

Arbuscular mycorrhizal fungal necromass: Rapid decomposition and texture-dependent formation of mineral-associated organic matter

Valentin B. Kurbel^{a,*}, Hana Hřelová^b, Maire Holz^c, Johanna Pausch^a, Jan Jansa^b

^a Agroecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany

^b Laboratory of Fungal Biology, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

^c Leibniz Center for Agricultural Landscape Research (ZALF), Müncheberg, Germany

ARTICLE INFO

Keywords:

Arbuscular mycorrhiza
Carbon sequestration
Mineral-associated organic matter
Necromass
Soil respiration
Stable isotopes

ABSTRACT

Fungal necromass represents a major source of soil organic carbon (C), partly stabilized as mineral-associated organic matter (MAOM). Mycorrhizal fungi, particularly arbuscular mycorrhizal fungi (AMF), are key contributors to soil C input, yet their necromass decomposition and role in MAOM formation remain poorly understood. We investigated, using a range of unsterile soils, (I) the fate of AMF necromass-C from *Rhizophagus irregularis* into MAOM, particulate organic matter (POM), and CO₂, (II) the influence of abiotic soil properties, and (III) effects on microbial communities, particularly the fungal-to-bacterial (F/B) ratio. Highly ¹³C-labelled (97 atom%) AMF necromass was incubated in six grasslands soils with contrasting textures and chemical properties. ¹³CO₂ efflux was measured five times. After 12 and 83 days, soils were fractionated to quantify ¹³C in MAOM and POM. Initially, 36.2 ± 12.5 % of necromass-C was found in MAOM, but this declined over time in most soils indicating the dynamic nature of MAOM. Total MAOM-C correlated positively, whereas necromass-derived ¹³C in MAOM correlated negatively with clay content, possibly due to varying sorption mechanisms. Soils with more clay showed less ¹³C loss from MAOM with time, suggesting higher stability. Necromass-derived CO₂ reached 33.1 ± 4.4 % after 12 days and 60.0 ± 8.5 % after 83 days, indicating rapid microbial utilization. Necromass addition altered microbial communities: Acidic soils showed increased fungal dominance, while alkaline soils showed little to no change. These microbial shifts coincided with altered SOM decomposition. In summary, AMF necromass contributes rapidly to MAOM but decomposes with time simultaneously fuelling respiration and altering microbial dynamics, thus influencing soil C in complex, texture-dependent ways.

1. Introduction

Soils represent the main terrestrial carbon (C) pool (Lehmann and Kleber, 2015). In the face of climate change, there is an increasing need for a mechanistic understanding of C-related soil processes to minimize losses from soil back to the atmosphere. A large fraction of C enters the soil via the symbiotic interaction between plants and mycorrhizal fungi, transporting assimilates belowground. Thereby, an estimated 13 Gt of CO₂ equivalents are at least temporally allocated by plants to mycorrhizal fungi per year (Hawkins et al., 2023). After cell death, fungal necromass, i.e., dead cells and compounds released from the cells (cf. Buckeridge et al., 2022a), provides a nutrient dense resource for soil organisms. During decomposition, part of it is respired back to the atmosphere, while the remaining necromass can be

stabilized within the soil matrix and thus, contributing to soil organic matter (SOM, Fernandez et al., 2016; Cotrufo et al., 2013; Liang et al., 2019). Microbial necromass in general, comprising bacterial besides fungal remnants, can contribute up to 50% of the stabilized SOM (Angst et al., 2021). Hereby, the role of fungi often exceeds bacterial contribution (Fu et al., 2025), with observed ratios of fungal necromass-C/bacterial necromass-C of >2.5 and fungal necromass contributions to SOC of >30% (Yang et al., 2022). Thus, knowledge on the long-term stabilization of fungal necromass in the soil is pivotal to understand the soil's capacity of C storage.

Soil organic matter can be operationally divided into particulate organic matter (POM) and mineral-associated organic matter (MAOM). The latter is defined as organic molecules bound onto mineral surfaces, via ligand exchange, cation bridging, hydrogen bonding or van der

* Corresponding author. Universitätsstraße 30, 95447, Bayreuth, Germany.

E-mail addresses: valentin.kurbel@uni-bayreuth.de (V.B. Kurbel), hrelsova@biomed.cas.cz (H. Hřelová), maire.holz@zalf.de (M. Holz), johanna.pausch@uni-bayreuth.de (J. Pausch), jansa@biomed.cas.cz (J. Jansa).

<https://doi.org/10.1016/j.soilbio.2026.110200>

Received 8 August 2025; Received in revised form 13 May 2026; Accepted 14 May 2026

Available online 26 May 2026

0038-0717/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Waals forces (Lavalée et al., 2020). In contrast to POM, MAOM is assumed to be more stable with stronger physicochemical protection and longer turnover times (Poeplau et al., 2018). While POM constitutes mostly of larger organic residues and macromolecules of plant origin, MAOM is mostly composed of molecules of microbial necromass origin (Kopittke et al. 2018, 2020; Jílková et al., 2025). Due to its longevity, MAOM can account for 70% of SOM (Georgiou et al., 2022) and, consequently, more global soil C is present as MAOM compared to POM, especially in grasslands and croplands (Liu et al., 2025). A high contribution of microbial necromass to MAOM was reported in numerous studies (Klink et al., 2022; Zhu et al., 2025; Six et al., 2006) and for bacteria, it was shown that necromass-C quickly enters the MAOM fraction already after three days (Buckeridge et al., 2022b). To date, studies investigating the stabilization of microbial necromass and the underlying drivers mostly focus on bacterial necromass (Buckeridge et al., 2020, 2022b; Kopittke et al., 2018). Therefore, despite the greater contribution of fungal over bacterial residues to MAOM (Ao et al., 2025; Vasilica et al., 2026a), we lack detailed knowledge on the conversion of fungal necromass to MAOM and its stability.

Formation of MAOM by microbial necromass-C is influenced both directly and indirectly by complex interactions involving a wide range of soil abiotic and biotic properties. Microbial necromass serves as nutrient source to cope with deficiencies (Cantor et al., 2023; Akroume et al., 2019). Thus, besides necromass quality (Beidler et al., 2020), the soil nutrient status (Pausch et al., 2024) influences the amount of necromass undergoing microbial recycling. Further, the composition of resident microbial community (Li et al., 2023) and the soil pH (Wang and Kuzyakov, 2024) can affect the efficiency of decomposition, a key step prior to the formation of MAOM (Liang et al., 2017). Lastly and most importantly, the availability of reactive surfaces plays a major role in MAOM formation and thus, the long-term stability of soil C. With increasing fine fraction, more reactive surfaces are present in the soil and thus, more binding places are available for molecules to form MAOM (Islam et al., 2022; Cotrufo et al., 2013; Barnard et al., 2025; Schweizer et al., 2021; Manzoni and Cotrufo, 2024; Kästner et al., 2021), leading to a positive relationship between soil C and soil clay content (Poeplau and Don, 2023). However, key factors controlling stabilization remain elusive for fungal necromass-C.

Moreover, fungal necromass can not only contribute to new MAOM formation but may also stimulate or suppress the decomposition rates of existing SOM through priming effects (PE, Kuzyakov et al., 2000) by serving as a resource for heterotrophic communities.

Arbuscular mycorrhizal fungi (AMF) remain largely underrepresented in studies on MAOM formation and stability. This is especially critical given that AMF associate with >70 % of all plant species (Brundrett and Tedersoo, 2019), especially with grasses and crops (van der Heijden et al., 2015), making up ~60 % of the land cover (Hawkins et al., 2023). The underrepresentation of AMF necromass is mainly due to challenges in AMF cultivation and necromass production for controlled laboratory studies: Unlike other mycorrhizal types or saprotrophic fungi, AMF obligatorily rely on plant partners, complicating their *in vitro* growth. Thus, detailed mechanistic understanding of the fate of AMF necromass in soil is still missing.

Aiming to close this knowledge gap we hypothesized that (I) besides being respired, AMF necromass is quickly converted from the POM pool to the MAOM pool, where it is continuously accumulating due to the stability of MAOM. Further, we hypothesize that (II) MAOM formation with AMF necromass is positively correlated with the fine fraction content of the mineral soil matrix. Lastly, we hypothesize (III) a higher SOM decomposition after the addition of AMF necromass as it serves as energy source, fuelling microbial processes.

To test this, we used root organ cultures to produce microbially clean, ^{13}C -enriched necromass of the AMF *Rhizophagus irregularis*. The necromass was then incubated in six different grassland soils that varied in soil properties, such as pH, clay content, the concentrations of bioavailable phosphorus (P) and nitrogen (N), and the ratio of fungi to

bacteria (F/B). We traced the necromass- ^{13}C incorporation in the soil fractions POM and MAOM and its loss as CO_2 efflux 12 and 83 days after necromass addition.

2. Material and methods

2.1. Necromass production

The necromass of *Rhizophagus irregularis* isolate BEG 236 was produced in compartmentalized monoxenic cultures with Ri-T DNA-transformed *Cichorium intybus* roots and liquid nutrient media as described previously (Rezanka et al., 2023), with the only difference that the sucrose (1% w/v) in the nutrient medium was replaced with ^{13}C -glucose (2% w/v). The glucose, purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA) was enriched by ^{13}C to more than 99 atom %. The cultures were grown for eleven months in a dark room at a constant temperature of 26 °C. Thereafter, biomass of the AMF was collected (as described in Rezanka et al., 2023), rinsed with deionized water, dried in a vacuum centrifuge for 48 h, yielding a total dry weight of 1.49 g. Then, it was frozen in liquid nitrogen and homogenized using pre-cooled ceramic mortar and pestle, before subsequent storing at -80 °C. The concentrations of C and N and the ratio $^{13}\text{C}/^{12}\text{C}$ of the necromass were measured by Flash (2000) elemental analyser coupled with isotope ratio mass spectrometer Delta V Advantage via ConFlo IV (Thermo Scientific, Waltham MA, USA). The isotopic composition of the CO_2 standard (gas cylinder) was calibrated using international standards CH-3 (cellulose), IAEA-600 (caffeine) and CO-8 (calcite) for $\delta^{13}\text{C}/^{12}\text{C}$ (all the standards were distributed by the International Atomic Energy Agency, Vienna, Austria). As a reference for the $^{13}\text{C}/^{12}\text{C}$ isotopic ratio measurement, VPDB was used ($R = 0.0111802$). Necromass N concentration was $1.19 \mu\text{mol mg}^{-1}$, its C concentration was $36.4 \mu\text{mol mg}^{-1}$, and atom % $^{13}\text{C}/^{12}\text{C}$ was 96.9. Thus the molar C/N ratio of the necromass was 30.6.

2.2. Experimental setup

Soil from six different grassland sites with contrasting soil properties (selected from the survey of Sun et al., 2025) across the Czech Republic were collected from the top 20 cm (Table 1). We focused on grassland soils as most of the grassland plant species are forming symbiosis with AMF or are non-mycorrhizal, avoiding interferences with other mycorrhizal types such as ecto- or ericoid mycorrhizas. The sites were not tilled for at least three years prior to sampling and were covered by natural vegetation (grasses + herbs) at all sites and by hawthorn bushes at site 6. Soil samples were collected from depth 0-20 cm by a spade from several spots in a perimeter of 10 m from the coordinates given in Table 1, sieved through 8 mm (fresh) and mixed to obtain one composite soil sample per site. By sieving, homogeneity of the sample was ensured and compaction was minimized. The gravimetric water contents were determined, and the soils were then air dried at room temperature for 2 weeks and sieved through 2 mm thereafter. A fungal necromass suspension was prepared by mixing 1.2 g of the dry AMF necromass in 30 mL deionized (DI) water. Forty g of the six soils (air dried) were each treated with 5 mL of the necromass suspension, before the soil samples were subdivided into six aliquots of 5.5 g (soil rewetted with the necromass suspension) each. These aliquots were each mixed with an amount of the respective air-dried soil corresponding to 15 g dry weight. Such soil aliquots were added (depending on the soil) with 2.6 to 3.6 mL water to achieve approx. 70% water holding capacity (determined gravimetrically, Table 1) and transferred to airtight 500 mL glass bottles. This two-stage mixing was implemented to ensure greater homogeneity of necromass enrichment to the soils, as preparing solid-solid mixtures is technically very challenging. This procedure resulted in the necromass amendment of 24.4 mg dry weight, mixed in soil corresponding to 20 g dry weight of each individual soil (correcting for the moisture of the air-dried soils, Table 1). Six replicate bottles per each soil were thus established. The bottles were equipped with a septum in the

Table 1

Properties of the six soils used in this study, all with n = 1. P: phosphorus. C/N: ratio of total organic carbon (C) and total nitrogen (N) concentrations. F/B: fungi-to-bacteria ratio.

Soil	Corresponding soil ID in Sun et al. (2025) ^a	Site (nearest village)	Latitude (°N)	Longitude (°E)	Soil type ^b	Sand (%) ^c	Silt (%) ^c	Clay (%) ^c	pH ^d	Bioavailable P (mg kg ⁻¹) ^e	Total organic C (%) ^f	C/N ^g	Mineral N (mg g ⁻¹) ^h	F/B ⁱ	Gravimetric water content of air dried soil (%) ^j	Gravimetric water content - 70% water holding capacity (%) ^k
1	25	Květuš	49.518	14.452	Haplic Cambisol	63.4	21.4	15.2	5.7	11.0	1.22	9.27	3.71	0.050	5.13	18.6
2	46	Rychnov u Nových Hradů	48.757	14.684	Haplic Cambisol	69.7	14.8	15.5	5.1	1.25	2.06	10.8	4.34	0.058	5.97	19.5
3	43	Pivonice u Pohorské vsi	48.664	14.648	Dystric Cambisol	51.3	25.4	23.3	5.4	1.16	3.19	10.0	13.5	0.030	23.7	45.0
4	4	Drahobuz	50.527	14.327	Calcaric Leptosol	49.8	18.6	31.6	8.0	7.73	2.98	16.4	6.22	0.046	6.42	20.0
5	5	Encovany	50.532	14.258	Calcaric Leptosol	11.6	37.8	50.6	7.9	0.40	7.83	26.7	8.18	0.057	7.54	26.4
6	16	Vojnice	50.416	13.940	Calcic Chernozem	9.4	23.9	66.7	7.9	3.45	4.91	22.0	5.96	0.045	9.41	26.2

^a See Sun et al. (2025).
^b FAO classification system, determined according to <https://mapy.geology.cz/pudy/#> (accessed 12 March 2026).
^c Determined gravimetrically after soil organic matter removal by hydrogen peroxide and dispersion (sodium hexametaphosphate) and sedimentation of soil particles in a water column for 3 h. Sand and silt sediment quickly and sand fraction was collected using analytical sieve (63 µm) after clay fraction was determined. Silt content was calculated as the difference between total weight of mineral soil, and the combined weights of sand and clay fractions.
^d Determined in a soil-water slurry 1:2.5 (w:w) after 1 h shaking at room temperature.
^e Water extractable P (soil:water slurry 1:10), determined after 18 h shaking at room temperature, filtering through 0.2 µm Teflon filter and colorimetry with Malachite Green (Ohno and Zibilske, 1991).
^f Determined by combustion CN analysis (Flash, 2000 elemental analyzer, Thermo FisherScientific, Waltham MA, USA) with combustion temperature set at 1020 °C (chromium oxide as a combustion catalyzer, reduced copper as a follow-up reduction step).
^g Both determined by combustion CN analyses as above.
^h Sum of NH₄⁺ and NO₃⁻ ions concentration in 2 M KCl extracts (1:10, weight:volume, shaken for 1 h and filtered through ashless paper filter), and determined by Berthelot and Griess reactions as described previously (Hood-Nowotny et al., 2010).
ⁱ Determined as the ratio of detected fungal internal transcribed spacer copies per mg soil to prokaryotic 16S gene copies per mg soil as detailed previously (Sun et al., 2025).
^j Determined gravimetrically before and after drying at 105 °C for 48 h.
^k water saturation capacity determined gravimetrically 2 h after full soil saturation and subsequent free-draining at room temperature.

lid to allow gas sampling. Additionally, six glass bottles with the same amount of each of the six fresh soils were prepared similarly as natural isotope abundance controls without necromass addition. These soils only received the same amount of water as the necromass treatments. The bottles were sealed and incubated in the dark at 20 °C over the experimental period during which half of the replicate bottles were harvested after 12 days and the second half after 83 days. Consequently, the experiment consisted of 72 experimental units (6 soil types $\times$ 2 treatments (necromass amended and unamended) $\times$ 2 harvest dates (12 and 83 days) $\times$ 3 replicates).

2.3. Gas measurements

Gas measurements were conducted two times during the incubation period for samples harvested after 12 days (day 6 and 12) and five times for samples harvested after 83 days (day 6, 12, 20, 48, and 83). Gas samples (0.3 mL) were collected through the septum with a syringe and analyzed using the GasBench II equipped with a single cold trap and coupled to the isotope ratio mass spectrometer as above to measure both, the headspace CO₂ concentration and its ¹³C isotopic composition in atom %. After taking the gas samples at day 20 and 48, the bottles were vented to prevent oxygen deficiencies: The bottles were opened for 3.5 h, during which fresh air was blown into them twice for 1 min each using a lab compressor (N816 Labport, KNF, Freiburg, Germany). Thus, for the samples harvested after 83 days, the total cumulative CO₂ and excess ¹³C over the 83 days was calculated as sum of the values obtained after 20, 48, and 83 days. For the replicates harvested after 12 days, the gas measurement at day 12 already represented cumulative values.

The ¹³C natural abundance (atom %) of the bulk soil of the six unamended controls per soil were averaged over both timepoints and used as background for calculating the atom % ¹³C-excess (APE) at all 5 gas samplings. Consequently, the ¹³C-excess mass (excess ¹³C, μmol) of the soil CO₂ efflux was calculated by multiplying the APE (atom % above the background) in the CO₂ with the total C of the CO₂ efflux (μmol , equation (1)). The ¹³C-excess mass was then referred to the ¹³C input as necromass to calculate the percentage recovery (% , equation (2)).

$$^{13}\text{C excess mass } [\mu\text{mol}] = \frac{C_{\text{total}} [\mu\text{mol}] * (^{13}\text{C}_{\text{sample}} [\text{atom \%}] - ^{13}\text{C}_{\text{control}} [\text{atom \%}])}{100} \quad (1)$$

$$^{13}\text{C recovery } [\%] = \frac{^{13}\text{C excess mass } [\mu\text{mol}]}{\text{necromass } ^{13}\text{C input } [\mu\text{mol}]} * 100 \quad (2)$$

To calculate the SOM-derived CO₂ efflux, a linear two-source isotopic mixing model was applied with the isotopic signature of the necromass and the SOM as two end members (equation (3)), where f_{SOM} represents the fraction of SOM-derived CO₂ in the total CO₂ efflux. Ultimately, f_{SOM} was multiplied with the measured cumulative CO₂ efflux to obtain the SOM-derived CO₂ efflux [$\mu\text{mol C}$].

$$f_{\text{SOM}} = \frac{^{13}\text{C}_{\text{CO}_2} [\text{atom \%}] - ^{13}\text{C}_{\text{necromass}} [\text{atom \%}]}{^{13}\text{C}_{\text{SOM}} [\text{atom \%}] - ^{13}\text{C}_{\text{necromass}} [\text{atom \%}]} \quad (3)$$

The effect of necromass on SOM decomposition was calculated as priming effect (PE, Kuzyakov et al., 2000). For this, the SOM-derived CO₂ efflux was subtracted from the total CO₂ efflux of the non-amended control samples (averaged across the three replicates). This difference was referred to the latter to calculate the relative PE in %.

2.4. Density fractionation and soil isotopic measurements

After 12 and 83 days, 3 replicates were harvested per soil. To separate the POM and MAOM fractions, a density-based fractionation was conducted. The detailed protocol can be accessed in (Vasilica et al., 2026b). In short, 3 g (modified compared to the protocol) of air-dried

soil were added to 25 mL sodium polytungstate (SPT). To disperse aggregates, the samples were treated with ultrasonication (450 J mL⁻¹, Sonopuls homogenisator HD4100, Bandelin, Berlin, Germany). MAOM and POM fractions were then separated based on their different densities in SPT (here, after dispersion of aggregates, POM comprises free and occluded POM). Samples were centrifuged (4000 rpm/approx. 1750 g, Rotina 380R, Hettich, Tuttlingen, Germany) and the floating POM was aspirated carefully, which was repeated four times. SPT was removed from POM and MAOM fractions with DI water and filtration (for POM) or centrifugation (for MAOM) until the conductivity of the DI water was <15 $\mu\text{S cm}^{-1}$ or <150 $\mu\text{S cm}^{-1}$ for POM and MAOM, respectively. No particle size fractionation was performed, i.e., the MAOM fraction included sand, silt, and clay sized particles. The mass recovery after fractionation (POM + MAOM) ranged from 80 to 106 %. Since low recovery rates were caused by MAOM losses during washing steps, the mass of MAOM was recalculated by subtracting the POM mass from the total SOM for all samples.

The separated soil fractions were freeze dried, weighed, and ground. The C content and ¹³C of POM and MAOM fractions, as well as of the total bulk soil (dried at 60 °C and ground) were analyzed with an elemental analyzer coupled to the mass spectrometer as above. As no fractionation was performed for unamended samples, ¹³C of bulk soil was used as natural abundance control for both SOM fractions. Although the natural ¹³C abundances of the individual fractions (POM and MAOM) are thought to differ from the bulk soil, this difference was shown to be minimal (~1 ‰, Klink et al., 2022; Yan et al., 2026), allowing the use of natural abundance values of the bulk soil. The necromass-¹³C recovery was calculated analogously to equations (1) and (2), with the respective values for the soil fractions. Total C recovery was calculated as percentage C in the individual soil fraction referred to the bulk soil C.

2.5. Analysis of the microbial community

Upon either of the destructive harvests, representative aliquots (2 g) of each of the incubated soils (individual bottles) were dried at 65 °C and milled by a ball mill (MM 200, Retsch, Haan, Germany) at 25 Hz for 2 min. DNA was extracted from 250 mg aliquots of the milled soils using DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany), while adding 20 billion copies of internal DNA standard (Thonar et al., 2012) to each sample before extraction. The recovery of the internal standard, using a TaqMan marker described previously (Thonar et al., 2012), and the abundance of fungi (ITS) and bacteria (16S), were analyzed as described previously (Dudáš et al., 2022). The concentrations of gene copies, measured by Lighcycler 480 II quantitative real-time PCR cycler (Roche, Rotkreuz, Switzerland) using Luna chemistry (New England Biolabs, Ipswich MA, USA) were corrected for DNA losses upon DNA extraction (measured by the internal DNA standard analyses) before further use. The ratio of soil fungi to bacteria (F/B) was calculated by dividing the fungal ITS copy counts by bacterial 16S copy counts per mg soil.

2.6. Statistics and visualisation

Two-way ANOVAs were used to test for statistical differences with two factor-levels (i.e., soil ID and timepoint) in Sigmaplot Version 15.0 (Systat Software Inc., Richmond, California, USA). In the case of heteroscedastic or non-normally distributed residuals, data was log-transformed. If this transformation did not yield improvement of the data distribution, an aligned rank transformed (ART) ANOVA was performed with the package ARTTool (Kay and Wobbrock, 2016; Wobbrock et al., 2011) in RStudio 4.3.0 (R Core Team, 2023). TukeyHSD was used as posthoc test for the ANOVA, while a posthoc test on estimated marginal means was performed after ART-ANOVA. The latter was performed with the emmeans package (Lenth and Piaskowski, 2026) in R. For testing the effects of different soil variables on C and ¹³C in soil fractions

and respiration, a multiple linear regression was performed in RStudio. For testing the effect of soil parameters on the temporal development of MAOM- ^{13}C (Δ recovery of necromass ^{13}C in MAOM), an individual linear regression analysis were conducted due to the limited sample size. The latter and figure creation were performed in Sigmaplot.

3. Results

3.1. Distribution of total C in POM and MAOM

Across all soils, more C was present relatively in MAOM ($69.2 \pm 10.9\%$) than POM ($24.8 \pm 9.0\%$, Fig. 1a and b). We observed the lowest total C recovery in MAOM for the two soils with the lowest clay content (soils 1 and 2). For soil 1, C in MAOM declined between the 12 and 83 day sampling (Fig. 1b), while the same was true for soil 2 and 4, yet not significant (Fig. 1b).

3.2. SOM-derived respiration

The six studied soils showed differences in CO_2 efflux, both in the basal respiration of controls without necromass addition (Fig. 2a, Supplementary Fig. S1a) and in the SOM-derived CO_2 from soils with necromass addition (Fig. 2b, Supplementary Fig. S1b). For both, soil 5 (soil with highest C content, Table 1) showed a higher respiration compared to all other soils – followed by soil 6 with the second highest C content. When comparing the SOM-derived CO_2 from the six soils that were amended with AMF necromass with the unamended controls of the respective soil, it became evident that the SOM-derived CO_2 changed after the necromass addition. This is also reflected in the PE (Fig. 2c, Supplementary Fig. S1c): In soils with a low pH (soils 2 and 3), a positive PE occurred, while in soil 4 with a high pH, the PE was negative.

3.3. Recovery of necromass-derived ^{13}C in POM, MAOM, and CO_2

After 12 days of incubation, $53.1 \pm 10.0\%$ of the ^{13}C that was added as AMF necromass was recovered in the soil fractions. More ^{13}C was recovered in the MAOM fraction ($36.2 \pm 12.5\%$ of the total necromass ^{13}C) compared to the POM fraction ($16.9 \pm 5.5\%$) with the exception of soil 6 (Fig. 3a and b). We found the ^{13}C recovery in the soil fractions to vary among the analyzed soils: in soils with clay contents $<30\%$ and

acidic pH (soils 1, 2, and 3), more MAOM- ^{13}C was formed. Analogously to the high relative MAOM formation with ^{13}C , least ^{13}C was recovered in the POM fraction in the soil with the lowest clay content (soil 1). Compared to the sampling after 12 days of incubation, the recovery of ^{13}C in the MAOM fraction of the soils was reduced after 83 days except for soil 6 (insignificant for soil 4). The reduction in the clay-poor soils 1 and 2 was by 23 % and 15 % of the ^{13}C input, respectively, while in soils 3 and 5, the reduction was around 6 %. In none of the soils, this was accompanied by a significant increase in POM ^{13}C .

Twelve days after the amendment of ^{13}C -enriched AMF necromass, $33.1 \pm 4.4\%$ of the added ^{13}C was recovered in the CO_2 efflux (Fig. 3c). This is in a similar range as the recovery in the MAOM fraction. However, after 83 days, $60.0 \pm 8.5\%$ of the necromass-derived ^{13}C was recovered in CO_2 , surpassing the recovery in the POM and MAOM combined ($46.5 \pm 5.6\%$ after 83 days). The cumulative ^{13}C recovery in CO_2 over 83 days differed between the soils with the three soils with an alkaline pH showing a lower percentage recovery of ^{13}C in the CO_2 respiration (Fig. 3, Table 3).

3.4. Effect of abiotic soil properties on C and ^{13}C in MAOM, POM and CO_2

As the six investigated soils differed in a variety of physico-chemical properties, we performed multiple correlations to investigate the driving factors of the distribution of C (Table 2) and necromass-derived ^{13}C (Table 3).

Total MAOM-C was explained by a mix of different soil parameters, with total C contributing the most after 12 and 83 days. At both timepoints, MAOM-C was correlated positively with soil clay content (Fig. 4a–c, Table 2). While C in POM was correlated with pH, N and P, all measured soil parameters were important to explain soil CO_2 efflux at the harvest after 12 days. After 83 days, C in POM was only correlated with mineral N, while the soil CO_2 efflux was correlated with pH and soil C.

The positive influence of the clay content on the MAOM-C fraction was inversed for necromass-derived ^{13}C , as less MAOM- ^{13}C was found in soils with a higher clay content (for both time points, Table 3, Fig. 4a,c). Importantly, despite this inverse effect, no direct correlations between MAOM-C and the formation of MAOM- ^{13}C was detected (Table 3, Fig. 4b,d).

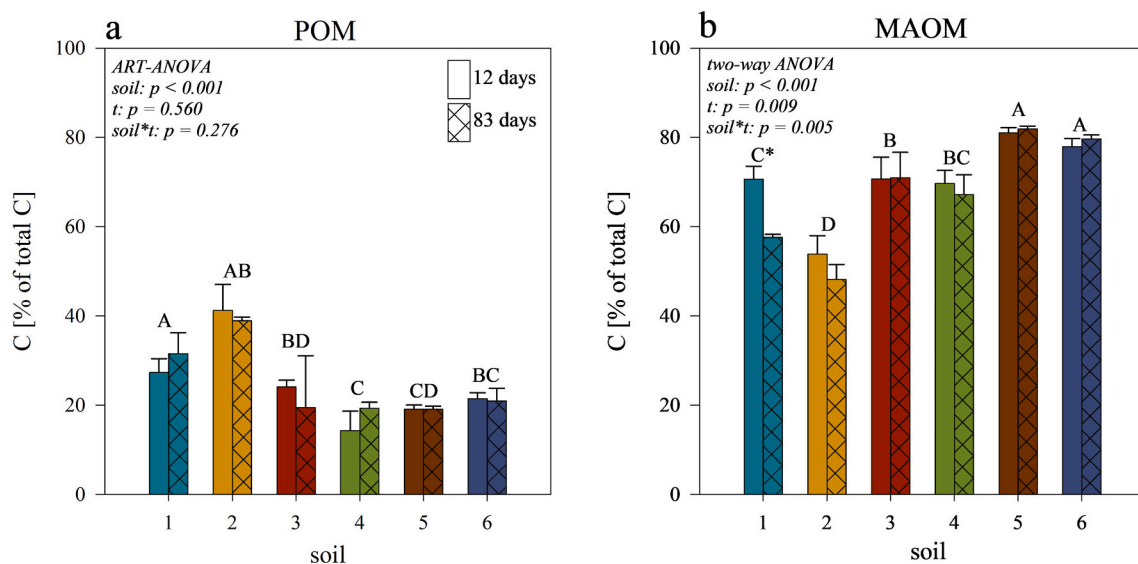


Fig. 1. Percentage of total C in the soil fractions particulate organic matter (POM, a) and mineral-associated organic matter (MAOM, b) relative to total C in bulk soil in six grassland soils after 12 days (solid bars) and 83 days (checkered bars) of incubation. Letters indicate significant differences between the soils, while asterisks display differences between the two timepoints of one soil ($p < 0.05$, a: aligned rank transformed ANOVA with pairwise comparison on estimated marginal means with Tukey adjustment as posthoc test, b: two-way ANOVA with TukeyHSD posthoc test).

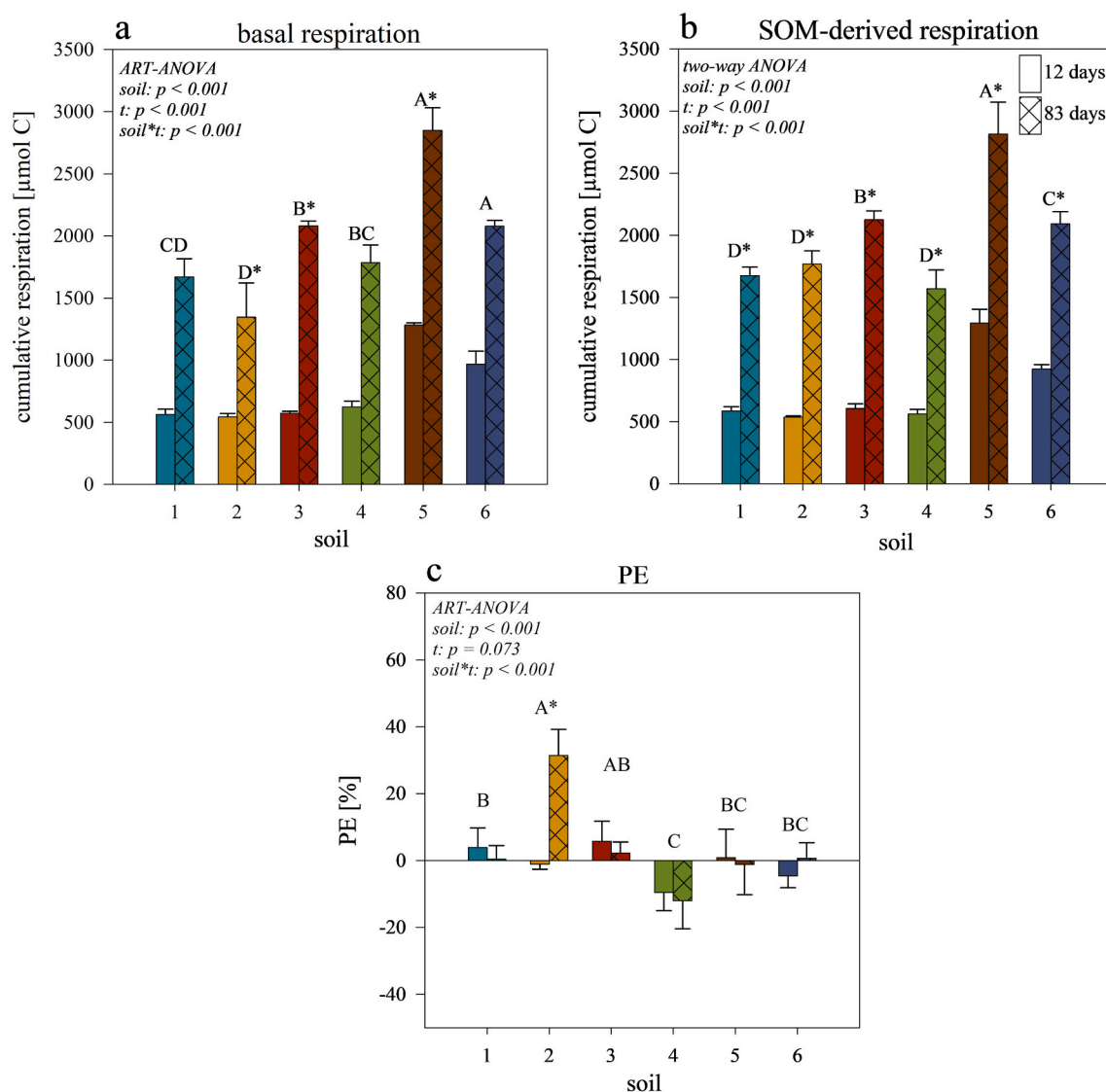


Fig. 2. Cumulative CO_2 efflux after 12 (solid bars) and 83 days (checked bars) of six grassland soils not amended with necromass (a), cumulative SOM-derived CO_2 efflux of soils the same set of soils after necromass addition (necromass-derived CO_2 excluded with a linear mixed model, b), and the resulting PE (priming effect, i.e. percentage change of SOM-derived CO_2 efflux after necromass addition, c). Letters indicate significant differences between the soils, while asterisks display differences between the two timepoints of one soil ($p < 0.05$, a, c: aligned rank transformed ANOVA with pairwise comparison on estimated marginal means with Tukey adjustment as posthoc test; b: two-way ANOVA with a TukeyHSD posthoc test).

For the incorporation of ^{13}C into the MAOM fraction, clay content was the only factor showing a significant correlation at both sampling times. While the recovery of necromass-derived ^{13}C in the POM fraction was positively correlated with the clay content of the soils and the overall C in POM at the first sampling, it was mainly explained by the pH at the second sampling. The ^{13}C - CO_2 efflux was explained mainly by the pH at both samplings, but most parameters contributed at the second sampling. For all described multiple correlations, the soil total N, C/N ratio, and F/B ratio were excluded due to collinearity.

The difference in the recovery of necromass-derived ^{13}C in MAOM between day 12 and 83, which was negative in all soils except soil 6, correlated with the clay content of the soils. With lower clay content, the difference in the ^{13}C recovery in MAOM between the two sampling points significantly increased (Fig. 5).

3.5. Effect of soil properties on the necromass-induced change in the ratio of fungi-to-bacteria (F/B)

After addition of the ^{13}C -labelled necromass, the soils exhibited a

change in the F/B ratio (Fig. 6). This change was positive in the soils 1 and 2 (acidic pH) and negative in soils 4 and 5 (alkaline pH). The F/B decreased for all soils 83 days compared to 12 days after the start of incubation, i.e., fungal growth decreased and/or bacterial growth increased between the two samplings, irrespective of whether the initial change after necromass addition was negative or positive. This trend, however, was not significant. The direction and magnitude of the change of F/B could be mainly explained by the soil pH, as the negative correlation between pH and $\Delta\text{F/B}$ had the highest explanatory power among the tested soil parameters (Table 4). N and C/N were excluded from the multiple linear regression analysis due to collinearity.

4. Discussion

4.1. Decomposition of AMF necromass

We used pure necromass of the AMF species *R. irregularis*, highly enriched in ^{13}C , to trace the fate of necromass-C in grassland soils with contrasting soil properties. We showed that already after 12 days,

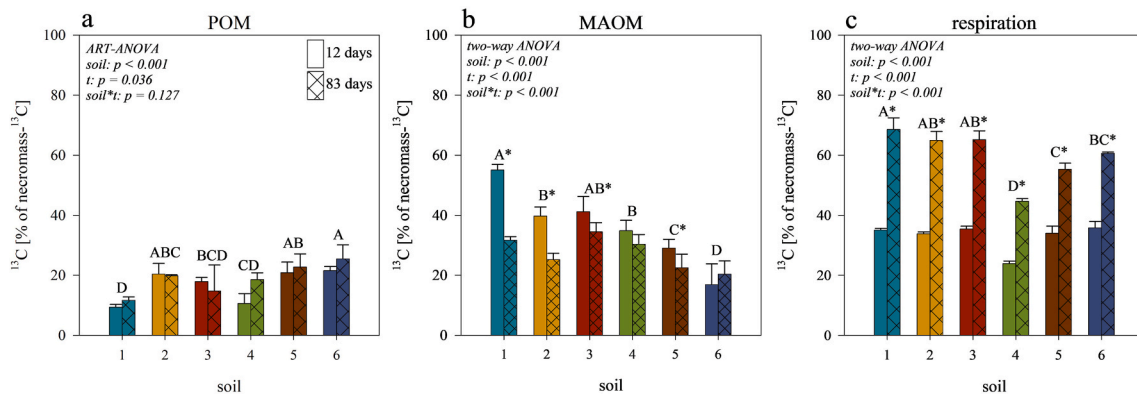


Fig. 3. Recovery of ^{13}C in the soil fractions POM and MAOM and the CO_2 efflux: Percentage of the ^{13}C added as arbuscular mycorrhizal fungal necromass that was recovered in the POM fraction (a), the MAOM fraction (b) and the CO_2 respiration (c) after 12 (solid bars) and 83 (checked bars) days in 6 grassland soils with varying properties. Letters indicate significant differences between the soils, while asterisks display differences between the two timepoints of one soil ($p < 0.05$, a: aligned rank transformed ANOVA with pairwise comparison on estimated marginal means with Tukey adjustment as posthoc test; b, c: two-way ANOVA with TukeyHSD posthoc test).

Table 2

Results of multiple correlations performed to investigate the impact of the explaining variables (displayed on the left) on the percentage of soil C present in particulate organic matter (POM) and mineral-associated organic matter (MAOM) as well as the cumulative CO_2 efflux of C across six grassland soils with varying properties. Numbers indicate the slope (correlation coefficient) of the individual contribution and asterisks indicate the level of significance (*** $p < 0.001$; ** $0.001 \leq p < 0.01$; * $0.01 \leq p < 0.05$).

	MAOM-C after 12 days (%)	POM-C after 12 days (%)	C- CO_2 after 12 days ($\mu\text{mol C}$)	MAOM-C after 83 days (%)	POM-C after 83 days (%)	C- CO_2 after 83 days ($\mu\text{mol C}$)
Clay (%)	0.294 ***		3.237 *	0.376 **		
pH	-3.638 **	-5.65 **	-118.552 ***			-249.691 **
Mineral N (extractable NH_4^+ + NO_3^- , mg g^{-1})	1.214 ***	-1.664 ***	-13.140 *	1.844 ***	-1.936 **	
Total C (%)	4.172 ***		179.777 ***	2.893 **		280.111 ***
Bioavailable P (mg kg^{-1})	2.382 ***	-1.234 **	26.767 ***	1.387 **		

Table 3

Results of the multiple correlations performed to investigate the impact of the explaining variables (displayed on the left) on the distribution of necromass derived ^{13}C into the soil fractions mineral-associated organic matter (MAOM), particulate organic matter (POM) and the CO_2 efflux across six grassland soils with varying properties. Numbers indicate the slope of the individual contribution and asterisks indicate the level of significance (*** $p < 0.001$; ** $0.001 \leq p < 0.01$; * $0.01 \leq p < 0.05$).

	MAOM- ^{13}C after 12 days (%)	POM- ^{13}C after 12 days (%)	^{13}C - CO_2 after 12 days (%)	MAOM- ^{13}C after 83 days (%)	POM- ^{13}C after 83 days (%)	^{13}C - CO_2 after 83 days (%)
Clay (%)	-0.647 ***	0.178 *	0.262 ***	-0.369 *		0.476 ***
pH			-6.98 ***		4.672 **	-12.225 ***
Mineral N (extractable NH_4^+ + NO_3^- , mg g^{-1})						
Total C (%)						2.050 *
Bioavailable P (mg kg^{-1})						1.535 **
MAOM-C (%)						
POM-C (%)		0.556 **			0.727 ***	0.296 *

around 30 % of the necromass-derived C was respired in most soils, indicating a fast initial decomposition. The total C loss over the incubation period of 83 days added up to 60 %. To date, no similar study was conducted using AMF necromass. However, similarly high ^{13}C -recovery rates in soil CO_2 were reported for ^{13}C -labelled bacterial necromass of *Escherichia coli* (Buckeridge et al., 2022b). The decomposition of organic molecules is influenced by their chemical composition, as preferably nutrient rich molecules are depolymerized, whereby CO_2 is released. Besides the N-content locked in the necromass (Brabcová et al., 2018), the melanin content was shown to have an influence on the decomposition of microbial necromass (Maillard et al., 2023). However, the decomposition might be influenced further by the exact chemical composition (Fernandez et al., 2016), hence the decomposition of necromass was shown to vary both, interspecifically (Dong et al., 2021) and intraspecifically (Maillard et al., 2023). Given the fast decomposition

shown in this study, AMF necromass seems to be a favourable source for soil microbes, which might be partly explained by rather low C/N ratio of 31 of the AMF, containing many N-rich compounds (Horsch et al., 2023) and the facilitated breakdown of necromass by fungal extracellular enzymes (Fernandez et al., 2016). However, studies on the exact chemical composition of AMF necromass are scarce (Horsch et al., 2023; Jansa et al., 2020) but would be valuable to further understand the necromass properties driving AMF decomposition.

4.2. Initial stabilization of AMF necromass-derived ^{13}C

At the first sampling point after 12 days, more than one third of the necromass-C was recovered in the MAOM fraction, which confirms an initial stabilization of necromass-C as part of our hypothesis (I). A fast retention of microbial necromass was also demonstrated for bacteria

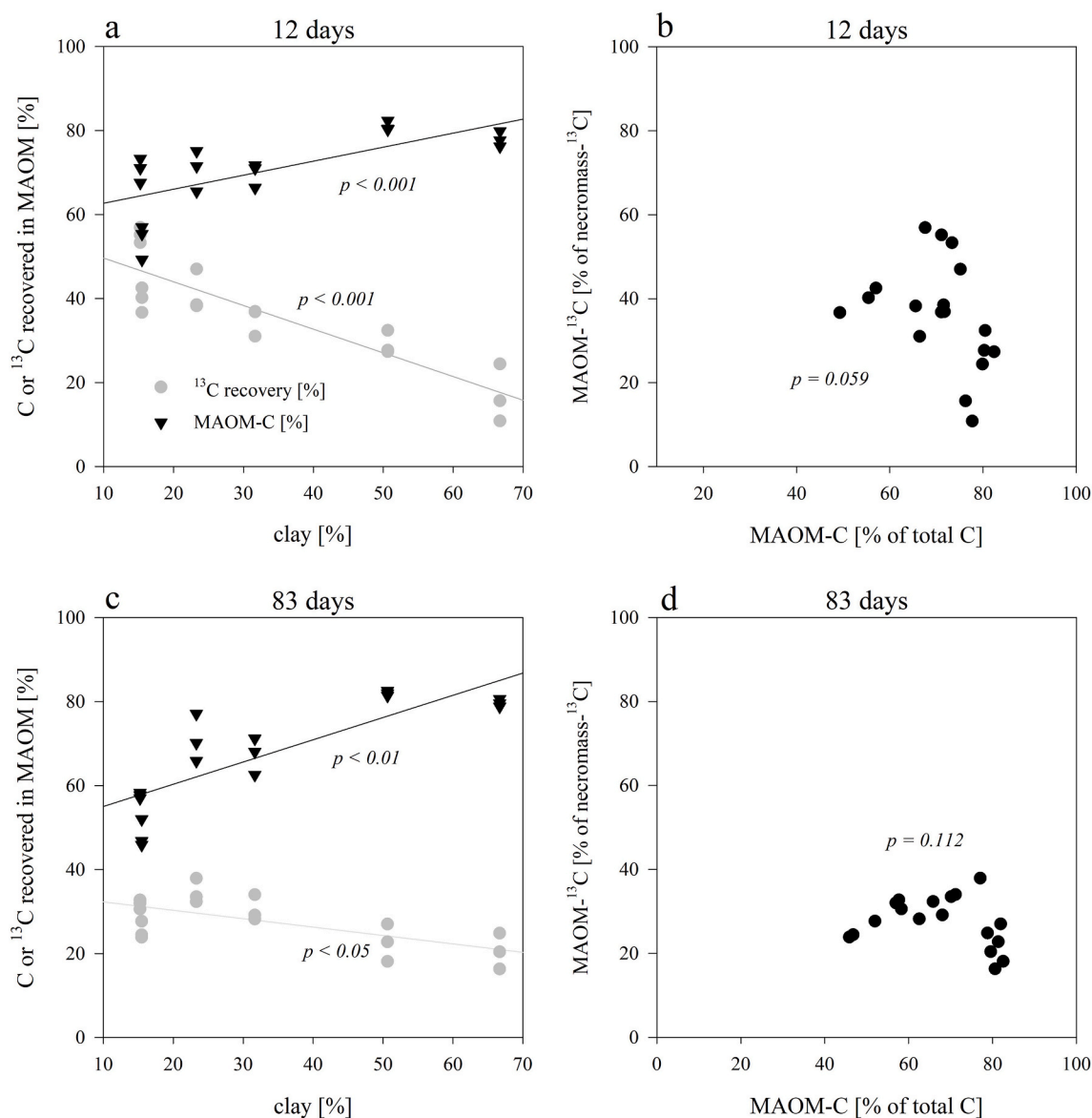


Fig. 4. Relationship between the clay content (%) and total C in mineral-associated organic matter (MAOM, blacktriangles) or arbuscular mycorrhizal fungal (AMF) necromass-derived ^{13}C (grey circles) 12 days (a) and 83 days (c) after the start of incubation. Relationship between necromass-derived ^{13}C in MAOM and total C in MAOM 12 days (b) and 83 days (d) after the start of the incubation. For a and c, displayed p-values in the graphs are extracted from a multiple linear regression analysis including all measured soil parameters while for b and d, p-values result from a simple linear regression.

(Buckeridge et al., 2020) and later specified as MAOM formation (Buckeridge et al., 2022b). This might indicate that microbial necromass has a potential to be stabilized over a longer time, thereby serving as important driver for soil C retention. However, studies investigating the efficiency of MAOM formation currently only exist for bacterial necromass (Buckeridge et al., 2020, 2022b). Although the contribution of AMF necromass to MAOM is recognized (Klink et al., 2022), studies confirming the efficiency and speed of MAOM formation were lacking so far for fungal, and particularly for AMF necromass. Thus, our findings extend the view that microbial necromass is a relevant factor in soil C storage given the fast stabilization of AMF necromass as MAOM. The fast stabilization also further emphasizes a high microbial affinity towards AMF necromass as MAOM formation may require prior microbially-driven depolymerization by exoenzymes (Sokol et al., 2019).

4.3. Effect of abiotic soil properties on the formation of MAOM

The use of six grassland soils that vary in basic soil properties, such as nutrients, pH, or texture allowed for correlation analysis to understand the main drivers of the fate of necromass in grassland soils. We hypothesized that the soil clay content is driving the MAOM formation positively. However, while we indeed found a positive correlation between the clay content of the soil and the percentage of soil C being present in MAOM, an inverse, thus negative correlation was observed between the clay content and the formation of MAOM with necromass-C.

Both, negative and positive correlations between clay and MAOM were reported in literature. A positive correlation is usually explained by the fact that higher clay contents provide greater surface areas for organic molecules to bind, thereby promoting the formation of MAOM (Cotrufo et al., 2019; Begill et al., 2025). For a negative correlation, in contrast, a clear explanation is not given to date. Zhang et al. (2025) explained a negative correlation between overall soil C and the clay

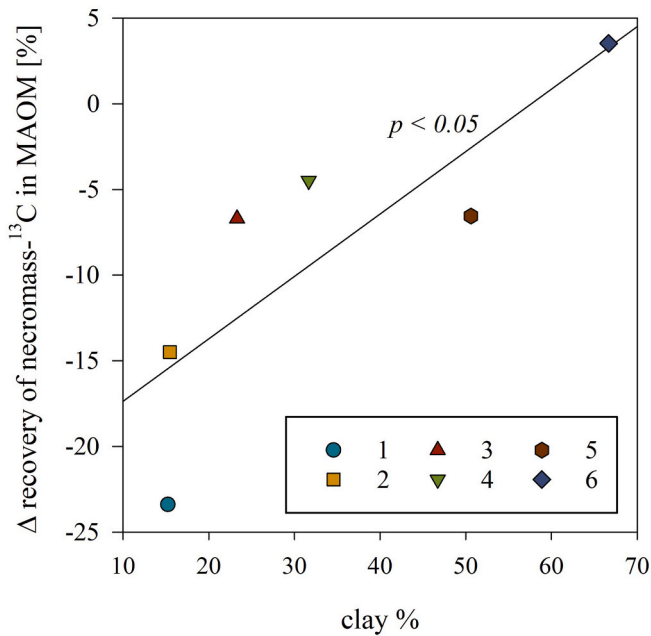


Fig. 5. Relationship between the clay fraction content and the change in arbuscular mycorrhizal fungal (AMF) necromass-derived ^{13}C recovered in the mineral-associated organic matter (MAOM) fraction between sampling after 83 days and after 12 days in 6 soils which differ in their clay contents. Displayed p-value results from a linear regression.

fraction with overlying effects of soil pH. This, however, cannot be used as explanation for our data due to the contrary effect on the overall C and the necromass-derived ^{13}C in the MAOM fraction. In other studies, it was discussed that MAOM was not only formed directly via binding of organic molecules to the mineral surface, but also through interactions among organic molecules themselves (i.e., organic-organic interactions), and thus binding on organics already associated with

mineral surfaces ('skyscraper', Begill et al., 2023; Schweizer et al., 2021; Kopittke et al., 2018). Begill et al. (2023) argue that such skyscrapers preferably form under limited reactive surface area, while under high availability of surfaces with increasing clay content, organic matter is distributed more sparsely with less pronounced formation of skyscrapers. For the latter, recent proof was found using NanoSIMS (Barnard et al., 2025). It is proposed that the binding of organic molecules onto organic matter is occurring more easily than on clay surfaces directly (Kang et al., 2024), which may be due to the formation of microbial hotspots around the organic patches. This was shown especially for microbial necromass (Buckeridge et al., 2020; Vogel et al., 2014). Thus, we suggest that AMF necromass is preferably binding on organic matter patches, which are less common under high clay content, explaining the observed negative correlation between the clay content and the formation of MAOM with AMF necromass in our study.

The phenomenon that both, negative and positive correlations between clay and MAOM-C exist in the same soil might be explained by the chemical composition of the specific organic compound affecting whether it preferably binds to the mineral surface directly or onto existing skyscrapers (Kopittke et al., 2018; Kleber et al., 2007; Kang

Table 4

Results of the multiple correlations performed to investigate the impact of the explaining variables (displayed on the left) on the difference in the fungal-to-bacterial (F/B) ratio between sampling at 12 days and sampling at 83 days relative to day 0 after amendment of arbuscular mycorrhizal fungal necromass to six grassland soils with varying properties. Numbers indicate the slope of the individual contribution and asterisks indicate the level of significance (***) $p < 0.001$; ** $0.001 \leq p < 0.01$; * $0.01 \leq p < 0.05$).

	F/B (day 12- day 0)	F/B (day 83 - day 0)
Clay (%)		<0.001 **
pH	-0.026 ***	< -0.001 ***
Bioavailable P (mg kg^{-1})	0.004 *	
Mineral N (extractable $\text{NH}_4^+ + \text{NO}_3^-$, mg g^{-1})	-0.003 *	< -0.001 ***

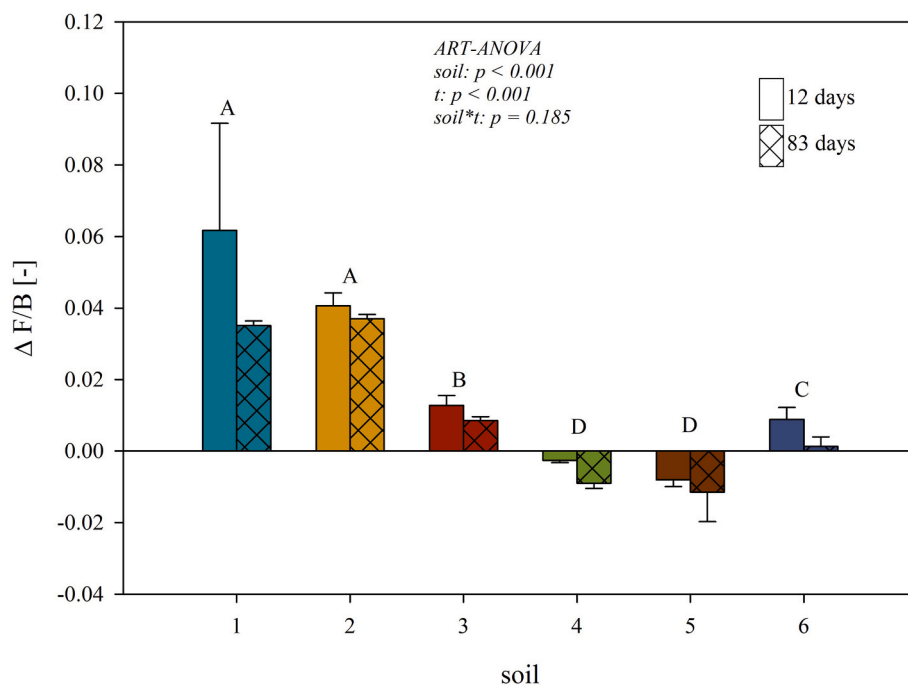


Fig. 6. Change of the fungi-to-bacteria ratio ($\Delta \text{F/B}$) in the soil 12 days (solid bars) or 83 days (checkered bars) relative to day 0 after the amendment of arbuscular mycorrhizal fungal necromass in six grassland soils with varying properties. Letters indicate significant differences between the soils (aligned rank transformed ANOVA with pairwise comparison on estimated marginal means with Tukey adjustment as posthoc test).

et al., 2024). For example, polysaccharides, which are abundant in AMF necromass (Horsch et al., 2023) were shown to play a major role in the formation of skyscrapers and even directly attributed to originate from microbial necromass (Kang et al., 2024). It therefore might be suggested that the accumulated MAOM-C already present in the soils consists of molecules, which are not binding onto skyscrapers as easily as the AMF necromass but bind on mineral surfaces directly favouring higher MAOM formation under high clay content. The discrepancy might also be supported by the lower C/N ratio of soils with a lower clay content, potentially indicating a higher contribution of necromass to SOM under lower clay contents given that microbial products usually show a lower C/N ratio compared to plant residues (Hall et al., 2020; Strickland and Rousk, 2010). Thus, although soil clay content was indeed the major driver for MAOM formation with necromass, we must reject our initial hypothesis (II) as this correlation is negative, contrary to our assumption.

In our study, the formation of MAOM with necromass (^{13}C -MAOM) was only explained by the soil texture. Nevertheless, more factors potentially influencing the dependency of MAOM formation with necromass on clay content could not be addressed in this study, but might be valuable to consider in the future: An increased clay content in the soil is also discussed to influence the microbial community composition (Cai et al., 2022), which is a major driver of MAOM formation (Zhao et al., 2024). Moreover, also the composition of the fine fraction of soil (i.e., kaolinite, illite, goethite) in combination with the exact chemical compound binding on the clay surface affects its binding affinity and strength (Bramble et al., 2024; Zhao et al., 2024; Konrad et al., 2025).

4.4. Temporal variability of necromass-C in the MAOM fraction

According to the view that C in the MAOM fraction is rather stable and can accumulate over longer time periods (Lavallee et al., 2020), we hypothesized that the necromass-C is accumulating in the MAOM fraction with time. However, we observed a decrease in the necromass-C content in MAOM (^{13}C -MAOM) already after 71 days (i.e., between the two sampling points) and thus need to reject this part of our initial hypothesis (I). This might indicate that the AMF necromass-C can be destabilised relatively easily from MAOM, for example by abiotic desorption or microbial utilization (Bölscher et al., 2025).

This could align with the proposed view that the AMF necromass in our study is preferentially forming organic-organic interactions: Within skyscrapers, a gradient of decomposability is proposed where organic molecules close to the mineral surfaces are protected to a greater extent than those positioned further away (Begill et al., 2023; Kleber et al., 2007; Schweizer, 2022). Assuming that AMF necromass-C is preferentially absorbing onto existing mineral-organic clusters, the decrease of necromass-derived C in the MAOM fraction with time can be attributed to a lower stability. Within the studied soils, a higher clay content was associated with a higher temporal stability of necromass-C. As the skyscrapers might be less common under high clay contents (Begill et al., 2023), AMF necromass may rather bind directly onto mineral surfaces and thus, be better protected from decomposition compared to low clay conditions. This could further explain the positive correlation between MAOM-C (not necromass- ^{13}C) and the clay content, as the higher stability of MAOM-C due to higher clay surface availability and thus, more direct sorption, could increase accumulation of C in the MAOM fraction over time. This would indicate that the turnover time of MAOM-C is determined by the substrate and the associated sorption mechanism and that AMF necromass is not necessarily well protected in the MAOM fraction.

4.5. Effect of necromass addition on SOM decomposition

The amendment of necromass caused significant changes in the soil microbial community, as expressed by alterations in the F/B ratio. While soils with a high pH showed a decrease in F/B after necromass addition

(i.e., more bacteria over fungi), F/B increased in more acidic soils. This suggests that necromass decomposition is not restricted to either fungi or bacteria, but soil properties, such as the pH, influence the composition of the microbial community growing on fungal necromass (Cantor et al., 2023; Kennedy and Maillard, 2023). Generally, fungi are considered to grow better under acidic conditions (Rousk et al., 2011), explaining the observed pattern.

We hypothesized that necromass addition would increase SOM decomposition. Indeed, the described change in the F/B can be connected to effects on SOM decomposition observed in this study, as fungi are usually seen as better decomposers (Koranda et al., 2014) due to their enzymatic repertoire (Liu et al., 2023): The soil with the lowest pH (soil 2), associated with an increased F/B, showed an enhanced SOM-derived CO_2 -efflux after amendment of AMF necromass. This positive PE, among other explanations, links the decomposition of new substrate to an additional need of nutrients fulfilled via the decomposition of additional SOM (Dijkstra et al., 2013) and was reported previously to correlate positively with soil F/B (Husain et al., 2024; Soares et al., 2017). However, this effect was only observed in one out of six soils, indicating that soil properties and existing microbial communities mainly control necromass-induced PE. Our hypothesis (III) thus cannot generally be supported.

Among the soils, soil 4, which was associated with the highest pH and a decrease in F/B after necromass addition, showed a negative PE, thus a decreased SOM-derived CO_2 . The main reason for negative priming is that the microbial community shifts to the more easily decomposable substrate (Kuzyakov et al., 2000) and is – in this case – preferentially decomposing the freshly added AMF necromass. The observation that this was only true for a soil with higher bacterial growth might emphasize the weaker potential to decompose complex SOM by bacteria compared to fungi. This shows that necromass can also indirectly influence C storage in the soil via changes in the soil microbial community – mediated by the initial soil pH.

5. Conclusion

Taken together, this study is the first to use very high ^{13}C -enriched AMF necromass (97 atom %) to investigate the fate of fungal necromass-C in soils with contrasting properties. Our results show high respiration rates and a quick formation of MAOM with AMF necromass-C. The formation of MAOM was mainly predicted negatively by the soil clay content, which was inverse to its correlation with total MAOM-C present in soil. In this discrepancy, the formation of mineral-organic-organic clusters ('skyscrapers'), the sorption preference of necromass-derived C on these clusters, and the associated stability might play a role. This could further explain the temporal variability of MAOM-C formed with necromass, as it decreased with time – most likely because the C bound in mineral-organic-organic clusters has a lower stability compared to direct mineral-organic interactions. Ultimately, the addition of necromass also resulted in changes in the F/B ratio and its associated decomposition patterns. The results collectively suggest a prominent role of AMF necromass in soil C cycling via direct and indirect effects and hence, demonstrates the necessity to consider fungal necromass, and especially mycorrhizal fungal necromass, in future soil C studies. Detailed insights into the identity and activity of the resident microbes following AMF necromass addition are certainly warranted.

Declaration of generative AI in scientific writing

During the preparation of the abstract, the authors used Chat-GPT-4o to increase its readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Valentin B. Kurbel: Data curation, Formal analysis, Writing – original draft. **Hana Hřselová:** Investigation, Writing – review & editing. **Maire Holz:** Formal analysis, Writing – review & editing. **Johanna Pausch:** Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. **Jan Jansa:** Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

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.

Acknowledgements

We would like to thank Angelika Mergner for the technical assistance during soil fractionation. Funding: This work was funded by the European Research Council under the ERC-starting Grant (J. Pausch, 101039716) 'Mycorrhizal Types and Soil Carbon Storage: A mechanistic theory of fungal mediated soil organic matter cycling in temperate forests (MYCO-SoilC)', by the Ministry of Education, Youth and Sports of the Czech Republic by the grant Talking microbes - understanding microbial interactions within One Health framework (CZ.02.01.01/00/22_008/0004597) and by the Czech Science Foundation (21-07247S). Support by the Czech Academy of Sciences (RVO 61388971) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2026.110200>.

References

- Akroume, E., Maillard, F., Bach, C., Hossann, C., Brechet, C., Angeli, N., Zellner, B., Saint-André, L., Buée, M., 2019. First evidences that the ectomycorrhizal fungus *Paxillus involutus* mobilizes nitrogen and carbon from saprotrophic fungus necromass. *Environmental Microbiology* 21 (1), 197–208. <https://doi.org/10.1111/1462-2920.14440>.
- Angst, G., Mueller, K.E., Nierop, K.G.J., Simpson, M.J., 2021. Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry* 156, 108189. <https://doi.org/10.1016/j.soilbio.2021.108189>.
- Ao, D., Wang, B., Wang, Y., Chen, Y., Liang, C., An, S., 2025. Arbuscular mycorrhizal fungi communities and glomalin mediate particulate and mineral-associated organic carbon formation in grassland patches. *Communications Earth & Environment* 6 (1), 553. <https://doi.org/10.1038/s43247-025-02492-x>.
- Barnard, N., Dalal, R.C., Weng, Z.H., Schweizer, S.A., Kopittke, P.M., 2025. The influence of fine fraction content on storage and retention of soil organic carbon in Vertisols of subtropical Australia. *Geoderma* 456, 117269. <https://doi.org/10.1016/j.geoderma.2025.117269>.
- Begill, N., Don, A., Poeplau, C., 2023. No detectable upper limit of mineral-associated organic carbon in temperate agricultural soils. *Global Change Biology* 29 (16), 4662–4669. <https://doi.org/10.1111/gcb.16804>.
- Begill, N., Poeplau, C., Meisenburg, H., Reibmann, C., Don, A., 2025. Different amounts of added litter do not affect long-term carbon mineralization and stabilization in topsoils and subsoils. *Journal of Plant Nutrition and Soil Science* 188, 925–938. <https://doi.org/10.1002/jpln.70002>.
- Beidler, K.V., Phillips, R.P., Andrews, E., Maillard, F., Mushinski, R.M., Kennedy, P.G., 2020. Substrate quality drives fungal necromass decay and decomposer community structure under contrasting vegetation types. *Journal of Ecology* 108 (5), 1845–1859. <https://doi.org/10.1111/1365-2745.13385>.
- Bölscher, T., Cardon, Z.G., Arredondo, M.G., Grand, S., Griffen, G., Hestrin, R., Herstin, R., Imboden, J., Jamoteau, F., Lacroix, E.M., Castro, S.P., Persson, P., Riley, W.J., Keiluweit, M., 2025. Vulnerability of mineral-organic associations in the rhizosphere. *Nature Communications* 16 (1), 5527. <https://doi.org/10.1038/s41467-025-61273-4>.
- Brabcová, V., Štursová, M., Baldrian, P., 2018. Nutrient content affects the turnover of fungal biomass in forest topsoil and the composition of associated microbial communities. *Soil Biology and Biochemistry* 118, 187–198. <https://doi.org/10.1016/j.soilbio.2017.12.012>.
- Bramble, D.S.E., Ulrich, S., Schöning, I., Mikutta, R., Brandt, L., Poll, C., Kandeler, E., Mikutta, C., Konrad, A., Siemens, J., Yang, Y., Polle, A., Schall, P., Ammer, C., Kaiser, K., Schrupf, M., 2024. Formation of mineral-associated organic matter in temperate soils is primarily controlled by mineral type and modified by land use and management intensity. *Global Change Biology* 30 (1), e17024. <https://doi.org/10.1111/gcb.17024>.
- Brundrett, M., Tedersoo, L., 2019. Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions. *New Phytologist* 221 (1), 18–24. <https://doi.org/10.1111/nph.15440>.
- Buckeridge, K.M., Creamer, C., Whitaker, J., 2022a. Deconstructing the microbial necromass continuum to inform soil carbon sequestration. *Functional Ecology* 36 (6), 1396–1410. <https://doi.org/10.1111/1365-2435.14014>.
- Buckeridge, K.M., La Rosa, A.F., Mason, K.E., Whitaker, J., McNamara, N.P., Grant, H.K., Ostle, N.J., 2020. Sticky dead microbes: rapid abiotic retention of microbial necromass in soil. *Soil Biology and Biochemistry* 149, 107929. <https://doi.org/10.1016/j.soilbio.2020.107929>.
- Buckeridge, K.M., Mason, K.E., Ostle, N., McNamara, N.P., Grant, H.K., Whitaker, J., 2022b. Microbial necromass carbon and nitrogen persistence are decoupled in agricultural grassland soils. *Communications Earth & Environment* 3 (1), 114. <https://doi.org/10.1038/s43247-022-00439-0>.
- Cai, Y., Ma, T., Wang, Y., Jia, J., Jia, Y., Liang, C., Feng, X., 2022. Assessing the accumulation efficiency of various microbial carbon components in soils of different minerals. *Geoderma* 407, 115562. <https://doi.org/10.1016/j.geoderma.2021.115562>.
- Cantorán, A., Maillard, F., Baldrian, P., Kennedy, P.G., 2023. Defining a core microbial necrobiome associated with decomposing fungal necromass. *FEMS Microbiology Ecology* 99 (9), 1–11. <https://doi.org/10.1093/femsec/fiad098>.
- Cotrufo, F.M., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience* 12 (12), 989–994. <https://doi.org/10.1038/s41561-019-0484-6>.
- Cotrufo, F.M., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* 19 (4), 988–995. <https://doi.org/10.1111/gcb.12113>.
- Dijkstra, F.A., Carrillo, Y., Pendall, E., Morgan, J.A., 2013. Rhizosphere priming: a nutrient perspective. *Frontiers in Microbiology* 4, 216. <https://doi.org/10.3389/fmicb.2013.00216>.
- Dong, W., Song, A., Yin, H., Liu, X., Li, J., Fan, F., 2021. Decomposition of microbial necromass is divergent at the individual taxonomic level in soil. *Frontiers in Microbiology* 12, 679793. <https://doi.org/10.3389/fmicb.2021.679793>.
- Dudáš, M., Pjevac, P., Kotianová, M., Gaňčáříková, K., Rozmoš, M., Hřselová, H., Bukovská, P., Jansa, J., 2022. Arbuscular mycorrhiza and nitrification: disentangling processes and players by using synthetic nitrification inhibitors. *Applied and Environmental Microbiology* 88 (20), e0136922. <https://doi.org/10.1128/aem.01369-22>.
- Řezanka, T., Hřselová, H., Kyselová, L., Jansa, J., 2023. Can cardiolipins be used as a biomarker for arbuscular mycorrhizal fungi? *Mycorrhiza* 33 (5–6), 399–408. <https://doi.org/10.1007/s00572-023-01129-1>.
- Fernandez, C.W., Langley, J.A., Chapman, S., McCormack, M.L., Koide, R.T., 2016. The decomposition of ectomycorrhizal fungal necromass. *Soil Biology and Biochemistry* 93, 38–49. <https://doi.org/10.1016/j.soilbio.2015.10.017>.
- Fu, H., Chen, H., Ma, Z., Liang, G., Chadwick, D.R., Jonas, D.L., Wanek, W., Wu, L., Ma, Q., 2025. Fungal necromass carbon dominates global soil organic carbon storage. *Global Change Biology* 31, e70413. <https://doi.org/10.1111/gcb.70413>.
- Georgiou, K., Jackson, R.B., Vindusková, O., Abramoff, R.Z., Ahlström, A., Feng, W., Harden, J.W., Pellegrini, A.F.A., Polley, H.W., Soong, J.L., Riley, W.J., Torn, M.S., 2022. Global stocks and capacity of mineral-associated soil organic carbon. *Nature Communications* 13 (1), 3797. <https://doi.org/10.1038/s41467-022-31540-9>.
- Hall, S.J., Ye, C., Weintraub, S.R., Hockaday, W.C., 2020. Molecular trade-offs in soil organic carbon composition at continental scale. *Nature Geoscience* 13 (10), 687–692. <https://doi.org/10.1038/s41561-020-0634-x>.
- Hawkins, H.-J., Cargill, R.I.M., van Nuland, M.E., Hagen, S.C., Field, K.J., Sheldrake, M., Soudzilovskaia, N.A., Kiers, E.T., 2023. Mycorrhizal mycelium as a global carbon pool. *Current Biology* 33 (11), R560–R573. <https://doi.org/10.1016/j.cub.2023.02.027>.
- Hood-Nowotny, R., Umana, N.H.-N., Inselbacher, E., Oswald-Lachouani, P., Wanek, W., 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. *Soil Science Society of America Journal* 74 (3), 1018–1027. <https://doi.org/10.2136/sssaj2009.0389>.
- Horsch, C.C.A., Antunes, P.M., Fahey, C., Grandy, A.S., Kallenbach, C.M., 2023. Trait-based assembly of arbuscular mycorrhizal fungal communities determines soil carbon formation and retention. *New Phytologist* 239 (1), 311–324. <https://doi.org/10.1111/nph.18914>.
- Husain, H., Keitel, C., Dijkstra, F.A., 2024. Fungi are more important than bacteria for soil carbon loss through priming effects and carbon protection through aggregation. *Applied Soil Ecology* 195, 105245. <https://doi.org/10.1016/j.apsoil.2023.105245>.
- Islam, MdR., Singh, B., Dijkstra, F.A., 2022. Stabilisation of soil organic matter: interactions between clay and microbes. *Biogeochemistry* 160 (2), 145–158. <https://doi.org/10.1007/s10533-022-00956-2>.
- Jansa, J., Šmilauer, P., Borovička, J., Hřselová, H., Forczek, S.T., Slámová, K., Řezanka, T., Rozmoš, Bukovská, P., Gryndler, M., 2020. Dead *Rhizophagus irregularis* biomass mysteriously stimulates plant growth. *Mycorrhiza* 30 (1), 63–77. <https://doi.org/10.1007/s00572-020-00937-z>.
- Jílková, V., Devetter, M., Al Haj Ishak Al Ali, R., Angel, R., Angst, G., Jandová, K., Libra, M., Starý, J., 2025. Tree species and soil depth affect microbial necromass contribution to soil organic matter in temperate forest soils, surpassing the impact of

- microbial and faunal community composition. *Functional Ecology* 39, 2219–2233. <https://doi.org/10.1111/1365-2435.70111>.
- Kang, J., Qu, C., Chen, W., Cai, P., Chen, C., Huang, Q., 2024. Organo-organic interactions dominantly drive soil organic carbon accrual. *Global Change Biology* 30 (1), e17147. <https://doi.org/10.1111/gcb.17147>.
- Kay, M., Wobbrock, J., 2016. Artool: 0.10.0 (V0.10.0). Zenodo. <https://doi.org/10.5281/zenodo.44586>.
- Kennedy, P.G., Maillard, F., 2023. Knowns and unknowns of the soil fungal necrobiome. *Trends in Microbiology* 31 (2), 173–180. <https://doi.org/10.1016/j.tim.2022.08.011>.
- Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: Self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry* 85 (1), 9–24. <https://doi.org/10.1007/s10533-007-9103-5>.
- Klink, S., Keller, A.B., Wild, A.J., Baumert, V.L., Gube, M., Lehnndorff, E., Meyer, N., Mueller, C.W., Phillips, R.P., Pausch, J., 2022. Stable isotopes reveal that fungal residues contribute more to mineral-associated organic matter pools than plant residues. *Soil Biology and Biochemistry* 168, 108634. <https://doi.org/10.1016/j.soilbio.2022.108634>.
- Konrad, A., Hofmann, D., Siemens, J., Stutz, K.P., Lang, F., Mulder, I., 2025. Microbial carbon use efficiency of mineral-associated organic matter is related to its desorbability. *Soil Biology and Biochemistry* 203, 109740. <https://doi.org/10.1016/j.soilbio.2025.109740>.
- Kopittke, P.M., Dalal, R.C., Hoeschen, C., Li, C., Menzies, N.W., Mueller, C.W., 2020. Soil organic matter is stabilized by organo-mineral associations through two key processes: the role of the carbon to nitrogen ratio. *Geoderma* 357, 113974. <https://doi.org/10.1016/j.geoderma.2019.113974>.
- Kopittke, P.M., Hernandez-Soriano, M.C., Dalal, R.C., Finn, D., Menzies, N.W., Hoeschen, C., Mueller, C.W., 2018. Nitrogen-rich microbial products provide new organo-mineral associations for the stabilization of soil organic matter. *Global Change Biology* 24 (4), 1762–1770. <https://doi.org/10.1111/gcb.14009>.
- Koranda, M., Kaiser, C., Fuchslueger, L., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S., Richter, A., 2014. Fungal and bacterial utilization of organic substrates depends on substrate complexity and N availability. *FEMS Microbiology Ecology* 87 (1), 142–152. <https://doi.org/10.1111/1574-6941.12214>.
- Kästner, M., Miltner, A., Thiele-Bruhn, S., Liang, C., 2021. Microbial necromass in soils—Linking microbes to soil processes and carbon turnover. *Frontiers in Environmental Science* 9, 756378. <https://doi.org/10.3389/fenvs.2021.756378>.
- Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* 32 (11–12), 1485–1498. [https://doi.org/10.1016/S0038-0717\(00\)00084-5](https://doi.org/10.1016/S0038-0717(00)00084-5).
- Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology* 26 (1), 261–273. <https://doi.org/10.1111/gcb.14859>.
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 528 (7580), 60–68. <https://doi.org/10.1038/nature16069>.
- Lenth, R., Piaskowski, J., 2026. Emmeans: estimated marginal means, aka Least-Square means. R package version 2.0.3. <https://rvinth.github.io/emmeans/>.
- Li, B., Li, Y., Fanin, N., Veen, G.F., Han, X., Du, X., Li, Y., Sun, Y., Li, Q., 2023. Stoichiometric imbalances between soil microorganisms and their resources regulate litter decomposition. *Functional Ecology* 37 (12), 3136–3149. <https://doi.org/10.1111/1365-2435.14459>.
- Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology* 25 (11), 3578–3590. <https://doi.org/10.1111/gcb.14781>.
- Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology* 2, 17105. <https://doi.org/10.1038/nmicrobiol.2017.105>.
- Liu, M., Zheng, S., Pendall, E., Smith, P., Liu, J., Li, J., Fang, C., Li, B., Nie, M., 2025. Unprotected carbon dominates decadal soil carbon increase. *Nature Communications* 16 (1), 2008. <https://doi.org/10.1038/s41467-025-57354-z>.
- Liu, Y., Dong, L., Zhang, H., Deng, Y., Hu, B., Wang, W., 2023. Distinct roles of bacteria and fungi in mediating soil extracellular enzymes under long-term nitrogen deposition in temperate plantations. *Forest Ecology and Management* 529, 120658. <https://doi.org/10.1016/j.foreco.2022.120658>.
- Maillard, F., Michaud, T.J., See, C.R., DeLancey, L.C., Blazewicz, S.J., Kimbrel, J.A., Pett-Ridge, J., Kennedy, P.G., 2023. Melanization slows the rapid movement of fungal necromass carbon and nitrogen into both bacterial and fungal decomposer communities and soils. *mSystems* 8 (4), e0039023. <https://doi.org/10.1128/mSystems.0039023>.
- Manzoni, S., Cotrufo, M.F., 2024. Mechanisms of soil organic carbon and nitrogen stabilization in mineral-associated organic matter – insights from modeling in phase space. *Biogeosciences* 21 (18), 4077–4098. <https://doi.org/10.5194/bg-21-4077-2024>.
- Ohno, T., Zibilske, L.M., 1991. Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Science Society of America Journal* 55 (3), 892–895. <https://doi.org/10.2136/sssaj1991.03615995005500030046x>.
- Pausch, J., Holz, M., Zhu, B., Cheng, W., 2024. Rhizosphere priming promotes plant nitrogen acquisition by microbial necromass recycling. *Plant, Cell and Environment* 47 (6), 1987–1996. <https://doi.org/10.1111/pce.14858>.
- Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J., Trigala, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., Nieder, R., 2018. Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – a comprehensive method comparison. *Soil Biology and Biochemistry* 125, 10–26. <https://doi.org/10.1016/j.soilbio.2018.06.025>.
- Poeplau, C., Don, A., 2023. A simple soil organic carbon level metric beyond the organic carbon-to-clay ratio. *Soil Use and Management* 39 (3), 1057–1069. <https://doi.org/10.1111/sum.12921>.
- R Core Team, 2023. R: a Language and Environment for Statistical Computing. R foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rousk, J., Brookes, P.C., Bååth, E., 2011. Fungal and bacterial growth responses to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. *FEMS Microbiology Ecology* 76 (1), 89–99. <https://doi.org/10.1111/j.1574-6941.2010.01032.x>.
- Schweizer, S.A., 2022. Perspectives from the Fritz-Scheffer Awardee 2021: soil organic matter storage and functions determined by patchy and piled-up arrangements at the microscale. *Journal of Plant Nutrition and Soil Science* 185 (6), 694–706. <https://doi.org/10.1002/jpln.202200217>.
- Schweizer, S.A., Mueller, C.W., Höschen, C., Ivanov, P., Kögel-Knabner, I., 2021. The role of clay content and mineral surface area for soil organic carbon storage in an arable toposequence. *Biogeochemistry* 156 (3), 401–420. <https://doi.org/10.1007/s10533-021-00850-3>.
- Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Science Society of America Journal* 70 (2), 555–569. <https://doi.org/10.2136/sssaj2004.0347>.
- Soares, M., Kritzberg, E.S., Rousk, J., 2017. Labile carbon 'primes' fungal use of nitrogen from submerged leaf litter. *FEMS Microbiology Ecology* 93 (9), fix110. <https://doi.org/10.1093/femsec/fix110>.
- Sokol, N.W., Sanderman, J., Bradford, M.A., 2019. Pathways of mineral-associated soil organic matter formation: integrating the role of plant carbon source, chemistry, and point of entry. *Global Change Biology* 25 (1), 12–24. <https://doi.org/10.1111/gcb.14482>.
- Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils – methods, controls, and ecosystem implications. *Soil Biology and Biochemistry* 42 (9), 1385–1395. <https://doi.org/10.1016/j.soilbio.2010.05.007>.
- Sun, D., Šmilauer, P., Pjevac, P., Rozmoš, M., Forczek, S.T., Kotianová, M., Hršelová, H., Bukovská, P., Jansa, J., 2025. Arbuscular mycorrhiza suppresses microbial abundance, and particularly that of ammonia oxidizing bacteria, in agricultural soils. *Frontiers in Microbiology* 16, 1671859. <https://doi.org/10.3389/fmicb.2025.1671859>.
- Thonar, C., Erb, A., Jansa, J., 2012. Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. *Molecular Ecology Resources* 12 (2), 219–232. <https://doi.org/10.1111/j.1755-0998.2011.03086.x>.
- van der Heijden, M.G.A., Martin, F.M., Selosse, M.-A., Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* 205, 1406–1423. <https://doi.org/10.1111/nph.13288>.
- Vasilica, V., Schöpf, H., Thaufelder, I., Wild, A., Pausch, J., 2026b. Density-based fractionation of soil organic matter. <https://dx.doi.org/10.17504/protocols.io.5jyl8q386l2w/v1>.
- Vasilica, Veronica, Guidi, Claudia, Abdalla, Khatab, Kurbel, Valentin B., Wild, Andreas J., et al., 2026a. Plant, mycorrhizal and saprotrophic fungal contributions to soil organic matter fractions differ between tree-mycorrhizal types. *Soil Biology and Biochemistry* 220, 110202. <https://doi.org/10.1016/j.soilbio.2026.110202>. <https://linkinghub.elsevier.com/retrieve/pii/S0038071726001215>.
- Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M., Kögel-Knabner, I., 2014. Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. *Nature Communications* 5 (1), 2947. <https://doi.org/10.1038/ncomms3947>.
- Wang, C., Kuzyakov, Y., 2024. Soil organic matter priming: the pH effects. *Global Change Biology* 30 (6), e17349. <https://doi.org/10.1111/gcb.17349>.
- Wobbrock, J.O., Findlater, L., Gergle, D., Higgins, J.J., 2011. The aligned rank transform for nonparametric factorial analyses using only ANOVA procedures. *CHI'11. Proceedings of the SIGCHI Conference on Human Factors in Computing Systems*, pp. 143–146. <https://doi.org/10.1145/1978942.1978963>.
- Yan, Y., Peng, Y., Shi, J., Huo, C., Wang, X., 2026. Depth-stratified isotopic and molecular signatures reveal decoupled soil organic matter dynamics following grassland-to-cropland conversion. *Soil and Tillage Research* 259, 107095. <https://doi.org/10.1016/j.still.2026.107095>.
- Yang, Y., Xie, H., Mao, Z., Bao, X., He, H., Zhang, X., Liang, C., 2022. Fungi determine increased soil organic carbon more than bacteria through their necromass inputs in conservation tillage croplands. *Soil Biology and Biochemistry* 167, 108587. <https://doi.org/10.1016/j.soilbio.2022.108587>.
- Zhang, M., Sayer, E.J., Ye, J., Yuan, Z., Lin, F., Hao, Z., Fang, S., Mao, Z., Jiang, P., Zhu, M., Wang, X., 2025. Tree mycorrhizal associations regulate relationships between plant and microbial communities and soil organic carbon stocks at local scales in a temperate forest. *Functional Ecology* 39, 1550–1566. <https://doi.org/10.1111/1365-2435.70035>.
- Zhao, J., Feng, X., Hu, J., He, M., Wang, S., Yang, Y., Chen, L., 2024. Mineral and microbial properties drive the formation of mineral-associated organic matter and its response to increased temperature. *Global Change Biology* 30 (12), e70004. <https://doi.org/10.1111/gcb.70004>.
- Zhu, Y., Hu, Y., Yao, X., Chen, G., Rai, J., Li, J., Zhang, D., Deng, Q., 2025. Estimating microbial necromass contribution to mineral-associated organic matter: comparison of stoichiometric and biomarker methods. *Plant and Soil* 517, 887–889. <https://doi.org/10.1007/s11104-025-07902-9>.