

The preference of nitrate uptake in Chinese prickly ash estimated by $\delta^{15}\text{N}$ values and cation concentrations

He-Chun Piao¹ · Si-Liang Li^{1,2} · Shi-Jie Wang¹ · She-Hong Li¹

Received: 1 March 2016 / Accepted: 7 January 2017 / Published online: 17 January 2017
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Abstract The nitrogen isotopic compositions of plant tissue could reflect its uptake of and preference for ammonium or nitrate. However, various factors may influence the field-collected $\delta^{15}\text{N}$ values under field condition, which causes the interpretation problematic. The spatial variation of nitrogen (N) concentrations and the isotopic compositions were investigated in the soils and tissues of Chinese prickly ash from the southwest China to the east China. The objectives were to investigate the variation in soil and tissue $\delta^{15}\text{N}$ values and N forms taken up by the plant. The leaf and root $\delta^{15}\text{N}$ values varied significantly in response to the pattern of soil $\delta^{15}\text{N}$ values. The difference in $\delta^{15}\text{N}$ values between the leaves and roots was 2.57‰ and may be caused by an increase in the transport of unassimilated NO_3^- and NH_4^+ to the leaves. Leaf nitrogen was significantly and positively correlated with leaf potassium and negatively related to leaf calcium. Because potassium is the favoured counter-cation for nitrate transport in the xylem, the enrichment of ^{15}N in leaf relative to root induced by preferred uptake of nitrate should be accompanied by significant and positive relationship of leaf nitrogen with leaf potassium concentrations. These results suggest that Chinese prickly ash prefers NO_3^- over NH_4^+ .

Keywords Preference · $\delta^{15}\text{N}$ values · Counter-cation · Nitrate · Ammonium · Chinese prickly ash

Introduction

Nitrogen (N) is one of the most important plant nutrients, and soil N availability is a major limiting factor for plant growth and survival. Although some species of plants are reliant on organic forms of N (Öhrlund and Näsholm 2004), the two major inorganic N forms for plant absorption in a soil solution are ammonium (NH_4^+) and nitrate (NO_3^-). Because the energetic, biochemical, and molecular features of NH_4^+ and NO_3^- have a different effect on plant metabolism (Luo et al. 2013, other references therein), the plants may exhibit a greater difference in their ability to take up and use NH_4^+ and NO_3^- as sources of inorganic N (Bown et al. 2010). McKane et al. (2002) have reported that the plant preference for a given N form was likely related to the most abundant N form in the soil at the plant growth location (Cao et al. 2015). However, increasing evidence shows that most species grow better with a mixture of NH_4^+ and NO_3^- compared to either source alone (Bown et al. 2010).

The plant tissue $\delta^{15}\text{N}$ is related to the isotopic ratio of the main N sources (Pascual et al. 2013) and can act as an integrated measure of nitrogen uptake and assimilation (Kalcsits et al. 2014, 2015). Therefore, the $\delta^{15}\text{N}$ values can be employed to reflect the N source used by plants (Tateno et al. 2005; Piao et al. 2012; Pascual et al. 2013; Brearley 2013; Tanaka-Oda et al. 2015). A measure of the difference in $\delta^{15}\text{N}$ between leaves and roots, i.e., $\delta^{15}\text{N}$ (leaf–root), has the potential to provide information on interspecific differences in the inorganic nitrogen source preference (Kalcsits et al. 2015). Under steady-state nitrogen conditions, differences between root and leaf $\delta^{15}\text{N}$ may reflect differences in the assimilation location (Kolb and Evans 2002; Kalcsits et al. 2014). However, much controversy

✉ Si-Liang Li
Siliang.li@tju.edu.cn

¹ State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China

² Institute of Surface-Earth System Science, Tianjin University, Tianjin 300072, China

exists about whether or not NH_4^+ is translocated in the xylem from roots to shoots (Schjoerring et al. 2002). Unassimilated root NH_4^+ , which also transports to leaves will cause an increase in leaf $\delta^{15}\text{N}$ values, similar to NO_3^- . Since unassimilated NH_4^+ is isotopically enriched relative to root-assimilated NH_4^+ (Kalcsits et al. 2015), it is difficult to conclude whether or not NO_3^- or NH_4^+ is taken up by the plants based only on the difference in $\delta^{15}\text{N}$ between the leaf and root.

Chinese prickly ash (*Zanthoxylum bungeanum*) is widely found in a transitional zone from the southwest China to the Shandong peninsula of the east China. Chinese prickly ash is a shrub species that is generally well defended and has survived and often flourished under systems subjected to anthropogenic disturbances, such as in karst degradation areas. Moreover, this species is a multipurpose horticultural plant that is becoming an integral part of the agriculture in agroforestry programmes in China (Li et al. 2009). It also plays an important role in the restoration of fragile ecosystems with poor soil because of its high drought resistance. The soil distributed highly heterogeneous in karst areas with extremely fragile environment and the economically backward in the southwest China (Wang et al. 2004; Liu 2007), facilitating Chinese prickly ash growth throughout this zone. Additionally, substantial land in central and eastern China has been planted with Chinese prickly ash due to water shortage and drought conditions (Shi et al. 2015). Evidence for the preference of NO_3^- by the prickly ash is its root architecture. It has been demonstrated that the external presence of NO_3^- triggers lateral root proliferation and stimulates the elongation of the mature roots in direct contact with NO_3^- (Kruse et al. 2010, other references therein). Furthermore, NO_3^- uptake has been shown to stimulate net K^+ uptake in various crop species, which suggests that the NO_3^- ion serves as a mobile accompanying anion during K^+ uptake and/or transport (Zhang et al. 2010).

The measurements of $\delta^{15}\text{N}$ (leaf–root) values, $\delta^{15}\text{N}$ (leaf–soil) values, and cation concentrations may provide useful benchmarks for determining the preferences of the inorganic N forms of NH_4^+ or NO_3^- for Chinese prickly ash. In this study, it was hypothesized that: (1) an increase of the difference in $\delta^{15}\text{N}$ values between the leaf and root, or between the leaf and soil, would be responsible for NO_3^- preferences in the plants; (2) consequently, there would be a highly significant and positive relationship between leaf N and leaf K concentrations. The present study was conducted to assess the combined use of nitrogen isotope ratios and leaf cation concentrations in Chinese prickly ash and determine their effect on the preferences of the N form taken up by this plant.

Materials and methods

Study sites

The Chinese prickly ash samples were collected from six sites in China (Fig. 1) and were divided into three groups. The first group consisted seedling samples collected from both Huajiang (HJ) and Zunyi (ZY) soils (named seedlings, $n = 44$). The second group included relative adult samples that were close to a period of ten years growth and collected from HJ and ZY (named adult 1, $n = 44$). Finally, the third group contained relative adult samples collected from Chongqing (CQ), Pingdingshan (PD), Zoucheng (ZC) and Laiwu (LW) soils (named adult 2, $n = 43$). The soil and tissue samples of the adult plants were collected along the climatic gradient from the southwest China with a relatively high mean annual temperature (MAT) and mean annual precipitation (MAP) to the Shandong peninsula of the east China with relatively low MAT and MAP. The Chinese prickly ash tissue samples were collected from the HJ, ZY, and CQ sites, which are located in subtropical areas with relatively high MAT and MAP. The other three sampling sites were located in temperate areas with relatively low MAT and MAP.

The soil types were categorized as clay–sandy or sandy–clay loams in the HJ, ZY, and CQ sites. The Chinese prickly ash in these areas had fine shallow roots and widely rooted in the top 1–5 cm. The PD, ZC and LW soils were sandy–clay loam or sandy soils where deeper roots were expected. The age of adult Chinese prickly ash samples used in this study was approximately 10 years. Tissue and soil samples from the HJ, ZY, and CQ sites were collected in late July, whereas the samples from the PD, ZC, and LW sites were collected in early August. The soil samples were

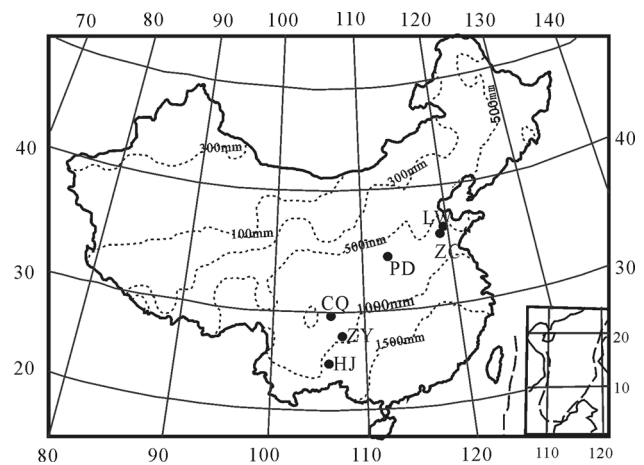


Fig. 1 Location of sampling sites in China, HJ (Huajiang), ZY (Zunyi), CQ (Chongqing), PD (Pingdingshan), ZC (Zoucheng), and LW (Laiwu). The dashed line shows spatial variation of annual rainfall

collected from 0 to 15 cm depths in six replicated plots surrounding the Chinese prickly ash, then mixed thoroughly, homogenized, and sieved (2 mm) to form a composite sample for each sampling site. Next, the available nutrients were analyzed. Chemical fertilizer was not added to any of the sampling soils of Chinese prickly ash plants during the sampling year.

Laboratory analysis

The plant samples for laboratory analysis were dried for 48 h at 60 °C and then ground with a mortar and pestle. Next, the organic carbon and total N content were determined using a CHNS autoanalyzer (PE 2400-II, Norwalk, CT, USA). Standard soil analysis methods were used to measure the soil extractable calcium, magnesium, and potassium with 1 M NH_4 OAC (Thomas 1982). The plant samples were digested with nitric–perchloric acid for subsequent potassium, calcium, and magnesium analysis using atomic absorption spectroscopy (PE900F, USA). The nitrogen isotopes were measured using a Euro EA 3000 elemental analyzer interfaced to an IsoPrime isotope ratio mass spectrometer (Elementar Analyse System GmbH, Germany). The pulverized sample containing 50 μg of N was weighed inside a tin capsule and wrapped for isotopic analyses. The samples were then arranged into the carousels of the elemental analyzer (EA 3000, Eurovector, Italy) for N_2 generation by combustion. The gas was then purified in a gas chromatographic column (Eurovector S.P.A. Milano, Italy) and passed directly to the inlet of a gas isotope ratio mass spectrometer (IRMS, Isoprime Ltd. UK). The ratio of heavy to light isotopes in the sample material (R_{sample}) was measured using the mass spectrometer as the deviation from the isotopic ratio of a standard (R_{standard}), where R denotes the ratio of stable N ($^{15}\text{N}/^{14}\text{N}$) isotopes, expressed in δ notation. For example, for nitrogen: $\delta^{15}\text{N}$ (‰) = $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where R_{sample} and R_{standard} are the $^{15}\text{N}:^{14}\text{N}$ ratios for the N of the sample and the standard. The atmospheric N ($\delta^{15}\text{N} = 0\text{‰}$) serves as an international standard for stable nitrogen isotope. The precision of the analyses was $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$.

Statistical analysis

To normalize the initial differences in soil and plant $\delta^{15}\text{N}$ values due to previous land management and soil age (Piao et al. 2012), the $\delta^{15}\text{N}$ (leaf–soil) value was used to estimate the preference of the N forms taken up by the plants. The $\delta^{15}\text{N}$ (root–soil) value was also used to estimate the preference of N forms taken up by plants. In any case of N assimilation, if the plant preferred NO_3^- , then $\delta^{15}\text{N}$ (root–soil) would also be significantly correlated with the leaf

potassium, which is similar to $\delta^{15}\text{N}$ (leaf–soil), because potassium is stimulated by NO_3^- uptake and transport. If the plant preferred NH_4^+ , unassimilated NH_4^+ in the leaves would also increase the difference in $\delta^{15}\text{N}$ values between the leaf and root; however, $\delta^{15}\text{N}$ (root–soil) would be not related to leaf potassium because K uptake is depressed by NH_4^+ . Therefore, both $\delta^{15}\text{N}$ (leaf–soil) and $\delta^{15}\text{N}$ (root–soil) values could be used to determine whether the plant preferred NO_3^- or NH_4^+ . Statistical analysis was conducted using SPSS software (SPSS Science, Chicago, USA). Significant differences between the mean of the $\delta^{15}\text{N}$ values, including $\delta^{15}\text{N}$ (leaf–root) and $\delta^{15}\text{N}$ (leaf–soil), in soil and tissue samples between seedling and adult species were determined by t test. Pearson correlation coefficients were performed to assess relationships between plant tissue and soil parameters, and linear regression was used to assess correlations between tissue parameters and soil parameters. For all statistical analyses, differences were considered significant at $P < 0.05$.

Results

Soil characteristics

The soil pH and extractable calcium concentrations did not vary significantly with climatic gradient. The soil organic carbon, soil nitrogen, and extractable magnesium and potassium concentrations were higher in subtropical soils than those in temperate soils for adult Chinese prickly ash samples (Table 1). The soil pH was significantly correlated with extractable calcium concentrations ($r = 0.59$, $P < 0.001$) but did not display a significant relationship with extractable magnesium and potassium concentrations.

Tissue $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$

Both soil and tissue $\delta^{15}\text{N}$ values decreased with increasing latitude. The soil $\delta^{15}\text{N}$ value decreased from $6.88 \pm 1.49\text{‰}$ in HJ with a lower latitude to $2.79 \pm 1.60\text{‰}$ in LW with a higher latitude, whilst the leaf $\delta^{15}\text{N}$ value decreased from 3.73 ± 1.38 to $-0.18 \pm 1.92\text{‰}$ for adult Chinese prickly ash (Fig. 2). The plant root and leaf $\delta^{15}\text{N}$ values were significantly correlated with the soil $\delta^{15}\text{N}$ values ($r = 0.80$, $P < 0.001$, $r = 0.74$, $P < 0.001$, respectively) (Fig. 3a, b) in both seedling and adult Chinese prickly ash ($n = 131$) samples.

Patterns among cations and $\delta^{15}\text{N}$

Leaf potassium was negatively and significantly related to the leaf calcium concentration ($r = -0.60$, $P < 0.001$,

Table 1 Mean concentration of organic carbon, total nitrogen, extractable cations, and pH in the soil of six sampling sites

| | Huajiang (HJ) | Zunyi (ZY) | Congqing (CQ) | Pingdingshan (PD) | Zoucheng (ZC) | Laiwu (LW) |
|---|---------------|-------------|---------------|-------------------|---------------|-------------|
| Soil pH (H ₂ O) | 7.31 (0.83) | 6.88 (1.18) | 6.84 (0.73) | 7.72 (0.24) | 6.58 (1.16) | 7.92 (0.27) |
| Soil org. C (mg g ⁻¹) | 25.5 (12.3) | 27.1 (8.6) | 37.1 (26.2) | 15.9 (6.0) | 11.8 (6.2) | 16.3 (10.1) |
| Soil N (mg g ⁻¹) | 3.36 (1.26) | 2.52 (1.54) | 1.68 (0.84) | 1.82 (0.84) | 1.54 (0.56) | 1.82 (0.84) |
| Soil extractable Ca (mg g ⁻¹) | 7.05 (2.12) | 4.09 (1.76) | 5.09 (1.32) | 5.57 (0.92) | 3.69 (1.92) | 5.21 (2.29) |
| Soil extractable Mg (mg g ⁻¹) | 0.53 (0.29) | 0.67 (0.29) | 0.34 (0.12) | 0.42 (0.15) | 0.44 (0.12) | 0.31 (0.14) |
| Soil extractable K (mg g ⁻¹) | 0.29 (0.23) | 0.32 (0.31) | 0.32 (0.38) | 0.33 (0.14) | 0.20 (0.13) | 0.26 (0.16) |

SD in brackets

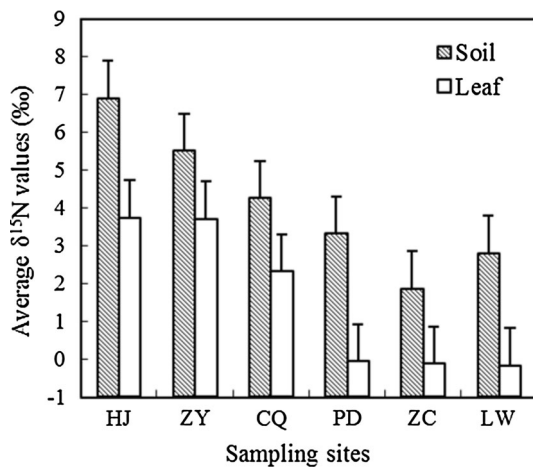
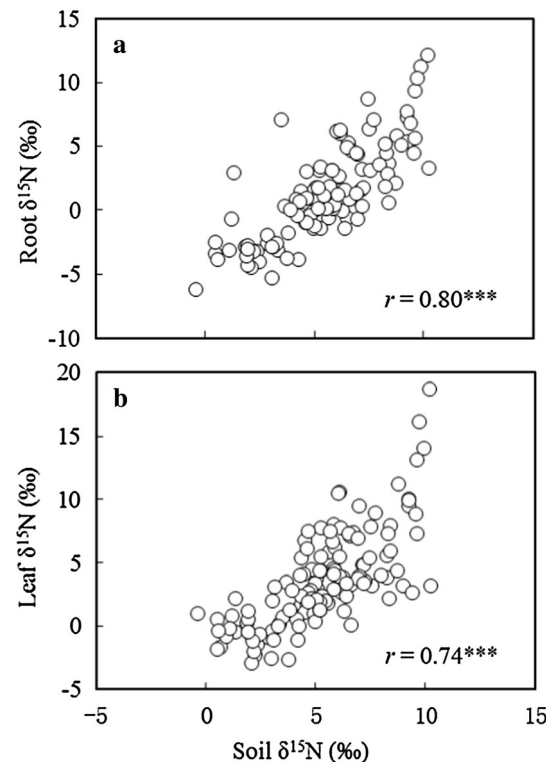
**Fig. 2** Average soil and leaf $\delta^{15}\text{N}$ values in different sampling sites. HJ (Huajiang; 25°43'N), ZY (Zunyi; 27°52'N), CQ (Congqing; 29°12'N), PD (Pingdingshan; 33°58'N), ZC (Zoucheng; 35°23'N), and LW (Laiwu; 36°11'N)

Table 2) and positively and significantly related to leaf N ($r = 0.57$, $P < 0.001$, Fig. 4a; Table 2). The leaf nitrogen content was negatively and significantly correlated with leaf calcium ($r = -0.42$, $P < 0.001$, Fig. 4b; Table 2), but not significantly related with leaf magnesium (Fig. 4c). However, leaf potassium was negatively and significantly correlated with leaf magnesium ($r = -0.34$, $P < 0.001$, Table 2) in all seedling and adult Chinese prickly ash samples.

The nitrogen concentrations were positively and significantly correlated with the $\delta^{15}\text{N}$ (leaf–root), $\delta^{15}\text{N}$ (leaf–soil), and $\delta^{15}\text{N}$ (root–soil) values, in all of the seedling and adult samples (Table 2). The potassium concentrations were positively and significantly correlated with the $\delta^{15}\text{N}$ (leaf–soil) and $\delta^{15}\text{N}$ (root–soil) values in all of the seedling and adult Chinese prickly ash samples (Table 2). The calcium content was negatively and significantly related to $\delta^{15}\text{N}$ (leaf–soil) and $\delta^{15}\text{N}$ (root–soil) values in all of seedling and adult Chinese prickly ash samples. The $\delta^{15}\text{N}$ (leaf–soil) values were negatively and significantly correlated with the leaf calcium concentrations ($r = -0.36$, $P < 0.001$) (Fig. 5a), and positively related to both leaf

**Fig. 3** Relationships of soil $\delta^{15}\text{N}$ with root $\delta^{15}\text{N}$ (a) and leaf $\delta^{15}\text{N}$ (b), *** $P < 0.001$ in all of both seedling and adult Chinese prickly ash

potassium concentrations ($r = 0.39$, $P < 0.001$) (Fig. 5b) and leaf nitrogen concentrations ($r = 0.50$, $P < 0.001$) (Fig. 5c) in adult 1 and adult 2 samples. The leaf N concentrations in the adult 2 samples were significantly and positively correlated with leaf potassium ($r = 0.70$, $P < 0.001$) and negatively correlated with leaf calcium ($r = -0.70$, $P < 0.001$).

Comparison of $\delta^{15}\text{N}$ and cations between seedling and adult Chinese prickly ash

The leaf $\delta^{15}\text{N}$ values were typically greater than the same values in the corresponding roots except for 12 out of the 131 samples, and the mean value of $\delta^{15}\text{N}$ (leaf–root) was 2.57‰

Table 2 Correlation coefficients (*r*) among cations and $\delta^{15}\text{N}$ in the soil, root, and leaf of Chinese prickly ash

| | K | Ca | Mg | $\delta^{15}\text{N}$ (leaf–root) | $\delta^{15}\text{N}$ (leaf–soil) | $\delta^{15}\text{N}$ (root–soil) |
|----|---------|----------|----------|-----------------------------------|-----------------------------------|-----------------------------------|
| N | 0.57*** | -0.42*** | -0.16 | 0.26** | 0.50*** | 0.34*** |
| K | | -0.60*** | -0.34*** | 0.037 | 0.39*** | 0.46*** |
| Ca | | | 0.091 | -0.062 | -0.36*** | -0.39*** |
| Mg | | | | -0.075 | -0.12 | -0.18 |

* $P < 0.05$
 ** $P < 0.01$
 *** $P < 0.001$

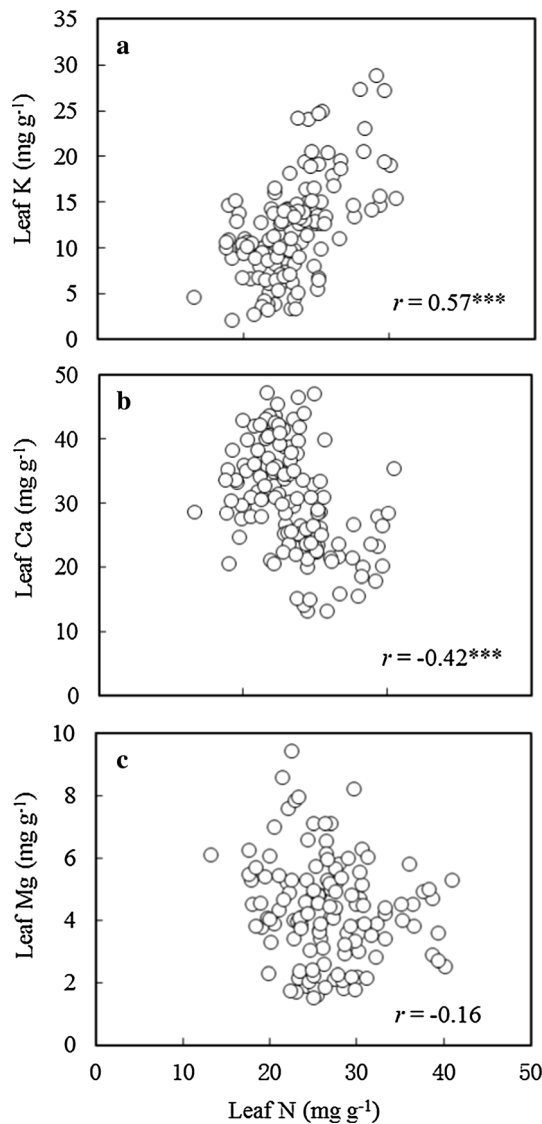


Fig. 4 Relationships between leaf K and leaf Ca (a), between leaf N and leaf Ca (b) and between leaf N and leaf K (c) in all of both seedling and adult Chinese prickly ash

for all seedling and adult Chinese prickly ash samples. Among them, the mean value of $\delta^{15}\text{N}$ (leaf–root) was 3.33‰ in the seedlings, whereas the mean value of $\delta^{15}\text{N}$ (leaf–root) was 1.87‰ in adult 1 (Table 3). Correspondingly, leaf

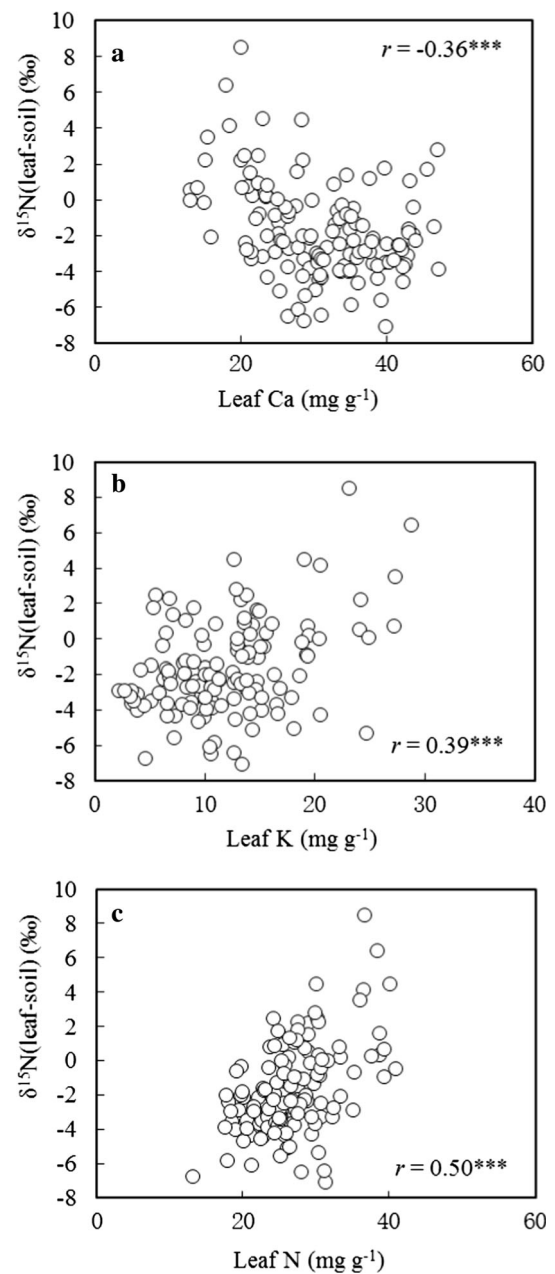


Fig. 5 Variations of $\delta^{15}\text{N}$ values with increasing leaf Ca (a), with leaf K (b), and with leaf N concentrations (c) in both seedling and adult Chinese prickly ash samples

Table 3 Comparison of mean values (mean \pm SD) of $\delta^{15}\text{N}$ and leaf cation concentrations between seedlings and adults of Chinese prickly ash collected in Zunyi and Huajiang areas

| Chinese prickly ash | Soil pH (H ₂ O) | Soil $\delta^{15}\text{N}$ (‰) | Leaf $\delta^{15}\text{N}$ (‰) | Root $\delta^{15}\text{N}$ (‰) | $\delta^{15}\text{N}$ (leaf–root) (‰) |
|---------------------|------------------------------|--------------------------------|--------------------------------|--------------------------------|---------------------------------------|
| Seedlings | 7.53 \pm 0.54 | 6.59 \pm 1.83 | 6.65 \pm 4.15 | 3.32 \pm 3.50 | 3.33 \pm 1.76 |
| Adult 1 | 7.10 \pm 1.03 | 6.19 \pm 1.67 | 3.66 \pm 1.88 | 1.68 \pm 2.18 | 1.87 \pm 2.25 |
| <i>t</i> test | $P < 0.05$ | NS | $P < 0.001$ | $P < 0.05$ | $P < 0.01$ |
| | Leaf N (mg g ⁻¹) | Leaf Ca (mg g ⁻¹) | Leaf K (mg g ⁻¹) | Leaf Mg (mg g ⁻¹) | $\delta^{15}\text{N}$ (leaf–soil) (‰) |
| Seedlings | 30.13 \pm 6.19 | 23.90 \pm 5.03 | 14.57 \pm 5.97 | 4.07 \pm 1.43 | 0.05 \pm 2.95 |
| Adult 1 | 23.23 \pm 3.92 | 35.10 \pm 5.98 | 8.36 \pm 3.77 | 5.18 \pm 1.59 | -2.54 \pm 2.10 |
| <i>t</i> test | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.01$ | $P < 0.001$ |

NS no significant

nitrogen (30.13 \pm 6.19 mg g⁻¹) and leaf potassium (14.57 \pm 5.97 mg g⁻¹) in the seedlings were significantly higher than leaf N (23.23 \pm 3.92 mg g⁻¹) and leaf potassium (8.36 \pm 3.77 mg g⁻¹) in the adult 1 samples (Table 3).

The leaf calcium (23.90 \pm 5.03 mg g⁻¹) and leaf magnesium (4.07 \pm 1.43 mg g⁻¹) concentrations in the seedlings were significantly lower than the leaf calcium (35.10 \pm 5.98 mg g⁻¹) and leaf magnesium (5.18 \pm 1.59 mg g⁻¹) content in adult 1 (Table 3). The mean value of $\delta^{15}\text{N}$ (leaf–root) was 2.43‰ in adult 2 samples. Correspondingly, leaf nitrogen (26.93 \pm 2.97 mg g⁻¹) and leaf K (13.35 \pm 4.63 mg g⁻¹) in adult 2 were higher than those in adult 1. By contrast, the leaf calcium (33.32 \pm 8.56 mg g⁻¹) and leaf magnesium (3.73 \pm 1.49 mg g⁻¹) in adult 2 were lower than those in adult 1.

Discussion

Variations in tissue $\delta^{15}\text{N}$ in plants along with soil $\delta^{15}\text{N}$

Variations in soil and plant $\delta^{15}\text{N}$ values are associated with changes in temperature and precipitation (Amundson et al. 2003; Härdtle et al. 2014). In this study, it was discovered that both soil- and tissue-enriched ¹⁵N isotopes in low latitude areas with a relatively high MAT and MAP were compared to those at high latitude. Thus, the values of $\delta^{15}\text{N}$ in soils and plants systematically decrease with decreasing temperature and precipitation. Therefore, the $\delta^{15}\text{N}$ values in soils and plants were higher in HJ with relatively high MAT and MAP than those in LW with relatively low MAT and MAP. Previous work has shown significant correlations of tree ring $\delta^{15}\text{N}$ with temperature and precipitation (Savard et al. 2009); however, Zeng et al. (2014) did not find such correlations in their study. The clay–sand loam in HJ soils had high $\delta^{15}\text{N}$ values, whilst the sandy–clay loam in ZY soils had low $\delta^{15}\text{N}$ values, which indicated that $\delta^{15}\text{N}$ values were

related to the clay content in soils. Soils with additional clay-sized particles generally have higher $\delta^{15}\text{N}$ values than sandy soils (Hobbie and Ouimette 2009). Fang et al. (2011) reported that the relatively high N and clay content of broadleaf forest soil has more open N cycling and ¹⁵N enrichment in both the soil and vegetation. In an early study of pastures, Ledgard et al. (1984) have reported that sand, silt, and clay fractions had differing ¹⁵N content, with clay containing approximately 3‰ more ¹⁵N than those in sand and silt (Hobbie and Ouimette 2009). In forest ecosystems, high plant tissue $\delta^{15}\text{N}$ values are associated with sites characterized by high N supply, high rates of both nitrification and nitrate leaching (Härdtle et al. 2014). In this study, the spatial pattern of N isotopic compositions of the soil suggested that climate and soil components may be key factors in shifting soil $\delta^{15}\text{N}$ values.

Similar spatial patterns of leaf $\delta^{15}\text{N}$ in plants and soil $\delta^{15}\text{N}$ were observed (Fig. 2). The N cycle contains a large number of fractionation steps, which in turn influence plant $\delta^{15}\text{N}$ values (Härdtle et al. 2014). Meyers et al. (2016) have demonstrated that soil $\delta^{15}\text{N}$ may reflect land use and soil disturbances based on the correlation between soil isotopes and ecological conditions. Large land disturbances may result in a greater availability of both ammonium and nitrate in soils and can eventually lead to an N pool enriched in ¹⁵N (Falxa-Raymond et al. 2012). However, the plant $\delta^{15}\text{N}$ generally reflects plant uptake of soil nitrogen regardless of various factors (Tanaka-Oda et al. 2015). In this study, the leaf and root $\delta^{15}\text{N}$ values increased significantly with increasing soil $\delta^{15}\text{N}$ (Fig. 3), which suggests that leaf and root $\delta^{15}\text{N}$ values were mainly impacted by the sources. Thus, the $\delta^{15}\text{N}$ values of plant tissue would be dominated by local soil N sources based on isotopic proof.

The preference of N form by Chinese prickly ash

All Chinese prickly ash plants show high values of $\delta^{15}\text{N}$ (leaf–root), as high as 2.57‰. Kalcsits et al. (2015) have

reported that aspen has a large $\delta^{15}\text{N}$ (leaf–root) value reaching 3.02‰ when grown with NO_3^- , which implies that plants may prefer nitrate. In contrast, there are no significant differences between the $\delta^{15}\text{N}$ values of leaves and roots for pine and spruce trees with either NO_3^- or NH_4^+ sources (Kalcsits et al. 2015). The value of $\delta^{15}\text{N}$ (leaf–root) was also small (−0.43‰) for Chinese fir (Piao et al. 2012) which indicated that Chinese fir preferred NH_4^+ over NO_3^- . Plant preferences for NO_3^- or NH_4^+ uptake appear to be associated with the prevailing inorganic N form in the natural habitat (Brix et al. 2002). The sources of N (NO_3^- , NH_4^+ or organic N) taken up by plants will influence the tissue N isotopic composition (Bustamante et al. 2004). Nitrate assimilation can occur in shoots and roots (Kolb and Evans 2002), and individual species differ in their capacity for leaf and root assimilation (Kolb and Evans 2002). Under many conditions, a significant proportion of nitrate assimilation occurs in shoots because the reducing power required for the assimilation processes comes from photosynthesis (Wang et al. 2012). Assuming that nitrate reductase activity fractionates against the heavier isotope, any unassimilated root nitrate should be enriched in ^{15}N compared with assimilated nitrate. Once this enriched pool is transported to the leaf for assimilation, the leaf $\delta^{15}\text{N}$ would become more enriched relative to the root (Kolb and Evans 2002), which results in higher leaf $\delta^{15}\text{N}$ values relative to root $\delta^{15}\text{N}$. When NH_4^+ is the N source, however, there is little difference in $\delta^{15}\text{N}$ between leaves and roots because NH_4^+ is immediately assimilated in the roots (Evans et al. 1996; Kolb and Evans 2002; Piao et al. 2012). Tcherkez and Hodges (2008) have suggested that an estimate of the actual fractionation for leaf nitrate reduction by nitrate reductase would be close to 5‰. Evans et al. (1996) obtained a difference of 5.8‰ in $\delta^{15}\text{N}$ between the leaf and root for nitrate-fed tomatoes. However, much controversy exists about whether or not NH_4^+ is translocated in the xylem from roots to shoots in previous studies (Schjoerring et al. 2002). Therefore, the preferences of N forms taken up by plants were not completely understood after only considering variations of $\delta^{15}\text{N}$ (leaf–root) values.

Potassium, an important nutrient for plants, shows a positive relationship with nitrogen in the plant tissue (Fig. 4). Root-to-shoot translocation and shoot homeostasis of K determine nutrient balance, growth, and stress tolerance of vascular plants (Drechsler et al. 2015). Triplett et al. (1980) demonstrated the close relationship between K^+ and NO_3^- uptake and transport, which was later confirmed by Ruffy et al. (1981). Potassium flow and partitioning can be transformed in response to the form of N (Szczerba et al. 2008; Zhang et al. 2010). NH_4^+ has been shown to reduce the primary influx of K^+ from the external environment and suppress its accumulation in plant tissues

(Szczerba et al. 2008; other references therein) because NH_4^+ and K^+ have similar charges and hydrated diameters (Zhang et al. 2010). Because K is the favoured counter-cation for the transportation of nitrate in the xylem, the high leaf $\delta^{15}\text{N}$ relative to root $\delta^{15}\text{N}$ induced by nitrate uptake preferred should be accompanied by relatively high concentrations of both leaf N and K. The values of $\delta^{15}\text{N}$ (leaf–root) decreased from 3.33‰ in seedlings (the highest), then to 2.43‰ in adult 2 and to lowest of 1.87‰ in adult 1 (the lowest). Correspondingly, the leaf N and K concentrations decreased, but leaf calcium increased with a variation of the $\delta^{15}\text{N}$ (leaf–root) (Fig. 5). The interspecific variation indicated that the proportion of NO_3^- relative to the NH_4^+ uptake ratio changed with growth status and soil conditions.

The tissues are generally enriched with ^{14}N relative to the soil (Fig. 2), which reflects the preference for lighter isotopes by plants. In the present study, the average value of $\delta^{15}\text{N}$ (leaf–soil) was −1.65‰ for Chinese prickly ash, which suggests the plant preference to take up light isotopes due to the enzymatic reactions (Piao et al. 2012). These results are consistent with the global pattern of $\delta^{15}\text{N}$ values between plants and soils (Amundson et al. 2003), which show a difference of 3–4‰ between surface bulk soil (0–10 cm) samples and leaves. Compared with the global database of Craine et al. (2009), the values were approximately 3‰ lower than might be expected as predicted by climate, foliar N concentration, and type of mycorrhizal association (Brearley 2013). Interestingly, $\delta^{15}\text{N}$ (leaf–soil) and $\delta^{15}\text{N}$ (root–soil) were positively and significantly correlated with both leaf N and K, and negatively correlated with leaf calcium for prickly ash samples (Fig. 5). However, these significant correlations did not occur for Chinese fir (Piao et al. 2012), which implies that the tissue cation distributions are associated with N forms taken up by plants. It is reported that the nitrate uptake increased in both leaf and root with K concentrations based on the pod experiments of nitrate-fed and ammonium-fed carob seedlings (Cruz et al. 1993). Therefore, despite nitrate reduction in the roots and leaves, the $\delta^{15}\text{N}$ (root–soil) value significantly correlated with leaf K concentrations, which suggests the plant preferred NO_3^- . In the present study, there is a significant relationship between the $\delta^{15}\text{N}$ (root–soil) values and leaf K concentrations in Chinese prickly ash.

Hernández-Gómez et al. (2015) reported that a bell pepper can take up higher amounts of NH_4^+ from external solutions of high NH_4^+ and higher concentrations of calcium, which results in higher root and leaf NH_4^+ concentrations. The nitrate uptake was lower compared to the use of Na^+ and K^+ as counter-cations and Ca^{2+} as the only nutrient cation, but nitrate translocation in the xylem is only stimulated by K^+ in wheat (Zhang and Forde 2000).

The increased shoot calcium and magnesium concentrations could be explained by charge compensation for the reduced amount of K (Drechsler et al. 2015). The small differences in $\delta^{15}\text{N}$ between the leaf and root, and enrichment of ^{15}N in roots relative to leaves were accompanied by an increase in the significant and positive relationship of leaf N with leaf calcium.

Conclusions

This study has investigated the nitrogen isotopic compositions of soil and economic plants and the cation content in plant tissues in China. Soil $\delta^{15}\text{N}$ values showed clear regional differences depending on climate and environmental conditions. The study has demonstrated that tissue $\delta^{15}\text{N}$ values of Chinese prickly ash are controlled by soil $\delta^{15}\text{N}$ values. In addition, the NO_3^- source and plant physiological processes were responsible for the large differences in $\delta^{15}\text{N}$ values between the leaf and root and were accompanied by high concentrations of both leaf N and K and low concentrations of leaf calcium. Therefore, it is concluded that Chinese prickly ash plants preferred NO_3^- over NH_4^+ . The results suggest that the plants have various strategies for N assimilation that are impacted by environmental conditions and physiological processes based on isotopic proof during growth. The results have provided important clues in the understanding of nitrogen cycling in various ecological systems.

Acknowledgements We thank anonymous reviewer for the helpful comments and criticisms. This study was financially supported by the National Natural Science Foundation of China (Grant No. 4121004), and the Ministry of Science and Technology of China through Grant No. 2013CB956700.

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