Ecological stoichiometry of plant leaves, litter and soils in a secondary forest on China's Loess Plateau (#43600)

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Ecological stoichiometry of plant leaves, litter and soils in a secondary forest on China's Loess Plateau

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Ecological stoichiometry can reveal nutrient cycles in soil and plant ecosystems and their interactions. However, the ecological stoichiometry characteristics of leaf-litter-soil system s of dominant grasses, shrubs and trees are still unclear as are their intrinsic relationship s during vegetation restoration. This study selected three dominant plant types of grasses (Imperata cylindrica (I. cylindrica) and Artemisiasacrorum (A.sacrorum)), shrubs (Sophora viciifolia (S. viciifolia) and Hippophae rhamnoides (H. rhamnoides)) and trees (Quercus liaotungensis (Q. liaotungensis) and Betula platyphylla (B. platyphylla)) in secondary forest areas of the Chinese Loess Plateau to investigate ecological stoichiometric characteristics and their intrinsic relationships in leaf-litter-soil systems. The results indicated that N concentration and N:P ratios in leaf and litter were highest in shrubland; leaf P concentration in grassland was highest and litter in forestland had the highest P concentration. Soil C, N and P concentrations were highest in forestland (P<0.05) and declined with soil depth. Based on the theory that leaf N:P ratio indicates nutritional limitation for plant growth, this study concluded that grass and shrub growth was limited by N and P element, respectively, and forest growth was limited by both of N and P elements. The relationships between the N concentration in soil, leaf and litter was not significant (P>0.5), but the soil P concentration was significantly correlated with litter P concentration (P<0.05). These finding enhance understandings of nutrient limitations in different plant communities during vegetation restoration and provide insights for better management of restoration.

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2 on China's Loess Plateau

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ABSTRACT

13	Ecological stoichiometry can reveal nutrient cycles in soil and plant ecosystems and their
14	interactions. However, the ecological stoichiometry characteristics of leaf-litter-soil systems of
15	dominant grasses, shrubs and trees are still unclear as are their intrinsic relationships during
16	vegetation restoration. This study selected three dominant plant types of grasses (Imperata
17	cylindrica (I. cylindrica) and Artemisia sacrorum (A. sacrorum)), shrubs (Sophora viciifolia (S.
18	viciifolia) and Hippophae rhamnoides (H. rhamnoides)) and trees (Quercus liaotungensis (Q.
19	liaotungensis) and Betula platyphylla (B. platyphylla)) in secondary forest areas of the Chinese
20	Loess Plateau to investigate ecological stoichiometric characteristics and their intrinsic
21	relationships in leaf-litter-soil systems. The results indicated that N concentration and N:P ratios
22	in leaf and litter were highest in shrubland; leaf P concentration in grassland was highest and
23	litter in forestland had the highest P concentration. Soil C, N and P concentrations were highest
24	in forestland (P<0.05) and declined with soil depth. Based on the theory that leaf N:P ratio
25	indicates nutritional limitation for plant growth, this study concluded that grass and shrub growth
26	was limited by N and P element, respectively, and forest growth was limited by both of N and P
27	elements. The relationships between the N concentration in soil, leaf and litter was not
28	significant (<i>P</i> >0.5), but the soil P concentration was significantly correlated with litter P
29	concentration (P <0.05). These finding enhance understandings of nutrient limitations in different
30	plant communities during vegetation restoration and provide insights for better management of
31	restoration.
32	
33	Key words: Plant community type; Leaf-litter-soil; Ecological stoichiometry; Nutrient elements;
34	Nutrient limitation
35	Abbreviations: Quercus liaotungensis: Q. liaotungensis; Betula platyphylla: B. platyphylla;
36	Sophora viciifolia: S. viciifolia; Hippophae rhamnoides: H. rhamnoides; Imperata
37	cylindrica: I. cylindrica; Artemisia sacrorum: A. sacrorum; Organic carbon: C; Total
38	nitrogen: N; Total phosphorus: P



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INTRODUCTION

42	ecosystem degradation (Luque et al., 2013). Soil nutrient losses greatly decreased soil quality
43	(Liu & Zhao, 1993), which seriously threatens the stability of ecosystems. Vegetation restoration
44	is a powerful approach for ecological restoration of degraded lands, as it can control soil erosion
45	and improve ecosystem functions and services (Godefroid et al., 2003; Zheng, 2006; Jiao et al.,
46	2012; Sauer et al., 2012; Zhao et al., 2015; Bienes et al., 2016). Vegetation restoration areas
47	currently cover approximately 0.20 billion ha worldwide and are being planted at a rate of 4.5
48	million ha per year (Zhao et al. 2015). Over time, vegetation restoration can improve soil quality
49	(Fu et al., 2010), and accelerate N and P cycling in plants and soils (Lü et al., 2012). Vegetation
50	restoration can affect the plant community composition, species diversity (Wang et al., 2011) and
51	form a diverse ecosystem of trees, shrubs, and herbs, which results in changes in nutrients
52	distribution in leaves, litter and soil (Parfitt, Yeates & Ross, 2005; Hobbie et al., 2006; John et
53	al., 2007; Jiao et al., 2013; Zhao et al., 2017). Several plant communities show significant
54	differences in nutrient allocation due to different plant species throughout vegetation restoration
55	(Warren & Zou, 2002; Schreeg et al., 2014; Deng et al., 2016). Therefore, it is necessary to
56	quality nutrient characteristics in the leaf-litter-soil system of dominant grasses, shrubs and trees,
57	as well as their intrinsic relationships during vegetation restoration.
58	Ecological stoichiometry describes the balance of energy and multiple chemical elements in
59	ecosystems (Elser et al., 2000), and has gradually become a method for studying the stability and
60	N/P limitations of degraded ecosystems (Güsewell, 2004; Han et al., 2005). Ecological
61	stoichiometry is also an effective tool to study the interactions between soils and plant, and their
62	nutrient cycles (<i>Elser</i> , 2006). C, N, and P cycles account for the transfer of nutrients between

Soil erosion remains a major global environmental problem, accelerating soil nutrient losses and

66 three nutrients interact with each other, and both N and P affect carbon fixation (*Han et al.*,

plant and soil. C is a key building block of structural substances, approximately supplying 50%

ecosystems and play important roles in several physiological and metabolic processes. These

of the dry biomass, whereas N and P are the major limiting elements of natural terrestrial



2005). The notion that leaf N:P ratio can be used to identify nutrient limitations for plant growth 67 has been widely confirmed in various plant communities (Koerselman & Meuleman, 1996; 68 Schreeg et al., 2014). The N:P ratio of plant leaves can be used to characterize the productivity 69 70 of terrestrial ecosystems, and it can also indicate which elements of the plant are limited, but this 71 relationship can change with changes in the environment (Güsewell, 2004). Thus, it provides a scientific basis for the rational allocation of vegetation to investigate nutrient limitation of N:P 72 73 ratio in the process of vegetation restoration. 74 Soil properties have a major influence on the structural and functional composition of a plant community, as well as on its succession and species diversity (Fu et al., 2010; Wang et al., 75 2011). Plants help to maintain stability of ecosystem elements. For example, carbon is fixed 76 77 through photosynthesis, and nutrients are gradually returned to the soil in the form of the litter 78 and root exudates. In the plant-soil ecosystem, litter serves as a main carrier of nutrients, linking plants and soil (Agren & Bosatte, 1998). The litter layer provides storage for ecosystems 79 nutrients and acts as a hub for material exchange between soil and plants, and is a natural source 80 of soil fertility (Agren et al., 2013). Nutrient supply in soil, plant growth demand, and litter 81 82 return to soil are nominally independent factors, but they also interact with each other, which leads to the complex relationships among nutrient concentrations in the plant-litter-soil systems 83 (Agren & Bosatte, 1998). Ecological stoichiometry provides an effective approach for observing 84 85 these relationships between nutrients in the plant-litter-soil systems and their characteristics in 86 ecological processes (*Elser et al.*, 2000). Thus, it is of theoretical and practical significance to analyze the ecological stoichiometric characteristics of leaf-litter-soil systems. 87 Due to its steep topography and erodible soil, coupled with long-term human activity, the 88 89 ecological environment of the Loess Plateau is extremely fragile, and has become one of the most severely eroded areas of China (Jiao et al., 2012; Zhao et al., 2015). In past centuries, the 90 91 majority of forestlands were destroyed to satisfy the food needs of the growing population, which resulted in severe soil erosion and land degradation. The Grain to Green Program (GTGP) 92 93 was implemented to control soil erosion and improve ecosystem degradation, with a main goal of



94	converting low-yield steep-slope cropiands into permanent vegetation cover (<i>stao et al.</i> , 2012;
95	Zhao et al., 2015). Vegetation restoration generated a diverse flora and reduced soil erosion,
96	raising interest in the characterization of this recovering ecosystem. For example, $An &$
97	Shangguan (2010) and Chai et al. (2015) studied leaf stoichiometric traits and concluded that the
98	growth of vegetation was N-limited at each secondary successional stage, according to the leaf
99	N:P threshold. Ai et al. (2017) observed that the slope aspect had various effects on plant and soil
100	C:N:P stoichiometry. Variations in vegetation types influenced soil C:N:P ratios, which were
101	higher in afforested lands than in slope croplands (Zhao et al., 2015; Deng et al., 2016; Zhao et
102	al., 2017). Jiao et al. (2013) studied soil stoichiometry during vegetation successional changes
103	and reported that soil N:P ratio increased with the vegetation restoration year. It was even
104	reported that forest age had a significant effect on C, N, P and K concentrations and their ratios
105	in plant tissues and soil (Li et al., 2013). Most previous studies addressed the stoichiometric
106	characteristics of soil system and vegetation communities, including forests and grasslands, as
107	well as litter individually or in both. However, the ecological stoichiometry of the plant-litter-soil
108	system as a whole has been rarely described (Zeng et al., 2017; Cao & Chen, 2017), and the
109	effects of dominant plant communities (tree, shrub, grass) during vegetation restoration on this
110	ecological stoichiometry remains poorly understood. Therefore, it is necessary to study such
111	stoichiometry characteristics and their relationships in leaf- litter- soil systems of dominant grass,
112	shrub and tree during vegetation restoration. This will provide a better understanding of nutrient
113	limitation in different plant communities during vegetation restoration and improve ecosystem
114	management. In addition, the majority of previous studies have focused on topsoil (Jiao et al.,
115	2013; Li et al., 2013; Zeng et al., 2016; Zeng et al., 2017), there is little information on
116	stoichiometry change with the soil profile (Zhao et al., 2015, Deng et al., 2016). Due to the depth
117	of thick loess on the Loess Plateau, the majority plant roots are distributed within the top 100 cm.
118	Therefore, it is important to investigate change of the stoichiometry of C, N and P with soil
119	profile depth.
120	Three dominant plant communities of grasses (Imperata cylindrica and Artemisia sacrorum),



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121	shrubs (Sophora viciifolia and Hippophae rhamnoides) and trees (Quercus liaotungensis and
122	Betula platyphylla) were selected in the Ziwuling secondary area of the Loess Plateau to
123	investigate ecological stoichiometry in the plant-litter-soil system and their intrinsic
124	relationships. The specific objectives of this study were to 1) determine leaf and litter C, N and P
125	concentrations and their ecological stoichiometry characteristics in six dominant plant species; 2)
126	investigate distributions of soil C, N, and P concentrations and ecological stoichiometry
127	characteristics in soil profile; 3) examine the relationships of ecological stoichiometry in leaf-
128	litter-soil system (C, N, and P); and 4) assess the limiting nutrient element for plant growth in the
129	six plant species. The effort will provide information about ecological stoichiometry and
130	theoretical support for enhancing vegetation and ecosystem restoration on the Loess Plateau.
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132	MATERIAL AND METHODS
133	Study site description
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	This study site was located at Fuxian County, Shanxi Province, China (35°5.4′ N, 109°8.9′ E), in
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are *Quercus liaotungensis* (climax forest community) and *Betula platyphylla* (early forest

community); dominant species for shrub are Sophora viciifolia and Hippophae rhamnoides, both

does not concur in same places; and main grass species are *Imperata cylindrica* and

Artemisia sacrorum (Zheng, 2006). The distribution area of the above mentioned six dominant



species occupies more than 70% of total area in the study site.

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Soil and plant sampling

According to our field investigation, there are 38 species in the study site, including 18 artificial species and 20 natural species, which cover five tree species, six shrub species, nine grass species. Moreover, the six tree, shrub, and grass species, i.e., *Quercus liaotungensis* and *Betula* platyphylla (forest communities), Sophora viciifolia and Hippophae rhamnoides (shrub communities) and Imperata cylindrica and Artemisia sacrorum (grass communities) are dominant species and their distribution area occupies more than 70% of total area in the study site. Other studies also reported that these six species are dominant species of natural vegetation succession (Zheng, 2006; Wang et al., 2010; Zhang and Shangguan 2016). Thus, these six species have been selected to investigate to ecological stoichiometry of plant leaf, litter and soil in a secondary forest on China's Loess Plateau. For each dominant species, three experimental sites (three replications) with a similar site condition including slope position (slope length, gradient and aspect), soil type and altitude were set up to collect samples. In addition, the distance within all experimental sites was within approximately 3 km, which reduced impacts of previous site condition. Plant leaves and soil samples were collected in late July 2016 when plants were in a vigorous growth period, and litter samples on the soil surface consisting of leaf fall over multiple years that were not decomposed were obtained in late October 2016. Table 1 shows the characteristics of these three plant types. Two plots with 10×10 m size were established in each experimental site of forest type, and the plots sizes for shrub and grass types were 5×5 m and 1×1 m, respectively. Ten to twenty complete expanded living and sun-exposed leaves were randomly collected from five to ten healthy individual plants per plot from shrubs or trees, and a total of 80 to 100 leave samples were collected. For each grass plot, all stems and leaves were completely cut from three 0.25 m² sampling areas. Leaves from each plot were evenly mixed and then put into a paper bag. Litter

samples were collected along the diagonal lines of three 1×1 m squares per plot, and mixed and



stored in paper bags. All samples of leaves and litter were carried back to the indoor laboratory for analysis.

Soil samples from a 100 cm-depth profile were collected using a 5-cm diameter to collect soil samples along an S-shaped line in each plot. Before each soil sample was collected, soil sampler was sterilized with ethanol to avoid cross-infection. Moreover, the 100 cm soil profile was divided into six layers (0-10, 10-20, 20-40, 40-60, 60-80, 80-100 cm), and soil samples from each layer were obtained from five points. The five soil samples of each layer were mixed evenly and stored in plastic bags, and then all soil samples (6 plant species × 3 experimental sites × 2 sample plots × 6 soil sample layers) were transported to the indoor laboratory.

Table 1

Sample analysis

Leaf and litter samples were oven dried at 70 °C for at least 48 h or more to reach a constant mass level, and then weighed. Dried plant samples were ground to a fine powder using a plant-sample mill (1093 Sample Mill, Foss, Sweden). Soil samples were air-dried and sieved using a 0.25 mm mesh. To determine C concentration in plant and soil, the Walkley-Black modified acid-dichromate FeSO₄ titration method was used (*Bao, 2000*), and the Kjeldahl method (KJELTE2300, Sweden) was applied to measure the total N concentration in plant and soil. The total P concentration in plant was measured by using a Spectrophotometer UV-2300 (Techcomp Com, Shanghai, China) after digestion with H₂SO₄ and H₂O₂, and the total P concentration in soil was determined by a spectrophotometer after wet digestion with H₂SO₄ and HClO₄ (*Bao, 2000*). Leaf, litter and soil C, N, P concentrations were expressed as g/kg on dry weight basis. The C:N:P ratios in leaves, litter and soil were computed as mass ratios.

Statistical analysis

All data are presented as mean \pm standard errors and tested for normality of distributions and homogeneity of variances before analysis. A one-way analysis of variance (ANOVA) was used to analyze the effects of the plant type on nutrients and stoichiometric characteristics in leaf,



litter and soil. Two-way ANOVAs were computed to analyze the effects of plant type, soil depth 202 and their interactions on soil C, N and P concentrations and their stoichiometry. 203 The linear regression analysis was used to test the relationship between C, N and P 204 205 concentrations in leaf, litter and soil. Pearson correlation was used to assess relationship between leaf, litter and soil stoichiometric characteristics. Differences were considered significant with a 206 P<0.05. All statistical analyses were determined with SPSS 19.0 software (SPSS, Inc., Chicago, 207 208 IL, USA). 209 **RESULTS** 210 Leaf and litter nutrients and ecological stoichiometry in dominant plant communities 211 The leaf C, N and P concentrations were different among plant communities (Table. 2). The C 212 concentration in leaf varied from 475 (grass) to 522 g/kg (forest), and was highest in B. 213 platyphylla and lowest in A. sacrorum. The leaf N concentration was 29.8 g/kg in shrub, and was 214 significantly greater than in forest and grass (P < 0.05), while the leaf P concentration with 1.80 215 g/kg was highest in grass. The leaf C:N ratio varied from 16.9 (shrub) to 47.3 (grass), and was 216 217 highest in I. cylindrica and lowest in H. rhamnoides. The leaf C:P ratio was significantly higher in O. liaotungensis and S. viciifolia than other species (P<0.05). The leaf N:P ratio varied from 218 6.12 (grass) to 22.6 (shrub) and was significantly higher in shrub than in grass and forest 219 (P < 0.05). 220 221 The C, N and P concentrations in litter were significantly affected by plant types (Table. 3). 222 The litter C concentration varied from 360 (shrub) to 413 (forest), and was significantly higher in forest than in grass and shrub (P<0.05). N concentrations showed a similar pattern between litter 223 and leaf, and were significantly highest in shrub (P<0.05). The litter P concentration varied from 224 0.51 (grass) to 0.97 g/kg (forest) and was highest in B. platyphylla and lowest in I. cylindrica. 225 The litter C:N and C:P ratios in grass were 52.9 and 735, respectively, and were significantly 226 higher than in forest and shrub (P<0.05). The litter N:P ratio varied from 12.5 (forest) to 24.2 227 (shrub), and was highest in H. rhamnoides and lowest in B. platyphylla (P < 0.05). 228



Table 2
Table 3
Soil nutrients and ecological stoichiometry in dominant plant communities and soil depths
Plant type and soil depth had significant effects on soil nutrients and their C:N:P ratios (Table. 4).
Soil C and N concentrations in forestland were greater than in shrubland and grassland at all soil
depths and both were highest in Q . liaotungensis and lowest in A . sacrorum ($P < 0.05$). Soil P
concentration in shrubland was lower than in grassland and forestland at every soil depth (P <0.05),
and there were no differences in B. platyphylla, Q. liaotungensis and A. sacrorum at 20-100 cm
soil depths. Soil C:N ratio in forestland was significantly higher than in shrubland and grassland
at both 0-10 and 10-20 cm soil depths (P <0.05), but there were no significant differences at 20-
100 cm soil depths (<i>P</i> >0.05). Soil C:P and N:P ratios in forestland was significantly higher than in
shrubland and grassland at both 0-10 and 10-20 cm soil depths (P<0.05), but both were highest in
shrubland at 20-100 cm soil depths (P <0.05).
The soil sampling depth significantly affected soil nutrients and their ratios (Table. 4 and Fig
1). Soil C and N concentrations significantly decreased with soil sampling depth. Soil C and N
concentrations decreased markedly from 10 to 40 cm of soil depth, and then slightly decreased
from 40 to 100 cm. Soil P concentration tended to stable with the soil sampling depth. Soil C:N
ratio fluctuated with depth, and soil C:P and N:P ratios had the same trend along the soil
sampling depth and decreased markedly from 10 to 40 cm of soil depth, and then slightly
decreased from 40 to 100 cm.
The results of the Two-way ANOVA analysis indicated that both plant type and soil depth
significantly affected the soil C, N and P concentrations and their stoichiometry (C:N, C:P and
N:P ratios). The interactions between plant type and soil depth significantly affected the soil C and
N concentrations and C:N, C:P and N:P ratios but not soil P concentration (Table 5).
Table 4



Table 5
Figure 1
Relationships between C, N and P concentrations and their characteristics of ecological
stoichiometry among leaf, litter and soil
There were significant correlations between leaf and litter for both N and P concentrations in
three plant community types (P <0.05) (Fig 2b, c). The relationships between the plant C
concentration and soil C concentration were significant in two soil layers (0-10 and 0-20) and the
profile (0-100 cm) (<i>P</i> <0.05) (Fig 3a, b, c and Fig 4a, b, c), while there were no significant
correlation between plant N concentration and soil N concentration (Fig 3d, e, f and Fig 4d, e, f).
In the three plant community types, there were no significant correlations between leaf P
concentration and soil P concentration (Fig 3g, h, i), but the soil P concentration was significant
correlated with litter P concentration in 0-10 cm soil depth (P<0.05) (Fig 3g).
For the three plant community types, leaf C:N and N:P ratios were positively correlated with
litter C:N and N:P ratios, respectively (P<0.05) (Fig 2d, f), while leaf C:P ratio was negatively
correlated with litter C:P ratio (P<0.05) (Fig 2e). Leaf C:P had a positive correlation with soil
C:P ratio at the 0-10 cm soil layer and over 0-100 cm soil profile (P<0.05) (Table 6), Leaf N:P
ratio had a positive correlation with soil N:P ratio at two soil layers (0-10 and 0-20 cm) and the
profile $(0-100 \text{ cm})(P<0.05)$ (Table 6); and there was significant correlation between leaf and soil
C:N ratio at the 0-10 and 0-20 cm soil layers (P <0.05) (Table 6). At the 0-10 cm soil layer, there
was a significant correlation between litter and soil C:N ratio (P<0.05) (Table 6), and litter C:P
ratios were negatively correlated with C:P ratios at two soil layers (0-10 and 0-20 cm) and the
profile (0-100 cm), and only in the profile (0-100 cm) did litter N:P ratio have a positive
correlation with soil N:P ratio (P < 0.05) (Table 6).
Figure 2
Figure 3



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281	Figure 4
282	Table 5

DISCUSSION

Impacts of dominant plant communities on leaf and litter nutrients and ecological

stoichiometry

As a key subsystem, plants have a vital function in governing the stability of terrestrial ecosystem. C, N and P are essential nutrients for plant (Han et al., 2005; John et al., 2007) and their interaction regulate plant growth (Güsewell, 2004). Litter is one main way for nutrients to return to the soil and is an important part of the forest ecosystem. The decomposition of plant litter replenishes soil nutrients to provide conditions for the adjustment and demand of the plant nutrients (Agren & Bosatte, 1998). There are differences in the types, quantity and utilization efficiency of absorbed nutrients in different plants types. In this study, the results indicated that leaf C, N and P concentrations differed across plant communities. The reason is that different plant communities has different adaptability to the environment, and possess different strategies of nutrient adaptation (Wright et al., 2004). In this study, leaf C concentration in forest species was significantly higher than in grass and shrub species while the leaf P in forest species was significantly lower than in grass species. An explanation may be that a large part of nutrients absorbed by trees is used to construct a protective structure, while grasses have a short life span, and high photosynthetic capacity and nutrient efficiency. The results are consistent with those of Wright et al. (2004), which reported that the leaf P concentration in herbaceous plants is significantly higher than in woody plants. Moreover, in this study, the C, N and P concentrations in plant leaves were higher than in the corresponding litter, which was consistent with previous studies (Pan et al., 2011; Zeng et al., 2017). Pan et al. (2011) showed that the C, N and P concentrations in the leaves of trees, shrubs and grasses were significantly higher than in litter, likely due to the reabsorption processes. Previous studies have shown that nutrients present in leaves are transferred to flowers, fruits, branches, and roots before leaf falling, thereby



307	preventing nutrients loss (Schreeg et al., 2014). The results showed that N and P concentrations
308	in litter varied greatly in different plant communities, and were significantly higher in trees than
309	in grasses. This is because tree and shrub are deep-rooted plants, and have the strong capability
310	of absorbing nutrients from multiple sources in the environment; while grasses have shallow
311	roots and rely more on the recycling of their own nutrients.
312	N and P elements are major limiting factors for plant growth in terrestrial ecosystems, and
313	the leaf N:P ratio could be used as an indicator to identify the limiting nutrient factors
314	(Koerselman & Meuleman, 1996; Güsewell, 2004). However, the threshold of N:P ratio is
315	affected by study area, plant growth stage and plant species (Güsewell, 2004). Güsewell (2004)
316	reported that a leaf N:P ratio between 10 and 20 was as a threshold to assess limitations in plant
317	growth. If the N:P ratio was > 20, plant growth was limited by P; if the N:P ratio was < 10, plant
318	growth was limited by N, and when the N:P ratio was between 10 and 20, plant growth was
319	limited by both P and N together. In this study, the leaf N:P ratio in S. viciifolia and H.
320	rhamnoides were 22.6 and 21.0, respectively, suggesting that shrub growth was P-limited. The
321	leaf N:P ratios in Q. liaotungensis and B. platyphylla were 14.3 and 12.9, respectively, indicating
322	that their growths were co-limited by both N and P. The leaf N:P ratios in A. sacrorum and I.
323	eylindrica were 6.12 and 9.93, respectively, indicating that grass growth was limited by N. The
324	results indicated that different plant communicates had different nutrient limiting elements,
325	which was consisted with previous studies (<i>Han et al., 2005</i>). The reason is that grass species (<i>I.</i>
326	cylindrica and A. sacrorum) is a shallow-rooted plant with a strong ability to absorb soil surface
327	nutrients, particularly it has a greater capacity of relocating its leaf P before leaf falling than
328	forest and shrub species, and it can more effectively utilize leaf P concentration to meet growth
329	demands. Moreover, the biochemistry of the grass organic structure determines that more
330	nitrogen is needed for growth. Therefore, grass species were less limited by P element than by N
331	element. In addition, the results indicated that the growth of shrub species was limited by P
332	element, which was similar to results reported by <i>Han et al.</i> (2005). This is because <i>S. viciifolia</i>
333	and <i>H. rhamnoides</i> are inherent species in vegetation restoration on the Loess Plateau and were





334	nitrogen-fixing plants, and the absorption on of N element is far greater than that of P element,
335	which results the shrub species to be limited by P element. Furthermore, the result showed that
336	leaf C:N and C:P ratios were lower than in litter, which is consistent with results reported by
337	McGroddy et al. (2004), indicating that the reabsorption capacity for C is lower than for N and P
338	Although leaf N:P ratio can effectively reflect N or P limitation, the importance of the N:P ratio
339	is mainly in its function as an indicator (Güsewell, 2004). If the leaf N:P ratio is to be used as an
340	index to evaluate both N and P nutrient supplies in the Loess Plateau, further diagnosis regarding
341	nutrient limitations should be conducted.
342	
343	Impacts of dominant plant communities on soil nutrient and ecological stoichiometry
344	Plants play an important role in improving soil fertility and contribute to the accumulation of soil
345	nutrients. Fu et al. (2010) found that vegetation restoration could improve the net fixation of C
346	and N and reduce their losses. However, the performance in soil quality recovery differed among
347	plant communities (Jiao et al., 2012; Zeng et al., 2016; Deng et al., 2016; Zhao et al., 2017). In
348	this study, soil C, N and P concentrations in forestland was greater than in grassland and
349	shrubland which is consistent with the previous results of <i>Jiao et al.</i> (2012) and <i>Qi et al.</i> (2015).
350	This result could be explained by a larger amounts of litter present in forestland, a more above-
351	ground litter and a higher volume of root exudates reaching the soil, resulting in higher nutrient
352	concentrations in the forestland than in other plant communities. Soil C and N concentrations
353	decreased with increasing of soil depth, while soil P concentrations were relatively stable with
354	depth, which was consistent with Wei et al. (2009). The reasons might be the influence of soil
355	parent material, the amount nutrient content of returning litter, the rate of decomposition, and
356	plant nitrogen fixation, absorption and utilization. With the increasing of the soil depth, the input
357	of organic matter gradually decreased (Nelson, Schoenau & Malhi, 2008). However, soil P is
358	mainly derived from rock weathering and leaching, and its mobility is very low, which caused
359	vertical variation of P along the soil profile to be relatively stable (Wei et al., 2009).
360	Soil C:N:P ratios are important indicators of organic matter composition, soil quality and



361	nutrient supply capacity (Bui & Henderson, 2013). In this study, soil C:N:P ratios among the
362	three plant communities was 16.9:1.7:1, 25.0:2.3:1 and 28.6:2.5:1 at the topsoil (0-10 cm),
363	respectively (Table 2), These values are substantially lower than the average global value of
364	186:13:1 (Cleveland & Liptzin, 2007). Since Loess soils are naturally low in C world wide and
365	the Loess Plateau has undergone a serious soil erosion prior to recent efforts at vegetation
366	restoration, resulting in a low C:N:P ratio. In this study, soil C:N ratio across different plant
367	communities and soil depths was approximately 10.8 in the Loess Plateau, which was similar to
368	the average level (11.9) in China (<i>Tian et al., 2010</i>), but lower than the world's average value of
369	13.3 (Cleveland & Liptzin, 2007). Previous studies showed that soil C:N ratio is negatively
370	correlated with the decomposition rate of organic matter, and low soil C:N ratio indicates that
371	organic matter is well decomposed (Zhao et al., 2015; Deng et al., 2016). The soil C:N ratio in
372	grassland, shrubland and forestland was 10.1, 10.8 and 11.7, respectively, implying that organic
373	matter had been completely decomposed. The soil C:N ratio in each plant community maintained
374	relative stability with increasing soil depth, which is consistent with previous studies (Tian et al.,
375	2010). This may be due to the same change dynamics in C and N. Soil C:P and N:P ratios in each
376	plant community decreased with increasing soil depth, which may be due to the difference in the
377	source of soil C, N and P. Furthermore, this study showed that soil C:P and N:P ratios in
378	forestland was higher than in shrubland and grassland in the topsoil depth, which may be due to
379	the fact that forest had more above-ground biomass than shrubland and grassland (Qi et al.,
380	2015).
381	Relationships between C, N and P concentrations and their characteristics of ecological
382	stoichiometry among leaf, litter and soil
383	Some previous studies have showed a strong correlation between leaf and soil nutrients (<i>Parfitt</i> ,
384	Yeates & Ross, 2005; Cleveland & Liptzin, 2007; Agren, 2008), while others found that there
385	was no correlation between N and P concentrations in leaf and soil (Ladanai, Ågren & Olsson,
386	2010; Yu et al., 2010). In this study, no significant correlation was found between soil N
387	concentration with leaf N concentration for three plant community types. One possible reason is



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that through long-term adaptation to the habitat, the N concentration in plant leaves in this region may be more affected by the attributes of the species than the limitation of soil nutrients. In addition, *Reich & Oleksyn*, (2004) showed that the mineral elements of plants are a combination of climate, soil nutrients and species composition. Other studies have suggested that soil temperature, soil water concentration, microbial activity and other factors have a greater impact on the mineral elements of plants (Chapin & Pastor, 1995; Güsewell, 2004). In this study, there was a significant correlation between litter N and P concentrations and their ratios with leaf N and P concentrations among the three plant types, indicating that the nutrients in litter were derived from plant leaves. In addition, a strong correlation between soil and litter for both C and P concentrations among the three plant types was observed. As a considerable portion of C and other nutrients elements in the litter could be released into the soil, such that litter was one of the main sources of soil nutrients (Agren et al., 2013). In general, this study showed that there is a close correlation between the concentrations of C, N and P and their ratios in leaf, litter and soil in three plant community types, which confirmed that C, N and P in the ecosystem were transported and transformed among plants, litter and soil (McGroddy, Daufresne & Heedin, 2004).

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CONCLUSION

This study analyzed C, N and P concentrations and their stoichiometric characteristics in leaf, litter and soil of three dominant plant types: grass (*I. cylindrica* and *A. sacrorum*)), shrubs (*S. viciifolia* and *H. rhamnoides*) and tree (*Q. liaotungensis* and *B. platyphylla*)) during vegetation restoration on the Loess Plateau of China. The results indicated that plant community type had significant effects on leaf, litter and soil nutrient concentrations, and their stoichiometry characteristics. Grass species had highest leaf P concentration and forest species litter had highest P concentration. Leaf C, N and P concentrations were higher than in litter and soil (*P*<0.05) and forest community type had highest soil nutrient concentrations at all soil layers and their ecological stoichiometries were highest in topsoil (*P*<0.05). In addition, soil C:N:P ratios in



415	all plant communities decreased with increasing soil depth. Soil P concentration and N:P ratio
416	had significant positive correlations with litter P concentration and N:P ratio for the three plant
117	community types (P <0.05), respectively. However, there were no significant correlations
118	between soil N, P concentrations and N:P ratio with leaf N and P concentrations and N:P ratio
119	(<i>P</i> >0.5), respectively. Based on the theory that leaf N:P ratio indicates nutritional limitation for
120	plant growth, this study concluded that plant growth of the forest community type $(Q$.
121	liaotungensis and B. platyphylla species) was co-limited by both of N and P elements, plant
122	growth of shrub community type (H. rhamnoides and S. viciifolia species) was limited by P
123	element and grass growth (I. cylindrica and A. sacrorum species) was limited by N element.
124	These results can provide a scientific basis for the reconstruction of degraded ecosystem on the
125	Loess Plateau of China.
126	
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141	Competing Interests
142	The authors declare that they have no competing interests.
143	



444 **Author Contributions**

- Zongfei Wang conceived and designed the field investigation, analyzed the data, wrote drafts of the paper and approved the final draft.
- Fenli Zheng helped with designing fieldwork, and contributed to writing the manuscript.

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Table captions

- Table 1. Characteristics of the three plant types.
- Table 2. Nutrient concentrations and characteristics of ecological stoichiometry in leaves of the
- three plant types.
- Table 3. Nutrient concentrations and characteristics of ecological stoichiometry in litter of the
- three plant types.
- Table 4. Profile distribution of soil nutrient concentrations and characteristics of ecological
- stoichiometry of the different plant types.
- Table 5 Two-way ANOVA results between-subject effects of plant type, soil depth and their
- interactions on the soil C:N:P ratios.
- Table 6. Correlations among ecological stoichiometry in leaf, litter and soil in 0-10/0-20 cm soil
- depths and 0-100 cm soil profile.



600	Figure captions
601	Fig. 1. Concentrations of soil C, N, P and their ecological stoichiometry in the different sampling
602	soil layers of the different plant species.
603	Fig. 2. Relationships between leaf and litter C: N: P stoichiometric characteristics

Fig. 3. Relationships between leaf and soil C, N and P concentrations in 0-10/0-20 cm soil depths

and 0-100 cm soil profile

606 Fig.4. Relationships between litter and soil C, N and P concentrations in 0-10/0-20 cm soil depths

and 0-100 cm soil profile

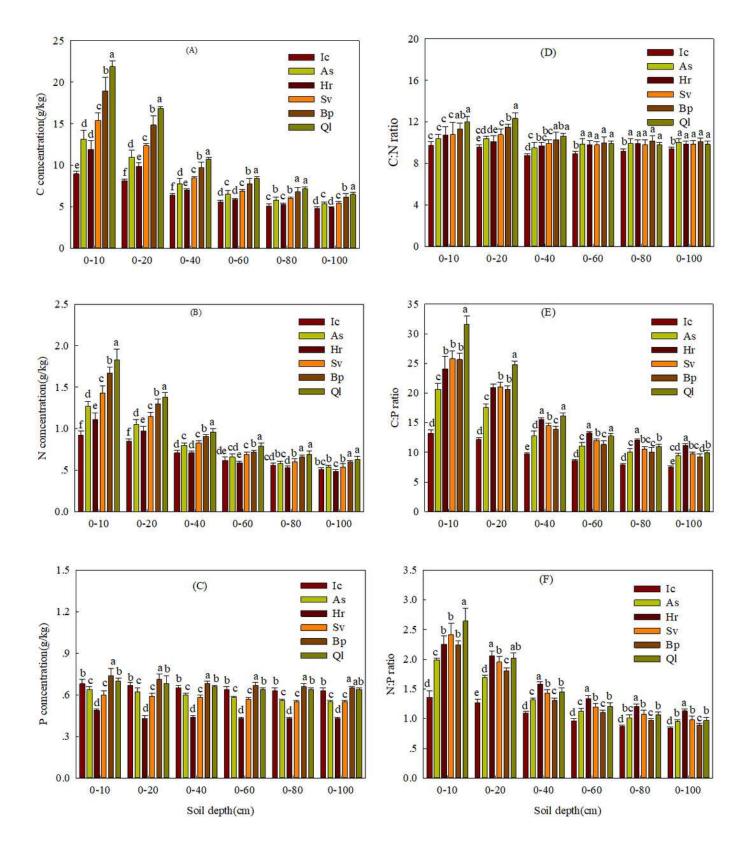
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Concentrations of soil C, N, P and their ecological stoichiometry in the different sampling soil layers of the different plant species

Bars indicates the standard errors (n=6). The lowercase letters above the bars indicate significant differences in different plant species at the same soil layers, and the capital letters represent significant differences in different soil layers at the same plant species (P< 0.05). Ic and As represent I. cylindrica and A. sacrorum, respectively; Hr and Sv represent H. rhamnoides and S. viciifolia, respectively; Bp and Ql represent B. platyphylla and Q. liaotungensis, respectively.

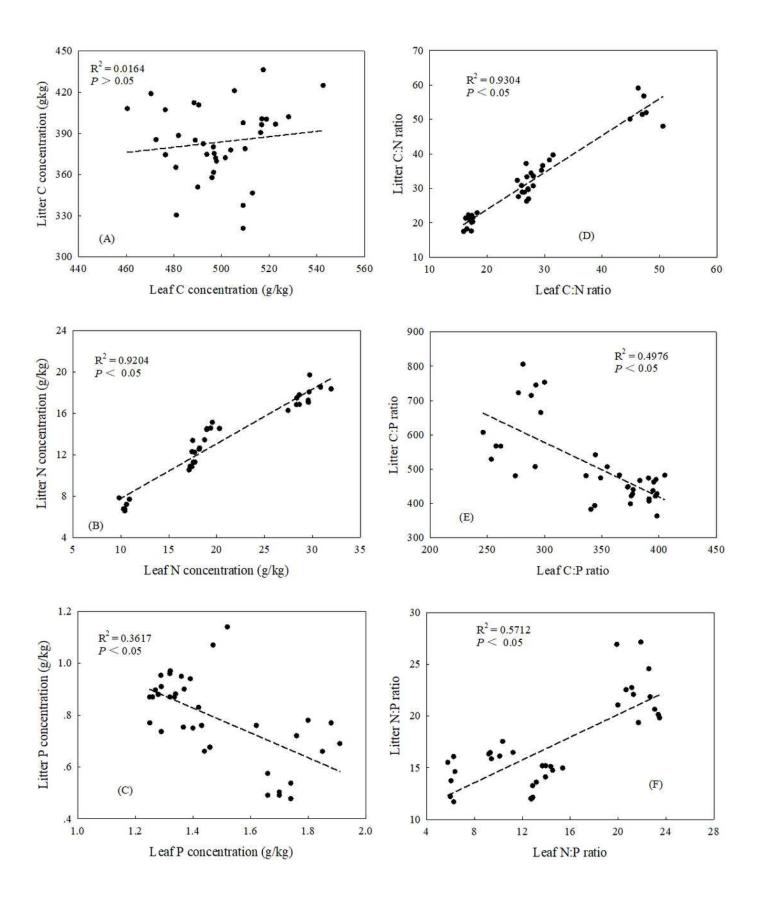






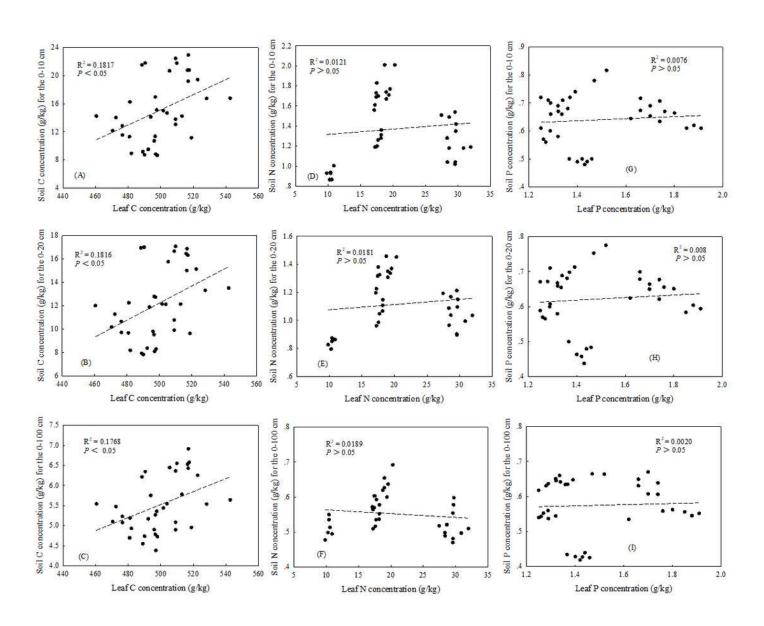
Relationships between leaf and litter C: N: P stoichiometric characteristics







Relationships between leaf and soil C, N and P concentrations in 0-10/0-20 cm soil depths and 0-100 cm soil profile





Relationships between litter and soil C, N and P concentrations in 0-10/0-20~cm soil depths and 0-100~cm soil profile

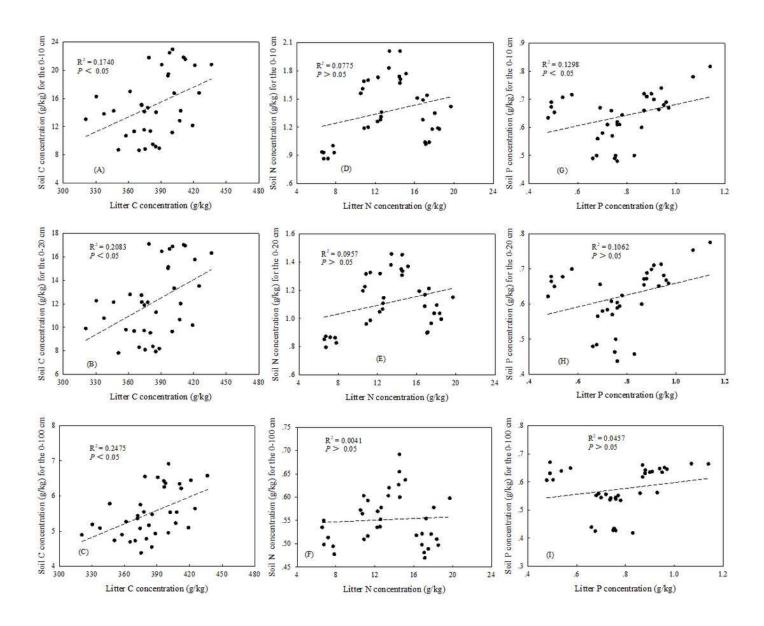




Table 1(on next page)

Characteristics of three plant types



Table 1 Characteristics of the three plant types.

Vegetation	Dominant plant	Abbreviation	Accompanying plant	Altitude	Coverag	Slope degree	Slope
types	species	Addreviation	species	(m)	e (%)	(°)	aspect
Forest	Quercus liaotungensis	Q. liaotungensis	C	1355	60	21-25	WS260°
roiest	Betula platyphylla	B. platyphylla	Carex lanceolata	1133	80	17-20	WS120°
Shrub	Sophora viciifolia	S. viciifolia	Stipa bungeana	1280	55	15-20	WS255°
Sillub	Hippophae rhamnoides	H. rhamnoides	Buddleja alternifolia	1332	75	15-17	WS45°
Grass	Imperata cylindrica	I. cylindrica	Artemisia giraldii	1310	70	10-12	WS259°
Giass	Artemisia sacrorum	A. sacrorum	Themeda japonica	1336	75	15-20	WS220°

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Table 2(on next page)

Nutrient concentrations and characteristics of ecological stoichiometry in leaf at the three plant types



1 Table 2 Nutrient concentrations and characteristics of ecological stoichiometry in leaves of the

2 three plant types.

Vegetation	Plant	С	N	P	CAN	C.D	N.D
types	species	/(g/kg)	/(g/kg)	/(g/kg)	C:N	C:P	N:P
Earnet	Q. liaotungensis	505±11.5Bb	18.7±0.98Ab	1.30±0.03Bc	27.1±0.86Ac	388±6.67Aa	14.3±0.55Ac
Forest	B. platyphylla	522±11.4Aa	$18.1 \pm 1.00 Ab$	1.40 ± 0.03 Ab	$29.0 \pm 2.04 Ab$	373±24.0Ab	12.9±0.15Bd
Shrub	S. viciifolia	499±9.62Ab	28.9±0.83Aa	$1.28 \pm 0.03 Bc$	17.3±0.23Ad	390±10.8Aa	22.6±0.82Aa
Snrub	H. rhamnoides	502±12.2Ab	29.8±1.24Aa	1.42±0.03Ab	16.9±0.77Ad	354±12.3Bb	21.0±0.97Ba
Canan	I. cylindrica	491±5.33Ab	10.4±0.32Bc	1.70±0.03Aa	47.3±1.73Aa	289±8.13Ac	6.12±0.21Bf
Grass	A. sacrorum	475±9.97Bc	17.9±0.38Ab	1.80±0.10Aa	26.6±0.78Bc	264±12.0Ac	9.93±0.71Ae

- Note: Bars indicate the standard errors (n=6). The lowercase letters above the bars indicate significant
- 4 differences in leaf at different plant types and the capital letters represent significant differences in leaf at the
- 5 same plant types of different species (P< 0.05).

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Table 3(on next page)

Nutrient concentrations and characteristics of ecological stoichiometry in litter at the three plant types



1 Table 3 Nutrient concentrations and characteristics of ecological stoichiometry in litter of the

2 three plant types.

Vegetation	Plant	С	N	P	C.N.	C-D	N.D
types	Species	/(g/kg)	/(g/kg)	/(g/kg)	C:N	C:P	N:P
Earast	Q. liaotungensis	398±11.5Aab	13.8±0.82Ab	0.92±0.04Aa	29.1±2.53Bc	433±29.1Ad	14.9±0.38Ac
Forest	B. platyphylla	413±15.4Aa	12.2±1.91Ac	0.97±0.10Aa	34.6±4.26Ab	431±35.3Ad	12.5±0.66Bd
Shrub	S. viciifolia	360±16.7Ac	17.5±1.12Aa	$0.75\pm0.06Ab$	20.7±1.48Ad	486±51.0Ac	23.5±1.52Aa
Shrub	H. rhamnoides	360±26.2Ac	17.7±0.56Aa	0.74±0.06Ab	20.4±2.00Ad	489±29.2Abc	24.2±2.27Aa
Casas	I. cylindrica	375±12.6Abc	7.12±0.48Bd	0.51 ± 0.03 Bc	52.9±3.84Aa	735±42.7Aa	14.0±1.61Bc
Grass	A. sacrorum	395±17.4Aab	12.0±0.69Ac	0.73±0.04Ab	33.0±2.43Bb	543±42.2Bb	16.5±0.53Ab

- Note: Bars indicate the standard errors (n=6). The lowercase letters above the bars indicate significant
- 4 differences in litter at different plant types and the capital letters represent significant differences in litter at the
- 5 same plant types of different species (P< 0.05).



Table 4(on next page)

Profile distribution of soil nutrient concentrations and characteristics of ecological stoichiometry at different community types



- 1 Table. 4 Profile distribution of soil nutrient concentrations and characteristics of ecological
- 2 stoichiometry of the different plant types.

Vegetation Community	Soil layer (cm)	C/(g/kg)	N/(g/kg)	P/(g/kg)	C:N	C:P	N:P
	0-10	21.9±0.69Aa	1.83±0.13Aa	0.70±0.02Ab	12.0±0.53Aa	31.5±1.49Aa	2.64±0.22Aa
	10-20	12.6±0.59Ba	0.99±0.02Ba	$0.66 \pm 0.03 Bab$	12.7±0.56Aa	19.1±1.48Ba	1.50±0.06Bb
O linetonemie	20-40	5.51±0.41Ca	0.61±0.06Ca	0.64±0.02BCa	9.13±0.6Ca	8.65±0.69Cb	0.91±0.09Cb
Q. liaotungensis	40-60	4.25±0.24Dab	0.49±0.04Da	0.62±0.02Cb	8.72±0.32Cc	6.88±0.27Dcd	0.79±0.06Dab
	60-80	4.04±0.25 Dab	0.43±0.03Da	0.63±0.02Ca	9.49±0.29BCa	6.40±0.32Dbc	0.67±0.03DEbc
	80-100	3.94±0.25 Da	0.40±0.06Da	0.63±0.02Ca	9.55±0.91Ba	6.28±0.18Dbc	0.64±0.07Ebc
	0-10	19.0±1.65Ab	1.67±0.70Ab	0.74±0.05Aa	11.3±0.57Aab	25.7±1.04Ab	2.27±0.07Ab
	10-20	11.3±0.65Bb	0.97±0.06Ba	0.69±0.03ABa	11.7±0.31Ab	16.2±0.42Bb	1.39±0.05Bc
B. platyphylla	20-40	5.46±0.41Ca	0.59±0.04Ca	0.66±0.03BCa	9.28±1.28Ca	8.32±0.64Cb	0.91±0.07Cb
Б. рішурпуни	40-60	4.48±0.53CDa	0.47±0.05Dab	0.65±0.03BCa	9.45±0.57Cabc	6.95±0.95Dabc	0.74±0.09Dc
	60-80	4.22±0.22Da	0.40±0.03Eab	0.64±0.06BCa	10.6±1.03ABa	6.71±1.02Dbc	0.63±0.07Ebc
	80-100	3.69±0.32Dab	0.37 ± 0.03 Eab	0.62±0.05Ca	9.93±0.94BCa	5.98±0.76Dbc	0.61±0.08Ec
	0-10	15.4±0.94Ac	1.43±0.0.9Ac	0.60±0.03Ac	10.8±1.17Ab	25.8±1.32Ab	2.41±0.19Ab
	10-20	9.58±0.69Bc	$0.89 \pm 0.02 Bb$	0.58±0.01Ac	10.8±0.92ABc	16.7±0.96Bb	1.55±0.05Bb
S. viciifolia	20-40	5.27±0.41Cab	0.57±0.03Ca	0.58±0.03Ab	9.26±0.35Ca	9.13±0.39Cb	0.99±0.04Cb
5. vicijona	40-60	3.98±0.38Dc	0.43±0.08Dabc	0.54±0.00Bc	9.48±1.16BCabc	7.38±0.70Dbc	0.80±0.15Dab
	60-80	3.52±0.29Dc	0.37±0.06Deb	0.52±0.00Bb	9.79±1.08ABCa	6.76±0.38Dbc	0.70±0.11Db
	80-100	3.40±0.20Dc	0.33±0.03Ec	0.52±0.01Bb	10.3±0.51ABCa	6.55±0.49Db	0.64±0.05Dbc
	0-10	11.9±1.12Ad	1.11±0.08Ae	0.49±0.01Ad	10.8±0.81Ab	24.1±2.07Ab	2.25±0.14Ab
	10-20	8.11±0.56Bd	$0.85 \pm 0.05 Bb$	0.45±0.03Bd	9.55±0.82ABd	18.1±1.94Ba	1.90±0.16Ba
H. rhamnoides	20-40	4.45±0.19Cc	0.48±0.01Cb	0.42±0.0.01Cc	9.31±0.39Ba	10.7±0.67Ca	1.15±0.05Ca
11. rnamnotaes	40-60	3.80±0.27CDc	0.38±0.03Dc	0.42±0.01Cd	10.1±1.08abAB	9.08±0.66CDa	0.91±0.08Da
	60-80	3.61±0.08Dc	0.35±0.03Db	0.42±0.01Cc	10.3±1.06ABa	8.61±0.64Da	0.84±0.08Da
	80-100	3.40±0.22Dc	0.36±0.04Dab	0.43±0.02Cc	9.56±1.04ABa	8.01±0.29Da	0.85±0.09Da
	0-10	13.2±1.05Ad	1.27±0.06Ad	0.64±0.02Ac	10.4±0.43Abc	20.7±1.01Ac	1.99±0.03Ac
	10-20	8.93±0.72Bc	$0.86 \pm 0.07 Bb$	0.60±0.03Bc	10.5±0.26Ac	14.8±0.47Bc	1.42±0.05Bc
I. cylindrica	20-40	5.03±0.53Cab	0.58±0.01Ca	$0.58 \pm 0.02 Bb$	8.72±0.90Bab	8.69±1.02Cb	1.00±0.05Cb
1. Cylinarica	40-60	4.20±0.27CDab	0.41±0.06Db	0.54±0.01Cc	10.5±1.23Aa	7.74±0.56CDb	0.75±0.10De
	60-80	3.70±0.37Dbc	0.37±0.03Dbc	0.52±0.02CDb	10.1±1.01Aa	7.09±0.61Db	0.71±0.07Db
	80-100	3.68±0.42Dab	0.36±0.05Dab	0.50±0.01Db	10.4±1.41Aa	7.34±0.76Da	0.72±0.10Db
	0-10	8.96±0.29Ae	0.92±0.02Af	0.68±0.03Ab	9.74±0.38ABc	13.2±0.67Ad	1.36±0.11Ad
	10-20	7.31±0.28Bd	0.78±0.05Bc	0.65±0.03Bb	9.46±0.40Bd	11.2±0.34Bd	1.19±0.05Bd
A сасмамим	20-40	4.74±0.37Cbc	0.59±0.06Ca	0.64±0.02BCa	8.02±0.31Cb	7.45±0.43Cc	0.93±0.07Cb
A. sacrorum	40-60	4.06±0.19Dab	0.45±0.04Dabc	0.62±0.01CDab	9.17±0.48Bbc	6.52±0.19Dd	071±0.06Dc
	60-80	3.60±0.36Ec	0.37±0.02Eb	0.61±0.02CDa	9.88±1.32ABa	5.90±0.46Ec	0.60±04Ec
	80-100	3.49±0.27Ec	0.34±0.03Ec	0.61±0. 02Da	10.4±0.54Aa	5.76±0.33Ec	0.55±0.03Ec

Note: Bars indicates the standard errors (n=6). The lowercase letters above the bars indicate significant differences in different plant species at the same

⁴ soil layers, and the capital letters represent significant differences in different soil layers at the same plant species (P < 0.05).



Table 5(on next page)

Correlations among ecological stoichiometry in leaf, litter and soil at 0-10, 0-20 and 0-100 cm soil depth



1 Table 5 Two-way ANOVA results between-subject effects of plant type, soil depth and their

2 interactions on the soil C:N:P ratios.

Factor	F(P)value									
1 actor	С	N	P	C:N	C:P	N:P				
Dlant tring	153	81.4	315	4.90	125	72.1				
Plant type	(<0.0001¶)	(<0.0001)	(<0.0001)	(0.0003)	(<0.0001)	(<0.0001)				
Cail danda	1999	1701	51.6	22.4	1859	1226				
Soil depth	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)				
Plant type \square	54.1	30.4	0.35	3.54	40.1	18.8				
Soil depth§	(<0.0001)	(<0.0001)	(0.0663)	(<0.0001)	(<0.0001)	(<0.0001)				

³ \$ Indicates the interaction between plant type and soil depth; \$ parentheses is P value.

4



Table 6(on next page)

Table 6 Correlations among ecological stoichiometry in leaf, litter and soil in 0-10/0-20cm soil depths and 0-100 cm soil profile.



Table 6 Correlations among ecological stoichiometry in leaf, litter and soil in 0-10/0-20cm soil

2 depths and 0-100 cm soil profile.

NI4i4				So	oil depth (c	m)			
Nutrient ratio		0-10		0-20			0-100		
14110	Soil C:N	Soil C:P	Soil N:P	Soil C:N	Soil C:P	Soil N:P	Soil C:N	Soil C:P	Soil N:P
Leaf C:N	-0.344*	-0.667**	-0.737**	-0.254	-0.732**	-0.880**	-0.381*	-0.880*	-0.813**
Leaf C:P	0.508**	0.693**	0.646**	0.672**	0.640**	0.466**	0.135	0.133	-0.016
Leaf N:P	0.329	0.643**	0.706**	0.261	0.678**	0.792**	0.214	0.676**	0.632**
Litter C:N	-0.395*	-0.708**	-0.758**	-0.303	-0.762**	-0.886**	-0.305	-0.874**	-0.809*
Litter C:P	-0.661**	-0.839**	-0.786**	-0.670**	-0.839**	-0.759**	-0.562**	-0.712**	-0.502**
Litter N:P	-0.092	0.183	0.313	-0.273	0.258	0.542**	-0.108	0.591**	0.721**

^{3 *} Correlation is significant at the 0.05 level (2-tailed).

^{4 **} Correlation is significant at the 0.01 level (2-tailed).