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The interaction between nitrogen and phosphorous is a strong predictor of intra-plant variation in nitrogen isotope composition in a desert species

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Abstract

Understanding intra-plant variations in δ^{15}N, which can be large, is essential for fully utilizing the role of δ^{15}N as an integrator of the terrestrial nitrogen cycle. Studying such variations can yield insights into nitrogen metabolisms by the plant as a whole or by specific plant organs. However, systematical evaluation of intra-plant variations in δ^{15}N and their relationship with organ nutrient contents is rare. We excavated whole plant architectures of *Nitraria tangutorum* Bobrov, a C_3 species that has an exceptional capability of fixing sands and building sand dunes, in two deserts in northwestern China. We systematically and simultaneously measured nitrogen isotope ratios and nitrogen and phosphorous contents of different parts of the excavated plants. We found that intra-plant variations in δ^{15}N of *N. tangutorum* were positively correlated with corresponding organ nitrogen (N) and phosphorous (P) contents. However, it was the N × P interaction, not N and P individually or their linear combination, that was the strongest predictor of intra-plant δ^{15}N. We hypothesized that this strong positive intra-plant δ^{15}N–N/P relationship was caused by fractionating gaseous N losses (volatilization) from plants and that the volatilization depended on the interaction between organ N and P contents. We also showed that root δ^{15}N increased with depth into soil. This pattern was similar to profiles of soil δ^{15}N reported by previous studies in different ecosystems although the exact relationship between root and soil profiles in δ^{15}N was not clear. Studies of intra-plant variations in δ^{15}N in different species, ecosystems, and climates and measurements of plant nitrogen volatilization and associated isotope fractionation are needed in order to determine the exact mechanisms responsible for the significant patterns first reported in this study.

1 Introduction

Nitrogen is frequently the most limiting nutrient in many terrestrial ecosystems, especially those in temperate and boreal regions (Vitousek, 1994). Consequently, nitrogen and its cycle are fundamental to ecosystem structure and functioning. As atmospheric CO_2 concentrations continue to rise due to anthropogenic fossil fuel emissions, the limiting effects of nitrogen on ecosystem productivity may become increasingly important (Luo et al., 2004; Thornton et al., 2007; Sun et al., 2014). Understanding the nitrogen cycle is essential to forecasting and predicting ecosystem dynamics in response to climate change. Plant nitrogen acquisition, transformation, and translocation are key steps in nitrogen cycling because they subsequently affect plant photosynthesis, growth and metabolism, and substrate supply for microbial activities. Many physical, biological and chemical processes that control plant nitrogen acquisition, transformation, and translocation discriminate against the heavier, rarer nitrogen isotope 15 (^{15}N) in favor of the lighter, more abundant nitrogen isotope 14 (^{14}N). As a result, the variations in the relative abundance of ^{15}N to ^{14}N, quantified as $\delta^{15}N$, of plants contain rich information about these processes (Högberg, 1997; Robinson, 2001; Evans, 2001; Dawson et al., 2002). For this reason, numerous studies have analyzed natural variations in plant $\delta^{15}N$ across disturbance and successional stages (e.g., Hobbie et al., 2000; Wang et al., 2007; Resco et al., 2011; Hyodo et al., 2013), climate and topoedaphic gradients (e.g., Austin and Sala, 1999; Schulze et al., 1998; Martinelli et al., 1999; Amundson et al., 2003; Craine et al., 2005, 2009; Bai et al., 2009), species (e.g., Cernusak et. 2009; Gubsch et al., 2011), nitrogen availability (Wigand et al., 2007; Mayor et al., 2014), types of mycorrhizal fungi (Hobbie and Hobbie, 2008; Hobbie and Högberg 2012) as well as other factors. These studies have demonstrated the power of using natural variations in $\delta^{15}N$ to understand processes controlling nitrogen cycling in terrestrial ecosystems.

Compared with the prolific studies on variations in $\delta^{15}N$ across ecological and climate gradients and species, relatively few studies have systematically evaluated intra-plant variations in $\delta^{15}N$. However, large intra-plant variations in $\delta^{15}N$ have been reported and such variations, if not accounted for, may confound interpretation of isotope variations at large scales (Evans, 2001). Studies that did examine intra-plant variations in $\delta^{15}N$ were often conducted in controlled environments. Many of such studies found that leaves of a plant tended to be enriched in ^{15}N compared with the roots of the same plant (Bergersen et al., 1988; Yoneyama and Kaneko, 1989; Evans et al., 1996; Kolb and Evans, 2002) and the difference can be as high as 7 ‰ which has the same magnitude as variations across ecological and climate gradients. Nevertheless, no foliar enrichment or mixed results have also been observed both in controlled experiments (Evans et al., 1996; Hobbie et al., 2008) and in natural environments (Dijkstra et al., 2003).

Several mechanisms have been proposed to explain intra-plant variations in δ^{15}N or lack thereof. The most commonly discussed mechanism involves the assimilation and transport of inorganic nitrogen of ammonium (NH_4^+) and nitrate (NO_3^-) within plants. NH_4^+ is toxic to plants (Britto and Kronzucker, 2002); once absorbed by roots, it is immediately assimilated in roots by the glutamine synthetase/glutamate synthase (GS-GOGAT) pathway. Thus organic nitrogen compounds derived from NH_4^+ ultimately result from a single assimilation event occurred in roots (Evans et al., 1996). In contrast, NO_3^- can be assimilated by nitrate reductase (NR) in roots, stems, and leaves. Consequently organic nitrogen compounds originated from NO_3^- may come from assimilation events that took place in different parts of the plant (Evans et al., 1996; Evans, 2001). Both the assimilation of NH_4^+ and NO_3^- discriminate against ^{15}N (Yoneyama et al., 2003; Karsh et al., 2012). But because a single assimilation event is responsible for all organic nitrogen compounds derived from NH_4^+, no intra-plant variation in δ^{15}N should occur for plants that have grown with NH_4^+ as the sole nitrogen source, assuming no other fractionation processes exist after NH_4^+ is assimilated. For NO_3^-, the situation is different. The discrimination by NR in roots leads to an enriched pool of unassimilated NO_3^-, which is then transported to other parts of the plant. Thus leaves and shoots should be enriched in ^{15}N as compared with roots when NO_3^- is the source of nitrogen. Besides this mechanism discussed above, other fractionating mechanisms such as differential losses of nitrogen from plant organs, reallocation and resorption of nitrogen have been suggested as potential causes of intra-plant variations in δ^{15}N (Evans, 2001). At present, it is not clear whether these mechanisms, if any, can adequately explain systematic intra-plant variations in δ^{15}N or which mechanism under what conditions may dominate intra-plant variations.

The objective of the present study was to shed light on the mechanisms controlling intra-plant variations in δ^{15}N by systematically and simultaneously analyzing variations in δ^{15}N and nutrient contents in different organs with excavated whole architectures of a desert species grown in natural conditions. The species used in this study was *Nitraria tangutorum* Bobrov, a C_3 shrub species endemic to northwestern deserts in China. *N. tangutorum* controls local landscape evolution, owing to its exceptional capability of fixing sands and building sand dunes known as nebkhas or coppice dunes around its extensive shoot and root systems (Baas and Nield, 2007; Lang et al., 2013; Li et al., 2013). This characteristic makes it relatively easy to excavate the whole plant including roots for isotope and nutrient analyses. Previously we studied intra-plant variations in carbon isotope composition of this species (Zhang et al., 2015). To our knowledge, this species has never been investigated for intra-plant variation in δ^{15}N, whether in cultures or in natural environments. We will demonstrate that intra-plant variations in δ^{15}N in *N. tangutorum* are highly related to organ nutrient contents and their interaction. More importantly, we will show that the intra-plant δ^{15}N – nutrient relationships are very similar to those reported for ecological and climate gradients and for cross-species comparisons even though the interpretation of the latter relationships cannot be applied to the former.

2 Materials and methods

2.1 *Nitraria tangutorum* Bobrov and the study sites

We previously described the biological and environmental characteristics of *N. tangutorum* and the study sites in detail (Zhang et al., 2015). For convenience and completeness, some of the information presented in Zhang et al. (2015) is repeated here. *N. tangutorum*, a spiny shrub species in the *Nitraria* genus of the Zygophyllaceae family, is endemic to the northwestern regions of China. Its distribution includes northeastern Tibet, Gansu, Qinghai, Xinjiang, western Inner Mongolia, western Ningxia, and northern Shaanxi. As a pioneer species with high tolerance for a variety of stresses, *N. tangutorum* is an ecologically important species and plays major roles in controlling desertification due to its exceptional capabilities in forming phytogenic nebkha dunes which prevent or slow down the movement of sands. The height of a *N. tangutorum* nebkha typically ranges from 1 to 3 m and some may reach 5 m. The base of a nebkha often has the shape of an ellipse with the major axis parallel to the local prevailing wind direction (Fig. 1).

The field work was carried out at two desert locations. The first study site was within an experimental area (40°24′ N, 106°43′ E) managed by the Experimental Center of Desert Forestry of the Chinese Academy of Forestry. This site is located in Dengkou County, Inner Mongolia Autonomous Region, China. Dengkou County is at the junction between the Hetao Plain and Ulan Buh Desert of the Mongolian Plateau in the middle reaches of the Yellow River. The mean annual temperature is 8.84° and the mean annual precipitation is 147 mm with 77.5 % of annual rainfall occurring from June to September (1983–2012 averages). The mean annual potential evaporation is 2381 mm (Li et al., 2013). The soil in the study region in general is sandy soil and gray-brown desert soil (Cambic Arenosols and Luvic Gypsisols in FAO taxonomy). The *N. tangutorum* nebkhas at the study site are formed on clay soils deposited by the Yellow River. Although the plant community is dominated by *N. tangutorum*, xerophytic species such as semi-shrub *Artemisia ordosica*, perennial grass *Psammochloa villosa*, and annual species *Agriophyllum squarrosum* and *Corispermum mongolicum* can also be found.

The second study site was within the Gansu Minqin Desert Ecosystem Research Station (38°34′ N, 102°58′ E), Minqin County, Gansu Province, China. Minqin County is located in the lower reaches of Shiyang River, surrounded by the Badain Jaran Desert in the west and north and the Tengger Desert in the east. The mean annual temperature is 8.87° and the mean annual precipitation is 117 mm with 73.1 % of annual rainfall occurring from June to September (1983–2012 averages). The mean annual potential evaporation is 2643 mm (Du et al., 2010). Thus the second study site is somewhat drier than the first site but with similar annual mean temperatures. The soil at the Minqin site is similar to that at the Dengkou site with sandy soil in the nebkhas and gray-brown desert soil between nebkhas. The native vegetation in the study area is usually dominated by shrubs and semi-shrubs with species such as *N. tangutorum* and *Calligonum mongolicum*. Experimental plots used in this study contained semi-fixed nebkha dunes developed by the growth of *N. tangutorum*. Typically in dry years, *N. tangutorum* is the only species growing in the nebkhas although in wet years, annual species such as *Agriophyllum squarrosum* and *Corispermum mongolicum* can also be found. Because the Minqin site is drier than the DengKou site, the nebkhas at the Minqin site are generally smaller and less populated with plants than at the Dengkou site. The rooting depth is deeper at the Minqin site than at the Dengkou site (see Table 1 in Zhang et al., 2015).

2.2 Excavation of *Nitraria tangutorum* nebkhas

In August 2012, three nebkhas were excavated at each study site. The geometrical and biometrical characteristics of the six nebkhas were summarized in Table 1 in Zhang et al. (2015). At the Dengkou site, the three nebkhas were excavated in a sampling area of 40 m × 40 m. At the Minqin site, nebkhas were generally much smaller. To ensure availability for analyses of sufficient biomass materials at this site, particularly the fine roots (see below), three sampling areas each with a dimension of 30 m × 30 m were established and three nebkhas from each sampling area were tentatively excavated. Two nebkhas from one sampling area and one from another were deemed to have sufficient amount of fine roots for analyses and were therefore excavated fully. The nebkhas were excavated by carefully teasing away the sands from the mounds to expose the root architecture of *N*. *tangutorum* with particular attention paid to preserving its fine roots and to distinguishing any roots from other plant species that may happen to grow in the same nebkhas. The roots of a *N*. *tangutorum* plant can be found inside the sand mounds as well as inside the clay layer that generally forms a plain on which the sand mounds rest. We therefore also excavated any roots inside the clay layer to a depth until no more roots could be found. Only biomass materials from *N. tangutorum* were harvested and any materials from all other species that may be present were excluded to ensure pure intra-plant analyses required by this study.

We separated the whole plant biomass into groups of leaves, stems, in-sand roots, and below-plain roots. The in-sand roots, which were roots found inside the nebkha sands but above the plain formed by the underlying clay layer, were further separated into in-sand fine roots (diameter ≤ 2 mm) and in-sand coarse roots (diameter > 2 mm). The same root diameter threshold was used to separate the below-plain roots, which were found inside the clay layer under the nebkha sands. Furthermore, the below-plain fine and coarse roots were grouped in a 20 cm depth increment from the plain surface. We did not separate the in-sand fine and coarse roots into layers because a nebkha has a cone shape on top, making a layer hard to define. Also we did not use a simple "below-ground" group because "ground" is not well defined in a nebkha-populated landscape and because there are large physical and chemical differences between sands and clay which may affect the isotope compositions of roots growing in them. Litter was rarely found on the nebkhas, presumably because strong winds at the study sites can easily blow away any litter produced. However, woody debris from dead ramets was present inside the sand mounds and was collected during excavation. Thus for each nebkha, we differentiated the following categories of *N. tangutorum* biomass for intra-plant isotope analyses: leaves, stems, in-sand fine roots (ISFR), in-sand coarse roots (ISCR), below-plain fine roots (BPFR) in 20 cm depth increments, and below-plain coarse roots (BPCR) in 20 cm increments, and woody debris (WD). Nutrient contents and nitrogen isotope compositions were measured separately for each category.

2.3 Measurements of nutrient contents and nitrogen isotope compositions with excavated biomass

All categories of *N. tangutorum* biomass (leaves, stems, ISFR, ISCR, BPFR in 20 cm increments, BPCR in 20 cm increments, and WD) from each excavated nebkha were dried to constant weight (60°, 48 h). The dry weight of biomass was determined with

0.01 g accuracy on an analytical scale. Dried materials were randomly sampled from each biomass category and ground to 80 mesh. The resultant powder was separated into six duplicates. Three duplicates were analyzed for carbon (C), nitrogen (N) and phosphorous (P) contents and the remaining three for isotope compositions. The C, N and P contents were measured in the Environmental Chemistry Analysis Laboratory in the Institute of Geographic Sciences and Natural Resources Research, the Chinese Academy of Sciences, Beijing, China. Total sample carbon and N were measured with the vario MACRO cube (Elementar Company, Germany). The analytical precision was better than 0.5 % Relative Standard Deviation (RSD). Total P was measured with the ICP-OES OPTIMA 5300DV (PE, USA). The analytical precision was better than 2 % RSD.

The nitrogen isotope compositions were analyzed at the Stable Isotope Ratio Mass Spectrometer Laboratory of the Chinese Academy of Forestry (SIRMSL, CAF), Beijing, China. The instrument used was a Delta V Advantage Mass Spectrometer (Thermo Fisher Scientific, Inc., USA) coupled with an elemental analyzer (FlashEA 1112; HT Instruments, Inc., USA) in the continuous flow mode. Isotope compositions were expressed using the delta notation (δ) in parts per thousand (‰): $\delta^{15}\mathrm{N}(‰) = [(R_{\mathrm{sample}})/(R_{\mathrm{standard}}) - 1] \times 1000$, where R is the molar ratio of ^{15}N to ^{14}N. The measurement applied the IAEA-600 standard (Caffeine) relative to atmosphere N_2. The analytical precision was better than 0.2 ‰ based on replicate measurements of the reference standard.

2.4 Statistical analyses

Two-way ANOVA analyses (organ by site) were performed with SPSS (Ver.17.0). C, N, and P contents, δ^{15}N, C/N ratios, N/P ratios, and C/P ratios were analyzed for differences between organs and between study sites. Tukey post-hoc tests were used to determine pairwise differences for significant effects ($P < 0.05$). Linear and multilinear regression analyses were used to determine the relationships between the organ δ^{15}N and nutrient contents. Due to the strong correlation between organ contents of different nutrients (Zhang et al., 2015) and therefore the potential presence of multicollinearity, we used stepwise regression to determine the most significant predictor(s) (including interaction) of intra-plant variations in δ^{15}N. Both forward and backward methods were used in the stepwise regression with F to Enter and F to Remove set at 4.0 ($P = 0.05$) and 3.9 ($P = 0.052$), respectively.

3 Results

3.1 Variations in δ^{15}N among plant organs and between study sites

For comparing δ^{15}N among plant organs and between sites (Fig. 2), we averaged the duplicate mean of each organ across the three nebkhas at each site. Results for comparisons of nutrient values were already presented in Zhang et al. (2015) and thus not repeated here. There were considerable variations in δ^{15}N values among plant organs and between study sites. At both the Dengkou and Minqin sites, leaves had positive δ^{15}N and were enriched in ^{15}N compared with corresponding stems and roots at the same site. Also at both sites, the δ^{15}N value of fine root followed the same order: ISFR < 1FR < 2FR < 3FR < 4FR; i.e., it increased with depth into soil. Here 1FR, 2FR, 3FR, and 4FR refer to fine roots in 0–20, 20–40, 40–60, and 60 to 80 cm soil depths, respectively. The same pattern was repeated for the δ^{15}N value of coarse root; the only exception was 2CR (coarse root at a soil depth of 20 to 40 cm) at the Dengkou site which dropped out of the general order. The δ^{15}N values of fine roots at the Dengkou site were consistently higher than the corresponding coarse roots both inside the nebkha sands and below the plain of the same site. In contrast at the Minqin site, the δ^{15}N values of fine roots were consistently less than the corresponding coarse roots except for the roots deep into the plain (40–80 cm) where the fine root was more enriched. At the Dengkou site, the stem had the lowest δ^{15}N while at the Minqin site, the ISFR had the lowest δ^{15}N. At both sites, the δ^{15}N value in the woody debris was greater than the corresponding stem although the difference was not statistically significant. The foliar δ^{15}N at the Dengkou site was higher than at the Minqin site. In fact, in all biomass categories investigated, the δ^{15}N value at the Dengkou site was greater than its corresponding counterpart at the Minqin site. The δ^{15}N values of plant organs at the Dengkou site were mostly positive while at the Minqin site, the values were mostly negative.

3.2 Intra-plant relationships between δ^{15}N and nutrient concentrations

Even though intra-plant and between-site variations in δ^{15}N were large, these variations were unified in relationships with organ nutrient contents (Fig. 3). The large intra-plant variations in δ^{15}N (~ 7 ‰ at the Dengkou site and 4 ‰ at the Minqin site) as well as in organ nitrogen and phosphorous contents facilitated regression analyses between these variables. Because the intra-plant relationships were not significantly different between the two study sites, we pooled the data from the two sites in regression analyses. We found that intra-plant variations in δ^{15}N were significantly correlated with the organ contents of carbon (Fig. 3a, $R^2 = 0.25$, $P < 0.0001$), nitrogen (Fig. 3b, $R^2 = 0.44$, $P < 0.0001$) and phosphorous (Fig. 3c, $R^2 = 0.40$, $P < 0.0001$) and with the organ ratios of carbon to nitrogen (Fig. 3d, $R^2 = 0.41$, $P < 0.0001$) and carbon to phosphorous (Fig. 3f, $R^2 = 0.25$, $P < 0.0001$). The correlations were positive with organ nitrogen and phosphorous contents but negative with the carbon content and the carbon to nitrogen and carbon to phosphorous ratios. No correlation with the organ nitrogen to phosphorous ratios was found (Fig. 3e).

Although intra-plant variations in δ^{15}N were significantly correlated with organ nitrogen and phosphorous contents, both forward and backward stepwise regressions consistently identified the interaction between nitrogen and phosphorous contents as the most significant predictor of intra-plant variation in δ^{15}N ($R^2 = 0.58$, $P < 0.0001$, Fig. 4). Adding organ nitrogen content or phosphorous content or both did not significantly improve the predictive ability of resultant equations.

Since fine roots differ from other organs in that fine roots are the primary organs for nitrate reduction, we re-calculated the organ δ^{15}N – nutrient relationships by removing all fine roots from the analyses and found that all correlations became stronger (compare Fig. 5 with Fig. 3). In addition, because leaves had considerably higher δ^{15}N and nutrient contents than other organs, we similarly re-calculated the correlations by removing leaves from the analyses to avoid a foliar domination of the obtained relationships. After the leaves were removed, all correlations were still significant (data not shown, but can be seen from Figs. 3–5). Furthermore, the removal of either leaves or fine roots did not alter the finding that the N × P interaction was the strongest predictor of intra-plant variations in δ^{15}N. Thus the intra-plant δ^{15}N – nutrient relationships appeared to be generic and independent of specific physiological or metabolic functions of particular plant organs.

4 Discussion

This study appears to be the first to report that the strongest predictor of intra-plant variation in δ^{15}N is the interaction between organ N and P contents rather than N or P themselves or their linear combination. To our knowledge, no previous studies have systematically evaluated relationships between intra-plant variations in δ^{15}N and organ N or P contents. However, a number of researchers have examined the correlations of foliar δ^{15}N with N across climate and ecological gradients or across species. The positive intra-plant correlation between δ^{15}N and N content reported in the present study is reminiscent of the foliar correlations reported in those previous studies. Using a dataset that contained over 11 000 plants worldwide, Craine et al. (2009) found that foliar δ^{15}N was positively correlated with foliar N. A subset of this dataset contained δ^{15}N, N and P measurements. Craine et al. (2009) subsequently analyzed this subset with a multilinear model that used N, P and their interaction as explanatory variables. It was not clear whether multicollinearity was controlled but Craine et al. found that after controlling for variations in N, foliar δ^{15}N decreased with an increase in P and in

N × P. We used the same model to fit our intra-plant dataset without consideration of multicollinearity and found that foliar δ^{15}N decreased with both N and P but increased with N × P. Thus controlling multicollinearity is important for ascertaining relationships between δ^{15}N and nutrient contents due to correlations between contents of different nutrients.

Positive foliar correlations of δ^{15}N with N have been reported in studies at smaller scales as well (e.g., Martinelli et al., 1999; Hobbie et al., 2000; Craine et al., 2005). In addition, Hobbie et al. (2008) reported a positive correlation for root tips. These positive correlations, which were all inter- rather than intra-plant in nature, are consistent with the reported experimental finding that an increase in soil nitrogen availability tends to lead to an increase in δ^{15}N of non-N-fixing plants (Wigand et al., 2007; Hobbie et al., 2008; Mayor et al., 2014). A hypothesis based on plant-mycorrhizal interactions has been advanced to explain this positive relationship (Hobbie et al., 2000; Craine et al., 2009; Hobbie and Högberg, 2012). Typically mycorrhizal fungi transfer isotopically depleted N to host plants. As soil N supply increases, the contribution from mycorrhizal symbionts to the total N budget of host plants may decrease, reducing the mycorrhizal dilution effect on the heavy isotope and resulting in a positive relationship of plant δ^{15}N with soil N supply. However, this explanation is only valid for δ^{15}N of the plant as a whole and cannot explain the positive relationship of intra-plant δ^{15}N with N and the interaction between N and P. In addition to the mycorrhizal hypothesis, a more general explanation for the N supply–plant δ^{15}N relationship involves the openness of the N cycle. This explanation hypothesizes that an increase in N supply promotes the openness of the N cycle and the increased openness results in higher losses of ^{14}N relative to ^{15}N from the system, leading to enrichment in ^{15}N in the remaining nitrogen pool. The openness typically refers to processes occurring in soil (e.g., N losses through denitrification via the release of N_2O and N_2 from soil which is a strong fractionating process, Mnich and Houlton, 2015). Clearly a soil-central N openness explanation is also not valid for the intra-plant δ^{15}N–N × P relationship reported in this study.

Another possibility to consider concerns the situation when nitrate is the source of N for plants. If soil supply of nitrate is low, all nitrate absorbed by roots may be assimilated in the roots and no enriched nitrate pool is left for transport to other parts of the plant. As soil supply of nitrate increases, the proportion of the nitrate pool that is unassimilated by roots and thus is available for transport to other parts of the plant may not only increase in size but also become more enriched in ^{15}N (a system cannot discriminate if all substrates are assimilated; discrimination generally increases with substrate availability, Evans, 2001). However, this possibility can only suggest that the difference in δ^{15}N between roots and the rest of the plant may increase with soil nitrate supply. It cannot account for the changes of δ^{15}N with organ N and P contents and their interaction within the plant.

We suggest that fractionating, N/P content-dependent gaseous N losses (volatilization) from plant organs are responsible for the positive intra-plant δ^{15}N–N/P relationship observed in this study. Volatilization of gaseous N from plant parts have long been reported (Farquhar et al., 1983; Sharpe and Harper, 1997). However quantifying the volatilization rate is difficult and determining the fractionation of volatilization process is especially challenging (Evans, 2001). Like many physical and biochemical processes, N volatilization from plant parts can be reasonably assumed to be fractionating with the end result of enriching plant parts in ^{15}N (Farquhar et al., 1983). It is also likely that part parts with higher N contents may volatilize N more readily (Wetselaar and Farquhar, 1980). If these two processes occur together, a positive intra-plant δ^{15}N–N relationship can be expected. However it remains a research task to ascertain roles of N × P interaction in the volatilization hypothesis. Major et al. (2014) showed that long-term additions of N and P reduced foliar δ^{15}N as compared with N or P addition alone in a lowland tropical rainforest. This experiment may not have direct implication for N × P interaction as a strong predictor of intra-plant variations in δ^{15}N.

This study also appears to be the first to systematically evaluate variations in root δ^{15}N with depth into soil. However, our finding that roots tend to become more enriched in ^{15}N deeper into soil is reminiscent of the general patterns of increasing soil δ^{15}N with depth as reported in previous studies (Hobbie and Ouimette, 2009; Gubsch et al., 2011; Szpak, 2014). Whether the profiles of root and soil δ^{15}N are related to each other and how they are related are also questions for future research.

5 Conclusions

A systematical evaluation of nitrogen isotope composition in the desert plant species *Nitraria tangutorum* Bobrov reveals that the magnitude of intra-plant variations in δ^{15}N is close to the highest value reported in previous studies (7 ‰, Fig. 3 and also Evans, 2001). These variations are positively correlated with corresponding organ N and P contents. However, it is the N × P interaction, not N and P individually or their linear combination, that is the strongest predictor of intra-plant δ^{15}N. While the positive correlation of intra-plant δ^{15}N with organ N resembles the δ^{15}N–N relationships reported in previous studies focusing on patterns across ecological and climate gradients and across species, explanations developed from these previous studies are not valid for the finding reported in the present study. We hypothesize that the positive intra-plant δ^{15}N–N/P relationship is caused by fractionating gaseous N losses (volatilization) from plants and that the volatilization depends on the interaction between organ N and P contents. We also report that root δ^{15}N increases with depth into soil. This pattern in root δ^{15}N is similar to profiles of soil δ^{15}N reported in previous studies although the exact relationship between root and soil profiles in δ^{15}N is not clear.

Knowledge of how plants acquire, transport and transform N is crucial for understanding how plants use this crucial resource for production and growth and how the terrestrial N cycle operates. Intra-plant variations in δ^{15}N are an important part of the N cycle and result from plant nitrogen metabolism which is organ specific and perhaps also affected by environmental conditions. The findings reported in the present study suggest that different mechanisms may operate at different scales to affect plant nitrogen isotope compositions and their relationships with nutrient availability. Alternatively, causes of variations in δ^{15}N, whether they are intra-plant, inter-species, or cross ecological and climate gradients, may differ from previously thought. Our findings suggest that studies into intra-plant variations in δ^{15}N and their mechanisms can yield deep insights into the N cycle of ecosystem and plant nitrogen metabolism and such studies have not be adequate in the past and are urgently needed.

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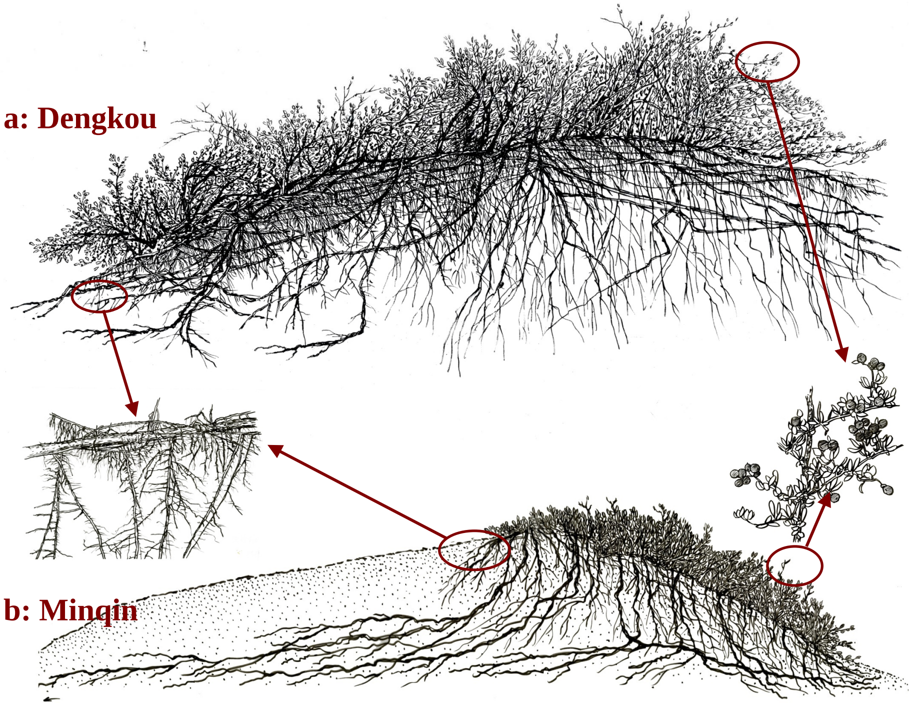

Figure 1. Pen drawings of typical nebkha formed by *Nitraria tangutorum* Bobrov at the Dengkou **(a)** and Minqin **(b)** study sites.

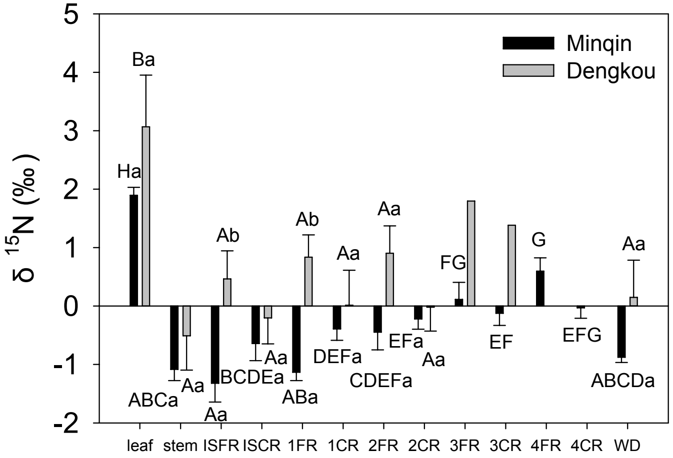

Figure 2. A comparison of δ^{15}N among different plant organs of *Nitraria tangutorum* Bobrov and between the Dengkou and Minqin study sites. The δ^{15}N value shown is averaged for each organ across the nebkhas excavated at the same site (Dengkou or Minqin). Upper-case letters denote ANOVA results within a study site (i.e., comparing δ^{15}N among different organs at the same site) and lower case letters between the two sites (i.e., comparing δ^{15}N of the same organ between the two sites). ISFR and ISCR stand for fine and coarse roots, respectively, in the sands of nebkhas. 1FR, 2FR, 3FR and 4FR stand for fine roots 0–20, 20–40, 40–60 and 60–80 cm depths, respectively, below the plains on which nebkhas rest. Similarly, 1CR, 2CR, 3CR and 4CR stand for coarse roots within these depth intervals. Fine and coarse roots are differentiated with a diameter threshold of 2 mm. Woody debris (WD) from dead ramets is also included in the figure. No ANOVA results for 3FR and 3CR at the Dengkou site as there was only one nebkha having roots between 40 to 60 cm. No roots were found below 60 cm at the Dengkou site.

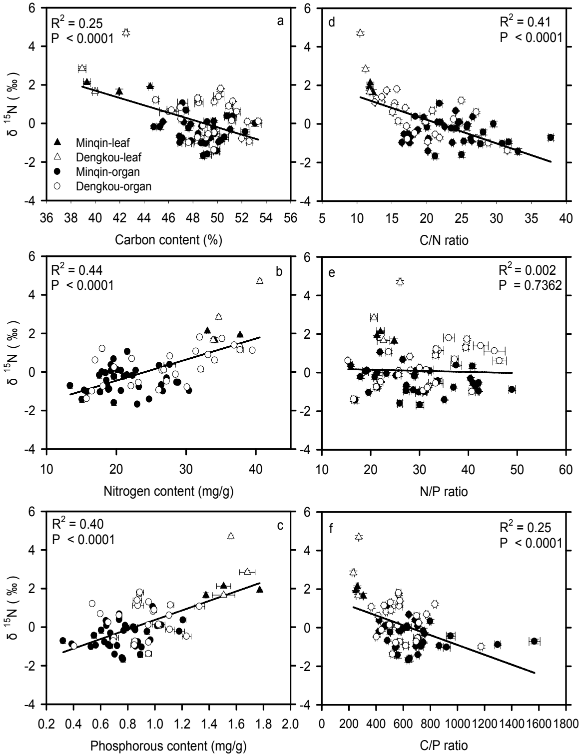

Figure 3. Changes of δ^{15}N as a function of organ contents of carbon **(a)**, nitrogen **(b)** and phosphorous **(c)** and of organ ratios of carbon to nitrogen **(d)**, nitrogen to phosphorous **(e)**, and carbon to phosphorus **(f)**. Filled and unfilled symbols represent organs at the Minqin and Dengkou site, respectively. Leaves are denoted by filled or unfilled triangles while other organs by filled or unfilled circles.

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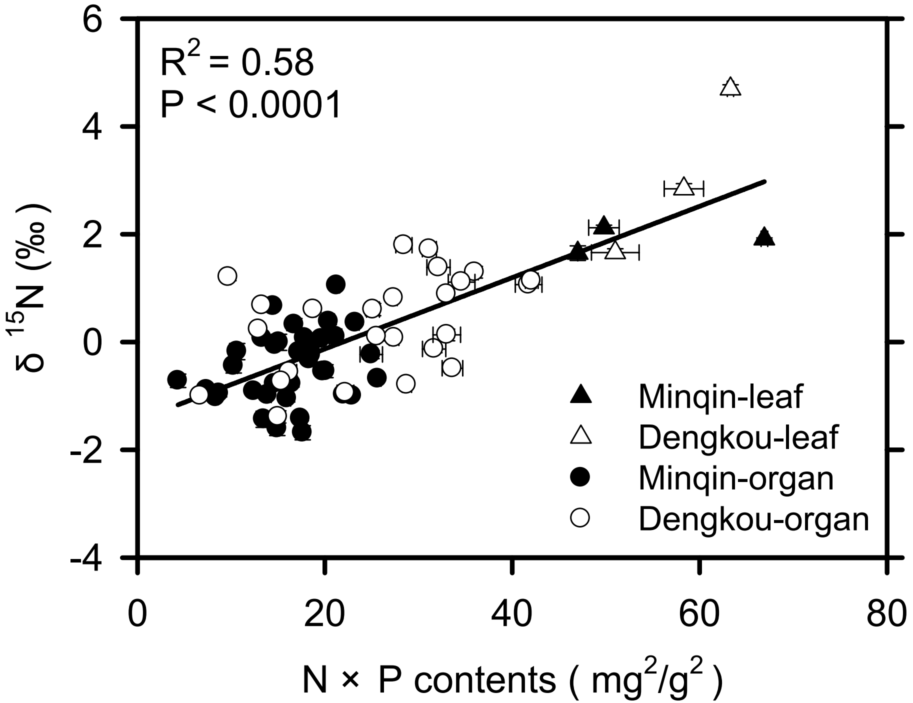

Figure 4. Changes of δ^{15}N as a function of the product of organ N × P contents. Filled and unfilled symbols represent organs at the Minqin and Dengkou site, respectively. Leaves are denoted by filled or unfilled triangles while other organs by filled or unfilled circles.

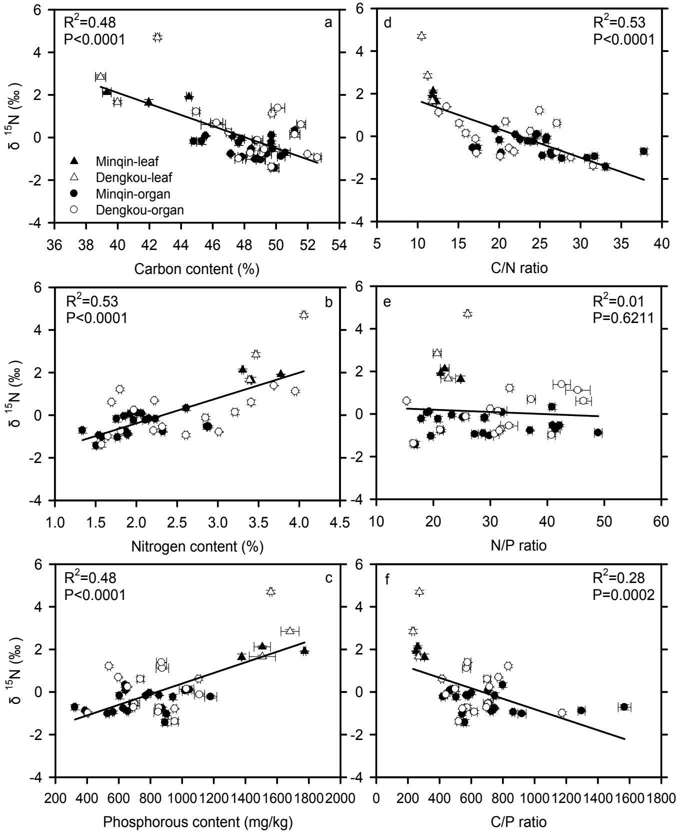

Figure 5. The same as Fig. 3 except that data of fine roots are not included.