



Seasonal changes in anecic earthworm cast properties reflect food source variability in Northern Vietnam

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ABSTRACT

The objective of this study was to assess seasonal variability of anecic earthworm cast properties in contrasting tropical ecosystems as it might provide information on the earthworms' feeding behavior during different times of the year. Fresh casts deposited at the soil surface by the anecic earthworm *Amyntas adexilis* and topsoil aggregates without trace of earthworm activity were collected at five dates across one year in meadow and woodland in northern Vietnam. We analysed elemental composition, stable isotopes, lignin signatures and rare earth elements (REEs) to investigate the changes in organic matter (OM) and mineral composition. Casts were consistently enriched in less degraded OM compared to topsoil aggregates, but their OM composition varied markedly throughout the year, with strong contrasts between habitats. In meadow, cast C content, C/N and lignin content fluctuated by 31, 23 and 65%, and their seasonal trajectories closely mirrored those of adjacent topsoil aggregates. In woodland, seasonal changes were much more pronounced and decoupled from those of the topsoil aggregates. Indeed, lignin content varied more than 14-fold, and isotopic signatures showed clear temporal shifts, indicating major seasonal changes in the OM type ingested by *A. adexilis*. In contrast, REE patterns remained stable across sampling dates in both ecosystems, suggesting that temporal changes in cast properties were primarily driven by food source variability rather than changes in feeding depth. In meadow, single-date estimates of C incorporated into casts ranged from 7.6 to 10.0 t C·ha⁻¹·yr⁻¹, whereas in woodland variability was much lower (1.3 to 1.5 t C·ha⁻¹·yr⁻¹).

We conclude that *A. adexilis* feeds mainly on topsoil. However, as OM composition of food sources may change with time, it is necessary to make multiple seasonal measurements of cast composition in some ecosystems to capture the full variability of earthworm effects on SOM dynamics under tropical condition. Moreover, ecosystem management should account for seasonal variability in earthworm activity, as it may directly affect soil carbon and nutrient dynamics.

1. Introduction

Earthworms may have many important impacts on (bio-)geochemical cycling in terrestrial ecosystems. In particular, they most probably drive soil organic matter (SOM) formation and turnover through (selective) ingestion of different organic matter (OM) and mineral types (Barois and Lavelle, 1986; Angst et al., 2019). Fresh earthworm casts are enriched in labile compounds and characterized by high decomposition

and mineralization rates after their deposition (Scheu, 1987; Van Groenigen et al., 2018). At the same time, fresh casts may also be enriched in OM stabilized for longer time periods through association with the mineral phase during gut passage (Song et al., 2025) and/or physical protection of ingested OM (Bossuyt et al., 2006). Cast ageing and associated release of nutrients impacting soil functioning (Decaëns et al., 1999) could depend on the composition of organic and inorganic cast constituents.

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Indeed, the effects of earthworms on SOM dynamics may vary among different ecosystems (Song et al., 2024), reflecting differences in OM sources, feeding strategies, migration behavior and mineral soil ingestion (Lavelle et al., 2004). Anecic earthworms, in particular, feed at the soil surface but inevitably ingest mineral soil while consuming litter. Since anecic earthworms simultaneously ingest fresh litter and more decomposed soil-derived OM, seasonal shifts in litter inputs or in the substrates encountered during foraging may change the relative proportions of these sources incorporated into their casts. Such changes are plausible because anecic species are known to occupy different soil depths at different times of the year (Baker et al., 1993; Jiménez and Decaëns, 2000), suggesting that their access to organic and mineral substrates may vary through time. Whether these depth shifts modify the amount or types of ingested OM and minerals, however, remains unknown.

A particularly important aspect is that several studies have shown that the physicochemical properties of casts can vary substantially over the course of a year. Indeed, in their seminal work, Sharpley and Syers (1977) demonstrated strong seasonal fluctuations in the proportions and release rates of organic and inorganic phosphorus from casts. Syers et al. (1979) reported clear seasonal patterns in ammonium- and nitrate-N concentrations within casts, indicating that microbial transformations within casts follow a pronounced annual cycle. More recently, Wang et al. (2021) documented month-to-month changes in TOC, TN, NH_4^+ -N and NO_3^- -N concentrations in casts over a full year in a subtropical forest. Together, these studies provide compelling evidence that cast properties may vary seasonally. However, none of these studies were conducted with anecic earthworms, although this ecological category is known to have a specific feeding behavior, which affects strongly the incorporation of fresh OM into deep mineral horizons (Don et al., 2008). As a result, the temporal patterns described for epigeic and endogeic species may not be extrapolated to anecics, whose feeding ecology and access to organic and mineral substrates differ fundamentally (Curry and Schmidt, 2007). Consequently, the seasonal dynamics and drivers of OM incorporated into anecic casts remain unknown, particularly in tropical environments. This knowledge gap is especially relevant for *Amyntas adexilis*, a highly prolific tropical anecic species whose casts represent a major pathway for the return of organic and mineral material to the soil surface (Bottinelli et al., 2021a). Even subtle seasonal shifts in the type or amount of OM incorporated into casts of this species could have substantial implications for OM cycling and accumulation in tropical soils, where climatic seasonality is pronounced.

Land use is also known to influence cast properties, as casts closely reflect the characteristics of the surrounding soil, the type of organic matter available to earthworms (Bottinelli et al., 2021b) and the prevailing microclimate (Song et al., 2024). Indeed, land-use differences in organic matter inputs or in microclimatic conditions in particular soil moisture and temperature could modify the depth at which anecic species forage and therefore the nature of the material they ingest. It is thus uncertain whether seasonal variability in anecic cast composition is consistent across land-use types, or whether it is influenced by land-use-driven differences in resource availability and/or feeding behavior.

The aim of this study was to assess how land use and sampling time affect the composition of casts produced by *Amyntas adexilis*, which is the only species depositing globular casts at soil surface of the study site, the watershed of Dong Cao in Northern Vietnam (Bottinelli et al., 2021a). Fresh casts and adjacent topsoil aggregates without recent earthworm activity distinguished by visual expert assessment were collected across four seasons in a woodland and a meadow and analysed using complementary biogeochemical tracers, including elemental composition, stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), lignin-derived phenols and rare earth elements (REEs). The C/N ratio, stable carbon and nitrogen isotope and lignin signatures provided information on OM origin and state of decomposition. REEs were used to trace the composition of the mineral fraction ingested by earthworms because their concentrations and patterns are primarily governed by parent material and soil

mineralogy (Ramos et al., 2016), and their distributions along soil profiles reflect pedogenic differentiation (Bispo et al., 2021). Owing to their minimal biological fractionation, REEs may provide a robust fingerprint of the composition of the mineral material incorporated into casts. We hypothesised that cast OM composition would vary seasonally because of changes in the quantity and nature of organic and mineral substrates encountered by earthworms during their migration to deeper layers in response to humidity changes. We expected that these dynamics would differ between the two land-use systems.

2. Materials and methods

2.1. Study fields and sampling

This study was conducted from January to October 2018 at the 'M-Tropics' long-term observatory catchment in Dong Cao village in northern Vietnam ($20^\circ 57'\text{N}$, $105^\circ 29'\text{E}$). The climate is subtropical humid and, in 2018, mean air temperature was 23°C and total rainfall was 2224 mm. The dry season (November–April) was characterized by lower precipitation (302 mm) and cooler air temperature (19°C), whereas the wet season (May–October) was characterized by higher precipitation (1922 mm) and warmer air temperature (27°C). The dominant soil type was Acrisol (WRB) (Podwojewski et al., 2008).

We selected two contrasting land use systems presenting large amounts of surface casts produced by the anecic *Amyntas adexilis*: a meadow and a woodland. The meadow has been covered for over 20 years by short grasses (mainly *Paspalum* and *Stachytarpheta*) and is regularly grazed by cattle and buffaloes. The woodland was formerly cultivated and afforested 14 years prior to sampling with two evergreen *Annonaceae* species, with an average tree height of 10 m and planting distances of 3–5 m, and shows no regular planting pattern. Cast production in 2018 reached 170 t ha^{-1} in the meadow and 35 t ha^{-1} in the woodland (Bottinelli et al., 2021a). Topsoil (0–5 cm) had an organic C content of 37 mg g^{-1} under meadow and 25 mg g^{-1} under woodland, a pH (KCl) between 3.8 and 3.9, and clay contents of 58% and 50%, respectively.

We considered two ecosystems: a meadow ($20^\circ 56.878'\text{N}$, $105^\circ 29.105'\text{E}$) and a woodland ($20^\circ 57.256'\text{N}$, $105^\circ 29.054'\text{E}$). In each ecosystem, we delineated a 100 m^2 area and established four plots ($50 \times 50\text{ cm}$) along a transect perpendicular to the slope. To account for heterogeneous cast production activity, in 2018, we sampled three plot replicates ($n = 3$) out of the four monitored plots at five dates (20 January, 17 March, 16 June, 28 July and 13 October) to cover contrasting seasonal conditions. At each replicated plot, we collected (i) all casts from the soil surface inside the plot, and (ii) control topsoil aggregates (2–7 cm depth) sampled within the same 100 m^2 area but outside the cast-collection plots, in locations without visible casts or burrow openings. Thereafter the samples were transported to the laboratory, air-dried, sieved at 2 mm, and ground to pass a $200\text{ }\mu\text{m}$ sieve prior to chemical analyses. Overall, 60 samples were analysed: 2 ecosystems $\times$ 5 dates $\times$ 3 replicates $\times$ 2 sample types (casts and control aggregates).

Soil temperature and soil water tension (0–250 cbar) were continuously monitored using sensors installed at 10 cm depth (Watermark sensors for tension). Across the five sampling dates, soil temperature ranged from 17.0 to 26.0°C in the meadow and from 17.7 to 25.4°C in the woodland, with the meadow being slightly warmer during the wet-season dates (June–July; difference $\leq 1^\circ\text{C}$) (Fig. 1). Soil water tension ranged from 4.8 to 144.8 cbar in the meadow and from 13.3 to 217.9 cbar in the woodland, and was consistently higher in the woodland (+5 to +73 cbar), indicating systematically drier topsoil conditions in the woodland across sampling dates. The strongest contrast occurred during the driest period.

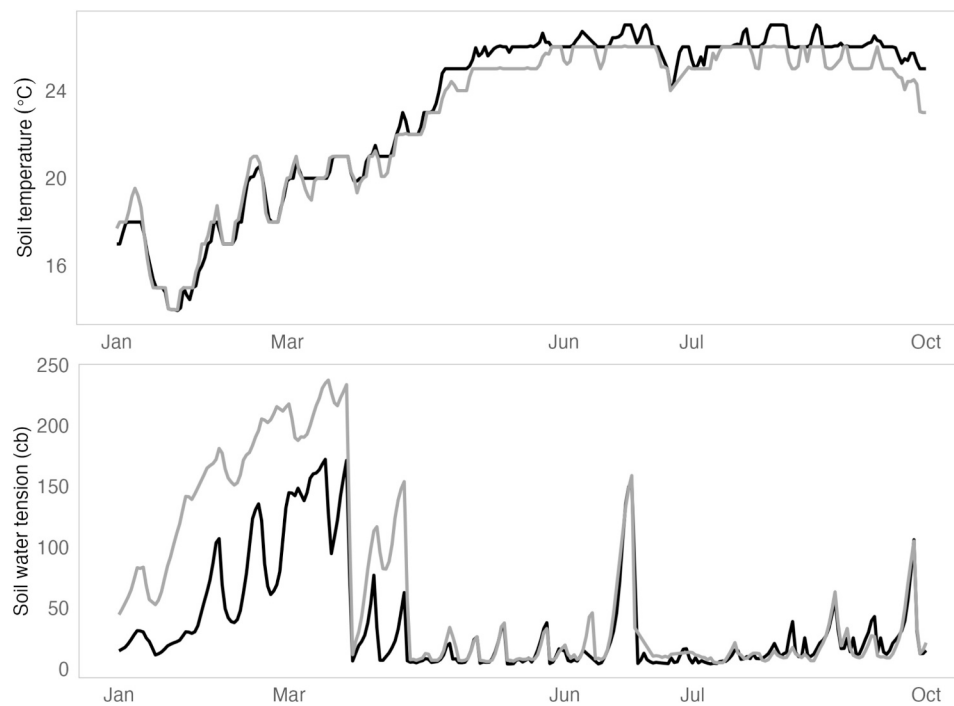


Fig. 1. Soil temperature and soil water tension at 10 cm depth in meadow and woodland during 2018; sampling dates were 20 January, 17 March, 16 June, 28 July and 13 October.

2.2. Elemental and isotopic composition of soil and cast samples

Total organic C and N content and stable isotope signatures $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured using an elemental analyzer Flash 2000 (Thermo Fisher Scientific, Bremen, Germany) coupled to an isotope ratio mass spectrometer Delta V Advantage (Thermo Fisher Scientific, Bremen, Germany). The C and N isotope ratios were expressed using the delta notation:

$$\delta \text{ sample } (\text{‰}) = \left[\frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \right] \times 1000 \quad (1)$$

where R is the isotopic ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample and reference gas, R_{sample} and R_{standard} , respectively. The stable isotope ratios were calculated relative to the Pee Dee Belemnite standard (PDB) for C and relative to atmospheric N_2 for N.

2.3. Lignin analysis of soil and cast samples

To determine lignin content and composition, we carried out alkaline CuO oxidation (Hedges and Ertel, 1982) of all samples collected at each sampling date from both locations. This method yielded quantitative information of both parameters, indicating (i) the presence of plant litter compounds and (ii) the state of their decomposition. About 500 mg of cast and soil aggregates were oxidized with 250 mg CuO and 2 M NaOH at 172 °C under N_2 for 2 h. After purification using solid-phase extraction with a C18 column (International Sorbent Technology) as suggested by Kögel and Bochter (1985), lignin phenols were quantified as trimethylsilyl derivatives by gas chromatography. We used a HP 6890 gas chromatograph (GC) equipped with a flame ionisation detector (FID) and a SGE BPX-5 column (50 m length, 0.25 mm inner diameter, 0.32 mm coating) for the separation of the individual monomers. The oven temperature rise was programmed from 100 °C (1 min isotherm) to 150 °C at +3 °C/min and from 150 to 300 °C at +1 °C/min. The detector temperature was kept at 350 °C. Helium was used as carrier gas (1 ml/min). This method yielded a suite of phenolic CuO oxidation products, including vanillyl (V), syringyl (S), and cinnamyl (C) units with aldehydic, ketonic, and acidic side chains. The sum of CuO oxidation

products (VSC) was used as an indicator of the total amount of lignin and the ratios between the acid and the aldehyde forms of the vanillyl and syringyl units ((Ac/Al)_V and (Ac/Al)_S) may indicate their degree of decomposition (Hedges et al., 1988). Analytical precision was 10% for VSC and 5% for ratios between phenolic CuO oxidation products.

2.4. Rare earth element analyses

Approximately 100 mg of finely ground fresh casts collected at the five sampling dates was combusted in a furnace at 600 °C for 2 h to remove OM. The mineral residue was transferred to Teflon beakers and digested using 2 mL of concentrated HNO_3 (16 N) and 2 mL of HF (27 N) on a hot plate at 105 °C for 24 h. After digestion, the beakers were opened and left at 95 °C overnight to evaporate acids. A second digestion was performed with 1 mL of HNO_3 (16 N) and 6 mL of HCl (6 N), followed by another evaporation step. The final residues were dissolved in 10 mL of HNO_3 (2 N) and left for 24 h before being transferred to 15 mL centrifuge tubes and diluted 1:100 in HNO_3 (0.5 N) prior to analysis. Concentrations of Lanthanum (La), Cerium (Ce), Praseodymium (Pr), Neodymium (Nd), Samarium (Sm), Europium (Eu), Gadolinium (Gd), Terbium (Tb), Dysprosium (Dy), Holmium (Ho), Erbium (Er), Thulium (Tm), Ytterbium (Yb) and Lutetium (Lu) were measured using an Agilent 7900 ICP-MS. Analytical accuracy was evaluated using the BCR-2 and BHVO-2 certified reference materials (United States Geological Survey, Reston, VA, USA). Measured values were in good agreement with certified concentrations, with a slight positive bias (~7.12% for BCR-2 and ~4.14% for BHVO-2).

Although the REE composition of soils is largely related to their parent material, changes in their composition might be related to pedogenic processes, which lead to their migration in the soil profile to their relative solubility, mobility and association with mineral and/or organic compounds (Laveuf and Cornu, 2009). The translocation of REE within the soil profile leads to their fractionation, the preferential migration of light REEs (La, Ce, Pr, Nd and Sm) or heavy REEs (Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) depending on the type of parent material and pedogenesis (Laveuf and Cornu, 2009). This results in changing ratios of light rare earth elements over heavy rare earth elements (LREE/HREE

ratio) in the soil profile especially in highly weathered tropical soils (Wu et al., 2022; own data see Fig. S1). We took advantage of these changes with depth, to assess whether cast mineralogy varied among sampling dates. This LREE/HREE ratio was used as an indicator of potential changes in the mineral soil fraction ingested by earthworms. We expected this ratio to change if earthworms fed at different soil depths.

2.5. Calculations and statistical analyses

Statistical analyses were performed in R (version 4.4.1) (R Core Team, 2024), and figures were generated using the *ggplot2* package (Wickham, 2016). For each of the two land-use types, differences in organic matter (OM) properties between earthworm casts and topsoil aggregates across sampling dates were analysed separately using a two-way ANOVA, with sample type (cast vs. topsoil aggregate), sampling date, and their interaction included as fixed effects. Homogeneity of variances was tested using Levene's test (*car* package) (Fox and Weisberg, 2019), and variables were Box–Cox transformed when necessary. ANOVA tables were computed using Type II sums of squares. Post-hoc comparisons were performed using estimated marginal means using the *emmeans* package (Lenth et al., 2023) with Tukey adjustment, either on sample type $\times$ sampling date combinations when the interaction was significant or on significant main effects otherwise. We determined annual carbon incorporation into casts by multiplying the total annual cast production (Bottinelli et al., 2021a) by the carbon contents of casts sampled at different times of the year. Because these values do not represent independent observations and they were not subjected to statistical testing. Temporal variation in rare earth elements was assessed using the LREE/HREE ratio. For this variable, one-way ANOVA was performed separately for each land-use type with sampling date included as the fixed factor, followed by Tukey-adjusted pairwise comparisons when significant. Because replicates were not tracked across sampling dates, they were treated as independent observations within each sampling date and were not included as repeated measures or random effects in the statistical models. Statistical significance was set at $p < 0.05$. A principal component analysis (PCA) was performed on centered and scaled OM variables using the *FactoMineR* package (Lê et al., 2008).

3. Results

3.1. C concentration, CN ratio and stable isotopic ratios

Clear contrasts in C concentration, C/N ratio, and isotopic signatures were observed between meadow and woodland topsoil aggregates (Fig. 2). On average, meadow topsoil aggregates showed higher C concentrations (41.2 mg g^{-1}) and higher C/N ratios (13.9) than those of woodland (25.3 mg g^{-1} and 11.9, respectively). Meadow samples also exhibited less depleted $\delta^{13}\text{C}$ values (-15.7‰) compared with woodland (-22.9‰), while $\delta^{15}\text{N}$ values were similar (6.9‰ – 7.0‰).

Cast elemental and isotopic composition consistently differed from topsoil aggregates for both land uses. In the meadow, sample type had a significant effect on C concentration ($p < 0.001$), $\delta^{15}\text{N}$ ($p < 0.001$) and $\delta^{13}\text{C}$ ($p < 0.001$). Casts contained on average 40% more C than topsoil aggregates, while $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were 16% and 22% lower. Woodland casts also differed from topsoil aggregates, with increases of 82% in C and decreases of 45% and 15% in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. C/N ratios did not differ significantly between casts and topsoil aggregates for either land use ($p > 0.05$). Temporal patterns varied between ecosystems. In the meadow, sampling date significantly affected C, C/N and $\delta^{15}\text{N}$ ($p < 0.001$) of casts, with a pronounced shift between June and July, whereas $\delta^{13}\text{C}$ remained stable across sampling dates ($p > 0.05$). In the woodland, no significant temporal variation was detected for casts' C, C/N, $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ ($p > 0.05$ for all parameters). No sample type $\times$ sampling date interaction was observed, indicating that differences between casts and topsoil aggregates remained similar across sampling dates in both

ecosystems.

Based on the annual cast production measured for each ecosystem, the annual amount of C incorporated into casts was estimated using cast C content measured at each sampling date. In the meadow, annual C incorporation ranged from 7.6 to $10.0 \text{ t C ha}^{-1} \text{ yr}^{-1}$, reflecting the strong seasonal variability in cast C content. In contrast, the amount of C incorporated into casts and its variability was much lower in the woodland, where estimates ranged from 1.3 to $1.5 \text{ t C ha}^{-1} \text{ yr}^{-1}$.

3.2. Lignin

Total lignin content (VSC), and lignin parameters of casts and topsoil aggregates are presented in Fig. 3 for meadow and woodland across sampling dates.

For topsoil aggregates, slight differences were observed between land uses. On average, meadow topsoil aggregates showed lower VSC values (0.70) than those of woodland (0.86). Mean (Ac/Al)_v ratios were similar between sites (1.12 in meadow and 1.06 in woodland), whereas (Ac/Al)_s values were lower for meadow topsoil aggregates (0.66) than for those of woodland (0.75).

In the meadow, sample type had a significant effect on VSC ($p < 0.05$), (Ac/Al)_v ($p < 0.05$), and (Ac/Al)_s ($p < 0.01$). On average, casts showed a 19% higher VSC and 21% and 26% lower (Ac/Al)_v and (Ac/Al)_s, than topsoil aggregates. Sampling date significantly affected VSC ($p < 0.05$) and (Ac/Al)_v ($p < 0.05$), with a slight shift between June and July. No sample type $\times$ sampling date interaction was detected for any of these variables ($p > 0.05$), indicating that cast–soil differences remained stable over time.

In the woodland, VSC, (Ac/Al)_v, and (Ac/Al)_s were all influenced by a significant sample type $\times$ sampling date interaction (VSC: $p < 0.01$; (Ac/Al)_v: $p < 0.05$; (Ac/Al)_s: $p < 0.001$), indicating that cast–topsoil differences varied across sampling dates. Increases in cast VSC occurred only in March, while no differences were detected at other dates. Casts showed lower (Ac/Al)_v values than topsoil only in October, whereas (Ac/Al)_s values were higher in casts than topsoil aggregates exclusively in October. Overall, the significant interaction reflected a greater temporal variability of cast properties compared with topsoil aggregates for all three lignin parameters.

3.3. Co-variation of OM quantity and quality in cast and topsoil aggregates

The PCA carried out using OM properties of casts and topsoil aggregates at the two sites is shown in Fig. 4. Cast and topsoil aggregates were clearly separated along PC1, which explained 45–50% of the variance. PC2 (22–24% of the variance) described the seasonal dynamics of OM properties. In the meadow, casts and topsoil aggregates followed similar seasonal trajectories: early-season samples (January, March, June) showed negative PC2 values, whereas late-season samples (July, October) showed positive values. Positive PC2 values were correlated with higher C/N ratios, (Ac/Al)_v and C content. In the woodland, casts showed a stronger seasonal variation than topsoil aggregates. Similar as in the meadow, PC2 opposed early-season woodland samples to late-season samples, but the amplitude of this contrast was larger. For woodland, positive PC2 values were correlated with higher C/N ratios and (Ac/Al)_s, whereas negative values were correlated to higher VSC contents.

3.4. Rare earth elements

LREE/HREE ratios showed clear differences between the two land-use types (Fig. 5). In the meadow, mean values averaged 1.15 and were slightly lower than those observed in the woodland, where the mean ratio reached 1.20. For both land uses, no significant temporal variation of the LREE/HREE ratio was detected ($p > 0.05$).

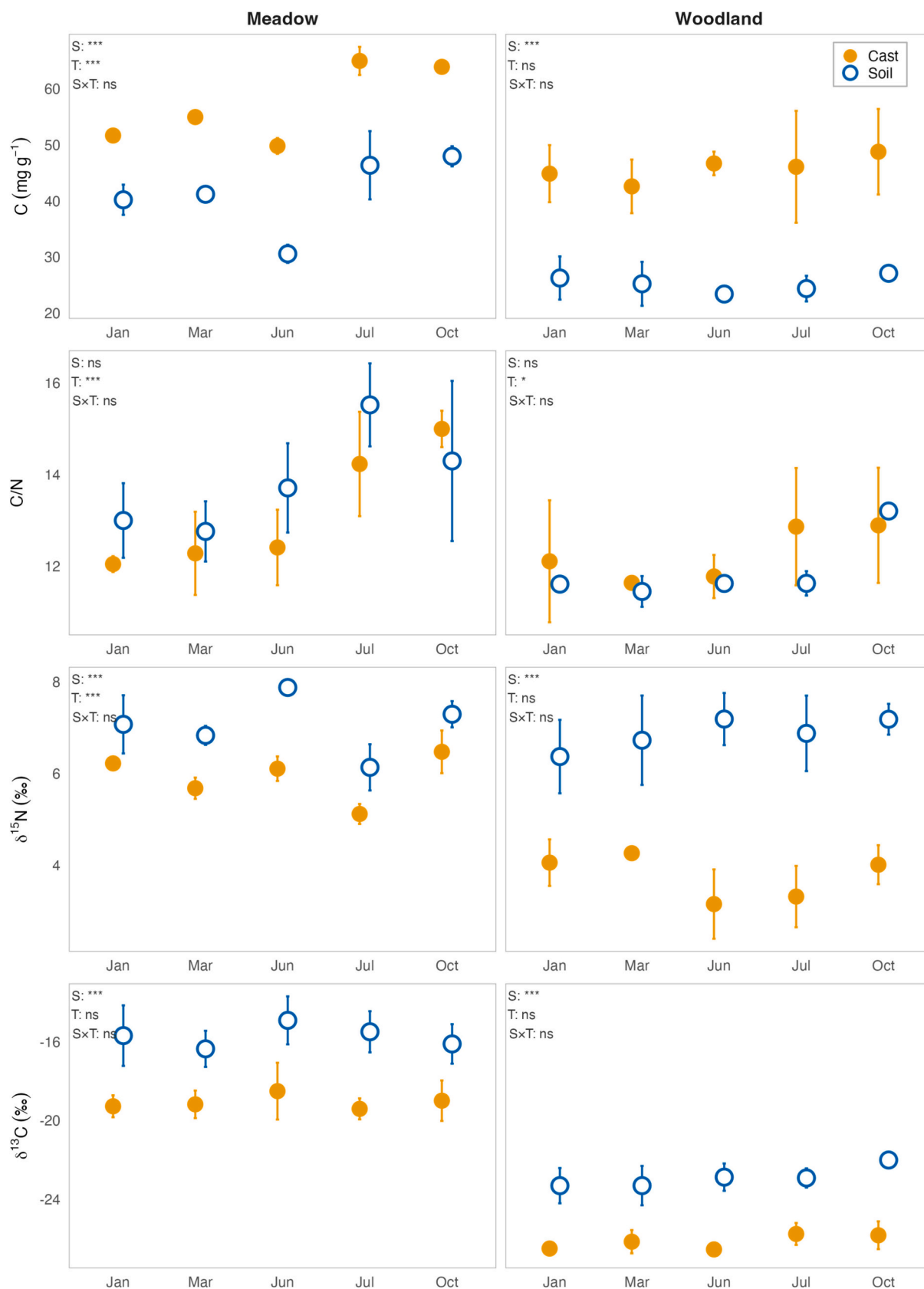


Fig. 2. Mean values ($\pm$ SD) of C, C/N, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ are shown for casts and topsoil aggregates at five sampling dates in the two landuse systems. Statistical outcomes of the linear models are reported in each panel: S = effect of sample type (topsoil aggregate vs. cast), T = effect of sampling date, and S $\times$ T = interaction between sample type and sampling date. Significance levels are indicated as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and ns = not significant.

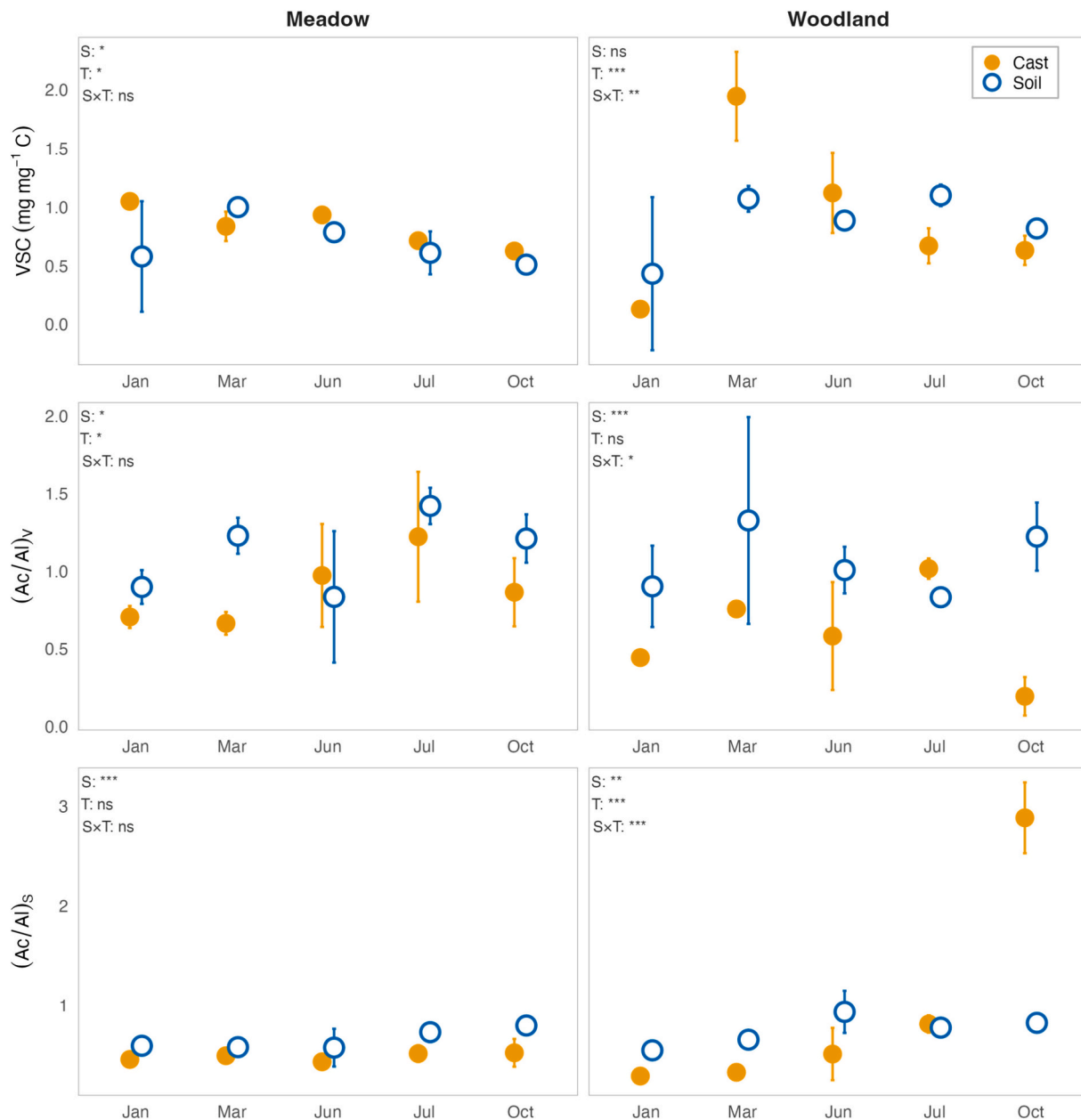


Fig. 3. Mean values ($\pm$ SD) of lignin content normalized by the C content (VSC) and acid/aldehyde ratios are shown for casts and topsoil aggregates at five sampling dates in the two landuse systems. Statistical outcomes of the linear models are reported in each panel: S = effect of sample type (topsoil aggregate vs. cast), T = effect of sampling date, and S $\times$ T = interaction between sample type and sampling date. Significance levels are indicated as ***p < 0.001, **p < 0.01, *p < 0.05, and ns = not significant.

4. Discussion

4.1. Organic matter composition in earthworm casts

For both land uses, the OM composition of the anecic *Amyntas adexilis* casts deposited at the soil surface was characterized by higher carbon concentrations, lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic ratios, higher lignin contents and lower (Ac/Al)_v and (Ac/Al)_s ratios than topsoil aggregates regardless the sampling date. Our data are in agreement with other studies showing that fresh casts contain high amounts of relatively undecomposed organic matter compared to surrounding soil aggregates (Guggenberger et al., 1996; Ngyen Hong et al., 2011; Vidal et al., 2019). This is the result of mixing of plant litter compounds and minerals within the earthworm gut system and ejection of casts with occluded and mineral associated organic matter compounds (Bossuyt

et al., 2006; Le Mer et al., 2020). Fresh casts were found to be sources of easily available carbon and nutrients (Van Groenigen et al., 2018). During cast ageing these labile compounds may be released and stabilized SOM compounds may accumulate due to the formation of micro-aggregates (Pulleman et al., 2005) and mineral-associated organic matter (Vidal et al., 2019; Angst et al., 2019). SOM dynamics during cast ageing and its role in biogeochemical cycling may be influenced by the nature of organic and mineral compounds incorporated into casts through earthworm activity (Vos et al., 2019), which might change throughout the year (Sharpley and Syers, 1977). In the following we used lignin parameters and rare earth elements to investigate if the sampling date influenced the composition of mineral and/or organic compounds of earthworm casts.

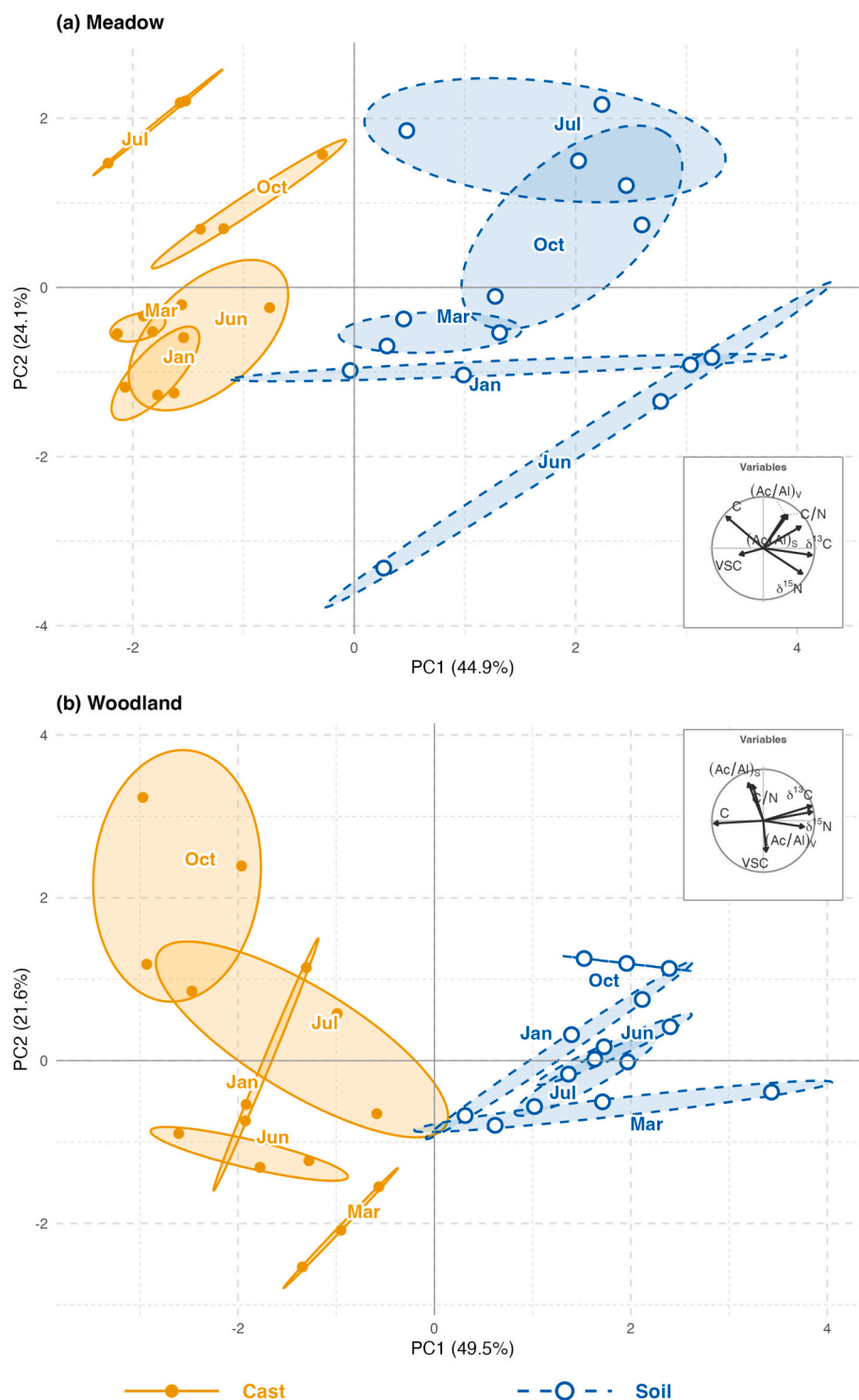


Fig. 4. Ordination biplots show the distribution of casts (yellow) and topsoil aggregates (blue) along the first two principal components for meadow (a) and woodland (b). Ellipses represent dispersion envelopes based on the covariance structure of the samples for each sampling date (January, March, June, July, October). Vectors indicate the direction and magnitude of the variables contributing to PC1 and PC2, including C concentration, C/N ratio, $\delta^{13}C$, $\delta^{15}N$, total lignin content (VSC), and the vanillyl and syringyl acid-to-aldehyde ratios ($(Ac/Al)_v$ and $(Ac/Al)_s$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.2. Contrasting effects of sampling dates on OM chemistry but not mineral composition

Under woodland, cast OM composition showed large variability between sampling dates, while OM properties of topsoil aggregates remained relatively stable. In contrast, under meadow, the OM

properties of casts and topsoil aggregates followed similar temporal trajectories across sampling dates. The LREE/HREE ratios did not show any shift towards lower values indicating that soil incorporated into casts originated from topsoil.

Our data are in agreement with studies indicating seasonal variation in earthworm cast composition, which was attributed to fluctuations in

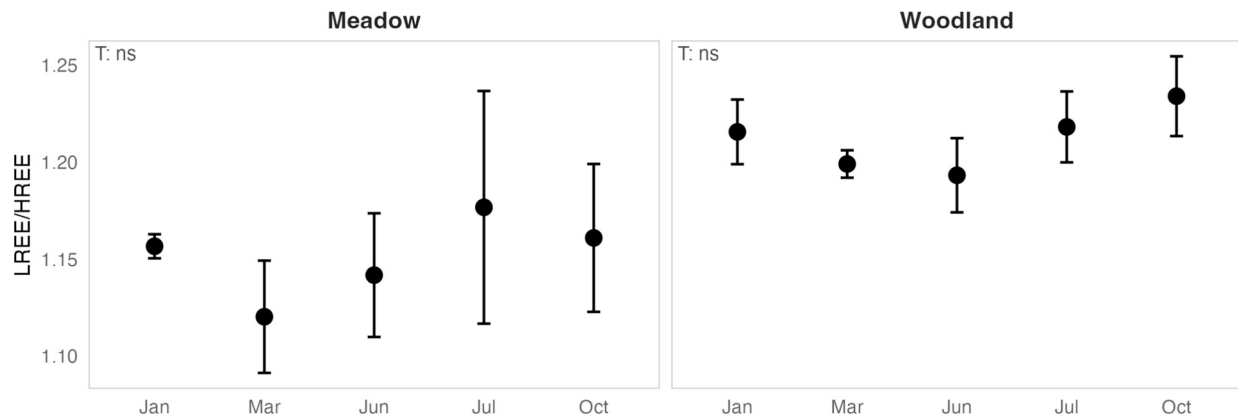


Fig. 5. Mean values ($\pm$ SD) of LREE/HREE ratios for casts samples at five sampling dates (January, March, June, July, October) in the meadow and woodland. Results of linear models are shown in each panel; T: ns indicates that the effect of sampling date was not statistically significant.

moisture, temperature and food supply (Sharpley and Syers, 1977). Indeed, anecic earthworms feed on fresh OM deposited at the soil surface as well as on soil material, which are subsequently mixed and transformed during gut transit and released as casts. As a result, the quantity and quality of OM in earthworm casts depend on both, the characteristics of the ingested soil and the availability and quality of fresh surface-derived OM inputs. Moreover, intra-annual variability in precipitation may influence earthworm foraging depth within the soil profile, as dry conditions can induce vertical migration into deeper soil layers (Baker et al., 1993; Morón-Ríos et al., 2010).

In woodland, which showed higher soil water tension than meadow from January to June, earthworms may have temporarily ingested soil material originating from deeper soil layers during dry periods. This interpretation is supported by PCA results showing that casts produced during this period were characterized by lower C/N ratios and higher lignin contents. Such characteristics may be indicative of root-derived and mineral-associated OM, which typically prevail in deeper mineral soil horizons (Rumpel and Kögel-Knabner, 2011). However, the similar LREE/HREE ratios measured throughout the year in both land uses suggest that earthworm feeding depth did not vary markedly over time. Consequently, the intra-annual variability of organic matter composition in casts under woodland is more likely driven by seasonal changes in the composition of surface-derived OM inputs. Indeed, seasonal litterfall dynamics controlled by temperature and precipitation have been reported in tropical forests (Zhang et al., 2014) and may influence leaf litter chemistry (Tonin et al., 2021).

At the meadow site, characterized by lower variability in soil moisture, OM properties of casts and topsoil aggregates varied similarly over time, suggesting a consistent contribution of soil-derived OM to both pools. In meadow topsoil, OM is largely root-derived. Seasonal changes in root litter inputs and rhizosphere activity may therefore drive temporal variability in OM composition in both casts and topsoil aggregates, potentially mediated by shifts in microbial communities in response to temperature and precipitation (Houlden et al., 2008). Altogether, these results suggest that *A. adexilis* maintained a broadly similar feeding behavior in both land use systems, with no marked seasonal shift in feeding depth, while the composition of the OM incorporated into casts varied according to land use and seasonal availability of organic resources.

The present study shows that intra-annual variability in earthworm cast composition may strongly affect estimates of carbon incorporation into casts, with contrasting patterns between land use systems. In the meadow, annual carbon incorporation estimates varied widely depending on the sampling date ($7.6\text{--}10.0\text{ t C}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$), whereas variability was much lower in the woodland ($1.3\text{--}1.5\text{ t C}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$). These values are substantial and indicative for the amount of carbon subject to processing and redistribution through earthworm activity. As

their annual variability can be high in some ecosystems, we suggest that one (cast) sampling date is insufficient to describe the earthworm effect on biogeochemical cycling under tropical conditions. In addition, the contrasting nature of OM ingested at different times of the year might influence the carbon allocation to specific OM compartments and therefore affect OM persistence (Lavalley et al., 2020). Overall, our findings highlight that anecic species may feed mainly on fresh OM deposited in topsoil and/or at the soil surface. As fresh OM may be altered by seasonal plant activity, capturing the true role of anecic earthworms in tropical biogeochemical cycles most probably requires temporally resolved sampling that accounts for ecosystem-specific variability in the OM consumed and its stabilisation pathways.

5. Conclusion

This study demonstrates that earthworm activity throughout the year is closely reflected in the composition of their casts, highlighting the importance of fresh OM characteristics incorporated following the feeding behavior of the anecic *A. adexilis*. Cast composition closely links with soil and vegetation parameters, which were contrasting in woodland and meadow ecosystems. Sampling date had a significant impact on the OM composition of *A. adexilis* cast in woodland, due to changing aboveground litter composition in response to seasonal litterfall. Meadow cast composition, characterized by intra-annual variability due to the plants' activity may reflect the earthworms' feeding within the rhizosphere. Land use thus strongly affects the composition of fresh casts throughout the year with potential implications for OM persistence. To improve assessments of earthworm impacts on biogeochemical cycling, we suggest adopting sampling designs considering multiple seasons within a year capturing ecosystem-specific temporal variations in resource availability.

CRedit authorship contribution statement

N. Bottinelli: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **T.T. Doan:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **X.H. Dang:** Writing – original draft, Investigation, Formal analysis, Data curation. **M.F. Benedetti:** Supervision, Resources, Methodology, Formal analysis. **P. Jouquet:** Writing – review & editing, Validation, Supervision, Investigation. **T.M. Tran:** Writing – review & editing, Supervision, Investigation. **C. Rumpel:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2026.107098>.

Data availability

Data will be made available on request.

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