Dear Dr. Sébastien Fontaine,

Thank you and the reviewers very much for the kind and irradiative comments on our manuscript entitled "*Nitrogen isotopic composition of plants and soil in an arid mountainous terrain: sunny slope versus shady slope*" (The revised title is "Nitrogen isotopic composition of plants and soil in an arid mountainous terrain: south slope versus north slope", bg-2017-313). These comments are valuable and very helpful for the improvement of our manuscript.

We have revised our manuscript after carefully reading the comments. The revised parts were marked in **red color** in the "marked-up manuscript version". The detailed replies or explanations on the comments were given on the following pages.

We wish this revised manuscript meet the demands for publication in "*Biogeosicences*". Thank you again!

Best regards

Yours sincerely,

Guoan Wang

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China Agricultural University

Answers to the questions:

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Reviewer #1:

1. Response to comment 1, The reasoning behind different results and the varying environmental factors determining them on two slopes is not clear. In addition, the correlation of various environmental factors with the δ^{15}N of leaf and soil is very ambiguous and unexplained. I would suggest to authors that the environmental factors and the response variables should be tested with principal component analysis(es) to get a clearer picture.

Answer: Special thanks to you for your good comment. According to your advice, we tested all variables using principal component analysis, the results was displayed in the following figure. In principal component analyses, PC1 and PC2 could represent soil conditions and plant traits (especially leaf N content), respectively. The results of principal component analyses (Fig. 4) seem consistent with correlation analyses (please see the following Tables 3 and 4). On the north slope, leaf N content had strong positive while leaf C/N had negative effects on leaf δ^{15}N, MAT and MAP also exerted influences on leaf δ^{15}N, however, soil factors almost did not affect leaf δ^{15}N except silt/clay ratio and soil moisture. Both MAT and MAP had large loadings on soil δ^{15}N, meanwhile, soil δ^{15}N increased with decreasing silt/clay ratio and increasing soil moisture. Compared with the north slope, representation of PC1 and PC2 on the south slope was clearer. Leaf δ^{15}N was primarily correlated with leaf C/N, soil δ^{15}N was significantly controlled by MAP and soil moisture, which might be due to arid environment on the south slope. Principal component analyses and correlation analyses both supported our argument that the relationships between leaf and soil δ^{15}N and environmental factors are localized (lines 216-218, 245-248, 250-252, 276-278, 616-621).

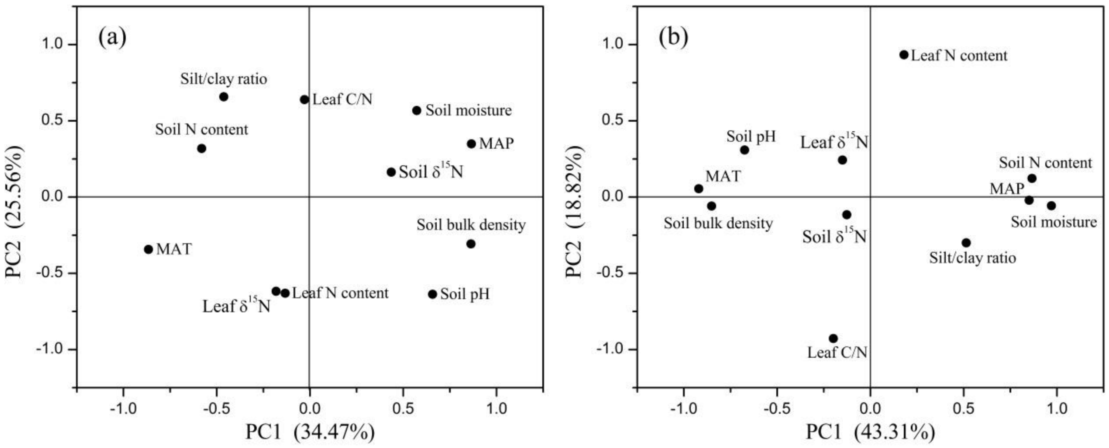

Fig 4. Variables loading on the first two principle components of the north (a) and south slope (b).

Table 3. Correlation analyses between leaf or soil δ^{15}N and influential factors on the north 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	**-0.271**	0.011	**0.388**	0.000
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 δ^{15}N and influential factors on the south slope of Mount Tianshan.

	Leaf δ^{15}N		Soil δ^{15}N	
	r	*P*	*r*	*P*
Leaf δ^{15}N	1	---	0.175	0.074
Soil δ^{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 $P < 0.05$.

2. Response to comment 2, The location of the two observatories on shady slope covers almost the whole range of the sampling gradient. However, on the sunny slope the two observatories merely cover half of the total gradient of the altitude sampled. How would the authors justify the use of climate data obtained from these observatories for the entire gradient of the altitude sampled and studied?

Answer: Your comment is right! In this paper, MAT and MAP were interpolated by two observations on each slope. We have to admit that the interpolated climatic data might be not very reliable, but we have no better ways to obtain more reliable climatic data. It is well known that this is also the greatest difficulty that the researchers studying global changes encounter. In fact, the case that two observations distributed at each slope is very rare in the world, and this is also one reason why we conducted the investigation here.

3. Response to comment 3, L48: localized is a better word that “local-dependent”.

Answer: Thanks, “local-dependent” has been changed to “localized” in revised manuscript (lines 48, 107, 321).

4. Response to comment 4, Various instead of varied.

Answer: Thanks, “varied” has been changed to “various” in revised manuscript (lines 316).

5. Response to comment 5, L316-320: Should the plant not discriminate against the heavier isotope during N uptake, even if it’s very low, thereby resulting in low leaf ^{15}N signature, when higher N uptake is the routine?

Answer: Sorry. We did not offer a clear explanation in the original manuscript. We did changes for this in the new version. The explanation is as follows. This is a widely accepted fact that plants are depleted in ^{15}N relative to its N sources because of ^{15}N discrimination, but in this paper, we meant that the plants grown in N-limited environments will enrich more ^{14}N compared with the plants in N-rich condition. The reason is that soil N transformations, such as NH_3 volatilization and NO_x emission are enhanced when soil N nutrient is rich, consequently, more ^{14}N losses from soil. This causes ^{15}N enrichment in soil, subsequently, plant δ^{15}N is more positive. Conversely, plants have more negative δ^{15}N values when soil N is limited because of weak soil N transformations and less ^{14}N loss (lines 331-339).

6. Response to comment 6, L336-340: This explanation presented here just says that cold temperature caused high leaf δ^{15}N on shady slope. But how ?

Answer: Sorry. We did not present a detailed mechanism for this (a positive effect of temperature on the north slope) in the manuscript, and the reason is that we are not sure about the mechanism. The probable mechanism is that higher temperature favors more complete plant nitrogen assimilation and transformation, which might decrease isotopic fractionation during N assimilation and transformation, then causes ^{15}N enrichment in plants. We will add the probable mechanism in the new version (lines 355-369, 511-512, 539-544).

Reviewer #2:

1. Response to comment 1, The paper would be clearer if the authors referred to north- and south-facing slopes, not sunny and shady. If this is wrong, the authors need to describe how a slope was determined to be either sunny or shady.

Answer: According to the reviewer’s comment, we have changed shady and sunny slope to north and south slope throughly in revised manuscript.

2. Response to comment 2, The authors interpolate mean annual temperature and mean annual precipitation for each site from measurements of MAT and MAP from 4 climate stations, two of which are sunny and two of which are shady. This is not valid. The authors sites are varying by a number of factors that cannot be "interpolated" from just 4 points. Stating that sunny sites are warmer than shady sites will need other data. One recommendation would be to simply remove the MAT and MAP regressions/correlations and examine other factors.

Answer: In this paper, MAT and MAP were interpolated by two observations on each slope. We have to admit that the interpolated climatic data might be not very reliable, but we have no better ways to obtain more reliable climatic data. In fact, the case that two observations distributed at each slope is very rare in the world. Although the obtained climatic data were not very reliable, we think it is necessary to remain the regressions/correlations between δ^{15}N and MAT and MAP in this paper. The reason is that, MAT and MAP effects on leaf and soil δ^{15}N at global scale are different from that at regional scale, this led to our argument (hypothesis) that environmental effects on leaf and soil δ^{15}N could depend on local environment, thus, a comparative study between the south and north slope with significant differently climate conditions was conducted. The regressions/correlations between δ^{15}N and MAT and MAP in this study did confirm our argument. If the MAT and MAP regressions/correlations were removed, our argument (hypothesis) will loss supports. Lacking reliable climatic data is a universal and most trouble for the researchers studying global change and biogeochemistry cycles. Although the regressions/correlations between δ^{15}N and MAT and MAP obtained in this study could be not perfect or reliable due to lacking accurate climatic data, we believe that the present study is also a small progress in science because we first put forward this argument (hypothesis), and confirm it. We hope more researchers to follow it.

Besides climatic data, vegetation types and species provide a strong support for the warmer climate on the south slope than the north slope. The main species occurred on the south slope were *Ephedra sinica*, *Stipa grandis*, *Stipa capillata*, *Achnatherum splendens*, *Nitraria tangutorum*, *Caragana sinica*, and *Suaeda glauca*, all these plants are typical xerophyte species, and they were not found on the north slope. On the north slope, the main species included *Kobresia myosuroides*, *Carex enervis*, *Poa annua* and *Thalictrum aquilegifolium*, they all are not xerophyte species. The information was added in the revised version (lines 139-140, 147-149).

3. Response to comment 3, The authors interpret the difference of leaf and soil delta 15N as "as the isotopic composition of plant-available N". There is no empirical evidence for this. Given the results of Craine et al. 2015 that examines global patterns of soil 15N, there is unlikely to be evidence that the signature of available N is controlled by soil delta 15N. Soil delta 15N at broad scales is likely simply an index of decomposition of the soil organic matter. Unless the authors have a reference to a graph that shows directly this relationship (delta 15N of available N vs. soil delta 15N) this statement is poorly supported.

Answer: Amundson et al. (2003) suggested that $\Delta\delta^{15}N_{\text{leaf-soil}}$ could be interpreted as the isotopic composition of plant-available N provided that isotopic discrimination does not occur during plant uptake and assimilation. In the present study, we found a highly correlation between leaf δ^{15}N and $\Delta\delta^{15}N_{leaf-soil}$ both on the two slopes, which is consistent with the result in Craine et al. (2009). As we all recognized, leaf δ^{15}N is a good indicator of plant N sources characteristics. Thus, the relationship between leaf δ^{15}N and $\Delta\delta^{15}N_{leaf-soil}$ could provide a powerful support for the suggestion in Amundson et al. (2003).

Besides, even though there was no direct evidence to support the relationship between δ^{15}N of bulk soil N and δ^{15}N of available N, the statement that soil δ^{15}N could be used to index the soil N availability had been widely accepted (McLauchlan et al., 2007; Högberg, 1997). The mechanism was that, high soil N availability leads to increased soil N transformation, such as nitrification, denitrification and NH_3 volatilization, which discriminates against ^{15}N and causes ^{15}N-enrichment in soil. Thus, ecosystems with high N availability exhibit high δ^{15}N values in soil (lines 303-314).

4. Response to comment 4, Figure 1 needs to redraw at a much larger scale, i.e over less total area. The points all overlap and it is not helpful to see where the sampling is.

Answer: Thank you for your advice, we have redrawn Figure 1 in revised manuscript (lines 568-575).

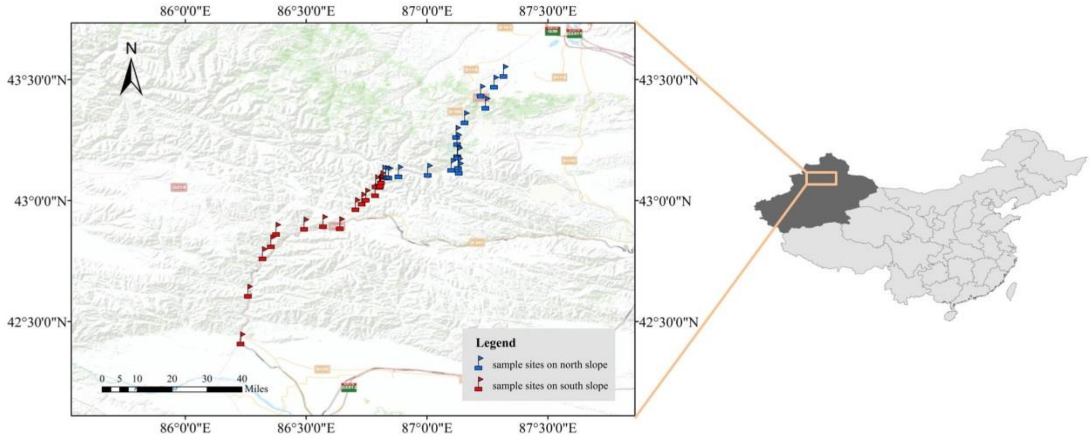

Fig 1. Sketch of study area. Locations of the sampling sites are indicated with points. A total of 17 sites (blue dots) were selected on the north slope, and 16 sites (red dots) on the south slope.

Nitrogen isotopic composition of plants and soil in an arid mountainous terrain: south slope versus north slope

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Abstract

Nitrogen cycling is tightly associated with environment. South slope of a given mountain could significantly differ from north slope in environment. Thus, N cycling should also be different between the two slopes. Since leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf-soil}$ ($\Delta\delta^{15}N_{leaf-soil}$ = 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 $\Delta\delta^{15}N_{leaf-soil}$ 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 south and north 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 $\Delta\delta^{15}N_{leaf-soil}$ on the south slope were greater than that on the north slope although the difference in soil $\delta^{15}N$ was not significant. The result confirmed our hypothesis and suggested that the south slope has higher soil N transformation rates and soil N availability than the north slope. Besides, this study observed that the significant influential factors of leaf $\delta^{15}N$ were temperature, precipitation, leaf N, leaf C/N, soil moisture and silt/clay ratio on the north slope, whereas on the south slope only leaf C/N was related to leaf $\delta^{15}N$. The significant influential factors of soil $\delta^{15}N$ were temperature, precipitation, soil moisture and silt/clay ratio on the north slope,

whereas on the south slope, MAP and soil moisture exerted significant effects. Precipitation exerted contrary effects on soil δ^{15}N between the two slopes. Thus, this study supported our argument that the relationships between leaf and soil δ^{15}N and environmental factors are localized.

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 (δ^{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 δ^{15}N and soil δ^{15}N ($\Delta\delta^{15}N_{\text{leaf-soil}}$ = leaf δ^{15}N – soil δ^{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 south slope may be significantly different from its north 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 south and north 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 south and north 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 south and north slopes of a given mountain could test the argument.

This study was conducted on the south slope and the north slope of Mount Tianshan. It is an ideal place for testing our hypotheses because its south slope differs greatly from its north 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 south slope differs from the north 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 localized.

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 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 north and south 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 north slope differs significantly from the south slope both in climate and vegetation. On the north 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 south 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 north 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). The main species on the north slope included *Kobresia myosuroides, Carex enervis, Poa annua* and *Thalictrum aquilegifolium.* A corresponding soil spectrum on the north 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 south 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 main species occurred on the south slope were *Ephedra sinica, Stipa grandis, Stipa capillata, Achnatherum splendens, Nitraria tangutorum, Caragana sinica,* and *Suaeda glauca.* The corresponding soil spectrum of the south 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 north slope, and 1,300 to 3,780 m a.s.l. on the south 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 north slope, including 72 herbs and 18 woody plants; 105 on the south 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 δ^{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). δ^{15}N, N and C contents in leaves, and δ^{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 δ^{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 δ^{15}N, soil δ^{15}N and $\Delta\delta^{15}N_{leaf\text{-}soil}$ between the north and south slopes. Leaf C/N was ln- transformed to improve data normality. The relationships between $\Delta\delta^{15}N_{leaf\text{-}soil}$ and leaf δ^{15}N were performed by the linear regression on the two slopes. Leaf and soil δ^{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 δ^{15}N included MAP, MAT, leaf N content, leaf C/N, soil δ^{15}N, soil N content, silt/clay ratio, soil moisture, soil bulk density and soil pH. The potential influential factors of soil δ^{15}N consisted of MAP, MAT, soil N content, silt/clay ratio, soil moisture and soil bulk density and soil pH. Finally, both principal component analysis (PCA) and correlation analysis were conducted to explore the complicated relationship among these factors and leaf or soil δ^{15}N.

3 Results

3.1 Comparisons of δ^{15}N in leaf and soil between the north and the south slopes

On Mount Tianshan, for all species pooled together, the arithmetic mean (mean ± SE) of leaf δ^{15}N were 0.5 ± 0.2‰ and 2.0 ± 0.2‰ for the plants grown on the north and the south slopes, respectively. One-way ANOVA suggested a significant difference for leaf δ^{15}N between the north and south slopes ($P < 0.001$) (Fig. 2a). The mean soil δ^{15}N of the north and south 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 $\Delta\delta^{15}N_{\text{leaf-soil}}$ was -3.6 ± 0.3‰ for the north slope and -2.4 ± 0.3‰ for the south slope, and one-way ANOVA suggested a significant difference in $\Delta\delta^{15}N_{\text{leaf-soil}}$ between the two slopes ($P = 0.003$) (Fig. 2c). In addition, this study showed that $\Delta\delta^{15}N_{\text{leaf-soil}}$ was positively related to $\delta^{15}N_{\text{leaf}}$ 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 north slope and south slope could be explained as a linear combination of all 10 independent variables, respectively ($P < 0.001$ for the north slope and $P = 0.005$ for the south slope) (Table

2). Among these influential factors, MAT, leaf N content correlated positively and leaf C/N, MAP, soil moisture and silt/clay ratio correlated negatively with leaf δ^{15}N on the north slope (Table 3). The results of PCA also showed that leaf N content had strong positive while leaf C/N had negative effects on leaf δ^{15}N, MAT and MAP also exerted influences on leaf δ^{15}N, however, soil factors almost did not affect leaf δ^{15}N except silt/clay ratio and soil moisture (Fig. 4a). Whereas on the south 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). Besides, on the south slope, PC1 and PC2 could almost represent soil conditions and plant traits, respectively, leaf δ^{15}N was affected strongly by leaf C/N (Fig. 4b).

Table 2

Table 3

Table 4

Fig. 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 north slope and south 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 north and south 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 north slope, $P < 0.001$ and $R^2 = 0.738$ for the south slope). Furthermore, compared to the adjusted R^2 values derived from the old regressions (adjusted $R^2 = 0.513$ for the north slope, adjusted $R^2 = 0.708$ for the south slope), the values of the new regressions were smaller or almost unchanged (adjusted $R^2 = 0.506$ for the north slope, adjusted $R^2 = 0.709$ for the south slope) (Table 2). Thus, the new multiple regressions indicated no effect of leaf nutrient traits on soil δ^{15}N. Among these factors, MAT, MAP, soil moisture and silt/clay were found to be significantly related to the soil δ^{15}N of the north slope (Table 3). The PCA showed that both MAT and MAP had large loadings on soil δ^{15}N, meanwhile, soil δ^{15}N increased with decreasing silt/clay ratio and increasing soil moisture (Fig. 4a). However, on the south slope, only MAP and soil moisture were found to play a significant and negative role in soil δ^{15}N (Table 4, Fig. 4b).

4 Discussion

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

On Mount Tianshan, leaf δ^{15}N and $\Delta\delta^{15}N_{leaf\text{-}soil}$ both showed higher values on the south slope than the north slope; soil δ^{15}N of the south slope was also more positive than that of the north 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 $\Delta\delta^{15}N_{leaf-soil}$ of the south slope could differ from those of the north slope. Greater leaf $\delta^{15}N$ on the south slope than north slope suggested that the south 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.

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

Amundson et al. (2003) suggested that $\Delta\delta^{15}N_{leaf-soil}$ could be interpreted as the isotopic composition of plant-available N provided that isotopic discrimination does not occur during plant uptake and assimilation. In the present study, we found a highly correlation between leaf $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf-soil}$ on each slope, which was consistent with the result observed by Craine et al. (2009). Additionally, leaf $\delta^{15}N$ has been widely considered as 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), because N isotopic discrimination would be negligible during nitrogen uptake and assimilation due to limited soil available N in most natural ecosystems (Högeberg et al., 1999). Thus, the significant correlated relationship between leaf δ^{15}N and $\Delta\delta^{15}N_{leaf\text{-}soil}$ could provide a powerful support for the suggestion in Amundson et al. (2003).

4.2 Influences of various factors on leaf δ^{15}N and soil δ^{15}N: south slope versus north slope

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

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. As we all know, when soil N availability is high and N nutrient is rich, soil N transformations, such as NH_3 volatilization and NO_x emission are enhanced, consequently, more ^{14}N losses from soil. This causes ^{15}N enrichment in soil, subsequently, plant $\delta^{15}N$ is more positive. Conversely, plants have more negative $\delta^{15}N$ values when soil N is limited because of weak soil N transformations and less ^{14}N loss. Thus, an increase in leaf C/N caused a decrease in nitrogen availability and ^{15}N-depletion in plants. 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 $\delta^{15}N$ might be caused by the positive relationship between leaf N content and leaf $\delta^{15}N$, 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 $\delta^{15}N$ on the north slope of Mount Tianshan.

MAP was observed to be significantly and negatively correlated with leaf $\delta^{15}N$ on the north slope; however, on the south 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 north slope, which was consistent with many previous studies (Amundson et al., 2003; Craine et al., 2009); whereas on the south slope the effect of MAT was not observed. The probable explanation for the observations was that climate on the north slope is very cold (the average MAT = -1.85 ℃), temperature is the key growth-limiting factor for plants. Previous studies consistently suggested that the key factor limiting plant growth generally also plays a dominant role in plant isotope discrimination (McCarroll and Loader, 2004; Winter et al., 1982; Xu et al., 2015), thus, temperature exerted an effect on leaf $\delta^{15}N$. However, on the south slope, 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. With respect to the positive effect of temperature on the north slope, the mechanism might be that higher temperature favors more complete plant nitrogen assimilation and transformation, this might decrease isotopic fractionation during N assimilation and transformation, then cause ^{15}N enrichment in plants.

On Mount Tianshan, soil $\delta^{15}N$ increased with increasing MAP on the north slope, while decreased with increasing MAP on the south 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 δ^{15}N can be estimated by the following equation:

$$\text{Soil } \delta^{15}\text{N} = \delta^{15}\text{N}_{\text{input}} + \varepsilon_{\text{G}} \times f_{\text{G}} + \varepsilon_{\text{P}} \times f_{\text{P}} \quad (1)$$

where $\delta^{15}\text{N}_{\text{input}}$ is the input δ^{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 factors of corresponding N losses processes, respectively. And

$$f_{\text{G}} + f_{\text{P}} = 1 \quad (2)$$

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 δ^{15}N is correlated positively with f_G and negatively with f_P based on eqn. (1) and (2). On the north slope, rainfall event may accelerate the gas losses (nitrification and denitrification processes) more than plant N uptake, while it may be opposite on the south slope. On the north slope, with increase in MAP, f_G increases and causes ^{15}N enrichment in soil; on the south slope, f_P increases with MAP, and results in ^{15}N depletion in soil.

The effects of silt/clay ratio on soil δ^{15}N might be driven by the indirect effects of silt/clay ratio on soil moisture and soil oxygen concentrations. The north slope is wetter than the south slope, and the north slope will prefer denitrification, while nitrification will be favored on the south slope. On the north 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 δ^{15}N.

5 Conclusion

We sampled plants and soils on the south slope and north slope of Mount Tianshan and measured their $\delta^{15}N$. South slope differs significantly in climate and environment from north slope. In the present study, leaf $\delta^{15}N$ and $\Delta\delta^{15}N_{\text{leaf-soil}}$ (leaf $\delta^{15}N$ – soil $\delta^{15}N$) of the south slope were more positive than that of the north slope, soil $\delta^{15}N$ of the south slope was also higher than that of the north slope although the difference between the two slopes was not significant. The results suggested that the south slope has higher soil N transformation rates and soil N availability relative to the north slope. In addition, among the potential influential factors, MAP, leaf C/N, soil moisture and silt/clay ratio had negative effects while MAT and leaf N content had positive effects on leaf $\delta^{15}N$ of the north slope; however, on the south 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, soil moisture and silt/clay ratio on the north slope, whereas on the south 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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Figures

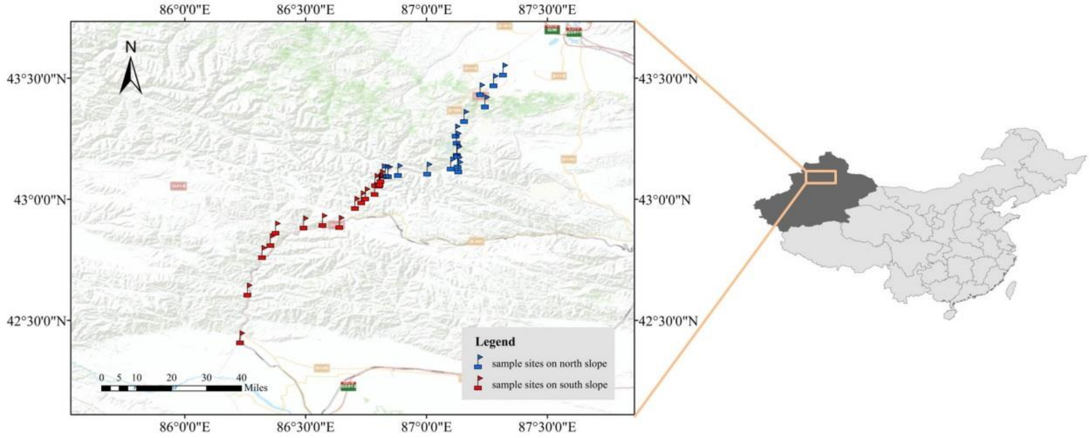

Fig 1. Sketch of study area. Locations of the sampling sites are indicated with points. A total of 17 sites (blue dots) were selected on the north slope, and 16 sites (red dots) on the south slope.

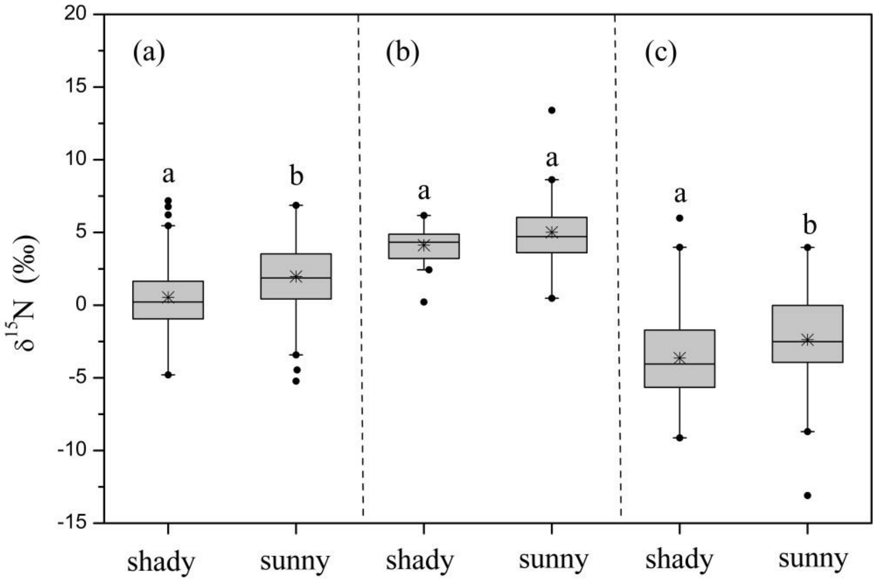

Fig 2. Differences in (a) leaf $\delta^{15}N$, (b) soil $\delta^{15}N$ and (c) $\Delta\delta^{15}N_{leaf\text{-}soil}$ between the north and south slopes of Mount Tianshan. Each box represents range of middle 50% of group values, the center lines and points within the boxes are median and mean values.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

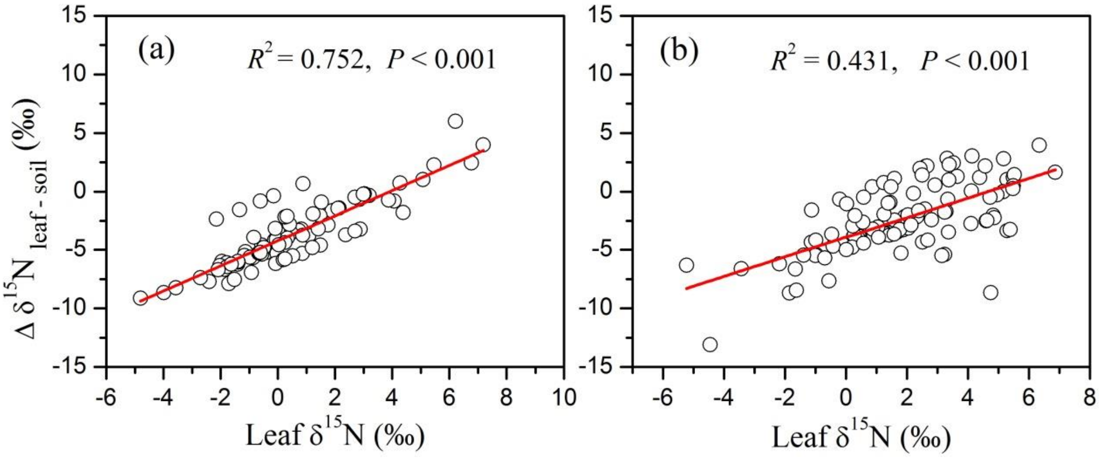

Fig 3. Relationships between $\Delta\delta^{15}N_{leaf-soil}$ and leaf $\delta^{15}N$ on the north slope (a) and the south slope (b) of Mount Tianshan.

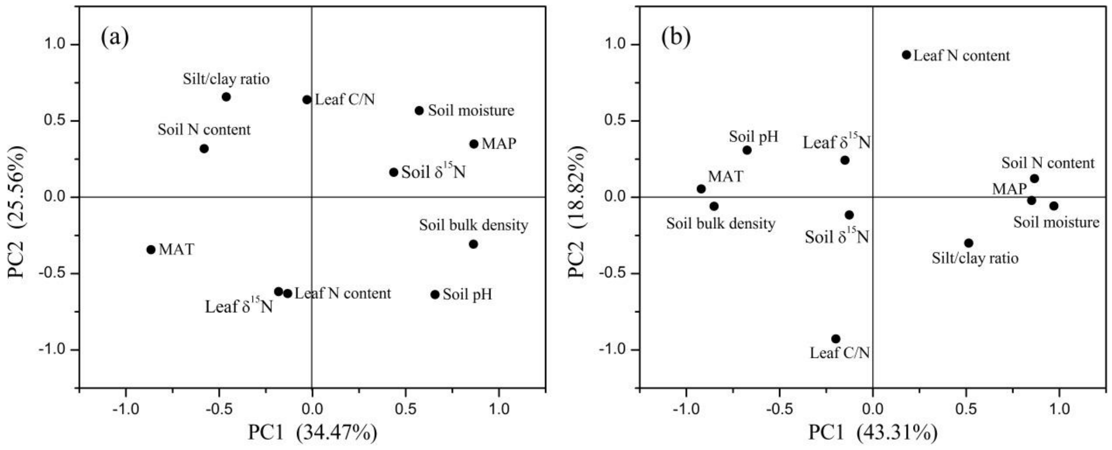

Fig 4. Variables loading on the first two principle components of the north (a) and south slope (b) of Mount Tianshan.

Tables

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

Meteorological observatories	Locations	MAT/℃	MAP/mm	Alt./m
WLMQ	north slope	6.9	269.4	918.7
MOS	north slope	-5.2	453.4	3539.0
BLT	south slope	6.6	208.4	1738.3
YQ	south 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.

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

Model	Dependent variable	North slope			South slope		
		R^2	Adjusted R^2	P	R^2	Adjusted R^2	P
1	Leaf δ^{15}N	0.458	0.388	< 0.001	0.234	0.150	0.005
2	Soil δ^{15}N	0.552	0.513	< 0.001	0.727	0.708	< 0.001
3	Soil δ^{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 δ^{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* δ^{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 north 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	**-0.271**	0.011	**0.388**	0.000
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 south 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 $P < 0.05$.