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The Role of Earthworms in Mediating Environmental Impacts on Mountain Ecosystem Functioning

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ABSTRACT

Aim: Soils host a quarter of all terrestrial species, and the pivotal role played by earthworms in soil ecosystem functioning is thought to be paramount. Here, we aimed to quantify the causal influence of changing environments, especially climate, soil conditions, and vegetation type and structure, on earthworm diversity and the subsequent impacts on ecosystem functions.

Location: French Alps.

Time Period: 2016–2021.

Major Taxa Studied: Earthworms and plants.

Methods: Along 17 elevational gradients, we sampled climate, soil conditions, plant relevés, ecosystem functions, and earthworm diversity from soil environmental DNA. Through a causal inference framework and structural equation modelling, we quantified how climate conditions structure soil conditions and vegetation structure along the gradients, how these three compartments shape earthworm diversity, and how, in turn, earthworm diversity modulates ecosystem functions in addition to direct environmental impacts.

Results: Vegetation was the most important driver of earthworm diversity, with acquisitive plant strategies contributing to an increase in earthworm diversity, followed by soil organic matter. While climate was important, its impact on earthworm diversity was only indirect, through cascading effects mediated by vegetation and soil. In addition to the abiotic drivers, earthworm diversity had an important effect on three out of the four studied ecosystem functions (i.e., carbon stock, plant primary productivity and carbon and nitrogen limitation of microbes). In closed habitats, earthworm diversity was positively linked to proxies of carbon stock and carbon availability, while in grasslands, it was positively linked to proxies of nitrogen availability. Interestingly, aboveground productivity was found to be independent of earthworm diversity.

Main Conclusions: Our study underscores the central role of earthworm diversity in linking environmental drivers to ecosystem functioning. We emphasise the importance of incorporating earthworm diversity in models aiming to elucidate the cascading effects of climate change across various ecosystem compartments, ultimately shaping ecosystem functioning.

1 | Introduction

Soils encompass a substantial part of the Earth's diversity (Johnson et al. 2016; Anthony et al. 2023), and play an essential role in nature's contribution to people (Guerra et al. 2021). The sustainability of soil health, a crucial aspect of ecosystem functioning, faces significant threats from the rapidly accelerating changes in climate and land use. Earthworms are recognised as paramount ecosystem engineers and guardians of soil health and function (Brown et al. 2003; Edwards and Arancon 2022), which highlights the critical importance of understanding how their diversity and distribution are affected by environmental changes, as these changes could have far-reaching consequences for overall ecosystem functioning (Rillig et al. 2019).

Yet, we do not have a clear picture of the drivers of earthworm diversity, nor do we understand how earthworm diversity affects ecosystem functioning, mostly due to the scarcity of consistent data at large spatial scales. In addition, research conducted at both regional (Rutgers et al. 2016) and global scales (Phillips et al. 2019) indicates that it is not appropriate to directly infer the response of earthworm diversity to environmental changes based solely on aboveground drivers. Recent advances in environmental DNA metabarcoding techniques now offer an opportunity to study earthworm communities over biogeographic scales (Pansu et al. 2015; Lilja et al. 2023), particularly when coupled with representative sampling across diverse habitats and steep gradients in climate, soil, and vegetation typical of mountain regions, allowing us to simultaneously study both aboveground and belowground drivers of earthworm diversity.

Earthworm diversity and distribution are influenced by a complex interplay between different environmental compartments including climate, soil, vegetation, habitat, and land use (Blouin et al. 2013; De Wandeler et al. 2016; Phillips et al. 2019). Divergent findings in studies attempting to identify the most important drivers of earthworm diversity may stem from variations in the inclusion of predictors and differences in spatial scales. Large-scale studies often highlight climate as a primary driver (Phillips et al. 2019; Li et al. 2023), while others that focus on specific habitats, such as forests (De Wandeler et al. 2016), or on a smaller spatial extent (Hoeffner et al. 2021) emphasise the importance of physico-chemical soil variables and vegetation. Studies at even smaller scales underline the role of environmental heterogeneity, for example in ecotones along a single elevation gradient (Gabriac et al. 2022).

Despite significant species-specific variability, earthworms overall tend to benefit from increasing temperature under optimal soil water content conditions. Conversely, climate extremes like drought and frost tend to have negative impacts on earthworm populations (Singh et al. 2019). It is crucial to note that changes in temperature and precipitation also influence vegetation and soil structure and functioning (Pugnaire et al. 2019), playing a role in either amplifying or buffering direct climatic impacts (Mariotte et al. 2016, see Figure 1.1).

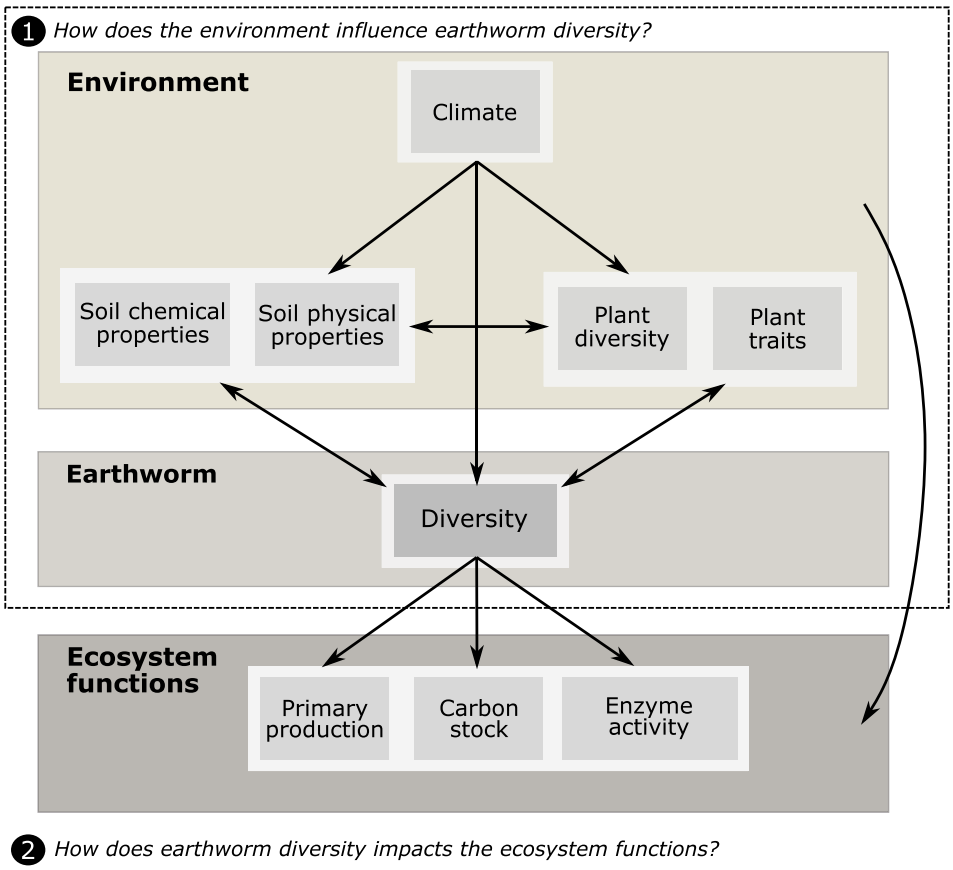

FIGURE 1 | Conceptual causal model depicting how earthworm diversity might mediate environmental impacts on ecosystem processes, tested through two subsequent questions.

For example, drought conditions directly reduce earthworm diversity and activity (Curry 2004; Singh et al. 2019) and indirectly affect them, in not always easy to anticipate directions, by altering soil properties (Tiunov et al. 2006; Nieminen et al. 2011). Therefore, considering the interactive effects between drivers (e.g., climate and vegetation) and the resulting cascading effects that these interactions may cause on earthworm diversity is crucial to predict the impact of climate change on earthworm diversity (Figure 1).

Soil conditions are also particularly important for earthworm diversity and can affect them directly or indirectly through the vegetation (Figure 1). Earthworms are thin-skinned invertebrates, which are highly exposed to soil physicochemical changes. The spatiotemporal distribution of earthworms is directly influenced by soil physical properties, where bulk density (Rossi 2003) and soil porosity (Bottinelli et al. 2010) positively contribute to earthworm diversity. Soil chemical properties, such as extreme pH, tend to negatively impact earthworm diversity (Lapied et al. 2009).

Previous studies underscore the significance of both climate and soil conditions in determining earthworm distribution and diversity (Figure 1). The interaction between these two broad drivers is likely to be particularly robust in mountain regions where climate and soil conditions exhibit considerable variability, exerting major influences on vegetation stratification, including forests, lands and grasslands. Plant diversity (Wardle et al. 1999; Zaller and Arnone 1999; Eisenhauer et al. 2009; Piotrowska et al. 2013) and specific plant functional traits, such as high leaf nitrogen content (Milcu et al. 2008), tend to be positively related to earthworm diversity. Yet, despite their importance, the relationship between vegetation diversity, functional structure, and earthworm diversity has not been much investigated so far. Ultimately, at biogeographical scales, we are missing studies that consider both direct and indirect effects of the different driver compartments on earthworm diversity.

Understanding the drivers of earthworm diversity is of importance given their role in ecosystem functioning (Figure 1.2; Bouché and Al-Addan 1997; Edwards 2004). Blouin et al. (2013) emphasised the key role of earthworms in water regulation, nutrient cycling and climate regulation. More particularly, earthworms can act as mediators between soil and climate and play an important role in soil carbon stocks (Martin 1991; Zhang et al. 2013), mineralisation processes, (Lavelle and Martin 1992) and soil decomposition activities (Scheu 2003; Ernst et al. 2009; Lubbers et al. 2017). Moreover, earthworms positively influence nitrogen mineralisation from organic matter (Van Vliet et al. 2007) partly by promoting bacterial activity (i.e., sleeping beauty paradox, Brown et al. 2000) and thus, improve primary productivity (Scheu 2003; van Groenigen et al. 2014) by forming readily available nitrogen resources for plants (Christensen 1988). They also lead to changes in soil structure and oxygenation (Sharma et al. 2017). However, so far, few studies have investigated the role of earthworm diversity in multiple ecosystem functions at large scales (Liu et al. 2019).

Although it is undisputed that earthworms play a central role in ecosystem functioning and that their diversity and distribution are affected by environmental change, there is a lack of studies that pinpoint how earthworms can act as a hub between environmental change and ecosystem functioning at macroecological scales. Here, we suggest piecing together different driver compartments on the one hand and multiple ecosystem functions on the other hand in a comprehensive study on earthworm diversity using environmental DNA metabarcoding along steep elevational gradients in the French Alps. To disentangle the drivers of earthworm diversity and the consequences on ecosystem functions, we need to integrate them in a conceptual causal framework that represents expected causal relationships between drivers and how they affect each other. Here, we built such a model through structural equation modelling (Figure 1) and tested it independently in grasslands and closed habitats (i.e., forests and heath). Specifically, we asked

1. How does climate influence earthworm diversity, and are these effects direct or mediated by soil and vegetation in different habitats? (Figure 1.1)
2. How important is earthworm diversity for key ecosystem functions compared to direct effects of climate, soil, and vegetation in different habitats? (Figure 1.2)

2 | Methods

2.1 | Study Site

We studied the effects of vegetation and abiotic soil variables on earthworm diversity and the subsequent cascading effects on ecosystem functioning along several elevational gradients in the French Alps within the Orchamp long-term monitoring programme (www.orchamp.osug.fr; Appendix S2), which encompasses high climatic, biological, pedological and geological variability (Thuiller et al. 2024). Each elevational gradient consists of four to nine plots with an average elevation difference between plots of 200 m. The plots within a gradient shared consistent exposure and slope, each measuring 30 m × 30 m. For the study, we used data collected between 2016 and 2021, which encompassed 17 elevational gradients with 45 grassland plots and 32 plots in closed habitats (i.e., a total of 77 plots). Among the total number of plots, 67 have been sampled twice across the whole time period, while the 10 others were sampled once. For each plot, we retrieved climatic data from the local meteorological model, measured abiotic soil properties, plant taxonomic and trait diversity, estimated earthworm diversity based on eDNA and measured ecosystem functions.

2.2 | Data and Summary Variables for the Statistical Analyses

2.2.1 | Climatic Variables

We calculated bioclimatic predictors for each plot using the SAFRAN-SURFEX/ISBA-Crocus-MEPRA model (Durand et al. 2009; Vernay et al. 2022) that accounts for weather and snow conditions based on large-scale topography. For each plot, we calculated the growing degree days (GDD, Choler 2018) and solar radiation to characterise heat accumulation, and freezing degree days (FDD) to characterise the intensity of freezing events during the year. The GDD and the FDD are the sums of average daily degrees above and below zero, respectively,

accumulated over the growing season each year, averaged over the period 1998–2018, and modelled in the first soil horizon (up to 10 cm depth, see Martinez-Almoyna et al. 2020). All variables described from now on are calculated as the annual average of each variable for a range of 10 years. We used a 10-year interval regardless of the exact sampling time to capture medium-term climatic conditions. The elevational gradients were chosen to represent the pedo-climatic conditions of the Alps, so each gradient—and thus each plot—can differ greatly in terms of climate and soil characteristics. As such, any slight misalignment between climate measurements and sampling years is unlikely to weaken the observed climatic effects. Several variables related to temperature have been calculated such as mean annual temperature (MAT), mean diurnal range which represents the difference between the highest temperature and the lowest temperature within 1 day, and the temperature annual range which is equivalent to the previous variables but calculated only on a monthly range. We also calculated mean annual temperature seasonality, which is the standard deviation of the monthly mean temperatures. Soil temperatures at 10 cm depth were also calculated. We also calculated the annual sum of precipitations and the precipitation seasonality, which represents the variation in monthly precipitation totals over the course of the year. Finally, the snow depth in each plot was calculated.

To limit multi-collinearity in the following analyses, we performed a PCA on these variables. The first axis summarised 52% of variation and corresponded to a temperature (and precipitation) gradient, with positive values being related to high temperature (Figure S1.1); this axis is referred to hereafter as "MAT". The second axis referred to a gradient of heat accumulation, with positive values being linked to strong heat accumulation (Figure S1.1), representing 22% of variation; this axis is called "GDD" hereafter. The third axis (12% of variation) was related to FDD, with positive values corresponding to high freezing events (Figure S1.2); named "FDD" hereafter.

2.2.2 | Soil Variables

In each plot, a soil description was done based on the World reference base (WRB, Anjos et al. 2015). Soil pits were dug to the parent material and each horizon was described and sampled for analysis. Bulk density (BD) was measured with a 100 cm^2 cylinder and the coarse fragment content (> 2 mm) was estimated (in % volume) at the fine scale with a 100 cm^2 cylinder (EG_weighted; Figure S1.3) and also visually from the soil horizon to account for larger stones or rocks (Svisual; Figure S3). If a BD measurement was not possible, the BD value of the nearest horizon was used (upper or lower horizon). Soil analyses were performed on 2 mm-sieved crushed soil samples (NF ISO 11464 standard) at each pedological horizon and the following measurements were retrieved: pH_{H2O} (NF ISO 10390), cation exchange capacity cobaltihexammine (NF ISO 23470) which is often used as a proxy of the capacity of soil to retain nutrients, bioavailable P by Olsen method (NF ISO 11263), grain size repartition (clay, silt, sand; NF X 31–107), organic carbon, total nitrogen (NF ISO 10693, NF ISO 10694, NF ISO 14235, NF ISO 13878).

Similarly to our approach with climate data, we reduced the dimensionality through a PCA. The first axis, which explained 36% of variation, represented soil organic matter and nitrogen content ("soil organic matter", hereafter). The second axis, termed "physical properties" hereafter, characterised a gradient of stoniness, summarising 23% of variation. Elevated values indicated pronounced soil stoniness, reflecting a higher concentration of coarse elements, while lower values were associated with increased soil bulk density. The third axis (17% of the variation) denoted a pH gradient and the soil saturation rate (S/T; Figure S1.4), with positive values corresponding to high pH and high S/T ("chemical properties", hereafter).

2.2.3 | Plant Variables

Plant species abundances were estimated during the peak vegetation and flowering period (between July and August) by professional botanists. They used a linear transect traversing each plot and the pin-point method (Jonasson 1988). Specifically, a 30-m-long transect was placed perpendicular to the slope in the middle of each plot. Every 20 cm along the transect, botanists placed two pinpoints: one 25 cm upslope and another 25 cm downslope from the transect. For each pinpoint, they recorded all touching plant individuals. In total, this method generated 300 pinpoint measurements per plot, with each point potentially involving multiple plant species contacts. Based on these data, we calculated plant species richness and inverse Shannon's diversity at plot level. However, given their high correlation, we only kept Shannon's plant diversity for further analyses, which reflects both the number of species in the plot (species richness) and their abundance distributions.

To represent the trait structure of the plant communities, we used five traits: plant height (hereafter "height"—associated to competitive strength), specific leaf area (SLA—associated to maximal photosynthetic rate), leaf dry matter content (LDMC—associated to investment in photosynthetic organs), leaf carbon content and leaf nitrogen content (LCC and LNC respectively—associated to maximal photosynthetic rate and resource economics, Cornelissen et al. 2003; Díaz et al. 2016). These traits were available for over 85% of the species from our in-house database, that is, our own measurements carried out over the last 20 years in the vicinity of the plots (for each species at least 10 individuals were sampled in similar conditions). To address the missing values, we supplemented data using TRY (Kattge et al. 2020) and then inferred values using the mice function with height information (covering 13% of SLA values, van Buuren and Groothuis-Oudshoorn 2011).

We then computed the community-weighted mean (CWM; Lavorel and Garnier 2002) for each of the 77 plant communities and each trait, but considering only the herbaceous species as traits of herbaceous and woody species are hardly comparable. We then performed a PCA. The first axis was mainly linked to SLA, leaf nitrogen content, leaf dry matter content and carbon/nitrogen ratio and the second axis was mainly linked to plant height. They represented 63% and 18% of the overall variation (see Figure S1.5). Following Reich (2014), we interpreted the first axis as a gradient from conservative (i.e., high leaf dry matter content and low leaf nitrogen content at low values) to acquisitive plant strategies, and the second axis as a competition

axis with positive values corresponding to taller and thus more light-competitive plants (Reich 2014).

2.2.4 | Earthworm Diversity

In each plot, soil samples for eDNA extraction were collected from three 2 m × 2 m subplots, randomly distributed across a linear transect. In each subplot, we randomly sampled 10 soil cores with a diameter of 5 cm. The soil cores were divided into two layers: the surface layer (ca. 1–8 cm in depth) and the subsurface layer (ca. 8–16 cm in depth), which could often be distinguished by differences in colour. Independently for the two layers, the 10 soil cores collected from each subplot were combined and thoroughly mixed, resulting in one biological sample per soil layer per subplot, thus yielding a total of six samples per plot. For each of these soil samples, we extracted DNA from a 15 g aliquot based on the procedure described in (Taberlet et al. 2012) and in Taberlet et al. (2018). Details on the molecular analyses carried out on the lab can be found in Calderón-Sanou et al. (2024). In short, oligochaeta eDNA was amplified using the 'olig01' DNA marker described in Taberlet et al. (2018) (16S mitochondrial rDNA region). The PCR conditions included an initial 10-min step at 95°C, followed by 40 cycles (30s at 95°C, 30s at 58°C, and 60s at 72°C), and a final elongation at 72°C for 7 min. Extraction and PCR blank controls were also included to check for contaminants. Illumina sequencing was performed on a HiSeq 2000 platform (2 × 125 bp paired-end reads). A standardised bioinformatic pipeline was then applied (Calderón-Sanou et al. 2020), using the OBITools software (Boyer et al. 2016) and the R package "metabaR" (Zinger et al. 2021), to remove contaminants and errors and to get the taxonomic composition in terms of molecular operational taxonomic unit (MOTU) of earthworms of each sample. For each MOTUs, we calculated the relative frequency of reads at each subplot (the two soil layers are averaged). Since species richness of MOTUs can give an over-optimistic estimation of diversity, we instead estimated earthworm diversity through the exponential Shannon diversity index that puts less weights on rarely observed sequences (Calderón-Sanou et al. 2020).

2.2.5 | Ecosystem Functions

We considered approximative measures for four ecosystem functions in our analysis, namely proxies for carbon and nitrogen limitation of microbes, carbon stock and plant primary production. We estimated carbon and nitrogen limitation of microbes via measures of the activity of different extracellular exoenzymes of microbial communities (EEA) targeting either carbon or nitrogen acquisition. EEAs depend on both the availability of the element they target and microbial biomass (Sinsabaugh et al. 2009). Thus, ratios of specific EEAs targeting either N or C and the sum of all measured EEAs indicate to what extent the element targeted by the specific EEAs is a limiting factor in the environment for microbial metabolism. Using standardised fluorimetric techniques (Bell et al. 2013), we determined the EEA of six different exoenzymes involved in the decomposition of carbon-rich substrates (EEC: α-glucosidase, β-1,4-glucosidase, β-D-cellobiosidase and β-xylosidase), and nitrogen-rich substrates (EEN: β-1,4-N-acetylglucosaminidase and leucine aminopeptidase). Soil samples were homogenised in a sodium acetate buffer solution using a Waring blender for 1 min. The resulting soil slurry was then added in duplicate to a 96-deep-well microplate containing 200 μL of specific substrates for each enzyme at the saturation concentration. The exoenzymatic activities were measured at pH 5 (average pH across the plots, similar to the meta-analysis conducted by Sinsabaugh et al. 2009). Standard curves were prepared for each duplicated soil sample by mixing 800 μL of soil slurry with 200 μL of 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC) in 96-deep-well microplates with concentrations ranging from 0 to 100 μM. The plates were then incubated at 25°C in the dark for 3 h on a rotary shaker (150 rpm) before being centrifuged at 2900 g for 3 min. Fluorescence measurements were taken on 250 μL of the supernatant using a microplate reader (Varioscan Flash, Thermo Scientific) with an excitation wavelength of 365 nm and an emission wavelength of 450 nm. The exoenzymatic activities were expressed as nmol.g.soil−1.h−1 after subtracting negative controls. We calculated the relative potential activity of exoenzymes targeting nitrogen (sum of all EEN/total EEA) and carbon (sum of all EEC/total EEA). In the rest of the article, we speak about nitrogen or carbon limitation when the relative potential activity of exoenzymes targeting nitrogen or carbon is high (Martinez-Almoyna et al. 2022).

Then, based on the measurement of bulk density (i.e., calculated as the weighted average of the bulk densities of the soil layers making up the focal 10 cm deep soil core, with the weight equal to the proportion of the respective soil layer in the soil core; g/cm3; BD) and the coarse elements (g/cm^3; CE; weighted average as above) contained in the cylinder of bulk density (2.65 corresponds to the quartz density), we assessed the fine soil density over the full soil profile (D_{fs}; Equation 1). Then, through the visual volumetric coarse fragments (%; *S*visual), we calculated the fine soil concentration (Cfs; Equation 2) and we finally calculated the carbon stock (SSOC) based on the soil carbon concentration (CSOC), the fine soil concentration (C_{fs}) and the soil thickness (T), according to Equation (3). This method is assumed to best estimate the real stocks of fine soil because the classic estimation methods clearly overestimate the stocks (Poeplau et al. 2017).

$$D_{fs} = \frac{\overline{Bd} \times 100}{100 - \frac{\overline{CE} \times 100}{2.65}} \quad (1)$$

$$C_{fs} = D_{fs} \times \left(1 - S_{visual}\right) \quad (2)$$

$$S_{soc} = C_{soc} \times C_{fs} \times T \times 100 \quad (3)$$

Finally, we used the normalised difference vegetation index (NDVI), which approximates chlorophyll activity and, when summed over the year, plant primary production. Estimates of the NDVI at a resolution of 250 m were derived from the MODIS time series (moderate resolution imaging spectroradiometer) bands 1 and 2; MOD09Q1: MODIS/Terra Surface Reflectance 8-Day L3 Global 250 m SIN Grid V006 satellite MODIS (Terra), available online: https://lpdaac.usgs.gov/products/mod09q1v006/. The preprocessing of NDVI time series followed Choler (2015) and the time-integrated NDVI (hereafter primary production) was then calculated for each year and each site as

the sum of the NDVI values above a threshold value (0.1) to consider only the snow-free period (see Choler 2015).

When combining all information together (plant surveys, soil eDNA, soil physico-chemical, climatic data and ecosystem functions) and removing subplots for which information was missing, we could finally work with a dataset of 233 subplots.

2.3 | From a Causal Inference Framework to Structural Equation Modelling

2.3.1 | Overview of the General Framework

To quantify both direct and indirect effects of environmental variables on earthworm diversity and ecosystem functions, we applied a causal inference framework (Grace and Irvine 2020; Arif and MacNeil 2023), a method increasingly used in ecological research for examining causal impacts of environmental changes (Fan et al. 2016; Schoolmaster et al. 2020; Arif et al. 2022). The first step in our framework is to construct a path model to represent current knowledge on the causal relationships among selected variables (Figure 1). This causal network was then evaluated globally using a structural equation model (SEM), which requires a directed acyclic graph (DAG) structure(Shipley 2000a, 2000b). In order to build this DAG, we set climate variables as top exogenous variables, assuming that they are independent of our other studied variables at the scale of our study. We set ecosystem functions as final response variables that do not influence other variables as our main research objective was to study responses of ecosystem functioning (Figure 1). We employed a two-step piecewise SEM approach (Lefcheck 2016) to test and refine this path model. In the first step, we preselected relevant pathways among soil, vegetation, and earthworm compartments to prevent loops within the DAG. To determine pathway directions, we paired these compartments (i.e., soil-vegetation, vegetation-earthworms and soil-earthworms) and applied independent generalised linear models to identify the most parsimonious paths, based on variable relationships (see Table S1.1). For each pair, we summed the explained variances (adjusted R^2) of all tested models for both directions and selected the pathway between the two compartments with the higher overall explained variance (Table S1.2). Note that pathways not chosen initially could be revisited in subsequent steps if they conformed to the acyclic structure.

In the second step, we tested an initial global model incorporating relevant paths from step one, with climate as exogenous variables and ecosystem functions as final responses. We then refined this model by (i) removing non-significant paths and (ii) adding paths not included in this initial global model but suggested as significant while not disrupting the DAG structure (Table S1.1). One pathway (earthworms to soil organic matter in closed habitats), whose R^2 for both directions was similar in step one, was reversed to better align with the global model support in step two. All piecewise SEM analyses were conducted using the piecewiseSEM function in R, with pathway significance assessed via d-separation tests.

2.3.2 | Nonlinearity

Since we had no prior expectations on the shape of the relationships, we first applied our SEM with generalised additive models (GAMs), constrained to fit no more complex relationships than quadratic curves. Once the variable and link detection was done, we then moved to a parametric model by 'parameterising' visually the estimated relationships estimated by the GAMs. As we constrained the GAMs to fit only quadratic curves we had three possibilities. Linear regressions were fitted by using $Y \sim X$, the symmetric quadratic curves by $Y \sim X^2$ and the non-symmetric quadratic curve by $Y \sim X + X^2$. Positive quadratic curves were modelled by a linear regression because of poor ecological explanations (only expected for stoniness which is more related to physical variables). Therefore, we have both linear and non-linear relationships within our final model.

2.3.3 | Estimation of Coefficients

We used the standardised coefficients extracted from the most parsimonious model (Grace et al. 2010) for the SEM interpretation. The direct effects were defined as the sum of the coefficients of each direct path. Indirect effects were calculated as the sum of the coefficients of each indirect path, where the effect for each indirect path is computed as the product of the standardised path coefficients along the path. We used the semEff packages (Murphy 2022) to assess these effects. Since grasslands and closed habitats are relatively different in terms of soil biodiversity (Calderón-Sanou et al. 2024) and difficult to compare in terms of vegetation structure, we built a separate structural equation model (SEM) for each of these two habitats.

All analyses were run in the R statistical environment version 4.2.1.

3 | Results

3.1 | How Is Earthworm Diversity Distributed?

We observed an overall significant but very small decline of earthworm Shannon diversity with increasing elevation ($R^2 = 0.08$, $p < 0.001$) in a linear regression model. This effect remained significant when focusing only on grasslands ($R^2 = 0.07$, $p < 0.01$) but disappeared when focusing on closed habitats ($R^2 = 0.01$, $p = 0.14$, Figure 2). Earthworm diversity was overall lower in grasslands than in closed habitats ($p < 0.01$; Figure 2). While earthworm diversity varied between the two habitat types, this was not necessarily the case for all environmental variables. In particular, mean annual temperatures, soil physical properties (i.e., coarse element) and plant community SLA tended to be higher in closed habitats than in grasslands, while the reverse was found for plant diversity (Figure S1.6). Ecosystem functions differed between the two habitat types, with plant primary production and relative nitrogen enzymatic activity being higher in closed habitats than in grasslands, with the latter having higher carbon stock and relative carbon enzymatic activity (Figure S1.6).

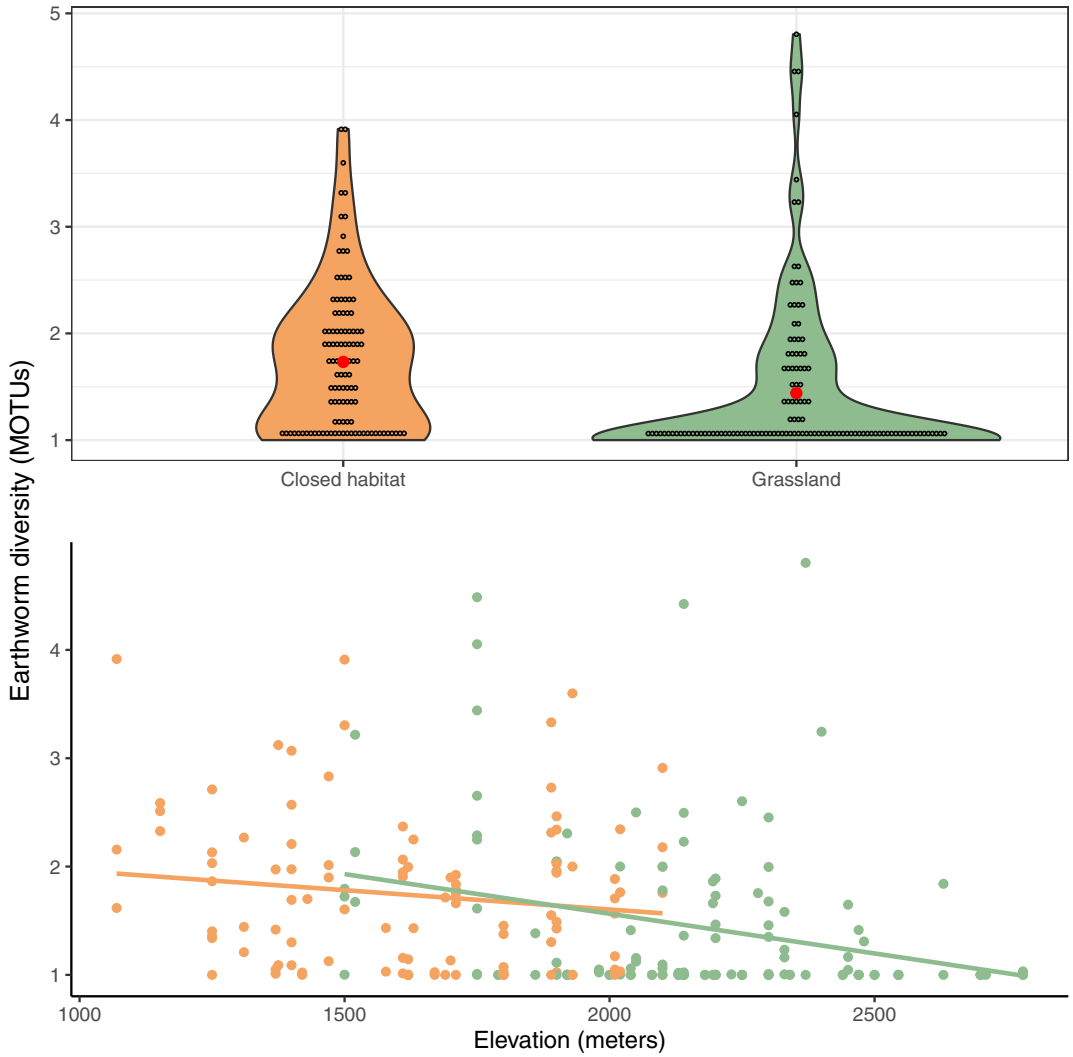

FIGURE 2 | Distribution of the earthworm alpha MOTUs diversity within each plot across habitats (top) and elevation (bottom). Closed habitats are represented by orange dots, while green dots correspond to grasslands. Red dots show mean values.

3.2 | How Do Climate, Soil and Vegetation Influence Earthworm Diversity?

In our piecewise SEM, environmental variables explained a substantial proportion of variation in earthworm diversity in both habitats ($R^2 = 0.24$ in closed habitats, $R^2 = 0.24$ in grasslands; Figure 3). Climate directly affected both the soil and vegetation but also indirectly affected vegetation through soil, with the exception of plant diversity impacting soil physical properties in grasslands (Figure 3). The relative importance of the different compartments was consistent between the two habitats (Figure 3), while the importance of single variables varied (Figure 4).

Overall, the primary direct driver of earthworm diversity was vegetation, followed by both the direct and indirect effects of soil, and subsequently climate. Interestingly, all the effects of climate on earthworm diversity were indirect, that is, passing through soil and/or vegetation pathways (Figure 4). Within closed habitats, SLA, plant diversity, and soil organic matter were the most important direct drivers of earthworm diversity, followed by the indirect effects of growing degree days and, to a lesser extent, soil physical properties and MAT (Figures 3 and 4). In grasslands, the direct effects of SLA (but not plant diversity) and combined direct and indirect effects of soil physical properties were the most important drivers of earthworm diversity, followed by the indirect effects of MAT and growing degree days.

3.3 | How Important Is Earthworm Diversity for Key Ecosystem Functions?

Our piecewise SEMs well-explained the variation in ecosystem functions in both habitats (explained variation between 0.26 and 0.56; Figure 3). In general, earthworms played pivotal roles in three of the four studied ecosystem functions. The importance of earthworm diversity on ecosystem functions varied with habitat and the focal ecosystem function (Figure 5). In grasslands, earthworm diversity had a significant effect on nitrogen enzymatic activity, explaining 55% of the total explained variation of this function (Figure 3), followed by climate (Figure 5). Higher earthworm diversity led to lower nitrogen limitation in soils (i.e., lower nitrogen rich enzymatic activity). In contrast, in closed habitats, higher earthworm diversity led to less carbon

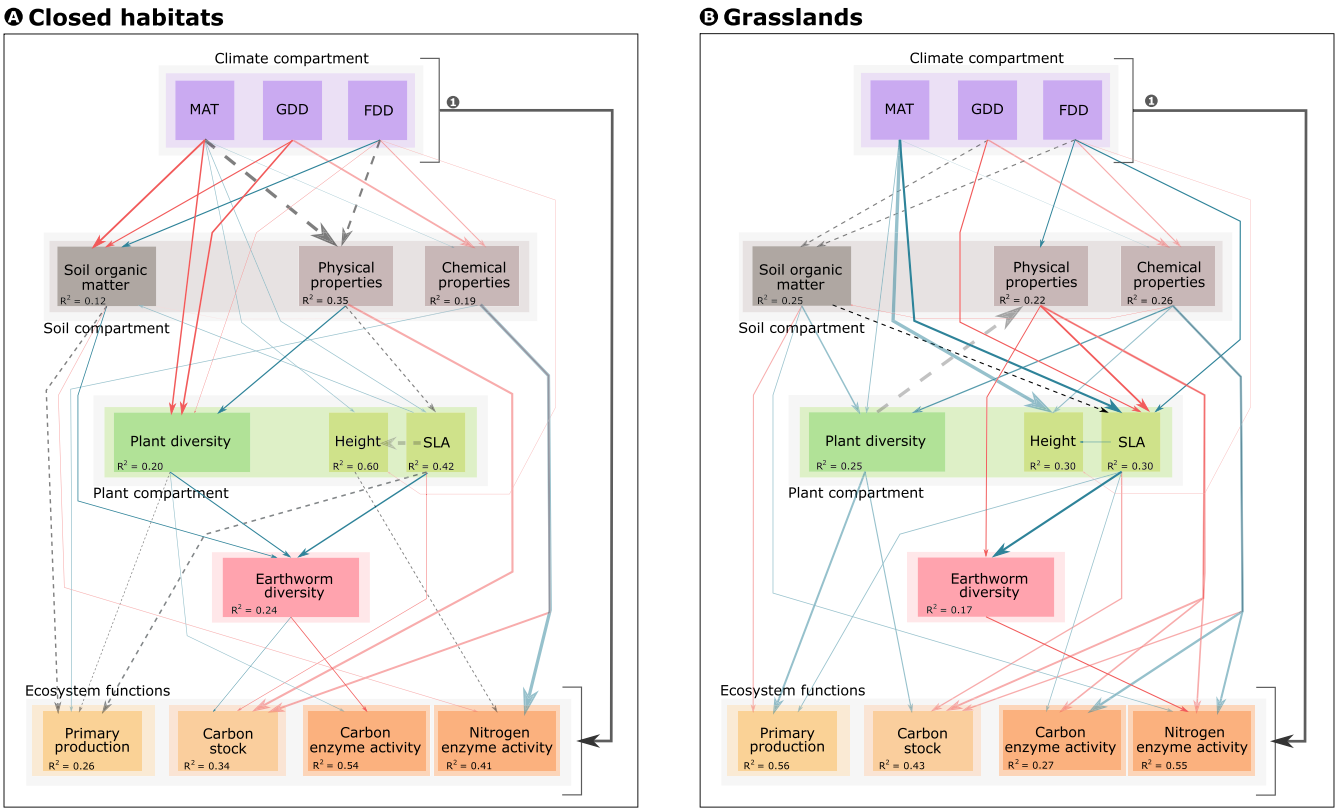

FIGURE 3 | Piecewise SEMs showing the overall dependencies between the environment, earthworm diversity and ecosystem functions for both closed (A) and grassland (B) habitats. The three environmental compartments are climate, soil and vegetation. Arrow sizes are proportional to the associated path coefficients. Blue and red arrows indicate a positive and negative relationship, respectively, and black dashed arrows represent a non-linear relationship. Arrows from climate to ecosystem functions are not shown individually but are represented by one single black arrow (1).

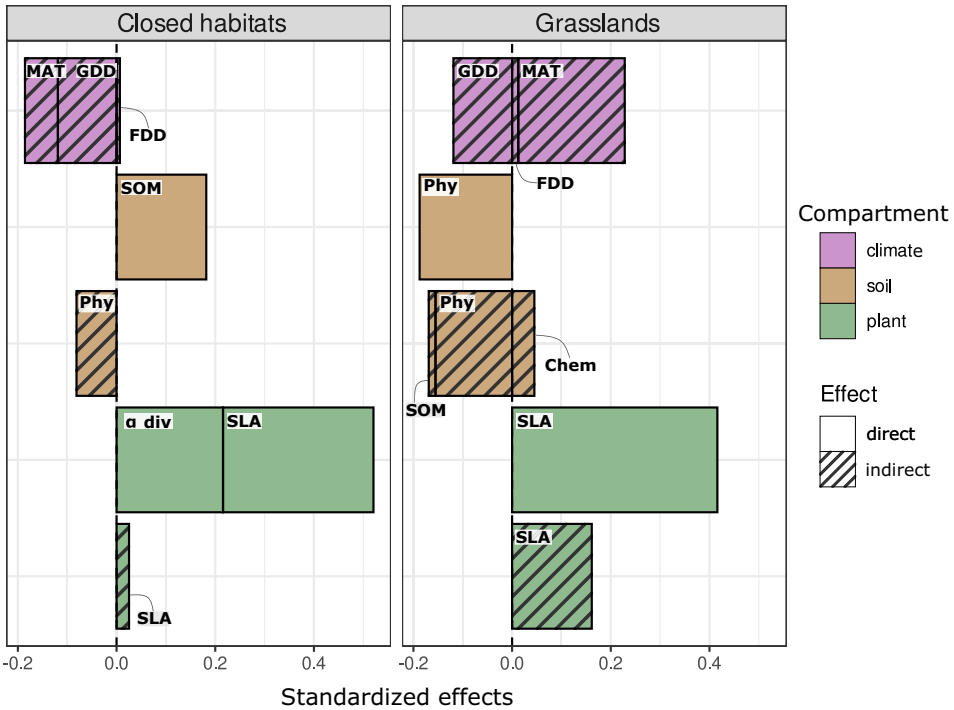

FIGURE 4 | Direct and indirect standardised effects of environment on earthworm diversity, extracted from the piecewise SEMs (Figure 3) for both closed (left) and grassland (right) habitats. Direct effects were computed as standardised model coefficients for each variable, while indirect effects resulted from the product of these direct effects along the causal pathways.

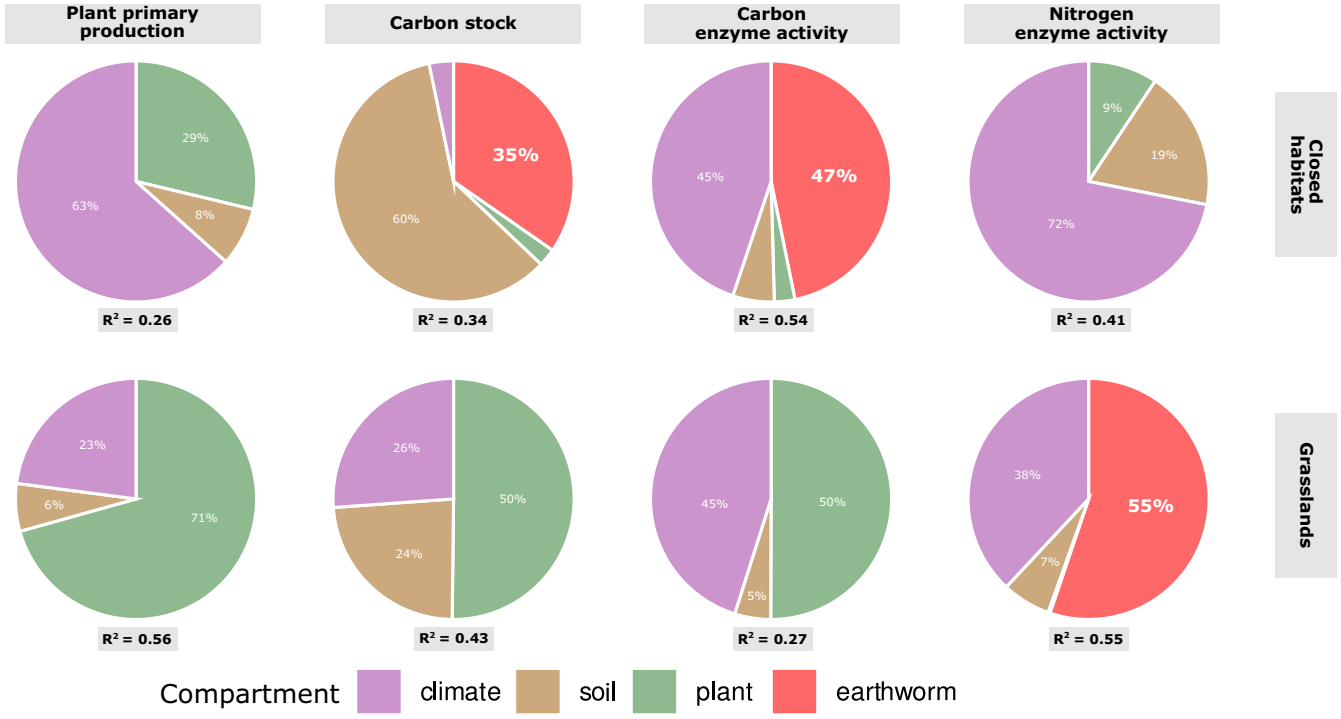

FIGURE 5 | Pie chart representing the effect of the environment and earthworm diversity on the ecosystem functions for both closed and grassland habitats. Each part is the sum of the direct, indirect and mediator effects of all variables.

limitation (i.e., reduced carbon rich enzymatic activity) and larger carbon stocks. Earthworm diversity explained 47% of the total explained variation of carbon enzyme activity, followed by climate, which explained 45% of variation, and it explained 35% of the total explained variation of carbon stock, followed by soil, which explained more than half of the variation. In contrast, in grasslands, earthworm diversity played no role in carbon related functions, which were rather explained by vegetation, climate and soil. Plant primary productivity, the only aboveground function we studied, was not affected by earthworm diversity but mostly by climate in closed habitats and by vegetation in grasslands.

4 | Discussion

Earthworms play essential roles in ecosystem functioning, yet understanding how their diversity responds to environmental changes—and how these shifts might impact ecosystem functions—remains a complex puzzle. Through causal reasoning and structural equation models, we address these questions and analyse the drivers and consequences of earthworm diversity along elevation gradients in the French Alps. Our study reinforces the importance of earthworm diversity as a mediator between environmental drivers and diverse ecosystem functions. We identified consistent direct and indirect environmental drivers of earthworm diversity across different habitats and demonstrated a significant impact of earthworm diversity on selected ecosystem functions.

We found a significant decline of earthworm diversity with increasing elevation, consistent with earlier studies (Bouché 1972). However, elevation alone explained only a minor fraction of this variation, whereas specific environmental variables accounted for nearly a quarter. This result underscores the importance of discerning environmental drivers in understanding macroecological patterns, enhancing both comprehension and predictive capabilities of models.

Moreover, our study allowed us to identify the relative importance of different ecosystem compartments in driving earthworm diversity, which was consistent across habitats. To address discrepancies in prior studies (Blouin et al. 2013; Singh et al. 2019), we emphasise the need to explicitly consider habitat-specific variables across all ecosystem compartments (i.e., climate, soil and vegetation), as well as their interdependencies. Our path model illustrates that climate exerts indirect effects on earthworm diversity, primarily mediated through changes in soil and vegetation. This approach revealed significant effects of all compartments—climate, soil and vegetation—on earthworm diversity. In both grasslands and closed habitats, vegetation emerged as the most consistently important direct driver, followed by soil and the indirect influence of climate. These results may reconcile the conflicting results in earlier studies, where smaller-scale studies emphasised a predominant effect of vegetation and/or soil (De Wandeler et al. 2016; Hoeffner et al. 2021), while large-scale studies identified climate as the primary driver (Phillips et al. 2019; Li et al. 2023). This disparity could be attributed to the crucial indirect effect of climate via vegetation and soil, often overlooked in large-scale studies. Recognising whether an effect is direct or indirect holds implications for conservation, particularly in the context of recent pronounced climate events like short periods of snow cover and summer droughts (e.g., 2022 in the French Alps). If the effects are predominantly indirect, they may not immediately impact earthworms but instead cascade through the soil and vegetation, creating a debt of extinction that may not manifest in the early years and decades.

While our study showed consistent impacts of the different ecosystem compartments on earthworm diversity, the most influential variables within each compartment differed between habitats. In closed habitats and grasslands, the dominance of plants with acquisitive trait strategies, such as high specific leaf area, increased earthworm diversity. This aligns with earlier studies emphasising the importance of highly acquisitive legume species due to their high nitrogen leaf and dead material content and easily decomposable litter (Milcu et al. 2008; Eisenhauer et al. 2009). However, the positive effect of acquisitive plants might also be influenced by soil properties, which our directional path model did not explicitly capture. In closed habitats, plant diversity, in addition to plant acquisitive strategies, strongly impacted earthworm diversity. This relationship, observed previously in mountains (Gabriac et al. 2022), may be attributed to the closed habitat category encompassing various successional stages, from mature forest to shrubby grasslands, and ecotones between grasslands and forests. Higher plant diversity in such transitional ecosystems may support higher earthworm diversity, influenced by successional dynamics (Bernier and Ponge 1994; Grossi and Brun 1997), a greater diversity of microhabitats (McCain and Grytnes 2010), or a richer trophic resource base (Gabriac et al. 2022).

Soil influences on earthworm diversity were found to be a complex interplay of direct and indirect effects. In closed habitats, soil organic matter (SOM) had a direct positive effect on earthworm diversity, consistent with studies highlighting SOM as a crucial resource for earthworms (El-Duweini and Ghabbour 1965; Hendrix et al. 1992). In these habitats, the lower quality and palatability of organic matter may drive earthworms to ingest more SOM than in grasslands (De Wandeler et al. 2016). However, our data could not definitively establish the direction of influence in closed habitats; while SOM influenced earthworm diversity, earthworms likely contributed to SOM accumulation as well (see Lavelle et al. 2004). The influence of soil physical properties, particularly stone content, emerged as an important driver of earthworm diversity in grasslands and, to a lesser extent, in closed habitats. Most studies investigating the relationship between earthworms and soil physical structure focused on the effect of the activity of earthworms on soil texture (e.g., coarse elements, Barrios 2007; Bottinelli et al. 2015), neglecting structural variables such as stone content. Indeed, while soil physical properties are often assessed through measures such as burrow density, bulk density, or soil aggregation, they can be influenced by earthworms (Lavelle and Spain 2002; Bohlen and Edwards 1995). Here, we thus measured soil physical properties through stone content, a variable that cannot be affected by earthworms, and demonstrated the importance of this variable to drive earthworm diversity. While soil stone content is rarely reported in studies, alpine grasslands, often characterised by shallow, rocky soils, may limit soil volume and resources, thereby increasing competition and reducing colonisation opportunities for earthworms (Singh et al. 2020). Unlike other studies (Edwards 2004), we found no clear relationship between soil pH and earthworm diversity. The probable reason is that the majority of our soils were acidic with low variability (pH between 4.5 and 6), preventing a meaningful test for the expected decline starting around pH 7.0 to 7.4 (El-Duweini and Ghabbour 1965; Bouché 1972; De Wandeler et al. 2016), considered optimal biological conditions for earthworms.

Climate impacts on earthworm diversity were indirect, primarily mediated by soil and vegetation, aligning with other studies (see also De Wandeler et al. 2016; Singh et al. 2019). In both closed habitats and grasslands, temperature (GDD and MAT) emerged as the most important climate variables. In closed habitats, indirect pathways of climate variables were predominantly via soil (mostly SOM) and vegetation (mostly plant diversity). In grasslands, indirect effects were primarily mediated via vegetation, with higher temperature promoting more acquisitive plants promoting higher earthworm diversity.

While climate, soil and plant variables have the greatest influence on ecosystem functions, earthworm diversity played a key role in three of the four functions studied, explaining approximately a quarter of the overall variance. Although the signal in favour of earthworm diversity was weaker compared to other variables, showing a significant effect in only three out of eight cases and explaining less than half of the variance for all tested ecosystem functions, this finding remains crucial. Indeed, in our study, we used empirical data at a large spatial scale. Consequently, capturing an effect of earthworm diversity on ecosystem functions, relative to other compartments, is a key finding. This result is consistent with previous studies showing that while environmental variation is important for ecosystem functioning (De Laender et al. 2016), a single group of species, earthworms, can explain a significant additional part of variation in ecosystem functions (Blouin et al. 2013; Sharma et al. 2017). Only plant primary productivity, the single above-ground function, was not directly affected by earthworm diversity but was reasonably well explained by other environmental drivers. As direct environmental effects on ecosystem functions are discussed elsewhere (Hautier et al. 2015; De Laender et al. 2016), here we focus on the role of earthworm diversity. The impacts of earthworms on ecosystem functions proved to be habitat-dependent. In closed habitats, earthworm diversity exhibited a positive correlation with increased carbon stocks and a reduction in carbon limitation for microbes. These habitats witness a substantial influx of epigeic organic matter, such as leaves and branches, resulting in a thick layer of hard-to-decompose organic matter. Epigeic and anecic earthworms facilitate the incorporation of this organic matter into the soil, thereby playing a key role in the dynamic carbon cycling and stabilisation within forests (Fahey et al. 2013). Earthworms provide a physical protection of soil organic carbon through the formation of soil aggregates (Bossuyt et al. 2005; Lubbers et al. 2013) and improve the availability of carbon from organic matter to microorganisms through their casts or burrows (Lavelle and Martin 1992; Bohlen and Edwards 1995). The positive effect of earthworm diversity on carbon stock and availability in closed habitats aligns with the suggestion of an earthworm-mediated carbon trap (Zhang et al. 2013). In contrast, carbon stock in grasslands appeared less influenced by earthworms. This could be attributed to lower accumulation of litter and higher soil aggregation with bacteria dominated cycles, rendering earthworm activity less efficient. In closed habitats, the positive correlation between earthworm diversity and soil organic carbon confirms our hypothesis. Higher earthworm diversity is associated with a reduction in the relative activity of carbon-targeting exoenzymes, attributed to the elevated soil organic carbon content, typical of less productive closed habitats. In contrast, grassland soils, known for their higher productivity and greater soil aggregate stability, exhibited a different trend.

Here, we observed that increased earthworm diversity alleviated nitrogen limitation for microbes, resulting in higher soil nitrogen content. This phenomenon may be linked to the dominance of bacteria as the primary energy channel in grasslands, as opposed to forests, leading to elevated bacteria-to-fungi ratios with higher earthworm diversity (Liu et al. 2019; Martinez-Almoyna et al. 2022; Calderón-Sanou et al. 2024). This, in turn, accelerates soil decomposition rates and enhances soil nitrogen availability. While extracellular enzymatic activities have been used repeatedly as an indicator for soil functioning (Nannipieri et al. 2002; Burns et al. 2013; Zuccarini et al. 2023), their measurement on extracted soil samples in the laboratory may be less accurate than in situ measurements of carbon and nitrogen fluxes. Nevertheless, the highly explained variability of these measures by environment and earthworms supports their use as indicators for ecosystem functions.

5 | Conclusions

In conclusion, our biogeographic study in the French Alps positions earthworm diversity as an important mediator between environmental drivers and ecosystem functions. Our findings emphasise the cascading effects of climate through changes in vegetation and soil on earthworm diversity, elucidated through a carefully constructed structural equation model. Variation in earthworm diversity, in turn, significantly explains the spatial variation in multiple ecosystem functions, particularly those associated with the carbon cycle in forests and the nitrogen cycle in grasslands. While our approach deepens the understanding of direct and indirect effects of climatic gradients on soil, vegetation, earthworms and multiple ecosystem functions, its potential as a predictive model for climate change scenarios warrants explorations.

Author Contributions

Romain Goury: conceptualization, formal analysis, methodology, writing – original draft and visualisation. **Tamara Münkemüller:** conceptualization, methodology, supervision and writing – review and editing. **Nicolas Bonfanti:** investigation, data curation and writing – review and editing. **Irene Calderón-Sanou:** data curation and writing – review and editing. **Jérôme Poulenard:** data curation and writing – review and editing. **Orchamp Consortium:** project administration. **Wilfried Thuiller:** conceptualization, funding acquisition, methodology, project administration, supervision and writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and R scripts used to produce the main results and figures can be found on zenodo: https://doi.org/10.5281/zenodo.15573402.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1. Appendix S2.**