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RESEARCH ARTICLE

Partner quality matters—overyielding in a maize/soybean mixture depends on the initiator of common mycorrhizal networks

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Abstract

Aims Cereal/legume intercropping has advantages in yield and nutrient uptake. However, how common mycorrhizal networks (CMNs), formed by arbuscular mycorrhizal fungi (AMF) play a role in those benefits has not been fully clarified. This study aimed to explore how CMNs initiated by different host plants affected plant performance and nutrient acquisition in a maize/soybean mixture.

Methods Microcosms with three compartments were used; these were separated by 30-µm nylon mesh. Two compartments were root compartments (RCs), and the third compartment was a hyphal compartment (HC). One RC was inoculated with the AMF *Rhizophagus irregularis* and the plant in this compartment was referred to as CMNs donor, and the plant in the other RC compartment as CMNs receiver.

Results Maize biomass was twice that of soybean. Nitrogen (N) and phosphorus (P) content of both maize and soybean were higher in the presence of CMNs compared with the treatment without AMF. When maize was the CMNs donor, shoot biomass, N and P content of the maize/soybean mixture were higher than the expected biomass, N and P content based on monocultures, suggesting overyielding. However, the overyielding was not observed when soybean was the CMNs donor.

Conclusion Overyielding in a maize/soybean mixture depends on the initiator of CMNs. With maize as CMNs donor, both species in the mixture benefited from CMNs compared with monocultures.

Keywords Common mycorrhizal networks · Maize/soybean mixture · Nitrogen and phosphorus content · Overyielding

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Introduction

Intercropping is cultivating two or more crop species simultaneously in the same field (Willey 1990). It is a sustainable agricultural practice, widely used in developing countries such as China (Li et al. 2021),

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Malawi (Njira et al. 2020), and Tanzania (Myaka et al. 2006), and developed countries. Intercropping aims at overyielding with lower external inputs (Renard and Tilman 2021). Although the managements of intercropping cost labor, intercropping has advantages in pest control (Iqbal et al. 2019), weed suppression (Hauggaard-Nielsen et al. 2001; Gu et al. 2021), and the acquisition of nutrients such as nitrogen (N), phosphorus (P), etc. (Wang et al. 2011). Previous studies have shown that aboveground and belowground interspecific interactions play critical roles in causing intercropping advantages (Hinsinger et al. 2011; Brooker et al. 2015). Belowground, interspecific interactions are influenced by soil biota such as bacteria and fungi (Zhang et al. 2020; Hu et al. 2021). Currently, the role of arbuscular mycorrhizal fungi (AMF) mediating interspecific interactions and causing overyielding is drawing increasing attention (Li et al. 2022b; Qiao et al. 2022).

AMF build mutualistic relationships with over 72% of plant species in terrestrial systems (Brundrett and Tedersoo 2018). AMF extends the volume of the nutrient depletion zone beyond the roots through their extraradical mycelium, and thereby acquire nutrients that are not accessed by plants. The host plants, in return for these nutrients, provide the AMF with a certain amount of carbon fixed through photosynthesis (Walder et al. 2012; Weremijewicz et al. 2016). Co-existing plant species are often interconnected by mycelial networks of AMF individuals called common mycorrhizal networks (CMNs) (Smith and Read 2008). CMNs can influence several ecosystem services (Alaux et al. 2021) by enhancing plant nutrient uptake (He et al. 2003) and improving plant resistance to abiotic (drought etc.) (Püschel et al. 2021) and biotic stresses (pests, pathogens) (Kadam et al. 2020).

Overyielding is the productivity benefit of multispecies cultures compared with monocultures, which can be generated by niche differentiation, complementary resource use, among species (Loreau and Hector 2001). Previous studies found that the presence of CMNs contributed to overyielding caused by enhanced N and P uptake in intercrops (Wang et al. 2019; Liu et al. 2021). Studies on pigeon pea/millet and chickpea/millet intercrops have shown that AMF symbioses with legumes play critical roles in producing intercropping benefits (Li et al. 2022a; Schütz et al. 2022). Two mechanisms can account for this. First, the hyphosphere recruits phosphate-solubilizing bacteria to mobilize P (Zhang et al. 2016, 2018) although AMF symbioses reduce the carboxylate exudation (Ryan et al. 2012). Second, legumes have a great demand for P for N_2 fixation, and symbiosis with AMF increases P acquisition and N_2 fixation, thereby alleviating competition for N between intercrops (Li et al. 2007, 2022a; Qiao et al. 2015; Wang et al. 2021b). To date, experimental results have shown contrasting results. The benefits of CMNs were affected by the size inequality of the interconnected partners. For instance, Walder et al. (2012) showed for a mixture of flax (*Linum usitasissimum* L.) and sorghum (*Sorghum bicolor* (L.) Moench) that the small-sized flax benefited more from the CMNs than the large-sized sorghum both in relative and absolute terms compared with monoculture. For N, flax acquired N at the expense of sorghum in the mixture. The CMNs therefore equalized to some extent the competitive inequality between both plants. However, a study by Weremijewicz and Janos (2013) with *Andropogon gerardi* Vitman implied that CMNs amplified competitive inequalities between different individuals, and the plant with large size benefited most from the CMNs.

Maize/soybean intercropping is prevalent in China and across the world due to the higher land use efficiency, N and P use efficiency, and economic profits compared with expected yields based on monocultured maize and soybean (Yang et al. 2017; Iqbal et al. 2019). Studies on maize/soybean intercropping have shown that dual inoculation of rhizobia and AMF increased AMF colonization of both maize and soybean and enhanced both N_2 fixation of soybean and N transfer from soybean to maize (Meng et al. 2015). A further study indicated increased photosynthesis by soybean but not by maize when inoculated with AMF and rhizobia in maize/soybean intercrops (Wang et al. 2016). Previous studies, investigating the effects of CMNs on nutrient uptake by intercrops, were always carried out by inoculating both species simultaneously. Under such conditions, we do not know for sure whether the roots of two species were actually connected by CMNs. Only in experimental designs where one species is inoculated and the companion species can only become mycorrhizal through that donor, can we know for sure that CMNs have built up between two neighboring species. In such designs we can also determine the extent to which the

nature of the donor (and of the receiver) determines symmetrical or asymmetrical benefits.

We conducted a greenhouse experiment to address the knowledge gaps mentioned above by using a maize/soybean mixture where either species acted as donor or receiver of the CMNs. We hypothesized that (1) maize/soybean mixture shows larger overyielding in biomass and N and P acquisition when mycorrhizal than in the absence of AMF; (2) CMNs initiated by maize cause more overyielding in biomass and N and P acquisition compared with CMNs initiated by soybean because maize forms more biomass and could invest more carbon to CMNs than soybean; (3) CMNs initiated by maize allow more N_2 fixation by soybean compared with CMNs initiated by soybean because the symbiosis with AMF of maize benefits N uptake by maize, which subsequently stimulates N_2 fixation by soybean.

Materials and methods

Growth substrate and microcosm setup

The soil was collected from Shangzhuang experimental station, Beijing, China (40°140′ N, 116°190′ E) with the following properties: soil pH 8.2 (soil: water, 1:5), 11.5 g kg^{-1} organic carbon, 2.6 mg kg^{-1} Olsen-P, 0.72 g kg^{-1} total N, 8.5 mg kg^{-1} available N ($NH_4^+ + NO_3^-$) and 32.3 mg kg^{-1} exchangeable K (Wang et al. 2021a). Texture was a silt loam. The soil was air-dried and sieved through a 2 mm sieve and then the soil was sterilized by γ-radiation (> 25 kGy) to eliminate indigenous microorganisms. The growth substrate was prepared by mixing sterilized soil and sand (w/w, 2:1).

A three-compartment growth microcosm (length × width × height = 20 × 13 × 10 cm) was used in the experiment (Fig. S1). The microcosm was made of polyvinyl chloride sheet sandwiched by 30-μm nylon mesh between the three compartments (two root compartments: RCs, and one hyphal compartment: HC) to allow fungal hyphae but not plant roots to enter the other compartments. Each RC contained 700 g growth substrate with another 20 g inoculum, while the HC contained 1600 g of growth substrate. The HC was set up to collect mycelium and measure the ^{13}C content of the CMNs mycelium to quantify the carbon investment by the intercropped maize and soybean to the CMNs. However, we did not manage to collect enough mycelium to analyze the ^{13}C content.

For ensuring the initial growth of maize and soybean, nutrient solutions were added in RCs and HC with the following concentration (mg kg^{-1} soil): 100 P as KH_2PO_4; 113 K as K_2SO_4; 43 Mg as $MgSO_4 \cdot 7H_2O$; 5.9 Fe as Fe-EDTA; 6.7 Mn as $MnSO_4 \cdot H_2O$; 10 Zn as $ZnSO_4 \cdot 7H_2O$; 2 Cu as $CuSO_4 \cdot 5H_2O$; 0.67 B as H_3BO_3; 0.17 Mo as $Na_2MoO_4 \cdot 5H_2O$. In addition, 100 mg kg^{-1} N as $Ca(NO_3)_2 \cdot 4H_2O$ was supplied only in the RCs. In other words, 70 mg P and 70 mg N were added in the RCs with maize and soybean, respectively, while 160 mg P was added in the HC.

Experimental design

The experiment was conducted from November 15th, 2021 to February 21st, 2022 (13 weeks) in a greenhouse at China Agricultural University (40°1′29"N,116°16′33"E) where both temperature and light are controlled. Light intensity was 400 ~ 1000 μmol photons m^{-2} s^{-1} with a time period from 8 am to 6 pm every day. The RCs were irrigated by deionized water every second day by weighting the whole microcosm to maintain soil moisture content of each compartment at about 75% of field capacity to ensure sufficient water supply for plant growth.

Maize (*Zea mays* L. cv. Zhengdan 958) and soybean (*Glycine max* (L.) Merrill cv. Jidou 12) were used to establish microcosms with monoculture (maize or soybean was grown in the two chambers of RC) and mixture (maize was grown in one chamber of RC and soybean in the other). Seeds were surface-sterilized with 10% H_2O_2 for 30 min, rinsed thoroughly in sterile distilled water and pre-germinated on filter paper. Then two pre-germinated seeds of uniform size were placed in each RC. Maize seedlings were thinned to one seedling in each RC 5 days after sowing, while soybean seedlings were thinned to one seedling 7 days after sowing.

One of the RCs was inoculated with the AM fungus *Rhizophagus irregularis* (*R. irregularis*, BGC JX04B; the model AM fungal strain (Stockinger et al. 2009)), or sterilized inoculum, yielding seven treatments (Fig. 1). *R. irregularis* was provided by the Beijing Academy of Agriculture and Forestry Sciences and further propagated by using maize as

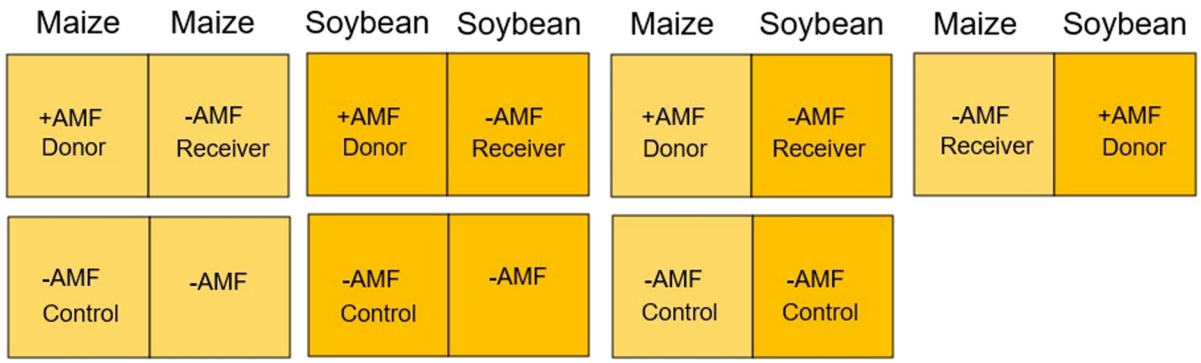

Fig. 1 Treatments of the root hyphal compartments (RCs). + AMF: one of the RCs was inoculated with AMF inoculum, -AMF: one of the RCs was inoculated with sterilized inoculum. Light orange and dark orange represent maize and soybean, respectively

host for four months to obtain enough AMF inoculum for our study. The seedings in the RC with AMF inoculation were inoculated with 25 g AMF inoculum (350 spores kg^{-1} soil). The seedlings in the RC without AMF inoculation were inoculated with 25 g inoculum sterilized at 121 °C for 30 min and received 20 mL of filtrate without AM fungal propagules filtered through a 30 µm nylon mesh sieve to balance the initial microbial communities (Singh et al. 2019; Liu et al. 2021). All treatments with soybean were inoculated with rhizobia (*Sinorhizobium fredii*; strain CCBAU 45436). The germinated soybean seeds were immersed in the rhizobium suspension (OD value = 1) for 30 min before sowing and 5 mL rhizobium suspension was added after sowing to each soybean compartment (Li et al. 2022a). The seedlings inoculated with AMF (+ AMF) were defined as donor plants and the neighbor seedlings with sterilized inoculum (-AMF) as receivers. The treatments with two RCs supplied with sterilized inoculum were non-mycorrhizal treatments. Each treatment was replicated four times and a total of 28 microcosms were established and randomly arranged (Fig. 1).

Harvest and sample analysis

Maize (tasseling period) and soybean (pod-forming stage) were harvested from all RCs in the four replicates of each treatment. Each plant was separated into shoot and root. Roots in the four replicates were washed with deionized water, and 50 root segments with a length of 1 cm were randomly selected for the determination of AMF colonization. Subsequently, the soybean root nodules were removed by cutting and stored in a 10 mL centrifuge tube for counting. After counting, the root nodules, remaining roots and shoots were dried at 105℃ for 30 min, and then at 70℃ for two days to constant weight before weighing.

Dry ashing method was used to determine shoot P concentration (Thomas et al. 1967). Briefly, 0.2 g of well-ground maize or soybean shoot samples were placed in a 25 mL porcelain crucible and placed in a muffle furnace for dry ashing. During dry ashing, the samples were heated to 180 °C, kept for half an hour to facilitate carbonization of the sample, then kept for 4 h in a muffle furnace at 450 °C. Two mL nitric acid with a concentration of 2 mol L^{-1} was added to the ash for digestion first, then 18 mL deionized water was added to dilute the solution to a total volume of 20 mL. After the above procedures, the solutions were filtered with P-free filter paper, then 1 mL filtrate was taken from the solution of each sample and diluted 10 times and vanadium molybdenum yellow colorimetric method was used for determination.

We assessed AMF colonization using the method from McGonigle et al. (1990) and Wang et al. (2022). Briefly, root clippings were immersed in 10% KOH, kept in 90℃ water bath for 30 min, then acidified in 2% HCl for 10 min and finally soaked in a plastic box containing 0.05% trypan blue in lacto-glycerol (lactic acid: glycerol: deionized water = 1:1:1) for 30 min in a 90℃ water bath. We then destained with lacto-glycerol at room temperature for 2 days and cut the root into 1 cm pieces. Fifteen pieces were placed on each microscope slide, and 10 visual fields were observed in each root piece, a total of 150 visual fields were scored for mycorrhizal colonization.

The mycorrhizal growth response (MGR) of plant shoot biomass, mycorrhizal N content response (MNR) and mycorrhizal P content response (MPR) were calculated as:

$$\text{MGR (MNR, MPR)} = \ln(\frac{\text{AM}}{\text{NM}})$$

where AM is plant shoot biomass, N content and P content in the mycorrhizal treatments and NM is plant shoot biomass, N content and P content, which are mean values of the corresponding non-mycorrhizal treatments (control treatments). Here we calculated the MGR, MNR and MPR of maize and soybean in each RC separately instead of taking the average of two RCs with AMF because we distinguished donor and receiver plant in the experiment i.e., we calculated the MGR, MNR and MPR of receiver or donor maize and receiver or donor soybean.

To assess the overyielding, the expected biomass and N, P content of maize/soybean mixture were calculated. With maize as donor, the expected value was calculated by maize as donor in monoculture + soybean as receiver in monoculture; When soybean as donor, the expected value was calculated by soybean as donor in monoculture + maize as receiver in monoculture.

Statistical analysis

We first tested, through two-way analysis of variance (ANOVA), for general effects of the mycorrhizal treatment (df = 2 for mycorrhiza, three levels of AMF inoculation: -AMF, donor, receiver; two levels of cropping system: monoculture and mixture). A second ANOVA tested for specific effects of being a donor or receiver of CMNs by omitting the -AMF treatments (df = 1, two levels of AMF inoculation: donor, receiver; two levels of cropping system: monoculture and mixture). These tests were carried out for maize and soybean separately. The first ANOVA was conducted for shoot biomass, N and P concentration, N and P content, N:P, nodule number and nodule weight, while the second ANOVA was conducted for the same parameters and additionally for AMF colonization, MGR, MNR and MPR. Before ANOVAs, the data were checked for homogeneity of variances with Levene's test and normality with Shapiro–Wilk test.

After ANOVAs, significant differences among treatments were tested by Tukey's honestly significant difference *post-hoc* test (Tukey HSD test). All statistical analyses were performed with SPSS 20.0 (IBM SPSS software), and figures were made with SigmaPlot 12.5 (Systat).

Results

AMF colonization

Regardless which crop was the CMNs donor, AMF colonization was observed in the roots of its neighbor, indicating that CMNs had been formed. The non-mycorrhizal treatments remained free of colonization. AMF colonization of maize was impacted by the interaction of CMNs and cropping system ($P = 0.002$), but not CMNs ($P = 0.068$) or cropping system ($P = 0.119$). Monocultured maize had similar colonization levels regardless whether maize was donor or receiver. The colonization of intercropped maize as donor was higher than that of intercropped maize as receiver. The colonization of soybean was not affected by CMNs ($P = 0.581$), cropping system ($P = 0.218$) or their interaction ($P = 0.393$) (Fig. 2; Table S2).

Shoot biomass, N content and P content

The first two-way ANOVA showed that maize shoot biomass was increased by CMNs ($P = 0.023$), cropping system ($P < 0.001$) and their interaction ($P = 0.036$) (Fig. S2a; Table S1). Soybean shoot biomass was only affected by the interaction of the cropping system and CMNs ($P = 0.017$), but not by cropping system ($P = 0.081$) or CMNs alone ($P = 0.130$) (Fig. S2b; Table S1). Mixing maize and soybean significantly increased maize biomass but not soybean compared with monoculture regardless of inoculating AMF, suggesting that maize was facilitated by the presence of soybean independent of mycorrhizal colonization or the identity of the donor (Fig. S2a, b; Table S1).

Shoot N concentration and N content of maize were affected by cropping system ($P = 0.041$ and 0.001, respectively) and the interaction of CMNs and cropping system ($P = 0.002$ and 0.001, respectively), but not CMNs ($P = 0.238$ and 0.082, respectively)

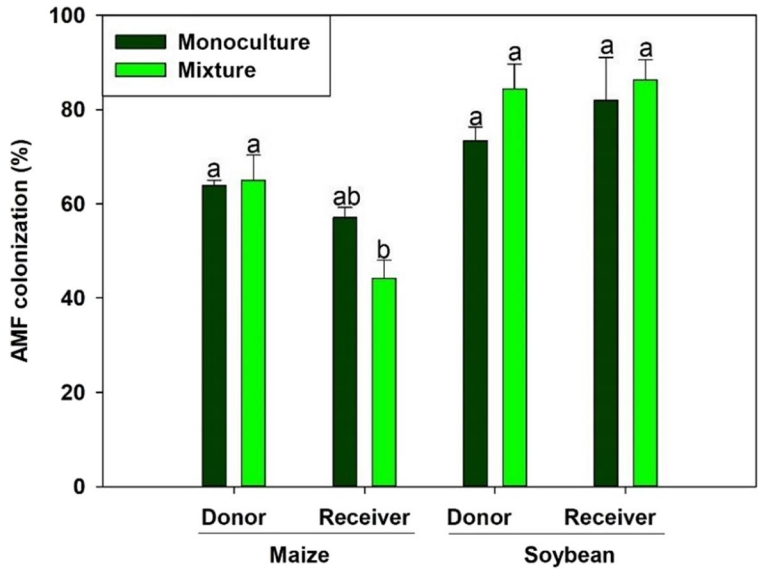

Fig. 2 AMF colonization of maize and soybean. Seedlings inoculated with AMF were defined as donor and neighboring seedlings with sterilized inoculum as receiver. Letters indicate significant differences among treatments for maize or soybean (Tukey HSD test, $P<0.05$). Bars represent mean value (%) ± standard error

(Fig. S2c, d; Fig. S3c, d; Table S1). However, shoot N concentration and N content of soybean was affected by CMNs (both $P<0.001$) and interaction between CMNs and cropping system ($P=0.007$ and 0.008, respectively), but cropping system had no significant effect on N concentration or N content ($P=0.382$ and 0.706, respectively) (Fig. S3c; Table S1). N contents of both maize and soybean were increased in mixture compared with that in monoculture with maize as donor. However, N contents of maize and soybean were similar in the mixture compared with that in the monoculture when plants were without CMNs or with soybean as donor.

The existence of CMNs and crop mixture increased P concentration of maize ($P<0.001$), while soybean shoot P concentration was affected by CMNs ($P<0.001$) and the interaction of CMNs and cropping system ($P=0.012$), but not by cropping system ($P=0.809$) (Fig. S3a, b; Table S1). Shoot P content of maize was enhanced by CMNs and cropping system (both $P<0.001$), but not affected by their interaction ($P=0.073$), and shoot P content of soybean was enhanced by CMNs ($P<0.001$) and the interaction of CMNs and cropping system ($P=0.013$), but not cropping system ($P=0.397$) (Fig. S2e, f; Table S1).

The second two-way ANOVA showed that the biomass, N and P content of maize and soybean (except the biomass of soybean) were significantly increased in maize/soybean mixture compared with monoculture with maize as donor (Fig. 3). However, the biomass, N and P content of maize and soybean (except the biomass of maize) were similar in maize/soybean mixture compared with monoculture with soybean as donor (Fig. 3). These results indicated that the enhanced N and P content of mixed maize and soybean compared with monocultures were dependent on the crop species that formed the CMNs (Fig. S2a, b).

N:P ratio

The first ANOVA showed that the presence of CMNs decreased the N:P ratio of maize regardless of monoculture or mixture ($P<0.001$, Fig. S4a, Table S1), but the presence of CMNs had no effect on the N:P ratio of soybean (Figs. S4b; S5b). The second ANOVA showed that the N:P ratios of maize and soybean were independent of the identity of donor or cropping system (Fig. S5). N:P ratios of maize were well below 10, indicating N-limitation and those of soybean were above 15, indicating P-limitation (Fig. S5a).

Mycorrhizal growth responses of biomass, N content and P content

The MGR of maize was affected by CMNs ($P=0.034$), cropping system ($P=0.003$) but not their interaction ($P=0.316$) (Fig. 4a; Table S2). The MNR of maize was affected by cropping system ($P=0.012$) and the interaction between cropping system and

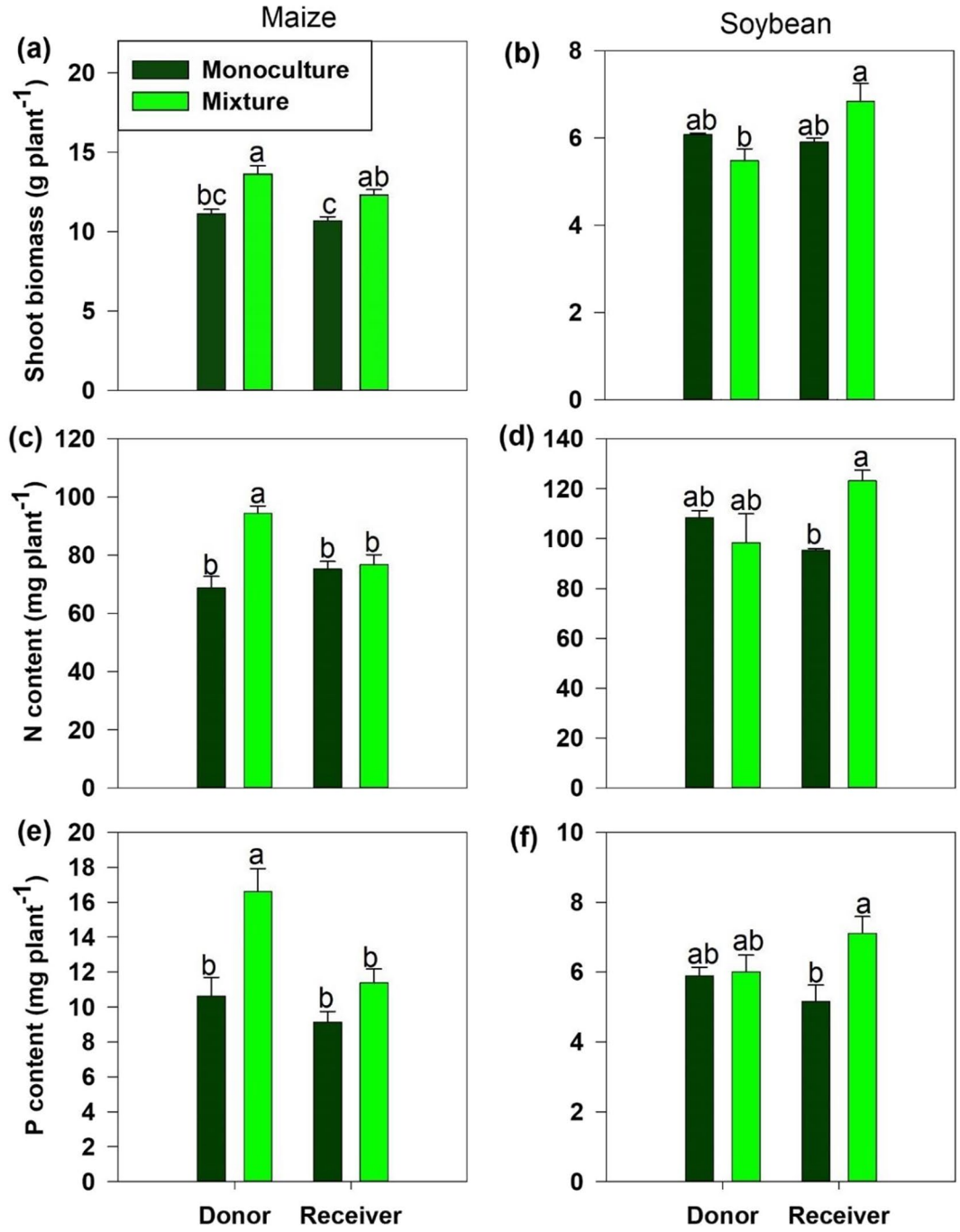

Fig. 3 Shoot biomass (**a**, **b**), N content (**c**, **d**) and P content (**e**, **f**) of maize and soybean in treatments with AMF inoculation. The seedlings inoculated with AMF were defined as donors while the neighboring seedlings with sterilized inoculum as receivers. Letters indicate significant differences among treatments for maize or soybean (Tukey HSD test, $P < 0.05$). Bars represent mean value (%) ± standard error

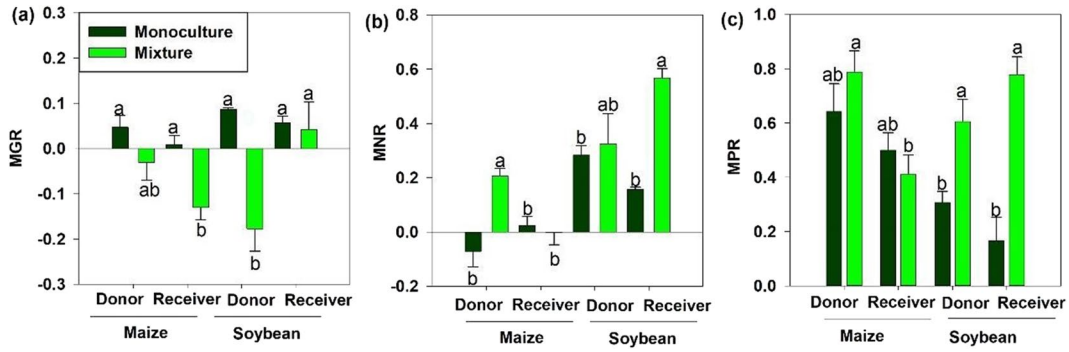

Fig. 4 Mycorrhizal growth response (MGR) of biomass (**a**), mycorrhizal N content response (MNR) (**b**) and P content response (MPR) (**c**) of maize and soybean in treatments with AMF inoculation. The seedling inoculated with AMF was defined as donors while the neighboring seedling with sterilized inoculum as receivers. Different letters indicate significant differences among treatments for maize or soybean (Tukey HSD test, $P<0.05$). Bars represent mean value (%) ± standard error

CMNs ($P=0.004$) (Fig. 4b; Table S2). However, the MPR of maize was only affected by the presence of CMNs ($P=0.007$) (Fig. 4c; Table S2). The MGR of soybean biomass was affected by CMNs ($P=0.036$), cropping system ($P=0.005$) and their interaction ($P=0.009$) (Fig. 4a; Table S2), but the MNR and MPR of soybean were affected by cropping system ($P=0.003$ and $P<0.001$, respectively) and the interaction between CMNs and cropping system ($P=0.011$ and 0.047, respectively) (Fig. 4b, c; Table S2).

There was no significant difference in MGR, MNR and MPR of maize either as donor or receiver. Soybean had similar results in monoculture treatments (Fig. 4a, b, c). In mixtures, the MNR and MPR of maize and the MGR of soybean were significantly higher with maize as donor (soybean as receiver) compared with soybean as donor. It should be noted that although the MNR and MPR of soybean was not significantly different with soybean as donor or receiver in mixture, soybean as receiver had higher MNR and MPR in mixtures than in monocultures. In addition, the MNR and MPR were usually positive (especially for soybean), but the MGR was often negative, suggesting a disconnect between nutrient acquisition and its translation in enhanced biomass.

Overyielding of shoot biomass, N content and P content

Overyielding in mixtures was asymmetrical. There was significant biomass overyielding in mixtures

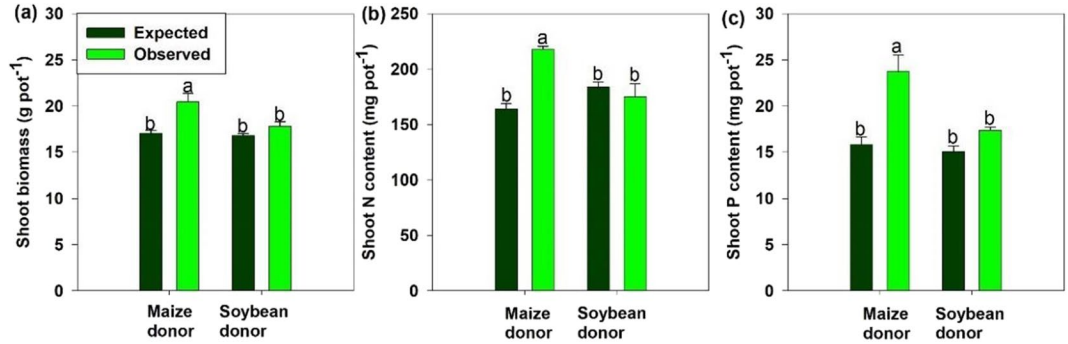

Fig. 5 Shoot biomass (**a**), N content (**b**) and P content (**c**) of maize and soybean at system level in treatments with AMF inoculation. The seedlings inoculated with AMF were defined as donors. Different letters indicate significant differences among treatments (Tukey HSD test, $P<0.05$). Bars represent mean value (%) ± standard error

without CMNs and with maize as donor, but no overyielding was observed with soybean as donor (Fig. 5a, Fig. S6a). For N content, there was overyielding in maize/soybean mixture with CMNs with maize as donor, but there was no overyielding without CMNs and with soybean as donor (Fig. 5b, Fig. S6b). For P content, there was overyielding in maize/soybean mixture with CMNs with maize as donor (Fig. 5c, Fig. S6c).

Nodule number and nodule weight of soybean

The nodule number of soybean was not significantly different among treatments regardless of CMNs or neighboring plant (Fig. S7a; Table S2), while nodule weight was increased when mixed soybean was receiver in the mixture compared with sole soybean (Fig. S7b; Table S2).

Discussion

Our study showed that CMNs formed by maize (CMNs donor) increased the biomass, N and P content of maize/soybean mixture compared with that expected based on monocultures. This overyielding in N and P content was caused by the higher N and P content of both mixed maize and mixed soybean compared with monocultures when maize was the CMNs donor. The N and P content of mixed soybean was enhanced by CMNs presence compared with the treatments without CMNs regardless of the CMNs donor; However, the N and P content of mixed soybean was similar with monocultured soybean when soybean was donor. Maize biomass was twice that of soybean in this study. The results showed that donor identity of CMNs unequally benefitted the N and P acquisition and content of crop species in the mixture. Both crop species benefitted in N and P content when the crop species with larger size (maize) was a CMNs donor but no benefits for both species when the crop species with smaller size (soybean) was a CMNs donor (Fig. 6).

Our results showed that the N and P content (Fig. S2) of maize/soybean mixture in the presence of CMNs were higher than in the treatments without CMNs, consistent with previous results that the presence of AMF in crop mixtures has positive effects on nutrient uptake compared with the treatments without AMF inoculation (Wang et al. 2016; Schütz et al.

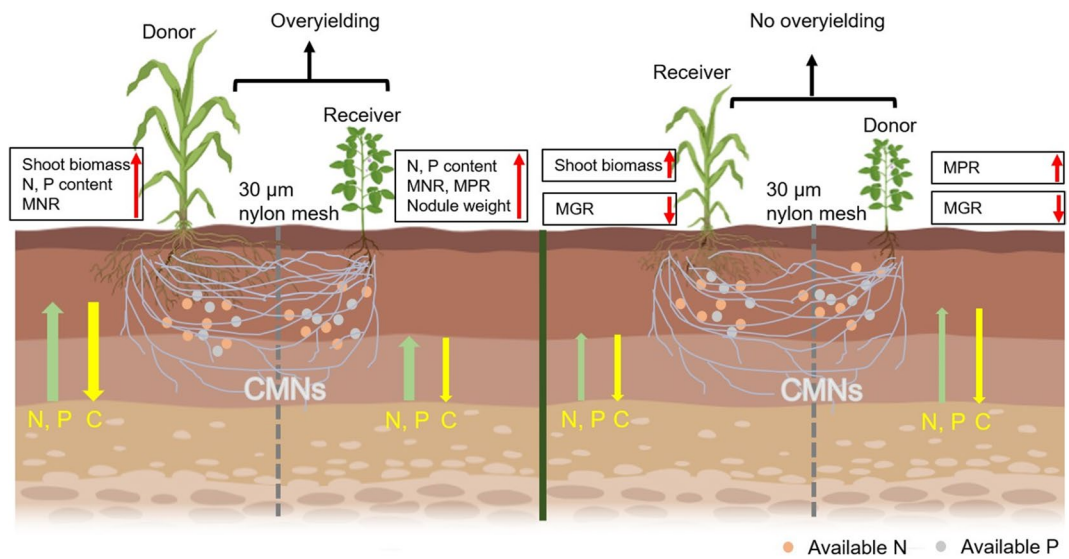

Fig. 6 Conceptual model to show how the common mycorrhizal networks (CMNs) initiated by maize (left panel) or soybean (right panel) mediated the accumulation of biomass, nitrogen (N) and phosphorus (P) in maize/soybean mixture compared with monocultures. The length of arrows represent the time of the AMF colonization in the crop species. The width of the yellow arrows represent the amount of carbon (C) input of crop species to the CMNs, and the width of the green arrows represent the amount of nutrient (N, P) acquisition

2022). However, we found that maize/soybean mixture showed overyielding in biomass but no overyielding in N and P content without CMNs, and maize/soybean mixture showed overyielding in biomass, N and P content with maize as donor. The explanation for this disconnect between nutrient acquisition and C gain could be that maize was co-limited by N and P without AMF (the average N:P of maize was 10.4), and maize became N-limited with AMF (the average N:P of maize was 6.25). That means that the additional P uptake could be luxury uptake, and the P uptake was not translated into biomass. In addition, the space limitation caused by the size of the microcosms could additionally limit the further growth of plants and potentially contribute to the observed lack of translation of improved P to growth.

Our second hypothesis was fully confirmed. The maize/soybean mixture with CMNs initiated by maize showed overyielding in biomass, N and P content but there was no overyielding with CMNs initiated by soybean in mixture. The overyielding of maize/soybean mixture with CMNs initiated by maize was caused by the higher N and P content by both mixed maize and mixed soybean compared with monocultured maize and monocultured soybean, respectively. Previous studies have shown that in symbiotic systems formed by CMNs, host plants with larger size generally exhibit stronger competitive ability than host plants with smaller size (Weremijewicz and Janos 2013; Weremijewicz et al. 2016) because larger host plants could provide more carbon to AMF, and reciprocally receive higher nutrients rewards from their fungal partner (Fellbaum et al. 2014; Walder and van der Heijden 2015). In the maize/soybean mixture, the average biomass of maize was about twice of soybean, and maize could maintain a more extensive and beneficial, hyphal network than soybean. Lower partner quality of soybean could also explain reduced root colonization of maize when soybean was the donor of CMNs (Fig. 2). Our results are consistent with Li et al. (2022a), who showed that the CMNs initiated by the species with higher biomass (chickpea in that study) than the other species (millet) promoted overyielding in nutrient uptake in millet/chickpea mixture. However, the causes could also be related to the different functional traits between maize and soybean since maize, a C4 plant, has stronger photosynthetic capacity than C3 soybean (Hatch 1987; Qiao et al. 2017), which allows maize to allocate large amount of carbon as the form of root exudates to mobilize sparingly soluble nutrients (Ma et al. 2022). Further studies on the causes related to functional traits such as root morphology or root exudates should be conducted to understand the host identity effect on the beneficial CMNs effect. Moreover, our results showed that the MNR and MPR content of mixed maize was higher when the CMNs were initiated by maize than when initiated by soybean, while the MNR and MPR of mixed soybean were not affected by donor species, indicating that the role of CMNs in promoting nutrient uptake is closely related to the initial host plant that has different plant functional traits (Toro et al. 2023). Together these data show that CMNs initiated by larger-biomass plants have a greater advantage in growth performance than the other species in the mixture, thereby exacerbating competition.

Our third hypothesis was supported by the increased nodule weight of mixed soybean in treatments with CMNs initiated by maize than without CMNs, indicating that the N_2 fixation capacity was enhanced with the dual inoculation of AMF and rhizobia, a result consistent with previous studies (Meng et al. 2015; Wang et al. 2016). Maize had a stronger ability to use soil available N than soybean, which reduced the amount of soil available N for soybean, and thus promoted soybean to rely more on N_2 fixation. In the maize/soybean mixture, the enhanced N_2 fixation of soybean reduced competition with maize for soil available N (Hauggaard-Nielsen and Jensen 2001; Raza et al. 2019; Du et al. 2020). The N:P of soybean with AMF was slightly lower compared with that without AMF. That suggests that the addition of AMF did to some extent alleviate P limitation of soybean, which enhanced the N fixation of soybean. However, the N:P ratios above 15 indicated that P remained the limiting nutrient for soybean, again emphasizing the need for balanced fertilization in intercropping. The results were consistent with previous studies that cereals in crop mixtures successfully compete for available N and thereby enhance N_2 fixation by soybean especially when mycorrhizal fungal activity supplies P to ensure N_2 fixation (Unger et al. 2016; Awaydul et al. 2019; Wipf et al. 2019). However, the N_2 fixation should be quantified using ^{15}N isotope labeling methods in further research to verify the enhancement of N_2 fixation caused by the CMNs initiated by maize.

Our study demonstrated that the N and P content and overyielding were influenced by the host identity that formed the CMNs in maize/soybean mixture. The mesh between root compartment and hyphal compartment could have reduced the access to nutrients in the non-mycorrhizal treatments (apart from mass flow from hyphal compartment to root compartment). This could have resulted in overestimating the mycorrhizal effect (the first ANOVA) but would not have impacted the mycorrhizal treatments (the second ANOVA to test the for specific effects of being a donor or receiver of CMNs), which was the main focus of our study. Future research should focus on to what extent overyielding in crop mixtures caused by CMNs is determined by plant identity or also by fungal identity (Walder et al. 2012) because field soils contain a diversity of AMF species. Further research should also focus on the difference in time for donor and receiver to become mycorrhizal, since the earlier the plant becomes mycorrhizal the more benefits could be derived from the CMNs. Our study increases understanding how root-microbe interactions contribute to overyielding in maize/soybean intercropping, which is now widely practiced in China. However, field studies are needed to understand the role of CMNs in the overyielding in nutrient uptake in intercropping. Additionally, it should be noted that although intercropping has advantages in yield and nutrient uptake compared with monoculture, mechanized production of intercropping remains a challenge, meaning that these advantages may come at the cost of more laborious management/harvesting.

Conclusion

In maize/soybean mixture, when maize, biomass of which was twice that of soybean, was the donor of the CMNs, N and P content of both maize and soybean was increased compared with monocultures, and overyielding in N and P content was observed. Thus, overyielding in a maize/soybean mixture depends on the initiator of CMNs. With maize, a C4 species that has larger biomass than soybean, as CMNs donor, both species in the mixture benefited from CMNs compared with monocultures.

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Author contributions Y.L, L.Z and C.L designed the experiment, Y.L, T.W.K, L.Z and C.L carried out data analysis and interpretation of results. All the authors reviewed the manuscript and contributed to the interpretation and manuscript revisions.

Declarations

Conflicts of interest The authors declare no competing interests.

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