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Interspecific interactions affect N and P uptake rather than N:P ratios of plant species: evidence from intercropping

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Abstract

Quantifying stoichiometry of crop N and P acquisition (i.e. removal from farmland) under different agronomic practices is essential for understanding nutrient budgets and optimizing N and P fertilizer application in agroecosystems. It is not clear how plant N and P uptake and N:P stoichiometry vary between monoculture and intercropping throughout an entire growing season under different N fertilization and mulching practices. Here, we addressed how plant interspecific competition for nutrients have affected the temporal dynamics of crop N and P uptake (and N:P ratios) in five cropping systems (wheat, maize and barley monocultures, and wheat/maize and barley/maize intercropping), under two N levels (0 and 225 kg N ha^{-1}) and two maize mulching treatments (with and without). Wheat and barley had greater N and P competitive ability than maize, leading to an increase in N and P uptake of wheat and barley and a decrease in N and P uptake of maize during co-growth stages in intercropping. N:P ratios of three crop species decreased with plant growth. Crop-level N:P ratios were either not affected by intercropping or did not change consistently with N fertilization while film mulching decreased maize N:P ratios. Community-level N:P ratios of the two intercrops were different from those of the corresponding monoculture at maturity. Because (i) the proportion of N and P removal from intercropping differs from monocultures, and (ii) N and P uptake by crops is decoupled under N fertilization and mulching, these findings may have practical implications for the nutrient budget of intercropping systems.

Keywords ecological stoichiometry, growth-rate hypothesis, intercropping system, interspecific competition, nitrogen addition, nutrient budget, plastic film mulch

种间相互作用影响植物氮和磷的吸收量而不是氮磷比

摘要：量化不同农艺措施下作物氮和磷吸收量(即从农田中移除的量)的化学计量特征对理解农田生态系统中的养分收支和优化氮、磷肥施用至关重要。目前还不清楚在不同的氮肥和覆膜措施下，单作和间作体系作物氮和磷吸收量以及氮磷化学计量特征随整个生长季的变化。本研究探讨了植物种间养分竞争如何对(1) 5种种植模式(小麦、玉米和大麦单作、小麦/玉米和大麦/玉米间作)，(2)两种施氮水平(0和225 kg

N ha⁻¹)和(3)两种玉米覆膜处理(覆膜和不覆膜)下的作物氮、磷吸收量(以及氮磷比)时间动态的影响。研究结果表明，小麦和大麦的氮、磷竞争能力强于玉米，导致间作体系共生期的小麦和大麦氮、磷吸收量相比于单作增加，而玉米氮、磷吸收量相比于单作减少。3种作物植株氮磷比随作物生长而降低。作物氮磷比不受间作的影响，也不随施氮呈现一致的变化，覆膜降低了玉米的氮磷比。两种间作体系群落水平的氮磷比在成熟期与相应单作不同。由于(1)间作从土壤移除的氮和磷的比例不同于单作，以及(2)作物对氮和磷的吸收在施氮和覆膜下均是不耦合的，这些发现可能对间作系统的养分收支有启示意义。

关键词：生态化学计量学，生长速率假说，间作系统，种间竞争，氮添加，养分收支，覆膜

INTRODUCTION

Nitrogen (N) and phosphorus (P) are the two most frequent limiting nutrients for plant growth in terrestrial ecosystems (Marschner 2011). N and P support key physiological functions in plants (Marschner 2011), and their relative abundance in plant tissues (i.e. N:P stoichiometry) plays a vital role in regulating ecological processes, including primary production (Elser *et al.* 2007), competitive interactions (Venterink and Güsewell 2010) and nutrient cycling (Peñuelas *et al.* 2013; Yu *et al.* 2021). However, our understanding of N:P stoichiometry across agroecosystems remains limited. Because nutrients are continuously removed with harvested crops from agroecosystems, new fertilizer inputs are needed to avoid soil nutrient deficiency. This however has led to unparalleled inputs of anthropogenic N and P fertilizers, which have caused a strong N–P imbalance in agroecosystems with profound effects on crop nutrient uptake and production (Peng *et al.* 2019; Peñuelas *et al.* 2013). Changes in crop N and P concentrations and N:P ratios under different management are often used to detect levels of N and P co-limitation and nutrient use efficiency (Greenwood *et al.* 2008; Güsewell 2004; van der Velde *et al.* 2014). This information on changes in crop N:P ratios may inform on what N:P ratios of fertilizer inputs are required to balance nutrient inputs and outputs (Maltais-Landry *et al.* 2016). Therefore, a better understanding of crop N and P uptake and N:P stoichiometry is essential to estimate nutrient budgets and optimize nutrient management in sustainable agroecosystems.

Intercropping, the simultaneous or sequential cultivation of at least two crops on the same land, has been long practiced in many different regions worldwide (Vandermeer 1992). Compared with monoculture, intercropping can produce significantly higher yields at lower environmental costs (Lithourgidis *et al.* 2011). Interspecific interactions play an important role on yield advantage of intercropping (Li *et al.* 2013). For example, faba bean (*Vicia faba*) tends to mobilize more inorganic P and organic P in soils, and facilitate P uptake by maize (*Zea mays* L.) in faba bean/maize intercropping (Li *et al.* 2007). Moreover, different crop species may have different nutrient requirements in time, space or quantity, which allows a more general efficient use of nutrients as a whole in intercropping (Zhang *et al.* 2017). For instance, despite strong competition for nutrients in wheat/soybean intercropping, there is a yield advantage of whole intercropping when the co-growth period of two crops is limited and soybean is released from competition with wheat at later growth stages (Li *et al.* 2001b). The nutrient capture of intercropped wheat and soybean is partitioned across a temporal scale, this temporal niche partitioning reduces interspecific competition and increases total nutrient capture of wheat/soybean intercropping (Li *et al.* 2001a, 2001b; Zhang *et al.* 2017).

Interspecific competition and facilitation can change plant growth rates (Zhang *et al.* 2015) and influence biomass allocation among plant organs (Zhang *et al.* 2013). The growth-rate hypothesis predicts that higher growth rates of organisms are related to higher [P] and lower N:P ratios (Elser *et al.* 2003). Changes of growth rate may influence plant N:P ratios in intercropping systems (Sterner and Elser 2002). In addition, changes in biomass allocation among organs may shift nutrient stoichiometry at the whole-plant level (Ågren 2008). However, it is still unclear whether interspecific interactions influence N:P ratios of crops in intercropping or whether crops show stoichiometric homeostasis.

N fertilization generally increases plant [N] and N:P ratios (Yuan and Chen 2015). However, evidence from empirical studies shows variable results with N additions not having any significant effect on plant N:P ratios (Kozovits *et al.* 2007; Novotny *et al.* 2007). Plastic film mulching is also widely practiced in dryland areas, because it can significantly increase yields by conserving soil warming and moisture (Zhang *et al.* 2019). Experimental and meta-analysis studies showed that plant N:P ratios can decrease with increasing soil-water content (Cernusak *et al.* 2010; Yuan and Chen 2015). A recent study also indicated that nitrogen fertilization and plastic film mulching showed opposite effects on crop N:P stoichiometry in a temperate agroecosystem, with N fertilization increasing, whereas film mulching decreasing crop N:P ratios (Ding *et al.* 2019). It remains unclear, however, whether N fertilization increases and film mulching decreases crop N:P ratios in intercropping systems.

Changes in plant N:P ratios in most studies are usually estimated only once or twice across the growing season (Elser *et al.* 2007; Novotny *et al.* 2007), and this limits our understanding of what potential biotic and abiotic factors might affect plant N:P stoichiometry (Ågren 2008). Plant [N], [P] and N:P ratios at the whole-plant level may shift with plant growth due to changes in biomass allocation among organs (Ågren 2008). For example, plant [N] and [P] decrease with plant growth because of (i) increases in non-photosynthetic/photosynthetic organ ratios, and (ii) increasing self-shading of leaves in lower position (Ziadi *et al.* 2008b). In addition, plant growth rate varies with age and size of individual plants (Zhang *et al.* 2015), thus potentially affecting N:P stoichiometry during ontogenetic development according to the growth-rate hypothesis (Ågren 2008; Elser *et al.* 2003).

Here, we hypothesized that (i) differences in interspecific competitive ability for nutrients would change crop N:P ratios, (ii) N fertilization would increase crop N:P ratios and (iii) film mulching would decrease crop N:P ratios in intercropping. To test these hypotheses, we (i) addressed how differences in plant interspecific competitive ability might affect the temporal dynamics of crop N and P uptake in wheat/maize and barley/maize intercropping, (ii) determine the temporal dynamics of [N], [P] and N:P ratios of three crop species throughout the entire growing season and (iii) identify the potential effects of N fertilization and film mulching on temporal dynamics of N:P stoichiometry throughout the growing season.

MATERIALS AND METHODS

Site description

The experiment was carried out in 2003 at the Baiyun Experimental Station (38°37′ N, 102°40′ E) in Gansu Province, China. Mean annual temperature is 7.7 °C at the site, mean annual precipitation 150 mm and potential evaporation 2021 mm. There are 170–180 days of frost-free period, and 5988 MJ m^{-2} $year^{-1}$ of total solar radiation. Before sowing, soil bulk density was 1.40 g cm^{-3}, pH was 8.8 and organic matter, total N, Olsen P and exchangeable-K contents in the top soil layer (0–20 cm) were 19.9, 1.18 g kg^{-1}, 17.3 and 233 mg kg^{-1}, respectively.

Experimental design

A three-factor experiment was performed with a randomized block design. The first experimental factor comprised five cropping systems: (i) monocultured maize (*Z. mays* L. cv. Zhongdan No. 2), (ii) monocultured wheat (*Triticum aestivum* L. cv. Long No.17), (iii) monocultured barley (*Hordeum vulgare* L. cv. Ganpi No. 3), (iv) intercropped wheat/maize and (v) intercropped barley/maize. All cultivars were commonly used on local farms. N fertilization level (0 and 225 kg N ha^{-1} applied as urea) was the second experimental factor, and mulching for maize only (i.e. maize mulched or not with PE film 80 cm wide × 0.008 mm thick) was the third experimental factor. Mulch was only used on maize strips in maize-related cropping systems (i.e. monoculture maize, wheat/maize and barley/maize intercropping), and was not used for monoculture wheat or barley according to local farmer's practice. The experiment was laid out as a 3-factorial design with a total of 16 treatments, including 5 (cropping systems) × 2 (N levels) + 3 (maize-related systems) × 2 (mulching treatments) (Table 1). Each treatment had 3 replicates, and the experiment included 48 experimental plots. Each plot was 5.5 m × 4.5 m.

Crop management and sample collection

The experiment had 24 intercropped plots each consisting of 3 alternating strips (1.5 m wide). Two maize rows alternating with six wheat or barley rows were cultivated in each strip (Supplementary Fig. S1). All intercropped combinations were arranged in a design where the density of a crop within an intercropping strip was the same as the corresponding monoculture on an equivalent land area (Supplementary Fig. S1). Densities were 8.55 × 10^4 plants ha^{-1} for maize and 7.50 × 10^6 plants ha^{-1} for wheat and barley.

Table 1: Experimental treatments for wheat, barley and maize in both monoculture and intercropping

	N rate (kg ha^{-1})					
Cropping	**24/3**	**5/5**	**25/5**	**23/7**	**Total**	**Film mulching**
Monocultured wheat	0	0	0	0	0	None
Monocultured barley	0	0	0	0	0	None
Wheat/maize	0	0	0	0	0	None
Barley/maize	0	0	0	0	0	None
Monocultured maize	0	0	0	0	0	None
Wheat/maize	0	0	0	0	0	Mulched maize
Barley/maize	0	0	0	0	0	Mulched maize
Monocultured maize	0	0	0	0	0	Mulched maize
Monocultured wheat	112.5	112.5	0	0	225	None
Monocultured barley	112.5	112.5	0	0	225	None
Wheat/maize	112.5	0	56.25	56.25	225	None
Barley/maize	112.5	0	56.25	56.25	225	None
Monocultured maize	112.5	0	56.25	56.25	225	None
Wheat/maize	112.5	0	56.25	56.25	225	Mulched maize
Barley/maize	112.5	0	56.25	56.25	225	Mulched maize
Monocultured maize	112.5	0	56.25	56.25	225	Mulched maize

Note: N fertilizer was applied four times between March and July 2003.

Dates of sowing were 26 March for wheat and barley, and 21 April for maize. The emergence dates were 8 April for wheat and barley, and 1–3 May for maize. N fertilizer was applied twice to wheat and barley monoculture, and three times to maize monoculture and intercropping according to local farmer's practice (Table 1). Each plot received an identical fertilization of 90 kg P_2O_5 ha^{-1}, applied as triple superphosphate. The film mulch for the mulching treatment was applied to the maize rows after sowing, and the film was cut where the maize was planted to ensure growth. All plots were flood irrigated seven times during whole season on 5 May, 25 May, 10 June, 30 June, 23 July, 5 August and 27 August, with an amount of 700 m^3 ha^{-1} each time. All plots were weeded manually over the growing season.

Wheat and barley in both monoculture and intercropping were sampled six times at intervals of 14 days: 8 May, 24 May, 7 June, 21 June, 5 July and 21 July. Maize plants matured at different times for the different treatments, and were sampled 8–10 times at intervals of 14 days: 24 May, 7 June, 21 June, 5 July, 21 July, 5 August, 19 August, 2 September, and the last one or two times were between 5 September and 20 September for different treatments. A subplot (0.3 m long × 1.5 m wide) was placed within one experimental plot at each harvest time, except for the first sampling of maize. Each subplot was 0.3 m distant from the previous subplot. Wheat and barley individuals were sampled within four rows for the monocultures and within six rows for the intercrops in the 0.45 m^2 subplot each time. Four maize plants (intercropped and monocultured) were sampled in the 0.45 m^2 subplots during the growth period, except 10 maize plants were randomly collected at the first sampling time. The whole aboveground plant biomass of three crop species was collected at each sampling. The micro-Kjeldahl method and the vanadomolybdate procedure were used to measure [N] and [P] of whole aboveground plant, respectively (Bao 2000). N (or P) uptake was calculated as the product between aboveground biomass and N (or P) concentration.

Calculations

Nutrient competitive ratio

Nutrient competitive ratio (NCR) was used to measure competitive ability in nutrient uptake by one species over the other in intercropping (Morris and Garrity 1993):

$$NCR_{ab} = RNU_a/RNU_b = \left(\frac{NU_{ia}}{NU_{sa}}\right) / \left(\frac{NU_{ib}}{NU_{sb}}\right) \quad (1)$$

Relative nutrient uptake (RNU) was defined as ratios of nutrient uptake by a crop species in intercropping to that in monoculture. Where NU_{sa} and NU_{sb} are N or P uptakes by crop 'a' and 'b' in monoculture, NU_{ia} and NU_{ib} are N or P uptakes by crop 'a' and 'b' in intercropping, respectively. Nutrient uptake by a crop species in intercropping and monoculture were based on the area actually occupied by this crop species (i.e. the same density). If RNU is greater than 1, nutrient uptake by a crop species is greater in intercropping than in monoculture. If NCR_{ab} is greater than 1, nutrient competitive ability by crop 'a' is greater than crop 'b' in intercropping.

Community-level N, P concentrations and N:P ratios at maturity

Community-level N and P concentration of intercropping systems were calculated as ratios of total N and P uptake to total biomass at maturity as follows:

$$\text{Community-level [N]} = \frac{[N]_{ia} \times B_{ia} \times F_a + [N]_{ib} \times B_{ib} \times F_b}{B_{ia} \times F_a + B_{ib} \times F_b} \quad (2)$$

$$\text{Community-level [P]} = \frac{[P]_{ia} \times B_{ia} \times F_a + [P]_{ib} \times B_{ib} \times F_b}{B_{ia} \times F_a + B_{ib} \times F_b} \quad (3)$$

where $[N]_{ia}$ and $[N]_{ib}$ are N concentration of crop 'a' and 'b' at maturity in intercropping, $[P]_{ia}$ and $[P]_{ib}$ are P concentration of crop 'a' and 'b' at maturity in intercropping, F_a and F_b are the proportions of the area occupied by crop species 'a' and 'b' in intercropping, and B_{ia} and B_{ib} are biomass of crop 'a' and 'b' at maturity in intercropping, respectively. Community-level N:P ratios were calculated as community-level [N] divided by community-level [P] at maturity.

Statistical analyses

Generalized linear mixed-effect models were used to test for potential effects of sampling date and N application on N and P competitive ratios (NCR_N and NCR_P) of wheat and barley relative to maize during co-growth stages. The values of NCR_N and NCR_P at different sampling dates were treated as repeated observations, with sampling date and N application as fixed factors and block as a random factor.

Mixed-effect models were also used to test for potential effects of sampling date and N application on relative N and P uptake (RNU_N and RNU_P) of wheat and barley. The values of RNU_N and RNU_P at different sampling dates were treated as repeated observations, with sampling date and N application as fixed factors and block as a random factor. The same approach was used for RNU_N and RNU_P of maize where the RNU_N and RNU_P measured at different sampling dates were treated as repeated observations, with sampling date, N application and film mulching as fixed factors, and block as a random factor.

Mixed-effect models were used to test for potential effects of sampling date, N application and cropping system on [N], [P] and N:P ratios of wheat and barley. The values of the three dependent variables measured at different sampling dates were treated as repeated observations, with sampling date, N application and cropping system as fixed factors, and block as a random factor. Mixed models were performed separately on variables measured in wheat and barley. The same method was used for maize where the three variables measured at different sampling dates were treated as repeated observations, with sampling date, N application, film mulching and cropping system as fixed factors, and block as a random factor.

We used *t*-tests to evaluate whether NCR_N, NCR_P, RNU_N and RNU_P at each sampling date differed significantly from 1. To test whether the [N], [P] and N:P differed among community-level at maturity, we performed one-way ANOVA analysis, and Tukey HSD test in *post hoc* multiple comparisons. All analyses were performed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA).

RESULTS

N and P competitive ratios, and relative N and P uptake

The N and P competitive ratios (NCR_N and NCR_P) of wheat (and barley) relative to maize always equaled or exceeded 1 during co-growth of two species in wheat/maize and barley/maize intercropping (Fig. 1). N fertilizer reduced the NCR_N and NCR_P of both wheat and barley relative to maize (Fig. 1; Supplementary Table S1). Relative N and P uptake (RNU_N and RNU_P) of wheat and barley were equal to or greater than 1 during co-growth of two species in the two intercropping systems (Fig. 2). N fertilization did not affect RNU_N and RNU_P of wheat and barley (Fig. 2; Supplementary Table S2).

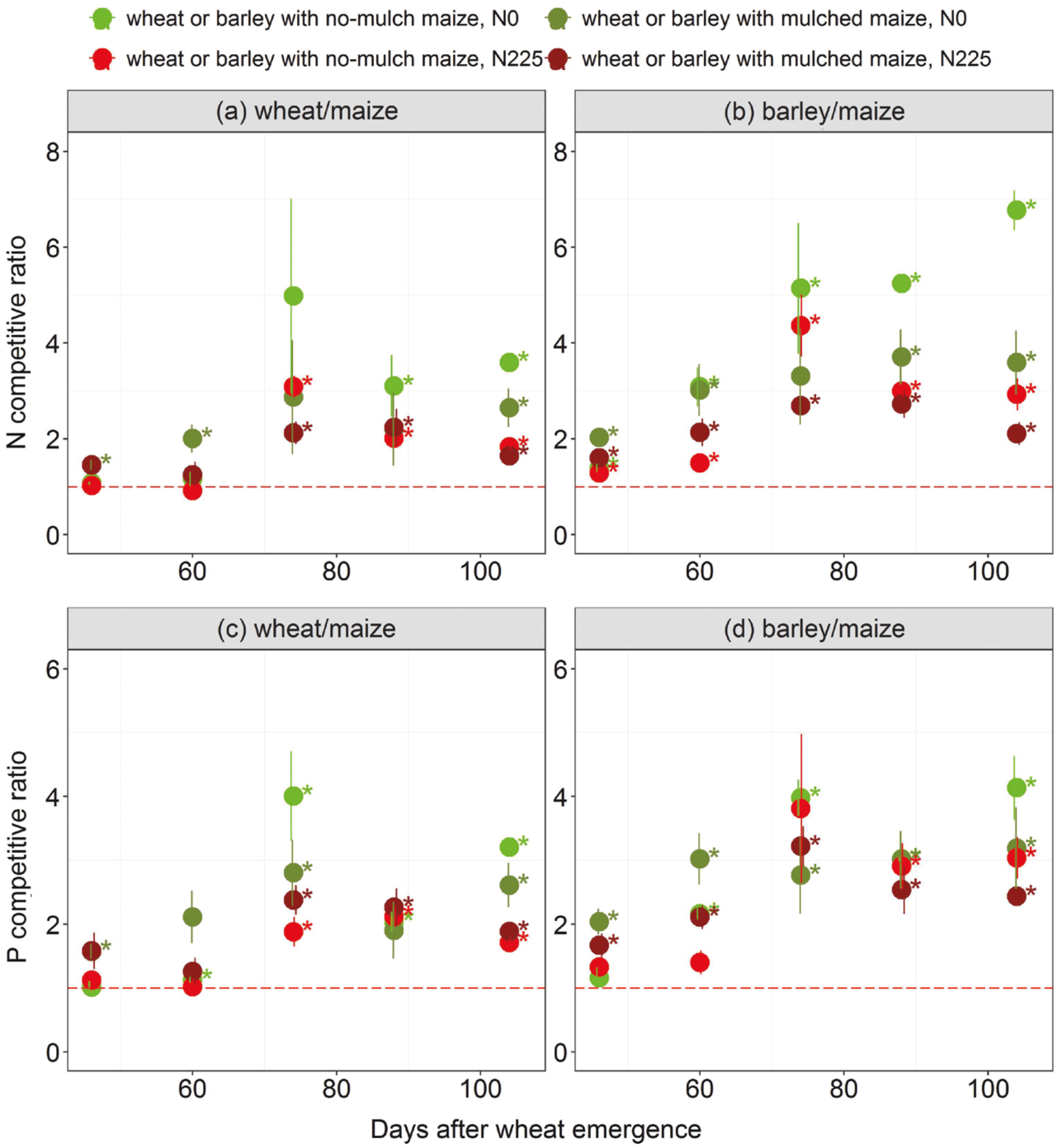

Figure 1: N and P competitive ratios of wheat (or barley) relative to maize during co-growth in wheat/maize (**a**, **c**) and barley/maize intercropping (**b**, **d**). Each data point represents mean ± SE of three replicates. Asterisks beside the points indicate significant differences between N or P competitive ratios and 1 using *t*-test.

Relative N uptake of maize ($RNU_{N\text{-maize}}$) was less than 1 in five or more of the eight treatments from second (7 June) to fifth (21 July) sampling time of maize (Fig. 2). After wheat or barley harvest, $RNU_{N\text{-maize}}$ was greater or close to 1 in three or more of the four treatments for mulched maize (Fig. 2). Relative P uptake of maize ($RNU_{P\text{-maize}}$) was less than 1 in four or more of the eight treatments from second (7 June) to sixth (5 August) sampling time of maize (Fig. 2). Since the seventh sampling event (19 August), $RNU_{P\text{-maize}}$ was closer to 1 in almost all treatments when maize was mulched or fertilized (Fig. 2). N fertilization and mulching increased RNU_N and RNU_P of maize (Fig. 2; Supplementary Table S3).

Temporal dynamics of N:P stoichiometry in aboveground biomass of three crop species

Sampling time significantly affected [N], [P] and N:P ratios of the three crop species (Tables 2 and 3). Specifically, [N] and [P] of wheat, barley and maize decreased with ontogenetic development (Supplementary Figs S2 and S3). Mean N:P ratios of wheat, barley and maize decreased from 15.9 on 8 May to 5.30 on 21 July, from 17.69 on 8 May to 5.96 on 21 July and from 17.65 on 24 May to 2.36 at final harvest, respectively, with plant development (Fig. 3).

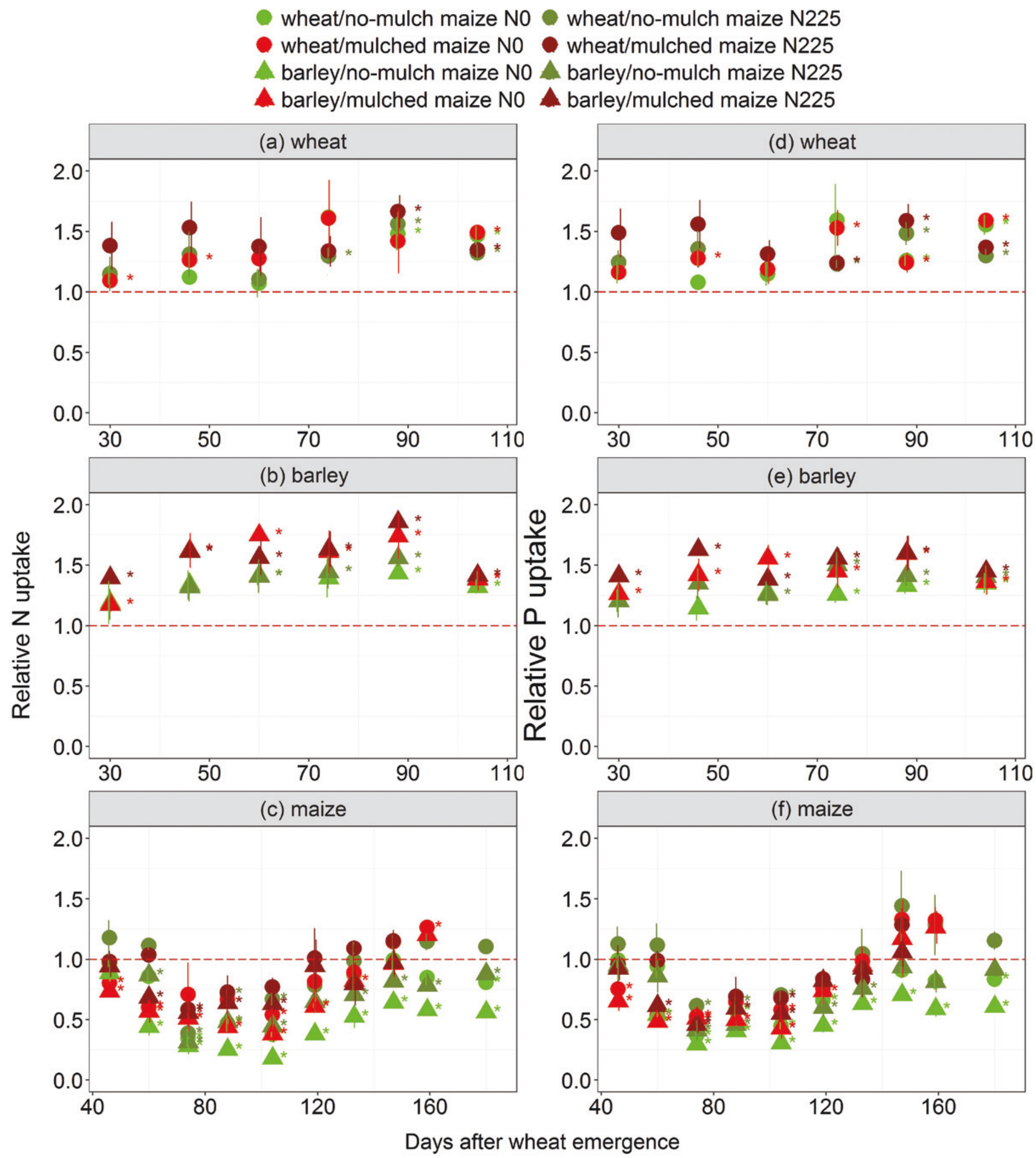

Figure 2: Relative N and P uptake of wheat (**a**, **d**), barley (**b**, **e**) and maize (**c**, **f**) across the growing season. Each data point represents mean ± SE of three replicates. Relative N and P uptake was defined as ratios of N and P uptake by a crop species in intercropping to that in monoculture. Asterisks beside the points indicate significant differences between relative N or P uptake and 1 using *t*-test.

Effects of cropping system, N fertilization and mulching on N:P stoichiometry of three crop species across growth stages

Intercropping significantly increased [N] and [P] whereas it did not influence the N:P ratio of wheat (Table 2). Intercropping significantly increased [N] but did not affect [P] or N:P ratio of barley. Intercropping did not have a significant effect on [N], [P] or N:P ratio of maize (Table 3).

N fertilization increased [N] of wheat, barley and maize (Tables 2 and 3). N fertilization increased [P] of wheat, but not that of barley and maize (Tables 2 and 3). N fertilization increased N:P ratio of barley and maize, but not that of wheat (Tables 2 and 3). Mulching increased [P] while contributed to decrease [N] and N:P of maize (Table 3).

Community-level N, P concentrations and N:P ratios at maturity

Community-level [N] in wheat/maize and barley/maize intercropping at maturity was 8.97% and 8.39% smaller than [N] in corresponding wheat and barley monoculture, respectively, and 25.07% and 29.73% greater than [N] of maize

Table 2: [N], [P] and N:P ratios of wheat and barley plants in response to changes in sampling date (D), N fertilization (N), cropping system (C) and their interactive effects

	Wheat			Barley		
Treatments	**N (%)**	**P (%)**	**N:P**	**N (%)**	**P (%)**	**N:P**
Monoculture, N0	2.053 ± 0.188	0.221 ± 0.010	9.248 ± 0.680	2.396 ± 0.257	0.258 ± 0.013	9.085 ± 0.795
With unmulched maize, N0	2.231 ± 0.222	0.234 ± 0.012	9.329 ± 0.609	2.523 ± 0.282	0.254 ± 0.013	9.599 ± 0.778
With mulched maize, N0	2.206 ± 0.223	0.233 ± 0.013	9.221 ± 0.557	2.593 ± 0.282	0.263 ± 0.014	9.556 ± 0.705
Monoculture, N225	2.324 ± 0.195	0.244 ± 0.010	9.450 ± 0.664	2.738 ± 0.290	0.264 ± 0.014	10.121 ± 0.797
With unmulched maize, N225	2.365 ± 0.224	0.250 ± 0.012	9.269 ± 0.568	2.813 ± 0.290	0.266 ± 0.015	10.269 ± 0.733
With mulched maize, N225	2.389 ± 0.229	0.248 ± 0.013	9.403 ± 0.555	2.869 ± 0.307	0.263 ± 0.016	10.583 ± 0.733
Significance						
Date (D)	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
N fertilization (N)	**0.000**	**0.000**	0.310	**0.000**	0.124	**0.000**
Cropping system (C)	**0.004**	**0.022**	0.734	**0.026**	0.898	0.063
D × N	**0.038**	0.473	0.120	**0.000**	0.175	0.056
D × C	**0.003**	**0.000**	0.119	0.533	**0.004**	0.170
N × C	0.119	0.261	0.585	0.599	0.886	0.646
D × N × C	0.857	0.594	0.742	0.531	0.285	0.402

Values are mean of all samples across growing season ± SE of three replicates. Bold value indicates $P < 0.05$.

Table 3: [N], [P] and N:P ratios of maize plants in response to changes in sampling date (D), N fertilization (N), mulching treatments (M), cropping system (C) and their interactive effects

Treatments	Maize		
	N (%)	P (%)	N:P
No mulch (monoculture, N0)	1.997 ± 0.198	0.235 ± 0.014	8.28 ± 0.584
No mulch (with wheat, N0)	1.918 ± 0.176	0.246 ± 0.014	7.725 ± 0.58
No mulch (with barley, N0)	1.843 ± 0.171	0.269 ± 0.016	6.964 ± 0.593
Mulch (monoculture, N0)	1.823 ± 0.235	0.281 ± 0.022	6.128 ± 0.359
Mulch (with wheat, N0)	1.76 ± 0.207	0.267 ± 0.014	6.269 ± 0.45
Mulch (with barley, N0)	1.712 ± 0.198	0.276 ± 0.015	5.955 ± 0.448
No mulch (monoculture, N225)	2.111 ± 0.19	0.241 ± 0.014	8.593 ± 0.563
No mulch (with wheat, N225)	2.192 ± 0.199	0.27 ± 0.017	8.091 ± 0.585
No mulch (with barley, N225)	2.186 ± 0.193	0.257 ± 0.014	8.326 ± 0.536
Mulch (monoculture, N225)	2.117 ± 0.243	0.299 ± 0.02	6.756 ± 0.453
Mulch (with wheat, N225)	2.105 ± 0.209	0.284 ± 0.014	7.108 ± 0.438
Mulch (with barley, N225)	2.085 ± 0.22	0.285 ± 0.016	7.097 ± 0.503
Significance			
Date (D)	**0.000**	**0.000**	**0.000**
N fertilization (N)	**0.000**	0.349	**0.000**
Mulch (M)	**0.000**	**0.000**	**0.000**
Cropping system (C)	0.881	0.057	0.154
D × N	**0.000**	0.391	**0.002**
D × M	**0.000**	**0.000**	**0.000**
D × C	**0.036**	**0.001**	0.589
N × M	0.981	0.679	0.745
N × C	**0.021**	0.965	0.054
M × C	0.432	**0.001**	**0.000**
D × N × M	0.882	0.343	0.650
D × N × C	**0.004**	0.434	0.162
D × M × C	0.359	**0.003**	0.068
N × M × C	0.455	0.941	0.576
D × N × M × C	0.289	0.079	0.239

Values are mean of all samples across growing season ± SE of three replicates. Bold value indicates $P < 0.05$.

monoculture, respectively (Fig. 4). Community-level [P] of wheat/maize intercropping at maturity was 22.25% greater than [P] of wheat monoculture and was not significantly different from [P] of maize monoculture. Community-level [P] of barley/maize intercropping at maturity was 17.88% greater than that of maize monoculture and was not significantly different from that of barley monoculture (Fig. 4). Community-level N:P ratios of wheat/maize and barley/maize intercropping at maturity were 25.09%

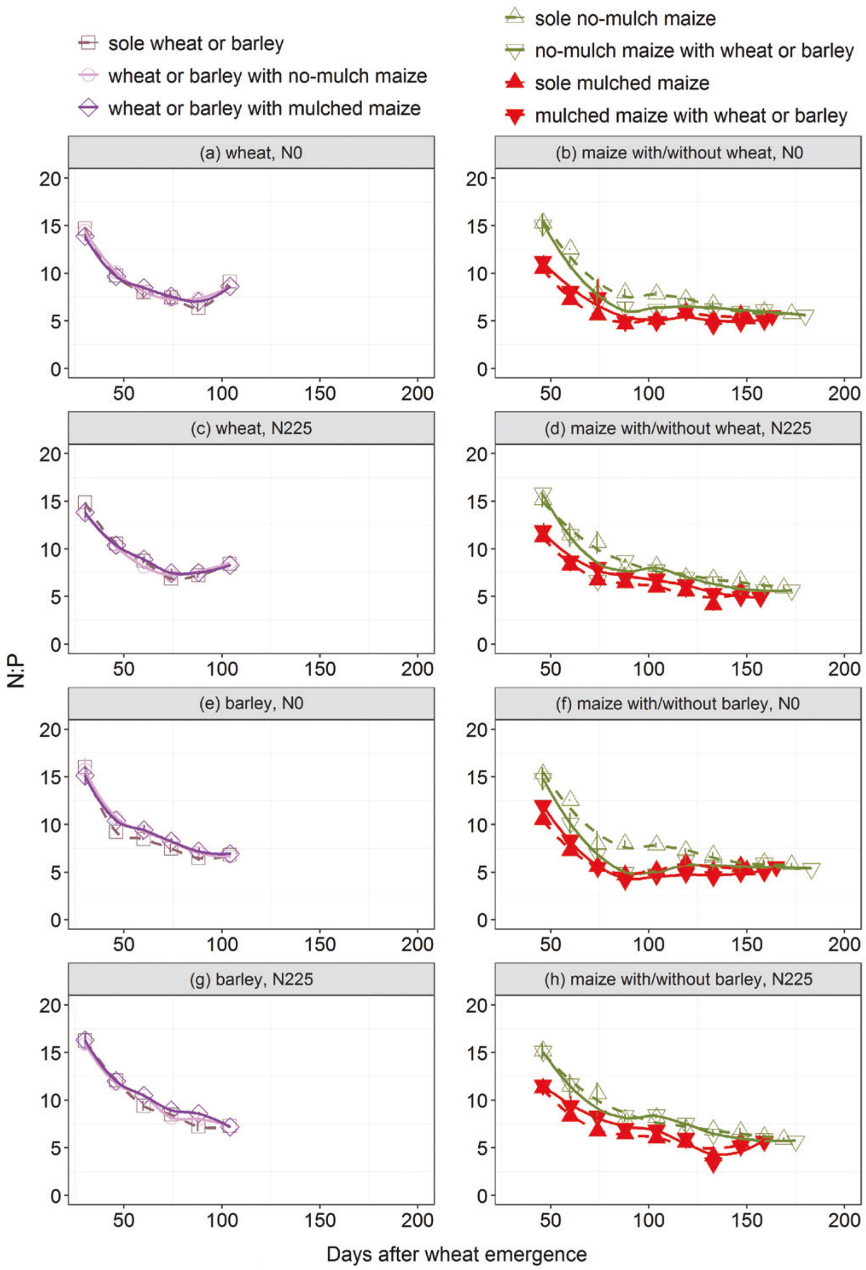

Figure 3: Changes in N:P ratios of wheat (**a**, **c**), barley (**e**, **g**) and maize (**b**, **d**, **f**, **h**) plants throughout the growing season across the three experimental treatments: cropping system, film mulching and N fertilization. Each data point represents mean ± SE of three replicates.

and 12.13% less than N:P ratios in corresponding wheat and barley monoculture, respectively, and were 16.95% and 10.29% greater than N:P ratios of maize monoculture, respectively (Fig. 4).

DISCUSSION

Our study shows that wheat and barley had greater N and P competitive ability compared with maize, this means that interspecific interactions increased N and P uptake of wheat and barley while decreasing nutrient uptake of maize in intercropping during co-growth stages. N and P uptake by intercropped maize increased rapidly after wheat and barley harvest, and exceeded N and P uptake of maize monoculture when mulched and/or fertilized. Plant [N], [P] and N:P ratios decreased with plant growth stage. Intercropping did not change N:P

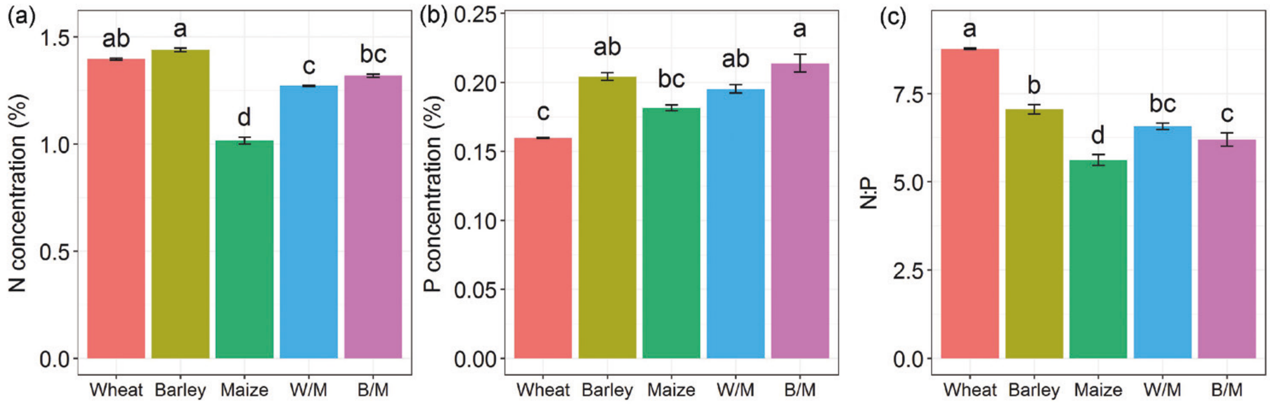

Figure 4: Community-level N (**a**), P concentrations (**b**) and N:P ratios (**c**) in wheat, barley and maize monoculture, wheat/maize (W/M) and barley/maize (B/M) intercropping at maturity.

ratios of the three crop species. Community-level [N], [P] and N:P ratios of wheat/maize and barley/maize intercropping were different from those of corresponding monocultures at maturity. N fertilization did not consistently affect crop-level N:P ratios whereas film mulching contributed to decrease N:P ratios of maize plants.

Effects of interspecific interactions on N and P uptake of crop species

The competitive ability of wheat and barley, as indicated by the NCRs, was greater than maize during the co-growth stage (Fig. 1). Previous studies showed how wheat and barley have higher competitive abilities for taking up nutrients (either from soil or fertilizer sources) in intercropping systems including wheat/maize or wheat/soybean (Li *et al.* 2001b) and barley/pea/oilseed rape tri-component intercrops (Andersen *et al.* 2004). The earlier emergence and larger initial size of wheat and barley seedlings compared with maize, possibly gave them an initial competitive advantage (Andersen *et al.* 2004; Li *et al.* 2001b). In addition, the competitive advantage of wheat and barley could be due to greater root extension of wheat and barley at the co-growth stage (Li *et al.* 2006). This competitive advantage of wheat and barley led to increased N and P uptake of wheat and barley, and decreased N and P uptake of maize during the co-growth stage (Fig. 2).

Maize has a longer growing season than wheat and barley. Because the inhibition by wheat or barley was released after their harvest and more space became available for growth and nutrient capture of maize in intercropping, N and P uptake of intercropped maize recovered and were higher than, or close to, those in monoculture maize when maize was mulched and/or fertilized (Fig. 2). Recovery or compensation of intercropped maize and soybean after wheat harvesting was also observed in wheat/maize or wheat/soybean strip intercropping (Li *et al.* 2001a). It has been shown that N fertilization reduces nutrient competition and mulching increases N and P uptake of maize (Zhang *et al.* 2017), these two effects have possibly alleviated the inhibition of wheat and barley on maize, and led to increased RNU_N and RNU_P of maize (Fig. 2; Supplementary Table S3).

Effects of interspecific interactions on N:P ratios at both species and community level

Interspecific interactions significantly changed N and P uptake of three crop species, in contrast to our first hypothesis, however, they did not significantly affect crop-level N:P ratios (Tables 2 and 3). Therefore, interspecific interactions did not decouple N and P uptake by crop species in intercropping. These results can be explained by stoichiometric homeostasis theory (Elser *et al.* 2010), which states that N:P stoichiometry should remain relatively constant in response to environmental fluctuations. Previous studies showed that plant diversity did not have any significant effect on the N:P ratios of five plant species in the BioCON experiment (biodiversity, CO_2, N), MN, USA (Novotny *et al.* 2007). A recent study also found that stem and root N:P ratio of the grass *Bothriochloa ischaemum* did not change across the mixture proportions with the legume *Lespedeza davurica* (2:10, 4:8, 6:6, 8:4, 10:2 and 12:0, grass:legume plants, respectively) under varying water and fertilizer supplies (Xu *et al.* 2016). Our study lasted for 1 year only, and this could be a too short period of time to detect significant effects of interspecific interactions on crop-level N:P ratios.

A recent study found that short-term intercropping did not affect soil fertility in terms of soil organic matter content, total N and Olsen P (Wang *et al.* 2015), suggesting that changes in N:P ratio of crop species are not likely to occur in the short term.

Community-level [N], [P] and N:P ratios of wheat/maize and barley/maize intercropping were different from those of corresponding monoculture at maturity (Fig. 4). Because interspecific interactions did not significantly affect crop-level N:P ratios, our results suggest that changes of community-level N:P ratios with species richness were mainly due to changes in species composition and biomass proportions. Recent studies show that changes in plant species diversity and composition influenced community-level aggregate nutrient ratios (Guiz *et al.* 2016). The distinct N:P ratio of each intercropped species would reduce interspecific competition and increase total resource capture (Sardans *et al.* 2021), which may be a potential factor contributing to yield advantage in intercropping. Because the proportion of N and P removal (i.e. uptake) from intercropping systems is different from monocultures, this study can have important implications for nutrient management and nutrient budgets across different farmlands.

General decreases in N:P ratios of crop species with crop growth

In our study, [N] and [P] of the three crop species decreased with crop growth (Supplementary Figs S2 and S3). These results can be explained by dilution effects, which indicate that plant [N] and [P] decrease as plants continue to grow (Greenwood *et al.* 1986; Ziadi *et al.* 2008b). The increasing proportion of nutrient-poor stems in total aboveground biomass compared with nutrient-rich leaves can produce a dilution effect of plant [N] and [P] (Greenwood *et al.* 1986). Daily temperatures were low in April, and increased from end of April to June in Northwest China. Higher temperature can increase microbial biomass and activities and stimulate phosphatase production, which could further contribute to increase soluble soil P concentration by enhancing mineralization of organic P or chemical decomposition of insoluble inorganic P (Rui *et al.* 2012). The improved soil P availability thus led to the peak [P] of three crop species at the second or third sampling times, then plant [P] decreased due to dilution effects (Supplementary Fig. S3). Increased soil temperatures due to mulching during or before the first sampling could increase soil P availability and therefore move the peak P concentration earlier in the growing season (Supplementary Fig. S3).

The N:P ratios of whole plants in the three crop species significantly decreased with crop growth (Fig. 3). Changes in biomass allocation among organs may contribute to this pattern. Firstly, N:P ratios are lower for stems than leaves, and stem:leaf ratios tend to increase with crop growth (Ågren 2008). Secondly, large amounts of nutrients are transferred from leaves and stems to seeds during reproductive growth, and stems and leaves have generally lower [P] and higher N:P ratios than seeds (Güsewell 2004). Thus shifts towards higher stem:leaf and seed:leaf ratios may determine decreases in N:P ratios during the growing season.

Effects of N fertilization on N:P ratios of crop species

Our results show that N fertilizer increased plant [N] of three crop species confirming findings from a previous meta-analysis, which also showed that N fertilization generally increases plant [N] (Yuan and Chen 2015). However, the effects of N fertilizer on plant [P] were highly variable for the three crop species (Tables 2 and 3). Inconsistent effects of N fertilization on P concentration were also observed for spring wheat (Ziadi *et al.* 2008a) or maize (Bélanger *et al.* 2012) across different sites or years. For example, N fertilization did not affect grain P concentrations of maize for 6 of the total 10 site-years, but decreased P concentrations in the remaining 4 site-years (Bélanger *et al.* 2012).

The response of N:P ratios to N fertilization was significant only when [N] and [P] were divergently affected by N fertilization (Bélanger *et al.* 2012). Different response of [P] led to inconsistent N fertilizer effects on N:P ratios of the three crop species in our study (Tables 2 and 3). Thus, these results did not fully support our second hypothesis that N fertilizer increased crop N:P ratios. A previous study also showed that N applications significantly increased maize grain N:P ratios at 4 of the 10 site-years but did not affected N:P ratios in the other 6 site-years (Bélanger *et al.* 2012). The inconsistent effects of N fertilizer on [P] or N:P ratio of three crop species were also perhaps due to species-specific plant responses to N inputs (Phoenix *et al.* 2003; Zhang *et al.* 2018), differences in N and P capture capacity from soils (Zhang *et al.* 2017) or the inconsistent application of N fertilizer in our study.

[N], [P] and N:P ratios in mulched maize plants

Film mulching in our study significantly reduced [N] and increased [P], thereby reducing the N:P ratios of maize plants (Table 3), which is consistent with our third hypothesis. The daily growth rate of maize significantly increased under film mulching (Zhang *et al.* 2015), suggesting that higher growth rate of mulched maize plants could be associated with high [P] and lower N:P ratios, as predicted by the growth-rate hypothesis (Elser *et al.* 2003). Also film mulching can effectively conserve soil moisture (Zhang *et al.* 2019) and recent experimental evidence shows that plant N:P ratios can decrease with increasing soil-water content or declining drought conditions (Cernusak *et al.* 2010; Ding *et al.* 2019; Yuan and Chen 2015). In addition, mulching increased soil temperature and further increased P availability, which could also explain high [P] and lower N:P ratios of mulched maize plants. Our findings suggest that less P fertilizer inputs are required under film mulching.

CONCLUSIONS

Our study is the first to apply the theoretical framework of N:P stoichiometry to intercropping. The results indicated that interspecific interactions affect N and P uptake rather than N:P ratios of plant species in intercropping. In addition, community-level N:P ratios of wheat/maize and barley/maize intercropping were different from those of corresponding monocultures at maturity. This study provides evidence from intercropping, which is similar to evidence gathered from grassland ecosystems (Guiz *et al.* 2016) where changes of community-level N:P ratios with species richness are mainly due to changes in species composition and biomass proportions in species-diverse communities. This study thus helps to understand the mechanisms underlying variations in plant N:P ratios in natural and agricultural ecosystems (Sterner and Elser 2002). In addition, our findings suggest that the proportion of N and P removal from farmland differs between intercropping and monocultures, and that N and P uptake by crops are decoupled under N fertilization and mulching. This nutrient decoupling can affect soil nutrient budgets within whole farms and it must be considered in future nutrient management strategies.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Figure S1: Diagrams of row arrangements for wheat, barley and maize in the monocultured and intercropped systems.

Figure S2: Changes of N concentration by wheat (**a**, **c**), barley (**e**, **g**) and maize (**b**, **d**, **f**, **h**) across growing season in relation to cropping system, film mulching and N fertilization.

Figure S3: Changes of P concentration by wheat (**a**, **c**), barley (**e**, **g**) and maize (**b**, **d**, **f**, **h**) across growing season in relation to cropping system, film mulching and N fertilization.

Table S1: The N and P nutrient competitive ratio (NCR_N and NCR_P) of wheat and barley relative to maize in response to changes in sampling date (D), N fertilization (N), mulching treatments (M) and their interactive effects during the co-growth stage.

Table S2: The N and P relative nutrient uptake (RNU_N and RNU_P) of wheat and barley in response to changes in sampling date (D), N fertilization (N) and their interactive effects across the growth stage.

Table S3: The N and P relative nutrient uptake (RNU_N and RNU_P) of maize in response to changes in sampling date (D), N fertilization (N), mulching treatments (M) and their interactive effects across the growth stage.

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