



## Spatial patterns of leaf carbon, nitrogen stoichiometry and stable carbon isotope composition of *Ranunculus natans* C.A. Mey. (Ranunculaceae) in the arid zone of northwest China



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### ABSTRACT

Leaf carbon concentrations (C%), nitrogen concentrations (N%), carbon–nitrogen ratio (C:N) and stable carbon isotope composition ( $\delta^{13}\text{C}$ ) are key foliar traits with great ecological importance, but few studies have attempted to document the pattern of leaf C%, N%, C:N and  $\delta^{13}\text{C}$  value for aquatic macrophytes. In this study, we examined the pattern of leaf C%, N%, C:N and  $\delta^{13}\text{C}$  value of *Ranunculus natans* collected from 26 sites across northwest China, and tried to explore how different environmental conditions affect leaf C%, N%, C:N stoichiometry, and  $\delta^{13}\text{C}$  value. Results showed that leaf N%, C:N ratios and  $\delta^{13}\text{C}$  varied significantly among the 26 *R. natans* collection sites, but leaf C% did not differ significantly. Our study found weak relationships between foliar N% and C:N of *R. natans* and altitude, latitude and longitude, which indicated that variability in foliar N%, C:N stoichiometry across diverse habitats may result from plant growth, development, metabolism, phenological and life history traits, rather than from variation in geographic environment. We also demonstrated that leaf  $\delta^{13}\text{C}$  values displayed a linear increase in altitudinal direction. The differences in  $\delta^{13}\text{C}$  values were likely caused by stomatal limitation rather than by nutrient-related changes in photosynthetic efficiency because  $\delta^{13}\text{C}$  values in *R. natans* were not correlated with foliar N concentrations. Our data support the previously proposed temperature–plant physiology hypothesis because there is a negative relationship between leaf N% of *R. natans* and temperature of water body where *R. natans* inhabits.

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### 1. Introduction

Plant carbon (C) and nitrogen (N) concentrations and stoichiometry are powerful indicators of diverse ecological processes (Zhang et al., 2013), such as species composition, distributions and abundances, population dynamics (Venterink et al., 2003; Ågren, 2004; Moe et al., 2005; Watanabe et al., 2007), plant–herbivore–predator relationships (Elser et al., 2000; Zhang et al., 2011), and the adaption to environment (Li et al., 2014). Due to these important applications, patterns of leaf stoichiometry and controlling factors in terrestrial and aquatic ecosystems have been widely studied at a various spatial scales in the past few decades (Duarte, 1992; Elser et al., 2000; Reich and Oleksyn, 2004; Han et al., 2005, 2011; He et al., 2006; Van de Waal et al., 2010; Anderson and Lockaby, 2011; Sardans et al., 2012; Qiu et al., 2013;

Li et al., 2013; Wu et al., 2014; Xia et al., 2014; Wang et al., 2015). Several recently observed patterns of foliar nutrient concentrations, based on literature data or field experiments, suggest the existence of significant trends in leaf N concentration along latitudinal, altitudinal and climatic gradients at local, regional and global scales (Reich and Oleksyn, 2004; Han et al., 2005; He et al., 2006; Wu et al., 2014). Several factors, such as climate, soil and plant growth form, are thought to be the primary factors influencing the leaf nutrient concentrations and stoichiometry (McGroddy et al., 2004; Reich and Oleksyn, 2004; Van de Waal et al., 2010; Wu et al., 2012; Chen et al., 2013). Two main hypotheses have been developed to explain these correlations. The biogeochemical hypothesis assumes that soil nutrient availability, which is influenced by precipitation through leaching effects, is the main driver of leaf nutrient concentration (McGroddy et al., 2004; Reich and Oleksyn, 2004). The plant physiology hypothesis proposes that plant metabolic processes are temperature sensitive, and that increases in N concentration can compensate for decreases in metabolic rate at low temperature (Reich and Oleksyn, 2004) or

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high latitude (Van de Waal et al., 2010). However, the influence of geographical and climatic factors on nutrient stoichiometry differs with scale studied. At the global scale, foliar N% increases from the warmer tropics to the cooler and drier mid-latitudes (McGroddy et al., 2004; Reich and Oleksyn, 2004; Kerkhoff et al., 2005). At regional or smaller spatial scales, insignificant correlations between geographical and climatic factors and leaf nutrient stoichiometry have been observed (Townsend et al., 2007; Zheng and Shangguan, 2007; He et al., 2008). These inconsistencies indicate that plant stoichiometry is far from clear. Also, to our knowledge, few studies have attempted to document the pattern of stoichiometry for aquatic macrophytes, despite aquatic higher plants being long-recognized as suitable models for the study of physiological variation (e.g., Lynn and Waldren, 2001; Greulich et al., 2001), due to wide distribution and limited genetic variation (Santamaría et al., 2003).

During plant development and growth, plant foliar C:N stoichiometry responds to environmental conditions and physiological constraints (Zhang et al., 2013). For different plant genera and species, the patterns of variation in nutrient concentrations differ substantially in relation to environment factors (He et al., 2006, 2008). Identifying patterns of climatic control of foliar physiological traits within genera or species should better allow us to ascribe causation of foliar stoichiometry to climate factors (He et al., 2008). However, previous studies were often conducted at large scales and plant species were taken as a whole regardless of inter-specific marked differences in nutrient stoichiometry. Recent studies have focused on leaf stoichiometry of a given genus (He et al., 2008; Gotelli et al., 2008; Wu et al., 2012), or species (Kang et al., 2011; Qiu et al., 2013; Li et al., 2014; Wu et al., 2014) in terrestrial plant, and its correlations with edaphic and climatic factors. These studies have demonstrated a non-linear leaf N %-latitude relationship at genus (Wu et al., 2012) or species (Kang et al., 2011; Wu et al., 2014) levels, which differ from the linear latitudinal decline for the global or regional flora reported in other studies (Reich and Oleksyn, 2004; He et al., 2008). The relationship between leaf stoichiometry and climatic, geographic variables for a single genus or species remains uncertain (Wu et al., 2012), and whether a stable range of stoichiometry exists for a given species also remain unclear (Townsend et al., 2007; Elser et al., 2010). Therefore, it is important to investigate the patterns of stoichiometric flexibility within plant species (Elser et al., 2010).

Analysis of stable carbon isotope composition ( $\delta^{13}\text{C}$ ) is an important tool in research on plant physiological ecology. Currently, data about foliar stable carbon isotopes is used to analyze intra-specific or inter-specific differences in photosynthetic and physiological characteristics (Ma et al., 2005; Li et al., 2007; Zheng and Shangguan, 2007), to understand water use efficiency under different environmental conditions (Körner et al., 1988) and to reveal significant functional changes in plant metabolism and responses to different environmental stressors. Plant  $\delta^{13}\text{C}$  is influenced by various abiotic factors such as precipitation (Ma et al., 2005; Zheng and Shangguan, 2007), humidity (Ma et al., 2005), soil moisture (Sun et al., 1996), light (Ehleringer et al., 1986), air temperature (Panek and Waring, 1995); atmospheric  $\text{CO}_2$  concentration (Ehleringer and Cerling, 1995), and barometric pressure (Marshall and Zhang, 1994). Many studies have reported that plant  $\delta^{13}\text{C}$  values correlate with altitude, latitude and longitude. However, the available data regarding plant  $\delta^{13}\text{C}$  values in response to these changes indicate considerable differences (Kelly and Woodward, 1995; Zhang and Marshall, 1995; Friend et al., 1989; Hultine and Marshall, 2000). For example, Hultine and Marshall (2000) reported an increase in  $\delta^{13}\text{C}$  values with altitudinal gradients whereas Friend et al. (1989) suggested that  $\delta^{13}\text{C}$  values either decreased or did not change at all with increasing altitude. These conflicting results highlight that further

studies are needed to understand how geographical factors influence plant  $\delta^{13}\text{C}$  values.

In the arid zone of northwest China, extreme aridity gradients exist over relatively short distances in both the west–east and north–south directions (Feng et al., 1989; Tang et al., 1992). Over such environmental transects, plants encounter a variety of microclimates differing in temperature, soil moisture and vapor pressure gradients, each of which may influence the variation of plant nutrient stoichiometry. *Ranunculus natans* is an ideal plant for this study because it has a wide ecogeographic range, exhibits a wide tolerance to different environmental conditions, and shows considerable morphological variation which correlates with local environmental conditions (Li and Yu, 2009). So, we hypothesized that (1) foliar C%, N%, C:N stoichiometry and  $\delta^{13}\text{C}$  values of *R. natans* in the arid zone of northwest China shift consistently along an environmental gradient, (2) temperature and temperature relative factors drive changes in plant foliar C%, N%, C:N stoichiometry and  $\delta^{13}\text{C}$  values. To address the hypotheses we aimed to: (1) describe variation of leaf C%, N%, C:N stoichiometry and  $\delta^{13}\text{C}$  values of *R. natans* among 26 sites; (2) assess the relationships between variation in leaf C%, N%, C:N stoichiometry and  $\delta^{13}\text{C}$  values and environmental, geographical, and climatic factors; (3) test temperature-plant physiological hypotheses and biogeochemical hypotheses. The study of conspecific populations distributed along clear geographical and environmental gradients such as gradients of water quantity, water availability and temperature can help to elucidate the physiological effects of natural selection and reveals different strategies of plant adaptive potential.

## 2. Materials and methods

### 2.1. Study area

The arid zone (35°30′–49°N, 73°–106°E) is a land-locked region located in northwestern China (Fig. 1). This area is surrounded by the Qinghai-Tibet plateau and many high mountains (Tang et al., 1992). The climate is generally water limited, and steppe biomes are prevalent. The annual rainfall in the arid zone is less than 250 mm, with some areas receiving less than 100 mm annually, but the annual evaporative capability is above 2000 mm. The mean annual temperature is 2–6 °C, with the maximum monthly mean temperature over 28 °C and the minimum monthly mean temperature below –16 °C, and the daily temperature fluctuates significantly (Feng et al., 1989).

### 2.2. Sampling and measurements

We studied living *R. natans* in the wild, from 26 different populations located in the arid zone of northwest China between August and September in 2004 (Fig. 1). The sampling locations ranged from 37° to 44°N, 75° to 109°E, and the altitude varied from 423 m to 3156 m above sea level. All samples were collected from robust, mature plants, growing in unshaded habitats. Sun-exposed and newly matured leaves (leaf blades) of three plants at each site were collected. At each site, latitude, longitude and altitude were recorded using the global positioning system. We also measured the pH, water temperature, conductivity, and salinity of each water body using an HORIBA U-10 Water Quality Checker (HORIBA, Japan).

Plants collected at each site were placed in paper envelopes, and dried in the sun. These samples were dried to constant mass at 60 °C for 72 h in the oven upon returning to the laboratory. All dried leaf samples of each plant were ground to a fine powder with a mortar in the laboratory. Total C and N concentration were determined on 5–6 mg of homogeneously ground material of each

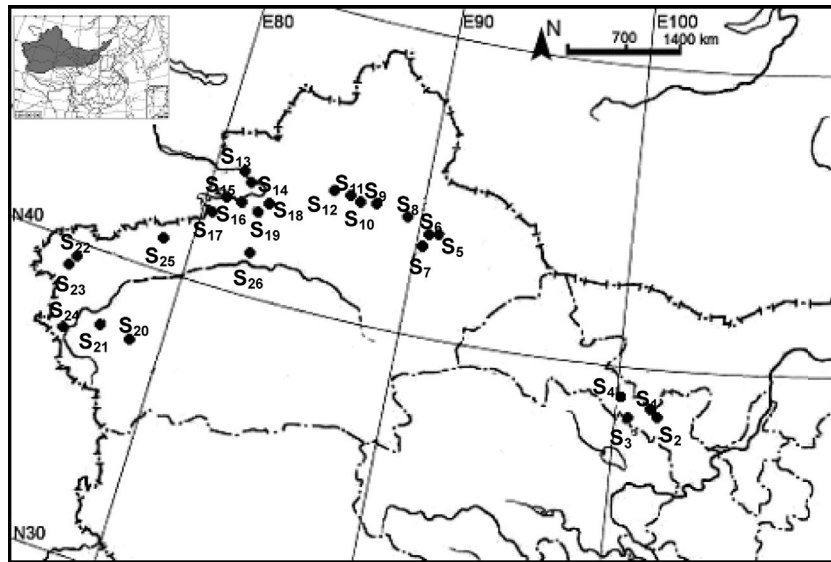


Fig. 1. Map showing the collection sites (labelled  $S_1$ – $S_{26}$ ) of *Ranunculus natans*, and its location in the arid zone of Northwest China.

plant using an elemental analyzer (NA2500, Carlo Erba Reagenti, Milan, Italy). Also, the  $\delta^{13}\text{C}$  values were measured with a Delta Plus (Finnigan MAT, Bremen, Germany) continuous-flow isotope ratio mass spectrometer coupled to a Carlo Erba NA2500 elemental analyzer (Carlo Erba Reagenti, Milan, Italy). Stable isotope ratios were expressed in denotation as parts per thousand (‰) deviation from the international standards according to the equation:  $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where X is  $^{13}\text{C}$  and R is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$ . The standard for carbon was Vienna Pee Dee Belemnite. The reference material for  $\delta^{13}\text{C}$  was carbonatite (IAEA–NBS18), supplied by the U.S. Geological Service (Denver, CO, USA) and certified by the International Atomic Energy Agency (Vienna, Austria). An internal working standard, urea ( $\delta^{13}\text{C} = -49.44\text{‰}$ ) was employed daily throughout the study. The average standard deviation of replicate measurements for  $\delta^{13}\text{C}$  was 0.3‰.

As all the sites were located in remote areas and direct measurements of climate were not possible, monthly mean temperature (MMT) and monthly precipitation (MP) for each site, were estimated according to climate data from the meteorological stations in each region in order to develop linear models for MMT and MP using latitude, longitude and altitude as predictors (Fang et al., 2001; Piao et al., 2003). The model formula is given by:

$$\text{MMT}(\text{MP}) = a \times \text{Latitude} + b \times \text{Longitude} + c \times \text{Altitude} + d$$

where  $a$ ,  $b$ ,  $c$ , and  $d$  are the regression coefficients.

According to estimated monthly mean temperature and monthly precipitation, mean annual temperature (MAT) and mean annual precipitation (MAP) of each site were calculated.

### 2.3. Data analysis

Statistical analysis was carried out using SPSS 17.0. For Leaf C%, N%, C:N and  $\delta^{13}\text{C}$ , the mean, standard error and coefficients of variation (CV, standard deviation/mean) were calculated. Analysis of variance was applied to determine the statistical significance of the differences of Leaf C%, N%, C:N and  $\delta^{13}\text{C}$  from different collection sites ( $P < 0.05$ ). Before performing one-way ANOVA, all data were tested for normality and homogeneity. Non-normal data were transformed ( $\log_{10}$ ) to obtain normality.

We used principal component analysis (PCA) as a preliminary correlation analyses to explore patterns of variation in leaf C%, N%, C:N and  $\delta^{13}\text{C}$  explained by the environmental, geographical and

climatic variables. All data were standardized by conversion to standard deviation units for the PCA. Data analyses were conducted using the computer program STATISTICA (Version 6.0 for Windows, StatSoft Inc., Tulsa, Oklahoma, USA). Univariate linear regression analyses (Pearson correlation) were applied to examine the effects of environmental variables on leaf nutrient concentrations and  $\delta^{13}\text{C}$  traits. The significance of all statistical analyses was tested at the  $P = 0.05$  level.

## 3. Results

### 3.1. Pattern and variation of leaf C%, N%, C:N and $\delta^{13}\text{C}$ of *R. natans* from 26 different sites

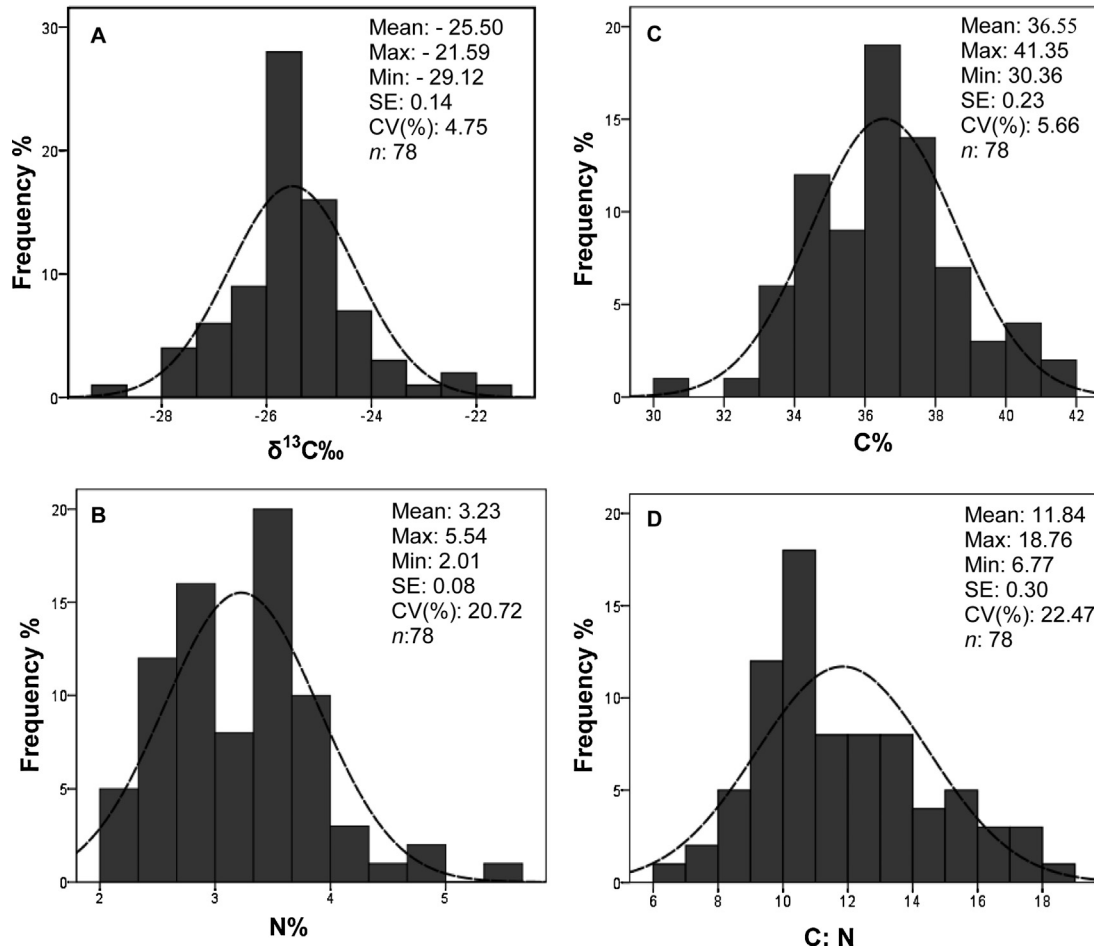
The frequency distribution of leaf C%, N%, C:N and  $\delta^{13}\text{C}$  is shown in Fig. 2. Leaf C%, N%, C:N and  $\delta^{13}\text{C}$  of *R. natans* varied substantially across the study areas. The mean leaf C% and N% were 36.55% and 3.23%, respectively, with a range of 30.36–41.35% for C% and 2.01–5.54% for N%. All  $\delta^{13}\text{C}$  values of samples varied from  $-29.12\text{‰}$  to  $-21.59\text{‰}$ , with a mean of  $-25.5\text{‰}$  and with a coefficient of variation of 4.75%, the smallest among four leaf traits measured. Leaf C:N varied from 6.77 to 11.84, and had the largest CV (22.47%). According to Sun et al. (2012), these variations can be roughly divided into three groups: constant ( $\delta^{13}\text{C}$ ;  $\text{CV} < 5\%$ ), low variability (C%;  $\text{CV} 5\text{--}10\%$ ), and high variability (N%, C:N;  $\text{CV} > 15\%$ ) (Fig. 2).

The C:N ratios were correlated with leaf C% and negatively with leaf N% for *R. natans* sampled in this survey, but the correlations between C:N and leaf N% were much higher than between C:N and leaf C% (Fig. 3). However, weak correlations between leaf N% and C% were observed in this study. Similarly,  $\delta^{13}\text{C}$  was generally not related to leaf N%, C% or C:N (Fig. 3).

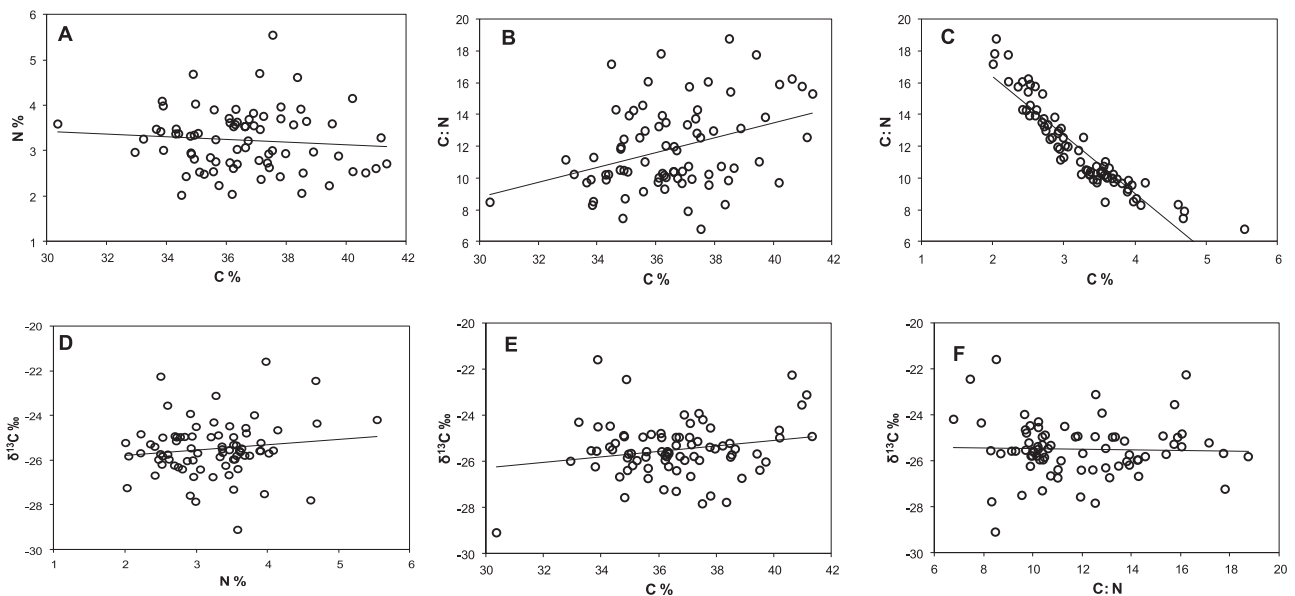
Leaf N%, C:N ratios and  $\delta^{13}\text{C}$  varied significantly among the 26 *R. natans* collection sites. According to  $F$ -values, foliar traits differed in the degree of variation described by the three variables.  $\delta^{13}\text{C}$  ranked first, followed by C:N ratios, leaf N% (Table 1). However, leaf C% did not differ significantly (Table 1).

### 3.2. Relationships between the leaf C%, N%, C:N and $\delta^{13}\text{C}$ and geographical and environmental factors

The leaf C%, N%, C:N and  $\delta^{13}\text{C}$  were partly affected by local habitat and environmental conditions. In the PCA, habitat and



**Fig. 2.** Frequency distributions of carbon isotope ratios ( $\delta^{13}\text{C}\text{‰}$ ) (A), nitrogen (N%) (B), carbon (C%) (C), and carbon and nitrogen ratio (C:N) (D) of *Ranunculus natans*, collected in Northwest China. Dashed lines indicate fitted log-normal curves, and frequency scale differs between  $\delta^{13}\text{C}$  and the rest. (Mean value: Mean; Maximum value: Max; Minimum value: Min; Standard error: SE; Coefficients of variation: CV %).



**Fig. 3.** Scatter plots showing the relationships among leaf carbon isotope ratios ( $\delta^{13}\text{C}\text{‰}$ ), nitrogen (N%), carbon (C%), and C:N ratio of *Ranunculus natans* across 26 sample sites. (A) N% and C% ( $r^2=0.008$ ,  $P=0.45$ ,  $n=78$ ), (B) C:N and C% ( $r^2=0.14$ ,  $P=0.001$ ,  $n=78$ ), (C) C:N and N% ( $r^2=0.86$ ,  $P<0.0001$ ,  $n=78$ ), (D)  $\delta^{13}\text{C}$  and N% ( $r^2=0.02$ ,  $P=0.20$ ,  $n=78$ ), (E)  $\delta^{13}\text{C}$  and C% ( $r^2=0.03$ ,  $P=0.18$ ,  $n=78$ ), and (F)  $\delta^{13}\text{C}$  and C:N ( $r^2=0.004$ ,  $P=0.61$ ,  $n=78$ ) are displayed.

**Table 1**

Results of the ANOVA (*F*-value) of leaf nitrogen (N%), carbon (C%), and carbon and nitrogen ratio (C:N) and carbon isotope ratios ( $\delta^{13}\text{C}_{\text{‰}}$ ), of *Ranunculus natans*, among the 26 collection sites in the study ( $n = 78$ ).

	d.f.	<i>F</i>	Sig.
N%	25	3.39	.000
C%	25	1.16	.322
C:N	25	3.96	.000
$\delta^{13}\text{C}_{\text{‰}}$	25	10.13	.000

d.f., degrees of freedom; *F*, *F*-value; Sig., significance level.

environmental conditions occupied different positions along the PC<sub>1</sub> and PC<sub>2</sub> axes (Fig. 4). Altitude, latitude, longitude, and  $\delta^{13}\text{C}$  influenced PC<sub>1</sub>, which explained 21.21% of total variance. PC<sub>2</sub>, which explained 18.82% of the total variance, was influenced primarily by leaf C%, N%, C:N and habitat variables such as water temperature (Fig. 4). Linear regression analyses indicated that water temperature was negatively related to leaf N%, and positively to C:N ratio.  $\delta^{13}\text{C}$  was significantly related to altitude and slightly related to water temperature (Table 2).

### 3.3. Relationships between the leaf C%, N%, C:N and $\delta^{13}\text{C}$ and climatic variables

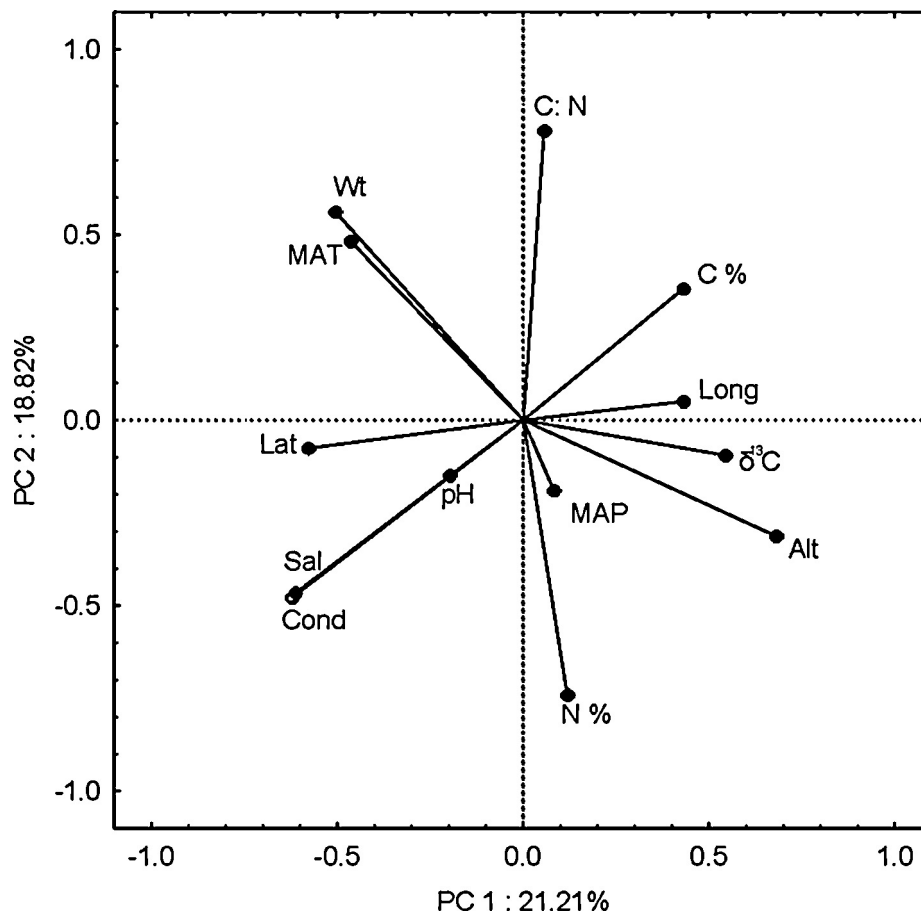
The results of the present study indicated that foliar  $\delta^{13}\text{C}$  values were highly and negatively correlated with the MAT and MAP. There were no significant correlations between leaf C%, N%, C:N and MAT and MAP (Fig. 5).

## 4. Discussion

### 4.1. Patterns of leaf C%, N% and C:N stoichiometry traits of *R. natans*

*R. natans* is widely distributed throughout the arid zone of northwest China and is one of the common emergent species in wetlands of north China (Li and Yu, 2009). Our study showed that concentrations of leaf C%, N%, and C:N ratio ranged narrowly (<1.2-fold for C%, and 2.8-fold for N% and C:N ratio) across *R. natans* collected in 26 sites in northwest China. Homeostasis constrains the elemental composition of individual species to a narrow range, regardless of chemical composition of the environment (Elser et al., 2010; Yu et al., 2011). Despite significant differences in leaf N% and C:N ratios among the 26 *R. natans* across northwest China, variances were substantially lower than those reported for herbs in Xinjiang (northwest China) and in the grassland biomes of China (He et al., 2006), which suggests that leaf C:N ratios are constrained to relatively stable range among *R. natans* populations.

The mean leaf C% of *R. natans* was 36.55%, nearly identical to that reported by Duarte (1992) for 11 aquatic angiosperms, to that reported by Xia et al. (2014) for 122 aquatic plant species in eastern China, and to that reported by Wang et al. (2015) for 154 aquatic plant species in Tibetan Plateau in China. However, the mean leaf C% was significantly lower than that of 103 herb species observed in Chinese grassland biomes by He et al. (2006). One possible explanation for this is that the low leaf C% in this emergent plant results from the relatively low contents of lignin and cellulose in aquatic plant (Santamaría et al., 2003).



**Fig. 4.** PCA based on environmental factors and leaf traits of *R. natans* from 26 sites. Environmental factors: Alt, altitude; Long, longitude; Lat, latitude; Sal, salinity; Cond, conductivity; Wt, water temperature; MAT, mean annual temperature and MAP, mean annual precipitation. Leaf traits: nitrogen, N%, carbon, C%, and C:N and carbon isotope ratios,  $\delta^{13}\text{C}$ .



**Table 2**

Pearson correlation coefficients between mean leaf N%, C% and C:N ratio and  $\delta^{13}\text{C}_{\text{‰}}$  of *R. natans* and environmental variables.

	Lat	Long	Alt	pH	Cond	Wt	Sal
N%	-0.04	0.01	0.003	-0.18	0.05	-0.29*	0.03
C%	-0.12	0.22	0.12	0.01	-0.23	-0.01	-0.22
C:N	-0.13	-0.06	-0.04	0.09	-0.13	0.22*	-0.11
$\delta^{13}\text{C}_{\text{‰}}$	-0.20	0.06	0.32**	-0.06	-0.21	-0.29*	-0.19

Lat, latitude; Alt, altitude; Long, longitude; Cond, conductivity; Wt, water temperature; Sal, salinity.

\*  $p < 0.05$ .

\*\*  $p < 0.001$ .

For leaf N%, our investigation showed that mean values of leaf N% of *R. natans* was 3.23%, significantly higher than that of the 11 aquatic angiosperms reported by Duarte (1992), that of the 122 aquatic plant species in eastern China reported by Xia et al. (2014), as well as the 154 aquatic plant species in Tibetan Plateau in China reported by Wang et al. (2015) respectively. However, our estimates were almost the same to 3.14% for another common emergent plant *Phragmites australis* in northern China as reported by Li et al. (2014), and close to 3.06% for 103 herb species in grassland biomes of China (He et al., 2006). Plant leaf N% is considered an important indicator of the adaptation of plants to abiotic factors in the environment (Li et al., 2014). Within taxonomic groups, the chemical compositions of leaves are partly affected by the intrinsic characteristics of vegetation such as physiological properties, and are also partly influenced by local habitat and environmental conditions (Rotundo and Westgate, 2009). As expected, significant differences were found among *R. natans* collected for Leaf N% and C:N ratio (Table 1). These intra-specific variations may reflect physiological adaptation to local environments because leaf nitrogen is strongly correlated to the concentration of leaf nitrogen compounds (RuBPCarbo-xylase and chlorophyll in particular) in photosynthesis (Evans, 1989) and can reflect the dynamics of net photosynthesis per individual (Reich et al., 1994). This stoichiometric mechanism could contribute to the wide distribution of *R. natans* whether in favorable or stressed environments. Surprisingly, leaf C% does not differ significantly in this study and one possible explanation for this is that carbon fulfils primarily a structural role (Anderson et al., 2004), because stable carbon content may be associated with leaf rigidity required for persistence through resistance to climatic and mechanical stress.

The C:N ratio in *R. natans* in the study also exhibited some differences with the corresponding value in both terrestrial plants and aquatic plants in China. The average C:N ratio of *R. natans* was 11.84, which is slightly lower than that observed for 154 aquatic plant species in Tibetan Plateau in China reported by (Wang et al., 2015), and significantly lower than that of 103 herb species in Chinese grassland biomes reported by (He et al., 2006) and that of 122 aquatic plant species in eastern China reported by (Xia et al., 2014). The lower C:N ratios observed in this study may be the result of the lower C concentration and higher N concentration (Fig. 2). Our study found that the correlations between C:N and leaf N% were much higher than between C:N and leaf C% (Fig. 3), which reflects that the variation in C:N ratio is primarily determined by leaf N%. However, the weak correlation between leaf N% and C% (Fig. 3) suggests the different, and important structural role of C, which uncouples it from metabolic related variation in the concentrations of N (Duarte, 1992).

#### 4.2. Relationships between leaf C%, N% and C:N stoichiometry traits and environmental variable for *R. natans*

Geographical and climatic factors can influence the variation of plant nutrient stoichiometry. Until now, the available data regarding leaf N% and C:N stoichiometry in response to

environmental changes indicate considerable differences (Elser et al., 2000; Reich and Oleksyn et al., 2004; Han et al., 2005, 2011; He et al., 2006, 2008; Van de Waal et al., 2010). Variability in foliar N%, C:N stoichiometry across diverse habitats emerges from two interacting processes: (1) macro-scale constraints caused by specific geographic environment (i.e., climate and soil), and (2) fundamental physiological constraints resulting from plant growth, development, metabolism, phenological and life history traits (McGroddy et al., 2004). Our findings indicate that in macro-scale foliar N% and C:N ratio do not directly correlate with geographical factors (latitude, longitude and altitude) (Table 2) or meteorological factors (MAT and MAP) (Fig. 5), which is in agreement with previous studies conducted in the grassland biomes of China (He et al., 2006). The weak relationships observed between foliar N% and C:N ratio and geographic and climatic variables may result from plant growth, development, metabolism, phenological and life history traits, rather than from the specific geographic environment. The data support the idea that climate affect leaf N% mainly through a change in plant species composition rather than via climate itself (He et al., 2006, 2008).

It is notable that the foliar N% and C:N ratio directly correlated with water temperature of habitat, despite the weak relationships found between foliar N% and C:N ratio and MAT (Table 2, Fig. 5). This may be because water temperature, rather than air temperature, can directly affect sediment temperature where aquatic plants grow. Moreover, at mid- to high-latitudes, the mean and variation in water temperatures are far more moderate than in air (Luning, 1993; Reynolds and Smith, 1995), leading to increasing disparity between air and water temperature in mean values and seasonal amplitude with increasing latitude. So, for aquatic plants, water body temperature is more important than MAT as an influence on plant physiology. Thus, our data support the previously proposed the temperature-plant physiology hypothesis that suggest plant metabolic processes are temperature sensitive, and that increases in N concentration can compensate for decreases in metabolic rate at low temperature (Reich and Oleksyn, 2004).

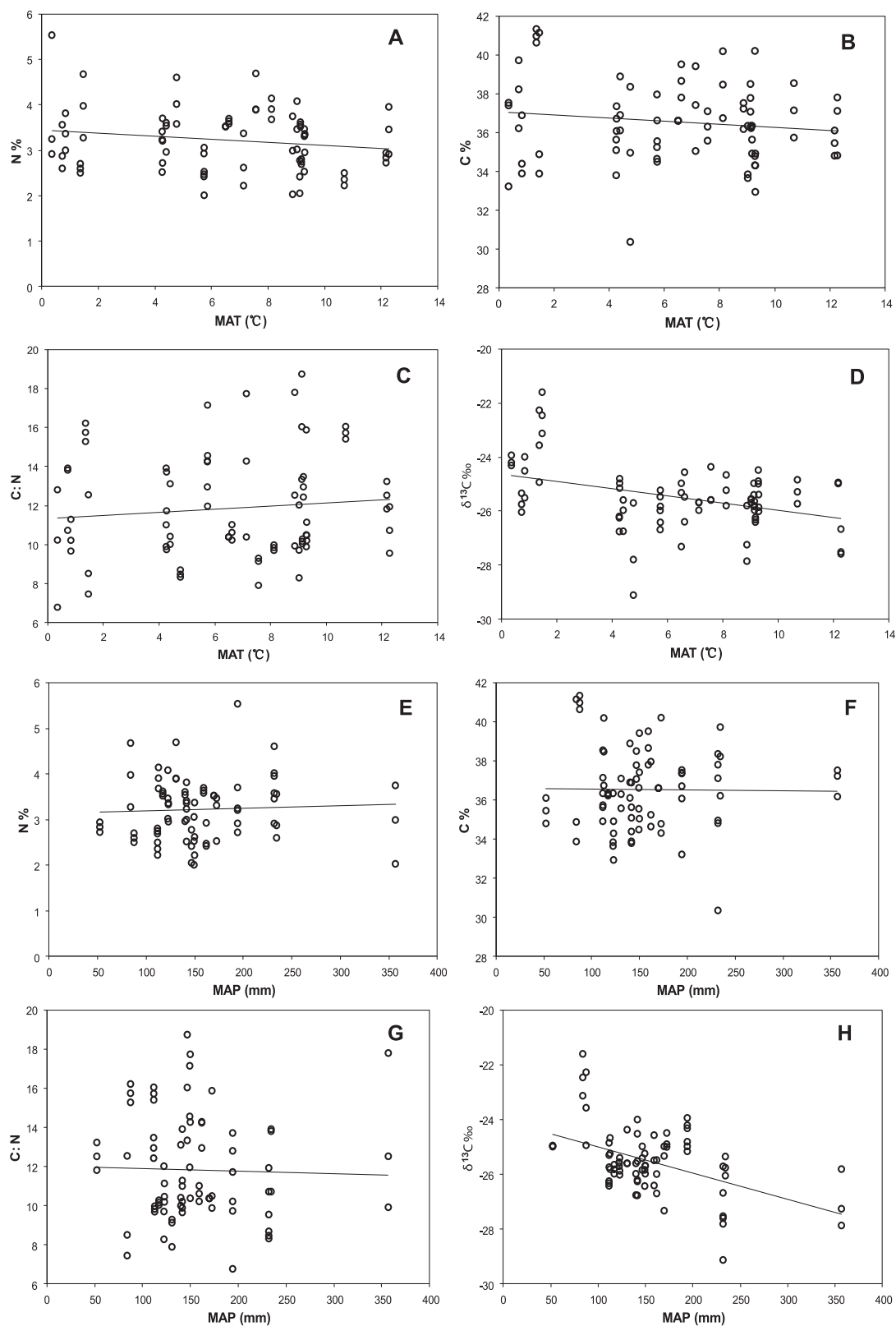
#### 4.3. $\delta^{13}\text{C}$ composition characteristics of *R. natans*

All  $\delta^{13}\text{C}$  values of the samples in this study ranged from  $-21.59\text{‰}$  to  $-29.12\text{‰}$ , with a mean of  $-25.50\text{‰}$  (Fig. 2). The  $\delta^{13}\text{C}$  values of *R. natans* were almost the same to  $-25.30\text{‰}$  for *Ranunculus japonicus* reported by Chen et al. (2007) in north China, and fell within the range from  $-23.63\text{‰}$  to  $-29.53\text{‰}$  of 20 species observed in the genus *Ranunculus* (Körner et al., 1991). Plants with different photosynthetic pathways ( $\text{C}_3$ ,  $\text{C}_4$  and CAM) have different foliar  $\delta^{13}\text{C}$  values because they have different primary carboxylation enzymes (Farquhar et al., 1989). Generally,  $\delta^{13}\text{C}$  values of  $\text{C}_3$  species vary from  $-20\text{‰}$  to  $-35\text{‰}$ , averaging  $-27\text{‰}$  (O'Leray, 1988). Therefore, it is confirmed that *R. natans* belong to  $\text{C}_3$  photosynthetic pathways.

Significant differences were found among collected *R. natans* for foliar  $\delta^{13}\text{C}$  values, which reflect significant functional changes in plant metabolism and responses to various environmental stressors. As expected, our study showed that foliar  $\delta^{13}\text{C}$  values in *R. natans* were not correlated with foliar C, N concentrations and C:N (Fig. 3). It is suggested that variations foliar  $\delta^{13}\text{C}$  values in *R. natans* were likely caused by stomatal limitation rather than by nutrient-related changes in photosynthetic capacity (Ma et al., 2005).

#### 4.4. Relationship between foliar $\delta^{13}\text{C}$ values and environmental factors

Environmental factors, such as precipitation, temperature, humidity, light, atmospheric pressure, air  $\text{CO}_2$  concentration,



**Fig. 5.** Relationship between mean annual temperature (MAT) and mean annual precipitation (MAP) and leaf carbon isotope ratios ( $\delta^{13}\text{C}$ ‰), nitrogen (N%), carbon (C%), and Carbon and nitrogen ratio (C:N). Linear fits for (A) leaf N% and MAT ( $r^2 = 0.03$ ,  $P = 0.13$ ,  $n = 78$ ), (B) leaf C% and MAT ( $r^2 = 0.02$ ,  $P = 0.23$ ,  $n = 78$ ), (C) C:N and MAT ( $r^2 = 0.02$ ,  $P = 0.36$ ,  $n = 78$ ), (D)  $\delta^{13}\text{C}$  and MAT ( $r^2 = 0.15$ ,  $P < 0.0001$ ,  $n = 78$ ), (E) leaf N% and MAP ( $r^2 = 0.003$ ,  $P = 0.65$ ,  $n = 78$ ), (F) leaf C% and MAP ( $r^2 = 0.0001$ ,  $P = 0.93$ ,  $n = 78$ ), (G) C:N and MAP ( $r^2 = 0.001$ ,  $P = 0.79$ ,  $n = 78$ ), (H)  $\delta^{13}\text{C}$  and MAP ( $r^2 = 0.16$ ,  $P < 0.0001$ ,  $n = 78$ ) are displayed.

and soil moisture can affect foliar gas exchange activity in plants and, thus, the  $\delta^{13}\text{C}$  composition of plants (Sparks and Ehleringer, 1997; Dawson et al., 2002). Temperature is one of the most important factors influencing carbon isotope fractionation (Körner et al., 1988) because temperature can directly affect enzyme activity of photosynthetic as well as stomatal conductance and  $\text{CO}_2$  assimilation (Körner et al., 1988). Numerous studies have demonstrated that there is a significant, negative correlation between temperature and plant  $\delta^{13}\text{C}$  value (O'Leray, 1988; Körner et al., 1988; Li et al., 2007; Zheng and Shangguan, 2007), while some field studies show the opposite trends (Stuiver and Braziunas, 1987; Ma et al., 2005). Our results demonstrated that temperature seems to be the dominant environmental factor to affect the variation of leaf  $\delta^{13}\text{C}$  value. The  $\delta^{13}\text{C}$  values of plants showed marked variation with the decreasing of both water temperature of its habitat and MAT (Table 2, Fig. 5). The increase of  $\delta^{13}\text{C}$  value may be associated with decreasing activities of leaf enzymes caused by low temperature (Guillemette and Stephen, 2001). Another importance reason for increase of plant  $\delta^{13}\text{C}$  value may be that cooler soil, as a result of reduced water temperature and air temperatures, inhibits stem sap flow, which increases the water potential gradient and induces partial stomatal closure (Smith et al., 1984).

Precipitation is another key factor that affects foliar  $\delta^{13}\text{C}$  value (O'Leray, 1988; Farquhar et al., 1989). Other research has shown that foliar  $\delta^{13}\text{C}$  values are negatively correlated with precipitation (Austin and Vitousek, 1998; Van de Water et al., 2002; Ma et al., 2005; Zheng and Shangguan, 2007). Interestingly, our study demonstrated that the foliar  $\delta^{13}\text{C}$  values of *R. natans* distributed across arid zone in northwest China were highly and negatively correlated with the MAP ( $P < 0.0001$ ; Fig. 5) although *R. natans* inhabits a wetland where water is not a limiting factor. One possible explanation for this is the effect of precipitation on relative humidity and moisture availability because there are significant differences in water availability for different wetlands due to low precipitation, high evaporation, high pH, salinity and conductivity of water body in the arid zone (Feng et al., 1989; Tang et al., 1992). Low precipitation, low air humidity and low soil water availability in wetland in the arid zone would lead to stomatal closure and decreases in stomatal conductance and interior carbon dioxide concentration (Ma et al., 2005), and then to the  $\delta^{13}\text{C}$  value increase.

Average  $\delta^{13}\text{C}$  values at each site in this study varied from  $-22.39\text{‰}$  to  $-27.54\text{‰}$  along the altitude gradient from 423 to 3156 m. The rate of change of  $\delta^{13}\text{C}$  value ( $+1.88\text{‰ km}^{-1}$ ) was much higher than the global trend of increasing  $\delta^{13}\text{C}$  ( $+1.2\text{‰ km}^{-1}$ ) with altitude (Körner et al., 1988) and the trend of increasing  $\delta^{13}\text{C}$  ( $+1.1\text{‰ km}^{-1}$ ) with altitude in the Qinghai-Tibet plateau (Li et al., 2007), which was probably related to plant species and sampling areas. Our findings indicated that the  $\delta^{13}\text{C}$  values of plants showed marked variation with the increasing of altitude ( $P < 0.001$ ; Table 2). There are two possible causes for high  $\delta^{13}\text{C}$  value at a high altitude (Körner et al., 1988). Firstly, the elevation of altitude induces a decrease of  $\text{CO}_2$  partial pressure, variations will lead to increases the  $\delta^{13}\text{C}$  values in plant tissues. Secondly, low temperature at higher altitude reduces the stomatal conductance and then decreases the ratio, therefore, the discrimination for  $^{13}\text{C}$  is relatively reduced (Körner et al., 1988). And decreasing activities of leaf enzymes during the process of photosynthesis at high elevation caused by low temperature is another important reason for increase of plant  $\delta^{13}\text{C}$  values (Guillemette and Stephen, 2001).

In this study, the foliar  $\delta^{13}\text{C}$  of this species were not related to the latitudes (Table 2), which was inconsistent with previous observations (Ma et al., 2005; Zheng and Shangguan, 2007). Different results in previous studies may be attributable to different sampling strategies used and the species investigated.

Different combinations and interactions of environmental and geographical factors in each local environment is another important reasons for offsetting the effects of latitude on  $\delta^{13}\text{C}$  values.

## 5. Concluding remarks

In this study, the weak relationships found between foliar N concentrations and C:N of *R. natans* in the arid zone of northwest China and altitude, latitude and longitude indicate variability in foliar N%, C:N stoichiometry across diverse habitats. These relationships may be a result of variation in plant growth, development, metabolism, phenological and life history traits, rather than from geographic environment variation. Also, our data support the previously proposed temperature-plant physiology hypothesis because there is a significantly negative relationship between leaf N% and temperature of water body where *R. natans* inhabits. Leaf  $\delta^{13}\text{C}$  values displayed a linear increase in altitudinal direction. The differences in  $\delta^{13}\text{C}$  values were likely caused by stomatal limitation rather than by nutrient-related changes in photosynthetic efficiency. Obviously, complex environmental factors including nutritional availability of nutrients such nitrogen and phosphorous likely play substantial role in physiological traits. Thus, further research is needed to perform to investigate the effects of other factors on leaf trait variation.

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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.2015.01.010>.

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