Depth-differentiated, multivariate control of biopore number under different land-use practices

Katharina Stolze [a], Andrew D. Barnes [b], Nico Eisenhauer [c,d], Kai U. Totsche [a,*]

[a] Hydrogeology, Institute of Geosciences, Friedrich Schiller University Jena, Jena, Germany
[b] Te Aka Mātuatua – School of Science, University of Waikato, Hamilton, New Zealand
[c] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
[d] Institute of Biology, University of Leipzig, Leipzig, Germany

ABSTRACT

Earthworms and (tap-)roots impact the soil structure by creating large biopores, affecting infiltration capacity, seepage, nutrient cycling, and soil aeration. Despite the importance of biopores for the functions of soils and the fact that several hundreds of biopores >2 mm in diameter may occur on one square meter of soil, knowledge on the interdependence of soil properties, land-use intensity, and biopore number is still rudimentary. In this study, we investigate the linkage of the number of biopores (>2 mm i.d.) with the earthworm community, root biomass, and soil properties, including pH, water content, soil organic carbon (SOC), as well as the land-use intensity (pasture vs. cropland) as a function of the soil depth (15, 30 and 50 cm). Hypothesized causal relationships among these factors were analyzed by piecewise structural equation modelling (SEM). We found various and novel linkages between roots, earthworms, biopores, and soil properties depending on soil depth. In topsoil (at 15 cm depth), roots directly affected the number of small-sized biopores, and anecic earthworms were related to medium-sized biopores. These effects diminished with depth. We identified land-use intensity as the factor preponderating the relations between biopores, root biomass, and earthworm number in the topsoil horizons, thereby masking other interactions among variables. This appeared as high multicollinearity among variables in the SEM of the topsoil. Land-use intensity effects were found to impact the whole soil profile but decreased with soil depth. To further elucidate the single effects of soil properties on biopore-forming biota and number of biopores in the topsoil, we excluded land-use intensity as a variable in subsequent analyses. Biopores increased with soil pH and soil water content but decreased with increasing SOC. Based on our SEM analysis, we conclude that the occurrence, frequency, and persistence of biopores are the consequence of intricate interdependencies between earthworm communities, roots, and site-specific soil properties, governed by land-use intensity.

1. Introduction

Earthworms and roots modify soil structure by two principal pathways: the formation of aggregates and the creation of biopores. Biogenic aggregates form during bioturbation by pressure supply, direct entangling of soil particles, or by excretion of mucus and mucilage as aggregation agents (Guhra et al., 2021). The coaction of roots and earthworms is quite intricate and cooperatively formed biogenic aggregates (Zangerlé et al., 2011) show increased stability (Kautz et al., 2010; Logsdon et al., 2013) and harbor more soil organic matter (SOM) (Fonte et al., 2012) compared to the bulk soil. Furthermore, earthworm activity was found to increase the availability of N and P in casts (De

Vleeschauwer and Lal, 1981; Le Bayon and Binet, 2006) via cutaneous (Guhra et al., 2020) and intestinal mucus (Brown et al., 2000), thereby facilitating overall nutrient uptake (Fonte et al., 2012) and increasing root biomass (Milleret et al., 2009). Though, roots and living plant parts may also serve as food source for earthworms, causing damage to growing roots (Brown et al., 2000). Yet, the ingestion of seeds may help plant dispersal and thereby shape plant communities (Forey et al., 2011).

Otherwise, earthworms and roots modify soil structure by creating biopores that change the porosity, severely affecting soil permeability, aeration, and hydraulic properties (Pires et al., 2017). Biopores are defined as roughly round-shaped channels >2 mm i.d. (Han et al., 2015;

Kautz et al., 2014), but may also be smaller if they are created by roots (Volkmar, 1996). Therefore, the role of biopores for aeration, infiltration, seepage, and solute transport was investigated intensively (Cey et al., 2009; Edwards et al., 1990; Ehlers, 1975; Pitkänen and Nuutinen, 1998; Uteau et al., 2013; Van Schaik et al., 2014). Particularly, anecic earthworms and taproots can build pores that reach several meters in depth (Kautz, 2015; Lee, 1985) and can pervade the soil profile down to the parent rock. Hence, large volumes of water and dissolved and colloidal matter, including microorganisms (Dibbern et al., 2014; Lehmann et al., 2021) can be piped during snowmelt or lasting precipitation (Edwards et al., 1990; Edwards et al., 1993; Lehmann et al., 2021; Shipitalo and Butt, 1999). This becomes obvious if one considers the impressive number of biopores with a diameter > 2 mm ranging from 27 and 1250 m^{-2} across various types of tillage systems and agricultural practices (Cey and Rudolph, 2009; Don et al., 2008; Pérès et al., 2010; Pitkänen and Nuutinen, 1997; Schneider et al., 2018; Van Schaik et al., 2014).

Variable diameter classes of biopores can be attributed to different earthworm species, functional groups (Pérès et al., 1998), and root types (Kautz, 2015). Endogeic earthworms (e.g., *Aporrectodea caliginosa*, *Aporrectodea rosea*) build small to medium-sized pores according to their diameter of ≤ 5 mm. They live in the top 30 cm of soil, feeding on soil organic matter (SOM), which they ingest during (predominantly) horizontal burrowing through the soil (Brown et al., 2000). Additionally, biopores of that size can be assigned to juvenile anecic earthworms (Pérès et al., 1998). Larger pores up to 9 mm are created by adult anecic earthworms (e.g., *Lumbricus terrestris*, *Aporrectodea longa*) (Pérès et al., 1998). In contrast to endogeic earthworms, anecic earthworms feed on plant residues, which they harvest at the soil surface and pull down into their permanent, vertical burrows (Curry and Schmidt, 2007). Consequently, these burrows are characterized by linings of mixtures of casts, plant residues, cutaneous mucus, and microbial biofilms (Brown et al., 2000). Also, plant roots create biopores of different diameters (Jarvis, 2007; Kautz, 2015) according to the rooting system, i.e., taproot or fibrous-root system. Fibrous roots generate biopores smaller than or around 1 mm in diameter (Edwards et al., 1988; Kautz, 2015) at favorable soil conditions, e.g., wet soils. However, if soils become dryer and harder to penetrate, they preferentially use existing larger biopores formed by taproots of, e.g., grass-clover, alfalfa, and lucerne, (Bodner et al., 2021; Logsdon et al., 2013). Those plant-derived biopores are in a similar diameter range as those of (anecic) earthworms (Angers and Caron, 1998; Kautz, 2015; Meek et al., 1989). Thus, established root systems have a strong influence on the distribution of earthworm burrows (Springett and Gray, 1997).

Previous studies have shown that earthworm and root abundance, growth, and activity depend on a variety of soil properties, including temperature, moisture, texture, bulk density, depth, pH, food availability (Curry, 2004; Edwards and Bohlen, 1996; Logsdon et al., 2013), and agricultural practices (Curry, 2004; Lavelle, 1988; Lucas et al., 2019). Studies investigating the frequency of biopores described the soil properties and the land-use intensity, as well as the distribution of the biopores with depth and focused on their function for soil hydraulics and aeration. Only a few studies have related the number of biopores > 2 mm i.d. to the abundance of earthworms (Pérès et al., 2010; Schneider et al., 2018; Van Schaik et al., 2014) and root systems (Han et al., 2015; Lucas et al., 2019). Van Schaik et al. (2014) showed that earthworm biomass and abundance were strongly correlated with the number of biopores in the first 30 cm of the soil. Similar studies (Pérès et al., 2010; Schneider et al., 2018) found no uniformly directed relationship between earthworm abundance and frequency of biopores across different land-use types, although the number of biopores varied between agricultural fields and pastures. Yet, these studies employed similar or the same soil groups. Thus, expectable variations in the soil properties are minor, and their effects on biopore frequency and earthworm abundance less pronounced and hidden by random variability and measurement uncertainty. Furthermore, a limited number of soil properties is considered in related studies, and the variation with depth is mainly limited to the topsoil (≈ first 30 cm soil depth). However, it is known that biopores reach into the subsoil and even the parent material. The number of biopores is hierarchically controlled by superordinated pedogenic and anthropogenic factors that impact soil properties, which may affect the abundance and biomass of earthworms and root biomass. It ultimately results in a changing number of biopores. However, earthworms and roots retroact on soil properties like pH, SOC, water content, and soil structure (Bottinelli et al., 2015; Logsdon et al., 2013; Vetterlein et al., 2020), resulting in intricate relationships. Consequently, entangling the mutual influences among these variables driving soil biopore formation and persistence is an ongoing challenge.

This study uses structural equation modeling to explore the intricate linkage between biopore number and typical biopore-forming biota, soil properties, and land-use intensity. Furthermore, we aim to unravel functional dependencies among these variables along a depth-differentiated gradient to examine the extensive effect of biopore-forming biota through the entire soil profile. For this, we investigate cropland and pasture sites, each at two different study sites with contrasting soil groups, in Thuringia, Germany, located within the Hainich Critical Zone Exploratory (CZE) of the Collaborative Research Center 1076 AquaDiva (Küsel et al., 2016).

2. Material and methods

2.1. Study site

This study was conducted on two sites within the framework of the Collaborative Research Center 1076 AquaDiva (Küsel et al., 2016). One site (HAI) is located within the Hainich Critical Zone Exploratory (CZE) along the Hainich low-mountain range in north-west Thuringia, Germany (latitude: 51°6′ N, longitude: 10°25′ E). The bedrock is built from lime- and mudstones alternations of the Upper Muschelkalk formations with partly quaternary loess covers. The main soil groups are Rendzic Leptosols, Cambisols, and Luvisols (Kohlhepp et al., 2017). Along the hillslope in the direction to the Unstrut valley, the local climate shows a decline in mean areal precipitation (<900 mm/a to < 600 mm/a) and an increase in mean air temperature (8 °C to 9.5 °C; Kohlhepp et al., 2017). The other site (HUM) is located in the Saale-Elster-Sandsteinplatte Observatory (SESO) along a hillscape in south-east Thuringia, Germany (Latitude: 50°46′ N, Longitude: 11°38′E). The parent material consists of weathered sand- and siltstones of the Triassic Buntsandstein formations. The main soil groups are Umbric Leptosols and Cambisols. The mean annual temperature is 8.0 °C, and the annual precipitation totals 525 mm (Kohlhepp et al., personal communication). According to the Köppen-Geiger classification, the climate of both sites used to be warm temperate, and fully humid with warm summers (Cfb; Kottek et al., 2006). On both sites, one pasture and one cropland were sampled in October 2017. Within four weeks before sampling, it cumulatively rained 130 mm in HUM with a mean temperature of 12 °C. During sampling, rainstorms with an additional precipitation rate of ≈ 340 mm occurred. In HAI, precipitation totaled 150 mm within four weeks before sampling, with a mean temperature of 12 °C (DWD Climate Data Center, 2018). The pastures are regularly mown and grazed by cattle (HAI) and sheep (HUM). At sampling, the cropland site HAI was planted with ryegrass (*Lolium* sp.). Formerly, the soil was cultivated with rape. In comparison, the cropland in HUM is cultivated following a winter wheat-rape rotation and plowed afterward.

2.2. Sampling design and sampling

According to Valckx et al. (2011), we employed a sampling design that minimizes the autocorrelation and representative sampling of the earthworm communities. Since we used a combination of hand-sorting and mustard extraction, a minimum sample number of at least ten samples corresponding to 6 m^2 were necessary to characterize the

earthworm communities (Valckx et al., 2011). Pits for earthworm extraction had an area of 0.25 m². Hence, at least 24 replicates were needed. Autocorrelation was prevented by allowing for a minimum distance of 20 m between sampling points. Since earthworm communities change rapidly throughout the year (Eisenhauer et al., 2009), the timeframe for the sampling campaign was short (2–3 weeks). Therefore, sampling plots were reduced to 12 plots per land-use site. Plots were randomly distributed across the study sites (Fig. 1).

2.2.1. Sampling of biopores and soil properties

The number of biopores and soil parameters were recorded in three depths: 15, 30, and 50 cm. Biopores were quantified by image analysis of frontal digital images at fixed illumination, distance, and pixel resolution. For this, plots of 1 m² in size were chosen at each site. We determined the vegetation cover in percentage for every plot and the proportions of herbs, legumes, and grasses. After vegetation harvesting, the soil was excavated to a depth of 15 cm. For digital imaging, a smooth and plane surface was prepared. The remaining soil particles were removed with brushes and a vacuum cleaner (Makita, DCL 182Z). Subsequently, images (with a spatial resolution of 350 μm/pixel and a field of view of 1.56 m² (1.25 × 1.25 m)) perpendicular to the prepared surfaces were taken with a digital reflex camera (Nikon D3200) at a constant distance. From the same depth, soil cores (Ø 53 mm, 100 cm³, Eijkelkamp, The Netherlands) were taken to determine the bulk density ($n = 5$), water content ($n = 5$), and root biomass ($n = 5$) as well as a composite sample for pH, C_{org} and N analyses. The same procedure was applied to all depths.

The earthworm communities of the plots were determined directly next to the pits for biopore number sampling. The excavated soil of a 0.25 m² pit (0.5 × 0.5 m²) was hand-sorted for endogeic earthworms. For exhaustive extraction of anecic earthworms, a mustard (Intermarkt, Koblenz) solution (Chan and Munro, 2001) was applied for 35 min. All earthworms were packed in plastic bags, immediately transferred to the lab, and stored in the fridge at 5 °C. According to Sims and Gerard (1999), earthworm species were identified with an incident light microscope (Leica Zoom 2000) and subsequently weighed with gut content. Species densities were totaled, and mean body size was calculated as mean biomass according to their functional group (Bouché, 1977). Juvenile earthworms were grouped according to the shape of their prostomium to *Lumbricus* spp. (tanylobous) or *Aporrectodea* spp. (epilobous) (Sims and Gerard, 1999).

2.3. Biopore analyses

Biopores were categorized according to their diameters in three different size classes "< 2 mm", "2–6 mm", and "> 6 mm". The smallest diameter class is attributed to roots and juvenile earthworms, 2–6 mm i. d. to adult endogeic and juvenile anecic earthworms, and the > 6 mm i. d. to adult anecic earthworms (Van Schaik et al., 2014). Corresponding to these diameters, biopores on each image were counted using the Fiji plug-in (ImageJ 1.51c; (Schindelin et al., 2012). Here, small sections of each image were selected to avoid artifacts due to shadows. Subsequently, the small sections were converted into binary images by using thresholds based on the histogram information of every image to differentiate between background and biopores. Next, marked pores were counted, and particle pixel areas were measured. Finally, one pixel length was attributed to a natural size with which it was possible to calculate pixel areas and compare those to actual pore diameters. We assumed biopores as circles for determining the areas and calculated the horizontal circular according to $A = \pi*r^2$. Areas corresponding to pore sizes smaller than 1 mm i.d. and >10 mm i.d. were excluded to eliminate artifacts and narrow the pore system to the spectrum of interest.

2.4. Soil analysis and root biomass

Soil rings (Eijkelkamp, Giesbeek, NL) were used to determine the bulk density and volumetric water content according to Pansu and Gautheyrou (2007). Composite samples were air-dried and passed through a 2 mm sieve. According to standard procedures (DIN/ISO 3019), the pH was determined in duplicate: 10 g of the soil were mixed with 25 ml of 0.01 M calcium chloride solution. Mixtures were shaken for one hour and left for one hour to allow for sedimentation. Subsequently, the pH was measured in the supernatant (probe: WTW SenTix 41, device: WTW pH 197i). For C and N analysis, samples were ground to powder with a mortar. The soil organic carbon content (SOC) was calculated as the difference between total carbon content (C_{tot}) and inorganic carbon (C_{in}). Inorganic carbon content was determined by ignition at 450 °C for 16 h (Don et al., 2008). C_{tot} and C_{in} of subsamples
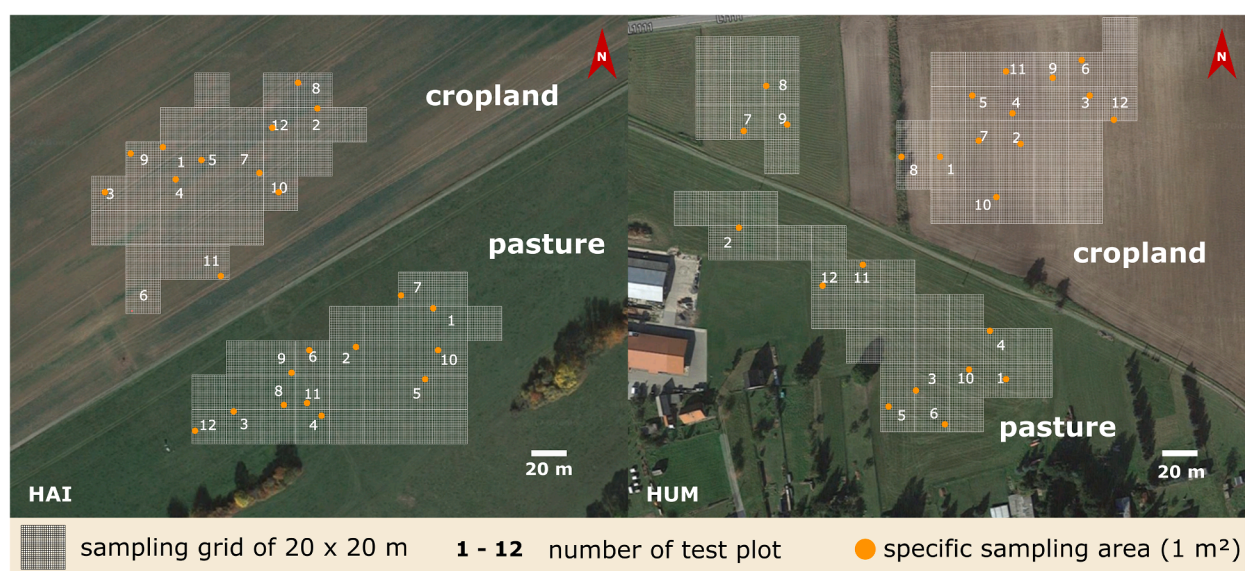

Fig. 1. Maps of study sites HAI (left) and HUM (right) showing the locations of pasture and cropland plots (orange dots). We used sampling grids of 20x20 m to avoid autocorrelation of earthworm communities, which were distributed across the sites. From these grids 12 were randomly chosen as experimental plots. Within the grids the specific sampling area of 1 m² was again randomly selected, and we then verified that a minimum distance of 20 m was maintained between the sampling areas. Maps of the study sites were obtained from Google Maps (Google LLC).

(ca. 2 mg) were analyzed in duplicates by dry combustion (Euro EA CHNS Elemental Analyser, EuroVektor, Italy).

Soil cores for root biomass determination were dispersed over a 1- and 0.063-mm sieve. The remaining roots were collected exhaustively, dried (40 °C, 48 h), and weighed.

2.5. Statistical analysis

For descriptive and multivariate statistics, we used R 4.0.3 (R Core Team, 2020). All parameters were initially tested for normal distribution using the Shapiro-Wilk test. Since assumptions of normality were not fulfilled, we used the Mann-Whitney-*U* test to assess variation in biopore classes (<2 mm, 2–6 mm, > 6 mm i.d.), soil properties (pH, SOC, water content), and biopore-forming biota (earthworms, root biomass) among the site and land use, as well as the Kruskal-Wallis test among depth. Post-hoc pairwise comparison was conducted to determine differences between pairs of means ($p < 0.05$).

2.5.1. Path modelling: Piecewise structural equation modelling

We used piecewise structural equation modelling (SEM) to account for the hierarchical structure of our sampling design. An advantage of piecewise SEM, which implements the 'd-sep' inferential test for multivariate causal models (Shipley, 2009), is that the presumably complex causal relationships can be established with relatively small sample sizes (Lefcheck, 2016), as long replication is adequate to estimate parameters for component models within the SEM. To create the model, we translated the pathways of the hypothetical model (Fig. 2) into linear equations. The test for goodness-of-fit is performed by Shipley's test of directed separation (Lefcheck, 2016). All identified independence claims, termed the basis set, are summarized into linear equations and solved as linear models. For this, we used the 'piecewiseSEM' package in R (Lefcheck, 2016). This method tests if all variables are conditionally independent, which means no relationships between unconnected variables are missing. All model probabilities, p_i, of the independence

claims (k) are combined, and Fisher's C is calculated as

$$C = -2 \sum_{i=1}^{k} \ln(p_i)$$

and subsequently compared to a chi-squared distribution with 2 k-degrees of freedom. If the resulting P-value from the chi-squared test is smaller than the specified α-level (α = 0.05), the model is rejected due to missing relationships. Hence, if P > 0.05, the model is considered appropriate for the data (Barnes et al., 2017; Lefcheck, 2016). For each identified SEM, we extracted standardized path coefficients, which indicate the relative strengths of each pathway when compared against each other (i.e., effect). Indirect effects are relationships between two variables linked by at least one other variable. Compound paths were calculated as the product of the standardized coefficients along the path.

Our model concept assumes that soils are complex natural objects affected by a hierarchy of factors. Based on this hierarchy, pedogenetic factors (bedrock-type) and land-use intensity are the predictors determining soil properties, e.g., pH, SOC, and the water content (response variables). The soil properties can also be predictor variables that influence the abundance and community composition of earthworms and plants (Lavelle et al., 2006; Lee, 1985; Leuschner and Hertel, 2003; Sasse et al., 2020; Singh and Sainju, 1998). They define the next hierarchical level. Earthworms and plants, however, may also contribute to a change of soil properties like pH, SOC, water content, and soil structure (Bottinelli et al., 2015; Vetterlein et al., 2020), potentially resulting in feedback loops. The next hierarchical level we defined was the formation of biopores in three different size classes by plants, especially roots and earthworms. The number of biopores is directly affected by plowing (Pérès et al., 2010). Thus, again, we need to add land-use intensity as a predictor (Fig. 2).

The linear models were checked for normality (Normal Q-Q plot and histograms of model residuals) and homogeneity of variance (residuals vs. fitted values). For linear models where parametric assumptions were not met, pH, water content, root biomass, earthworm biomass and densities, and SOC were log-transformed, and the number of biopores square-root-transformed. We used the variance inflation factor (VIF) to test for multicollinearity of predictor variables, whereby values above 4 indicated the presence of multicollinearity (Zuur et al., 2010) and led to erratic path coefficients < -1 and > 1. Consequently, variables with a VIF value > 3 in component models were removed. This was the case in the 15 cm SEM where we removed land use as a factor as it generally resulted in high multicollinearity among variables.

3. Results

3.1. Biopores

Total biopore numbers were significantly higher (p < 0.001) in HAI (918 ± 742) compared to HUM (428 ± 320 m^{-2}). The same was found for the diameter classes < 2 mm (p < 0.001), 2–6 mm (p < 0.001), and > 6 mm i.d. (p < 0.001) (Fig. 3). There was no significant difference of biopores between pasture and cropland, except for biopores > 6 mm i.d., which was higher in cropland than in pasture (p < 0.05). However, the total number of biopores (15 cm: 1057 ± 689 m^{-2}; 30 cm: 506 ± 445 m^{-2}; 50 cm: 282 ± 204 m^{-2}), and number of biopores within each size class decreased with depth (p < 0.01) (Fig. 3).

3.2. Soil properties

3.2.1. Volumetric water content

We recorded a higher volumetric water content in HAI (34 ± 7 %) than in HUM (17 ± 6 %; p < 0.001), with no difference between land use types (pasture: 22 ± 11 %, cropland: 25 ± 9 %) or between depths (15 cm: 27 ± 7 %, 30 cm: 23 ± 10 %, 50 cm: 20 ± 13 %) (Fig. 4A).
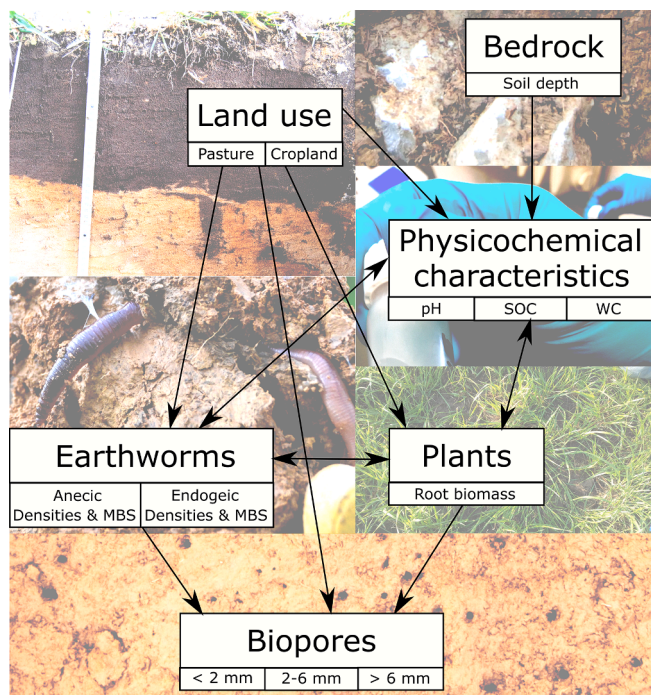
Fig. 2. Model scheme for hierarchical path analysis of interdependencies of soil properties like pH, soil organic carbon (SOC), and water content (WC), land use, earthworm densities and mean body size (MBS), plant root biomass, and biopores (diameter classes < 2 mm, 2–6 mm, and > 6 mm). Arrows indicate hypothesized relationships between sets of variables.
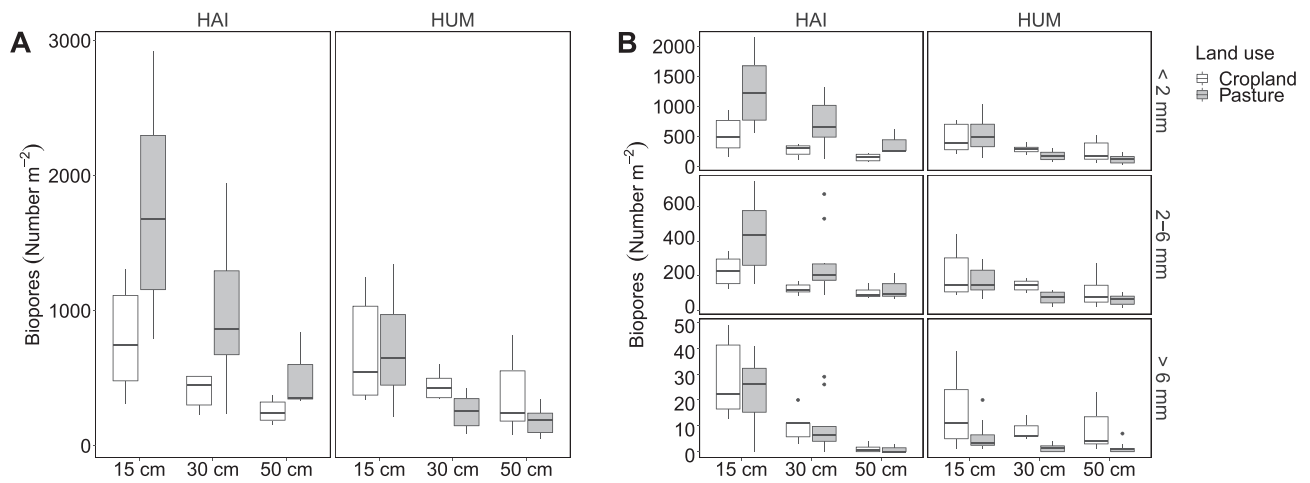
Fig. 3. The number of all biopores (A) and the number of biopores within each diameter size class (B) as a function of soil depth, study sites (Hainich (HAI), Hummelshain (HUM), land use type cropland (white), and pasture (grey).
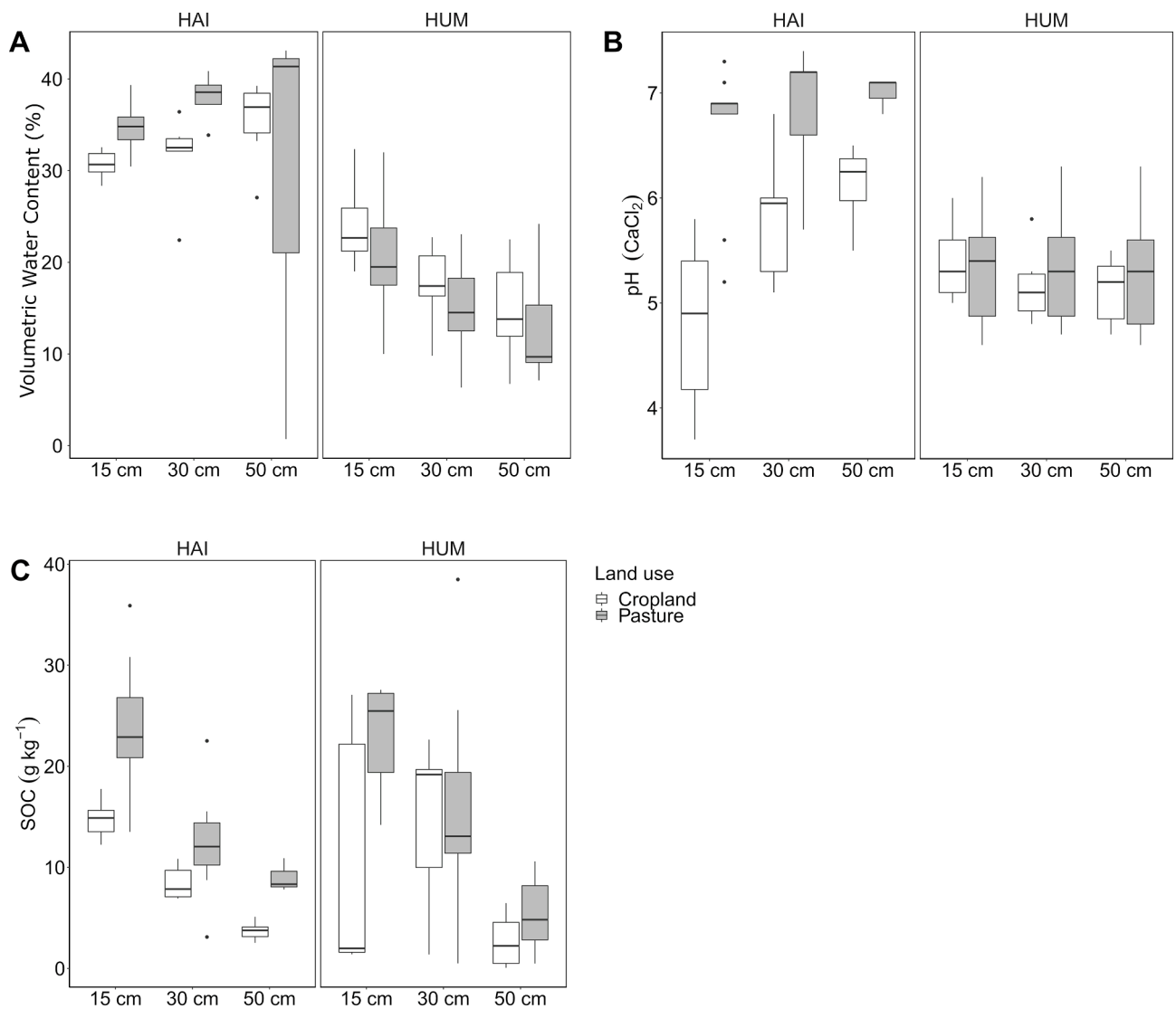

Fig. 4. Change of the volumetric water content (A), the pH (B), and the soil organic carbon (SOC) content with increasing depth and across the two study sites, Hainich (HAI) and Hummelshain (HUM), and the two land-use types cropland (white) and pasture (grey).

3.2.2. Soil pH

The soil pH of HAI (6.2 ± 0.9) was significantly higher compared to HUM (5.3 ± 0.4; p < 0.001). We also found a higher pH on the pastures (5.9 ± 0.9) than on the cropland sites (5.4 ± 0.6; p < 0.05). The Kruskal-Wallis test revealed no difference of soil pH with increasing soil depth (15 cm: 5.6 ± 0.9; 30 cm: 5.8 ± 0.9; 50 cm: 5.6 ± 0.7) (Fig. 4B).

3.2.3. Soc

We found no difference in SOC content between HAI (13.47 ± 8.06 g kg^{-1}) and HUM (12.86 ± 10.09 g kg^{-1}). Significantly higher SOC contents were recorded for the pasture sites (15.76 ± 9.30 g kg^{-1}) compared to the cropland (9.15 ± 7.70 g kg^{-1}; p < 0.001), which decreased with soil depth (15 cm: 19.56 ± 8.63 g kg-1; 30 cm: 13.38 ± 7.66 g kg-1; 50 cm: 4.76 ± 3.23 g kg-1; p < 0.001) (Fig. 4C).

3.2.4. Soil depth

The soil depth (i.e., the mean distance of the soil-regolith interface to the surface) varied strongly in space on the HAI site (42.7 ± 20.1 cm), while being more even on the HUM site with 48.4 ± 4.2 cm. In particular, the less-mature soils (Rendzic Leptosols) of the HAI pasture site resulted in shallow soil profiles with mean depths of 30.7 ± 10.7 cm. In contrast, the Luvisols found on the cropland site exhibited mean depths of 66.7 ± 8.2 cm. In HUM, no differences in soil profile depths between the land use types were detected (pasture: 49.3 ± 4.2 cm; cropland: 46.9 ± 3.8 cm).

3.3. Biopore-forming biota

3.3.1. Earthworm communities

Overall, we found nine different earthworm species, belonging to the three functional groups (Bouché, 1977), endogeic (Aporrectodea caliginosa, Aporrectodea rosea, Allolobophora chlorotica, Octolasion cyaneum, and Octolasion tyrtaeum), anecic (Lumbricus terrestris, and Aporrectodea longa), and epigeic earthworms (Lumbricus castaneus, and Lumbricus rubellus). Earthworm densities, biomass, and species were distributed differently across the study sites and land use types (Table 1). While the community composition was similar for HAI and HUM, croplands had fewer species than pasture, especially at HUM. However, at HUM, two endogeic species, A. chlorotica and O. tyrtaeum, were absent but were present at HAI, whereas L. rubellus only appeared at HUM.

Endogeic earthworm densities were significantly higher in HAI (364 ± 249 Ind. m^{-2}) compared to HUM (188 ± 152 individuals (Ind.) m^{-2}; p < 0.001). Likewise, we found higher earthworm densities in pastures (321 ± 205 Ind. m^{-2}) than in croplands (174 ± 216 Ind. m^{-2}; p < 0.001) (Fig. 5A). Similarly, the densities of anecic earthworms were significantly higher in HAI (77 ± 37 Ind. m^{-2}) compared to HUM (21 ± 25 Ind. m^{-2}; p < 0.001). However, we found no difference in earthworm densities between land-use types (pasture: 49 ± 39 Ind. m^{-2}, cropland: 43 ± 45 Ind. m^{-2}) (Fig. 5A).

Furthermore, we detected a higher mean body size of endogeic earthworms in HAI (0.5 ± 0.3 g) than in HUM (0.5 ± 0.2 g; p < 0.05) as well as on the pastures (0.6 ± 0.2 g) compared to the croplands (0.4 ± 0.3 g; p < 0.001) (Fig. 5B). The mean body size of anecic earthworms was significantly higher in HAI (2.6 ± 0.5 g) compared to HUM (1.2 ± 0.7 g; p < 0.001). Additionally, the mean body size of earthworms on pastures (1.8 ± 0.9 g) was lower than on croplands (2.4 ± 0.9 g; p < 0.001) (Fig. 5B).

3.3.2. Plant communities and root biomass

On pasture sites, we found different proportions of grasses (e.g., *Alopecurus geniculatus*, *Elymus caninus*), herbs (e.g., *Capsella bursa-pastoris*, *Ficaria* spp., *Galium* spp., *Taraxacum*), and legumes (e.g., *Trifolium* spp.) which varied between HAI and HUM and land use types. The vegetation covered between 80 and 100 % of plot areas except for HAI cropland, where we recorded only 60–70 %. While we detected higher

proportions of grasses (59 ± 17 %) and herbs (26 ± 14 %) in HUM pastures compared to HAI pastures (grasses: 49 ± 21 %, herbs: 17 ± 9 %), the proportion of legumes was higher in HAI (20 ± 16 %) than in HUM (3 ± 5 %). We determined only one grass species on HAI cropland plots which covered 63 ± 5 %, whereas HUM cropland plots were covered by small grasses (39 ± 12 %) and herbs (12 ± 9 %). In HUM, pasture (4 ± 3 %) and cropland (43 ± 13 %) sites were additionally covered by moss, which we did not find in HAI.

We detected no difference in root biomass between HAI (16.2 ± 17.8 g m^{-2}) and HUM (12.5 ± 17.6 g m^{-2}). However, the root biomass of pastures (20.7 ± 20.6 g m^{-2}) was significantly higher compared to the croplands (5.2 ± 6.0 g m^{-2}; p < 0.001). Furthermore, with increasing soil depth, we found a notable decline in root biomass (p < 0.001) (15 cm: 24.7 ± 20.9 g m^{-2}; 30 cm: 10.9 ± 13.0 g m^{-2}; 50 cm: 2.4 ± 2.6 g m^{-2}) (Fig. 5C).

3.4. Piecewise SEM

Across all depths, we found appropriate model structures (*p* > 0.05; Table 2), where variables of all hierarchical levels affected the number and size of biopores (Fig. 6). At 15 cm depth, we found direct positive effects of root biomass and mean body size of anecic earthworms on biopores < 2 mm i.d. Furthermore, the anecic earthworm densities directly affected biopores of 2–6 mm i.d. Additionally, pH had a strong positive effect on biopores < 2 mm and 2–6 mm i.d. In contrast, we found a substantial reduction of biopores < 2 mm i.d. and 2–6 mm i.d. in response to SOC content. We also detected several indirect effects of soil parameters such as SOC content, pH, water content, and soil depth on the number of biopores. For instance, a higher SOC content led to an

Table 1

Earthworm communities of the study sites with density (Ind. m^{-2}) and biomass (g m^{-2}; in brackets).

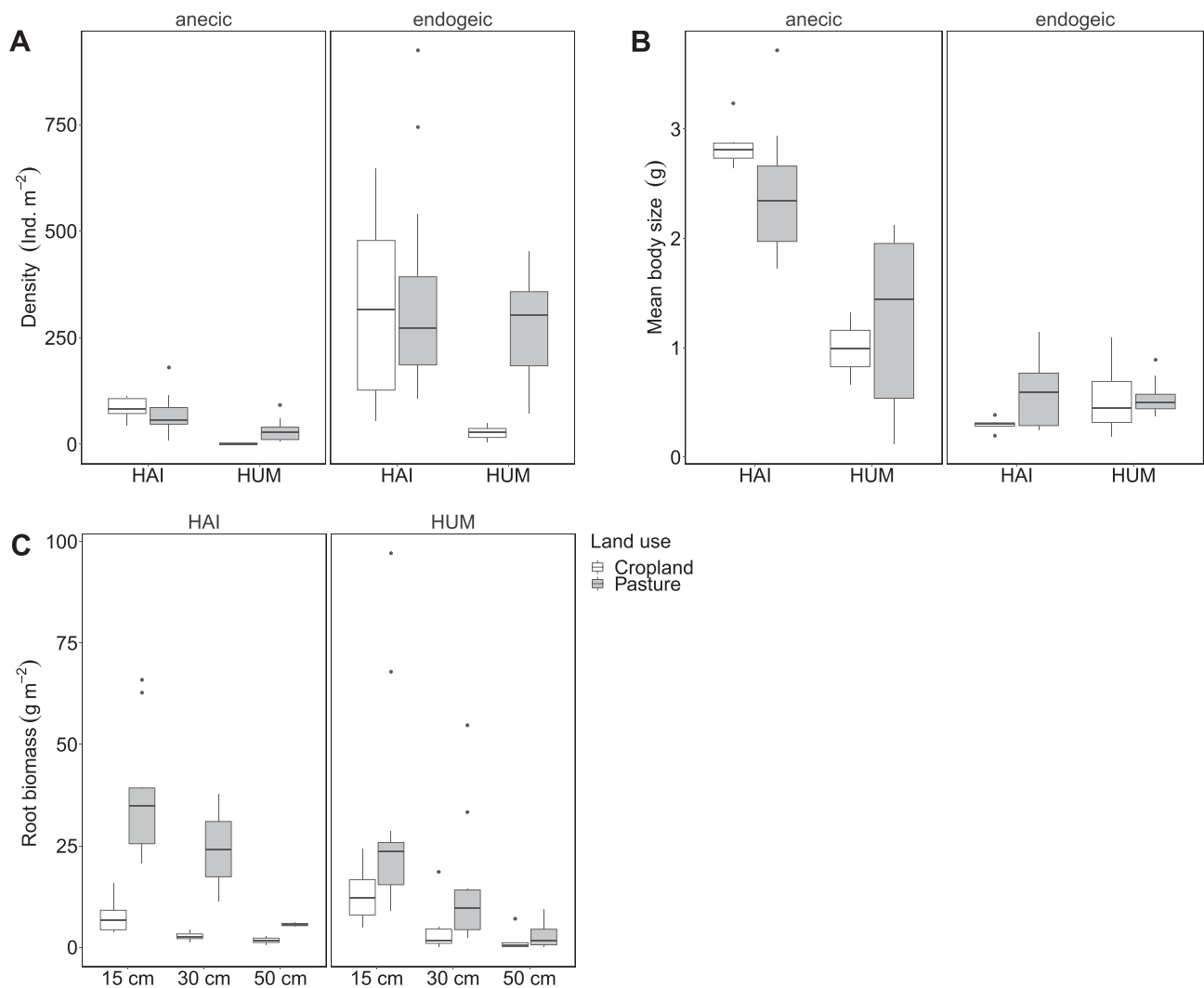|	HAI		HUM	
	Pasture	Cropland	Pasture	Cropland
Total earthworms	449 ± 256 (182 ± 78)	410 ± 236 (272 ± 96)	313 ± 136 (153 ± 74)	29 ± 19 (15 ± 13)
Anecic earthworms	72 ± 46 (91 ± 49)	84 ± 27 (207 ± 107)	31 ± 26 (30 ± 27)	1 ± 2 (1 ± 2)
Lumbricus terrestris	12 ± 6 (55 ± 30)	33 ± 24 (148 ± 126)	9 ± 7 (25 ± 21)	
Aporrectodea longa	7 ± 6 (3 ± 2)		4 ± 0 (3.2 ± 0.2)	
Endogeic earthworms	370 ± 250 (89 ± 46)	323 ± 235 (65 ± 42)	268 ± 126 (115 ± 68)	27 ± 16 (12 ± 11)
Aporrectodea caliginosa	100 ± 58 (46 ± 26)	33 ± 23 (14 ± 10)	66 ± 49 (55 ± 45)	10 ± 7 (9 ± 9)
Aporrectodea rosea	28 ± 17 (8 ± 5)	10 ± 7 (1.7 ± 0.8)	11 ± 5 (2 ± 1)	
Allolobophora chlorotica	4 (1)			
Octolasion cyaneum	8 ± 6 (18 ± 13)	4 (6)	7 ± 6 (11 ± 10)	
Octolasion tyrtaeum	8 (12)			
Epigeic earthworms	7 ± 8 (2 ± 2)	3 ± 3 (0.9 ± 1.2)	14 ± 10 (7 ± 6)	
Lumbricus castaneus	13 ± 7 (4 ± 2)	5 ± 2 (2 ± 1)	4 ± 0 (0.9 ± 0.1)	
Lumbricus rubellus			15 ± 9 (9 ± 6)	8
Juveniles				
Aporrectodea spp.	236 ± 207 (23 ± 18)	280 ± 255 (48 ± 38)	191 ± 95 (55 ± 27)	22 ± 10 (6 ± 4)
Lumbricus spp.	62 ± 43 (39 ± 22)	51 ± 33 (59 ± 32)	27 ± 26 (14 ± 14)	4 ± 0 (4 ± 2)
Octolasion spp.	4 (3)	8 (6)		
Diversity indices				
Shannon-Index	1.1 ± 0.3	1.02 ± 0.27	0.96 ± 0.34	0.06 ± 0.19
Evenness	0.7 ± 0.2	0.85 ± 0.09	0.7 ± 0.2	

Fig. 5. Distribution of anecic and endogeic earthworm density (A) and mean body size (B) across the study sites Hainich (HAI) and Hummelshain (HUM). The root biomass (C) for the study sites as a function of soil depth and land-use type cropland (white) and pasture (grey).

Table 2
Test results of the piecewise SEMs of the hierarchical systems for all three depths. The C statistics' p-value > 0.05 indicates that no relationships between variables are missing.

	Fisher's C	d.f.	p-value
15 cm	44.43	72	0.996
30 cm	59.07	100	1
50 cm	49.42	72	0.981

indirect increase in biopores < 2 mm i.d., via root biomass and the mean body size of endogeic earthworms. Furthermore, soil pH had a negative indirect effect on biopores < 2 mm via root biomass. Several indirect effects of soil depth on biopores of < 2 mm and 2–6 mm i.d. were also detected, ranging from a weak positive effect via pH and root biomass (0.13) to a medium negative effect (-0.46) via pH.

At 30 cm soil depth, the most substantial direct effects of land use were found on endogeic earthworm densities (-0.68) and mean body size (-0.84), as well as on biopores with 2–6 mm (0.47) and > 6 mm i.d. (0.78). A direct negative effect of land use was detected on root biomass and a positive effect on soil water content. We found substantial increases in the densities of anecic and endogeic earthworms with soil depth, a positive effect of soil water content on the mean body size of anecic earthworms, and a positive effect of pH on endogeic earthworm
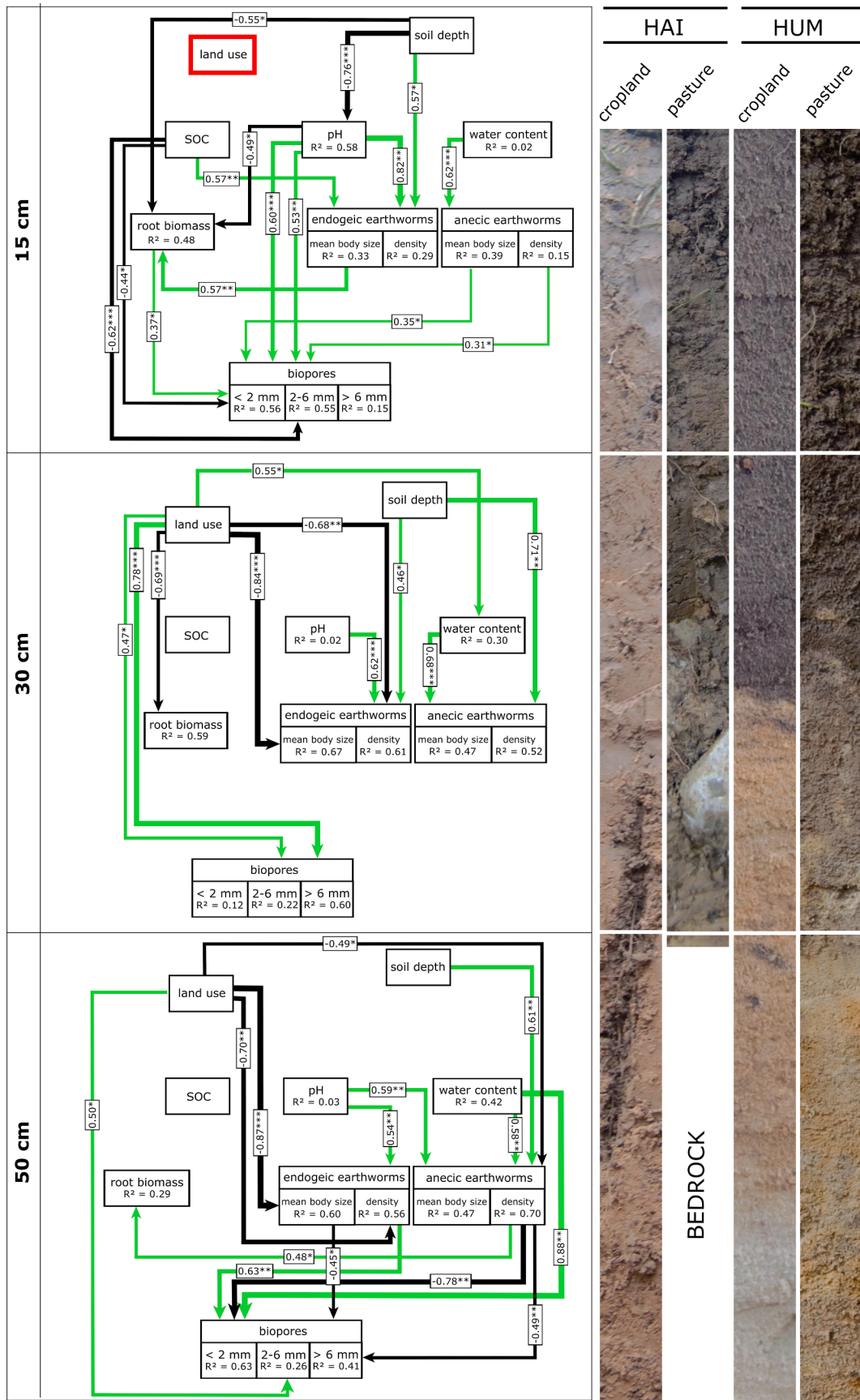
densities.

At 50 cm depth, the number of biopores was impacted directly and indirectly by variables on all hierarchical levels. We found direct solid positive effects of endogeic earthworm densities, land use, and soil water content on biopores < 2 mm. In contrast, there was a strong negative effect of anecic earthworm densities on biopores < 2 mm. Surprisingly, our model also revealed that greater endogeic earthworm body size and anecic earthworm densities reduced biopores > 6 mm. Land use exhibited both indirect positive and indirect negative effects on biopores. We found a negative effect via endogeic earthworm densities on the biopores < 2 mm i.d., in addition to contrasting positive indirect effects via anecic earthworm densities on biopores < 2 mm and > 6 mm. An increase of biopores < 2 mm was found as an indirect effect of pH via endogeic earthworm densities. Negative indirect effects of soil depth on biopores < 2 mm and > 6 mm i.d. were found via the densities of anecic earthworms.

4. Discussion

4.1. Biopore distribution

We found a maximum of 2925 biopores m^{-2} (HAI, pasture, 15 cm) and a minimum of 53 biopores m^{-2} (HUM, pasture, 50 cm), which varied enormously across plots and study sites. This high number of

Fig. 6. Hierarchical structural equation models testing for the causes of variation in soil biopore densities at 15, 30, and 50 cm soil depth. Significant positive pathways between the soil parameters (soil depth, soil organic carbon (SOC), pH, and water content), biopore-forming biota (endogeic and anecic earthworm densities and mean body size as well as root biomass), and the number of biopores (biopores) are indicated with green arrows and significant negative pathways with black arrows. If an arrow ends at a box with combined variables, the relationship only counts for the small box indicated by the arrow. The proportion of variation explained by predictors for each response variable (multiple R-squared) is denoted as R^2, and standardized path coefficients on the arrows indicate the relative strength of the pathway. Significance levels of pathways are *$p < 0.1$, **$p < 0.001$, ***$p < 0.0001$. The land-use variable in the 15 cm model was removed due to high multicollinearity and therefore marked in red to highlight the difference to the other models. Images on the right highlight the variability of the soil profiles between sites and land use types. White lines indicate the depth steps.

biopores exceeds the number of previous studies dealing with agricultural soils (Cey et al., 2009; Don et al., 2008; Ehlers, 1975; Pérès et al., 2010; Pitkänen and Nuutinen, 1997; Van Schaik et al., 2014), which had on average between 300 and 1000 biopores m^{-2}. However, the high variability of biopore numbers is in line with those investigations and depend on a variety of factors, such as food resource availability, pH, water content, and soil structure (Curry, 2004; Edwards and Bohlen, 1996; Lavelle, 1988; Logsdon et al., 2013). With increasing depth, the total number of biopores decreased. Furthermore, in the topsoil, roots positively affected small biopores, which disappeared at 30 and 50 cm depth. This is in line with the declining number of small biopores < 2 mm i.d. of up to 50% with depth increment due to a decreasing influence of roots. Additionally, a reduction of the biopore number can be caused by roots that occupy preexisting pores to grow into deeper soil horizons (Lucas et al., 2019). However, the decrease in soil depth was more pronounced in HAI than in HUM. On the HUM site, the variability of larger biopores (2–6 mm, > 6 mm) in 30 and 50 cm was smaller than in 15 cm, and their mean number did not decrease to the same extent in the subsoil as from topsoil to subsoil. An increasing number of large biopores with increasing depth can be explained by a lesser to no destruction of biopores below the plow pan (Capowiez et al., 2009). Furthermore, earthworm-formed structures like casts or burrows are physically more stable than bulk soil aggregates, enabling those structures to outlast their creators and be reused by other earthworms (Bastardie et al., 2005; Hagedorn and Bundt, 2002). This would lead to decoupling the number of biopores and earthworm densities in the long term. Schneider et al. (2018) described this effect as a "time delay" between the measured earthworm abundances and the counted biopores.

We found no significant effect of anecic earthworms of both densities and mean body size on biopores > 6 mm i.d. across all models. Next to anecic earthworms, taproots of fodder crops are known to leave biopores after decay in diameters similar to those created by earthworms (Athmann et al., 2017; Athmann et al., 2013; Kautz, 2015) and increase the number of biopores in agricultural fields (Kautz et al., 2014). These biopores enable roots of other plant species, e.g., oilseed rape, to acquire nutrients and water from the pore wall, even in subsoil horizons (Athmann et al., 2019; Han et al., 2015). Especially at the cropland of HUM, we found higher numbers of biopores than at the pasture site, which may be due to the taproots of fodder crops.

The 15 cm model revealed a significant positive effect of anecic earthworm mean body size on biopores < 2 mm i.d., and of anecic earthworm densities on biopores between 2 and 6 mm i.d. We attribute this to communities with a high number of juvenile and small individuals (>50 % juvenile earthworms in HAI and HUM; data of relative proportions not shown) with lower mean body mass and diameter than adult earthworms (Tab. 1). According to Schneider et al. (2018), juvenile earthworms should be included in the consideration of earthworm-biopore relationships as they may contribute to biopore formation in the topsoil. In addition, juvenile *Lumbricus terrestris* are reported to act more like epigeic earthworms, i.e., living in the litter layer or topsoil, and hence the biomass attributed to the adult *L. terrestris* correlates more strongly with large macropores than earthworm densities which may also include juvenile earthworms in many studies (Van Schaik et al., 2014).

Furthermore, natural decay of biopores or tillage may result in a diminishment and destruction of earthworm burrows, leading to

different ratios of the number of biopores per earthworm (Van Schaik et al., 2014). Consequently, direct proportionality between earthworm abundance and macropore cannot be mandated, as already stated (Capowiez et al., 2009). This is reflected in the absent relationship in the SEMs between anecic earthworms and large biopores. A significant pathway between endogeic earthworms and biopores could only be found in the 50 cm depth model, where earthworm densities positively affected biopores < 2 mm i.d. It is widely assumed that endogeic earthworms mainly live in the topsoil (Brown et al., 2000). But, endogeic earthworms burrow into deeper horizons to escape periods of drought or frost (Edwards and Bohlen, 1996; Lee, 1985). Hence, burrows of those estivation phases can persist in deeper horizons. Additionally, endogeic earthworms refill their burrows with excreted casts (Brown et al., 2000), which leads to a decrease in the actual pore diameter. Furthermore, through deeper incorporation of OM in the soil horizons by plowing, the densities of endogeic earthworms might be underestimated in the subsoil through a higher availability of food resources (Nuutinen, 1992).

In addition to the direct effects of endogeic earthworms on the number of biopores, indirect effects were also identified in the models, as endogeic earthworms positively affected root biomass. Endogeic earthworms can break up larger aggregates, reducing bulk density and releasing mineralizable C. Furthermore, fresh cast material contains plant-available nutrients (Blouin et al., 2013; Jouquet et al., 2006; Six et al., 2004), like K, consequently facilitating root penetration. Similarly, a direct positive effect of anecic earthworm densities on root biomass was identified at 50 cm depth. With increasing soil depth, burrows of anecic earthworms are preferential channels for root growth (Han et al., 2015; Kautz, 2015) as they also provide nutrients (Andriuzzi et al., 2016; Don et al., 2008) and contribute to gas exchange and water transport between top- and subsoil (Kautz, 2015). On the other hand, earthworms also feed on roots and root residues (Curry and Schmidt, 2007; Lavelle, 1988). As we did not detect this negative effect of earthworms, this interaction might be superposed by the beneficial effects that earthworms can have on root growth (Scheu, 2003; Van Groenigen et al., 2014).

4.2. Interdependencies of soil properties

HUM's groundwater-distant, less aggregated sandy soils showed low water contents, which decreased with soil depth. This might be one reason for the low densities of earthworms compared to the loamy and more drought-resistant soils in HAI that developed from loess deposits and limestone-mudstone-alternations (Kohlhepp et al., 2017). In contrast, soil water content and the densities of earthworms in the HAI sites were higher. However, deep-burrowing earthworms like *L. terrestris* demand sufficient depth of aerobic soil (Curry and Cotton, 1983). This feature was described in HAI soils as oxidative hydromorphic attributes compared to soils in HUM, where oxidative and reductive pedogenetic features were observed, indicating temporarily anaerobic conditions (Kohlhepp et al., 2017; Lehmann et al., personal communication). In general, earthworms require a certain level of soil water to maintain their cutaneous respiration and coelomic hydrostatic pressure (Lavelle, 1988).

Furthermore, the ambient soil water content affects their activity (Edwards and Bohlen, 1996), cast excretion (Scheu, 1987), food consumption (Amador et al., 2005; Daniel, 1991), and body mass (Amador

et al., 2005). During dry-out periods, earthworms respond with quiescence and escape into deeper soil horizons (Lavelle, 1988). Soil moisture regimes are affected by soil texture, where clay-richer soils are poorly drained at lasting field-moist conditions and may turn anaerobic. In contrast, sandy soils drain more freely and rapidly, yet they are more susceptible to drought. This may lead to reduced densities of earthworms in sandy soils.

Furthermore, the distribution of earthworms is tightly linked to soil depth according to their ecological functional group-specific digging behavior. This linkage is also reflected in the SEM models at 30 and 50 cm depth, where soil depth directly affected the anecic earthworm densities. Additionally, this points to the underlying cause of the low abundance of anecic earthworms on the HAI pasture since the dominant soils are shallow Rendzic Leptosols, in contrast to the cropland site that had Luvisols with more thick soil profiles.

The positive effect of pH on the earthworm communities was especially strong for endogeic earthworms and was additionally present in 50 cm for anecic earthworms. The optimum pH range among species is between 5.0 and 7.4 (Curry, 2004). Across both study sites, soil pH covered the lower boundary with pH around 5 at the HUM site and HAI cropland and the upper limit with pH up to 7 on HAI pasture and cropland topsoil. The influence of soil pH may be another reason for the lower earthworm densities on the acidic HUM site than the neutral to slightly alkaline HAI site. Additionally, the calculated standardized effects are relatively high, especially in the topsoil (Fig. 6), emphasizing the importance of pH for the abundance of earthworms. This observation coincides with results from experimental studies that have found a reduced tolerance of endogeic earthworms compared to anecic earthworms against low pH (Schrader, 1994). Observational studies also support the dominant effect of soil pH on earthworms at larger spatial scales (e.g., Fisichelli et al., 2013; Phillips et al., 2019; Rutgers et al., 2016). We found the highest number of endogeic earthworms on the HAI pasture site, exhibiting the most increased soil pH (around 6.8). Again, this relation is reflected in the SEMs, where soil pH directly affected the endogeic earthworm densities across the whole soil profile, which points to sensitivity to pH changes.

An effect on the mean body size of the anecic earthworms was only observed in the 50 cm depth SEM. This may be explained by a tolerance of anecic earthworms like *Lumbricus terrestris* against acidic pH (Schrader, 1994). This species has calciferous glands that allow calcium carbonate excretion to elevate the pH to a circumneutral range (Lee, 1985; Schrader, 1994). Hence, they can alter the physicochemical properties of their inhabited burrow in comparison to the bulk soil (Andriuzzi et al., 2013; Lipiec et al., 2015; Schrader, 1994). In contrast, endogeic earthworms, such as the most common species *Aporrectodea caliginosa*, do not have such glands due to their humus- and non-litter-based diet (Lee, 1985). Hence, these species, which also burrow continuously through the soil, might respond more sensitive to changes in pH to an acidic milieu than species like *Lumbricus terrestris*. A recent study by Lehmann et al. (2021) reported soil seepage pH below 3 on HAI pasture and cropland sites in summer. This might affect earthworms in different ways as the quiescence of endogeic earthworms may be reinforced to escape the acidic pore water or are, due to dormancy in the summer months, not affected at all.

Soil organic carbon (SOC) originates from the decomposition of plant litter and faunal tissues, microbial bio- and necromass, and various excretions and exudates of soil organisms and roots, which become incorporated in soil aggregates (Guhra et al., 2021; Totsche et al., 2018). As endogeic earthworms feed on such aggregates (Lee, 1985), SOC content affects the densities of endogeic earthworms. The SEM of soils supports this at 15 cm depth, where a direct positive effect of the SOC content on the mean body size of endogeic earthworms was found. For example, Hendrix et al. (1992) reported a significant positive correlation between SOC ($\leq$3%) and earthworm densities considering different soil textures, landscape positions, vegetation types, and management histories. In contrast, a negative relationship between SOC (about 5%) and

endogeic earthworms was found by Palm et al. (2013) for loess soils of a narrow catchment area. Yet, the sampling depth of the latter study was limited to the top 5 cm of the topsoil. As endogeic earthworms burrow to mean depths of up to 30 cm, the results of this study represent only a minor part of their foraging domain. In turn, anecic earthworms preferentially consume the SOC of the first 5 cm, which might explain the positive relationship in the analysis of Palm et al. (2013). In contrast, we found no effect in the model for soils at 15 cm depth, indicating that the SOC content at 15 cm does not play a significant role for anecic compared to endogeic earthworms. In contrast, no relations between SOC content and earthworms, in general, were found below 30 cm.

However, we found direct effects of pH, SOC, and soil water content on the number of biopores in the 15 cm and 50 cm depth models. SOC had an immediate negative effect on biopores. We explain this by diverse SOC sources that potentially influence the number and size of biopores. For instance, organic coatings of pores and SOC-enriched casts can decrease pore diameters or even occlude pores (Brown et al., 2000; Hagedorn and Bundt, 2002; Leue et al., 2010), and hence reduce biopore numbers. In turn, soil pH and water content had a direct positive effect on the number of biopores. Soil water holding capacity is contingent upon, among other factors, the texture-dependent soil structure and pore size distribution (Rabot et al., 2018). The pore system of fine-textured soils, as present in HAI, is organized hierarchically. It consists of primary pores between the textural grains and secondary pores between aggregates and macropores related to the biological activity (Lipiec et al., 2015). The biotic influence of structure formation includes the burrowing activity of soil fauna and stabilization through the root system (Oades, 1993). Earthworms can influence the soil structure by egesting casts and creating biopores. Thereby, up to 40–50 t ha^{-1} y^{-1} cast material can be deposited on the soil surface and even more belowground (Lee, 1985). Those casts can be considered (macro-)aggregates, commonly enriched in clay and organic matter (Schrader and Zhang, 1997). They are characterized by higher stability than the bulk soil aggregates when aged or dried (Shipitalo and Protz, 1988) and a different pore size distribution (Blanchart et al., 1999; Lipiec et al., 2015). Hence, the water retention capacity of soils can be enhanced by earthworm activity (Blanchart et al., 1999; Lipiec et al., 2015). Therefore, the substantial direct effect of soil water content on the number of biopores can be attributed to soil structure on the one hand, where a distinct pore system and stable aggregates can be maintained by organo-mineral associations formed by biogenic OM and clay minerals (Guhra et al., 2021), for instance in soils of the HAI.

On the other hand, earthworm activity plays a significant role in soil structure and biopore formation by affecting the water retention characteristics of the soil. Hence, the emerging feedback loops between water content, biopores, earthworms, and soil structure may lead to the observed pathways. However, our analysis cannot reflect such reciprocal effects because piecewise SEM cannot model feedback loops (Lefcheck, 2016). Schneider et al. (2018), finding similar direct effects of soil moisture and clay content on the biopore density, also drew this conclusion. Furthermore, the effect is more pronounced in the soils of the HAI, since burrow construction is fostered in structured and wet soils, where pores can be widened, and soil material can be easily ingested and egested (Oades, 1993). On the other hand, this was not the case in the sand-textured soils of the HUM site with a pore-system that allows for a more rapid and pronounced drainage, lower field capacity, and lower moisture contents, and the presence of free, sharp-edged quartz grains, which might injure earthworms (Lee, 1985).

The potential feedback loops between earthworms, soil structure, and soil water content may also play a role in the effect of soil pH, which had a direct positive effect on the number of biopores. As already discussed above, earthworms, e.g., *L. terrestris*, can actively affect their environment by excretion of calcium carbonate and cutaneous mucus. Furthermore, the carbonate content on the HAI pasture site is higher due to the limestone bedrock in comparison to the HAI cropland or the HUM site, resulting in higher pH values. Additionally, the weathering of

limestone bedrock leads to soil groups rich in residual clay. Hence, the well-aggregated soil structure may lead to higher stability of biopores and subsequently higher numbers of biopores (Oades, 1984). Thus, we interpret the direct positive effect of the pH on the number of biopores as a combined impact of soil type and soil structure.

4.3. Effect of land-use intensity

In addition to natural soil characteristics, land-use affects soil properties, vegetation, root biomass, and earthworm communities. These interrelations became apparent in the 15 cm SEM where the land use resulted in high multicollinearity among variables indicating a significant management effect between pasture (grazing) and cropland (plowing). Furthermore, especially for the first few centimeters, Horel et al. (2015) reported that conventional agriculture adversely impacts soil structure, hydraulic conductivity, bulk density, and water retention capacity. They further showed that tillage destroyed macroaggregates and, ultimately, resulted in the loss of soil carbon. Thus, these properties and changes have massive consequences for the living biota. Therefore, we decided to remove land-use as a predictor in the 15 cm SEM to explore further the effects of individual soil properties on biopore-forming biota and the number of biopores.

For instance, we found no anecic earthworms on the cropland site in HUM, which we attributed to frequent disturbance of the topsoil. Land use practices like plowing can have harmful and beneficial effects on earthworms, depending on the functional groups (Chan, 2001). In particular, anecic earthworms are adversely affected by plowing due to the destruction of their vertical burrows in the plowing horizon (Curry, 2004). Hence the abundance (Capowiez et al., 2009) and burrowing activity (Ernst and Emmerling, 2009) of anecic earthworms are reduced. However, an adverse effect of land-use intensity for anecic earthworms could only be verified in the 50 cm model. In turn, endogeic earthworm mean body size and densities were negatively affected by the land-use intensity at 30 and 50 cm depths. We attribute this to a reduced vegetation cover, which leads to a reduced input of litter and SOM, the primary food source of endogeic earthworms (Curry, 2004; Edwards, 1983).

Similarly, root biomass was negatively affected due to the reduced vegetation cover. In addition, a closer look at the diversity of the earthworm communities revealed a negative effect of invasive soil management, especially for the cropland site in HUM. This is in line with Smith et al. (2008), who attributed species richness and community composition to the changes in land-use intensity. However, the differences in earthworm communities between management types are marginal for the HAI site. We explain this by the recently stopped active management of the cropland site. Spurgeon et al. (2013) demonstrated by *meta*-analyses that transitions to lower intensity land use to increase the abundances and complexity of earthworm communities at relatively short periods of time.

Unexpectedly, an increase of land-use intensity had a positive effect on the number of biopores in 30 and 50 cm depth, especially on pores between 2 and 6 mm and > 6 mm i.d. This observation may be due to the inherited biopores below the plow pan by tap roots of crops such as, for instance, alfalfa plants (Angers and Caron, 1998). After root decay, large biopores remain protected from destruction by plowing due to a mean plowing depth of 25 cm. Similarly, earthworm burrows can outlast below the plow pan since the organic matter coatings of biopores stabilize the pore walls and thus, increase their persistence against different stresses (Kay and VandenBygaart, 2002). However, Pérès et al. (2010) found that there was no relationship between earthworm and biopore number regardless of land-use practices. They thus postulated that the biopore number is more an indicator for the activity of biopore-forming biota (and consequently soil quality) rather than their abundance.

5. Conclusion

Using a hierarchical structural equation modelling approach, we revealed relationships and mutual effects between biopores, biopore-forming biota, and soil properties. We found that the number of biopores was directly affected by earthworms and roots, land-use intensity, and soil properties. The effects of biopore-forming biota on the numbers of biopores diminished or even inverted with increasing soil depth. In deeper soil, the availability of earthworm food sources decreases; thus, the burrowing activity, especially of endogeic earthworms, is limited to the upper soil horizons. However, we found evidence that endogeic earthworms also impact the biopore system in the subsoil as they reside in deeper horizons under stress conditions, i.e., summer drought and winter frost. The missing relationships between anecic earthworms and large biopores substantiate that the burrow systems created by earthworms can outlast for extended periods, exceeding the lifetimes of earthworms that created the burrows. Hence, with time a dynamic interplay between burrow formation, utilization, destruction, and stabilization evolves, which results in the decoupling of direct relationships between earthworm abundance and biopore number in mature soils. From our results, we conclude that the biopore frequency is related to biopore-forming biota activity rather than their quantities in soils.

In the topsoil, soil properties and the formation of biopores by earthworms and roots are especially prone to alteration due to tillage. The strong influence of land-use intensity is punctuated by the SEM modeling results independent of the compared soil types. To explore the intricate relationships between the other factors, we excluded land-use intensity as it was highly collinear with other predictors and masked interactions among further investigated parameters. We infer the governing role of land-use intensity in the topsoil, which dominates all other parameters like soil properties and soil biota. The massive anthropogenic impact was also demonstrated by its ubiquitous effect across the subsoil horizons, like the adverse effects of intensive land use on biopore-forming biota in 30 cm and 50 cm depth. This exemplifies how sensitive the soil system, including its inhabitants, responds to anthropogenic manipulations. The results accentuate the need for sustainable and low-impact agricultural practices, e.g., no-tillage, to preserve soil structure, soil quality, and soil health. The cultivation of taproot-forming crops may positively affect the formation of biopores and consequently promotes bypass flow and soil aeration on arable fields. Properties like soil pH and water content were tightly linked to the number of biopores, highlighting their role in forming and stabilizing biopores. Hence, the number of biopores can be utilized as an indicator for soil structure, stability, and thus as a proxy for soil quality. Therefore, linear relationships depicted by SEM analyses are valuable to acquire functional relations between predictor and response variables utilized in the provided conceptional approach.

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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