



Carbon, nitrogen and phosphorus stoichiometry in differently aged lucerne stands during flowering

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ABSTRACT

This study aimed to test the hypothesis that stoichiometric ratios of C, N and P change with plant growth during flowering. The C:N, C:P and N:P varied without solid trends during flowering. The C:N was significantly different only between cuts, while the C:P and N:P were influenced by age, cut and their interaction. The C:N and C:P were higher in the 1st cut than in the 2nd cut, while for N:P, it was opposite. No solid trends were observed with the effects of age on both C:P and N:P. The N and P were closely correlated with C:N, C:P and N:P. Conclusively, stoichiometric ratios of C, N and P varied during flowering and changed with stand age and cut.

Key words: Alfalfa, C:N, C:P, Flowering, Growth stage, N:P.

INTRODUCTION

Nitrogen (N) and phosphorus (P), two of the most important limiting factors for plant growth, are influenced not only by abiotic factors, but also change with plant development. However, past studies focusing only on sole elements gave no full description on this.

Ecological stoichiometry (ES) has provided an integrative solution to measure changes in C, N and P within a plant at a time (Sternner and Elser, 2002). Changes in C:N and C:P influence C fixation and exhaustion in diverse ecosystems, thus making both ratios important indices to assess N or P use efficiency. The N:P is an efficient indicator to express N or P limitation in soils to plant growth (Güsewell, 2004; Reich and Oleksyn, 2004; Zhang *et al.*, 2004). For different functional groups and genus of plants, ES of elements is various (McGroddy *et al.*, 2004) mainly due to different genotypes. While during a plant life, nutrient concentration within the plant is the consequence of nutrition availability in soils and requirement of plants (Yang *et al.*, 2013). It is reasonable that the ratios of nutrients in the plant tend to be constant finally in a specific situation (Chapin III *et al.*, 2011; Michaels, 2003) and may vary as the environment changes (Reich and Oleksyn, 2004). At present, most knowledge about the change in ES of elements is derived from studies across space, while only few are touched from the viewpoint of chronosequence. Yang and Luo (2011) found plant tissue C:N increased significantly with stand age in forest ecosystems, while Clinton *et al.* (2002) found no significant variation over age sequence in a New Zealand forest. Pioneering work has also been done on ES change within one year. Ågren (2008) found that C:N and C:P

increased with plant growth in the growing season. Wang *et al.* (2013) showed preliminarily C:N change with lucerne growth at the vegetation stages, and Wang *et al.* (2014, 2015) showed N:P decreased and then increased with stand age of lucerne. However, there was no effort put into the study on ES change during plant flowering.

Lucerne (*Medicago sativa*) is one of the most important legumes in the world. To date, rare study has been focused on changes in the ratios of C, N and P during lucerne flowering. In the present study, we tested the hypothesis that stoichiometric ratios of C, N and P should change with lucerne growth during flowering. It aimed to figure it out 1) how stoichiometric ratios of C, N and P changed as lucerne grew during flowering, 2) whether they were different among stand ages, cuts or organs, and 3) what the relationships were among concentrations and ratios during flowering.

MATERIALS AND METHODS

Description of the study site: The study area locates at the Qingyang experimental station (35°40' N, 107°51' E, 1298 m above sea level) on the Loess Plateau of northwestern China. The average annual precipitation is 561 mm and 70% of this total usually falls in July, August and September. The average annual evaporation is 1504 mm. The soil is a Heilu soil (Entisol of FAO classification), a sandy-loam with 70% silt and 23% clay, representing the major cropping soil in this area.

A local variety of lucerne (*Medicago sativa* L. cv. Longdong) was planted in the station and there are different stands established in 2001, 2002, 2004 and 2005. No irrigation and fertilisers were applied to the fields. General

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Plant sampling: Plant sample was taken at the flowering stage of each cut in 2009 when the ages of the stands were 5 (established in 2005), 6 (established in 2004), 8 (established in 2002) and 9 years (established in 2001). The detailed sampling dates were: June 1 (early flowering) and 15 in the 1st cut, July 10 (early flowering), 17 and 24 in the 2nd cut. There were three separate fields for each stand. In each of three replicates, at least 10 shoots were taken for the stand of one age. Plant samples were then oven-dried at 105°C for 10 min and then at 80°C for at least 48 h. Then leaves and stems were ground separately into uniformly fine powder to pass through a 1.0 mm sieve.

The ecological stoichiometric ratios of C, N and P were calculated as organic C vs. total N (C:N), organic C vs. total P (C:P) and total N vs. total P (N:P).

RESULTS AND DISCUSSION

There was also no regular trend of C:P change during flowering (Fig 1B), while C:P change was, to some extent, opposite to what total P concentration did, indicating

Table 1: Characteristics of leaf C:N, C:P and N:P of differently aged lucerne stands during flowering

Age (year)	Cut	C:N			C:P			N:P					
		Mean (± SD)	Max	Min	CV (%)	Mean (± SD)	Max	Min	CV (%)	Mean (± SD)	Max	Min	CV (%)
5	1 st	14.5 ± 2.2	16.9	10.3	15.0	176 ± 30c	225	126	16.9	12.1 ± 0.8b	13.3	10.7	6.7
	2 nd	10.4 ± 1.8	13.3	8.5	17.2	129 ± 15b*	155	100	11.5	12.8 ± 2.9b	16.3	8.3	22.3
6	1 st	16.0 ± 0.6	16.9	15.2	3.9	232 ± 23ab	259	191	9.8	14.1 ± 1.0ab	15.4	12.6	7.2
	2 nd	11.2 ± 2.2	15.9	8.7	20.0	189 ± 39a*	280	133	20.6	17.8 ± 5.8a*	31.2	10.8	32.8
8	1 st	15.3 ± 0.8	16.2	14.2	5.1	204 ± 39b	240	129	19.2	13.2 ± 2.1b	14.8	9.1	15.7
	2 nd	10.9 ± 2.6	16.3	8.1	23.8	176 ± 24a*	227	149	13.8	16.3 ± 4.0a*	23.6	11.9	24.7
9	1 st	16.2 ± 0.6	17.3	15.8	3.8	281 ± 51a	375	227	18.2	17.1 ± 3.2a	23.7	14.2	18.7
	2 nd	10.5 ± 1.9	13.6	8.1	18.3	155 ± 21a*	190	113	13.6	15.4 ± 3.8a	20.0	8.6	25.0
Average (cut)	1 st	15.4				/				/			
	2 nd	10.7*				/				/			
Age			P > 0.05				P < 0.001						P < 0.001
Cut			P < 0.001				P < 0.001						P < 0.001
Age × Cut			P > 0.05				P < 0.01						P < 0.05

Different lowercase letters show significant differences among ages of the same cut. The asterisk (*) shows a significant difference between cuts of the same age or between cuts of averaged ages.

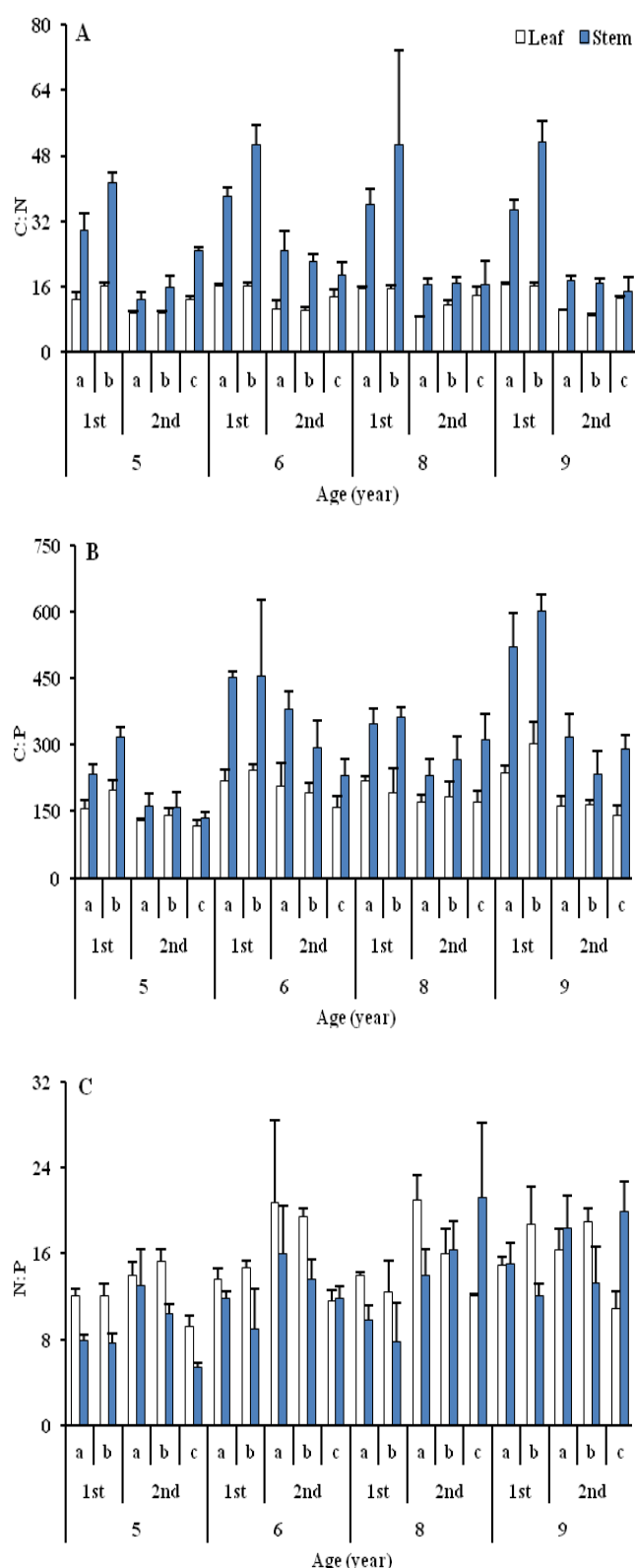


Fig 1: Changes of C:N, C:P and N:P in the leaf and stem during flowering of differently aged lucerne stands

Under the x axis, a, b and c refer to growth stages on 1 and 15 June of the 1st cut (1st) and 10, 17 and 24 July of the 2nd cut (2nd).

that there might be a close relationship between them. Generally, leaf C:P was lower than stem. Age, cut and their interaction influenced C:P significantly ($P < 0.001$, $P < 0.001$ and $P < 0.01$; Table 1). Leaf C:P was ranging from 100 to 375 with max and min measured in the 1st cut of 9 year stand and the 2nd cut of 5 year stand. The C:P in the 1st cut was higher than that in the 2nd cut. The CV of leaf C:P was 9.8%–20.6% with max and min in 6 year stand. The CV in the 1st cut was higher than that in the 2nd cut (6 year stand as an exception).

The N:P changed heavily without a regular trend during flowering (Fig 1C). In the stand of 5 years, it hardly changed in the 1st cut, while in the 2nd cut, leaf N:P increased and then decreased, and stem N:P decreased. The N:P in the 1st cut of 6 year stand increased in the leaf but decreased in the stem, and in the 2nd cut, it decreased. For 8 year stand, it decreased in the 1st cut, and in the 2nd cut, decreased in the leaf but increased in the stem. Leaf N:P of 9 year stand increased in the leaf and decreased in the stem in the 1st cut, while in the 2nd cut, it increased and then decreased in the leaf and changed contrarily in the stem. On most occasions, leaf N:P was higher than stem. There were significant effects of age, cut and their interaction ($P < 0.001$, $P < 0.001$ and $P < 0.05$) on leaf N:P (Table 1). Leaf N:P was ranging from 8.3 to 31.2 with max and min values measured in the 2nd cut of the stands of 6 and 5 years, respectively. The N:P was lower in the 1st cut than in the 2nd cut. The CV of leaf N:P was 6.7%–32.8% with max and min values in the 2nd cut of 6 year stand and the 1st cut of 5 year stand. The CV in the 1st cut was lower than that in the 2nd cut.

Correlations of stoichiometric ratios with concentrations or ratios of C, N and P during lucerne flowering:

There were negative correlations between C:N and total N concentration (Fig 2A), and total P concentration (Fig 2B). Negative correlations were also observed between C:P and total N concentration (Fig 2C), and total P concentration (Figure 2D). There was a positive correlation of N:P with total N concentration (Fig 2E), while with total P concentration, there was a negative but weak correlation (Fig 2F). In addition, during lucerne flowering, C:N positively correlated with C:P (Fig 2G), and negatively correlated with N:P (Fig 2H). No significant correlations were observed in other cases.

Effects of stand age, cut and growth stage on stoichiometric ratios of C, N and P during lucerne flowering:

In this study, leaf C:N averaged 12.0–12.8 of 5–9 year stands during flowering, which was higher than 10.6 with 1 year stand in the field (Wang and Yang, 2011) but lower than those in other legumes (He *et al.*, 2006). Mixed samples from early to late flowering were used in this study, which leads to the increase in C:N, compared to the previous study (Wang and Yang, 2011). While more legumes, especially tree- and shrub-like species and older plants were

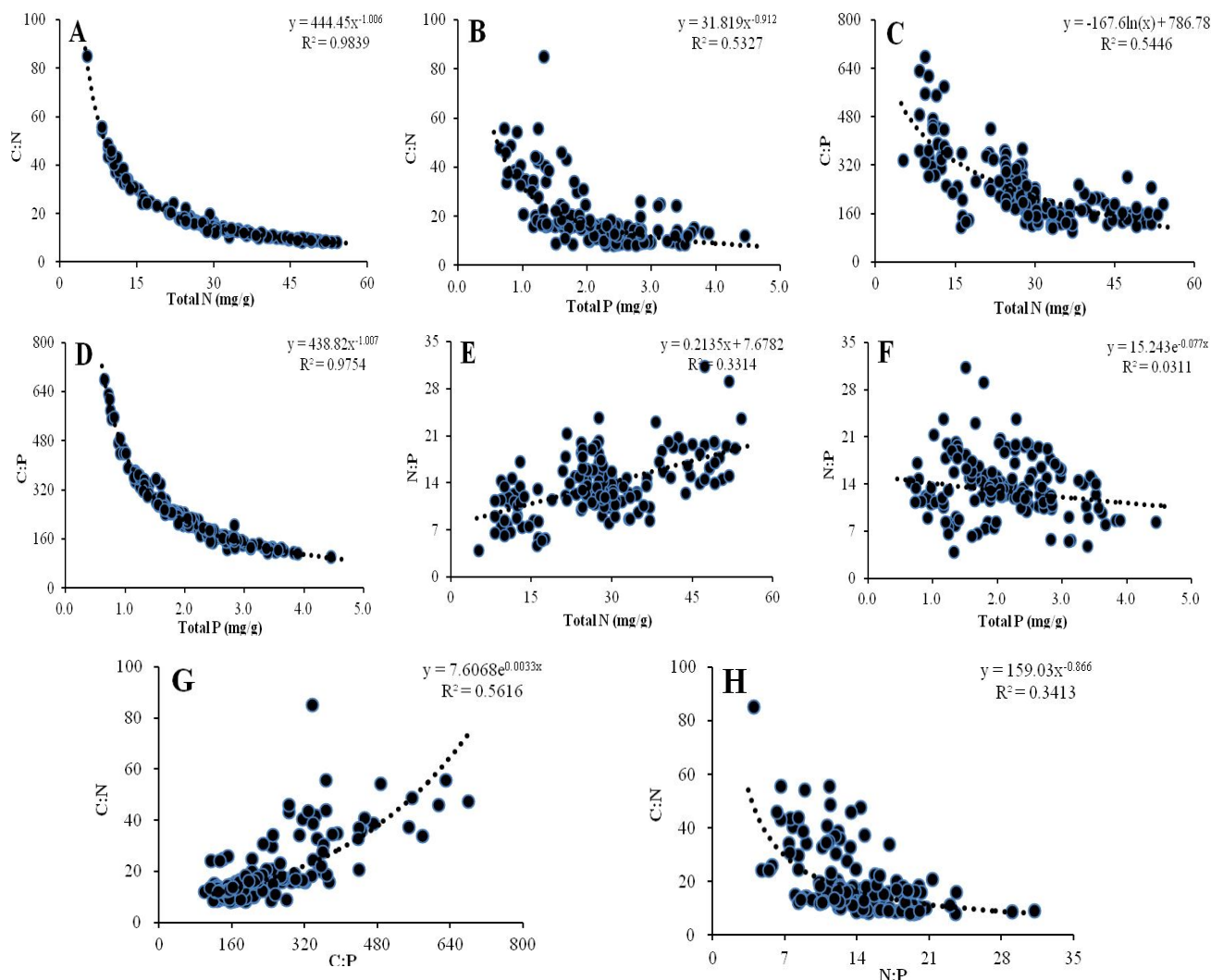


Fig 2: Correlations of stoichiometric ratios with concentrations or ratios of C, N and P during lucerne flowering
Only correlations with significance are shown with equations and R^2 . All leaf and stem data were used for analyzing the correlations.

sampled in Fang group study (He *et al.*, 2006), which results in C:N elevation. These values were much lower than those of other species from larger scales (Elser *et al.*, 2000; He *et al.*, 2006; McGroddy *et al.*, 2004; Zheng and Shangguan, 2007). Generally leguminous species has the lowest C:N than other species (Cui *et al.*, 2010) due to more N accumulation. There was significant cut effect on leaf C:N of lucerne, in agreement with Wang *et al.* (2013). Generally C:N is well constrained in leaves (McGroddy *et al.*, 2004) and much more restricted by N and P as there were significant and strong correlations of C:N with N and P. In addition, leaf C:N was much lower than stem C:N, in accord with previous reports (Wang and Yang, 2011; Wang *et al.*, 2013).

In this study, leaf C:P ranged from 148–205 of 5–9 year stands during flowering. These values found in lucerne were much lower than those gathered from larger scales (Elser *et al.*, 2000; McGroddy *et al.*, 2004; Zheng and Shangguan, 2007). Age, cut and their interaction influenced

C:P significantly. Considering that C is relatively stable, we assume P is affected more by age and cut, which proves true in this study. Significant and strong correlations of C:P with P and N had also shown that C:P was controlled by P and N but not C. Additionally, C:P in the leaf was lower than that in the stem, which should be attributed to more C accumulation in the stem.

Leaf N:P in this study was 12.5–16.3 of 5–9 year stands during flowering. No significant difference was found between this study and previous report (Wang *et al.*, 2014) and between lucerne and other species from larger scales (Elser *et al.*, 2000; He *et al.*, 2008; McGroddy *et al.*, 2004; Reich and Oleksyn, 2004; Townsend *et al.*, 2007; Zheng and Shangguan, 2007). This result differs a lot from what we knew from a semi-arid grassland of China (Cui *et al.*, 2010), in which the legume shrub showed the highest foliar N:P. However, in most cases lucerne leaf N:P was higher than the global values, possibly resulting from the very high

N concentration of leguminous lucerne with strong BNF. Dramatically, leaf N:P of other legumes (He *et al.*, 2008) and tropical rainforests (Townsend *et al.*, 2007) were higher than those of lucerne leaf. The leguminous species shares a common capability of BNF so is higher in N:P. The tropical rainforests live with a short leaf lifespan so leaf N concentration is high and they live in tropical soils, which leads easily to P limitation (Reich and Oleksyn, 2004). Cut effect was significant on total N:P concentration. There was a significant effect of age on leaf total P concentration, in accordance with Wang *et al.* (2014). These cut and age effects are mostly from the change in N concentration since there was a stronger correlation of N:P with N than P. Additionally, N:P in leaves was greater than in stems due to more N accumulation in the leaf.

The N or P limitation to lucerne growth during flowering:

The physiological concentration requirements of N and P for adequate plant growth are 15 and 2 mg/g, respectively (Han *et al.*, 2011; Marschner, 2011). In this study, the concentrations of N and P in the leaves of lucerne showed there were more than enough N and P for growth during flowering. It suggested that there should be no N or P

limitation to lucerne growth at this stage. However, according to the principles stated in previous literatures (Güsewell *et al.*, 2003; Koerselman and Meuleman, 1996; Olde Venterink *et al.*, 2003; Zhang *et al.*, 2004), there was an obvious indication that N or P limitation to plant growth occurred in this study area (Wang *et al.*, 2014), though the optimal N:P is 10 for plants (Linder, 1995). Considering lucerne is able to use symbiotically fixed N (with very strong BNF) and is less dependent on soil mineral N, the finding on N limitation will not always be the case for lucerne. Since one nutrient that is more stable and less sensitive to environmental gradients would be more easily a limiting factor to plant growth (Han *et al.*, 2011), P is possibly a limitation to the growth of lucerne in this area due to its low mobility in the arid soils.

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