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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

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ABSTRACT

Sulphur-containing amino acids (i.e. Cysteine (Cys) and methionine (Met)) constitute an important proportion of the soil organic sulphur. However, detailed information regarding the microbial transformation of Cys and Met at a molecular level remain poorly characterized. To trace the fate of carbon (C) and sulphur (S) derived from Cys and Met in an agricultural grassland soil, a ^{14}C and ^{35}S dual-isotopic labelling approach was adopted. We also investigated whether their mineralization was affected by manipulating C (added as glucose), nitrogen (N), phosphorus (P) and S (added as NH_4NO_3, KH_2PO_4 and K_2SO_4) availability in soil solution. Our results showed that over a 7-day incubation period, 67.2–89.2% of the ^{14}C derived from Cys and Met was respired as $^{14}CO_2$, 2.7–19.5% had been immobilized in the soil microbial biomass; while the recovery of ^{35}S in soil solution ranged from 6.4 to 9.9%, with the reminder retained in the soil microbial biomass. Overall, our results indicated that soil microbial communities possess a high capacity to utilize Cys and Met. Furthermore, using the ^{14}C and ^{35}S dual-labelling technique, we found that C and S derived from Cys and Met were microbially mineralized and immobilized at different rates, indicating that the cycles of these two elements were temporally decoupled at the molecular level. The addition of glucose-C increased $^{14}CO_2$ respiration from Cys and Met after 7 d, while in comparison inorganic N, P and S addition had less effect on ^{14}C and ^{35}S partitioning.

1. Introduction

A mean stoichiometric carbon (C): nitrogen (N): phosphorus (P): sulphur (S) ratio of 10,000: 833: 200: 143 has been reported for a wide range of global soils (Kirkby et al., 2011). Consequently, changes in the abundance of one element are often balanced by increases or decreases in another. Over the next few decades, it is predicted that global C and N inputs to soil will increase due to the need to improve plant productivity and food security (He et al., 2021). Thus, terrestrial ecosystems are expected to develop a higher demand for other nutrients (i.e. P and S) stripped from the soil system at harvest (Jones et al., 2013). Typically, N, P, and S are seen as the major nutrients that are most likely to limit plant and microbial growth (Fermoso et al., 2019), with their availability in soil a key constraint for the productivity of terrestrial ecosystems (Marschner and Rengel, 2012; Vitousek and Howarth, 1991).

More than 90% of the soil's S reserves are bound within soil organic matter (SOM; Eriksen, 2009) with most of this held within protein moieties. In most agroecosystems, the decomposition and mineralization of organically bound S is mediated by the soil microbial community, thereby contributing to the release of S for plant nutrition (Sahrawat, 1981; Kertesz and Mirleau, 2004; Wang, 2021). This is especially the case at the peak of the growing season, or when there is a shortfall in the supply of sulphate (Freney et al., 1975). Acid hydrolysis has revealed that amino acids represent the largest pool of soil organic matter (Stevenson, 1982). Within this amino acid pool, S-containing cysteine (Cys) and methionine (Met), constitute the most important reserve of combined C, N and S in soil (Scott et al., 1981) and they also constitute the majority of organic S in plant tissues (Allaway and Thompson, 1966).

After uptake, the amino acid-C taken up by the soil microbial community is partitioned into two pools (Jones et al., 2009) associated with either catabolic (i.e. respiration) or anabolic processes (i.e. cell growth and maintenance). The catabolic production of CO_2 is normally characterized by an initial rapid phase (<3 h) followed by a slower phase that continues for several weeks (Boddy et al., 2007; Farrell et al., 2011;

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Wilkinson et al., 2014a). Amino acid-N held in exposed amino groups can be released extracellularly through the action of deaminases, after which the NH_4^+ can be taken up and assimilated (Jansson and Persson, 2015). Alternatively, mineralization of amino acid-N occurs via the indirect route in which organic N compounds are taken up intact by soil microorganisms, assimilated and then any excess N is released as NH_4^+ back into the soil (Finzi and Berthrong, 2005; Barraclough et al., 2015). Similarly, based on the stoichiometric relationship between C, N and S, it is suggested that both biological and biochemical mineralization processes are involved in the mineralization of organic S (David et al., 1983; McGill and Cole, 1981).

From the current knowledge of organic S decomposition processes in soils, it is possible that microbial demand for C is the dominant driver for both biochemical and biological mineralization of organic S (Ghani et al., 1992). Therefore, the supply of a readily available C source to the soil may have a considerable effect on organic S mineralization. If concentrations of labile C in the soil are too low to meet microbial demand, mineralization of organic S would take place, and during this process greater S excretion is likely. Conversely, in the presence of high amounts available C in soil, immobilization of sulphate is likely to occur. Similarly, since sulphatase activity is related to concentrations of its end-product (sulphate), when sulphate concentrations are too low to meet microbial demand, sulphatase enzymes are used to hydrolyse sulphate esters. Conversely, addition of sulphate to the soil may hinder the mineralization or soil native organic S. By experimentally manipulating the amounts and the availability of C, N and S in soils, we aim to improve our understanding of what regulates the fate of Cys and Met in soil.

The specific objectives of this study were to (1) compare the short-term concurrent mineralization of C-, N- and S-derived from Cys and Met over a 168-h incubation period; and (2) assess the effect of organic C and inorganic nutrient availability on microbial mineralization and utilization of Cys and Met. This study was designed to provide valuable information for future modelling of S transformations and nutrient coupling in soil. Further, the information will improve our fundamental mechanistic understanding of S cycling with the aim of designing better fertiliser management regimes to address S deficiency.

2. Materials and methods

2.1. Study sites and soil sampling

Replicate batches of soil samples ($n = 3$) were collected in May 2018 from the Ah horizon (0–10 cm) of an unfertilized grassland field at Abergwyngregyn, Gwynedd, UK (53°14′N 4°11W). On return to the laboratory, the soil was sieved (<2 mm) to remove stones, fine roots, and other plant debris, and then stored at < 4 °C prior to further analysis. 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 (EC) were determined in 1:5 (w/v) soil-to-distilled water extracts using standard electrodes. Soil bulk density was estimated by taking intact cores (100 cm^3) from the field followed by oven drying and stone correction (Rowell, 2014). Microbial biomass C and N (MBC/N) were determined by the chloroform-fumigation extraction procedure of Voroney et al. (2007). Briefly, the amount of DOC and DON 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 K_{EC} and K_{EN} extraction factors of 0.35 and 0.5, respectively (Voroney et al., 2007). For NO_3^- and NH_4^+ analysis, the soil was extracted with 0.5 M K_2SO_4 (1:5 w/v) and NO_3^- in the extracts determined colorimetrically on a Synergy MX microplate reader using the vanadate procedure of Miranda et al. (2001), while NH_4^+ was determined colorimetrically using the salicylate procedure of Mulvaney (1996). Extractable P was extracted using a 0.5 M acetic acid (1:5 w/v) shaken for 1 h (200 rev min^{-1}; 20 °C), then centrifuged for 10 min at 3220 *g* before passing through a Whatman 42 filter (Quevauviller, 2007). 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. All values are reported on a dry soil weight basis (Table S1).

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), 0.45 μm syringe filtered 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 (DOS plus sulphate; TDS) and other major cations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Varian 710 ES, 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 sulphur (MBS) content was estimated from the differences between total dissolved S concentrations in the fumigated and unfumigated extracts using an extraction efficiency conversion factor (Ks) of 0.35 (Saggar et al., 1981; Wu et al., 1994).

2.2. Incubation experiments

Each experimental unit comprised 5 g of field-moist soil held in individual 50 cm^3 polypropylene centrifuge tubes with a screw cap. All radiolabelled substrates were made up in 1 ml of deionized water, before being uniformly applied to the soil surface dropwise using a pipette. The final soil water content is 40.8% (on a soil dry weight basis). All centrifuge tubes were set up at the same time under the same experimental conditions. The incubation study consisted of two parallel experiments, one involving ^{14}C and the other using ^{35}S.

Briefly, soil in each unit was spiked with 1 ml of ^{14}C-labelled or ^{35}S-labelled Cys or Met (1 mM; 0.3 kBq ml^{-1}; PerkinElmer Inc, Waltham, MA) either in the presence or absence of glucose-C or inorganic nutrients (N, P and S). Glucose (+G treatment) was applied to soil at a rate of 360 mg C kg^{-1} soil, 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. Glucose addition was equivalent to ca. 50% of the native microbial biomass C content. NPS application rates were chosen to represent common UK field fertiliser application rates (DEFRA, 2016), which are equivalent to 30 kg NPS/ha.

In this study, a non-fertilized grassland soil was chosen to avoid the effects of long-term fertilization on soil microbial community structure and functioning. We chose Cys and Met as model substrates as C-bonded S as they represent the dominant low molecular weight (LMW) dissolved organic S compounds entering soil (Yeoh and Watson, 1982; Fitzgerald et al., 1988; Yazzie et al., 1994). The concentration of Cys and Met (1 mM) was chosen to reflect those likely to occur in the rhizosphere upon lysis of root cells.

The harvest scheme for Cys is as follows (The same harvest scheme was applied on Met):

(1) Rapid 14C mineralization determination: high temporal resolution measurements in the first 24 h
   - ^{14}C-Cys (6 units), NaOH traps were changed and collected hourly up to 24 h, all units were destructively harvested and soil from three units was extracted with 0.5 M K_2SO_4 at 24 h, soil in the other 3 units were fumigated before extraction;
   - ^{14}C-Cys with glucose addition (6 units), same as above;
   - ^{14}C-Cys with NPS addition (6 units), same as above;

(2) Medium-term ^{14}C mineralization determination: Measurements of mineralization over longer term with lower frequency
   - ^{14}C-Cys (48 units), all units were separated in 8 groups, at 6, 12, 48, 72, 96, 120, 144 and 168 h separately, one group (6 units) were destructively harvested, NaOH traps were collected

from each unit, and soil from three units was extracted with 0.5 M K_2SO_4, soil in the other 3 units were fumigated before extraction;
- ^{14}C-Cys with glucose addition (48 units), same as above;
- ^{14}C-Cys with NPS addition (48 units), same as above;

(3) ^{35}S mineralization measurement
- ^{35}S-Cys (54 units), all units were separated in 9 groups, at 6, 12, 24, 48, 72, 96, 120, 144 and 168 h separately, one group (6 units) were destructively harvested, soil from three units was directly extracted with 0.01 M $CaCl_2$, soil in the other 3 units were fumigated before extraction;
- ^{35}S-Cys with glucose addition (54 units), same as above;
- ^{35}S -Cys with NPS addition (54 units), same as above;

The incubation lasted for 168 h, as previous studies have shown that the response of the microbial biomass was relatively fast (several hours to several days) when labile substrates are added to soil (Blagodatskaya and Kuzyakov, 2013; Blagodatskaya et al., 2007, 2009). The incubation was carried out at room temperature (21 ± 1 °C) in the dark, in the absence of plants. All treatments were conducted in triplicate. Samples for microbial biomass C, N and S determination (MBC, MBN, MBS) were taken before application of the experimental treatments.

2.3. Partitioning of the ^{14}C from Cys and Met into different compartments

After application of the ^{14}C-labelled amino acid solution to the soil surface, ^{14}C either remained in solution, or was taken up by the microbial community. After uptake, the amino acids can be used either for microbial growth (synthesis of new cells; ^{14}C-biomass) or for production of energy ($^{14}CO_2$). Therefore, we determined the partitioning of added ^{14}C isotope into a range of pools as follows:

- $^{14}C_O$ (^{14}C added per unit at time zero): determined by counting the ^{14}C-substrate stock solution (kBq);
- $^{14}C_{uf}$ (^{14}C remaining in soil solution; %): ^{14}C in the 0.5 M K_2SO_4 extract of non-fumigated soils (kBq)/$^{14}C_O \times 100$;
- $^{14}CO_2$ partitioning ($^{14}CO_2$ of total ^{14}C added; %): $^{14}CO_2$ trapped in NaOH/$^{14}C_O \times 100$;
- $^{14}C_{MB}$ (^{14}C retained in soil microbial biomass; %): ($^{14}C_O - CO_2$ - $^{14}C_{uf}$)/$^{14}C_O \times 100$;
- $^{14}C_{FE}$ (^{14}C recovered in soil microbial biomass that could be determined by fumigation extraction method; %): (^{14}C in the 0.5 M K_2SO_4 extract of fumigated soils - ^{14}C in the 0.5 M K_2SO_4 extract of non-fumigated soils) × 100/0.35/$^{14}C_O$;
- $^{14}C_{FNON\text{-}FE}$ (^{14}C in soil microbial biomass that could not be determined by fumigation extraction method; %): $^{14}C_{MB}$ - $^{14}C_{FE}$;
- $^{14}C_{efficiency}$ (^{14}C use efficiency): $^{14}C_{MB}/^{14}C_O$;

2.3.1. Microbial mineralization of ^{14}C-labelled Cys and Met

After addition of ^{14}C labelled amino acid solution to the soil surface, a 6 ml polypropylene tube containing 1 M NaOH (1 ml) was placed inside the incubation vessels above the soil to trap respired $^{14}CO_2$. These NaOH traps were changed hourly up to 24 h and then 48, 72, 96, 120, 144 and 168 h after substrate addition to quantify microbial respiration rates. After removal, the amount of $^{14}CO_2$ in the 1 M NaOH traps was determined by liquid scintillation counting using a Wallac 1404 scintillation counter with automated quench correction (Wallac EG&G, Milton Keynes, UK) and Optiphase HiSafe 3 alkali-compatible scintillation fluid (PerkinElmer Inc., Waltham, MA).

2.3.2. Determination of microbial biomass ^{14}C

Microbial biomass ^{14}C was measured by the $CHCl_3$ fumigation incubation method (Voroney et al., 2007). Briefly, at nine different time points (6, 12, 24, 48, 72, 96, 120, 144 and 168 h) after substrate addition, 5 g of soil was exposed to chloroform vapour for 24 h. After removal of the fumigant, the soil was extracted with 25 ml of 0.5 M K_2SO_4 (30 min, 200 rev min^{-1}) to recover any ^{14}C-label remaining in the soil solution or held on soil exchange surfaces. The extracts were centrifuged (4000 rev min^{-1}, 5 min), and ^{14}C in the supernatant determined by liquid scintillation counting as described previously. A non-fumigated control was extracted alongside the fumigated counterparts. The ^{14}C contained in the microbial biomass was calculated as the differences of ^{14}C recovered in the fumigated and non-fumigated samples, adjusted with the extraction factor of 0.35.

2.4. Partitioning of the ^{35}S into different compartments

Similar to the approach taken above for ^{14}C, the partitioning of ^{35}S was determined as follows:

- $^{35}S_O$ (^{35}S added per unit at time zero): determined by counting the ^{35}S-substrate solution (kBq);
- $^{35}S_{uf}$ (^{35}S remaining in soil solution; %): ^{35}S in the 0.01 M $CaCl_2$ extract of non-fumigated soils/$^{35}S_O$;
- $^{35}S_{MB}$ (^{35}S in soil microbial biomass; %): Microbial biomass $^{35}S = (^{35}S_O - ^{35}S_{uf}) \times 100/^{35}S_O$;
- $^{35}S_{FE}$ (^{35}S in soil microbial biomass that could be determined by fumigation extraction method; %): (^{35}S in the 0.01 M $CaCl_2$ extract of fumigated soils - ^{35}S in the 0.01 M $CaCl_2$ extract of non-fumigated soils) × 100/0.35/$^{35}S_O$;
- $^{35}S_{FNON\text{-}FE}$ (^{35}S in soil microbial biomass that could not be determined by fumigation extraction method; %): $^{35}S_{MB}$ - $^{35}S_{FE}$;

At nine different time points (6, 12, 24, 48, 72, 96, 120, 144 and 168h), ^{35}S remaining free in the soil was extracted by adding 25 ml of 0.01 M $CaCl_2$ solution to the soil. The soils were then extracted on an end-over-end shaker (30 min, 200 rev min^{-1}), and subsequently centrifuged (5 min, 4000 rev min^{-1}). The supernatant was then analysed for total ^{35}S activity by liquid scintillation counting as described previously. To separate organic-S and sulphate-S in the soil extract, a $BaCl_2$ turbidimetric/precipitation approach was used (Combs et al., 1998). Briefly, 10 ml of the centrifuged extract was mixed with 10 ml of 1 M $BaCl_2$ on an end-over-end shaker for 10 min at 200 rev min^{-1}. Subsequently, the mixture was centrifuged (5 min, 4000 rev min^{-1}) and the supernatant analysed for organic ^{35}S (sulphate having precipitated as $BaSO_4$). The difference between ^{35}S activity in soil solution before and after $BaSO_4$ precipitation was considered as sulphate generated from mineralization of the added amino acids.

To estimate microbial biomass-S, chloroform fumigation-extraction was used as described above for microbial biomass ^{14}C, 25 ml of 0.01 M $CaCl_2$ was used to extract ^{35}S remaining in fumigated soil. ^{35}S retained in the microbial biomass was calculated as the differences in $CaCl_2$ extractable ^{35}S between fumigated and non-fumigated samples, adjusted with an extraction factor of 0.35 (Voroney et al., 2007). All ^{35}S data were corrected for radioactive decay relative to the start of the incubation (half-life of 87.4 d; Holtzhauer, 2006; Zoon, 1987).

2.5. Microbial biomass analysis

The chloroform fumigation-extraction procedure used above was used to determine microbial biomass (C_{mic}, N_{mic}). In this case, total dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) in the 0.5 M K_2SO_4 extracts from both the fumigated and un-fumigated samples were analysed with a Multi N/C 2100 analyser (AnalytikJena, Jena, Germany). C_{mic} and N_{mic} were determined as the difference of DOC or TDN concentrations between the fumigated and non-fumigated samples, corrected by the extraction factor 0.35 (K_{EC}) and 0.5 (K_{EN}), respectively (Vance et al., 1987; Wu et al., 1990; Joergensen and Olfs, 1998).

2.6. Statistical and data analysis

2.6.1. Half-life of ^{14}C-amino acid mineralization in soil

By difference, the amount of respired $^{14}CO_2$ was used to calculate the ^{14}C remaining in soil and the microbial biomass pools (% of total added available to soil microbes). Previous studies have demonstrated that LMW substrate mineralization occurs in two distinct phases (Saggar et al., 1996; Chotte et al., 1997; Hill et al., 2008; Glanville et al., 2012; Wilkinson et al., 2014b). In this study, the mineralization of Cys and Met to $^{14}CO_2$ was described by a double first order exponential decay equation:

$$y_1 = (a_1 \times \exp_1^{tb}) + (a_2 \times \exp^{-}{}^{b}_{2}) \quad \text{(Eqn1)}$$

Where y_1 the amount of ^{14}C remaining in the soil and microbial biomass, t is time, b_1 is the exponential coefficient describing the primary mineralization phase, b_2 is the exponential coefficient describing the secondary mineralization of the microbial biomass, while a_1 and a_2 represent the size of two pools. Half-lives (the time in which radioactivity drops to half of its original value) of the soil/microbial biomass pools can therefore be defined as:

$$t_{½} = \ln(2)/\ b_1 \text{ or } t_{½} = \ln(2)/\ b_2 \quad \text{(Eqn2)}$$

2.6.2. Half-life of amino acid-^{14}C and ^{35}S depletion in soil

To determine the half-life ($t_{½}$) of amino acid-^{14}C and ^{35}S depletion in the soil solution, a double first order exponential decay equation (Eqn. (1)) was fitted to the experimental data separately.

Where y_1 is the ^{14}C or ^{35}S remaining in soil solution, b_1 and b_2 the exponential coefficient describing the rate of amino acid-^{14}C or ^{35}S depletion by soil microbial community, a_1 and a_2 describes the sizes of pools and t is time. The half-life of the soil solution pools a_1 and a_2 can therefore be calculated according to Eqn. (2).

^{35}S counts in this study were decay-corrected to the start of the incubation (half-life of ^{35}S is 87.4 d) according to the following equation (Korsun, 2018):

$$N_{(t)} = N_0 \times \exp^{-0.693 * t/87.4} \quad \text{(Eqn3)}$$

Where t is time (d), $N_{(t)}$ represents the specific radioactivity of ^{35}S at time t (d), N_0 represents the radioactivity of ^{35}S added at time 0.

2.6.3. Half-life of ^{14}C and ^{35}S in microbial biomass in soil

Generally, a single first order exponential decay equation fitted well to the data (based on the amount of ^{14}C or ^{35}S-labelled C_{FE} and S_{FE} at each time point where:

$$A_t = A_0 \times \exp^{-kt} \quad \text{(Eqn4)}$$

Where A_0 and A_t are the content of $^{14}C_{FE}$ or $^{35}S_{FE}$ at times $t = 0$ and t, respectively. The initial time point of t was set at 24 h after substrate addition, as this was the time at which an approximate equilibrium was attained between exchangeable soil C and ^{14}C, soil S and ^{35}S. In addition, the $^{14}C_{FE}$ and $^{35}S_{FE}$ reached the peak and started to decline at this time. k is the decay rate, therefore the turnover time is calculated by Eqn. 6:

$$t_{½} = \ln(2)/\ k \quad \text{(Eqn5)}$$

All treatments were replicated three times. All statistical analyses were carried out using SPSS v25.0 (SPSS Inc., Chicago, IL, USA), with $p < 0.05$ used as the upper limit for statistical significance. The exponential decay curves for ^{14}C and ^{35}S were fitted to the experimental results using SigmaPlot v13.0 (SPSS Inc., Chicago, IL), and the adjusted R^2 for the curves as well as the significance of each parameter were calculated.

3. Results

3.1. ^{14}C-labelled amino acid mineralization in soil

The kinetics of $^{14}CO_2$ evolution was biphasic for all treatments and the rate constants (b_1 and b_2) for these two phases are presented in Table 1. This $^{14}CO_2$ evolution from the soil was best described by a double first order exponential equation; Fig. 1; $r^2 > 0.99$), which is in line with previous studies (Scow et al., 1986; Boddy et al., 2008; Glanville et al., 2012; Mariano et al., 2016). Overall, mineralization of the added ^{14}C-labelled amino acid by the microbial community was extremely rapid, suggesting that soil microbes are severely C-limited. In contrast to the half-life for the first phase of mineralization (12.8 ± 4.9 h), the half-life for the second phase was significantly longer, with an average value of 16.3 ± 9.3 d. Generally, the addition of glucose-C or nutrients did not affect the two-phase microbial respiration pattern of Cys or Met.

Within the first 48 h, the proportion of Cys-^{14}C mineralized was higher than that of Met. According to our calculation, the mineralization rate for Met for the first 10 h after substrate addition remained relatively constant, ranging from 292 ± 31 to 309 ± 12 μg ^{14}C-Met DW soil kg^{-1} h^{-1}, which is lower than that from Cys, ranging from 297 ± 71 to 699 ± 79 μg ^{14}C-Cys DW soil kg^{-1} h^{-1}. From 10 h onwards, the mineralization rates of Cys decreased dramatically to slightly below that of Met.

3.2. Amino acid-^{14}C and ^{35}S depletion from soil

3.2.1. Amino acid-^{14}C depletion from soil

Overall, the depletion of both ^{14}C-labelled Cys and Met from soil solution was extremely rapid (Fig. 2). For example, after 3 h, 81.2 ± 0.6% of the added ^{14}C-Cys had been taken up by the microbial community, whereas in the same time period 74.4 ± 0.5% of the ^{14}C-Met was taken up. In addition, after 24 h, the content of 0.5 M K_2SO_4-extractable ^{14}C in the soil had declined to <10% of the applied amino acid-^{14}C, indicating that >90% of the added amino acid-^{14}C was consumed by the microbial community. This rapid disappearance of substrate-^{14}C is consistent with other studies (Vinolas et al., 2001; Jones et al., 2018) (see Fig. 3).

3.2.2. Amino acid-^{35}S depletion from soil

Similarly, ^{35}S-Cys and ^{35}S-Met depletion from the soil solution was extremely rapid, with a much higher proportion of ^{35}S-Met removed from the soil solution than ^{35}S-Cys. After only 6 h, the total $CaCl_2$ extractable ^{35}S (SO_4^{2-}-S + org-S) derived from Cys and Met treatments was 52.1 ± 1.2 and 25.5 ± 4.1%, respectively, suggesting around 47.9% and 74.5% of the Cys-^{35}S and Met-^{35}S was removed from soil solution by microorganisms. 72 h after substrate addition, the total extractable ^{35}S content significantly decreased, by more than half, and then remained consistently low (<15% of total added) until the end of the experiment. The depletion of ^{35}S-Cys and ^{35}S-Met from soil solution was best described by a double first order exponential decay equation ($r^2 > 0.80$ in all cases; Eq. (1); Fig. 2).

3.3. Soil microbial biomass C and N

Within 9 h of substrate addition, C_{mic} increased from 782 ± 52 mg C kg^{-1} DW soil (Control soil) to 1006 ± 20 mg C kg^{-1} soil (1 mM Cys treatment) and 917 ± 67 mg C kg^{-1} soil (1 mM Met treatment), respectively (Fig. 4). Similarly, N_{mic} increased from 63.3 ± 13.2 (Control soil) to 69.4 ± 7.9 mg N_{mic} kg^{-1} DW soil and 66.5 ± 1.1 mg N_{mic} kg^{-1} soil within 9 h for the Cys and Met treatments individually. Thereafter, both C_{mic} and N_{mic} decreased gradually to the level seen before amino acid addition. An increase in C_{mic} concentrations due to glucose addition were significant ($p < 0.01$) for the whole incubation period for both Cys and Met, whereas the addition of NPS did not affect C_{mic} or N_{mic} to a significant extent ($p > 0.05$).

Table 1
Kinetic coefficients of double first order exponential decay models describing the depletion of ^{14}C-Cys or Met from soil by mineralization over a 168 h-incubation period in the absence (control) or presence of either glucose (+G) or nutrients (+NPS). a_1 and a_2 are estimated pool sizes for the fast and slow phases of substrate mineralization C pools, and b_1 and b_2 are the rate constants for the fast and slow phases of mineralization, respectively. $t_{½}$ values are the half-times for pool a_1 and a_2 determined from b_1 and b_2. Values represent means ± SEM ($n = 3$). The R^2 represents the goodness of fit of the experimental data to the kinetic model.

Substrate	Treatments	a_1	b_1	a_2	b_2	a_1 $t_{½}$ (h)	a_2 $t_{½}$ (d)	R^2
Cys	+G	65.1 ± 2.7	0.07 ± 0.003	30.4 ± 2.9	0.004 ± 0.0010	12.8 ± 5.3	7.3 ± 3.3	0.99
	Control	62.7 ± 0.9	0.08 ± 0.002	34.8 ± 1.1	0.001 ± 0.0003	9.2 ± 8.4	23.1 ± 5.2	0.99
	+NPS	44.7 ± 2.0	0.08 ± 0.006	51.7 ± 2.2	0.003 ± 0.0003	9.5 ± 1.4	10.7 ± 1.6	0.99
Met	+G	43.0 ± 0.6	0.04 ± 0.004	42.3 ± 0.9	0.04 ± 0.004	17.3 ± 1.7	0.7 ± 0.1	0.99
	Control	72.9 ± 1.5	0.04 ± 0.001	28.4 ± 1.6	0.003 ± 0.0005	18.6 ± 5.9	29.6 ± 7.3	0.99
	+NPS	49.3 ± 2.0	0.07 ± 0.005	53.4 ± 2.2	0.003 ± 0.0001	9.3 ± 1.0	10.7 ± 3.9	0.99

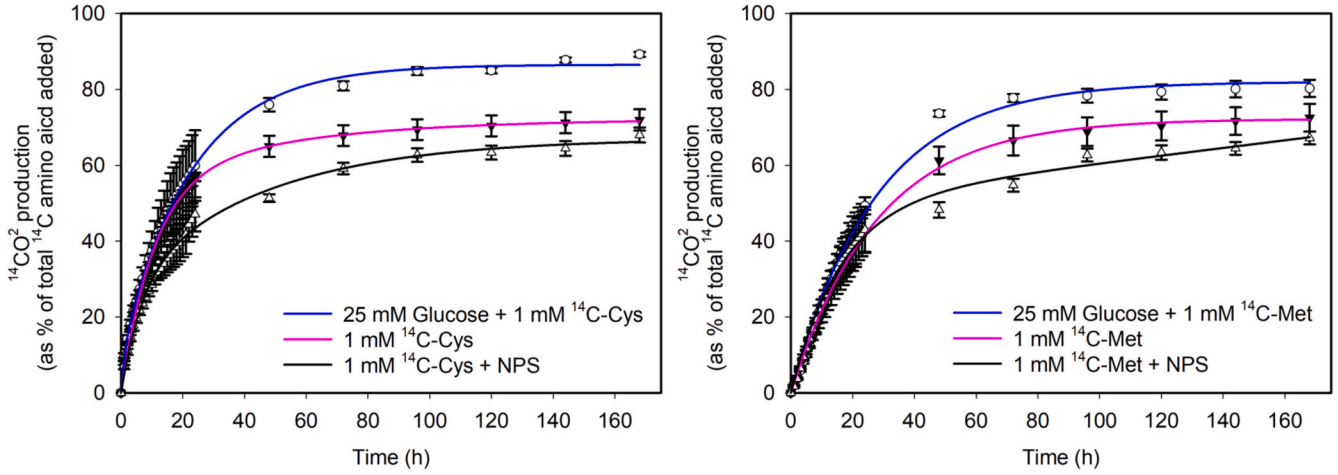

Fig. 1. Cumulative $^{14}CO_2$ evolution from ^{14}C-labelled Cys or Met (1 mM) in a grassland soil. Lines represent fits of a double first order exponential equation to the experimental data. Values represent means ± SEM ($n = 3$).

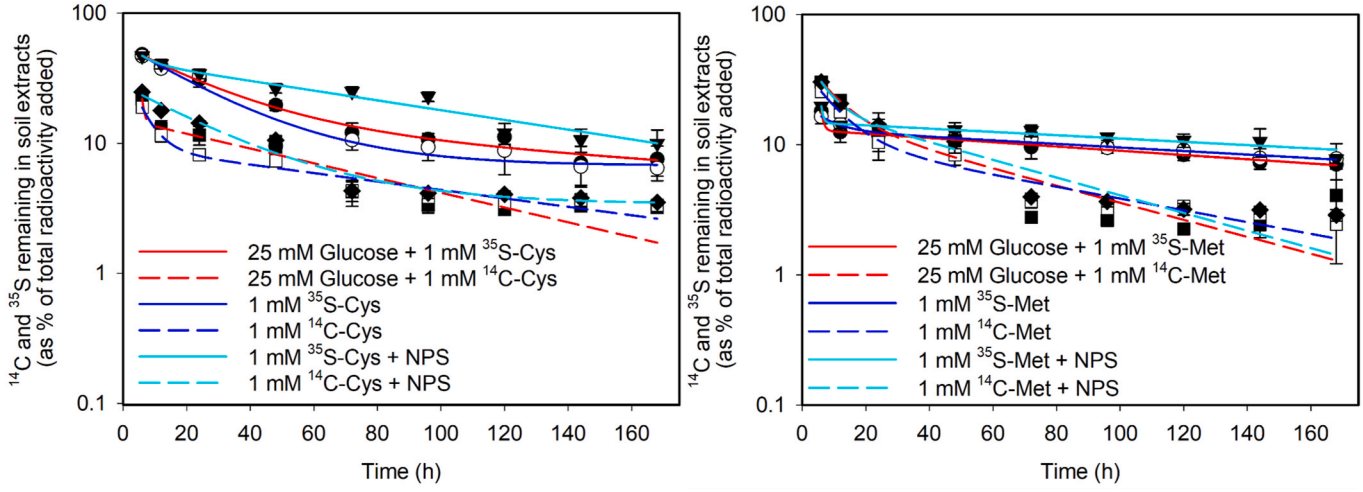

Fig. 2. Recovery of ^{14}C and ^{35}S remaining in soil extracts after addition of Cys or Met, compared to the initial amount added at time zero (100%). Values represent means ± SEM ($n = 3$). Lines represent fits of a double first order exponential decay equation to the experimental data ($r^2 > 0.80$ in all cases; Eq. (1)).

3.4. *Turnover of ^{14}C and ^{35}S in microbial biomass*

The turnover of $^{14}C_{FE}$ and $^{35}S_{FE}$ in the microbial biomass was estimated from the decline of radioisotope in the soil microbial biomass over time. In this study, between 24 and 168 h was chosen to calculate this because isotope incorporation into the microbial biomass between this time-period followed a clear first order exponential decay pattern (Table 2 and Table 3). For the ^{14}C treatment, we focused solely on the production of ^{14}C that was incorporated into microbial biomass, the respired ^{14}C was excluded.

3.4.1. *^{14}C tracer incorporation into microbial biomass and turnover*

24 h after substrate addition, as much as 30% of the ^{14}C-Cys was recovered in $^{14}C_{FE}$, while for ^{14}C-Met, a slightly higher amount (37%)

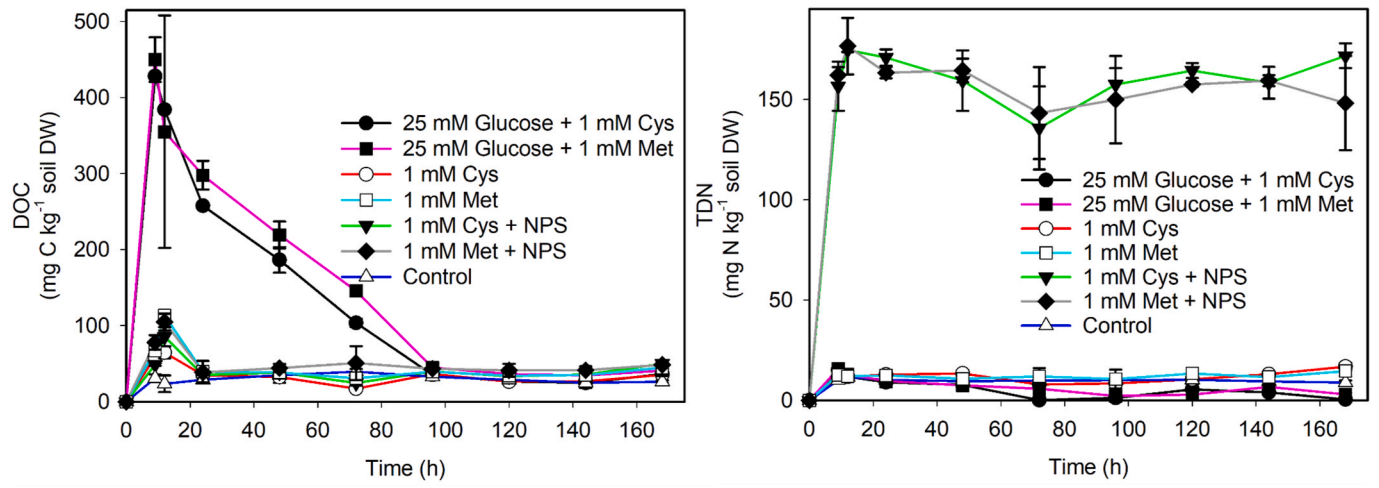

Fig. 3. Dissolved organic carbon (DOC; mg C kg^{-1} DW soil) and N (TDN; mg N kg^{-1} DW soil) concentrations in grassland soil extracts in the absence (control) or presence of either glucose (+G) or nutrients (+NPS). Values represent means ± SEM (n = 3).

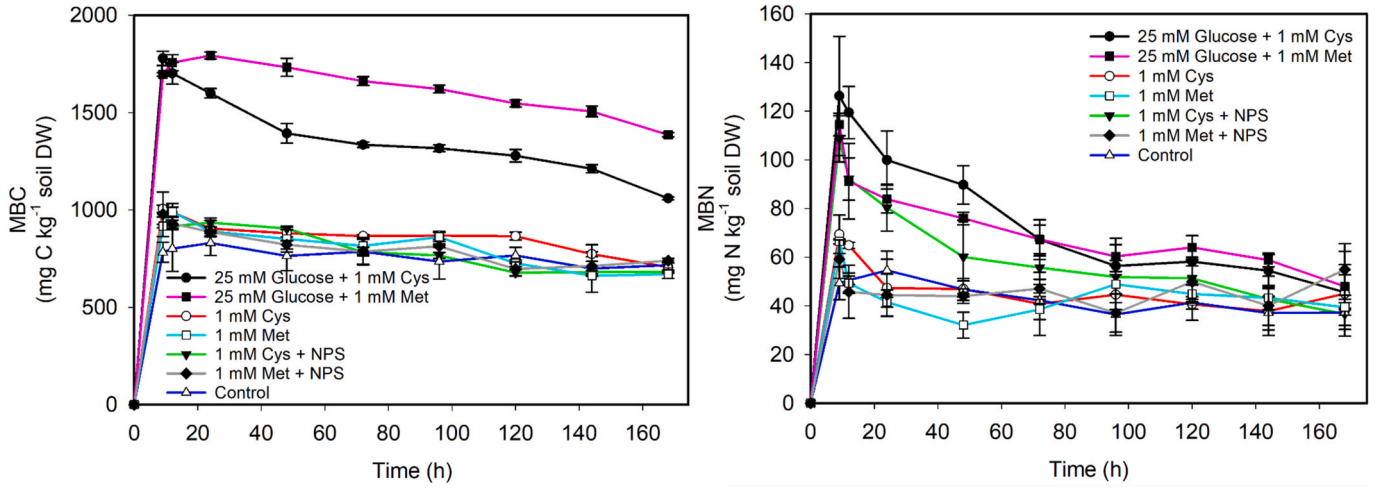

Fig. 4. Microbial biomass carbon (MBC; mg C kg^{-1} DW soil) and N (MBN; mg N kg^{-1} DW soil) concentrations in grassland soil extracts in the absence (control) or presence of either glucose (+G) or nutrients (+NPS). Values represent means ± SEM (n = 3).

Table 2
Turnover of soil microbial biomass ^{14}C derived from ^{14}C-Cys or Met in the absence (control) or presence of either glucose (+G) or nutrients (+NPS). A first order exponential decay equation (Eqn. (1)) was fitted to the experimental data. Values represent means ± SEM (n = 3). The R^2 represents the goodness of equation fit of the experimental data to the model.

Substrate	Treatments	A_0-^{14}C	K–^{14}C	$t_{½}$ (d)	R^2
Cys	+G	21.3 ± 2.8	0.013 ± 0.002	3.7 ± 0.8	0.92
	Control	35.8 ± 7.5	0.008 ± 0.003	6.2 ± 0.5	0.69
	+NPS	39.6 ± 3.9	0.008 ± 0.001	6.5 ± 2.2	0.90
Met	+G	42.4 ± 5.8	0.013 ± 0.002	4.3 ± 0.8	0.91
	Control	49.8 ± 7.4	0.009 ± 0.002	6.3 ± 1.4	0.83
	+NPS	56.3 ± 5.9	0.009 ± 0.001	6.7 ± 1.6	0.91

Table 3
Turnover of soil microbial biomass ^{35}S-Cys or Met derived from ^{35}S-Cys or Met in the absence (control) or presence of either glucose (+G) or nutrients (+NPS). A first order exponential decay equation (Eqn. (1)) was fitted to the experimental data. Values represent means ± SEM (n = 3). The R^2 represents the goodness of equation fit of the experimental data to the kinetic model.

Substrate	Treatments	A_0-^{35}S	K–^{35}S	$t_{½}$ (d)	R^2
Cys	+G	32.2 ± 6.2	0.013 ± 0.003	3.6 ± 1.5	0.83
	Control	43.3 ± 5.5	0.006 ± 0.002	7.4 ± 2.0	0.74
	+NPS	63.5 ± 6.7	0.004 ± 0.001	10.4 ± 2.6	0.73
Met	+G	50.7 ± 3.5	0.005 ± 0.001	7.8 ± 0.6	0.80
	Control	80.8 ± 9.0	0.002 ± 0.001	10.1 ± 3.2	0.67
	+NPS	82.4 ± 5.9	0.003 ± 0.001	15.7 ± 1.4	0.71

was recovered in this pool. The microbial biomass decline was best fitted with an exponential decay equation (Eq. (1)). From this, the calculated half-life for $^{14}C_{FE}$-Met in the biomass was 6.3 d; this was quite similar to that of $^{14}C_{FE}$-Cys (6.2 d). The ^{14}C-Cys and Met recovery in the microbial biomass was close to those from glucose and NPS amended soils, indicating that C or NPS addition did not significantly change the incorporation of ^{14}C-Cys or Met into the soil microbial biomass.

3.4.2. *^{35}S tracer incorporation into microbial biomass and turnover*

3 h after substrate addition, 38.2% of ^{35}S-Cys was recovered in the microbial biomass, while over the same period 67.6% was recovered from the ^{35}S-Met. This result is consistent with $^{35}S_{uf}$ data, where an

opposite trend was found for the two amino acids depletion from soil solution. Clearly, ^{35}S in the microbial biomass was quite labile and active as $^{35}S_{FE}$ turned over rapidly. Specifically, the turnover time of $^{35}S_{FE}$ was longest in soil amended with NPS (10.4 and 15.6 d for Cys and Met, respectively), followed by the control soil (7.4 and 10.1 d), and soil amended with glucose (3.5 and 7.8 d). Overall, the turnover of $^{35}S_{FE}$-Cys was faster than for $^{35}S_{FE}$-Met (Fig. 5, Table 3).

3.5. Inorganic nitrogen (NH_4^+ and NO_3^-) release from Cys and Met decomposition

After a preliminary lag phase of about 24 h, the production of NH_4^+ reached a maximum for all treatments (Fig. 6). During this ammonification period, there was a sharp rise in the rate of CO_2 loss from the soil, indicating an extensive breakdown of the amino acids. The course of ammonification was followed by nitrification, with ca. 70% of the NH_4^+-N being converted to NO_3^--N.

3.6. ^{35}S-sulphate release from Cys and Met decomposition

As sources of soil sulphate, our results showed that Cys is more readily mineralized than Met (Fig. 2). By applying the $BaCl_2$ precipitation method to separate organic and inorganic forms of S, we found that the majority of ^{35}S radioactivity in soil solution was present as sulphate, indicating that immediately after addition of ^{35}S-Cys and Met, ^{35}S was mineralized and released back to the soil as sulphate. This sulphate pool was then gradually incorporated into the microbial biomass or was present as non-extractable organic sulphur compounds.

4. Discussion

4.1. Short term concurrent mineralization of C, N and S derived from Cys and Met

Our results suggested that Cys and Met were both rapidly mineralized to CO_2, NH_4^+, NO_3^-, and SO_4^{2-} in our grassland soil. The short half-lives of ^{14}C and ^{35}S in different compartments found in this study is in line with other studies (Geisseler and Horwath, 2014; Ma et al., 2017). The proportion of amino acid-^{14}C and ^{35}S incorporated into the microbial biomass showed a similar trend through the incubation: $^{14}C_{FE}$ from Cys and Met reached a maximum before our first sampling (3 h after substrate addition), while $^{35}S_{FE}$ reached maximum with a slight lag ($^{35}S_{FE}$ from Cys reached a peak after 9 h, while $^{35}S_{FE}$ from Met reached a peak after 6 h). This indicates that instead of being mineralized outside microbial cell, Cys and Met were taken up intact into the microbial cells.

We provided clear evidence of successive breakdown of added Cys and Met by soil micro-organisms. A rapid loss of amino acid from soil solution was associated with an increase of free NH_4^+ and SO_4^{2-}, as well as a sharp rise in $^{14}CO_2$ release, indicating a thorough deconstruction of added Cys and Met. Subsequently, the ammonium-N produced by the breakdown of the S-containing amino acids in soil becomes available to the nitrifier community leading to NO_3^- production (Verma et al., 2018; Fujii et al., 2020; Ma et al., 2021). After 168 h, 77.3–80.0% of the added amino acid-N had been mineralized to NH_4^+ and NO_3^-. S derived from both Cys and Met was rapidly converted to sulphate, and this conversion was almost complete within 24 h after substrate addition. This is also in line with previous studies (Meena, 2018). Some of the newly released sulphate was then converted to soil organic S through subsequent immobilization by the microbial biomass (Barraclough et al., 2015; Verma et al., 2018). However due to the lack of sensitive and specific methods for detection, identification and estimation of individual organic S compounds remain uncertain and represents an area for future research.

4.2. Cys is a more readily available C and S source than met

Our results indicated that a higher proportion of ^{14}C-Cys was partitioned into microbial catabolic processes while a higher proportion of ^{14}C-Met was partitioned into microbial anabolic processes. Similarly, a higher proportion of ^{35}S-Cys was released as sulphate while a higher proportion of ^{35}S-Met was recovered in the microbial biomass. This difference could be due to the internal metabolic control of amino acid transport and synthesis pathways for individual amino acid by soil micro-organisms. Studies of amino acid uptake and utilization in pure microbial cultures have shown that amino acids are transported into microbial cells according to amino acid group (e.g. neutral, basic, acidic) and isomer-specific membrane transporters (Popova et al., 2003; Tilsner et al., 2005), and therefore metabolized differentially (Bender, 2012). Generally, Cys is converted to pyruvate and sulphate as end products (Freney, 1960), while Met is used to produce a variety of biosynthetic intermediates, S from Met is metabolized using Cys as an intermediate, with the remainder of Met C skeleton converted to propionyl-CoA (Brandt, 2003). In addition, it is possible that the short term availability of Met for soil micro-organisms mineralization is reduced by its metabolic fate of immobilization, since Met could be rapidly incorporated into soil organic matter via the formation of acid-labile peptide bonds (Fitzgerald and Andrew, 1984), as well as into microbial protein (Nader and Walker, 1970). Due to these differences, Cys and Met possess

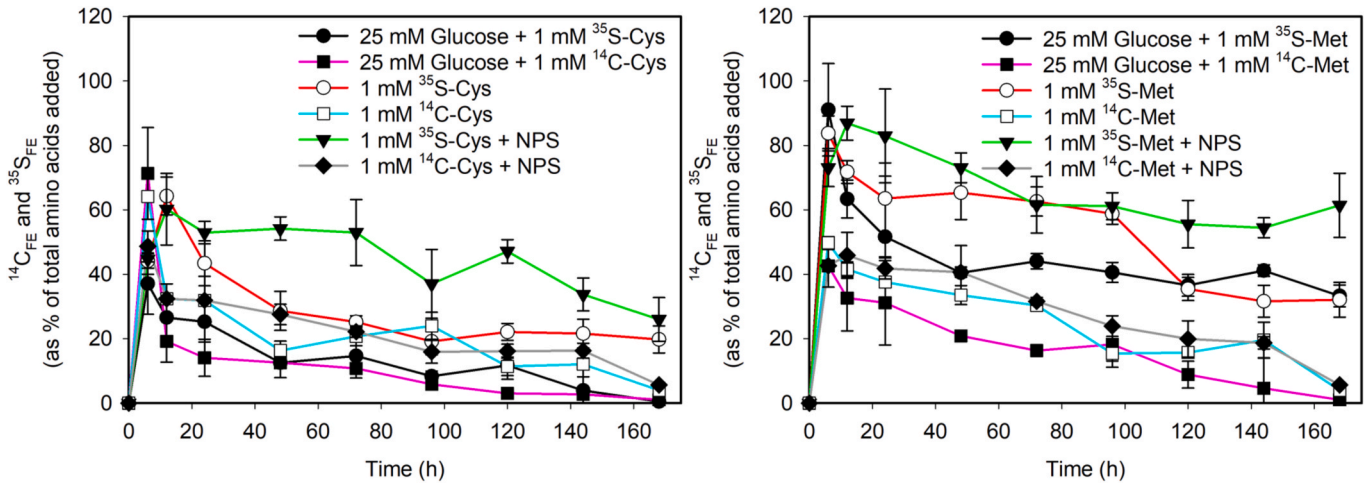

Fig. 5. Fraction of ^{14}C-C_{FE} and ^{35}S-S_{FE} in soil following the addition of Cys and Met to soil in the absence (control) or presence of either glucose (+G) or nutrients (+NPS). Values represent means ± SEM (n = 3). Lines represent ^{14}C-microbial biomass and ^{35}S-microbial biomass obtained by the fumigation-extraction method, adjusted with the extraction factor of 0.35.

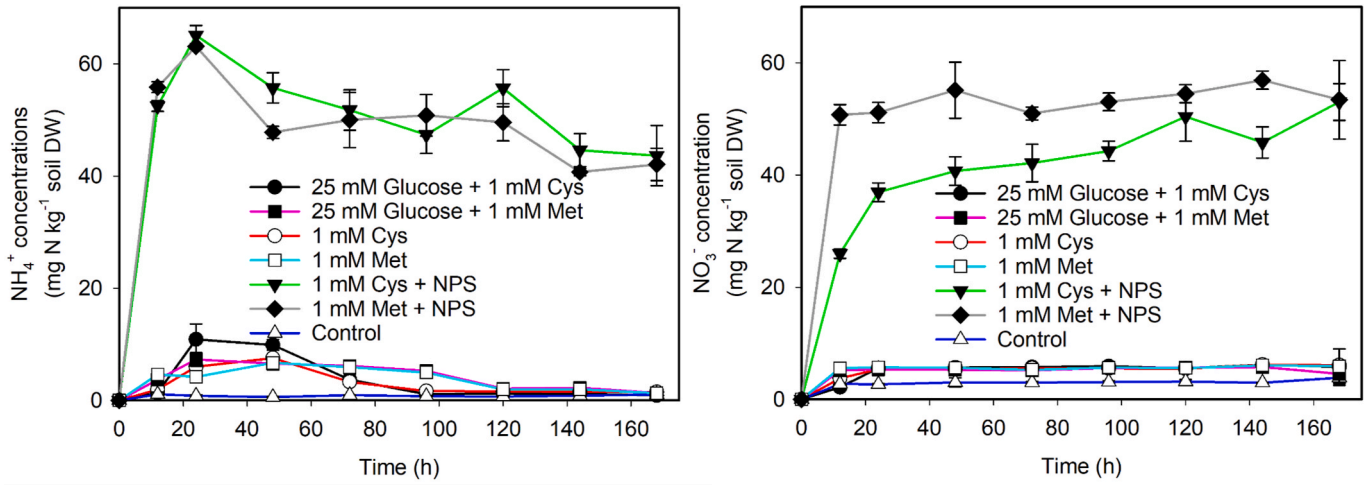

Fig. 6. Generation of ammonium and nitrate from added Cys and Met (1 mM) in soil extracts over a 168 h period. Data expressed on a dry soil weight basis. Values represent means ± SEM (n = 3). Control treatments were included where substrate solution was replaced with distilled water.

clear differences as energy sources for soil micro-organisms.

4.3. Effects of manipulating nutrient availability on mineralization of Cys and Met

Glucose addition promoted greater mineralization of both Cys and Met, while it had less effect on the proportion of ^{14}C incorporated in microbial biomass, resulting in a more complete utilization of both amino acids. Glucose is labile LMW organic compound, which can be easily and immediately assimilated by micro-organisms (Fischer et al., 2010). Therefore addition of glucose is expected to rapidly increase the soil microbial biomass (Schneckenberger et al., 2008) thus resulting in a higher mineralization of Cys and Met. In contrast, nutrient (NPS) addition had less effect on Cys and Met mineralization. We ascribe the lack effect of NPS addition to the low C/N ratio of these amino acids (C/N ratio = 3 for Cys and 5 for Met), compared to a C:N:S ratio of 8:1:0.25 found in bacterial cells (Fagerbakke et al., 1996). Generally, it is reported that the critical point of the C/N ratio with respect to mineralization/immobilization is around 25 (Kumar and Goh, 1999). Materials with a narrow C/N ratio (below 25) are expected to result in net mineralization, whereas materials with wider C/N ratio favour immobilization due to the abundance of C relative to N (Kumar and Goh, 2003).

4.4. Mysterious "missing" ^{35}S

It is also interesting to note that by the end of the incubation there was incomplete recovery of the total ^{35}S added in both amino acids (total recovery <100%). It is possible that this may be associated with analytical errors, including incomplete mixing and sub-sampling of the soil samples. However, some of this 'missing' S could also be due to volatile S emissions from soil (Banwart and Bremner, 1975; Brown et al., 2021), or incorporation into organic matter or absorbed by mineral colloids (Gustafsson et al., 2015; Heinze et al., 2021) and therefore not extractable by 0.01 M $CaCl_2$ in our study. Previous studies have shown that some soils have a substantial capacity for sorption of volatile sulphur suggesting that a heating step may be needed to induce their desorption from soil prior to capture (Banwart and Bremner, 1975; Ko and Chu, 2005; Ko et al., 2006).

5. Conclusions

The decomposition of S-containing amino acids (Cys and Met) to CO_2, NH_4^+, NO_3^-, and SO_4^{2-} was extremely rapid in grassland soil. This is also in agreement with previous studies on the breakdown of LMW organic matter in soil (Roy and White, 2013; Tanikawa et al., 2013; Creamer et al., 2014). The loss of C, N and S from the amino acids is likely to be as a result of mineralization and their later use in the biosynthesis of new cellular biochemicals. e.g. fatty acids, purines, pyrimidines, amino sugars, etc. The different distribution pattern of ^{14}C and ^{35}S in microbial catabolic and anabolic process indicate that C, N and S mineralization from Cys and Met by soil microbials was temporally decoupled at the molecular level.

^{14}C and ^{35}S radio isotopic analysis demonstrated that Cys and Met functioned as nutrient substrates to different extents, and clear differences exist in the degree of C, N and S utilization by soil micro-organisms for this purpose. The evidence is conclusive that there is rapid interconversion of organic and inorganic S forms in the soil, such as mineralization (the transformation of organic S into sulphate) and immobilization (incorporation of sulphate into soil organic compounds or soil microbial biomass). These mineralization and immobilization processes in soil are tightly linked to soil microbial activity, which is regulated by the supply of energy and nutrients.

CRediT authorship contribution statement

Conceptualization, DW, DLJ; Experimentation, DW; Data curation and analysis, DW; Writing of first draft, DW; Review DW, TG, DLJ, DRC; Supervision, TG, DRC, DLJ; Funding acquisition, DLJ, TG, DW.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Deying Wang reports financial support was provided by China Scholarship Council.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108906.

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