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Consequences of subtropical land-use intensity for the abundance and diversity of earthworm ecological categories

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ABSTRACT

Understanding how soil biodiversity, especially of macrofauna like earthworms, responds to land-use intensity is crucial for developing sustainable land-use strategies. This work is a two-year field investigation of earthworm community responses to increasing land-use intensity, from undisturbed fallow land to actively cultivated agricultural lands (including fallow land, tea plantation, orange plantation, camphora plantation, synthetic fertilizer-amended cropland, compost-amended cropland, and vermicompost-amended cropland) in a subtropical region. Earthworm abundance and diversity increased with land-use intensity, likely due to the compensatory effects of organic amendments, which improve the habitat and resource availability, thereby alleviating the potential negative impacts of tillage and harvesting. Notably, earthworm abundance was higher in cropland (70 ind. m^{-2}) than in other land-use types, such as fallow (4 ind. m^{-2}) and plantation (22 ind. m^{-2}). Greater earthworm abundance was associated with higher soil pH and more food resources, as indicated by high microbial biomass carbon (C), the humification index, and the particulate organic C fraction. Anecic and endogeic earthworms increased more than epigeic earthworms from fallow lands to plantations and croplands, reflecting their ecological adaptability to the soil conditions in managed lands with higher land-use intensity. This suggests that soil ecological restoration practices may enhance the role of earthworms related to soil structure dynamics and carbon sequestration. Our study provides empirical evidence that soil macrofauna have ecological adaptations to cope with agricultural intensification across landscapes.

1. Introduction

Anthropogenic transformation from undisturbed natural ecosystems to managed lands is a major driver of contemporary biodiversity loss (Cardinale et al., 2012). Land-use intensification is an important global environmental change, exerting pressure on soil fauna and the ecosystem functions they provide (Allan et al., 2015). Earthworms, as prominent members of the soil fauna, are referred to as 'nature's plough' for their ability to modify soil porosity and aggregation through their burrowing and casting activities (Briones and Schmidt, 2017). Their community dynamics are modulated by human disturbances, nitrogen inputs, biomass removal, and changes in vegetation diversity associated with land-use intensification (Edwards and Arancon, 2022). Crucially, interspecific responses to land-use intensity are mediated by functional traits embedded within ecological niches, which determine taxon-specific tolerance thresholds to environmental disturbances (Smith et al., 2008). Although the impacts of land-use intensification are often negative for earthworm communities (Ahmed et al., 2022; Weldmichael et al., 2020), this effect exhibits context-dependency, modulated by management regime and biome-specific conditions (Singh et al., 2020). For instance, agricultural practices, such as adding organic amendments, planting cover crops, and reclaiming and replanting fallow land, can promote earthworm colonization and growth (Emmerling et al., 2021). However, the response of earthworms to these practices is largely determined by their dispersal capacity, phenotypic plasticity, and environmental adaptability, as well as geographic factors, including

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climate, soil type, and local farming practices (Carnovale et al., 2015; Chan, 2001; Hoeffner et al., 2021; Singh et al., 2020). Given the critical role of earthworms in soil and ecosystem services, it is essential to understand how earthworms respond to land-use intensity in a specific context is essential for effective management of soil biodiversity.

Earthworms are generally categorized ecologically as epigeic, endogeic, and anecic, based on their morphological traits and the soil layers they inhabit and the modification they make through soil engineering activities (Edwards and Arancon, 2022). Epigeic earthworms are small-sized species that live within the litter layer on the soil surface. They have relatively high reproductive rates and actively participate in litter decomposition and soil nutrient cycling, with little or no burrowing ability. Endogeic earthworms are intermediate-sized species that create non-permanent, discontinuous burrows without preferential orientation in upper mineral soil layers, primarily consuming particulate and mineral materials. Anecic earthworms are large-sized species that construct vertical permanent burrows, incorporate litter into deeper soil layers and bring mineral soil to the surface. These ecological categories determine their varying vulnerability to anthropogenic interferences. Epigeic earthworms, with their restricted burrowing capacity, are more vulnerable to habitat changes than endogeic and anecic earthworms (Edwards and Arancon, 2022). Mechanical disturbances like inversion tillage could disturb the surface and expose epigeic earthworms to the abrasion and predators, resulting in injury or death (Briones and Schmidt, 2017). However, their r-selected life history strategies, characterized by high mobility and accelerated reproductive rates, may confer resilience through rapid recolonization post-disturbance (Edwards and Arancon, 2022). Land-use intensification imposes two interrelated stresses on earthworm communities. Vegetation simplification reduces litter heterogeneity (Sala et al., 2000), leaving fewer resources for litter-dependent epigeic and anecic earthworms. In addition, soil acidification disrupts pH-sensitive biochemical processes in endogeic and anecic earthworms (Duddigan et al., 2021; Gossner et al., 2016). Due to the species-specific life histories, habitat preferences, feeding strategies, and burrowing capacities, the responses of earthworm ecological categories to land-use intensity may differ greatly and potentially alter soil functions (Smith et al., 2008). Understanding how these distinct earthworm ecological categories respond to varying land-use intensity is essential for developing sustainable soil management strategies that enhance soil health and ecosystem services.

The effects of land-use intensity on the species abundance, community composition, and spatial distribution of earthworms are well documented for tropical and temperate regions, but limited research has been done in subtropical areas (Phillips et al., 2019). The lack of information on earthworms in subtropical areas is a critical knowledge gap, for two reasons. First, subtropical regions experience high temperatures and abundant rainfall, fostering the growth of aboveground vegetation, which may provide abundant litter and food resources for earthworms (Xu et al., 2013). Vegetation diversity may disproportionately benefit epigeic earthworms, which live within surface litter, while anecic and endogeic earthworms rely on organic matter within the soil. However, local factors may not regulate the diversity of aboveground and belowground communities in the same way (Decaëns, 2010). The loss of key vegetation functional groups may be more important than the vegetation abundance and diversity (Buchkowski et al., 2024; Eisenhauer et al., 2009). Second, subtropical regions have a long history of agricultural activity, potentially filtering the more sensitive species (Balmford, 1996), which means that shifting land-use to intensive agriculture may have a relatively small effect on earthworms. The anecic and endogeic earthworms, in particular, possess larger body sizes and adaptive life-history traits, allowing them to withstand agricultural disturbances more effectively than epigeic earthworms (Edwards and Arancon, 2022). Moreover, long-term reliance on synthetic fertilizers has exacerbated soil acidification and accelerated soil degradation in this region (Guo et al., 2010). In contrast, applying organic amendments increases the soil organic matter and alleviates acidification, supports multiple ecosystem services, including food and feed provision, while contributing to soil restoration (Chen et al., 2022; Neuenkamp et al., 2024; Scotti et al., 2015). These contrasting fertilization practices shape earthworm communities by altering soil conditions, with synthetic fertilizers exacerbating environmental stress while organic amendments create a more favorable habitat. Thus, land-use intensity is expected to have a disproportionate effect across earthworm ecological categories in subtropical areas.

Based on a two-year field investigation, we explored how increasing land-use intensity, from the fallow lands to plantations and croplands, influences the abundance and diversity of earthworms in a subtropical region of China. We calculated the land-use intensity index based on

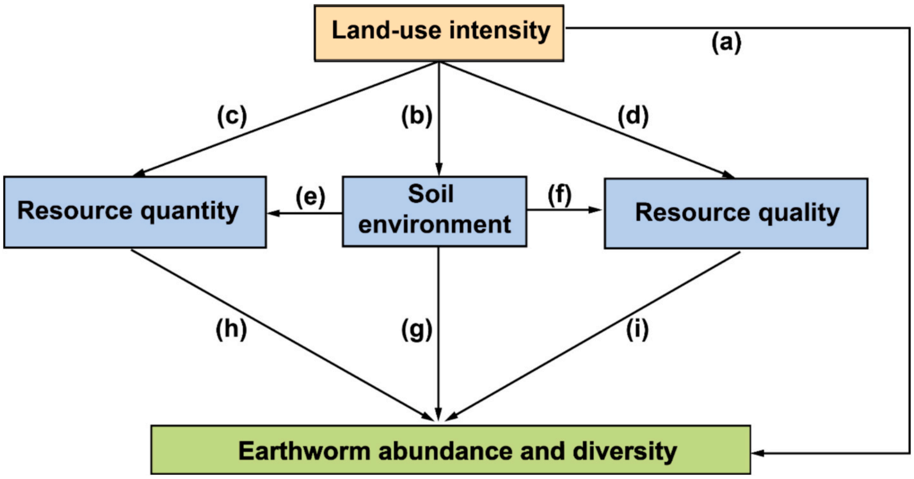

Fig. 1. An a-priori conceptual model illustrates how land-use intensity affects the abundance and diversity of earthworms through direct and indirect influences of soil environment, resource quantity and quality in subtropical ecosystems. We expect human disturbances to land-use, such as tillage, will expose earthworms to harsh environmental conditions and predators, causing direct injury or death (a; Briones and Schmidt, 2017). Further, land-use intensity changes the diversity of above-ground vegetation, which directly affects the soil environment and food resource of earthworm habitats, resulting in different community compositions (b, c, and d; Eisenhauer et al., 2007; Gudeta et al., 2022). Finally, land-use intensity indirectly modifies the resource quantity and quality by changing the soil environment, such as soil water content, aggregates and pH (e and f; Karami et al., 2012; Mulder and Elser, 2009). These changes in soil environment conditions, resource quantity and quality can directly impact earthworm colonization (g, h and i; Hoeffner et al., 2021), with effects depending on earthworm ecological category (Huang et al., 2020).

degree of agricultural activities (Gossner et al., 2016; Peters et al., 2019). According to the *a-priori* models (Fig. 1), we hypothesized that: 1) increasing land-use intensity will reduce abundance and diversity of earthworms by disrupting the soil environment and reducing the quality and quantity of food resources; and 2) compared with the anecic and endogeic earthworms, the abundance and/or diversity of epigeic earthworms will decrease with increasing land-use intensity, as they are more susceptible to surface soil degradation.

2. Material and methods

2.1. Sampling site description

The study area is located at the Jiangxi Institute of Red Soil, Jiangxi Province, China (116°20′24″ E, 28°15′30″ N). This region has a typical subtropical climate with distinct wet and dry seasons. The mean annual temperature ranges from 17.7 to 18.5 ℃, and mean annual precipitation of 1537 mm. The soil is a dryland red soil developed from Quaternary red clay, classified as Acrisols and Ferralsols according to the World Reference Base (IUSS Working Group WRB, 2014).

The land-use intensity gradient included seven land-use types. These were fallow land (None), tea plantation (Compost addition, 10,000 kg ha^{-1} $year^{-1}$, and synthetic fertilizer addition, N: 100 kg ha^{-1} $year^{-1}$, P_2O_5: 150 kg ha^{-1} $year^{-1}$, K_2O: 120 kg ha^{-1} $year^{-1}$), orange plantation (Compost addition, 12,000 kg ha^{-1} $year^{-1}$, and synthetic fertilizer addition, N: 150 kg ha^{-1} $year^{-1}$, P_2O_5: 200 kg ha^{-1} $year^{-1}$, K_2O: 150 kg ha^{-1} $year^{-1}$), camphora plantation (Compost addition, 5,000 kg ha^{-1} $year^{-1}$), synthetic fertilizer-amended cropland (Synthetic fertilizer addition, N: 255 kg ha^{-1} $year^{-1}$, P_2O_5: 375 kg ha^{-1} $year^{-1}$, K_2O: 300 kg ha^{-1} $year^{-1}$), compost-amended cropland (Compost addition, 15,000 kg ha^{-1} $year^{-1}$) and vermicompost-amended cropland (Vermicompost addition, 15,000 kg ha^{-1} $year^{-1}$). These land-use types were classified according to agricultural activities, vegetation types and literature descriptions (Foley et al., 2005). Contaminated zones were excluded to ensure representative sampling. Fallow land refers to abandoned cropland now dominated by shrubs and grasses, mainly of the Asteraceae, Poaceae, and Fabaceae families, with no trees or anthropogenic disturbances. Tea plantations consist of neatly trimmed bushes, approximately 1.5 m high with no herbaceous understory. Orange and camphora plantations are approximately 3 m and more than 10 m tall, respectively, with rich herbaceous layers. These are managed plantations that are harvested annually for their leaves, fruits and woody materials. The double-cropping system included local varieties of winter-rapeseed (*Brassica napus* L.) from November to May and summer-sweet potato (*Ipomoea batatas* L.) from June to October. All land-use types had been established for over five years.

2.2. Land-use intensity calculation

Land-use intensity (LUI) index was calculated from four components (Erb et al., 2013; Peters et al., 2019): nitrogen inputs, human disturbance, biomass removal, and variation of vegetation structure (Table S1). Nitrogen inputs were primarily calculated based on the total amount of nitrogen derived from synthetic fertilizer and organic amendments. A distinction between these two sources was implemented to address their distinctly different effects on soil properties and ecosystem functions. Specifically, the continuously applied synthetic fertilizers were well-documented to induce soil acidification and amplify nutrient leaching cascades, ultimately degrading soil health (Guo et al., 2010; Maaz et al., 2021). In contrast, organic amendments were recognized as an effective ecologically extensive practice for restoring soil health (Neuenkamp et al., 2024). Given these contrasting impacts between synthetic fertilizers and organic amendments, the nitrogen applied in organic amendments was assigned a negative weighting factor (−1) in the LUI calculation. The final nitrogen input was determined by summing the standardized values from both sources. Human disturbance was calculated by averaging standardized estimates of tillage, harvest, irrigation, spraying, and weeding frequency (Allan et al., 2014; Erb et al., 2013). Disturbance frequency was based on information provided by local landowners and land managers' records. Biomass removal was the percentage of the standing biomass removed per year, including the removal by picking, pruning, and harvesting (Peng et al., 2023; Peters et al., 2019). All estimates depended on repeat visits to each study site (more than 10 visits per site), and by cross-checking personal estimates with information provided by local landowners. Variation in vegetation structure was obtained as the coefficient of variation of normalized difference vegetation index (NDVI) estimated by Google Earth Engine using Sentinel-2 data with a spatial resolution of 10 m × 10 m. Specifically, variation in vegetation structure was deduced from the interannual mean (January 2021 to December 2022) and the coefficient of variation (CV) of NDVI. While the mean NDVI partially represents the net primary productivity of vegetation (Wu et al., 2023), values were similar across land-use types in this study. For example, the interannual mean NDVI was 0.37 in fallow lands, 0.41–0.45 in plantations, and 0.21 in croplands. The CV of NDVI is an accepted indicator of the heterogeneity of habitats associated with land-use changes (Oindo and Skidmore, 2002; Pettorelli et al., 2005), and it was adopted in this study to reflect the influence of land use on habitat heterogeneity. All four components were standardized before averaging them for the LUI index. Standardization was done difference in individual measures from the minimum of these measures, divided by the range of all measurements where $y_{i\text{-standardization}} = (y_i - \text{minimum}(y))/(\text{maximum}(y) - \text{minimum}(y))$. This generated LUI values between 0 and 1.

The LUI varied across land-uses, with the lowest LUI in fallow lands < plantations (moderate LUI) < cultivated croplands (highest LUI, Table 1). This LUI index is similar to land-use intensity metrics described in the literature (Gossner et al., 2016; Peters et al., 2019). Variation partitioning analysis and Venn diagrams (Borcard et al., 1992) were used to quantify the relative importance of different factors in regulating earthworm abundance, biomass, and diversity. These factors included soil habitat factors (environmental conditions, resource quantity, and resource quality), and the combination of LUI components (nitrogen inputs, human disturbance, biomass removal, variation of vegetation structure). The analyses were conducted with the *varpart* function in the R package *vegan* (Philip, 2003). As the original variance partitioning method allows only four explanatory matrices (groups of statistical predictors), we adopted a two-step approach to resolve this limitation. First, variation was partitioned into two explanatory tables (soil habitat factors and the combination of LUI component factors) to quantify the unique and shared contributions of these factors. Second, the shared variation attributed to the combination of LUI components was further partitioned into its four components (nitrogen inputs, human disturbance, biomass removal, and variation of vegetation structure), with their unique and shared fractions distinguished. Our results confirmed that composite LUI index explained more variance that any individual LUI component (Fig. S6).

Table 1
Land-use intensity (LUI), soil environment, resource quantity, and resource quality in subtropical ecosystems. LUI values were calculated from four components: nitrogen inputs, human disturbance, biomass removal, and variation of vegetation structure (Supplementary Table S1) observed in subtropical ecosystems.

	Land-use types						
	Fallow land	Camphora plantation	Tea plantation	Orange plantation	Vermicompost-amended cropland	Compost-amended cropland	synthetic fertilizer-amended cropland
LUI	0.00 ± 0.0000 e	0.00 ± 0.0005 e	0.16 ± 0.0007 d	0.22 ± 0.0009 c	0.67 ± 0.0198 b	0.69 ± 0.0198 b	1.00 ± 0.0000 a
Soil environment							
pH	4.2 ± 0.3 bc	4.2 ± 0.5 bc	3.9 ± 0.3 c	4.6 ± 0.5 ab	5.2 ± 0.9 a	5.5 ± 0.5 a	4.1 ± 0.1 bc
SWC (%)	20.0 ± 2.1 c	19.1 ± 2.3 c	27.7 ± 3.9 a	25.2 ± 3.1 ab	22.2 ± 2.9 bc	21.7 ± 2.3 bc	21.7 ± 2.0 bc
MWD (mm)	142 ± 8 c	173 ± 26 a	155 ± 16 abc	168 ± 19 ab	153 ± 7 abc	152 ± 11 bc	147 ± 11 bc
SC (%)	33.0 ± 5.0	25.0 ± 13.0	30.2 ± 6.1	27.8 ± 9.6	27.0 ± 4.2	29.2 ± 5.0	32.6 ± 5.7
Resource quantity							
SOC (g kg^{-1})	9.56 ± 0.63 bc	10.2 ± 0.67 abc	8.94 ± 0.81 c	9.88 ± 0.65 abc	11.19 ± 2.13 a	10.93 ± 1.09 ab	9.46 ± 0.44 c
DOC (mg kg^{-1})	5.02 ± 0.40 b	8.02 ± 1.55 a	5.71 ± 0.96 b	7.76 ± 1.42 a	5.45 ± 0.59 b	8.48 ± 1.73 a	5.25 ± 1.89 b
MBC (mg kg^{-1})	710 ± 74 ab	540 ± 97 c	628 ± 129 bc	521 ± 86 c	766 ± 70 a	801 ± 137 a	532 ± 79 c
Resource quality							
f_{poc} (%)	32.5 ± 4.5 c	32.8 ± 4.6 abc	32.8 ± 4.6 abc	32.7 ± 4.6 bc	33.6 ± 4.5 abc	39.8 ± 7.6 ab	39.9 ± 5.6 a
C: N	9.54 ± 0.76	9.49 ± 0.73	10.11 ± 0.74	9.55 ± 0.76	9.47 ± 0.38	10.13 ± 1.72	9.88 ± 1.39
BIX	0.94 ± 0.07 ab	0.69 ± 0.12 c	0.81 ± 0.16 bc	0.67 ± 0.10 c	0.93 ± 0.11 ab	0.81 ± 0.18 bc	1.02 ± 0.12 a
HIX	4.16 ± 0.23 b	2.6 ± 0.53 c	3.6 ± 0.73 b	2.53 ± 0.54 c	1.75 ± 1.31 c	5.8 ± 0.62 a	4.52 ± 0.44 b

Note: all values are means ± SD (n = 10). Means in rows followed by different letters denote a significant difference among land-use types ($p < 0.05$). SWC, soil water content; MWD, mean weight diameter of aggregates; SC, silt–clay fractions; SOC, soil organic carbon; DOC, dissolved organic carbon; MBC, microbial biomass carbon; f_{poc}, the ratio of particulate organic carbon to soil organic carbon; C: N, the ratio of soil organic carbon to total nitrogen; BIX, the biological index of dissolved organic matter; HIX, the humification index of dissolved organic matter.

2.3. *Earthworm sampling and identification*

Earthworm populations were sampled from April-May 2021 and April-May 2022, following Eggleton et al. (2009), due to the humid soil conditions and favorable temperature in the study area at this time of year. Five sites were selected as replicates within each land-use type, and these were > 100 m apart to reflect the spatial variation within the land-use area. At each site, three soil monoliths were extracted. A combination of electrical octet (Schmidt, 2001) and hand sorting methods (Stroud, 2019) was used to extract earthworms from each monolith, which covered an area of 1 m × 1 m and a depth of 20 cm, and were spaced 10——20 m. The collection process and methods followed the protocol described by Liu et al. (2022).

Earthworm species were identified from their morphological features and molecular biology methods. Collected earthworms were rinsed with clean water, patted dry, then grouped and counted according to their dermal color and pigmentation. Next, earthworms were anesthetized in a 10 % ethanol solution, straightened, and fixed. Each individual's weight (± 0.1 g) and body length (± 0.1 cm) were measured simultaneously. Earthworm samples were initially preserved in a 70 % ethanol solution with periodic changes until solution remained clear. Subsequently, they were transferred to a 70 % ethanol solution for long-term preservation.

Preserved earthworm samples were identified to species level using morphological methods based on Chen (1956), Sims & Easton (1972) and Blakemore (2008). Diagnostic taxonomic characteristics were analyzed through dissection, including prostomium shape, spermathecal pore (number and location), male pore (shape), clitellum position (shape and location), prostate gland (location and shape) and caeca (shape and size). Specimens that could not be identified from morphological features were sent for molecular identification (Sun et al., 2017). Specifically, DNA was extracted from tail muscle tissue using Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech). PCR amplification of mitochondrial COI genes was performed in a 20 μL reaction using TransTaq® High Fidelity PCR SuperMix I (TransGen Biotech) and specific primers under standard thermal cycling conditions. Sequences were conducted by Beijing Genomics Institute (Chongqing, China), and blasted through NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

After all earthworms were identified to the species level, they were assigned to three ecological categories (Table 2): anecic, endogeic, and epigeic, based on skin coloration, body length, skin pigmentation, and from literature descriptions (Bottinelli et al., 2020). The following earthworm community attributes were calculated: abundance (number of individuals per unit area, ind. m^{-2}); biomass (biomass per unit area, g m^{-2}); body length (cm); and species richness (number of species). Species richness was used to assess earthworm diversity.

Table 2
Summary of the family and ecological categories of earthworm species present in each land-use type (A, B, C, D, E, F, G) at each survey time (2021 and 2022) in subtropical ecosystems. Land-use types were: A = fallow land; B = camphora plantation; C = tea plantation; D = orange plantation; E = vermicompost-amended cropland; F = compost-amended cropland; G = synthetic fertilizer-amended cropland. Occurrence of earthworm species was scored 0 if no individuals of the species were present, 1 if it was present in 2021, 2 if it was present in 2022, and 3 if it was present in both years.

Species	**Family**	**Ecological categories**	**Occurrence**						
			A	**B**	**C**	**D**	**E**	**F**	**G**
Metaphire californica	*Megascolecidae*	Endogeic	0	3	0	3	3	3	3
Drawida japonica	*Moniligastridae*	Endogeic	3	3	0	3	3	3	3
Eisenia fetida	*Lumbricidae*	Epigeic	3	3	3	3	3	3	3
Metaphire guillelmi	*Megascolecidae*	Anecic	0	3	0	3	3	3	3

2.4. Soil sampling and analysis

During earthworm sampling, five in-situ soil samples (0–20 cm) from each sampling site were collected, and mixed as a composite sample. A separate intact soil core (0–20 cm) was taken to determine aggregate properties. Composite soil samples were sieved ($<$ 2 mm) to remove stones, plant debris and roots. The samples were then divided into two subsamples, one subsample was air-dried for analyzing soil physicochemical properties and soil organic carbon (SOC) composition, and the second subsample was stored at 4 ℃ for analyzing soil available nutrients and microbial biomass.

Intact soil was coarsely sieved ($<$ 2 mm) and separated into three aggregate-size classes through a nest of two sieves (0.25 and 0.053 mm): Macroaggregates (MA) $>$ 0.25 mm, Microaggregates (MI) 0.053–0.25 mm and Silt-clay fractions (SC) $<$ 0.053 mm using an electric vibrating screen. The mean weight diameter (MWD) was calculated based on the proportion of aggregates in each size fraction. Soil water content (SWC, %) was measured on 10 g fresh soil that was dried at 105 ℃ for 48 h. Soil pH (H_2O) was measured using a pH meter in soil slurries with a soil: deionized water ratio of 1:2.5 (*w/v*). Extractable ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) contents were extracted with 2 M KCl using a soil: solution ratio at 1:5 (*w/v*) and the extracts were quantified on a continuous flow analyzer (Auto-Analyzer AA3, Germany). Mineral nitrogen (Min-N) was calculated as the sum of NH_4^+-N and NO_3^--N. Dissolved organic matter (DOM) was extracted with ultrapure water (soil: solution = 1:5 (*w/v*)) and filtered (Whatman No. 45) before analysis on a total C analyzer (Elementar, Germany) for dissolved organic carbon (DOC). The absorption and fluorescent spectra of DOM were measured using the fluorescence spectrometer (Cary eclipse fluorescence spectrophotometer, Agilent Technologies). The fluorescence index (FI; McKnight et al., 2001), humification index (HIX; Zsolnay, 2003), and biological index (BIX; Huguet et al., 2009) were calculated using excitation-emission fluorescence matrix spectroscopy as follows:

$$FI = \frac{F_{E_X370,E_M450}}{F_{E_X370,E_M500}} \tag{1}$$

$$HIX = \frac{\sum F_{E_X254,E_M435-E_X254,E_M480}}{\sum F_{E_X254,E_M300-E_X254,E_M345}} \tag{2}$$

$$BIX = \frac{F_{E_X310,E_M380}}{F_{E_X310,E_M430}} \tag{3}$$

SOC was fractionated into particulate organic carbon (POC) and mineral-associated organic carbon (MAOC; Lavallee et al., 2020). The ratio of POC to SOC, represented as f_{poc}, was an indicator of the resource quality of organic substrates for earthworms. A subsample of the air-dried soil (10 g) was shaken for 18 h with 30 mL of sodium hexametaphosphate solution, and then thoroughly rinsed through a 53 μm sieve, to separate the POC fraction ($>$ 53 μm) and the MAOC fraction ($<$ 53 μm), POC fraction was collected, dried to constant mass, and passed through a 0.149 mm sieve to measure its carbon content. The MAOC concentration was the difference between SOC and POC concentrations. Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the fumigation-extraction method (Vance et al., 1987). Specifically, a 10 g sample of fresh soil was extracted with 50 mL 0.5 M K_2SO_4 after fumigation for 24 h with or without $CHCl_3$, filtered through Whatman No. 45 filter paper and analyzed with a total C analyzer (Elementar, Germany). MBC and MBN were then calculated based on the difference between fumigated and non-fumigated samples using conversion factors ($K_C = 0.45$, $K_N = 0.54$; Brookes et al., 1985; Wu et al., 1990).

2.5. Statistical analysis

Soil habitat consists of the soil environment, resource quantity, and resource quality (Edwards and Arancon, 2022; Tumolo et al., 2023). We assumed the soil environment was represented by the SWC, pH, SC, and MWD of aggregates (Rantalainen et al., 2004). Resource quantity was represented by the DOC, MBC, and SOC concentrations (Cotrufo and Lavallee, 2022; Edwards and Arancon, 2022). Resource quality was indicated by the soil total nitrogen, the SOC to TN ratio, the f_{poc}, and the FI, BIX, and HIX of DOM (Table 1; Tumolo et al., 2023).

The effects of time (sampling year), land-use type, and their interactions on earthworm abundance, biomass, and diversity were tested with linear mixed-effects models using the *lmer* function from the *lme4* package in R (Bates et al., 2014), with block as a random effect (y ∼ time × land-use type + (1 | block)). Significant differences among land-use types were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's HSD tests for post-hoc mean comparisons. The correlations between soil parameters (including environment condition, resource quantity, and resource quality) and earthworm abundance, biomass, and diversity were tested using Mantel correlations.

To evaluate the relative importance of soil environment, resource quantity, and resource quality in influencing earthworm abundance and diversity, we used a model selection procedure based on corrected Akaike's information criterion (AICc; ΔAICc $<$ 2) to identify the best predictors. We used the *dredge* function in the R package *MuMIn* (Bartoń, 2023) to generate a set of models comprising all possible combinations of the initial predictors. These models were subsequently ranked based on the Akaike information criterion (AIC) fitted with maximum likelihood in R. We selected all models with ΔAIC $<$ 2 and applied the model averaging approach to estimate parameters and associated *p*-values using the function *model.avg*. Prior to analysis, all predictor variables were transformed into z-scores, and variance inflation factors were calculated to assess the multicollinearity of predictor variables using the R package *Hmisc* (Harrell, 2014).

Additionally, we fitted structural equation models (SEMs) using piecewise structural equation modeling (piecewiseSEM, Lefcheck, 2016) to evaluate the associations between land-use intensity (LUI) and the abundance, biomass, and diversity of earthworms in our study. These models accounted for soil environment (SWC, pH, SC, and MWD), resource quantity (DOC, MBC and SOC), and resource quality (soil C: N, BIX, HIX, and f_{poc}). This SEM method allows for testing the overall fit of a multifaceted hierarchical network, estimating indirect effects, and considering random effects. The SEM analyses were based on an *a-priori* conceptual model that contained all hierarchical pathways posited in Fig. 1. For that, we divided all measured variables included in this model into 'composite variables', simplified by progressively filtering the measured variables. These analyses were conducted using *piecewiseSEM*, and *lme4* packages (Bates et al., 2014; Lefcheck, 2016). We assessed the overall model fit using direct separation tests based on Fisher's *C* statistic, with the model being accepted where the associated $p > 0.05$. The total effect of a predictor is the sum of the standardized path coefficient of the direct effect and the sum of the indirect effects. Statistical analyses were done with R 4.3.3 software (R Development Core Team, 2022).

3. Results

3.1. Earthworm abundance, biomass and diversity

A total of 2805 individual earthworms were collected, representing 3 families, 4 species, and classified as anecic, endogeic, and epigeic (Table 2). All three earthworm ecological categories were present in croplands, orange and camphora plantations (Table 2). Endogeic and epigeic earthworms were in fallow land, while only the epigeic earthworms were found in the tea plantation (Table 2).

The abundance, biomass, and diversity of earthworms were significantly influenced by land-use types (Table S2). Earthworm abundance and biomass decreased significantly from cropland (13–107 ind. m^{-2}, 4.3–45.5 g m^{-2}) to fallow land (4 ind. m^{-2}, 1.7 g m^{-2}) and plantation (3–35 ind. m^{-2}, 0.6–14.3 g m^{-2}, $p < 0.05$; Fig. 2a and S2a). Specifically, earthworm abundance was the highest in croplands with organic

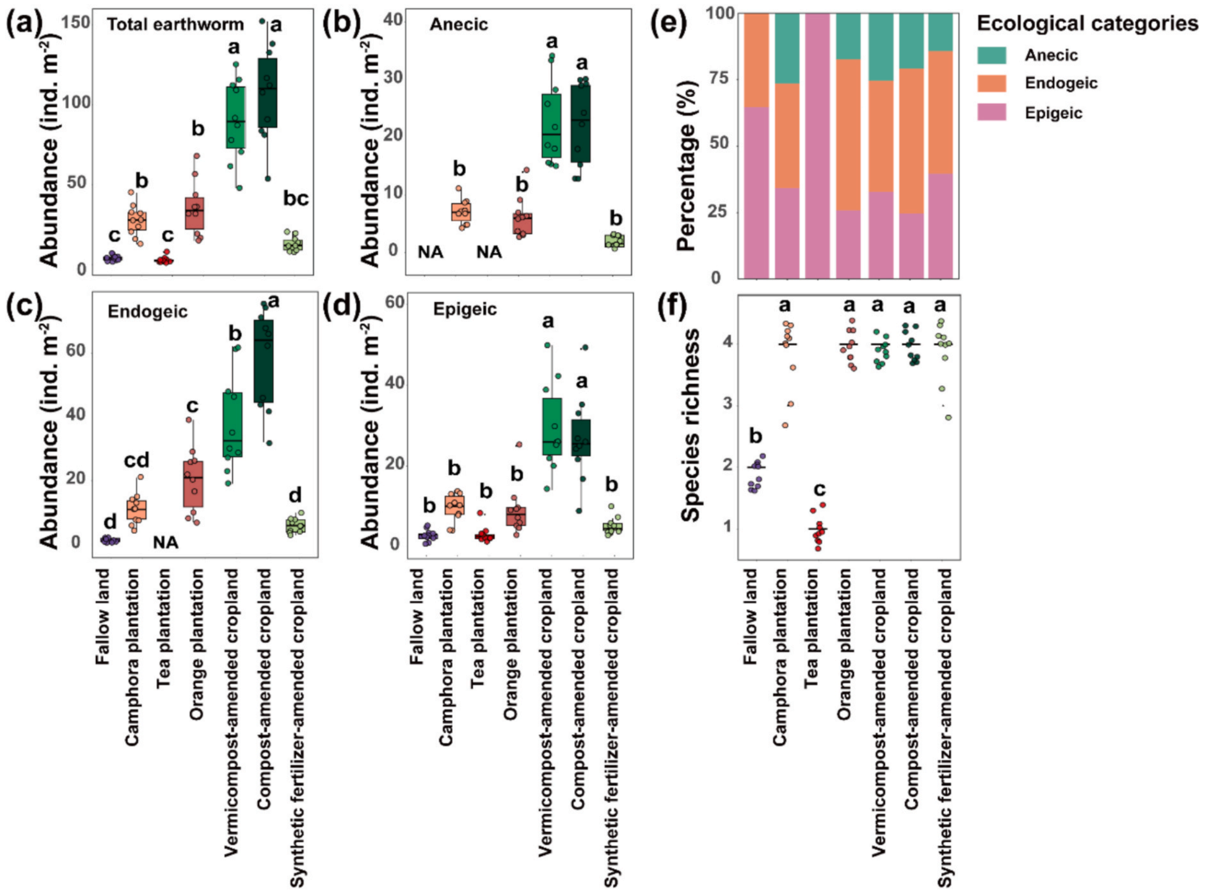

Fig. 2. Effects of land-use types on the abundance of (a) total, (b) anecic, (c) endogeic, and (d) epigeic earthworms, as well as (e) the percentage of ecological categories and (f) earthworm diversity in subtropical ecosystems. Boxplots show the median (horizontal line), first and third quartile (rectangle). Lowercase letters represent significant differences between land-use changes by Post hoc Tukey's HSD tests at $p < 0.05$.

amendments, followed by orange and camphora plantations, and the lowest in synthetic fertilizer-amended cropland, fallow land, and tea plantation (Fig. 2a). The same trend was observed for earthworm biomass (Fig. S2a). Overall, earthworm abundance in croplands was 2 times and 16-fold higher than that in plantations and fallow land, respectively. Organic amendments, i.e., compost and vermicompost, increased earthworm abundance by 6 times and 8 times, respectively, compared with synthetic fertilizers in cropland (Fig. 2a). Anecic and epigeic earthworms were most abundant in the croplands with organic amendments, and were significantly different from the synthetic fertilizer-amended croplands (Fig. 2b and 2d). Endogeic earthworms were more abundant in compost-amended cropland, followed by the vermicompost-amended cropland and plantations, and were lowest in the fallow land and synthetic fertilizer-amended cropland (Fig. 2c). Most of the earthworms were endogeic (about 39 %) and epigeic (about 46 %; Fig. 2e). Earthworm diversity was highest in croplands, orange and camphora plantations, followed by fallow land, and lowest in tea plantation (Fig. 2f).

3.2. *Linking environmental factors to earthworm abundance, biomass and diversity*

Earthworm abundance and biomass correlated significantly with variables describing the soil habitat, including soil pH, SOC, soil C: N ratio, POC, and HIX of DOM (Fig. 3 and S3). Earthworm abundance was positively associated with organic amendment input and land-use intensity, but negatively with NDVI (Fig. S4). Earthworm diversity was significantly correlated with SWC and soil total nitrogen (Fig. 3). The model incorporating soil environment, resource quantity and quality explained 73 % and 52 % (adjusted R^2) of the total variation in earthworm abundance and diversity, respectively (Fig. 4). Earthworm abundance was best predicted by soil pH, DOC, f_{poc}, and HIX of DOM (Fig. 4a). Soil environment was responsible for 36 %, while resource quantity and quality were responsible for 27 % and 37 % of explained variance in earthworm abundance ($R^2 = 0.731$). Earthworm diversity was more strongly related to the soil environment, with 61 % of variability attributed to the SWC and soil pH, and the remaining variation due to resource quantity (27 %) and resource quality (12 %, Fig. 4b).

Total earthworm abundance and the proportions in ecological categories responded to ecosystem factors like land-use intensity, soil environment, and resource quantity and quality, and these factors explained 67–79 % of the variation by SEM analysis (Fig. 5a and S5). Land-use intensity directly influenced the earthworm abundance, with indirect effects through soil environment, resource quality and resource quantity. Earthworm abundance was directly and indirectly affected by soil environment, which influenced the quality and quantity of their food resources. The same pattern was observed for earthworm biomass (Fig. 5b). Land-use intensity and soil environment directly influenced earthworm diversity, rather than through changes in resource quantity or quality (Fig. 5c). The standardized path coefficients of these variables suggest that soil environment and resource quality were the first and second most important factors associated with earthworm abundance, biomass and diversity, more so than resource quantity or land-use intensity (Fig. 5).

4. Discussion

This study provides a systematic, two-year investigation of land-use

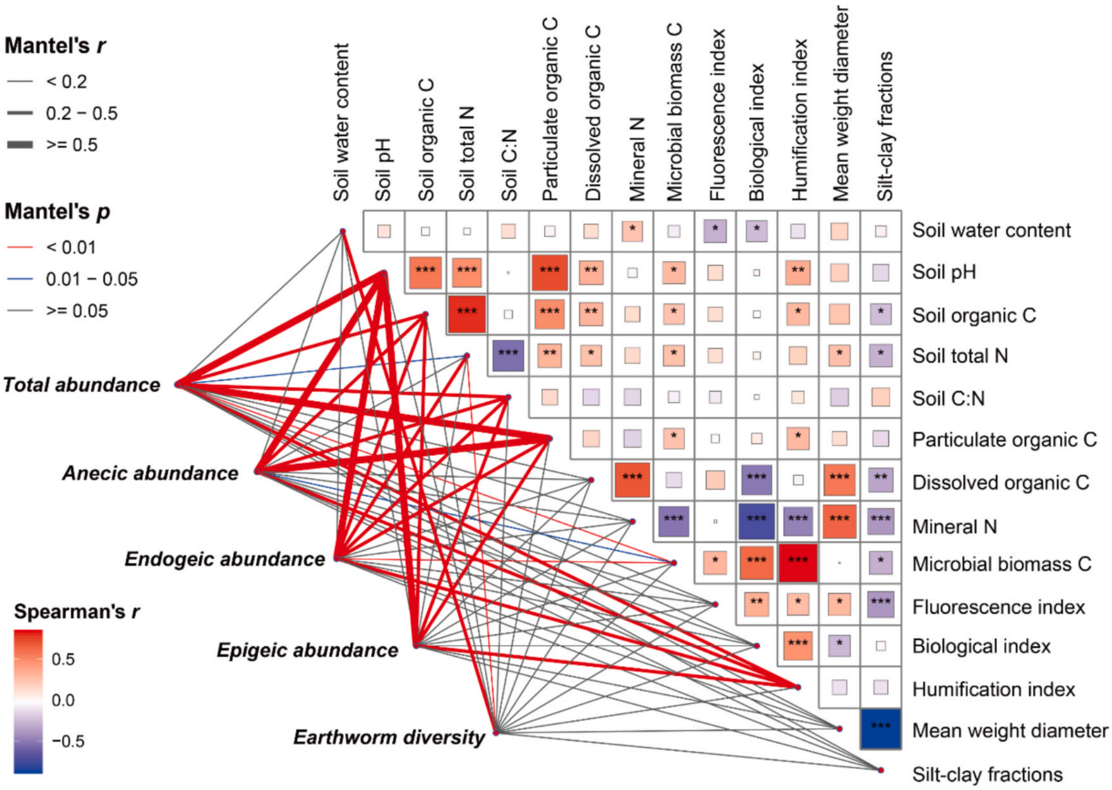

Fig. 3. Environmental drivers of earthworm abundance and diversity in subtropical ecosystems. Pairwise comparisons of environmental factors are shown, with a color gradient denoting the Spearman's correlation coefficient. Earthworm abundance and diversity were related to each environmental factor by Mantel tests. Edge width corresponds to Mantel's *r* statistic for the corresponding distance correlations, and edge color denotes the statistical significance based on 9999 permutations.

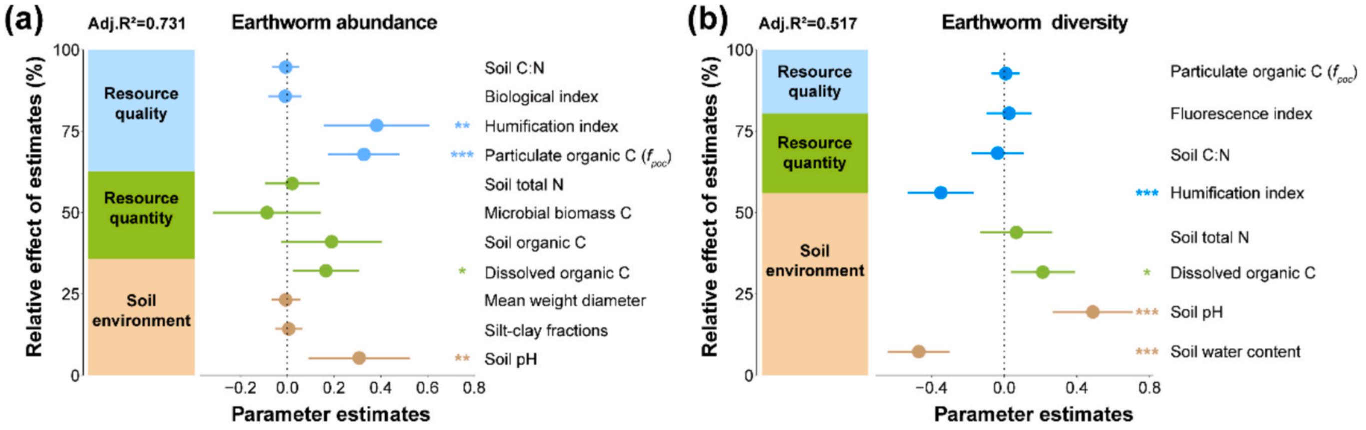

Fig. 4. Relative effects of multiple predictors on earthworm (a) abundance and (b) diversity in subtropical ecosystems. The averaged parameter estimates (standardized regression coefficients) of the model predictors are shown with their associated 95 % confidence intervals along with the relative importance of each predictor, expressed as the percentage of explained variance for earthworm abundance and diversity. The best model is selected based on the AICc. The relative effect of the predictors is calculated as the ratio between the parameter estimate of the predictor and the sum of all parameter estimates, and it is expressed as a percentage. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

intensity on earthworm communities in a subtropical region. Our findings revealed that croplands with organic amendments, as well as orange and camphora plantations, supported greater abundance and biomass of earthworms than fallow lands. The response of earthworm abundance and diversity varied across ecological categories, with anecic and endogeic earthworms potentially having narrower habitat requirements or greater sensitivity to environmental conditions than epigeic earthworms. Furthermore, we found that the soil environment conditions and resource quality were key drivers shaping earthworm communities. These results suggest that implementing management practices that enhance soil quality could alleviate the negative impacts of land-use intensification while promoting earthworm abundance and diversity.

Contrary to our first hypothesis, higher earthworm abundance and diversity were observed in more intensively managed lands, likely due to the addition of organic amendments rather than land-use intensity itself. Applying organic amendments and maintaining vegetative cover improves the soil environment conditions and provides food resources (Yahyaabadi et al., 2018), thereby stimulating earthworm growth and reproduction. Another possibility was that cultivation (i.e., tillage)

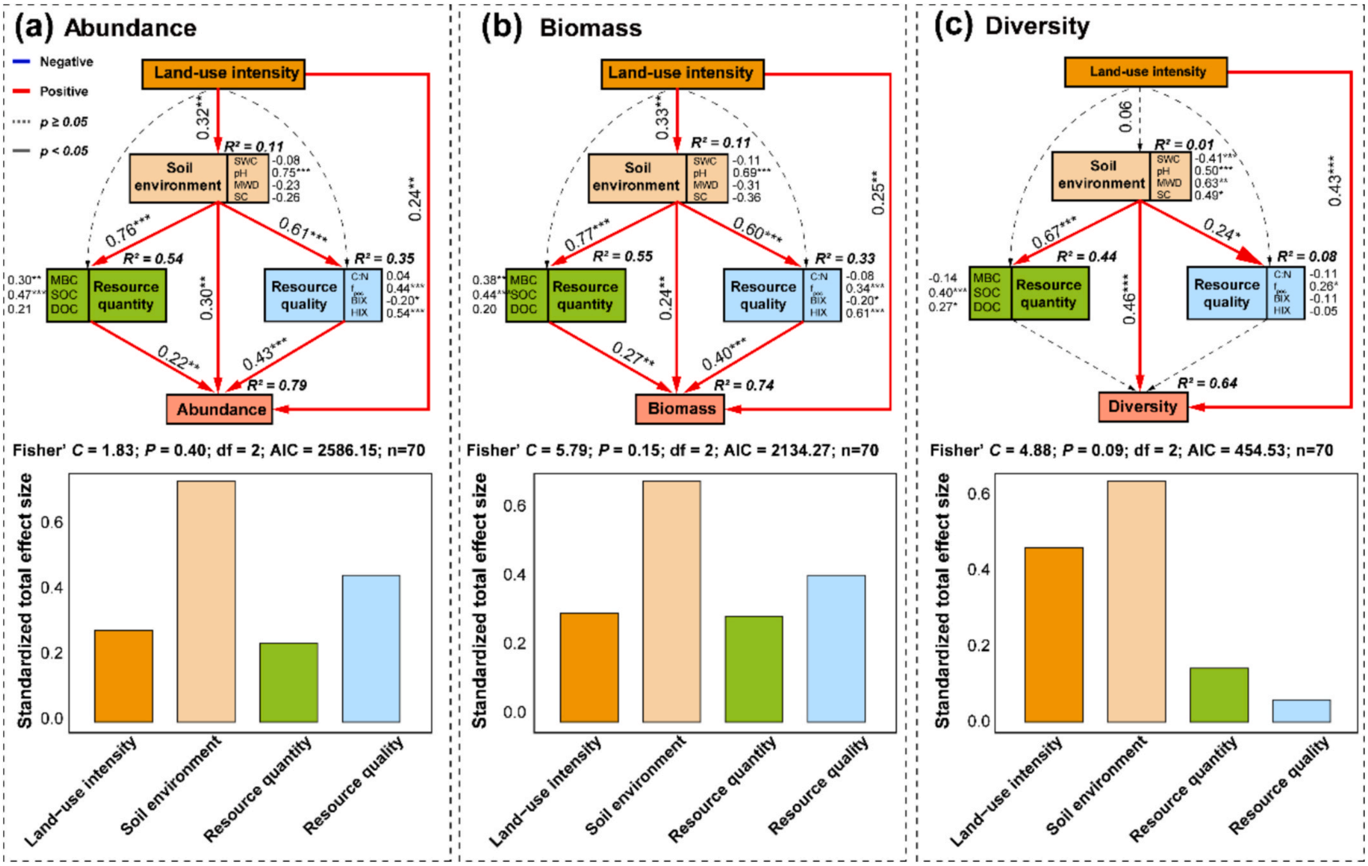

Fig. 5. Direct and indirect drivers of earthworm (a) abundance, (b) biomass, and (c) diversity. piecewiseSEM accounting for the direct and indirect effects of land-use intensity, soil environment, resource quantity and quality on the abundance, biomass, and diversity of earthworms in subtropical ecosystems. Standardized total effect size calculated by summing the direct and indirect effects derived from the model. Numbers adjacent to arrows are path coefficients which represent the directly standardized effect size of the relationship. R^2 represent the proportion of variance explained by all predictors. Significance levels of each predictor are $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. *df*, degree of freedom; AIC, Akaike information criterion; n = 70 independent soil samples; SWC, soil water content; MWD, mean weight diameter of aggregates; SC, silt–clay fractions; MBC, microbial biomass carbon; SOC, soil organic carbon; DOC, dissolved organic carbon; C: N, the ratio of soil organic carbon to total nitrogen; BIX, biological index of dissolved organic matter; HIX, the humification index of dissolved organic matter; f_{poc}, the ratio of particulate organic carbon to soil organic carbon.

aerated the soil and incorporated more food resources into deeper layers, making it more accessible to anecic and endogeic earthworms (Kladivko, 2001). Although previous studies have reported negative effects of land-use intensity on earthworms (Briones and Schmidt, 2017; Smith et al., 2008), these effects are likely site-specific, related to an indirect fragmentation of earthworm habitats that depends on soil types. Our findings suggest that earthworm activities are constrained in the soil habitat of fallow lands in this subtropical region. Agricultural management in plantations and croplands modified the structure of red soil, introduced a large amount of palatable organic material as a food resource, and established a favorable habitat for anecic and endogeic earthworms. Specifically, our study revealed that earthworm abundance in subtropical croplands with organic amendments could reach up to 90–107 ind. m^{-2}, aligning with global reports (Phillips et al., 2019). Generally, organic amendments support more earthworms than synthetic fertilizers do because of improving food resources and also the soil environment such as soil aggregation, pH buffering and other physicochemical properties (Lapied et al., 2009; Whalen et al., 1998; Yahyaabadi et al., 2018). As an effective strategy for ecological restoration in agricultural field, the application of organic amendments enhances soil organic matter, which improves soil conditions and provides a baseline level of organic substrates for feeding earthworms (Edwards and Arancon, 2022; Larney and Angers, 2012; Neuenkamp et al., 2024). However, the benefits of organic amendments for earthworms depend upon the organic material and land-use types. For example, croplands amended with compost supported more endogeic earthworms than those amended with vermicompost, likely because compost is more palatable to endogeic earthworms (Blanchet et al., 2016), which primarily feed on organic matter. Additionally, only epigeic earthworms were present in tea plantations, with fewer individuals and less species richness than in croplands and other plantations. Perhaps only epigeic earthworms can tolerate the tea plantation habitat, which has acidic pH, low soil organic matter, and a sparse litter layer containing polyphenol-rich litter from tea trees (Arafat et al., 2020). This remains to be confirmed, for example, by testing the feeding rates of epigeic, endogeic and anecic earthworms on tea leaf litter.

Consistent to our second hypothesis, we observed that an uneven distribution of earthworm ecological categories across land-use types. Specifically, epigeic earthworms were present across all land-use types, whereas anecic and endogeic earthworms were absent in fallow lands or tea plantations. This indicates that anecic and endogeic earthworms may have narrower habitat requirements or be more sensitive to specific environmental conditions than epigeic earthworms. We propose that tolerance of acidic soils explains the widespread distribution of epigeic earthworms in this subtropical region (Ma et al., 1990), while anecic and endogeic earthworms are less tolerant to acid soils (Duddigan et al., 2021). Variation in epigeic abundance across land-use types was attributed to the soil pH, but it was also related to differences in organic matter content, thickness of the surface layer, compaction, and vegetation diversity (Buchkowski et al., 2024; Gudeta et al., 2022). Another possibility is that their shorter generation times, compared to anecics and endogeics, may enhance their ability to recover in disturbed environments (Edwards and Arancon, 2022). Furthermore, epigeic earthworms, such as *Eisenia fetida* could be inoculated into soils through vermicompost applications, as they are actively propagated during the vermicomposting process (Ali et al., 2015). This likely contributes to shifts in earthworm community composition and structure by increasing the dominance of epigeic species in amended soils. Earthworm species that reside in upper mineral soil layers (endogeics) or build vertical burrows (anecics) were most abundant under moderate land-use intensity, such as in croplands or plantations with organic amendments. This suggests that anecics and endogeics are more sensitive to intensive land-use than epigeic earthworms, and rely on beneficial agricultural activities, such as organic inputs. This is supported by the increase in endogeic species under deep ploughing, particularly when there is a larger food supply (Chan, 2001). Anecic species construct burrows, permanent or semipermanent structures extending up to 1 m or more that can be inherited by their offspring (Grigoropoulou et al., 2008), which may help anecics to tolerate tillage in the plough layer (generally $<$ 20 cm deep; Briones and Schmidt, 2017). Although tillage may be damaging to earthworms (Briones and Schmidt, 2017), it may reduce soil mechanical resistance and improve the soil environment conditions for earthworms in subtropical regions (i.e., in acidic soils with low pH and compact structure; Arrázola-Vásquez et al., 2022). Furthermore, organic residues mixed into topsoil may compensate for tillage-induced destruction of biogenic structures (pores and tunnels) because they regenerate the soil structure and increases the food resources (Lapied et al., 2009). The observed relationships between earthworm abundance and soil physicochemical variables under varying land-use intensity could guide management practices that aimed at increasing soil pH and enhancing the availability of food resources to support soil biodiversity.

Our results indicate that the soil habitat improved with increasing land-use intensity in the current subtropical region, in contrast to other regions (Ahmed et al., 2022; Carnovale et al., 2015). This improvement was attributed to the organic amendments spreading in intensive land-use practices, as confirmed by the improvement of soil organic matter level and soil structure in croplands and plantations. Moreover, the positive correlation between earthworm abundance and the amount of applied organic amendments suggests that increased inputs enhance earthworm proliferation under intensive management. Specifically, the soil environment, rather than the quantity or quality of food resources, was the main factor influencing earthworm abundance and diversity in this subtropical region. Soil pH was influential, and it followed the same trend as land-use intensity, increasing gradually from fallow land to plantations and croplands. Notably, the application of organic amendments in croplands significantly enhanced soil pH, potentially alleviating the stress experienced by earthworms in acidic soil conditions. Additionally, we observed that earthworm abundance was negatively correlated with the normalized difference vegetation index. Although this index partially reflects the primary productivity of vegetation (Wu et al., 2023), it may also indicate the litter layer thickness in plantations. Given the negative relationship between litter layer thickness and earthworms (Buchkowski et al., 2024), it would be informative to directly measure the litter chemical composition and litter layer thickness to explain the responses of earthworm ecological categories to land-use intensity in future studies.

Our study provides empirical evidence that organic amendments can alleviate the negative effects of land-use intensity on earthworm abundance and diversity in a subtropical region. Still the gain in earthworm abundance with increasing land-use intensity depended on ecological categories and agricultural practices. Overall, the soil environment, particularly soil pH, influenced the earthworm community response, and may be more important than the quality and quantity of food resources in subtropical regions. This should be validated in the future by investigating the functional responses of earthworms in other locations with broader environmental gradients. Such work will confirm the optimal soil habitat for earthworm ecological categories, based on soil conditions, food resources, and biotic stresses, including viruses, predators, and competitors.

CRediT authorship contribution statement

Xishuai Liu: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Bingbing Wan:** Writing – review & editing. **Dingyi Wang:** Writing – review & editing. **Xiaoxu Qi:** Writing – review & editing. **Yan Du:** Writing – review & editing. **Jun Jiang:** Writing – review & editing. **Xiaoyun Chen:** Writing – review & editing. **Feng Hu:** Writing – review & editing. **Manqiang Liu:** Writing – review & editing, Project administration. **Joann K. Whalen:** 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2025.117270.

Data availability

Data will be made available on request.

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