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Original Research Paper

Water-use Efficiency of Typical Afforestation Tree Species in Liaoning, P.R. China and Their Response to Environmental Factors

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ABSTRACT

This study aimed to make a reasonable use of forests in Liaoning province. By using C-isotope (leaf $\delta^{13}C$ value) as an index for long-term water-use efficiency (WUE), we evaluated the WUE of three typical afforestation tree species, namely, *Pinus sylvestris* var. *mongolica*, *Pinus tabulaeformis*, and *Quercus mongolica*. We then determined their response to environmental factors, such as annual average precipitation, annual average air temperature, altitude, and soil nutrient. Results of linear regression analysis indicate that (1) the $\delta^{13}C$ values of the three tree species were -30.37‰ to -23.51‰, and the $\delta^{13}C$ values in their leaves decreased with the longitudes. (2) The $\delta^{13}C$ values in the leaves of the three tree species were significantly influenced by the annual average precipitation and altitude, i.e., the $\delta^{13}C$ values decreased significantly with the annual average precipitation and increased with altitude, although the $\delta^{13}C$ values in their leaves were less influenced by the annual average air temperature. (3) Except soil nutrients, such as calcium contents, which showed no obvious relationship with $\delta^{13}C$ of *Q. mongolica*, *P. sylvestris* and *P. tabulaeformis* were significantly affected by soil nutrients. This work was the first to investigate the WUE of the three tree species mentioned above. This study also explored the main factors influencing WUE. Overall, our findings may facilitate future scientific studies on WUE based on $\delta^{13}C$, and these studies will be beneficial to environmental protection.

INTRODUCTION

The climatic conditions within the Liaoning region are characterized by a typical summer drought (western Liaoning) and abundant sunshine and elevated temperature (eastern Liaoning). These environmental constraints promote the use of irrigation and encourages forestation, raising important concerns on the sustainability of the current afforestation. With the increase in temperature and worsening water scarcity in the near future in these areas as predicted by global climate models, water deficiency may increasingly become a limiting factor in afforestation activities. The current and the predicted changes in climatic conditions reinforce the need to improve the water-use efficiency (WUE).

Researchers have demonstrated that carbon isotope composition (^{13}C) of dry mass is a representative parameter of WUE in various plant species under different environmental conditions (Yu et al. 2004, Zheng et al. 2008, Picon-Cochard et al. 2006, Tan et al. 2009, Kaminski et al. 2014). The concept of WUE always reflects a balance between gains (carbon acquisition or crop yield) and costs (water consumed by transpiration and water applied) (Cernusak et al. 2007, Gouveia et al. 2009, Li et al. 2010, Wang et al. 2013). This balance can be measured at the level of the leaves in terms of instantaneous fluxes of CO_2 and water vapour, as well as at the plant levels, although in a wider context; this concept also applies to the entire agricultural systems (Nicotra et al. 2008, Kenney et al. 2014, Yaghi et al. 2013, Fernandes et al. 2016). At the leaf level, the rate of net CO_2 assimilation (AN), transpiration (E), and stomatal conductance for water vapour (g_s) allows the determination of "instantaneous water-use efficiency (AN/E, WUEinst)" and "intrinsic water-use efficiency (AN/gs, WUEi)" (Bota et al. 2016, Medrano et al. 2015, Tomás et al. 2014). At this level, WUE can be easily measured but always under short intervals (instantaneous gas-exchange measurements) (Herrero-Langreo et al. 2013, Chen et al. 2012, Skubel et al. 2015).

In this study, *Pinus sylvestris* var. *mongolica*, *Pinus tabulaeformis*, and *Quercus mongolica* were selected on the basis of their typical ecological characteristics, mainly on their contribution to environmental protection, wind sheltering, and drought resistance. Historically, large-area afforestation of *P. sylvestris*, *P. tabulaeformis*, and *Q. mongolica* was introduced in Liaoning Province in the 1970s. The afforested area increased until 2015, with the three species dominating the farmland shelter-belt system in northeastern China, offering significant benefits to the environment, economy and society.

The WUE of a plant is a function of multiple factors, including physiological characteristics of genotype and soil characteristics, such as soil nutrient and meteorological conditions (Gouveia et al. 2009, Li et al. 2010, Wang et al. 2013, Herrero-Langreo et al. 2013, Chen et al. 2012, Skubel et al. 2015). Thus, to understand the effect of the environment on the WUE of plants, researchers have devoted considerable attention on both environmental and economic forest tree species. However, related studies have seldom focused on *P. sylvestris*, *P. tabulaeformis*, and *Q. mongolica*, given their response to environmental factors in Liaoning area in China. This work aims to study (1) the WUE of these three tree species used in afforestation and (2) the effects of the major meteorological factors and soil nutrients on WUE. Our results are beneficial to obtain further understanding on the WUE mechanisms of tree species in Liaoning Province and provide a scientific basis for vegetation restoration in the future.

MATERIALS AND METHODS

Study area: The study area is located in Liaoning Province, which lies in the southern region of northeast China (40°37' 49.1' N-42°8' 21.4' N, 118°39' 34.6' E-125°19' 17.8' E). A low mountainous and hilly area is found on both sides of the eastern and western Liaoning, and the middle region of the province is plain, which lies in the middle and downstream of the Liaohe River. The study site receives the continental monsoon climate of central temperate zone and is located in the staggered flora band in Changbai Mountain, northern China, and Inner Mongolia province. Moreover, the study site is rich in plant species, and vegetation flourishes better in this area.

The study site harbours three typical forest types, namely, the artificial pure forest of *P. tabulaeformis* and *P. sylvestris* and natural forest of *Q. mongolica*, which served as research objects. The plots were setup in eastern Liaodong Mountain and western Liaoning hills on July-September 2014. The sample area is 20 m × 20 m, and the aboveground investigation includes measurement of the diameter of the long-boled trees, tree height, and characteristics of underbrush and herbs. Leaves of the long-boled tree species were also collected. During the experiment, we carefully selected the trees of the same height and adret leaves to avoid the dry and withered leaves, which are exposed to strong light condition; diseased plants and damaged branchlets were not selected. Considering the principles above, we collected 20-30 healthy leaves in each small branch, mixed them together as one sample, stored in bags with good permeability, and then brought to the laboratory for carbon isotope ($\delta^{13}C$) measurement. Triplicate samples were obtained from each plot. Soil nutrients, namely, organic carbon (C), total nitrogen (N), total phosphorus (P), effective N, effective P, and calcium (Ca) contents, were measured in the laboratory in accordance with the rules mentioned above (soil depth: 0-15 cm). The general condition in the study area is summarized in Table 1.

Determination of leaf $\delta^{13}C$: As stated in the introduction, the several means of WUE, the forms are taking as follows, the Equation (1), and (2) are gas exchanged based measured WUE:

$$WUE_{ins\tan\tan eous} = \frac{A}{E} \quad ...(1)$$

$$WUE_{instrinsic} = \frac{A}{g} \quad ...(2)$$

Where A is the net photosynthetic rate expressed in μmol CO_2 $m^{-1}.s^{-1}$, E is the transpiration rate expressed in mmol H_2O $m^{-2}.s^{-1}$, and g is the stomatal conductance to water vapour expressed in mol H_2O $m^{-2}.s^{-1}$.

Eqs. (3) and (4) were integrated using the measured WUE:

$$WUE_{biomass} = \frac{DryMatter}{\text{(evapo) transpiredwater}} \quad ...(3)$$

$$WUE_{yield\,(\text{waterproductivity})} = \frac{Matteryield}{\text{(evapo) transpiredwater}} \quad ...(4)$$

Eq. (5) was estimated using the measured WUE:

$$^{13}C_{estimation} = \frac{A}{gratio} \quad ...(5)$$

Where $^{13}C_{estimation}$ is the carbon isotope discrimination.

In this study, we used Eq. (6) to calculate $\delta^{13}C$:

$$\delta^{13}C = [(Rsam./Rstd.)-1] \times 1000‰ \quad ...(6)$$

Where Rsam. is $^{13}Cs/^{12}Cs$ (see Eq. (7)), and Rstd. is the international standard material (PDB, South Carolina carbonate rocks, see Eq. (8). The carbon isotope ratio of the plant dry matter ($\delta^{13}C$) is determined during photosynthesis based on the differential diffusion of the carbon isotopes ^{13}C and ^{12}C between the atmosphere and the chloroplast:

$$Rsam./\% = \frac{^{13}C}{^{12}C} \quad ...(7)$$

Table 1: The general situation in the study area.

Region	Coenotype	Latitude/Altitude (m)	Plot density (trees/ha)	DBH (cm)	Plots	Origin
Western Liaoning	*P. sylvestris*	41°50'-42°02'/232-757	1209±633	14.65±3.77	10	Artificial forest
	P. tabulaeformis	40°54'-42°03'/180-801	1370±645	13.86±3.71	12	Artificial forest
	Q. mongolica	40°46'-41°54'/481-871	1150±594	13.16±4.69	4	Natural forest
Eastern Liaoning	*P. sylvestris*	41°18'-42°18'/171-528	1172±452	18.33±5.43	12	Artificial forest
	P. tabulaeformis	41°19'-42°15'/155-504	710±316	22.85±6.31	10	Artificial forest
	Q. mongolica	40°37'-42°12'/348-552	1533±292	14.98±2.91	12	Natural forest

$$Rsam./\%=\frac{^{13}C}{^{12}C}PDB \qquad ...(8)$$

The equations above show that the less negative $\delta^{13}C$ represents higher WUE. Analysis of the composition of carbon isotopes in different plant tissues allows determination of the average leaf WUE. Therefore, discrimination against ^{13}C is a widely used indicator of WUE and water stress in different plant species.

Statistical analysis: Data on mean air temperature and mean precipitation were collected from the study sites in western Liaoning, and the corresponding results were obtained as follows: Lingyuan (0.5-15.6 °C and 267.41-513.33 mm), Jianping (3.5-15.6 °C, 242.33-646.22 mm), Fuxin (2.6-15.2 °C, 250.33-447.75 mm), Huanren (1.9-13.3°C, 524.58-787.66 mm), and Qingyuan (4.3-13.3 °C, 481.16-764.66 mm); in Eastern Liaoning: Xiuyan (4.1-14.7 °C, 374.58-737.08 mm), Benxi (4.5-14.1 °C, 529.08-758.05 mm), and Dandong (5.3-14.1 °C, 484.33-1048.11 mm). Furthermore, data on precipitation and temperature (data for approximately 10 years) were obtained from local meteorological stations. Correlation and variance analyses of all the data were conducted using SPSS 17.0 software (IBM Inc., NC, USA). Data processing and plotting were completed using OriginPro 9.0 software (OriginLab Inc., Northampton, MA, USA).

RESULTS AND ANALYSIS

Spatial difference in WUE of tree species in Liaoning Province: The results showed that the WUE of *P. sylvestris*, *P. tabulaeformis*, and *Q. mongolica* slightly varied ($P > 0.05$); with $\delta^{13}C$ values as index, the variation ranged from -30.37% to -25.10%, -30.32% to -24.07%, and -29.85% to -23.51%, respectively. Overall, the WUE of *P. sylvestris* was slightly lower than that of *P. tabulaeformis* and *Q. mongolica* both in eastern and western Liaoning. However, the WUE of all trees in western Liaoning was higher than that of all the trees in eastern Liaoning ($P < 0.01$) (data not shown). Our results are the opposite of previous findings (Gouveia et al. 2009, Li et al. 2010, Wang et al. 2013), and this disparity is possibly related to the extent of the study area. Previous studies often selected the same geographical features, such as precipitation and elevation, which can easily and better determine interspecific relationships. By contrast, we used a wide range of sampling sites, and environmental factors may exert high influence on different species. Given the arid climatic conditions in western Liaoning, the tree species in the area may display high WUE.

Relationship between the factors affecting climate and the WUE of the tree species: Fig. 1 demonstrates that $\delta^{13}C$ of the tree species is significantly and negatively correlated with the annual average precipitation. As the annual average precipitation increased, $\delta^{13}C$ decreased. Furthermore, the annual average precipitation substantially influenced *P. sylvestris*. Compared with the effect of the annual average precipitation on the $\delta^{13}C$ of *P. tabulaeformis* and *Q. mongolica* ($P < 0.05$), a stronger relationship was observed between the $\delta^{13}C$ of *P. sylvestris* and the annual average precipitation ($P < 0.01$).

The annual average air temperature slightly influenced the $\delta^{13}C$ values of these plant species ($P > 0.05$) (Fig. 2), indicating that the annual average air temperature exerts less influence on the WUE of these tree species.

Fig. 3 shows that the $\delta^{13}C$ value of plant species presented similar trends of altitude to that of the annual average air temperature, and the $\delta^{13}C$ values highly increased with increasing altitude, indicating that *P. sylvestris* was significantly influenced by altitude ($P < 0.01$).

Precipitation considerably influenced the WUE of different plant species. Our result demonstrates that with increasing average annual precipitation, the $\delta^{13}C$ values of different plant species decreased. Researchers have revealed that when the average annual precipitation was 400-1019 mm, the $\delta^{13}C$ is significantly and negatively correlated with the average annual precipitation but positively correlated when the average annual precipitation was over 1019 mm (data not shown) (Gouveia et al. 2009, Herrero-Langreo et

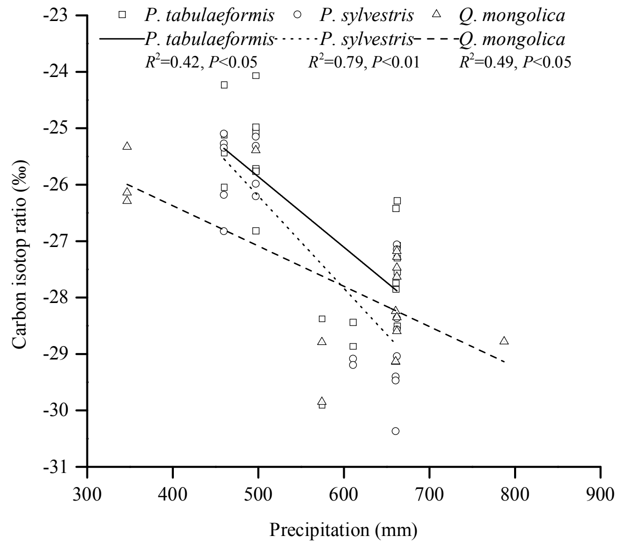

Fig. 1: Relationship between $\delta^{13}C$ values of plants and annual average precipitation.

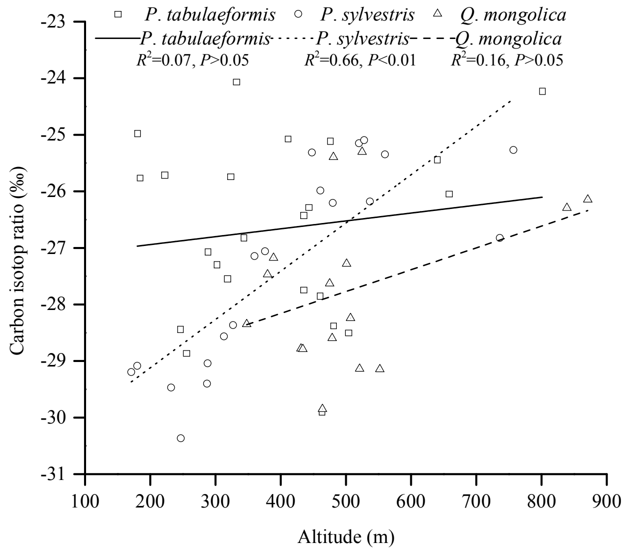

Fig. 3: Relationship between $\delta^{13}C$ values of plant species and altitude.

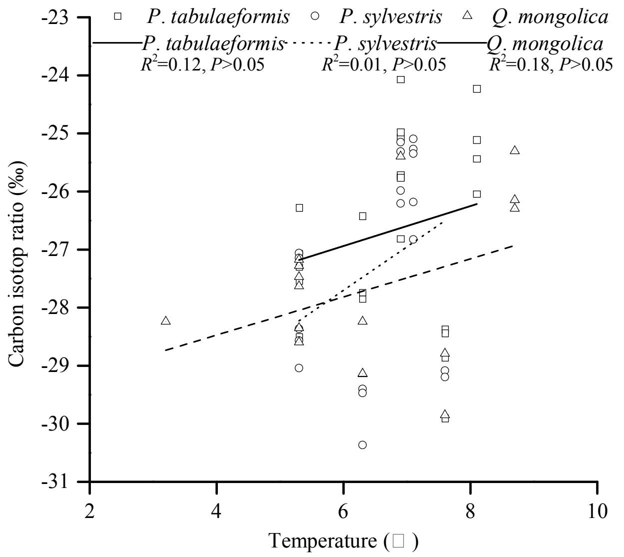

Fig. 2: Relationship between $\delta^{13}C$ values of the tree species and annual average air temperature.

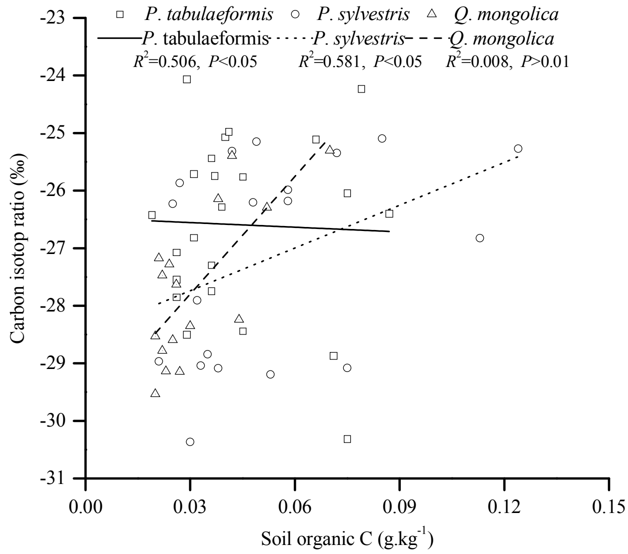

Fig. 4: Relationship between $\delta^{13}C$ of plant species and soil organic carbon.

al. 2013, Skubel et al. 2015). In our research site, the average annual precipitation was 300-800 mm, indicating a significantly negative correlation between the $\delta^{13}C$ value of different plant species and the annual average precipitation; this result is consistent with the findings of the studies mentioned above. Moreover, the annual average precipitation is the most essential environmental factor influencing the $\delta^{13}C$ value of different plant species.

The relationship of the WUE of *P. sylvestris* and *P. tabuliformis* with rainfall is more obvious than that of the WUE of *Q. mongolica* with rainfall possibly because of the low amount of rainfall in the arid and semiarid areas in western Liaoning. Moreover, *P. sylvestris* and *P. tabuliformis* (also maximum stomatal closure) can avoid transpiration moisture loss. In addition, low amount of rainfall reduces the entry of CO_2 into the leaf, which is the opposite condition for normal photosynthesis, forcing the plant to reduce CO_2 recognition capabilities. Thus, the $\delta^{13}C$ values are relatively high. Moreover, the leaf is thin and blade-like in shape, which are conducive characteristics for internal stor-

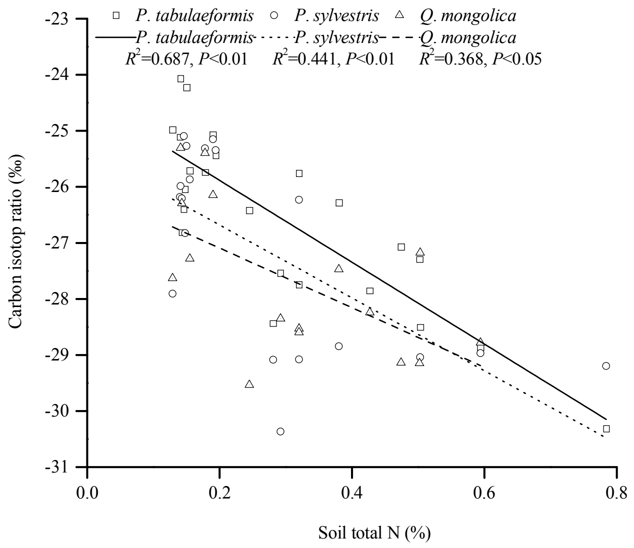

Fig. 5: Relationship between δ^{13}C of the plant species and soil total N.

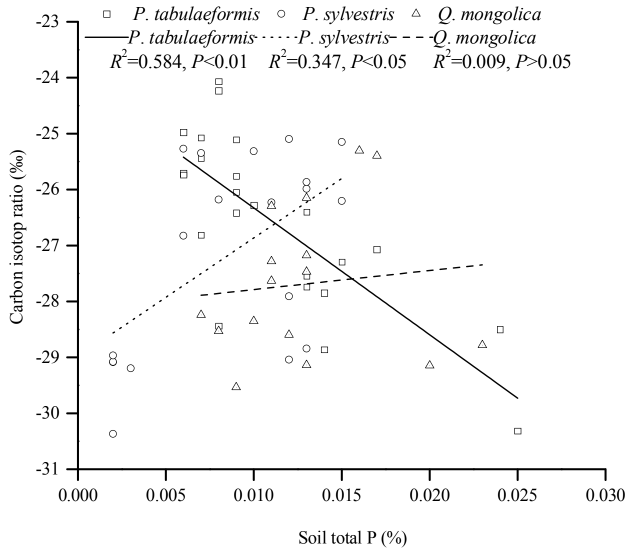

Fig. 6: Relationship between δ^{13}C of plant species and soil total P.

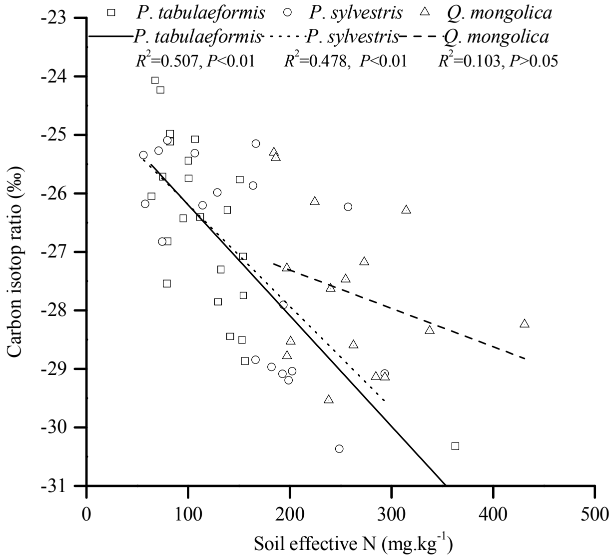

Fig. 7: Relationship between δ^{13}C of the plant species and soil effective N.

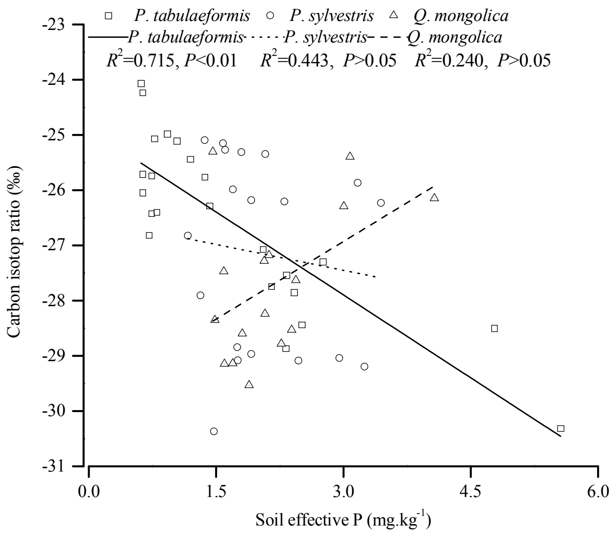

Fig. 8: Relationship between δ^{13}C of plant species and soil effective P.

age and water-loss reduction, as well as essential for increasing the moisture absorption rates by roots of groundwater to achieve higher water use.

Temperature is an important environmental factor that can significantly affect C isotope fractionation. Temperature directly affects the activities of photosynthetic enzymes and indirectly influences stomatal conductance and CO_2 density, further affecting the δ^{13}C values. The relationship between temperature and plant leaf δ^{13}C values is complex. No uniform conclusions have been drawn to explain this relationship. Our experimental results indicate that the carbon isotope ratios (δ^{13}C) of the three species were less affected by temperature, and the correlation is not significant ($P > 0.05$). Thus, the δ^{13}C values of the three tree species are influenced mainly by precipitation followed by temperature.

Relationship between soil nutrient and WUE of the tree species: Fig. 4 shows the relationship between δ^{13}C of the tree species and soil organic C, and the results show that the leaf δ^{13}C values of the trees are negatively correlated with soil organic C. In addition, the leaf δ^{13}C values of *P. tabulaeformis* and *P. sylvestris* were significantly and nega-

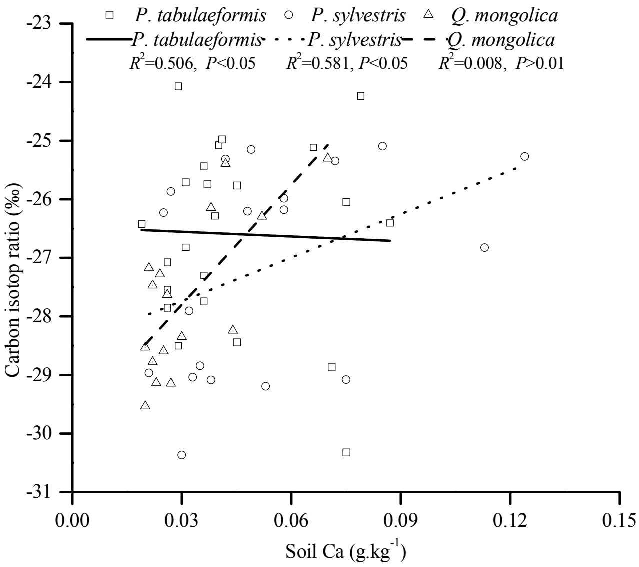

Fig. 9: Relationship between δ^{13}C of plants species and soil Ca content.

tively correlated with soil organic C ($P < 0.05$), whereas no obvious relationship exists between the δ^{13}C values of *Q. mongolica* and soil organic C ($P > 0.05$).

Fig. 5 shows the relationship between δ^{13}C of the tree species and soil total N, and the results indicate that the δ^{13}C values of the tree species are significantly and negatively correlated with the soil total N ($P < 0.05$).

Fig. 6 shows that the δ^{13}C value of *P. tabulaeformis* is significantly and negatively correlated with the soil total P ($P < 0.05$). By contrast, the δ^{13}C values of *P. sylvestris* and *Q. mongolica* are significantly and positively correlated with the soil total P ($P < 0.05$ and $P < 0.01$, respectively).

Fig. 7 shows the relationship between δ^{13}C of the tree species and soil effective N, and the results show that the leaf δ^{13}C values of the tree species were negatively correlated with the soil effective N; however, the correlation of the δ^{13}C values of *P. tabulaeformis* and *Q. mongolica* and soil effective N is significant ($P < 0.01$).

Fig. 8 illustrates the correlation between the ^{13}C values of different plant species and soil effective P. The result reveals the negative correlation between the δ^{13}C value of *P. tabulaeformis* and soil effective P ($P > 0.05$). In addition, the δ^{13}C value of *P. sylvestris* and soil availability P are significantly and positively correlated ($P < 0.01$).

Fig. 9 shows that no obvious relationship exists between the δ^{13}C value of *Q. mongolica* and soil Ca ($P > 0.05$). By contrast, the δ^{13}C values of *P. tabulaeformis* and *P. sylvestris* are significantly and positively correlated with the soil Ca ($P < 0.05$).

On the basis of the results above, we concluded that the δ^{13}C values of the three tree species and all the soil nutrient contents are higher in eastern Liaoning than in western Liaoning. In addition, soil C, N, and effective P are the main factors that mostly influenced the trees. These two elements are massively produced from aboveground and underground decomposition. Moreover, soil C, N, and P can reflect soil use and properties, such as soil quality, clay contents, and soil type. Thus, clarifying the relationship between soil nutrient content and leaf δ^{13}C values will help us realize the different land uses and conducted to plantations.

CONCLUSION

This work aims to determine the WUE of three typical environmental protection trees, namely, *P. sylvestris*, *P. tabulaeformis*, and *Q. mongolica*, and their responses to environmental factors, such as the annual average precipitation, annual average air temperature, altitude, and soil nutrient contents. The results of our experiments are described as follows:

1. The δ^{13}C values of the above three tree species were -30.37‰ to -25.10‰, -30.32‰ to -24.07‰, and -29.85‰ to -23.51‰, respectively. The δ^{13}C values in the leaves of the three tree species decreased with longitudes.

2. Statistical analysis shows that the δ^{13}C values of the three tree species were considerably higher in the western part than in the eastern part of Liaoning province.

3. Further analysis shows that the influence of altitude on WUE was considerably more robust in *P. sylvestris* than in the two other tree species ($P < 0.01$). In addition, more significant effects of precipitation on WUE were found in *P. sylvestris* and *P. tabulaeformis* than those in *Q. mongolica*. In summary, identifying interspecies differences in WUE based on leaf δ^{13}C, which is significantly affected by environmental factors (e.g., rainfall and altitude), is difficult.

4. The leaf δ^{13}C values of *P. tabulaeformis*, *P. sylvestris*, and *Q. mongolica* were negatively correlated with soil organic C and soil total N. By contrast, total P was positively correlated with the δ^{13}C values of *P. sylvestris* and *Q. mongolica*, and the δ^{13}C values of *Q. mongolica* was significantly and positively correlated with the soil effective P. The δ^{13}C values of *Q. mongolica* were not obviously correlated with soil Ca, whereas those of *P. sylvestris* and *P. tabulaeformis* were significantly and positively correlated with the soil Ca content.

This work is the first investigation on the WUE of three typical afforestation trees. However, the limitation of this study implies that more influencing factors, such as relative humidity, solar radiation, and plant nutrients, should have been included. Inclusion of these factors would be the direction of our future studies. Overall, the above results can provide useful information that is useful for better afforestation and environmental protection in Liaoning Province.

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