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Subtropical forest macro-decomposers rapidly transfer litter carbon and nitrogen into soil mineral-associated organic matter

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A R T I C L E I N F O

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A B S T R A C T

Background: Forest soils in tropical and subtropical areas store a significant amount of carbon. Recent frameworks to assess soil organic matter (SOM) dynamics under evolving global conditions suggest that dividing bulk SOM into particulate and mineral-associated organic matter (POM vs. MAOM) is a promising method for identifying how SOM contributes to reducing global warming. Soil macrofauna, earthworms, and millipedes have been found to play an important role in facilitating SOM processes. However, how these two co-existing macrofaunae impact the litter decomposition process and directly impact the formation of POM and MAOM remains unclear.
Methods: Here, we set up a microcosm experiment, which consisted of 20 microcosms with four treatments: earthworm and litter addition (E), millipedes and litter addition (M), earthworm, millipedes, and litter addition (E+M), and control (only litter addition) in five replicates. The soil and litter were sterilized prior to beginning the incubation experiment to remove any existing microbes. After incubating the samples for 42 days, the litter properties (mass, C, and N contents), soil physicochemical properties, as well as the C and N contents, and POM and MAOM ^{13}C abundance in the 0–5 and 5–10 cm soil layers were measured. Finally, the relative influences of soil physicochemical and microbial properties on the distribution of C and N in the soil fractions were analyzed.
Results: The litter mass, C, and N associated with all four treatments significantly decreased after incubation, especially under treatment E+M (litter mass: −58.8%, litter C: −57.0%, litter N: −75.1%, respectively), while earthworm biomass significantly decreased under treatment E. Earthworm or millipede addition alone showed no significant effects on the organic carbon (OC) and total nitrogen (TN) content in the POM fraction, but joint addition of both significantly increased OC and TN regardless of soil depth. Importantly, all three macrofauna treatments increased the OC and TN content and decreased the ^{13}C abundance in the MAOM fraction. More than 65% of the total variations in the distribution of OC and TN throughout the two fractions can be explained by a combination of soil physicochemical and microbial properties. Changes in the OC distribution in the 0–5 cm soil layer are likely due to a decrease in soil pH and an increase in arbuscular mycorrhizal fungi (AMF), while those in the 5–10 cm layer are probably caused by increases in soil exchangeable Ca and Mg, in addition to fungi and gram-negative (GN) bacteria. The observed TN distribution changes in the 0–5 cm soil likely resulted from a decrease in soil pH and increases in AMF, GN, and gram-negative (GP) bacteria, while TN distribution changes in the 5–10 cm soil could be explained by increases in exchangeable Mg and GN bacteria.
Conclusions: The results indicate that the coexistence of earthworms and millipedes can accelerate the litter decomposition process and store more C in the MAOM fractions. This novel finding helps to unlock the processes by which complex SOM systems serve as C sinks in tropical forests and addresses the importance of soil macrofauna in maintaining C-neutral atmospheric conditions under global climate change.

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1. Introduction

Soils comprise the largest carbon pool in the terrestrial biosphere. As such, its ability to fix CO_2 is an essential consideration given the concern over global climate change and the initiative to counteract warming conditions (Bradford et al., 2016; Jackson et al., 2017). However, due to an incomplete understanding of soil organic matter (SOM) stabilization and destabilization mechanisms, as well as its functional complexity, numerous uncertainties exist in the global model that is used to predict SOM fate under changing environments (Rossel et al., 2019; Lehmann et al., 2020; Chen et al., 2021; Chandel et al., 2023). Recent research suggests that dividing bulk SOM into a mineral-associated organic matter (MAOM) fraction and a particulate organic matter (POM) fraction enables SOM dynamics to be accurately demonstrated, as POM and MAOM can be better linked to the litter decomposition process and the associated soil carbon (C) transformation processes (Cotrufo et al., 2019; Lavallee et al., 2020; Gill et al., 2021; Li et al., 2023). The POM fraction is predominantly comprised of partly decomposed lightweight plant fragments, while the MAOM fraction consists primarily of those that are protected through their association with minerals (Lavallee et al., 2020). Importantly, the different turnover rates of POM and MAOM may significantly affect SOM dynamics, yet most current microbial models employed for simulating SOM cycling do not consider these differences (Angst et al., 2023; Chandel et al., 2023). Therefore, understanding POM and MAOM behavior during litter decomposition is critical for assessing and predicting SOM dynamics (Lyu et al., 2023).

More than 60% of the plant biomass is returned to soils via the litter decomposition process. Moreover, the litter decomposition process contributes to one of the largest C fluxes in terrestrial ecosystems and strongly affects soil C sequestration (Wardle et al., 2004; Craig et al., 2022). Although litter decomposition processes are largely co-controlled by soil fauna and microorganisms, fewer empirical studies have been conducted on the former than the latter (González and Seastedt, 2001; Bradford et al., 2002; Snyder and Hendrix, 2008; Liang et al., 2017; Potapov et al., 2022). This research gap is problematic, as soil fauna functions particularly that of typical macrofauna (i.e., earthworms and millipedes), can not be neglected when building a global C model (Bradford et al., 2002; Chandel et al., 2023). Earthworms and millipedes are typical soil macrofauna in forest ecosystems and these organisms have similar body sizes and feeding habits (Potapov et al., 2021). Millipedes and epigeic/anecic earthworms mainly feed on litter—they each demonstrate unique behavior that impacts the soil processes differently (Potapov et al., 2022). Specifically, millipedes generally reside on the soil's surface, while earthworms inhabit the surface and middle/lower subsoil layer (Seeber et al., 2006). Moreover, the structure and nutrient content of their faeces differ due to earthworms and millipedes digesting plant residues in dissimilar quantities. Millipede fecal pellets usually contain more organic matter than earthworm casts and may lead to a bottom-up effect on soil microorganisms (Scheu and Schaefer, 1998; Joly et al., 2018). Given the functional and behavioral differences between millipedes and earthworms, it is reasonable to suspect that they have different and unique impacts on C and N distribution in the POM and MAOM fractions.

While previous studies have investigated the regulating effects of soil macrofauna on the litter decomposition process and soil carbon sequestration, few have made or evaluated the direct link between the litter decomposition process and C and N distribution in POM and MAOM fractions (Tian et al., 1995; Gergocs and Hufnagel, 2016; Sauvadet et al., 2016; Angst et al., 2023). For example, numerous studies have been conducted investigating earthworm-microbe interactions and the effects of earthworms on plant growth, soil C biogeochemical cycling, and soil structure, making earthworms among the most prolifically investigated macrofauna (Edwards et al., 2013; Pant et al., 2017; Vidal et al., 2023). Recent studies conducted using lab incubational experiments showed that earthworms can convert labile plant C compounds into stabilized C pools by increasing its microbial necromass content, without altering the bulk soil C contents. These results further elucidated the central role of earthworms in forming and stabilizing microbial necromass during litter decomposition (Angst et al., 2019, 2022). However, how earthworm interaction with other soil macrofauna (e.g., millipedes) affects C and N distribution during litter decomposition has seldom been directly investigated (El-Wakeil, 2015; Macias et al., 2019). In theory, the C and N content in POM may increase in response to macrofauna crushing large litter debris into smaller particles during the first stage of the litter decomposition process (Gill et al., 2021; Craig et al., 2022). In addition, bioturbation may alter the soil microenvironment and mineral properties, e.g., Fe, Ca, Mg, and Al contents, and further affect SOM cycling (Arrazola-Vasquez et al., 2022; Vidal et al., 2023). In essence, experiments designed to clarify the relationship between different soil macrofauna species and C and N distribution in soil fractions should be conducted.

Tropical and subtropical forests comprise 56% of earth's forested area and are vital for soil biodiversity and providing C storage to combat global warming (Andreas, 2020; Dong et al., 2023). The macrofauna (i.e., earthworms and millipedes) in these forest ecosystems generally constitute most of the total soil animal biomass and substantially contribute to soil food-web functioning (Bar-On et al., 2018). Nevertheless, the direct effects of soil macrofauna on C and N distribution in tropical and subtropical forests are still poorly understood (Gongalsky, 2021). Herein, sterilized litter and soils were used in a 42-day soil macrofauna incubational experiment that was designed to test whether macrofauna can affect the C and N distribution in POM and MAOM fraction. We hypothesized that the presence of soil earthworms or millipedes would transform the litter-derived C and N into POM and MAOM fractions. However, their effects on litter decomposition and transformation would be more pronounced under combined addition than sole addition. Our research objectives were to (I) investigate the effects of adding macrofauna (earthworms, millipedes, or both) to sterilized soil on litter properties and C and N distributions in POM and MAOM fractions; and (II) determine the relative influence of soil physicochemical factors and microbial properties on the C and N distributions in two fractions in the presence of macrofauna.

2. Materials and methods

2.1. Materials preparation and experimental set-up

This study was performed in the South China Botanical Garden, Chinese Academy of Sciences (23°13′ N, 113°28′ E). The area exhibits a monsoon climate, with a mean annual temperature and a mean annual precipitation of 23 °C and 1,761 mm, respectively. A common species of earthworm (*Eisenia fetida*) and millipede (*Spirobolus walkeri*) were selected as the macrofauna for this investigation (Potapov et al., 2022; Liu et al., 2023). Both macrofauna are soil detritivores that live below the litter layer and feed on partially decomposed leaf litter. Utilized soils were collected in the garden. The soil is classified as Red Soil, according to the Chinese Soil Classification system, and is characterized by the following properties: pH = 5.0, moisture = 10%, total carbon (TC) ~14.9 $g \cdot kg^{-1}$, and total nitrogen (TN) = 0.94 $g \cdot kg^{-1}$. All soil samples were passed through a 2-mm screen to remove visible roots, litter, and rocks. The partially decomposed *Juglans regia* leaves were collected and mixed, washed in tap water, autoclaved, and then dried in a 60 °C oven. The litter total C and N concentrations were 330.5 and 19.5 $g \cdot kg^{-1}$, respectively.

Prior to being added to the experimental microcosms, all collected macrofauna were incubated for ≥10 days in an incubational box to ensure they adapted to the lab environment and remained at ontogenetic stages. 20 microcosms were built and situated in the laboratory. The microcosms were then randomly selected to be treated with one of the four potential treatments: earthworm and litter (E), millipedes and litter (M), earthworm, millipedes, and litter (E+M), and the control (only litter), such that each treatment was replicated in five microcosms

(Fig. 1). The PVC column (25 cm depth and 15 cm diameter) was dug on the tab, and the soil, litter, and macrofauna were placed in an orderly fashion. The soil was packed to a depth of ~12 cm. To ensure enough living space for the soil macrofauna, 6 earthworms were used for treatment E, 3 millipedes for treatment M, and 6 earthworms + 3 millipedes for treatment E+M. All fresh soils were sterilized to kill existing microbes in an autoclave pot at 126 °C and 0.142 MPa for 40 min. Thus, the direct effects of soil microbes on litter decomposition were primarily eliminated, enabling the experiment to provide direct evidence regarding how soil macrofauna influences C and N distribution in different SOM fractions. The microcosm experiment was executed for 42 days under lab temperatures and humidity. To avoid the organic matter being repeatedly recycled, we stopped the experiment when we did not see any big litter fraction in the E+M hole. More detailed information related to the materials and experimental set-up can be found in Liu et al. (2023).

2.2. Litter and soil sampling and analysis

After 42 days of incubation, the litter on the soil's surface was carefully collected using tweezers and oven-dried to a constant weight. Subsequently, litter samples were ground to powder in preparation for the C and N analyses. Next, the earthworms and millipedes in the 0–5 and 5–10 cm soil layers were collected using the hand-collection method, and the total macrofauna mass of each treatment was weighed and recorded. Prior to being weighed, the macrofauna collected from each hole were wiped with a brush to remove the soil and litter on the macrofaunal body. In addition, the earthworms' and millipedes' faeces products were carefully collected with a spoon immediately after the macrofauna were removed. Notably, no earthworm faeces were observed in the E+M holes, as most of it had been eaten by millipedes. Finally, the soil in the hole was mixed and stored in the cold room until it was analyzed. In total, 40 soil samples (4 treatments × 5 replicates × 2 soil layers), 20 litter samples (4 treatments × 5 replicates), and 15 samples of macrofauna faeces (3 treatments × 5 replicates) were collected for analysis.

The soil suspension pH (1:2.5, soil/water) was measured with a pH meter. Soil moisture was measured using fresh soil that was oven-dried at 105 °C. The total carbon (TC) and nitrogen (TN) of litter and soil were determined using a C–N analyzer with isotope probes (Analytik-Jena multiN/C 3100; Germany). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined with extracts from fresh soil using 0.5 $mol \cdot L^{-1}$ K_2SO_4. Furthermore, MBC and MBN were determined with a Multi N/C 21,005 (Analytik-Jena AG). The biomass of fungi, bacteria, GP bacteria, GN bacteria, and Actinomycetes (Act) was indicated by the phospholipid fatty acids (PLFAs), which were extracted from freeze-dried soil and analyzed using a gas chromatograph (Agilent 6850, MIDI V.6.2; Agilent USA; Grayston et al., 2004). The concentrations of soil available Fe (AvFe) and Al (AvAl) and exchangeable Ca (ExCa) and Mg (ExMg) were measured via extracts created from 10 g of dried soil and 25 ml of 0.5 $mol \cdot L^{-1}$ $BaCl_2$. The elements were further measured with an inductively coupled plasma optical emission spectrometer (Thermo Electron Corporation, USA). More details associated with these processes can be found in Liu et al. (2023).

2.3. Physical fractions

The bulk soil was divided into POM and MAOM fractions using a wet-sieving method (Lavallee et al., 2020; Niu et al., 2022). Soil fraction with particles ranging in size from 0 to 53 μm was defined as MAOM, while that with particle sizes from 53 to 2,000 μm was defined as POM. The division was accomplished using the following procedure (Lavallee et al., 2020): First, ~20 g of air-dried soil was weighed in a 250 ml jar. To overcome hydrophobicity and ensure the soil being completely dispersed, each soil sample was sprayed with water. Next, 200 ml of dilute sodium hexametaphosphate solution (0.5%) and ~4 g beads were added to the jar, after which the jar was shaken for 18 h at room temperature. Then, the dispersed soil was sieved several times using distilled water and a 53 μm screen. The POM was the fraction left on the screen while the MAOM was the fraction through the screen. All POM and MAOM samples were oven-dried to constant weight. After weighing the mass, the fractional samples were ground and analyzed for the concentrations of OC and TN. Furthermore, the natural abundance of stable C isotopes in the POM and MAOM fractions were measured (GC Agilent 6890, Thermo Finnigan Delta plus^{XP}) to better quantify the amount of litter-derived C that enters the two fractions (Mendez-Millen et al., 2014).

2.4. Statistical analysis

The dataset included four soil physicochemical properties, eight soil microbial properties, four litter properties, and three macrofaunal properties. Before performing the analysis, the data set was tested using the “Bartlett. Test” and “Q-Q plot” functions in the “Car” package, which confirmed that the data met the analysis of variance (ANOVA) homogeneity and normality assumption. Two-way ANOVA and one-way

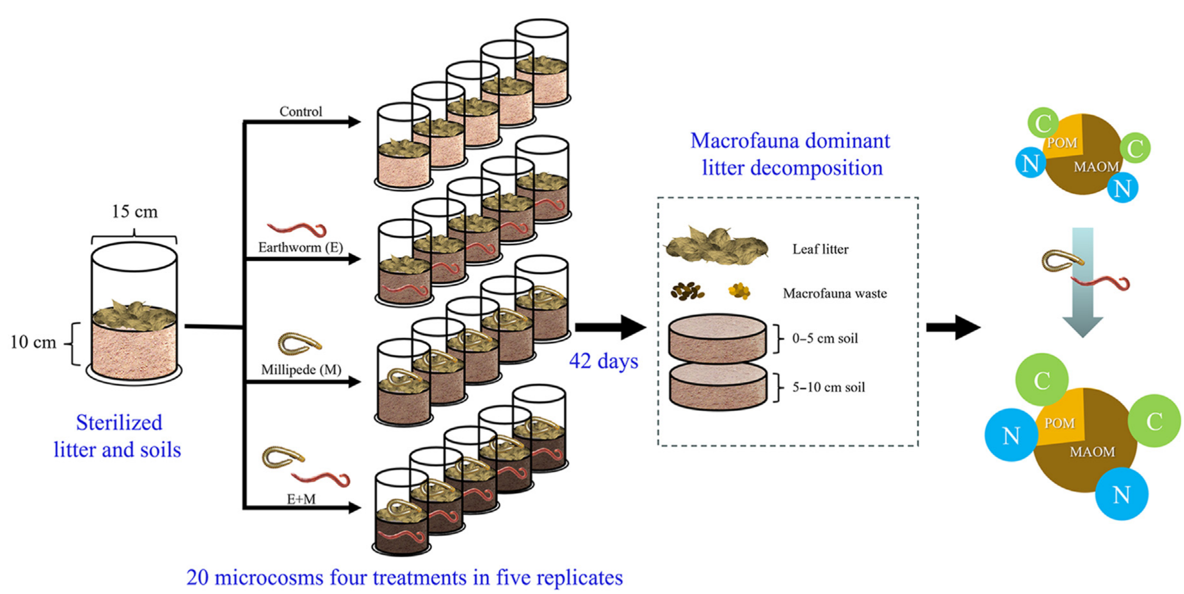

Fig. 1. Diagram of the experimental microcosm in the lab.

ANOVA were performed to test the effects of treatments and depth on the litter variables, soil physicochemical properties, and microbial properties. In addition, one-way ANOVA, followed by the Tukey HSD test, was performed to test the differences of these indicators among treatments in the 0–5 and 5–10 cm soil layers. It should be noted that one-way ANOVA was used to compare the differences of three macrofaunal properties among treatments. Variation partitioning analysis was conducted to show the relative influences of soil physicochemical properties and microbial properties on the C and N distribution in the POM and MAOM fractions. Redundancy analysis (RDA) and hierarchical partitioning analysis were further employed to indicate the specific explanation (represented as %) using the "Vegan" and "rdacca.hp" packages (Lai et al., 2022). A Pearson correlation coefficient was used to show the correlation between the soil physicochemical and microbial variables.

3. Results

3.1. Soil physicochemical properties

Soil ExCa, ExMg, AvAl, AvFe, POM-mass, and MAOM-mass were significantly affected by the macrofauna treatments, yet only ExCa, ExMg, and MAOM-mass were significantly affected by soil depth (Table S1). Specifically, when compared to the control, ExCa was significantly lower with the E and E+M treatments in the 0–5 and 5–10 cm soil layers, while AvAl was significantly lower with the E+M treatment (Table S2). Moreover, ExMg was significantly higher with the M and E+M treatments in the 0–5 cm and under E and E+M treatments in the 5–10 cm soil (Table S2). The POM-mass (13.14∼14.18 g) was significantly higher than MAOM-mass (5.54∼7.60 g) regardless of treatments and soil depths, and the MAOM-mass significantly increased from 6.0 ± 0.1 g (Control) to 6.7 ± 0.2 g (E and M treatments), and from 6.0 ± 0.1 g (Control) to 6.8 ± 0.1 g (E+M treatment) in the 0–5 cm soil depth (Table S2).

3.2. The C and N concentrations in the litter, macrofauna faeces, and POM and MAOM fractions

As compared with the control, litter mass under the E, M, and E+M treatments was significantly reduced by an average of 0.50, 1.94, and 7.10 g ($p < 0.001$), respectively (Fig. 2a). Litter C significantly decreased by 57.0% under E+M treatment, while litter N significantly decreased by 48.6%, 53.5%, and 75.1% under the E, M, and E+M treatments, respectively (Fig. 2b and c). Only the earthworm biomass under the E treatment significantly decreased after incubation (from 5.34 to 2.79 g) (Fig. S1a). The total C and N concentrations in macrofauna faeces under the M treatment were significantly higher than that under E and E+M treatments. The OC, TN, and ^{13}C concentrations in the POM and MAOM fractions were significantly affected by the macrofauna treatments, while only POM-TN was affected by soil depth (Fig. 2). The E and E+M treatments significantly increased the POM-TN in the 0–5 cm soil and MAOM-OC in the 0–5 and 5–10 cm soil layers, but decreased POM-^{13}C in the 0–5 cm soil layer (Fig. 3a–d, e). Regardless of soil depth, the E+M treatment significantly increased POM-OC and all macrofauna treatments increased MAOM-TN (Fig. 3b and c). In addition, adding macrofauna significantly decreased MAOM-^{13}C in the 0–5 cm soil, but the decreasing effects were only significant under M and E+M treatments (Fig. 3f). Moreover, the percent POM-TN (27.7%∼41.6%) and POM-OC (31.4%∼45.9%) were always lower than the percent MAOM-TN and MAOM-OC regardless of macrofauna treatments and soil depth, whereas the POM-C/N values were always higher than the MAOM-C/N values (Fig. S2).

3.3. Dominant controls on the distribution of C and N in soil fractions

Despite the fact that the C and N variations observed in all the soil layers in POM and MAOM fractions were driven by soil physicochemical and microbial properties, when compared, soil microbial properties more strongly affected the variations in the 0–5 cm soil and had less impact in the 5–10 cm soil (Fig. 4, Fig. S3). RDA further indicated that soil pH and AMF significantly affect the OC distributions in the 0–5 cm soil layer and explained 20.2% and 25.9% of the total variations, respectively (Fig. 5a and b). In the 5–10 cm soil layer, soil ExMg, ExCa, GN bacteria, and fungi were the significant factors, explaining 41.6%, 17.4%, 15.7%, and 23.7% of the total variation, respectively (Fig. 5c and d). For the TN distribution, soil pH, AMF, GN bacteria, and GP bacteria were the most significant variables in the 0–5 cm soil, contributing 20.3%, 20.1%, 15.5%, and 15.2% to the total variation, respectively (Fig. 6a and b). In the 5–10 cm soil layer, soil ExMg and GN bacteria explained 23.3% and 17.4% of the total variation, respectively (Fig. 6c and d). In the 5–10 cm soil layer, soil pH and AMF consistently had significant positive correlations with OC and TN in the POM and MAOM fractions in the 0–5 cm soil layer, while the positive correlations with soil ExMg, ExCa, and fungi were also observed (Fig. S5). Similarly, the positive correlations of OC and TN in soil fractions with pH, AMF, GN bacteria, and GP bacteria were observed in the 0–5 cm soil and with ExMg and GN bacteria in the 5–10 cm (Fig. S5).

4. Discussion

4.1. Impacts of earthworms on the C and N distribution in the POM and MAOM fractions

Previous studies have shown that across the globe, earthworms serve as physical ecosystem engineers that can, directly and indirectly, reduce three times the amount of litter mass than other plots devoid of earthworms in forest ecosystems (Angst et al., 2019; Huang et al., 2020). In contrast to the previous results and our hypothesis, results obtained herein showed no significant decrease in litter mass or litter C amount in

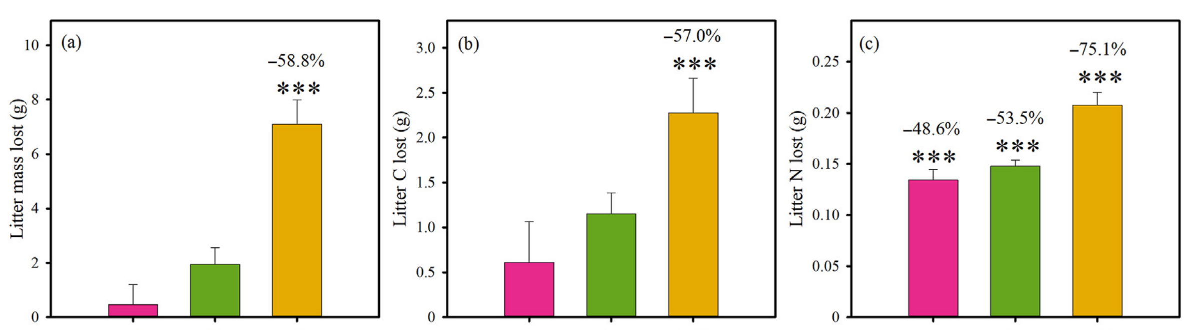

Fig. 2. Effects of earthworms (E), millipedes (M), and both (E+M) addition on litter mass loss and carbon (C) and nitrogen (N). Values are the difference between control and macrofauna addition treatment ($n = 5$). The symbol *** indicates the significant difference at $p \leq 0.001$, while the number on the bar shows the changed proportion relative to the control.

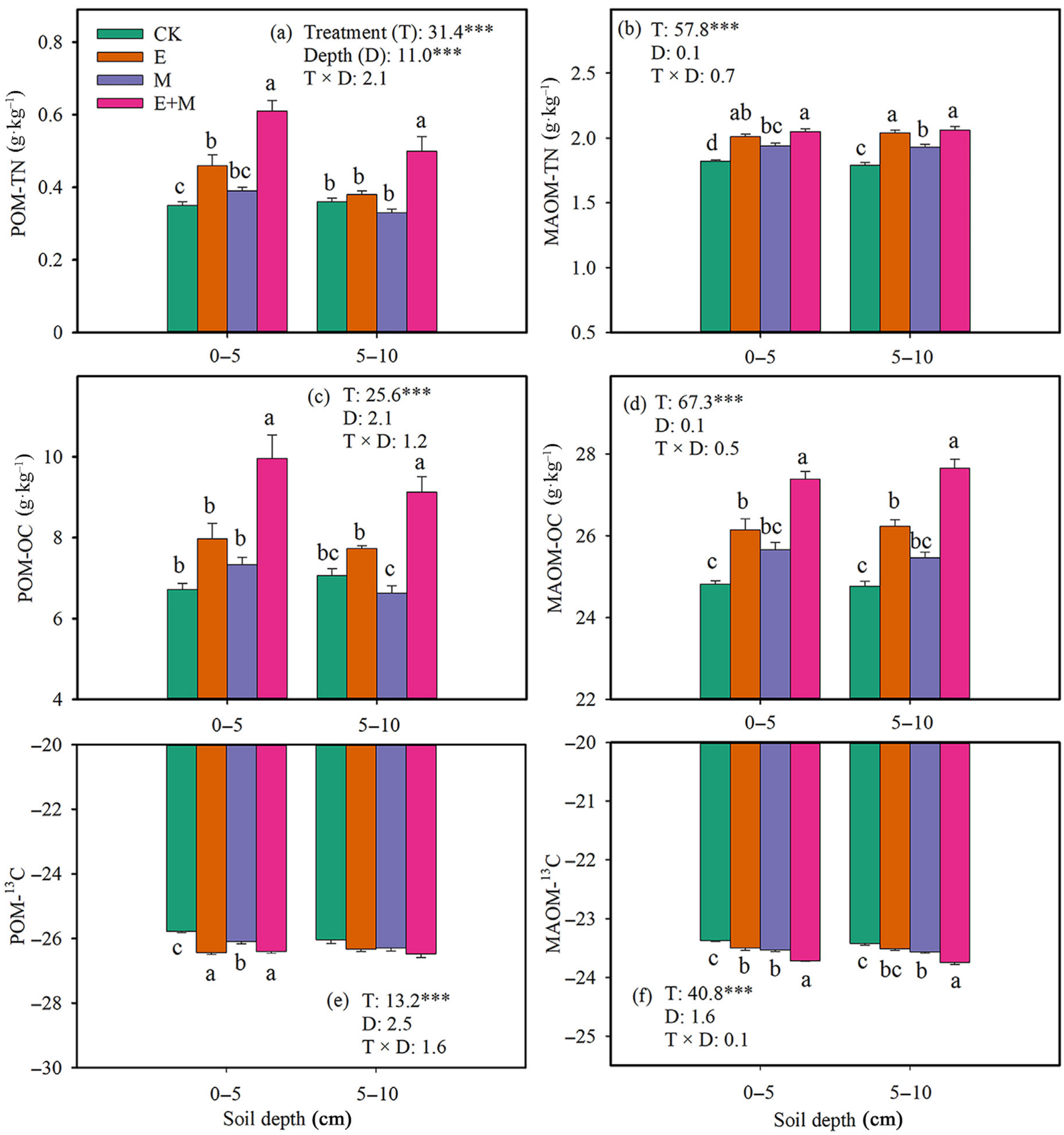

Fig. 3. Effects of earthworms (E), millipedes (M), and both (E+M) addition on the distributions of organic carbon (OC) and total nitrogen (TN) and ^{13}C values in particulate organic matter (POM) and mineral-associated organic matter (MAOM). Values are presented as mean values and stand errors ($n = 5$). Different letters denote significant differences revealed by the Tukey test among treatments in the 0–5 and 5–10 cm soil layers. Two-way ANOVA results for the OC and TN as affected by macro-decomposers (treatment, T), depth (D), and their interactions (T × D) were attached (F values; Symbol *** indicated the significant effect at $p \leq 0.001$).

samples where earthworms were present but depicted a significant decrease in litter N amount (Fig. 2). Potential explanations for this inconsistency include: 1) Different studies use varying pretreatment methods for leaf litter. Many previous studies cut the leaf litter into small fragments or used leaf powder prior to incubating, which enables the earthworms to feed on the litter more easily (Yang et al., 2020; Vidal et al., 2023). Herein, intact leaf litter was used to simulate natural conditions better. The degree of litter fragmentation after incubation decreased as follows: E+M > M > Control. 2) Experimental duration is likely the second factor, as leaf litter does not decompose within a short period. In addition, the significant decrease in litter N may have resulted from earthworms tending to prefer the litter components with relatively high N contents (e.g., carbohydrates, proteins, and other smaller non-structural ones) (Hendriksen, 1990; Edwards et al., 2013; Cotrufo et al., 2015).

The OC and TN contents in POM and MAOM fractions increased with earthworm addition regardless of soil depth, but the impacts were only significant in MAOM fractions (Fig. 3). On one hand, POM predominantly originates from leaf litter; thus, the dynamics of C and N in POM usually depend on the input of structural compounds with relatively high C:N ratios (Cotrufo et al., 2015; Lavallee et al., 2020). The small changes in litter mass may be the main reason of significant increases in C and N were not observed in the POM fraction. On the other hand, MAOM is predominantly created from microbial processes and consists of numerous microbial products (e.g., microbial polysaccharides, muramic acid, and other amino sugars). In this sense, earthworms might use the leaching materials to favor the MAOM formation. The changes in litter mass and C and N contents also support this explanation. Additionally, these results support the previous opinion, which indicates earthworm activity can facilitate the shift from a POM to an MAOM-dominated soil

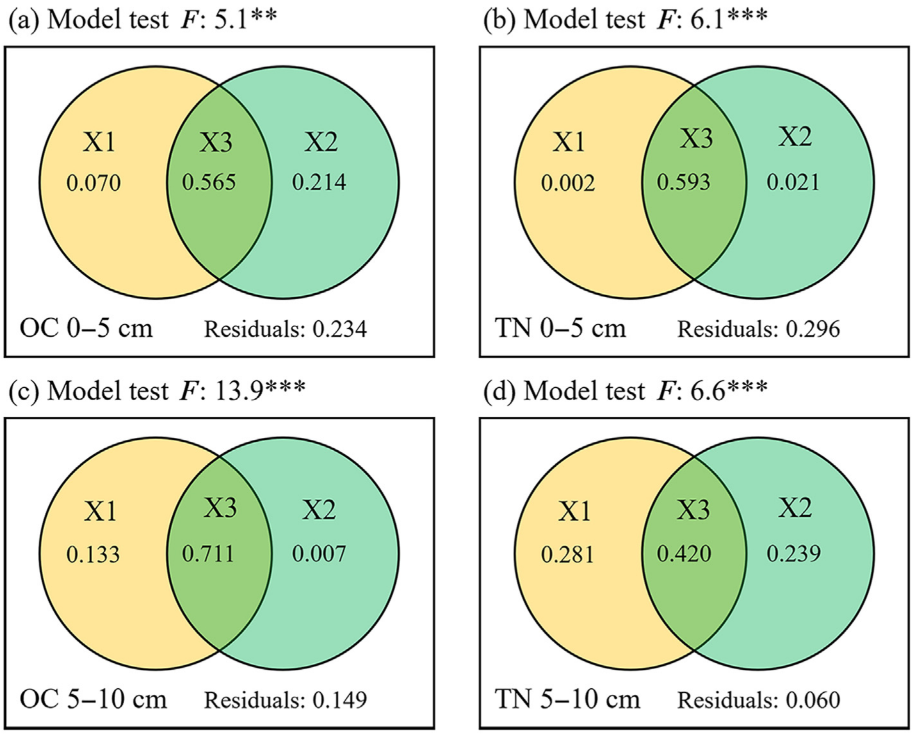

Fig. 4. Variation partitioning analyses for the distribution of organic carbon (OC) and total nitrogen (TN) in POM and MAOM fractions. The total variation was further partitioned into the following fractions: pure impact of eight soil physicochemical properties (X1), including soil pH, moisture, total carbon (TC), total nitrogen (TN), available Fe (AvFe), available Al (AvAl), exchangeable Ca (ExCa), exchangeable Mg (ExMg); pure effect of eight soil microbial properties (X2), including microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), fungi PLFAs, GN, GP, AMF, Act, and F:B ratios (Fungi:Bacteria); and combined effects of soil physicochemical and microbial properties (X3).

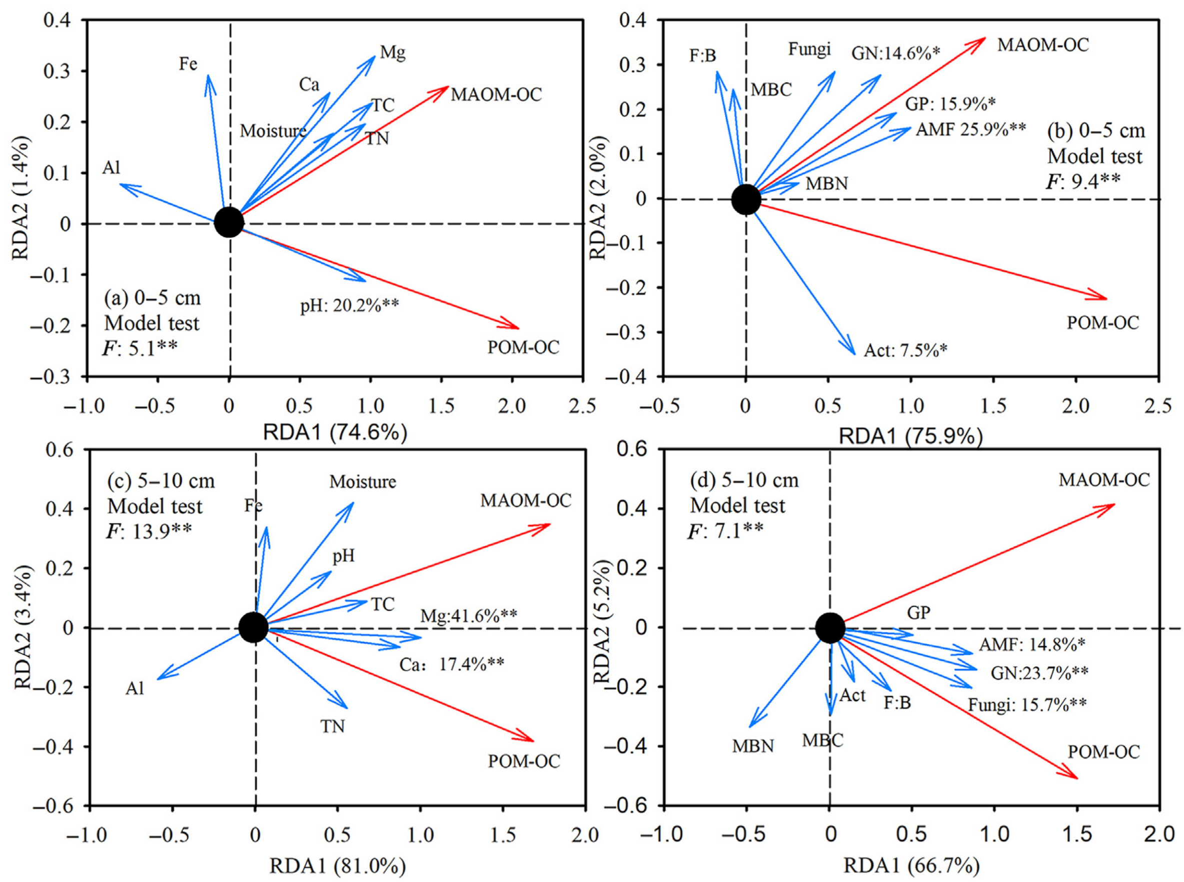

Fig. 5. Relationships of organic carbon in POM and MAOM fractions with soil property variables and soil microbial properties at different soil depths based on RDA. Red arrows denote OC distribution in two fractions, while blue arrows represent soil property variables. While *, **, and *** indicate significant effects of these variables at $p \leq 0.1$, 0.05, and 0.01, respectively, the values show the relative importance of each variable.

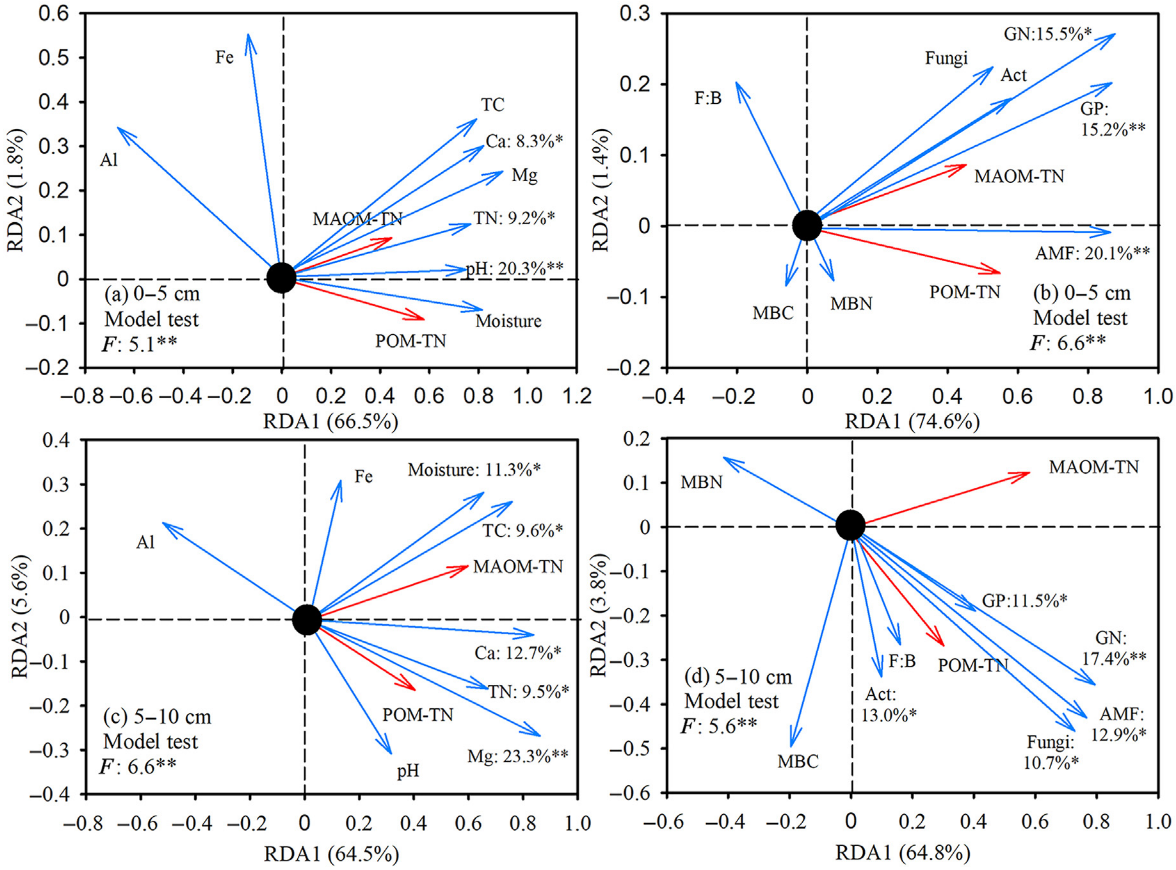

Fig. 6. Relationships of total nitrogen in POM and MAOM fractions with soil property variables and soil microbial properties at different soil depths based on RDA. See notes in Fig. 5.

system (Le-Mer et al., 2021; Vidal et al., 2023). In other words, the presence of earthworms can increase SOM stability. Moreover, we suspect that earthworms can directly facilitate C and N transformation process even under conditions where no litter is input conditions because of earthworm feed on fine soils (Blouin et al., 2013; Pelosi et al., 2014).

4.2. Impact of the millipedes and earthworms on the C and N distribution in the fractions

Results from this study demonstrated that adding millipedes decreased the litter mass, as well as litter C and N concentrations, which is consistent with our hypothesis. Moreover, adding millipedes (M) was more effective at decreasing the aforementioned variables than earthworms (E), but less effective than adding earthworms and millipedes (E+M). This phenomenon may reflect the relative importance of earthworms and millipedes at different stages during litter decomposition (Frouz et al., 2004; Špaldoňová and Frouz, 2014; Yang et al., 2020). At the early stage, millipedes may have a stronger impact than earthworms, as millipedes can directly break down both intact leaf and fragment litter, while millipede faeces can better support earthworms and microbes as time progresses. The fact that the highest values of TC and TN were found in the macrofaunal faeces supports this explanation (Fig. S1b). Consequently, the TN and OC significantly increased under the M and E+M treatments. In addition, all macrofaunal treatments decreased the ^{13}C abundance and the ^{13}C abundance in the MAOM fraction was generally lower than that in the POM fraction, indicating that more litter C entered into MAOM fractions because of the lower ^{13}C values of litters relative to the soils (Rochette et al., 1999; Mendez-Millen et al., 2014).

As discussed above, the results showed that macrofauna significantly altered the distribution of C and N in the POM and MAOM fractions. Moreover, soil physicochemical and microbial properties jointly explained most of the total variations, yet microbial properties were more influential in the surface soils, while physicochemical properties were the more dominant influence in the subsoils. Pearson correlation and redundancy analyses indicated that soil pH, AMF, ExMg, ExCa, fungi, GN bacteria, and GP bacteria were important moderators (Figs. 5 and 6, Figs. S4 and S5). In this study, when values from the two soil depths were averaged, the presence of earworms, millipedes, and both increased the pH by 0.16, 0.17, and 0.40 units (Liu et al., 2023), ExCa by 17%, 9%, and 27%, ExMg by 40%, 19%, and 66%, respectively (Table S2). The increased soil pH may alleviate the negative impacts of soil acidification on microbial activities (Bahram et al., 2018; Niu et al., 2021). Higher ExCa and ExMg contents in soil solution can facilitate the accumulation of C and N in the MAOM fraction by the transformation process of sorption and desorption (Kleber et al., 2021; Lugato et al., 2021). Our previous studies also indicated that high concentrations of mineral elements (i.e., Ca, Mg, and Al) generally favor MAOM formation and found higher concentrations of Al, Ca, and Fe in the MAOM fraction relative to in the POM fraction (Niu et al., 2022). In addition, the E, M, and E+M treatments all remarkably increased AMF by 21%, 22%, and 67%; fungi by 7%, 30%, and 51%; GP bacteria by 24%, 15%, and 44%; and GN bacteria 15%, 12%, and 31%, respectively (Niu et al., 2021). The inconsistent impacts of varying macrofauna on different microbial functional groups also affect the C and N distribution in the two fractions, as different microbiomes provide varying functionality. For instance, GP bacteria would prefer to assimilate recalcitrant C fractions and NO_3^--N especially under the conditions of low C and NH_4NO_3 inputs, while AMF can directly assimilate the intact mobilized oligopeptides in soil solution (Bajwa and Read, 1985; Verbaendert et al., 2011).

4.3. Implications and limitations of this research as related to the forest C-neutral

The current study addressed the importance of the interactive effects of earthworms and millipedes on the C and N distribution in soil fractions during litter decomposition. This incubational experiment provided direct evidence for the key point that the presence of soil macrofauna can facilitate SOM sequestration and store more C in the MAOM fraction. This finding is novel and timely because the role of soil macrofauna in forest C sequestration has not been fully interrogated in the functional C models (Gongalsky, 2021). Nevertheless, several limitations should be considered when interpreting these results. First, the impact of soil macrofauna on litter decomposition is highly dependent on litter quality (Sauvadet et al., 2016; Vidal et al., 2023). A recent study evaluated the key role of litter quality in C distribution between POM and MAOM and further suggested that regardless of low- (high C:N) and high- (low C:N) quality litter trends to replenish the MAOM-C pools in a subtropical forest (Lyu et al., 2023). Therefore, the qualitative relationship between litter quality, soil macrofauna activities, and the formation of POM and MAOM needs to be reassessed. Second, even though this study addressed the importance of soil macrofaunal biodiversity and microbes from the macrofaunal gut, the differences between the earthworm gut and the millipede gut were not directly identified. Thus, variances in the microbial community among different macrofauna (also including different species of earthworm or millipede) and their potential impacts on litter decomposition remain unclear (Toyota and Kimura, 2000; Huang et al., 2020; Gongalsky, 2021; Potapov et al., 2022).

5. Conclusion

The results of this study indicate that the presence of macrofauna can remarkably promote litter decomposition, thereby decreasing litter mass, and C and N contents, especially under the E+M treatment. As a consequence, the OC and TN contents in the POM and MAOM fractions significantly increased under the E+M treatment. While the OC and TN contents in MAOM fractions also significantly increased under the E and M treatments, the combined impacts of earthworms and millipedes (E+M) on the distribution of C and N in the two fractions was stronger than that of earthworms and millipede addition alone. Soil physicochemical and microbial properties can explain most of the total variations of C and N distribution, but microbial properties more heavily impact the surface soil. The current work highlights the direct relationships between litter decomposition, soil macrofauna (including microbes from macrofauna), and the redistribution of C and N in POM and MAOM fractions, which will aid the C-neutral atmospheric conditions under global warming in forest ecosystems.

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Availability of data and materials

Supplementary data to this article can be found in supporting materials and data are also available from the authors upon reasonable request.

CRediT authorship contribution statement

Guoxiang Niu: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tao Liu:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Zhen Zhao:** Software, Methodology, Investigation, Formal analysis, Data curation. **Xuebing Zhang:** Software, Investigation, Data curation. **Huiling Guan:** Investigation, Formal analysis, Data curation. **Xiaoxiang He:** Writing – review & editing, Visualization, Software, Funding acquisition. **Xiankai Lu:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

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References

Andreas, N., 2020. Tropical forests lost to land grabbing. Nat. Geosci. 13, 460–462.
Angst, G., Mueller, C., Prater, I., Angst, Š., Frouz, J., Jílková, V., Peterse, F., Nierop, K., 2019. Earthworms act as biochemical reactors to convert labile plant compounds into stabilized soil microbial necromass. Commun. Biol. 2, 441.
Angst, G., Frouz, J., van-Groenigen, J., Scheu, S., Kögel-Knabner, I., Eisenhauer, N., 2022. Earthworms as catalysts in the formation and stabilization of soil microbial necromass. Global Change Biol. 28, 4775–4782.
Angst, G., Mueller, K., Castellano, M., Vogel, C., Wiesmeier, M., Mueller, C., 2023. Unlocking complex soil systems as carbon sinks: multi-pool management as the key. Nat. Commun. 14, 2967.
Arrazola-Vasquez, E., Larsbo, M., Capowiez, Y., Taylor, A., Sandin, M., Iseskog, D., Keller, T., 2022. Earthworm burrowing modes and rates depend on earthworm species and soil mechanical resistance. Appl. Soil Ecol. 178, 104568.
Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., Huerta-Cepas, J., Medema, M.H., Maltz, M.R., Mundra, S., Olsson, P.A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., Bork, P., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237.
Bajwa, R., Read, D.J., 1985. The biology of mycorrhiza in the Ericaceae: IX. Peptides as nitrogen sources for the ericoid endophyte and for mycorrhizal and non-mycorrhizal plants. New Phytol. 101, 459–467.
Bar-On, Y.M., Phillips, R., Milo, R., 2018. The biomass distribution on Earth. Proc. Natl. Acad. Sci. USA 115, 6506–6511.
Blouin, M., Hodson, M., Delgado, E., Baker, G., Brussaard, L., Butt, K., Dai, J., Dendooven, L., Peres, G., Tondoh, J., Cluzeau, D., Brun, J., 2013. A review of earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 64, 161–182.
Bradford, M.A., Tordoff, G.M., Eggers, T., Jones, T.H., Newington, J.E., 2002. Microbiota, fauna, and mesh size interactions in litter decomposition. Oikos 99, 317–323.
Bradford, M.A., Wieder, W.R., Bonan, G.B., Fierer, N., Raymond, P.A., Crowther, T.W., 2016. Managing uncertainty in soil carbon feedbacks to climate change. Nat. Clim. Change 6, 751–758.
Chandel, A.K., Jiang, L., Luo, Y., 2023. Microbial models for simulating soil carbon dynamics: a review. JGR Biogeosciences 128, e2023JG007436.
Chen, L., Fang, K., Wei, B., Qin, S., Feng, X., Hu, T., Ji, C., Yang, Y., 2021. Soil carbon persistence governed by plant input and mineral protection at regional and global scales. Ecol. Lett. 24, 1018–1028.
Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nat. Geosci. 8, 776–779.
Cotrufo, M.F., Ranalli, M.C., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nat. Geosci. 12, 989–994.
Craig, M., Gryer, K., Beidler, K., Brzostek, E., Frey, S., Grandy, A., Liang, C., Phillips, R., 2022. Fast-decaying plant litter enhances soil carbon in temperate forests but not through microbial physiological traits. Nat. Commun. 13, 1229.
Dong, Y., Agathokleous, E., Liu, S., Yu, Z., 2023. Demographic changes in China's forests from 1998 to 2018. For. Ecosyst. 10, 100094.
Edwards, C.A., Arancon, N., Bohlen, P.J., Hendrix, P., 2013. Biology and Ecology of Earthworms. Springer, New York.
El-Wakeil, K.F.A., 2015. Effects of terrestrial isopods (crustacea: oniscidea) on leaf litter decomposition processes. J. Basic Appl. Zool. 69, 10–16.

Frouz, J., Pižl, V., Tajovský, K., Balík, H., Starý, J., Lukesšová, A., Šourková, M., 2004. The role of saprophagous macro-fauna on soil formation in reclaimed and non-reclaimed post-mining sites in Central Europe. Int. J. Ecol. Environ. Sci. 30, 257–261.
Gergocs, V., Hufnagel, L., 2016. The effect of microarthropods on litter decomposition depends on litter quality. Eur. J. Soil Biol. 75, 24–30.
Gill, A., Schilling, J., Hobbie, S., 2021. Experimental nitrogen fertilization globally accelerates, then slows decomposition of leaf litter. Ecol. Lett. 24, 802–811.
Gongalsky, K.B., 2021. Soil macrofauna: study problems and perspectives. Soil Biol. Biochem. 159, 108281.
Gonzälez, G., Seastedt, T.R., 2001. Soil fauna and plant litter decomposition in tropical and subalpine forests. Ecology 82, 955–964.
Grayston, S.J., Campbell, C.D., Bardgett, R.D., Mawdsley, J.L., Clegg, C.D., Ritz, K., Griffiths, B.S., Rodwell, J.S., Edwards, S.J., Davies, W.J., Elston, D.J., Millard, P., 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Appl. Soil Ecol. 25, 63–84.
Hendriksen, N.B., 1990. Leaf litter selection by detritivore and geophagous earthworms. Biol. Fertil. Soils 10, 17–21.
Huang, W., González, G., Zou, X., 2020. Earthworm abundance and functional group diversity regulate plant litter decay and soil organic carbon level: a global meta-analysis. Appl. Soil Ecol. 150, 103473.
Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G., Pineiro, G., 2017. The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. Annu. Rev. Ecol. Evol. 48, 419–445.
Joly, F., Coq, S., Coulis, M., Nahmani, J., Hättenschwiler, S., 2018. Litter conversion into detritivore faeces reshuffles the quality control over C and N dynamics during decomposition. Funct. Ecol. 32, 2605–2614.
Kleber, M., Bourg, I.C., Coward, E.K., Hansel, C.M., Myneni, S.C.B., Nunan, N., 2021. Dynamic interactions at the mineral–organic matter interface. Nat. Rev. Earth Environ. 2, 402–421.
Lai, J., Zou, Y., Zhang, J., Peres-Neto, P., 2022. Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. Methods Ecol. Evol. 13, 782–788.
Lavallee, J., Soong, J., Cotrufo, M., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biol. 26, 261–273.
Le-Mer, G., Jouquet, P., Capowiez, Y., Maeght, J., Tran, T.M., Doan, T.T., Bottinelli, N., 2021. Age matters: dynamics of earthworm casts and burrows produced by the anecic *Amynthas khami* and their effects on soil water infiltration. Geoderma 382, 114709.
Lehmann, J., Hansel, C.M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., Nunan, N., Reichstein, M., Schimel, J., Torn, M., Wieder, W., Kögel-Knabner, I., 2020. Persistence of soil organic carbon caused by functional complexity. Nat. Geosci. 13, 529–534.
Li, X., Zhang, Q., Feng, J., Jiang, D., Zhu, B., 2023. Forest management causes soil carbon loss by reducing particulate organic carbon in Guangxi, Southern China. For. Ecosyst. 10, 100092.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2, 17105.
Liu, T., Wang, Z., Guan, H., Zhong, B., He, X., Wang, Y., Qi, Y., Yan, W., Lu, X., 2023. Soil macrofauna disperse and reconstruct soil nematode communities: takeaways from a microcosm study. Forests 14, 748.
Lugato, E., Lavallee, J.M., Haddix, M.L., Panagos, P., Cotrufo, M.F., 2021. Different climate sensitivity of particulate and mineral-associated soil organic matter. Nat. Geosci. 14, 295–300.
Lyu, M., Homyak, P.M., Xie, J., Peñuelas, J., Ryan, M.G., Xiong, X., Sardans, J., Lin, W., Wang, M., Chen, G., Yang, Y., 2023. Litter quality controls tradeoffs in soil carbon decomposition and replenishment in a subtropical forest. J. Ecol. 111, 2181–2193.
Macias, A., Marek, P., Morrissey, E., Brewer, M., Short, D., Stauder, C., Wickert, K., Berger, M., Metheny, A., Stajich, J., Boyce, G., Rio, R., Panaccione, D., Wong, V., Jones, T., Kasson, M., 2019. Diversity and function of fungi associated with the fungivorous millipede, *Brachycybe lecontii*. Fungal Ecol. 41, 187–197.
Mendez-Millen, M., Nguyen Tu, T., Balesdent, J., Derenne, S., Derrien, D., Egasse, C., Thongo M'Bou, A., Zeller, B., Hatté, C., 2014. Compound-specific ^{13}C and ^{14}C measurements improve the understanding of soil organic matter dynamics. Biogeochemistry 118, 205–223.
Niu, G., Hasi, M., Wang, R., Wang, Y., Geng, Q., Hu, S., Xu, X., Yang, J., Wang, C., Han, X., Huang, J., 2021. Soil microbial community responses to long-term nitrogen addition at different soil depths in a typical steppe. Appl. Soil Ecol. 167, 104054.
Niu, G., Liu, L., Wang, Y., Guan, H., Ning, Q., Liu, T., Rousk, K., Zhong, B., Yang, J., Lu, X., Han, X., Huang, J., 2022. Effects of decadal nitrogen addition on carbon and nitrogen stocks in different organic matter fractions of typical steppe soils. Ecol. Indic. 144, 109471.
Pant, M., Negi, G., Kumar, P., 2017. Macrofauna contributes to organic matter decomposition and soil quality in Himalayan agroecosystems, India. Appl. Soil Ecol. 120, 20–29.
Pelosi, C., Barot, S., Capowiez, Y., Hedde, M., Vandenbulcke, F., 2014. Pesticides and earthworms. A review. Agron. Sustain. Dev. 34, 199–228.
Potapov, A.M., Rozanova, O.L., Semenina, E.E., Leonov, V.D., Belyakova, O.I., Bogatyreva, V.Y., Degtyarev, M.I., Esaulov, A.S., Korotkevich, A.Y., Kudrin, A.A., Malysheva, E.A., Mazei, Y.A., Tsurikov, S.M., Zuev, A.G., Tiunov, A.V., 2021. Size compartmentalization of energy channeling in terrestrial belowground food webs. Ecology 102, e03421.
Potapov, A.M., Beaulieu, F., Birkhofer, K., Bluhm, S., Degtyarev, M., Goncharov, A., Gongalsky, K., Klarner, B., Korobushkin, D., Liebke, D., Maraun, M., Mc Donnell, R., Pollierer, M., Schaefer, I., Shrubovych, J., Semenyuk, I., Sendra, A., Tuma, J., Tumova, M., Vassilieva, A., Chen, T., Geisen, S., Schmidt, O., Tiunov, A., Scheu, S., 2022. Feeding habits and multifunctional classification of soil-associated consumers from protists to vertebrates. Biol. Rev. 97, 1057–1117.
Rochette, P., Flanagan, L.B., Gregorich, E.G., 1999. Separating soil respiration into plant and soil components using analyses of the natural abundance of carbon-13. Soil Sci. Soc. Am. J. 63, 1207–1213.
Rossel, R., Lee, J., Behrens, T., Luo, Z., Baldock, J., Richards, A., 2019. Continental-scale soil carbon composition and vulnerability modulated by regional environmental controls. Nat. Geosci. 12, 547–552.
Sauvadet, M., Chauvat, M., Fanin, N., Coulibaly, S., Bertrand, I., 2016. Comparing the effects of litter quantity and quality on soil biota structure and functioning: application to a cultivated soil in Northern France. Appl. Soil Ecol. 107, 261–271.
Scheu, S., Schaefer, M., 1998. Bottom-up control of the soil macrofauna community in a beechwood on limestone: manipulation of food resources. Ecology 79, 1573–1585.
Seeber, J., Scheu, S., Meyer, E., 2006. Effects of macro-decomposers on litter decomposition and soil properties in alpine pastureland: a mesocosm experiment. Appl. Soil Ecol. 34, 168–175.
Snyder, B.A., Hendrix, P.F., 2008. Current and potential roles of soil macroinvertebrates (earthworms, millipedes, and isopods) in ecological restoration. Restor. Ecol. 16, 629–636.
Špaldoňová, A., Frouz, J., 2014. The role of *Armadillidium vulgare* (isopoda: oniscidea) in litter decomposition and soil organic matter stabilization. Appl. Soil Ecol. 83, 186–192.
Tian, G., Brussaard, L., Kang, B.T., 1995. Breakdown of plant residues with contrasting chemical compositions under humid tropical conditions: effects of earthworms and millipedes. Soil Biol. Biochem. 27, 277–280.
Toyota, K., Kimura, M., 2000. Microbial community indigenous to the earthworm *Eisenia foetida*. Biol. Fertil. Soils 31, 187–190.
Verbaendert, I., Boon, N., De Vos, P., Heylen, K., 2011. Denitrification is a common feature among members of the genus *Bacillus*. Syst. Appl. Microbiol. 34, 385–391.
Vidal, A., Blouin, M., Lubbers, I., Capowiez, Y., Sanchez-Hernandez, J., Calogiuri, T., van-Groenigen, J., 2023. The role of earthworms in agronomy: consensus, novel insights and remaining challenges. Adv. Agron. 181, 1–78. https://doi.org/10.1016/bs.agron.2023.05.001.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van der putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Yang, X., Shao, M., Li, T., 2020. Effects of terrestrial isopods on soil nutrients during litter decomposition. Geoderma 376, 114546.