INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–089/2015/17–6–1179–1185
DOI: 10.17957/IJAB/15.0042
http://www.fspublishers.org

Full Length Article

Influence of Nitrogen Fertilization on Wheat, and Soil Carbon, Nitrogen and Phosphorus Stoichiometry Characteristics

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Abstract

Nitrogen fertilizer is the most commonly fertilizer for increasing grain yield of wheat, but its effects on wheat carbon (C), nitrogen (N) and phosphorus (P) availability are not clear yet. This field experiment was conducted to evaluate the C, N and P stoichiometry characteristics of winter wheat and soil and their relationship during the anthesis and harvest stages under nitrogen fertilization (0, 180, 360 kg ha^{-1}) in Northwest China. We found that N fertilization could increase N contents, C: P and N: P ratios and decrease P contents, C: N ratio in wheat. During anthesis, the highest N and P contents were found in the flag leaf and culm, respectively and at harvest, N and P contents were higher in the grain. N and P contents in all tissues of wheat were related, and more importantly, a significant relationship with the soil N and P contents was found, these results could help us understand the nutritional balance regulation in wheat under nitrogen fertilization. © 2015 Friends Science Publishers

Keywords: C, N, P stoichiometry; Nitrogen fertilizer; Winter wheat; Soil; Ontogeny

Introduction

Carbon (C), nitrogen (N) and phosphorus (P) are the three main elements in living organisms (Michaels, 2003). Their ratios defined as nutrient stoichiometry could as a tool for analyzing the balance between the nutrition elements required by organisms and affects nutrient cycling (Sterner and Elser, 2002). Soil C: N: P stoichiometry patterns could enhance our understanding of nutrient cycling and biological processes in terrestrial ecosystems (Tian *et al*., 2010; Yuan *et al*., 2011; Ding et al., 2014). Soil N: P dynamics directly reflect the availability of N and P, but are influenced by biotic and abiotic factors as well (Jiao *et al*., 2013; Farooq *et al*., 2014). Leaf C: N: P stoichiometry patterns could reflect plant nutrient balance and it is affected by many factors, especially plant species and the growth environment (Sardans *et al*., 2011).

To date, numerous stoichiometric studies have been conducted in grassland, forest and marine ecosystems (Elser and Hassett, 1994). However, agronomic studies of crop C: N: P stoichiometry are lacked (Sadras, 2006). Wheat (*Triticum aestivum* L.) is the most important food crops in the world and its output affects world food security. Nitrogen is the most commonly used fertilizer because of its large effect on the grain yield (Sadras, 2006). Nitrogen fertilization could alleviate N limitation in soil and increase plant productivity (Bai *et al*., 2010). However, it also can affect other nutrition absorbing leading nutrient imbalance and the effects of nitrogen fertilization on crop C: N: P patterns and balance in farmland are inconsistent. In some experiments, increased N fertilizer applications resulted in higher grain N concentrations, whereas grain P concentrations were not consistently affected (Osborne *et al*., 2004), or a decrease was reported (Bélanger *et al*., 2012). Plants fix inorganic C through photosynthesis and increased N and P availability can affect the plant growth (Xia and Wan, 2008; Perring *et al*., 2009) and primary productivity (Bai *et al*., 2010). Plant absorb N and P comes from the soil, so soil N and P strongly influences plant nutrition, but the responses of plants to N and P supply were different. Gusewell (2004) reported that soil N and P contents could cause 50-fold variations in biomass N: P, but Bowman *et al*. (2003) found that no significant relationship was observed between soil and foliar nutrient. Soil C: N can affects N and P availability by change the activity of soil microorganisms. These contrasting results show that we should more further studies to understand the relationship between soil nutrients and plant nutrient contents. Besides, leaf ontogeny also could affects leaf stoichiometry (Zhang *et al*., 2013), over the growing season, leaf nutrient content is higher in early growth and decreases with the rapid expansion of the leaves, then increases to a stable period when the leaves mature and finally decreases as senesces (Wu *et al*., 2013). The growth period of winter wheat is long and the nutrition levels differences in different growth stages, anthesis and harvest stages are very

To cite this paper: Zhong, Y., W. Yan, X. Xu and Z. Shangguan, 2015. Influence of nitrogen fertilization on wheat, and soil carbon, nitrogen and phosphorus stoichiometry characteristics. *Int. J. Agric. Biol*., 17: 1179–1185

important to wheat yield and the report about stoichiometry characteristics in different ontogeny stages is rarely, so it is necessary to investigate the nutritional changes in anthesis and harvest stage.

In this study, we analyzed the nutrient allocation patterns among the different parts of winter wheat under different nitrogen fertilization rates. Measurements were taken during the anthesis and harvest stage in 2013 and 2014. Our aims were to investigate (1) the impacts of different rates of N fertilization on the stoichiometry characteristics of wheat and soil; (2) the stoichiometry differences between the two growth stages and (3) the relationship between wheat N and P stoichiometry and soil nutritional characteristics. Our results could help us understanding the nutritional regulation balance in wheat and help guiding fertilization.

Materials and Methods

Experimental Site and Climatic Conditions

The study was set up in an experimental field at the Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi (34°17'56''N, 108°04'7''E), where located at the southern boundary of the Loess Plateau, the experimental site belong to a temperate and semi-humid zone with a mean annual temperature of 13°C and mean annual precipitation of 632 mm, and approximately 60% of precipitation occurs between July and September. Selected soil physical and chemical properties at 0–20 cm layer before fertilization are presented in Table 1.

Experimental Design

The study was performed following a randomized block design with 3-replicate N treatments and cultivated with winter wheat (*Triticum aestivum* cv. Changhan No. 58). N was applied at three rates, 0, 180 and 360 kg ha^{-1} (termed N0, N180 and N360, respectively). The plots had an area of 2×3 m and each had twenty 15 cm-spaced rows of wheat to which 90 seeds were sown. Wheat was sown on October 10, 2012 and October 12, 2013 and harvested on May 26, 2013 and June 3, 2014 and the seeding rate was 130 kg ha^{-1}. Prior to wheat sowing, fertilizer was evenly spread on the soil surface and incorporated into the upper 20 cm of soil by chiseling. N was supplied as urea and P was applied as super phosphate (75 kg P_2O_5 ha^{-1}). During the study, the soil was never irrigated, weeds were regularly removed and there was no tillage during the growth stage, all these field management practices were the same in the treatment plots.

Measurements

During the anthesis and harvest stages in 2013 and 2014, five soil samples was randomly taken from each treatment plot, to a depth of 20 cm, using a soil drilling sampler (5 cm inner diameter), and fully mixed. Ten plants of wheat were also collected in each plot. The soil sample was air-dried before the determination of soil chemical properties. Sieved the soil samples through a 2 mm screen and removed the roots and other debris. Wheat samples taken at the anthesis stage were divided into four groups: flag leaf, second leaf, ear and the culm and samples taken at harvest were divided into two groups: grain and straw (including leaves). Samples were oven dried at 60°C for 72 h for subsequent nutrient content analyses.

The soil and plant C contents were assayed by dichromate oxidation (Nelson *et al*., 1982), N contents in the soil and wheat were assayed using the Kjeldahl method (Bremner *et al*., 1996) and P contents in the soil and wheat were measured using the Mo–Sb colorimetric method, after H_2SO_4–$HClO_4$ digestion (Parkinson and Allen, 1975). The tissue P concentration in plant samples shall be analyzed colorimetrically by the molybdenum blue method after H_2SO_4-H_2O_2 digestion (Bao, 2000).

Calculation of C, N and P Stoichiometry and Statistics

Shapiro-wilk test was used to test the normality of data. The data were natural log transformed when needed, for meet the assumption of normal distribution of data and homogeneity of variances (Cui *et al*., 2010). C, N and P stoichiometry were used the mass ration of nutrition content. After data processing, we found the two years data showed similar trends, we average the data for further analysis. One-way ANOVA was performed to test significance. Significant differences were evaluated at the 95% confidence level. We used the Pearson correlation coefficient to test the associations between the leaf nutrients (C, N, P) within and across the wheat organs. Linear regression was used to analyze relationships between leaf nutrients and soil nutrients (N, P) of wheat in two growth stages. All statistical analyses were performed using the software program SPSS, ver. 17.0 (SPSS Inc., Chicago, IL, USA).

Results

C, N and P Content of Wheat and Soil at the two Growth Stages

The C contents were consistent throughout the different wheat parts (Fig. 1 a, b). The highest C contents were found in the second leaf, with 369.38, 355.94 and 389.20 (mg g^{-1}), respectively, for the N0, N180 and N360 treatments. The C content in the N180 treatment was significantly lower than the N360 treatment in the flag leaf, second leaf and culm. However, when wheat was grown to harvest, there were no significant differences among the N treatments.

The N content was the highest in the flag leaves, with contents of 24.90, 35.50 and 35.83 mg g^{-1} in N0, N180 and N360, respectively. At the same N treatment level, the N content decreased in the order: flag leaf, second leaf, ear and culm at anthesis (Fig. 1c) and the N content in the grain was higher than in the straw at harvest (Fig. 1d). The N content significantly increased with nitrogen fertilization for all plant parts and for both growth stages, but there was no difference between N180 and N360 treatments.

In contrast, at anthesis, P content was the highest in the culm, with contents of 2.70, 2.44 and 2.05 mg g^{-1} for N0, N180 and N360, respectively (Fig. 1e). The P content at harvest was lower for all plant parts than at anthesis. For any given part, the P content significantly decreased with N fertilization, but there was no significant difference between the N180 and N360 treatments.

At the anthesis stage, soil C content (Fig. 2a) in the N0 was significantly higher than N treatments. Soil N content (Fig. 2c) was significantly higher in the N360 treatment, but no significant difference between the N0 and N180 treatments. The P contents (Fig. 2e) were significantly higher in N treatments than in the N0 treatments, but no significant difference between the N180 and N360 treatments. However, the C and P content in the soil showed no significant differences between any of the treatments at the harvest stage.

C, N and P Stoichiometry Characteristics of Wheat and Soil

The C: N ratio was higher in the culm for both growth stages (Fig. 3 a, b), with culm C: N ratios of 71.41, 42.29 and 42.68 for N0, N180 and N360, respectively, at anthesis and 103.29, 84.42 and 73.32 for straw, respectively, at harvest. The C: N was significantly higher in N0 than in N180 and N360 treatments. However, there was no difference between N180 and N360. The C: P trend was the opposite to the C: N ratio at anthesis (Fig. 3 c, d). The C: P ratio was the highest in the flag leaf in N treatments. However there were no significant differences in N treatments, except the culm and straw at harvest. The N: P ratio (Fig. 3 e, f) had a similar trend to C: P, in that the highest N: P ratio was in the flag leaf at anthesis. N application significantly increased the N: P ratio, but there was no significant difference between N180 and N360.

The stoichiometry characteristics of the soil at the two growth stages are shown in Fig. 2 (b, d, f). The C: N and C: P significantly decreased as the nitrogen rate rose at anthesis. However, the N: P was significantly higher in N360 than N0 and N180 treatments.

The Relationships of Nutrient Contents between Wheat and Soil

In general, there were positive correlated between N and P contents across the different plant tissues, but no significant relationships between C content and N and P (Table 2). Correlations for the N contents between the second leaf and flag leaf (r=0.94, $P < 0.01$) and culm (r=0.91, $P < 0.01$) were higher. In addition, correlations for P contents between the flag leaf and ear (r=0.83, $P < 0.01$) at anthesis and grain (r = 0.80, $P < 0.001$) at harvest were also higher. Interestingly, we found that the P content in the second leaf had no significant relationship with the N content of any of the other plant parts. In contrast, the P content in the flag leaf at anthesis and in straw at harvest have significant relationships with the N contents of the other plant parts.

Table 1: Selected soil physical-chemical properties measured in 0-20 cm soil before fertilization

Property	Value
Taxonomy	Eum-Orthic Anthrosols
Texture	
2000-50 μm (g kg^{-1})	64
50-2μm (g kg^{-1})	694
<2 μm (g kg^{-1})	342
Bulk density (g cm^{-3})	1.23
pH	8.25
Water holding capacity (%)	23.6
Available N (mg kg^{-1})	25.10
Available P (mg kg^{-1})	7.90

Selected soil physical-chemical properties in 0-20 cm soil before fertilization were showed in table

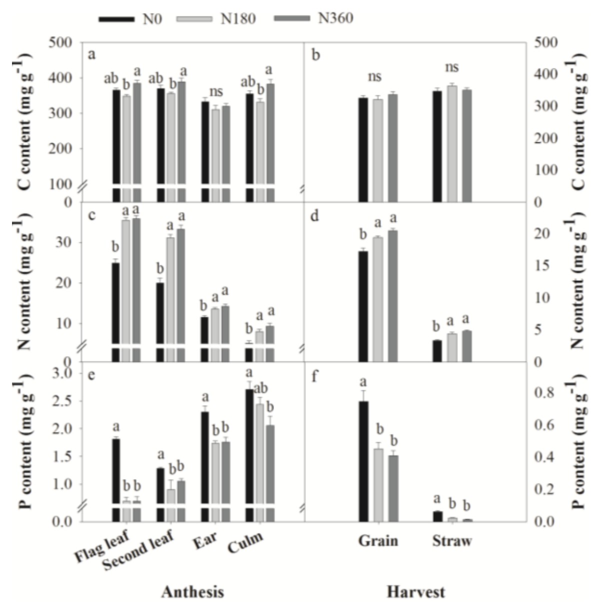

Fig. 1: Effects of nitrogen fertilization on C, N and P contents in different wheat tissues at two different growth stages

Values are means of three replicates for each treatment. Different letters indicate significant differences between N treatments at $P < 0.05$; ns means $P > 0.05$

The liner regression between wheat tissues and soil is shown in Fig. 4. The N contents in all plant tissues at both growth stages increased significantly as the soil N contents rose ($P < 0.05$) (Fig. 4a, c), with r^2 = 0.32, 0.35, 0.43. 0.26, respectively, in the flag leaf, second leaf, ear and culm at anthesis; and r^2 = 0.37 and 0.23, respectively in the grain and straw at harvest. The P contents in the flag leaf, second leaf and ear were significantly negatively correlated with the

Table 2: The correlation matrix (r value) for the C, N and P contents in different wheat tissues

X-variable	Y-variable	C content	N content						P content				
		All parts	Flag leaf	Second leaf	Ear	Culm	Grain	Straw	Flag leaf	Second leaf	Ear	Culm	Grain
C content	All parts	ns											
	Flag leaf												
	Second leaf		0.94**										
N content	Ear		0.81**	0.80**									
	Culm	ns	0.85**	0.91**	0.71**								
	Grain		0.74**	0.78**	0.71**	0.74**							
	Straw		0.71**	0.73**	0.63**	0.67**	0.79**						
	Flag leaf		-0.84**	-0.83**	-0.67**	-0.64**	-0.79**	-0.66**					
	Second leaf		-0.19	-0.15	-0.14	0.11	-0.34	-0.28	0.56*				
P content	Ear		-0.69**	-0.65**	-0.42	-0.47*	-0.56*	-0.51*	0.81**	0.55*			
	Culm	ns	-0.47	-0.55*	-0.29	-0.52*	-0.38	-0.47*	0.47*	0.03	0.47*		
	Grain		-0.69**	-0.69**	-0.69**	-0.43	-0.56*	-0.61**	0.83**	0.64**	0.79**	0.44	
	Straw		-0.86**	-0.86**	-0.70**	-0.74**	-0.76**	-0.74**	0.80**	0.36	0.78**	0.65**	0.77**

ns: The correlation is insignificant at P > 0.05. *The correlations are significant at P < 0.05. **The correlations are significant at P < 0.01

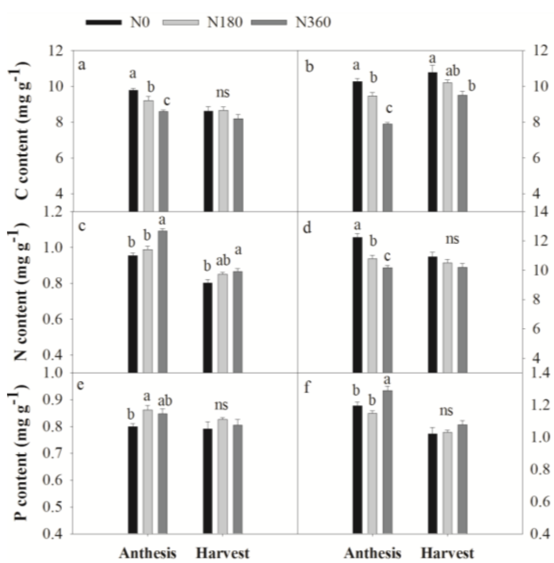

Fig. 2: Effects of nitrogen fertilization on C, N and P contents and stoichiometry patterns in the 0–20 cm soil layer at two different growth stages

Values are the means of three replicates for each treatment. Different letters indicate significant differences between N treatments at P < 0.05; ns means P > 0.05

soil P contents (P < 0.05), with r^2 = 0.47, 0.47 and 0.35, respectively. The P contents in the culm at anthesis and the grain and straw at harvest did not significantly affect by soil P contents (P > 0.05).

Discussion

Carbon contents changed little at both growth stages whereas N and P contents varied in different plant parts. These differences suggested that the C contents in wheat are more stable than N and P, consistent with He *et al.* (2006) and Yang *et al.* (2011), who reported that C was usually relatively stable in plants. N fertilization significantly increased N concentrations in wheat (Fig. 1 c, d), which was observed in other studies (Ruohomaki *et al.*, 1996; Tomassen *et al.*, 2003; Esmeijer-Liu *et al.*, 2009). At harvest, the grain N and P contents were higher, indicated that N and P in other parts of wheat transferred to the grain. Previous studies reported that 67–102% of plant N, 64–100% of P present at harvest were accumulated at anthesis (Clarke *et al.*, 1990) and over 70% of the total N uptake was transferred to the grain at maturity (Waldren and Flowerday, 1979). The grain N content significantly increased as N application increase, consistent with studies by Bélanger *et al.* (2012) and Jiao *et al.* (2013), and the lowest N and P in straw at harvest mainly due to the senescence (Wu *et al.*, 2013).

Kerkhoff *et al.* (2006) reported that flowers and seeds are rich in mitochondria, which have a higher N and P contents. However, the present study showed that P contents were higher in the N0 treatment in both anthesis and harvest stages, consistent with Perring *et al.* (2009) who found that nitrogen fertilizer could decrease plant P contents and suggested that grain P concentrations may be decreased significantly with increasing N addition (Alfoldi *et al.*, 1994; Osborne *et al.*, 2004; Bélanger *et al.*, 2012). These different grain N and P concentration responses to N fertilization could be due to variations in soil N levels (Li *et al.*, 2014).

C, N and P concentrations in the soil are crucial for ecosystem sustainability and productivity (Ågren, 2008; Li *et al.*, 2013). C is mainly derived from soil organic matter, which can stabilize soil structure against erosion and enhance soil nutrient availability (Wang *et al.*, 2008). The C, N and P contents in the soil at anthesis were different among N treatments, which might caused by the different N fertilization and the absorption ability of wheat under the different N treatments. However, at the harvest stage, the C and P contents lower than anthesis stage and showed no significant differences between N treatments (Fig. 2). The C content decreased mainly leading by the soil respiration reporting in our previous study (Shao *et al.*, 2014). The soil P content decreased mainly due to the wheat absorption during two stages. The soil N content differences between N0 and N360 mainly caused by the large amount of N fertilizer applied, and the lack of any significant differences

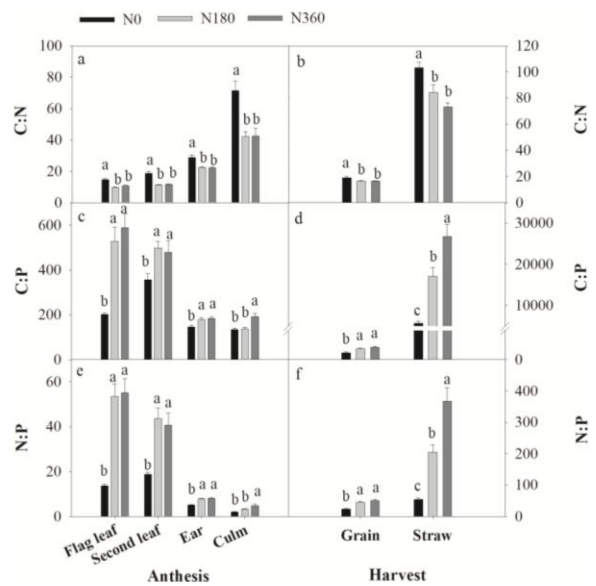

Fig. 3: Effects of nitrogen fertilization on C, N and P stoichiometry patterns for wheat tissues at two different growth stages

Values are the means of three replicates for each treatment. Different letters indicate significant differences between N treatments at P < 0.05; ns means P > 0.05

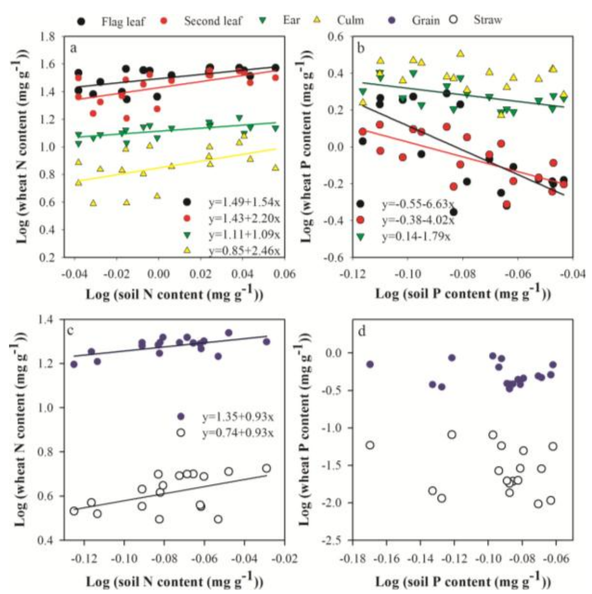

Fig. 4: The relationships of soil N and P availability on the N and P contents of wheat

The data was log10-transformed; the regression line is shown when the slope is significant (P < 0.05) and not shown when the slope is not significant (P > 0.05)

between N0 and N180 were mainly due to the absorption of wheat.

In this study, N fertilization caused a higher plant N concentration, but little change in the C concentration, which caused a lower C: N ratio in plant. Yang *et al.* (2011) reported that changes in C: N did not produce strong correlations with C dynamics, but were negatively associated with corresponding changes in N content. Reduced C: N ratios was a universal plant response when they were subjected to enriched N conditions in various ecosystems (Novotny *et al.*, 2007). The C: N was higher in the culm mainly due to the lower N contents and consistent with Norby *et al.* (2001) who showed that an increase in C: N ratio mainly caused by the decrease of N. The leaf N: P in the study was38.78, which was higher than the ratios for other grain crops reported by Sadras (2006). The higher leaf N content and N: P may reflect the high N availability in the N treatments (Li *et al.*, 2014). In this study, the N: P ratios were significantly higher in the N treatments, due to the greater availability of N than P, consistent with previous studies (Craine *et al.*, 2008; Cui *et al.*, 2010). The N treatments had higher C: P ratios than the non-N treatment, but no significant differences were found between N treatments at anthesis. The C: P ratio had a similar trend to the N: P. Maximum N: P was in the flag leaf, which caused by the lower P content. The higher C: P ratios in the N treatments were probably caused by the decrease in P as N fertilizer application and consistent with the results reported by Cui *et al.* (2010) for N addition in a temperate steppe ecosystem. N and P content decreased from anthesis to harvest stages, leading C: N and C: P ratios increase in harvest. This changes in the C: nutrient element mass ratio with the ontogenetic development of plants could be attributable to a more rapid formation of supportive, element-poor tissues (stems) in older plants compared to metabolically more active, element-rich tissues (leaves) (Ågren, 2008; Yang *et al.*, 2011).

Changes in total C, N and P concentrations in the soil inevitably led to alterations in the nutrient stoichiometric relationships with wheat (Ågren, 2008; Tian *et al.*, 2010). In Chinese soils, the mean C: N: P ratio is 134: 9: 1, but there is a high spatial heterogeneity and large variations at different soil depths (Tian *et al.*, 2010). N addition to the soil led to a decrease in soil C and increased P content at anthesis, which caused a significant decrease of C: N and C: P and an increase of N: P ratios.

The N and P contents in all wheat tissues co-varied positively (Table 2). According to our results, the C contents in plant tissue had no significant relationship with N and P contents and may be due to C was the fundamental element, so the plant maintain a stable level of C in order to perform the normal function. The relationships between N and P in plant tissues were shown in flag leaf, grain and straw. These results suggest that the flag leaf, which was responsible for plant growth, had a very strong relationship with nutrition in wheat and the C: N: P of the flag leaf could be an important indicator of the nutritional condition of wheat. The consistency of the N and P patterns may reflect the general constraints and allocation rules governing the partitioning of nutrients among plant tissues (Kerkhoff *et al.*, 2006).

Elements such as C, N and P were coupled in soils (Tian *et al*., 2010). Plants obtain their N and P through root (Chapin, 1980), therefore, soil N and P availability should affected plant N and P contents (Chen *et al*., 2011; Han *et al*., 2011; Han *et al*., 2012). The relationship between wheat and soil during the two stages was showed in Fig. 4. A significant ($P<0.05$) positive correlation was observed between the soil N and aboveground parts but a significant ($P<0.05$) negative linear correlation between soil P and flag leaf, second leaf and ear. The relationship between plant and soil was also reported by Li *et al*. (2014), who suggested that N and P levels were significantly related to soil nutrient levels in reeds (*Phragmites australis*). The higher r^2 in the flag leaf, second leaf and ear for the N content relationship may indicate that wheat was allocating more nutrients to metabolic and reproductive tissues (e.g., the leaf and ear) than to structural tissues (e.g., the culm) in order to ensure the key plant physiological activities (Li *et al*., 2014). However, N increases in the leaves probably leads to a P decrease. The N and P in the leaves is also transferred to the ear via the culm, which suggests that the culm probably performs a buffering function for storing N and P in plants as reported by Li *et al*. (2014). Furthermore, the relationship between plant and soil in anthesis was stronger than harvest, may be due to the vigorous growth and a considerable ability to absorb nutrients at anthesis.

Conclusion

This study suggests that N fertilization may increase the N content and decrease the P content in wheat and decreases C: N ratio and increases the C: P and N: P ratios. The results help us understanding the nutritional balance regulation and provide guidance for nitrogen fertilization.

Acknowledgements

The study was sponsored by the National Natural Science Foundation of China (41390463) and the National Sci-Tech Support Program of China (2015BAD22B01).

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(Received 24 January 2015; Accepted 13 May 2015)