Mycorrhizal Networks: Common Goods of Plants Shared under Unequal Terms of Trade[1][W][OA]

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Plants commonly live in a symbiotic association with arbuscular mycorrhizal fungi (AMF). They invest photosynthetic products to feed their fungal partners, which, in return, provide mineral nutrients foraged in the soil by their intricate hyphal networks. Intriguingly, AMF can link neighboring plants, forming common mycorrhizal networks (CMNs). What are the terms of trade in such CMNs between plants and their shared fungal partners? To address this question, we set up microcosms containing a pair of test plants, interlinked by a CMN of *Glomus intraradices* or *Glomus mosseae*. The plants were flax (*Linum usitatissimum*; a C_3 plant) and sorghum (*Sorghum bicolor*; a C_4 plant), which display distinctly different $^{13}C/^{12}C$ isotope compositions. This allowed us to differentially assess the carbon investment of the two plants into the CMN through stable isotope tracing. In parallel, we determined the plants' "return of investment" (i.e. the acquisition of nutrients via CMN) using ^{15}N and ^{33}P as tracers. Depending on the AMF species, we found a strong asymmetry in the terms of trade: flax invested little carbon but gained up to 94% of the nitrogen and phosphorus provided by the CMN, which highly facilitated growth, whereas the neighboring sorghum invested massive amounts of carbon with little return but was barely affected in growth. Overall biomass production in the mixed culture surpassed the mean of the two monocultures. Thus, CMNs may contribute to interplant facilitation and the productivity boosts often found with intercropping compared with conventional monocropping.

Arbuscular mycorrhizal fungi (AMF) inhabit the soils of virtually all terrestrial ecosystems, forming symbiotic associations with most plants (Parniske, 2008; Smith and Read, 2008). The host plants incur substantial carbon costs to sustain this symbiosis (Jakobsen and Rosendahl, 1990), but in return, they obtain multiple benefits from the fungal partners, above all, the provision of mineral nutrients. AMF may supply up to 90% of the host plant's nitrogen and phosphorus requirements (Smith and Read, 2008). Moreover, AMF are important determinants of plant community structure and ecosystem productivity (Grime et al., 1987; van der Heijden et al., 1998), and they represent a crucial asset for sustainable agriculture (Rooney et al., 2009). Typically, AMF exhibit little host specificity; a single individual may form a common mycorrhizal network (CMN) between several coexisting plant individuals, even from different species (Whitfield, 2007; Smith and Read, 2008; Bever et al., 2010). Such CMNs may be enlarged through hyphal fusion of conspecific AMF (Giovannetti et al., 2004). The functionality of CMNs formed by the fusion of two individual fungal networks by hyphal anastomoses has been demonstrated by tracing nutrient allocation between individual host plants upon the fusion of their associated CMNs (Mikkelsen et al., 2008).

The potential role and importance of CMNs is most apparent in the case of mycoheterotrophic plants. These plants connect themselves to an existing CMN to receive both carbon and mineral nutrients (Bidartondo et al., 2002; Courty et al., 2011). There is an ongoing debate over whether carbon transfer through CMNs may also occur among autotrophic plants (Bever et al., 2010; Hodge et al., 2010). This is of a certain academic interest, but it may obscure a more general and obvious question arising from recent literature (Hodge et al., 2010; Hammer et al., 2011; Kiers et al., 2011; Smith and Smith, 2011; Fellbaum et al., 2012): What are the terms of trade between plants and their shared fungal partners? Put another way, what is the "investment" of a given plant into a CMN (in the currency of assimilated carbon), and what is the "return of investment" in terms of mineral nutrients provided by the CMN? Indeed, different cocultivated plants benefit differently from their CMN, depending on the AMF species involved, and these differences significantly affect plant coexistence (Zabinski et al., 2002; van der Heijden et al., 2003; Wagg et al., 2011). However, until now, the relationship between the carbon investment and the nutritional benefit of different plants engaged in a CMN has never been assessed.

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To address the terms of trade in a CMN experimentally, we established a model system consisting of two plant individuals growing side by side in compartmented microcosms (Fig. 1). The roots of the plants were confined to their respective "root hyphal compartments" (RHCs). In the treatments with AMF inoculation, however, the plants were able to connect through CMN in the "hyphal compartment" (HC) or in the "label-hyphal compartment" (LHC). We assessed the carbon investments of the single plants into the CMN through stable isotope tracing. To this end, we chose the C_3 plant flax (*Linum usitatissimum*) and the C_4 plant sorghum (*Sorghum bicolor*) for our experiments. Due to the different isotope fractionation during C_3 versus C_4 carbon fixation, these two species display distinctly different carbon isotope ratios (δ ^{13}C approximately 33‰ for flax and approximately 14‰ for sorghum). This difference in the ^{13}C signature of C_3 and C_4 plants has been widely used to track carbon flows in mycorrhizal symbioses (Allen and Allen, 1990; Fitter et al., 1998). The plants were grown either in "monocultures," as a pair of identical plant species, or in a "mixed culture," as a pair of different plant species. We used two different AMF species in the experiments for inoculation, *Glomus intraradices* and *Glomus mosseae* (recently renamed *Glomus irregulare* [*Rhizophagus irregularis*] and *Funelliformis mosseae*, respectively [Schüssler and Walker, 2010]). The chosen experimental setup allowed us to harvest the bulk of the CMN in the HC (Fig. 1) and to estimate the respective carbon investment of the two plants into the CMN through the analysis of the δ ^{13}C of isolated AMF hyphae or, with higher precision, of the AMF-specific fatty acid C16:1ω5 (Olsson and Johnson, 2005). We estimated the return of investment with respect to nitrogen and phosphorus for each of the two plants using ^{15}N and ^{33}P as tracers added to the LHC (Fig. 1). As a control, we also grew two monocultures and a mixed culture without any AMF inoculation.

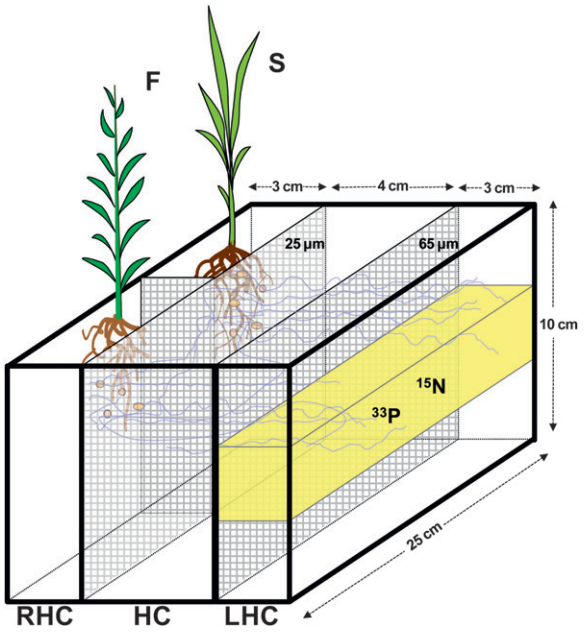

Figure 1. Compartmented microcosms to study the role of CMNs in monocultures and mixed culture. Microcosms, consisting of two plant individuals, were set up in compartmented containers subdivided by nylon mesh screens (25 and 65 μm, respectively, as indicated). Both types of screens are pervious for fungal hyphae but not for roots and allow the separation into two RHCs, a HC, and a LHC for supplying ^{15}N and ^{33}P labels. The plants used were flax (F) and sorghum (S) either as a pair of conspecific plants (F:F, S:S) as a model of monoculture or in combination (F:S) as a model of a mixed culture.

RESULTS

Impact of CMNs on Monocultures and Mixed Culture

A first experiment in the compartmented microcosms (Fig. 1) demonstrated that in mixed culture with sorghum, flax grew poorly in the absence of AMF (Supplemental Table S1). Its growth was significantly enhanced (almost by a factor of 3), however, in the presence of a CMN formed by *G. intraradices* (Fig. 2; compare center top and bottom). Growth of sorghum, in contrast, was not significantly affected by the presence or absence of a CMN (Fig. 2). Comparing the growth performance of monoculture versus mixed culture of flax and sorghum in a CMN, respectively, was equally striking (Fig. 2, bottom): flax profited substantially (+46% more biomass) from a neighboring sorghum, whereas sorghum was only marginally negatively (−7%) affected by the mixed culture growth with flax as neighbor. Thus, the biomass increase of flax did not happen at a relevant expense of the neighboring sorghum. Apparently, the two plants had different terms of trade with the CMN of *G. intraradices*, resulting in an overall higher productivity of the mixed culture, compared with the mean of the two monocultures of flax and sorghum (5.97 ± 0.18 g versus 5.36 ± 0.14 g, respectively [P = 0.039], amounting to an 11% overall biomass increase by mixed culturing).

The carbon investment of the two plants into the CMN was quantified through analysis of the carbon isotope composition (δ ^{13}C; for definition, see "Materials and Methods") of extracted AMF hyphae (Supplemental Fig. S1A). The hyphal material obtained from the flax monoculture had a δ ^{13}C value of approximately 27‰ (i.e. slightly higher than the δ ^{13}C of the host plant [approximately 33‰]). Hyphae from the sorghum monoculture displayed a δ ^{13}C of approximately 13‰, very close to the value of sorghum plants (δ ^{13}C = approximately 14‰). Interestingly, the δ ^{13}C of the hyphal material from the mixed culture was also very close to that of the sorghum monoculture, indicating that around 80% of the carbon invested into the CMN originated from sorghum (Supplemental Fig. S1A).

In the mixed culture, the return of investment in terms of nutrient uptake by the plants, measured as the relative uptake of ^{33}P and ^{15}N from the LHC (Fig. 1), was similarly unbalanced, but in the opposite sense. In the mixed culture, flax obtained the lion's share of both nutrients (i.e. in the range of approximately 80%, compared with about

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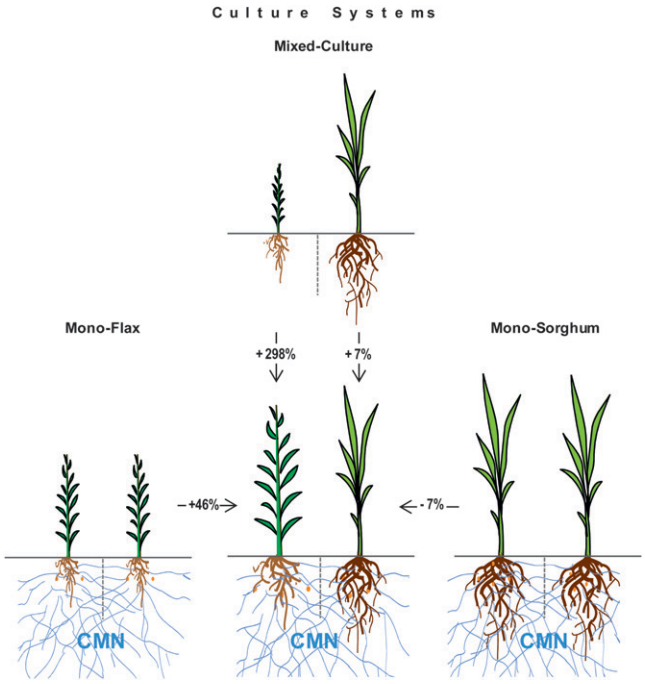

Figure 2. Impact of a CMN in monocultures and mixed culture. The presence of a CMN of *G. intraradices* strongly enhanced the biomass production of flax in mixed culture with sorghum. Sorghum was not significantly affected by the presence of a CMN in the mixed culture with flax. The flax plants grew significantly faster in the flax/sorghum mixed cultures than in the flax monocultures. Conversely, the growth of sorghum was only marginally influenced by the culture system.

approximately 20% for sorghum; Supplemental Fig. S1, B and C).

To confirm and extend these findings, we conducted a second experiment with two different AMF (*G. intraradices* and *G. mosseae*). As in the first experiment, biomass accumulation and phosphorus and nitrogen contents of the flax plants were higher when grown in a CMN together with a neighboring sorghum, irrespective of the fungal species (Fig. 3, A, C, and E). In contrast, the growth performance of sorghum was not affected (Fig. 3B). As a consequence, the overall productivity in the mixed culture was, again, higher than in the combined monocultures. Despite the absence of any significant growth effect, sorghum also seemed to benefit from the AMF, as indicated by the significant increase in phosphorus and nitrogen contents (except nitrogen with *G. intraradices* in mixed culture; Fig. 3, D and F). Growth-limiting factors at the time of labeling (e.g. constraints of rooting space) might have led to a surplus or "luxury" carbon as well as a reduced sink strength for soil nutrients.

An AMF-Specific Fatty Acid as a Biomarker for the Plants' Carbon Investment

In order to quantify the carbon investments into the CMN more precisely, we selectively analyzed the carbon isotopic composition of the AMF-specific fatty acid (C16:1ω5) in the lipid fraction obtained from the HC (Fig. 1). This way, potential contamination of the hyphal material by nonsymbiotic fungi or other microorganisms can be excluded. Indeed, confirming its use as a marker for AMF, we found C16:1ω5 exclusively in the microcosms inoculated with AMF. As expected, the C16:1ω5 in the HC (Fig. 1) of the monocultures inoculated with *G. intraradices* or *G. mosseae* displayed a similar carbon isotopic signature as their host plants, confirming that the AMF rely on the carbon of their symbiotic partners. The fact that the biomarker δ ^{13}C values were consistently lower by approximately 2‰ than those of the host is likely due to the small but measurable and constant carbon isotope discrimination during carbon transfer from the plants to the lipids of the arbuscular mycorrhizal fungi (Fig. 4). Remarkably, in the mixed culture, the δ ^{13}C values for the extraradical mycelium of both *G. intraradices* and *G. mosseae* were much closer to the δ ^{13}C of sorghum than to that of flax in monoculture, roughly confirming our initial finding that the carbon invested into the CMN of the mixed culture was derived approximately 70% from sorghum and only approximately 30% from flax, independent of the fungi involved (Fig. 4).

Nutritional Benefit Gained via CMNs

The nonmycorrhizal systems did not take up any ^{33}P and relatively little ^{15}N (Fig. 5, A and B), indicating that at the time scale of the experiments, nutrient mobilization due to diffusive processes or mass flow did not play any significant role (Fig. 5, C and D). Thus, virtually all ^{33}P and the bulk of ^{15}N acquired by the plants engaged in the CMN must have come from the fungal partners. Indeed, in monocultures with AMF, both flax and sorghum were able to retrieve substantial amounts of ^{33}P and ^{15}N (Fig. 5, F:F and S:S). Interestingly, *G. mosseae* delivered about twice as much ^{33}P to sorghum than *G. intraradices* (Fig. 5B, S:S), whereas similar amounts were delivered to flax by both fungi (Fig. 5A, F:F). As a control, we also compared the relative uptake of ^{33}P and ^{15}N for the two plant individuals grown in monoculture. In all cases, nutrient acquisition by the two plants was comparable (Supplemental Fig. S2, F:F and S:S).

When comparing the acquisition of the isotopically labeled nutrients through the CMN in monocultures and mixed culture, we observed marked differences depending on the fungal species involved. With a CMN formed by *G. intraradices*, flax received more than twice as much ^{33}P, and also a little more ^{15}N, in mixed culture than in monoculture (Fig. 5, A and C, gray columns). In contrast, sorghum obtained much less ^{33}P and ^{15}N in mixed culture than in monoculture (Fig. 5, B and D, gray columns), indicating that flax used the CMN of *G. intraradices* highly efficiently for nutrient uptake, at the expense of sorghum. This corroborates the results of the first experiment (Supplemental Fig. S1, B and C) and becomes particularly apparent when the data are plotted as relative uptake

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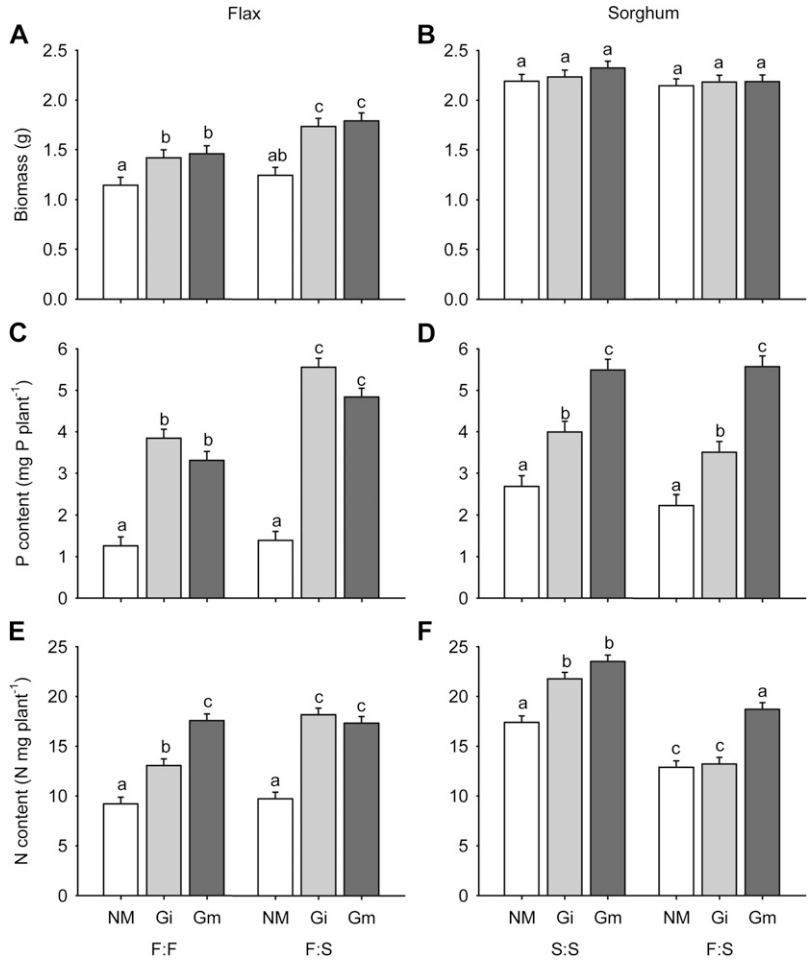

Figure 3. Impact of a CMN on plant growth performance and nutrient uptake in monocultures or mixed culture. A, C, and E, Performance of flax in monoculture (F:F) or mixed culture (F:S). B, D, and F, Performance of sorghum in monoculture (S:S) or mixed culture (F:S). Data represent means ± SE (n = 6). Different lowercase letters above the bars indicate significant differences ($P \leq 0.05$) among treatments according to planned contrast analysis; mean comparisons were treated separately for both plant species. NM, Nonmycorrhizal control; Gi, *G. irregulare*; Gm, *G. mosseae*.

values (Supplemental Fig. S2): 94% of the ^{33}P and 80% of the ^{15}N supplied to the plant pair via the CMN was secured by flax. In contrast, with a CMN formed by *G. mosseae*, flax did not benefit significantly from a neighboring sorghum (Fig. 5, A and C, black columns). At the same time, sorghum did not suffer intensively from the neighboring flax, although there was still a significantly reduced uptake of nutrients in mixed culture compared with monoculture (Fig. 5, B and D, black columns). In terms of relative uptake, there was no significant difference between flax and sorghum in the mixed culture (Supplemental Fig. S2). Thus, the respective nutrient return to flax and sorghum strongly differed between the two AMF: flax was much more efficient than sorghum in exploiting the CMN of *G. intraradices*, whereas in symbiosis with *G. mosseae*, the two plants exploited the CMN on equal terms, although sorghum invested much more carbon than flax (Fig. 4).

Mycorrhizal Root Colonization and Hyphal Length Density

Both flax and sorghum were well colonized by AMF, with total colonization ranging between 40% and 62% and arbuscular colonization ranging between 31% and 43% (Supplemental Table S2). For flax, colonization was similar in monoculture and mixed culture with both AMF species. As for sorghum, both root colonization and arbuscular colonization were significantly reduced in mixed culture, compared with the monoculture, indicating that sorghum plants were less engaged in the symbiosis in the presence of neighboring flax plants. The extraradical hyphal length density in the hyphal compartment was slightly higher in the sorghum monoculture than in the flax monoculture, although it was statistically significant only in the case of *G. mosseae* (Supplemental Fig. S3). In the mixed culture, hyphal length density was intermediate.

DISCUSSION

Uneven Terms of Trade in a CMN

Our results (for a graphical synopsis, see Fig. 6) emphasize the importance of the terms of trade within a CMN as a driver for the coexistence of mycorrhizal plants in ecosystems. In our mixed-culture experiments, sorghum, as the plant with the higher biomass, consistently provided the bulk of carbon to both tested fungal partners, investing at least twice as much into

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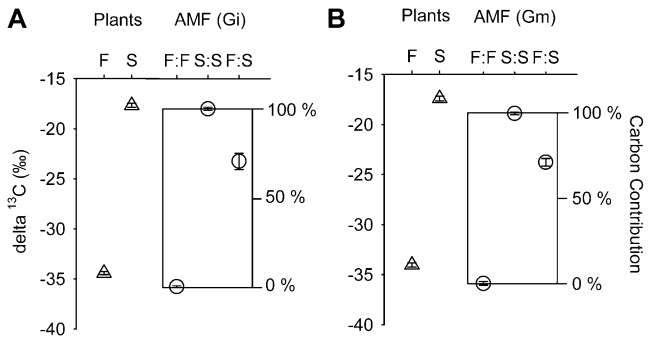

Figure 4. Carbon investment of two plants sharing a CMN. δ ^{13}C values of plant tissue (triangles; means ± SE, $n = 36$) differ between flax (F; C_3 plant; approximately 34‰) and sorghum (S; C_4 plant; approximately 17‰). The δ ^{13}C values of the AMF-specific fatty acid C16:1ω5 (circles; means ± SE, $n = 6$) were approximately 2‰ below the values of the corresponding host plants in the hyphal networks of *G. intraradices* (Gi; A) or *G. mosseae* (Gm; B) when assessed in monocultures (F:F, S:S). In the mixed culture (F:S), the carbon contribution of sorghum is approximately 70% in both investigated hyphal networks.

the CMN as flax. However, the nutritional benefit to the two host plants strongly depended on the fungus involved: in the case of *G. intraradices*, flax might be viewed as a "cheater" on sorghum, acquiring 80% to 90% of the total labeled nitrogen and phosphorus provided by the CMN, whereas the acquisition of labeled nitrogen and phosphorus was more balanced in the case of *G. mosseae* (Fig. 6). Obviously, in our experiments, carbon investment and nutritional benefit were not tightly linked. This stands in contrast to recent findings where the resource exchange in the symbiosis of plants with AMF appeared to rely on reciprocal "fair trade" (Javot et al., 2007; Pietikainen and Kytoviita, 2007; Kiers et al., 2011; Fellbaum et al., 2012). At least with lower levels of root colonization, sorghum did express a negative response to the diminished nutritional benefit in mixed culture with flax, so that a certain reciprocity of investment and nutritional benefit also became apparent in our system. It has been proposed that the symbiosis between plants and AMF is based on the exchange of "luxury goods" (Kiers and van der Heijden, 2006). Hence, CMNs can exist without causing significant additional costs to either partner, especially when the cost of carbon is negligible for the main carbon donor. This appeared to be the case for sorghum, which dominated (approximately 60% by biomass weight) in our mixed cultures, or more obviously, for large trees supporting small mycoheterotrophic plants (Courty et al., 2011).

In natural plant communities, the demand for "AMF services" such as soil nutrient acquisition, and, vice versa, the availability of luxury goods such as a surplus of carbon, are expected to dynamically change for the different plants, depending on their strategies to respond to environmental cues and their specific life-history traits with consecutive phases of vegetative growth, maturation, senescence, etc. Thus, CMNs supposedly function as dynamic "marketplaces" in biodiverse ecosystems, where the symbionts involved and apparently organized in networks of plant-AMF assemblages (Montesinos-Navarro et al., 2012) can offer luxury goods in exchange for more limited resources. As a consequence, these trades can only weakly be reciprocal, dependent on transient sink strengths and the efficiency of exchanges at the various symbiotic interfaces, which can differ for different

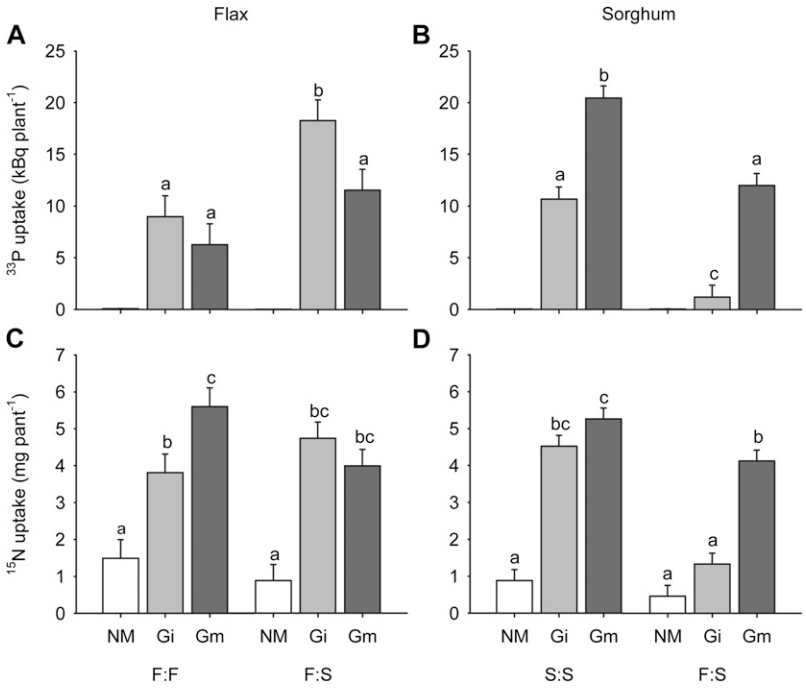

Figure 5. CMN-mediated uptake of nitrogen and phosphorus in plants sharing a CMN. Total ^{33}P (A and B) and ^{15}N (C and D) uptake of individual flax (A and C) and sorghum (B and D) is shown. Plants were grown in monoculture as pairs of identical plant species (F:F, S:S) or in mixed cultures as pairs of different plant species (F:S) and inoculated with a nonmycorrhizal control (NM), with *G. irregulare* (Gi), or with *G. mosseae* (Gm). Values are means ± SE ($n = 6$). Mean comparisons are treated separately for both plant species. Different lowercase letters above the bars indicate significant differences ($P \leq 0.05$) among treatments according to planned contrast analysis.

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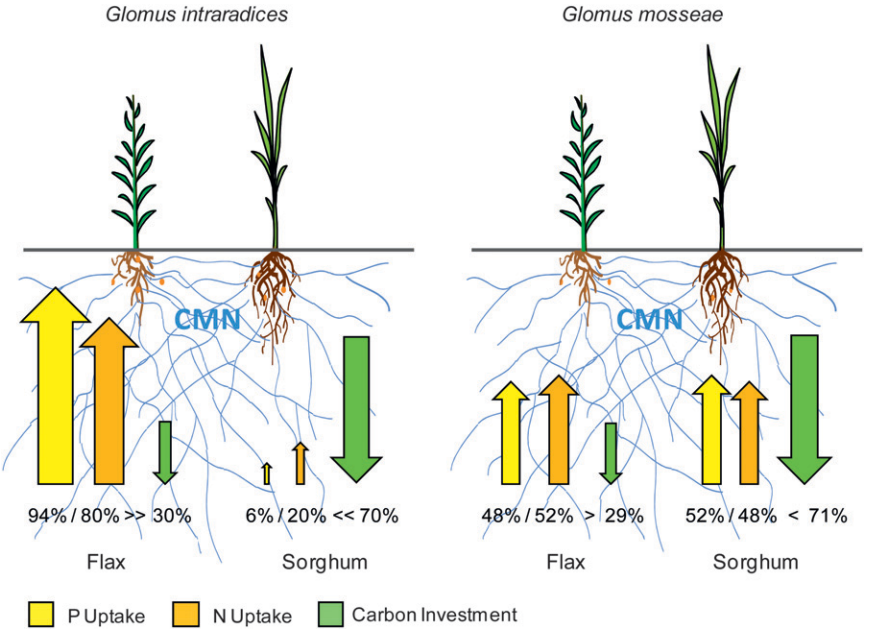

Figure 6. Terms of trade in CMNs between flax and sorghum formed by *G. intraradices* and *G. mosseae*. In this scheme, the carbon investment of the plants is depicted by green arrows. In both CMNs, sorghum invested more than twice as much than flax in terms of carbon. The return, in the form of the nutrients phosphorus (P) and nitrogen (N), is illustrated by the yellow and orange arrows, respectively. In the CMN formed by *G. intraradices*, the return was extremely uneven; flax obtained 80% to 94% of the nutrients delivered by the CMN, and sorghum, the main investor, obtained only 6% to 20%. In the CMN formed by *G. mosseae*, both flax and sorghum received an approximately equal share of the nutrients delivered by the CMN, but because flax invested less than half as much carbon compared with sorghum, it still benefited from its neighbor.

plant-fungus combinations (Klironomos, 2003; Helgason et al., 2007). This is evident in our mixed cultures, where sorghum, in return for a similar expenditure of carbon, received much more phosphorus from *G. mosseae* than from *G. intraradices*, whereas for flax it was the inverse. This difference in functional compatibility between host plants and fungal partners was also displayed in the monocultures, where sorghum acquired more phosphorus from *G. mosseae* than from *G. intraradices* whereas flax acquired marginally more phosphorus from *G. intraradices*. With regard to our findings with the mixed cultures, it would be challenging to monitor the trading of the two plants in the CMN over their whole life cycle and/or under changing sink-source relationships, elicited for instance by a change of the light regimes or by leaf clipping. To this end, the $\delta^{13}C$ values of the respired CO_2 in the hyphal compartment could be monitored.

We still lack a detailed understanding of what exactly controls the observed asymmetry in carbon investment and the return of nutrients in the investigated mycorrhizal symbioses. It is likely, however, that the regulation of fungal and plant transporters at the interface between the two organisms plays a role (Parniske, 2008; Smith and Smith, 2011). With respect to phosphate transfer from the fungus to the plant, the plant's AMF-inducible phosphate transporters appear to be crucial (Javot et al., 2007).Vice versa, with respect to carbon transfer from the plant to the fungus, the symbiosis-induced sugar transporter of *Glomus* species might be of similar importance (Helber et al., 2011).

Sharing Luxury Goods Maximizes Productivity

Our experimental data demonstrate that an unbalanced use of the CMN not only can increase the growth of an individual plant such as flax but also the overall productivity of our two-plant model ecosystem by sharing the benefit of a luxury good (carbon provided by sorghum) between sorghum and flax (Kiers and van der Heijden, 2006). There are other possibilities for plants of different functional groups to jointly profit from the CMN as a common good (e.g. by taking advantage of the proficiency of legumes to fix nitrogen in symbiosis with rhizobia [Jalonen et al., 2009] or the capacity to lift water by deep rooting [Egerton-Warburton et al., 2007]). By the complementary use of different resources in biodiverse ecosystems, plants may cooperatively maintain CMNs without causing exorbitant costs to any of the partners joined in the network. This may explain how the presence of AMF promotes the productivity and diversity of plant communities (van der Heijden et al., 1998).

CONCLUSION

Traditional agricultural systems, which have emerged over millennia globally at multiple locations, usually display a high biodiversity engendered by meticulously planned mixed culturing practices, including agroforestry (Jalonen et al., 2009; Bainard et al., 2011). Such biodiverse agroecosystems, such as the diverse cereal-legume intercropping systems traditionally used in Asia (Li et al., 2007) and in Africa (Snapp et al., 2010), have been shown repeatedly to be more efficient and often also more productive than conventional monocropping systems (Hauggaard-Nielsen and Jensen, 2005; Perfecto and Vandermeer, 2010; Hinsinger et al., 2011). This is currently ascribed to effects such as complementary resource use, improved resilience and yield stability under stress conditions, or pest and pathogen control by facilitation of

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antagonists (Altieri, 2002). We propose that, in addition, such biodiverse agroecosystems were unwittingly developed by resource-poor farmers to make maximal use of CMNs. A revival of favorable intercropping systems, considering the extensive experience and knowledge of indigenous communities in combination with ongoing efforts to better comprehend the intricacies of the CMNs, may help to boost productivity in a sustainable way and, thus, contribute to satisfying the increasing global demand for food (Godfray et al., 2010).

MATERIALS AND METHODS

Organisms and Microcosms

The two host plants used were flax (*Linum usitatissimum* 'Agatha') and sorghum (*Sorghum bicolor* 'Pant Chari-5'). The two fungal partners, both of the genus *Glomus* (phylum Glomeromycota), were *Glomus intraradices*, strain TERI commercial (Mathimaran et al., 2008), and *Glomus mosseae*, strain ISCB 22, both kept in our fungal strain collection.

Pairs of plants were planted into microcosms ($25 \times 10 \times 10$ cm^3) with four compartments, as illustrated in Figure 1, and grown under controlled conditions (16 h of light [220 μE m^{-2} s^{-1}] at 25°C and 8 h of dark at 20°C, constant relative aerial humidity of 65%). The RHC and HC were separated by a 25-μm nylon mesh (Lanz-Anliker) sandwiched between two 500-μm fiberglass meshes (Tesa), allowing fungal hyphae but not plant roots to enter the HC. The HC and LHC were separated by a 65-μm nylon mesh. All compartments were filled with sterile (120°C, 20 min) growth substrate consisting of a mixture of Terragreen (American aluminum oxide, oil-dry U.S. special, type III R, 0.125 mm; Lobbe Umwelttechnik), sand (quartz sand from Alsace, 0.125–0.25 mm; Kaltenhouse), and Loess from a local site (5:4:1, w/w/w). The substrate had the following chemical properties: pH (water) approximately 6, organic carbon < 0.5 g kg^{-1}, P_2O_5 (Na acetate) = 3 mg kg^{-1}, P_2O_5 (double lactate) = 35 mg kg^{-1}, K_2O (Na acetate) = 45 mg kg^{-1}, K_2O (double lactate) = 47 mg kg^{-1}, clay content $< 5\%$. The chemical parameters were measured in the laboratory of F.M. Balzer (http://www.labor-balzer.de).The RHCs were inoculated with a 2-g (approximately 100 spores per compartment) inoculum of one of the *Glomus* strains or with 2 g of sterilized (120°C, 20 min) inocula as a nonmycorrhizal control. In addition, the RHCs received 10 mL of a microbial wash to equalize microbial communities (Koide and Elliott, 1989). This wash was prepared by wet sieving 100 g of each inoculum through a 32-μm sieve and a paper filter (FS 14 1/2; Schleicher & Schuell), yielding a final volume of 1 L. The LHC (Fig. 1) was filled with 325 g of growth substrate including a 100-g layer labeled with 500 kBq $^{33}PO_4^{3-}$ (Hartmann Analytic) and ^{15}N (100 mg $^{15}NH_4{}^{15}NO_3$ [Cambridge Isotope Laboratories]) placed in the middle of the LHC. This compartment was attached to the microcosm after 8 weeks of cultivation. The voluminous HC provided a diffusive barrier for ^{33}P and ^{15}N isotopes toward the RHC and, thus, minimized direct uptake by root hairs that may have reached into the HC by passing the nylon net during the experiment. The microcosms were watered with distilled water twice a week in the RHCs and HC and thereby adjusted to equal soil water content of 90% field capacity by weighing. In addition, every week during the first 8 weeks of cultivation, the RHC was amended with 8 mL of a phosphorus-free Hoagland solution (Gamborg and Wetter, 1975; Zabinski et al., 2002).

Experimental Design

In each of the two RHCs, a single plant was grown, yielding microcosms with monocultures (a pair of identical plants) or a mixed culture (one flax and one sorghum plant). In the preliminary experiment, the plants were inoculated either with *G. intraradices* or with the sterilized control inoculum. In the second experiment, growth experiments were also conducted with *G. mosseae*. The microcosms were harvested after 12 weeks of growth.

Plant Growth Performance and Symbiotic Interaction

Roots were washed thoroughly, excess moisture was removed, and fresh weight was determined. Two subsamples were weighed, one of which was then used for the determination of root dry weight. The other aliquot was cleared using a 10% KOH solution and stained in trypan blue for mycorrhizal structure identification inside the root (Phillips and Hayman, 1970). The percentage of root length occupied by hyphae, arbuscules, and vesicles was estimated for each subsample by a modified line intersection method (McGonigle et al., 1990). A minimum of 50 line intersections per root sample were scored for AMF. Shoot and root samples were dried for 24 h at 105°C and weighed separately; the sum corresponds to the "total biomass" indicated in the figures. Dried shoots and roots were ground at 30 Hz in a mixer mill (MM2224; Retsch). Aliquots of 2 mg were weighed for elemental analyses. Nitrogen and carbon concentrations were determined using an ANCA elemental analyzer/mass spectrometer (Europa Scientific). The phosphorus concentration of shoots and roots was measured using the molybdate blue method on a Shimadzu UV-160 spectrophotometer (Shimadzu Biotech) after acid digestion (Murphy and Riley, 1962). The substrate of the HC was stored at −20°C. A subsample of 50 g was used for hyphal length density measurements, determined by the grid-line intersection method (Jakobsen et al., 1992).

Nutrient Gain of the Mycorrhizal Network

Plant ^{33}P contents were measured using a Packard 2000 liquid scintillation counter (Hewlett-Packard). The ^{15}N content of plants was analyzed with an ANCA mass spectrometer (Europe Scientific). Relative ^{33}P and ^{15}N uptake was calculated by dividing the uptake of individual plants by the total uptake of both plants of the microcosm.

Carbon Contribution to the Mycorrhizal Network

The carbon isotope composition of plant shoots and roots and of hyphal biomass was determined using an ANCA isotope ratio mass spectrometer. Extraradicle hyphae were extracted from the HC by a wet sieving method (Johansen et al., 1996). The recovered hyphae were dried in a DNA SpeedVac (Savant) prior to bulk mass spectrometric analysis. For compound-specific analyses of the AMF-specific fatty acid C16:1ω5, lipid extraction was carried out according to previously described methods (Elvert et al., 2003; Niemann et al., 2005). Briefly, total lipid extracts were obtained by suspending and sonicating 25 g of freeze-dried substrate of the HC in organic solvents of decreasing polarity. Internal standards (*n*-nonadecanol and *n*-nonadecanoic acid) of known concentration and carbon isotopic composition were added prior to extraction. Total lipid extracts were saponified with a methanolic KOH solution (6%). After extraction of the neutral fraction from this mixture, fatty acids were methylated using a boron trifluoride solution (14% BF_3 in methanol), yielding fatty acid methyl esters. The double bond positions of monounsaturated fatty acids were determined by analyzing the dimethyl disulfide adducts of fatty acids (Moss and Lambert-Fair, 1989). The carbon isotopic composition of C16:1ω5 was determined by gas chromatography-isotope ratio mass spectrometry. All stable carbon isotope ratios presented here are reported in the conventional δ notation with respect to the Vienna Pee Dee Belemnite standard. The relative carbon contribution of sorghum to the hyphal network in the mixed culture was calculated on the basis of the mixing model with two end members as follows: relative carbon contribution = [δ ^{13}C F:F − δ ^{13}C F:S/δ ^{13}C F:F − δ ^{13}C S:S] (see Fig. 1 legend for definitions; Peterson and Fry, 1987).

Statistical Analysis

Experiment 1 was set up in a randomized block design where each treatment was replicated four times. Mean comparisons among treatments were performed by independent paired *t* tests for dry weight and relative uptake of ^{33}P and ^{15}N of the two individual plants.

Experiment 2 was set up in a randomized block design including two temporal blocks with a time lag of 4 weeks. Each block contained three replicates, with a resulting total of six replicates per treatment. An ANOVA was performed on the total biomass, on the phosphorus and nitrogen content, and on the total and arbuscular colonization for each plant species separately, where the two latter parameters were arcsine transformed to fit the assumption of normal distribution. The ANOVA was based on the three-factor culture system (with two levels), AMF (with three levels), and block (with two levels). Pairwise comparisons between the treatments were done with planned contrast analysis. Independent paired *t* tests were performed to analyze whether the means of the relative uptake of ^{33}P and ^{15}N of the two individual plants differed significantly from each other. An ANOVA with the factor treatment (nine levels) and block (two levels) was executed on the fungal parameter

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hyphal length density. $P \leq 0.05$ was considered as representing a significant difference.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Carbon investment and nutrient return.

Supplemental Figure S2. Nutrient return with *G. intraradices* and *G. mosseae*.

Supplemental Figure S3. Hyphal length density.

Supplemental Table S1. Dry weight after harvest.

Supplemental Table S2. Root colonization by AMF.

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