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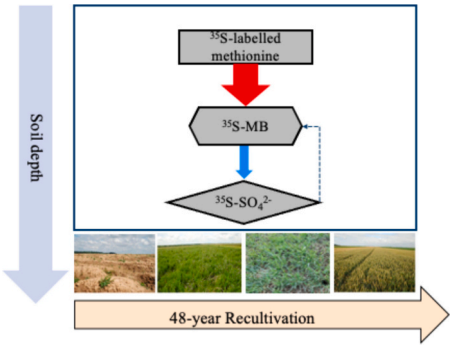
HIGHLIGHTS

- Depletion of ^{35}S-labelled methionine in reclaimed soil profile was evaluated.
- Surface soil SOC and TS content improved significantly after recultivation.
- Immobilisation of organic ^{35}S (^{35}S-MB) in subsurface soils significantly decreased over recultivation.
- Microbial organic S metabolism to sulphate ($^{35}S\text{-}SO_4^{2-}$) decreased significantly in older soil profile.
- Long-term conventional agricultural management had minimal effect on organic S decomposition.

GRAPHICAL ABSTRACT



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ABSTRACT

The significance of sulphur (S) availability for crop yield and quality is highlighted under the global S deficiency scenario. However, little is known about the temporal trend in belowground organic S mineralisation when restoring land to productive agricultural systems, particularly for the deeper soil parts. Therefore, we investigated the decomposition of ^{35}S-labelled methionine in surface (0–30 cm) and subsurface soil (30–60 cm and 60–90 cm) over a 48-year recultivation chronosequence (sampled after1, 8, 14, 24 and 48 years). Soil total sulphur (TS) significantly ($p < 0.05$) increased in surface soil but not in subsurface soils after 48 years of recultivation. Overall, the immobilisation of ^{35}S-methionine (^{35}S-MB) in subsurface soils relative to year 1 significantly decreased over the chronosequence but did not change in the surface samples. The ^{35}S-MB values in subsurface soils were positively corrected with soil carbon (C) stoichiometry (Pearson correlation, $p < 0.05$), suggesting the immobilisation of methionine was likely constrained by microbial C demand in deep soil. Compared to year 1, $^{35}S\text{-}SO_4^{2-}$ released from ^{35}S-methionine significantly declined throughout the older ($\geq$ 8 years) soil profiles. Significant ($p < 0.05$) changes in the organic ^{35}S partition (^{35}S immobilisation and ^{35}S released as sulphate) were observed in year 8 after the soil was recultivated with N-fixing alfalfa or fertilisers.

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Whereas, after that (≥ 14 years), soil organic S partition remained affected when conventional tillage and agricultural crops dominated this site. Indicating that the effect of recultivation on organic S decomposition depends on the manner of recultivation management. Our study contributes to an improved understanding of amino acid S and organic S mineralisation under severe anthropogenic disturbance.

1. Introduction

Sulphur (S) availability in agricultural soil plays an essential role for crop yield quantity and quality, as S is an essential nutrient required for the synthesis of essential amino acids and chlorophyll, as well as the fixation of nitrogen (N) by leguminous plants (Freney and Williams, 1983; Eriksen et al., 2004; Eriksen, 2009). However, due to (1) the application of low-S-containing fertilisers; (2) the decreasing use of S-containing fungicides and pesticides; (3) increasing S removal with the harvest biomass of high-yielding crops (Scherer, 2001; Eriksen et al., 2004); and (4) the reduction in the atmospheric S deposition (Irwin et al., 2002), S deficiency in plants has been common over the last four decades and is considered a constraint for crop production and quality worldwide (Eriksen et al., 2004; Girma et al., 2005; Schonhof et al., 2007; Mascagni et al., 2008), particularly in most Western European countries (Aas et al., 2019). The supply of S has received increased attention since S deficiency symptoms in plants have been observed more frequently (Singh et al., 2020; Ma et al., 2023; Wang et al., 2024). However, there is limited information on the response of belowground microbial-mediated S cycling to anthropogenic disturbance under this S deficiency scenario.

In soils, organic S accounts for >90 % of total S (Ghani et al., 1992; Ghani et al., 1993; Yang et al., 2007). Typically, organic S represents the main S pool that can be mineralised to inorganic S by microorganisms and then contribute to the S nutrition of plants (Zhou et al., 1999). It is thus regarded as a non-directly available S source for plants. However, the significance of organic S has recently been emphasised as about 3 % S-containing amino acid can be utilised by plants directly as an S source (Ma et al., 2021b). Sulphur-containing amino acids (i.e., cysteine, methionine) represent a significant fraction of organic S and the main metabolite used for S mineralisation and utilisation (Dong et al., 2017). Their decomposition reflects microbial S demand and organic S cycling, and can be traced effectively using ^{35}S isotope labelling (Fitzgerald and Watwood, 1988; Ma et al., 2021c; Wang et al., 2023b). Recent evidence revealed that the decomposition of S-containing amino acids includes three key processes as the incubation time increases: firstly, amino acids are immobilised by microorganisms and retained as microbial biomass, secondly, amino acids are mineralised by microorganisms and released into the soil as sulphate, finally, sulphate is reused by microorganisms (Ma et al., 2020; Ma et al., 2021a). Additionally, the addition of excess carbon (C) or N and S has little effect on the microbial immobilisation of S-containing amino acids as low molecular weight organic S compounds are preferred and immobilised by microbes rapidly. However, the added substrate levels have a major effect on the second process (microbial release of S as sulphate) (Ma et al., 2020). In particular, as an essential amino acid, methionine is abundant in biological organisms, as a product and reactant in S-containing metabolic pathways for biomass breakdown and transformation (Fitzgerald and Watwood, 1988; Wang et al., 2023b). Methionine is a good model of more complex organic S compounds and cysteine interconverts with methionine through the transsulfuration pathway. Methionine can thus adequately trace the overall metabolism of organic S (Fitzgerald and Watwood, 1988; Bin et al., 2017).

Despite the importance of organic S, research on low-molecular-weight S-containing amino acids is still lacking. In particular, the microbial decomposition of S-containing amino acids (e.g. methionine) in ecosystems highly affected by human activities is still poorly understood. Recultivation after lignite mining effectively recovers the soil function, structure and fertility by reducing high levels of soil compaction, acidity, and limitations in nutrient availability and the imbalance of soil microbial processes (Allison et al., 2005; Čížková et al., 2018; Feng et al., 2019). This is mainly attributed to the increase in soil organic carbon contents (Graham and Haynes, 2004; Ge et al., 2021), the increase in microbial biomass (Singh et al., 2001), and the diversity of soil microbial communities (Chodak et al., 2009) in the years after recultivation. As soil S mineralisation and immobilisation processes are typically mediated by microbial community (Dedourge et al., 2003; Kertesz and Mirleau, 2004) and are closely related to the supply of organic C, N, and S substrates (Riffaldi et al., 2006; Niknahad Gharmakher et al., 2009), it is reasonable to assume that the recultivation process will affect soil organic S mineralisation in reclaimed soil by regulating soil nutrient status and microbial activity.

During the mining process, the original soil profiles are fully destroyed, at the beginning of the recultivation the soil material is restructured to a depth of 2 m from homogeneous substrate (Feng et al., 2019). Over longer periods after recultivation, the soil materials begin to develop into different layers, which is manifested in differentiations of post-mining material into genetic horizons that differ in biological and physical properties (Lucas et al., 2019; Kozłowski et al., 2022). Generally, soil C and N content in topsoil are higher than in the deeper soil, with >50 % of soil nutrient stock of the soil profile being located in the topsoil (Ahirwal et al., 2017), and decreasing C and N content with increasing soil depths (Liu et al., 2017; Smal et al., 2019). However, the response of soil phosphorus and S cycling to recultivation in the subsurface horizons has no clear consistent trends due to the complexity of reclaimed soil (Šourková et al., 2005; Zhang et al., 2022). In particular, it remains unclear whether the response of S-containing amino acids (e.g. methionine) decomposition in the deeper parts of the soil to recultivation differs from that in surface soil.

Long-term observations are required to analyse recultivation effects, and soil chronosequences have the potential to deliver important insights into the effect of soil management practices and the development of soil microbial and physico-chemical properties over the course of recultivation activities (Roy et al., 2017; Lucas et al., 2019). Therefore, based on an agricultural chronosequence, we aimed to explore how S-containing amino acids decomposed in reclaimed soil profile after different recultivation periods by using ^{35}S isotope labelling. Our main objectives were to: (1) quantify soil S stocks in the reclaimed soil profile after different years of recultivation; and (2) identify the effects of recultivation age on organic S partitioning characteristics in the soil profile.

2. Materials and methods

2.1. Experimental site and design

The study was carried out near Inden, Germany (50°52′44.6″N 6°19′04.4″E), in a post-mining agricultural chronosequence, where decades of open-cast mining process resulted in a > 50-year chronosequence of restored agricultural soils (Lucas et al., 2019; Roy et al., 2022). The average annual temperature is 9.8 °C, with an average annual precipitation of 829 mm. The details of the open-cast mining process are described in Clayton et al. (2021). The dilution ratio of arable soil to loess material was roughly 1:5 in this site according to comparisons of the soil organic carbon (SOC) content before and after removal (Reichel et al., 2017). Former topsoil and deeper parts of the original soil profile, including loess parent material, were mixed for agricultural recultivation. The recultivation process started three

months after soil substrate deposition (Fig. 1b). The recultivation involved a three-phase management. In the first three years (phase 1, Fig. 1c), reclaimed soils were fertilised with 200 kg ha^{-1} per year mineral fertilisers (with a mass ratio of N: P_2O_5: K_2O = 15:15:15) and cultivated with alfalfa (*Medicago sativa* L.). At the end of phase 1, 40 t ha^{-1} green-cutting compost was applied. In the following two years after reclamation (phase 2, Fig. 1d), winter barley was cultivated, and 167 kg N, 150 kg P_2O_5, and 120 kg K_2O were applied every year to recover soil nutrient contents to pre-mining levels. Finally, the reclaimed mining fields were returned to the local farmers (phase 3, Fig. 1e), fields are then conventionally managed with a sugar beet - winter wheat - winter barley crop rotation. In addition, the fields are ploughed to a depth of 30 cm, and standard agricultural practices were followed, in accordance with the German Fertiliser Ordinance (Roy et al., 2017; Clayton et al., 2021; Zhao et al., 2022).

2.2. Soil sampling and nutrient determination

We selected five fields that were restored in 1971, 1995, 2005, 2011, and 2018 (Fig. 1a, Table S1). Soil samples were collected in June 2019, the corresponding recultivation age was 48 years, 24 years, 14 years, 8 years, and 1 year, respectively. We used a sampling scheme with four within-field replicates, which has been shown to be statistically valid in previous studies of the same chronosequence (Roy et al., 2017; Zhao et al., 2022). Four points (vertices of a square with a side length of 50 m) were selected from each field, and soil samples were taken at three depths (0–30 cm, 30–60 cm, 60–90 cm) at each sampling point using a soil auger (⌀ 3.0 cm). Plant roots and stones were removed and the soil was passed through a 2 mm sieve for incubation. Sub-samples were ground and passed through a 0.15 mm sieve to determine the soil nutrient content. Soil total nitrogen (TN) and total sulphur (TS) contents were determined by elemental analysis (vario EL cube; Elementar, Hanau, Germany) (Li et al., 2019). And SOC contents in soil samples were analysed by elemental analysis after acid fumigation with 32 % HCl using the procedure of Walthert et al. (2010).

2.3. Decomposition of ^{35}S-labelled methionine

To recover the soil microbial activity, approximately 200 g air-dried soil samples (< 2 mm) of each recultivation age and soil depth were incubated at 60 % water-holding capacity under room temperature (22 °C) for seven days (Chen et al., 2020; Mo et al., 2021), resulting in soil moisture of 18 % after the pre-incubation (determined by oven drying of a subsample at 80 °C for 48 h). During the incubation, we set 5 different time points (6 h, 24 h, 48 h, 96 h, 144 h) to trace the dynamics of organic S decomposition. Therefore, 300 units of soil samples (including 5 recultivation ages, 3 soil depths, 5 incubation time points, and 4 replicates) were prepared. Specifically, for each unit, 5 g of soil samples were placed in a sterile 50 ml polypropylene centrifuge tube, 1 ml 100 μM ^{35}S-labelled methionine (0.75 kBq ml^{-1}; PerkinElmer Inc., Waltham, MA) was uniformly applied onto the soil surface. At each time point, 60 units (5 recultivation ages, 3 soil depths, 4 replicates) of soil samples were destructively harvested and extracted with 25 ml 0.01 M $CaCl_2$ (200 rpm for 30 mins). The extracts were then centrifuged (18,000 *g*, 5 mins) and ^{35}S activity in 1 ml supernatant was identified as $^{35}S_{CaCl2}$. To determine the ^{35}S-SO_4^{2-} content, 0.75 ml $BaCl_2$ was added to 0.75 ml of the $CaCl_2$ extract to precipitate the $^{35}SO_4^{2-}$ (Wang et al., 2023b), and the suspensions were centrifuged at 18000 *g* for 5 min and the ^{35}S activity in 1 ml suspension was identified as $^{35}S_{CaCl2+BaCl2}$. Due to the precipitation of $BaSO_4$, the differences in ^{35}S activity between the $CaCl_2$ extractions ($^{35}S_{CaCl2}$) and $BaCl_2$ treatments ($^{35}S_{CaCl2+BaCl2}$) were taken as the amount of ^{35}S-SO_4^{2-} derived from labelled methionine, this method was found feasible and used by Ma et al. (2021a) and Wang et al. (2023b):

$$^{35}S - SO_4{}^{2-} = {}^{35}S_{CaCl2} - {}^{35}S_{CaCl2+BaCl2}.$$

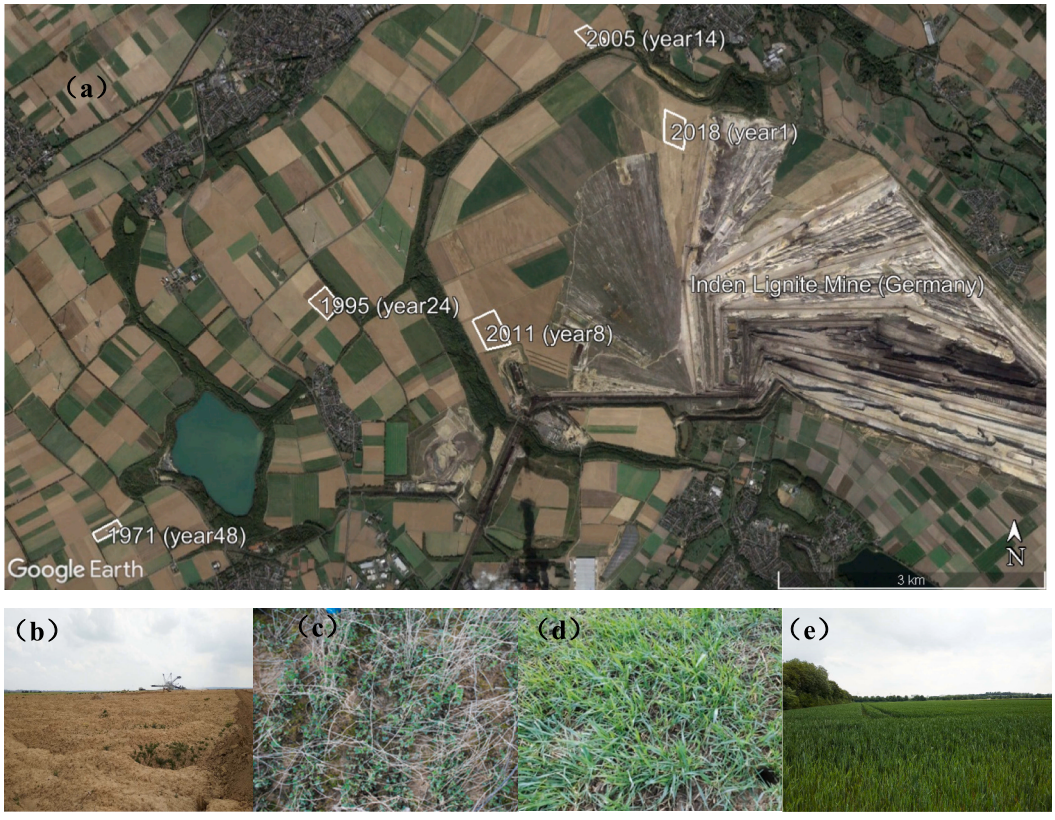

Fig. 1. Spatial distribution of sampling fields across the recultivation chronosequences (a) and the pictures of the fields under different phases of the recultivation periods. The beginning of the recultivation (b), phase 1 of the recultivation (c), phase 2 of the recultivation (d), phase 3 of the recultivation (e).

^{35}S activity was measured by a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK) after mixing 4 ml Hisafe 3 scintillation cocktail (Fisher Scientific, Loughborough, UK). The ^{35}S activity was calculated as the percentage of the total ^{35}S added.

$$^{35}S\ \text{activity}\ (\%) = \frac{Measured\ 35S\ activity\ (kBq)}{Total\ 35S\ decay(kBq)} \times 100.$$

Total $^{35}S_{decay}$ indicated the corrected ^{35}S activity after natural radioactive decay, where t is the time (h).

$$\text{Total}^{35}S_{decay}\ (\text{kBq}) = 0.75\ (kBq) \times \left(exp^{\frac{t}{24}} \Big/ 87.51 \right)$$

Due to the very low sorption of methionine and sulphate to the solid phase (Rothstein, 2010; Ma et al., 2022; Wang et al., 2024), the difference between the total added ^{35}S-methionine and the ^{35}S extracted by $CaCl_2$ at each time point was thus considered as the ^{35}S that cannot be extracted and immobilised in the microbial biomass (^{35}S-MB) (Ma et al., 2021a; Wang et al., 2023b).

$$^{35}S - MB = {}^{35}S_{total} - {}^{35}S_{CaCl2}$$

2.4. Statistical analysis

Using SPSS Statistics 22 software (SPSS Inc., Chicago, USA), one-way ANOVA (Duncan test; $p < 0.05$) was carried out to compare S partitioning to ^{35}S-MB and ^{35}S-SO_4^{2-} at different recultivation ages and three soil depths. Two-way ANOVA analysis was conducted to analyse the effects of the recultivation period and soil depths and their interactions on methionine decomposition at each time point during the incubation. Pearson correlation analysis was conducted to investigate the links between soil chemical properties and the decomposition of methionine. Figures were mapped using the "ggplot2" package in R 4.2.1.

3. Results

3.1. Soil C, N, S content and their stoichiometric ratios after different years of recultivation

After 48-year recultivation, soil TN, SOC and TS contents in surface soil (0–30 cm) significantly increased by 199 %, 187 % and 55 %, respectively (Table 1). The SOC to TN ratio (SOC:TN), SOC to TS ratio (SOC:TS) and TN to TS ratio (TN:TS) changed from 7.83, 23, 2.93 to 7.56, 42 and 5.62, respectively. Notably, a significant increase in SOC, TN and TS contents and their stoichiometric ratios was observed in year 8 ($p < 0.05$).

In subsurface soils (30–60 cm, 60–90 cm), relative to year 1, in year 8, soil TN and SOC contents significantly increased by 26 % and 22 % in 30–60 cm and by 26 % and 11 % in 60–90 cm (Table 1). After that, the SOC and TN contents decreased gradually along the soil chronosequence and reached the level of the beginning of the recultivation (year 1) at age 48. The SOC:TN and SOC:TS ratios significantly decreased over 48-year recultivation, while recultivation had no significant impact on subsurface TS content and TN:TS ratio. Additionally, soil SOC, TN, TS contents and their stoichiometry in surface soil were significantly higher than in the subsurface soils in older soils (recultivation age > 1 year).

3.2. Immobilisation of ^{35}S (^{35}S-MB) after different years of recultivation

The content of ^{35}S in microbial biomass (^{35}S-MB) peaked in <48 h at all soil depth, afterwards ^{35}S-MB declined and was released as ^{35}S-SO_4^{2-} (Fig. 2). Overall, the recultivation age had no significant effect on ^{35}S-MB in surface soil, but ^{35}S-MB in the subsurface soils decreased gradually along the soil chronosequence (Fig. 2B, C). Specifically, relative to age 1 (52.1 % on average), the ^{35}S-MB decreased to 41.6 % (age 8), 42.4 % (age 14), 41.9 % (age 24) and 39.3 % (age 48) as an average of the two subsurface depth intervals after 144 h incubation (Fig. 2, Fig. S1e). Additionally, the ^{35}S-MB in older (recultivation age > 1 year) surface soils were significantly higher than in the subsurface soils (Fig. S1a).

Soil depth had a greater effect on the ^{35}S-MB than the recultivation age (Table 3, Fig. S1a). Pearson correlation analysis (Table 4) showed that in the subsurface soils, the ^{35}S-MB was positively corrected with SOC:TN and SOC:TS ratios with higher r values ($p < 0.05$) during the first hours of incubation (< 48 h).

3.3. ^{35}S released as sulphate (^{35}S-SO_4^{2-}) after different years of recultivation

Within 24 h, <10 % of ^{35}S-SO_4^{2-} was released from ^{35}S-methionine in surface soils (Fig. 3). Overall, the released ^{35}S-SO_4^{2-} amount significantly decreased in the older soil profiles. Specifically, in year 1, after 144 h incubation, >30 % of the ^{35}S-methionine was released as ^{35}S-SO_4^{2-} throughout the profile, but the amount of released ^{35}S-SO_4^{2-} decreased significantly ($p < 0.05$) to 9 % (0–30 cm in year 8), 4 % (30–60 cm in year 14) and 13 % (60–90 cm in year 14), respectively (Fig. 3) and no significant changes in ^{35}S-SO_4^{2-} release were observed at recultivation ages >14 years. An effect of depth on the ^{35}S-SO_4^{2-} release was observed at recultivation age 8 (Fig. 3, Fig. S2c, d, e), when the released ^{35}S-SO_4^{2-} amounts (in 144 h) in the subsurface soils (44 % in 30–60 cm, 35 % in 60–90 cm, Table 2) were significantly higher than ^{35}S-SO_4^{2-} in the surface soil (9.31 %).

Table 1
Soil TN, SOC and TS contents and their stoichiometric ratios in soil profiles after different years of recultivation.

Soil depth (cm)	Recultivation age	TN (g kg^{-1})	SOC (g kg^{-1})	TS (g kg^{-1})	SOC:TN	SOC:TS	TN:TS
0–30	1	0.32 ± 0.00cA	2.49 ± 0.06cA	0.11 ± 0.004dA	7.83 ± 0.16aA	23.0 ± 0.97bA	2.93 ± 0.13cA
	8	0.64 ± 0.06bA	5.36 ± 0.52bA	0.13 ± 0.004cA	8.42 ± 0.24aA	41.4 ± 2.77aA	4.91 ± 0.30abA
	14	0.58 ± 0.03bA	4.84 ± 0.41bA	0.13 ± 0.003bcA	8.33 ± 0.33aA	36.5 ± 2.70aA	4.37 ± 0.22bA
	24	0.71 ± 0.02bA	6.02 ± 0.36abA	0.15 ± 0.005bA	8.53 ± 0.57aA	41.5 ± 2.05aA	4.91 ± 0.30abA
	48	0.95 ± 0.08aA	7.15 ± 0.50aA	0.17 ± 0.009aA	7.56 ± 0.24aA	42.3 ± 0.82aA	5.62 ± 0.24aA
30–60	1	0.31 ± 0.01bA	2.44 ± 0.07abA	0.11 ± 0.002aA	7.86 ± 0.07aA	22.2 ± 0.38abA	2.83 ± 0.07aA
	8	0.39 ± 0.01aB	2.97 ± 0.23aB	0.14 ± 0.026aA	7.51 ± 0.31aB	23.5 ± 4.08aB	3.10 ± 0.49aB
	14	0.31 ± 0.03bB	2.19 ± 0.33abB	0.12 ± 0.001aB	6.90 ± 0.55abB	18.6 ± 2.63abB	2.66 ± 0.22aB
	24	0.32 ± 0.03bB	2.04 ± 0.31bB	0.14 ± 0.025aA	6.29 ± 0.40bcB	15.5 ± 3.49abB	2.41 ± 0.40aB
	48	0.32 ± 0.02bB	1.80 ± 0.22bB	0.14 ± 0.029aAB	5.57 ± 0.41cB	13.7 ± 1.04bB	2.53 ± 0.32aB
60–90	1	0.31 ± 0.02bA	2.50 ± 0.09abA	0.10 ± 0.001aA	8.16 ± 0.26aA	24.2 ± 1.12abA	2.97 ± 0.19bA
	8	0.39 ± 0.02aB	2.78 ± 0.17aB	0.11 ± 0.009aA	7.09 ± 0.19abB	25.9 ± 2.20aB	3.66 ± 0.29aB
	14	0.30 ± 0.04bB	1.92 ± 0.36bcB	0.11 ± 0.002aC	6.40 ± 0.43bB	18.0 ± 3.07bcB	2.77 ± 0.33bB
	24	0.30 ± 0.02bB	1.86 ± 0.25bcB	0.11 ± 0.007aA	6.19 ± 0.37bcB	16.3 ± 1.46cB	2.61 ± 0.10bB
	48	0.29 ± 0.03bB	1.51 ± 0.35cB	0.11 ± 0.008aB	5.00 ± 0.67cB	13.8 ± 1.98cB	2.75 ± 0.06bB

Different lower and upper letters indicate significant differences among the different recultivation ages and depths at $p < 0.05$. Values represent means ± SEM. TN: total nitrogen, SOC: soil organic carbon, TS: total sulphur, SOC:TN: total organic carbon to total nitrogen ratio, SOC:TS: total organic carbon to total sulphur ratio, TN:TS: total nitrogen to total sulphur ratio.

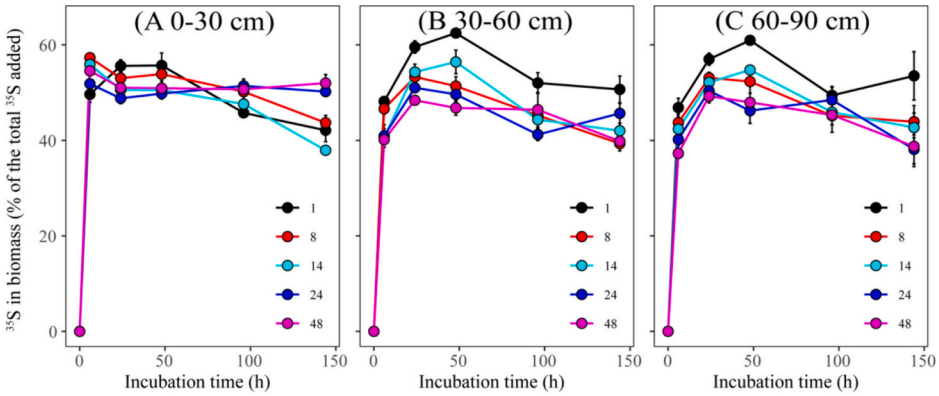

Fig. 2. The time course of the proportion of ^{35}S immobilised in soil microbial biomass in the reclaimed 0–30 cm (A), 30–60 cm (B), and 60–90 cm (C) soil after different years of recultivation. Data are means ± SEM, $n = 4$.

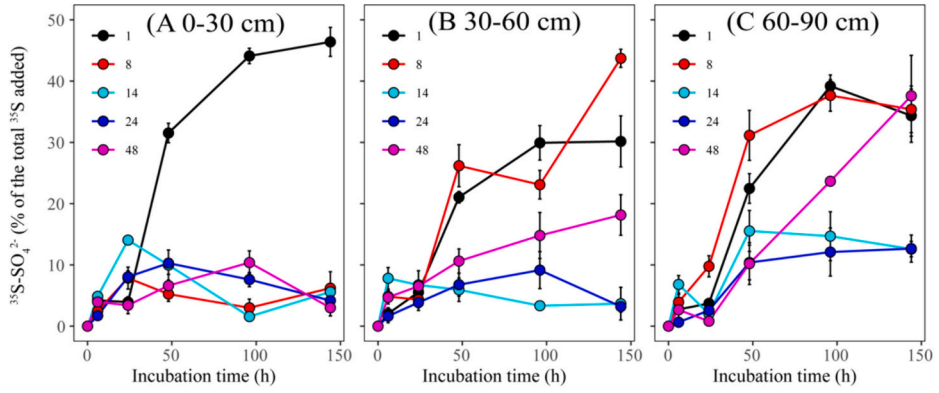

Fig. 3. The time course of the proportion of ^{35}S-SO_4^{2-} released from ^{35}S-labelled methionine in the reclaimed 0–30 cm (A), 30–60 cm (B), and 60–90 cm (C) soil after different years of recultivation. Data are means ± SEM, n = 4.

Recultivation age had a greater impact on the ^{35}S-SO_4^{2-} than soil depth or the interaction effects of the recultivation age and soil depth (Table 3). In surface soil, the ^{35}S-SO_4^{2-} was negatively corrected with SOC, TN, and TS contents, and SOC:TS and TN:TS ratios at 48 h, 96 h and 144 h (Table 4). However, in subsurface soils, the correlation between ^{35}S-SO_4^{2-} and SOC, TN, TS and their stoichiometric ratios were weaker than in the surface soils.

4. Discussion

4.1. Recultivation age affects the soil C, N and S content in the soil profile

Recultivation age is the main driving force for the restoration of soil properties and microbial communities in mining areas, while agricultural management, e.g. fertilisation, can shorten the recovery time of the reclaimed soil (Wang et al., 2020). In our study, the impacts of recultivation on soil C, N and S were mainly manifested in two different ways: in the first 8 years (phases 1 and 2, the soil was recultivated with alfalfa and mineral fertiliser was applied), recultivation significantly improved soil nutrient status in the surface soil. This is consistent with earlier studies, which showed that recultivation age had a positive relationship with soil nutrient content in soil chronosequences (Shrestha and Lal, 2010; Huang et al., 2011; Zhao et al., 2013; Liu et al., 2017). As reported by Reichel et al. (2017) in the same area as our study, a highly structured and stable microbial food web has been established after 3 years of alfalfa cultivation, which further benefits nutrient and SOC storage. This is mainly attributed to the application of compost or the cultivation of alfalfa with narrow C:N ratios, both of which are believed to accelerate the accumulation of soil organic matter and nitrogen by boosting microbial communities (Pihlap et al., 2019; Otremba et al., 2020).

However, after the field was returned to the local farmers (recultivation >8 years), the continuous conventional agricultural management did not further improve soil TN and TS content, even leading to the decline of SOC and nutrient contents in subsurface soils. This finding was supported by results from Mihelič et al. (2024), who found that SOC levels remained constant under long-term conventional tillage. This can be attributed to the following reasons: (i) greater C mineralisation may be potentially induced by the larger disturbance from conventional tillage (Liu et al., 2023), and (ii) according to the findings from previous studies in this field (Roy et al., 2017; Roy et al., 2022), long-term conventional farming may limit the development of functional fungi (e.g. arbuscular mycorrhizal) correlating positively with nutrient content, i.e. with the decline nutrient contents also arbuscular mycorrhizal richness declines, resulting in less C accumulated belowground. However, iii) during conventional management, the slight decline of SOC along the chronosequence in subsoils might also result from crop harvest without extra carbon input (Table 1). The underlying mechanisms that control soil C content in these deep layers of the reclaimed agricultural systems require further study. In addition, the significantly lower SOC content, SOC:TN and SOC:TS ratios (Table 1) in subsurface soils indicated less available C for microbial growth relative to surface soil. The subsurface soil is less densely rooted and also receives less input from crop residues, leading to C limitation as reported in cultivated (Schneider et al., 2020) and agroforestry systems (Siegwart et al., 2023). It can be concluded that long-term conventional farming may not benefit C and S accumulation relative to recultivation with alfalfa and fertilisers, especially in subsurface soils.

Table 2
Effects of soil depth and the recultivation age on ^{35}S in microbial biomass (^{35}S-MB) and ^{35}S-SO_4^{2-} production at each incubation time after the ^{35}S-methionine addition.

Organic S partition	Depths (cm)	Recultivation age (Year)	Incubation time (h)				
			6	24	48	96	144
		1	49.6 ± 1.65 c A	55.6 ± 1.28 a A	55.7 ± 2.61 a B	45.8 ± 0.41 b B	42.1 ± 2.40 b A
		8	57.3 ± 0.32 a A	53.0 ± 1.07 ab A	53.9 ± 0.96 ab A	50.2 ± 1.50 ab A	43.7 ± 1.54 b A
^{35}S-MB	0–30	14	55.9 ± 2.09 a A	50.6 ± 1.21 b A	50.5 ± 1.87 ab A	47.6 ± 1.59 ab A	37.9 ± 0.69 b A
		24	51.9 ± 0.35 bc A	48.8 ± 1.12 b A	49.8 ± 0.66 b A	51.4 ± 1.40 a A	50.2 ± 1.02 a A
		48	54.6 ± 0.73 ab A	51.0 ± 2.03 b A	50.9 ± 1.55 ab A	50.7 ± 2.15 ab A	52 ± 1.78 a A
		1	48.2 ± 0.47 a A	59.6 ± 1.29 a A	62.5 ± 0.97 a A	52.0 ± 2.17 a A	50.7 ± 2.81 a A
		8	46.6 ± 1.14 a B	53.3 ± 0.71 b A	51.4 ± 1.95 bc A	45.2 ± 1.31 ab B	39.3 ± 1.52 b A
^{35}S-MB	30–60	14	40.6 ± 1.87 b B	54.3 ± 1.65 b A	56.4 ± 2.46 b A	44.3 ± 2.53 ab A	42.0 ± 1.53 b A
		24	40.9 ± 2.36 b B	51.0 ± 0.79 bc A	49.6 ± 1.62 c A	41.2 ± 1.25 b B	45.7 ± 2.09 ab A
		48	40.2 ± 1.63 b B	48.4 ± 0.98 c A	46.8 ± 1.54 c A	46.4 ± 3.61 ab A	39.8 ± 1.36 b B
		1	46.9 ± 1.97 a A	57 ± 1.27 a A	61.0 ± 0.43 a AB	49.4 ± 1.86 a AB	53.5 ± 5.07 a A
		8	43.7 ± 0.80 ab C	53.1 ± 1.17 ab A	52.3 ± 2.44 b A	45.2 ± 0.39 a B	43.9 ± 3.40 ab A
^{35}S-MB	60–90	14	42.4 ± 3.08 abc B	52.2 ± 2.02 b A	54.8 ± 0.86 ab A	46.0 ± 2.64 a A	42.7 ± 3.01 ab A
		24	40.2 ± 1.28 bc B	50.4 ± 1.37 b A	46.2 ± 1.05 b A	48.5 ± 2.61 a AB	38.1 ± 3.05 b B
		48	37.3 ± 1.02 c B	49.3 ± 1.39 b A	47.96 ± 4.40 b A	45.3 ± 3.62 a A	38.7 ± 4.24 b B
		1	4.23 ± 0.75 ab A	3.93 ± 0.57 a A	31.5 ± 1.58 a A	44.1 ± 1.26 a A	46.4 ± 2.36 a A
		8	2.47 ± 0.85 bc A	7.86 ± 1.79 a A	5.24 ± 1.18 b B	3 ± 1.42 c C	9.31 ± 2.69 b B
^{35}S-SO_4^{2-}	0–30	14	4.87 ± 0.72 a A	14.1 ± 0.62 a A	9.97 ± 2.46 b A	1.57 ± 0.53 c B	5.60 ± 0.40 b A
		24	1.72 ± 0.15 c AB	8.05 ± 0.97 a A	10.3 ± 0.31 b A	7.61 ± 1.15 b A	4.18 ± 1.26 b B
		48	3.96 ± 0.71 abc A	3.42 ± 1.38 a A	6.62 ± 1.84 b A	10.4 ± 1.91 b B	3.01 ± 1.33 b B
		1	2.04 ± 0.96 b A	5.55 ± 0.99 a A	21.1 ± 0.94 a B	29.9 ± 2.82 a B	30.2 ± 4.17 b B
		8	4.83 ± 0.70 b A	4.33 ± 1.75 a A	26.2 ± 3.43 a A	23.1 ± 2.33 ab B	43.7 ± 1.48 a A
^{35}S-SO_4^{2-}	30–60	14	7.81 ± 1.76 a A	6.73 ± 0.56 a A	5.94 ± 1.90 b A	3.34 ± 0.44 d B	3.67 ± 2.67 d A
		24	3.28 ± 1.08 ab A	3.86 ± 0.83 a AB	6.77 ± 1.87 b A	9.15 ± 3.02 cd A	3.21 ± 0.91 d B
		48	4.72 ± 1.09 ab A	6.52 ± 2.53 a A	10.6 ± 1.98 b A	14.8 ± 3.74 bc B	18.1 ± 3.31 c AB
		1	2.73 ± 1.47 ab A	3.71 ± 0.81 b A	22.5 ± 2.42 ab B	39.2 ± 1.82 a A	34.4 ± 4.34 a B
		8	3.92 ± 1.26 ab A	9.77 ± 1.72 a A	31.1 ± 4.08 a A	37.7 ± 2.56 a A	35.4 ± 3.80 a A
^{35}S-SO_4^{2-}	60–90	14	6.82 ± 1.44 a A	2.03 ± 0.61 b A	15.5 ± 3.35 b A	14.7 ± 3.99 bc A	12.6 ± 1.24 b A
		24	0.64 ± 0.23 b B	2.58 ± 1.59 b B	10.4 ± 2.83 b A	12.1 ± 3.92 c A	12.7 ± 2.21 b A
		48	2.67 ± 1.60 ab A	0.81 ± 0.28 b A	10.2 ± 3.41 b A	23.7 ± 0.19 b A	37.6 ± 6.60 a A

Different lowercase and uppercase letters indicate significant differences among different recultivation periods and three depths at $p < 0.05$, respectively. Values represent means ± SEM.

Table 3
Two-way ANOVA analysis of ^{35}S-labelled methionine depletion in soils after different years of recultivation.

	Time after ^{35}S-methionine addition (h)	*F*-test		
		Soil depth	Recultivation age	Soil depth*Recultivation age
^{35}S-SO_4^{2-}	6	1.374	5.946**	1.014
	24	2.712	1.483	1.734
	48	5.079*	23.334**	8.550**
	96	30.435**	71.127**	10.634**
	144	15.183**	47.016**	10.958**
^{35}S-MB	6	81.456**	6.171**	4.151**
	24	1.62	16.200**	1.078
	48	0.476	14.797**	1.851
	96	2.792	0.822	2.039
	144	0.562	3.310*	4.064**

Value with * and ** represent significance at $p < 0.05$ and $p < 0.01$, respectively.

4.2. Effects of recultivation age on the immobilisation of ^{35}S-labelled methionine (^{35}S-MB)

Methionine is a low-molecular-weight source of organic S, and the decomposition of methionine includes immobilisation and mineralisation processes. Immobilisation is the initial process for methionine decomposition as most of the methionine is taken up by microbes quickly (Fitzgerald and Watwood, 1988; Wang et al., 2023a), while with increasing incubation time, S content in microbial biomass decreased and was released as sulphate (Ma et al., 2023; Wang et al., 2023b). Our results indicated that despite significant changes in SOC content in the surface soil after recultivation, the microbial uptake of organic S was mainly independent of recultivation age. This can be explained by findings from Ma et al. (2020) and Ma et al. (2021a), who found that the short-term microbial uptake of organic S is determined by the amino acid structure and is less affected by the addition of extra C and nutrients.

However, immobilisation of ^{35}S-methionine in subsurface soils was lower than in the surface soil and was significantly decreased along the soil chronosequence. Especially the short-term immobilisation of organic S into microbial biomass was highly affected by soil depth rather than recultivation age (Table 3). In our study, the microbial uptake of organic S in subsurface soils was highly correlated with soil C content (Table 4), and ^{35}S-MB decreased with decreasing amounts of SOC. The availability of C as the energy source that controls microbial growth is one of the key factors governing S cycling (Niknahad Gharmakher et al., 2009). As a result, factors that change the C cycling influence S turnover (McGill and Cole, 1981; Kumar et al., 2022). Here, subsurface soils became C limited as SOC content declined with increasing recultivation age, which further led to the decrease in ^{35}S-MB. Our assumption is supported by research at the same site as our study (Clayton et al., 2021), which showed that at SOC levels below 1 %, the microbial biomass was out of stoichiometric equilibrium with a lower carbon immobilisation efficiency due to high maintenance respiration. In

Table 4
Pearson correlation analysis between soil properties and ^{35}S-labelled methionine decomposition in soil profiles.

Soil depth (cm)	Variables	^{35}S-MB (r value)					^{35}S-SO_4^{2-} (r value)				
		6 h	24 h	48 h	96 h	144 h	6 h	24 h	48 h	96 h	144 h
	TN	0.418	−0.341	−0.299	0.630**	0.764*	−0.274	−0.085	−0.792**	−0.619**	−0.855**
	SOC	0.480*	−0.306	−0.316	0.672**	0.772**	−0.280	−0.045	−0.835**	−0.705**	−0.860**
	TS	0.287	−0.298	−0.296	0.465*	0.792**	−0.116	−0.110	−0.658**	−0.489*	−0.770**
	SOC:TN	0.255	0.128	−0.059	0.197	0.024	0.007	0.188	−0.177	−0.317	−0.203
	SOC:TS	0.601**	−0.312	−0.346	0.734**	0.640**	−0.346	0.047	−0.890**	−0.844**	−0.875**
0–30	TN:TS	0.513*	−0.390	−0.348	0.706**	0.652**	−0.350	−0.021	−0.857**	−0.757**	−0.845**
	TN	0.367	0.066	0.033	0.301	−0.323	0.229	−0.311	0.320	0.055	0.471
	SOC	0.644**	0.416	0.428	0.444	−0.045	0.200	−0.186	0.456	0.348	0.593*
	TS	−0.333	−0.221	−0.225	−0.120	−0.181	0.172	−0.552*	−0.265	−0.229	−0.271
	SOC:TN	0.758**	0.667**	0.717**	0.497*	0.266	0.045	−0.063	0.495	0.558*	0.588**
	SOC:TS	0.752**	0.506*	0.478*	0.452	0.037	0.011	0.167	0.580*	0.499	0.677**
30–60	TN:TS	0.533*	0.253	0.185	0.277	−0.136	−0.033	0.272	0.502*	0.345	0.585*
	TN	0.499*	0.43	0.245	0.151	0.182	0.324	0.641**	0.573*	0.361	0.028
	SOC	0.692**	0.678**	0.557*	0.257	0.459	0.315	0.647**	0.594*	0.493*	0.026
	TS	−0.088	0.136	0.020	0.177	0.028	0.011	−0.151	−0.136	−0.174	−0.064
	SOC:TN	0.695**	0.780**	0.775**	0.309	0.698**	0.266	0.442	0.436	0.486*	−0.022
	SOC:TS	0.757**	0.688**	0.603**	0.215	0.517*	0.338	0.726**	0.714**	0.619**	0.077
60–90	TN:TS	0.543*	0.367	0.225	0.067	0.206	0.324	0.713**	0.710**	0.523*	0.134

Correlation coefficient (r) with * and ** represent significance at $p < 0.05$ and $p < 0.01$, respectively. TN: total nitrogen, SOC: soil organic carbon, TS: total sulphur, SOC:TN: soil organic carbon to total nitrogen ratio, SOC:TS: soil organic carbon to total sulphur ratio, TN:TS: total nitrogen to total sulphur ratio.

conclusion, the increase in SOC in surface soil had little effect on microbial immobilisation of low-molecular-weight S-containing amino acids, but ^{35}S-MB was negatively affected by the decrease of SOC content in subsurface soils when the SOC is under a very low level over recultivation.

4.3. Effects of recultivation age on the released sulphate (^{35}S-SO_4^{2-}) in the soil profile

In the present study, a decline of sulphate release was observed both in surface soil and subsurface soils along the chronosequence. A significant decrease in ^{35}S-SO_4^{2-} was observed from year 1 to year 8, while no significant change beyond 14 years of recultivation occurred when conventional tillage and agricultural crops dominated this site. It indicated that the change in ^{35}S-SO_4^{2-} is mainly attributed to the effects from the early phases of recultivation management (alfalfa and adding fertilisers) and the subsequent conventional agricultural management had little effect on the microbial mineralisation of organic S into sulphate.

However, the changes in ^{35}S-SO_4^{2-} still differed between surface soil and subsurface soil. This was consistent with the finding from Fitzgerald and Andrew (1984), suggesting that the conversion process of methionine-S to sulphate depends on the incubation time and soil depths. Compared to the decline of sulphate release in surface soil after 8 years of recultivation, the decline of ^{35}S-SO_4^{2-} in subsurface soils was observed only after 14 years of recultivation, indicating that a lag in microbial response to the recultivation processes in subsurface soils. Additionally, Pearson correlation analysis indicated that the underlying controls of the declined ^{35}S-SO_4^{2-} release were different in surface and subsurface soils. In surface soil, the ^{35}S-SO_4^{2-} was highly correlated with soil SOC content and nutrient status. We, therefore, assume that the mineralisation of organic S into inorganic sulphate resulted from biological S mineralisation, which is driven by the microbial need for organic C to provide energy (Scherer, 2009). Higher SOC content in surface soils indicated a higher C: nutrient ratio (Table 1), which can induce microbial immobilisation of excess C. Further, according to ecological stoichiometry theory (Mooshammer et al., 2014; Sterner and Elser, 2002), to maintain microbial stoichiometric equilibrium under excess C immobilisation (microbial biomass C:S ratio), S-containing methionine will be primarily immobilised as biomass and accordingly, less S was released as sulphate from methionine in older surface soils. Our assumption was supported by the finding from Ma et al. (2021c), showing that microbial decomposition of organic S is driven by C demand rather than S demand. However, relative to surface soil, the release of sulphate was less correlated with soil C availability or nutrient content in subsurface soils, which was similar to the finding from Moradi et al. (2020), who found that soil depth highly explained the variation of soil microbial biomass under reclamation, and soil C is not the only limiting factor for the microbial community due to the complexity of subsurface reclaimed soil. Two hypotheses can explain the variation in ^{35}S-SO_4^{2-} in subsurface soils: firstly, sulphate release may be regulated by other geochemical properties (e.g. soil phosphorus content), as a previous study on this site showed that the N to phosphorus stoichiometry is crucial to the arbuscular mycorrhizal fungi richness (Roy et al., 2017) and thus likely also microbial activity; secondly, environmental factors, such as parent material, climate, and vegetation, determine ecosystem structure and function across biomes (Delgado-Baquerizo et al., 2020). For example, McFarland et al. (2019) found that the short-term (in 50 h) partitioning of low molecular weight organic compounds across chronosequence was regulated by mineralogical effects on microbial access to the low molecular weight organic compounds. Therefore, as a result of soil horizon formation after recultivation, ^{35}S-SO_4^{2-} in deeper soil layers was more dependent of soil chemical properties (e.g. mineralogical status) compared to surface soils. Our findings suggest greater attention should be paid to the deeper subsoil to fully understand the global S dynamics.

In our study, chronosequences and associated space-for-time substitutions were applied to the Inden (Germany) chronosequence of reclaimed lignite mining sites to examine the long-term effect of recultivation age on soil organic S cycling. Chronosequences are mostly used to examine ecosystems with convergent successional trajectories, and the recultivation age was typically found to be the dominant driver of the variations in soil properties and microbial responses (Rosinger and Bonkowski, 2021). But the application of chronosequence should be carefully considered in research studies, as chronosequences are least suited for implying successional dynamics when sites have different vegetation histories or when dealing with traits that are more variable and unpredictable, such as species composition and abundance (Walker et al., 2010). In our study, due to the variability of human intervention, the conventional management applied by local farmers in the third phase of the recultivation could potentially influence our results.

5. Conclusion

The alfalfa or fertilisers (first 8 years) significantly increased SOC, TN and TS contents in surface soil, but not in deeper subsurface soils. The controls on the ^{35}S immobilisation (^{35}S-MB) were different in surface

soil (0–30 cm) and subsurface soils (30–60 cm and 60–90 cm) along the 48-year recultivation chronosequence. The ^{35}S-MB in subsurface soils significantly decreased over recultivation attributed to the microbial C limitation resulting from declining SOC in the chronosequence. The ^{35}S-MB in surface soil was independent of recultivation age as most of the methionine (> 50 %) was utilised by microbes in 24 h. Conversely, the $^{35}S\text{-}SO_4^{2-}$ changes were dependent on the recultivation age rather than soil depths. The significant decrease in $^{35}S\text{-}SO_4^{2-}$ in the whole soil profiles is mainly attributed to the effects from the early phases of recultivation management (i.e., alfalfa and adding fertilisers). Recultivation management has the potential to affect ecosystem S cycling, but the effectiveness of the restoration depends on the specific management practices employed. Relative to conventional agricultural management, the application of fertiliser or functional plants has a greater effect on microbial-medicated organic S decomposition. Our study using ^{35}S isotope approach filled the research gap in S-containing amino acid decomposition in those recultivated ecosystems highly affected by human activities. It provides novel insights into understanding the microbial-regulated S cycling in European countries with low S deposition or within systems that are highly susceptible to S deficiency.

CRediT authorship contribution statement

Qiqi Wang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Sara L. Bauke:** Writing – review & editing, Supervision, Data curation. **Deying Wang:** Methodology, Data curation. **Yi Zhao:** Writing – review & editing, Resources. **Rüdiger Reichel:** Resources, review and editing. **Davey L. Jones:** Writing – review & editing, Supervision, Resources, Methodology. **David R. Chadwick:** Writing – review & editing, Supervision, Resources. **Albert Tietema:** Writing – review & editing, Supervision. **Roland Bol:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Aas, W., Mortier, A., Bowersox, V., Cherian, R., Faluvegi, G., Fagerli, H., Hand, J., Klimont, Z., Galy-Lacaux, C., Lehmann, C.M.B., Myhre, C.L., Myhre, G., Olivie, D., Sato, K., Quaas, J., Rao, P.S.P., Schulz, M., Shindell, D., Skeie, R.B., Stein, A., Takemura, T., Tsyro, S., Vet, R., Xu, X., 2019. Global and regional trends of atmospheric sulfur. Sci. Rep. 9 (1), 953. https://doi.org/10.1038/s41598-018-37304-0.

Ahirwal, J., Maiti, S.K., Satyanarayana Reddy, M., 2017. Development of carbon, nitrogen and phosphate stocks of reclaimed coal mine soil within 8 years after forestation with Prosopis juliflora (Sw.) dc. Catena 156, 42–50. https://doi.org/10.1016/j.catena.2017.03.019.

Allison, V.J., Miller, R.M., Jastrow, J.D., Matamala, R., Zak, D.R., 2005. Changes in soil microbial community structure in a tallgrass prairie Chronosequence. Soil Sci. Soc. of Am. J. 69 (5), 1412–1421. https://doi.org/10.2136/sssaj2004.0252.

Bin, P., Huang, R., Zhou, X., 2017. Oxidation resistance of the sulfur amino acids: methionine and cysteine. Biomed. Res. Int. 2017, 9584932. https://doi.org/10.1155/2017/9584932.

Chen, X., Xia, Y., Rui, Y., Ning, Z., Hu, Y., Tang, H., He, H., Li, H., Kuzyakov, Y., Ge, T., Wu, J., Su, Y., 2020. Microbial carbon use efficiency, biomass turnover, and necromass accumulation in paddy soil depending on fertilization. Agric. Ecosyst. Environ. 292 https://doi.org/10.1016/j.agee.2020.106816.

Chodak, M., Pietrzykowski, M., Niklińska, M., 2009. Development of microbial properties in a chronosequence of sandy mine soils. Appl. Soil Ecol. 41 (3), 259–268. https://doi.org/10.1016/j.apsoil.2008.11.009.

Čížková, B., Woś, B., Pietrzykowski, M., Frouz, J., 2018. Development of soil chemical and microbial properties in reclaimed and unreclaimed grasslands in heaps after opencast lignite mining. Ecol. Eng. 123, 103–111.

Clayton, J., Lemanski, K., Bonkowski, M., 2021. Shifts in soil microbial stoichiometry and metabolic quotient provide evidence for a critical tipping point at 1% soil organic carbon in an agricultural post-mining chronosequence. Biol. Fertil. Soils 57 (3), 435–446. https://doi.org/10.1007/s00374-020-01532-2.

Dedourge, O., Vong, P.-C., Lasserre-Joulin, F., Benizri, E., Guckert, A., 2003. Immobilization of Sulphur-35, microbial biomass and arylsulphatase activity in soils from field-grown rape, barley and fallow. Biol. Fertil. Soils 38 (3), 181–185. https://doi.org/10.1007/s00374-003-0646-x.

Delgado-Baquerizo, M., Reich, P.B., Bardgett, R.D., Eldridge, D.J., Lambers, H., Wardle, D.A., Reed, S.C., Plaza, C., Png, G.K., Neuhauser, S., 2020. The influence of soil age on ecosystem structure and function across biomes. Nat. Commun. 11 (1), 1–14.

Dong, Y., Silbermann, M., Speiser, A., Forieri, I., Linster, E., Poschet, G., Allboje Samami, A., Wanatabe, M., Sticht, C., Teleman, A.A., Deragon, J.M., Saito, K., Hell, R., Wirtz, M., 2017. Sulfur availability regulates plant growth via glucose-TOR signaling. Nat. Commun. 8 (1), 1174. https://doi.org/10.1038/s41467-017-01224-w.

Eriksen, J., 2009. Soil sulfur cycling in temperate agricultural systems. Adv. Agron. 102, 55–89.

Eriksen, J., Thorup-Kristensen, K., Askegaard, M., 2004. Plant availability of catch crop sulfur following spring incorporation. J. Plant Nutr. Soil Sci. 167 (5), 609–615. https://doi.org/10.1002/jpln.200420415.

Feng, Y., Wang, J., Bai, Z., Reading, L., 2019. Effects of surface coal mining and land reclamation on soil properties: a review. Earth Sci. Rev. 191, 12–25. https://doi.org/10.1016/j.earscirev.2019.02.015.

Fitzgerald, J.W., Andrew, T.L., 1984. Mineralization of methionine Sulphur in soils and forest floor layers. Soil Biol. Biochem. 16 (6), 565–570.

Fitzgerald, J.W., Watwood, M.E., 1988. Amino-acid metabolism in forest soil-isolation and turnover of organic matter covalently labelled with 35 S-methionine. Soil Biol. Biochem. 20, 833–838.

Freney, J.R., Williams, C.H., 1983. The sulfur cycle in soil. In: Ivanov, M.V., Freney, J.R. (Eds.), The Global Biogeochemical Sulphur Cycle. Scientific Committee on Problems of the Environment (SCOPE) 19. Wiley, Chichester, pp. 129–201.

Ge, B., Yang, R., Yang, L., Jiang, S., Tang, B., 2021. Changes in soil macrofaunal communities along soil age gradient under centuries of cultivation after coastal reclamation. Catena 200, 105170.

Ghani, A., Mclaren, R.G., Swift, R.S., 1992. Sulfur mineralization and transformations in soils as influenced by additions of carbon. Nitrogen and Sulfur. Soil Biol. Biochem. 24 (4), 331–341. https://doi.org/10.1016/0038-0717(92)90193-2.

Ghani, A., McLarren, R., Swift, R., 1993. Mobilization of recently-formed soil organic Sulphur. Soil Biol. Biochem. 25 (12), 1739–1744.

Girma, K., Mosali, J., Freeman, K., Raun, W., Martin, K., Thomason, W., 2005. Forage and grain yield response to applied sulfur in winter wheat as influenced by source and rate. J. Plant Nut. 28 (9), 1541–1553. https://doi.org/10.1080/01904160500203259.

Graham, M.H., Haynes, R.J., 2004. Organic matter status and the size, activity and metabolic diversity of the soil microflora as indicators of the success of rehabilitation of mined sand dunes. Biol. Fertil. Soils 39 (6), 429–437. https://doi.org/10.1007/s00374-003-0713-3.

Huang, Y.M., Michel, K., An, S.S., Zechmeister-Boltenstern, S., 2011. Changes in microbial-community structure with depth and time in a chronosequence of restored grassland soils on the loess plateau in Northwest China. J. Plant Nutr. Soil Sci. 174 (5), 765–774. https://doi.org/10.1002/jpln.201000397.

Irwin, J.G., Campbell, G., Vincent, K., 2002. Trends in sulphate and nitrate wet deposition over the United Kingdom: 1986–1999. Atmos. Environ. 36 (17), 2867–2879.

Kertesz, M.A., Mirleau, P., 2004. The role of soil microbes in plant Sulphur nutrition. J. Exp. Bot. 55 (404), 1939–1945. https://doi.org/10.1093/jxb/erh176.

Kozłowski, M., Otremba, K., Tatuśko-Krygier, N., Komisarek, J., Wiatrowska, K., 2022. The effect of an extended agricultural reclamation on changes in physical properties of technosols in post-lignite-mining areas: a case study from Central Europe. Geoderma 410. https://doi.org/10.1016/j.geoderma.2021.115664.

Kumar, U., Cheng, M., Islam, M.J., Maniruzzaman, M., Nasreen, S.S., Haque, M.E., Rahman, M.T., Jahiruddin, M., Bell, R.W., Jahangir, M.M.R., 2022. Long-term

conservation agriculture increases sulfur pools in soils together with increased soil organic carbon compared to conventional practices. Soil Tillage Res. 223 https://doi.org/10.1016/j.still.2022.105474.
Li, T., Liu, H., Wang, R., Lü, X.-T., Yang, J., Zhang, Y., He, P., Wang, Z., Han, X., Jiang, Y., 2019. Frequency and intensity of nitrogen addition alter soil inorganic sulfur fractions, but the effects vary with mowing management in a temperate steppe. Biogeosciences 16 (14), 2891–2904. https://doi.org/10.5194/bg-16-2891-2019.
Liu, X., Bai, Z., Zhou, W., Cao, Y., Zhang, G., 2017. Changes in soil properties in the soil profile after mining and reclamation in an opencast coal mine on the loess plateau. China. Ecol. Eng. 98, 228–239. https://doi.org/10.1016/j.ecoleng.2016.10.078.
Liu, X., Song, X., Li, S., Liang, G., Wu, X., 2023. Understanding how conservation tillage promotes soil carbon accumulation: insights into extracellular enzyme activities and carbon flows between aggregate fractions. Sci. Total Environ. 897, 165408 https://doi.org/10.1016/j.scitotenv.2023.165408.
Lucas, M., Schlüter, S., Vogel, H.-J., Vetterlein, D., 2019. Soil structure formation along an agricultural chronosequence. Geoderma 350, 61–72. https://doi.org/10.1016/j.geoderma.2019.04.041.
Ma, Q., Wen, Y., Pan, W., Macdonald, A., Hill, P.W., Chadwick, D.R., Wu, L., Jones, D.L., 2020. Soil carbon, nitrogen, and Sulphur status affects the metabolism of organic S but not its uptake by microorganisms. Soil Biol. Biochem. 149 https://doi.org/10.1016/j.soilbio.2020.107943.
Ma, Q., Kuzyakov, Y., Pan, W., Tang, S., Chadwick, D.R., Wen, Y., Hill, P.W., Macdonald, A., Ge, T., Si, L., Wu, L., Jones, D.L., 2021a. Substrate control of Sulphur utilisation and microbial stoichiometry in soil: results of (13)C, (15)N, (14)C, and (35)S quad labelling. ISME J. 15 (11), 3148–3158. https://doi.org/10.1038/s41396-021-00999-7.
Ma, Q., Pan, W., Tang, S., Sun, X., Xie, Y., Chadwick, D.R., Hill, P.W., Si, L., Wu, L., Jones, D.L., 2021b. Maize and soybean experience fierce competition from soil microorganisms for the uptake of organic and inorganic nitrogen and Sulphur: a pot test using 13C, 15N, 14C, and 35S labelling. Soil Biol. Biochem. 157 https://doi.org/10.1016/j.soilbio.2021.108260.
Ma, Q., Tang, S., Pan, W., Zhou, J., Chadwick, D.R., Hill, P.W., Wu, L., Jones, D.L., 2021c. Effects of farmyard manure on soil S cycling: substrate level exploration of high- and low-molecular weight organic S decomposition. Soil Biol. Biochem. 160 https://doi.org/10.1016/j.soilbio.2021.108359.
Ma, Q., Xu, M., Liu, M., Cao, X., Hill, P.W., Chadwick, D.R., Wu, L., Jones, D.L., 2022. Organic and inorganic sulfur and nitrogen uptake by co-existing grassland plant species competing with soil microorganisms. Soil Biol. Biochem. 168, 108627.
Ma, Q., Tou, C., Tang, S., Wang, D., Pan, W., Wu, L., Jones, D.L., 2023. Biodegradation and bioavailability of low-molecular-weight dissolved organic Sulphur in soil and its role in plant-microbial S cycling. Plant and Soil. https://doi.org/10.1007/s11104-023-06387-8.
Mascagni, H.J., Harrison, S.A., Padgett, G.B., 2008. Influence of sulfur fertility on wheat yield performance on alluvial and upland soils. Commun. Soil Sci. Plant Anal. 39 (13–14), 2133–2145. https://doi.org/10.1080/00103620802135328.
McFarland, J.W., Waldrop, M.P., Strawn, D.G., Creamer, C.A., Lawrence, C.R., Haw, M. P., 2019. Biological and mineralogical controls over cycling of low molecular weight organic compounds along a soil chronosequence. Soil Biol. Biochem. 133, 16–27. https://doi.org/10.1016/j.soilbio.2019.01.013.
McGill, W.B., Cole, C.V., 1981. Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26 (4), 267–286.
Mihelič, R., Pintarič, S., Eler, K., Suhadolc, M., 2024. Effects of transitioning from conventional to organic farming on soil organic carbon and microbial community: a comparison of long-term non-inversion minimum tillage and conventional tillage. Biol. Fertil. Soils 60 (3), 341–355. https://doi.org/10.1007/s00374-024-01796-y.
Mo, F., Zhang, Y.-Y., Liu, Y., Liao, Y.-C., 2021. Microbial carbon-use efficiency and straw-induced priming effect within soil aggregates are regulated by tillage history and balanced nutrient supply. Biol. Fertil. Soils 57 (3), 409–420. https://doi.org/10.1007/s00374-021-01540-w.
Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Front. Microbiol. 5, 22. https://doi.org/10.3389/fmicb.2014.00022.
Moradi, J., John, K., Vicentini, F., Veselá, H., Vicena, J., Ardestani, M.M., Frouz, J., 2020. Vertical distribution of soil fauna and microbial community under two contrasting post mining chronosequences: sites reclaimed by alder plantation and unreclaimed regrowth. Global Ecology and Conservation 23. https://doi.org/10.1016/j.gecco.2020.e01165.
Niknahad Gharmakher, H., Machet, J.M., Beaudoin, N., Recous, S., 2009. Estimation of sulfur mineralization and relationships with nitrogen and carbon in soils. Biol. Fertil. Soils 45 (3), 297–304.
Otremba, K., Kozłowski, M., Tatuśko-Krygier, N., Pająk, M., Kołodziej, B., Bryk, M., 2020. Impact of alfalfa and NPK fertilization in agricultural reclamation on the transformation of Technosols in an area following lignite mining. L. Degrad. Dev. 32 (3), 1179–1191. https://doi.org/10.1002/ldr.3781.
Pihlap, E., Vuko, M., Lucas, M., Steffens, M., Schloter, M., Vetterlein, D., Endenich, M., Kögel-Knabner, I., 2019. Initial soil formation in an agriculturally reclaimed open-cast mining area - the role of management and loess parent material. Soil Tillage Res. 191, 224–237. https://doi.org/10.1016/j.still.2019.03.023.
Reichel, R., Hänsch, M., Brüggemann, N., 2017. Indication of rapid soil food web recovery by nematode-derived indices in restored agricultural soil after open-cast lignite mining. Soil Biol. Biochem. 115, 261–264. https://doi.org/10.1016/j.soilbio.2017.08.020.
Riffaldi, R., Saviozzi, A., Cardelli, R., Cipolli, S., Levi-Minzi, R., 2006. Sulphur mineralization kinetics as influenced by soil properties. Biol. Fertil. Soils 43 (2), 209–214. https://doi.org/10.1007/s00374-006-0095-4.
Rosinger, C., Bonkowski, M., 2021. Soil age and soil organic carbon content shape biochemical responses to multiple freeze–thaw events in soils along a postmining agricultural chronosequence. Biogeochemistry 155 (1), 113–125. https://doi.org/10.1007/s10533-021-00816-5.
Rothstein, D.E., 2010. Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils. Soil Biol. Biochem. 42 (10), 1743–1750. https://doi.org/10.1016/j.soilbio.2010.06.011.
Roy, J., Reichel, R., Bruggemann, N., Hempel, S., Rillig, M.C., 2017. Succession of arbuscular mycorrhizal fungi along a 52-year agricultural recultivation chronosequence. FEMS Microbiol. Ecol. 93 (9) https://doi.org/10.1093/femsec/fix102.
Roy, J., Reichel, R., Bruggemann, N., Rillig, M.C., 2022. Functional, not taxonomic, composition of soil Fungi reestablishes to pre-mining initial state after 52 years of Recultivation. Microb. Ecol. https://doi.org/10.1007/s00248-022-02058-w.
Scherer, H.W., 2001. Sulphur in crop production — invited paper. Eur. J. Agro. 14, 81–111.
Scherer, H.W., 2009. Sulfur in soils. J Plant Nutrition and Soil Science 172 (3), 326–335. https://doi.org/10.1002/jpln.200900037.
Schneider, F., Amelung, W., Don, A., 2020. Origin of carbon in agricultural soil profiles deduced from depth gradients of C:N ratios, carbon fractions, δ13C and δ15N values. Plant and Soil 460 (1–2), 123–148. https://doi.org/10.1007/s11104-020-04769-w.
Schonhof, I., Blankenburg, D., Müller, S., Krumbein, A., 2007. Sulfur and nitrogen supply influence growth, product appearance, and glucosinolate concentration of broccoli. J. Plant Nutr. Soil Sci. 170 (1), 65–72. https://doi.org/10.1002/jpln.200620639.
Shrestha, R.K., Lal, R., 2010. Carbon and nitrogen pools in reclaimed land under forest and pasture ecosystems in Ohio, USA. Geoderma 157 (3–4), 196–205. https://doi.org/10.1016/j.geoderma.2010.04.013.
Siegwart, L., Piton, G., Jourdan, C., Piel, C., Sauze, J., Sugihara, S., Bertrand, I., 2023. Carbon and nutrient colimitations control the microbial response to fresh organic carbon inputs in soil at different depths. Geoderma 440. https://doi.org/10.1016/j.geoderma.2023.116729.
Singh, K., Mandal, T., Tripathi, S., 2001. Patterns of restoration of soil physciochemical properties and microbial biomass in different landslide sites in the Sal forest ecosystem of Nepal Himalaya. Ecol. Eng. 17 (4), 385–401.
Singh, S., Kumar, A., Kumar, M., Arvind, Bahuguna A., Sharma, S., Rai, S., Anal, A.K.D., Kumar, S., Kumari, N., Rajput, J., Sharma, R.B., Gangwar, A., Kumar, V., Kumari, N., Rai, V.K., Shahi, B., Mishra, R., Singh, A.K., Rai, A., 2020. Sulphur in soils and plants: An overview. International Research Journal of Pure and Applied Chemistry 66-70. https://doi.org/10.9734/irjpac/2020/v21i1030209.
Smal, H., Ligęza, S., Pranagal, J., Urban, D., Pietruczyk-Popławska, D., 2019. Changes in the stocks of soil organic carbon, total nitrogen and phosphorus following afforestation of post-arable soils: a chronosequence study. For. Ecol. Manage. 451 https://doi.org/10.1016/j.foreco.2019.117536.
Šourková, M., Frouz, J., Šantrůčková, H., 2005. Accumulation of carbon, nitrogen and phosphorus during soil formation on alder spoil heaps after brown-coal mining, near Sokolov (Czech Republic). Geoderma 124 (1–2), 203–214. https://doi.org/10.1016/j.geoderma.2004.05.001.
Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere, First ed. Princeton University Press, Princeton NI, USA.
Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of chronosequences in studies of ecological succession and soil development. J. Ecol. 98 (4), 725–736. https://doi.org/10.1111/j.1365-2745.2010.01664.x.
Walthert, L., Graf, U., Kammer, A., Luster, J., Pezzotta, D., Zimmermann, S., Hagedorn, F., 2010. Determination of organic and inorganic carbon, δ13C, and nitrogen in soils containing carbonates after acid fumigation with HCl. J. Plant Nutr. Soil Sci. 173 (2), 207–216. https://doi.org/10.1002/jpln.200900158.
Wang, D., Chadwick, D.R., Hill, P.W., Ge, T., Jones, D.L., 2023a. Rapid microbial uptake and mineralization of 14C-labelled cysteine and methionine along a grassland productivity gradient. Soil Biol. Biochem. 180 https://doi.org/10.1016/j.soilbio.2023.109022.
Wang, D., Chadwick, D.R., Hill, P.W., Ge, T., Jones, D.L., 2023b. Tracing the mineralization rates of C, N and S from cysteine and methionine in a grassland soil: a 14C and 35S dual-labelling study. Soil Biol. Biochem. 177 https://doi.org/10.1016/j.soilbio.2022.108906.
Wang, Q., Bauke, S.L., Döring, T.F., Yin, J., Cooledge, E.C., Jones, D.L., Chadwick, D.R., Tietema, A., Bol, R., 2024. Soil pH and phosphorus availability regulate Sulphur cycling in an 82-year-old fertilised grassland. Soil Biol. Biochem. 194 https://doi.org/10.1016/j.soilbio.2024.109436.
Wang, X., Li, Y., Wei, Y., Meng, H., Cao, Y., Lead, J.R., Hong, J., 2020. Effects of fertilization and reclamation time on soil bacterial communities in coal mining subsidence areas. Sci. Total Environ. 739, 139882 https://doi.org/10.1016/j.scitotenv.2020.139882.
Yang, Z., Singh, B.R., Hansen, S., Hu, Z., Riley, H., 2007. Aggregate associated sulfur fractions in long-term (>80 years) fertilized soils. Soil Sci. Soc. Am. J. 71 (1), 163–170. https://doi.org/10.2136/sssaj2006.0242.
Zhang, N., Huang, S., Lei, H., Lei, X., Liu, P., Yan, J., 2022. Changes in soil quality over time focusing on organic acid content in restoration areas following coal mining. Catena 218. https://doi.org/10.1016/j.catena.2022.106567.
Zhao, Y., Reichel, R., Herbst, M., Sun, Y., Brüggemann, N., Mörchen, R., Welp, G., Meng, F., Bol, R., 2022. Declining total carbon stocks in carbonate-containing

agricultural soils over a 62-year recultivation chronosequence under humid conditions. Geoderma 425. https://doi.org/10.1016/j.geoderma.2022.116060.
Zhao, Z., Shahrour, I., Bai, Z., Fan, W., Feng, L., Li, H., 2013. Soils development in opencast coal mine spoils reclaimed for 1–13 years in the west-northern loess plateau of China. Eur. J. Soil Biol. 55, 40–46. https://doi.org/10.1016/j.ejsobi.2012.08.006.
Zhou, W., Li, S.T., Wang, H., He, P., Lin, B., 1999. Mineralization of organic sulfur and its importance as a reservoir of plant-available sulfur in upland soils of North China. Biol. Fertil. Soils 30 (3), 245–250. https://doi.org/10.1007/s003740050615.