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# Variation in foliar $\delta^{15}$ N among oriental oak (*Quercus variabilis*) stands over eastern China: Patterns and interactions

Hongzhang Kang <sup>a,b,\*</sup>, Chunjiang Liu <sup>a,c</sup>, Wenjuan Yu <sup>d</sup>, Lili Wu <sup>a</sup>, Dongmei Chen <sup>a</sup>, Xiao Sun <sup>a</sup>, Xiaoping Ma <sup>a</sup>, Haibo Hu <sup>e</sup>, Xueling Zhu <sup>f</sup>

<sup>a</sup> School of Agriculture and Biology, Shanghai Jiao Tong University, Dongchuan Rd. 800, Shanghai 200240, PR China

<sup>b</sup> Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, People's Republic of China, Dongchuan Rd. 800, Shanghai 200240, PR China

<sup>c</sup> SJTU Research Centre for Low Carbon Agriculture, Dongchuan Rd. 800, Shanghai 200240, PR China

<sup>d</sup> Instrumental Analysis Center of Shanghai Jiao Tong University, Dongchuan Rd. 800, Shanghai 200240, PR China.

<sup>e</sup> Xiashu Ecological Research Station, Nanjing Forestry University, Longpan Rd. 159, Nanjing, Jiangsu 210037, PR China

<sup>f</sup> Baotianman National Leveled Nature Reserve Management Bureau, Tuanhe Rd. 54, Tuandong, Chengguan Town, Neixiang, Henan 474350, PR China

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### ABSTRACT

The patterns of variation in natural abundance of foliar and soil ( $^{15}N$ ) (expressed as  $\delta^{15}N$ ) related to geographical and climatic variables frequently vary over plant species or plant function groups. It is essential to identify the pattern of foliar  $\delta^{15}$ N for specific plant species which has a widespread distribution and to explore the underlying mechanisms in the context of environmental change such as global warming and anthropogenic nitrogen deposition. Oriental oak (Quercus variabilis Bl.), characterized by cork bark, is a widely distributing deciduous broadleaf tree species that can be found in temperate and subtropical areas of East Asia. In this study, the variations in foliar and soil <sup>15</sup>N were investigated based on the data of leaf and soil samples collected in 22 stands over eastern China (from 24 to 41° N in latitude and 102 to 123° E in longitude), and the mechanisms were discussed. The results showed that for oriental oak stands, mean foliar and soil  $\delta^{15}$ N were -4.8% and 2.0‰, respectively, indicating an evident <sup>15</sup>N enrichment in the soils. The average foliar  $\delta^{15}$ N value of oriental oak in warm temperate forest was significantly lower (p < 0.01), while a higher enrichment factor was also observed in temperate forests, than that in the subtropical areas. Over the eastern China, the foliar  $\delta^{15}$ N increased linearly and significantly with increasing mean annual precipitation (MAP) (R<sup>2</sup> = 0.481 and p < 0.001) and mean annual temperature (MAT) ( $R^2 = 0.285$  and p = 0.01) and decreasing pH value of rain water ( $R^2 = 0.333$  and p = 0.005). With these results in eastern China, there are great implications in understanding how nitrogen dynamics of ecosystems responds to warming, drought and acid rain.

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### 1. Introduction

During the past two decades, increasing attention has been paid to examine the large-scale pattern of natural abundance of nitrogen ( $\delta^{15}$ N) in plants and soils in terrestrial ecosystems and to explore the underlying mechanisms in the context of global change (Amundson et al., 2003; Craine et al., 2009; Handley et al., 1999; Martinelli et al., 1999; Pardo and Nadelhoffer, 2009; Pardo et al., 2006). This is due to the fact that variation in  $\delta^{15}$ N in plants and soils within a broad geographical area is closely related to climate (Amundson et al., 2003; Handley et al., 1999), mycorrhizal fungi (Dijkstra et al., 2008; Kahmen et al., 2008; Lilleskov et al., 2008; Mayor et al., 2009; Pörtl et al., 2007), plant functional groups (Evans, 2001; Swap et al., 2004), dynamics of nitrogen (N) and carbon (C) of different compartments in ecosystems (Schulze et al., 1998, 1999), and anthropogenic N deposition (Boggs et al., 2007; Pardo et al., 2006, 2007a, 2007b; Sah, 2005). However, there has been no coherently general pattern of variation in plant  $\delta^{15}$ N with some environmental variables. For instance, based on meta-analysis, Amundson et al. (2003) showed that for terrestrial plants (especially for non N2-fixing ones) as a whole, foliar  $\delta^{15}$ N increased with increasing temperature and decreasing precipitation on a global scale. Handley et al. (1999) also showed that foliar  $\delta^{15}$ N was negatively correlated with rainfall. Contrary to Handley et al. (1999) and Amundson et al. (2003), Pardo et al. (2006) demonstrated that there was a positive relationship between foliar  $\delta^{\rm 15}N$  and precipitation in a range of 500–1800 mm across northeastern North America, Colorado, Alaska, southern Chile and Europe. With a field investigation across boreal Finland, Sah et al. (2006) demonstrated that  $\delta^{15}$ N in Scots pine (*Pinus sylvestris*) needles in higher latitude areas was significantly higher than that in lower latitude areas, indicating that  $\delta^{15}N$ in Scots pine needles decreased with increasing temperature on a regional scale. We argue that these confusions might be attributed to difference in plant physiological traits, environmental variables, and geographical scales in question. Discrimination occurs because the rate

<sup>\*</sup> Corresponding author at: School of Agriculture and Biology, Shanghai Jiao Tong University, Dongchuan Rd. 800, Shanghai 200240, PR China. Tel.: +86 21 34206605; fax: +86 21 34206603.

E-mail address: kanghz@sjtu.edu.cn (H. Kang).

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of reaction of molecules bearing the lighter isotope is faster than those bearing the heavier isotope and as a result, the product of a reaction is depleted in the heavier N isotope (<sup>15</sup>N). And because of the kinetic isotope effect, the natural abundance of <sup>15</sup>N in the substrate can become gradually enriched during the N cycling processes if the reaction is incomplete. Fractionation during plant uptake of N can be large particularly when the supply of N is high relative to plant demand which may lead to the volatilization of NH<sub>3</sub> or denitrification. In both cases the <sup>15</sup>N that remained in the substrate may increase over time and contribute to isotopic fractionation (Högberg, 1997). So, different N pools in an ecosystem can have characteristic  $\delta^{15}N$  signatures. In this context, it is necessary to explore the pattern of variation in foliar  $\delta^{15}N$ for specific plant species across its distribution range and to seek relevant affecting factors (Evans, 2001). This can further deepen our understanding of the relationship between plant  $\delta^{15}N$  and influencing factors in ecosystems along an environmental gradient.

In plant ecosystems, the status of nitrogen, carbon and the isotopes in plants and soils are closely associated due to nutrient cycling. It is intriguing to reveal the relationship between plant  $\delta^{15}N$  and the  $\delta^{15}N$ and N concentration of soils where the plants grow (Nadelhoffer and Fry, 1994). Amundson et al. (2003) concluded that plant  $\delta^{15}N$  values were more negative than those of soils, and the enrichment factor ( $\delta^{15}Nsoil - \delta^{15}Nplant$ ) increased with decreasing mean annual temperature (MAT). Craine et al. (2009) pointed out that foliar  $\delta^{15}N$ increased with increasing foliar N concentrations and decreasing foliar phosphorus (P) concentrations; they also showed that from independent landscape-level to regional-level, foliar  $\delta^{15}N$  increased with increasing N availability. This implicates that plant species may display a diverse of dependences of  $\delta^{15}N$  on other nutrients.

Anthropogenic N deposition exerts a strong impact on the chemical and biochemical properties of plants and soils in terrestrial ecosystems. Across a gradient of nitrogen deposition in northeastern U.S., Pardo et al. (2007a, 2007b) showed that foliar  $\delta^{15}$ N was strongly correlated with N deposition, and was also positively correlated with net nitrification potential and negatively correlated with soil C:N ratio. In this sense, plant foliar  $\delta^{15}$ N could be used as a useful indicator in reflecting the response of plants to anthropogenic N deposition in an area (Emmett et al., 1998; Garten and Hanson, 1990; Koopmans et al., 1997; Liu et al., 2007; Pardo et al., 2007a; Sah, 2005; Zechmeister et al., 2008). Meanwhile, it might be used to evaluate the effect of atmospheric N deposition and nitrogen saturation in forest ecosystems.

Oriental oak (Quercus variabilis Bl.) is a common deciduous broadleaf tree in East Asia (24° to 42° N and 96° to 140° E), and the oak not only supplies raw material for timber market and industry (e.g. cork, fodder and bioethanol), but also ecologically functions in China, Korea and Japan. For its distribution area in mainland China, mean annual temperature (MAT) ranges from 7.2 to 23.6 °C, and mean annual precipitation (MAP) ranges from 411 to 2000 mm (Wang et al., 2009). With such a broad geographical distribution and climatic gradient, oriental oak stands provide an ideal situation of examining the pattern of variation in physiological traits, including natural abundance of isotope nitrogen, for a species at a regional scale. In this study, our main objectives are 1) to characterize  $\delta^{15}$ N of live leaves and soils in different oriental oak stands across eastern China based on a systematic investigation, 2) to demonstrate the patterns of variation in foliar  $\delta^{15}$ N in relation to climatic factors (MAT and MAP) and geographical coordinates, and 3) to examine the relationship between foliar  $\delta^{15}N$ (soil  $\delta^{15}$ N) and the pH value of rain water as to survey on the status of anthropogenic N deposition across eastern China.

### 2. Methods

### 2.1. Study stands

Based on the literature concerning geographical distribution of oriental oak (Wang et al., 1985) and the specimen information in Chinese Virtual Herbarium (CVH) (Wang et al., 2009), the distribution area of oriental oak was delimited. The data of totally 22 sample stands were collected in this study (Fig. 1). All the sample stands used in this study are natural secondary forests which have not been disturbed by direct human activities (such as cutting, fertilization, litter collection and grazing) and wildfires for the last five decades.

The whole study area extended from the temperate areas in northeastern and northern China through the subtropical areas in central and southern China. In the temperate part of the study area (e.g. Beijing City, Liaoning and Hebei Provinces), the temperate deciduous broadleaf forests with oriental oak as the dominant species cover most of this area. In these forests, the soils are typically entisols (USDA Soil Taxonomy). In the middle part of the study area (e.g. Hubei and Jiangsu Provinces), the climate transits from the temperate to the subtropical, the deciduous and evergreen broadleaf forests share this area, and the soils are typically inceptisols. In the southern part of the study area (Jiangxi, Guangxi, and Fujian Provinces, etc.), the zonal vegetation is typically subtropical, evergreen and broadleaf with oriental oak grows as a company species. The soils are typically ultisols in the subtropical China.

Geographically, the study area covered from 24 to 41° N in latitude and from 102 to 123° E in longitude (Fig. 1). The site located in Fengyang, Anhui Province (32.64° N, 117.56° E) had the lowest altitude (28 m above sea level, ASL) while the site in Kunming, Yunnan Province had the highest altitude (1955 m ASL). Across the study area, MAT ranged from 8.7 to 21.8 °C and MAP ranged from 495 to 1739 mm (Table 1).

### 2.2. Collection of sample leaves and soils and chemical analysis

Leaf samples were collected from late July to early Sept. in 2008 when the leaves had been fully developed. Generally, the leaf sampling was firstly carried out in northern temperate areas, and then towards southern subtropical areas. At each site, a  $20 \times 20$  m sampling plot was fixed in a south-facing slope within a stand. The information of latitude, longitude and altitude was determined with GPS (Thales, USA). Within the plot, five dominant trees were selected as the sample trees. Leaves were cut with a pole pruner from the topmost after climbing the trees. About 250 g fresh leaves were picked from 2 to 3 small branches. The leaf samples were then dried at 60 °C for 72 h in the laboratory.

In order to collect soil samples systematically, the plot was divided into five sub-plots and five sampling points were selected in each sub-plot. The soils were collected from the layer of 0–10 cm in depth at each sampling point and then mixed as a composite sample for a sub-plot. In this way, five soil samples were obtained from each site.

For leaf and soil samples, the  $\delta^{15}N$  and N concentrations were determined with Finnigan MAT Delta V advantage (Thermo Finnigan, USA) in Centre for Isotope Analysis, Chinese Academy of Forestry, China. The nitrogen stable isotopic composition is expressed in  $\delta$  (‰) (Robinson, 2001):

$$\delta^{15}N(\text{\%}) = \left(R_{sample} \,/\, R_{std} \!-\! 1\right) \times 1000,$$

where  $R_{sample}$  and  $R_{std}$  are the  ${}^{15}N/{}^{14}N$  ratios of the sample and ambient air as a standard, respectively.

#### 2.3. Environmental data of sample sites

Mean annual temperature (MAT, °C) and mean annual precipitation (MAP, mm) were achieved from China Meteorological Data Sharing Service System, Chinese Natural Resources Database and the local weather stations. These climate data were the average of observation data during 30 years. The annual mean pH values of rain water were collected from Quality Report on the Environment in

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Fig. 1. Distribution map of sample oriental oak stands in eastern China.

China, 2008 (Ministry of Environmental Protection of the People's Republic of China, 2009).

### 2.4. Data processing

The relationship of foliar and soil  $\delta^{15}$ N against environmental and geographical variables was described with single normal linear models. Statistical differences were tested with a *t*-test for unequal variance. In order to show relative effects of geographical variables on foliar and soil  $\delta^{15}$ N, latitude, longitude and altitude were standardized before regression analysis was made. All statistical and regression analyses were conducted with software SigmaStat (Systat Software, Inc.).

### 3. Results

# 3.1. Spatial distribution of foliar and soils $\delta^{15}N$ in relation to geographical variables

For oriental oak investigated in eastern China, mean foliar  $\delta^{15}$ N was -4.8%, ranging from -6.9 to -1.9%; mean foliar N concentration was 17.9 mg g<sup>-1</sup>, ranging from 14.8 to 21.2 mg g<sup>-1</sup> (Table 1). Compared with foliar  $\delta^{15}$ N, soil  $\delta^{15}$ N (2.0‰) was much more positive (Table 1). Enrichment factor ( $\delta^{15}$ N<sub>soil</sub>  $-\delta^{15}$ N<sub>foliar</sub>) ranged from 4.3 to 9.9‰, with a mean of 6.8‰. At these sites, mean soil N concentration was 1.9 mg g<sup>-1</sup>, ranging from 1.0 to 3.2 mg g<sup>-1</sup> (Table 1).

### Table 1

Statistical summary of  $\delta^{15}N$  and N concentrations of leaves and soils, climatic factors (MAT and MAP), pH value of rain water and geographical location (latitude, longitude, and altitude) for 22 oriental oak stands over eastern China. For  $\delta^{15}N$ , N concentration and enrichment factor, the coefficient of variation (%) was also calculated.

	Mean	Min	Max	SD	CV(%)
Leaf $\delta^{15}$ N (‰)	-4.8	-6.9	-1.9	1.3	27
Leaf N (mg $g^{-1}$ )	17.9	14.8	21.2	1.8	10
Soil δ <sup>15</sup> N (‰)	2	-0.7	4.4	1.5	78
Soil N (mg $g^{-1}$ )	1.9	1.0	3.2	0.7	35
Enrichment factor (‰)	6.8	4.3	9.9	1.6	24
MAP (mm)	989	495	1739	-	-
MAT (°C)	14.6	8.7	21.8	-	-
pH value of rain water	5.6	4.5	6	-	-
Latitude (°)	-	24.43	40.25	-	-
Longitude (°)	-	102.45	122.96	-	-
Altitude (m)	-	28	1955	-	-

Foliar  $\delta^{15}$ N of oriental oak was negatively correlated with the geographical variable (latitude, p < 0.01, Table 2). The average foliar  $\delta^{15}$ N value of oriental oak in subtropical areas was  $-4.3 \pm 1.3\%$ . (n = 14), which was significantly greater (p < 0.01) than the warm temperate forest value of  $-5.7 \pm 0.8\%$  (n = 8) (Fig. 2A). The average concentration of nitrogen in oriental oak leaves in subtropical forests was  $18.2 \pm 2.0\%$  (n = 14), which was higher, but not significantly different from the average value of  $17.3 \pm 1.4\%$  (n = 8) found in the warm temperate forests (Fig. 2B). Also, the average N concentration and  $\delta^{15}$ N in soils of oriental oak forests were higher, but not significantly, in the subtropical forests, compared with those in the warm temperate ones (Fig. 2C and D). In the warm temperate forests, the soils had a higher enrichment factor than that in the subtropical ones (p = 0.06) (Fig. 2E).

### 3.2. Variation in foliar and soil $\delta^{15}N$ with environmental factors

With increasing MAP and MAT, foliar  $\delta^{15}$ N increased significantly (p < 0.01) (Table 2, Fig. 3) in oriental oak forests across eastern China. The soil  $\delta^{15}$ N values significantly increased with increasing MAP (p < 0.05), but had not significant relationship with MAT. The N concentrations of both soils and leaves had an increase trend with increasing MAP, but the relationships were not significant (Table 2). In eastern China, MAP and MAT are both related to latitude (Table 2), which leads to an indirect positive relation between MAP and MAT.

Simple normal linear regression analyses showed that foliar  $\delta^{15}N$  and N concentrations were negatively correlated with the pH value of rain water (Fig. 4) in oriental oak forests. Similarly with the increasing of pH value of rain water, soil  $\delta^{15}N$  and N concentrations and enrichment factor were decreased, though their relationships were all not significant (Table 2, Fig. 4).

### 4. Discussion

### 4.1. The influence of species and geographical scale

In a meta-analysis by Pardo et al. (2006), foliar  $\delta^{15}N$  and N concentrations were c. -1% (ranging from -7 to 2%) and about 25 mg g<sup>-1</sup> (ranging from 15 to 34 mg g<sup>-1</sup>), respectively, for hardwood tree species. Along a nitrogen deposition gradient in northeastern US, foliar  $\delta^{15}N$  and N concentrations ranged from -4.5 to 0.5‰ and 17 to 30 mg g<sup>-1</sup> (Pardo et al., 2007b). Compared with those average data for hardwood tree species (Pardo et al., 2007b), oriental

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### Table 2

Pearson correlation coefficients among leaf  $\delta^{15}$ N, enrichment factor (EF), environmental and geographical variables (latitude, LAT; longitude, LON; and altitude, ALT).

	Leaf $\delta^{15}\text{N}$	Leaf N	Soil N	Soil $\delta^{15}N$	EF	LAT	LON	ALT	MAP	MAT
Leaf N	0.048									
Soil N	0.123	0.335								
Soil $\delta^{15}N$	0.345	-0.253	0.015							
EF	$-0.477^{*}$	-0.275	-0.085	0.661**						
LAT	$-0.529^{**}$	-0.022	-0.070	-0.303	0.139					
LON	0.241	0.146	0.176	0.053	-0.144	0.500**				
ALT	-0.114	-0.268	-0.150	0.249	0.324	$-0.425^{*}$	$-0.768^{**}$			
MAP	0.693**	0.197	0.344	0.403*	-0.177	$-0.686^{**}$	0.174	-0.046		
MAT	0.534**	0.095	0.051	0.087	-0.345	-0.825**	-0.317	0.027	0.612**	
pH	-0.577**	-0.538**	$-0.366^{*}$	-0.185	0.287	0.424*	$-0.394^{*}$	0.317	$-0.798^{**}$	$-0.492^{*}$

Note: \*p<0.05; \*\*p<0.01.

oak had much more negative mean value of foliar  $\delta^{15}N(-4.8\%)$ , but a similar range (-6.9 to -1.9‰); and the mean foliar N concentration of oriental oak was also lower (17.9 mg g<sup>-1</sup>, ranging from 14.8 to 21.2 mg g<sup>-1</sup>).

At a regional scale, spatial patterns of ecological phenomenon often associate with geographical coordinates due to the differences in natural (e.g. climate, soils) and anthropogenic (human management, N deposition, etc.) factors controlling ecological processes among diverse regions. Therefore, it is of great importance to show the relationships between ecosystem  $\delta^{15}$ N and geographical variables. In a global data set, soil  $\delta^{15}$ N displayed a positive relationship with latitude (p<0.001, r=0.47, and n=47) and a negative relationship with precipitation (p<0.03, r=-0.29, n=52); as a result (Handley et al., 1999), latitude and rainfall being independent variables in a



Fig. 2.  $\delta^{15}$ N and N concentrations of leaves and soils in oriental oak forests across warm temperate and subtropical areas. Error bars represent one standard deviation.

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**Fig. 3.** Variation of leaf  $\delta^{15}$ N with mean annual precipitation (MAP, mm) and mean annual temperature (MAT, °C) in oriental oak stands across the eastern China. For the regression equation between leaf  $\delta^{15}$ N and MAP (A), R<sup>2</sup>=0.481 and p<0.001; for the regression equation between leaf  $\delta^{15}$ N and MAT (B), R<sup>2</sup>=0.285 and p=0.01.

multiple regression model accounted for 49% of the variation in whole soil  $\delta^{15}$ N, and foliar  $\delta^{15}$ N was more strongly related to rainfall than was whole soil  $\delta^{15}$ N, but, no significant relation was found between foliar  $\delta^{15}$ N and geographical variables (Handley et al., 1999). Contrary to the finding of Handley et al. (1999), however, our study showed an obvious relationship between foliar  $\delta^{15}$ N and geographical variables at a regional scale.

Microbial growth is often carbon limited. Under these circumstances, microorganisms break down the dissolved organic nitrogen (DON) by secreting exoenzymes, use the carbon skeleton to support their energy requirements for growth and maintenance, and secrete NH<sub>4</sub><sup>+</sup> into the soil. This process is commonly known as nitrogen mineralization. Environmental conditions that promote microbial activity enhance both gross and net nitrogen mineralization (Chapin et al., 2002). Net nitrogen mineralization rates are therefore generally higher in subtropical than in temperate forest soils. In tropical or subtropical ecosystems, plant and microbial growth are frequently limited by nutrients other than nitrogen, and their demand for nitrogen is low, so nitrifiers have ready access to NH<sub>4</sub><sup>+</sup>. Consequently, nearly all nitrogen that is mineralized in these systems is immediately nitrified. In contrast, nitrification is frequently less than 25% of net mineralization in temperate ecosystems (Vitousek and Matson, 1984). Subtropical ecosystems have a similar status to tropical ones. In view of the above, subtropical forests should have more open N cycles than temperate forests with both inputs and outputs of N large relative to N cycling within systems. This implies that subtropical forests should in general be more <sup>15</sup>N enriched than most temperate forests.

The ectomycorrhizae on oriental oak secret proteases and other enzymes to attack organic nitrogen compounds. The fungus then absorbs the resulting amino acids and transfers them to the plant (Read, 1991). NH<sup>+</sup><sub>4</sub> in soils may be another main N source for oriental oak as the fungus can only use  $NH_4^+$ –N rather than  $NO_3^-$ –N, directly. The foliar  $\delta^{15}$ N value is a record of the inorganic N that the plant has taken up. In our study, foliar  $\delta^{15}$ N in warm temperate forests were more negative than those in subtropical ones. Several mechanisms may be possible: (1) Amundson et al. (2003) figured that increasing temperature from temperate to subtropical ecosystems could intensify microbial competition with plants for N, resulting in a relatively low concentration of mineral N available as NH<sub>4</sub><sup>+</sup>. (2) Plant and microbial growth are frequently limited by nutrients other than N in tropical and subtropical ecosystems, and their demand for N is low, so nitrifiers can transform  $NH_4^+$  produced by mineralization to  $NO_3^$ through nitrification nearly 100%. As discussed above,  $NO_3^-$ -N cannot be used directly by oriental oak, then, the ectomycorrhizae may break down more DON to acquired amino acids for the nutrient demand of oriental oak. In contrast, in many unpolluted temperate deciduous forests, nitrifier populations are often too small in infertile soils, and nitrification is only a small proportion of net mineralization (e.g. 0 to 4%). Even when ecosystems receive increasing nitrogen deposition, the fraction of nitrification can only increase to 25% (McNulty et al., 1990). Thereupon more  $^{15}$ N-depleted NH<sup>+</sup> were taken up by oriental oak from soils, resulting in more negative foliar  $\delta^{15}$ N in temperate forests. Högberg (1997) reckoned that plant N sources in northern latitudes may be depleted in <sup>15</sup>N through mycorrhizal fungi/plant interactions. (3) As presumed above, the plant can take up more <sup>14</sup>N enriched NH<sup>+</sup><sub>4</sub> in temperate forests than in subtropical ones. Is the further isotopic discrimination taking place during NH<sup>+</sup><sub>4</sub> uptake? There is presently disagreement over the important issue of assimilation-based N isotope discrimination (Amundson et al,



**Fig. 4.** Variation in leaf and soil  $\delta^{15}$ N and the N concentrations with the pH value of rain water in oriental oak stands across the eastern China. For the regression equation between leaf  $\delta^{15}$ N and pH (A), R<sup>2</sup>=0.333 and p=0.005; for the regression equation between leaf N and pH (B), R<sup>2</sup>=0.289 and p=0.01.

2003). No research about isotope discrimination during oriental oak nitrogen uptake has been reported. Clearly, this is an area deserving additional work. Anyway, isotopic discrimination during  $NH_4^+$  uptake can also make foliar  $\delta^{15}N$  more negative, if it happens.

### 4.2. Environmental controls over foliar and soil $\delta^{15}N$

As discussed in the introduction, there is a bewilderment that the patterns of variation in foliar  $\delta^{15}$ N are not consistently in relation to climatic factors at a regional or global scale. An extensive global synthesis by Handley et al. (1999), demonstrated a negative linear relationship between foliar  $\delta^{15}$ N and annual rainfall across a wide range of ecosystems. The study by Schulze et al. (1998) is another example of the generality of the pattern of decreasing foliar  $\delta^{15}$ N with increasing annual precipitation, independent of nitrogen-fixing status of species, or large differences in other climatic factors such as temperature. Based on the data from some review papers (Amundson et al., 2003; Craine et al., 2009; Handley et al., 1999) and the results in this study, there are several factors that can modify the simple and unified patterns of foliar  $\delta^{15}N$ with climate at a regional scale shown by Handley et al. (1999), Amundson et al. (2003) and Craine et al. (2009): the differences among plant species (Evans, 2001; Robinson, 2001; Robinson et al., 1998), the variation brought by differing scales (Austin and Sala, 1999; Houlton et al., 2006, 2007; Sah et al., 2006), the fractionation caused by the N mineralization via soil microorganisms (Dijkstra et al., 2008; Kahmen et al., 2008; Pörtl et al., 2007), and the influence from anthropogenic N deposition (Amundson et al., 2003; Boggs et al., 2007; Emmett et al., 1998; Pardo et al., 2006, 2007a, 2007b), etc.

In this study, the trend of variation in foliar  $\delta^{15}N$  of oriental oak with temperature was the same, but the precipitation pattern was opposite, to that for all plants as a whole at a global scale (Amundson et al., 2003; Craine et al., 2009; Handley et al., 1999). In comparison to the plant at humid region, the more negative foliar  $\delta^{15}$ N in arid areas indicated more isotope fractionation at lower precipitation. Two mechanisms may be responsible. First, factors that improve the soil moisture environment for microbial activity enhance net nitrogen mineralization. Rates of mineralization are positively related to precipitation. However, the improved microbial activity also enhances their competition with the plants for NH<sup>+</sup><sub>4</sub>. Second, nitrification rates are slow in dry soils primarily because thin water films restrict NH<sub>4</sub><sup>+</sup> diffusion to nitrifiers (Stark and Firestone, 1995). Then, dry soils reserve more <sup>15</sup>N-depleted NH<sup>+</sup><sub>4</sub> which can be used by the plant. In contrast, in relatively moist soils, faster nitrification rates bring less available  $NH_4^+$  for plants, and more DON will be absorbed through the action of ectomycorrhizae for the nutrient demand of oriental oak, during which the inorganic  $\delta^{15}$ N value of leaves has little change. These may provide some reasonable explanations why annual rainfall input is an important component controlling ecosystem nitrogen cycling (Austin and Sala, 1999).

The positive relationship between foliar  $\delta^{15}N$  and mean annual temperature may also be indirectly related to nitrification rates. The activity of nitrifying bacteria is very sensitive to temperature, and its activity usually increases with increasing temperature. In this way, in warmer forests, more  $NH_4^+$  which is easier to be taken up by oriental oak are transformed to  $NO_3^-$  which is relatively more difficult to be utilized by oriental oak, leading to foliar  $\delta^{15}N$  more approaching zero. However, as mentioned earlier, net mineralization caused by saprophytic microorganisms in soils will be enhanced as temperature increases, and subtropical soils are commonly more fertile than temperate ones. The synergistic effect of these factors may be a roughly, but not strictly positive relationship between foliar  $\delta^{15}N$  and mean annual temperature.

In terms of the pattern of plant foliar  $\delta^{15}$ N at a regional scale, foliar  $\delta^{15}$ N-temperature pattern of oriental oak was inconsistent with that of Scots pine (Sah et al., 2006). There may be two reasons. First, a difference in N metabolic physiology exists between Scots pine (an

evergreen conifer) and oriental oak (a deciduous broadleaf tree). For instance, broadleaf trees usually have higher foliar N concentration than conifers probably caused by different N-form assimilations and the participation of mycorrhizal fungi in N cycling for conifers. Secondly, the climate gradient of Scots pine is within the boreal area, while that for oriental oak stretches from the temperate to subtropical areas. In Sah's et al. (2006) case, the foliar  $\delta^{15}$ N-temperature pattern was similar to that of the first flat-part (<5 °C) shown by Craine et al. (2009); however, in this study, the variation in foliar  $\delta^{15}$ N-temperature of oriental oak was conformed to that of the second part (8–20 °C).

The use of the <sup>15</sup>N enrichment factor of vegetation provided means by which initial differences in soil  $\delta^{15}$ N values could be normalized for effects of land management practices, soil age and climate (Emmett et al., 1998). Amundson et al. (2003) showed that globally, plant  $\delta^{15}$ N values were more negative than soils, but the difference between soil and foliar increases with decreasing MAT. In our study, the enrichment factor of oriental oak was smaller in the subtropical forests than in the warm temperate forests. Nitrogen in most subtropical forests is relatively more available and cycles faster than in most temperate forests, leading to larger differences of the  $\delta^{15}$ N value between soils and leaves in temperate forest than in subtropical ones. In addition, the relative change in the <sup>15</sup>N abundance of the plant is greater than that in the soil, as soils have a large pre-treatment 'memory' due to the large, relatively inactive, soil N store (Emmett et al., 1998). Plant <sup>15</sup>N abundance thus reflects the more active soil nitrogen in soil solution or on ion exchange sites and therefore, the more recent history of N dynamics at a site (Johannisson and Högberg, 1994).

Along a northeastern to southeastern transect, there is a high to low gradient of rain water pH value (Ministry of Environmental Protection of the People's Republic of China, 2009), implying a low to high trend of N deposition. Meanwhile, N cycling in tropical or subtropical forests is faster than in temperate forests, releasing more  $NO_x$  to the atmosphere settling more  $NO_3^-$  to the land and contributing to a lower pH of rain water. These may interpret why the foliar and soil  $\delta^{15}N$  and N concentrations were increased with the decreasing of pH value of rain water, though the mechanisms and consequences of acidity in regulating nitrification rates are uncertain.

### 5. Conclusions

The large-scale patterns of plant  $\delta^{15}$ N in relation to environmental factors are not consistent due to the differences in plant species, geographical scales, or anthropogenic nitrogen deposition. Our results reveal the regional patterns of foliar  $\delta^{15}$ N among oriental oak stands across eastern China and their interactions, and confirm the hypothesis that climate, especially precipitation, exerts the first-order control on foliar  $\delta^{15}$ N. These results have great implications in understanding the response of plants to climate change in eastern China. Based on our study and those cases discussed above, one aim of our future research is to pinpoint the patterns of specific tree species which is widely distributed in order to accurately understand the patterns of variation in foliar  $\delta^{15}$ N and to clarify the underlying mechanisms.

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