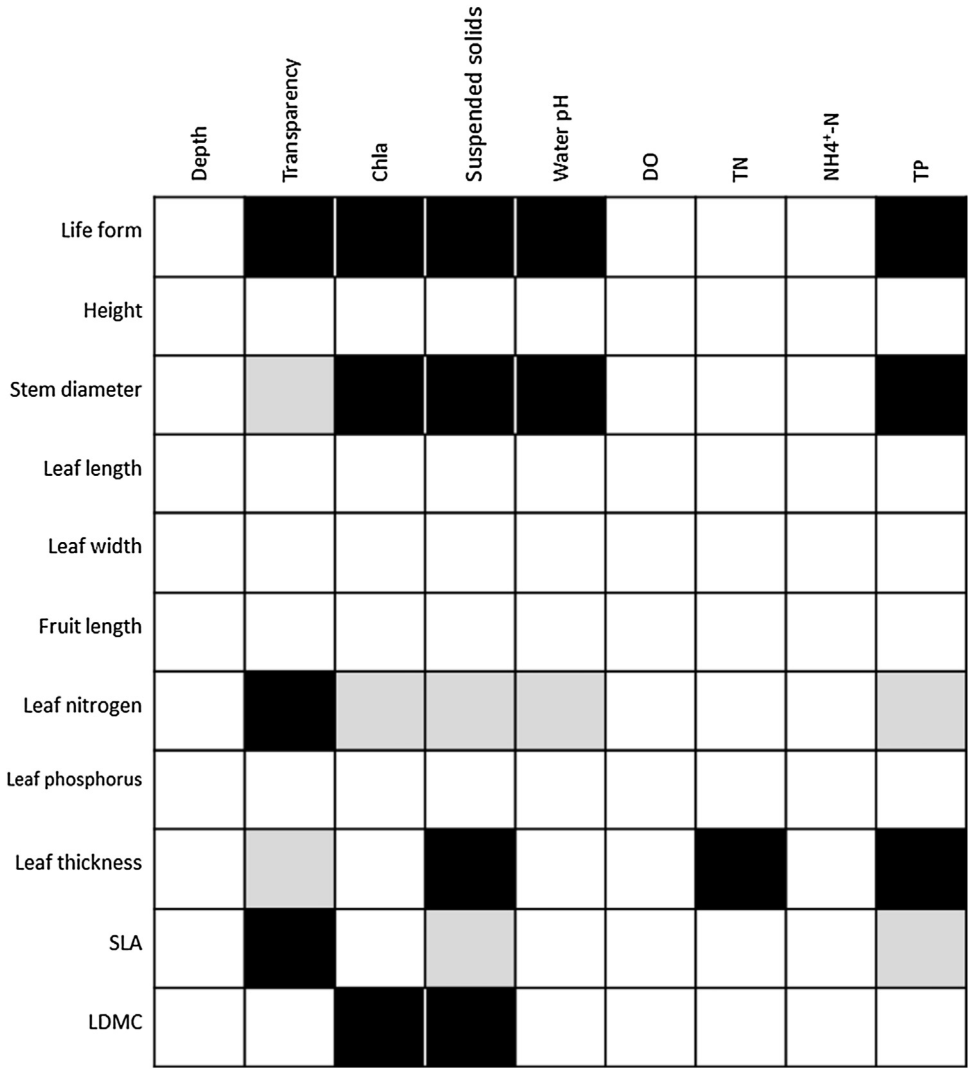

Fig. 4. Results of the Fourth-Corner tests. Significant ($P < 0.05$) positive and negative associations are represented by black and grey cells respectively. Non-significant associations are in blank.

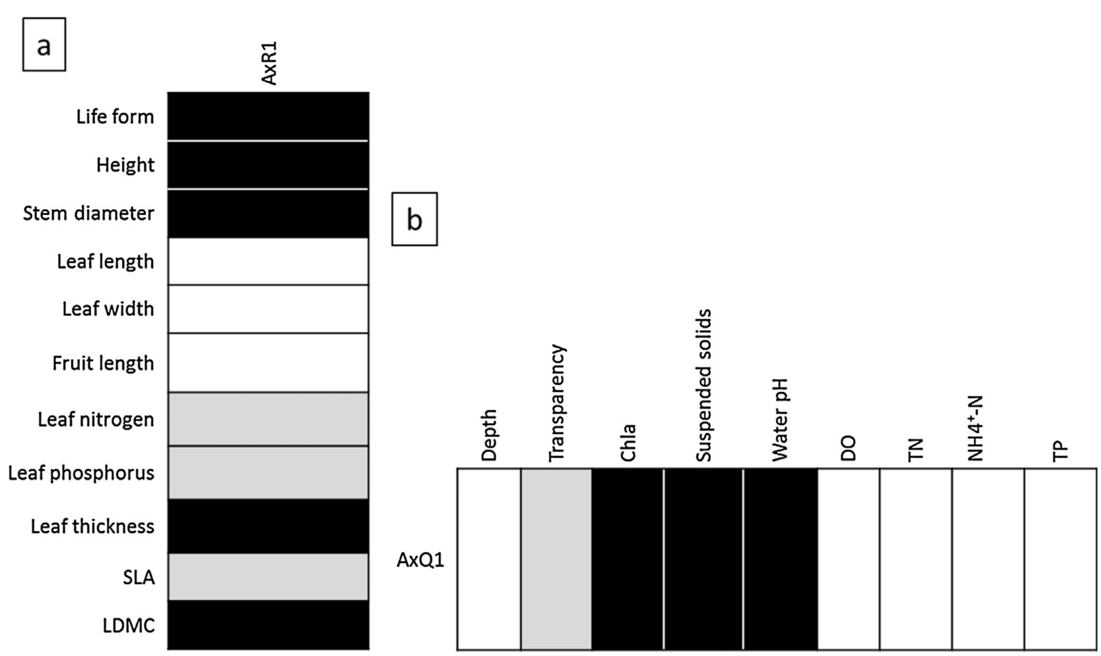

Fig. 5. Combination of RLQ and Fourth-corner results. (a) Fourth-corner tests between the first two RLQ axes for environmental gradients (AxR1/AxR2) and traits. (b) Fourth-corner tests between the first two RLQ axes for trait syndromes (AxQ1 and AxQ2) and environmental variables. Significant ($P < 0.05$) positive and negative associations are represented by black and grey cells respectively. Non-significant associations are in blank. The results for second RLQ axis (AxR2/AxQ2) are not shown here because of no significant relationships were detected.

limiting factors for macrophytes distribution in many freshwater lakes. According to a meta-analysis of 73 Danish lakes, on average, 1 m increase in water transparency resulted in the addition of 1.6 macrophyte species (Fischer et al., 2013). Based on the discussion above, improving water transparency via TP mitigation would be the prior method for the purpose of macrophytes restoration. However, in our study, the ratios of nitrogen to phosphorus (N:P) in 55% of the sites are lower than 7.2 (Redfield Ratio). According to Redfield Ratio, nitrogen is theoretically the limiting factor for algal in a eutrophic lake when N:P ratio in water is less than 7.2, otherwise phosphorus is the limiting factor (Redfield, 1958). That is to say, nitrogen is also an important limiting factor for algal growth in Dianshan lake and should be properly managed.

As for the macrophyte traits, the life form is the most important traits that responds to environmental gradients (contributed 15.7% to total inertia in RLQ analysis). Life form has been previously reported to reflect different eutrophic stages and in turn affect the surrounding environment such as sediment resuspension (Nurminen, 2003; Nurminen and Horppila, 2009). In our study, submerged macrophytes are mostly found in the habitat with higher transparency, lower TP and lower suspended solids. This result supports the idea that light limitation mainly affects the characteristics of submerged macrophytes communities (Zhang et al., 2012), and transparency is an important threshold for the maintenance and the recovery of submerged macrophytes (Wang et al., 2005). Emergent macrophytes are more tolerant to higher nitrogen and phosphorus environment with a higher Chla and suspended solids level. A trend shifting from submerged life form to emergent and floating life form has also been reported from a long-term observational study of Dianshan Lake (Shi et al., 2011).

Leaf nitrogen is accounted as the second important traits responding to transparency, especially for submerged macrophytes, which indicates that leaf nitrogen is a trait that strongly correlated with light use efficiency. From previous studies, leaf nitrogen and leaf phosphorus were integral to leaf and plant physiological functioning especially the light utilization (Wright et al., 2004). SLA shows the similar trend with leaf nitrogen. Based on previous studies, SLA was a good indicator for vegetation community and strategies (Poorter and De Jong, 1999; Reich et al., 1999).

From the results of Fourth-Corner analysis, significant specific trait-environment relationships are identified. Especially, the light condition associated environmental variables significantly relate to macrophyte traits such as life form, leaf nitrogen, SLA, leaf thickness and stem diameter. As a proxy indicator of the photosynthetic capacity, leaf nitrogen respond to most of the environmental variables relating to light availability in this case. Leaf nitrogen and SLA have similar correlations to environment can be explained by the positive relationships between SLA, photosynthetic capacity and leaf nitrogen content of macrophytes (Fu et al., 2014). From another study on the *Mentha aquatica* L., higher SLA showed higher light absorption efficiency (Enriquez and Sand-Jensen, 2003) which also indicates that SLA is an important traits responding to the light availability.

Different from many studies showing that the SLA increased with lower light availability in terrestrial ecosystems (Feng et al., 2007; del Pino et al., 2015) or aquatic ecosystems (Enriquez and Sand-Jensen, 2003; Fu et al., 2012), SLA responds to the light condition by showing a significant positive correlation to transparency ($p < 0.01$) and significant negative correlation to suspended solids in our case. This is most because of submerged macrophytes with higher leaf nitrogen and SLA commonly occurr in sites with clear water, while emergent macrophytes with lower leaf nitrogen and SLA occupy sites with turbid water. Moreover, submerged macrophytes, no matter erect ones or rosette ones, prefer to extend their stems or shoots height in response to shading (Fu et al., 2012), and habitat filtering along light gradient mainly has effects on traits related to plant size (i.e. shoot height, ramet size), which characterize the ability for light harvesting and space occupation (Fu et al., 2014). Thus, submerged macrophytes with higher SLA usually have higher leaf nitrogen content and show a high photosynthetic rate and fast growth, leading to great advantage in biomass accumulation and elongation of shoots at a given light availability, which make them more abundance in sites with greater light condition (Fu et al., 2015).

The almost opposite trend of SLA and leaf thickness in response to environment is in accordance with the fact that SLA and leaf thickness are found negatively correlated when the leaf dry matter content is similar (Wilson et al., 1999). Leaf thickness increases with a better nutrient availability and has a significant positive correlation with TN and TP (Meziane and Shipley, 1999), and contributes additively to the structure-level toughness and tends to be affected by lack of light sources (Wilson et al., 1999; Kitajima and Poorter, 2010).

5. Conclusion

In this study, RLQ and Fourth-corner method provided a potential alternative to determine the trait-environment associations in aquatic system. This novel application of the two methods to aquatic system helped to improve our understanding of the impacts of eutrophication on macrophytes and the underlying responding processes from the view of plant functional traits. Clear associations between macrophyte traits and environmental gradients were detected. In the current situation, water phosphorus associated more to macrophyte traits than nitrogen. Therefore, phosphorus should be given priority for the future macrophyte conservation and restoration considering environmental filter effect. Among all traits indices, the life form is the most important one that corresponds to the environment. Since the environment gradients shape the macrophyte community compositions through different aspects of macrophyte traits, we predict that submerged macrophyte communities will be further threatened with the degraded water quality.

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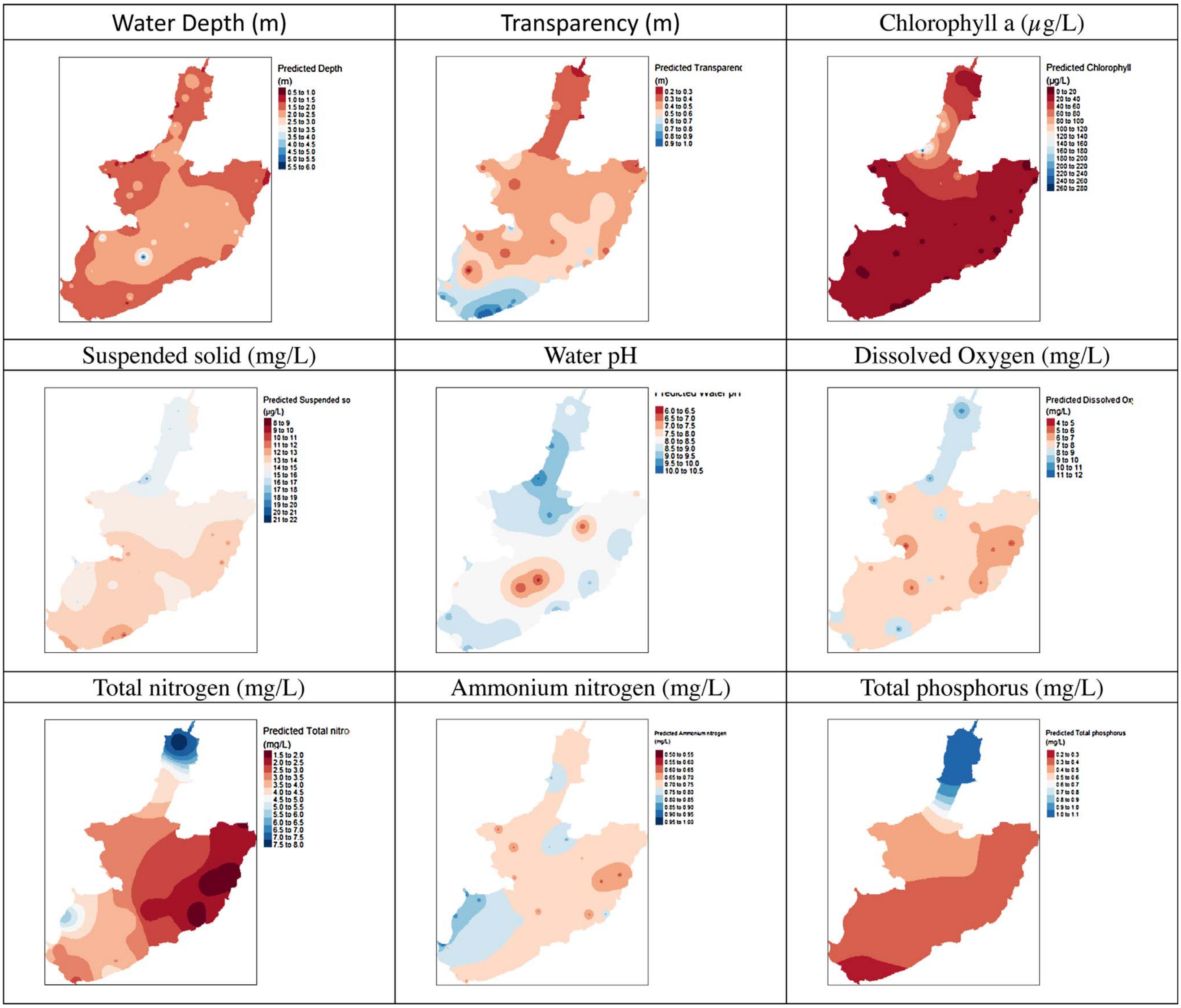
Appendix A. The inverse distance weighted (IDW) interpolation maps of the 9 environmental variables of Dianshan lake



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