

# Rapid nitrogen transfer in the *Sorghum bicolor*-*Glomus mosseae* arbuscular mycorrhizal symbiosis

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We have recently identified two genes coding for ammonium transporters (AMT) in *Sorghum bicolor* that were induced in roots colonized by arbuscular mycorrhizal (AM) fungi. To improve our understanding of the dynamics of ammonium transport in this symbiosis, we studied the transfer of soil-ammonium-derived <sup>15</sup>N to *S. bicolor* plants via the *Glomus mosseae* fungal mycelium in compartmented microcosms. The <sup>15</sup>NH<sub>4</sub><sup>+</sup>-containing hyphal compartment was inaccessible to the roots in the plant compartment. <sup>15</sup>N label concentrations significantly increased in plant roots and leaves already 48 h after exposure of the AM fungus to the <sup>15</sup>NH<sub>4</sub><sup>+</sup> substrate, attesting an efficient symbiotic N transfer between the symbiotic partners and further highlighting that AM symbiosis represents an important component of plant nitrogen nutrition.

We are interested in the arbuscular mycorrhizal (AM) symbiosis<sup>1,2</sup> using *Glomus mosseae*-inoculated sorghum (*Sorghum bicolor*) as a model system. Recently, we described the expanded family of ammonium transporters (AMTs) in sorghum.<sup>3</sup> We could demonstrate that two AMTs, *SbAMT3;1* and *SbAMT4*, are upregulated in mycorrhizal plants (AM-inducible AMTs) and that *SbAMT3;1* is localized at the plant-fungus interface. However, the biological significance of ammonium transport during symbiotic interaction remained elusive.

Nitrate and ammonium represent the main sources of bioavailable inorganic N in soils. Albeit their high mobility in soils, fixed N-depletion (in the vicinity of the root system) is a common environmental constraint plants have to cope with.<sup>2</sup> Different studies estimated that AM fungi delivered between 30% and 42% of the N taken up by the plant.<sup>4,5</sup> Mitigation of N deficiency through the interaction with AM fungi may play a particularly important role under drought-stress conditions or in marginal soils where nutrient supply is strongly limited,<sup>1</sup> environmental conditions that are often pertinent to the growth of sorghum. In fact, sorghum can grow under more arid conditions than most other grain crops, making it an existential source of food, feed or fiber for many farmers living in the semi-arid tropics of Africa, Asia and South America.<sup>6</sup>

To determine the importance of AM fungi in N nutrition of sorghum, we set up an experiment using compartmented microcosms (Fig. 1), where one plant and one hyphal compartment are connected, but separated by two 21 μm nylon meshes

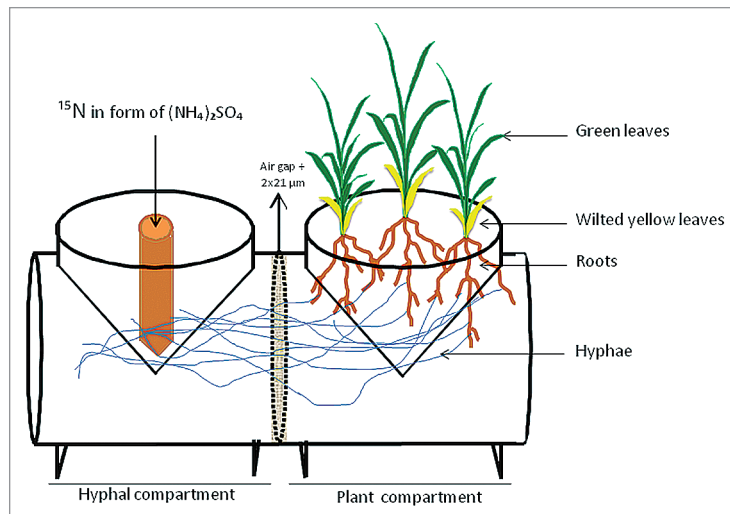
and an air gap in between. The air gap was created by placing two 5 mm plastic meshes between the two 21 μm nylon meshes. The two compartments were filled with sterile (120°C, 20 min) growth substrate consisting of a mixture of Terragreen (American aluminum oxide, oil-dry US special, type III R, 0.125 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). Sorghum [*S. bicolor* (L.) Moench], cv Pant-5 seedlings were inoculated with a 2 g (approximately 100 spores) inoculum of *G. mosseae* ISCB13 or with 2 g of sterilized (120°C, 20 min) inoculum as a non-mycorrhizal control. In the center of the hyphal compartment, a 21 μm nylon mesh bag of 15 ml was inserted and kept empty until introduction of the <sup>15</sup>N labeled substrate 12 wk after inoculation. Then, the nylon mesh bag was filled with 13 g of sand including 10 mg of <sup>15</sup>N ammonium sulfate (Cambridge Isotope Laboratory). Two ml of water were added to wet the <sup>15</sup>N-spiked sand without inducing mass flow. The microcosms were irrigated with distilled water twice a week. In addition, the compartments were amended weekly with 8 mL of a Hoagland solution.<sup>3</sup> Plants were grown under controlled conditions (16 h of light at 28°C and 8 h of dark at 15°C, constant relative aerial humidity of 65%).

A total of 40 microcosms were prepared. Four compartmented microcosms were harvested separately 0, 12, 24, 48 and 72 h after <sup>15</sup>N-labeling. From the plant compartments, roots, green parts of the leaves and wilted, yellow leaves were harvested separately. In the mycorrhizal systems, the AM fungal

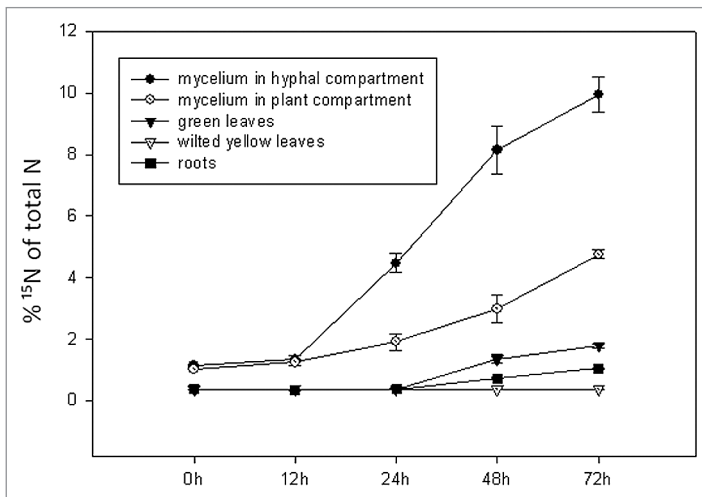
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**Figure 1.** Compartmented microcosms. One plant and one hyphal compartment are separated by two 21  $\mu\text{m}$  nylon meshes and an air gap to prevent mass flow. A 21  $\mu\text{m}$  nylon mesh bag of 15 ml was inserted in the center of the hyphal compartment and filled with 13 g of sand including 10 mg of  $^{15}\text{N}$  ammonium sulfate 12 wk after inoculation.



**Figure 2.** Time course experiment on the transfer of  $(^{15}\text{NH}_4)_2\text{SO}_4$  as N source from the soil to *Sorghum bicolor* plants via *Glomus mosseae* ISCB13 mycelium.  $^{15}\text{N}$  was measured in different tissues after 0, 12, 24, 48 and 72 h of labeling: roots, green leaves and wilted, yellow leaves of plants, as well as in the AM fungal mycelium from the plant and from the hyphal compartment. Values are the means of four replicates.

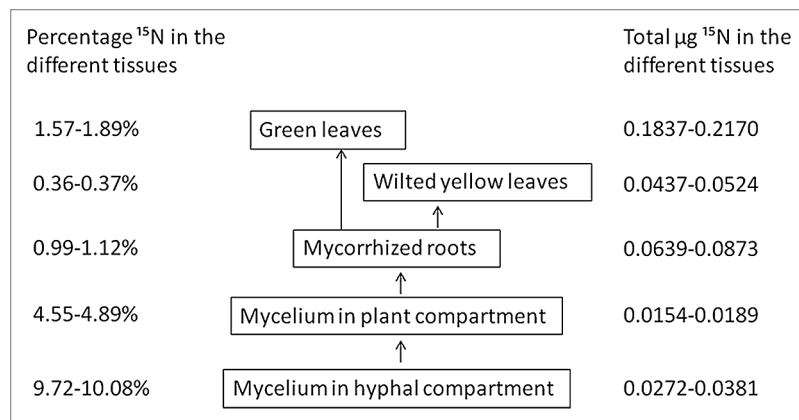
mycelium was harvested both in the plant and in the hyphal compartment. The total amount of N and  $^{15}\text{N}/^{14}\text{N}$  ratio of plant materials were determined using an elemental analyzer (EA) and a ThermoFinnigan DeltaV Advantage Continuous-Flow EA-IRMS, respectively. For N isotope analyses, up to 2 mg of bulk sample was combusted at 1,030°C, combustion gases were passed through a reduction column (650°C) and produced  $\text{N}_2$  gas was then purified and transferred to the IRMS. Isotope values were calibrated using internal EDTA and ammonium oxalate standards, both of which had previously been calibrated against international standards.

Transfer of excess (i.e., beyond natural abundance)  $^{15}\text{N}$  to the non-mycorrhizal plants was undetectable (Table S1). On the other hand,  $^{15}\text{N}$ -label was found already 24 h after spiking in the AM fungal mycelium from both the hyphal and the plant compartment, indicating rapid ammonium uptake by AM fungi (Fig. 2; Table S1). Transfer of mycorrhizal N to the host plant is indicated by the increase of  $^{15}\text{N}$  in the plants. In fact, the  $^{15}\text{N}$  turnover from the substrate pool to the plant was significantly higher in the mycorrhizal compared with the non-mycorrhizal plant systems after 48 h (Table S1). Label  $^{15}\text{N}$  was present both in roots and in green leaves, indicating a substantial transfer to the entire plant, except the wilted yellow leaves, which did not display any marked increase in  $^{15}\text{N}/^{14}\text{N}$  (Figs. 2 and 3; Table S1). The  $^{15}\text{N}$  concentrations continued to increase between 48 and 72 h, indicating ongoing transfer during the the whole experimental period and accumulation in the plant (Figs. 2 and 3; Table S1).

AM fungi are able to take up N in form of nitrate, ammonium or amino acids from the soil far away from the plant<sup>5,7</sup> and they are believed to transfer it to the arbuscules, through the extraradical mycelium, in the form of arginine.<sup>8</sup> There, they may produce ammonium and deliver it to the plant, which is then taken up by plants' AMTs induced upon colonization by AM-inducible AMTs at the symbiotic interface. Our data underline the effectiveness of AM fungi in the uptake of soil ammonium and symbiotic N transfer. It still remains to be seen what role the AM-inducible AMTs play during symbiotic N incorporation.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.



**Figure 3.** Percentage of  $^{15}\text{N}$  and total  $^{15}\text{N}$  ( $\mu\text{g}$ ) in the different tissues of mycorrhized sorghum plants harvested in the compartmented microcosms after 72 h of labeling. Values represent the mean of four replicate treatments.

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### Supplementary Material

Supplementary material may be found here: <http://www.landesbioscience.com/journals/psb/article/25229/>

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