



# The Effect of Climatic Factors on Leaf Traits of a Non-Leguminous Nitrogen Fixing Species *Hippophae tibetana* (Schlecht.) Along the Altitudinal Gradient in the Eastern Tibetan Plateau, China

Xiaowei Li<sup>(\*\*)</sup> and Kun Sun<sup>\*†</sup>

\*College of Life Science, Northwest Normal University, Lanzhou 730070, China

\*\*Key Laboratory for Restoration and Reconstruction of Degraded Ecosystems in North-western China, Ningxia University, Ningxia 750021, China

†Corresponding author: Kun Sun

Nat. Env. & Poll. Tech.

Website: [www.neptjournal.com](http://www.neptjournal.com)

Received: 14-02-2015

Accepted: 05-05-2015

## Key Words:

Leaf traits

Nitrogen-fixing plant

*Hippophae tibetana*

Climatic factors

Tibetan plateau

## ABSTRACT

Plant leaf traits and their changes along environmental gradients, reflect their environmental adaptability. Few studies have examined the relationship between the intraspecific leaf-trait and the climate on the Tibetan Plateau. We determined the leaf traits of *Hippophae tibetana* Schlecht., a non-legume species with symbiotic N fixation (SNF), at 25 sites on the Tibetan Plateau and explored the relationship between leaf traits and the local climate. These leaf traits included leaf length/leaf width ratio (LL/LW), leaf mass per area (LMA), leaf nitrogen (N), phosphorus (P) and potassium (K) concentrations. We found that (i) N was negatively related to LMA, leaf nitrogen, P, and K were positively correlated with each other and the leaf LL/LW ratio was positively related to leaf P; (ii) leaf N and P increased, while N:P ratio and LMA decreased with the increase in annual mean temperature (MAT), and the Leaf K and LL/LW increased with increasing annual mean precipitation (MAP). Our results showed that the leaf traits of *H. tibetana* on the Tibetan Plateau can be modified by the climate, and factors on Tibetan Plateau can exert a strong evolutionary pressure on leaf traits at the intra-specific levels. Out of our expectation, the growth of *H. tibetana* can be limited by P and K.

## INTRODUCTION

Green leaves are fundamental to the function of ecosystems and the changes in their traits can indicate their adaptation to the environment (Fahn & Cutler 1992, Wright et al. 2004a). Leaf stoichiometry traits such as nitrogen (N), phosphorus (P) content, N:P ratio, leaf mass per area (LMA), and shape (length/width ratio) are fundamental leaf traits, and their interrelations and changes along environmental gradients on a large scale may help to understand the pattern of plant functional traits in relation to the climate and plant evolution (Wright et al. 2004a, He et al. 2006, Osnas et al. 2013). However, previous studies on the relationships between leaf traits and the environment, mainly based on mean functional trait values of species, but ignored the intraspecific variability in plant leaf traits (Cécile et al. 2010). Intraspecific functional variability of leaf traits can indicate the response and adaptation of the plant to the environmental (McIntyre et al. 1999) and can be chosen according to the studied system and selected traits and species, as well as on the study objectives (Cécile et al. 2010).

Many studies showed similar patterns in the relationships between leaf traits, and between leaf traits and environment factors at the population (Wright et al. 2004a, Qi et al. 2009),

biome (He et al. 2006) and global scale (Reich & Oleksy 2004, Wright et al. 2004a, 2005a, 2005b).

Reich & Oleksyn (2004) observed that the global pattern of leaf N and P concentrations were obviously influenced by the climate. In addition, the temperature-plant physiology hypothesis was put forward to explain the variability of geographical patterns in plant N and P concentrations (Reich & Oleksyn 2004), which was based on the point that all plant metabolic processes are temperature sensitive, predicts that leaf N and P concentrations should increase and compensate for the low efficiency of metabolic rate at low temperatures. Leaf N:P ratio can be used to diagnose plant nutrient limitation, N: P ratio < 14 generally indicating N limitation, and a N:P > 16 suggesting P limitation (Koerselman & Meuleman 1996).

Leaf N concentration was found to be negatively correlated with LMA (Wright et al. 2005a). The LMA is a key trait in plant growth (Lambers & Poorter 1992) and an important indicator of plant growth strategies (Grime 2001, Westoby et al. 2002). Two opposite functional strategies can be distinguished from the range of leaf trait variation that defines the leaf economics spectrum (Wright et al. 2004b). Species in arid, semi-arid or cold regions tend to have higher

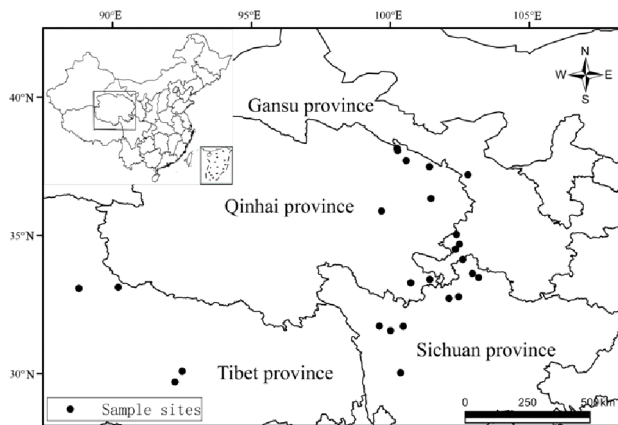


Fig. 1: Sampling sites of *Hippoph tibetana* in this study.

LMA (Schulze et al. 1998, Fonseca et al. 2000, Niinemets 2001, Wright 2001, 2004b, 2005a), lower leaf N concentration (Wright 2004a, He et al. 2006) and higher life-span (Wright 2004a). Leaves associated with high LMA have thick leaf blade, small and thick-walled cells to continue leaf functions under very cold conditions (Wright 2004b). Species with low LMA tend to have higher photosynthetic capacity per unit leaf mass (Field & Mooney 1986, Reich et al. 1997, Wright et al. 2001, Niinemets 2005), higher leaf N concentration (Field & Mooney 1986, Reich et al. 1997, Diemer 1998) and lower lifespan (Wright 2004a).

Recent global data compilations have revealed the modulation of climate on plant traits (Wright et al. 2005b). For example, He et al. (2006) reported that on the Tibetan Plateau, the modulation of climate on leaf traits mainly occurred at the intra and interspecific levels. These studies showed that the climate is one of the important factors that control the intraspecific variability of leaf traits on the Tibetan Plateau. This suggested that those wide distributed species with the area of Tibetan Plateau can provide a unique opportunity to study the relationship between intraspecific leaf traits and the climate.

The Tibetan Plateau is characterized with high-altitude, strong radiation, cold climate (very low mean annual temperature) and is one of the most sensitive areas to respond to global climate change (Liu et al. 2000, Yang et al. 2011) and has had tremendous impact on the evolution and the development of species, making it a centre of differentiation for new species (Sun & Zheng 1998). Thus, the Tibetan Plateau is an ideal place for studying plant traits change in relation to the climate. However, no studies have detected the relationship between leaf traits of any individual species and the climate on the Tibetan Plateau.

*Hippophae tibetana* Schlecht. is an endemic shrub

occurring on the Tibetan Plateau and a nitrogen-fixing plant which has a symbiotic relationship with *Frankia* sp. (Lian et al. 2000). This species grows mainly on sandy soils by river banks or along river beds, mountain slopes, valleys and alpine meadow and occupies a wide range of habitats from 2800m to 5200m (a.s.l.) on the Plateau. It is the major pioneer tree species for artificial reforestation (Lian et al. 2000). In such a wide geographical area, there is a pronounced gradient of climate (mean annual temperature MAT and mean annual precipitation MAP), providing a unique opportunity to study the leaf traits of this species.

In this study, we explored the patterns of the leaf traits (N, P, K, N:P ratio, leaf shape and LMA) of *H. tibetana* in relation to the climate in twenty-five nature populations. Three aims were posed: (1) What are the relationships between leaf stoichiometry traits (N, P, K, N:P ratio) of *H. tibetana* and the climate on eastern Tibetan Plateau; (2) Whether the theory of the leaf economics spectrum could be supported by the relationship between leaf traits of *H. tibetana* and the climate; (3) Whether the environmental factors of the Tibetan Plateau can exert strong evolutionary pressure on leaf traits at the intraspecific levels.

## MATERIALS AND METHODS

**Study area:** The study area was located on the Tibetan Plateau. Twenty-five natural populations of *H. tibetana* were investigated spanning a wide geographical range of 29°-38°N in latitude, 88° to 103°E in longitude, and 2869 to 4860m a.s.l. in altitude (Fig. 1). Climate variation in the distribution area of *H. tibetana* is represented by a MAT range of -5.87 to 4.53°C, and a MAP range of 222 to 766 mm. Within the distribution area, MAP is positively correlated with MAT ( $r^2 = 0.514$ ,  $p < 0.0001$ ,  $n = 25$ ).

**Sampling and measurement:** Leaf samples were collected according to the protocol of Cornelissen et al. (2003). Every 15 fully expanded sun leaves in the east, south, west and north directions were collected from each natural population during the plant growing season (August-September 2010) and were mixed together to represent the leaves of the population. On the day of collection, the leaf area of 45 leaves of each population was measured immediately with a portable leaf-area meter (AM200; ADC Bioscientific Limited, Herts, UK). The plant samples were dried for 48 h at 70°C to a constant weight. Their dry masses were measured on a semi-analytical balance. LMA was determined by the ratio of dry mass and leaf area. Then the dry leaf was ground with a porcelain mortar into a uniformly fine powder and sieved through a 100-mesh screen before chemical analysis. For N, P and K determination, the samples were digested in a solution of  $H_2SO_4-H_2O_2$ , and then N concentrations were meas-

ured using a fully automatic azotometer (UDK159, VELP, Italy), P concentrations were measured colorimetrically by molybdenum blue spectrophotometry (6505 UV spectrophotometer, Jenway, Staffordshire, UK) and K concentrations were measured by flame photometer (ZL-5100 atomic absorption spectrophotometer, USA). All the chemical determinations were repeated three times with the same samples.

**Climatic data:** For each sample site, monthly mean temperature and precipitation values (means over 1950-2000) were extracted from a global climate database according to the geographical coordinates of each sample site. MAT and MAP were calculated using these monthly climate data.

**Data analysis:** The normality of the distribution in values of the measured plant trait variables (leaf N, P, K, N:P ratio, LMA and LL/LW) was tested using the Kolmogorov-Smirnov test. A regression analysis was conducted to quantify the relationship between leaf trait variables. As MAP is positively correlated with MAT within the distribution area, the collinearity tests were used to detect the presence of a collinearity relationship between the leaf trait variables and climate (MAP, MAT). A stepwise regression method was then conducted to quantify the relationship between the leaf

trait variables and the climate.

A principal component analysis (PCA) was completed to comprehensively define the relationships between leaf trait variables and the interrelated changes in altitude and climate. The figures and statistical analysis were completed with Sigma Plot (Systat Software Inc. 2005) and SPSS (SPSS Inc. 2008).

## RESULTS

**Leaf traits of *H. tibetana* on eastern Tibetan Plateau:** Leaf nitrogen, P and K concentrations of *H. tibetana* averaged 28.41 mg/g, 1.66 mg/g, and 19.15 mg/g, respectively; and their variations synchronized (positively correlated) across the region (Fig. 2a, b, c, Table 1). The correlation coefficient between leaf N and P ( $r^2 = 0.324$ ,  $p = 0.003$ ) was much higher than that between leaf N and K ( $r^2 = 0.174$ ,  $p = 0.038$ ) and that between leaf P and K ( $r^2 = 0.320$ ,  $p = 0.003$ ). Leaf N:P ratio averaged 17.85, and it was negatively correlated with leaf P and K (Fig. 2f,g Table 1). LMA was 94.20 g/m<sup>2</sup> on average (range: 127.41 to 77.12 g/m<sup>2</sup>), decreasing with the increasing leaf N (Fig. 2d, Table 1). Leaf LL/LW ratio was 5.04 on average and it was positively correlated with leaf P (Fig. 2e, Table 1).

**Variation in leaf traits with climatic variables:** Leaf N and P increased, while LMA and N:P ratio decreased with the increase of MAT (Table 2). Leaf K and Leaf LL/LW ratio increased with the increase of MAP (Table 2).

**Integrative relationships among leaf N, P, K, N:P ratio, LMA, LL/LW ratio, altitude, and climatic factors MAT and MAP:** Relationships among the leaf nutrient concentrations (N, P, K and N:P), altitude, and climatic factors (MAT and MAP) were tested with principle component analysis (PCA). The loadings of all the variables in the PCA explain their interrelated changes (Fig. 3). The first principal component (PC1) explained 50.4% of the variance. It showed that *H. tibetana* located at high altitude region, where LMA was high and MAP, MAT and LL/LW were low (left side of PC1 in Fig. 3), had low leaf N concentration. In contrast, *H. tibetana* located at low altitude regions, where LMA was low and MAT, MAP, and LL/LW were high (right side along PC1), had high leaf N concentration. These results indicated that plant N (or N-fixation by *Frankia*) was favoured by habitats at low altitude or high MAT. The second principal component explained 16.7% of the total variance and showed that changes in leaf N:P ratio was determined by leaf P and K.

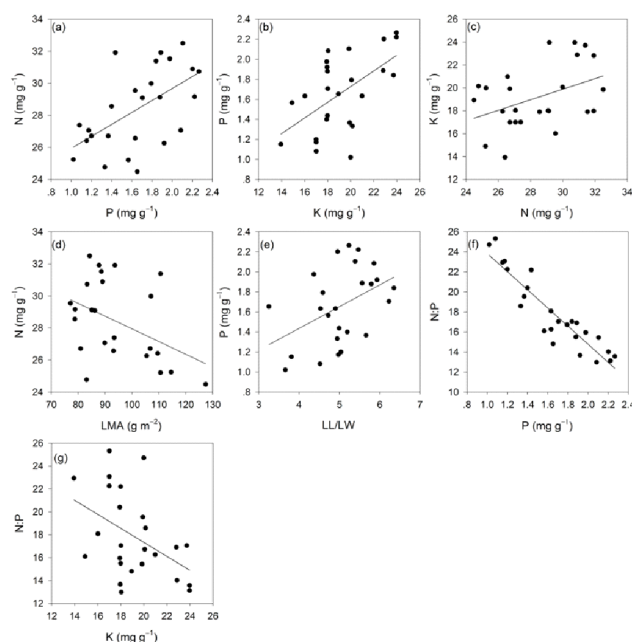


Fig. 2: Leaf trait relationships of *Hippophae tibetana*.

The lines are the significant regression between two variables with  $p < 0.05$ . (a) leaf P and N ( $r^2 = 0.324$ ,  $p = 0.003$ ,  $n = 25$ ); (b) leaf K and P ( $r^2 = 0.320$ ,  $p = 0.003$ ,  $n = 25$ ); (c) leaf N and K ( $r^2 = 0.174$ ,  $p = 0.038$ ,  $n = 25$ ); (d) leaf LMA and N ( $r^2 = 0.184$ ,  $p = 0.032$ ,  $n = 25$ ); (e) leaf LL/LW and P ( $r^2 = 0.197$ ,  $p < 0.028$ ,  $n = 25$ ); (f) leaf P and N:P ( $r^2 = 0.874$ ,  $p < 0.0001$ ,  $n = 25$ ); (g) leaf K and N:P ( $r^2 = 0.202$ ,  $p = 0.024$ ,  $n = 25$ ).

## DISCUSSION AND CONCLUSION

**Leaf traits of *H. tibetana* on eastern Tibetan Plateau:** The relationships among leaf N, P, and K of *H. tibetana* on local scale were similar to that on global scale (Wright et al. 2004a,

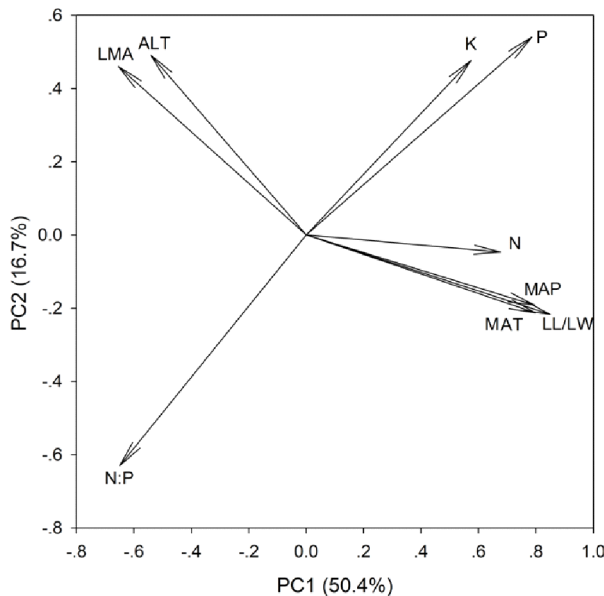


Fig. 3: Loadings of plant leaf traits (N, P, K, N:P, LMA, LL/LW), altitude (ALT), and climatic factors (MAT, MAP) in a principal component analysis of the sampling sites. The first and second principal components, PC1 and PC2, explain 50.4% and 16.7% of the variance in the data respectively. ALT - altitude, MAP - mean annual precipitation, MAT - mean annual temperature, N - leaf N, P - leaf P, K - leaf K, N:P - leaf N:P ratio, LMA - leaf mass per area, LL/LW - leaf length/leaf width ratio, PC1 - first principal component, PC2 - second principal component.

He et al. 2006). Leaf N, P and K were highly and positively correlated with one another, which showed that they were coordinate elements. The higher correlation between leaf N and P than that of leaf N and K, and that of leaf P and K suggested that N has different functions or development origins.

The magnitude of  $N_2$  fixed by *H. tibetana* (a N-fixing plant), can be limited by the availability of P in terrestrial ecosystems (Hungate et al. 2004). An N:P ratio of *H. tibetana* > 16 indicates P limitation at a species level (Wassen et al. 1995, Koerselman & Meuleman 1996).

Out of our expectations, leaf K significantly influenced N:P ratio of *H. tibetana*. Potassium plays an important role as an activator of many enzymes in the process of photosynthesis and respiration; K is also an important contributor to the osmotic potential of cell and in stomatal control (Sailsbury & Ross 1991). It suggested that the growth of *H. tibetana* can be limited by P and K. Similar results were reported that the growth of plants in herbaceous wetlands can be limited by N and P as well as K (Verhoeven et al. 1996, Olde Ven-terink et al. 2001, Olde Venterink et al. 2003).

**Variations in leaf traits along an environmental gradient:** The environment condition on the Tibetan Plateau was harsh and characterized by low MAT, low air pressure, high

wind speed, and high UV-B radiation (Sun & Zheng 1998). Environmental factors can exert strong evolutionary pressure on plant physiology. Low MAT is the key limited factor of plant growth on the Tibetan Plateau. The first principal component indicated that leaf N of *H. tibetana* (or N-fixation by *Frankia*) was favoured by habitats at low altitude or high MAT (Fig. 3). Leaf N and P concentrations in *H. tibetana* were positively corrected with MAT. These relationships did not support the temperature-plant physiology hypothesis (TPPH). According to which leaf N and P concentrations should increase and can compensate for the low efficiency of metabolic rate at low temperature (Friend & Woodward 1990, Reich & Oleksyn 2004). *Hippophae tibetana* can take up N, from both, soil and air through biological N fixation (Lian et al. 2000). Plant N-fixation is affected markedly by soil temperatures (Sprent 1979, Reddell et al. 1985, Danso et al. 1992). This view is supported by the observation of Zhang et al. (2010), which reported that the size of *H. tibetana* root nodules decreases significantly with increasing altitude. Therefore, leaf N was positively correlated with MAT, which is also consistent with that of the flora of the Tibetan Plateau reported by He et al. (2006) and that of Chinese sea buckthorn (*Hippophae rhamnoides* L. subsp. *sinensis* Rousi) among *Hippophae* L. by Li et al. (2014).

The leaf N:P ratio of *H. tibetana* increased significantly with decreasing MAT, unlike the pattern of leaf N:P ratio changing with MAT in other plant species reported by Reich & Oleksyn (2004) and Yang et al. (2007) at global and regional level, respectively. Our results showed that leaf N:P ratio was determined largely by leaf P.

Though the N-MAT relationship did not support the TPPH, the N-LMA relationship is consistent with the leaf economics spectrum (Wright et al. 2004a, He et al. 2006). Leaf mass per area (LMA) is related to the plant carbon assimilation capacity. Species that occur in cold and high UV-B radiation regions build thick small blades and led to high LMA (Schulze et al. 1998, Fonseca et al. 2000, Niinemets 2001, Wright 2001, 2004b, 2005a). These traits are beneficial to postpone leaf function and maintain appropriate leaf temperature and high photosynthetic rate (Givnish & Vermeij 1976, Dolph & Dilcher 1980, Cunningham et al. 1999, GregoryWodzicki 2000, Guo et al. 2013). But this limits photosynthetic capacity and leads to low N and P due to leaves with high mass per area having longer diffusion paths from stomata to chloroplasts or greater internal shading of lower chloroplasts (Parkhurst 1994, Green & Kruger 2001, Wright 2004a, He et al. 2006). Our results suggested that LMA were negatively correlated with MAT. The first principal component showed that the populations of *H. tibetana* in high MAT or low altitude area have low LMA, high leaf

Table 1: The N, P and K, LAM, LL/LW, and N:P ratio in the leaf of *H. tibetana* in the Tibetan Plateau.

	N (mg/g)	P (mg/g)	K (mg/g)	N:P ratio	LMA (g/m <sup>2</sup> )	LL/LW
Arithmetic mean	28.41	1.66	19.15	17.85	94.20	5.04
Geometric mean	28.31	1.62	18.97	17.50	93.33	4.98
CV (%)	8.74	22.81	14.31	20.91	14.23	15.24
Maximum	32.50	2.27	23.96	25.33	127.41	6.35
Minimum	24.49	1.02	13.93	12.98	77.12	3.25
P (K-S) test	0.66	0.99	0.41	0.36	0.23	0.92
Number of samples	25	25	25	25	25	25

CV, coefficient of variation; P (K-S), Kolmogorov-Smirnov test. N - leaf nitrogen, P - leaf phosphorus, K - leaf potassium, LMA - leaf mass per area, LL/LW - leaf length/leaf width ratio, N:P - leaf N:P ratio.

Table 2: Stepwise regression analyses between leaf traits and climate factor.

Leaf traits	Stepwise regression equations	Multiple R	P
N (mg/g)	$N = 0.378MAT + 28.081$	0.436	0.030
P (mg/g)	$P = 0.070MAT + 1.601$	0.520	0.008
K (mg/g)	$K = 0.006MAP + 15.774$	0.400	0.047
N:P ratio	$N:P = -0.618MAT + 18.381$	0.463	0.020
LMA (g/m <sup>2</sup> )	$LMA = -2.948MAT + 96.707$	0.615	0.001
LL/LW	$LL/LW = 0.004MAP + 3.183$	0.785	0.000

K-leaf K, MAP-mean annual precipitation, MAT-mean annual temperature, N-leaf N, N:P-leaf N:P ratio, P-leaf P, LMA-leaf mass per area, LL/LW-leaf length/leaf width ratio. Significant relationships were at  $P < 0.05$ .

N contents. In contrast, populations of *H. tibetana* in low MAT or high altitude areas have high LMA, low leaf N concentrations.

Leaf shape is one of the most diverse traits in plant species, and the variation in leaf shape is likely to have evolutionary and ecological consequences (Nicotra et al. 2011). Leaf length/width ratio is usually used to quantify leaf shape. Leaf shape is closely related to water stress (Jacobs 1999, Zhang et al. 2012). In order to adapt to drought, *H. tibetana* built thick leaf blade, small and thick-walled cells to continue leaf function under very dry conditions. The LL/LW ratio of *H. tibetana* was positively correlated with MAP, which suggested leaf shape of *H. tibetana* was sensitive to MAP.

In conclusion, the relationships between leaf stoichiometry traits and MAT in this study were inconsistent with those reported previously on the global scale. It showed that SNF plants have different relationships between leaf stoichiometry traits and MAT. The N-LMA relationship is consistent with the global and local pattern, providing additional support for the leaf economics spectrum theory at intraspecific level. Leaf traits of *H. tibetana* such as leaf N, P, K, LL/LW ratio and LMA, can be modified by the climate on the Tibetan Plateau. It suggested that the environmental factors on Tibetan Plateau can exert a strong evolutionary pressure on

leaf traits at the intraspecific level.

## ACKNOWLEDGEMENTS

Authors would like to thank Dahua Yu for technical assistance in analysis of plant samples. This work was financially supported by National Natural Science Foundation of China (NSFC, No. 31160046 and 31270429).

## REFERENCES

- Cécile, H.A., Wilfried, T., Nigel, G.Y., Rolland, D., Serge, A. and Sandra, L. 2010. A multi-trait approach reveals the structure and the relative importance of intra-vs. interspecific variability in plant traits. *Functional Ecology*, 24: 1192-1201.
- Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., Ter Steege, H., Morgan, H.D., Van Der Heijden, M.G. A., Pausas, J.G. and Poorter, H. 2003. A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, 51: 335-380.
- Cunningham, S.A., Summerhayes, B. and Westoby, M. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecol. Monogr.*, 69(4): 569-588.
- Danso, S.K.A., Bowen, G.D. and Sanginga, N. 1992. Biological nitrogen fixation in trees in agro-ecosystems. *Plant and Soil*, 141: 177-196.
- Diemer, M. 1998. Leaf lifespans of high-elevation, a seasonal andean shrub species in relation to leaf traits and leaf habit. *Global Ecol. Biogeogr. Lett.*, 7: 457-65.
- Dolph, G.E. and Dilcher, D.L. 1980. Variation in leaf size with respect to climate in Costa Rica. *Biotropica*, pp. 91-99.
- Fahn, A. and Cutler, D.F. 1992. *Xerophytes*. Berlin. Gebrüder Borntraeger.
- Field, C. and Mooney, H.A. 1986. *On the Economy of Plant Form and Function* (ed. Givnish, T. J.). Cambridge Univ. Press, Cambridge, pp. 25-55.
- Fonseca, C.R., Overton, J.M., Collins, B. and Westoby, M. 2000. Shifts in trait combinations along rainfall and phosphorus gradients. *J. Ecol.*, 88: 964-977.
- Friend, A. D. and Woodward, F. I. 1990. Evolutionary and ecophysiological responses of mountain plants to the growing environment. *Advances in Ecological Research*, 20: 59-124.
- Givnish, T.J. and Vermeij, G.J. 1976. Sizes and shapes of liane leaves. *Am. Nat.*, 110(975): 743-778.
- Green, D.S. and Kruger, E.L. 2001. Light-mediated constraints on leaf function correlate with leaf structure among deciduous and evergreen tree species. *Tree Physiol.*, 21: 1341-1346.
- Gregory-Wodzicki, K.M. 2000. Relationships between leaf morphology and climate, Bolivia: implications for estimating paleoclimate from fossil floras. *Paleobiology*, 26(4): 668-688.

- Grime, J.P. 2001. *Plant Strategies, Vegetation Processes and Ecosystem Properties*. 2nd ed., Chichester: Wiley.
- Guo, X., Guo, W., Luo, Y., Tan, X., Du, N. and Wang, R. 2013. Morphological and biomass characteristic acclimation of trident maple (*Acer buergerianum* Miq.) in response to light and water stress. *Acta Physiologiae Plantarum*, 35(4): 1149-1159.
- He, J.S., Wang, Z.H., Wang, X.P., Schmid, B., Zuo, W.Y., Zhou, M., Zheng, C.Y., Wang, M.F. and Fang, J.Y. 2006. A test of the generality of leaf trait relationships on the Tibetan Plateau. *New Phytologist*, 170: 835-848.
- Hungate, B.A., Stiling, P.D., Dijkstra, P., Johnson, D.W., Ketterer, M.E., Hymus, G.J., Hinkle, C.R. and Drake, B.G. 2004. CO<sub>2</sub> elicits long-term decline in nitrogen fixation. *Science*, 304: 1291.
- Jacobs, B.F. 1999. Estimation of rainfall variables from leaf characters in tropical Africa-Palaeogeogr. *Palaeoclimat. Palaeoecol.*, 145: 231-250.
- Koerselman, W. and Meuleman, A.F.M. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *J. Appl. Ecol.*, 33: 1441-1450.
- Lambers, H. and Poorter, H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research*, 23: 187-261.
- Li, X.W., Sun, K. and Li, Y.H. 2014. Variation in leaf nitrogen and phosphorus stoichiometry in the nitrogen-fixing Chinese sea-buckthorn (*Hippophae rhamnoides* L. subsp. *sinensis* Rousi) across northern China. *Ecol. Res.*, 29(4): 723-731.
- Lian, Y.S. 2000. *Plant Biology and Chemistry of the Genus Hippophae L.* Gansu Science and Technology Press, Lanzhou (in Chinese).
- Liu, X.D. and Chen, B.D. 2000. Climatic warming in the Tibetan Plateau during recent decades. *Int. J. Climatol.*, 20: 1729-1742.
- McIntyre, S., Lavorel, S., Landsber, J. and Forbes T.D.A. 1999. Disturbance response in vegetation-towards a global perspective on functional traits. *Journal Vegetation Science*, 10(5): 621-630
- Nicotra, A.B., Leigh, A., Boyce, C.K., Jones, C.S., Niklas, K.J., Royer, D.L. and Tsukaya, H. 2011. The evolution and functional significance of leaf shape in the angiosperms. *Functional Plant Biology*, 38: 535-552.
- Niinemets, U. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, 82: 453-469.
- Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Warton, D.I. and Westoby, M. 2005. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography*, 14: 411-421.
- Olde Venterink, H., Van der Vliet, R.E. and Wassen, M.J. 2001. Nutrient limitation along a productivity gradient in wet meadows. *Plant and Soil*, 234:171-179.
- Olde Venterink, H., Wassen, M.J., Verkroost, A.W.M. and De Ruiter, P.C. 2003. Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. *Ecology*, 84(8): 2191-2199.
- Osnas, J.L.D., Lichstein, J.W., Reich, P.B. and Pacala, S.W. 2013. Global leaf trait relationships: mass, area, and the leaf economics spectrum. *Science*, 340: 741-744.
- Parkhurst, D. F. 1994. Diffusion of CO<sub>2</sub> and other gases inside leaves. *New Phytol.*, 126: 449-479.
- Qi, J., Ma, K.M. and Zhang, Y.X. 2009. Leaf-trait relationships of *Quercus liaotungensis* along an altitudinal gradient in Dongling Mountain, Beijing. *Ecological Research*, 24:1243-1250.
- Reddell, P., Bowen, G.D. and Robson, A.D. 1985. The effects of soil temperature on plant growth, nodulation and nitrogen fixation in *Casuarina cunninghamiana*. *New Phytologist*, 101: 441-450.
- Reich, P.B. and Oleksyn, J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences, USA*, 101: 11001-11006.
- Reich, P.B., Walters, M.B. and Ellsworth, D.S. 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences, USA*, 94:13730-13734.
- Sailsbury, F.B. and Ross, C.W. 1991. *Plant Physiology*. 4th edn, Washington Publishing, Belmont.
- Schulze, E.D., Williams, R.J., Farquhar, G.D., Schulze, W., Langridge, J., Miller, J.M. and Walker, B.H. 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. *Aust. J. Plant Physiol.*, 25: 413-425.
- Sprent, J. I. 1979. *The Biology of Nitrogen Fixing Organisms*. McGraw-Hill, London, pp. 196.
- Sun, H.L. and Zheng, D. 1998. Formation, evolution and development of the Qinghai-Xizang (Tibetan) Plateau. Guangzhou, China: Guangdong Science and Technology Press (in Chinese).
- Verhoeven, J.T.A., Koerselman, W. and Meuleman, A.F.M. 1996. Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. *Trends in Ecology and Evolution*, 11: 494-497.
- Wassen, M.J., Olde Venterink, H.G.M. and De Swart, E.O.A.M. 1995. Nutrient concentrations in mire vegetation as a measure of nutrient limitation in mire ecosystems. *Journal of Vegetation Science*, 6: 5-16.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. and Wright, I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, 33: 125-159.
- Wright, I.J., Groom, P.K., Lamont, B.B., Poot, P., Prior, L.D., Reich, P.B., Schulze, E.D., Veneklaas, E.J. and Westoby, M. 2004a. Leaf trait relationships in Australian plant species. *Functional Plant Biology*, 31: 551-558.
- Wright, I.J., Reich P.B., Cornelissen, J.H.C., Falster, D.S., Garnier, E., Hikosaka, K., Lamont, B.B., Lee, W., Oleksyn, J., Osada, N., Poorter, H., Villar, R., Warton, D.I. and Westoby, M. 2005a. Assessing the generality of global leaf trait relationships. *New Phytologist*, 166: 485-496.
- Wright, I.J., Reich P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee T., Lee, W.J., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. and Villar, R. 2004b. The worldwide leaf economics spectrum. *Nature*, 428: 821-827.
- Wright, I.J., Reich, P. B., Cornelissen, J.H.C., Falster, D.S., Groom, P. K., Hikosaka, K., Lee, W., Lusk, C.H., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Warton, D.I. and Westoby M. 2005b. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography*, 14: 411-421.
- Wright, I.J., Reich, P.B. and Westoby, M. 2001. Strategy-shifts in leaf physiology, structure and nutrient content between species of high and low rainfall, and high and low nutrient habitats. *Function Ecology*, 15: 423-434.
- Yang, K., Huang, J. H., Dong, D., Ma, W. H., He, J. S. 2010. Canopy leaf N and P stoichiometry in grassland communities of Qinghai-Tibetan Plateau, China. *Chinese Journal of Plant Ecology*, 34, 17-22.
- Yang, X.G., Zhang, T.J., Qin, D.H., Kang, S.C. and Qin, X. 2011. Characteristics and changes in air temperature and glacier's response on the north slope of Mt. Qomolangma (Mt. Everest). *Arctic, Antarctic and Alpine Research*, 43(1): 147-160.
- Zhang, A.M., Niu, S.Q., Sun, K., Cao, W.X. and Da, W.Y. 2010. Study on the root nodules features of *Hippophae* and *Frankia* isolation. *Grassland and Turf*, 30:43-46.
- Zhang, Y.X., Equiza, M.A., Zheng, Q. and Melvin, T.T. 2012. Factors controlling plasticity of leaf morphology in *Robinia pseudoacacia* L. II: the impact of water stress on leaf morphology of seedlings grown in a controlled environment chamber. *Ann. Forest Sci.*, 69: 39-47.