

Article

Leaf and Soil $\delta^{15}\text{N}$ Patterns Along Elevational Gradients at Both Treelines and Shrublines in Three Different Climate Zones

Xue Wang ^{1,*}, Yong Jiang ², Haiyan Ren ³, Fei-Hai Yu ¹  and Mai-He Li ⁴ 

¹ Institute of Wetland Ecology & Clone Ecology/Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou 318000, China

² Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

³ College of Grassland, Resources and Environment, Key Laboratory of Grassland Resources of the Ministry of Education, Key Laboratory of Forage Cultivation, Processing and High Efficient Utilization of the Ministry of Agriculture, and Key Laboratory of Grassland Management and Utilization of Inner Mongolia Autonomous Region, Inner Mongolia Agricultural University, Hohhot 010011, China

⁴ Forest Dynamics, Swiss Federal Research Institute WSL, Zuercherstrasse 111, CH-8903 Birmensdorf, Switzerland

* Correspondence: xuewang@tzc.edu.cn; Tel.: +86-136-2662-0017

Received: 16 May 2019; Accepted: 2 July 2019; Published: 3 July 2019



Abstract: The natural abundance of stable nitrogen (N) isotope ($\delta^{15}\text{N}$) in plants and soils can reflect N cycling processes in ecosystems. However, we still do not fully understand patterns of plant and soil $\delta^{15}\text{N}$ at alpine treelines and shrublines in different climate zones. We measured $\delta^{15}\text{N}$ and N concentration in leaves of trees and shrubs and also in soils along elevational gradients from lower altitudes to the upper limits of treelines and shrublines in subtropical, dry- and wet-temperate regions in China. The patterns of leaf $\delta^{15}\text{N}$ in trees and shrubs in response to altitude changes were consistent, with lower values occurring at higher altitude in all three climate zones, but such patterns did not exist for leaf $\Delta\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$. Average $\delta^{15}\text{N}$ values of leaves (-1.2‰) and soils (5.6‰) in the subtropical region were significantly higher than those in the two temperate regions (-3.4‰ and 3.2‰ , respectively). Significant higher $\delta^{15}\text{N}$ values in subtropical forest compared with temperate forests prove that N cycles are more open in warm regions. The different responses of leaf and soil $\delta^{15}\text{N}$ to altitude indicate complex mechanisms of soil biogeochemical process and N sources uptake with environmental variations.

Keywords: ^{15}N natural abundance; nitrogen cycling; treeline; shrubline; altitude

1. Introduction

Variation in natural abundance of the stable isotope ^{15}N ($\delta^{15}\text{N}$) in plants and soils can reflect N cycling in ecosystems [1] because it is related to the isotope compositions of N inputs and outputs and the internal N transformations [2]. Moreover, $\delta^{15}\text{N}$ can reflect the degree of the ‘openness’ of the N cycles, with higher values indicating greater N losses and a more open N cycling [3,4]. Thus, assessing patterns of $\delta^{15}\text{N}$ may help understand the process of N cycling in ecosystems [1,5].

Many studies have examined the variations of spatial patterns of $\delta^{15}\text{N}$ in plants and soils [6–9] and showed correlations of $\delta^{15}\text{N}$ and environmental factors. Elevation appears to be a major influence on leaf and soil $\delta^{15}\text{N}$ due to its natural environmental variations, such as soil water and temperature. At lower elevations where temperature tends to be higher, $\delta^{15}\text{N}$ values are likely to be higher [10,11]. Since litter decomposition and N mineralization can be accelerated by a direct increase in temperature at lower elevations [12]. However, an increasing trend or no change of $\delta^{15}\text{N}$ with elevational gradients

has also been observed in some regions [4,13]. This is because not only the local microclimate but the primary physiological and biogeochemical processes regulate the N transformations in soils [2]. The soil processes including N mineralization, nitrification, denitrification and NH_3 volatilization all discriminate against ^{15}N and lead to different leaf and soil $\delta^{15}\text{N}$ signatures [7]. In addition, mycorrhizal fungi types and various N uptake sources can influence leaf $\delta^{15}\text{N}$ signatures [14–16]. Therefore, the mechanisms of elevational response for $\delta^{15}\text{N}$ could be complex.

In particular, at high elevations in alpine ecosystems, where are expected to be active in N dynamics with the increasing global warming [17]. The distributions of plant species on alpine ecosystems are constrained by upper altitude limits, resulting in markedly boundaries such as treelines and shrublines [18,19]. Although temperature has been suggested as a primary driver underlying the formation of such boundaries [20], low soil nutrient availability may also be responsible for reduced growth of trees and shrubs at their upper limits [21]. A recent study found that the growth of trees and shrubs in an alpine ecosystem increased with slightly increased nutrient availability [22], which in turn suggesting the potential increasing nutrient dynamics with the expansion of trees or shrubs. Thus, understanding changes of N cycling at alpine ecosystems is particularly important as both alpine treelines and shrublines may shift in the face of global climate change [23–25]. The signature of $\delta^{15}\text{N}$ at alpine ecosystems could serve as a powerful signal for potential effects of climate change on N cycling. For instance, experimental warming increased foliar $\delta^{15}\text{N}$ in the Swiss Alps [26], indicating an opening of N cycles with climate warming. However, we still do not fully understand the patterns of $\delta^{15}\text{N}$ in plants and soils at high altitudes of alpine ecosystems and whether plant and soil $\delta^{15}\text{N}$ at alpine treelines and shrublines show similar patterns.

We sampled leaves of trees and shrubs and soils at the upper limits of alpine treelines and shrublines and the lower altitudes in three different climate zones (subtropical, dry-temperate and wet-temperate) and measured their $\delta^{15}\text{N}$ values. Specifically, we address the following questions: (1) Do leaf and soil $\delta^{15}\text{N}$ patterns change with climatic zones? We hypothesized that leaf and soil $\delta^{15}\text{N}$ values are higher in subtropical zones than in temperate zones as temperature is higher and available N is richer in subtropical forests than in temperate forests. (2) Do leaf and soil $\delta^{15}\text{N}$ vary with altitude? We hypothesized that leaf and soil $\delta^{15}\text{N}$ would decrease with altitude as temperature decreases with increasing altitude. (3) Do leaf $\delta^{15}\text{N}$ values of trees and shrubs respond differently to changing altitude? We hypothesized that the response of leaf $\delta^{15}\text{N}$ to altitude was similar in trees and shrubs as the distribution of trees and shrubs are both restrained to the cold temperature.

2. Materials and Methods

2.1. Site Description and Sample Collection

The study sites were three mountains in China (Balang Mts., Qilian Mts. and Changbai Mts.), located in three climate zones (summarized in Table 1, Figure 1): Balang Mts. ($102^{\circ}52'–103^{\circ}24' \text{ E}$, $30^{\circ}45'–31^{\circ}25' \text{ N}$) are located in Wolong Nature Reserve in southwestern China. The climate is subtropical, with the mean annual precipitation of about 995 mm and the annual mean temperature of 12.8°C (measured at 1920 m a.s.l. according to the data from Wolong Nature Reserve Authority) [27]. Qilian Mts. ($102^{\circ}58'–103^{\circ}01' \text{ E}$, $37^{\circ}14'–37^{\circ}20' \text{ N}$) are located in northwestern China. Its climate is dry-temperate, with the mean annual precipitation of about 435 mm and the annual mean temperature of 0.6°C (measured at 2787 m a.s.l. by the Qilian weather station) [28]. Changbai Mts. ($126^{\circ}55'–129^{\circ}00' \text{ E}$, $41^{\circ}23'–42^{\circ}36' \text{ N}$) are located in northeastern China, with wet-temperate climate. For Changbai Mts., the mean annual precipitation increases from 1000 to 1100 mm and annual mean temperature decreases from -2.3 to -3.8°C at the altitude from 1950 m to 2000 m a.s.l [29].

Table 1. The characteristics of sampling sites.

Mountain	Climate Zone	MAT (°C)	MAP (mm)	Longitude (E)	Latitude (N)	Species	Life Form	Elevation (m) a.s.l.) *	Height (m)
Balang	Sup-tropical	12.8	995	102°58′	30°51′	<i>Abies faxoniana</i> Rehder & E.H.Wilson	Tree	2860	14 ± 1.7
								3290	9.2 ± 0.9
								3670	11.5 ± 0.4
				102°45′	30°53′	<i>Quercus aquifolioides</i> Rehd. et Wils.	Shrub	2840	4.9 ± 0.1
								3160	2.8 ± 0.4
								3590	2.9 ± 0.2
Qilian	Dry-temperate	0.6	435	100°17′	38°34′	<i>Picea crassifolia</i> Kom.	Tree	2540	13.7 ± 0.9
								2870	15.5 ± 1.4
								3250	11.2 ± 0.5
				100°18′	38°31′	<i>Salix gilashanica</i> C. Wang, P.Y. Fu	Shrub	3000	1.4 ± 0.2
								3250	1.2 ± 0.0
								3550	1.5 ± 0.2
Changbai	Wet-temperate	−2.3--3.8	1000	128°04′	42°03′	<i>Betula ermanii</i> Cham.	Tree	1700	18.9 ± 5.2
								1860	13 ± 0.8
								2030	6.4 ± 4.5
				128°04′	42°02′	<i>Vaccinium uliginosum</i> Linn.	Shrub	1430	0.5 ± 0.0
								2000	0.2 ± 0.0
								2380	0.1 ± 0.0

* The elevations showed from low to high for each tree and shrub are presented as “lower”, “middle” and “upper”.

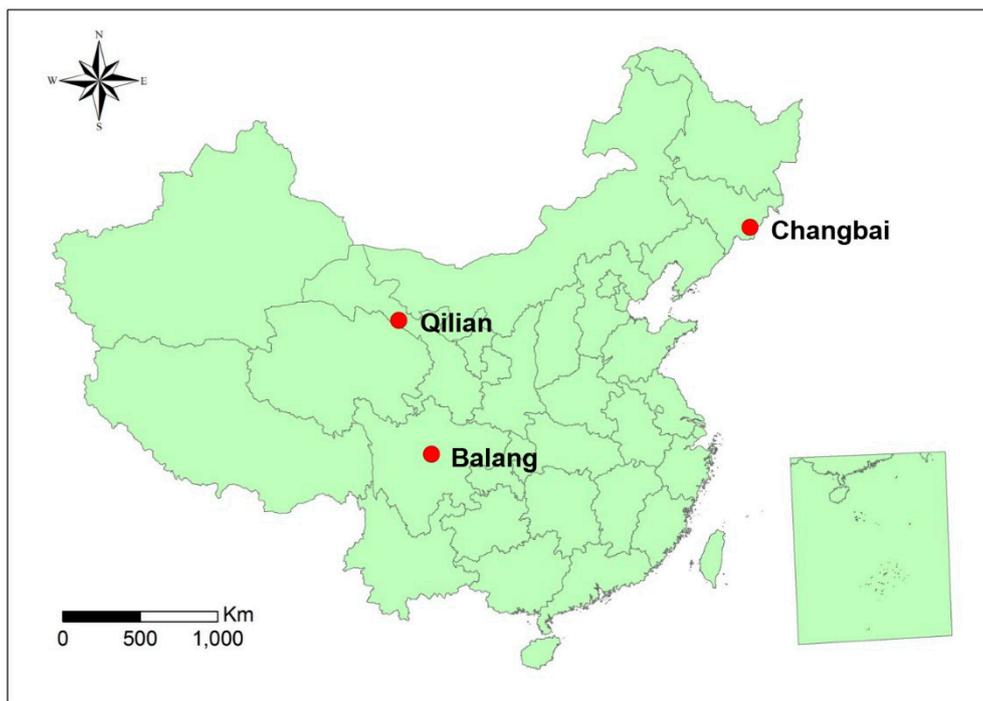


Figure 1. Location of three sampling sites of Balang Mt. (subtropical), Qilian Mt. (dry-temperate) and Changbai Mt. (wet-temperate).

In this study, the alpine treelines and shrublines were defined as the upper limits of individuals without visible disturbance and suppression. For Balang Mts., the treeline species was *Abies faxoniana* Rehder & E.H.Wilson and the shrubline species was *Quercus aquifolioides* Rehd. et Wils., and the altitude ranged from 2840 to 3670 m asl., (Table 1). For Qilian Mts., the treeline species was *Picea crassifolia* Kom. and the shrubline species was *Salix gilashanica* C. Wang, P.Y. Fu, and the altitude ranged from 2540 to 3540 m (Table 1). For Changbai Mts., the treeline species was *Betula ermanii* Cham. and the shrubline species was *Vaccinium uliginosum* Linn., and the altitude ranged from 1430 to 2380 m (Table 1). The soil was classified as Umbric Cryic Cambisols on Balang Mts., Calcaric Ustic Cambisols on Qilian Mts. and Andic Gelic Cambisols on Changbai Mts. [30].

At each site, leaf samples from the treeline and shrubline species were collected at three altitudes, i.e., the upper limit, the middle altitude and the lower altitude. At each altitude, soils (0–10 cm depth) under the canopy of the sampled trees or shrubs were collected after removing the layer of soil organic matter. They passed through a 2 mm sieve to remove stones and plant residues. At each altitude from each site, leaf samples were collected from six trees or shrubs, and a composite soil sample was collected from three randomly locations around corresponding trees or shrubs selected.

2.2. Chemical Analysis

Samples of leaves were ground into a fine powder after being oven-dried at 65 °C for 48 h, and then stored for the measurement of ^{15}N values and N concentration. For each soil sample, a fresh subsample was used for testing soil inorganic N (IN, $\text{NO}_3^- + \text{NH}_4^+$), and the remaining part was air dried and then milled to powder for the measurement of soil organic carbon (SOC), total carbon (TC), total N (TN) and ^{15}N abundance.

The ^{15}N abundance in leaves and soils, and concentrations of leaf N, soil TN and soil TC were analyzed in an elemental analyzer (Elementar Vario MICRO cube, Hanau, Germany) coupled with a stable isotope ratio mass spectrometer (Isoprime 100, Stockport, UK). Calibrated

DL-alanine ($\delta^{15}\text{N} = -1.7\text{‰}$), glycine ($\delta^{15}\text{N} = 10.0\text{‰}$) and histidine ($\delta^{15}\text{N} = -8.0\text{‰}$) were used as the internal standards.

$$\delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 10^3, \quad (1)$$

where R_{sample} is the $^{15}\text{N}/^{14}\text{N}$ ratio of the sample and R_{standard} is the $^{15}\text{N}/^{14}\text{N}$ ratio of the atmospheric N_2 [31]. Precision of duplicate measurements was $<0.2\%$.

The soil IN was determined colorimetrically using an Auto Continuous Flow Analyzer (Bran and Luebbe, Norderstedt, Germany) after extracting fresh soil samples in 1 M KCL. For subtropical and wet temperate mountain zones, SOC was determined on ground soils using an elemental analyzer (Vario MACRO Cube, Elementar, Germany). For the dry temperate mountain, the ground soil samples were treated with 12 M HCl to remove inorganic C before organic C determination on the elemental analyzer [32]. Soil pH was determined in a 1:5 (soil:water) solution (w/v).

2.3. Statistical Analyses

The normality of the distribution and the homogeneity of the data were checked (Kolmogorov–Smirnov test) before statistical analyses. We used a three-way ANOVA to test the effects of life form (trees vs. shrubs), altitude (upper, middle and lower) and climatic zone (Balang Mts., Qilian Mts. and Changbai Mts.) on leaf and soil $\delta^{15}\text{N}$, leaf $\Delta\delta^{15}\text{N}$ (calculated as $\delta^{15}\text{N}_{\text{leaf}} - \delta^{15}\text{N}_{\text{soil}}$), leaf N, soil inorganic N, soil total N and soil C:N. Multiple comparisons of means were conducted with Duncan's significant difference to test the different responses between altitudes. The correlations between variables were investigated with Pearson's correlation coefficient. All statistical analyses were performed using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. $\delta^{15}\text{N}$ in Leaves and Soils

$\delta^{15}\text{N}$ values varied significantly in different climate zones (sites; Table 2). Altitude and life form had significant effects on $\delta^{15}\text{N}$ in leaves and soils (Table 2). The two- and three-way interactions among variables also significantly influenced the $\delta^{15}\text{N}$ in leaves and soils (Table 2). Leaf $\delta^{15}\text{N}$ values of both trees and shrubs were lower at the upper limits than at lower altitudes in all three climate zones (Table 2, Figure 2a–c). There was no significant difference for leaf $\delta^{15}\text{N}$ between trees and shrubs in subtropical and dry-temperate forests, but leaf $\delta^{15}\text{N}$ of shrub (-1.3‰) was significantly higher than that of tree (-4.2‰) in wet-temperate forest (Table 2, Figure 2c). Leaf $\delta^{15}\text{N}$ was significantly different among climate zones ($p < 0.05$, Figure 2a–c), with that average $\delta^{15}\text{N}$ values of leaves in dry-temperate (-4.2‰) were lower than those in subtropical (-1.2‰) and wet-temperate (-2.8‰), but there was no difference of leaf $\delta^{15}\text{N}$ between subtropical and wet-temperate forest (Figure 2a–c).

Table 2. Three-way ANONAs of the effects of climate zones (site), altitude, life form and their interactions on Leaf $\Delta\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{leaf}} - \delta^{15}\text{N}_{\text{soil}}$), and $\delta^{15}\text{N}$ and N concentration in leaves and soils.

Source		Leaf $\delta^{15}\text{N}$	Soil $\delta^{15}\text{N}$	Leaf $\Delta\delta^{15}\text{N}$	Leaf N	Soil IN	Soil TN	Soil C:N
	df	F	F	F	F	F	F	F
Site (S)	2	83.4 ***	44.2 ***	26.0 ***	790.6 ***	27.7 ***	4.5 *	45.3 ***
Life form (L)	1	17.8 ***	7.7 **	0.2 ^{ns}	1776.8 ***	19.8 ***	0.0 ^{ns}	2.2 ^{ns}
Altitude (A)	2	26.7 ***	1.4 ^{ns}	8.2 ***	7.7 ***	0.8 ^{ns}	1.1 ^{ns}	4.1 *
S × L	2	4.7 *	2.5 ^{ns}	1.5 ^{ns}	1.0 ^{ns}	4.1 *	4.4 *	30.5 ***
S × A	4	4.6 **	5.7 ***	12.8 ***	7.3 ***	0.6 ^{ns}	5.8 ***	1.1 ^{ns}
L × A	2	32.3 ***	6.5 **	15.3 ***	450.9 ***	0.5 ^{ns}	0.8 ^{ns}	8.6 ***
S × L × A	4	6.7 ***	10.0 ***	11.0 ***	6.6 ***	3.8 **	3.6 **	12.7 ***

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ^{ns} $p > 0.05$.

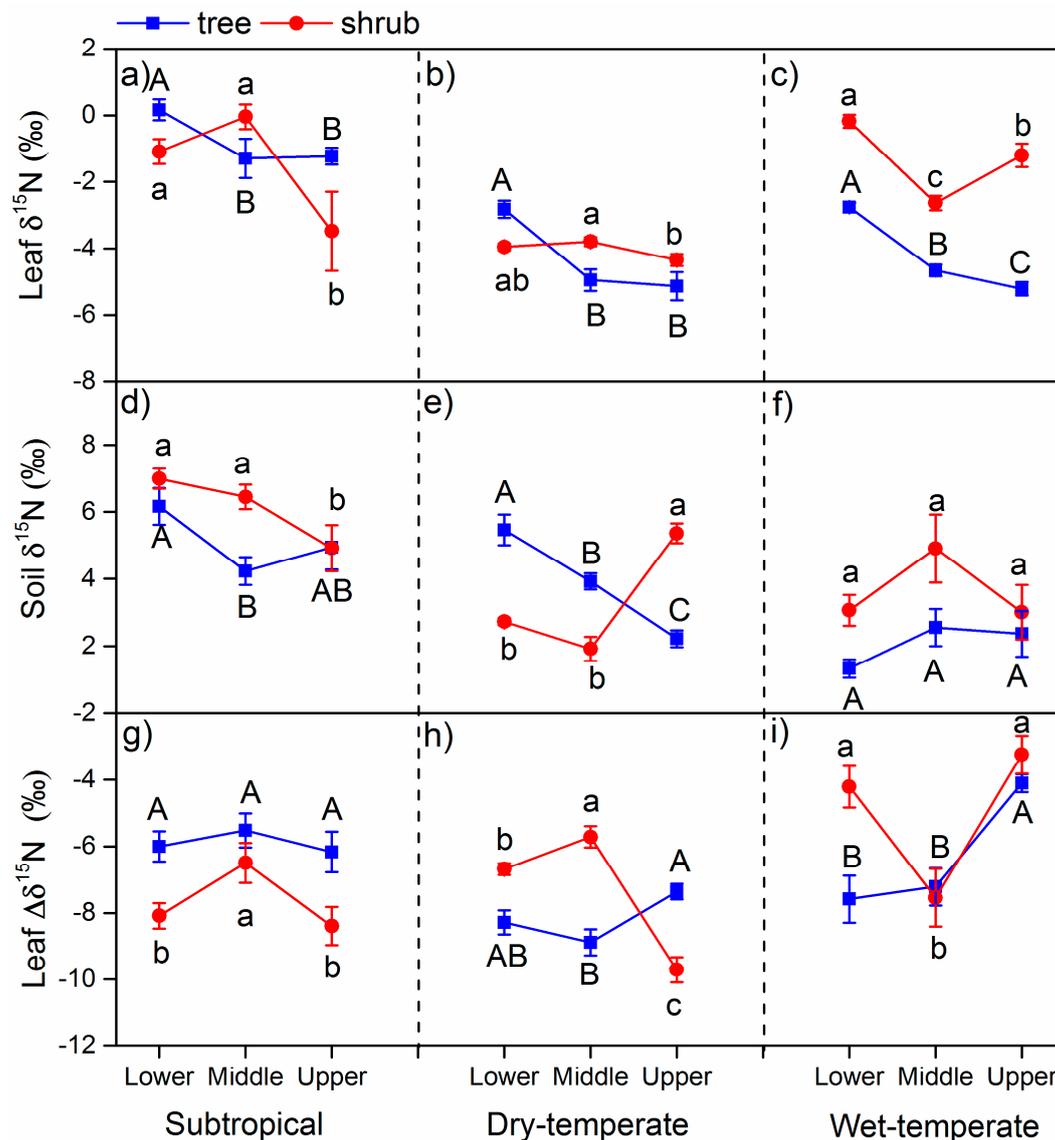


Figure 2. Leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and leaf $\Delta\delta^{15}\text{N}$ (i.e., $\delta^{15}\text{N}_{\text{leaf}} - \delta^{15}\text{N}_{\text{soil}}$) of trees and shrubs along the altitude in subtropical (a,d,g), dry-temperate (b,e,h) and wet-temperate mountain forest (c,f,i), different lower case letters indicate the difference for shrub $\delta^{15}\text{N}$ between altitudes and upper case letters indicate the difference for tree $\delta^{15}\text{N}$ between altitudes.

The increasing altitude significantly decreased the value of soil $\delta^{15}\text{N}$ in subtropical forest but had no effect on that in wet-temperate forest (Table 2, Figure 2d–f). Soil $\delta^{15}\text{N}$ under the tree canopy decreased, however soil $\delta^{15}\text{N}$ under shrub canopy increased, with increasing altitude in dry-temperate forest because of the significant interaction between life form and altitude (Table 2, Figure 2e). In wet-temperate forest, soil $\delta^{15}\text{N}$ was significantly higher under shrub canopy than under tree canopy (Table 2, Figure 2f). The soil $\delta^{15}\text{N}$ in subtropical forest (5.6‰) was higher than those in dry-temperate (3.6‰) and wet-temperate forests (2.9‰) and there was no difference for soil $\delta^{15}\text{N}$ between dry- and wet-temperate forests (Figure 2d–f).

Altitude had a significant effect on leaf $\Delta\delta^{15}\text{N}$ values (i.e., the difference of leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$), however, this effect was changed by site and life form due to the significant two-way and three-way interactions (Table 2). For example, leaf $\Delta\delta^{15}\text{N}$ values for shrubs were lower at the upper limits than at lower elevations, whereas $\Delta\delta^{15}\text{N}$ values for trees tended to be higher at the upper limits in dry-temperate forest (Figure 2h). No significant difference for leaf $\Delta\delta^{15}\text{N}$ values of trees and shrubs was found between the upper limits and lower elevations in subtropical forest, but leaf $\Delta\delta^{15}\text{N}$ values

for trees tended to be higher than those for shrubs in the subtropical forest (Figure 2g). Leaf $\Delta\delta^{15}\text{N}$ values were all negative for all trees and shrubs across the climate zones (Figure 2g–i).

3.2. N Concentrations in Leaves and Soils and Soil C:N Ratios

N concentrations in leaves varied in different climate zones (Table 2). Altitude had the significant effect on leaf N for dry- and wet-temperate forests but had no effect on that for subtropical forest (Table 2, Figure 3). Leaf N concentrations of shrubs were significantly higher than those of trees in both dry- and wet-temperate forests (Figure 3b–c). Average leaf N in wet-temperate forest was among the highest, and followed by dry-temperate forest and then subtropical had the least leaf N (Figure 3).

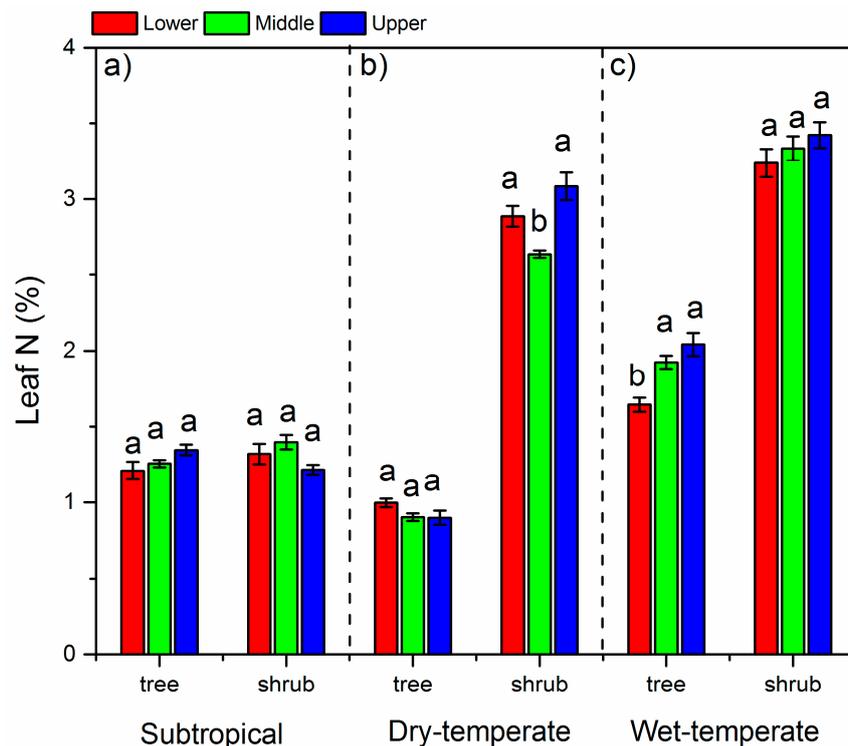


Figure 3. Leaf N of trees and shrubs along the altitude in subtropical (a), dry-temperate (b) and wet-temperate mountain forest (c), different lower case letters indicate the difference for leaf N concentration between altitudes.

Different climate zones had different soil total inorganic N (IN) and TN concentrations and soil C:N ratios (Table 2). There were significant two-way interactions of site and life form and three-way interactions of site, altitude and life form (Table 2). The response of soil available N to altitude was more sensitive in subtropical and dry-temperate forests than in wet-temperate where soil IN showed no change with increasing altitude (Figure 4a–c). Soil IN under tree canopy decreased but those under shrub canopy increased with increasing altitude in subtropical and dry-temperate forests, except that IN under shrub canopy in dry-temperate forest stabilized with increasing altitude (Figure 4a,b). The increasing altitude decreased soil TN under tree canopy but increased soil TN under shrub canopy in subtropical forest (Figure 4d). The increasing altitude increased soil TN under tree canopy but had no effect on soil TN under shrub canopy in dry-temperate forest, which was opposite in wet-temperate forest (Table 2, Figure 4e, f). Soil IN was lower but soil TN was higher in dry-temperate forest than those in subtropical and wet-temperate forests ($p < 0.05$, Figure 4). The soil C:N ratio under tree canopy increased but that under shrub canopy decreased with increasing altitude (Table 2; Figure 4h,i). Soil C:N ratio in wet-temperate forest (17) was the highest, followed by dry-temperate forest (15) and then subtropical forest (13; $p < 0.05$, Figure 4g–i).

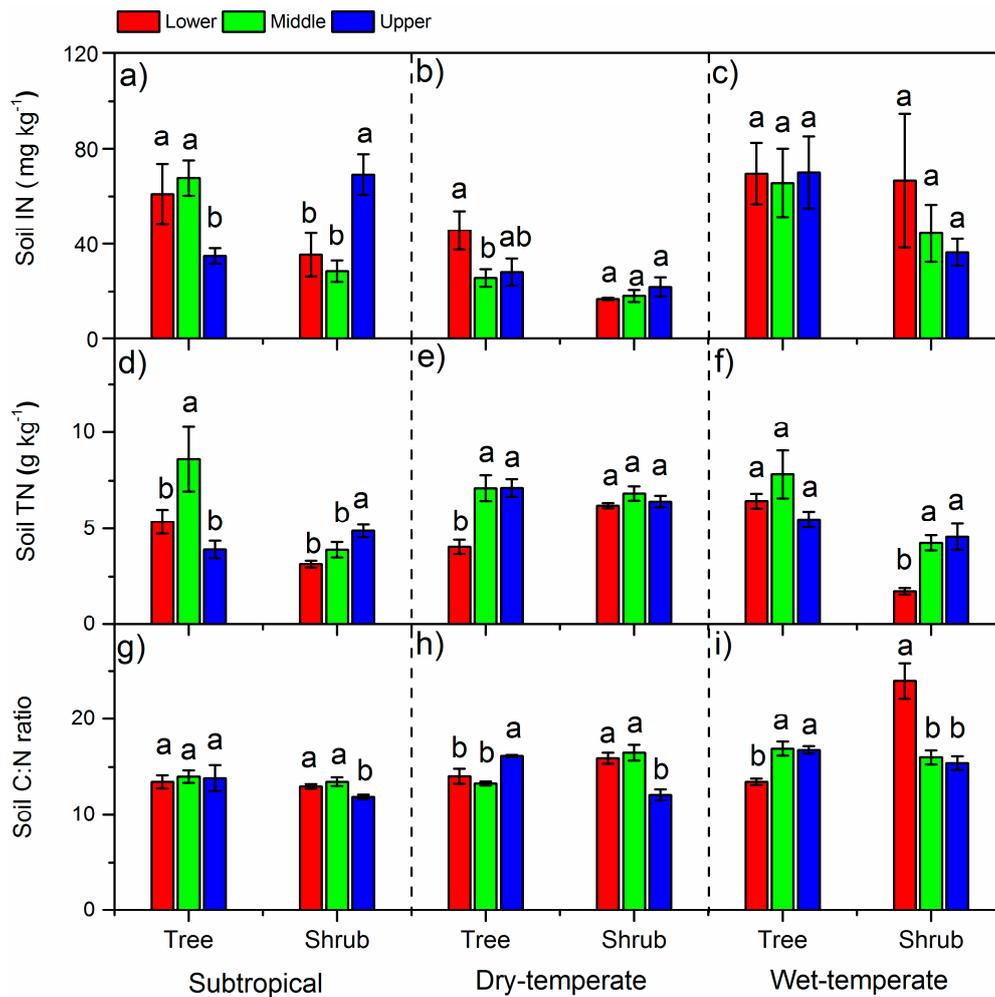


Figure 4. Soil IN, TN and C:N ratio under the canopy of trees and shrubs along the altitude in subtropical (a,d,g), dry-temperate (b,e,h) and wet-temperate mountain forest (c,f,i), lower case letters indicate the difference for the parameters between altitudes.

3.3. Correlations between $\delta^{15}\text{N}$ and Parameters in Leaves and Soils

Across all sampling mountains and plant types, both leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ were negatively correlated with SOC and soil TN (Figure 5b–c); leaf $\delta^{15}\text{N}$ was negatively affected by pH (Figure 5a), however, soil $\delta^{15}\text{N}$ was negatively related to soil C:N (Figure 5d). Both leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ were significantly correlated with leaf N in wet-temperate mountain forest (Table 3). Soil IN had a positive effect on leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ in dry-temperate mountain forest (Table 3).

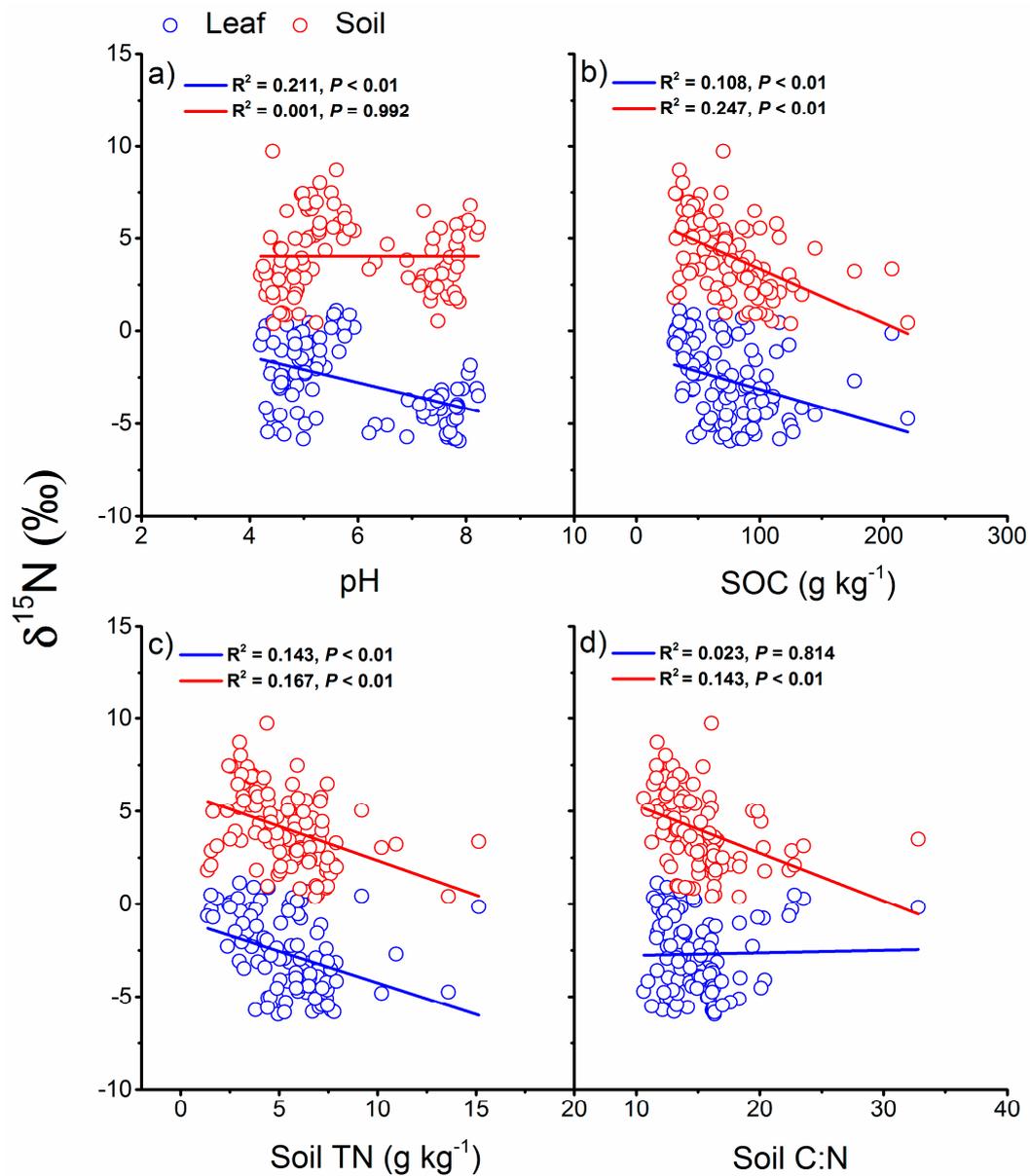


Figure 5. Relationships of $\delta^{15}\text{N}$ in leaves and soils and soil physicochemical parameters across three sampling sites.

Table 3. Correlation analyses (R values) of leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ with leaf N and soil physicochemical parameters for each sampling site.

	Leaf N	Soil IN	Soil TN	Soil pH	SOC	Soil C:N
Subtropical						
Leaf $\delta^{15}\text{N}$	0.249	-0.292	0.058	-0.439 **	0.036	-0.015
Soil $\delta^{15}\text{N}$	0.072	-0.432 **	-0.508 **	-0.089	-0.555 **	-0.393 *
Dry-temperate						
Leaf $\delta^{15}\text{N}$	0.172	0.496 **	-0.484 **	0.115	-0.340 *	0.006
Soil $\delta^{15}\text{N}$	-0.05	0.476 **	-0.464 **	0.404 *	-0.578 **	-0.373 *
Wet-temperate						
Leaf $\delta^{15}\text{N}$	0.682 **	-0.144	-0.629 **	-0.123	-0.612 **	0.402 *
Soil $\delta^{15}\text{N}$	0.459 **	-0.031	-0.433 **	-0.087	-0.388 *	0.099

** $p < 0.01$, * $p < 0.05$.

4. Discussion

4.1. The $\delta^{15}\text{N}$ and N in Different Climate Zones

The average $\delta^{15}\text{N}$ values in leaves and soils for subtropical forest was higher than the averages for both dry-and wet-temperate forests (Figure 2, $p < 0.05$). This finding is opposite to the synthesis of leaf $\delta^{15}\text{N}$ values in eastern Asian forests conducted by Fang et al. [33], in which no difference of leaf $\delta^{15}\text{N}$ values was found among tropical, subtropical and temperate forests. The opposite results may be due to the differences of the site size and species selected, since we only selected one mountain and two species in each climate zone. However, our result is consistent with the global compilation of Martinelli et al. [34], in which average leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ values in tropical forests were respectively 6.5‰ and 8‰ higher than those in temperate forests. Similarly, another two global compilations have found the positive correlations of leaf and soil $\delta^{15}\text{N}$ with increasing MAT gradients when MAT was more than $-0.5\text{ }^{\circ}\text{C}$ and $9.8\text{ }^{\circ}\text{C}$, respectively [11,35].

The higher mean annual temperature in subtropical mountain forest might contribute to higher leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ (Table 1). Since temperature has been suggested to be a critical factor to regulate the $\delta^{15}\text{N}$ by influencing the processes of soil mineralization, nitrification and denitrification [17,26]. These processes all fractionate nitrogen, in which more ^{15}N -depleted nitrogen can lose and consequently the nitrogen remaining is ^{15}N -enriched [2]. Previous studies also suggested that the $\delta^{15}\text{N}$ was related to soil N [36] because higher soil N concentrations can accelerate the rates of microbial N transformations [37], which probably resulted in greater potential for losses of ^{14}N and therefore higher ^{15}N retention in leaves and soils [36]. Subtropical forest has been suggested to have more available N in soils [34], however, both soil available N and total N in the subtropical forest in our study were not higher, and we even found the negative relationship between $\delta^{15}\text{N}$ and soil TN across the climate zones (Figure 5c), indicating that soil N is not the main cause for the higher $\delta^{15}\text{N}$. In addition, $\delta^{15}\text{N}$ has also suggested to be associated with N sources [2]. Atmospheric N deposition are the main sources for both plant and soil N pools. From the review of N deposition in China by Liu et al. [38], subtropical forest had a higher N deposition than temperate forest, which might cause plant $\delta^{15}\text{N}$ depletion because the deposited N is usually ^{15}N -depleted [39,40]. Therefore, more atmospheric N should lead to ^{15}N more depleted in leaves and soils. However, enhanced N deposition could also increase the soil N availability, which may further stimulate soil N biogeochemical process and thereafter lead to more enriched ^{15}N in soils.

4.2. Variation of $\delta^{15}\text{N}$ and N Along the Altitude

Although the relationship of leaf $\delta^{15}\text{N}$ and altitude was not linear in all the three climate zones, we found that leaf $\delta^{15}\text{N}$ of both trees and shrubs was consistently lower at the upper limit than at lower altitude. Unlike the patterns of leaf $\delta^{15}\text{N}$ along the altitude, the response of leaf $\Delta\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ to altitude was climate zone-dependent, showing no consistent trend for the three climate zones. Previous studies have observed a significant relationship between $\delta^{15}\text{N}$ and altitude. For example, Sah and Brume [41] found leaf $\delta^{15}\text{N}$ of *Pinus roxburghii* was negatively correlated with altitude (ranging from 1200 to 2200 m a.s.l.) in a pine forest in Nepal. Similarly, leaf $\delta^{15}\text{N}$ was more negative at higher altitude, which has been observed in Gongga Mountain in southwest China, ranging from 1100 to 4900 m a.s.l. [42]. However, leaf $\delta^{15}\text{N}$ decreased with altitude from 400 to 1350 m a.s.l. and then increased above 1350 m a.s.l. in Dongling mountain [43]. The previous study of Vitousek et al. [4] also found that there was no relationship between leaf $\delta^{15}\text{N}$ and altitude.

The altitudes in the present study ranged from 2840 to 3670 m a.s.l., 2540 to 3550 m a.s.l. and 1430 to 2380 m a.s.l. in subtropical, dry-temperate and wet-temperate mountain forests, respectively (Table 1). The lower leaf $\delta^{15}\text{N}$ at higher altitude might be attributed to decreased temperature induced by high altitude because cold temperature could weaken the activity of soil microbes and thereafter reduce the N uptake for plants and soil N loss from ammonia volatilization and other gas N losses (such as N_2O , NO), and thereby more ^{14}N -enriched retains in soils [41]. However, the response of leaf

$\Delta\delta^{15}\text{N}$ values to elevation was inconsistent in three climate zones. The negative $\Delta\delta^{15}\text{N}$ values for all trees and shrubs indicated the mineral N uptake during ^{15}N discrimination processes, and different plants discriminate against ^{15}N via associations with mycorrhizal fungus, which deliver ^{15}N -depleted N to plants [14,44]. For soil $\delta^{15}\text{N}$ along the altitude, however, not only temperature but also other factors tended to have effect on soil $\delta^{15}\text{N}$ in our study areas (Figure 2f). In general, soil $\delta^{15}\text{N}$ could index long-term dynamics of N cycling, and other factors, such as, soil pH, SOC and soil C:N ratio, might co-regulate soil $\delta^{15}\text{N}$ (Table 3, Figure 5).

4.3. Variation of $\delta^{15}\text{N}$ and N between Life Forms

Across the climate zones, leaf $\delta^{15}\text{N}$ of both shrubs and trees were more depleted at the upper limits than at lower altitudes. This result indicates that in spite of different species, the depleted $\delta^{15}\text{N}$ at higher altitude might be a general pattern.

However, the values of $\delta^{15}\text{N}$ varied between life forms in our study, especially the significant difference of $\delta^{15}\text{N}$ between shrub and tree in wet-temperate mountain forest. Different $\delta^{15}\text{N}$ values indicated different use of N sources in the same regions. The shrub *Vaccinium uliginosum* is a typical ericoid mycorrhizal plant, which could be more depleted in ^{15}N [11,15,45]. Since ericoid plants have been considered to be more reliant on mycorrhizal fungi and thereafter obtained more depleted ^{15}N [46]. However, *Vaccinium uliginosum* was more enriched than *Betula ermanii* (ectomycorrhizal plant) in wet-temperate forest and even other plants (ectomycorrhizal plants or arbuscular mycorrhizal plants) selected in both subtropical and dry-temperate forest in the present study. This is consistent with the study of $\delta^{15}\text{N}$ among life forms conducted by Schulze et al. [47], in which *Vaccinium* was more enriched than other plants at the northern treeline of Alaska. The authors attributed it to more organic N (normally ^{15}N enriched) uptake by ericoid mycorrhizae. In addition, leaf $\delta^{15}\text{N}$ in trees were not more enriched than in shrubs at their corresponding upper limits (i.e., treeline and shrubline), although the shrublines are higher than the treelines. It has been suggested that higher leaf N concentrations tend to higher leaf $\delta^{15}\text{N}$ [48,49]. For instance, from the synthesis of the global patterns of N isotope compositions, a large proportion of the variation in leaf $\delta^{15}\text{N}$ was explained by leaf N concentrations, although only occurring above a mean annual temperature (MAT) of $-0.5\text{ }^{\circ}\text{C}$ [11]. In the present study, higher leaf $\delta^{15}\text{N}$ in shrub might be attributed to its higher leaf N concentration (Table 3). However, higher leaf N in shrub in dry-temperate did not lead to its higher leaf $\delta^{15}\text{N}$. This suggests that $\delta^{15}\text{N}$ is species- and site-determined in our study.

5. Conclusions

Our results showed that leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ were higher in subtropical forest than in dry- and wet-temperate forests. Leaf $\delta^{15}\text{N}$ of both treeline and shrubline species in three climate zones decreased with increasing altitude, whereas the response of leaf $\Delta\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ to altitude varied in different climate zones. $\delta^{15}\text{N}$ values differed between trees and shrubs in different climate zones. Different responses of leaf and soil $\delta^{15}\text{N}$ to altitude indicate the complexity of soil biogeochemical process and N sources uptake along with environmental variations. Higher $\delta^{15}\text{N}$ values in subtropical forest indicate that N cycles are more open in warm regions. The nutrient-related effect can also explain the patterns of $\delta^{15}\text{N}$, but their effects are species- and site-dependent. Overall, the patterns of N isotopes give insights into understanding the potential climate and edaphic influence on N cycles in high-latitude and high-altitude ecosystems.

Author Contributions: Funding acquisition, X.W. and M.-H.L.; Methodology, F.-H.Y.; Supervision, Y.J. and M.-H.L.; Writing—original draft, X.W.; Writing—review & editing, H.R. and F.-H.Y.

Funding: This work was funded by Research and Innovation Initiatives of Taizhou University (2017PY033), the National Natural Science Foundation of China (41371076), and Sino-Swiss Science and Technology Cooperation (SSSTC) program (EG 06-032015).

Acknowledgments: We would like to thank Yun-Ting Fang for the comments on earlier versions of the manuscript. We appreciate the help of Xiao-bin Li on the field sampling.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Robinson, D. $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle. *Trends Ecol. Evol.* **2001**, *16*, 153–162. [[CrossRef](#)]
2. Högberg, P. ^{15}N natural abundance in soil-plant systems. *New Phytol.* **1997**, *137*, 179–203. [[CrossRef](#)]
3. Austin, A.; Vitousek, P.M. Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia* **1998**, *113*, 519–529. [[CrossRef](#)] [[PubMed](#)]
4. Vitousek, P.M.; Shearer, G.; Kohl, D.H. Foliar ^{15}N natural abundance in Hawaiian rainforest: Patterns and possible mechanisms. *Oecologia* **1989**, *78*, 383–388. [[CrossRef](#)] [[PubMed](#)]
5. Handley, L.L.; Austin, A.T.; Stewart, G.R.; Robinson, D.; Scrimgeour, C.M.; Raven, J.A.; Schmidt, S. The ^{15}N natural abundance ($\delta^{15}\text{N}$) of ecosystem samples reflects measures of water availability. *Aust. J. Plant. Phys.* **1999**, *26*, 185–199.
6. Mizutani, H.; Kabaya, Y.; Wada, E. Linear correlation between latitude and soil ^{15}N enrichment at seabird rookeries. *Naturwissenschaften* **1991**, *78*, 34–36. [[CrossRef](#)]
7. Craine, J.M.; Brookshire, E.N.J.; Cramer, M.D.; Hasselquist, N.J.; Koba, K.; Marin-Spiotta, E.; Wang, L. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil* **2015**, *396*, 1–26. [[CrossRef](#)]
8. Gavazov, K.; Hagedorn, F.; Buttler, A.; Siegwolf, R.; Bragazza, L. Environmental drivers of carbon and nitrogen isotopic signatures in peatland vascular plants along an altitude gradient. *Oecologia* **2016**, *180*, 257–264. [[CrossRef](#)] [[PubMed](#)]
9. Bai, E.; Boutton, T.W.; Liu, F.; Wu, X.B.; Archer, S.R.; Hallmark, C.T. Spatial variation of the stable nitrogen isotope ratio of woody plants along a topographic gradient in a subtropical savanna. *Oecologia* **2009**, *159*, 493–503. [[CrossRef](#)] [[PubMed](#)]
10. Amundson, R.; Austin, A.T.; Schuur, E.A.G.; Yoo, K.; Matzek, V.; Kendall, C.; Uebersax, A.; Brenner, D.L.; Baisden, W.T. Global patterns of the isotopic composition of soil and plant nitrogen. *Glob. Biogeochem. Cycles* **2003**, *17*, 1031. [[CrossRef](#)]
11. Craine, J.M.; Elmore, A.J.; Aidar, M.P.M.; Bustamante, M.; Dawson, T.E.; Hobbie, E.A.; Kahmen, A.; Mack, M.C.; McLaughlan, K.K.; Michelsen, A.; et al. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol.* **2009**, *183*, 980–992. [[CrossRef](#)] [[PubMed](#)]
12. Rustad, L.; Campbell JLMarion, G.; Norby, R.; Mitchell, M.; Hartley, A. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **2001**, *126*, 543–562. [[CrossRef](#)] [[PubMed](#)]
13. Yi, X.F.; Yang, Y.Q. Enrichment of stable carbon and nitrogen isotopes of plant populations and plateau pikas along altitudes. *J. Anim. Feed Sci.* **2006**, *15*, 661–667. [[CrossRef](#)]
14. Hobbie, E.A.; Peter, H. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. *New Phytol.* **2012**, *196*, 367–382. [[CrossRef](#)] [[PubMed](#)]
15. He, X.H.; Xu, M.G.; Qiu, G.Y.; Zhou, J.B. Use of ^{15}N stable isotope to quantify nitrogen transfer between mycorrhizal plants. *J. Plant Ecol.* **2009**, *2*, 107–118. [[CrossRef](#)]
16. Michelsen, A.; Schmidt, I.K.; Jonasson, S.; Quarmby, C.; Sleep, D. Leaf ^{15}N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. *Oecologia* **1996**, *105*, 53–63. [[CrossRef](#)] [[PubMed](#)]
17. Chang, R.; Wang, G.; Yang, Y.; Chen, X. Experimental warming increased soil nitrogen sink in the Tibetan permafrost. *J. Geophys. Res. Biogeosci.* **2017**, *122*, 1870–1879. [[CrossRef](#)]
18. Dial, R.J.; Smeltz, T.S.; Sullivan, P.F.; Rinas, C.L.; Timm, K.; Geck, J.E.; Tobin, S.C.; Golden, T.S.; Berg, E.C. Shrubline but not treeline advance matches climate velocity in montane ecosystems of south-central Alaska. *Glob. Chang. Biol.* **2016**, *22*, 1841–1856. [[CrossRef](#)]
19. Myers-Smith, I.H.; Hik, D.S. Climate warming as a driver of tundra shrubline advance. *J. Ecol.* **2018**, *106*, 547–560. [[CrossRef](#)]
20. Körner, C. A re-assessment of high elevation treeline positions and their explanation. *Oecologia* **1998**, *115*, 445–459. [[CrossRef](#)]

21. Sullivan, P.F.; Ellison, S.B.Z.; McNown, R.W.; Brownlee, A.H.; Sveinbjornsson, B. Evidence of soil nutrient availability as the proximate constraint on growth of treeline trees in northwest Alaska. *Ecology* **2015**, *96*, 716–727. [[CrossRef](#)] [[PubMed](#)]
22. Mohl, P.; Morsdorf, M.A.; Dawes, M.A.; Hagedorn, F.; Bebi, P.; Viglietti, D.; Freppaz, M.; Wipf, S.; Korner, C.; Thomas, F.M.; et al. Twelve years of low nutrient input stimulates growth of trees and dwarf shrubs in the treeline ecotone. *J. Ecol.* **2019**, *107*, 768–780. [[CrossRef](#)]
23. Myers-Smith, I. Shrub Line Advance in Alpine Tundra of the Kluane Region: Mechanisms of Expansion and Ecosystem Impacts. *Arctic* **2007**, *60*, 447–451. [[CrossRef](#)]
24. Harsch, M.A.; Hulme, P.E.; McGlone, M.S.; Duncan, R.P. Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecol. Lett.* **2009**, *12*, 1040–1049. [[CrossRef](#)] [[PubMed](#)]
25. Hagedorn, F.; Shiyatov, S.G.; Mazepa, V.S.; Devi, N.M.; Grigor'ev, A.A.; Bartysh, A.A.; Fomin, V.V.; Kapralov, D.S.; Terent'ev, M.; Bugman, H.; et al. Treeline advances along the Urals mountain range—Driven by improved winter conditions? *Glob. Chang. Biol.* **2014**, *20*, 3530–3543. [[CrossRef](#)] [[PubMed](#)]
26. Dawes, M.A.; Schleppei, P.; Hattenschwiler, S.; Rixen, C.; Hagedorn, F. Soil warming opens the nitrogen cycle at the alpine treeline. *Glob. Chang. Biol.* **2017**, *23*, 421–434. [[CrossRef](#)] [[PubMed](#)]
27. Wang, A.; Wang, X.; Tognetti, R.; Lei, J.P.; Pan, H.L.; Liu, X.L.; Jiang, Y.; Wang, X.Y.; He, P.; Yu, F.H.; et al. Elevation alters carbon and nutrient concentrations and stoichiometry in *Quercus aquifolioides* in southwestern China. *Sci. Total Environ.* **2018**, *622*, 1463–1475. [[CrossRef](#)] [[PubMed](#)]
28. Qiang, W.Y.; Wang, X.L.; Chen, T.; Feng, H.Y.; An, L.Z.; He, Y.Q.; Wang, G. Variations of stomatal density and carbon isotope values of *Picea crassifolia* at different altitudes in the Qilian Mountains. *Trees* **2003**, *17*, 258–262.
29. Yu, D.; Wang, Q.; Liu, J.; Zhou, W.; Qi, L.; Wang, X.; Zhou, L.; Dai, L. Formation mechanisms of the alpine Erman's birch (*Betula ermanii*) treeline on Changbai Mountain in Northeast China. *Trees* **2014**, *28*, 935–947. [[CrossRef](#)]
30. WRB, I.W.G. *World Reference Base for Soil Resource 2014, International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2014.
31. Mariotti, A. Atmospheric nitrogen is a reliable standard for natural ¹⁵N abundance measurements. *Nature* **1983**, *303*, 685–687. [[CrossRef](#)]
32. Wang, R.; Dungait, J.A.J.; Creamer, C.A.; Cai, J.; Bo, L.; Xu, Z.; Zhang, Y.; Ma, Y.; Yong, J. Carbon and nitrogen dynamics in soil aggregates under long-term nitrogen and water addition in a temperate steppe. *Soil Sci. Soc. Am. J.* **2015**, *79*, 527–535. [[CrossRef](#)]
33. Fang, Y.; Koba, K.; Yoh, M.; Makabe, A.; Liu, X. Patterns of foliar delta ¹⁵N and their control in Eastern Asian forests. *Ecol. Res.* **2013**, *28*, 735–748. [[CrossRef](#)]
34. Martinelli, L.A.; Piccolo, M.C.; Townsend, A.R.; Vitousek, P.M.; Cuevas, E.; McDowell, W.; Robertson, G.P.; Santos, O.C.; Treseder, K. Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. *Biogeochemistry* **1999**, *46*, 45–65. [[CrossRef](#)]
35. Craine, J.M.; Elmore, A.J.; Wang, L.; Augusto, L.; Baisden, W.T.; Brookshire, E.N.J.; Cramer, M.D.; Hasselquist, N.J.; Hobbie, E.A.; Kahmen, A.; et al. Convergence of soil nitrogen isotopes across global climate gradients. *Sci. Rep.* **2015**, *5*, 8280. [[CrossRef](#)] [[PubMed](#)]
36. Pardo, L.H.; Templer, P.H.; Goodale, C.L.; Duke, S.; Groffman, P.M.; Adams, M.B.; Boeckx, P.; Boggs, J.; Campbell, J.; Colman, B. Regional Assessment of N Saturation using Foliar and Root ¹⁵N. *Biogeochemistry* **2006**, *80*, 143–171. [[CrossRef](#)]
37. Booth, M.; Stark, J.E. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol. Monogr.* **2005**, *75*, 139–157. [[CrossRef](#)]
38. Liu, X.; Duan, L.; Mo, J.; Du, E.; Shen, J.; Lu, X.; Zhang, Y.; Zhou, X.; He, C.; Zhang, F. Nitrogen deposition and its ecological impact in China: An overview. *Environ. Pollut.* **2011**, *159*, 2251–2264. [[CrossRef](#)] [[PubMed](#)]
39. Garten, C.T.J. Nitrogen isotope composition of ammonium and nitrate in bulk precipitation and forest throughfall. *Int. J. Environ. Anal. Chem.* **1992**, *47*, 33–45. [[CrossRef](#)]
40. Houlton, B.Z.; Edith, B. Imprint of denitrifying bacteria on the global terrestrial biosphere. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21713–21716. [[CrossRef](#)] [[PubMed](#)]
41. Sah, S.; Brume, R. Altitudinal gradients of natural abundance of stable isotopes of nitrogen and carbon in the needles and soil of a pine forest in Nepal. *J. For. Sci.* **2003**, *49*, 19–26. [[CrossRef](#)]

42. Liu, X.-Z.; Wang, G. Measurements of nitrogen isotope composition of plants and surface soils along the altitudinal transect of the eastern slope of Mount Gongga in southwest China. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 3063–3071. [[CrossRef](#)] [[PubMed](#)]
43. Liu, X.Z.; Wang, G.A. Nitrogen isotope composition characteristics of modern plants and their variations along an altitudinal gradient in Dongling Mountain in Beijing. *Sci. China* **2010**, *53*, 128–140. [[CrossRef](#)]
44. Hobbie, E.A.; Colpaert, J.V. Nitrogen Availability and Colonization by Mycorrhizal Fungi Correlate with Nitrogen Isotope Patterns in Plants. *New Phytol.* **2003**, *157*, 115–126. [[CrossRef](#)]
45. He, X.H.; Critchley, C.; Bledsoe, C. Nitrogen Transfer Within and Between Plants Through Common Mycorrhizal Networks (CMNs). *Crit. Rev. Plant Sci.* **2003**, *22*, 531–567. [[CrossRef](#)]
46. Hobbie, E.A.; Jumpponen, A.J. Foliar and fungal $^{15}\text{N}:$ ^{14}N ratios reflect development of mycorrhizae and nitrogen supply during primary succession: Testing analytical models. *Oecologia* **2005**, *146*, 258–268. [[CrossRef](#)]
47. Schulze, E.D.; Chapin, F.S.; Gebauer, G. Nitrogen nutrition and isotope differences among life forms at the northern treeline of Alaska. *Oecologia* **1994**, *100*, 406–412. [[CrossRef](#)]
48. Pardo, L.H.; McNulty, S.G.; Boggs, J.L.; Duke, S. Regional patterns in foliar ^{15}N across a gradient of nitrogen deposition in the northeastern US. *Environ. Pollut.* **2007**, *149*, 293–302. [[CrossRef](#)]
49. Kang, H.; Liu, C.; Yu, W.; Wu, L.; Chen, D.; Sun, X.; Ma, X.; Hu, H.; Zhu, X. Variation in foliar $\delta^{15}\text{N}$ among oriental oak (*Quercus variabilis*) stands over eastern China: Patterns and interactions. *J. Geochem. Explor.* **2011**, *110*, 8–14. [[CrossRef](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).