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Nitrogen isotopic composition of plants and soil in an arid mountainous terrain: sunny slope versus shady slope

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Abstract

Nitrogen cycling is tightly associated with environment. Sunny slope of a given mountain could significantly differ from shady slope in environment. Thus, N cycling should also be different between the two slopes. Since leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ ($\Delta\delta^{15}N_{leaf\text{-}soil}$ = leaf $\delta^{15}N$ – soil $\delta^{15}N$) could reflect the N cycling characteristics, we put forward a hypothesis that leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ should differ across the two slopes. However, such a comparative study between two slopes has never been conducted yet. In addition, environmental effects on leaf and soil $\delta^{15}N$ derived from studies at global scale were often found to be different from that at regional scale. This led to our argument that environmental effects on leaf and soil $\delta^{15}N$ could depend on local environment. To confirm our hypothesis and argument, we measured leaf and soil $\delta^{15}N$ on the sunny and shady slopes of Mount Tianshan. Remarkable environment differences between the two slopes provided an ideal opportunity for our test. The study showed that leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ on the sunny slope were greater than that on the shady slope although the difference in soil $\delta^{15}N$ was not significant. The result confirmed our hypothesis and suggested that the sunny slope has higher soil N transformation rates and soil N availability than the shady slope. Besides, this study observed that the significant influential factors of leaf $\delta^{15}N$ were temperature, precipitation, leaf N, leaf C/N and silt/clay ratio on the shady slope, whereas on the sunny slope only leaf C/N was related to leaf $\delta^{15}N$. The significant influential factors of soil $\delta^{15}N$ were temperature, precipitation and silt/clay ratio on the shady slope, whereas on the sunny slope MAP

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and soil moisture exerted significant effects. Precipitation exerted contrary effects on soil $\delta^{15}N$ between the two slopes. Thus, this study supported our argument that the relationships between leaf and soil $\delta^{15}N$ and environmental factors are local-dependent.

1 Introduction

In natural terrestrial ecosystem, nitrogen (N) is not only the most required element, but also is usually a key limiting resource for plants (Vitousek et al., 1997), thus, studying N cycling is of vital importance. The variations of nitrogen isotope ratio ($\delta^{15}N$) in plants and soil are tightly associated with many biogeochemical processes including N mineralization, ammonia volatilization, nitrification, denitrification (Högberg, 1997; Houlton et al., 2006). Mineralization produces available N, including ammonium and nitrate, which are the substrates for ammonia volatilization, nitrification and denitrification. During these processes, gaseous N loss is more likely to be depleted in ^{15}N, which will cause the remaining N pool and subsequently plants to enrich ^{15}N (Högberg, 1997). Additionally, the difference between leaf $\delta^{15}N$ and soil $\delta^{15}N$ ($\Delta\delta^{15}N_{leaf\text{-}soil}$ = leaf $\delta^{15}N$ – soil $\delta^{15}N$), which is also named enrichment factor (Emmett et al., 1998), was also suggested to be an indicator of ecosystem N cycling (Charles and Garten, 1993; Kahmen et al., 2008; Fang et al., 2011), and it was also reported to be correlated with soil N transformation rates (N mineralization or nitrification rates) (Garten and Van Miegroet, 1994). Thus, nitrogen isotopes have been widely applied in studies of terrestrial ecosystem N cycling (Handley et al., 1999;

Evans, 2001; Robinson, 2001; Hobbie and Colpaert, 2003; Houlton et al., 2007).

For a given mountain, its sunny slope may be significantly different from its shady slope in climate and environment. It is well known that ecosystem N cycling is associated with climatic and environmental conditions (Amundson et al., 2003; Craine et al., 2009; Yang et al., 2013; Zhou et al., 2016), thus, ecosystem N cycling should vary across sunny and shady slopes. Since leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ could reflect and indicate ecosystem N cycling, differences in leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ were expected to appear between sunny and shady slopes. Comparisons on leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ across two slopes of a mountain would provide a good insight into the response of terrestrial ecosystem N cycling to climate and environment. However, to our knowledge, such a comparative study has never been conducted yet.

Most of the published works consistently suggested that leaf $\delta^{15}N$ increased with increasing mean annual temperature (MAT) and decreasing mean annual precipitation (MAP) at large regional or global scales (Austin and Sala, 1999; Amundson et al., 2003; Craine et al., 2009). However, in contrast to the commonly reported patterns, leaf $\delta^{15}N$ was found to be negatively related to MAT in some Asian regions, e.g., in Inner Mongolian (Cheng et al., 2009) and eastern China (Sheng et al., 2014). Relative to plant $\delta^{15}N$, soil $\delta^{15}N$ has been little addressed. Some studies demonstrated that soil $\delta^{15}N$ decreased with increasing MAP and decreasing MAT at the global scale (Amundson et al., 2003; Craine et al., 2015b). However, studies based on local or region scale showed inconsistent results with the global patterns. Cheng et al. (2009)

reported that soil $\delta^{15}N$ increased with decreasing MAT in Inner Mongolian. Sheng et al. (2014) showed that the soil $\delta^{15}N$ in tropical forest ecosystems were ^{15}N-depleted than in temperate forest ecosystems of eastern China. Yang et al. (2013) found that soil $\delta^{15}N$ did not vary with either MAT or MAP on the Tibetan Plateau. Wang et al. (2014) revealed a second-order polynomial relationship between soil $\delta^{15}N$ and aridity index across arid and semi-arid regions. The above inconsistent observations led to our argument that the relationships between environmental factors and leaf $\delta^{15}N$ or soil $\delta^{15}N$ would depend on local environment. Comparisons on the effects of climatic and environmental factors on leaf $\delta^{15}N$ and soil $\delta^{15}N$ between sunny and shady slopes of a given mountain could test the argument.

This study was conducted on the sunny slope and shady slope of Mount Tianshan. It is an ideal place for testing our hypotheses because its sunny slope differs greatly from its shady slope in climatic and environmental conditions (Deng et al., 2015; Zhang et al., 2016). The first objective of the present study was to confirm our hypothesis that the sunny slope differs from the shady slope in leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$. The second objective was to test our argument that environmental effects on leaf $\delta^{15}N$ and soil $\delta^{15}N$ are local-dependent.

2 Materials and methods

2.1 Study area

Mount Tianshan is one of the largest seven mountains over the world. It has a total length of 2500 km straddling four countries including China, Kazakhstan, Kyrgyzstan

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and Uzbekistan. In China, Mount Tianshan stretches 1700 km along the east-west direction in the Xinjiang Uygur Autonomous Region and covers about 570,000 square kilometers and accounts for one third of the whole area. Mount Tianshan divides Xinjiang into two parts, the south of Tianshan is the Tarim Basin and the north is the Dzungaria Basin.

This study was conducted along an elevation transect on the shady and sunny slopes on eastern Mount Tianshan (42.43° – 43.53°N, 86.23° – 87.32°E) (Fig.1). Mount Tianshan is characterized by an arid mountainous climate; vertical variations in temperature and precipitation are very pronounced, temperature decreases and precipitation increases with altitude on both slopes. The shady slope differs significantly from the sunny slope both in climate and vegetation. On the shady slope, the annual mean temperature (MAT) ranges from -6.40 ℃ to 3.90 ℃ with the average temperature of -1.85 ℃, and the annual mean precipitation (MAP) ranges from 314 mm to 472 mm with the average precipitation of 402 mm. While on the sunny slope, the MAT varies from -5.65 ℃ to 9.23 ℃ with the average temperature of 1.03 ℃, and the MAP varies from 124 mm to 308 mm with the average precipitation of 246 mm. There were four meteorological observatories along our elevation transects, two on either slope of Mount Tianshan (Table 1).

Fig. 1

Table 1

Intact and continuous vertical vegetation and soil spectrums can be observed along the two slopes. On the shady slope from bottom to top, vegetation spectrum consists of upland desert (800–1100 m), upland steppe (1100–2500 m), frigid coniferous forest (1800–2700 m), subalpine meadow (2500–3300 m), alpine meadow (3000–3700 m), alpine sparse vegetation and a desert zone (3700–3900 m), and an alpine ice-and-snow zone (> 3900 m). A corresponding soil spectrum on shady slope includes brown calcic soil (800–1100 m), chestnut soil (1100–2500 m), mountain grey cinnamon forest soil (1800–2700 m), subalpine meadow soil (2500–3300 m), alpine meadow soil (3000–3700 m) and chilly desert soil (> 3700 m). While on the sunny slope, it includes upland desert (1300–1800 m), arid upland steppe (1800–2600 m), subalpine steppe (2600–2800 m), alpine meadow and cushion plants (2800–3800 m), an alpine desert zone (3800–4000 m), and an alpine ice-and-snow zone (> 4000 m). The corresponding soil spectrum of sunny slope consists of sierozem (1300–1800 m), chestnut soil (1800–2800 m), alpine meadow soil (2800–3800 m), and chilly desert soil (> 3800 m).

2.2 Plants and soil sampling

An altitudinal transect of 1,564 to 3,800 m above sea level (a.s.l.) was set on the shady slope, and 1,300 to 3,780 m a.s.l. on the sunny slope. Few human habitats distribute along the two transects. Plant and soil samples were collected in July of 2014. To minimize the influences of human activities, light regime, or location within the canopy, the sampling was restricted to open sites that are far from the major roads and human habitats.

Plants and soil were collected along the two transects at altitudinal intervals of about 100 m. Almost all plant species that we found at each sampling site were collected, and at each site, 5–7 individual plants of each species were collected and the same number of leaves was sampled from each individual plant. For shrubs and herbs, the uppermost leaves of each individual plant were collected; for tree species, 8 leaves were collected from each individual, 2 leaves were collected at each of the 4 cardinal directions from the positions of full irradiance, about 8–10 m above the ground. The leaves from the same species of each site were combined into one sample. Excluding N-fixing plants and mosses, a total of 90 plant samples were collected from shady slope, including 72 herbs and 18 woody plants; 105 on the sunny slope, covering 85 herbs and 20 woody plants.

Surface soils (0–5cm) were collected after removing the litter layer at each sampling site. At each location, one composite soil sample was prepared by combining six subsamples randomly taken within a radius of 20 m. Sample was used to determine soil index including $\delta^{15}N$, N content, silt/clay ratio, pH and particle size. In addition, at each sampling site, we also collected another three soil samples using a ring, which were used to measure soil bulk density and moisture.

2.3 Laboratory measurements

Plant and soil samples were air-dried in the field and then in the laboratory. The soil samples were sieved through a 2 mm sieve to remove stones and plant residues. Plant leaves and about 5 g sieved soil samples were then ground into a fine powder using a steel ball mixer mill MM200 (Retsch GmbH, Haan, Germany). $\delta^{15}N$, N and C contents in leaves, and $\delta^{15}N$ and N contents in soil were measured on a Delta^{Plus} XP mass spectrometer (Thermo Scientific, Bremen, Germany) coupled with an automated elemental analyzer (Flash EA1112, CE Instruments, Wigan, UK) in a continuous flow mode at the Stable Isotope Laboratory of the College of Resources and Environmental Sciences, China Agricultural University. For this measurement, we obtained standard deviations of less than 0.1% for C and N contents and less than 0.15‰ for $\delta^{15}N$ among replicates of the same sample.

The measurements of soil pH and soil particle size (clay, silt and sand content) were determined using the sieved soil samples. Soil pH was measured using the pH electrode in soil water suspension, with soil to water ratio of 1:2.5 (10 g soil and 25 mL deionized water removing carbon dioxide). Soil particle size (clay, silt and sand content) was analyzed using a particle size analyzer (Malvern Masterizer 2000, UK) after removing the calcium carbonates and organic matter. Soil moisture and bulk density were determined after oven drying at 105 ± 2 ℃ to a constant weight. Soil moisture of each sample was the difference between its wet and dry weight divided by its dry weight. Soil bulk density was the dry weight divided by the certain volume of the ring.

2.4 Statistical analysis

The MAT and MAP data of each sampling elevation used in the statistical analyses were generated by interpolation based on the climatic data derived from the four meteorological observatories distributed along the altitudinal transect. Statistical analyses were conducted by SPSS software (SPSS for Windows, Version 20.0, Chicago, IL, USA). One-way analysis variance (ANOVA) was used to compare leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ between the shady and sunny slopes. Leaf C/N was ln- transformed to improve data normality. The relationships between $\Delta\delta^{15}N_{leaf\text{-}soil}$ and leaf $\delta^{15}N$ were performed by the linear regression on the two slopes. Leaf and soil $\delta^{15}N$ were firstly analyzed by multiple linear regressions against all potential influential factors using ordinary least square (OLS) estimation. The potential influential factors of leaf $\delta^{15}N$ included MAP, MAT, leaf N content, leaf C/N, soil $\delta^{15}N$, soil N content, silt/clay ratio, soil moisture, soil bulk density and soil pH. The potential influential factors of soil $\delta^{15}N$ consisted of MAP, MAT, soil N content, silt/clay ratio, soil moisture and soil bulk density and soil pH. Finally, correlation analyses were conducted to explore the effects of these factors on leaf $\delta^{15}N$ and soil $\delta^{15}N$.

3 Results

3.1 Comparisons of $\delta^{15}N$ in leaf and soil between the shady and the sunny slopes

On Mount Tianshan, for all species pooled together, the arithmetic mean (mean ± SE) of leaf $\delta^{15}N$ were 0.5 ± 0.2‰ and 2.0 ± 0.2‰ for the plants grown on the shady and the sunny slopes, respectively. One-way ANOVA suggested a significant difference for leaf $\delta^{15}N$ between the shady and sunny slopes ($P < 0.001$) (Fig. 2a). The mean soil

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δ^{15}N of the shady and sunny slope were 4.1 ± 0.4‰ and 5.0 ± 0.8‰, respectively. One-way ANOVA showed that sampling slope exerted no significant effect on soil δ^{15}N (P = 0.290) (Fig. 2b). The mean $\Delta\delta^{15}N_{leaf\text{-}soil}$ was -3.6 ± 0.3‰ for the shady slope and -2.4 ± 0.3‰ for the sunny slope, and one-way ANOVA suggested a significant difference in $\Delta\delta^{15}N_{leaf\text{-}soil}$ between the two slopes (P = 0.003) (Fig. 2c). In addition, this study showed that $\Delta\delta^{15}N_{leaf\text{-}soil}$ was positively related to $\delta^{15}N_{leaf}$ on the two slopes (P < 0.001, Fig. 3).

Fig. 2

Fig. 3

3.2 The relationships between leaf δ^{15}N and potential influential factors

A multiple regression of leaf δ^{15}N against potential influential factors including soil δ^{15}N, MAT, MAP, leaf N content, leaf C/N, soil N content, soil moisture, soil pH, soil bulk density and silt/clay was conducted. The statistical analyses showed that 45.8% and 23.4% of the variability in leaf δ^{15}N on the shady slope and sunny slope could be explained as a linear combination of all 10 independent variables, respectively (P < 0.001 for the shady slope and P = 0.005 for the sunny slope) (Table 2). Among these influential factors, MAT, leaf N content correlated positively and MAP, leaf C/N, and silt/clay ratio correlated negatively with leaf δ^{15}N on the shady slope (Table 3). Whereas on the sunny slope, only leaf C/N was found to have a negative effect on leaf δ^{15}N, MAP correlated marginally and negatively with leaf δ^{15}N (Table 4).

Table 2

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Table 3

Table 4

3.3 The relationships between soil δ^{15}N and potential influential factors

Multiple regressions analysis with soil δ^{15}N as a dependent variable and MAT, MAP, soil N content, silt/clay ratio, soil moisture, soil bulk density and soil pH as independent variables were conducted separately for the shady slope and the sunny slope. The statistical analyses showed that the regressions were very significant on both slopes (P < 0.001 for the both slopes). The seven factors in total accounted for 55.2% and 72.7% of soil δ^{15}N variance on the shady and sunny slope, respectively (Table 2). Considering the potential link between soil N and plant N, new multiple regressions including leaf δ^{15}N, leaf N and leaf C/N were performed on the two slopes. Compared to the old multiple regressions, the new regressions did not exhibit changes in R^2 and P values on both slopes (in the new regressions P < 0.001 and R^2 = 0.563 for the shady slope, P < 0.001 and R^2 = 0.738 for the sunny slope). Furthermore, compared to the adjusted R^2 values derived from the old regressions (adjusted R^2 = 0.513 for the shady slope, adjusted R^2 = 0.708 for the sunny slope), the values of the new regressions were smaller or almost unchanged (adjusted R^2 = 0.506 for the shady slope, adjusted R^2 = 0.709 for the sunny slope) (Table 2). Thus, the new multiple regressions indicated no effect of leaf nutrient traits on soil δ^{15}N. Among these factors, only MAT, MAP and silt/clay were found to be significantly related to the soil δ^{15}N of the shady slope. The soil δ^{15}N was observed to increase with increasing MAP and decreasing MAT and silt/clay ratio on the shady slope (Table 3). However, on the sunny slope, only MAP and soil moisture were found to play a significant and negative role in soil $\delta^{15}N$ (Table 4).

4 Discussion

4.1 Differences in leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ between the sunny and the shady slopes

On Mount Tianshan, leaf $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ both showed higher values on the sunny slope than the shady slope; soil $\delta^{15}N$ of the sunny slope was also more positive than that of the shady slope although the difference was not significant. The results confirmed our hypothesis that, for a given mountain, the leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ of the sunny slope could differ from those of the shady slope. Greater leaf $\delta^{15}N$ on the sunny slope than shady slope suggested that the sunny slope had higher soil N availability and higher soil N transformation rates (N mineralization or nitrification rates) (Garten and Van Miegroet, 1994; McLauchlan et al., 2007). Increasing soil N transformation rates led to an increase in soil available N. Meanwhile, increasing soil N transformation rates could result in more ^{15}N enrichment in soil available N sources, and consequently plant $\delta^{15}N$ increased, because N transformation processes discriminate against ^{14}N.

$\Delta\delta^{15}N_{leaf\text{-}soil}$ was greater on the sunny slope than the shady slope. This result also suggested that the sunny slope has higher N availability and N mineralization or nitrification rates relative to the shady slope, because previous studies reported that

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$\Delta\delta^{15}N_{leaf-soil}$ increased with increasing soil N transformation rates (N mineralization or nitrification rates) and N availability (Garten and Van Miegroet, 1994; Kahmen et al., 2008; Cheng et al., 2010).

In most natural ecosystems, where soil available N is limited or plant N demand exceeds soil nitrogen supply, N isotopic discrimination would be negligible during nitrogen uptake and assimilation (Högeberg et al., 1999). Thus, leaf $\delta^{15}N$ is a good approximation to $\delta^{15}N$ of soil available nitrogen sources in natural ecosystems (Virginia and Delwiche, 1982; Cheng et al., 2010; Craine et al., 2015a), and $\Delta\delta^{15}N_{leaf-soil}$ was interpreted as the isotopic composition of plant-available N (Amundson et al., 2003). So, both leaf $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf-soil}$ are good indicators of available N, and the positive relationship is expected to exist between leaf $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf-soil}$. The results that $\Delta\delta^{15}N_{leaf-soil}$ was highly correlated with leaf $\delta^{15}N$ on the two slopes of Mount Tianshan provided a powerful support for the above viewpoints. The observed positive relationship between $\Delta\delta^{15}N_{leaf-soil}$ and leaf $\delta^{15}N$ suggested that significant difference in $\Delta\delta^{15}N_{leaf-soil}$ between the sunny and shady slopes was mainly due to the significant difference in leaf $\delta^{15}N$ between two slopes.

4.2 Influences of varied factors on leaf $\delta^{15}N$ and soil $\delta^{15}N$: sunny slope versus shady slope

The regression and correlation analyses showed that each factor did not exert completely identical effect on leaf $\delta^{15}N$ and soil $\delta^{15}N$ across the two slopes, this provided powerful support for our argument that the influences of environmental

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factors on leaf δ^{15}N and soil δ^{15}N are local-dependent.

Leaf C/N may play a role in regulating biogeochemical cycles of carbon and nitrogen in natural ecosystems (Luo et al., 2004), or, conversely, soil biogeochemistry and plant physiology also cause the shifts in leaf C/N stoichiometric characters (Reich and Oleksyn, 2004; Yang et al., 2011). In this study, leaf C/N was negatively correlated with leaf δ^{15}N on both slopes of Mount Tianshan. The result was similar to the finding by Pardo et al. (2006), in which leaf δ^{15}N and root δ^{15}N both decreased with forest floor C/N. A negative correlation between leaf C/N and δ^{15}N was also reported for the fine roots in Glacier Bay (Hobbie et al., 2000). Two possible reasons were responsible for the pattern observed in the present study. First, the increase in leaf C/N might be caused by enhanced photosynthesis, which would aggravate the limit in nitrogen nutrients and result in a decrease in nitrogen availability, consequently, plants would deplete ^{15}N. Second, leaf C/N usually was considered to be negatively correlated with leaf N contents because leaf C contents always keep relative stable (Tan and Wang, 2016). The relative stability of leaf C was also observed in this study. The negative relationship between leaf C/N and leaf δ^{15}N might be caused by the positive relationship between leaf N content and leaf δ^{15}N, which has been reported by many studies (Chen et al., 2015; Zhang et al., 2015; Craine et al., 2012; Pardo et al., 2006; Craine et al., 2009; Martinelli et al., 1999). This study also found a positive relationship between leaf N content and leaf δ^{15}N on the shady slope of Mount Tianshan.

MAP was observed to be significantly and negatively correlated with leaf δ^{15}N on the shady slope; however, on the sunny slope the relationship was just marginally significant. A negative relationship between leaf $\delta^{15}N$ and MAP was reported in many previous studies (Austin and Sala, 1999; Handley et al., 1999; Robinson, 2001; Amundson et al., 2003; Craine et al., 2009). The decrease in leaf $\delta^{15}N$ with increasing precipitation could be associated with decreased gaseous N loss in wetter regions (Houlton et al., 2006).

MAT played a positive effect in the leaf $\delta^{15}N$ of the shady slope, whereas on the sunny slope the effect of MAT was not observed. The probable explanation for this observation was that the climate on the shady slope is very cold (the average MAT = -1.85 ℃), temperature is the key growth-limiting factor for plants, thus, temperature exerted an effect on leaf $\delta^{15}N$. However, on the sunny slope the climate is relatively warm except those sites with higher altitudes, and usually, temperature does not limit plant growth, thus, leaf $\delta^{15}N$ was not related to temperature.

On Mount Tianshan, soil $\delta^{15}N$ increased with increasing MAP on the shady slope, while decreased with increasing MAP on the sunny slope. Soil $\delta^{15}N$ could be determined by the balance of the N input or output processes and corresponding isotopic fractionation factors (Brenner et al., 2001; Bai and Houlton, 2009; Wang et al., 2014). Considering that the leaching loss could be neglected on both slopes because of the dry environment, soil $\delta^{15}N$ can be estimated by the following equation:

$$\text{Soil } \delta^{15}N = \delta^{15}N_{input} + \varepsilon_G \times f_G + \varepsilon_P \times f_P \quad (1)$$

where $\delta^{15}N_{input}$ is the input $\delta^{15}N$; f_G and f_P are the fraction of gas losses and net plant N accumulation out of total N losses (%), respectively; ε_G and ε_P are the fractionation

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factors of corresponding N losses processes, respectively. And

$$f_G + f_P = 1 \quad (2)$$

ε_G varies from 16‰ to 30‰ (Handley et al., 1999; Robinson, 2001); ε_P is between 5‰ and 10‰ (Handley et al., 1999; Evans, 2001), thus, in general, $\varepsilon_G > \varepsilon_P$, and soil $\delta^{15}N$ is correlated positively with f_G and negatively with f_P based on eqn. (1) and (2). On the shady slope, rainfall event may accelerate the gas losses (nitrification and denitrification processes) more than plant N uptake, while it may be opposite on the sunny slope. On the shady slope, with increase in MAP, f_G increases and causes ^{15}N enrichment in soil; on the sunny slope, f_P increases with MAP, and results in ^{15}N depletion in soil.

The effects of silt/clay ratio on soil $\delta^{15}N$ might be driven by the indirect effects of silt/clay ratio on soil moisture and soil oxygen concentrations. The shady slope is wetter than the sunny slope, and the shady slope will prefer denitrification, while nitrification will be favored on the sunny slope. On the shady slope, with increase in silt/clay ratio, soil oxygen concentration increases and this inhibits soil denitrification, consequently, ^{15}N depletion in soil would be resulted in, thus silt/clay ratio showed a negative relationship with soil $\delta^{15}N$.

5 Conclusion

We sampled plants and soils on the sunny slope and shady slope of Mount Tianshan and measured their $\delta^{15}N$. Sunny slope differs significantly in climate and environment from shady slope. In the present study, leaf $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ ($\delta^{15}N_{leaf} - \delta^{15}N_{soil}$)

of the sunny slope were more positive than that of the shady slope, soil $\delta^{15}N$ of the sunny slope was also higher than that of the shady slope although the difference between the two slopes was not significant. The results suggested that the sunny slope has higher soil N transformation rates and soil N availability relative to the shady slope. In addition, among the potential influential factors, MAP, leaf C/N and silt/clay ratio had negative effects while MAT and leaf N content had positive effects on leaf $\delta^{15}N$ of the shady slope; however, on the sunny slope, only leaf C/N played a negative role in leaf $\delta^{15}N$. For soil $\delta^{15}N$, the significant influential factors were MAT, MAP and silt/clay ratio on the shady slope, whereas on the sunny slope, MAP and soil moisture exerted significant effects. Interestingly, MAP was found to exert contrary effects on soil $\delta^{15}N$ between the two slopes. This indicated that environmental influences on leaf $\delta^{15}N$ and soil $\delta^{15}N$ are local-dependent.

Data availability. There is no underlying material and related items in this paper. All data will be provided in the Supplement.

Competing financial interests. The authors declare no competing financial interests.

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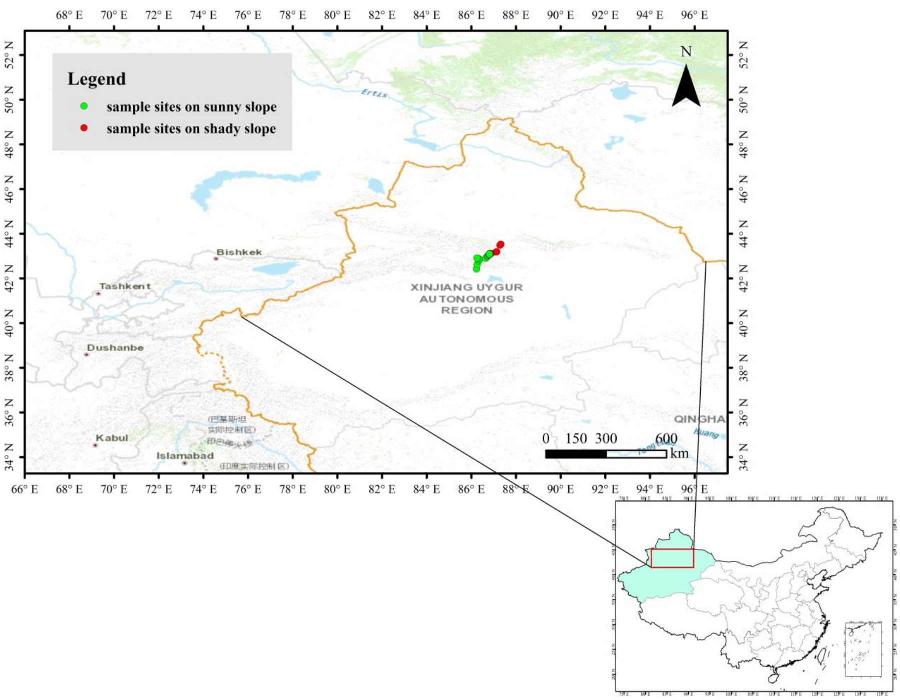

Fig 1. Sketch of study area. Locations of the sampling sites are indicated with points. A total of 17 sites (red dots) were selected on the shady slope, and 16 sites (green dots) on the sunny slope.

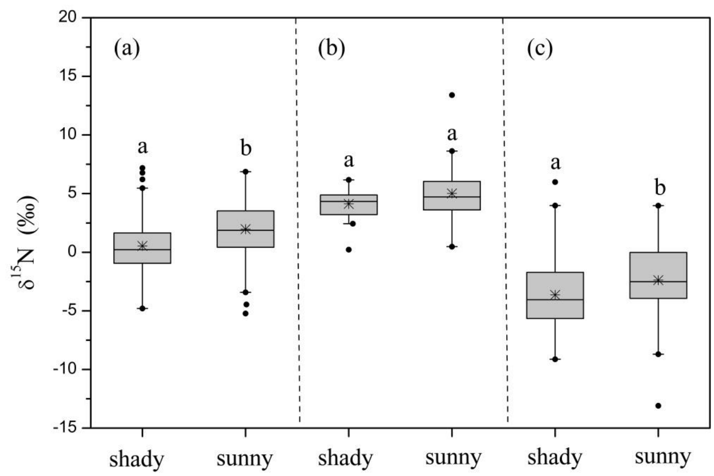

Fig 2. Differences in (a) leaf $\delta^{15}N$, (b) soil $\delta^{15}N$ and (c) $\Delta\delta^{15}N_{leaf\text{-}soil}$ between the shady and sunny slopes of Mount Tianshan. Each box represents range of middle 50% of group values, the center lines and points within the boxes are median and mean values. Whiskers are outside 25% each, and dots are outliers.

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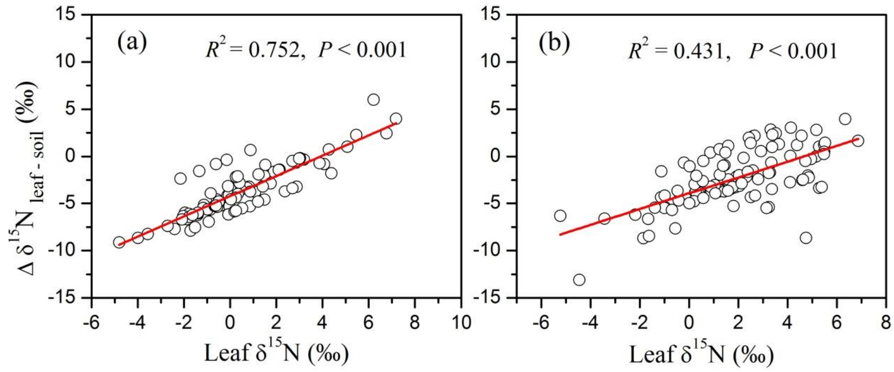

Fig 3. Relationships between $\Delta\delta^{15}N_{leaf\text{-}soil}$ and leaf $\delta^{15}N$ on the shady slope (a) and the sunny slope (b) of Mount Tianshan.

Tables

Table 1. Climate data from the meteorological observatories in the research area

Meteorological observatories	Locations	MAT/℃	MAP/mm	Alt./m
WLMQ	shady slope	6.9	269.4	918.7
MOS	shady slope	-5.2	453.4	3539.0
BLT	sunny slope	6.6	208.4	1738.3
YQ	sunny slope	8.4	73.3	1055.8

Abbreviation: WLMQ, Wulumuqi Meteorological Observatory; MOS, Mountain Observation Station of the Tianshan Glaciological Station, Chinese Academy of Sciences; BLT, Baluntai Meteorological Observatory; YQ, Yanqi Meteorological Observatory; MAT, annual mean temperature; MAP, annual mean precipitation.

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Table 2. Multiple linear regressions of leaf $\delta^{15}N$ and soil $\delta^{15}N$ based on ordinary least square (OLS) estimation.

Model	Dependent variable	Shady slope			Sunny slope		
		R^2	Adjusted R^2	P	R^2	Adjusted R^2	P
1	Leaf $\delta^{15}N$	0.458	0.388	< 0.001	0.234	0.150	0.005
2	Soil $\delta^{15}N$	0.552	0.513	< 0.001	0.727	0.708	< 0.001
3	Soil $\delta^{15}N$	0.563	0.506	< 0.001	0.738	0.709	< 0.001

Note: In the model-1, independent variables were MAT, MAP, leaf N content, leaf C/N, soil $\delta^{15}N$, soil N content, silt/clay, soil moisture, soil bulk density and soil pH. In the model-2, independent variables were MAT, MAP, soil N content, silt/clay, soil moisture, soil bulk density and soil pH. In the model-3, besides all variables in Model-2, leaf $\delta^{15}N$, leaf N content and leaf C/N were also included in independent variables.

Table 3. Correlation analyses between leaf or soil δ^{15}N and influential factors on the shady slope of Mount Tianshan.

	Leaf δ^{15}N		Soil δ^{15}N	
	r	*P*	*r*	*P*
Leaf δ^{15}N	1	---	-0.120	0.264
Soil δ^{15}N	-0.120	0.264	1	---
MAT	**0.266**	0.012	**-0.385**	< 0.001
MAP	**-0.272**	0.010	**0.387**	< 0.001
Leaf N content	**0.340**	0.001	-0.090	0.397
Leaf C/N	**-0.452**	< 0.001	-0.036	0.739
Soil N content	-0.048	0.659	0.088	0.408
Soil moisture	0.005	0.962	-0.061	0.565
Soil pH	0.162	0.132	0.070	0.513
Soil bulk density	-0.056	0.604	0.145	0.174
Silt/clay ratio	**-0.236**	0.027	**-0.370**	< 0.001

Note: the r values were in bold when P < 0.05.

Table 4. Correlation analyses between leaf or soil $\delta^{15}N$ and influential factors on the sunny slope of Mount Tianshan.

	Leaf $\delta^{15}N$		Soil $\delta^{15}N$	
	r	*P*	*r*	*P*
Leaf $\delta^{15}N$	1	---	0.175	0.074
Soil $\delta^{15}N$	0.175	0.074	1	---
MAT	0.157	0.109	0.115	0.244
MAP	-0.168	0.087	**-0.203**	0.038
Leaf N content	0.119	0.229	-0.073	0.459
Leaf C/N	**-0.228**	0.021	0.062	0.533
Soil N content	-0.173	0.078	0.014	0.888
Soil moisture	-0.141	0.150	**-0.229**	0.019
Soil pH	0.04	0.686	-0.138	0.161
Soil bulk density	0.151	0.125	0.041	0.679
Silt/clay ratio	-0.07	0.477	-0.004	0.964

Note: the r values were in bold when P < 0.05.