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Stoichiometry patterns of leaf carbon, nitrogen and phosphorous in aquatic macrophytes in eastern China



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ABSTRACT

Ecological stoichiometry focuses on the balance of chemical elements and can enhance our understanding of the adaptive strategies of plants. However, few studies concerning the stoichiometry patterns of leaf carbon (C), nitrogen (N) and phosphorous (P) concentrations in aquatic macrophytes across latitudinal gradients have been reported. We collected 681 samples from 213 sites in eastern China, including 122 species from 55 genera and 35 families, to analyse variations in leaf C, N and P and their relationships with latitude, mean annual temperature and mean annual precipitation. The leaf C, N and P concentrations were significantly different among the four investigated life forms, but no significant differences were detected between seed plants and ferns. The N:P ratios were well constrained in different functional groups. The C:P and N:P ratios found in aquatic macrophytes in the present study were lower than previous findings, likely due to higher concentration of P in the surrounding environments. As the temperature increased, the leaf N concentration and leaf N:P ratio increased, whereas the leaf P concentration decreased. Furthermore, temperature was the key factor which can affect the stoichiometry patterns observed in widely distributed species directly and also indirectly at the community level. The slope of the regression line of the log 10-transformed N concentrations against the log 10-transformed P concentrations found in the seed plants of aquatic macrophytes was conserved and was greater than the slope of 2/3 reported for terrestrial plants, suggesting that the leaf N concentration increases faster relative to leaf P in aquatic than terrestrial plants.

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1. Introduction

Ecological stoichiometry provides a very effective way to enhance our understanding of the elemental composition and growth of organisms from a molecular level to a global scale level as well as our understanding of the ecological interactions of organisms with the surrounding environment, for example, in terms of metabolomics and genomics (Elser et al., 1996, 2000b, 2010), growth rates (Ågren, 2008; Vrede et al., 2004), population dynamics (Andersen et al., 2004), food web trophic structures (Elser et al., 2000a; Sterner et al., 1992) and biogeochemistry (Sterner and Elser, 2002; Sardans et al., 2012a,b). In these processes, the most important macroelements are carbon (C), nitrogen (N) and phosphorous (P). C, N and P play important roles in the formation of carbohydrates, lignin, cellulose, proteins, nucleotides and other useful substances associated with the regulation of organismal growth

rates, which are particularly impacted by N and P (Ågren, 2004; Elser et al., 2010; Sterner and Elser, 2002; Van de Waal et al., 2010). N and P are the two major limiting elements and can frequently affect photosynthesis (Reich et al., 1997), respiration (Tjoelker et al., 1999), individual growth rates (Ågren, 2008; Matzek and Vitousek, 2009), primary production (Howarth, 1988), community composition and species richness (Bedford et al., 1999; Welle et al., 2003).

In terrestrial ecosystems, many studies have suggested that the patterns of elements are also affected by latitude and large-scale climatic factors, such as temperature and precipitation, which can influence stoichiometry patterns in plants by changing the elemental composition of plant organs and altering the physical and chemical conditions of surrounding habitats (Han et al., 2011; He et al., 2006, 2008; Reich and Oleksyn, 2004; Santiago et al., 2004; Wright et al., 2005). Some studies have also shown that differences in stoichiometry can be caused by growth forms (Reich and Oleksyn, 2004; Reich et al., 1997), nutrient acquisition and conservation mechanisms (Aerts and Chapin, 1999), photosynthetic pathways (Halsted and Lynch, 1996), plant strategies (Aerts and Chapin, 1999), phylogenetic groups (Han et al., 2005; Kerkhoff

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et al., 2006; Güsewell, 2004; Zhang et al., 2012) and soil substrate age (Treseder and Vitousek, 2001; Vitousek et al., 1995). Many hypotheses have been proposed to explain these phenomena related to plant stoichiometry patterns, including the following: (1) the temperature–plant physiological hypothesis, which indicates that plants from colder and high-altitude habitats exhibit higher leaf N and P concentrations because temperature can directly influence the rate of physiological processes (e.g., photosynthesis and respiration) and prompt plants to undergo acclimation and adaptation (Oleksyn et al., 1998; Tjoelker et al., 1999; Weih and Karlsson, 2001); (2) the biogeochemical hypothesis, which suggests that the biogeochemistry of the soil can affect leaf N and P concentrations via soil N and P availability because temperature can influence the activity of microorganisms, which show lower activity in colder habitats, such that soil N and P availability and, thus, leaf N and P concentrations are lower (Aerts and Chapin, 1999; Vitousek and Farring, 1997); and (3) the growth rate hypothesis, which is related to the N and P levels and N:P ratio in surrounding environments and organisms and suggests that organisms with higher growth rates exhibit higher P concentrations and a lower N:P ratio (Ågren, 2008; Enríquez et al., 1996; Main et al., 1997; Matzek and Vitousek, 2009).

In freshwater ecosystems, previous studies of aquatic macrophytes stoichiometry have focused on the contents of mineral elements in individuals on a limited scale (Atkinson and Smith, 1983; Jackson and Kalff, 1993; Jackson et al., 1991; Li et al., 2013; Zimba et al., 1993). In recent years, researchers have paid more attention to phytoplankton, zooplankton and fish in freshwater and marine ecosystems and also studied nutrient cycling in food webs in detail (Elser et al., 2000a, 2007; Loladze and Elser, 2011; Sterner et al., 1992). However, few studies have been conducted on the large-scale patterns of aquatic macrophytes stoichiometry (Ambasht, 1991; Demars and Edwards, 2007; Duarte, 1992; Fernández-Aláez et al., 1999; McJannet et al., 1995; Xing et al., 2013).

In the present study, we collected 681 samples from 213 sites across eastern China, including 122 species from 55 genera and 35 families as well as from four life forms (emergent plants, freely floating plants, floating leaved plants and submerged plants) (Cronk and Fennessy, 2001) to analyse the large scale patterns of C:N:P stoichiometry in aquatic macrophytes and their relationships with latitude, mean annual temperature and mean annual precipitation. We also aimed to determine whether the patterns varied among different functional groups by life form or phylogenetic group and to explore the possible reasons underlying such patterns. Specifically, three main questions were addressed: (1) how variable is the C:N:P stoichiometry of aquatic macrophytes; (2) what are the relationships of the leaf N and P concentrations and N:P ratio with latitude and temperature; and (3) what are the differences in the relationships between the leaf N and P concentrations in different groups by life form and phylogenetic group?

2. Materials and methods

2.1. Study region

Topographically, the study region, located in eastern China, is mostly composed of plains and hills and shows a mean elevation below 500 m. Many rivers and lakes are also present in this region: more than 800 lakes (area > 1 km²) are distributed around rivers including the Yangtze, Yellow, Songhua and Heilongjiang, accounting for approximately 28% of the total number of lakes in China (Wang and Dou, 1998). The climate of this region is quite variable from north to south, with the annual precipitation ranging from 300 to more than 3000 mm and the annual temperature varying

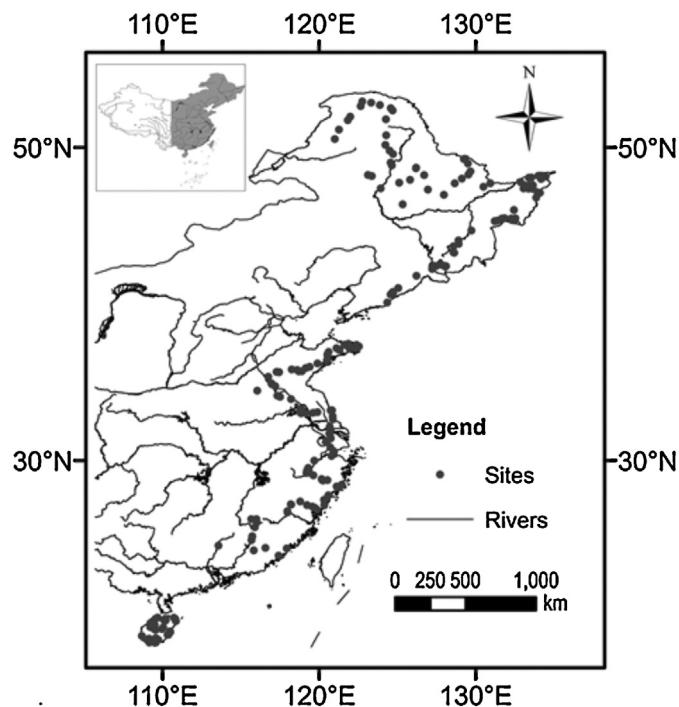


Fig. 1. Geographical locations of the sampling sites.

from -5 to 26°C . Because of the diversity of climates and habitats, the aquatic macrophytes in this region are very diverse.

2.2. Field sampling

Leaf sample collection was conducted during the growing seasons (early July to late September) of 2009 and 2010. We collected the leaves of aquatic macrophytes, avoiding fertilised plants and planted sites, recorded the geographic information (latitude, longitude and altitude) for each site, and wrapped the leaves of the dominant species with paper and air-dried them. During this process, the epiphytic algae and mud on the surface of the leaves were removed very carefully, especially for samples of submerged plants. The detailed geographical locations and climate data for the sample sites are shown in Fig. 1 and Table 1, respectively.

2.3. Laboratory analysis

The collected aquatic macrophytes samples were dried in an oven at 70°C for 72 h in the laboratory, and the dried samples were then ground into a powder before the elemental analysis. The leaf C and N concentrations were determined from approximately 2.0 mg of the homogenously ground material of each sample using a CHNS/O Elemental Analyzer (EA3000, EuroVector, Italy). The total P concentration was determined via the molybdate–ascorbic acid

Table 1
Description of the study region.

	Eastern China (study region) 213
Number of sites	213
Number of sampled species	122
Latitude ($^{\circ}$)	18.35–52.94
Longitude ($^{\circ}$)	108.68–134.47
Altitude (m)	124
Mean annual precipitation (mm)	1055.99
Mean annual temperature ($^{\circ}\text{C}$)	12.69

Arithmetic means are shown for the altitude, mean annual precipitation and mean annual temperature.

method with a total phosphorus analyser (IL-500P, Hach® Company, Loveland, USA) after digestion with a microwave reaction system (Multiwave 3000, Anton Paar® GmbH, Austria) (Kuo, 1996).

2.4. Dataset

In total, we collected 681 samples from 213 sites in rivers, streams, lakes, ponds, bogs and other small water bodies across eastern China, including 122 species from 55 genera and 35 families as well as four life forms. The climate data for each site were calculated using linear models, which were established from the averaged data for the past 50 years from 680 climate stations, using the geographical data (latitude, longitude and altitude) as variables (Fang et al., 2001; He et al., 2006; Piao et al., 2003). The leaf C, N and P concentrations are presented in units of mg g^{-1} dry weight, and the C:N, C:P, and N:P ratios are presented on a mass basis.

2.5. Data analysis

We analysed the data at two levels: (1) using all of the data together (i.e., treating all observations equally) and (2) subdividing the dataset into different life forms and phylogenetic groups. We calculated the arithmetic means of the leaf C, N and P concentrations and the C:N, C:P and N:P ratios for each life form (emergent plants, freely floating plants, floating leaved plants and submerged plants) and each phylogenetic group (seed plants and ferns). The seed plants were further divided into two groups (dicotyledons and monocotyledons) (see Appendix S1). All of the elemental concentration data for the plant species were first log 10-transformed. The differences between different life forms and phylogenetic groups were then compared using one-way ANOVA. Pearson correlations among the C, N and P concentrations and the C:N, C:P and N:P ratios were also calculated for all 681 observations. We used the total dataset and the data averaged by each life form and phylogenetic group to perform linear regressions with temperature, precipitation and latitude. The mean temperature and precipitation values were also calculated, and the differences between these values were compared using one-way ANOVA. All of the above statistical analyses were conducted with SPSS 13.0 software (SPSS Inc., Chicago, IL, USA, 2004).

We also used standardised major axis regression (SMA) (Warton et al., 2006) to fit a line for the log 10-transformed N concentrations against the log 10-transformed P concentrations and compared the slopes of these lines among the four life forms and between seed plants and ferns as well as between monocotyledons and dicotyledons. The above analyses were conducted with the SMATR computer package, version 2.0 (Falster et al., 2006).

3. Results

3.1. Patterns of the leaf stoichiometry traits of aquatic macrophytes

The obtained leaf C, N and P concentrations ranged from 225 to 487 mg g^{-1} , 6.53 to 59.4 mg g^{-1} and 0.63 to 8.63 mg g^{-1} , respectively. The C:N ratio ranged from 7.12 to 63.1, the C:P ratio ranged from 38.7 to 648, and the N:P ratio ranged from 1.94 to 49.3. The arithmetic means for all plant species combined were 370 mg g^{-1} for C, 25.9 mg g^{-1} for N and 3.28 mg g^{-1} for P, and the means were 17.1 for the C:N ratio, 149 for the C:P ratio and 9.50 for the N:P ratio (Table 2). The coefficients of variation (CV) for the C, N and P concentrations and the C:N, C:P and N:P ratios were 18.1, 40.8, 48.8, 51.4, 62.8 and 62.8%, respectively. Furthermore, the mean C:N:P mass ratio was 113:8:1.

The mean C concentration in the submerged plants was 337 mg g^{-1} , which was significantly lower than the concentrations found in the other three life forms. The highest mean N and P concentrations were observed in freely floating plants, whereas the lowest means were observed in emergent plants. The mean C:N and C:P ratios in emergent plants were significantly higher than in the other life forms. No significant differences in elemental concentrations and ratios were detected between the seed plants and ferns in this study. The mean N concentration recorded in monocotyledons was lower than in dicotyledons, although the C:N ratio was higher; however, no significant differences were observed for the other variables. Furthermore, the mean N:P ratio did not differ among the four life forms and phylogenetic groups (Table 2).

3.2. Relationships between leaf element concentrations and ratios

Strong correlations were observed between elemental concentrations and elemental ratios. The leaf C concentrations exhibited strong negative correlations with the leaf P concentrations but positive correlations with leaf N concentrations, and the leaf N concentrations exhibited strong positive correlations with leaf P concentrations. The leaf C:N and C:P ratios presented strong negative correlations with leaf N and leaf P concentrations and positive correlations with leaf C concentrations, whereas the leaf N:P ratios displayed strong negative correlations with leaf P concentrations and positive correlations with leaf C and N concentrations (Table 3).

3.3. Relationships between leaf stoichiometry traits and temperature (latitude) and precipitation

The leaf C, N and P concentrations and C:N, C:P and N:P ratios were significantly correlated with latitude and temperature across all species (Figs. 2 and 3). Although the relationship between the leaf P concentration and precipitation was not significant ($p=0.098$), the other variables (leaf C, leaf N and the C:N, C:P, and N:P ratios) were significantly correlated with precipitation (Figs. 2g-i and 3g-i). The leaf C and N concentrations and C:P and N:P ratios all decreased as latitude increased and temperature decreased, with the opposite pattern observed for the leaf P concentration and C:N ratio. The leaf C and N concentrations and C:P and N:P ratios increased as precipitation increased, whereas the leaf C:N ratio decreased.

3.4. Critical relationship between leaf N and P concentrations

For all of the pooled observations ($n=681$), the leaf N concentration increased as the leaf P concentration increased ($p<0.001$, $r^2=0.149$; Fig. 4 and Table 4), showing a scaling exponent of 0.842 (95% CI, 0.786–0.903). In each of the four life forms, a strong positive correlation was detected between the leaf N and P concentrations. The largest scaling exponent was obtained for emergent plants (0.960), and the smallest for submerged plants (0.686). In the four phylogenetic groups, significantly positive correlations were detected between leaf N and P concentrations, except in ferns. The scaling exponents calculated for seed plants, monocotyledons and dicotyledons were 0.838, 0.847 and 0.810, respectively (Fig. 4 and Table 4).

4. Discussion

4.1. Stoichiometry patterns of aquatic plants and the interactions of leaf C, N and P concentrations

In the present study, the mean C:N:P ratio was approximately 291:17:1, and the median ratio was approximately 320:18:1 in

Table 2
Leaf C, N and P concentrations and C:N, C:P and N:P ratios on a mass basis.

Plant group	n	C (mg g ⁻¹)	N (mg g ⁻¹)	P (mg g ⁻¹)	C:N	C:P	N:P
All species	681	369 ± 1.87	25.9 ± 0.40	3.28 ± 0.06	17.1 ± 0.34	149 ± 3.57	9.50 ± 0.23
<i>Life form</i>							
Emergent plants	304	392 ± 2.41a	24.0 ± 0.65c	3.00 ± 0.09b	20.5 ± 0.62a	170 ± 5.40a	9.35 ± 0.31a
Freely floating plants	24	382 ± 8.83a	31.6 ± 2.36a	3.70 ± 0.41a	13.6 ± 1.04b	138 ± 16.0b	9.94 ± 0.80a
Floating leaved plants	102	381 ± 3.86a	28.7 ± 1.08ab	3.41 ± 0.16ab	15.4 ± 0.63b	150 ± 10.8ab	10.7 ± 0.78a
Submerged plants	251	337 ± 2.64b	26.6 ± 0.55bc	3.52 ± 0.10ab	14.0 ± 0.31b	123 ± 5.02b	9.15 ± 0.37a
<i>Phylogeny</i>							
Ferns	23	372 ± 11.1a	22.2 ± 2.09a	2.95 ± 0.33a	19.4 ± 1.58a	149 ± 11.5a	8.58 ± 0.23a
Seed plants	658	370 ± 1.89a	26.0 ± 0.41a	3.29 ± 0.06a	17.0 ± 0.34a	149 ± 3.68a	9.53 ± 0.96a
Monocotyledons	422	371 ± 2.35a	25.4 ± 0.50b	3.31 ± 0.08a	17.8 ± 0.48a	150 ± 4.50a	9.20 ± 0.27a
Dicotyledons	236	367 ± 3.20a	27.2 ± 0.72a	3.25 ± 0.10a	15.6 ± 0.42b	147 ± 6.37a	10.1 ± 0.43a

The number of species (n) and mean values (mg g⁻¹) (±S.E.) are shown. Differences between life forms were tested using one-way ANOVA with a Duncan post hoc test of significance. Differences between seed plants and ferns or between monocotyledons and dicotyledons were tested using independent-samples t tests. Different letters indicate significant differences at p < 0.05.

Table 3
Pearson correlation among leaf element concentrations.

	C	N	P	C:N	C:P	N:P
C	1.00					
N	0.223**	1.00				
P	-0.166**	0.379**	1.00			
C:N	0.148**	-0.791**	-0.385**	1.00		
C:P	0.364**	-0.261**	-0.788**	0.391**	1.00	
N:P	0.277**	0.349**	-0.549**	-0.264**	0.720**	1.00

Significant correlations are presented in bold.
** p < 0.01.

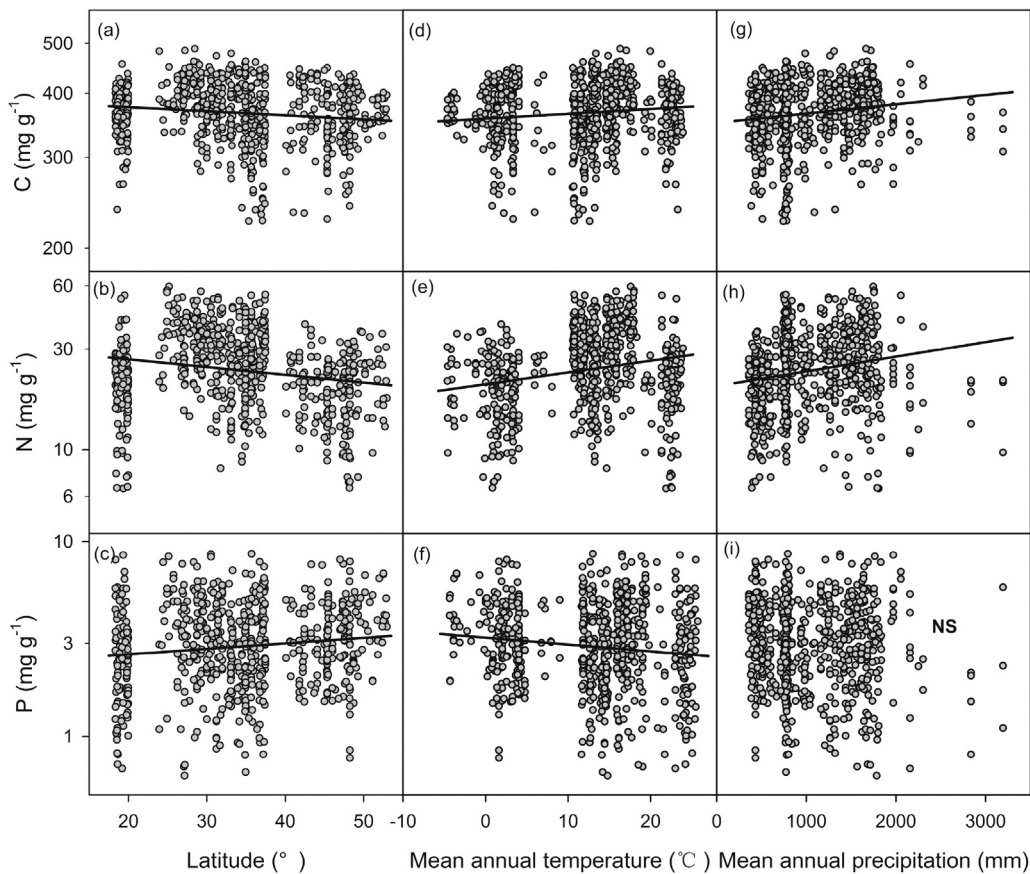


Fig. 2. Relationships between leaf C, N and P concentrations and latitude, mean annual temperature (MAT) and mean annual precipitation (MAP) across eastern China. Data points indicate every observation of C, N and P within the sampling sites. Linear regressions are shown for (a) latitude and leaf C concentrations ($r^2 = 0.016$, $p < 0.001$), (b) latitude and leaf N concentrations ($r^2 = 0.035$, $p < 0.001$), (c) latitude and leaf P concentrations ($r^2 = 0.015$, $p < 0.01$), (d) MAT and leaf C concentrations ($r^2 = 0.013$, $p < 0.01$), (e) MAT and leaf N concentrations ($r^2 = 0.049$, $p < 0.001$), (f) MAT and leaf P concentrations ($r^2 = 0.015$, $p < 0.01$), (g) MAP and leaf C concentrations ($r^2 = 0.024$, $p < 0.001$), (h) MAP and leaf N concentrations ($r^2 = 0.037$, $p < 0.001$) and (i) MAP and leaf P concentrations (no significant relationship, $p = 0.098$).

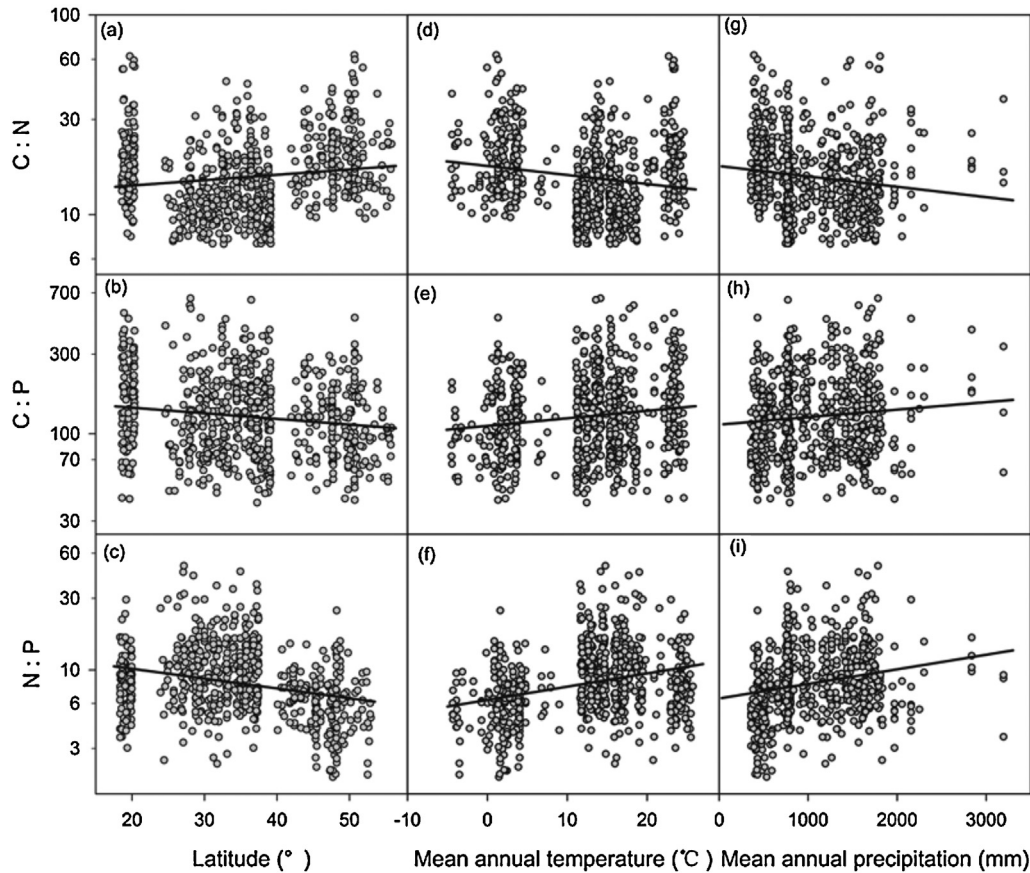


Fig. 3. The leaf C:N, C:P and N:P ratios with respect to latitude, mean annual temperature (MAT) and mean annual precipitation (MAP) across eastern China. Data points indicate every observation within the sampling sites. Linear regressions are shown for (a) latitude and leaf C:N ratios ($r^2 = 0.022$, $p < 0.001$), (b) latitude and leaf C:P ratios ($r^2 = 0.021$, $p < 0.001$), (c) latitude and leaf N:P ratios ($r^2 = 0.074$, $p < 0.001$), (d) MAT and leaf C:N ratios ($r^2 = 0.034$, $p < 0.001$), (e) MAT and leaf C:P ratios ($r^2 = 0.021$, $p < 0.001$), (f) MAT and leaf N:P ratios ($r^2 = 0.091$, $p < 0.001$), (g) MAP and leaf C:N ratios ($r^2 = 0.021$, $p < 0.001$), (h) MAP and leaf C:P ratios ($r^2 = 0.010$, $p < 0.05$) and (i) MAP and leaf N:P ratios ($r^2 = 0.048$, $p < 0.001$).

atomic terms. Both the mean and median ratios were obviously lower than the following previously reported ratios: mean and median ratios of 700:35:1 and 550:30:1, respectively, in marine plants (Atkinson and Smith, 1983); mean ratios of 704:26:1 and 790:22:1 in aquatic and emergent angiosperms, respectively (Fernández-Aláez et al., 1999), and median ratio of 570:29:1 in freshwater aquatic vascular plants (Demars and Edwards, 2007). These lower C:P and N:P ratios were most likely caused by high

phosphorous levels. In the present study, the mean P concentration found in aquatic macrophytes was approximately two times greater than the value reported by Fernández-Aláez et al. (1999) and one and a half times the value reported by Demars and Edwards (2007) (Table 2). Aquatic macrophytes stoichiometry can be impacted by the characteristics of the surrounding environment, such as sediment nutrients (Jackson et al., 1991; McJannet et al., 1995), water nutrients (Ambasht, 1991; Xing et al., 2013)

Table 4

Summary of the standardised major axis (SMA) regression results for the investigated plant groups.

Plant group	<i>n</i>	Intercept	Exponent	Lower CI	Upper CI	r^2	<i>p</i> -Value
All species	681	0.959	0.842	0.787	0.903	0.149	<0.001
<i>Life forms</i>							
Emergent plants	304	0.924	0.960a	0.866	1.07	0.158	<0.001
Freely floating plants	24	1.12	0.700ab	0.510	0.962	0.466	<0.001
Floating leaved plants	102	1.06	0.767ab	0.634	0.929	0.057	0.016
Submerged plants	251	1.06	0.686b	0.611	0.771	0.129	<0.001
<i>Phylogeny</i>							
Ferns	23	0.902	0.956				0.112
Seed plants	658	0.989	0.838	0.781	0.900	0.150	<0.001
Monocotyledons	422	0.978	0.847a	0.777	0.924	0.188	<0.001
Dicotyledons	236	1.01	0.810a	0.716	0.916	0.088	<0.001

The standardised major axis intercept (scaling constant) and scaling exponent (slope); lower and upper 95% CI of the exponent and r^2 values are shown. Significant differences ($p < 0.05$) in the slopes between groups are indicated with different letters. The *p*-values indicating the significance of the relationships between the log₁₀-transformed N concentrations against the log₁₀-transformed P concentrations are also shown; significant correlations between leaf N and leaf P concentrations are presented in bold. *n*, number of observations.

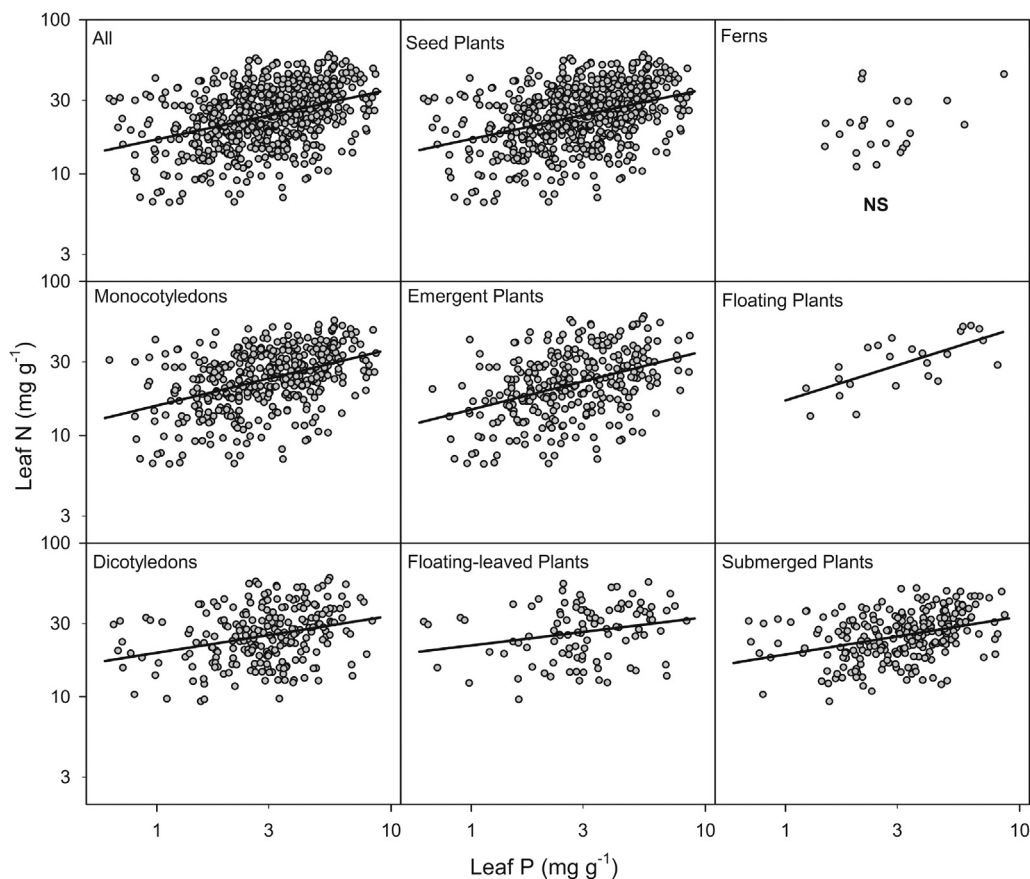


Fig. 4. Relationships between the log 10-transformed N concentrations against the log 10-transformed P concentrations for all of the data pooled and for the plants grouped by life form (emergent plants, freely floating plants, floating leaved plants and submerged plants) and plants grouped by phylogeny (ferns, seed plants, monocotyledons and dicotyledons). The details of these relationships, calculated through standardised major axis (SMA) regressions, are presented in Table 4.

and water depth (Li et al., 2013). Thus, leaf element traits can reflect differences among environments (Van de Waal et al., 2010). Many studies have also suggested that most of the variability in C:N:P stoichiometry could be explained by the taxon and species composition (Demars and Edwards, 2007, 2008). However, different environments induce different species compositions (He et al., 2006; Lacoul and Freedman, 2006a; Stock and Verboom, 2012). In our study region, the eutrophication of many water bodies has been caused by local anthropogenic pollution, with the total phosphorous concentration being particularly high (Jin et al., 2005; Wu et al., 1999). Therefore, this result could be explained by aquatic macrophytes absorbing excess P from the surrounding environment.

The obtained leaf C concentrations were significantly related to the leaf N and leaf P concentrations (Table 3), consistent with the study conducted by Fernández-Aláez et al. (1999) in aquatic angiosperms and emergent angiosperms. C acquisition and use can be regulated by N and P because N-rich enzymes and P-rich RNA and ATP are involved in a series of reactions, such as respiration (Tjoelker et al., 1999) and photosynthesis (Reich et al., 1997).

4.2. Relationships between leaf N and P concentrations and temperature (latitude)

The present study indicated that leaf N increased as the temperature increased (or latitude decreased) (Fig. 2b and e), supporting the previously proposed temperature–biogeochemical hypothesis (Reich and Oleksyn, 2004). The soil total N increases from north to south in this study region (0.93 ± 0.29 to 1.50 ± 0.67 mg g⁻¹), except in northeast China (2.58 ± 0.77 mg g⁻¹) (Zhu et al., 1997).

Warm temperatures can accelerate the decomposition and mineralisation of organic matter in soil and water by affecting metabolic processes and microbial activity, which can increase the availability of N. In contrast, low temperature can suppress N movement and plant N uptake (Aerts and Chapin, 1999). Hence, the total available N in northeast China is 0.16 mg g⁻¹ (Zhang et al., 2007), which is likely lower than in other regions of China. In addition, the total available N concentration is lower in high precipitation regions of terrestrial ecosystems, which is caused by slow decomposition and high leaching loss. N is therefore carried to rivers and lakes, increasing the load of water-soluble nitrates that are available for uptake by aquatic macrophytes (Austin and Vitousek, 1998; Neff et al., 2003; Reynolds and Davies, 2001). Thus, available N is expected to increase in aquatic habitats due to the N load in the surrounding environment, likely resulting in the observed pattern of leaf N concentration.

The leaf P concentration increased as the temperature decreased (or latitude increased) (Fig. 2c and f), which was consistent with the temperature–plant physiological hypothesis. The load of P in the surrounding environment of aquatic habitats, such as streams, rivers and lakes, may be in dissolved, colloidal or particle form, and not every form of P can be taken up by aquatic macrophytes (Reynolds and Davies, 2001). Therefore, in aquatic ecosystems, the patterns of leaf P concentration are likely caused by the adaption and physiological acclimation of plants to low temperature and cold habitats, which enhances the activity of enzymes and thereby accelerates metabolism and growth rates (Oleksyn et al., 1998; Reich and Oleksyn, 2004). This adaption and physiological acclimation could also result in higher leaf P concentrations in colder

habitats or high latitude regions. In addition, the observed patterns of the leaf P concentration were in agreement with the pattern of soil P in China (Jiang et al., 1979). In eastern China, the soil P content decreases from north to south and can be divided into four classes: >0.70, 0.52–0.70, 0.35–0.52 and <0.35 mg g⁻¹ (Han et al., 2005; Jiang et al., 1979). The soil P also affects the pattern of leaf P concentration found in aquatic macrophytes because the leaf P concentration can reflect P availability (Aerts and Chapin, 1999).

The leaf N:P ratios recorded in this work increased as temperature increased (or latitude decreased) (Fig. 3c and f). This pattern may be a direct result of the increased leaf N concentrations and decreased leaf P concentrations associated with increased temperatures (or decreased latitude). As such, this pattern could provide robust evidence supporting the growth rate hypothesis (GRH), which suggests that plants in colder regions display higher growth rates and metabolic rates because of the shorter growing season and that they therefore need to produce more P-rich rRNA to support the increased protein synthesis and growth rates (Austin and Vitousek, 1998; Chapin, 1980; Elser et al., 1996, 2000b; Kerkhoff et al., 2006; Sardans et al., 2012b).

These patterns of leaf N and P concentrations and N:P ratios associated with temperature (latitude) are not only affected by environmental factors but also influenced by the species composition (Demars and Edwards, 2007; Demars and Edwards, 2008; Frost and Hicks, 2012) and phylogenetic effects (Broadley et al., 2004; Kerkhoff et al., 2006; Stock and Verboom, 2012). In the present study, the species composition and the mean annual temperature explained 29.7–35.8% and 1.37–9.11%, respectively (data not shown), of the observed variation in the leaf stoichiometry patterns of the aquatic plants. In general, the distribution and abundance of aquatic macrophytes is controlled by environmental factors, especially temperature (Crow, 1993; Heino, 2002; Lacoul and Freedman, 2006a,b). Thus, temperature was the key factor which can affect the stoichiometry patterns found in widely distributed species (*Hydrilla verticillata* and *Myriophyllum spicatum*) directly and can also have an indirect impact at the community level.

4.3. Critical relationship between leaf N and leaf P concentrations

The results of the SMA showed that the scaling slopes of the log₁₀-transformed N concentrations against the log₁₀-transformed P concentrations were not conserved across the four investigated life forms of aquatic macrophytes. The scaling slope for the emergent plants was greater than for the other three life forms. However, in the seed plants, the scaling slope was conserved, showing a value of 0.838, which was greater than the value found for 2500 species of seed plants (0.676) by Reich et al. (2010), likely caused by higher leaf N and leaf P concentrations (Fig. 4 and Table 4). In the present study, the obtained leaf N and P concentrations were 26.0 and 3.29 mg g⁻¹, respectively. However, in the study by Reich et al. (2010), these concentrations were 17.1 and 1.57 mg g⁻¹, respectively. Furthermore, the scaling slope was greater in aquatic macrophytes than in terrestrial plants, which suggested that the leaf N concentration increases faster relative to P in aquatic than terrestrial plants.

5. Conclusions

This study deepens our understanding of the C:N:P stoichiometry patterns of aquatic macrophytes and their relationships with latitude, temperature and precipitation on a large scale in eastern China. As global warming and worldwide eutrophication continue to occur and aquatic macrophytes play a key role in the functioning of inland waters, exploring C:N:P patterns and their driving forces

can contribute to inland water management policies. We found the following results: (1) the C:N:P ratios of aquatic macrophytes were lower than previous studies because of excess P in the surrounding environments; (2) leaf N concentrations decreased as temperature decreased (or latitude increased), supporting the temperature-biogeochemical hypothesis, due to the greater amount of available N caused by leaching action, the soil N pattern and microbial activity; in contrast, leaf P concentrations increased as temperature decreased (or latitude increased), which was in agreement with the temperature–plant physiological hypothesis because of the soil P pattern and the adaption and physiological acclimation of plants to low temperatures and cold habitats. The leaf N:P ratio increased as temperature increased (or latitude decreased), which supports the growth rate hypothesis (GRH). In addition, thus, temperature was the key factor which can affect the stoichiometry patterns found in widely distributed species (*H. verticillata* and *M. spicatum*) directly and can also have an indirect impact at the community level. (3) The scaling slope for the log₁₀-transformed N concentrations versus the log₁₀-transformed P concentrations in seed plants was conserved, showing a value of 0.838 (greater than 2/3), which was caused by the higher N and higher P concentrations found in the aquatic macrophytes and suggested that the leaf N concentration increases faster relative to P in aquatic than terrestrial plants. The present study broadens our knowledge of the stoichiometry patterns of leaf elements in aquatic macrophytes on a large scale. Nevertheless, we only focused on three factors (latitude, temperature and precipitation), and other factors, such as soil nutrients and water physicochemical characteristics (e.g., water nutrients and light availability), should be studied in future works.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2014.06.018>.

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