Arbuscular mycorrhiza fungi colonisation stimulates uptake of inorganic nitrogen and sulphur but reduces utilisation of organic forms in tomato

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Abstract

Arbuscular mycorrhizal fungi (AMF) form symbioses with most plants, potentially improving their growth and nutrient assimilation activities. Cysteine (Cys) and methionine (Met) are nitrogen (N) and sulphur (S)-containing amino acids. Compared with phosphate and N, limited attention has been paid to the role of AMF in low molecular weight organic S acquisition. To explore the uptake and relative contributions of organic and inorganic N and S to plants, and the role of AMF in S uptake, a study was conducted based on ^{14}C, ^{35}S, ^{13}C, ^{15}N quad labelling using a mutant tomato genotype with highly decreased AMF symbiosis capacity. Tomato roots can uptake limited amounts of added Met and Cys (<1.77%) as indicated by ^{14}C and ^{13}C labelling results under fierce competition with soil microorganisms. After uptake for 6 h, 10.0–14.8% of N and 1.4–6.1% of S derived from added Cys and Met was utilized by plants, mainly in inorganic N and S forms derived from Cys and Met decomposition. Met and Cys could be important S sources (Met: 3.0–9.8%, Cys: 8.8–22.0%) for plants; however, they have negligible roles in N nutrition (~1%). Tomato uptake of inorganic S derived from Cys decomposition was much higher than that derived from Met, as higher ratios of S-Cys were released as SO_4^{2-} from microorganisms. Even with artificial addition of AMF, most of the added Met and Cys were utilized by Gram-negative bacteria, as indicated by ^{13}C-PLFA biomarkers. AMF reduced host plant uptake of organic N and S, but stimulated plant N uptake

from Met and Cys, which was mainly inorganic N following mineralization. AMF not only utilize organic carbon from host plants but also capture soil organic matter to satisfy their energy demands. In the spaces where both root and AMF occur, AMF colonization decreased tomato ^{35}S uptake from Cys, Met, and SO_4^{2-} by 24.6%, 20.6%, and 11.0%, respectively, when compared with in the mutant genotype reduced colonization capacity; in contrast, AMF colonization increased ^{35}S-Cys uptake by 118.7% from areas that roots could not reach. Overall, AMF enhanced host plant N uptake, but reduced organic N uptake under competition with plant roots for S in the rhizosphere, but stimulate plant S uptake by extraradical mycelia.

Keywords: Arbuscular mycorrhizal fungi; soil organic nitrogen; soil organic matter decomposition; sulphur cycling; microbial decomposition

1. Introduction

Nitrogen (N) and sulphur (S) are nutrients essential for plant growth and development. Previous studies have paid relative less attention to S compared to N, due to rather adequate atmospheric and fertilizer S inputs to soil. Recently however, plant S deficiency has emerged globally, mainly owing to a considerable decrease in sulphur dioxide emissions following strict air regulations, high S demand by high yielding plants, application of fertilizers with limited S contents, and reduced S return via farmyard manure (Piotrowska-Długosz et al., 2017; Vermeiren et al., 2018). Excluding inorganic N and S, mainly in the forms of ammonium-N (NH_4^+-N), nitrate-N (NO_3^--N), and sulphate (SO_4^{2-}), plants can partially utilize organic N and S forms, such as amino acids (Ganeteg et al., 2017; Ma et al., 2018), short peptides (Farrell et al., 2013; Hill et al., 2019), quaternary ammonium compounds (Warren, 2013), as well as large molecular proteins (Paungfoolonhienne et al., 2008), bypassing microbial decomposition of

organic matter into inorganic N or S.

Owing to their high content in soil solution and complete absorption and transport system in plants roots (Jones et al., 2005; Nasholm et al., 2009), amino acids are highly available to plants, especially in some cold ecosystems with relatively low mineralization rates (Hill et al., 2019; Nasholm et al., 1998). Amino acids can still be absorbed by plants in warmer agricultural ecosystems under inorganic N fertilizer application and high mineralization rates; however, their role is much weaker than that of inorganic N under such conditions (Ganeteg et al., 2017; Ma et al., 2021). Consequently, plants tend to absorb various forms of N or S to avoid intense competition with microbes and other co-existing plants to satisfy their N or S nutrient demands (Ma et al., 2021).

Cysteine (Cys) and methionine (Met) are unique amino acids, which contain both N and S. Plants can absorb Met and Cys under low SO_4^{2-} supply in most natural and agricultural soils (Ma et al., 2021). Although both Cys and Meth contain one molar N and one molar S, there are considerable differences in their utilization by soil microorganisms and plant roots (Ma et al., 2020; Ma et al., 2020). For example, Cys is more readily mineralized into SO_4^{2-} than Met, and the SO_4^{2-} derived from Cys is highly bioavailable to plant roots (Fitzgerald and Watwood, 1988; Ma et al., 2020). In addition, higher proportions of S from Met are retained in microbial biomass (MB) for protein synthesis than from Cys, and relatively limited S is released as SO_4^{2-} from Met compared to from Cys (Ma et al., 2021). Furthermore, potato can metabolize high Cys amounts but not Met, even though Met is a precursor of volatile compounds both in plants and microorganisms (Maggioni and Renosto, 1977). However, maize and soybean can utilize higher amounts of intact Met than Cys, even if its N/S contribution is much lower than its

inorganic N/S contribution (Ma et al., 2021). Whether plants can access such amino acids and their importance to plant S and N nutrition, in addition to their competitive utilization by plants roots and soil microbes remains unclear.

Arbuscular mycorrhizal fungi (AMF), which are associated with approximately 72% of vascular plants in various ecosystems, play an important role in sustainable agricultural development and in natural ecosystems (Brundrett and Tedersoo, 2018; Qin et al., 2020; Rillig et al., 2018). The metabolism and transfer of carbon (C), N, phosphorus (P), and S are vital for resource reallocation and nutrient balance between fungi and host plants. The carbohydrates derived from plant photosynthesis (5–25%, mainly as hexose) are transported to fungi as energy sources to support growth and nutrient uptake, whereas NH_4^+-N is released from fungi to facilitate plant growth, leading to the establishment of a mutually beneficial relationship (Lang et al., 2021; Rillig et al., 2018; Zhou et al., 2020). Plant roots can obtain nutrients directly through root hairs and epidermis, and by AMF hyphae in cortical cells of root, where hyphal coils or arbuscular mycorrhiza form symbiotic interfaces (Thirkell et al., 2020). The functional interplay between plants and AMF in nutrient uptake activities has important implications for plant nutrition and field nutrient management activities.

Plant hosts benefit from AMF by taking up the P, N, S, micronutrients, and water transferred from the soil. Fungal extraradical mycelia take up inorganic N rapidly and incorporate 90% of it into arginine, which is then transported into intraradical mycelia (Jin et al., 2005). Subsequently, arginine is broken down in intraradical mycelia, with urea and ornithine release, and further decomposed into NH_4^+-N by urease and ornithine aminotransferase (Jin et al., 2005). AMF absorb N either predominantly or exclusively in the

form of NH_4^+-N (Veresoglou et al., 2012). Moreover, studies have shown that AMF can immobilise N from organic sources. Plants uptake ^{15}N from patches (organic matter labelled with ^{13}C and ^{15}N) by AMF symbionts, without ^{13}C transfer, suggesting that organic N is not transferred to the plant in an intact form (Hodge et al., 2010; Hodge and Fitter, 2010; Leigh et al., 2009). Organic N absorption by mycorrhizal plants involves multiple steps, including uptake and breakdown of soil organic matter by mycorrhizal fungi, internal transformation, and transfer of the N to the host plant (Talbot and Treseder, 2010). AMF might increase the transfer of decomposed inorganic N to plants, as a result of competition with soil microorganisms and its effective spatial exploitation (Smith and Smith, 2011).

Compared to P and N, limited attention has been paid to the role of AMF in plant S acquisition. Studies have demonstrated that AMF symbiosis can improve plant S absorption by upregulating the expression of sulphate transporter genes by plant roots (e.g. MtSULTR 1.2 and MtSULTR 1.1) (Sieh et al., 2013), in turn relieving S deficiency in plants (Wu et al., 2018). AM symbiosis can also increase plant S absorption through S direct uptake and transport via extraradical mycelia (Gigolashvili and Kopriva, 2014; Wu et al., 2018). In addition, AMF colonization could increase Met and Cys uptake, which could improve plant access to a N/S from amino acids; however, whether the amino acids were utilized by intact or mineralized N remains unclear (Whiteside et al., 2012).

To explore the uptake and relative contributions of organic and inorganic N/S to plants, and the role of AMF in the absorption of various forms of N/S aby host plants, two cultivation tests were conducted based on ^{14}C, ^{35}S, ^{13}C, ^{15}N quad labelling. We hypothesised that 1) plants could uptake some intact Cys and Met, but most of them would be decomposed into inorganic

N and S by soil microorganisms; 2) AMF increases plant uptake of inorganic N and S due to its large absorption area, but decreases plant uptake of organic N/S, due to AMF also requiring high C amounts; 3) AMF enhance plant growth by enhancing root access to nutrients.

2. Materials and methods

2.1 Soil collection

Brown Earth soil was sampled (0–10 cm) from a grassland at Henfaes Agricultural Research Station, Abergwyngregyn, Bangor, UK (53°14′N, 4°01′W). The soil was air-dried to a water content of 20%, and the stones, vegetation, and earthworms removed by passing through a 4-mm sieve. Soil basic properties (Table S1) were detected as described previously (Ma et al, 2021).

2.2 Plant cultivation and AMF colonization

Two tomato genotypes (Lycopersicon esculentum L.) were used in the present study: 1) mutant tomato with highly decreased AMF symbiosis capacity, named rmyc (reduced mycorrhizal colonization), and 2) a closely related wild type named MYC (Zhou et al., 2020). The biomass of the two genotypes under various circumstances was similar, with or without mycorrhizae, indicating that the mutation associated with mycorrhiza colonization had limited effects on other plant metabolic processes (Cavagnaro et al., 2004; Zhou et al., 2020). Using the genotypes above, we could explore the effects of AMF on organic and inorganic N/S uptake without soil sterilization (non-mycorrhizal control), and on soil microbial community structure (Zhou et al., 2020). *Funneliformis mosseae* (formerly Glomus mosseae), a type of representative AMF, which associates extensively with plants and is present in soil was collected (Cheng et al., 2021). The mycorrhizal and rhizobial symbiotic inoculants were

obtained from Plantworks Ltd (Kent, United Kingdom) in a mixture of substrate, spores, hyphae, and infected root fragments (Cheng et al., 2021). To improve AMF colonization potential, the microgranules were mixed with the soil prior to plant cultivation.

2.3 Competitive uptake of N and S by plants under various AMF symbiosis

Tomato seeds of the test samples (MYC and rmyc) were sown into culture dishes for 5 d, and one germinated seed was sown into a pot (20 pots for each genotype; five labelling mixtures × four replicates). The pots contained 400 g soil (9-cm height, 9-cm top width, 7-cm bottom width), mixed with 0.2 g mycorrhizal symbiotic inoculants (pot test).

After cultivation in pots for 82 d, 20-ml of mixed organic and inorganic S/N sources, which were separately labelled, were injected into soils 10 times (2 ml each time) with a 10-cm long syringe needle. The 2 ml solution was gradually injected into soil to ensure sure the labelled materials were rapidly and uniformly deposited. To test whether the injected solution was uniformly distributed, a similar amount of blue ink was injected into the soil, and the colour separated uniformly through the soil within seconds. The injected solution included five labelled mixtures: ^{15}**NH**$_4^+$**-**^{35}**SO**$_4^{2-}$-Met-Cys; NH_4^+-SO_4^{2-}-^{13}**C,**^{15}**N,**^{35}**S-Met**-Cys; NH_4^+-SO_4^{2-}-^{14}**C-Met**-Cys; NH_4^+-SO_4^{2-}-Met-^{13}**C,**^{15}**N,**^{35}**S-Cys**; NH_4^+-SO_4^{2-}-Met-^{14}**C-Cys**. The concentrations of Cys, Met, SO_4^{2-}, and NH_4^+ were all 50 μM (L-$^{13}C_5$,^{15}N-Met, L-$^{13}C_3$,^{15}N-Cys, 99.8%, Aldrich; ^{35}S:7.9-8.2 kBq ml^{-1}; ^{14}C: 3.3-3.5 kBq ml^{-1}). The low concentrations of amino acids used in the present study were similar to their concentrations in soil solution when microbial or root cells lyse (Jones et al., 2005).

NH_4^+-N was selected as a representative inorganic N, as its contents were much higher than those of NO_3^--N in the test soil. Such a selective labelling mechanism can separate the uptake

and relative contributions of organic and inorganic S and N by plants. ^{13}C and ^{15}N dual-labelling can distinguish the N uptake from intact Cys and Met from N uptake from mineralized Cys and Met (Ganeteg et al., 2017); ^{14}C and ^{35}S radioactive labelling will also separate the S absorbed as intact molecular S or SO_4^{2-} following decomposition. A fan was in operation in the greenhouse to accelerate air flow and prevent photosynthetic assimilation of $^{13}CO_2$ or $^{14}CO_2$ released from the soil. Unlabelled Cys-Met-SO_4^{2-}-NH_4^+ (20 ml) was injected into four pots of each tomato genotype as a blank sample to detect the radioactive and stable isotope ratios, and the root lengths colonized by MYC and rmyc AMF were detected as previously described (Cheng et al., 2021). Prior to the injection of labelled solutions, the soil background concentrations of soluble Met, Cys, NH_4^+, and SO_4^{2-} were detected as described previously (table S1) (Ma et al., 2021).

After 6 h, the tomato roots were separated from soil by shaking gently, washed with 0.01M $CaCl_2$ for 1 min, and then washed thoroughly using distilled water to remove soil traces on root surfaces. The shoots and roots were separated and freeze-dried using a Labconco Freeze-Dry System (Labconco Corp., Kansas City, MO, USA) before being ground to fine powder using a ball mill, Retsch MM301 Mixer Mill (Haan, Germany). The ^{14}C assimilated into plant tissues were combusted in an OX400 Biological Oxidiser (Harvey Instruments Corp., Hillsdale, NJ, USA); the liberated $^{14}CO_2$ was captured in Oxosol Scintillant (National Diagnostics, Atlanta, GA, USA) and ^{14}C activity was measured using a Wallace 1404 liquid scintillation counter (Wallace EG&G, Milton Keynes, UK) after mixing with 4 ml Scintisafe 3 scintillation cocktail (Fisher Scientific, Loughborough, UK). To detect ^{35}S in plant tissues, 200-μg plant powder was extracted using 1.5 ml SOLUENE 350 (PerkinElmer) for 24 h, centrifuged for 5 min at

5000*g*, and the ^{35}S activity in the extracts (0.4 ml) detected using the liquid scintillation counter. The C and N contents, and ^{13}C and ^{15}N abundance in plants were detected using an Elemental Analysis-Stable Isotope Mass Spectrometer (IsoPrime100, Isoprime Ltd., Cheadle Hulme, UK).

The soil in each pot was mixed thoroughly, and three portions prepared (5 g per portion): one portion was extracted using 25 ml 0.01M $CaCl_2$ (^{35}S-labeled) or 25 ml 1M KCl (^{14}C-labeled) to detect the labelled Met, Cys, and SO_4^{2-} left in soil solution and the $^{35}SO_4^{2-}$ produced after decomposition; one portion was used to detect the ^{14}C and ^{35}S immobilized into MB using the fumigation-extraction method, as follows. Soil (5 g) was fumigated by adding 1-ml alcohol-free $CHCl_3$ for 24 h. After removing the residual $CHCl_3$ by vacuuming for 1 h, it was extracted using 25 ml 0.01M $CaCl_2$ or 1M KCl to detect the ^{35}S and ^{14}C immobilized in MB (Vong et al., 2004). ^{35}S extracted using 0.01M $CaCl_2$ and ^{14}C extracted using 1M KCl in fumigated (portion 2) and non-fumigated soil samples (portion 1) were measured as described above, and MB-S and MB-C were calculated using a conversion factor of 2.86 for S (Vong et al., 2004), and 2.22 for C (Jenkinson et al., 2004). The last portion was used to detect soil moister by oven-drying at 105°C for 24 h. After adding extract solution, they were shaken at 180 rpm for 1 h, and centrifuged at 6000*g* for 15 min. Subsequently, 0.5 ml of purified water or 0.5 ml 1M $BaCl_2$ was added to 1 ml 0.01 M $CaCl_2$ extracts (unfumigated sample), and then centrifuged at 18,000*g* for 5 min. ^{35}S activity was detected and the difference between water and $BaCl_2$ addition was the activity of SO_4^{2-} produced from labelled Cys and Met. The $BaCl_2$ added would precipitate SO_4^{2-} into $BaSO_4$, but would have limited effects on S-containing amino acids in soil.

In addition, the phospholipid fatty acids (PLFAs) in ^{13}C-Cys/Met-treated soil were

extracted and tested as stated previously (Ma et al., 2018). Freeze-dried soil (2 g) was extracted twice using an 11.4-ml chloroform/methanol/citrate buffer (1:2:0.8 v/v/v, 0.15 M, pH 4.0), and phospholipids were separated using silica acid columns (Supelco, Bellefonte, PA, USA). After phospholipid methylation, PLFA methyl esters were identified using a gas chromatograph (GC 7890A; Agilent, Santa Clara, CA, USA) and fitted using a MIDI Sherlock microbial identification system v.6.2B (MIDI, Newark, DE, USA). The $\delta^{13}C$ in individual PLFAs was tested by gas chromatography combustion isotope ratio mass spectrometry (Ma et al., 2018). Anteiso- and iso-branched fatty acids were considered indicators of Gram-positive bacteria (G+), whereas cyclopropyl and monounsaturated fatty acids were considered indicators for Gram-negative bacteria (G-). Saturated straight-chain fatty acids were considered non-specific PLFAs indicators that exist in microorganisms. Specifically, the 16:1 w5c is considered an indicator of AMF.

2.4 AMF uptake of organic N/S from areas root cannot reach (pot + mesh test)

To explore how AMF colonization influence host plant uptake of organic N and S out of reach of the root system, we conducted a pot cultivation test with mesh that prevented the expansion of root (pot + mesh test). The pot contained 1150 g soil (a dual compartment device, inner pot, 400 g, outer pot, 750 g, Fig. 1), mixed with 0.575 g mycorrhizal symbiotic inoculants. The inner pot (planting compartment) contained only the skeleton and was wrapped with fine nylon mesh (25 μm). Seeds of tomato (Myc and Rmyc) were sown in culture dishes for 5 d, and one germinated seed was sown into the inner pot (eight pots for each genotype; two labelling solutions × four replicates). The tomato roots could grow in the inner pot, but could not pass the mesh, whereas AMF ectophypha could pass the mesh. After cultivation in pots for

82 d, 50 ml of 50 μM ^{13}C,^{15}N,^{35}S-Cys, or ^{14}C-Cys was injected into soils 10 times (5 ml for one time) with a 10 cm long syringe needle, gradually injected 5 ml solution in the inner wall of the outer pot when raise up, to make sure the labelled materials were rapidly and uniformly separated (L-$^{13}C_3$,^{15}N-Cys, 99.8%, Aldrich; ^{35}S:8.6 kBq ml^{-1}; ^{14}C: 3.6 kBq ml^{-1}). In this test, only Cys was added, due to similar effects of AMF observed for Cys and Met. After uptake for 6 h, the tomato was harvested and the ^{14}C, ^{35}S activity and ^{13}C, ^{15}N abundance were detected as stated above. The ^{14}C and ^{35}S retained in MB, and the $^{35}SO_4^{2-}$ produced from added Cys in soils from both inner and outer pots, were determined as stated above.

2.5. Calculations

The uptake of ^{13}C by grasses from the labelled Met or Cys was calculated using equation (1) (similar with ^{15}N) (Ma et al., 2021):

$$^{13}C_{\text{uptake ratio}} = C_{Total-C}\ (A_s - A_c)\ /^{13}C_{Total}\ (1)$$

where $^{13}C_{uptake\ ratio}$ is the ratio of ^{13}C uptake from the labelled Met or Cys; $C_{Total\text{-}C}$ is the total C of the tomato; A_c is the abundance of ^{13}C in the 'blank' seedlings; A_s is the abundance of ^{13}C in the ^{13}C- Met/Cys treated tomato; and $^{13}C_{Total}$ is the total amount of ^{13}C added to the soil.

The uptake of ^{14}C by tomato from the labelled Met or Cys was calculated using equation (2) (similar with ^{35}S):

$$^{14}C_{\text{uptake ratio}} = (A_s - A_c)\ /^{14}C_{Total}\ (2)$$

where $^{14}C_{uptake\ ratio}$ is the ratio of ^{14}C absorbed from the labelled Met or Cys; A_c is the ^{14}C activity in the tomato treated with unlabelled solution; A_s is the ^{14}C activity in the ^{14}C-Cys/Met-treated tomato, and $^{14}C_{Total}$ is the total activity of ^{14}C added to the soil.

The uptake of ^{15}N by tomato after microbial mineralisation ($^{15}N_{\text{uptake ratio-min}}$) was calculated as the ^{15}N uptake minus the ^{13}C uptake (intact Met or Cys uptake) using equation 3

(the same for ^{35}S uptake after mineralisation).

$$^{15}N_{\text{uptake ratio-min}} = {}^{15}N_{\text{uptake ratio}} - {}^{13}C_{\text{uptake ratio}} \quad (3)$$

The uptake amounts of N and S (derived from N/S initially present in the original soil [table S1] and additions, μmole pot^{-1}) using equation (5) (the same for S, 1.5 means 1.5 μmole of labelled N/S added to one pot):

$$N_{uptake} = {}^{15}N_{uptake\ ratio} * 1.5 * \frac{(Content_{soil} + 1.5)}{1.5} \quad (4)$$

The contributions of N (% of total N uptake) from organic or mineralized Met and Cys, and NH_4^+ were calculated using equation (6) (the same for S):

$$N_{\text{contribution}} = N_{uptake} / (N_{uptake-Cys} + N_{uptake-Met} + N_{uptake-NH_4^+}) * 100 \quad (5)$$

where $N_{uptake\text{-}Met}$ is the N uptake amount of Met (organic and inorganic N after mineralisation), and similarly for Cys and NH_4^+.

2.5. Statistical analyses

Data are presented as means ± SE. The Shapiro-Wilk test was used to assess normality before applying the t-test to assess differences between the two genotypes. Figures were illustrated using Origin 8.1 (OriginLab, Northampton, MA, USA).

3. Results

3.1 AMF colonization and plant biomass

The root lengths colonized by AMF under MYC were 19.8% and only 1.0% under rmyc after culture for 82 d (Table S1, the plant in the pot test). In the pot tests, the biomass of tomato under MYC was significantly reduced by 8.6% compared with rmyc. However, in the pot+mesh tests, plant growth in the two genotypes was similar (Fig. 2).

3.2 Plant uptake of organic and inorganic N/S as indicated by ^{13}C, ^{15}N, ^{14}C, and ^{35}S

In the pot tests, 0.58–1.26% of Cys and Met added was utilized by tomato roots after addition

268 for 6 h, as indicated by ^{13}C abundance, and similar uptake amounts (0.67–1.77%) were also
269 indicated by ^{14}C activity. In addition, 10.03–14.82% of N derived from Cys and Met was
270 utilized by plants, and minimal differences were observed among Cys, Met, and NH_4^+ (Fig. 3).
271 Tomato absorbed 4.58–6.07% of S derived from Cys, while it only accounted for 1.43–1.80%
272 S from Met, and much higher of S was absorbed from SO_4^{2-} (9.93–11.16%) (Fig. 4).
273 AMF colonization reduced intact Met and Cys uptake significantly, as shown both by ^{13}C and
274 ^{14}C labelling in both pot and pot + mesh tests (Fig. 3, 4). However, it increased ^{15}N uptake from
275 Cys by 27.2% when compared with rmyc in the pot test, which increased by 15.7% in the pot
276 + mesh tests. In the pot tests, AMF colonization decreased ^{35}S uptake from Cys, Met, and SO_4^{2-}
277 by 24.6%, 20.6%, and 11.0%, respectively, when compared with rmyc. In contrast, AMF
278 colonization increased ^{35}S uptake from Cys by 118.7% when compared with rmyc in the pot +
279 mesh test (Fig. 4).
280 In addition, most of the ^{13}C and ^{14}C of Cys and Met absorbed by tomato roots were transported
281 to leaves at 6 h, and AMF colonization deceased the transportation of $^{13}C/^{14}C$ in the pot tests
282 significantly; however, there were limited effects on ^{15}N and ^{35}S transportation (Fig. S1 and
283 S2).

284 **3.3 Organic and inorganic N/S contributions in pot test**

285 In the pot tests, tomato utilized SO_4^{2-} as their main S source (68.2–88.2%), and S uptake
286 from Cys (8.8–22.0%) and Met (3.0–9.8%) played important roles in plant S nutrition. NH_4^+
287 was the main N source for tomato growth, which accounted for 98.7–98.9%, and the N
288 contribution from Cys and Met (intact and inorganic N derived from organic N decomposition)
289 accounted for only ~1%. The intact Cys and Met uptake accounted only 0.06–0.09% and 0.06–

290 0.12% of the total N uptake, respectively. AMF colonization decreased intact Cys and Met, and
291 the inorganic S (derived from Cys and Met decomposition) uptake significantly, but greatly
292 increased SO_4^{2-} uptake, by 22.7%, when compared with rmyc (Fig. 5).

293 **3.4 C and S tracing in microorganisms**

294 In the pot tests, approximately half of ^{35}S from Cys and Met was retained in MB, and
295 41.2–43.0% of Cys, and 20.5–22.8% of Met was released as SO_4^{2-}. Lower ratios of added SO_4^{2-}
296 were retained in MB. In the pot+mesh tests, 3.3–5.1 of ^{14}C, and 8.1–10.1% of ^{35}S from Met
297 were found in MB in the inner pot soil (Fig. 6).

298 AMF colonization increased the ^{14}C from Cys and Met retained in MB in the pot tests.
299 However, in the pot+mesh tests, AMF colonization reduced ^{14}C-MB in the outer pot soil, but
300 increase it in the inner pot soil. Much higher ^{14}C from Met (48.4–66.4%) was retained in MB
301 compared with Cys (14.2–19.1%).

302 **3.5 Active microorganisms in utilizing Cys and Met**

303 Most of the added Cys and Met were utilized by G-, as indicated by ^{13}C-PLFA biomarker, and
304 much higher ratios of ^{13}C were obtained from Met than from Cys. The AMF biomarker of
305 16:1 w5c was significantly higher under MYC cultivation than under rmyc cultivation ($P <$
306 0.001) (Fig. 7).

307

308 **4. Discussion**

309 **4.1 Tomato uptake of N and S derived from Cys and Met**

310 Plant roots can absorb limited amounts of supplemented Met and Cys under severe
311 competition with soil microorganisms. In pot experiments, two tomato genotypes could utilize

only less than 1.77% of Met and Cys supplement after 6 h, indicated by both ^{13}C and ^{14}C labelling. The direct uptake of amino acids may be favourable to plants from an energy use perspective; it can save the energy required to assimilate NH_4^+ and SO_4^{2-} into amino acids (Franklin et al., 2017), and plants roots possess a complete absorption system with a great capacity to absorb and metabolise the amino acids (Ma et al., 2017; Ma et al., 2017; Nsholm et al., 2009). For example, glycine acts is an important N source for pak choi (*Brassica chinensis* L.) in sterile environments, accounting for 19.0–33.1% of the total N uptake from mixed N sources (glycine: NH_4^+: NO_3^-=1:1:1) (Ma et al., 2017). However, in soil environments, most of the Cys and Met were decomposed into inorganic N and S within minutes to hours, and further utilized by plant roots, similar to other organic N forms, such as alanine and glycine (Kuzyakov and Xu, 2013). After uptake for 6 h, 10.03–14.82% of N and 1.43–6.07% of S derived from Met and Cys were utilized by plants, indicating that plants capture high amounts of inorganic N and S after organic matter decomposition. S uptake by tomato roots as inorganic S derived from Cys was much higher than that derived from Cys, as higher ratio of S-Cys was released as SO_4^{2-} from microorganisms.

Considering soil N and S contents, Met and Cys could be important S sources for plants but play negligible roles in N nutrition. In the pot test, tomato utilized SO_4^{2-} as its main S source (68.2-88.2%). S uptake from Cys (8.8–22.0%) and Met (3.0–9.8%) are also crucial in plant S nutrition due to lower contents of SO_4^{2-} in soil solution. NH_4^+ was the main N source for tomato growth, which accounted for 98.7–98.9% of the N, and the N contributions of Cys and Met (intact and inorganic N derived from organic N decomposition) were only ~1%, due to the presence of high amounts of inorganic N in the agricultural soil (Ma et al., 2021). The uptake of intact Cys and Met accounted for only 0.06–0.09%, and 0.06-0.12% of the total N uptake.

The contributions of intact Met and Cys based on $^{13}C/^{14}C$ labelling could have been overestimated due to the uptake of other labelled C from $^{13}C/^{14}C$-Met/Cys in the soil, such as $H^{13}CO_3^-$ and $^{13}CO_3^{2-}$ (Nasholm et al., 2009); furthermore, the Cys can be oxidised to cystine before plant uptake. Conversely, the contributions of intact Met and Cys could have been underestimated since the ^{13}C or ^{14}C released from the leaves as CO_2 were not measured, and a study has shown that the labelled C can be released after root uptake for 4 h (Ma et al., 2020). In addition, soil available Met and Cys could be higher than the levels detected, as high amounts were adsorbed onto soil particles and organic matter, and can be utilized by plant roots (Cao et al., 2013). In addition, plant roots can access higher proportions of soil soluble amino acids when their concentrations are high, as microbial decomposition decreases under high amino acid concentrations (Hill et al., 2019; Jones et al., 2005). After clover and earthworm decomposition, amino acid concentrations could be as high as 2.7 mM and 45.3 mM, respectively, which can be accessed by root uptake (Hill et al., 2019). The concentrations of amino acids in rhizosphere were much higher than in soil solution, and may play more important roles in plant S nutrition, and plant utilization of soil-dissolved organic S may primarily take place in organic matter-rich patches in soil.

4.2 How soil microorganisms utilize Cys and Met

Soil microorganisms are strong competitors for low molecular weight organic S in soil. Microbes are C limited but not S or N limited in well-aerated soils, and microbe utilization of Met and Cys have been demonstrated to be driven by C demand but not S demand (Ma et al., 2020). Microbial utilization of Met and Cys includes three major processes: first, they are integrated into MB following uptake, which occurs with seconds to hours. Rapid Met and Cys uptake implies that limited intact Met and Cys could be captured by roots, as has been demonstrated previously for other amino acids, such as glycine and alanine (Ganeteg et al.,

2017; Kuzyakov and Xu, 2013; Ma et al., 2018). Secondly, the N, C, and S in the MB are released as NH_4^+, CO_2, and SO_4^{2-}, respectively (in the present study, 41.2–43.0% of ^{35}S-Cys, and 20.5–22.8% of ^{35}S-Met was released as SO_4^{2-}), and the inorganic ions are further utilized by plant roots; lastly, part of the inorganic N and S can be utilized again by microbes to satisfy their nutrient demands, which also compete with plants for the inorganic nutrients (Ma et al., 2021).

When Met and Cys are assimilated into MB, the metabolic process is dominated by their original molecular structures (Manzoni et al., 2012; Xu et al., 2014). In the pot test, 41.2–43.0% of ^{35}S-Cys, and 20.5–22.8% of ^{35}S-Met were released as SO_4^{2-}. Under continuous sampling, a study revealed that Cys was largely mineralised, whereas a high proportion of Met was assimilated into MB (Fitzgerald et al., 1988; Romero et al., 2014). Met may decompose mainly into methanethiol, α-ketobutyrate, and NH_4^+, and Cys may be transferred to NH_4^+, pyruvate, and H_2S via *L*-Cys desulfydrase; the H_2S is further oxidised into SO_4^{2-} (Takagi and Ohtsu, 2016). Cys may represent a good source of plant available S considering its high SO_4^{2-} after microbial decomposition; conversely, Met may be a better source of S for microbes as high S amounts are retained in MB to maintain its growth (Ma et al., 2020). In addition, even with AMF supplementation, most of Met and Cys added was utilized by G- bacteria, as indicated by ^{13}C-PLFA biomarkers, and much higher ratios of ^{13}C were from Met. Compared with slowly growing microorganisms, such as G+ positive bacteria and fungi, the fast-growing G-bacteria captured most of the added amino acids (Lazcano et al., 2013). However, in the Antarctic, G+ bacteria are the primary competitors for peptides and amino acids (Broughton et al., 2015), since G+ is the dominant species in the cold ecosystem, and G- is the major active microbe in

the warmer ecosystems (Ma et al., 2018).

4.3 Effect of AMF on plant uptake of organic and inorganic N/S

AMF colonization increased nutrient competition in the rhizosphere, but stimulated nutrient transportation. In the pot tests, plant roots and AMF mainly competed for nutrients; however, in the pot+mesh tests, the extraradical mycelia of AMF can transport nutrients absorbed from the areas that roots cannot reach. In the pot tests, the biomass of tomato under AMF colonization (MYC) was significantly reduced, by 8.6%, when compared with in the rmyc, which indicated that AMF might compete with plants for nutrients, or the transportation of C from plants to AMF could also reduce plant growth. However, in the pot+mesh tests, the growth of the two plant genotypes was similar (Fig. 2), and the extraradical mycelia absorbed nutrient and transported them from areas that roots could not accessed to the host plants. AMF increase grain yields by 16% based on a meta-analysis, which was associated with nutrient uptake from areas roots cannot reach (Zhang et al., 2019).

In the present study, AMF reduced host plant uptake of organic N/S, but stimulated plant N uptake. Based on quantum dots labelling, AM colonization increased the uptake of Met and Cys, and other neutral and positively-charged amino acids, such as lysine, phenylalanine, arginine, histidine, and asparagine, however, whether they were utilized as intact forms or inorganic N after mineralisation remains unclear (Whiteside et al., 2012). AMF might increase the capacity of plants to compete for organic N against other microbes (Whiteside et al., 2012). However, we showed that AMF colonization reduced intact Met and Cys uptake significantly in both pot test and pot + mesh tests, indicating that the increase uptake of N from Met and Cys under AM colonization was mainly associated with inorganic N after mineralization, but not

organic forms, as indicated by ^{14}C and ^{13}C labelling. AMF not only utilize organic C from host plants but also capture soil organic matter to satisfy their energy demands. Nevertheless, AMF increased ^{15}N uptake from Cys by 27.2% when compared to rmyc in the pot test, and increased by 15.7% in the pot + mesh test. AMF transfer substantial N to their host plants from organic matter (Leigh et al., 2009). Therefore, AMF enhanced host plant N uptake, but reduced organic N uptake.

AMF colonization increased the ^{14}C from Met and Cys retained in MB in the pot test. Much higher amounts of ^{14}C from Met (48.4-66.4%) were retained in MB compared with Cys (14.2–19.1%). With AMF addition, fungal microbial communities were slowly growing relative to bacteria, resulting in lower $^{14}CO_2$ release and higher C retention in the fungi (Lazcano et al., 2013). In addition, C:N:S stoichiometry for fungi and bacteria are 38:9:1 and 105:11:1, respectively (Kirkby et al., 2011), implying that fungi have greater S demands than bacteria. As AMF has also been suggested to reduce the rhizosphere priming effect on soil organic matter decomposition, which might be due to lower metabolic rates of AMF, and lower ratios of C were released as CO_2 (Zhou et al., 2020). However, in the pot+mesh tests, AMF colonization reduced ^{14}C-MB in the outer pot soil, but increased it in the inner pot soil, indicating that the extraradical mycelia transported the C in AMF organisms, although it is not utilized by host plants.

AMF colonization has been shown to increase plant S acquisition by up-regulating the expression of low affinity SO_4^{2-} transporter genes, such as MtSULTR2.2 and MtSULTR2.1 (Sieh et al., 2013), and high affinity SO_4^{2-} transporters, such as MtSULTR1.2 and MtSULTR1.1, in plant roots (Giovannetti et al., 2014; Sieh et al., 2013). AMF colonization

can also stimulate plant S absorption through transport of S via extraradical mycelia and direct uptake (Giovannetti et al., 2014). However, in the pot tests, AMF colonization decreased tomato ^{35}S uptake from Cys, Met, and SO_4^{2-}, by 24.6%, 20.6%, and 11.0%, respectively, when compared with rmyc. In contrast, AMF colonization increased ^{35}S uptake from Cys by 118.7 when compared with rmyc in the pot + mesh tests, indicating that the AMF has high S demand, and plant roots face fierce competition with AMF for S in the rhizosphere, whereas AMF could stimulate plant S uptake from the areas that root could not access. In addition, even AMF have been shown to stimulate plant S uptake; however, in soil environments, soil microorganisms influence soil S bioavailability, which in turn determines the amount of S transported to host plants.

5. Conclusion

Plant roots can absorb limited amounts of Met and Cys supplemented to growth media when facing fierce competition from soil microorganisms for N and S. After uptake for 6 h, 10.03–14.82% of N and 1.43–6.07% of S derived from Met and Cys were utilized by plants, mainly in the forms of inorganic N and S after mineralisation. S uptake by tomato as inorganic S derived from Cys was much greater than that derived from Met, as higher ratios of S-Cys were released as SO_4^{2-} from microorganisms. Considering soil N and S contents, Met and Cys could be important S sources for plants but they play negligible roles with regard to N nutrition. Even with artificial addition of AMF, most of the added Met and Cys were utilized by G- bacteria as indicated by ^{13}C-PLFA biomarker. AMF reduced host plant uptake of organic N and S, but stimulated plant N uptake. Under AMF supplementation, fungi exhibited

447 relatively slow growth when compared to bacterial growth, resulting in lower $^{14}CO_2$ release
448 and higher C retention in fungal biomass. AMF has high S demand, and plant roots face
449 fierce competition with AMF for S in the rhizosphere; nevertheless, AMF can stimulate plant
450 S uptake from areas that roots cannot access.

451 **Acknowledgements**

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Figure captions

Fig. 1. Photograph (A) and schematic diagram (C) of tomato cultivation, and simplified model of soil methionine and cysteine cycling (B). Two genotypes of tomato were cultivated in pots with or without 25-μm nylon mesh. Processes of methionine and cysteine cycling, which could be affected by Arbuscular mycorrhizal fungi colonization included (1) root uptake as intact molecules; (2) immobilisation of C, N and S in the microbial biomass; (3) release of SO_4^{2-} and NH_4^+ by soil microorganisms; (4) absorption of SO_4^{2-} and NH_4^+ by the plant roots.

Fig. 2. The root and shoot biomass of two genotypes, rmyc (reduced mycorrhizal colonization capacity) and wild type (MYC), under pot tests and pot + mesh tests. A significant difference between total biomass (shoot and root) was observed between the two genotypes under pot tests. Values are mean ± standard error of 20 replicates in pot tests (five labelling treatments × four replicates), and eight replicates for pot + mesh tests.

Fig. 3. ^{13}C (A) and ^{15}N (B) uptake derived from cysteine, methionine, and NH_4^+ in two genotypes, rmyc (reduced mycorrhizal colonization) and wild type (MYC), under pot tests and pot +mesh tests. Values represent mean ± standard error of four replicates. The differences between MYC and rmyc were separately analysed using t-tests. *, $p < 0.05$.

Fig. 4. ^{14}C (A) and ^{35}S (B) uptake derived from cysteine, methionine, and SO_4^{2-} in two genotypes, rmyc (reduced mycorrhizal colonization) and wild type (MYC) under pot test and pot +mesh test. Values represent mean ± standard error of four replicates. The differences between MYC and rmyc were separately analysed using t-tests. *, $p < 0.05$; **, $p < 0.01$.

Fig. 5. Uptake and contribution of S (A, B) and N (C, D) from intact amino acids and inorganic N/S, derived from the added and native Met/Cys/NH_4^+/SO_4^{2-} in soil by two tomato genotypes, rmyc reduced mycorrhizal colonization) and wild type (MYC) under pot tests and pot + mesh tests calculated from ^{13}C,^{15}N labelling, and ^{14}C,^{35}S labelling. Values are mean ± standard error

of four replicates. Met: methionine; Cys: cysteine; IN: inorganic nitrogen; IS: inorganic sulphur.

Fig. 6. Tracing ^{14}C from methionine and cysteine in microbial biomass (A), and tracing ^{35}S in microbial biomass (B) and $^{35}SO_4^{2-}$ released (C) in pot tests and pot + mesh tests after cultivation of two tomato genotypes, rmyc (reduced mycorrhizal colonization) and wild type (MYC). Values are mean ± standard error of four replicates.

Fig. 7. Ratio of ^{13}C labelled in phospholipid fatty acids (PLFAs) in soils after cultivation of two tomato genotypes, rmyc (reduced mycorrhizal colonization) and wild type (MYC) under pot tests. Values are mean ± standard error of four replicates.

Fig. S1. ^{13}C (A, B) and ^{15}N (C, D) uptake and transportation rates in two genotypes, rmyc (reduced mycorrhizal colonization) and wild type (MYC) under pot tests and pot +mesh tests. Values are mean ± standard error of the mean of four replicates. *, $p < 0.05$.

Fig. S2. ^{14}C (A, B) and ^{35}S (C, D) uptake and transportation rates in two genotypes, rmyc (reduced mycorrhizal colonization) and wild type (MYC) under pot tests and pot +mesh tests. Values are mean ± standard error of the mean of four replicates. *, $p < 0.05$; **, $p < 0.01$.

Figures

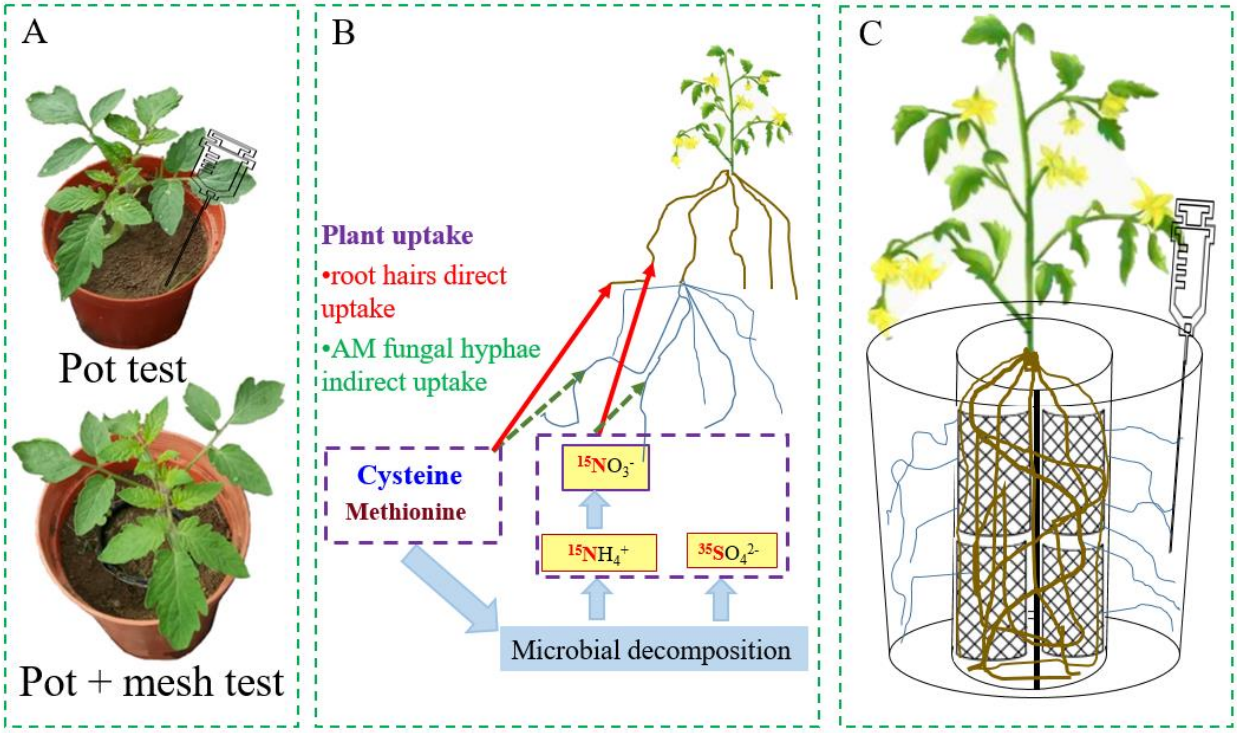


Figure 1

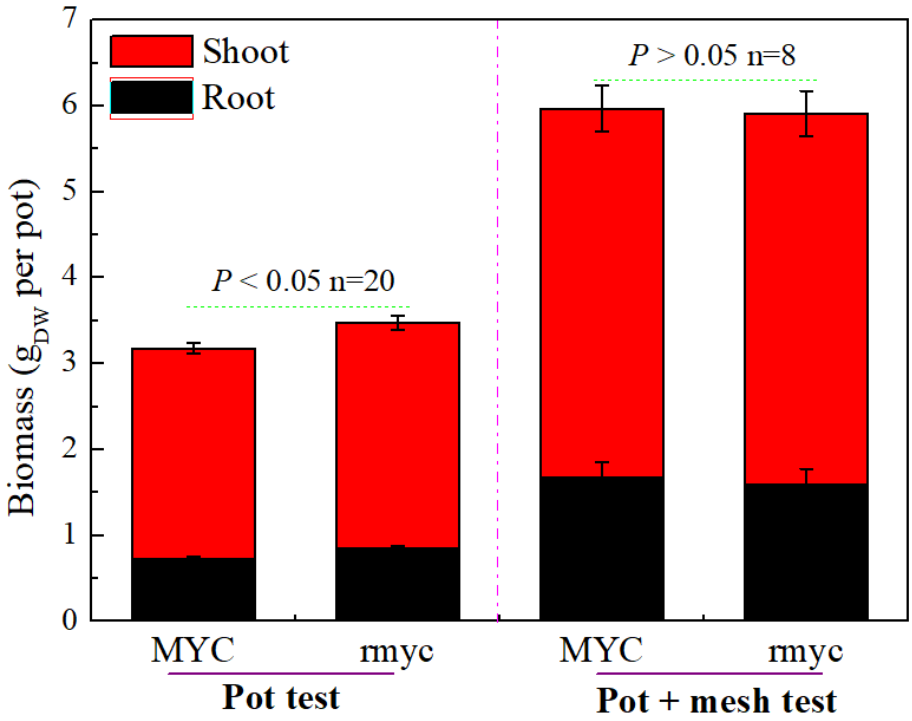

Shoot
Root
P > 0.05 n=8
P < 0.05 n=20
Biomass (g_DW per pot)
0
1
2
3
4
5
6
7
MYC
rmyc
MYC
rmyc
Pot test
Pot + mesh test

Figure 2

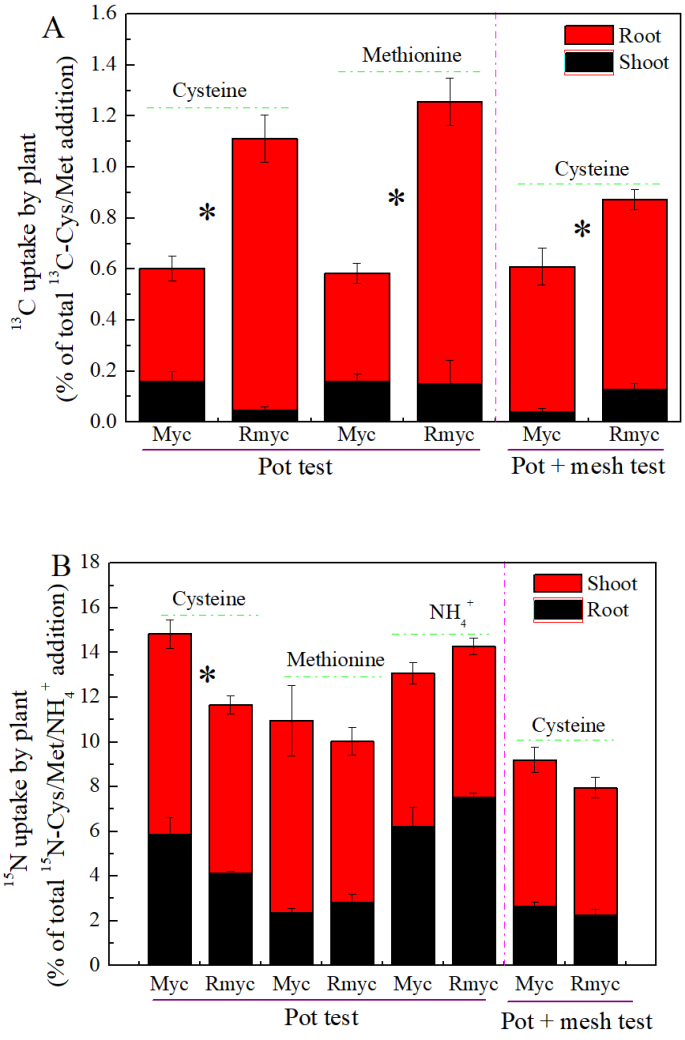

A
^{13}C uptake by plant
(% of total ^{13}C-Cys/Met addition)
Root
Shoot
Cysteine
Methionine
Cysteine
*
*
*
Myc
Rmyc
Myc
Rmyc
Myc
Rmyc
Pot test
Pot + mesh test
B
^{15}N uptake by plant
(% of total ^{15}N-Cys/Met/NH_4^+ addition)
Shoot
Root
Cysteine
Methionine
NH_4^+
Cysteine
*
Myc
Rmyc
Myc
Rmyc
Myc
Rmyc
Myc
Rmyc
Pot test
Pot + mesh test

Figure 3

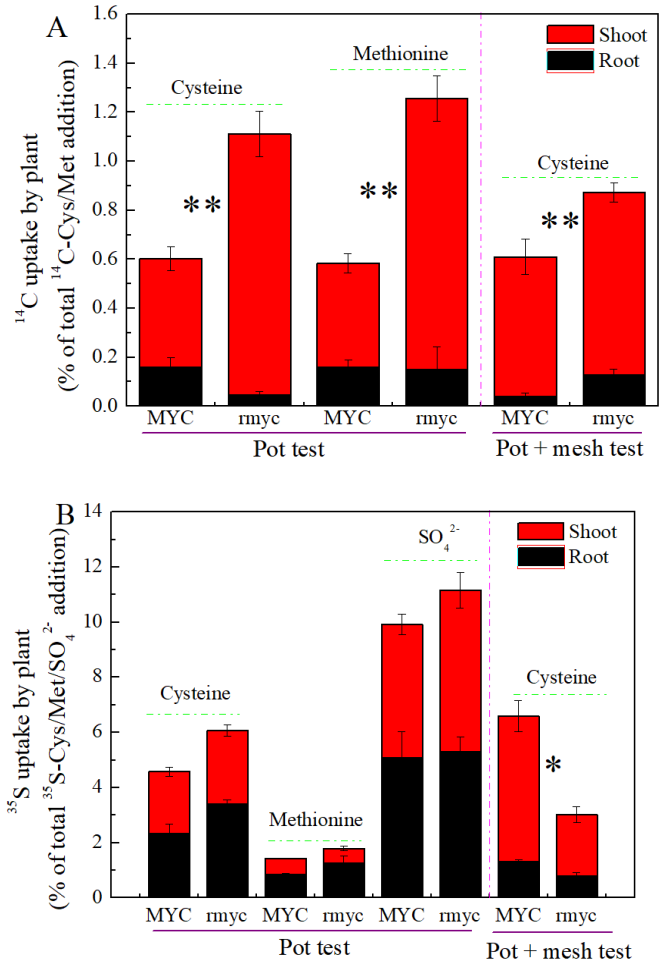
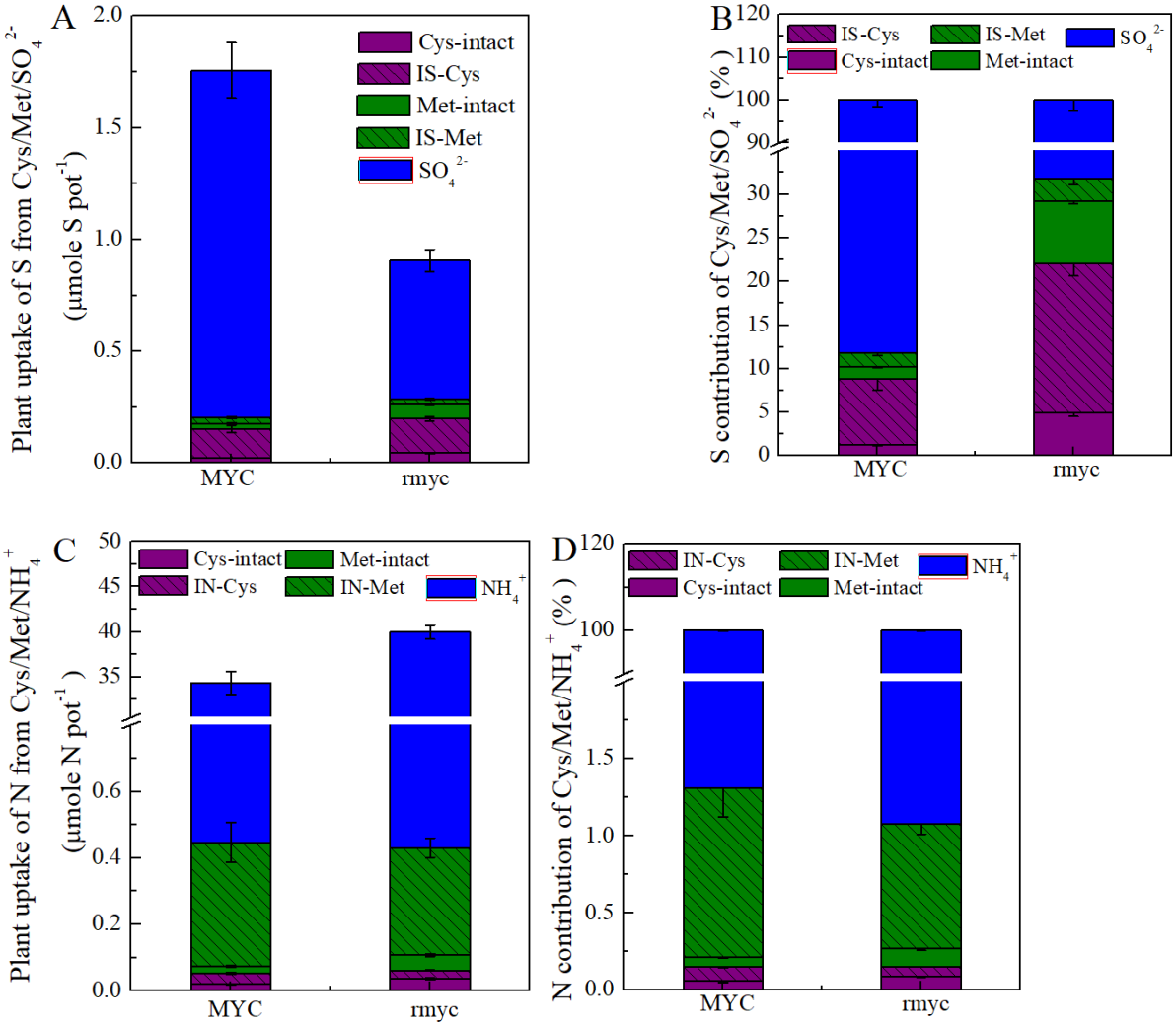

A
^{14}C uptake by plant
(% of total ^{14}C-Cys/Met addition)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Shoot
Root
Cysteine
Methionine
Cysteine
**
**
**
MYC
rmyc
MYC
rmyc
MYC
rmyc
Pot test
Pot + mesh test
B
^{35}S uptake by plant
(% of total ^{35}S-Cys/Met/SO_4^{2-} addition)
14
12
10
8
6
4
2
0
Shoot
Root
SO_4^{2-}
Cysteine
Methionine
Cysteine
*
MYC
rmyc
MYC
rmyc
MYC
rmyc
MYC
rmyc
Pot test
Pot + mesh test

Figure 4

696



697

698 **Figure 5**

699

700

701

702

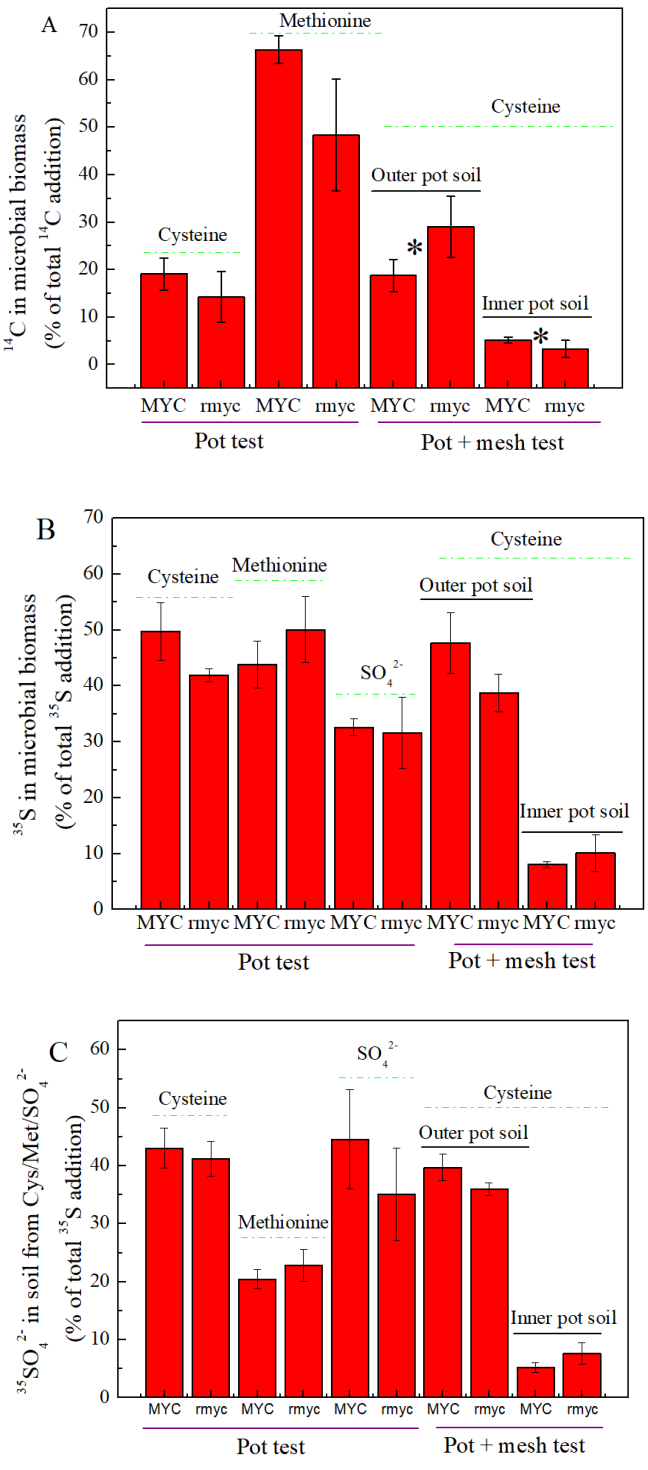

A
^{14}C in microbial biomass
(% of total ^{14}C addition)
Methionine
Cysteine
Outer pot soil
Inner pot soil
Cysteine
*
*
MYC rmyc MYC rmyc MYC rmyc MYC rmyc
Pot test
Pot + mesh test
B
^{35}S in microbial biomass
(% of total ^{35}S addition)
Cysteine
Methionine
SO_4^{2-}
Cysteine
Outer pot soil
Inner pot soil
MYC rmyc MYC rmyc MYC rmyc MYC rmyc MYC rmyc
Pot test
Pot + mesh test
C
$^{35}SO_4^{2-}$ in soil from Cys/Met/SO_4^{2-}
(% of total ^{35}S addition)
SO_4^{2-}
Cysteine
Methionine
Cysteine
Outer pot soil
Inner pot soil
MYC rmyc MYC rmyc MYC rmyc MYC rmyc MYC rmyc
Pot test
Pot + mesh test

Figure 6

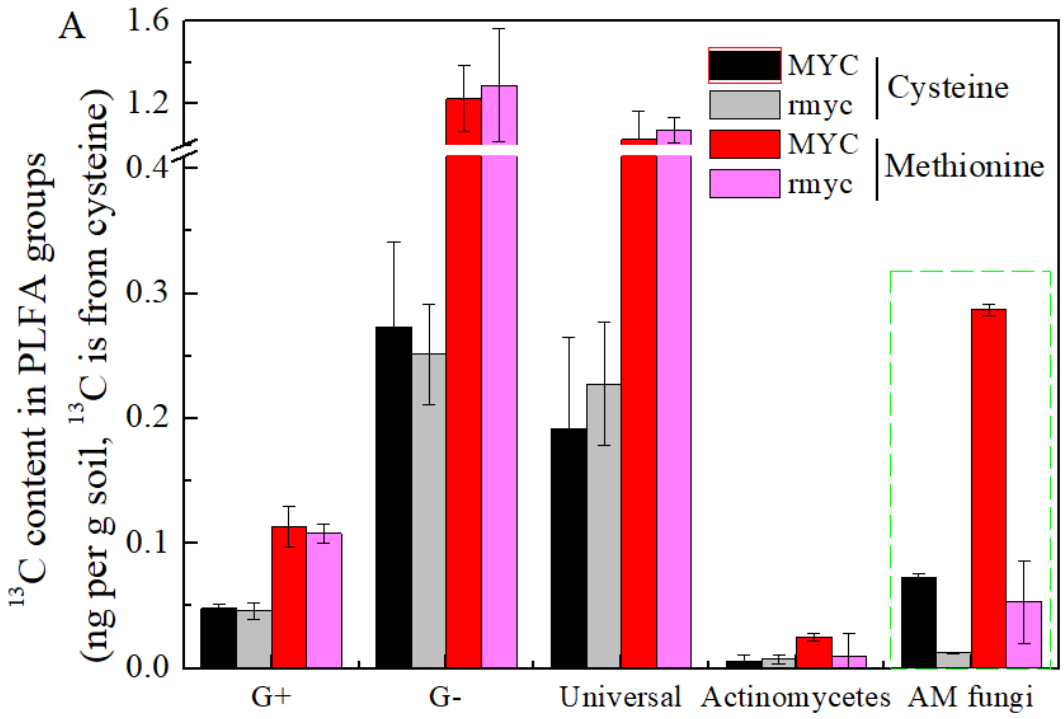

A
1.6
1.2
0.4
0.3
0.2
0.1
0.0
^{13}C content in PLFA groups
(ng per g soil, ^{13}C is from cysteine)
MYC
rmyc
Cysteine
MYC
rmyc
Methionine
G+
G-
Universal
Actinomycetes
AM fungi

Figure 7

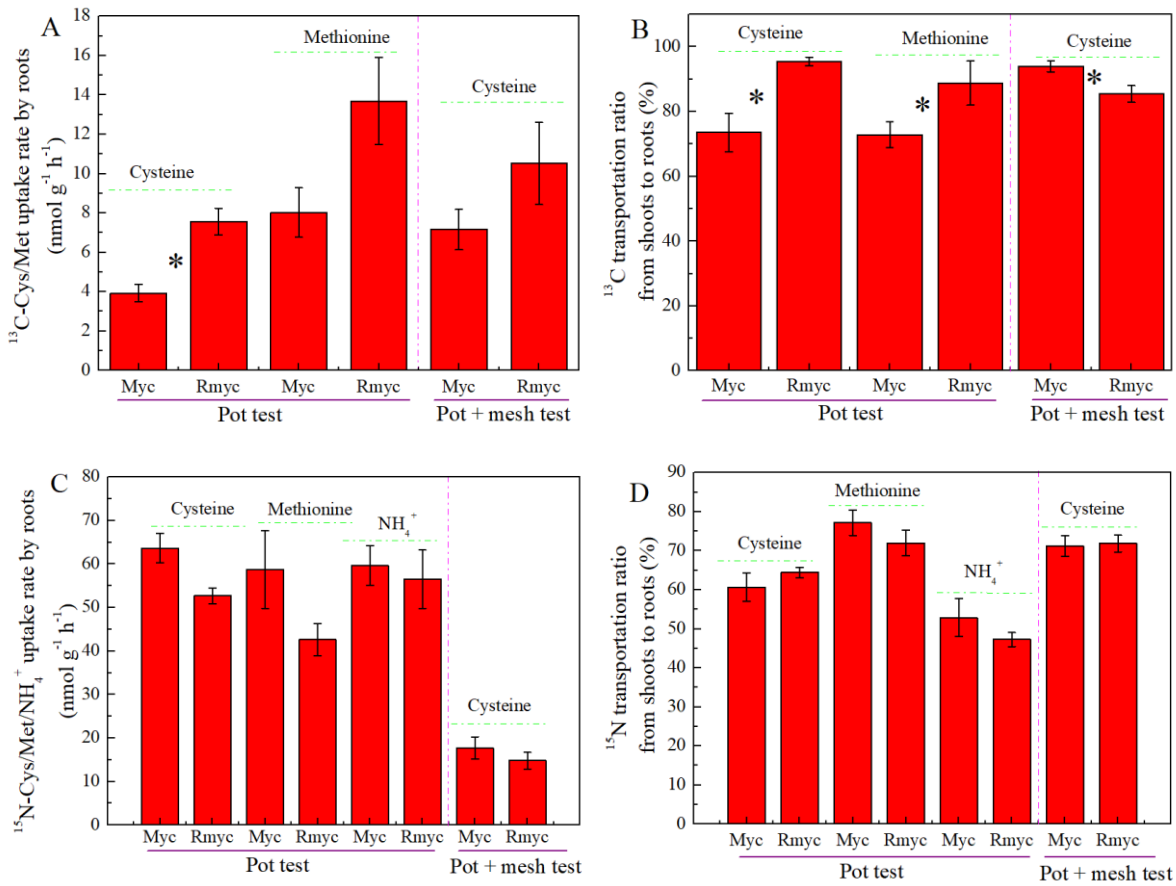

A
^{13}C-Cys/Met uptake rate by roots (nmol g^{-1} h^{-1})
Cysteine
Methionine
Cysteine
Myc
Rmyc
Pot test
Pot + mesh test
B
^{13}C transportation ratio from shoots to roots (%)
C
^{15}N-Cys/Met/NH$_4^+$ uptake rate by roots (nmol g^{-1} h^{-1})
NH$_4^+$
D
^{15}N transportation ratio from shoots to roots (%)

Figure S1

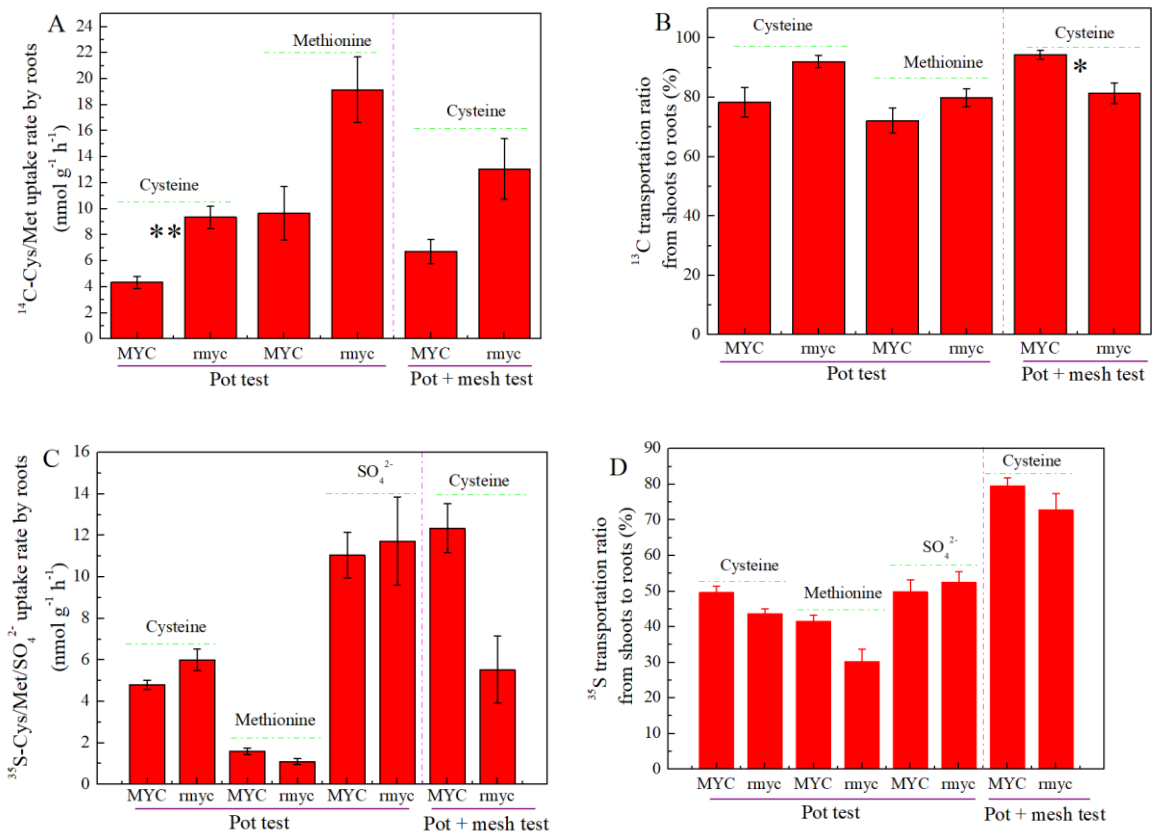

A
^{14}C-Cys/Met uptake rate by roots (nmol g^{-1} h^{-1})
Cysteine
Methionine
Cysteine
**
MYC
rmyc
MYC
rmyc
MYC
rmyc
Pot test
Pot + mesh test
B
^{13}C transportation ratio from shoots to roots (%)
Cysteine
Methionine
Cysteine
*
MYC
rmyc
MYC
rmyc
MYC
rmyc
Pot test
Pot + mesh test
C
^{35}S-Cys/Met/SO_4^{2-} uptake rate by roots (nmol g^{-1} h^{-1})
Cysteine
Methionine
SO_4^{2-}
Cysteine
MYC
rmyc
MYC
rmyc
MYC
rmyc
MYC
rmyc
Pot test
Pot + mesh test
D
^{35}S transportation ratio from shoots to roots (%)
Cysteine
Methionine
SO_4^{2-}
Cysteine
MYC
rmyc
MYC
rmyc
MYC
rmyc
MYC
rmyc
Pot test
Pot + mesh test

Figure S2

Table Captions

Table S1. Soil soluble methionine, cysteine, NH_4^+, and SO_4^{2-} contents after the cultivation of the two genotypes named rmyc (reduced mycorrhizal colonization) and wild type (MYC) under pot tests, and root lengths colonized by arbuscular mycorrhizal fungi.

	Cysteine (mg kg^{-1})	Methionine (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})	NH_4^+ (mg kg^{-1})	Root lengths colonized by AMF (%)
MYC	0.15±0.02	0.21±0.02	1.13±0.05	3.09±0.10	19.8±4.9
rmyc	0.14±0.03	0.22±0.03	1.22±0.06	3.30±0.11	1.0±0.3