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The relationships and sensibility of wheat C:N:P stoichiometry and water use efficiency under nitrogen fertilization

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ABSTRACT

A field study was conducted to investigate the variations in the carbon:nitrogen:phosphorus (C:N:P) stoichiometry of winter wheat (*Triticum aestivum* L.) and its relationships with wheat growth rate (r) and water use efficiency (WUE) under different rates of N fertilization. The results indicated that the growth rate and WUE of wheat significantly increased with N fertilization and were strongly correlated with the N and P contents and C:N:P ratio. The C content in wheat was relatively stable, regardless of N fertilization, and the N contents were higher in the N fertilization treatments. In addition, the P contents were higher in the treatments without N fertilization, and changes in the C:P and N:P ratios mainly depended on changes in P. The response ratio of the N:P ratio in straw was higher than that of the other investigated traits and largely depended on changes in the plant P content due to N addition.

Keywords: grain yield; evapotranspiration; wheat production; nutrient availability; macroelement

Carbon (C), nitrogen (N) and phosphorus (P) are the three main elements that exist in living organisms (Michaels 2003). The balance of these nutrients can influence plant growth rates, and the C:N:P stoichiometry in ecosystems has been widely studied. Many studies have been conducted in grassland, forest, and marine ecosystems (Elser and Hassett 1994), as well as in crop fields (Sadras 2006). Globally, wheat (*Triticum aestivum* L.) is one of the most important food crops, and wheat production affects the world's food security. Currently, water stress and nutrient deficits are the main factors that limit primary crop production (Zand-Parsa et al. 2006). Thus, fertilization has been widely applied to increase the water use efficiency (WUE) of wheat (Fan et al. 2005). Nitrogen fertilizers are the most common fertilizers and are known to affect grain yield (Sadras 2006), C:N:P allocation patterns and WUE in wheat (Fan et al. 2005).

Several studies have demonstrated that supplying N enhances plant productivity by increasing evapotranspiration (ET) and can improve WUE (Li et al. 2014, Zhong and Shangguan 2014). In addition, N fertilization influences nutrient allocation and can enhance the photosynthetic capacity of leaves, resulting in the accumulation of more dry matter and increasing the yields of the grain, which is mainly composed of carbohydrates. Yang et al. (2011) reported that N addition resulted in lower C:N ratios in plant tissues. Thus, the changes in nutrition caused by N fertilization must influence growth rates and the WUE. However, the relationships between stoichiometry patterns, crop growth rates and WUE are not well understood.

This study was conducted to determine the stoichiometry patterns, growth rates and WUE of winter wheat under three rates of N fertilization. We aimed to determine the relationships between wheat stoichiometry patterns and WUE and detect their sensitivity to N addition. To our knowledge, this is the first attempt to detect relationships between WUE and C:N:P ratios and consider the sensitivities of these traits in wheat in a farmland ecosystem. In addition, this study could provide

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useful information for WUE research in wheat regarding N fertilization.

MATERIAL AND METHODS

Experimental site and climatic conditions. This study was set up in an experimental field at the Institute of Soil and Water Conservation of Northwest A&F University, Yangling, Shaanxi. The site is located on the southern boundary of the Loess Plateau, which has a temperate and semi-humid climate with a mean annual temperature of 13°C and a mean annual precipitation of 632 mm.

Experimental design. This study was performed using 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 N/ha (referred to as N0, N180 and N360, respectively). The plots covered areas of 2 × 3 m with a guard row of 0.5 × 2 m, and the seeding rate was 130 kg/ha. Immediately before sowing the wheat, fertilizer was evenly spread on the soil surface and incorporated into the upper 15 cm of the soil via chiseling. N was applied as urea and P was applied as super phosphate (33 kg P/ha).

Measurements. Basic information regarding the soil physical and chemical properties at a depth of 0–20 cm in the study site were measured before fertilization (Table 1). The soil particle size distribution was determined using the Mastersizer 2000 (Malvern, UK) laser diffractometer. The soil bulk density was measured using a soil bulk sampler. The soil pH was measured using a pH meter after shaking the soil water (1:2.5 w/v) suspension for 30 min. Available N was determined by a micro-diffusion technique after alkaline hydrolysis and available P (Olsens's P) was extraction following the method of Olsen et al. (1954).

In all plots, the volumetric soil water content was measured every 10 cm at depths of 0–300 cm by using a neutron moisture meter (CNC100, Super Energy, Nuclear Technology Ltd., Beijing, China). The soil water content was measured the first week of every month during wheat growth. Monthly precipitation data were obtained from local weather station.

Wheat was sown on October 10, 2012 and October 12, 2013 and harvested on May 26, 2013 and June 3, 2014. During the elongation stage (which corresponds with growth stage 30 BBCH) and harvest stage (which corresponds with 89 BBHC) (Lancashire et al. 1991) in 2013 and 2014, the aboveground portions of ten wheat plants were collected from each plot. All samples were dried for 0.5 h at 105°C before drying in an oven at 60°C until a constant mass was achieved to prepare the samples for subsequent nutrient content analyses. After the dry mass was measured in two stages, the samples were broken down into two parts at the harvest stage: grain and straw. The C, N and P contents in the wheat and soil were assayed as reported by Bao (2000).

Calculations and statistics. The ET of winter wheat was calculated using the following equation:

$$ET = \Delta S + P + I - R - D \quad (1)$$

Where: ΔS – change in soil water storage; P – precipitation; I – irrigation rate; R – surface runoff; D – deep water percolation (all in mm). Because no irrigation was used, I = 0. Precipitation during the growing season of 2013 was 238.9 mm and 2014 was 287 mm, with negligible surface runoff. At the study site, deep-water percolation did not occur.

The WUE was defined as follows:

$$WUE = Y/ET \quad (2)$$

Where: Y – grain yield (kg/ha).

The wheat growth rate was calculated based on the following model that was reported by Ågren (2004):

$$r = [\ln(M2) - \ln(M1)]/t \quad (3)$$

Where: $\ln(M_2)$, $\ln(M_1)$ – natural logarithms of plant dry mass at the end (harvest stage) and beginning (elongation stage) of the experiment; t – duration of the experiment, a t of 63 days was used in this study.

Table 1. Basic information of soil physical and chemical properties at 0–20 cm layer in the study site before fertilization

Property	Value
Taxonomy	Eum-Orthic Anthrosols
Texture	
2000–50 μm (g/kg)	64
50–2 μm (g/kg)	694
< 2 μm (g/kg)	342
Bulk density (g/cm^3)	1.23
pH	8.25
Water holding capacity (%)	23.60
Available nitrogen (mg/kg)	25.10
Available phosphorus (mg/kg)	7.90

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We calculated the response ratios (RR) to reflect changes in the C, N, P content, C:N, C:P, and N:P ratios, the growth rate, grain yields (Y) and WUE between the non-N and N treatments. The RR is defined as the ratio of the mean value of a given variable in the experimental treatment (X_E) to that in the control treatment (X_C) (Hedges et al. 1999):

$$RR = \ln(X_E) - \ln(X_C) \quad (4)$$

We used Wilcoxon signed rank tests to determine if the mean response ratios were significantly different than zero.

After data processing, two years of data showed similar trends; thus, we averaged the data for further analysis. A one-way ANOVA was performed to test for statistical significance, and significant differences were evaluated at the 95% confidence level. We used Pearson's correlation coefficients to test for associations between the C:N:P stoichiometry and the other traits. Linear regressions were used to analyze the relationships between nutrients and WUE. All statistical analyses were conducted using the SPSS 17.0 software (SPSS Inc., Chicago, USA).

RESULTS

Growth rates, grain yields, WUE and C:N:P stoichiometry of wheat under different N fertilization rates. The addition of N fertilizer significantly increased the growth rates, grain yield and WUE in wheat. However, no significant differences in growth rate or WUE were observed between the N180 and N360 treatments (Table 2). The C contents showed no differences in grain or straw at the harvest stage, and the N contents in the grain and straw significantly increased with N fertilization. However, no significant differences were observed between the N180 and N360 treatments. In contrast, the P content significantly decreased with N fertilization, and no significant differences were found between the N180 and N360 treatments (Figures 1a,b,c). The C:N ratio was significantly higher in the N0 treatment than in the N180 and N360 treatments for the same plant part, and no significant difference was observed between the N180 and N360 treatments (Figures 1d,e,f). The trend of the C:P ratio contrasts that of the C:N ratio, with significant differences observed under different N treatments in each plant part. The N:P ratio displayed a trend that was similar to that of the C:P ratio, and the highest N:P ratio was observed in the straw in the N360 treatment.

Response ratios of the C, N, P content and stoichiometry, growth ratio, yield and WUE under different N fertilization rates. The largest response ratio was detected between the N360 and N0 treatments, and the smallest response ratio was obtained between the N360 and N180 treatments (Figure 2). The absolute value of response ratios for all of the traits recorded in the three treatments were significantly greater than zero between N180 and N0 and between N360 and N0, except for the C content. In all three treatments, the P content, C:P and N:P ratios in the straw were significantly higher than those in the grain. Compared with the N0 treatment, the response ratios of C:P and N:P in N180 were significantly greater than those of the other traits (Figure 2a). N:P ratio in the N360 treatment was significantly greater compared with the N0 treatment. However, compared with N180, the N360 treatment was not significantly different regarding the P content and C:P and N:P ratios, which were greater than the other traits.

Relationships between the C:N:P stoichiometry and other traits. The growth rate, yield and WUE are strongly and significantly correlated with the C:N:P stoichiometry and N, P content (Table 3).

Table 2. Evapotranspiration, growth rate, grain yield and water use efficiency (WUE) of wheat under three nitrogen (N) treatments

Nitrogen (kg N/ha)	Soil water consumption (mm)	Evapotranspiration (mm)	Growth rate (g/g/day)	Grain yield (kg/ha)	WUE (kg/m^3)
0	243.47 ± 6.9	482.37 ± 6.9	0.028 ± 0.0007^b	2223 ± 86^c	0.44 ± 0.006^b
180	279.21 ± 13.2	518.11 ± 13.2	0.032 ± 0.0010^a	5012 ± 87^b	0.97 ± 0.021^a
360	297.28 ± 21.8	536.18 ± 21.8	0.034 ± 0.0020^a	6026 ± 193^a	1.12 ± 0.050^a

The presented values are the means of six replicates from two year data (3-replicate plots per year) with standard errors for each treatment. Different letters within the same column indicate statistical significance at $P < 0.05$

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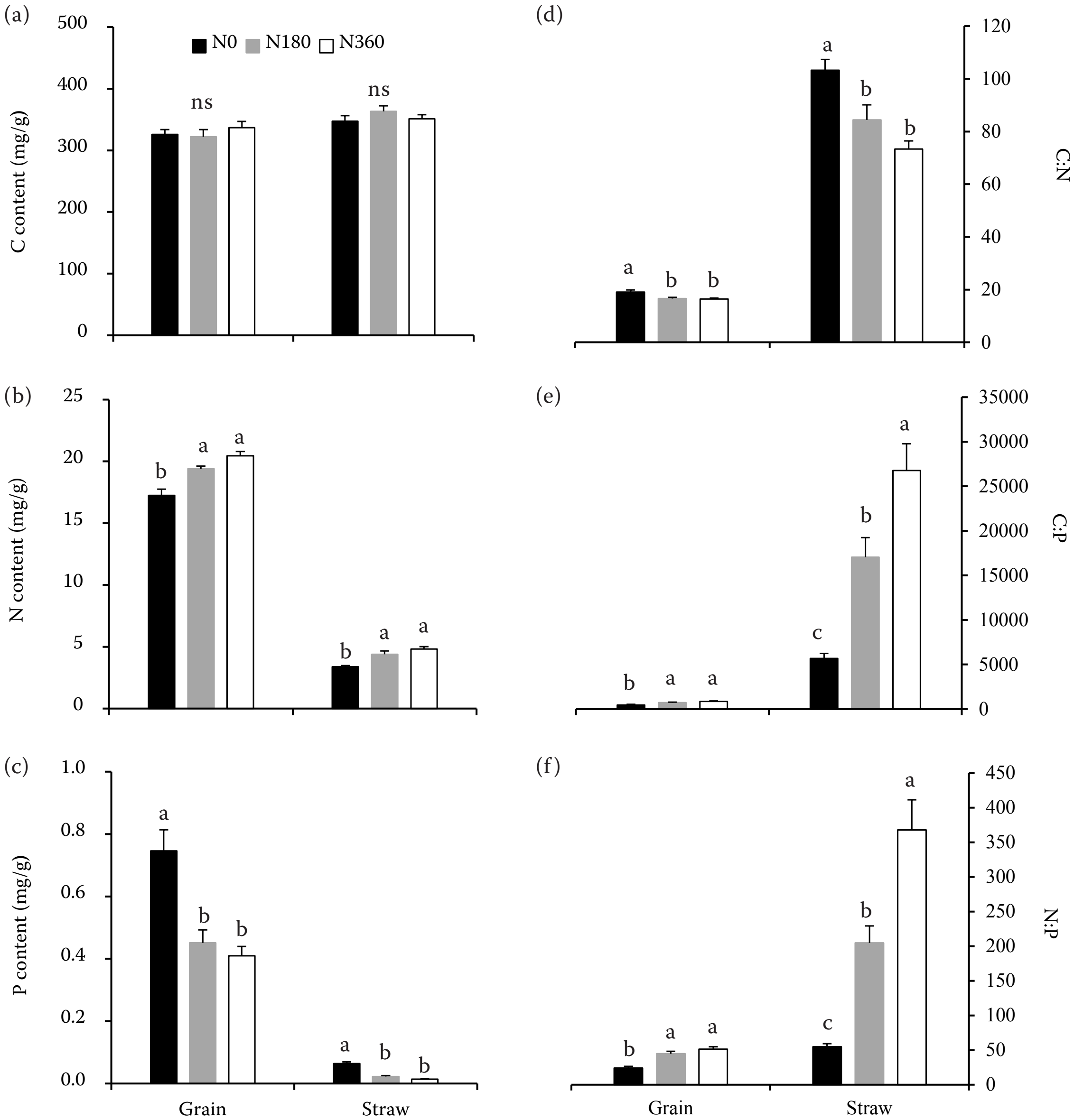

Figure 1. Effects of nitrogen (N) fertilization on the carbon (C), nitrogen and phosphorus (P) contents and stoichiometry patterns of wheat. Values are the means of six replicates from two year data (3-replicate plots per year) with standard errors. Different letters indicate statistical significance at $P < 0.05$ in different N treatments. N0 – 0, N180 – 180, N360 – 360 kg N/ha; ns – not significant

The N content showed a positive correlation with yield and WUE, and P content showed a negative correlation. The strongest relationships between the WUE and stoichiometry were found for the C:P and N:P ratios in straw. The C:N:P stoichiometry between the plant organs was also significantly related, except for the C:N ratios in the grain with straw and the C:P ratio in the grain. The N:P ratios in the grain ($y = 9.6 + 35.7x$, $P < 0.0001$) and straw ($y = -115 + 381x$, $P < 0.0001$) were linearly and positively correlated with WUE (Figure 3) and explained 71% and 66% of the WUE, respectively.

DISCUSSION

As expected, N fertilization significantly increased the growth rate, yield and WUE of wheat. In the N180 and N360 treatments, ET increased by 7.33% and 11.16% and yield increased by 125% and 171% (Table 2), respectively, which significantly enhanced the WUE of wheat. However, the growth rate and WUE were not significantly different between the N180 and N360 treatments. These results confirmed the findings obtained in previous studies that showed N fertilization

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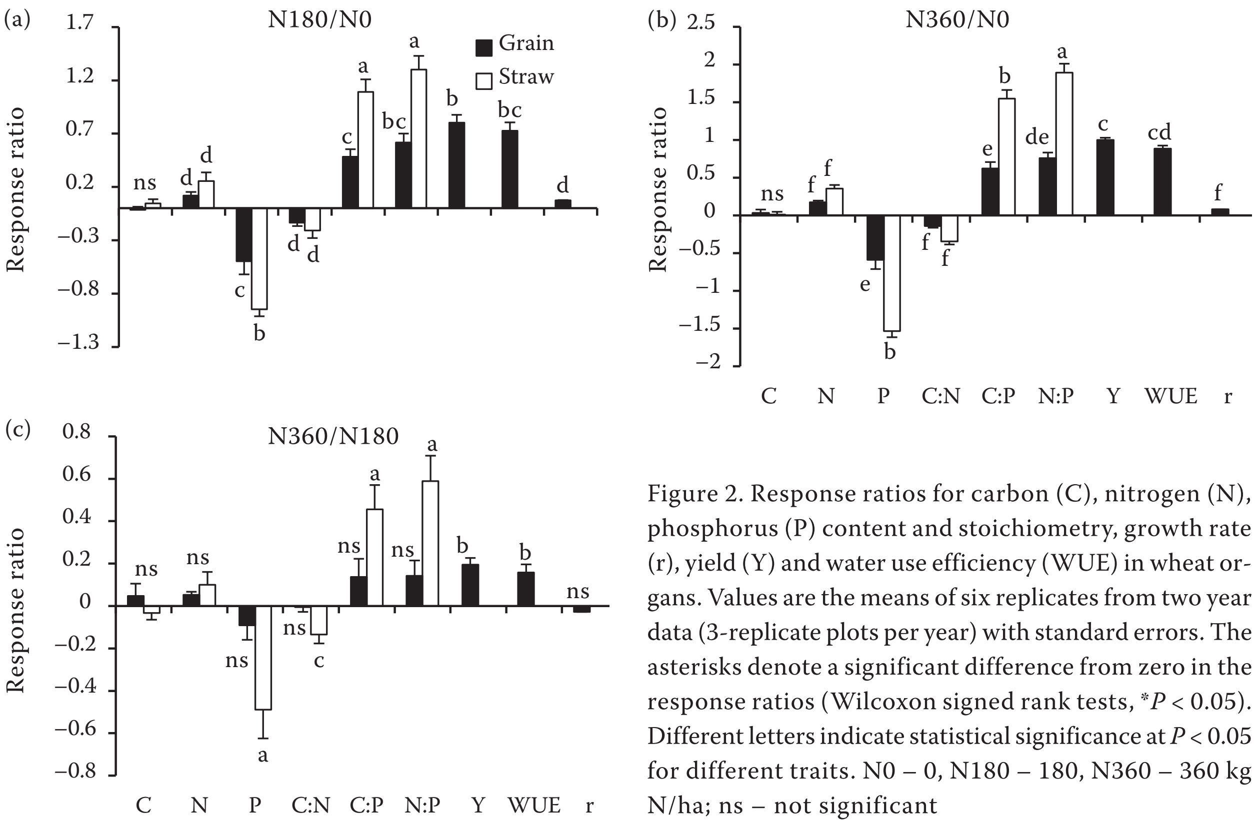

Figure 2. Response ratios for carbon (C), nitrogen (N), phosphorus (P) content and stoichiometry, growth rate (r), yield (Y) and water use efficiency (WUE) in wheat organs. Values are the means of six replicates from two year data (3-replicate plots per year) with standard errors. The asterisks denote a significant difference from zero in the response ratios (Wilcoxon signed rank tests, *$P < 0.05$). Different letters indicate statistical significance at $P < 0.05$ for different traits. N0 – 0, N180 – 180, N360 – 360 kg N/ha; ns – not significant

can increase crop growth and WUE and that applying too much N has no benefit (Zhong and Shangguan 2014). The C contents were consistent in different parts of wheat, and the N and P contents were higher in the wheat grains than in the straw. These results indicate that the C contents are more stable in wheat than N and P, primarily because C is the main basic element present in the

Table 3. Correlation matrix (*r* value) for the growth rate (r), yield, water use efficiency (WUE) and carbon (C), nitrogen (N), phosphorus (P) contents and stoichiometry of wheat

		r	Yield	WUE	C		N		P		C:N		C:P		N:P
					grain	straw	grain	straw	grain	straw	grain	straw	grain	straw	grain
Yield		0.58*													
WUE		0.58*	0.99**												
C	grain	−0.08	0.08	−0.02											
	straw	0.29	0.00	−0.03	0.31										
N	grain	0.70**	0.73**	0.69**	0.20	0.18									
	straw	0.68**	0.70**	0.65**	0.38	0.12	0.79**								
P	grain	−0.45	−0.80**	−0.78**	−0.11	−0.40	−0.56*	−0.59**							
	straw	−0.64**	−.90**	−0.85**	−0.16	−0.33	−0.74**	−0.74**	0.87**						
C:N	grain	−0.67**	−0.57*	−0.59**	0.52*	0.08	−0.73**	−0.4	0.38	0.50*					
	straw	−0.61**	−0.72**	−0.69**	−0.26	0.16	−0.76**	−0.95**	0.49*	0.65**	0.43				
C:P	grain	0.38	0.80**	0.77**	0.33	0.38	0.59**	0.62**	−0.93**	−0.79**	−0.27	−0.52*			
	straw	0.60**	0.90**	0.89**	0.14	0.12	0.74**	0.64**	−0.67**	−0.87**	−0.52*	−0.65**	0.79**		
N:P	grain	0.52*	0.87**	0.84**	0.15	0.32	0.72**	0.66**	−0.95**	−0.84**	−0.51*	−0.58*	0.96**	0.85**	
	straw	0.64**	0.92**	0.90**	0.17	0.02	0.75**	0.76**	−0.63**	−0.85**	−0.53*	−0.77**	0.78**	0.98**	0.84**

*$P < 0.05$; **$P < 0.01$

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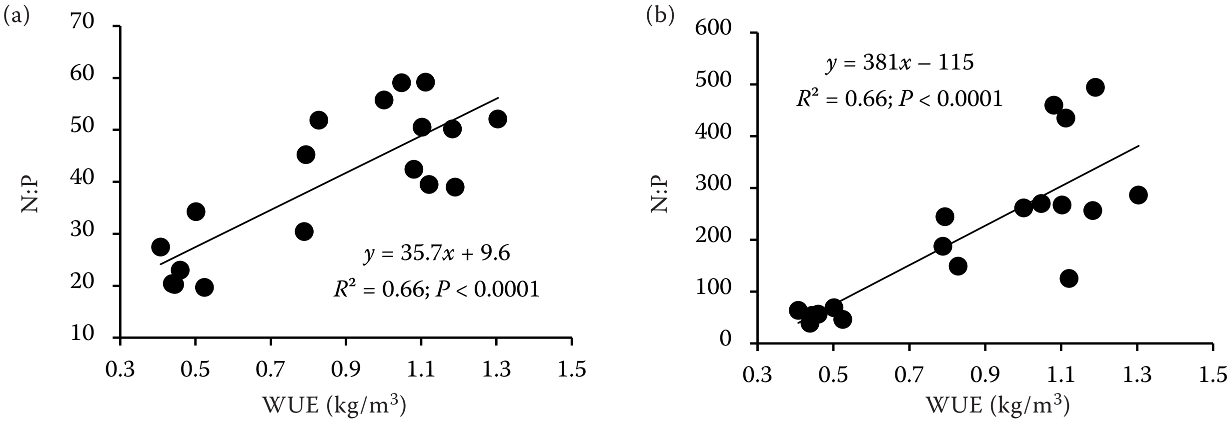

Figure 3. Relationships of nitrogen:phosphorus (N:P) with water use efficiency (WUE) in grain and straw. The linear relationship between N:P ratio and WUE in grain (a) and straw (b) are shown by the equation (n = 18)

plant. The C content remains relatively stable in the ecosystem, as reported by Yang et al. (2011). Fertilization with N significantly increased the N content of the wheat, which corresponded with the results of Delogu et al. (1998), who indicated that N fertilization increased the uptake of N by wheat. The N content of the grain significantly increased as the N application was increased, but the P content remained significantly lower in the N treatments in both parts of the plant, mainly because the P concentration decreased with increasing N fertilization rates (Ziadi et al. 2008).

According to our results, N:P response ratio was higher than the other response ratios, which indicated that N:P ratio was more sensitive to N treatment. This could be explained by the increased N content and decreased P content in wheat when N was added. And the decreased P content may be related with the general soil characteristic that low available P content and high soil pH in this study area, however, these effects need a further study. The C:N ratio was less sensitive to N fertilization due to the stability of C. Overall, C provides the structural basis of plants and accounts for 50% of a plant's dry mass. Consequently, C is less influenced by N fertilization. The higher C:P ratio mainly resulted from differences in P in the N treatments. Therefore, we concluded that the N:P ratio was the most sensitive of the examined N treatment ratios. When we tested the relationships between the N and P contents (Figure 4), the P content was a function of N, and the absolute value of the slope (2.13 for grain and 2.79 for straw) was greater than 1. This result indicates that the P content changes faster than the N content and agrees with the patterns observed by Güsewell (2004), who suggested that the leaf N:P ratio primarily involves variations in leaf P rather than leaf N.

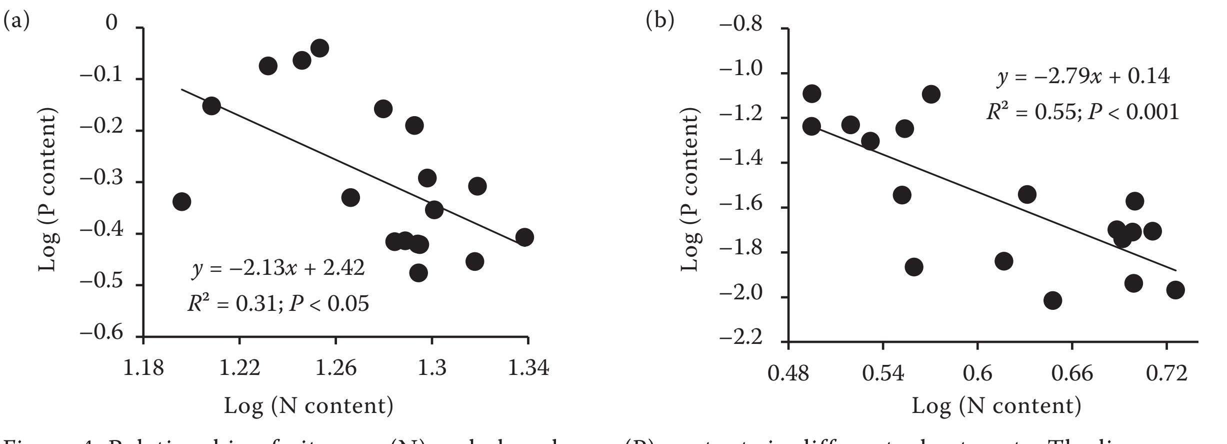

Figure 4. Relationship of nitrogen (N) and phosphorus (P) contents in different wheat parts. The linear regression equations for the N and P contents in grain (a) and straw (b) of wheat are shown (n = 18)

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The C:N:P stoichiometry is strongly and significantly correlated with crop yields, WUE and growth ($P < 0.01$), and the strongest relationships between the growth rate and WUE regarding the C:N:P stoichiometry were found for the C:P and N:P ratios in the straw (Table 3). The regression analysis results for the N:P ratios in the grain and straw and the WUE (Figure 3) are consistent with the findings of Cernusak et al. (2010), who demonstrated that the N:P ratio is strongly and positively related to WUE. However, negative correlations with the leaf N:P ratios were observed previously in some vascular plant species (Güsewell 2004, Cernusak et al. 2010). This difference potentially resulted from the application of N and different soil nutrition conditions, which resulted in a greater N:P ratio in this study.

In conclusion, the growth rate and WUE of wheat significantly increased with N fertilization and were strongly correlated with the N and P contents and C:N:P ratio. N fertilization increased the N content and decreased the P content in wheat. The N:P ratio was positively correlated with WUE, and its response ratio in straw was higher than that of the other investigated traits, which suggests that N:P could be used as an index to evaluate plant growth. Moreover, our findings built the relationships between C:N:P nutrition pattern to wheat WUE under N addition.

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