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SHORT COMMUNICATION

Competition for two sulphur containing amino acids (cysteine and methionine) by soil microbes and maize roots in the rhizosphere

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Abstract

The factors regulating potential acquisition of sulphur (S)-containing amino acids by plant roots from the rhizosphere remain poorly understood. Using radio tracer (^{14}C and ^{35}S), we studied the competition for two S-containing amino acids (i.e., cysteine (Cys) and methionine (Met)) within 24 hours (h), by the rhizosphere microbial community and maize plants (*Zea mays* L.). Our results showed that the capture of Cys and Met by the maize plants was much lower, with only <10% of the added amino acid-^{14}C or ^{35}S captured by the plant, compared to the rhizosphere microbial community (76.9%) on average. We suggest that this could be a result of relatively high availability of inorganic N and S in soil solution, the lack of transmembrane for amino acids on maize root cells, as well as the rapid turnover of Cys and Met by soil microbes in the rhizosphere. The addition of inorganic S, significantly reduced plant capture of Cys and Met-^{14}C by maize plants in the rhizosphere but had little effect on the capture of Cys and Met-^{35}S ($p<0.05$). Overall, our results imply that (1) Cys and Met are available carbon (C), nitrogen (N) and S sources for maize plants, potentially contributing to total plant N and S demand under certain conditions; (2) Utilization of Cys and Met by maize roots in the rhizosphere is independent of inorganic S availability; (3) Increased amino acid concentration led to higher capture by both plants and soil microbes, but had little effect on the competition success on either side.

Keywords Cysteine · Methionine · Plant-microbial competition · Maize · Rhizosphere · Radiotracer

Introduction

S is an essential nutrient for plants, being required for the biosynthesis of essential amino acids (i.e., cysteine (Cys) and methionine (Met); Kopriva et al. 2019), oligopeptides (e.g. glutathione and phytochelatins; Zenda et al. 2021), vitamins (e.g. biotin, thiamine), enzyme co-factors (e.g. Fe-S clusters), and a variety of secondary metabolites (e.g. glucosinolates and alliins; Maruyama-Nakashita and Ohkama-Ohtsu 2017). An adequate supply of S is therefore essential to ensure optimal crop growth (Koprivova and Kopriva 2016), however, S deficiency is becoming more widespread in agricultural systems over the past several decades (Webb et al. 2016; Aula et al. 2019; Pariasca-Tanaka et al. 2020). This deficiency is mainly caused by 1) Typically low S use efficiency in agriculture (< 25 %; Eriksen 2009); 2) A major reduction in global anthropogenic SO_2 emissions due to a decrease in fossil fuel burning and greater regulatory controls on S release to the atmosphere (Hinckley et al. 2020); 3) The adoption of low S-containing fertilizers and greater crop yields.

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Traditionally, plant roots have been considered poor competitors with soil microbes for organic nutrients in soil (Moran-Zuloaga et al. 2015; Jacoby et al. 2017), and they can only acquire S in its inorganic form (SO_4^{2-}; Prasad and Shivay 2018). However, there is growing evidence that plants can also take up a range of nutrients (e.g. N, P, Fe) held in low molecular weight (LMW) organic forms (Chatzistathis et al. 2017; Yao et al. 2020; Gatiboni et al. 2021). Likewise, LMW soil organic S such as S containing amino acids is likely to be bioavailable N and S nutrient source for plants (Ma et al. 2021; Wang et al. 2021, 2023). It is assumed that the selective uptake and incorporation of N, and presumably S, from amino acids via a wide range of H^+ fuelled amino acid and sugar co-transporters (Perchlik and Tegeder 2017; Tegeder and Masclaux-Daubresse 2018) requires less energy in comparison to the assimilation of NO_3^-, NH_4^+ of SO_4^{2-} (Franklin et al. 2017). In addition, the uptake of amino acids from the rhizosphere is an important to recapture C lost in root exudation, this could prevent excessive microbial growth in the rhizosphere and simultaneously reduce nutrient loss to the soil (Jones et al. 2009).

The potential importance to plants of S containing amino acids capture from soil is likely to depend on the level of competition between the root and the rhizosphere microbial community (Xu et al. 2008). Based on studies of other organic N solutes, we expect that this competition will be particularly strong when concentrations of organic S are low in the soil solution (Owen and Jones 2001). This competition could be influenced by a range of abiotic factors (Kuzyakov and Xu 2013). Besides factors (the intensity of photosynthetically active radiation, temperature, soil moisture etc.) that could affect the partitioning of nutrients between roots and soil microbes via altering delivery of nutrients to root surfaces, the external supply of easily available nutrients to the rhizosphere could also play an important role. The supply of easily available inorganic N, S source may weaken the rhizosphere competition for Cys and Met, due to as a result of alternative N and S supply to plants. On the other hand, the supply of easily available organic C source into the rhizosphere may lead to an increase in microbial abundance, activity and growth, therefore promote increased plant capture of Cys and Met due to the provision of an alternative C supply to the microbial community.

In the present study, we investigated the competition for S-containing amino acids between maize (*Zea mays* L.) and microorganisms in the rhizosphere (^{14}C, ^{35}S). We chose maize as the model plant as it is known to possess a high demand for both N and S (Sutar 2017) and is capable of taking up exogenously applied amino acids (Moran-Zuloaga et al. 2015). Soil used in our study was collected from a low-altitude, high productivity site, soil from this site is C, N and S limited therefore strong competition for available nutrients in the rhizosphere may occur. We hypothesized that (I) Plant roots are poor competitors for externally applied amino acids compared to soil micro-organisms; (II) Elevated C availability would promote increased plant capture of Cys and Met, while N and S addition would reduce Cys and Met.

Materials and methods

Soil sampling and chemical characterisation

An agricultural soil (Eutric Cambisol) was collected from the Ah horizon (0-10 cm depth) of a temperate *Lolium perenne* L. dominated grassland site located at Abergwyngregyn, Gwynedd, North Wales (53°14'N, 4°01'W). Approximately 1 kg of soil was collected from three randomly positioned replicate plots, located 2 meters apart, within our study site (5×5 m^2). In terms of N availability, the site is characterised as being spatially homogenous (Shaw et al. 2016). The soil was then placed in gas permeable plastic bags and transferred immediately to the laboratory. These samples represented the three independent replicates for all following experiments. On return to the laboratory, this crumb structured, sandy clay loam textured soil was sieved to pass 2 mm before being stored at 4 °C until required for experimentation.

Soil moisture content was assessed by oven drying soil at 105 °C, and soil organic matter as estimated by weight loss-on-ignition at 450 °C for 16 h. Soil pH and electrical conductivity were analysed in a 1:5 (w/v) soil: deionised water suspension using standard electrodes. To determine the levels of available N, fresh soil (5 g) was shaken (200 rev min^{-1}, 15 min) with 0.5 M K_2SO_4 (25 ml), the suspension centrifuged (10,000 *g*, 15 min) and the supernatant retained for analysis. The concentration of NH_4^+ in the extracts was determined colorimetrically on a Synergy MX micro-plate reader using the salicylic acid method of Mulvaney (1996), while NO_{3-} was determined colorimetrically using the vanadate procedure of Miranda et al. (2001). Extractable P was extracted using a 0.5 M acetic acid (1:5 w/v) shaken for 1 hour (200 rev min^{-1}; 20°C), then centrifuged for 10 min at 3220 *g* before passing through a Whatman 42 filter (Quevauviller 1998). P was then analysed by the colorimetric method of Murphy and Riley (1962). Free amino acids and hydrolysable protein was determined by the *o*-phthaldialdehyde fluorescence method of Jones et al. (2002) using a Cary Eclipse fluorimeter.

Microbial biomass C and N (MBC/N) were determined by the chloroform-fumigation extraction procedure of (Voroney et al. 2007). Briefly, the amount of dissolved organic C (DOC) and total dissolved N (TDN) was determined before and after $CHCl_3$ fumigation (48 h) with 0.5 M K_2SO_4 extracts (30 min, 200 rev min^{-1}) using a TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan;(Brookes

et al. 1985)). Extraction efficiency conversion factors (K_{eC} and K_{eN}) of 0.35 and 0.50 were used to calculate MBC and MBN, respectively (Voroney et al. 2007).

To determine the levels of available S, soil was shaken (200 rev min^{-1}, 15 min) with distilled water (1:5 w/v). All extracts were centrifuged (8000 *g*, 10 min), filtered by 0.45 µm syringe and frozen at -20°C prior to analysis. The concentration of sulphate and other major anions in the extract was determined by ion chromatography (IC; Dionex corporation, ICS 2100, USA; Zhao and McGrath 1994) according to ISO 10304-1:2009. Total dissolved S (TDS) and other major cations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Varian 710ES, Agilent Technologies, USA) according to ISO 11885:2016. Dissolved organic S (DOS) concentrations were calculated from the difference between TDS and sulphate (David and Mitchell 1985). Microbial biomass S (MBS) content was estimated from the differences between total dissolved S concentrations in the fumigated and unfumigated extracts, extraction efficiency conversion factors (K_{eS}) of 0.35 were adopted (Saggar et al. 1981; Wu et al. 1994).

Maize plants growth

Maize plants were grown in soil-filled mesocosms (rhizotubes) as described in Ström et al. (2002). The rhizotubes were constructed from nylon tube (200 mm long, 8 mm diameter) which expanded over a 0.5 cm span to a 50 mm long, 20 mm diameter section which was used to hold the seed. Holes (0.5 mm diameter) were pierced at 10 mm intervals down the length of the main rhizotube to ensure aeration. Before the addition of the pre-germinated plant, the mesocosms were filled with 15 ± 0.5 g of soil to a bulk density of 1.16 g cm^{-3} to reflect that in the field. After 7 days (d) of plant growth, all of the soil volume contains primary and secondary roots and was effectively classed as rhizosphere soil.

Competition for Cys and Met in the rhizosphere (using ^{14}C and ^{35}S)

Just after germination, uniform maize seedlings ($n = 54$) were transplanted into individual mesocosms (rhizotubes; Fig. S1). When the maize roots were ca. 20 cm long (7 d after transplantation; three leaf stage), a solution containing ^{14}C-or ^{35}S-labelled solution of Cys or Met (0.1 mM; 0.25 ml) was injected at four different points along the rhizotubes through the pre-drilled aeration holes. This enabled a uniform labelling of the soil along the root length. Individual mesocosms were then transferred to transparent gas-tight polypropylene containers (11×8 cm base and 27 cm high; Lock & Lock Ltd., Seoul, Republic of Korea) with removable caps. Each container cap had a hole drilled in the middle (ca. 1 cm diameter), to allow physical separation of the plant shoot and root compartments. The shoot and root compartments were isolated by a non-phytotoxic silicon paste (Kuzyakov and Siniakina 2001).

To determine the influence of nutrient availability on plant capture of free Cys and Met from soil solution, glucose (360 mg C kg^{-1} soil), NH_4Cl (30 mg N kg^{-1} soil) or Na_2SO_4 (30 mg S kg^{-1} soil) was added to the soil surface together with the ^{14}C or ^{35}S labelled amino acid solution individually. Glucose addition was equivalent to ca. 50 % of the native microbial biomass C, whereas inorganic N, P and S (+NPS treatments) were added as 100 mg N (NH_4NO_3), 30 mg P (KH_2PO_4), 30 mg S (K_2SO_4) per kg soil separately. NPS application rates were chosen to represent common UK field fertiliser application rates (Wray 2016). A control treatment was included where labelled Cys or Met was applied in distilled water alone.

In addition, we hypothesized that plant-microbial competition for Cys and Met would be diminished at high concentrations of free amino acid in the rhizosphere. To test this hypothesis, plant-microbial competition for Cys and Met in the rhizosphere was investigated using ^{14}C labelling at three amino acid concentrations (0.1 mM, 1 mM, 10 mM; PerkinElmer Inc, Waltham, MA). This range was chosen to reflect the free amino acid concentrations naturally present in rhizosphere soil solution or which arise from the lysis of a root or microbial cell (Dietz et al. 1990; Jones et al. 2005).

For ^{14}C treatments, 1 M NaOH traps (5 ml) were placed in the shoot and root compartment separately, to recover $^{14}CO_2$ from the root and shoot compartments. The NaOH traps were collected and analysed after 24 h. The efficiency of the NaOH traps was > 98% (as determined by collecting $^{14}CO_2$ generated from adding excess 0.1 M HCl to 0.001 M $NaH^{14}CO_3$). After 24 h, all the rhizotubes were destructively harvested by splitting the rhizo-tubes vertically with a razor blade, allowing the separation of the soil, shoot and root components. Rhizosphere soil (5 g) was shaken with 0.5 M K_2SO_4 (25 ml, 20 min, 200 rev min^{-1}), centrifuged (3800 *g*, 5 min). The amount of ^{14}C in the K_2SO_4 extracts and NaOH traps was then mixed with HiSafe 3® scintillation cocktail (PerkinElmer, Waltham, MA) and measured by a Wallac 1404 liquid scintillation counter (Wallac EG&G, UK) with automated quench correction. The roots were rinsed with 0.01 M $CaCl_2$ for 30 s before being further washed with distilled water to visually remove all adhering soil. Plant shoots and roots were oven-dried (105 °C for 30 min, 80 °C for 24 h) respectively, after which ^{14}C content was measured with an OX-400 Biological Sample Oxidizer.

For the ^{35}S parallel labelling experiments, rhizotubes were destructively harvested as described above. The amount of ^{35}S in plant tissues was determined by dissolving oven-dried plant tissues in Soluene-350 (40 °C, 4 h; PerkinElmer Inc.) followed by liquid scintillation counting as described

above. ^{35}S in soil was extracted with 0.01 M $CaCl_2$ (1:5 w/v) followed by liquid scintillation counting as described above.

Microbial biomass ^{14}C and ^{35}S were determined by chloroform-fumigation extraction procedure (Voroney et al. 2007). Briefly, soil was extracted with 0.5 M K_2SO_4 and 0.01 M $CaCl_2$ separately (1:5 w/v; 30 min; 200 rev min^{-1}), the amount of ^{14}C and ^{35}S in soil extracts before and after $CHCl_3$ fumigation (48 h) were then measured by liquid scintillation counting. Extraction efficiency conversion factors (Kec and Kes) of 0.35 and 0.35 were used to calculate $MB^{14}C$ and $MB^{35}S$, respectively (Wu et al. 1994; Voroney et al. 2007)

Statistics and data analysis

All statistical analyses were carried out in in IBM SPSS statistics v25.0 (IBM UK ltd., Portsmouth, UK). Graphs and curve fitting were produced using SigmaPlot 13.0 (Systat software Inc., London). All results are presented in figures and tables as mean ± SEM. $p < 0.05$ used as the upper limit for statistical significance.

Results

Competition for ^{14}C-Cys and Met (0.1 mM) as affected by nutrient amendment

A rapid utilization of Cys and Met was seen in the mesocosms containing both soil microbes and maize roots, with only <10 % of the amino acid-^{14}C recovered in the soil extracts after 24 h (Table 1). Plant roots were poor competitors for free Cys and Met in the rhizosphere (0.1 mM), <10% of amino acid-^{14}C was recovered from plant shoot, root and shoot respiration. In contrast, a much larger proportion of Cys and Met-^{14}C was recovered in the soil microbial biomass (37 ± 5.1 %, 33 ± 5.9 %, respectively), along with 47 ± 0.4, 37 ± 0.5% evolved via microbial respiration. The total uptake of ^{14}C-Cys and Met by

Table 1 Percentage partitioning of ^{14}C label in different compartments after the introduction of ^{14}C-Cys or Met (0.1 mM, 1 mM, and 10 mM) into the maize rhizosphere over a 24h incubation period. To explore the effects of readily available organic C, inorganic N or S on this rhizosphere competition, an excess of glucose, NH_4Cl or Na_2SO_4 was supplied when Cys and Met was added at 0.1 mM, $p < 0.05$ was used as the upper limit for statistical significance among these treatments. 0.1 mM was selected for further analysis as this is the closet concentration to those naturally present in soil solution. Values represent means ± SEM ($n = 3$). Average plant shoot dry weight is 86.5 mg, and average plant root dry weight is 56.9 mg.

(% of total ^{14}C added)		Cys					
		0.1 mM				1 mM	10 mM
		^{14}C-Cys	^{14}C-Cys + N	^{14}C-Cys + S	^{14}C-Cys + G	^{14}C-Cys	^{14}C-Cys
Plant	Shoot respiration	4.9 ± 0.3a	2.0 ± 0.4b	2.6 ± 0.7b	5.7 ± 0.7a	4.2 ± 0.5	2.2 ± 0.2
	Shoot	1.6 ± 0.1a	1.2 ± 0.02b	1.0 ± 0.1b	1.1 ± 0.2b	1.1 ± 0.3	0.7 ± 0.1
	Root	0.5 ± 0.2ab	0.4 ± 0.1b	0.4 ± 0.02b	0.6 ± 0.1a	0.3 ± 0.04	0.1 ± 0.02
	Plant capture	7.4 ± 0.6a	3.6 ± 0.5b	3.9 ± 0.8b	7.3 ± 0.8a	5.6 ± 0.4	3.1 ± 0.3
Soil	Microbial biomass	37 ± 5.1b	33 ± 1.8bc	49 ± 7.8a	27 ± 1.3c	31 ± 3.9	19 ± 2.4
	Microbial respiration	47 ± 0.4b	41 ± 1.1c	39 ± 0.7c	58 ± 2.1a	38 ± 3.1	20 ± 2.8
	Remained in solution	2.3 ± 0.9	5.5 ± 3.7	6.1 ± 2.9	2.2 ± 0.5	9. 5 ± 2.9	29 ± 3.5
	Microbial capture	84 ± 5.1c	74 ± 1.3b	88 ± 7.3ac	84 ± 2.9c	69 ± 3.7	40 ± 3.2
Total recovery (% of total ^{14}C added)		93 ± 4.7	83 ± 3.8	98 ± 10	94 ± 3.6	84 ± 6.1	72 ± 3.3
(% of total ^{14}C added)		Met					
		0.1 mM				1 mM	10 mM
		^{14}C-Met	^{14}C-Met + N	^{14}C-Met + S	^{14}C-Met + G	^{14}C-Met	^{14}C-Met
Plant	Shoot respiration	2.1 ± 0.3a	1.4 ± 0.1b	1.4 ± 0.2b	1.9 ± 0.1a	1.6 ± 0.1	0.4 ± 0.1
	Shoot	5.4 ± 0.3a	3.4 ± 0.5b	3.4 ± 0.4b	5.8 ± 1.5a	4.5 ± 0.6	1.4 ± 0.1
	Root	3.0 ± 0.4	2.7 ± 0.2	3.0 ± 0.5	2.5 ± 0.5	0.7 ± 0.1	0.5 ± 0.1
	Plant capture	10 ± 0.4a	7.5 ± 0.6b	7.8 ± 0.5b	10 ± 1.1a	6.8 ± 0.7	2.3 ± 0.2
Soil	Microbial biomass	33 ± 5.9	42 ± 9.0	32 ± 3.6	30 ± 8.4	32 ± 1.1	24 ± 3.9
	Microbial respiration	37 ± 0.5b	33 ± 0.4c	31 ± 0.6c	42 ± 2.1a	32 ± 4.8	15 ± 1.4
	Remained in solution	6.4 ± 0.8	6.9 ± 1.1	9.4 ± 1.9	9.3 ± 2.6	19 ± 2.9	38 ± 4.9
	Microbial capture	70 ± 6.3	75 ± 9.1	63 ± 4.2	71 ± 8.4	64 ± 6.1	40 ± 3.7
Total recovery (% of total ^{14}C added)		87 ± 7.0	89 ± 8.7	80 ± 3.9	91 ± 9.3	90 ± 2.8	80 ± 5.1

the microbial community was therefore estimated to be around 80% of total ^{14}C added. A caveat to this is that we could not account for root respiration, however, we predict this to only contribute a small amount to soil $^{14}CO_2$ evolution based on the sterile, plant-only ^{14}C partitioning results (Wang 2021).

In general, the addition of N or S did not significantly influence the accumulation of ^{14}C-Cys and Met into maize root, but significantly reduced translocation of ^{14}C to the plant shoot and thus the production of $^{14}CO_2$ by the shoot ($p < 0.05$). As a result, the addition of N and S led to a significant reduction of total capture of Cys and Met-^{14}C by maize plants ($p < 0.05$; Table 1). In contrast, the addition of glucose-C to the soil did not seem to have a significant influence on plant capture of either amino acid. In addition, addition of N and S caused a significant reduction in the amount of soil microbial respiration ($p < 0.05$), whilst it resulted in a significant increase in soil microbial respiration ($p < 0.05$).

Competition for ^{14}C-Cys and Met as affected by substrate concentrations (0.1, 1 and 10 mM)

Higher amino acid concentrations in the rhizosphere increased availability of the amino acids, therefore allowing greater plant and microbial capture (Table 1). Therefore, plant and microbial capture of ^{14}C-Cys and Met from rhizosphere increased with applied amino acid concentrations by the end of incubation (24 h) in absolute terms. When added at highest concentration (10 mM), plant capture rates of Cys and Met were 3.9 ± 0.4 and 2.9 ± 0.2 nmol amino acid-^{14}C (cm root)$^{-1}$, while microbial capture were 65 ± 5.3 and 66 ± 6.2 nmol amino acid-^{14}C (g soil DW)$^{-1}$, separately. However, increased amino acid concentration had unnoticeable effect on competitive success on either plant or rhizosphere microbes ($P>0.05$). At all three concentrations (0.1, 1 and 10 mM) adopted in our study, only a small proportion of amino acid-^{14}C was captured by maize (<10%), with around 10-fold captured by soil microbes.

Competition for ^{35}S-Cys and Met as affected by SO_4^{2-} amendment

The ^{35}S label results revealed that among all three S sources (added at 0.1 mM), SO_4^{2-} is the preferred S source for maize plants, with around 30 % of the added SO_4^{2-} incorporated into plant biomass within 24 h. This was higher than that of both S-containing amino acids (3 times higher than that of Cys, 5 times higher than that of Met). In contrast, amino acid-S was the preferred S source by soil microbes in comparison to SO_4^{2-}, with more than 40 % of the added ^{35}S derived from amino acids retained in soil microbial biomass, while merely 17 % of the $^{35}SO_4^{2-}$ was recovered in the soil microbial biomass. SO_4^{2-} addition imposed little effect on plant-microbial competition for Cys or Met (Table 2).

More ^{35}S from Cys, Met and SO_4^{2-} was translocated to plant shoot than that retained in root, indicating that all 3 S sources in our study are readily plant available S nutrients, after root uptake they were distributed through the whole plant level to optimize performance.

Discussion

Co-location of ^{14}C and ^{35}S labels appears reasonable evidence for intact uptake of Cys and Met by maize from the rhizosphere (Fig. S2), this greatly expands our understanding of terrestrial S cycling, as previously it was assumed that low molecular weight S compounds such as S containing amino acids must be extracellularly cleaved to inorganic forms before plant uptake. However, soil microbes out competed maize roots for both Cys and Met (Tables 1 and 2). We propose this poor performance of maize roots compared to soil microbes in amino acid competition is in part due to the complete coverage of the rhizotubes by soil microbes, higher microbial surface: soil volume ratio left them a particularly advantageous position to access added amino acids when they pass towards plant root. In addition, soil microbes are capable of moving towards hot-spots of substrate (Kuzyakov and Blagodatskaya 2015), while plant roots are more static. Furthermore, Cys and Met are excellent C, N and S sources

Table 2 Percentage partitioning of ^{35}S-Cys, Met or $Na_2{}^{35}SO_4$ (0.1 mM) between maize tissues and soil microorganisms in the rhizosphere over a 24 h incubation period. Values represent means ± SEM ($n = 3$). $p < 0.05$ was used as the upper limit for statistical significance.

(% of total ^{35}S added)		^{35}S-Cys	^{35}S-Cys + Na_2SO_4	^{35}S-Met	^{35}S-Met + Na_2SO_4	^{35}S-Na_2SO_4
Plant	Shoot	6.8 ± 0.9b	7.2 ± 1.1b	3.5 ± 0.8b	2.8 ±0.4b	29 ± 7.6a
	Root	2.8 ± 0.6b	2.6 ± 0.4b	2.4 ± 0.5b	1.7 ±0.2b	8.7 ± 1.2a
	Plant capture	9.6 ± 1.1b	9.8 ± 1.5b	5.9 ± 1.2b	4.6 ± 0.5b	31 ± 7.5a
Soil	Microbial biomass	52 ± 1.3ab	41 ± 15b	62 ± 2.5a	53 ± 7.3ab	17 ± 7.6c
	Remaining in solution	18 ± 0.5a	17 ± 1.4a	5.9 ± 1.0c	5.1 ± 0.6c	14 ± 1.0b
Total recovery (% of total ^{35}S added)		79 ± 0.2	68 ± 15	73 ± 3.9	62 ± 7.7	69 ± 6.7

for rhizosphere microbial community (Wang 2021), leaving soil microbes a favourable position to utilize these amino acids, while only part of added Cys and Met were available to maize roots.

Rhizosphere competition shifted from maize root dominant to microbial success when different forms of S were supplied (i.e., inorganic vs. organic). Our results showed that maize plants recovered more ^{35}S than soil microbes in the inorganic S treatment, while soil microbes recovered more ^{35}S than maize plants in the organic S (^{35}S-Cys and Met) treatments. We ascribe this to the fact that heterotrophic soil microbes demand C and N as well as S for their maintenance and growth, whereas autotrophic plants mainly require N and S from soil (Takahashi 2010; Gupta and Germida 2021). This is further supported by a significant reduction ($p<0.05$) in the rate of Cys and Met-^{14}C uptake by plants occurred when inorganic N and S was supplied to the rhizosphere. In addition, microbial utilization of amino acids may influence their availability to plant roots (Jones et al. 2005) and thus the degree of competition between plant roots and the soil microbial community (Sauheitl et al. 2009). However, our results revealed that increased amino acid concentrations in the rhizosphere did not favour maize or soil microbes in the competition success.

However, root performance in the rhizosphere could be different as a function of crop species (Ma et al. 2021) and root morphology (Wang et al. 2006), therefore it could improve in the case of species with higher S requirements, plant genotypes with root systems characterized by thinner and longer secondary roots. In addition, diurnal dynamics of plants could also affect the plant-microbial competition for nutrients in the rhizosphere via C allocation (Liu et al. 2021), since rhizosphere microbes are primarily limited by available C (Kuzyakov and Xu 2013), the competition for LMW organic S sources is expected to be reduced when plants provide labile C to rhizosphere microbes via rhizodeposition during the daytime compared to nighttime. Our study points out the clear need for studies of plant acquisition of low molecular weight S containing compounds (i.e., oligopeptides and S-containing secondary metabolites) under field conditions, where amino acid concentrations are lower.

Conclusions

Maize plants are capable of taking up intact Cys and Met from the rhizosphere, and soil microbes overwhelmingly outcompete maize plants for these organic S nutrients within short-term. Higher Cys and Met concentrations in the rhizosphere promoted their capture by both soil microbes and maize in absolute terms but had little effect on the competition success by rhizosphere microbes. Newly assimilated ^{14}C and ^{35}S derived from Cys and Met by plant roots were rapidly translocated to shoot. Translocation of ^{14}C derived from Cys and Met declined when external inorganic S was supplied, but in general Cys and Met uptake is independent of the inorganic N, S conditions in the rhizosphere.

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Data availability Data will be made available on request.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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