



Temporal Dynamics in Soil Carbon and Glomalin-Related Soil Proteins Under Sewage Sludge-Derived Biochar and Compost Treatments

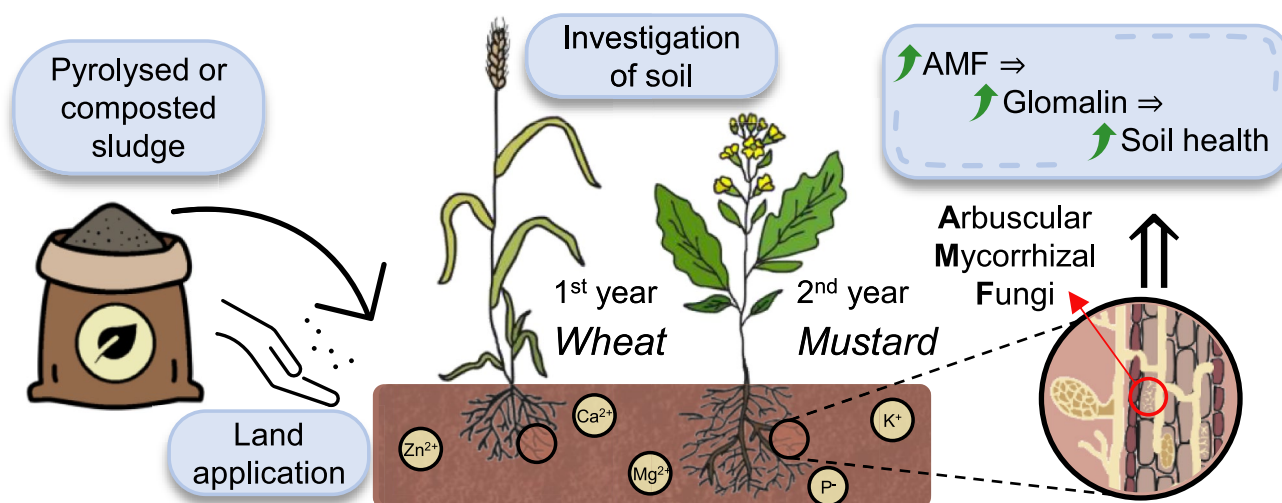
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Abstract

Sewage sludge recycling through composting or pyrolysis offers potential to enhance soil biological activity and health indicators, yet field-based evidence across different soil conditions and crop rotations remains limited. This two-year field trial evaluated the effects of composted sludge and sludge char (applied at 5 tons per hectare) on soil glomalin, permanganate oxidisable carbon (POXC), total carbon (TC), pH, effective cation exchange capacity (ECEC), and Mehlich 3 extractable nutrients (phosphorus, potassium, calcium, Magnesium, sulphur, iron, Manganese, Zinc) in two contrasting agricultural soils. The studied soils differed substantially in carbon (3-fold), phosphorus (2-fold), and nitrogen (2-fold). Winter wheat (*Triticum aestivum* L., cv. Viriato) followed by mustard (*Sinapis alba* L., cv. Zlata) was used to evaluate glomalin accumulation under mycorrhizal host and non-mycorrhizal conditions. Both amendments increased easily extractable glomalin (EEG) during wheat cultivation, with greater responses at the site with lower phosphorus and a lower carbon-to-nitrogen (C:N) ratio (27% for sludge char and 13% for compost). TC and POXC increased significantly within the first 12 months at both sites. Neither amendment altered pH or nutrient extractability significantly compared to the unamended control. Under non-mycorrhizal mustard, glomalin continued to significantly increase where the C:N ratio and phosphorus levels were lower, indicating that phosphorus availability and the C:N ratio control arbuscular mycorrhizal fungi (AMF) activity. These findings demonstrate that sewage sludge recycling can enhance soil biological activity in phosphorus-limited soils, particularly when combined with mycorrhizal crop rotations, although long-term carbon persistence, AMF colonisation rates, and plant nutritional status require further investigation.

Graphical Abstract



Keywords Arbuscular mycorrhizal fungi · Nutrient availability · Permanganate oxidisable carbon · Pyrolysis · Sludge char · Soil health

1 Introduction

The growing volumes of sewage sludge from wastewater treatment plants present both significant environmental management challenges and potential opportunities for its beneficial use in agricultural resource recovery (Kowalski et al. 2024). When appropriately treated, sewage sludge can serve as a valuable source of organic matter and plant nutrients (Marchuk et al. 2023). Among available treatment strategies, composting and pyrolysis offer distinct pathways for converting sludge into safer, more stable soil amendments (Wickramasinghe et al. 2024). Composting facilitates microbial transformation of organic matter into humus-like substances, while pyrolysis thermochemically converts biomass into biochar, reducing contaminants and producing carbon-rich, recalcitrant products (Hsu and Lo 2001; Moško et al. 2020). However, the effects of these sludge treatments on soil health and carbon dynamics under field conditions and crop rotations remain inadequately understood.

Soil health assessment has evolved to incorporate biological markers alongside traditional physicochemical indicators. Among these biological markers, glomalin-related soil proteins (GRSP) produced by arbuscular mycorrhizal fungi (AMF) have gained considerable attention due to their role in soil aggregation and carbon sequestration (Rillig 2004; Schindler et al. 2007). The AMF extend hyphal networks extensively beyond the root system, facilitating the absorption of nutrients, such as phosphorus and micronutrients, from the soil and their transfer to plants in exchange for photosynthetically derived carbon (Shukla et al. 2025). Through fungal biomass accumulation and glomalin production, this symbiotic relationship influences soil organic matter dynamics (Zhou et al. 2024). Easily extractable glomalin (EEG) and total glomalin (TG) serve as proxies for AMF activity and are regarded as an indicator of the soil organic matter quality (Černý et al. 2024; Wright and Upadhyaya 1998). Additionally, permanganate-oxidizable carbon (POXC) provides a sensitive measure of labile organic carbon, reflecting early changes in soil organic matter in response to management practices (Verma et al. 2010; Weil et al. 2003).

Despite growing interest in sludge-derived amendments, limited understanding exists regarding how sludge char and compost influence glomalin levels, labile carbon, and nutrient availability under field conditions involving crop rotation. These effects may vary with site-specific soil characteristics. This study addressed the research question of how composted and pyrolysed sewage sludges, compared to untreated control soil, affect glomalin-related soil protein levels, labile carbon fractions, and nutrient availability over time under different cropping sequences and soil conditions. We hypothesised that application of

treated sewage sludge would significantly enhance GRSP levels, POXC, total carbon (TC), and nutrient availability compared to untreated control soil. We further hypothesised that these effects would vary depending on sludge treatment type, cropping sequence, and background soil properties of the experimental sites.

The objectives of this study were: (i) compare the effects of compost and sludge char on GRSP fractions under field conditions; (ii) evaluate the influence of these treatments on POXC and TC, along with the availability of essential plant nutrients including phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), manganese (Mn), and zinc (Zn) over a two-year period; (iii) investigate how crop rotation involving an AMF host (wheat) followed by a non-mycorrhizal species (mustard) influences the response of soil to sludge application; and (iv) assess whether the treatment effects are consistent across two agricultural sites with contrasting soil physicochemical properties.

2 Materials and Methods

2.1 Sewage Sludge Treatments

Two treatment methods were applied to anaerobically stabilised dewatered sludges obtained from wastewater facilities across the Czech Republic. For both treatments, 50 kg sludge samples were prepared through preliminary air drying over several weeks. Pyrolysis treatment was conducted at temperatures between 600–650 °C under an inert nitrogen atmosphere. The process incorporated forced evacuation systems to remove primary pyrolysis byproducts, including condensate and gaseous compounds. All pyrolysis experiments were performed at the Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic. Composting treatment was carried out at the composting facility operated by Kobra Údlice, s.r.o. (Chomutov district, Czech Republic). The composting mixture consisted of 50 kg dewatered sludge (containing 17–27% dry matter), 30 kg grass, and 10 kg wood chips to ensure proper homogenisation and carbon-to-nitrogen (C:N) balance. The composting process was conducted in garden composting containers with a 300 L capacity over a three-month period. Temperature monitoring throughout the process revealed a maximum temperature of 52 °C maintained for one week. Although larger commercial compost heaps typically achieve higher temperatures for extended periods, temperatures exceeding 50 °C are generally considered sufficient for basic sludge sanitisation.

Table S1 presents the basic characteristics and major alkali element concentrations of both raw and treated sludge

samples. The pyrolysed sludge material exhibited a notable pH increase compared to the original material. This pH elevation can be attributed to the thermal decomposition of acidic functional groups and the concurrent release of alkaline elements (Ca, Mg, K, Na) from organic components during the high-temperature pyrolysis process (Weber and Quicker 2018).

2.2 Experimental Sites and Sample Collection

This study was conducted as an exploratory field trial to evaluate the effects of composting and pyrolysis sludge under real agricultural conditions. Treated sludge amendments were applied to two agricultural field sites in north-western Bohemia, Czech Republic (Fig. 1). Site 1 (50°26'29.26" N, 13°29'09.55" E) was located in the Bohemian coal mining region and was characterised by higher TC content (3.50%) and greater effective cation exchange capacity (ECEC) of 27.9 cmol(+) kg⁻¹. Site 2 (50°36'41.6" N, 14°25'09.5" E) was situated in an area without known industrial or mining

impacts and exhibited lower TC content (1.63%) and ECEC value (15.4 cmol(+) kg⁻¹), despite a higher total nitrogen concentration, which was approximately 2-fold than that of site 1 (Table S2). The experimental plots included three treatment groups: (i) sludge char, (ii) composted sewage sludge, and (iii) control soil without any amendments, each having an area of 1 m² (n=5). Compost or sludge char was applied to the top layer (~15 cm) and thoroughly mixed with the soil. No further tillage occurred after treatment application throughout the two-year study period. The subplots were spaced sufficiently apart to prevent interaction. The application rate was 0.5 kg dry matter per subplot, aligning with the maximum permissible dose of 5 tons of sludge per hectare specified in Decree No. 273/2021 Coll. (2021) on the details of waste management valid in the Czech Republic.

Winter wheat (*Triticum aestivum* L., cv. Viriato) was cultivated during the first growing season following sludge application. This species was selected for its well-established capacity to form AMF, making it an ideal model plant for investigating AMF community dynamics in amended



Fig. 1 Geographic location of the two agricultural field sites used in this study in the Bohemian region, Czech Republic. Site 1 (50°26'29.26" N, 13°29'09.55" E) is located in an area historically influenced by coal mining activities, while Site 2 (50°36'41.6" N, 14°25'09.5" E) is in

an area without known industrial or mining impacts. The inset map shows the position of both sites within the Czech Republic in a Central European context. Major cities and regional boundaries are indicated for spatial reference

agricultural systems (Lehnert et al. 2017). Soil sampling was conducted at two phenologically significant developmental stages: six months post-sowing, coinciding with peak vegetative growth, and twelve months post-sowing, corresponding to physiological maturity. In the second year, mustard (*Sinapis alba* L., cv. Zlata), a non-mycorrhizal crop, was introduced into the same plots to examine how rotational cropping with contrasting mycorrhizal capacities influences soil responses to amendments. The seeds were supplied by Oseva, a.s. (Czech Republic) and used at both sites due to their suitability for cultivation under a wide range of climatic conditions. Five soil subsamples were collected from each plot and mixed into one sample in a plastic bag. Each sample was homogenised, air-dried, and sieved through a 2 mm stainless steel sieve.

2.3 Soil Sample Analysis

Soil pH was measured using demineralised water (DW) in a 1:5 (v/v) ratio. ECEC was determined using the BaCl_2 extraction method proposed by Schwertfeger and Hendershot (2009). ECEC was calculated as the sum of exchangeable cations (Ca, Mg, K, Na, Al, Fe, Mn).

Glomalin is an indicator of the biological and chemical health of the soil and is associated with the overall soil health status (Balík et al. 2020; Wright and Upadhyaya 1998). Glomalin was detected in two fractions (EEG and TG). The analyses were performed according to Wright and Upadhyaya (1998) procedure. The EEG was determined as follows: 8 mL of 20 mmol L^{-1} sodium citrate (pH 7) was added to 1 g of air-dried ground soil. The mixture was autoclaved at 121 °C for 30 min, followed by centrifugation at 5000 g for 10 min. The TG content was determined as follows: 8 mL of 50 mmol L^{-1} sodium citrate (pH 8) was added to 1 g of ground air-dried soil, followed by autoclaving at 121 °C for 60 min and centrifugation at 5000 g for 15 min. The centrifugation of the supernatant of the same sample was repeated five times until there was no visible red-brown colour. After extraction, the concentration of glomalin was determined via the Bradford dye-binding method (Balík et al. 2022) at 595 nm via a TECAN microplate reader. Bovine serum albumin was used to generate the calibration curve.

POXC fraction was determined according to the method described by Lucas and Weil (2012). Potassium permanganate solution oxidises the easily accessible soil carbon fraction via the conversion of Mn^{7+} to Mn^{2+} . Therefore, a more significant decrease in the purple colour can be observed, which is proportional to the POXC, measured by absorbance at 550 nm (Agilent, Cary 60 UV–Vis). TC was measured via a TOC-L CPH carbon analyser (Shimadzu-SSM 5000A, Japan).

After 24 months of initial application of treated sewage sludge, the soils from both sites were analysed to determine the concentrations of plant-available nutrients. The analysis followed the Mehlich 3 extraction procedure, a standard method for assessing nutrient availability (P, K, Ca, Mg, S, Fe, Mn, Zn) in soils (Zbiral 2016) and measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP 7000 series, Thermo Scientific). All the soil analyses were performed in triplicate.

2.4 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (Version 29, IBM Corp., Armonk, NY, USA). Treatment effects were evaluated using the Kruskal–Wallis H test, a nonparametric alternative to one-way ANOVA. When significant differences were detected ($p < 0.05$), pairwise post hoc comparisons were conducted using Dunn's test with Bonferroni correction. The results were presented graphically using Grapher (Version 25, Golden Software, LLC).

3 Results and Discussion

3.1 Effect of Sludge Treatments on Soil pH and Cation Exchange Capacity

At site 1, soil pH changed only within a narrow range during the 24-month monitoring period. In the unamended control soil, pH rose slightly from 7.44 to 7.78, and in the compost-amended soil, pH ranged between 7.35 and 7.68, while soils amended with sludge char varied between 7.36 and 7.50 (Fig. 2a). The control plots consistently had slightly higher median pH than compost or sludge char treatments, though these differences were not statistically significant at each sampling point. This indicates that neither compost nor biochar application dose produced a significant change in pH at site 1 compared with the unamended control soil. The small increase observed across all treatments, including the control, could be due to natural temporal variation, possibly reflecting seasonal shifts in soil carbonate equilibria and base cation availability rather than an amendment effect. Other studies have similarly observed minimal pH changes following organic amendments. Agegnehu et al. (2016) reported no significant pH response to biochar or compost amendments in soil with total organic carbon of 2.01% and cation exchange capacity of 5.4 cmol(+) kg^{-1} , despite a higher application rate (25 tons per hectare). Similarly, Balkrishna et al. (2024) reported that organic amendments derived from sewage sludge did not cause major pH changes when applied at recommended doses, although the specific application rate was not explicitly stated.

Fig. 2 Soil pH measured at 6, 12, and 24 months after sludge amendment at site 1 (a) and site 2 (b) under control, composted sludge, and sludge biochar treatments. Each boxplot shows the median, and the whiskers represent the minimum to maximum. At each sampling time within a site, treatments with different letters are significantly different (Kruskal–Wallis ANOVA with Bonferroni correction, $p < 0.05$)

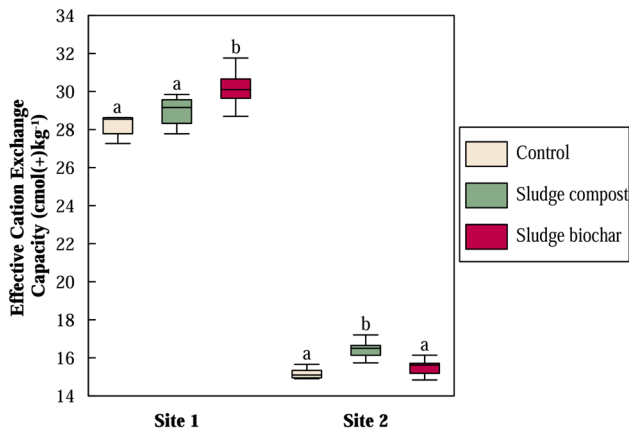
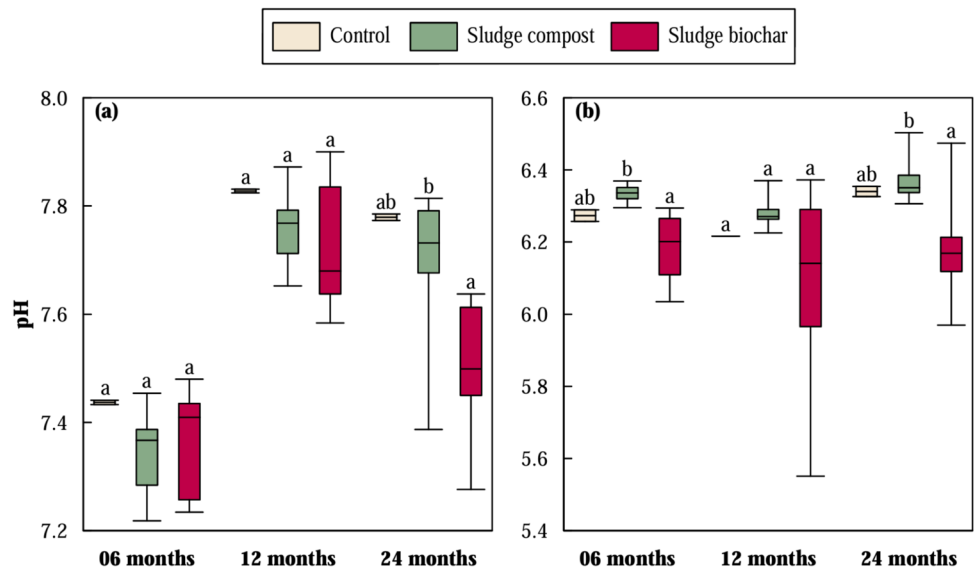


Fig. 3 Effective cation exchange capacity (ECEC) of soils measured 24 months after sludge amendment at sites 1 and 2 under control, composted sludge, and sludge biochar treatments. Boxplots show the median and the minimum-to-maximum range for each treatment. Within each site, treatments with different letters are significantly different (Kruskal–Wallis ANOVA with Bonferroni correction, $p < 0.05$)

At site 2, effects of the amendment on pH were more significant between composted and sludge char (Fig. 2b). At 6 months, compost significantly increased the mean pH by 0.15 units relative to the sludge char. By 12 months, there was no significant difference in the pH among the treatments. At 24 months, the compost treatment maintained a higher pH level than the control and sludge char, while the biochar treatment significantly decreased the pH compared to the compost treatment. The improvement of pH level by compost treatment in slightly acidic site 2 soil (initial soil $\text{pH}_{(\text{DW})}$ 6.20 / $\text{pH}_{(\text{CaCl}_2)}$ 5.47) reflects the release of alkaline cations and organic anions, consistent with reports that composted sewage sludge can raise pH in acidic soils. Almási et al. (2025) observed pH increase from 4.58 to 5.49–5.97 in acidic soil amended with sewage sludge compost, and

Cely-Vargas et al. (2024) similarly reported a pH increase to approximately 8.0 in soil with an initial pH of 5.6 following sludge compost amendments. The decline in pH under the sludge char treatment is consistent with studies showing that biochar may lose its liming effect over time due to leaching of soluble bases and surface oxidation, leading to the formation of acidic functional groups (Liu et al. 2025; Liu and Chen 2022; Yao et al. 2010).

The ECEC showed clear site-specific patterns (Fig. 3). At site 1, the sludge char treatment significantly increased ECEC by 7% relative to the control, whereas compost had no significant increase. The improvement under sludge char reflects both the high surface area of the biochar and the gradual development of oxygen-containing functional groups during ageing, as well as interactions with the clay and organic matter of the soil, which can form organo-mineral complexes with additional exchange sites (Agegnehu et al. 2016; Laird et al. 2010). This delayed but persistent effect is consistent with findings that the contribution of sludge char to ECEC often becomes more pronounced over time as its surface chemistry evolves (Lu et al. 2025).

At site 2, the opposite pattern emerged. Compost significantly increased ECEC by 8% relative to the control at the end of 24 months, whereas sludge char had no significant increase. This increase can be attributed to the input of humic and fulvic substances with high intrinsic exchange capacity, which is particularly beneficial in soils with low native ECEC (Khan et al. 2023; Tiwari et al. 2023). Moreover, the higher ECEC observed in composted sludge treatments at site 2 compared to sludge char can be attributed to the more negative charges developed on clay particles and organic matter surfaces due to the pH increase (Fig. 2b). In contrast, the lack of sludge char effect suggests that in soils with low organic matter, surface charge development

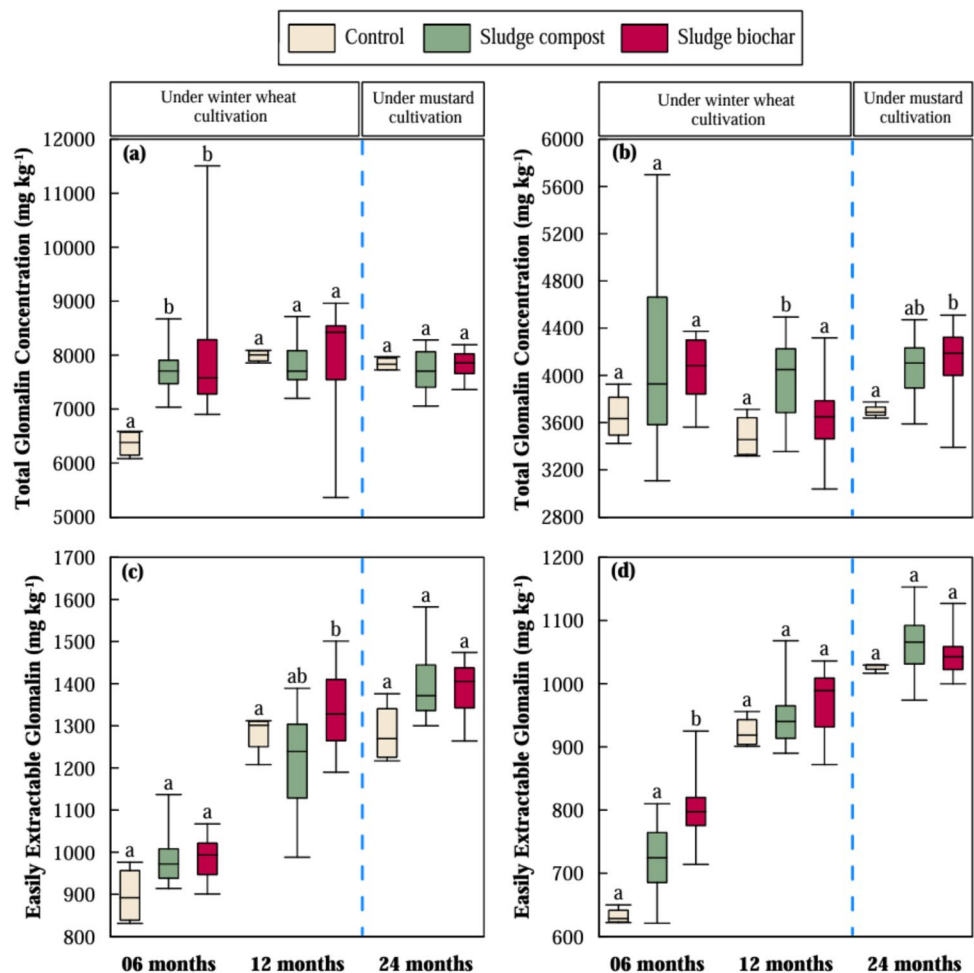
of sludge char is insufficient within two years to produce a measurable ECEC change, particularly under conditions with higher leaching potential.

3.2 Glomalin-Related Soil Proteins Under Winter Wheat Cultivation

Six months after sludge application, GRSP levels under winter wheat (an AMF host species) showed distinct differences between treatments (Fig. 4). Both compost and sludge char applied plots exhibited significantly higher TG concentrations than unamended control soil at site 1 (Fig. 4a), while the increase in TG at site 2 was not statistically significant compared to the control (Fig. 4b), possibly indicating that a single season was insufficient to substantially alter the entrenched glomalin pool in a less carbon-rich soil. The relative increase in EEG compared to the control soil differed between sites after 6 months of treatment application. At site 2, where lower initial total phosphorus (606 mg kg^{-1}) content was observed, composted sludge and sludge biochar increased EEG by 13% and 27%, respectively. At site 1, where the higher initial phosphorus

content (1460 mg kg^{-1}) was observed, EEG increased by approximately 10% for both treatments. These results suggest that sludge amendments have a more pronounced impact on glomalin accumulation where total phosphorus levels are lower, consistent with the findings that high soil phosphorus availability can suppress AMF colonisation and glomalin accumulation (Singh et al. 2022). At the end of the winter wheat season (12 months after soil amendments), both EEG and TG had further increased in all pots, including the controls, likely due to the winter wheat cultivation through its mycorrhizal associations (Gałazka et al. 2018; Wilkes et al. 2021). At the end of the winter wheat period, sludge char yielded the highest TG and EEG concentrations at both sites, except the TG concentration at site 2 (Fig. 4b), where composted sludge treatment significantly increased the TG. Interestingly, biochar amendments in other studies have shown variable effects on AMF. De Figueiredo et al. (2019) reported that sludge char can indirectly increase glomalin by improving soil moisture and habitat for fungi, whereas others found excessive biochar can impede fungal growth (Barna et al. 2020). The contrasting EEG responses between compost and sludge char in our results highlight

Fig. 4 Concentrations of total glomalin (TG) at sites 1 (a) and 2 (b), and the easily exchangeable glomalin (EEG) concentrations at sites 1 (c) and 2 (d) in soil. Samples were collected 6 and 12 months after sludge application (during winter wheat cultivation) and 24 months after application (during a mustard crop). Treatments include a control (no sludge), composted sludge, and sludge biochar. The boxplots show median values, with whiskers indicating the minimum and maximum observed. For each sampling time, treatments marked with different letters are significantly different (Kruskal–Wallis ANOVA followed by Bonferroni correction, $p < 0.05$)



the importance of amendment type. Composted sludge provided an immediate boost to the soil food web, fuelling AMF growth and glomalin exudation, whereas sludge char contributed more to the stable carbon framework of soil with a slower biological response. However, this needs to be confirmed by further long-term research on soils with different soil properties. Overall, the wheat cultivation period demonstrated that sewage sludge amendments can augment soil glomalin, especially the easily extractable fraction. Therefore, the sludge-amended soils had a richer glomalin pool than the control soils. By increasing glomalin through amendments, soil aggregation, porosity, and water retention have been enhanced in soils through the glueing effect, thereby reducing erosion and runoff (Shukla et al. 2025; Wright and Upadhyaya 1998).

3.3 Glomalin-Related Soil Proteins Under Mustard Cultivation

In the second year, white mustard crops were grown, which do not form AMF symbioses. By 24 months (approximately one year after the wheat harvest), soil glomalin levels had changed in ways indicative of this shift in crop biology (Fig. 4). Sludge-amended plots still tended to have slightly higher TG than the control (a possible residual effect of the previous year's inputs). Under mustard cultivation, site 2 showed relative increases in total glomalin of 11% and 9% for sludge char and composted sludge, respectively, with sludge char showing a significant increase compared to the unamended control. In contrast, site 1 showed no significant changes in total glomalin. These differential responses reflect inherent differences in soil properties between sites. Site 2 has approximately twofold higher total nitrogen, substantially lower total phosphorus, and a lower C:N ratio compared to site 1. These properties may provide more favourable conditions for AMF biomass maintenance even in the absence of an active mycorrhizal host. Lower soil phosphorus has been reported to decrease functional traits of AMF (Singh et al. 2022), while soil C:N ratio is a key variable negatively affecting AMF colonisation (Kowal et al. 2022), supporting the differential glomalin responses observed between sites. EEG concentrations at both sites were maintained with slight increases under non-mycorrhizal host plant cultivation, suggesting the persistence of this labile glomalin fraction. This observation is supported by recent work by Quintana-Estera et al. (2025) on cover cropping with mustard, which found that no-tillage soils supported the retention of glomalin, aligning with our no-tillage experimental results. In our study, over a full mustard growing season, the decline in glomalin is even more evident in control soils at site 1. Glomalin in soil is relatively stable, with reported residence times of decades

(Balík et al. 2022). However, it is not inert; some turn-over occurs as soil microbes slowly decompose the protein or incorporate it into humified organic matter. The easily extractable portion is likely the most susceptible to such degradation once fresh inputs cease (Quintana-Estera et al. 2025). Consistent with this, the EEG of the unamended control soil at site 1 (Fig. 4c) dropped more sharply than TG over the mustard period.

Crucially, the absence of an AMF host in the mustard crop meant that no new glomalin was being produced to offset these losses. Unlike wheat, mustard roots do not harbour AMF (Cosme et al. 2018), so the AMF population in soil likely diminished over the course of the second year due to a lack of a symbiotic partner. Any AMF that persisted (e.g. as spores or dormant hyphae) would have had minimal activity and thus minimal EEG secretion (Fig. 4c and d). In our case, a legacy effect was observed, which is a potential remnant of the previous year's fungal contributions. Interestingly, we noted that sludge char plots tended to maintain glomalin slightly better through the mustard period than compost plots. For example, TG in sludge char-amended soil decreased less than in the compost-amended soil at both sites, resulting in sludge char plots having equal or even marginally higher TG than compost plots by 24 months (Fig. 4a and b). This could be due to the sludge char offers a protective matrix that adsorbs or physically protects glomalin, making it less accessible to decomposition. There is an analogy here to no-tillage systems, where recalcitrant glomalin accumulates at the surface due to reduced disturbance and improved protection in aggregates (Quintana-Estera et al. 2025). Our results indicate that the rotation of mycorrhizal and non-mycorrhizal host plants after sludge application can be done under no-tillage systems without prominent harm to the glomalin pool and, therefore, soil health.

However, our results also raise caution and counsel holistic management. The decline of glomalin during the mustard period is a reminder that one-time interventions have transient effects if not supported by continuous good practices. It would be wise to reintroduce a mycorrhizal crop to rebuild AMF networks. This could maintain the glomalin momentum and further stabilise the added organic matter.

3.4 Labile Fraction of Soil Carbon and the Total Carbon Dynamics

The treatment effects on soil TC were statistically significant at 6 months at site 1 and, at 12 months at site 2, though they were no longer significant at 24 months (Fig. 5). At 24 months, the relative increase in TC compared to unamended control was approximately 3% for both treatments at site 1, while at site 2 it was 5% and 3% for sludge char and composted sludge, respectively. These results

Fig. 5 Soil total carbon (TC) measured at 6, 12, and 24 months after sludge amendment at site 1 (a) and site 2 (b) under control, composted sludge, and sludge biochar treatments. Each boxplot shows the median, and the whiskers represent the minimum to maximum. At each sampling time within a site, treatments with different letters are significantly different (Kruskal–Wallis ANOVA with Bonferroni correction, $p < 0.05$)

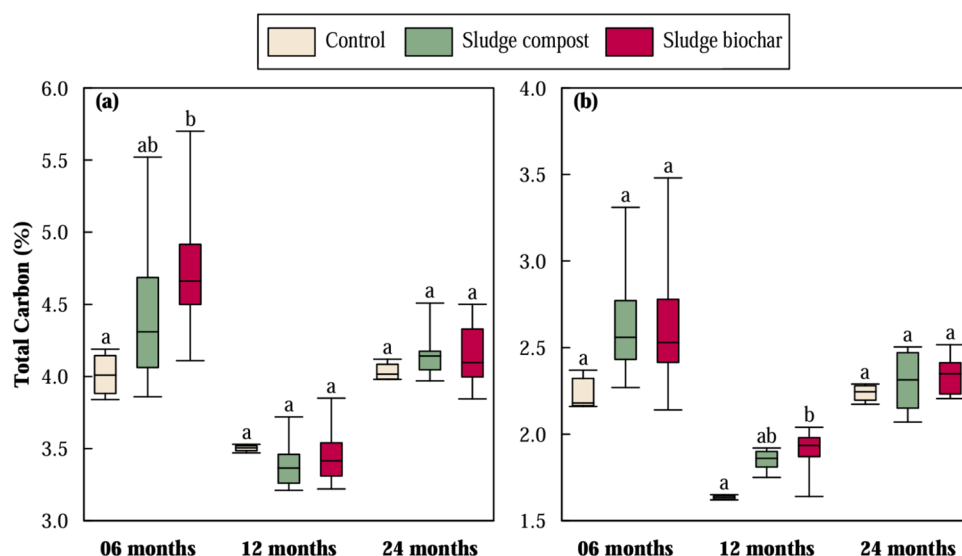
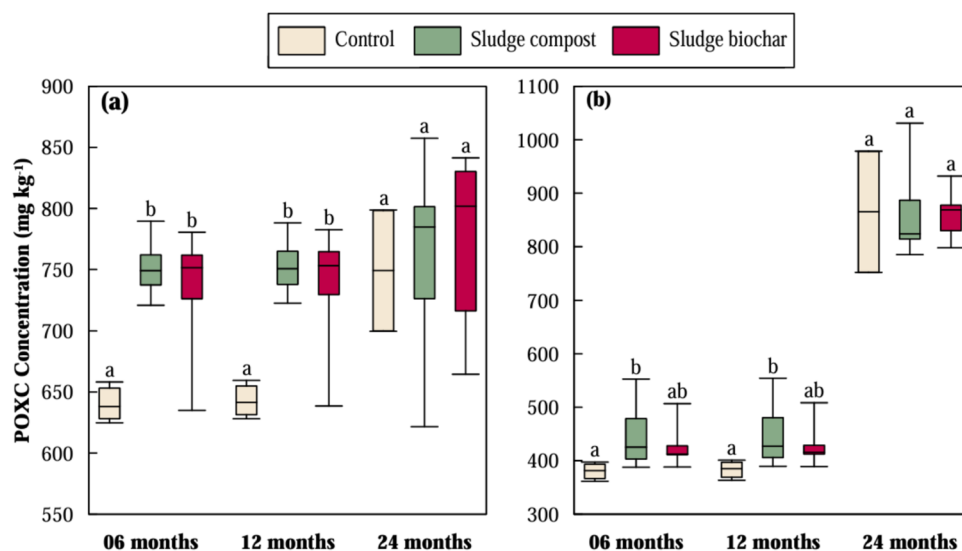


Fig. 6 Permanganate oxidizable carbon (POXC) measured at 6, 12, and 24 months after sludge amendment at site 1 (a) and site 2 (b) under control, composted sludge, and sludge biochar treatments. Each boxplot shows the median, and the whiskers represent the minimum to maximum. At each sampling time within a site, treatments with different letters are significantly different (Kruskal–Wallis ANOVA with Bonferroni correction, $p < 0.05$)



suggest that sludge char provided a more persistent carbon input that was less prone to decomposition, particularly in the less carbon-rich soil at site 2 (Fig. 5b).

The greater residual TC with sludge char aligns with its known recalcitrance. While traditional compost carbon is largely mineralised over time, pyrolysis converts labile organic matter into more stable aromatic forms (Jakubus et al. 2025). In our 2-year field study, higher residual TC in the sludge char treatment is consistent with prior findings that biochar amendments increase soil carbon stocks relative to unamended controls (Jakubus et al. 2025; Zhao et al. 2023). However, without characterisation of carbon fractions or stabilisation mechanisms, we cannot fully assess the long-term persistence or climate relevance of these increases. Our results suggest that sludge char may offer potential for short-term carbon retention

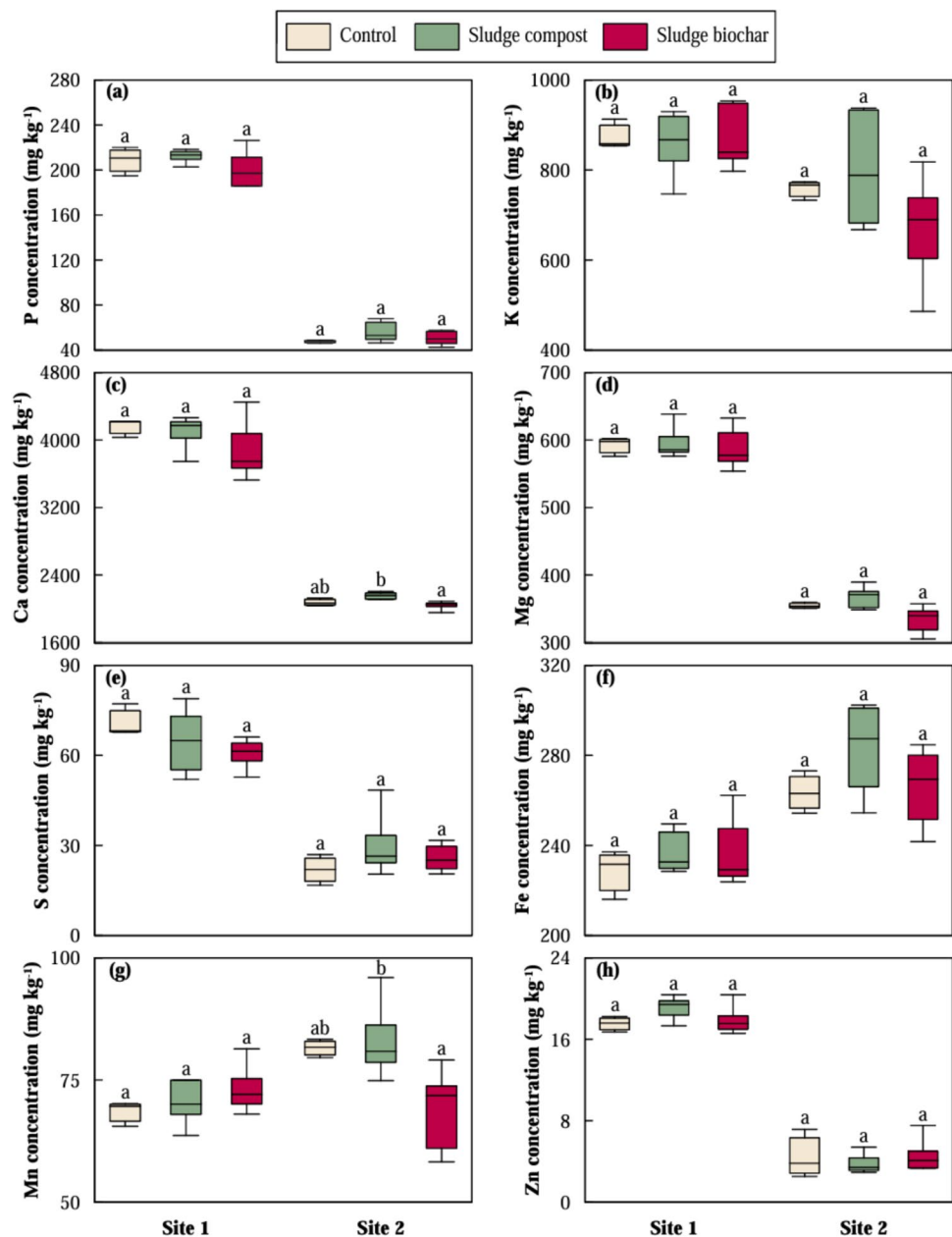
in agricultural soils, needing further investigation into its stability and fate over longer time scales.

Unlike TC, the labile carbon pool of POXC responded differently to the two treatments (Fig. 6). Composted sludge significantly increased POXC relative to the control at both sites during the first 12 months of the amendments, indicating an enrichment of readily oxidizable organic fractions. In contrast, the sludge char had a significantly increased POXC only at site 1 compared to the control (Fig. 6a). At 24 months, neither treatment showed any significant increase compared to the control in both sites. At site 2, where less organic matter is present, composted sludge led to the largest POXC gains during the first year (reflecting the influx of easily oxidizable organic matter from the compost), whereas sludge char in that soil showed only a marginal POXC increase that was not statistically significant. However, at the end of

24 months, the POXC from the composted amended plots became the lowest, even though this decrease is not statistically significant (Fig. 6b). These results point to fundamental differences in carbon quality between the amendments. Compost contains partially decomposed, humified organic compounds that are still accessible to microbial oxidation, thereby raising the POXC fraction (often termed “active” or labile carbon). Biochar, on the other hand, consists mostly of stable polyaromatic carbon structures that are not easily oxidised by the mild KMnO_4 solution used in the POXC assay. In our experiment, the lack of POXC response to sludge char at site 2 during the first year of the amendments is in line with the technical report by Aswin and Sanyal

(2025) that carbon inputs rich in labile components, such as compost, elevate POXC more effectively than inputs dominated by stable carbon, such as biochar. The authors emphasise that biochar’s carbon is largely resistant to microbial attack and does not significantly contribute to the readily available carbon pool, whereas compost provides simple organic molecules (e.g. carbohydrates, amino acids) that boost POXC and fuel microbial activity. The application of treated sewage sludge at 5 t ha^{-1} resulted in statistically significant increases in TC and POXC only during the first year of treatment application at sites 1 and 2. Neither TC nor POXC were significantly increased by treatments after 24 months (Figs. 5 and 6).

Fig. 7 Available plant nutrient concentrations in soils 24 months after sludge amendment under control, composted sludge, and sludge biochar treatments. The eight panels **a**, **b**, **c**, **d**, **e**, **f**, **g**, and **h** represent phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) as macronutrients, and iron (Fe), manganese (Mn), and zinc (Zn) as micronutrients, respectively. Each boxplot depicts the median and the full range of values for each treatment. For each nutrient, treatments with different letters are significantly different (Kruskal–Wallis ANOVA with Bonferroni correction, $p < 0.05$)



3.5 Available Plant Nutrient Concentrations after 2 Years from the Sludge Amendments

Across both sites, extractable Ca, Fe, K, Mg, P, S, and Zn did not differ significantly between the control and sludge-amended plots (Fig. 7). Comparisons between amendment types showed lower Ca and Mg in the sludge char plots than in the compost plots, with significant differences for Ca at site 2. Potassium was higher in the control than in the sludge char plots, although not significantly different from the control when all treatments were considered together. The behaviour of K fits expectations for sewage sludge. Sewage treatment commonly depletes K from the solid phase because much of the K remains in the aqueous stream (Ndoung et al. 2023). Pyrolysis can further slow K release and reduce leaching losses, improving apparent K use efficiency while lowering the risk of groundwater contamination (Fachini et al. 2022). Manganese showed the clearest separation at site 2, where sludge char plots had lower extractable Mn than both the control and the composted sludge plots (Fig. 7g). For Fe, mean concentrations tended to be higher under composted sludge than under sludge char, though neither differed significantly from the control (Fig. 7f).

These patterns are consistent with known effects of sludge pyrolysis on nutrient speciation and sorption. Phosphorus in sludge-derived biochar is predominantly present as relatively insoluble calcium and aluminium phosphates, which limit its immediate plant availability (Kwapinski et al. 2021). However, a minor fraction may occur as more soluble orthophosphates. This fraction could contribute to a small but rapidly available P pool shortly after application. Biochar's high surface area and functional groups further enhance nutrient retention, moderating solution concentrations and promoting slow release, which can be advantageous for contaminant stabilisation in remediation contexts (Mitzi et al. 2024; Nguyen et al. 2023). Although pyrolysis increased total nutrient contents in our sludge char (Table S1), nutrient availability typically declines once sludge is pyrolysed at about 420 °C or higher (Mercl et al. 2020). Our sludge char was produced at 600 to 650 °C, which is consistent with the reduced short-term availability observed here. At both sites, plant nutrient concentrations were generally higher under composted sludge than under sludge char, with a significant increase only for Mn at site 2 (Fig. 7g). A recent study reported that converting sludge to biochar reduced the water extractable P fraction and slowed P release relative to unpyrolysed sludge (Lustosa Filho et al. 2025). While a meta-analysis has shown that biochar applications can, on average, increase soil available P through direct inputs and pH or sorption effects (Glaser and Lehr 2019), we did not observe a large increase at the relatively modest application rate of 5 t ha⁻¹ over the two-year observation period.

This study did not investigate the AMF community composition or fungal biomarkers in soils. Therefore, the mechanisms underlying these responses are unclear. Future research should directly assess plant nutritional status, AMF colonisation rates, and fungal biomarkers to better understand the relationship between nutrient availability and glomalin production under different sludge amendment types.

4 Conclusions

Sewage sludge amendments enhanced soil glomalin, with strong site-specific impacts driven by the carbon-to-nitrogen ratio and phosphorus availability. Sludge char and compost increased easily extractable glomalin during wheat cultivation, with greater responses at the site with lower phosphorus and higher nitrogen. Under non-mycorrhizal mustard, glomalin was preserved at both sites; however, it continued to increase significantly only where the carbon-to-nitrogen ratio and phosphorus levels were lower, indicating that phosphorus availability and the carbon-to-nitrogen ratio control arbuscular mycorrhizal fungal activity. Total carbon and permanganate-oxidisable carbon increased significantly only during the first year; by 24 months, neither treatment maintained significant changes. These findings support sewage sludge recycling as a sustainable strategy in phosphorus-limited soils when combined with appropriate crop rotation. Future research should characterise carbon fraction stability, measure arbuscular mycorrhizal fungi colonisation rates and plant nutritional status, and validate findings across broader contexts.

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Data Availability Data will be made available on request.

Code Availability Not applicable.

Declarations

Ethical Approval Not applicable. This study did not involve human participants, animal subjects or sensitive data.

Consent to Participate Not applicable.

Consent for Publication This study does not include any individual data requiring consent for publication.

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.

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