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SHORT NOTE

Kinetics of NH_4^+ uptake by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*

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Abstract The kinetics and energetics of $^{15}NH_4^+$ uptake by the extraradical mycelium of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* were investigated. $^{15}NH_4^+$ uptake increased with increasing substrate concentration over the concentration range of 0.002 to 25 mM. Eadie–Hofstee plots showed that ammonium (NH_4^+) uptake over this range was biphasic. At concentrations below 100 μM, NH_4^+ uptake fits a Michaelis–Menten curve, typical of the activity of a saturable high-affinity transport system (HATS). At concentrations above 1 mM, NH_4^+ influx showed a linear response typical of a nonsaturable low-affinity transport system (LATS). Both transport systems were dependent on external pH. The HATS and, to a lesser extent, the LATS were inhibited by the ionophore carbonylcyanide *m*-chlorophenylhydrazone (CCCP) and the ATP-synthesis inhibitor 2,4-dinitrophenol. These data indicate that the two NH_4^+ transport systems of *R. irregularis* are dependent on metabolic energy and on the electrochemical H^+ gradient. The HATS- and the LATS-mediated $^{15}NH_4^+$ influxes were also regulated by acetate. This first report of the existence of active high- and low-affinity $NH4^+$ transport systems in the extraradical mycelium of an arbuscular mycorrhizal fungus and provides novel information on the mechanisms underlying mycosymbiont uptake of nitrogen from the soil environment.

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Introduction

Although nitrogen (N) is present in the soil as a complex mixture of organic and inorganic compounds, ammonium (NH_4^+) and nitrate (NO_3^-) are by far the main sources for nutrition of most species of higher plants (Williams and Miller 2001). Plants are in fact completely dependent on N availability in the soil solution for their growth and productivity (Gobert and Plassard 2008). N is often the major limiting macronutrient for plants because the concentrations of these two ions in the soil solution are generally low and fluctuant; therefore, the plants have developed strategies to increase their capacity for N mobilization. One strategy is the symbiotic association with arbuscular mycorrhizal (AM) fungi which play a crucial role in the growth and development of the plant.

In contrast to phosphorus, fewer studies have considered the role of AM fungi in N acquisition because the greater mobility of NH_4^+ and especially of NO_3^- ions in soil, compared to phosphate, led to the assumption that little benefit was to plants from enhanced N uptake. However, there is increasing evidence that AM fungi have the potential to take up and transfer significant amounts of N to the host plant (George et al. 1992; Johansen and Jensen 1996). Total N uptake by the extraradical mycelia (ERM) has been observed to account for 21 % to over 50 % of total root N in different mycorrhizal in vitro systems (Toussaint et al. 2004; Govindarajulu et al. 2005; Jin et al. 2005). Moreover, Tanaka and Yano (2005) reported that 75 % of the N in leaves of mycorrhizal maize was taken up by the ERM of *Glomus aggregatum*. In addition, AM fungi can apparently also transfer N from one plant to another (Bethlenfalvay et al. 1991; Cheng and Baumgartner 2004; He et al. 2009) and can enhance

decomposition of and increase N capture from complex organic material in soil (Hodge et al. 2001; Leigh et al. 2009).

The ERM of AM fungi are able to take up and assimilate various forms of N, such as NH_4^+ (George et al. 1992; Frey and Schüepp 1993; Johansen et al. 1996), NO_3^- (Tobar et al. 1994; Bago et al. 1996; Johansen et al. 1996), and amino acids (Hawkins et al. 2000; Hodge et al. 2001) from their surroundings, and to translocate N to the intraradical mycelium and then release N to the host plants (Govindarajulu et al. 2005; Jin et al. 2005). Although AM fungi are able to take up both NO_3^- and NH_4^+, a clear preference for NH_4^+ has been demonstrated (Villegas et al. 1996; Toussaint et al. 2004), which is explained, at least in part, by the extra energy the fungus must expend in reducing NO_3^- to NH_4^+ before it can be incorporated into organic compounds (Marzluf 1996). N transfer to the plant may also be higher when NH_4^+, rather than NO_3^-, is supplied to the fungal hyphae (Tanaka and Yano 2005), even though N is likely transferred from the external to the internal AM hyphae as arginine (Govindarajulu et al. 2005; Cruz et al. 2007; Tian et al. 2010) before transfer to the plant as NH_4^+ (Guether et al. 2009). However, very little is known about the mechanisms of NH_4^+ uptake in AM fungi.

Until now, two genes encoding NH_4^+ transporters have been described from Glomeromycota genomes: *GintAMT1* (López-Pedrosa et al. 2006) and *GintAMT2* (Pérez-Tienda et al. 2011). Both genes were identified in *Glomus intraradices* and functionally complement corresponding mutant yeast strains. The apparent K_m of GintAMT1 has been evaluated in yeast to be in the micromolar range characteristic of a high-affinity NH_4^+ transporter. While the existence of multiple genes suggests the existence of different NH_4^+ transport systems in AM fungi, the kinetics and energetics of NH_4^+ transport in these organisms have not yet been determined. The aim of the present work was to study the mechanisms of NH_4^+ transport in the AM fungus *Rhizophagus irregularis* (syn *G. intraradices*) through the physiological characterization of the NH_4^+ uptake systems operating in the ERM. Data provided in this study show, for the first time, the existence of active high- and low-affinity NH_4^+ transport systems in the ERM of an AM fungus.

Materials and methods

Arbuscular mycorrhizal monoxenic cultures

Arbuscular mycorrhizal monoxenic cultures consisted of Ri T-DNA (*Agrobacterium rhizogenes*)-transformed carrot (*Daucus carota* L. clone DC2) roots colonized with *R. irregularis* (synonym of *G. intraradices* DAOM 197198; Krüger et al. 2012). Cultures were established in bicompartmental Petri plates to separate the root compartment (RC) from the hyphal compartment (HC) (St-Arnaud et al. 1996). Cultures were started by placing a mycorrhizal carrot root segment in the RC containing M medium (Chabot et al. 1992). Petri plates were incubated in the dark at 24 °C until the HC, which contained M medium without sucrose (M-C medium), was profusely colonized by the fungus (approximately 6 weeks). The content of the HC was then removed and replaced by liquid M-C medium (15 ml). The mycelium was allowed to colonize this medium over the subsequent 2 weeks. Petri dishes were examined regularly, and roots were trimmed as required to prevent crossing into the HC. Only cultures with vigorous roots and densely colonized HC were selected for the experiments.

NH_4^+ uptake measurements

NH_4^+ uptake by the ERM was determined by measuring the influx of $^{15}NH_4^+$ (Cerezo et al. 2001). ERM from the HC compartment of six monoxenic cultures were used for each measurement. Briefly, the culture medium of the HC was replaced by fresh medium 24 h before performing measurements. Then, the medium was replaced by 0.1 mM $CaSO_4$, and the ERM were incubated 1 min at room temperature. This solution was replaced by $^{15}NH_4^+$ influx solution: N-free liquid M-C medium with $[^{15}N](NH_4)_2SO_4$ (99 at.% ^{15}N; Cambridge Isotope Laboratories, Inc.) at different concentrations. After 3 min incubation in the influx solution, ERM were incubated 1 min in cold 0.1 mM $CaSO_4$. ERM were then collected with forceps, dried with filter paper, placed in cleaned tin capsules, and oven dried for 48 h at 65 °C. The ^{15}N content of the ERM was determined by mass spectrometry (Clarkson et al. 1996) using an integrated system for continuous-flow isotope ratio mass spectrometry (Euro-EA elemental analyzer, EuroVector S.P.A.) and isoprime mass spectrometer (GV Instruments Ltd.). The values of the ERM ^{15}N influx are expressed in micromolal ^{15}N (gram ERM dry weight (DW))$^{-1}$ per hour. The experiments were repeated at least three times, and the mean ± SE is shown ($n \geq 3$).

Kinetics of NH_4^+ influx

The kinetics of NH_4^+ uptake as a function of external NH_4^+ concentration ($[NH_4^+]_o$) were measured in ERM with $[^{15}NH_4^+]_o$ ranging from 2 μM to 25 mM. Eadie–Hofstee diagrams were obtained by plotting NH_4^+ flux (v) versus the NH_4^+ flux–substrate concentration ratio (v/S). The K_m and V_{max} values were determined for the saturable portion of NH_4^+ uptake from the Lineweaver–Burk transformation using SigmaPlot software (Jandel Scientific).

External pH effect

Prior to the $^{15}NH_4^+$ influx measurements, the liquid medium of the HC (pH 5.5) was replaced with fresh liquid M-C

medium adjusted at pH values of 4.5, 5.5, 6.5, and 7.5 with 10 mM MES-TRIS, and the ERM were incubated during 24 h at 25 °C. The $^{15}NH_4^+$ influx solutions, containing either 10 μM or 2 mM $[^{15}NH_4^+]_o$ to assay, respectively, the activities of the high- and low-affinity transport systems, were adjusted to the same pH values than the M medium with 10 mM MES-TRIS.

Metabolic inhibitor studies

$^{15}NH_4^+$ influx measurements were performed after 30 min incubation in M-C liquid medium supplemented, or not, with the inhibitors dissolved in ethanol (2 ml/l medium) to obtain final concentrations of 10 μM CCCP or 50 μM 2,4-dinitrophenol (2,4-DNP). The control plates only received the medium with ethanol. The HATS and LATS activities were measured in 10 μM and 2 mM $[^{15}NH_4^+]_o$ influx solutions, respectively.

Effect of acetate

Prior to the $^{15}NH_4^+$ influx measurements, the liquid medium of the HC was replaced with fresh liquid M-C medium supplemented with 4 mM acetate during 48 h at 25 °C. The HATS and LATS activities of the acetate-treated ERM were measured in 10 μM and 2 mM $[^{15}NH_4^+]_o$ influx solutions, respectively.

Statistical analysis

Statistical analysis was carried out using the IBM SPSS 18.0 software support. The data are expressed as means ± SE. Mean values were compared by a Fisher's least significant difference (LSD) test. Differences were taken into account only when they were significant at least at the 5 % level. All experiments were repeated at least three times.

Results

Kinetics of $^{15}NH_4^+$ uptake by the *R. irregularis* ERM

$^{15}NH_4^+$ uptake increased with increasing substrate concentration over the concentration range of 0.002 to 25 mM (Fig. 1a). Over this range, uptake did not appear to saturate and, thus, did not conform to simple Michaelis–Menten kinetics. Eadie–Hofstee plots (v against v/S) showed that uptake over the 0–25 mM range was biphasic (Fig. 1b), indicating the existence of two components in the uptake of NH_4^+ by *R. irregularis*. When the external $^{15}NH_4^+$ concentrations were below 100 μM (Fig. 1c), NH_4^+ uptake fits a Michaelis–Menten curve typical of the activity of a saturable high-affinity transport system. However, at concentrations above 1 mM, the uptake rate was directly proportional to the external $^{15}NH_4^+$ concentrations, indicating the action of a

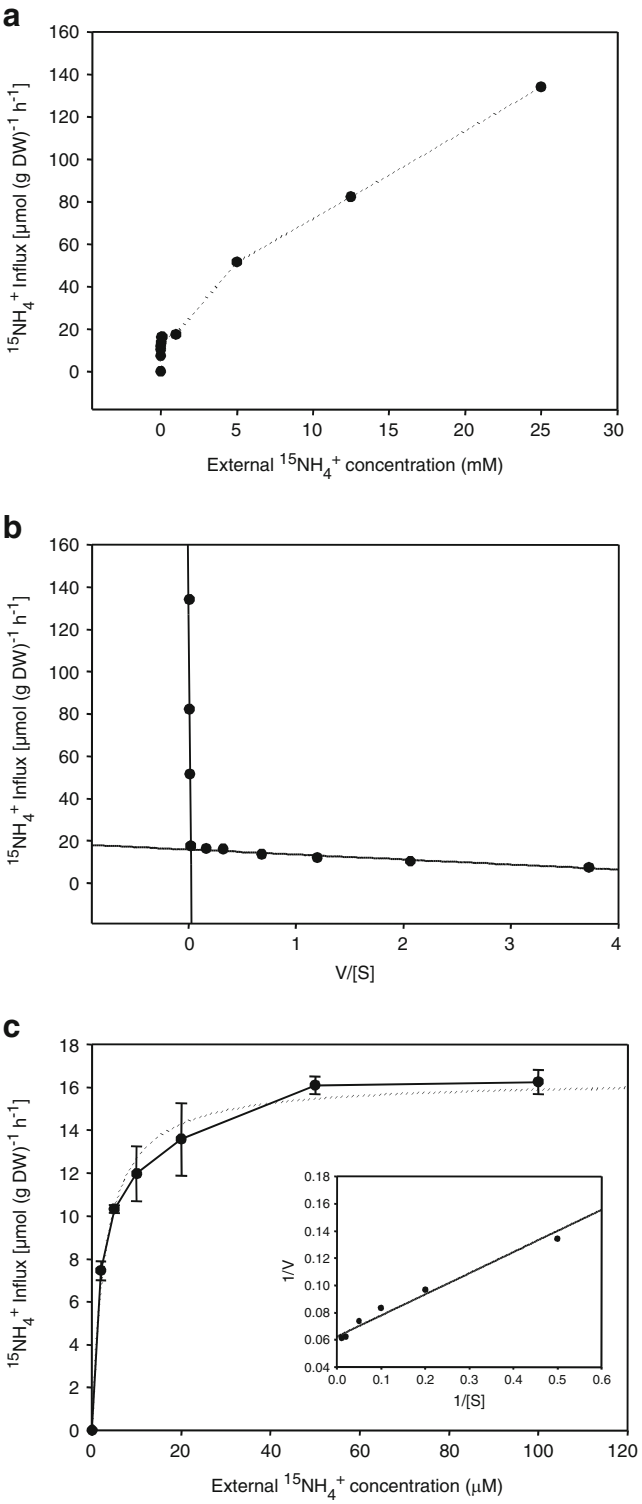

Fig. 1 Uptake of $^{15}NH_4^+$ by *R. irregularis* ERM as a function of NH_4^+ concentration: (**a**) NH_4^+ uptake in the range 0.002–25 mM, (**b**) Eadie–Hofstee plot of $^{15}NH_4^+$ uptake in a substrate range of 0.002 to 25 mM, and (**c**) NH_4^+ uptake in the range 2–100 μM, *inset* shows the corresponding Lineweaver–Burk plot. Values are means ± SE ($n \geq 3$)

nonsaturable LATS. Values for Michaelis–Menten parameters of the HATS were estimated from the Lineweaver–Burk transformation within the range 0–100 μM (Fig. 1c). The estimated K_m concentration was 2.53±0.25 μM with a V_{max} of 16.58±0.81 μmol (g ERM DW)$^{-1}$h^{-1}.

Energetics of $^{15}NH_4^+$ uptake

The two components of the biphasic system of NH_4^+ uptake of *R. irregularis* were further characterized by assessing their sensitivity to external pH and metabolic inhibitors. External $^{15}NH_4^+$ concentrations of 10 μM and 2 mM were selected to assay the activity of the HATS and LATS, respectively. Although both the HATS- and the LATS-mediated $^{15}NH_4^+$ influxes were dependent on external pH, maximum activity of the HATS was observed at pH 4.5, while influx by the LATS increased with external pH reaching the maximum activity at pH 8.5 (Fig. 2a).

The effect of the protonophore CCCP and of the ATP synthesis inhibitor 2,4-DNP on $^{15}NH_4^+$ uptake was also tested. CCCP is a compound that depletes the proton-motive force by increasing H^+ influx and, thereby, induces acidification of the cytosol (Kasianowicz et al. 1984). Both the HATS- and the LATS-mediated $^{15}NH_4^+$ influxes were reduced after the application of the metabolic inhibitors, although the HATS was more sensitive than the LATS. Relative to the control treatments, the HATS was reduced 70 and 86 % by CCCP and 2,4-DNP, respectively, while inhibition of the LATS by both inhibitors was about 40 % (Table 1).

Regulation of $^{15}NH_4^+$ uptake by acetate

To determine if a carbon supply could regulate NH_4^+ influx, the *R. irregularis* ERM was exposed to acetate, a carbon form taken up and assimilated via acetyl-CoA and the glyoxylate cycle by the ERM (Pfeffer et al. 1999). Exposure of the fungus to 4 mM acetate for 48 h activated $^{15}NH_4^+$ influx mediated by both the HATS and LATS (Fig. 2b).

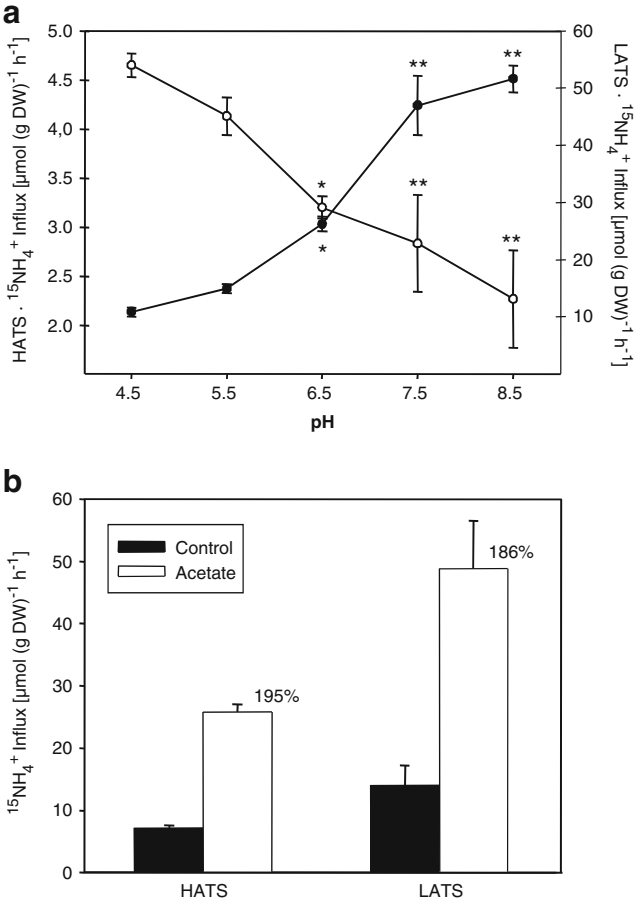

Fig. 2 **a** Effect of pH on $^{15}NH_4^+$ influx into *R. irregularis* ERM. $^{15}NH_4^+$ uptake was measured in influx solution containing 10 μM $^{15}NH_4^+$ for the HATS (*empty circle*) or 2 mM $^{15}NH_4^+$ for the LATS (*filled circle*) at various pHs. Values are means ± SE (n≥3). *P<0.05 and **P<0.01, statistically significant in comparison to the control value at pH 5.5, according to Fisher's LSD test. **b** Effect of acetate on $^{15}NH_4^+$ uptake by *R. irregularis* ERM. $^{15}NH_4^+$ influx measurements were performed 48 h after incubation of the ERM in fresh liquid M-C medium supplemented, or not, with 4 mM acetate. The HATS and LATS activities were measured in 10 μM and 2 mM $^{15}NH_4^+$ influx solutions, respectively. Values are means ± SE (n≥3)

Discussion

The external AM fungal mycelium is the fungal phase which is in contact with the soil and thus responsible for nutrient acquisition and transport to the internal mycelium inside the roots before any transfer to the plant occurs. However, despite the obvious importance of the ERM in nutrient acquisition, current knowledge of the nutrient uptake processes, taking place in the ERM, is poor. The obligate biotrophic nature of these fungi, which makes it impossible to cultivate them in the absence of a host root, and the traditional cultivation systems of AM, which add to the difficulty of working with AM ERM from soil, have hampered these studies. These two problems have been overcome with the use of AM monoxenic cultures in two-compartmented Petri dishes (St-Arnaud et al. 1996). Although such an in vitro system is an artificial system for studying AM physiology (especially the role of the host plant), it has proven useful for investigating fungal transport (Rufyikiri et al. 2005) since it eliminates the complications arising from the uptake and metabolism of nutrients by other microorganisms and avoids the diffusion of soluble nutrients between the compartments. In this work, using the in vitro system and isotopically labeled NH_4^+, it has been possible to demonstrate for the first time that uptake of NH_4^+ by the ERM of *R. irregularis* is mediated by at least two functionally distinct systems, a low-affinity nonsaturable component and a high-affinity saturable component.

Table 1 Effect of various inhibitors on $^{15}NH_4^+$ influx into *R. irregularis* ERM

Treatment	HATS		LATS	
	μmol (g ERM DW)$^{-1}$ h^{-1}	Level without inhibitor (%)	μmol (g ERM DW)$^{-1}$ h^{-1}	Level without inhibitor (%)
Control	6.94±0.46	100	25.62±1.70	100
CCCP	2.08±0.22	30	11.53±0.55	45
2,4-DNP	0.98±0.13	14	10.55±0.59	41

$^{15}NH_4^+$ influx measurements were performed after 30 min incubation in fresh liquid M-C medium supplemented, or not, with 10 μM CCCP or 50 μm 2,4-DNP. The HATS and LATS activities were measured in 10 μM and 2 mM $^{15}NH_4^+$ influx solutions, respectively. Data are means of three independent experiments ± SE

The concentration-dependent kinetics of NH_4^+ uptake show that, at concentrations below 1 mM NH_4^+, uptake is mediated by a saturable transport system whose kinetic parameters are typical of a high-affinity and low-capacity transport system (von Wirén and Merrick 2004). The HATS of *R. irregularis* with a K_m value of 2.53±0.25 μM has a very high affinity for NH_4^+. Transport systems with very high affinity for NH_4^+ have also been reported in other fungi, such as *Laccaria bicolor* (K_m 6 μM; Jongbloed et al. 1991), *Saccharomyces cerevisiae* (K_m 1–10 μM; Marini et al. 1994, 1997), and *Agaricus bisporus* (K_m 3.7 μM; Kersten et al. 1999). Given that these systems are adapted to transport at low concentrations of external NH_4^+, the saturable HATS of *R. irregularis* should allow the ERM to absorb sufficient N (NH_4^+) from very low levels in the soil. Comparison of values for Michaelis–Menten parameters of the HATS with those obtained in several plant species shows that the K_m of the HATS of *R. irregularis* is at least fivefold less than that of various investigated plant species whose K_m values ranged between 10 and 170 μM (Glass and Siddiqi 1995). Although caution must be taken when comparing kinetic values from different studies, these data suggest that the NH_4^+ HATS of *R. irregularis* has higher affinity than that of plants, which might account for the increased NH_4^+ uptake of mycorrhizal roots (Frey and Schüepp 1993). At concentrations above 1 mM, the uptake rate by the *R. irregularis* ERM was directly proportional to the external $^{15}NH_4^+$ concentrations, indicating the action of a nonsaturable LATS. However, given that NH_4^+ concentrations in soil solutions rarely exceed 50 μM (Marschner 1995) and that the LATS contribute to total uptake only at substrate concentrations of >1 mM, uptake mediated by the LATS is likely to be of little importance in most natural soils.

As in other filamentous fungi investigated so far, uptake of NH_4^+ by *R. irregularis* displays characteristic features of active transport. Inhibition of the HATS and, to a lesser extent, of the LATS by the ionophore CCCP and the ATP synthesis inhibitor DNP indicates that, although a diffusion-like component contributes to total uptake, both transport systems are dependent on metabolic energy and on the electrochemical H^+ gradient. Similar inhibitory effects of CCCP and 2,4-DNP have been demonstrated for NH_4^+ uptake by other fungi, such as *Paxillus involutus* (Javelle et al. 1999). Inhibition of the *R. irregularis* NH_4^+ transport systems by CCCP indicates that NH_4^+ uptake by the ERM is dependent on the activity of a plasma membrane H^+-ATPase and supports the occurrence of a secondary transport system, such as NH_4^+/H^+ symport (Ortiz-Ramirez et al. 2011). Previous studies have shown that AM fungi have several H^+-ATPases expressed in their ERM (Ferrol et al. 2000; Requena et al. 2003). Energization required for NH_4^+ uptake could be performed by one of these isozymes.

Our kinetic studies, however, do not establish whether NH_4^+ transport is mediated by a single transporter or whether it results from the activity of multiple transport proteins. In a previous work, we have shown that *G. intraradices*, synonym of *R. irregularis*, expresses two genes in the ERM, *GintAMT1* (López-Pedrosa et al. 2006) and *GintAMT2* (Pérez-Tienda et al. 2011) that encode NH_4^+ permeases belonging to the ammonium transporter (AMT)/methylamine permease (MEP)/rhesus protein family (Andrade and Einsle 2007). The apparent K_m of GintAMT1 has been evaluated in yeast to be 26 μM, characteristic of a high-affinity NH_4^+ transporter, and its transport activity was dependent on the ATPase activity. Therefore, the observed active component of $^{15}NH_4^+$ uptake that obeyed Michaelis–Menten kinetics at concentrations below 100 μM is likely mediated by GintAMT1. Although the kinetic properties of GintAMT2 could not be determined, it was also proposed to encode a high-affinity NH_4^+ transporter that was functionally different from *GintAMT1*. Moreover, a new as yet uncharacterized NH_4^+ transporter has recently been identified in the *G. intraradices* transcriptome (Tisserant et al. 2012). Multiplicity of NH_4^+ transporters is also found in other fungi. For example, NH_4^+ uptake by *S. cerevisiae* involves at least three permeases (Marini et al. 1994, 1997) and four in *Aspergillus nidulans* (Monahan et al. 2002, 2006). The presence of at least three ammonium transporters with different affinities has been reported for the ectomycorrhizal fungus *Hebeloma cylindrosporum* (Javelle et al. 2001, 2003), and genome database screening has also identified 16 putative AMT/MEP systems in the ectomycorrhizal fungus *L. bicolor* (Lucic et al. 2008). Functional and electrophysiological analyses of the different members of the *R. irregularis* NH_4^+ transporter gene family will enable identification of the transport protein responsible for the active transport activity of the LATS and will provide further insights into the energetic coupling of the different *R. irregularis* AMTs.

Exposure of the fungus to acetate, a carbon form taken up by the ERM (Pfeffer et al. 1999), activated $^{15}NH_4^+$ influx mediated by the HATS and LATS. In this sense, it has recently been reported that an exogenous supply of glucose to germinated spores of *G. intraradices* also enhances uptake and metabolism of different exogenous nitrogen sources (Jin et al. 2011). Since in the symbiotic stage AM fungi obtain their carbon mainly within the host root, activation of the ERM NH_4^+ uptake by acetate suggests that the plant might regulate fungal N influx by providing carbon to the fungus. This hypothesis is supported by the recent report that the carbon supply from the plant to the fungus across the symbiotic interface is a key trigger for N uptake and transport in the symbiosis (Fellbaum et al. 2012). However, these authors found that the uptake of acetate via the ERM had no significant effect on N transport to the root. These data suggest that the extra N taken up by the ERM as a consequence of the activation of N influx by acetate is retained in the fungus for its own metabolism.

In conclusion, data provided in this study show, for the first time, the existence of active high- and low-affinity NH_4^+ transport systems in the ERM of an AM fungus and provide novel information on the mechanisms of nitrogen uptake by AM fungi from the soil environment. Although, in recent years, there has been a significant increase in our understanding of the physiological and molecular mechanisms of nitrogen transport in the AM symbiosis, further research is needed to identify the transport protein responsible of the active transport activity of the LATS and to determine the regulatory mechanisms of nitrogen transport in the symbiosis.

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References

Andrade SLA, Einsle O (2007) The Amt/Mep/Rh family of ammonium transport proteins. Mol Membr Biol 24:357–365

Bago B, Vierheilig H, Piche Y, Azcón-Aguilar C (1996) Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. New Phytol 133:273–280

Bethlenfalvay GJ, Reyes-Solis MG, Camel SB, Ferrera-Cerrato R (1991) Nutrient transfer between the root zones of soybean and maize plants connected by a common mycorrhizal mycelium. Physiol Plant 82:423–432

Cerezo M, Tillard P, Gojon A, Primo-Millo E, Garcia-Agustin P (2001) Characterization and regulation of ammonium transport systems in *Citrus* plants. Planta 214:97–105

Chabot S, Bécard G, Piché Y (1992) Life cycle of *Glomus intraradix* in root organ culture. Mycologia 84:315–321

Cheng X, Baumgartner K (2004) Arbuscular mycorrhizal fungi-mediated nitrogen transfer from vineyard cover crops to grapevines. Biol Fert Soils 40:406–412

Clarkson D, Gojon A, Saker L, Wiersema P, Purves J, Tillard P, Arnold G, Paans A, Vaalburg W, Stulen I (1996) Nitrate and ammonium influxes in soybean (*Glycine max*) roots: direct comparison of ^{13}N and ^{15}N tracing. Plant Cell Environ 19:859–868

Cruz C, Egsgaard H, Trujillo C, Ambus P, Requena N, Martins-Loucao MA, Jakobsen I (2007) Enzymatic evidence for the key role of arginine in nitrogen translocation by arbuscular mycorrhizal fungi. Plant Physiol 144:782–792

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bücking H (2012) Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 109:2666–2671

Ferrol N, Barea JM, Azcón-Aguilar C (2000) The plasma membrane H^+-ATPase gene family in the arbuscular mycorrhizal fungus *Glomus mosseae*. Curr Genet 37:112–118

Frey B, Schüepp H (1993) Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. New Phytol 124:221–230

George E, Häussler K-U, Vetterlein D, Gorgus E, Marschner H (1992) Water and nutrient translocation by hyphae of *Glomus mosseae*. Can J Bot 70:2130–2137

Glass ADM, Siddiqi MY (1995) Nitrogen absorption by plant roots. In: Srivastava HS, Singh RP (eds) Nitrogen nutrition in higher plants. Associated Publishing Company, New Delhi, pp 21–56

Gobert A, Plassard C (2008) The beneficial effect of mycorrhizae on N utilization by the host-plant: myth or reality? In: Varma A (ed) Mycorrhiza: state of the art, genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics. Springer, Berlin, pp 209–240

Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Guether M, Neuhauser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P (2009) A mycorrhizal-specific ammonium transporter from *Lotus japonicus* acquires nitrogen released by arbuscular mycorrhizal fungi. Plant Physiol 150:73–83

Hawkins HJ, Johansen A, George E (2000) Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant Soil 226:275–285

He X, Xu M, Qiu GY, Zhou J (2009) Use of ^{15}N stable isotope to quantify nitrogen transfer between mycorrhizal plants. J Plant Ecol 2:107–118

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299

Javelle A, Chalot M, Soderstrom B, Botton B (1999) Ammonium and methylamine transport by the ectomycorrhizal fungus *Paxillus involutus* and ectomycorrhizas. FEMS Microbiol Ecol 30:355–366

Javelle A, Rodríguez-Pastrana BR, Jacob C, Botton B, Brun A, Andre B, Marini AM, Chalot M (2001) Molecular characterization of two ammonium transporters from the ectomycorrhizal fungus *Hebeloma cylindrosporum*. FEBS Lett 505:393–398

Javelle A, Morel M, Rodríguez-Pastrana BR, Botton B, Andre B, Marini AM, Brun A, Chalot M (2003) Molecular characterization, function and regulation of ammonium transporters (Amt) and ammonium-metabolizing enzymes (GS, NADP-GDH) in the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Mol Microbiol 47:411–430

Jin H, Pfeffer PE, Douds DD, Piotrowski E, Lammers PJ, Shachar-Hill Y (2005) The uptake, metabolism, transport and transfer of

nitrogen in an arbuscular mycorrhizal symbiosis. New Phytol 168:687–696

Jin HR, Jiang DH, Zhang PH (2011) Effect of carbon and nitrogen availability on metabolism of amino acids in germinating spores of arbuscular mycorrhizal fungi. Pedosphere 21:432–442

Johansen A, Jensen ES (1996) Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. Soil Biol Biochem 28:73–81

Johansen A, Finlay RD, Olsson PA (1996) Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 133:705–712

Jongbloed RH, Clement JMAM, Borst-Pauwels GWFH (1991) Kinetics of NH_4^+ and K^+ uptake by ectomycorrhizal fungi. Effect of NH_4^+ on K^+ uptake. Physiol Plant 83:427–432

Kasianowicz J, Benz R, McLaughlin S (1984) The kinetic mechanism by which CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) transports protons across membranes. J Membr Biol 82:179–190

Kersten MASH, Arninkhof MJC, Op Den Camp HJM, Van Griensven LJLD, Van Der Drift C (1999) Transport of amino acids and ammonium in mycelium of *Agaricus bisporus*. Biochim Biophys Acta 1428:260–272

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970–984

Leigh J, Hodge A, Fitter AH (2009) Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. New Phytol 181:199–207

López-Pedrosa A, González-Guerrero M, Valderas A, Azcón-Aguilar C, Ferrol N (2006) GintAMT1 encodes a functional high-affinity ammonium transporter that is expressed in the extraradical mycelium of *Glomus intraradices*. Fungal Genet Biol 43:102–110

Lucic E, Fourrey C, Kohler A, Martin F, Chalot M, Brun-Jacob A (2008) A gene repertoire for nitrogen transporters in *Laccaria bicolor*. New Phytol 180:343–364

Marini AM, Vissers S, Urrestarazu A, Andre B (1994) Cloning and expression of the MEP1 gene encoding an ammonium transporter in *Saccharomyces cerevisiae*. EMBO J 13:3456–3463

Marini AM, Soussi-Boudekou S, Vissers S, Andre B (1997) A family of ammonium transporters in *Saccharomyces cerevisiae*. Mol Cell Biol 17:4282–4293

Marschner H (1995) Mineral nutrition of higher plants. Academic, London

Marzluf GA (1996) Regulation of nitrogen metabolism in mycelial fungi. In: Brambl R, Marzluf GA (eds) The mycota III: biochemistry and molecular biology. Springer, Berlin, pp 357–368

Monahan BJ, Fraser JA, Hynes MJ, Davis MA (2002) Isolation and characterization of two ammonium permease genes, meaA and mepA, from *Aspergillus nidulans*. Eukaryot Cell 1:85–94

Monahan BJ, Askin MC, Hynes MJ, Davis MA (2006) Differential expression of *Aspergillus nidulans* ammonium permease genes is regulated by GATA transcription factor AreA. Eukaryot Cell 5:226–237

Ortiz-Ramirez C, Mora SI, Trejo J, Pantoja O (2011) PvAMT1;1, a highly selective ammonium transporter that functions as H^+/NH_4^+ symporter. J Biol Chem 286:31113–31122

Pérez-Tienda J, Testillano PS, Balestrini R, Fiorilli V, Azcón-Aguilar C, Ferrol N (2011) GintAMT2, a new member of the ammonium transporter family in the arbuscular mycorrhizal fungus *Glomus intraradices*. Fungal Genet Biol 48:1044–1055

Pfeffer PE, Douds DD, Becard G, Shachar-Hill Y (1999) Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. Plant Physiol 120:587–598

Requena N, Breuninger M, Franken P, Ocón A (2003) Symbiotic status, phosphate, and sucrose regulate the expression of two plasma membrane H^+-ATPase genes from the mycorrhizal fungus *Glomus mosseae*. Plant Physiol 132:1540–1549

Rufyikiri G, Kruyts N, Declerck S, Thiry Y, Delvaux B, Dupré de Boulois H, Jones E (2005) Uptake, assimilation and translocation of mineral nutrients in monoxenic cultivation systems. In: Declerck S, Strullu DG, Fortin JA (eds) In vitro culture of mycorrhizas. Springer, Berlin, pp 201–215

St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA (1996) Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an in vitro system in the absence of host roots. Mycol Res 100:328–332

Tanaka Y, Yano K (2005) Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. Plant Cell Environ 28:1247–1254

Tian C, Kasiborski B, Koul R, Lammers PJ, Bucking H, Shachar-Hill Y (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiol 153:1175–1187

Tisserant E, Kohler A, Dozolme-Seddas P, Balestrini R, Benabdellah K, Colard A, Croll D, Da Silva C, Gomez SK, Koul R, Ferrol N, Fiorilli V, Formey D, Franken P, Helber N, Hijri M, Lanfranco L, Lindquist E, Liu Y, Malbreil M, Morin E, Poulain J, Shapiro H, van Tuinen D, Waschke A, Azcón-Aguilar C, Bécard G, Bonfante P, Harrison MJ, Küster H, Lammers P, Paszkowski U, Requena N, Rensing SA, Roux C, Sanders IR, Shachar-Hill Y, Tuskan G, Young JPW, Gianinazzi-Pearson V, Martin F (2012) The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate. New Phytol 193:755–769

Tobar R, Azcon R, Barea JM (1994) Improved nitrogen uptake and transport from ^{15}N-labeled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. New Phytol 126:119–122

Toussaint JP, St-Arnaud M, Charest C (2004) Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an in vitro compartmented system. Can J Microbiol 50:251–260

Villegas J, Williams RD, Nantais L, Archambault J, Fortin JA (1996) Effects of N source on pH and nutrient exchange of extramatrical mycelium in a mycorrhizal Ri T-DNA transformed root system. Mycorrhiza 6:247–251

von Wirén N, Merrick M (2004) Regulation and function of ammonium carriers in bacteria, fungi, and plants. In: Boles E, Krämer R (eds) Topics in current genetics, vol 9: molecular mechanisms controlling transmembrane transport. Springer, Berlin Heidelberg, pp 95–120

Williams L, Miller A (2001) Transporters responsible for the uptake and partitioning of nitrogenous solutes. Annu Rev Plant Phys 52:659–688