

Article

Variation of Oriental Oak (*Quercus variabilis*) Leaf $\delta^{13}\text{C}$ across Temperate and Subtropical China: Spatial Patterns and Sensitivity to Precipitation

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Academic Editor: Steven Jansen

Received: 24 March 2015 / Accepted: 25 June 2015 / Published: 30 June 2015

Abstract: The concentration of the carbon-13 isotope (leaf $\delta^{13}\text{C}$) in leaves is negatively correlated with the mean annual precipitation (MAP) at large geographical scales. In this paper, we explain the spatial pattern of leaf $\delta^{13}\text{C}$ variation for deciduous oriental oak (*Quercus variabilis* Bl.) across temperate and subtropical biomes and its sensitivity to climate factors such as MAP. There was a 6‰ variation in the leaf $\delta^{13}\text{C}$ values of oak with a significant positive correlation with latitude and negative correlations with the mean annual temperature (MAT) and MAP. There was no correlation between leaf $\delta^{13}\text{C}$ and altitude or longitude. Stepwise multiple regression analyses showed that leaf $\delta^{13}\text{C}$ decreased 0.3‰ per 100 mm increase in MAP. MAP alone could account for 68% of the observed variation in leaf $\delta^{13}\text{C}$. These results can be used to improve predictions for plant responses to climate change and particularly lower rainfall.

Keywords: carbon isotope; oak; precipitation; latitude; *Quercus variabilis*

1. Introduction

Natural abundance of the carbon-13 isotope (^{13}C) can be affected by environmental factors such as the partial pressure of CO_2 and O_2 [1], the irradiance level [2–4] and air temperature [5]. However, water availability is a predominant determinant of changes in the carbon-13 isotope at regional and global scales [1,6–8]. This is due to the fact that water supply affects the stomatal conductance and photosynthesis of plants, which changes $^{13}\text{C}/^{12}\text{C}$ ratios in the synthesized carbohydrates. The isotopic composition of carbon ($\delta^{13}\text{C}$) in leaves has also been correlated with leaf specific area and nitrogen concentration [9,10], water deficit [11] and water use efficiency (WUE) [12–14]. Hence the spatial pattern of leaf $\delta^{13}\text{C}$, arising from changes in water availability, is of physiological and ecological interest particularly in the context of climate change.

At large geographical scales, precipitation has often been used as a surrogate of the effect of water availability on leaf $\delta^{13}\text{C}$. A significant negative correlation is found between leaf $\delta^{13}\text{C}$ and mean annual precipitation (MAP) at a global scale [1,6]. The same relationship has also been found at regional scales in different climatic conditions, for instance, in northern temperate Australia [15], both C_3 and C_4 vegetation in Southern Africa [16], *Quercus suber* in Mediterranean Portugal [17] and *Metrosideros polymorpha* in tropical Hawaii [10]. In China, there have been studies on a range of terrestrial plants in rainforests [18], in arid north and northeast China areas [19,20] and on high mountains [21,22]. However these studies do not focus on the driving forces or the effects on leaf $\delta^{13}\text{C}$ variation at a species level across biomes. Changes in leaf $\delta^{13}\text{C}$ are well correlated with spatial environmental variables, such as latitude (LAT), longitude (LON) and altitude (ALT), due to their co-variation with precipitation [1,23–27]. Shestakova *et al.*, (2014) investigated the spatial pattern of carbon isotope discrimination for three deciduous oaks and one evergreen oak along an aridity gradient, showing that the values for evergreen oak was primarily related to temperature, whilst the spatial pattern for deciduous oaks was primarily dependent on precipitation [28]. The total range of $\delta^{13}\text{C}$ values was 4.4‰ and 3.1‰ for *Quercus pubescens* and *Quercus ilex*, respectively, in Southern France with different levels of water availability [29]. Klein *et al.*, also report that stomatal conductance and water use affect plant $\delta^{13}\text{C}$ for *Quercus calliprinos* in Israel [30]. By contrast, Donovan *et al.*, found no significant differences in $\delta^{13}\text{C}$ among three *Quercus* species in the Southeastern USA [31]. Hence different *Quercus* species show a variety of $\delta^{13}\text{C}$ responses at a regional scale; understanding the reasons for these variations could help determine how different species will respond to climate change and in particular increased drought conditions. An improved understanding of variations in $\delta^{13}\text{C}$ for selected species can also be used to build isoscapes [32] and spatial models of precipitation [33].

Oriental oak (*Quercus variabilis* Bl.) is a deciduous broadleaf tree that is relatively abundant across temperate and subtropical areas in East Asia (24° N to 42° N; 96° E to 140° E), including the eastern part of Mainland China, Taiwan and Zhoushan islands, as well as Korea and Japan [34]. *Q. variabilis* is found at altitudes ranging from sea-level to 2000 m above sea level (a.s.l.) in subtropical China. The associated forests are important for timber and cork production and ecosystem services, such as carbon

sequestration, and water and soil conservation. Within the distribution area in Mainland China, mean annual temperature (MAT) and MAP range from 7.2 °C to 23.6 °C and 410 mm to 2000 mm, respectively [34]. This provides an ideal situation for studying spatial patterns of leaf $\delta^{13}\text{C}$ variation within a single widespread species. The present study aims (1) to show the spatial pattern of variation in leaf $\delta^{13}\text{C}$ of *Q. variabilis* within its distribution range across temperate-subtropical biomes and (2) to assess the correlation relationships between leaf $\delta^{13}\text{C}$ and climate variables.

2. Materials and Methods

2.1. Study Areas and Sample Stands

According to the previous information of spatial distribution of *Q. variabilis* from the Chinese Virtual Herbarium [34,35], 25 sample stands were identified in Mainland China with a range of the longitude from 102° E to 123° E and latitude ranging from subtropical areas 24° N to temperate areas 41° N (Figure 1). For each site, temperature and precipitation data over a period of 30 years were collected from the Chinese meteorological data sharing service system, the Chinese Natural Resources Database and local weather stations. MAT and MAP ranged from 8.8 °C to 20 °C and 495 mm to 1850 mm, respectively (Supplementary Table S1).

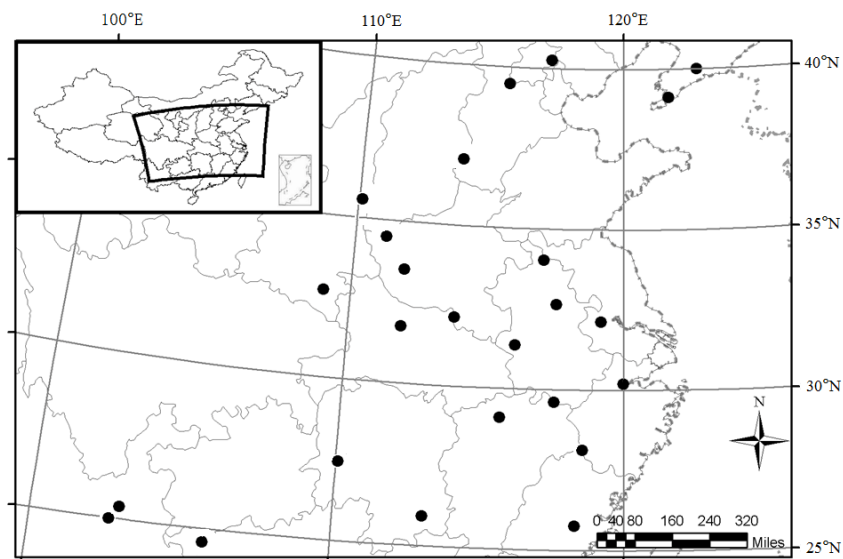


Figure 1. Distribution map of sample *Quercus variabilis* stands sampled across eastern China in 2008.

Each sample stand comprised of natural secondary forests judged to have been unaffected by fire, tree cutting, fertilization, litter collection, or grazing during the last two decades. In the temperate areas, such as the Liaoning, Beijing and Hebei provinces, *Q. variabilis* is typically the dominant tree species within a temperate deciduous broadleaf forest located in entisol soils. In the central areas, such as the Henan, Hubei and Jiangsu provinces, the climate varies from warm temperate to subtropical, and the climax vegetation is mixed deciduous and evergreen broadleaf forest in ceptisol soils. In southern areas, such as the Jiangxi, Guangxi and Fujian provinces, *Q. variabilis* forms a co-dominant tree species within subtropical evergreen broadleaf forests in acidic, aluminum-rich, ultisol soils.

2.2. Sampling

Leaf samples were collected between late July and early September in 2008. At each site, a sampling plot (20 m × 20 m) was delimited in the middle position of a south-facing slope, where a global positioning system (Thales Navigation, Santa Barbara, CA, USA) was used to determine LAT, LON and ALT. Five dominant trees in each sample plot were measured in terms of height and diameter at breast height (Supplementary Table S1). From a sample tree, approximately 250 g of fresh leaves were picked from three to four small branches at the central south-facing part of crown. Leaf samples were dried at 60 °C for 72 h. The sample leaves were ground and sieved through a 60 mesh sieve (0.25 mm diameter) for chemical analysis.

2.3. Chemical Analyses

The $\delta^{13}\text{C}$ and C content of leaf samples were determined using a Finnigan MAT Delta V advantage mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the Center for Isotope Analysis, Chinese Academy of Forestry, Beijing, China. The carbon stable isotopic composition was expressed as $\delta^{13}\text{C}$ (‰) = $((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$, where $\delta^{13}\text{C}$ is the isotope ratio in parts per million (‰), $R_{\text{sample}}/R_{\text{standard}}$ are the $^{13}\text{C}/^{12}\text{C}$ molar abundance ratios of leaf material and the PDB (Pee Dee Belemnite) standard, respectively [36].

2.4. Statistical Analyses

Simple linear regression was used to describe the patterns of association between leaf $\delta^{13}\text{C}$ and climate variables. To assess the influence of climatic factors on leaf $\delta^{13}\text{C}$, we performed a stepwise multiple regression analysis. Pearson correlation coefficients were calculated to explore relationships among climate variables. Stepwise multiple regression analysis was performed in SPSS 16.0 (IBM, Chicago, IL, USA); all other tests were carried out by SigmaPlot10.0 software (Systat Software, Inc., Richmod, CA, USA).

3. Results

MAP of the sampled *Q. variabilis* populations ranged from 495 mm to 1850 mm; the MAT ranged from 8.8 °C to 20.0 °C. The mean value for leaf $\delta^{13}\text{C}$ of the 25 *Q. variabilis* populations across eastern China was $-27.4\text{‰} \pm 0.33\text{‰}$, with a coefficient of variation of 5.73% (Table 1). The lowest value of -30‰ was found at Dehua (DH), Fujian, where MAP was 1850 mm. The highest value of -24‰ was found at Anming (AN) and Kunming (KM), Yunnan, where MAP was approximately 1000 mm. Similar leaf $\delta^{13}\text{C}$ values were found in Sanmenxia (SM), Henan (MAP = 495 mm) and Pinggu (PG), Beijing (MAP = 542 mm) (Supplementary Table S1).

Table 1. Statistics of mean annual precipitation (MAP), mean annual temperature (MAT), the carbon content (C) and the relative concentration of carbon 13 in the leaves ($\delta^{13}\text{C}$) of 25 *Quercus variabilis* across eastern China.

	Mean	Standard Error	Maximum	Minimum	Coefficient of Variation(%)
MAP (mm)	1065	75	1850	495	38
MAT ($^{\circ}\text{C}$)	14.7	0.5	20	8.8	20
C (mg g^{-1})	493.1	3.4	519.1	462.5	3.4
$\delta^{13}\text{C}$ (‰)	-27.4	0.3	-24.3	-30.4	5.7

Leaf $\delta^{13}\text{C}$ was significantly and positively correlated with latitude ($r^2 = 0.48$, $p = 0.0003$), and significantly and negatively with MAT ($r^2 = 0.40$, $p = 0.0012$) and MAP ($r^2 = 0.675$, $p < 0.0001$) (Figure 2). There was no significant trend with altitude and longitude. By stepwise multiple regression analyses, MAP alone could account for 67.5 % of the variation in leaf $\delta^{13}\text{C}$ (Table 2).

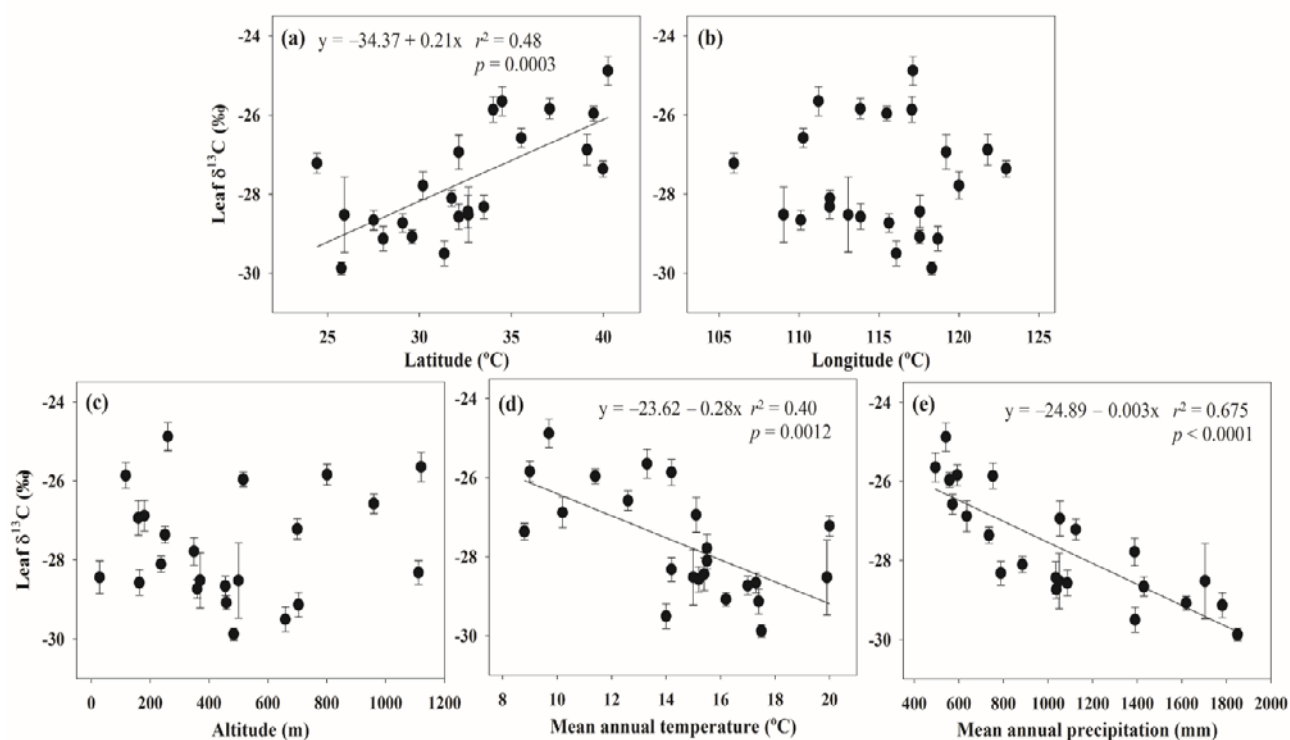


Figure 2. Linear regressions of the relationship between leaf $\delta^{13}\text{C}$ and five environmental variables: (a) latitude; (b) longitude; (c) altitude; (d) mean annual temperature; and (e) mean annual precipitation ($n = 23$, except for the sites at Anning and Kunming).

Table 2. A simple stepwise multiple regression of leaf $\delta^{13}\text{C}$ (‰) against mean annual precipitation, and a full model describing leaf $\delta^{13}\text{C}$ (‰) in terms of latitude (LAT), longitude (LON), altitude (ALT), mean annual temperature (MAT), mean annual precipitation (MAP) ($n = 23$, excluding the populations at Anning and Kunming).

Methods	Equation	R^2	p
Full model	Leaf $\delta^{13}\text{C} = -29.475 - 0.005 \times \text{LAT} + 0.038 \times \text{LON} + 0.000 \times \text{ALT} + 0.032 \times \text{MAT} - 0.003 \times \text{MAP}$	0.680	< 0.0001
Stepwise	Leaf $\delta^{13}\text{C} = -24.889 - 0.003 \times \text{MAP}$	0.675	< 0.0001

4. Discussion

Large variations of leaf $\delta^{13}\text{C}$ have been observed for many species across their distribution range, showing plant adaptations to environmental change. In this study, the range of *Q. variabilis* leaf $\delta^{13}\text{C}$ changed from -30‰ to -24‰ , which is greater than within-country variations reported for other oak species. For example, evergreen oak species, *Q. suber* leaf $\delta^{13}\text{C}$ varied from -28.58‰ to -24.58‰ in Mediterranean Portugal [17], and the range of *Q. ilex* $\delta^{13}\text{C}$ values was 3.1‰ for *Q. ilex* southern France [29]. For deciduous oak species, ranges for leaf $\delta^{13}\text{C}$ of 1.9‰ , 1.5‰ , 0.7‰ , and 4.4‰ have been reported for *Q. faginea*, *Q. humilis*, *Q. petraea* [28] and *Q. pubescens* [29], respectively. Plant carbon isotope discrimination is an ecophysiological property that can reflect the capacity of a species to cope with climatic stressors and, ultimately, define their potential distribution. Therefore, the large range in the $\delta^{13}\text{C}$ values for *Q. variabilis* population in this study demonstrates strong plasticity in response to the environment [37]. According to Castillo *et al.*, spatial differences in carbon isotope levels at a regional scale can be used to model an area of similar precipitation [33]. This study, which focuses on an individual species at a large regional scale, demonstrates how carbon isotope can be used to develop isoscape models and thereby refine our understanding of biogeochemical cycles [32].

Previous work has demonstrated that leaf $\delta^{13}\text{C}$ can be related to spatial variables, such as latitude, altitude, MAT and MAP [1,3,6,24,26]. This paper shows that leaf $\delta^{13}\text{C}$ of a selected species has a significant positive correlation with latitude and a negative correlation with MAT and MAP, with no significant correlation with altitude and longitude (Figure 2). Based on a Pearson correlation analysis, latitude is negatively correlated with MAT ($R = -0.86$, $p < 0.0001$) and MAP ($R = -0.73$, $p < 0.0001$) (Supplementary Table S2). Although longitude was also negatively correlated with altitude, there was no correlation of altitude with MAT and MAP ($R = -0.74$, $p < 0.0001$) (Supplementary Table S2). This suggests that the spatial variation of leaf $\delta^{13}\text{C}$ is primary caused by differences in MAT and MAP, which vary with latitude.

Although both MAT and MAP show significant influence on leaf $\delta^{13}\text{C}$, MAP alone accounted for 67.5% of the variation in leaf $\delta^{13}\text{C}$ (Table 2). Ferrio *et al.*, in a study of *Q. ilex* in Spain, also showed that 59% of the variation in leaf $\delta^{13}\text{C}$ could be explained by changes in precipitation [38]. Although this study focused on the response to mean annual precipitation, there is an argument that, for deciduous trees, a stronger relationship may be obtained from the mean precipitation in the growing season than over a calendar year.

In this paper, we used stepwise multiple regression analyses to minimize the problems with collinearity, for example MAT is positively correlated with MAP ($r = 0.76$, $p < 0.0001$). Our results suggest that

MAP is the strongest predictor for leaf $\delta^{13}\text{C}$, in line with other observations [6,9]. The soil water content determines the leaf stomatal conductance and photosynthesis, and thereby the water use efficiency (WUE) of plants [39–41]. Environmentally induced increases in WUE, such as under drought, are expected to be accompanied by decreases in carbon isotope discrimination, because under drought, changes in p_i/p_a (ratio of intercellular to atmospheric partial pressures of CO_2) and WUE will be largely due to stomatal closure [42].

The values of leaf $\delta^{13}\text{C}$ for the population at the Anning and Kunming sites in the Yunnan province were -24.8‰ and -24.4‰ , respectively. This is higher than in other sites. The two sites were excluded for the regression analyses because we assumed that these high values were not caused by climate factors. Yunnan province has some of the highest soil phosphorus concentrations in China [43]. For example the soil P concentration at Anning and Kunming was 1.7 g kg^{-1} , and 1.2 g kg^{-1} , respectively, compared to $0.16\text{--}0.47\text{ g kg}^{-1}$ at the other sites [44]. P-rich soils, in addition to being high in phosphorus, contain relatively high levels of phosphate-associated elements such as Ca, Mg, Fe and Al, which can cause high concentrations N, P, Ca, Fe and Zn in the leaf. The concentrations of these elements in the Yunnan province were higher than those at P-deficient sites ($p < 0.05$) [44]. Organisms need to maintain the balance of internal element concentrations with respect to the environment (*i.e.*, homeostasis) [45]. Zhou *et al.*, also reported that element ratios were more stable than individual element concentrations regardless of P-rich and P-deficient sites [44]. Hence plants grown in the P-rich soil can fix more CO_2 through photosynthesis to improve the carbon content of plant bodies in order to maintain homeostatic regulation. The process can result in decreased fractionation and high leaf $\delta^{13}\text{C}$. An alternative explanation is that the two sites are located in the high altitude mountains of a subtropical area, characterized by lower temperatures and precipitation levels than other sites at a subtropical latitude (Supplementary Table S1). Low temperature and precipitation can decrease photosynthesis, transpiration and leaf conductance, resulting in a high value for leaf $\delta^{13}\text{C}$ [36].

5. Conclusions

Deciduous *Q. variabilis*, distributed across temperate-subtropical biomes, is one of the most extensive tree species in eastern Asia. The range of the values of leaf $\delta^{13}\text{C}$ for *Q. variabilis* across 25 populations was as great as 6‰ (from -30.4‰ to -24.4‰). At a large regional scale, leaf $\delta^{13}\text{C}$ is positively correlated with latitude and negatively correlated with MAT and MAP; there is no correlation between leaf $\delta^{13}\text{C}$ and either altitude or longitude. MAP, which is partly determined by changes in latitude, could explain 68% of the regional variation in leaf $\delta^{13}\text{C}$ at a large regional scale. The leaf $\delta^{13}\text{C}$ decreased by 0.3‰ per 100 mm increase in MAP. These results can be used to model spatial precipitation and improve our understanding of the potential for plant adaptation to climate change and dry conditions.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (NSFC, 31270640, 31070532, 30800138, and 30671674). We would like to thank Xiao Sun, Pisheng Zhou, Zhicheng Liu, Lili Wu, Dongmei Chen, Jing Wang, Zaijun Xin, Zhe Wang, Yongfu Li and Xinghu He for their assistance in the field work and laboratory analyses. We gratefully acknowledge the anonymous reviewers for their valuable suggestions for improving the manuscript.

Author Contribution

Chunjiang Liu, Paul J. Burgess and Hongzhang Kang designed the experiments and analysis; Yanhua Zhu, Xuan Zhou and Baoming Du performed the experiments; Shan Yin analyzed the data; Baoming Du wrote the paper together with Chunjiang Liu.

Conflicts of Interest

The authors declare no conflict of interest.

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