Article

Stable carbon isotope ($\delta^{13}C$) composition in leaf of *Pinus sylvestris* var. *mongolica* in Hulunbuir and its relationship with ecological stoichiometry and environmental factors

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Abstract: The decline in *Pinus sylvestris* var. *mongolica* in the introduction area has had a high profile in recent years. For the ecological restoration, management and silvicultural design of Mongolian pines in the introduction area, it is necessary to conduct a comprehensive and in-depth study on the ecological adaptation mechanism of Mongolian pines in provenances. The ecological process of water and nutrient accumulation as well as the influence of environmental factors on the photosynthetic physiology are the key to revealing the ecological adaptation mechanism of Mongolian pines. According to the differences of climate in the distribution area of Mongolian pines, sampling sites were set up, the effects of environmental factors on leaf $\delta^{13}C$ and the relationship between leaf $\delta^{13}C$ and nutrient content were analyzed. The results showed that leaf $\delta^{13}C$ values were ranging from - 29.7 ‰ to - 23.76 ‰. The ecological stoichiometry, including LC (522.81 $mg \cdot g^{-1}$), LN (16.04 $mg \cdot g^{-1}$), LP (1.19 $mg \cdot g^{-1}$) and L-N:P (13.56), indicated that leaf photosynthesis and water use efficiency is greatly affected by environmental conditions, Mongolian pines had strong ability of carbon fixation, and its growth was obviously restricted by nitrogen. Although there was no significant correlation between $\delta^{13}C$ with stoichiometric parameters in leaf, photosynthesis was the key link in the process of carbon fixation. It also showed that *Pinus sylvestris* var. *mongolica* was a stomatal limited plant. Leaf $\delta^{13}C$ had significant correlation with climatic factors. VPD is the dynamic factor affecting the photosynthetic physiological process in leaves. Air and soil moisture are the dominate factors affecting the leaf stomatal conductance and determines leaf $\delta^{13}C$ value, while other factors indirectly affect leaf $\delta^{13}C$ by its impact on relative humidity or soil water content. Soil phosphorus content affected by clay is a key factor affecting soil water availability and soil nutrient cycling. Photosynthetic process in leaf is the dynamic process affecting the nutrient accumulation.

Keywords: *Pinus sylvestris* var. *mongolica*; stable carbon isotope ($\delta^{13}C$); ecological stoichiometry; environmental factors

1. Introduction

Pinus sylvestris var. *mongolica* (Mongolian pines) is an evergreen coniferous tree species, which is widely distributed in temperate zone, cold temperate zone, semi-arid and semi humid region, owing to the characteristics of its cold, drought and barren resistance. It is one of the main sand fixation afforestation tree species in the "Three North Shelterbelt". Since 1955, it has been introduced from its provenence (Hulunbuir) to many other places in North China. At present, the early introduction areas (such as Horqin sandy land) of Mongolian pines show signs of decline, such as withered shoots, death and other phenomena, while the Mongolian pines forest in the provenance is in a relatively healthy growth state. Therefore, it is very necessary to study the ecological adaptation mechanism of Mongolian pines in the provenance. In the late 1980s, in order to explore the decline mechanism of Mongolian pines forest in the introduction area, a lot of research has been carried out in China. Combined with the research results in the recent 40 years, it was found that with the increase of age and density of trees, the demand for environmental resources such as water and nutrients increased, and the supply capacity gradually decreased [1-6]. The restriction on the metabolic process and growth of trees is the main factor that causes the decline of Mongolian pines. Therefore, the water and nutrient cycling process is the key process to reveal the ecological adaptation mechanism of Mongolian pines, while climate and soil are the main factors affecting the process. Currently, the research on the ecological adaptation mechanism of Mongolian pines has been approved by most scholars from the perspective of the relationship between Mongolian pines and environmental water conditions, and water use characteristics [2,6-9]. It is an effective method to study the water use process of Mongolian pines by using new techniques such as stable isotopes. However, there is a lack of research on the coupling relationship between water use, nutrient cycling and other key ecological processes of the Mongolian pines forest in the provenance, especially on the correlation between water transport process and photosynthesis, transpiration process, and nutrient accumulation and cycling process. With the aggravation of seasonal drought in this area, will the natural forest of Mongolian Pine in Hulunbuir decline and similar to the plantation in the introduction area under the background of global climate change? Is it sustainable? These questions will eventually become the challenges of forest ecosystem management and decision-making in semi-arid areas. How plants respond to drought stress is the key to revealing the ecological adaptation mechanism of plants, but similar studies are relatively lacking.

Carbon cycle is the key to study the effects of global change on biogeochemical cycle and adaptation of terrestrial ecosystems. Plants participate in carbon cycle through photosynthetic carbon fixation process [10]. Leaf $\delta^{13}C$ can indicate photosynthetic pathway and water use efficiency. Leaf $\delta^{13}C$ has the characteristics of changing with environmental gradient or spatial scale [11-16]. Transpiration is a dynamic physiological process affecting photosynthesis and metabolism of plants, which is accompanied by CO_2 absorption [17] and water transport, i.e. carbon water balance process [15, 18]. The process of water movement in plants promotes the accumulation, circulation and metabolism of nutrients in the atmosphere, soil and plants, as well as the vegetative growth of plants. The supply of water and nutrients in terrestrial ecosystems can affect the photosynthetic fixation of carbon [16, 19, 20]. However, in different regions, different species have different adaptation strategies towards the environment, and the influence of environmental water and nutrients on plant $\delta^{13}C$ are quite different [21]. From the point of view of environmental water and nutrients, there are few studies on the ecological adaptation mechanism of Mongolian pines.

Leaf nutrient content and ecological stoichiometry can indicate the characteristics of nutrient accumulation and vegetative growth of plants. Leaf $\delta^{13}C$ and ecological stoichiometry are plant traits [23, 24], which are the performance of environmental adaptation strategies. Because the process of water cycle in plants drives the process of nutrient accumulation, leaf $\delta^{13}C$ and ecological stoichiometry must be related. Some studies have confirmed that leaf nutrient and ecological stoichiometry are closely related to photosynthetic carbon sequestration and water use efficiency [25-29]. For example, leaf nitrogen content has a limiting effect on photosynthetic process [30-33], In addition, leaf $\delta^{13}C$ and nitrogen had a coordinated variation relationship [34-41]. However, some studies showed that leaf $\delta^{13}C$ was not correlated with leaf nitrogen content, which might be related to stomatal limitation [42, 43] and photosynthetic nitrogen use efficiency [44, 45]. Under the influence of regional scale, species and ecological process, the correlation of leaf $\delta^{13}C$ with nutrient and ecological stoichiometry is different, which means that the coordinated variation relationship between leaf water use and nutrient accumulation varies in different regions and species. However, there are few studies in similar areas. Therefore, the analysis of the coordinated variation between leaf $\delta^{13}C$ and ecological stoichiometry, as well as the impacts of environmental factors on leaf $\delta^{13}C$, will help to clearly identify the water and nutrient cycle mechanism of Mongolian pines, and further to clarify the adaptation strategy of Mongolian pines to the natural environment of Hulunbuir. So as to provide the scientific basis for the design of forest management plan in the introduction area in the future, and to provide the strong theoretical support for the forest management in the context of global change.

2. Materials and Methods

2.1. Study area

The climate type in Hulunbuir is temperate continental monsoon climate, with a long cold winter and a short warm summer. The annual mean temperature is about ranging from - 4.4℃ to 3.3℃, and the annual precipitation is about ranging from 235 mm to 538 mm. The precipitation is mainly concentrated in summer. Affected by the Greater Khingan Mountains and the East Asian monsoon, the precipitation decreases from east to west, forming the climate characteristics of semi-humid to semi-arid transition dominated by water conditions. *Pinus sylvestris* var. *mongolica* belongs to the Siberian coniferous forest vegetation type. It is distributed in the transition area from the western slope of Greater Khingan Mountains to Hulunbuir plateau, which mainly has a banded distribution along the sand belt on the banks of Yimin River and Hailar River. It extends northward from Handagai to Hailar along Yimin River, and then bends westward and extends to Wangong (47 ° - 49 ° N, 119 ° - 120 °E) (Figure 1). In addition, there are some sporadic distribution in such areas as Manzhouli and Moerdaoga.

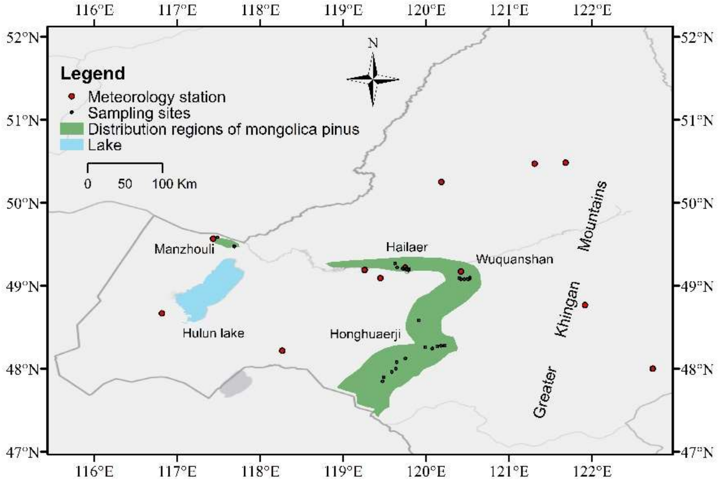

Figure 1. Sampling sites in the distribution regions of Mongolian pines in Hulunbuir, China.

2.2. Sample collection and test methods

According to the distribution of *Pinus sylvestris* var. *mongolica* forest, four sampling sites including Manzhouli, Hailar, Wuquanshan and Honghuaerji were selected for sampling from May to June in 2019. In each sample plot, leaf samples were collected from individual Mongolian pines trees randomly selected from 1.8-2 m above the ground. In order to avoid the interference of light (sun / shade) on the uneven growth of plants, leaves were collected from different directions around the individual trees to form a mixed sample of leaves. A total of 55 leaf samples were collected. The leaves were brought back to the laboratory in a pollution-free paper bag, and cleaned with distilled water, and then the samples were put into the oven and dried at 55 ℃. Then the leaves were grinded and sifted, from which about 2mg grinded leaves were taken out and put into tin capsules for testing. The $\delta^{13}C$ value of leaves was measured by a mass spectrometer MAT253. The average standard deviation of repeated measurement of $\delta^{13}C$ was 0.2 ‰. At the same time, the ecological stoichiometric parameters of leaf samples were determined and calculated, including leaf organic carbon (LC), nitrogen (LN), phosphorus (LP), potassium (LK), organic carbon nitrogen ratio (L-C:N) and nitrogen phosphorus ratio (L-N:P). Soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), soil texture, soil moisture and soil pH were tested in 13 soil profiles collected in the Mongolian pines forest.

2.3. Meteorological data

Due to the limited number of meteorological stations and their discrete distribution, except Hailar, Manzhouli and Wuquanshan (which are close to Yakeshi, and the meteorological data were from Yakeshi meteorological station), Honghuaerji was lack of meteorological station observation data, which was unable meet the requirements for the correlation analysis of climatic factors and plant traits in the sample plot. It needs the mean value of climate data during from May to June in 2019 and the location of existing meteorological stations in Hulunbuir. The spatial interpolation model was used to estimate the climate data in Honghuaerji. The meteorological information of the sampling sites was calculated. The model formula [46] is as follows:

$$\mathrm{F} = \mathrm{a} \times \mathrm{latitude} + \mathrm{b} \times \mathrm{longitude} + \mathrm{c} \times \mathrm{altitude} + \mathrm{d} \qquad (1)$$

Where F is the monthly mean value of climate factors, and a, b, c and d are the regression coefficients. According to the estimated mean climate value of flowering period (from May to June) of 2019, including temperature, precipitation, sunshine hours, relative humidity and saturated vapor pressure deficit (VPD), the climate data of sampling sites in Honghuaerji were estimated.

2.3. Statistical analysis method

In this paper, statistical analysis methods are used, including one-way ANOVA, linear correlation and path analysis. The statistical analysis is conducted by means of spss17.0. Among them, one-way ANOVA is mainly used to preliminarily judge the effects of environmental factors (on sampling sites) on leaf $\delta^{13}C$ and ecological stoichiometry (LC, LN, LP, LK, L-C:N, L-N:P). Linear correlation analysis is mainly used to analyze the variation characteristics of leaf $\delta^{13}C$ with the environmental factors (climate factors and soil factors), and the correlation between leaf $\delta^{13}C$ and ecological stoichiometry. Path analysis is mainly used to analyze the impact of environmental climate and soil factors on leaf δ^{13}C, as well as the impact of leaf δ^{13}C on ecological stoichiometry.

3. Results

3.1. The Characteristics of Leaf δ^{13}C and ecological stoichiometry

3.1.1. The δ^{13}C composition and its ecological stoichiometric features

The δ^{13}C values in leaves of Mongolian pines ranged from - 29.7 ‰ to - 23.76 ‰, with an average of - 27.29 ‰ (Figure 2 (a)). The frequency distributions of LC, LN, LP, LK, L-C:N ratio and L-N:P ratio are shown in Figure 2 (b-g). Leaf carbon content LC (mean value ± SD = 522.81 ± 30.86 mg.g^{-1}; range: 473.69 - 628.35 mg.g^{-1}), leaf nitrogen content LN (mean value ± SD = 16.04 ± 2.39 mg.g^{-1}; range: 12.58 - 23.74 mg.g^{-1}); leaf phosphorus content LP (mean value ± SD = 1.19 ± 0.12 mg.g^{-1}; range: 0.96-1.51 mg.g^{-1}); and leaf potassium content LK (mean value ± SD = 3.44 ± 0.59 mg.g^{-1}; range: 2.44 - 4.82 mg.g^{-1}). Leaf carbon nitrogen ratio (L-C:N) (mean value ± SD = 33.09 ± 3.82; range: 22.38 - 42.89), and nitrogen phosphorus ratio (L-N:P) (mean value ± SD = 13.56 ± 1.78; range: 10.34 - 17.91).

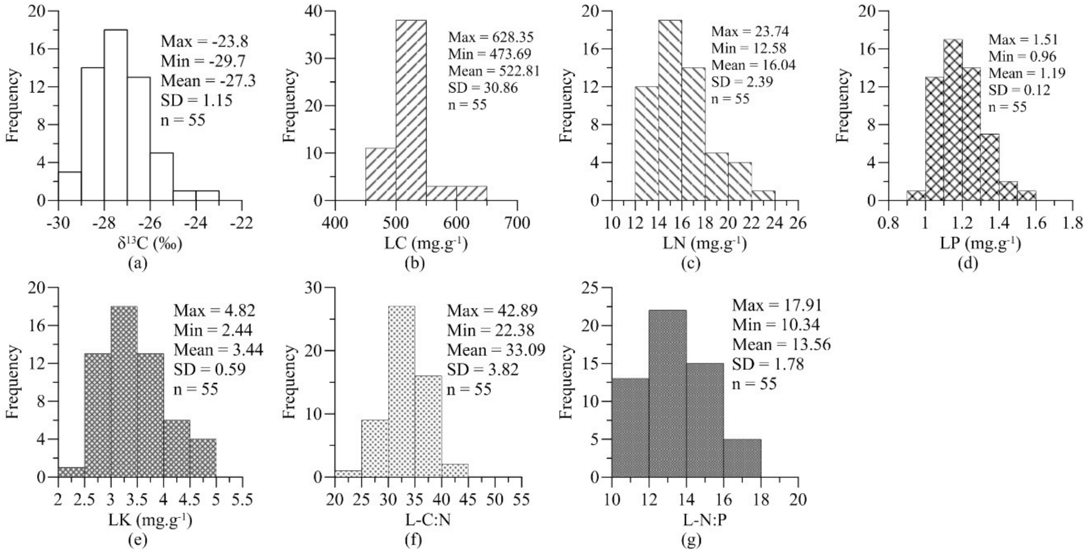

Figure 2. Histogram of δ^{13}C and ecological stoichiometry in leaves.

(**a**) leaf δ^{13}C; (**b**) Leaf organic carbon content (LC); (**c**) Leaf nitrogen content (LN); (**d**) Leaf phosphorus content (LP); (**e**) Leaf potassium content (LK); (**f**) The ratio of organic carbon to nitrogen content in leaf (L-C:N); (**g**) The ratio of nitrogen to phosphorus content in leaf (L-N:P).

There were significant differences in leaf δ^{13}C, LP and LK from the four sampling sites, but no significant differences in LC, LN, L-C: N and L-N: P (Table 1).

Table 1. Analysis of variance of leaf δ^{13}C and ecological stoichiometry in four sampling sites.

Leaf traits index	Abbreviation	*d.f.*	*F*	Sig.
Leaf stable carbon isotope	δ^{13}C	3	6.232	0.001**
Leaf organic carbon content	LC	3	0.554	0.648
Leaf nitrogen content	LN	3	0.651	0.586
Leaf phosphorus content	LP	3	4.649	0.006**
Leaf potassium content	LK	3	3.143	0.033*
The ratio of organic carbon to nitrogen content in leaf	L-C:N	3	0.514	0.675
The ratio of nitrogen to phosphorus content in leaf	L-N:P	3	0.836	0.480

**stand that the reliability test passed 99%; * stand that the reliability test passed 95%.

3.1.2 The characteristics of correlation between leaf δ^{13}C and ecological stoichiometry

The results (Figure 3) showed that there was no significant correlation between leaf δ^{13}C and LC, LN, LP, LK and L-C:N, and there was a positive correlation between leaf δ^{13}C and L-N:P. With the increase of leaf δ¹³C, LC ($p = 0.096$) and LN($p = 0.464$) showed curve changes. There were negative correlations between δ¹³C and LP ($p = 0.163$), LK ($p = 0.120$), and L-C:N ($p = 0.396$) and positive correlation between δ¹³C and L-N:P ($p = 0.035$) in leaf.

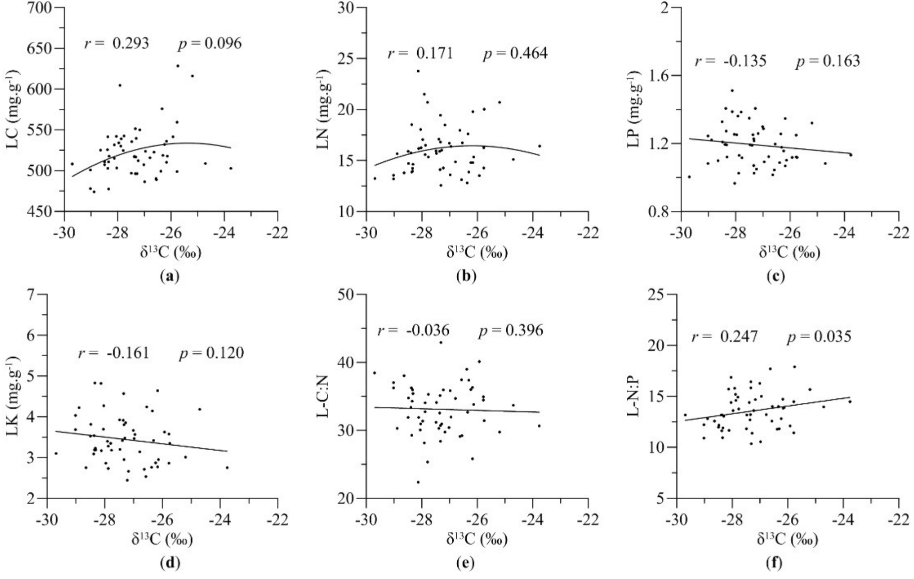

Figure 3. Correlation between ecological stoichiometry and δ¹³C in leaves.

(**a**) Organic carbon contents (LC) and δ¹³C in leaves; (**b**) Nitrogen contents (LN) and δ¹³C in leaves; (**c**) Phosphorus contents (LP) and δ¹³C in leaves; (**d**) Potassium contents (LK) and δ¹³C in leaves; (**e**) The ratios organic carbon to nitrogen in leaves (L-C:N) and δ¹³C; (**f**) The ratios nitrogen contents to phosphorus in leaves (L-C:N) and δ¹³C.

3.2 Correlation between leaf δ¹³C and environmental factors

3.2.1 Correlation between leaf δ¹³C and climatic factors

Figure 4 showed that leaf δ¹³C were significantly positively correlated with the mean temperature ($p = 0.010$), the sunshine hours ($p = 0.000$) and VPD ($p = 0.005$) in the flowering period. Leaf δ¹³C were significantly negatively correlated with precipitation ($p = 0.000$) and relative humidity ($p = 0.000$) in the flowering period.

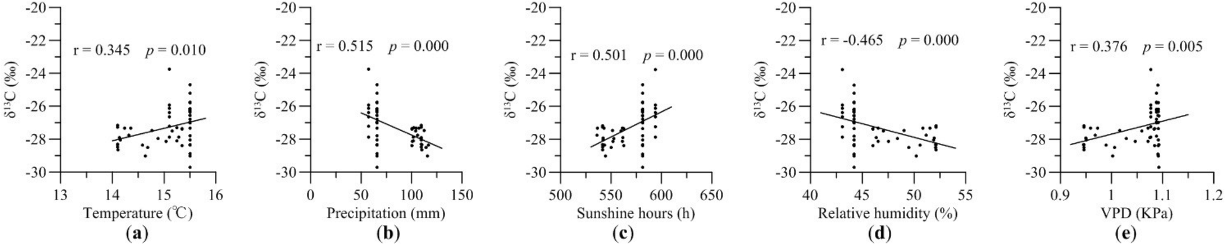

Figure 4. Correlation between leaf δ¹³C and climatic factors.

(**a**) Correlation between leaf δ¹³C and temperature; (**b**) Correlation between leaf δ¹³C and precipitation; (**c**) Correlation between leaf δ¹³C and sunshine hours; (**d**) Correlation between leaf δ¹³C and relative humidity; (**e**) Correlation between leaf δ¹³C and saturated vapor pressure deficit (VPD).

3.2.2 Correlation between leaf δ¹³C and soil factors

The analysis of correlation between leaf δ¹³C and soil factors (Figure 5) showed that except for significant negative correlation with soil moisture (SWC) ($p = 0.022$), leaf δ¹³C was negatively correlated with SOC, TN, TP, clay content and silt content, and they did not pass 95% of significance tests. There was a significant positive correlation between leaf δ¹³C and pH ($p = 0.038$), and there was a positive correlation between leaf δ¹³C and TK and soil sand content, but it did not pass 95% of reliability tests.

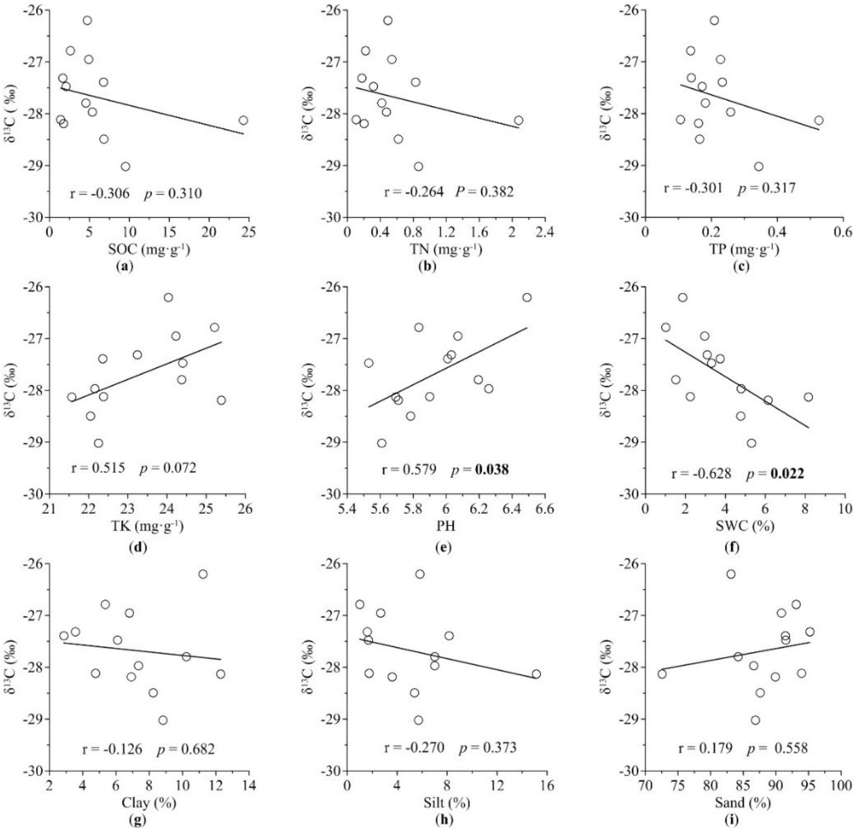

Figure 5. Correlation between leaf δ13C and soil factors.

(a) Leaf δ13C and soil organic carbon content; (b) Leaf δ13C and soil total nitrogen content; (c) Leaf δ13C and soil total phosphorus content; (d) Leaf δ13C and soil total potassium content; (e) Leaf δ13C and soil PH; (f) Leaf δ13C and soil water content; (g) Leaf δ13C and soil clay content; (h) Leaf δ13C and soil silt content; (i) Leaf δ13C and soil sand content.

4. Discussion

4.1 δ13C composition and ecological stoichiometric features in leaves of Pinus sylvestris var. mongolica

Generally speaking, the δ13C values of C_3 species ranged from - 35 ‰ to - 20 ‰, with an average of -27 ‰ [17]. Therefore, *Pinus sylvestris* var. *mongolica* belongs to C_3 photosynthetic pathway. In Horqin sandy land of North China, the δ13C values in leaves of Mongolian pines reported by Li et al. [47] ranged from- 30.37 ‰ to - 25.10 ‰. The δ13C values in leaves of Mongolian pines in our study are almost similar to it. Due to the change of environmental factors such as collecting time and place, air humidity and soil moisture, the δ13C value of Mongolian pines leaves in this study area is slightly different from the above results.

Compared with Chinese and global databases [48-51], the average LC of Mongolian pines leaves in Hulunbuir as higher than that of the forest transects in North and South China [48] and the world [50, 51], indicating that Mongolian pines has higher carbon sequestration capacity. The average content of LN and LP was lower than that of the forest transects in North and South China, China and the world, indicating that the growth of Mongolian pines restricted seriously by nitrogen and phosphorus deficiency. The average value of L-C:N (33.09) was higher than that of the forest transects in North and South China and the world; and the average value of L-N:P was 13.56, which was lower than the level at home and abroad. L-N:P ratio is generally considered as an indicator of plant growth, metabolism and nutrient limitation. If it is less than 14, it is deemed that nitrogen limitation is significant [52, 53], indicating that the vegetative growth of Mongolian pines is mainly limited by nitrogen.

Analysis of variance showed that there were significant differences in δ13C, LP and LK of leaves from different sampling sites, indicating that leaf δ13C, LP and LK were greatly affected by environmental conditions. LC and LN both came from CO_2 and N_2 in atmosphere, with a small range. The differences of CO_2 and N_2 concentration in the atmosphere were small. There was no significant difference in LC and LN of leaves from different sampling sites.

4.2 Relationship between leaf $\delta^{13}C$ and ecological stoichiometry

There was a positive correlation between leaf $\delta^{13}C$ and L-N:P, indicating that photosynthetic carbon fixation of leaves could promote vegetative growth. In the study of the coordinated relationship between plant growth and soil water in the loess region, it was found that when plants were under non phosphorus (P) limitation, there was a significant positive correlation between water use efficiency of plant and leaf nitrogen phosphorus ratio (N:P) [54], which was similar to the conclusion of this study. There was no significant correlation between leaf $\delta^{13}C$ and LC, LN, LP, LK, etc., which indicated that the change of leaf $\delta^{13}C$ value of Mongolian pines might be caused by stomatal limitation rather than the change related to the nutrient of photosynthetic capacity. In addition, it may be related to the distribution of nutrients in plants. In a word, in addition to photosynthesis and transpiration, the factors affecting nutrient accumulation in leaves are more complex.

Figure 6 showed that when $\delta^{13}C$ affects LC, the value of standardized path coefficient was -0.378, and this path presented a significant level of 0.05 ($p < 0.05$), indicating that $\delta^{13}C$ had a significant negative impact on LC, that is, the larger the $\delta^{13}C$ value was, the smaller the LC was. In semi-arid areas, stomatal conductance was the key factor affecting the size of leaf $\delta^{13}C$. The air saturated vapor pressure deficit was large, and the leaf transpiration was strong. Due to drought stress, some stomata closed, CO_2 assimilation rate decreased, and carbon fixation decreased, so $\delta^{13}C$ increased and LC decreased. The normalized coefficients of the interaction between LC and LN as well as between LN and LP were both positive, which indicated that LC, LN and LP had a positive coordinated variation relationship. The normalized coefficient of the interaction between LC and LK was negative, which indicates that LC and LK had a negative coordinated variation relationship. LC had a significant positive effect on L-C:N, while LN had a significant negative effect on L-C:N. LN had a significant positive effect on L-N:P, while LP had a significant negative effect on L-N:P. The normalized coefficient of the interaction between LP and LK was positive, which indicated that LP and LK had a positive coordinated variation relationship. The contents of carbon, nitrogen, phosphorus, potassium and any other nutritive element in leaves had no significant effect on $\delta^{13}C$.

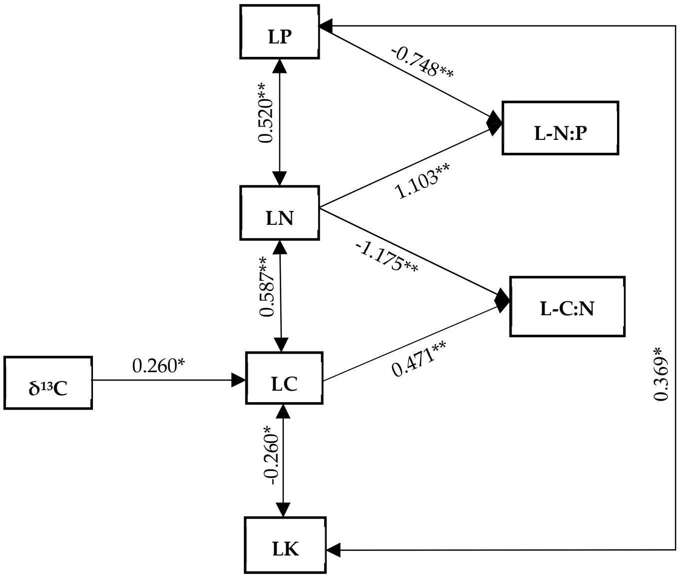

Figure 6. A Path-analysis of the effect of leaf $\delta^{13}C$ on ecological stoichiometry.

$\delta^{13}C$: Leaf $\delta^{13}C$; LC: Leaf organic carbon content; LN: Leaf nitrogen contents; LP: Leaf phosphorus contents; LK: Leaf potassium contents; L-C:N: The ratio organic carbon to nitrogen in leaf; L-N:P: The ratio nitrogen content to phosphorus in leaf.

4.3 Effects of climate factors on leaf $\delta^{13}C$

Leaf $\delta^{13}C$ was positively correlated with light (sunshine), heat (temperature) and VPD, and negatively correlated with precipitation and relative humidity, that is, with the increase of light and heat, VPD inside and outside the leaf chamber increased, and the leaf $\delta^{13}C$ increased (positive); with the increase of precipitation and relative humidity, the leaf $\delta^{13}C$ of Mongolian pines decreased (negative). Leaf $\delta^{13}C$ had a strong correlation with annual mean value of temperature, precipitation, sunshine hours and the average value of growing season. The correlation of climate factors in that year was significant, which indicated that the photosynthetic physiological state of Mongolian pines was the result

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of long-term joint action of light, heat and water. Climate was significantly dominant factors, especially in terms of relative humidity and VPD.

The influence of climate factors on leaf δ¹³C mainly depended on regional water status and VPD, which was the direct factor affecting the photosynthesis transpiration process. VPD was the driving force of transpiration [55], which directly affected stomatal conductance, photosynthesis and stable carbon isotope fractionation. In semi-arid areas, the smaller the VPD, the larger the stomatal conductance, and the smaller the leaf δ¹³C; on the contrary, the larger the VPD, the smaller the stomatal conductance, and the larger the leaf δ¹³C, because stoma is closed in order to reduce the adverse impact of transpiration on plants. Other factors have indirect effects on leaf δ¹³C mainly through their effects on VPD. Figure 7 shows that the value of leaf δ¹³C depends on the value of VPD. VPD had a significant positive impact on leaf δ¹³C, and the normalized path coefficient was 0.377 ($p < 0.01$), that is, the larger the VPD was, the more positive the δ¹³C was. VPD was affected by temperature and relative humidity. Temperature had a significant positive effect on VPD ($p < 0.01$), and the normalized path coefficient was 0.624. The relative humidity had a significant negative effect on VPD ($p < 0.01$), and the normalized path coefficient was -0.388. Relative humidity was mainly affected by precipitation and air temperature. Precipitation had a significant positive effect on relative humidity ($p < 0.01$), and the normalized path coefficient is 0.495. Temperature had a significant negative effect on relative humidity ($p < 0.01$), and the normalized path coefficient was -0.536. Sunshine duration had indirect impact on VPD through its impact on air temperature, and further affecting the value of leaf δ¹³C. Sunshine duration had a significant positive effect on air temperature ($p < 0.01$), and the normalized path coefficient was 0.828.

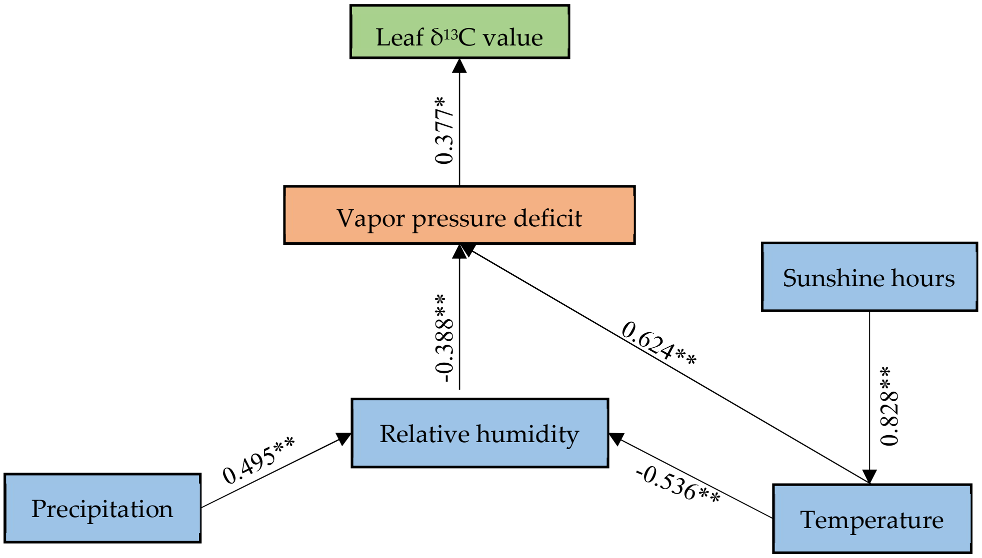

Figure 7. A Path-analysis of the effect of soil factors on leaf δ¹³C value.

4.4 Effects of soil factors on leaf δ¹³C

Similar to previous studies [56], leaf δ¹³C was negatively correlated with soil moisture. There were negative correlation between leaf δ¹³C and soil organic carbon, nitrogen and phosphorus content, but the correlation was not significant, indicating that soil organic carbon, nitrogen and phosphorus nutrient content mainly affected plant growth through the impact on soil moisture, and there was a more complex relationship between plant and soil nutrient accumulation and transformation, and its driving mechanism still needs to be further studied. Leaf δ¹³C was positively correlated with soil potassium content. The smaller the soil potassium content is, the stronger the leaching effect is, the smaller the pH is, and the greater the soil water content is. The smaller the water stress on *Pinus sylvestris* var. *mongolica* is, the larger the leaf stomatal conductance is, and the smaller the leaf δ¹³C is. Therefore, in semi- humid and semi-arid areas, the soil pH value and potassium content can indicate the degree of dryness and wetness in a sense.

Path analysis results showed that leaf δ¹³C value mainly depended on soil moisture content (Figure 8). Soil water content (SWC) had a significant negative effect on leaf δ¹³C ($p < 0.01$), and the normalized path coefficient was -0.597. TP had a significant positive effect on soil water ($p < 0.01$). Clay had a significant positive effect on TP ($p < 0.01$). TP had a significant positive effect on TN ($p < 0.01$). TN had a significant positive effect on SOC ($p < 0.01$). TP had a significant negative effect on TK ($p < 0.01$). In addition, SWC had a negative effect on soil pH, though which did not pass the significance test of $p < 0.01$.

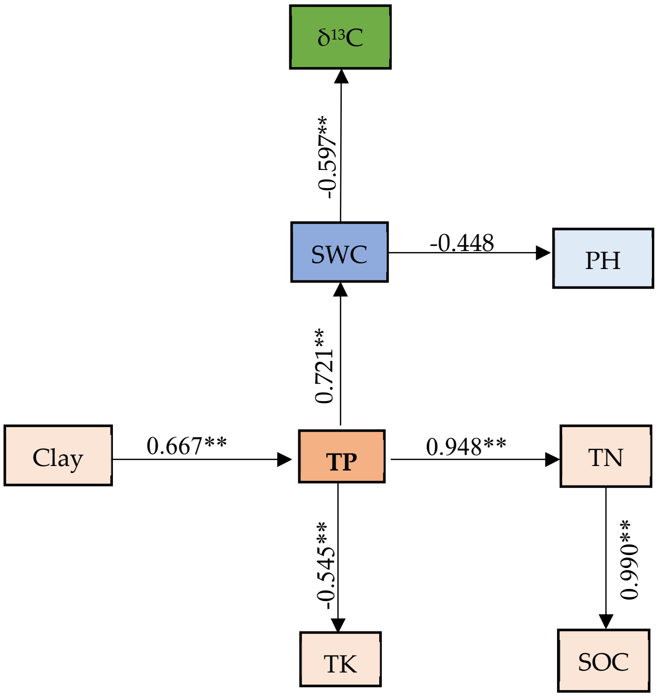

Figure 8. A Path-analysis of the effect of climate factors on leaf δ13C.

δ13C: Leaf δ13C; SWC: Soil water content; SOC： Soil organic carbon content; TN: Soil nitrogen content; TP: Soil phosphorus content; TK: Soil potassium content; PH: Soil PH; Clay: Soil clay content.

Soil water is the main source of available water for plant, which is the key factor affecting the leaf δ13C. SWC mainly depends on the amount of precipitation and soil water holding capacity. TP plays an important or indicative role in soil water availability [57], which is the major limiting factor for the vegetative growth of plants. Its availability is related to soil particle size and climate factors [57]. Similar to the conclusion of previous studies [16, 59], TP plays an important role in influence on leaf δ13C. Soil phosphorus mainly exists in aggregates. The content of soil clay determines the release of phosphorus in clay minerals. Soil clay content is one of the final products of rock physical weathering and biochemical weathering, which directly affects soil texture, porosity, etc., and further affects soil water holding capacity. However, clay content had no direct effect on soil water in this study. SWC was indirectly affected by TP. TP plays a crucial role in nutrients such as nitrogen (TN), organic carbon (SOC) and potassium (TK) in soil. The higher the content of soil phosphorus, the higher the content of soil carbon and nitrogen, the lower the content of soil potassium, the lower the pH, the stronger the acidity, the stronger the water holding capacity of soil. The more conducive to the photosynthetic physiology and growth process of Mongolian pines; otherwise, it is not conducive to the photosynthetic physiology and growth of Mongolian pines. Therefore, soil moisture is the key factor affecting photosynthesis transpiration process, growth, and leaf carbon and nitrogen accumulation process of Mongolian pines. The release and decomposition of soil phosphorus is the key biogeochemical process affecting soil water availability, plant growth and soil nutrient accumulation of the Mongolian pines forest.

Soil water was transported to the leaves through xylem conduit, which determines the degree of drying and wetting inside the leaf chamber. Atmospheric water content determines the degree of drying and wetting outside the leaf chamber, and VPD directly determines the transpiration intensity of leaves. Leaf transpiration had a direct impact on leaf stomatal conductance. Before the optimal stomatal conductance reached, the larger difference of saturated vapor pressure, the greater the transpiration intensity is, the more vigorous the photosynthesis is, and the smaller the leaf δ13C is. In the area or period of drought stress, VPD is large. In order to avoid the physiological damage caused by transpiration, the leaves of Mongolian pines developed thick cuticle and stoma deeply stuck in tissues, and even closed parts of stoma, abscised old leaves, resulting in decreased stomatal conductance, reduced photosynthesis and increased δ13C of leaves. Therefore, in the area or period with higher humidity, the stronger the transpiration, the stronger the photosynthesis, and the smaller the leaf $\delta^{13}C$. while in the area or period of drought stress, the stronger the transpiration, the weaker the photosynthesis, and the larger the leaf $\delta^{13}C$. The value of leaf $\delta^{13}C$ was impacted by optimal stomatal conductance. Under the influence of dynamic process (transpiration process), there was a positive and negative feedback mechanism in leaf photosynthesis.

In Hulunbuir, the difference of site conditions is obvious under the dominance of water. The precipitation increases gradually from west to east. The soil moisture is more in the east and less in the west. The eastern area is close to the Greater Khingan Mountains, with good water condition, large stomatal conductance, strong photosynthesis and transpiration process, and negative leaf $\delta^{13}C$, so Mongolian pines is in a good growth state with higher stand density and stronger ability of carbon and nitrogen fixation. The spatial coupling of soil moisture, clay and soil organic carbon, nitrogen and phosphorus showed the characteristics of high mountainous in the east and forests in the north, and low sand and sparse forest in the west. The spatial difference of atmospheric precipitation is the dominant factor affecting this coupling relationship.

5. Conclusions

The value of $\delta^{13}C$ in leaves of Mongolian pines mainly depends on the process of photosynthesis and transpiration. Mongolian pines had a strong ability of carbon fixation, and its growth was significantly restricted by nitrogen. Under the influence of photosynthesis transpiration process, the leaves promote the absorption and balance of nutrient elements, and then affect the growth rate of Mongolian pines. The photosynthesis drives nutrient accumulation in leaves. The greater the photosynthetic intensity of leaves, the smaller the $\delta^{13}C$ of leaves, the more obvious the carbon fixation effect, and the greater the content of nitrogen and phosphorus of leaves, indicating that the photosynthetic process of leaves had a significant role in promoting the nutrient accumulation process of leaves. Therefore, the ecological stoichiometry and $\delta^{13}C$ in leaves had a certain coordinated variation feature. However, the correlation is not linear, which may be related to stomatal limitation or nutrient distribution in plants. Water condition was the key factor among the environmental factors effecting the leaf $\delta^{13}C$ of Mongolian pines, and further affecting its growth-metabolism process. The water mainly came from air and soil water.

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