# Species-specific interaction affects organic nitrogen uptake during intercropping of four important crop species: A useful index for selecting appropriate intercropping combination

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## ARTICLE INFO

## ABSTRACT

Intercropping has been an important practice for improving field production and its various aspects have been thoroughly investigated. However, only few studies have explored it from the perspective of organic nitrogen (N) uptake, especially under the rapid development of organic and no-tillage farming worldwide. In this study, two cereals (*Triticum aestivum* and *Zea mays*) and two legumes (*Glycine max* and *Pisum sativum*) were mono- and intercropped using microcosm experiments in a greenhouse, with a total of 10 combinations. The uptake pattern of *in situ* $^{15}$N-labelled glycine by plants in each combination was investigated. The total biomass of wheat increased 1.6 times when intercropped with soybean, compared to its monoculture, but not at the expense of reduced soybean growth. Root:shoot ratios of maize increased 1.3 times when intercropped with pea compared with its monoculture. Intercropping with soybean decreased the uptake rates of $^{15}$N-labelled glycine by wheat compared to its monoculture. Although intercropping with other species increased the root:shoot in maize, it did not increase the glycine-derived $^{15}$N uptake rates. We conclude that interactions between agricultural plants are species-specific and can alter the uptake of N-derived from organic forms. These findings indicate that wheat-soybean intercropping can be a good choice for organic and no-tillage farming to improve production with the potential to reduce the application of synthetic N fertilizers.

## 1. Introduction

Improving crop production to promote the development of sustainable agriculture has been gradually attracting considerable attentions worldwide, especially because of the various threats posed by drought (Godfray et al., 2010; Ding et al., 2018; Kuhla et al., 2021), acidification caused by nitrogen (N) deposition and fertilization (Guo et al., 2010; Tian and Niu 2015), and tillage-mediated erosion (Van Oost et al., 2000). To cope with such threats and increase crop production, various practices including no-tillage and organic farming have been extensively developed worldwide (Rigby and Cáceres 2001; Triplett and Dick 2008; Knapp and van der Heijden 2018). Additionally, many crop species from the same or different families are often planted together to improve productivity by increasing biodiversity (Malézieux et al., 2009). Certain co-existing crops could belong to the same plant family and occupy the same niche, thus often require nearly the same essential nutrients for their maintenance, growth and reproduction (Silvertown 2004). Thus, co-existing crops intensively compete for resources, thereby significantly affecting the productivity of agricultural fields (Gebru 2015; Trinder et al., 2021). Accordingly, cereal-legume intercropping has been observed as an important practice for a long time through taking complete advantages of interspecific facilitation between grasses and legumes (Zhang and Li 2003; Gebru 2015; Liu et al., 2020b).

Nitrogen (N) is a major limiting element for plant growth in most terrestrial ecosystems (Lebauer and Treseder 2008). In mixed-crop agroecosystems, legumes are applied as an intercropping species for increasing the soil available N because of their capacity to fix atmospheric N to ammonia via their symbiotic rhizobia and supply their

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neighbors with available N (Dixon and Kahn 2004; De Bruijn 2015). Field evidence has demonstrated the synchrony between legume N release and corn demand (Stute and Posner 1995). Moreover, numerous studies have quantified the N transfer from legumes to associated plants by mainly focusing on their benefits to the associated species (Martin et al., 1991; Jiang and Zhai 2000; Thilakarathna et al., 2016). However, studies have also demonstrated that legumes may compete with cereals for N at low levels of available N (Chamberlin et al., 1985). Thus, intercropping could be less advantageous if the intercropped plant species aggressively forage for soil resources in the same niche. Therefore, clarifying how interactions between plant species affect their N uptake from soils is a prerequisite to better understand intercropping systems (Malézieux et al., 2009).

In agricultural soil, mineral N content (i.e. $NH_4^+$ and $NO_3^-$) is generally higher than in natural ecosystems due to chemical fertilization (Savci 2012). Although numerous studies have focused on mineral N to improve crop production (Basosi et al., 2014; Liu et al., 2018; Plaza-Bonilla et al., 2021), many agricultural crop species such as wheat, maize, and rice have the capacity to directly take up intact amino acids from soil solution (Näsholm et al., 2000; Xu et al., 2008; Sauheitl et al., 2009). However, soil organic N has been overlooked but it is potentially significant contribution to crop nutrition (Farzadfar et al., 2021). In the past decades, no-tillage and organic farming has rapidly expanded worldwide, and has been regarded as a sustainable alternative to reduce the dependence on chemical fertilization and decrease environmental pollution risks (Garg and Balodi 2014; Miśniakiewicz et al., 2021). Bio-organic amendments with reduced amounts of chemical additions can improve soil fertility, crop yield, and its quality (Ye et al., 2020). In organic farming, N contents often increase due to the application of organic fertilizers and wastes or straw returning. Therefore, it is necessary to investigate organic N uptake by agricultural species to understand plant nutrition in these agricultural systems (Farzadfar et al., 2021).

Besides intercropping of crops from various families, many crop species from the same family (e.g. Poaceae or Leguminosae) are also mixed. Because of the apparent benefits of legume-cereal intercropping, studies have increasingly focused on their interaction mechanisms. However, few studies have been conducted to simultaneously compare the combined interactions between cereals and legumes, between two cereals, and between two legumes. In this study, the effects of interactions between agricultural plant species on organic N uptake were evaluated by selecting two cereals of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), and two legume species of soybean (*Glycine max* L.) and pea (*Pisum sativum* L.). Totally 10 combinations of mono- or intercropping systems (see in Materials and Methods) were designed using microcosm experiment. *In situ* [15]N labelling was used to detect the plant N uptake under each combination. In particular, as one of the most abundant amino acids in soil, ^{15}N-labelled glycine was used as a representative of organic N (Näsholm et al., 2000). Considering the generally believed positive effects of legumes on the co-existing crop species (Ofori and Stern 1987), we hypothesized that (1) intercropping with legumes (i.e. soybean and pea) can increase the biomass of cereals (i.e. wheat and maize) compared to monocultures. On the contrary, intercropping plants from the same family does not foster this interspecific facilitation; (2) the interspecific facilitation between legumes and cereals decreases the glycine uptake by cereals, because the co-existing legume species supplies a portion of N.

## 2. Materials and methods

### 2.1. Soil and species

Soil used in this study was collected from the upper 10 cm layer in a farmland in Beijing (48°42′44″N, 9°11′24″E), China, and contained 0.2% clay, 51.4% silt, and 48.4% sand. Soil was air-dried and sieved through a 2 mm sieve before the experiment. The details of other physical and chemical properties, including soil organic carbon (C), total N, C:N, pH, electrical conductivity, and contents of $NH_4^+$, $NO_3^-$ and glycine are listed in Table 1. Four agricultural species, wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) were used in this study. These species were all collected locally in Beijing and suitable for the planting in this soil and climate. The cultivars used in this study were Luyuan No. 22 (maize), Shengdou No. 5 (soybean), Jingdong No. 8 (wheat), and Zhongwan No. 8 (bean).

### 2.2. Experimental design

Microcosm experiment was designed in a greenhouse. Same-sized pots (10 cm in height and 10 cm in diameter) were filled with 500 g of air-dried soil. Several seeds from the same species or different species were sown in each pot according to the corresponding treatments. After germination, the seedlings were allowed to grow for one week. Finally, the pots were thinned to maintain two seedlings with desired spacing. The effect caused by various plant sizes was avoided by using seedlings of similar sizes for the same species. The two co-planted species were either identical or different, constituting in total 10 combinations (Table 2), that is, two individual seedlings from the same species (wheat-wheat, maize-maize, soybean-soybean, and pea-pea) and two seedlings from different species (wheat-maize, wheat-soybean, wheat-pea, maize-soybean, maize-pea, and soybean-pea). Three replicates were set for each combination, with six replicates for wheat-wheat treatments. Thus, 33 pots were established. After planting, the microcosms were randomly placed in a common garden.

The planted microcosms were cultivated in a greenhouse, which was set to 14 h light with an intensity of 800 μmol m$^{-2}$ s$^{-1}$. The day- and nighttime temperatures in the greenhouse were $27 \pm 1$ °C and $22 \pm 1$ °C, respectively. Soil moisture was maintained at 60% of the water-holding capacity by watering daily. All microcosms were inoculated with *Bradyrhizobium japonicum* strain USDA 110, when seeds were sown for 7 days to guarantee root nodule formation.

### 2.3. [15]N labelling, harvesting and measurement

Fifty days after the seeds were sown, 50 mL 10 mg N L$^{-1}$ labelling solution containing ^{15}N-labelled glycine (95.0% enrichment) was added into the soil, which corresponded to 1.0 μg N g$^{-1}$ soil. After 24 h, ^{15}N-labelled microcosms were randomly destructively sampled.

Plant roots were carefully separated from the soil and rinsed with water immediately, and then soaked in 0.5 mM CaCl$_2$ solution for 30 min to maintain the functions and integrity of the roots (Warren and Adams 2007). The roots were then rinsed again with distilled water, following which the roots and leaves were microwaved for 90 s under 600 W, and dried at 60 °C for 48 h. Dried biomass weight was determined, following which the samples were ground into a fine powder using a ball mill (MM200, Haan, Retsch).

The N content and ^{15}N/^{14}N ratio in the dry-ground sample were measured by a continuous-flow gas isotope ratio mass spectrometry with an elemental analyser (EA 1110, CE Instruments, Milan, Italy) and a ConFlo III (Finnigan MAT, Bremen, Germany). Atmospheric N$_2$ was used as the reference standard for N isotopic analyses, with a δ^{15}N value defined as 0‰. The standard deviation of the repeated measurements of laboratory standards was ±0.15‰.

### 2.4. Calculation and statistical analysis

Total biomass was calculated as the sum of the plant shoot and root biomass, and the root:shoot biomass ratio was calculated. The effects of intercropping of wheat, maize, soybean and pea on shoot biomass, root biomass, total biomass and root:shoot ratio were calculated individually using one-way ANOVA.

Competitive response (CR) was calculated to demonstrate the

**Table 1**

Physical and chemical properties of the original soil used in this study. SOC: soil organic carbon (%), TN: total nitrogen (%), EC: electrical conductivity ($\mu s\ cm^{-1}$), $NH_4^+$: ammonium content ($mg\ kg^{-1}$), $NO_3^-$: nitrate content ($mg\ kg^{-1}$), Gly: glycine content ($mg\ kg^{-1}$). Mean and SE (standard error) are calculated based on three replicates.

| Parameter | SOC | TN | C:N | pH | EC | $NH_4^+$ | $NO_3^-$ | Gly |
|---|---|---|---|---|---|---|---|---|
| Mean | 1.50 | 0.11 | 13.96 | 7.37 | 152.33 | 2.27 | 1.50 | 0.14 |
| SE | 0.03 | 0.00 | 0.09 | 0.03 | 4.63 | 0.10 | 0.02 | 0.01 |

**Table 2**

Experimental design for the 4 species of 10 combinations. The 4 species are maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.) and pea (*Pisum sativum* L.). These species were planted by 10 different combinations, with two individual seedlings from the same species or different species in each pot. In the table, W, M, S, and P represent the four species of wheat, maize, soybean, and pea, respectively.

| Species | Wheat | Maize | Soybean | Pea |
|---|---|---|---|---|
| Wheat | W–W | | | |
| Maize | W–M | M–M | | |
| Soybean | W–S | M-S | S–S | |
| Pea | W–P | M-P | S–P | P–P |

competitive ability of species based on the total biomass of each plant species under each intercropping treatment. As shown in equation (1), $CR_{ij}$ under the intercropping regime was calculated as the ratio of the biomass of species i when intercropped with species j ($Y_{ij}$) to the biomass of species i when grown in monoculture regime ($Y_{ii}$) (Sun et al., 2020). When $CR_{ij} > 1$, species i responds more positively to intercropping than to monoculture. When $CR_{ij} < 1$, species i responds more negatively to intercropping than to monoculture.

$$CR_{ij} = \frac{Y_{ij}}{Y_{ii}} \quad (1)$$

Atom percent excess (APE) was calculated as the difference of atm% $^{15}N$ between $^{15}N$ labelled and control plants. The amount of $^{15}N$ glycine uptake in the shoots or roots (GUA, glycine uptake amount, $\mu g$) was calculated by multiplying the corresponding shoot or root N content ($\mu mol\ N\ g^{-1}$ dry mass), APE and the relative molecular mass of $^{15}N$ (g $mol^{-1}$), as shown in equation (2).

$$GUA\ (\mu g) = N\ content\ (\mu mol\ g^{-1}) \times APE \times biomass\ (g)$$
$$\times 15(g\ mol^{-1}) \quad (2)$$

$^{15}N$-glycine uptake rates by plants (GUR, $\mu g\ g^{-1}$ d. w. root $h^{-1}$) were calculated by adding shoot GUA and root GUA, and then dividing the sum by labeling time (in hours) and root biomass (Liu et al., 2020a). Because of the rapid amino acid turnover in soil and that $^{15}N$ -labelled glycine was used over 24 h, it is hard to estimate the uptake of intact glycine in this study. Therefore, uptake rates were referred to as that glycine-derived $^{15}N$, including the uptake of intact and mineralized glycine. The difference of glycine uptake rates between different co-existing species by each crop of wheat, maize, soybean and pea was analyzed using one-way ANOVA separately. Residuals of the model were checked for normality and homogeneity, and if assumptions were met, Tukey's test was performed at $P < 0.05$. Statistical calculations were performed and the figures were made by R (R version 4.1.1, R core team, Vienna).

## 3. Results

### 3.1. Shoot and root biomass

Shoot biomass of maize and soybean under all cropping combinations was 0.7–0.8 g per plant, and that of wheat and pea was 0.4 g per plant. Root biomass of maize under all cropping combinations was 0.4 g per plant, while that for wheat, soybean, and pea was 0.1 g per plant.

When intercropped with other species, the shoot and root biomass of wheat varied , but not for maize, soybean and pea (Table S1). Shoot and root biomass of wheat when intercropped with soybean were larger than under monoculture (Fig. 1, p<0.05). Shoot biomass of wheat when intercropped with maize and pea were similar to monoculture and intercropped with soybean. Root biomass of wheat when intercropped with pea was similar to monoculture and lower than intercropped with soybean (Fig. 1).

### 3.2. Total biomass and root:shoot ratio

Total biomass of maize and soybean under all cropping combinations was 1.0–1.1 g per plant, and that for wheat and pea were 0.5 g per plant. Total biomass of wheat varied, whereas the total biomass of other species (i.e. maize, soybean, and pea) presented a similar pattern in the intercropping systems (Table S2). When intercropped with soybean, the total biomass of wheat was larger than in monoculture system (Fig. 2, p<0.05). The total biomass of wheat was not effected by intercropped maize or pea. Interactions between two species from the same family demonstrated similar patterns. Interactions between wheat and maize neither increased the wheat biomass nor changed maize biomass compared to its monoculture. Similarly, interactions between soybean and pea did not change the total biomass compared to their monocultures (Fig. 2).

Root:shoot ratios of maize were different between the four cropping regimes, but those of wheat, soybean, and pea observed remained stable (Table S2), i.e., the root:shoot ratios of wheat, soybean, and pea under all cropping regimes maintained 0.2, whereas those for maize were 0.5 (Fig. 2). For maize, root:shoot ratios when intercropped with pea were higher than under monoculture (p<0.05).

### 3.3. Competitive response

Intercropping with other three species increased the competitive response of wheat compared to its monoculture (Fig. 3), andthe positive effect was particularly strong when intercropped with soybean. Competitive responses of maize and soybean presented a general positive or negative pattern (higher or lower than 1) when intercropped with other species, e.g., when intercropped with wheat, the competitive response of soybean was less than that during its monoculture (Fig. 3). The competitive responses of pea when intercropped with other species were lower than 1, indicating the negative effects of intercropping compared to that of monocropping.

### 3.4. Glycine uptake rates

The uptake rates of glycine-derived $^{15}N$ for the species of wheat, maize and pea revealed significant differences among various cropping combinations (Table S3). Glycine uptake rates by the four species ranged from 1.8 to 16.4 $\mu g$ $^{15}N$ $g^{-1}$ d. w. root $h^{-1}$. When intercropped with maize and pea, the glycine-derived $^{15}N$ uptake rates by wheat were higher than those when intercropped with soybean (Fig. 4). For maize, the uptake rates of glycine-derived $^{15}N$ under monoculture were higher than those under other the three cropping combinations, with significant differences only observed between monocropping and intercropping with pea (Fig. 4). Interactions between two species from the same family exerted various effect on glycine-derived $^{15}N$ uptake. Interactions
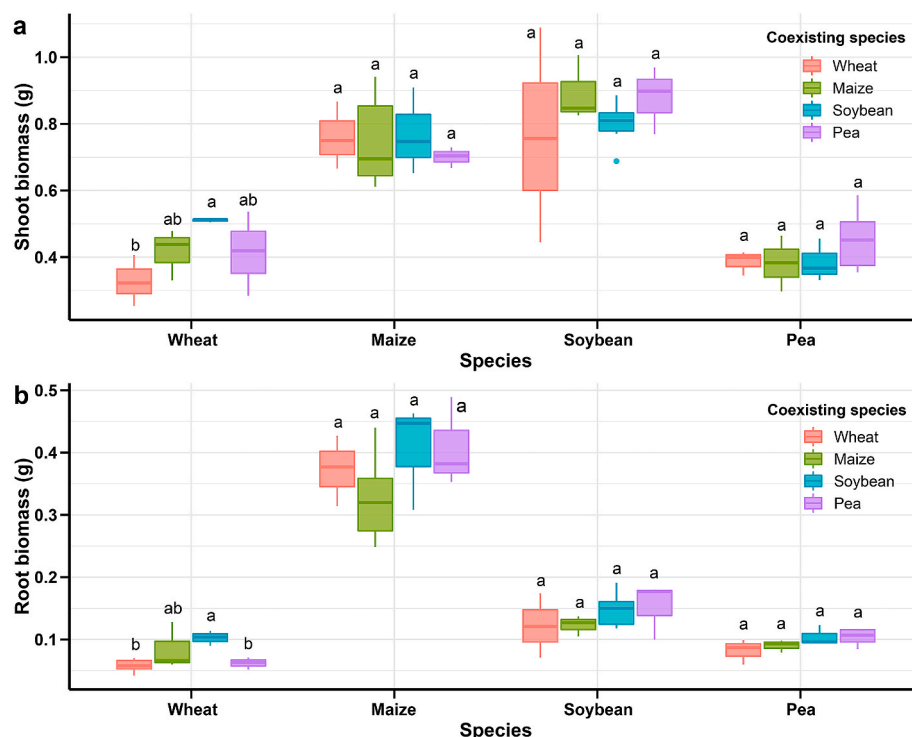
**Fig. 1.** Shoot (a) and root (b) biomass for wheat, maize, soybean and pea under each mono- and intercropped system. The different coexisting species is distinguished by colors. Different letters above the bars indicate significant differences between the treatments at $P < 0.05$ level. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
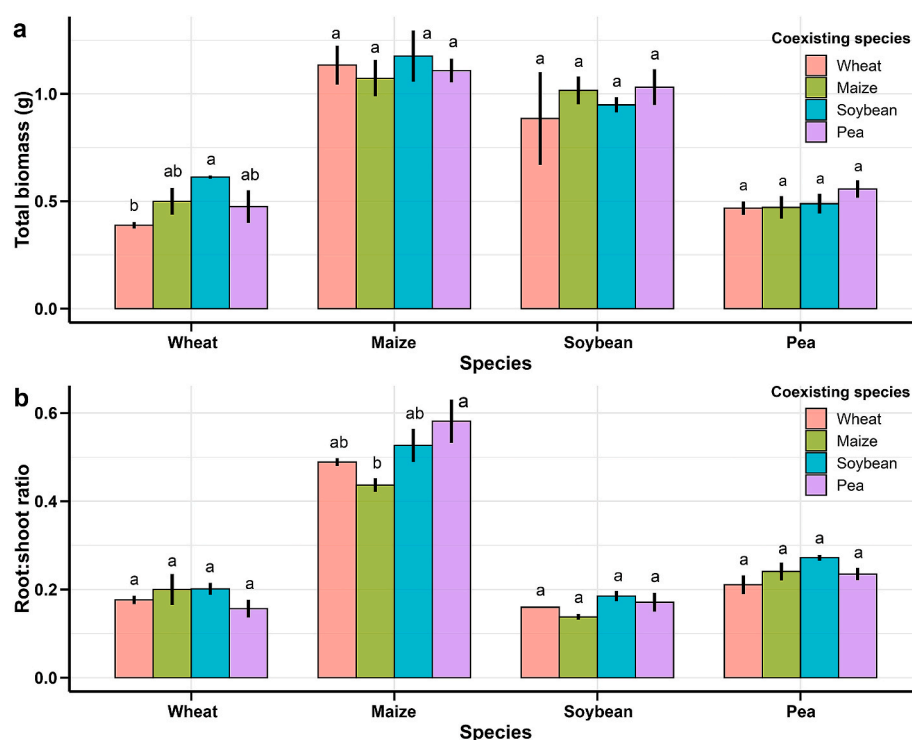


**Fig. 2.** Total individual biomass (a) and ratios of root to shoot biomass (b) of four agricultural species under the 10 planting combinations. Values are presented by mean ± SE of three replicates for intercropped system; six replicates for monocropping maize, soybean and pea; and 12 replicates for monocropping wheat. Letters above the bars indicate significant differences between the treatments at $P < 0.05$ level.

between wheat and maize did not alter the glycine-derived $^{15}N$ uptake by wheat and maize compared with their monocropping. Similarly, interactions between soybean and pea also did not change the glycine-

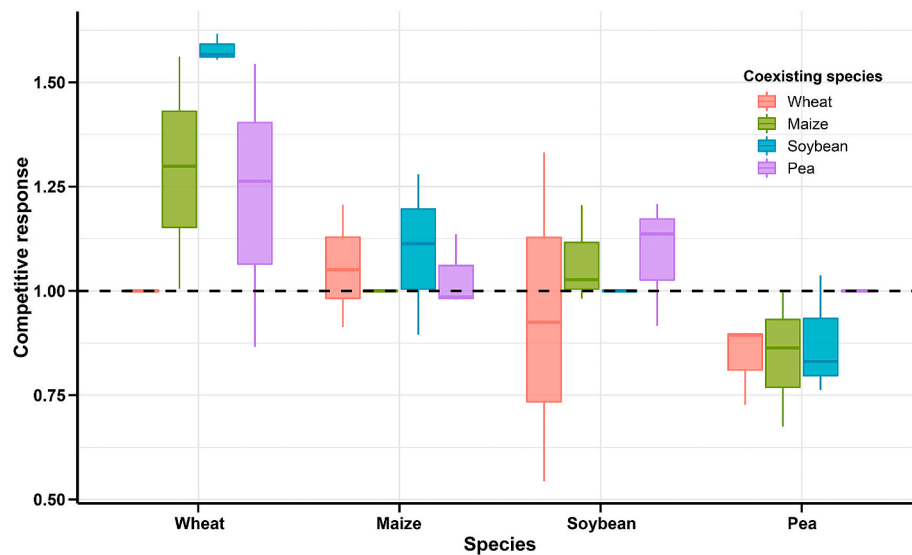derived $^{15}N$ uptake by soybean and pea (Fig. 4).

**Fig. 3.** Competitive response for wheat, maize, soybean and pea under each mono- and intercropped system. The different coexisting intercropping species is distinguished by colors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
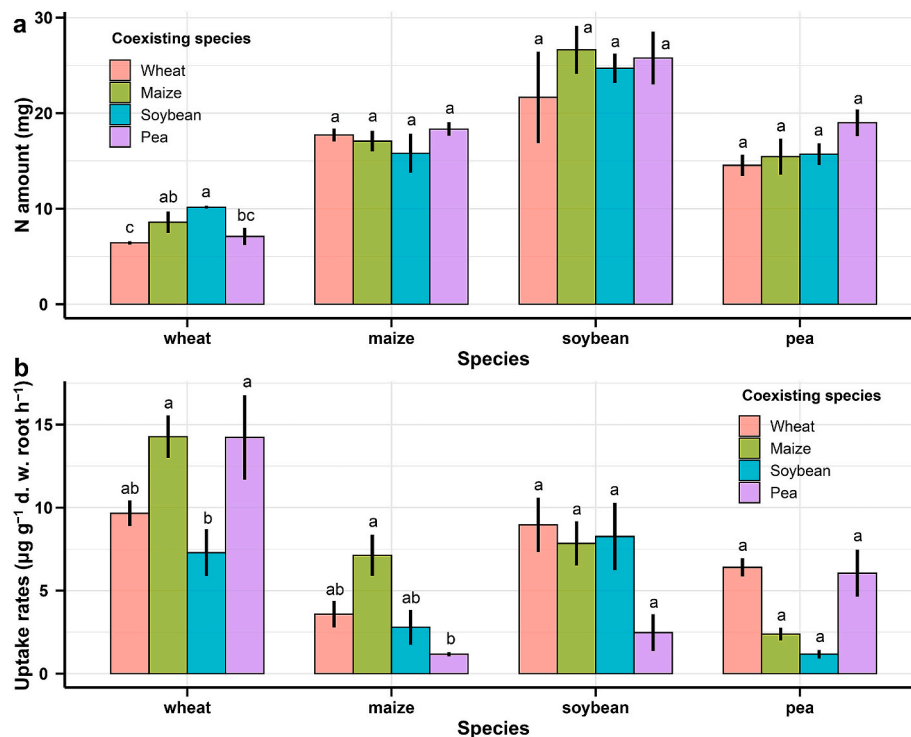


**Fig. 4.** Nitrogen (N) amount (a) and glycine-^{15}N uptake rates (b) of four agricultural species under 10 planting combinations. Values are presented by mean ± SE of three replicates for intercropped system, six replicates for monocropping maize, soybean and pea, and 12 replicates for monocropping wheat. Different letters above bars indicate significant differences between the treatments at $P < 0.05$ level.

## 4. Discussion

To investigate the effects of intercropping from the perspective of organic N uptake, two cereals (wheat and maize) and two legumes (soybean and pea) were mono- and intercropped with each other, and *in situ* labelled by ^{15}N-glycine. Results showed that intercropping with soybean can increase the biomass of wheat and decrease its uptake of glycine-derived ^{15}N, which only partly supports our first but approves our second hypothesis.

Numerous studies have suggested that interspecific competition

often affects plant growth and biomass allocation in nutrient-poor environments (Aerts 1999; Poorter et al., 2012; Sun et al., 2020; Rehling et al., 2021). In this study, an increase in biomass by cereals when intercropped with legume is not a general pattern, but species specific (i. e. only significant when intercropping for wheat and soybean intercropping ). Despite no typical increase in biomass, biomass production was stable for mono- and intercropped legumes and cereals (Figs. 1 and 2a), which was similar to previous field observations (Liu et al., 2018). This partly demonstrates the positive cooperation between intercropped legumes and cereals.

Symbiotic rhizobium in legumes can fix $N_2$ from the atmosphere, but legumes are always less competitive than intercropped cereals in N use efficiency (Jensen et al., 2020). Intercropping with soybean increased the shoot, root, and total biomass of wheat (Figs. 1 and 2a), which is consistent with field observations and presents the benefits of facilitative interactions between these intercropped species (Zhang and Li 2003). In the intercropping system, soybean provides wheat with excess available N via niche differentiation in resource utilization (Liu et al. 2018, 2020a, 2020b). This is demonstrated by the increased N amount in wheat when intercropped with soybean (Fig. 4a). Interactions between intercropped plant species can also induce higher rhizosphere priming and accelerate mineralization of soil organic matter, thus increasing N availability in soil (Pausch et al., 2013). Competitive responses by wheat under intercropping combinations were generally higher than those during monoculture, which was especially high when intercropped with soybean (Fig. 3). This also reflects the positive effects of soybean on wheat growth (Zhang and Li 2003). Particularly, the root: shoot ratios of wheat remained stable among different combinations, indicating the increase in wheat biomass by intercropped with soybean is balanced in both above- and belowground parts. In contrast, intercropping with other species did not affect the total biomass of maize, but increased biomass allocation to their root system, especially during the maize-pea intercropping (Fig. 2). This indicates that the effects of interactions between plant species on plant growth demonstrate distinct patterns and are species specific. Importantly, the positive effects on wheat by intercropping with soybean was not observed at the expense of a decrease in growth of the intercropped soybean.

Our results show that interspecific facilitation does not always occur between cereals and legumes. This is also the case for interactions between two species from the same family, which satisfies our first hypothesis, mainly because plants from the same family can always occupy the same nutrient niche and compete fiercely when intercropped. Although it is assumed that complementarity in their root structure for soil resources (Knörzer et al., 2009; Li et al., 2016; Wang et al., 2018) could be conducive for interspecific facilitation between plants from the same family, no noticeable facilitation was observed in this study. The detailed mechanisms responsible for this activity necessitates further investigation in the future to improve the knowledge on intercropping systems for sustainable agriculture.

Consistent with previous studies (Näsholm et al., 2000; Okamoto and Okada 2004; Xu et al., 2008), the four targeted agricultural species (i.e., wheat, maize, soybean and pea) show different capacity to take up organic-derived N from soil (Fig. 4b). Therefore, interspecific interactions can affect $^{15}N$-glycine uptake by plants, which strongly depends on species identification. As glycine was only labelled by $^{15}N$, the results only reflect the glycine-derived $^{15}N$ to represent the organic N uptake patterns. Between the four agricultural crops, $^{15}N$-glycine uptake rates increased only in wheat when it was intercropped with maize or pea (Fig. 4b, higher but not significant at $P < 0.05$ compared to monoculture, but significant compared to intercropping with soybean). This is ascribed to the facilitation of interactions between wheat-maize or what-pea (Zhang and Li 2003). The mechanisms behind these facilitative interactions could be attributed to the greater lateral deployment of roots and increased root length density (Li et al., 2006). In contrast, intercropped with soybean strongly reduced the uptake rates of glycine-derived $^{15}N$ by wheat (Fig. 4b). This could be ascribed to the extra N supplies by soybean via biological $N_2$ fixation by the associated rhizobia (Jensen et al., 2020). This assumption is supported by the higher N amount in wheat body when intercropped with soybean (Fig. 4a). The transfer of available N from soybean to wheat potentially decreases its demand of wheat for glycine-derived $^{15}N$. Moreover, intercropping did not alter the uptake of glycine-derived $^{15}N$ by both intercropped crops from the same family (Fig. 4b, p>0.05). These results indicate that soybean could need other N sources, e.g., biological N fixation and uptake of mineral N, but this needs further explorations.

In nutrient-limited environments, plants can efficiently acquire nutrients by altering their allocation to different organs (Yan et al., 2016). Intercropped maize consumes less glycine-derived $^{15}N$ than under monoculture (Fig. 4a), and more biomass is allocated to roots under these intercropped combinations (Fig. 2b). A possible explanation is that not all roots efficiently participate in N uptake, particularly in the case of more pillar roots and less absorptive roots, because absorptive roots with root hairs play a major role in this process (Peterson and Farquhar 1996; Gilroy and Jones 2000). Additionally, our previous study showed that maize prefers to take up N in the form of $NO_3^-$ (Xu et al., 2008), which may explain the low uptake of glycine-derived $^{15}N$. .

## 5. Conclusions

Interspecific interactions can affect organic N uptake by agricultural plants, which strongly depend on the species identity. Intercropping with soybean increased the biomass of wheat but decrease its N uptake from organic forms. Intercropping did not produce interspecific facilitation between crops from the same family in the perspective of biomass and uptake of $^{15}N$-derived from glycine. Under intercropping combinations, root:shoot ratios of maize increased, but the organic N uptake decreased. These findings indicate that interactions between interspecies for organic N uptake are species-specific, which can be a useful index for selecting appropriate combination of intercropping species to improve crop production.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2021.100460.

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