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

Wang, Deying; Wang, Jinyang; Chadwick, Dave; Ge, Tida; Jones, Davey L.

Biology and Fertility of Soils

DOI:
10.1007/s00374-023-01724-6

Published: 05/04/2023

Peer reviewed version

Uptake of intact cysteine and methionine as a sulphur source by young maize roots under hydroponic conditions

Deying Wang · Jinyang Wang · David R. Chadwick · Ryan M. Mushinski · Tida Ge · Davey L. Jones

D. Wang (✉) · D.R. Chadwick · D.L. Jones
School of Natural Sciences, Bangor University, Gwynedd, LL57 2UW, UK
e-mail: afpa89@bangor.ac.uk

J. Wang
Key Laboratory of Green and Low-carbon Agriculture in Southeastern China, Ministry of Agriculture and Rural Affairs, Jiangsu Key Laboratory of Low Carbon Agriculture and GHGs Mitigation, College of Resources and Environmental Sciences, Nanjing Agricultural University, 210095 Nanjing, China

R.M. Mushinski
School of Life Sciences, The University of Warwick, Coventry, CV4 7AL, UK

T. Ge
State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Key Laboratory of Biotechnology in Plant Protection of Ministry of Agriculture and Zhejiang Province, Institute of Plant Virology, Ningbo University, Ningbo 315211, China

D.L. Jones
SoilsWest, Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch University, 90 South Street, Murdoch WA 6150, Australia

Abstract
Purpose: A critical question is whether plants can acquire sulphur (S)-containing amino acids (i.e., cysteine (Cys) and methionine (Met)) without prior mineralisation via microorganisms to sulphate, and if so, how does this compare with direct sulphate uptake?
Methods: To address this, we measured the influx of three S compounds (Cys, Met and sulphate) by maize (*Zea mays* L.) in sterile hydroponic culture. Plants were then labelled with either ^{14}C or ^{35}S at ecologically relevant concentrations (100 µM) over a short period (24 h). Uptake of intact Cys and Met was estimated by the ratio of ^{14}C to ^{35}S incorporation into plant tissues. Efflux of ^{35}S-compounds was also estimated by monitoring the increase of ^{35}S in the root bathing solution after pre-feeding maize roots with each S compound. In addition, a split root system was used to explore S incorporation and translocation within the host maize plant. All experiments in this study were conducted on 10 days old maize plants (cultured from seeds to three leaf stage) in 10% strength S free Long Ashton solution.
Results: Sulphate was the preferred S source by maize, with a two-fold greater S accumulation compared to that from Cys or Met. In addition, 62 % of Cys and 59 % of Met was taken up intact by maize roots, even when sulphate was available. A large proportion of the S taken up was rapidly translocated to the shoot preventing loss in root exudation. This indicates that Cys and Met could theoretically constitute a significant proportion of a maize plant's S supply, particularly under S-limiting conditions. The efflux of ^{35}S from Cys, Met and sulphate were in the same form they were taken up, indicating that efflux occurred via passive leakage.
Conclusion: We present direct evidence for the rapid intact influx and efflux of dissolve organic sulphur (DOS) by maize plants. Our results indicated that maize plants are very effective in cycling S compounds at the whole-plant level.

Keywords Cysteine · Dissolved organic sulphur · Methionine · Influx · Efflux · Radiotracer

Abbreviations
N- nitrogen; S- sulphur; Cys- cysteine; Met- methionine; DOS- dissolved organic sulphur; C- carbon; s-second(s); min- minutes(s); h- hour(s); d- day(s); IRMS- isotope ratio mass spectrometry

Acknowledgements
This work was supported by the China Scholarship Council (Grant number: 201606510012).

Introduction

Sulphur (S) is an essential element for plants, insufficient S supply could affect crop yield and quality, caused by S requirement for S containing amino acids, protein and enzyme synthesis (Koprivova and Kopriva 2016). Soil S occurs in both organic and inorganic forms: sulphate is generally much less abundant (Bohn et al. 2015), and the most common form of inorganic S and can be divided into sulphate in soil solution, adsorbed sulphate and mineral sulphur; while up to 98% of total soil S may be present as organic compounds, associated with a heterogeneous mixture of plant residues, animals and soil microorganisms (Freney 1986). It is generally believed that S is predominantly taken up by plant roots in an inorganic form (i.e., sulphate;(Prasad and Shivay 2018). In actively growing plants, sulphate is then transported in the xylem via a selective distribution/redistribution system to the expanding leaves (Anderson and Fitzgerald 2003), where assimilation into organic S takes place in the light (Takahashi 2010; Takahashi et al. 2011). It should be noted that in the case of N, many plants are opportunistic, and capable of taking up a range of organic N forms, such as simple forms:amino acids and oligopeptides (Weigelt et al. 2005; Gallet-Budynek et al. 2009; Ge et al. 2009; Czaban et al. 2016; Song et al. 2016; Zhu et al. 2019), depending on the prevailing conditions in the soil (Moreau et al. 2019). However, only a few studies have focused on S-containing amino acids (Ma et al. 2021; Wang 2021).

Cysteine (Cys) and methionine (Met) represent an important proportion (8-15%) of soil organic S (Scott et al. 1981; Zenda et al. 2021). Cys is the first reduced S product resulting from the sulphate assimilation pathway (Li et al. 2020), while both Cys and Met play important roles in the growth and development of plant cells (Wirtz and Droux 2005; Kopriva et al. 2019; Narayan et al. 2022). Evidence has been presented that Cys can be actively transported into cultured tobacco cells (Harrington and Smith 1977), where it can be rapidly metabolized, with the final products being pyruvate, ammonium and S-sulfocysteine (Tishel and Mazelis 1966). The transport of Met into excised plant roots has also been studied, with observations suggesting that the same membrane transport system can take up both Cys and Met as other free amino acids (Wright 1962). Therefore, direct evidence is required to determine if S-containing amino acids could be taken up intact by plant roots and their quantitative contribution to plant S demand compared to sulphate (Fig. S1).

In previous studies, plant nutrient uptake is often studied in simplified systems, such as excised roots in hydroponic culture (Jia et al. 2020; Bai et al. 2022). This technique has been used extensively and has provided valuable information about uptake rates of specific nutrients at the molecular and cellular level. Some researchers, however, have argued that excised roots may artificially increase the loss of nutrients from roots and thereby inhibit net uptake (Lucash et al. 2007), resulting in an unrealistic estimation of root uptake. In addition, excising roots could alter the source-sink relationships within the plant, which may give feedback on root membrane transport systems and repress uptake if the above-ground sink is removed. It is therefore essential to study nutrient uptake in intact plants.

Root systems of plants not only import water and nutrients from the soil solution but also release low and high-molecular-weight compounds back to the environment (More et al. 2020). The translocation of organic compounds from leaves, and the release of root exudates such as sugars, amino acids and organic acids by roots, are particularly important when plants are growing in nutrient-deficient soils or when plant species have a very low capacity for reducing nutrients in their roots (Atkins and Smith 2007; Carvalhais et al. 2011). Amino acids are generally considered the second most abundant class in terms of the total amount exuded by plant root systems, after sugars (Iannucci et al. 2021). Depending on the cause and mechanisms, amino acid release from roots may include active transport (Badri et al. 2009; Lesuffleur and Cliquet 2010) and passive diffusion (Rroço et al. 2002; Vives-Peris et al. 2020a). Passive diffusion of amino acids is driven primarily by the large concentration gradient between the cytoplasm of root cells (e.g., 1 – 10 mM) and the outside soil solution (0.1 – 10 µM;(Jones and Darrah 1993; Moore et al. 2003; Phillips et al. 2004) while active transport of amino acids is mediated by proteins located in the root plasma membrane and can release amino acids against the electrochemical potential gradient into the soil solution (Okumoto et al. 2004; Vives-Peris et al. 2020b).

We conducted four hydroponic experiments using maize as a model plant. Dual labelled (^{14}C, ^{35}S) Cys and Met was supplied to young maize under hydroponic conditions, since it has been proved that some plants can utilize amino acids as sources of N for growth and development (Moran-Zuloaga et al. 2015), we hypothesized that (1) S containing amino acids (Cys and Met), as sources of C, N and S, could also be taken up intact by maize even at field-relevant amino acid concentrations (100 µM). In addition, it is known that amino acid uptake by plant roots involves selective proton-coupled amino acid transporter, and these transporters act on the specificity of substrates (Yao et al. 2020), but relatively little is known about how S containing amino acids might be transported, we hypothesized that (2) Cys and Met would not affect the uptake of each other, due to being taken up by separate transporters. Moreover, a few physiological studies indicated that amino acid efflux from roots was via passive diffusion, while other researchers

argue that amino acid efflux may rely on dedicated root transporters, we hypothesized that (3) Cys and Met efflux from maize roots in hydroponic conditions is a passive process, due to them being low molecule weight, relatively hydrophobic.

Materials and methods

Plant material and nutrient solution

Maize (*Zea mays* L.) seeds were sterilized in 2 % sodium hypochlorite (1 min) and rinsed twice with sterile distilled water (Cuero et al. 1986; Sauer and Burroughs 1986). The seeds were soaked for 24 h in sterile deionized water and allowed to germinate on moist filter paper at room temperature (ca. 20 °C) under sterile conditions. After 48 h, each seedling was transferred into individual microcosms. Each microcosm consisted of 25 ml polypropylene containers filled with 20 ml of full-strength S-free Long Ashton nutrient solution (Hewitt 1952; Smith et al. 1983). The composition of the full-strength nutrient solution used in this study was as follows (g 10 L^{-1}) $MgCl_2 \cdot 6H_2O$, 3.05; KCl, 1.49; $CaCl_2 \cdot 2H_2O$, 5.88; $NaH_2PO_4.2H_2O$, 2.92; $Na_2HPO_4.12H_2O$, 0.47; H_3BO_3, 0.86; $MnCl_2.H_2O$, 0.30; $ZnCl_2$, 0.03; $CuCl_2.2H_2O$, 0.06; $Na_2MoO_4.2H_2O$, 0.005; FeEDTA, 0.33; MES buffer, 0.19; $NaNO_3$, 3.40; NH_4Cl, 2.14. This study replaced all sulphate nutrient salts with chloride salts, so plants were expected to be S deficient once the nutrient reserve from the seeds was exhausted. After adding an individual seedling, the microcosms were placed in a climate-controlled cabinet with a 16 h photoperiod maintained at 25 ± 0.5 °C. 7 d after transplanting, plants were transferred to 10 %-strength S-free Long Ashton solution for a further 3 d. All experiments were conducted on 10 days old maize plants in 10 % strength S-free hydroponic solution, with three fully expanded leaves on the main shoot.

At the three-leaf stage, there are three elongated leaves, and the tip of the fourth leaf appears at the centre of the leaf whorl (Fig. S2). We chose this growth stage for the following experiments because this stage is a pivot point of maize growth from heterotrophic growth (i.e., growth relying on seed reserves) to autotrophic growth (i.e., growth relying on photosynthesis after the exhaustion of seed reserves) (Cooper and MacDonald 1970)(Hanway 1966). All three compounds were chosen to reflect possible organic (Cys, Met) and inorganic (Na_2SO_4) S compounds typically released and exposed to plant roots during the breakdown of soil organic matter.

To ensure and maintain sterile conditions, all nutrient stock solutions, deionized water and containers (polypropylene vials, syringes, pipette tips, etc.) used in this experiment were autoclaved (121 °C, 30 min) before experimentation. In addition, roots were rinsed with sterile 10 %-strength Long Ashton nutrient solution (to remove any exoenzymes and exudates) before being exposed to $^{35}S/^{14}C$-labelled substrates (L-[1-^{14}C]-Cys, L-[^{35}S]-Cys, L-[1-^{14}C]-Met, L-[^{35}S]-Met, [^{35}S]-SO_4^{2-}; 0.3 kBq ml^{-1}; American Radiolabeled Chemicals, St. Louis, MO, USA). Experiments were carried out during daylight hours with plants exposed to the same light intensity and temperatures described above. Potential influence of light on nutrient solution was excluded by tightly wrapping the root compartment with aluminium foil.

Experiment 1: Plant uptake and partitioning of ^{14}C-Cys and Met

Briefly, 18 uniform maize plants (10 days old) were rinsed with sterile 10 %-strength Long Ashton nutrient solution before being placed into individual 25 ml polypropylene containers filled with 20 ml of 10 %-strength S-free Long Ashton nutrient solution. Experiment 1 included the following six treatments; three replicates of each treatment were performed:

(a) ^{14}C-Cys
(b) ^{14}C-Cys + Met
(c) ^{14}C-Cys + Na_2SO_4
(d) ^{14}C-Met
(e) ^{14}C- Met + Cys
(f) ^{14}C- Met + Na_2SO_4

The concentration of each S compound in the final nutrient solution was 100 µM (i.e., in treatment b, the concentration of Cys and Met were 100 µM separately), which was in the range of previously reported amino acid concentrations in soil solution (Jones and Darrah 1994; Johnson and Pregitzer 2007). After injection of labelled material(s) into the nutrient solution (0.3 kBq ml^{-1}), the plant-solution system was immediately transferred into a 2 L translucent sealable plastic container (Lock & Lock; Really Useful Products Ltd, West Yorkshire, UK; Fig. S3).

After 24 h, ^{14}C activity in each compartment was determined separately. Plant roots and shoots were destructively harvested. $^{14}CO_2$ evolution from plant tissues was trapped by placing a NaOH solution vial (1 M, 10 ml) inside the

container. This harvest period was chosen to ensure that sterility was maintained as well as ensuring that all the S in the external medium was not depleted during the incubation period (Barber and Gunn 1974; Gaume et al. 2001).

Plant materials were first rinsed with 0.01 M $CaCl_2$ for 30 s to remove any isotope adhering to the plant surface. Plant shoots and roots were then oven dried (80 °C, 24 h), weighed and ground to powder separately. ^{14}C activity in the plant tissues (shoots and roots) was determined with an OX-400 Biological Sample Oxidizer (RJ Harvey Instrument Corp., Hillsdale, NJ). The liberated $^{14}CO_2$ from the oxidizer was captured in Oxosol scintillation fluid (National Diagnostics, Hessle, UK) and then quantified by liquid scintillation counting using a Wallac 1404 scintillation counter with automated quench correction (Wallac EG&G, Milton Keynes, UK). The amount of ^{14}C-amino acid remaining in the nutrient solution alongside the amount of plant respiration ($^{14}CO_2$ trapped in NaOH solution) was also determined by liquid scintillation counting, as described above.

Experiment 2: Plant uptake and partitioning of ^{35}S-Cys, Met and SO_4^{2-}

27 uniform maize plants were selected for experiment 2. Maize germination, transplanting and nutrient provision were the same as described in Experiment 1, except that ^{14}C was replaced with a ^{35}S tracer, and no NaOH traps were required (Fig. S3). The concentration of each ^{35}S-labelled compound in the final nutrient solution was 100 µM and the chase period was 24 h, at which time the incorporation of ^{35}S into plant tissues was determined. Experiment 2 included nine treatments as follows, and three replicates of each treatment were performed:

(a) ^{35}S-Cys
(b) ^{35}S-Cys + Met
(c) ^{35}S-Cys + Na_2SO_4
(d) ^{35}S-Met
(e) ^{35}S-Met + Cys
(f) ^{35}S-Met + Na_2SO_4
(g) ^{35}S-$Na_2{}^{35}SO_4$
(h) ^{35}S-$Na_2{}^{35}SO_4$ + Cys
(i) ^{35}S-$Na_2{}^{35}SO_4$ + Met

Similar to experiment 1, the concentration of each S compound in the final nutrient solution was 100 µM (i.e., in treatment b, the concentration of Cys and Met were 100 µM separatelyAfter injection of labelled material(s) into the nutrient solution (0.3 kBq ml-1), the plant-solution system was immediately transferred into a 2 L translucent sealable plastic container (Lock & Lock; Really Useful Products Ltd, West Yorkshire, UK; Fig. S3). In treatments a, b, c, d, e and f, to determine the liberation of inorganic ^{35}S from Cys or Met (e.g., from exudation or exoenzyme activity) in the nutrient solution during the course of the experiment, the nutrient solution at the end of the experiment was divided equally into two parts. Half of the nutrient solution was directly used for ^{35}S quantification (i.e., sulphate mineralized from Cys or Met, plus organic ^{35}S) by liquid scintillation counting. The remaining half was shaken (200 rev min^{-1}; 5 min) with the same volume of 0.1 M $BaCl_2$ (10 ml) and centrifuged (4000 rev min^{-1}; 5 min) to remove any sulphate by precipitation (i.e., as $Ba^{35}SO_4$) from the nutrient solutions, leaving the organic-S (^{35}S-Met and ^{35}S-Cys) in solution. As described above, the amount of ^{35}S in the resultant solutions was determined by liquid scintillation counting.

The amount of ^{35}S incorporated into the plant material was also determined. Plant materials were first rinsed with 0.01 M $CaCl_2$ for 30 s to remove any isotope adhering to the plant surface. Plant material was then divided into roots and shoots, weighed and dried (80 °C, 24 h), and ground to powder prior to further measurements. To determine the total amount of ^{35}S incorporated into each plant tissue, 40 mg of the powdered sample was placed in glass vials, and 1 ml of Soluene-350 (PerkinElmer Life Sciences, Inc) added. The vials were then capped and incubated (40°C, 4 h) until the samples were fully digested and almost colourless. In our case, this eliminated the presence of pigments and chlorophyll, which may cause quenching and inaccurate readings on the scintillation counter (Gibson 1980; Smith and Lang 1987; Thomson and Temple 2020). The amount of ^{35}S was then determined by liquid scintillation counting, as described above.

Experiment 3: Efflux of ^{35}S-labelled Cys, Met and $^{35}SO_4^{2-}$ from maize roots

9 uniform sterile maize plants were removed from the 10 %-strength Long Ashton nutrient solution and transferred into the open barrel of individual 25 ml polypropylene syringes with a two-way stopcock connected at the bottom (Fig. S4). Each syringe was filled with 20 ml of one isotopically labelled S compound (i.e., ^{35}S-Na_2SO_4, ^{35}S-Cys or ^{35}S-Met; 100 µM) in 10 %-strength Long Ashton S-free nutrient solution. This simple axenic system facilitated the collection of root-derived ^{35}S efflux and minimized root damage and overestimation of efflux (Ayers and Thornton 1968).

After being transferred to the new nutrient solution, maize plants were supplied with each ^{35}S compound for 1 h in the external root bathing medium. After 1 h, the labelled nutrient solution was removed by opening the valve at the bottom of each syringe, and the plants were rinsed with 0.01 M $CaCl_2$ for 30 s to remove any isotope adhering to the roots. The syringe was then refilled with 20 ml of non-^{35}S-labelled 10 %-strength Long Ashton nutrient solution (bathing solution). This root bathing solution was collected and replaced every 10 min over an 80 min period. The amount of ^{35}S label present in the collected solutions in either an organic or inorganic form was determined using the 0.1 M $BaCl_2$ precipitation procedure described above. This enabled the efflux of both sulphate and organic S from maize roots to be determined. ^{35}S efflux from roots (S_{efflux}) was expressed as nmol (g root DW)$^{-1}$ determined by the increase of ^{35}S in the bathing solution between the start (T_0) and the end (T_t) of the sampling period, where R is dry root biomass, and T denotes sampling time.

$$S_{efflux} = (T_t - T_O) \div (R \times T) \quad \text{(Eqn. 1)}$$

Many studies have investigated the efflux of low molecular weight (MW) organic solutes and ions (e.g., K^+, Cl^-, sugars, etc.) after pre-loading plant roots. This efflux process typically involves three distinct root compartments: the apoplast, cytoplasm and vacuole (Thoiron et al. 1981; Saftner et al. 1983). In this study, we discounted the fast exchanging (<1 min) apoplast compartment, due to failure to collect bathing solution in the first 5 min. Here we fitted a mathematical model to the experimental efflux data (Rauser 1987), in which the leakage of S compounds to the outer bathing solution was considered as the sum of two diffusional processes from the cytoplasm and vacuole to the root bathing medium:

$$y = a \times (1 - e^{-c \times t}) + b \times (1 - e^{-d \times t}) \quad \text{(Eqn. 2)}$$

Where y is the accumulated ^{35}S washed out of plant roots into the bathing solution, a and b are the sizes of the S storage pool in the cytoplasm and vacuole, respectively, and c and d are the exponential coefficients describing the rate of ^{35}S release from these pools into the external root bathing solution. The half-life ($t_{½}$) of each pool can then be calculated as follows:

$$t_{1/2} = \ln(2)/c$$
$$t_{1/2} = \ln(2)/d \quad \text{(Eqn. 3)}$$

The initial volume of different S compounds in the two compartments, A and B, can be calculated by:

$$A = b - d/c \times b$$
$$B = a + d/c \times b \quad \text{(Eqn. 4)}$$

Experiment 4: A split root system to explore the cycling of amino acid S and $^{35}SO_4^{2-}$ between shoots and roots in young maize plants

In experiment 4, 15 uniform maize plants were selected, and the roots of each maize plant were split approximately equally between two separate containers of nutrient solution (Fig. 1). At the start of the experiment, one of the root compartments was exposed to a radioisotope solution (i.e., either ^{35}S-Cys, ^{14}C-Cys, ^{35}S-Met, ^{14}C-Met or ^{35}S-Na_2SO_4; 100 µM). Roots in this compartment were termed 'donor' roots, while the other root compartment was immersed into the unlabelled 10 %-strength Long Ashton nutrient solution, and roots in this compartment were termed 'receiver' roots. The 'donor root' compartment was used for the labelling, and the 'receiver root' compartment was used for determining the internal S cycling and subsequent release of radioisotope into the nutrient solution.

Each experimental unit was placed inside a 2 L translucent sealable plastic container (Lock & Lock; Really Useful Products Ltd, West Yorkshire, UK; Fig. 1). After 24 h, the shoots were removed, and the roots harvested and rinsed with 0.01 M $CaCl_2$ for 30 s to remove any surface isotope contamination. The nutrient solution from both compartments was collected to determine isotope depletion by the donor root and isotope exudation from the receiver root. As described above, ^{35}S and ^{14}C in the plants and solutions was determined by liquid scintillation counting.

For ^{14}C treatments, 1 M NaOH trap (10 ml) was placed inside the 2 L plastic container beside the maize plant to catch any $^{14}CO_2$ evolved. For the ^{35}S treatments, no NaOH traps were used.

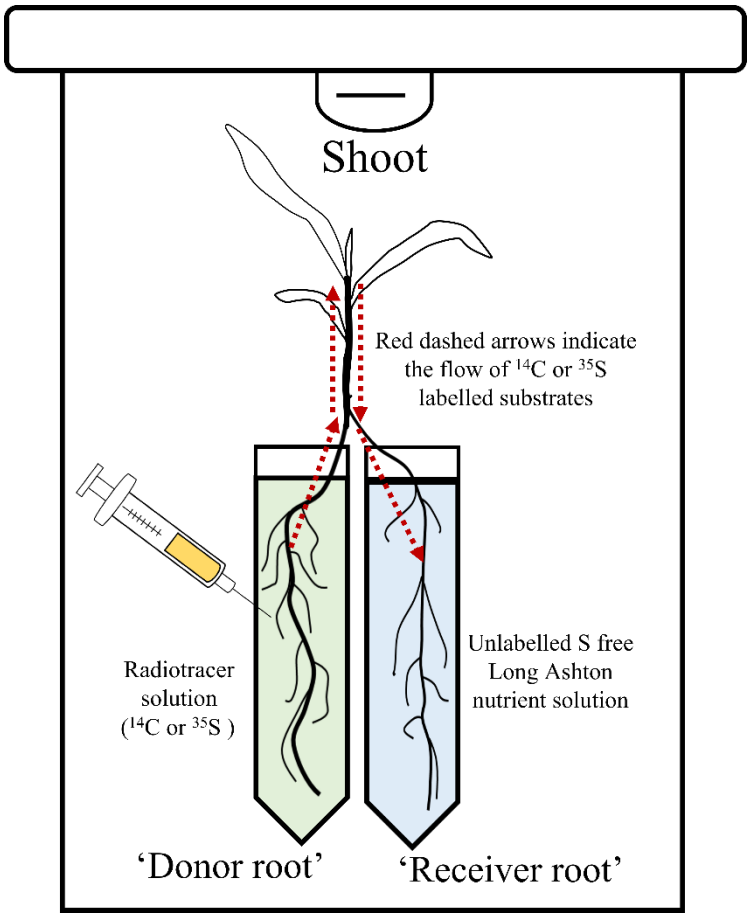

Fig. 1. Schematic representation of the experimental apparatus showing the maize plant growing in a split root system with only 'donor root' exposed to radioisotope. This system was allowed to develop over a 24 h-period. For the ^{14}C treatment, NaOH traps were placed inside Lock & Lock plastic containers to capture any $^{14}CO_2$ respiration from maize.

Statistics and data analysis

All experiments were carried out in triplicate. Plants with similar shoot heights (ca. 12 cm) and root lengths (ca. 9 cm) were selected for our experiments. When calculating root S influx, it was assumed that the efflux of the S compounds was minimal during the exposure period. Similarly, it was assumed that S uptake was minimal during the exposure period for the calculation of S efflux. The relative contribution of amino acid-S taken up in an intact form into maize plant (in percent) was calculated using the $^{14}C/^{35}S$ excess ratio in plant samples relative to the $^{14}C/^{35}S$ ratio of applied Cys and Met tracer separately (the $^{14}C/^{35}S$ ratios of applied Cys and Met are 1, because only one carbon atom of Cys and Met is labelled with ^{14}C, and only one sulphur atom is labelled with ^{35}S). Amino acid efflux was expressed in nmol (g root DW)$^{-1}$ h^{-1} on a dry root biomass weight basis, as the increase of amino acid-^{35}S increase in the bathing solution between the start and the end of experiment (80 min). All data analysis was carried out in IBM SPSS Statistics v25 (IBM UK Ltd., Portsmouth, UK). Graphs and curve fitting were produced using SigmaPlot v13.0 (Systat Software Inc., London). The results are presented as means ± SEM (n = 3), and significant differences are discussed at the $p < 0.05$ level.

Results

Plant uptake and partitioning of ^{14}C-labelled Cys and Met

In experiment 1, the results indicated that maize roots rapidly took up both ^{14}C-Cys and Met, after which the amino acid-C was incorporated into both new cell biomass and utilized for respiration. A similar amino acid incorporation

rate was recorded for both amino acids: 203.4 ± 35.7 nmol ^{14}C (g root DW)$^{-1}$ h^{-1} for Cys and 191.9 ± 30.7 nmol ^{14}C (g root DW)$^{-1}$ h^{-1} for Met. However, the partitioning of ^{14}C among the different plant compartments varied for the two amino acids. Overall, a higher proportion of ^{14}C-Cys was partitioned into plant respiration, while a higher proportion of ^{14}C-Met was partitioned into plant biomass ($p < 0.05$). Based on our calculation, 5.8 ± 0.7 % and 3.7 ± 0.2 % of the added Cys and Met-^{14}C were respired by the maize plants, respectively, whereas 6.7 ± 0.3 % and 11 ± 0.5 % were incorporated into plant biomass (shoot plus root tissues), respectively.

Overall, ^{14}C recovered in plant shoots, roots, solution and CO_2 evolution from the root-solution system exceeded 80 % for all treatments. The highest amount of ^{14}C recovered in the ^{14}C-Cys among all three compartments (shoots, roots and respiration) was for $^{14}CO_2$ evolution, constituting 5.8 ± 0.7 % of ^{14}C-Cys input. Only a small fraction of the ^{14}C derived from Cys was retained in plant roots after uptake (2.6 ± 0.1 %), while a larger proportion (4.1 ± 0.2 %) was transported to the shoots. Total plant utilization of ^{14}C derived from Met (15 ± 0.4 %) was similar to that from Cys (13 ± 0.8 %); however, the highest ^{14}C partitioning for Met was found in the shoots (6.3 ± 0.3 %), followed by the roots (4.5 ± 0.5 %) and respiration (3.7 ± 0.2 %).

Plant uptake of Cys and Met decreased in the presence of each other. Cys supply led to a decrease in Met partitioning into respiration, shoot tissue and root tissue by 29 ± 10 %, 49 ± 13 %, and 58 ± 11 % respectively, while Met supply led to a decrease in Cys incorporation into respiration, shoot tissue and root tissue by 48 ± 12 %, 42 ± 10 %, and 55 ± 6.7 %, respectively. In contrast, plant uptake of Cys and Met was not markedly affected by the presence of sulphate ($p < 0.05$, Fig. 2). This implies that Cys and Met may be a more favourable source of S even under situations where plants could access sulphate.

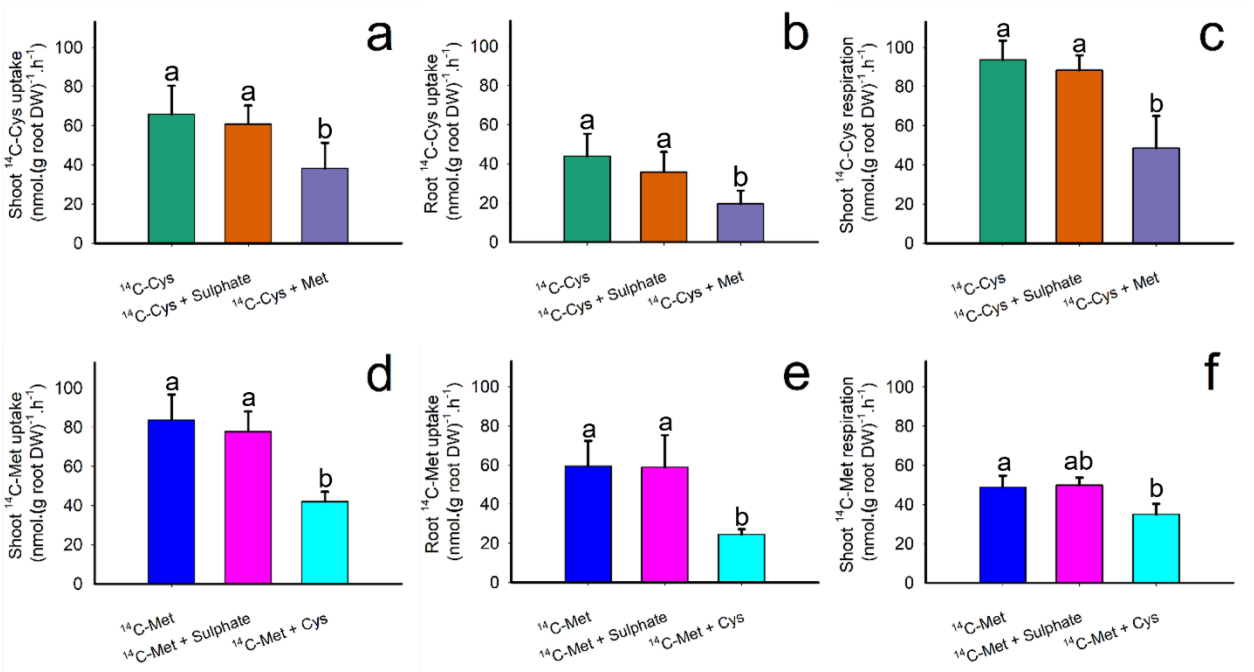

Fig. 2. Partitioning of ^{14}C label after the introduction of ^{14}C-Cys or Met to maize plants under sterile hydroponic conditions for 24 h a) ^{14}C-Cys recovered in the shoots; b) ^{14}C-Cys recovered in the roots; c) ^{14}C-Cys respiration from the maize plant; d) ^{14}C-Met recovered in the shoots; e) ^{14}C- Met recovered in the roots; f) ^{14}C-Met respiration from the maize plant. Bars and lines represent mean ± SEM ($n = 3$). Different lowercase letters note significant differences ($p < 0.05$ was used as the upper limit for statistical significance).

Uptake and partitioning of ^{35}S-labelled Cys, Met and SO_4^{2-}

The uptake of all three S forms was similar in that the labelled S taken up was not retained in root tissues but was rapidly transported to the shoots. However, there were striking differences in the ability of the maize plants to utilize these three different S compounds.

Overall, 10 ± 1.4 %, 10 ± 0.8 %, and 12 ± 1.1 % of the added ^{35}S were recovered in the plant roots from Cys, Met and sulphate, respectively (Fig. 3). However, in terms of transportation to shoot tissue, sulphate was more mobile than

both amino acids as only 12 ± 0.9 % and 13 ± 1.9 % of Cys and Met was detected in shoot tissues, whereas a significantly greater proportion (34 ± 2.2 %) of the added sulphate ($p < 0.05$) was found in the shoots.

Cys and Met uptake rate by the whole plant was 347 ± 15 and 390 ± 54 nmol ^{35}S (g root DW)$^{-1}$ h^{-1}, respectively, over this 24 h sampling period. Cys and Met resulted in significantly decreased levels of both root uptake and shoot transportation in the presence of each other ($p < 0.05$; Fig. 3). Cys supply effectively decreased root uptake and shoot transportation of Met by 35 % and 30 %, respectively ($p < 0.05$), while Met supply effectively decreased root uptake and transportation of Cys by 42 % and 30 %, respectively ($p < 0.05$).

The retention of both amino acids in the roots was unaffected by the presence of sulphate ($p > 0.05$), but transportation of ^{35}S-Cys and ^{35}S-Met to the shoot was markedly decreased in the presence of sulphate by 30 % and 21 % ($p < 0.05$), respectively (Fig. 3). This resulted in significant inhibition of total Cys uptake when sulphate was present ($p < 0.05$), although it proved non-significant for Met. In contrast, root ^{35}S-sulphate uptake and transportation to shoots were markedly decreased by 33 % and 47 % in the presence of Cys, and by 23 % and 34 % in the presence of Met, respectively, indicating that there was a downregulation of inorganic S uptake by organic S compounds.

As described above, 0.1 M $BaCl_2$ was introduced to remove any sulphate (i.e., as $Ba^{35}SO_4$), enabling us to separate organic and inorganic S forms in the solutions. According to our results, after 24 h, > 80 % of the ^{35}S in the nutrient solution remained in the forms they were injected initially, indicating that the majority of ^{35}S-Cys, Met and $^{35}SO_4^{2-}$ had not been mineralized or degraded by the time we sampled.

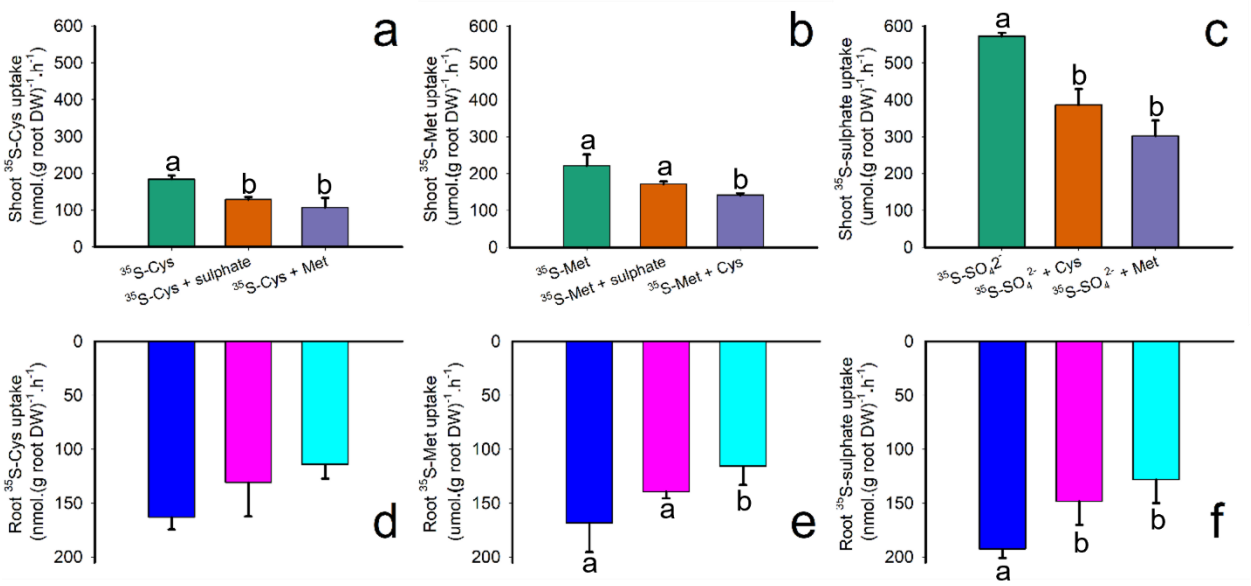

Fig. 3. Partitioning of ^{35}S label after the introduction of ^{35}S-Cys, Met or SO_4^{2-} to maize plants under sterile hydroponic conditions for 24 h a) ^{35}S-Cys recovered in the shoots; b) ^{35}S-Met recovered in the shoots; c) ^{35}S-SO_4^{2-} recovered in the shoots; d) ^{35}S-Cys recovered in the roots; e) ^{35}S-Met recovered in the roots; f) ^{35}S-SO_4^{2-} recovered in the roots. Values represent means ± SEM ($n = 3$). Different lowercase letters note significant differences ($p < 0.05$ was used as the upper limit for statistical significance).

Uptake of intact Cys and Met

To determine whether Cys and Met were taken up as intact molecules or as inorganic compounds after enzymatic or microbial degradation, results of the uptake techniques (i.e., via ^{14}C and ^{35}S labelling) were combined (Fig. 4). The co-location of ^{14}C and ^{35}S appears a reasonable measure of uptake of intact Cys and Met by plants roots under sterile hydroponic conditions. The ratio of $^{14}C/^{35}S$ incorporation into plant shoot and root indicated that at least 62 % of Cys and 59 % of Met were taken up intact. This is based on our assumption that: intact amino acid uptake is implied if the slope of the correlation of ^{14}C to ^{35}S excess in the plant tissue is the same as in the parent amino acid compounds fed to the plants. ^{14}C to ^{35}S ratio of parent Cys and Met is 1:1 in our study, due to only one carbon atom of Cys and Met was labelled with ^{14}C.

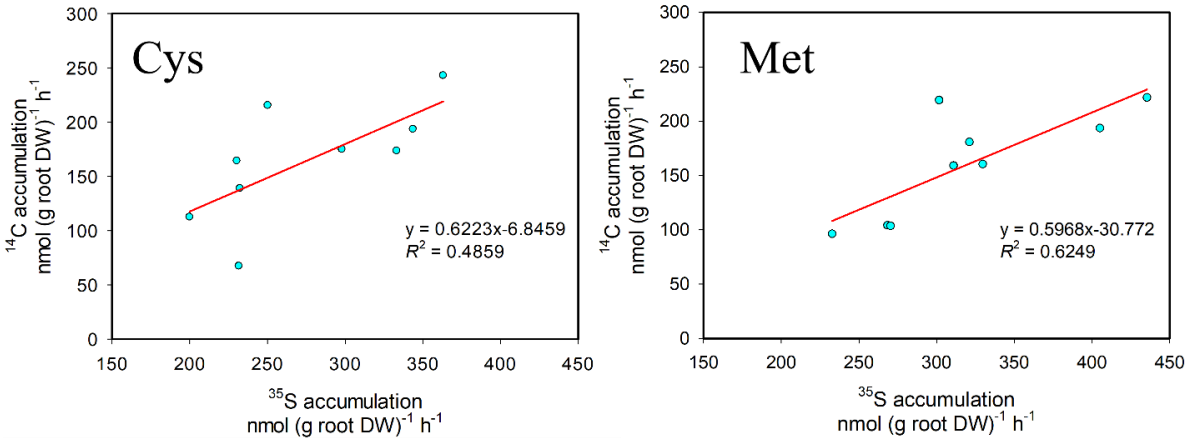

Fig.4. The relationship between the total accumulation of ^{14}C and ^{35}S radiotracer (nmol. (g root DW)$^{-1}$. h^{-1}) by maize roots after 24 h of exposure to the labelled solution containing 100 µM Cys or Met. *n* = 9. Lines indicate a linear regression for 1-^{14}C, ^{35}S Cys and Met: ^{14}C excess = 0.622 ^{35}S excess for Cys; ^{14}C excess = 0.598 ^{35}S excess for Met.

Efflux of ^{35}S labelled-Cys, Met and SO_4^{2-} from maize roots.

The results showed that of the ^{35}S taken up by the plant, 37, 28, and 28 % Cys, Met and sulphate-S was recovered in the root bathing medium within 80 min, respectively. The results showed that Cys and Met effluxes were in a similar range, between 1.0 ± 0.1 and 4.2 ± 0.3 µmol (g root DW)$^{-1}$ h^{-1} for Cys, and 0.7 ± 0.2 and 3.2 ± 0.1 µmol (g root DW)$^{-1}$ h^{-1} for Met (Fig. 5), indicating rapid efflux of low molecular S compounds within a short monitoring period. The release of sulphate was similar to those of the Cys and Met, with rates ranging from 1.2 ± 0.1 to 4.2 ± 0.3 µmol (g root DW)$^{-1}$ h^{-1}.

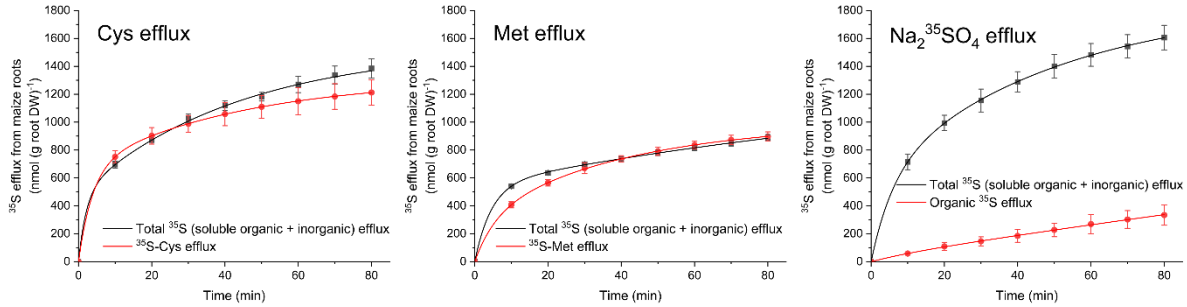

Fig. 5. Cumulative efflux of added ^{35}S-Cys, Met or sulphate per unit (g) dry mass of maize roots. Prior to measuring efflux, plants were pre-treated with radioisotopes for 60 min. Efflux was determined by measuring the increase of radioisotope in the root bathing medium solutions. $BaCl_2$ was applied to separate organic and inorganic S in solution. Data represent means ± SEM (*n* = 3). Lines represent fits of a double first-order exponential decay equation to the experimental data (r^2 > 0.99 in all cases; Eq. (2)).

The application of $BaCl_2$ allowed the separation of organic and inorganic S in the root exudates. The results revealed that the efflux of all three S compounds was mainly in the form they were taken up, suggesting efflux of low molecular weight compounds in a short period occurs via passive leakage. This is in line with previous studies, which suggested that amino acid efflux is generally regarded as not carrier-mediated but occurs by passive leakage (Jones and Darrah 1993; Paynel et al. 2001) and could be recaptured by roots.

The release of ^{35}S into the bathing solution indicated two distinct compartments, which may be interpreted as two pools: the cytoplasmic and vacuole compartments (Cooper and Clarkson 1989; Paynel et al. 2001). The rate of ^{35}S release from the roots decreased sharply over the course of the efflux period. A double first-order exponential decay equation fitted well to the efflux data (R^2 > 0.99; Fig. 5). This predicted that the half-life for the slower exchanging

compartment (vacuole) were 0.7, 2.6, and 0.5 h for Cys, Met and sulphate, respectively (Table. 1), while half-lives for the faster-exchanging compartment (cytoplasm) were 3.0, 3.1 and 3.7 mins for Cys, Met and sulphate, respectively. Based on calculation from Eqn. 4, the cytoplasmic S pool was estimated to range from 0.58 to 0.71 µmol (g root DW)$^{-1}$, which was smaller than their concentration in the vacuole, ranging from 1.0 to 1.1 µmol g^{-1} root DW.

Table 1. Parameters of amino acid-^{35}S (Cys and Met) and sulphate-^{35}S release from intact maize roots. Efflux data was fitted to a double first order exponential decay model (Eqn. 2: $y = a \times (1 - e^{-c \times t}) + b \times (1 - e^{-d \times t})$. The parameters *a* and *c* represent the ^{35}S held in the cytoplasm and vacuole, respectively, while *b* and *d* are the efflux constants for these two pools, respectively. The leakage of S compounds to the outer bathing solution was considered as the sum of two diffusional processes from the cytoplasm and vacuole to the root bathing medium: where *y* is the accumulated ^{35}S washed out of plant roots into the bathing solution, *a* and *b* are the sizes of the S storage pool in the cytoplasm and vacuole, respectively, and *c* and *d* are the exponential coefficients describing the rate of ^{35}S release from these pools into the external root bathing solution. Values represent means ± SEM (*n* = 3).

Substrates	**Pool *a***	**Pool *b***	**$t_{½}$ (fast pool) min**	**$t_{½}$ (slow pool) h**	**A (nmol. (g root DW)$^{-1}$)**	**B (nmol. (g root DW)$^{-1}$)**	**R^2**
Cys	588 ± 17	1107 ± 187	3.0 ± 0.6	0.7 ± 01	1029 ± 179	665 ± 20	0.99
Met	556 ± 24	1124 ± 355	3.1 ± 0.4	2.6 ± 0.9	1102 ± 354	578 ± 25	0.99
Na_2SO_4	560 ± 74	1287 ± 46	3.7 ± 0.3	0.5 ± 0.1	1142 ± 47	705 ± 62	0.99

Cycling of sulphur compounds between maize shoot and root via a split root system

Over 50 % of the ^{14}C tracer taken up by the donor root was cycled through the whole plant. The fraction of isotope tracer in each compartment (donor root, shoot, receiver root) is shown in Table. 2. By the end of this incubation experiment, ≤ 10 % of ^{14}C-Cys from the donor root was transported and retained in the shoot, while a much higher proportion (nearly 50 %) was respired from the shoot, ^{14}C-Cys partitioning in the receiver root reached a similar level as the donor root (approximately 20 %). Similarly, ^{14}C-Met was cycled from the donor root to the whole plant, with a higher proportion retained in the shoot and a lower proportion respired compared to ^{14}C-Cys.

The distribution pattern for ^{35}S differed from that of ^{14}C in that a higher proportion of S was retained in the donor root tissue. Nearly half of the ^{35}S taken up from the nutrient solution was retained in the donor root, and ca. 40 % was transported to the shoot, from where less than half was subsequently translocated to the receiver root. One possible explanation for the difference in ^{35}S and ^{14}C distribution is that after being taken up by the donor roots, amino acids are metabolized (deaminated, transaminated, etc.) prior to being transported to the shoots (Warren 2012). Donor roots had taken up 2.1 ± 0.3, 2.4 ± 0.2 and 9.1 ± 0.5 µmol. (g root DW)$^{-1}$ of ^{35}S-Cys, Met and Na_2SO_4 in total within 24 h. Overall, the actual amount of sulphate cycled from the nutrient solution to the donor root was around three times higher than that of Cys and Met.

By the end of the 24 h cycling period, however, negligible amounts of radioactivity were observed in the nutrient solution in the receiver root compartment.

Table 2. Translocation and utilization of three sulphur compounds by maize plants over a 24 h period analysed via the split root system. Both root compartments received the same amount of nutrient solution. Three independent measurements from replicate plants were made for each treatment. Values represent means ± SEM (*n* = 3).

Partitioning of ^{14}C or ^{35}S in each compartment	**^{14}C-Cys**	**^{35}S-Cys**	**^{14}C-Met**	**^{35}S-Met**	**^{35}S-Na_2SO_4**
$^{14}CO_2$ partitioning (% ^{14}C taken up)	47 ± 9.3		32 ± 2.9		
Shoot tissue (% ^{14}C taken up)	6.0 ± 1.9	21 ± 5.2	11 ± 1.4	29 ± 2.5	21 ± 2.5
Donor root (% ^{14}C taken up)	23 ± 3.1	64 ± 2.7	32 ± 1.6	49 ± 3.2	63 ± 1.9
Receiver root (% ^{14}C taken up)	24 ± 5.0	16 ± 3.2	24 ± 1.8	22 ± 2.9	16 ± 3.7

Discussion

Previously, dual-labelled (^{13}C, ^{15}N) compounds have been used to estimate amino acid uptake in plants (Wei et al. 2015; Enggrob et al. 2019). In these experiments, uptake of intact amino acids is implied if the slope of the correlation of ^{13}C to ^{15}N excess in the plant tissue is the same as in the parent amino acid compounds fed to the plants. In this present study, dual radio-isotope labelling: ^{14}C and ^{35}S were used to estimate uptake of intact amino acids. ^{14}C labelling has been used in studies of soil-free systems (Pratelli et al. 2016; Oburger and Jones 2018). A ^{14}C tracer was chosen in this study to eliminate the problem of ^{13}C dilution by the high ^{12}C content in plant tissues, as ^{13}C isotope can be strongly diluted in plant tissues making it difficult to detect ^{13}C in bulk plant tissues. A ^{14}C tracer approach allows estimation of the incorporation of amino acid-^{14}C into plant tissues, as well as the amino acid-^{14}C loss in the form of $^{14}CO_2$ produced during deamination and breakdown of the C skeleton in the tricarboxylic acid cycle (Näsholm and Persson 2001), or in processes relating to photorespiration (Bauwe et al. 2010).

Data from experiment 2 revealed that plants utilized sulphate preferentially over Cys and Met. This faster ^{35}S-sulphate uptake compared to Cys and Met could be explained by faster sulphate transport from root to shoot tissue, as similar values for root retention of all three S sources were obtained (Fig. 3). In addition, organic S supply has a negative effect on sulphate uptake. This agrees with the widely accepted view that initial root uptake of sulphate is energy dependent through a proton/sulphate coupled co-transport in the plasma membrane of root cells and is well adjusted to the S status of the plant (Davidian and Kopriva 2010a). When other organic sulphur sources (Cys or glutathione) are provided to the plant, sulphate uptake is repressed in a negative feedback loop (Hawkesford et al. 2003; Davidian and Kopriva 2010b; Noctor et al. 2011), while during S starvation, uptake is enhanced by activating the expression of high-affinity sulphate transporters (Maruyama-Nakashita et al. 2004).

It is well documented that the influx of amino acids involves proton-coupled amino acid transporters (Bush 1993; Delrot et al. 2000). The observation that both Cys and Met inhibit the uptake of each another supported our hypothesis that these amino acids enter root cells via the same transport system. The results clearly showed that a considerable proportion of the supplied amino acids might have been absorbed intact; this was illustrated by plots of excess ^{14}C against excess ^{35}S in plant material, with 62 % and 59 % of Cys and Met taken up intact separately (Fig. 4). This uptake pathway could be important in providing an alternative S source to plants and in recapturing amino acids previously lost in root exudates or when plants are directly adjacent to decomposing organic matter. However, it should be noted that the lower concentrations of Cys and Met in soil solutions *in situ*, as well as the intense competition between plant roots and rhizosphere microorganisms for nutrient (Owen and Jones 2001) may limit the actual contribution of these compounds to plant nutrition. It is also not clear whether plant roots are capable of taking up other dissolved organic S forms (e.g., peptides and proteins). These may also play an important role in the N and S dynamics, where inorganic N and S are inadequate for plant growth.

A higher proportion of ^{35}S (experiment 2) than ^{14}C (experiment 1) derived from both amino acids in plant material was detected in the plant tissue. The discrepancy between expected and measured ratios of ^{14}C to ^{35}S may be explained by several possibilities. First, the difference in measurement of ^{14}C and ^{35}S from biological samples (^{14}C was measured by dry combustion, while ^{35}S was measured by wet digestion) could have led to different recovery rates of the two radiotracers. Second, under the action of enzymes released by plant roots, part of added amino acids could have been degraded in the nutrient solution to inorganic compounds ($^{14}CO_2$, NO_3^-, NH_4^+ and $^{35}SO_4^{2-}$) prior to being taken up independently (Jones et al. 2005). This rapid enzymatic degradation of amino acids may contribute to a higher ^{35}S recovery in plant materials due to the fast uptake rates of sulphate by maize roots (Astolfi et al. 2004). Some $^{14}CO_2$ may also have been lost in respiration during the washing and drying of the root and shoot material. In addition, the rapid post-uptake metabolism of amino acids may also explain the anomalous relationships between ^{14}C and ^{35}S.

A complication about quantifying intact amino acid uptake is that theoretically, the same correlation in isotope enrichment could still arise if amino acids were broken down (e.g., by carbon-sulphur lyases) to inorganic forms in the nutrient solution before being taken up independently. In this short plant uptake experiment, considering the efforts to minimize microbial growth in nutrient solution before the conduction of experiments, it is likely that the pre-mineralization of Cys and Met was negligible. In addition, previous studies have addressed the importance of carbon-sulphur-lyases in the degradation of amino acids to inorganic compounds, among which methionine gamma-lyase degrades Met to a-keto acids, ammonia and thiols (Rébeillé et al. 2006; Goyer et al. 2007; Huang et al. 2014), while D-cysteine desulfhydrase degrades Cys to pyruvate, sulphide and ammonia (Riemenschneider et al. 2005). Therefore, it is possible that enzymatic transformation of Cys and Met took place during our experiment, and the breakdown products of these metabolites were taken up separately by maize roots; however, to our knowledge, these enzymes do not exist extracellularly. Stronger evidence from applying compound-specific ($^{13}C/^{34}S$) isotope ratio mass spectrometry (IRMS) could be used to examine this further.

The contribution of S containing amino acids to plant S nutrition under field conditions could be lower than what is reported in this study, this is due to the lower Cys and Met concentration *in situ*, the physico-chemical sorption mainly by association to clay particles in soil aggregates, as well as a lower bioavailability of Cys and Met to plants caused by rapid microbial uptake, decomposition (Wang et al. 2023a, b). The rhizosphere plant-microbial competition for Cys and Met was studied in a mesocosms containing both soil microbes and maize roots, soil microbes overwhelmingly outcompeted maize plants for Cys and Met within short term (Wang et al. 2023c). However, root performance in the rhizosphere competition could be different as a function of crop species, root morphology, inorganic N and S supply, diurnal dynamics and other factors. Therefore, field studies should be carried out in the future to explore contribution of organic S compounds to plant S nutrition.Rapid efflux of intact S compounds was detected from maize roots in experiment 3. Rapid sampling was used to minimize the negative effect of root re-capture of the compounds lost from root efflux. Considering S influx across the plasma membrane is an energy-dependent process, it is therefore surprising that S appears to leak out again rapidly. We therefore assume that after uptake, each compound transitorily accumulates in the cellular compartments (cytoplasm, vacuole) that are sensitive to efflux. Once they enter a complex reductive metabolic pathway, they are less likely to leak out again. Even so, the total amino acid efflux in the present study may have been underestimated since amino acids could be re-absorbed by plant roots, and these re-absorbed amino acids would not have been detected in efflux. In addition, high exogenous amino acid concentrations applied may have stimulated influx and diminished efflux, which is assumed to be concentration dependent.

Conclusions and outlook

We have presented direct experimental evidence that under hydroponic conditions, maize could directly take up dissolve organic sulphur in the form of free amino acids (e.g., cysteine and methionine), Cys and Met entered root cells through the same transport system. This uptake pathway could be important in providing an alternative sulphur source to plants, and in recapturing amino acid loss in root exudates. Root sulphur efflux results indicated that two distinct compartments were involved, with the vacuole being the slower releasing compartment and the cytoplasm being the smaller storage compartment, which is in line with previous studies (Bell et al. 1994; Hawkesford 2008). Root efflux of cysteine, methionine and Na_2SO_4 was via passive leakage. We also provided evidence for the rapid redistribution of sulphur within the plant following root uptake via a split root system.

References

Anderson JW, Fitzgerald MA (2003) Sulphur distribution and redistribution in vegetative and generative plants. In: Sulphur in plants. Springer, pp 113–134. https://doi.org/10.1007/978-94-017-0289-8_6

Astolfi S, Zuchi S, Cesco S, et al (2004) Influence of iron nutrition on sulphur uptake and metabolism in maize (*Zea mays* L.) roots. Soil Sci Plant Nutr 50:1079–1083. https://doi.org/10.1080/00380768.2004.10408577

Atkins CA, Smith PMC (2007) Translocation in legumes: Assimilates, nutrients, and signaling molecules. Plant Physiol 144:550–561. https://doi.org/10.1104/pp.107.098046

Ayers WA, Thornton RH (1968) Exudation of amino acids by intact and damaged roots of wheat and peas. Plant Soil 28:193–207. https://doi.org/10.1007/BF01880238

Badri D V., Quintana N, El Kassis EG, et al (2009) An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. Plant Physiol 151:2006–2017. https://doi.org/10.1104/pp.109.147462

Bai B, Zhang S, Suo X, et al (2022) Selenite uptake by *Medicago sativa* L. roots. Grassl Sci 68:328–335. https://doi.org/10.1111/grs.12367

Barber DA, Gunn KB (1974) The effect of mechanical forces on the exudation of organic substances by the roots of cereal plants grown under sterile conditions. New Phytol 39–45. https://doi.org/10.1111/j.1469-8137.1974.tb04604.x

Bauwe H, Hagemann M, Fernie AR (2010) Photorespiration: players, partners and origin. Trends Plant Sci 15:330–336. https://doi.org/10.1016/j.tplants.2010.03.006

Bell CI, Cram WJ, Clarkson DT (1994) Compartmental analysis of 35SO2-4exchange kinetics in roots and leaves of a tropical legume *Macroptilium atropurpureum* cv. siratro. J Exp Bot 45:879–886. https://doi.org/10.1093/jxb/45.7.879

Bohn HL, Barrow NJ, Rajan SSS, Parfitt RL (2015) Reactions of inorganic sulfur in soils. In: Sulfur in Agriculture. wiley, pp 233–249

Bush DR (1993) Proton-coupled sugar and amino acid transporters in plants. Annu Rev Plant Physiol Plant Mol Biol 44:513–542. https://doi.org/10.1146/annurev.pp.44.060193.002501

548 Carvalhais LC, Dennis PG, Fedoseyenko D, et al (2011) Root exudation of sugars, amino acids, and organic acids by
549 maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. J Plant Nutr Soil Sci 174:3–11.
550 https://doi.org/10.1002/jpln.201000085
551 Cooper CS, MacDonald PW (1970) Energetics of Early Seedling Growth in Corn (*Zea mays* L.) 1. Crop Sci 10:136–
552 139. https://doi.org/10.2135/cropsci1970.0011183x001000020003x
553 Cooper HD, Clarkson DT (1989) Cycling of amino-nitrogen and other nutrients between shoots and roots in cereals—
554 a possible mechanism integrating shoot and root in the regulation of nutrient uptake. J Exp Bot 40:753–762.
555 https://doi.org/10.1093/jxb/40.7.753
556 Cuero RG, Smith JE, Lacey J (1986) The influence of gamma irradiation and sodium hypochlorite sterilization on
557 maize seed microflora and germination. Food Microbiol 3:107–113. https://doi.org/10.1016/S0740-0020(86)80034-
558 X
559 Czaban W, Jämtgård S, Näsholm T, et al (2016) Direct acquisition of organic N by white clover even in the presence
560 of inorganic N. Plant Soil 407:91–107. https://doi.org/10.1007/s11104-016-2896-z
561 Davidian JC, Kopriva S (2010) Regulation of sulfate uptake and assimilation - The same or not the same? Mol Plant
562 3:314–325. https://doi.org/10.1093/mp/ssq001
563 Delrot S, Atanassova R, Maurousset L (2000) Regulation of sugar, amino acid and peptide plant membrane
564 transporters. Biochim Biophys Acta - Biomembr 1465:281–306. https://doi.org/10.1016/S0005-2736(00)00145-0
565 Enggrob KL, Jakobsen CM, Pedersen IF, Rasmussen J (2019) Newly depolymerized large organic N contributes
566 directly to amino acid uptake in young maize plants. New Phytol 224:689–699. https://doi.org/10.1111/nph.16070
567 Freney JR (1986) Forms and reactions of organic sulfur compounds in soils. Sulfur in agriculture 27:207–232
568 Gallet-Budynek A, Brzostek E, Rodgers VL, et al (2009) Intact amino acid uptake by northern hardwood and conifer
569 trees. Oecologia 160:129–138. https://doi.org/10.1007/s00442-009-1284-2
570 Gaume A, Mächler F, Frossard E (2001) Aluminum resistance in two cultivars of *Zea mays* L.: Root exudation of
571 organic acids and influence of phosphorus nutrition. Plant Soil 234:73–81. https://doi.org/10.1023/A:1010535132296
572 Ge T, Song S, Roberts P, et al (2009) Amino acids as a nitrogen source for tomato seedlings: The use of dual-labeled
573 (13C, 15N) glycine to test for direct uptake by tomato seedlings. Environ Exp Bot 66:357–361.
574 https://doi.org/10.1016/j.envexpbot.2009.05.004
575 Gibson JAB (1980) Modern techniques for measuring the quenching correction in a liquid scintillation counter: a
576 critical review. In: Physical Aspects. Academic press, INC., pp 153–172. https://doi.org/10.1016/b978-0-12-549901-
577 9.50021-1
578 Goyer A, Collakova E, Shachar-Hill Y, Hanson AD (2007) Functional characterization of a methionine γ-lyase in
579 arabidopsis and its implication in an alternative to the reverse trans-sulfuration pathway. Plant Cell Physiol 48:232–
580 242. https://doi.org/10.1093/pcp/pcl055
581 Hanway J (1966) How a corn plant develops.; Spec. Publ. 48. Iowa Agric Home Econ Exp Stn Publ 48:1–18
582 Harrington HM, Smith IK (1977) Cysteine Transport into Cultured Tobacco Cells. Plant Physiol 60:807–811.
583 https://doi.org/10.1104/pp.60.6.807
584 Hawkesford MJ, Buchner P, Hopkins L, Howarth JR (2003) Sulphate Uptake and Transport. In: Sulphur in Plants.
585 Springer, pp 71–86. https://doi.org/10.1007/978-94-017-0289-8_4
586 Hawkesford MJ (2008) Uptake, distribution and subcellular transport of sulfate. Sulfur Metab phototrophic Org 15–
587 30. https://doi.org/10.1007/978-1-4020-6863-8_2
588 Hewitt EJ (1952) Sand and water culture methods used in the study of plant nutrition.
589 https://doi.org/10.2134/agronj1952.00021962004400120018x
590 Huang T, Joshi V, Jander G (2014) The catabolic enzyme methionine gamma-lyase limits methionine accumulation
591 in potato tubers. Plant Biotechnol J 12:883–893. https://doi.org/10.1111/pbi.12191
592 Iannucci A, Canfora L, Nigro F, et al (2021) Relationships between root morphology, root exudate compounds and
593 rhizosphere microbial community in durum wheat. Appl Soil Ecol 158:103781.
594 https://doi.org/10.1016/j.apsoil.2020.103781
595 Jia X, Huangfu C, Hui D (2020) Nitrogen Uptake by Two Plants in Response to Plant Competition as Regulated by
596 Neighbor Density. Front Plant Sci 11:1–13. https://doi.org/10.3389/fpls.2020.584370
597 Johnson RM, Pregitzer KS (2007) Concentration of sugars, phenolic acids, and amino acids in forest soils exposed to
598 elevated atmospheric CO_2 and O_3. Soil Biol Biochem 39:3159–3166. https://doi.org/10.1016/j.soilbio.2007.07.010
599 Jones DL, Darrah PR (1994) Amino-acid influx at the soil-root interface of Zea mays L. and its implications in the
600 rhizosphere. Plant Soil 163:1–12. https://doi.org/10.1007/BF00033935
601 Jones DL, Darrah PR (1993) Influx and efflux of amino acids from *Zea mays* L. roots and their implications for N
602 nutrition and the rhizosphere. Plant Soil 155/156:87–90. https://doi.org/10.1007/bf00024990

603 Jones DL, Healey JR, Willett VB, et al (2005) Dissolved organic nitrogen uptake by plants - An important N uptake
604 pathway? Soil Biol Biochem 37:413–423. https://doi.org/10.1016/j.soilbio.2004.08.008
605 Koprivova A, Kopriva S (2016) Sulfur metabolism and its manipulation in crops. Journal of Genetics and Genomics
606 43:623–629. https://doi.org/10.1016/j.jgg.2016.07.001
607 Kopriva S, Malagoli M, Takahashi H (2019) Sulfur nutrition: Impacts on plant development, metabolism, and stress
608 responses. J Exp Bot 70:4069–4073. https://doi.org/10.1093/jxb/erz319
609 Li Q, Gao Y, Yang A (2020) Sulfur homeostasis in plants. Int J Mol Sci 21:1–16.
610 https://doi.org/10.3390/ijms21238926
611 Lucash MS, Eissenstat DM, Joslin JD, et al (2007) Estimating nutrient uptake by mature tree roots under field
612 conditions: Challenges and opportunities. Trees - Struct. Funct. 21:593–603. https://doi.org/10.1007/s00468-007-
613 0160-0
614 Ma Q, Hill PW, Chadwick DR, et al (2021) Competition for S-containing amino acids between rhizosphere
615 microorganisms and plant roots: the role of cysteine in plant S acquisition. Biol Fertil Soils 57:825–836.
616 https://doi.org/10.1007/s00374-021-01572-2
617 Maruyama-Nakashita A, Nakamura Y, Yamaya T, Takahashi H (2004) Regulation of high-affinity sulphate
618 transporters in plants: Towards systematic analysis of sulphur signalling and regulation. J Exp Bot 55:1843–1849.
619 https://doi.org/10.1093/jxb/erh175
620 Moore JC, McCann K, Setälä H, Ruiter, Peter C de (2003) Top-down is bottom-up: does predation in the rhizosphere
621 regulate aboveground dynamics? Ecology 84:846–857. https://doi.org/10.1890/0012-
622 9658(2003)084[0846:tibdpi]2.0.co;2
623 Moran-Zuloaga D, Dippold M, Glaser B, Kuzyakov Y (2015) Organic nitrogen uptake by plants: reevaluation by
624 position-specific labeling of amino acids. Biogeochemistry 125:359–374. https://doi.org/10.1007/s10533-015-0130-3
625 More SS, Shinde SE, Kasture MC (2020) Root exudates a key factor for soil and plant: An overview. Pharma Innov J
626 8:449–459.
627 Moreau D, Bardgett RD, Finlay RD, et al (2019) A plant perspective on nitrogen cycling in the rhizosphere. Funct
628 Ecol 33:540–552. https://doi.org/10.1111/1365-2435.13303
629 Narayan OP, Kumar P, Yadav B, et al (2022) Sulfur nutrition and its role in plant growth and development. Plant
630 Signal Behav 00: https://doi.org/10.1080/15592324.2022.2030082
631 Näsholm T, Persson J (2001) Plant acquisition of organic nitrogen in boreal forests. Physiol Plant 111:419–426.
632 https://doi.org/10.1034/j.1399-3054.2001.1110401.x
633 Noctor G, Queval G, Mhamdi A, et al (2011) Glutathione. In: The Arabidopsis Book, vol 9, American Society of Plant
634 Biologists. https://doi.org/10.1199/tab.0142
635 Oburger E, Jones DL (2018) Sampling root exudates – Mission impossible? Rhizosphere 6:116–133.
636 https://doi.org/10.1016/j.rhisph.2018.06.004
637 Okumoto S, Koch W, Tegeder M, et al (2004) Root phloem-specific expression of the plasma membrane amino acid
638 proton co-transporter AAP3. J Exp Bot 55:2155–2168. https://doi.org/10.1093/jxb/erh233
639 Owen AG, Jones DL (2001) Competition for amino acids between wheat roots and rhizosphere microorganisms and
640 the role of amino acids in plant n acquisition. Soil Biol Biochem 33:651–657. https://doi.org/10.1016/S0038-
641 0717(00)00209-1
642 Paynel F, Murray PJ, Bernard Cliquet J (2001) Root exudates: A pathway for short-term N transfer from clover and
643 ryegrass. Plant Soil 229:235–243. https://doi.org/10.1023/A:1004877214831
644 Phillips DA, Fox TC, King MD, et al (2004) Microbial products trigger amino acid exudation from plant roots. Plant
645 Physiol 136:2887–2894. https://doi.org/10.1104/pp.104.044222
646 Prasad R, Shivay YS (2018) Sulphur in Soil, Plant and Human Nutrition. Proc Natl Acad Sci India Sect B - Biol Sci
647 88:429–434. https://doi.org/10.1007/s40011-016-0769-0
648 Pratelli R, Boyd S, Pilot G (2016) Analysis of amino acid uptake and translocation in arabidopsis with a low-cost
649 hydroponic system. J Plant Nutr Soil Sci 179:286–293. https://doi.org/10.1002/jpln.201500464
650 Rauser WE (1987) Compartmental Efflux Analysis and Removal of Extracellular Cadmium from Roots. Plant Physiol
651 85:62–65. https://doi.org/10.1104/pp.85.1.62
652 Rébeillé F, Jabrin S, Bligny R, et al (2006) Methionine catabolism in Arabidopsis cells is initiated by a γ-cleavage
653 process and leads to S-methylcysteine and isoleucine syntheses. Proc Natl Acad Sci U S A 103:15687–15692.
654 https://doi.org/10.1073/pnas.0606195103
655 Riemenschneider A, Wegele R, Schmidt A, Papenbrock J (2005) Isolation and characterization of a D-cysteine
656 desulfhydrase protein from Arabidopsis thaliana. FEBS J 272:1291–1304. https://doi.org/10.1111/j.1742-
657 4658.2005.04567.x

Rroço E, Kosegarten H, Mengel K (2002) Importance of plasmalemma H+-ATPase activity for N losses from intact roots of spring wheat (*Triticum aestivum* L.). Eur J Agron 16:187–196. https://doi.org/10.1016/S1161-0301(01)00123-X

Saftner RA, Daie J, Wyse RE (1983) Sucrose Uptake and Compartmentation in Sugar Beet Taproot Tissue. Plant Physiol 72:1–6. https://doi.org/10.1104/pp.72.1.1

Sauer DB, Burroughs R (1986) Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76:745–749. https://doi.org/10.1094/phyto-76-745

Scott NM, Bick W, Anderson HA (1981) The measurement of sulphur‐containing amino acids in some scottish soils. J Sci Food Agric 32:21‐24. https://doi.org/10.1002/jsfa.2740320105

Smith GS, Johnston CM, Cornforth IS (1983) Comparison of nutrient solutions for growth of plants in sand culture. New Phytol 94:537–548. https://doi.org/10.1111/j.1469-8137.1983.tb04863.x

Smith IK, Lang AL (1987) Decoloration and solubilization of plant tissue prior to determination of ^{3}H, ^{14}C, and ^{35}S by liquid scintillation. Anal Biochem 164:531–536. https://doi.org/10.1016/0003-2697(87)90529-X

Song S, Li G, Sun G, et al (2016) Uptake Kinetics of Different Nitrogen Forms by Chinese Kale. Commun Soil Sci Plant Anal 47:1372–1378. https://doi.org/10.1080/00103624.2016.1178279

Takahashi H, Kopriva S, Giordano M, et al (2011) Sulfur Assimilation in Photosynthetic Organisms: Molecular Functions and Regulations of Transporters and Assimilatory Enzymes. Annu Rev Plant Biol 62:157–184. https://doi.org/10.1146/annurev-arplant-042110-103921

Takahashi H (2019) Sulfate transport systems in plants: functional diversity and molecular mechanisms underlying regulatory coordination. J Exp Bot 70:4075–4087. https://doi.org/10.1093/jxb/erz132

Thoiron A, Thoiron B, Demarty M, Thellier M (1981) The compartmental analysis of sulphate transport in cells of. Biochim Biophys Acta - Biomembr 644:24–35. https://doi.org/10.1016/0005-2736(81)90054-7

Thomson J, Temple S (2020) Sample preparation techniques for liquid scintillation analysis. In: Handbook of Radioactivity Analysis. Elsevier Inc., pp 803–860. https://doi.org/10.1016/b978-0-12-814397-1.00007-8

Tishel M, Mazelis M (1966) Enzymatic degradation of L-cystine by cytoplasmic particles from cabbage leaves [26]. Nature 211:745–746. https://doi.org/10.1038/211745a0

Vives-Peris V, de Ollas C, Gómez-Cadenas A, Pérez-Clemente RM (2020) Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep 39:3–17. https://doi.org/10.1007/s00299-019-02447-5

Wang D (2021) Turnover of S-containing amino acids (cysteine and methionine) in grassland soils and their availability to plants. Bangor University (United Kingdom)

Wang D, Chadwick DR, Hill PW, et al (2023a) Tracing the mineralization rates of C, N and S from cysteine and methionine in a grassland soil: A ^{14}C and ^{35}S dual-labelling study. Soil Biol Biochem 177:. https://doi.org/10.1016/j.soilbio.2022.108906

Wang D, Chadwick DR, Hill PW, et al (2023b) Rapid microbial uptake and mineralization of ^{14}C-labelled cysteine and methionine along a grassland productivity gradient. Soil Biol Biochem 180:109022. https://doi.org/10.1016/j.soilbio.2023.109022

Wang D, Wang J, Chadwick DR, et al (2023c) Competition for two sulphur containing amino acids (cysteine and methionine) by soil microbes and maize roots in the rhizosphere. Biol Fertil Soils. https://doi.org/10.1007/s00374-023-01724-6Wei L, Chen C, Yu S (2015) Uptake of organic nitrogen and preference for inorganic nitrogen by two Australian native Araucariaceae species. Plant Ecol Divers 8:259–264. https://doi.org/10.1080/17550874.2013.871656

Weigelt A, Bol R, Bardgett RD (2005) Preferential uptake of soil nitrogen forms by grassland plant species. Oecologia 142:627–635. https://doi.org/10.1007/s00442-004-1765-2

Wirtz M, Droux M (2005) Synthesis of the sulfur amino acids: Cysteine and methionine. Photosynth Res 86:345–362. https://doi.org/10.1007/s11120-005-8810-9

Wright DE (1962) Amino acid uptake by plant roots. Arch Biochem Biophys 97:174–180. https://doi.org/10.1016/0003-9861(62)90061-9

Yao X, Nie J, Bai R, Sui X (2020) Amino acid transporters in plants: Identification and function. Plants 9:1–17. https://doi.org/10.3390/plants9080972

Zenda T, Liu S, Dong A, Duan H (2021) Revisiting sulphur—the once neglected nutrient: It's roles in plant growth, metabolism, stress tolerance and crop production. Agric 11:. https://doi.org/10.3390/agriculture11070626

Zhu F, Dai L, Hobbie EA, et al (2019) Uptake patterns of glycine, ammonium, and nitrate differ among four common tree species of northeast China. Front Plant Sci 10:1–11. https://doi.org/10.3389/fpls.2019.00799

Statements & Declarations

Funding
This work was supported by China Scholarship Council (Grant number: 201606510012).

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Deying Wang. The first draft of the manuscript was written by Deying Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability
Data will be made available on request.