Faculté des bioingénieurs

Effects of stand-specific composition on earthworm communities in the Arboretum of Tervuren

Comparative approach between broadleaves and conifers

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Avant - Propos

Au cours de la rédaction de ce mémoire, certains choix méthodologiques ont été posés et méritent d'être explicités. L'usage d'outils d'intelligence artificielle générative s'est limité à des opérations de reformulation, dans le but d'améliorer la clarté et la fluidité du propos. Son utilisation a également été sollicitée lors des analyses statistiques en tant qu'outil d'aide à l'utilisation du logicel RStudio et à la correction des scripts. Cet usage suit strictement les recommandations faites par l'UCLouvain dans sa note sur l'utilisation des intelligences artificielles génératives du 6 juillet 2023.

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Introduction

Temperate forests play a key role in climate regulation, carbon storage and ecosystem services. Today, however, these ecosystems are facing strong pressure from climate change, with impacts already noticeable in Belgium. In the face of these disturbances, forest resilience is becoming a major challenge, and one that requires a better understanding of the interactions between vegetation, soil and soil fauna. As ecosystem engineers, earthworms can influence the physical and chemical properties of forest soils, through litter decomposition, nutrient recycling and soil structuring. These functions directly support soil fertility and carbon storage, playing a role in the ability of forests to mitigate the effects of climate change. The composition of forest stands, and in particular the distinction between broadleaves and coniferous trees, modulates microclimate and soil conditions, through litter quality, decomposition rate, pH and nutrient availability. These conditions influence the composition and biomass of earthworm communities.

The main objective is to determine the differences in earthworm communities, in terms of abundance, biomass and species distribution, between coniferous and broadleaved tree species. The second objective is based on the ability of trees to modify their environment through litter, soil and microclimate characteristics and the influence these characteristics have on earthworm communities. The research is based on an experimental set-up installed in the arboretum of Tervuren (Belgium), in which 14 monospecific stands were selected. This unique common garden provides a wide range of tree species, exotic and native, coming from various regions of the world. These trees grew up in particular conditions, climate, soil forming processes and soil types, being homogenous inside the arboretum and allowing direct comparison between tree species. The earthworm sampling was conducted in the field during the months of September and October. The collected earthworms were then identified in the laboratory after preservation in ethanol.

After presenting the background and objectives, this document describes the experimental set-up, and the methods used to process and analyze the data. The main results are then presented, highlighting the correlations observed between the different variables. The effect of tree species is examined, both in its direct dimension on earthworm communities, and in its indirect effects via changes in abiotic properties. Finally, all these results are discussed in the last part of the paper.

State of the art

1. The importance of forests in the context of climate change

1.1. Implications of climate change for forests

Forests are central to the functioning of terrestrial ecosystems, covering approximately 31% of the Earth's land surface (FAO 2020). Among them, temperate forests, located in eastern North America, northeastern Asia, and central and western Europe, represent 16% of the global forested area. They play key roles in climate regulation, biodiversity conservation, and provision of ecosystem services. However, these ecosystems are facing major challenges due to climate change (FAO 2020).

In the short term, the rise in atmospheric carbon dioxide (CO_2) concentrations and temperatures may show positive effects on trees by enhancing photosynthesis and stimulating growth, to some extent (Leakey et al. 2009; Zhu et al. 2017). Nevertheless, climate change is expected to cause substantial negative impacts on forests, ultimately outweighing these initial benefits (Ciais et al. 2005; Mina et al. 2022). The rising frequency and intensity of extreme events such as droughts, heatwaves, floods and storms (Lindner et al. 2010), combined with the growing spread and outbreaks of pests (Lindner et al., 2010; Volney and Fleming, 2000), pose serious threats to forest resilience and their capacity to function as carbon sinks (Mina et al. 2022). Furthermore, due to their long lifespan, trees possess a limited ability to adapt rapidly to swift environmental changes (Lindner et al. 2010).

Therefore, the need for resilient forests, capable of facing climate change and its consequences, is of paramount importance. A resilient forest refers to a persistent system and its ability to preserve its structures and functions against change, here climate change (Mina et al. 2022).

1.2. Specific focus on temperate forests

Although climate change occurs at a global scale, its impacts vary across regions, including forest ecosystems. In Belgium and across the temperate oceanic zone, mean annual temperatures are rising and are expected to be 2.5-3.5°C higher in the coming years (Lindner et al. 2010). Extreme events such as droughts and heatwaves are becoming more frequent and severe, and pest and disease outbreaks are also on the rise (IRM - Rapport climatique 2020; Lindner et al. 2010). A notable example is the 2018 bark beetle crisis, which was especially damaging for spruce trees in Europe, including Belgium, and was driven by particularly hot and dry weather conditions (Saintonge et al. 2021). In response to these changes, plants must adapt quickly or migrate. Unfortunately, such adaptations require time, whereas climate changes are occurring at an accelerated pace (Gray and Hamann 2013; Zhu et al. 2012). Observed rates of plant migration average around 500 meters per year, but projections suggest that migration rates of 3,000 - 5,000 meters per year are necessary for their survival under current climate change scenarios, approximately ten times faster than observed (Aitken et al. 2008; Davis and Shaw 2001).

To meet these challenges and sustain forest resilience, management strategies must be adapted. This includes both forest management practices and the selection of the tree species, whether native or exotic. It has been shown that tree diversity has a very positive impact on the resilience of forests to climate change, improving the survival and growth of trees in mixed-species stands (Paquette et al. 2018). The extent of the impacts of climate change also depends on the resilience of the society and natural ecosystems affected by those (Schröter et al. 2005).

Furthermore, with ongoing climate change, and especially the rising of temperatures, some exotic tree species that were previously unable to survive in a region may now thrive in these regions. In fact, climate change may lead to a shift in the spatial distribution of trees (Boucher-Lalonde, Morin et Currie 2012) to higher altitudes (Lenoir et al. 2008) and latitudes (Cramer et al. 2001). As mentioned earlier, the effects of climate change are already visible in Belgium and some species that were well-adapted a few years ago, are now struggling and must be replaced or complemented with exotic species. This deliberate human intervention, planting exotic species to accelerate their migration beyond natural rates, is referred to as assisted migration. Hence, this process can be of paramount importance in the survival of forests. It may help prevent species extinction while sustaining ecological services and forest functions but also biodiversity (Aubin et al. 2011; Schwartz et al. 2012; Ste-Marie et al. 2011; Winder et al. 2011). However, this assisted migration must be implemented with caution as some introduced species may become invasive and outcompete native species, already weakened (Vitt et al. 2010).

Temperate forests play crucial roles, especially for human societies. Some of these roles are commonly referred to as ecosystemic services, defined as "the benefits that people obtain from ecosystems, the direct and indirect contributions of ecosystems to human wellbeing" (TEEB, 2010). Forests therefore provide a wide range of such services: cultural services (e.g., recreation and education), regulating services (such as water cycling, climate regulation and soil conservation), provisioning services (including timber and food production), and finally supporting services (such as nutrient cycling and photosynthesis) (Reid 2005).

1.3. Influence of forests on the carbon cycle

Ecosystems can influence and regulate the climate through carbon storage, with the soil being one of the most important carbon reservoirs. In fact, organic compounds in soils contain more carbon than the combination of the atmosphere and vegetation (Jobbágy & Jackson 2000). In forest ecosystems, approximately 44% of the total carbon is stored in the first meter of soil (Pan et al. 2011). A potential strategy to mitigate the CO_2 concentrations in the atmosphere, and therefore climate change, is to increase the amount of organic carbon sequestered in forest soils, enhancing the role of European forests as carbon sinks (Lal 2005).

The amount of carbon stored in the forest floors and soils can be influenced by forest management practices. Tree species, through the quantity and quality of litter they produce, as well as thinning regimes, can modify the composition the forest floor, enhance the stimulation of decomposition and impact the carbon storage (Jandl et al. 2007). Consequently, tree species are also able to define the rate of carbon accumulation and its distribution along the soil profile (Jandl et al. 2007). These considerations on the role of tree species in carbon storage underline the importance of studying their differentiated effects on soil and litter. In this context, experimental plantations such as arboreta represent valuable tools.

1.4. Construction of arboreta and tree planting trails outside native ranges

An arboretum can be defined as "A place with an orderly, documented, labeled, collection of living plants, that is open to the general public, with collections used principally for research and education" (Watson et al. 1993). Experiments conducted in arboreta are often referred to as “common garden experiments”. This term is used to describe experimental designs where organisms from different origins are grown together in a shared environment with uniform conditions (Berend et al. 2019). In Belgium, when the Arboretum of Tervuren was initially established, its aim was to evaluate the ability of exotic species, mainly from North America and Asia, to adapt to the local environmental conditions of Belgium, to assess their wood production potential, and to determine whether they could expand the still relatively limited range of European tree species (Royal Trust 2020). Nowadays, the arboretum serves as a valuable tool for identifying which tree species are most likely to adapt to future conditions in this region and how.

2. Impacts of tree species on earthworm communities

The presence of a tree species, native or introduced, in a specific area depends on various abiotic and biotic factors such as climate, soil type, soil properties, water regime, and understory vegetation. In return, it is also well established that they influence the environment in which they grow. This influence can be seen through creation of a microclimate induced by the canopy cover (De Frenne et al. 2019), the quality and quantity of litter that falls to the ground (Pastor et al. 1993; Berendse 1998) and the modification of soil characteristics (Reich et al. 2005).

2.1. Influence of trees on microclimate

Trees have the ability to modify the temperature and moisture parameters under forest cover, at the forest floor and therefore, within the soil (De Frenne et al. 2019). These local conditions, which differ from the regional climate, define what is called the microclimate, with effects depending on the tree species, growth and mortality (Jucker et al. 2018). This influence is mostly due to their canopy cover, through its height, density and roughness (Hardwick et al.

2015). The microclimate created by forests influences all organisms living underneath the canopy cover and serves as a thermal buffer, limiting important temperature variations (De Frenne et al. 2013; Frey et al. 2016; Jucker et al. 2018; Scheffers et al. 2013; Senior et al. 2018). Therefore, forests can reduce the air warming and cooling under their canopy compared to surrounding open areas (De Frenne et al. 2019). Moreover, areas close to the ground are protected by tree stems and canopy from solar radiation (von Arx et al., 2013). Air temperature influences soil temperature, which is affected by a few factors including air temperature and solar radiation, both influenced by the canopy cover (Paul et al. 2004). The spatial arrangement of trees also drives shading, air mixing and evapotranspirative cooling (Geiger et al. 2003; von Arx et al. 2013; Zellweger et al. 2019).

Soil moisture is affected by forest cover through canopy interception, root water uptake, litter layer retention and soil infiltration. The precipitation regime can be modified by the tree canopy as it intercepts and redirects precipitation, therefore modifying the quantity of water that comes to the forest floor (Demir et al. 2024). Water infiltration in the soil is influenced, among others, by tree roots which creates macropores enhancing infiltration (Lange, Lüescher & Germann 2009).

Plant litter plays a key role in regulating soil moisture, acting as a barrier that reduces evaporation by limiting the diffusion of water vapor and lowering soil temperature, thus promoting water retention. It also improves infiltration by protecting the soil from the direct impact of raindrops (Facelli & Pickett 1991). Hence, it helps reducing runoff, although in some cases very dense litter can increase the latter or cause lateral runoff. The overall effect varies according to the characteristics of the litter (deciduous leaves or conifer needles), the shading of the canopy cover, the type of rainfall, the condition of the litter (sometimes hydrophobic when dry) and its spatial distribution, which creates soil moisture heterogeneity (Facelli & Pickett 1991). Finally, some water can be retained and evaporated directly by the litter, which can reduce water availability for plants (Facelli & Pickett 1991).

According to Peng et al. (2022), the soil macrofauna is affected by the microclimate created by trees not only through temperature and moisture modifications but also as it influences their food resource availability.

2.2. Influence of trees on soils and nutrient cycle

Trees grow into the soil and therefore rely on it for their survival, but they are not completely passive in their interactions with it. In fact, they can modify certain soil properties such as its structure, through their roots, or its pH (Mueller et al. 2012). For instance, the leaf litter of some coniferous species is composed of less amounts of exchangeable base cations which contributes to soil acidification (Reich et al. 2005). Several studies have also shown that a reduction of the soil solution pH was caused by evergreen gymnosperms (Augusto et al. 2015). Some causes were determined to explain this acidification: (1) the chemical composition of the litterfall being more acidic and with less nutrients (Hansen et al. 2009), (2) different nutrient absorption rates (de Schrijver et al. 2012), (3) different production of acid exudates in the rhizosphere, (4) dissimilar production of organic acids during litter decomposition (Augusto et al. 2015). However, this acidification remains relative and does not imply that soils are always acidic under coniferous stands and never under deciduous stands. Soils can become more acidic due to atmospheric deposition of acidifying pollutants, regardless of the tree type (Augusto et al. 2015).

2.3. Influence of trees on litter decomposition

2.3.1. Definition of forest litter

Forest above-ground litter is predominantly constituted of coniferous needles or deciduous leaves depending on the type of tree (Kögel-Knabner 2002). Branches, bark and fruits usually represent a small part of this litter (20 - 40% in coniferous stands according to Millar, 1974). The predominant organic compounds found in plant litter are polysaccharides, cellulose and hemicelluloses, and lignin (Kögel-Knabner 2002). The percentages and composition of these structural compounds depend on the litter type, differing between species, tissues, and tree type. The compounds show different degradability. The leaf litterfall composition therefore influences the decomposition rates (Kögel-Knabner 2002)

2.3.2. Definition of litter decomposition

The reduction of litter to its elemental chemical constituents (CO_2 and nutrients) is what decomposition refers to. This consists of several processes occurring simultaneously including breakdowns and transformation of the chemicals into afresh materials (Prescott & Vesterdal, 2021). First, decomposition involves physical processes. The detritivores, such as earthworms, break down the litter into smaller pieces and consume it. These physical processes increase the contact surface making it more accessible to microbial decomposition. Then, this allows microorganisms (bacteria and fungi) to perform chemical processes. The smaller pieces of organic matter are reduced and mineralized by those organisms into basic inorganic molecules (Aerts 1997).

This process is crucial for plants and other organisms relying on the nutrient cycle because it allows nutrients to return to the soil after the leaf fall or the death of the vegetation. This constitutes the recycling of the nutrients (Krishna & Mohan 2017). Fast litter decomposition is associated with less forest floor mass and rapid return of the nutrients to the plants, meeting their nutrient needs quicker. On the opposite, a slow decomposition process leads to higher organic matter and nutrients stocks in the soils, causing an accumulation of forest floor (Krishna & Mohan 2017).

Beyond nutrient cycling, litter decomposition plays a crucial role in carbon dynamics within forest ecosystems. The balance between litter input and decomposition influences soil carbon storage and its function as a carbon sink or source. Higher temperatures can accelerate decomposition, to a certain level, leading to increased CO_2 emissions and potentially reinforcing climate change through positive feedback mechanisms (Davidson & Janssens, 2006). However, the rate of decomposition is also regulated by litter quality and soil microbial communities, which can modulate carbon release independently of climate effects (Bradford et al., 2016).

In soils, the primary resources for organic matter formation are provided by plant litter materials mainly the tree leaves and understory vegetation if present (representing less the 5% of the litter inputs in temperate forests according to Kögel-Knabner (2002). Litter is therefore characterized by its quantity, chemical composition and properties. These factors are fundamental and they control the formation of soil organic matter (Swift et al., 1979; Scholes et al., 1997). The secondary resource being the microbial residues and exudates (Kögel-Knabner, 2002).

2.3.3. Factors influencing litter decomposition

Litter decomposition rates are influenced by biotic and abiotic factors. According to Aerts (1997), the most important one is the (micro)climate, through temperature and moisture, even though its impact is indirect. The rates of decomposition tend to be higher when the temperatures are higher because it increases the microbial activity in the soil (Krishna & Mohan 2017). This factor, the climate, and the disturbances due to climate change, influence the composition of the vegetation, the species. This will therefore later determine the litter composition (Prescott 2010).

The other abiotic factors affecting litter decomposition are soil chemical and physical properties. The principal physical properties, porosity, water retention, surface area, permeability, and nutrient dynamics, are conditioned by soil texture. The chemical properties are mostly pH, cation exchange capacity, organic matter content and nutrients with the organic matter content being the prevailing factor (Krishna & Mohan 2017).

When the abiotic factors are constant between sites, the biotic factors, litter traits and soil organisms, become the predominant factors (Aerts, 1997). Other studies (Tao et al. 2019 ; Prescott 2010 ; Cornwell et al. 2008) stated that the litter chemistry is in fact the predominant factor regarding the decomposition rates. Coûteaux et al. (1995) nuances and said that biotic factors are actually dominant when the climatic factors are favorable.

The leaf litter quality is determined by the nitrogen concentrations, carbon/nitrogen (C/N) ratio and phenol/lignin concentrations. Litter decay is strongly correlated with the initial ratios of C/N, lignin /N, or lignin/cellulose where the decomposition rate is higher when the C:N ratio and lignin content are lower and calcium (Ca) content is higher (Castellano et al. 2015).

Finally, the organisms living in the soil, the soil biota including microfauna and macrofauna, and their abundance must be taken into account (Aerts 1997; Coûteaux et al. 1995; Swift et al. 1979). Among microfauna, the decomposers that have the possible greater impact on decomposition are fungi. They are followed by bacteria (Krishna & Mohan 2017). Litter decomposition in greatly impacted by the decomposer community (Swift et al. 1979; Heal et al. 1997). Earthworms are part of this decomposer community and play a crucial part in litter decomposition, but this is not the only role they play.

2.4. Influence of trees on soil macrofauna

Tree species have different litter composition and the quantity that falls to the ground also depends on the tree type. Therefore, they affect the quantity and quality of litter that lies on the forest floor and is available for decomposers (Pastor et al. 1993; Berendse 1998). Even though soil conditions might not be favorable for earthworm presence, it might be compensated by favorable quality litter (Schelfhout et al. 2017). However, the positive effects of litter quality are lower than with nutrient-rich soil (Cesarz et al. 2016).

An important driver of earthworm presence seems to be the Ca concentration of the leaf litter. Reich et al. (2005) found that trees with higher concentration of Ca in their leaf litter hosted more numbers and species of earthworms. A conclusion also found by Hobbie et al. (2006).

Another study, conducted by Schelfhout et al. (2017), observed differences in the effect of litter Ca between ecological groups and that tree species don't affect earthworm groups and species equally. Indeed, when it wasn't an explanatory factor for the presence of epigeic species, it showed a positive effect on anecic species density. But this effect was even more positive when soil Al concentrations were low. They also concluded that the build-up of the forest floor caused by lower rates of litter decomposition and higher concentration of soil exchangeable Al was a consequence of the absence of burrowing species and then negatively impacted the earthworm communities. This absence of anecic earthworms in the first place was attributed to low concentrations of Ca in litter.

3. Importance of earthworms in litter decomposition

3.1. Description of earthworms

Earthworms are invertebrates belonging to the phylum *Annelida* and the subclass *Oligochaeta* (Earthworm Biology | Earthworm Society of Britain n.d.). They represent a key component of the soil macrofauna, with approximately 6,000 species identified worldwide (Jänsch et al. 2013). As saprophagous organisms, earthworms feed primarily on decaying organic matter (Earthworm Biology | Earthworm Society of Britain n.d.).

Marcel Bouché (1977) established an ecological classification system for earthworm species that remains widely used today. This system groups species into ecological categories based on morphological traits, habitat preferences, and feeding habits. There are three major ecological categories that are epigeic, endogeic and anecic.

Epigeic earthworms are the smallest and inhabit the litter layer or the uppermost layers of the soil. They mainly consume decomposing plant material (De Wandeler et al. 2016). Endogeic species are of intermediate size and live in horizontal burrows within the soil matrix (De Wandeler et al. 2016). As geophagous organisms, they ingest and process large amounts of soil, mixing it with organic material, carrying out bioturbation. Their diet consists largely of humified soil organic matter and decaying roots (Schelfhout et al. 2017). Anecic earthworms are the largest, they create deep vertical burrows that extend further into the soil. They feed on leaf litter, which they actively drag into their burrows (De Wandeler et al. 2016). They enhance the input of organic matter to the soil as they incorporate litter into deeper soil layers (Binkley 1995; Prescott 2010; Quideau et al. 1998; Vesterdal et al. 2013).

Although these ecological categories are still widely used, they are sometimes oversimplified or misapplied. Bouché (1977) originally defined seven categories, with epigeic, endogeic, and anecic species positioned at the three vertices of a conceptual triangle. While some species fit clearly within one category, others exhibit intermediate traits, with different life stages or individuals falling into multiple categories. For instance, some species can be classified as epi-anecic, displaying characteristics of both epigeic and anecic species. Furthermore, it is essential to recognize that Bouché's classification defines ecological groups rather than functional categories. It is therefore important to interpret with caution the presence of species observed on a study site, as their belonging to an ecological group does not necessarily reflect their functional role in the ecosystem (Capowiez et al. 2022).

3.2. Factors influencing earthworm communities

Depending on the scale, regional, local or continental, the factors influencing earthworm presence and abundance differ in nature and impact (De Wandeler et al. 2016). When regional and continental scales are considered, the main factors explaining the earthworm presence and abundance are climate, land use history and dispersal possibilities of the earthworms (De Wandeler et al. 2016). On the local scale, which is the scale this research focus on, their presence is mostly conditioned by vegetation and soil characteristics: soil texture, pH, base saturation, soil moisture content, organic matter content, nutrient content and aluminum toxicity (Desie et al. 2020; Lavelle et al. 1997).

The key factors influencing earthworm presence also depend on the ecological group to which the earthworms belong. Epigeic and anecic species are primarily driven by litter traits, while endogeic species are more influenced by soil characteristics, according to Schelfhout et al. (2017). Muys et Granval (1992) also stated that soil acidity was negatively affecting especially burrowing species.

Where leaf litter calcium has been proved to enhance earthworm presence, an increase in the soil exchangeable aluminum (Al) concentration in the soil could negatively influence their biomass (Desie et al. 2020; Peng et al. 2022). High exchangeable Al concentrations in soil, when pH values are lower than 5, could inhibit earthworm growth and cocoon production as base cations are removed from the CEC and replaced by Al^{3+} (Schelfhout et al. 2017). Some earthworm species such as *Lumbricus sp*., anecic species, require important Ca contents because they need to produce $CaCO_3$ through their calciferous gland. This allows the reduction of CO_2 in their blood and the regulation of blood pH (Hobbie et al. 2006). Therefore, some species are absent in forest floor with litter poor in Ca except if there is a local enrichment of Ca in the soil due, for instance, to a limestone layer (Ponge et al. 1999).

It has been widely discussed that the pH was one of the most important factors explaining earthworm presence and biomass. In fact, some species are completely absent in acidic soils (Jänsch et al. 2013) and growth and development tend to not be favored by low pH values (Edwards & Arancon 2022). One of the reasons is that in acidic soils, aluminum is found in its soluble form, Al^{3+}, which is a toxic form for earthworms. However, some species thrive in acidic soil and others tolerate a large range of pH values (Edwards & Arancon 2022).

Soil moisture is another important factor as earthworms are mainly constituted of water (Grant 1955) and their growth can be limited with low moisture rates in soils (Baker & Whitby 2003a, b; Berry & Jordan 2001). Nevertheless, the water requirements vary between species and regions, and they haven't been determined for all earthworm species (Edwards & Arancon 2022). Earthworms are able to avoid dry soils by moving to a close area with more moisture. If they fail, they will start to lose the water from their body in order to survive (Roots 1956).

Being poikilothermic organisms, earthworm behavior and activity are directly affected as well by the temperature of their environment, the temperature of the soil. However, the effects differ from one species to another. They each possess a survival range on temperature outside which they can't live. The soil coverage is very important to keep temperatures suitable for them in the soil. If the temperatures are too high and the soil too dry, earthworms can temporarily migrate into the deeper layers of the soil to find lower temperatures and higher moisture. If the unfavorable conditions persist, they can stay in an inactive state that is called "quiescence". As soon as the environmental conditions become suitable again, they can be active. At the same time each year, earthworm usually remain in another inactive state: "obligatory diapause". This state occurs every year at the same time period and is independent of current environmental conditions (Edwards & Arancon 2022).

3.3. Roles of earthworms in forest ecosystems

Not only the earthworm communities are influenced by their environment, they can also modify it to a certain level. A few studies observed cause-effect relationships between earthworms and soil properties (Bohlen & Edwards 1995; Cesarz et al. 2016; De Wandeler et al. 2016; Schelfhout et al, 2017). Others found the existence of a positive feedback loop between earthworms and their environment (Desie et al. 2020).

Earthworms are considered to be ecosystem engineers as they play a role in important processes shaping the environment (Gonzalez et al 2003). They perform bioturbation, mostly endogeic and anecic species, which is made by the physical movement of soil by the earthworms. Some species, belonging to the anecic ecological category, carry litter and organic matter from the top layer of the soil to the deeper layers through vertical burrows (De Wandeler et al. 2016).

They also enable and control the rates of organic matter decomposition which later provides more nutrients for plants. Therefore they have an indirect influence on primary production (De Wandeler et al. 2016; Jänsch et al. 2013). Even though microbial decomposition doesn't need the earthworm presence to occur, it is more effective when they are as they provide broken-down plant residue, which are dispersed underneath the surface and distributed among the soil profile (Curry 2004).

3.4. Consequences of climate change on earthworm populations

The survival of the earthworm communities will depend on the dispersion capacity of the earthworm species. As their natural environment become unsuitable for them to live, the temperatures being too high and the soil being too dry added to the more frequent and severe droughts across Europe, some earthworm species have the capacity to relocate to other places. They will try to find more suitable living conditions with more moisture and lower temperatures. These species are then capable of colonizing new sites and modify the ecological processes happening (Fourcade & Vercauteren 2022). Species can also adapt locally and migrate to deeper soil layers where temperatures are lower and moisture rates higher (Fourcade & Vercauteren 2022; Singh et al. 2019). However, the activity of earthworms can be limited by very high temperatures, restraining their ability to travel either vertically or horizontally (Singh et al. 2019).

The impact of climate change on earthworms is therefore mainly indirect. Changes in temperature, soil moisture and vegetation composition might induce changes in earthworm communities by modifying their environment which can become unsuitable for them (Singh et al. 2019).

As earthworms are very sensitive to soil temperature, they can be negatively affected by temperatures outside of their tolerance range, the temperatures being lower or higher (Singh et al. 2019). According to Bohlen & Edwards (1995), the ideal temperatures range between 10°C and 40°C. Outside of this range, earthworms are still able to survive but their activity and other needed processes are limited but some species are still able to survive. This ability to survive depends on the species and where they thrive. For example, some species lives in northern countries where temperatures can be very low, and others thrive in arid environment with high temperatures (Edwards & Arancon, 2022; Singh et al. 2019).

Objectives and research question

The main objective of this master's thesis is to characterize the composition of earthworm communities, considering their abundance, biomass, species, and ecological categories, under tree stands with different species compositions. The study site selected for this purpose is the Arboretum of Tervuren, located in Belgium. To achieve this objective, 14 monospecific stands within the arboretum were carefully selected.

This research is guided by the following main research question: *Under similar initial site conditions, can we observe differences between a range of tree species, broadleaves and conifers, through their impact on microclimate, soil, and leaf litterfall characteristics, in terms of earthworm community composition*?

This research question assumes that site conditions, including climate, soil texture, and soil formation processes (pedogenesis), are homogeneous across all considered stands.

Several hypotheses were defined: (1) Broadleaved stands host a larger and more diverse earthworm community than coniferous stands, (2) Tree species influence the composition and structure of earthworm communities through their effects on soil characteristics (pH, base cation content, exchangeable Ca and Al concentrations), leaf litterfall characteristics (C:N ratio, Ca and Al concentrations) and microclimate (soil temperature and moisture), (3) High soil exchangeable Al (and/or leaf litterfall Al) concentrations negatively affect earthworm presence, while high Ca (and/or leaf litterfall Ca) concentrations have a positive impact.

The next figure aims to visually represent the different compartments and interactions considered in the objectives and hypotheses of this master thesis.

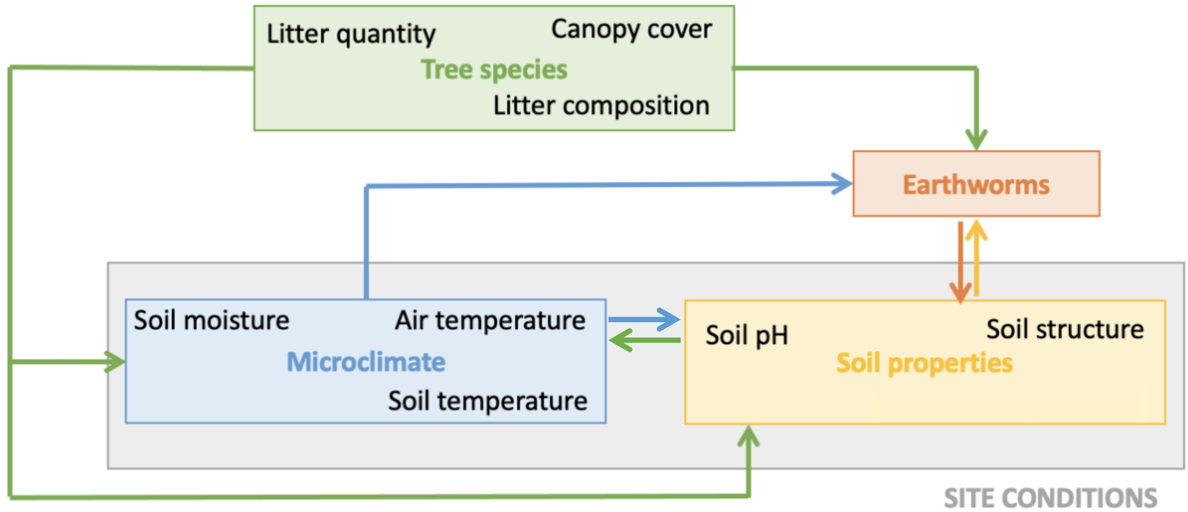


Figure 1: Conceptual scheme representing the factors influencing earthworm communities considered for this research.

Materials and methods

1. Research framework

This research is part of the doctoral thesis conducted by Joachim López, entitled: "Disentangling effects of litter quality and biotic soil regulation on litter decomposition processes in forests of the northern hemisphere using a synthetic community approach". The thesis aims to assess the relationships between the above-ground vegetation and decomposition processes in a unique common garden, the Arboretum of Tervuren.

This thesis studies how the tree species composition influences litter decomposition in the initial phase. It aims to distinguish between the direct effects of litter inputs and the indirect effects of the decomposition environment (microclimate, soil, biota). Tree diversity, abiotic conditions and decomposer communities are characterized to explain decomposition variations. Non-linear structural equation models are then used to identify causal relationships between above- and below-ground processes.

2. Description of the study area

The Arboretum of Tervuren is a unique botanical collection located in central Belgium, on the outskirts of Brussels (Figure 2). It is an integral part of the Sonian Forest, a 5,000-hectare periurban forest situated southwest of the capital. The arboretum belongs to the Royal Trust and is a part of its public patrimony (Royal Trust 2020).

2.1. Historical and scientific significance

The arboretum was established in 1902 under the direction of Professor Charles Bommer with the primary goal of exhibiting and studying tree species from around the world in a controlled environment. While its initial purpose was scientific research, the site has evolved to fulfill additional roles, including species conservation, education, and recreation (imearth-adm 2023).

Unlike conventional arboreta, the Arboretum of Tervuren is both a geographical and forest arboretum. The geographical designation refers to the spatial organization of tree species according to their natural regions of origin, while the forest aspect highlights the fact that the arboretum was planted as a forest ecosystem and is maintained using silvicultural management practices (Royal Trust 2020).

Figure 2. Map showing the geographical location of the Arboretum of Tervuren within Belgium (red dot).

2.2. Composition and structure

With more than 700 tree species and approximatively 30,000 tree individuals on a surface of 120 ha, the Arboretum of Tervuren is one of the most important in Europe. The vast majority of tree species originate from the northern hemisphere, with only one collection representing species from the southern hemisphere (Royal Trust 2020).

The arboretum is structured into two major sections: the Ancient World or Eurasian continent, which groups species from Europe, North Africa, and Asia, and the New World or American continent, which includes species from North and South America (Royal Trust 2020).

Each of these sections is divided into 20 distinct geographical subgroups, making a total of 40 groups, each representing a different forest ecosystem. This layout allows for comparative ecological studies, offering a natural laboratory to investigate species adaptation, forest dynamics, and ecosystem functioning (Royal Trust 2020).

2.3. Site conditions

The Belgian climate is temperate maritime, meaning that temperatures in the country are usually moderate. It is also distinguishable by predominant southerly to westerly winds, substantial cloud cover and recurrent precipitation. The summer months are characterized by relatively cool and humid conditions, while winters are marked by milder temperatures and increased precipitation (World Bank Climate Change Knowledge Portal s. d.). The average temperature in Tervuren was 10.9 °C and the average annual precipitation was approximately 781.3 mm between 1991 and 2020 (IRM - Climat dans votre commune 2020).

Figure 3 shows on which soil type, according to the Belgian classification, the selected plots are located. Except for one plot, number 7, all plots are located on the same soil type: Aba0(b). According to the Carte numérique des sols de Wallonie, this soil type refers to loamy soil with favorable drainage, spotted textural B horizon, thick A horizon (>40 cm).

Plot 7 is located on a Abc0 soil type meaning a loamy soil with favorable drainage, strongly spotted textural B horizon, thick A horizon (>40cm). The only difference between the two soil types lies in the traces of hydromorphy that are more present in the Abc0 soil.

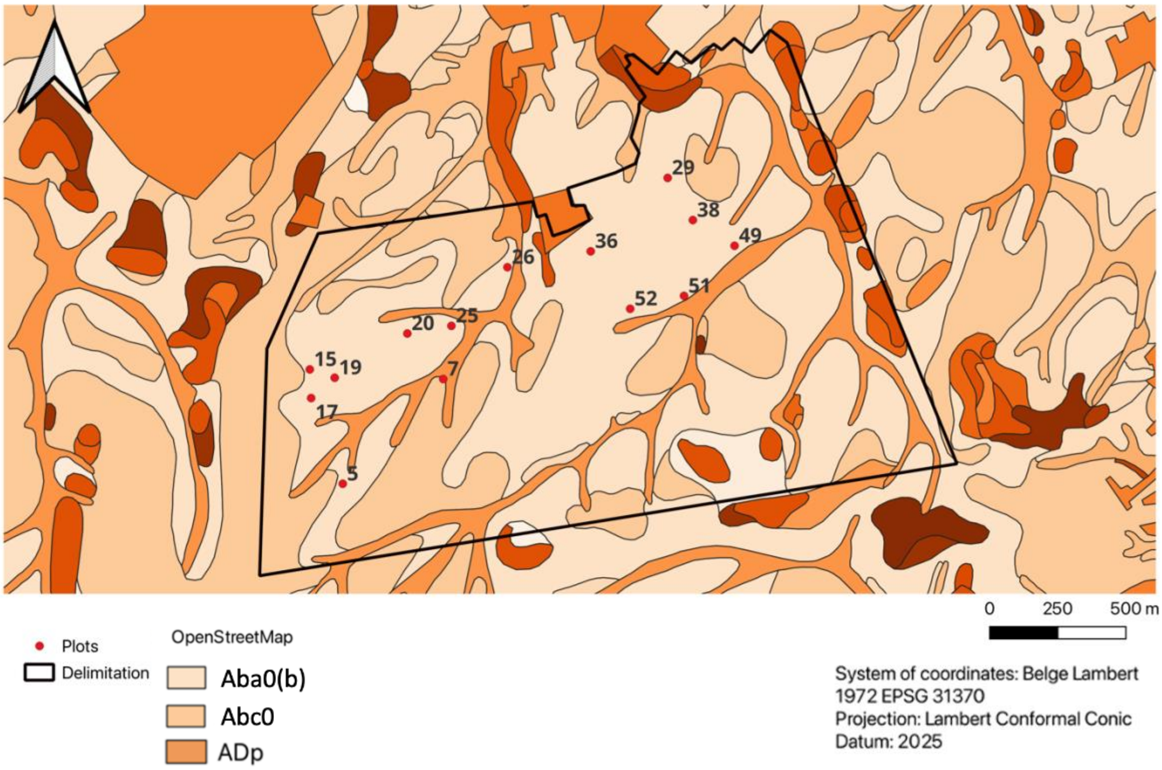


Figure 3: Map of the soil types (Belgian classification) found in the studied site and the localization of sampling plots.

3. Sampling approach

3.1. Method

The sampling of the earthworm community can be carried out using various methods, including chemical extraction, hand sorting, formalin application, electric octet, or onion extraction (Singh et al. 2016).

For this research, a combined approach was used, involving chemical extraction with a mustard solution supplemented by hand sorting. This mustard method is based on the release of allyl isothiocyanate through the enzymatic breakdown of glucosinolates in mustard flour which irritates the mucosal membranes of earthworms (Singh et al. 2016).

This method was selected because, although it can be less efficient than full hand sorting, it is particularly suitable for comparative studies like this one, as it is less time-consuming and labor-intensive (Singh et al. 2016). The mustard extraction method is also cheap and relatively easy to implement. The hand sorting method was also applied, but only on a small portion of the sampling area.

3.2. Field sampling design

The sampling was conducted in 14 distinct stands within the Arboretum of Tervuren. For each of these stands, data on soil characteristics such as organic carbon content, and stand characteristics were available, having been collected during the earlier stages of Joachim Lopez's doctoral thesis. During the same period as this master thesis, data on microclimate and leaf litterfall composition were also collected for all stands.

The selected stands are monocultures, each dominated by a different tree species shown in Table 1, allowing for a comparative analysis of their respective influences on earthworm communities. These stands, or plots, are distributed across both the American and European sections of the arboretum, as illustrated in Figure 4, ensuring a broad representation of tree species from different biogeographical origins.

Table 1: List of plots, dominant tree species, species type, dominant age, mean diameter at a height of 1.3 m, stem density, and basal area.

Plot id	**Dominant species**	**Type**	**Dominant age (years)**	**Mean diameter at 1.30m (cm)**	**Stem density (N/ha)**	**Basal area (m^2/ha)**
5	*Thuja plicata*	Coniferous	125	62.409	314	102.495
7	*Calocedrus decurrens*	Coniferous	120	55.675	265	79.418
15	*Juglans cinerea*	Broadleaved	117	28.785	363	28.960
17	*Tsuga canadensis*	Coniferous	121	69.805	98	44.565
19	*Betula alleghaniensis*	Broadleaved	54	24.762	521	29.604
20	*Acer saccharinum*	Broadleaved	123	43.509	236	50.873
25	*Quercus rubra*	Broadleaved	75	31.756	373	47.308
26	*Tilia americana*	Broadleaved	75	32.092	246	22.407
29	*Quercus robur*	Broadleaved	123	32.149	275	32.291
36	*Pinus nigra subsp. Laricio*	Coniferous	65	39.703	383	50.123
38	*Fagus sylvatica*	Broadleaved	45	19.924	914	38.773
49	*Cryptomeria japonica*	Coniferous	60	41.585	521	75.090
51	*Larix kaempferi*	Coniferous	100	56.966	138	37.427
52	*Juglans mandshurica*	Broadleaved	117	28.607	305	36.848

The stands were selected with the aim to have both coniferous and broadleaved tree types. Monospecific stands that were at least 40 years old and with available data on the soil characteristics, microclimate, and leaf litterfall characteristics were prioritized. The stands were chosen in order to cover several geographical zones of the arboretum, as shown in the next figure, they are referred as their plot number. A study plot consisted of a center point with a 18m radius in which measurements took place. This next figure provides an overview of the geographical zoning in the Arboretum, illustrating the spatial distribution of the different tree communities into the geographical zones. The American continent is located in the left part of the map while the Eurasian continent is on the right.

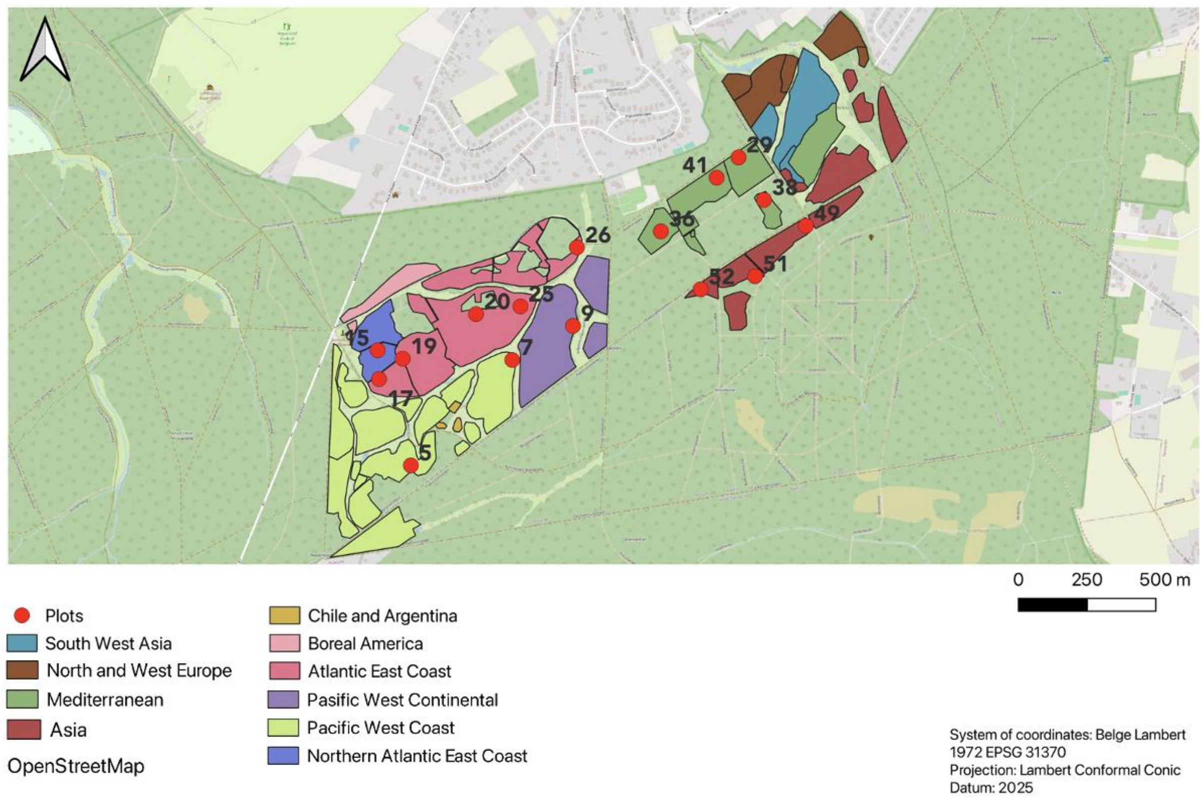


Figure 4: Map showing the positioning of the sampling plots in the Arboretum of Tervuren in the geographical zones.

To ensure replicability and spatial consistency, three sampling spots were defined within each stand, therefore being pseudo-replicates forming a triangular arrangement. One spot was oriented north, another southeast, and the last southwest in relation to the center of the plot. To avoid potential biases linked to root influence and microenvironmental variations, the sampling spots were positioned at a sufficient distance from tree stems and pathways following the recommendations of Muys et al. (2013). Each sampling spot covered a 0.5 m² area, delineated by a wooden frame placed on the ground.

Given the need for heavy and bulky equipment during fieldwork, the student was always accompanied by at least a technician or the co-supervisor to assist in transporting and setting up the necessary materials at the sampling sites. The sampling was conducted during the months of September and October 2024. For each plot, a datasheet containing the following information was completed: date of sampling, name of samplers, plot number, pseudo-replicate, weather conditions, soil conditions, vegetation conditions and comments.

At each sampling spot, if ground vegetation was present, it was removed. The litter and organic layers were then manually examined for earthworms. If no earthworms were found in these layers in the first replicate, the remaining two replicates were not screened for them. This can be attributed to the very time-intensive nature of scanning individual leaves and organic matter and that almost no earthworms were found in this layer. Once the surface was cleared of vegetation, litter, and organic material, the site was ready for mustard extraction (Muys et al., 2013).

The mustard powder was mixed with water in two separate jerrycans: one containing 60 g of mustard powder in 20 L of water (3g/L), and the other with 60 g of mustard powder in 10 L of water (6g/L). The application was done in two times, first the least concentrated solution and then the higher concentration with a 10-minute interval after each application.

Just before application, the mustard solution was stirred to ensure homogeneity. It was then evenly distributed over the 0.5 m^2 sampling area. After each application, the earthworms that emerged inside the wooden frame within the 10-minute interval were carefully collected using plastic tweezers and placed into a storage container filled with 70% ethanol for further identification.

Ten minutes after the final mustard application, a soil sample was taken from the center of the sampling area. This sample measured 25 × 25 cm^2 and extended to a depth of 20 cm. Using a spade, the sample was extracted and placed on a white sorting table, where it was hand-sorted with plastic gloves and tweezers to retrieve any remaining earthworms.

The earthworms collected were stored separately in two labeled containers per replicate, both filled with 70% ethanol. One pot containing the earthworms collected using the mustard method and the other one containing the earthworms found during the hand-sorting. If earthworms were found in the litter layer, this was notified in the field datasheet, but they were placed in the same container as those extracted from the soil using the mustard method.

The earthworms stored in the ethanol containers were then placed in a cool chamber with a room temperature of 4°C in Louvain-la-Neuve until they were identified.

4. Laboratory analyses

Prior to the identification, the earthworms were counted and weighted (to calculate the fresh biomass) using a scale with a precision of 0.01 g during the month of November 2024. The entire earthworms in the container were dried using a paper towel, weighted together and then quickly taken back into the ethanol before they could dry out. This allowed to estimate the fresh biomass for each method in every pseudo-replicate.

The identification of earthworms was conducted during the months of March and April 2025 using a binocular microscope and the taxonomic key of Sims & Gerard (1999) implemented in an excel file provided by KULeuven (see Appendix 1.). Each earthworm was placed in a petri dish with ethanol 70%, to prevent them from drying out, and then carefully observed.

The earthworms were determined to the species level or to the genus level if the individuals were juveniles and/or too complicated to identify. This allowed to determine the number of earthworms for each species, when possible, and each stage (juveniles and adults). Some juveniles weren't developed enough to be identified either to the species or genus level, they remained unidentified. As juveniles, they were lacking the clitellum, a kind of "belt" or "ring", which is a glandular swelling (Sims & Gerard 1999), that is essential for the identification. They also weren't looking close to another already identified adult specimen, making the determination unfeasible.

The main morphological characteristics that were examined to identify the earthworm species were the presence or absence of a purple pigment, the shape of the prostomium which is the "mouth" of the earthworm, the position of the clitellum and the spatial arrangement of their bristles, called setae. All these morphological traits are represented in Figure 5.

The setae can be widely, closely or distant paired as shown in Figure 6. This could be well observed when turning the earthworm with the plastic tweezers and light coming from above the petri dish. Two variations of the prostomium were considered, tanylobous and epilobous (Figure 7).

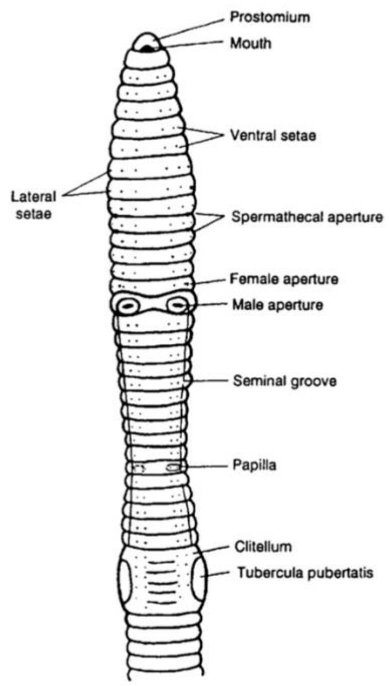


Figure 5: Ventral view of the anterior region of *Lumbricus terrestris* (Edwards & Arancon 2022).

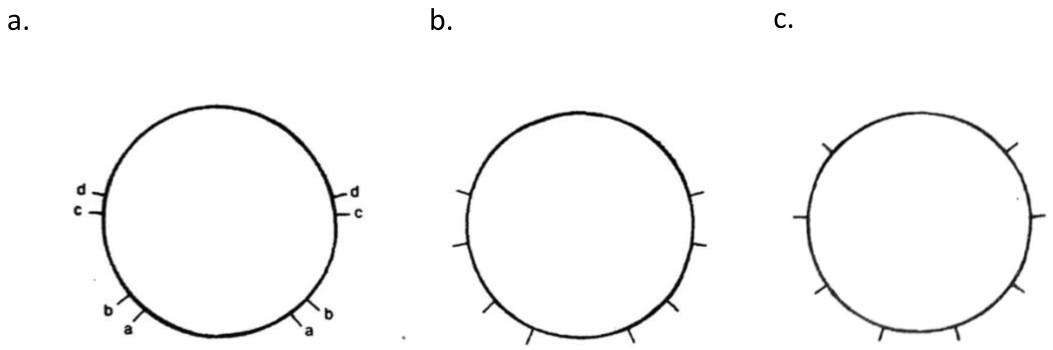


Figure 6: Arrangements of the setae of earthworms: (a) Closely paired (b) Widely paired (c) Distant (Sims & Gerard 1999).

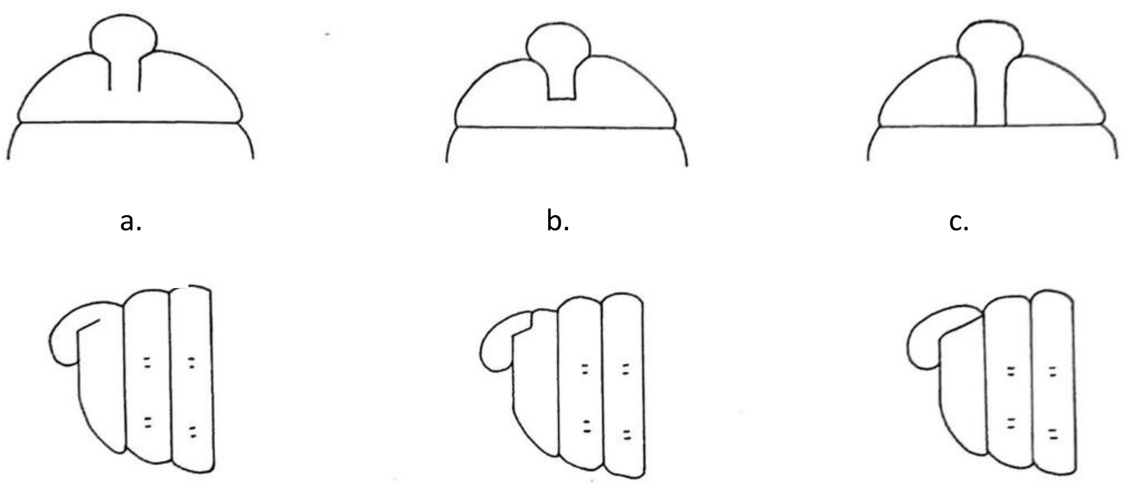


Figure 7: Various forms of prostomium: (a), (b) Epilobous (c) Tanylobous (Sims & Gerard 1999).

5. Data visualization and analyses

5.1. Data processing

All statistical analyses were performed using RStudio (version 4.4.3), primarily employing functions from the `dplyr` (Wickham et al 2023) and `tidyverse` packages for data manipulation and visualization.

For each plot, replicate, and method used, data on the total number of earthworms and their fresh mass was available. Hence, for example, in plot 5, first replicate, there were two values of the number of earthworms collected (and for the mass as well). The first value was the number of earthworms collected while using the mustard extraction method and the second value while using the hand sorting method.

Before further analyses, the area-weighted sum was calculated for both density (earthworms/m^2) and fresh biomass (g/m^2) according to the next equations. This sum was calculated in order to have the density and fresh biomass of earthworms for each pseudo-replicate combining the two methods.

Equation 1:

$$Density = \frac{(Number\ of\ earthworms_{Mustard} + Number\ of\ earthworms_{Hand\ sorting} \times \frac{Surface_{Mustard}}{Surface_{Hand\ sorting}})}{Surface_{Mustard}}$$

Equation 2:

$$Biomass = \frac{(Mass_{Mustard} + Mass_{Hand\ sorting} \times \frac{Surface_{Mustard}}{Surface_{Hand\ sorting}})}{Surface_{Mustard}}$$

The surface used for mustard extraction was 0.5 m^2, and the hand sorting was performed on a 0.0625 m^2 area located within it. The number of earthworms per m^2 will then be referred to as the earthworm density and the term biomass will be used to refer to the fresh biomass.

5.2. Descriptive analyses

The average biomass and density of each plot were determined by taking the mean value of the three pseudo-replicates. The standard deviations were calculated as well, with the aim to show the variability between the pseudo-replicates inside the plots. These values of the mean density/biomass and standard deviations were added in bar plots.

The mean biomass and density were also calculated for each tree type, broadleaves and conifers. Normality of the data was assessed using the Shapiro-Wilk test. As the assumption of normal distribution was not met for at least one group for the biomass, the non-parametric

Mann-Whitney U test was used to assess differences in earthworm biomass between broadleaved and coniferous stands. For the density, a student's t-test was conducted.

The earthworms were separated into two life stage categories: juveniles and adults and the determined species were assigned to an ecological category. Each earthworm species belongs to an ecological category (epigeic, anecic or endogeic) as described in the *State of the art,* Section 3.1. The repartition is shown in table 2. Five species were epigeic species, two were endogeic species and one was an anecic species. This classification is based on the one made by Bottinelli et al. (2020).

Table 2: List of the ecological category to which each earthworm species belongs to.

Species	**Ecological category**
Lumbricus castaneus	Epigeic
Dendrobaena octaedra	Epigeic
Dendrobaena pygmaea	Epigeic
Dendrodrilus rubidus sp.	Epigeic
Lumbricus sp (*L. castaneus* or *L. rubellus*)	Epigeic
Aporrectodea rosea	Endogeic
Octolasion cyaneum	Endogeic
Lumbricus terrestris	Anecic

5.3. Diversity indexes and NMDS

The Shannon Diversity Index is a measure that aims to quantify the diversity within a community and here coniferous and broadleaved tree species are compared. This index considers both abundance and evenness of species. It is calculated using the following equation.

Equation 3:

$$H = -\sum_{j=1}^{S} p_i \ln p_i$$

Where H is the Shannon Diversity Index, S is the number of species and p_i is the proportion of the species in the sample (in terms of earthworm density).

The Simpson Index, a diversity index as well, also aims to determine the difference in species diversity based on the species richness and evenness. The following equation is used to calculate this index.

Equation 4:

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

Where D is the Simpon index, n is the total number of organisms of a particular species and N is the total number of organisms of all species

The extended evenness, which reflects the balance in the distribution of individuals between species, was computed based on the Shannon diversity index and species richness using the equation 5.

Equation 5:

$$E = \frac{H}{\ln{(S)}}$$

Where E is the extended evenness, H is the Shannon index and S is the number of species.

To further explore potential differences in earthworm community composition between broadleaved and coniferous stands, a beta diversity analysis using Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarity was performed. The community matrix used in this analysis was constructed from the summed densities of each earthworm species per plot. This approach allows to capture differences in species composition and community structure across plots. The NMDS projects these multivariate ecological distances into a two-dimensional space to visually assess clustering patterns related to tree type. Statistical testing of community composition differences between broadleaved and coniferous plots was performed with PERMANOVA (using the `adonis2` function from the `vegan` package (Oksanen et al., 2025)), based on the same Bray-Curtis dissimilarity matrix.

5.4. Regression analyses

Several soil, plot, and leaf litterfall characteristics were considered to better understand the factors influencing earthworm communities. Soil pH-H_2O, pH-$BaCl_2$, exchangeable calcium (Ca^{2+} in cmolc/kg), exchangeable aluminum (Al^{3+} in cmolc/kg), carbon content (%) and base cation content (sum of K^+, Ca^{2+}, Mg^{2+} and Na^+ expressed in cmolc/kg) were measured in autumn 2022 (Di Francesco 2023) to characterize soil acidity and chemical composition for the following depths: 0-5 cm, 5-10 cm, 10-20 cm, 50 cm, 75 cm, 100 cm. The carbon stock was also measured in g/m^2 for the forest floor and in the first 5, 10 and 20 cm of the soil. A value of the total carbon stock was also calculated. The soil depth considered in the regression analyses is between 0-5cm and this for all soil parameters (Appendix 4.).

A forest inventory, characterizing local forest composition (species, diameter, shrub and herb cover), was performed in spring 2022 (Torremans 2022). Annual litterfall was monitored by regularly collecting the contents of fixed litter traps (three buckets with diameter 33cm), positioned on the forest floor and 5 meters from the plot center in the directions 0° (North), 120° and 240°. Samples were dried, sorted and weighed in the laboratory for the period 2022-2023 to assess the leaf turnover rate (Appendix 5.).

The leaf turnover rate indicates the rate at which leaves, and other plant debris are renewed and decomposed in an ecosystem. It is calculated by taking the annual litterfall and dividing it by the persistent organic layer, using the following equation:

Equation 6:

$$Leaf\ Turnover\ Rate = \frac{Yearly\ litterfall}{Persistent\ ectorganic\ layer}$$

In addition, concentrations of nitrogen, carbon, phosphor, calcium and aluminum (mg/kg sample) in leaf litterfall were considered. These parameters were calculated for fresh leaves that were collected during summer 2024 for the occurring species in the plots and chemically analyzed through an ICP analyzer (Appendix 3.).

Data visualization was conducted using the `ggplot2` package. Regression equations and R^2 values were added to the plots using the `ggpmisc` package (Aphalo 2024). Linear relationships were fitted with the base `lm()` function and visualized using `geom_smooth()` with 95% confidence intervals.

Local microclimate, daily values of air temperature at the soil surface and 15cm above the soil surface (°C), soil temperature (measured 8 cm deep in the soil) (°C), and soil moisture (Volumetric Water Content measured between 0 and 14 cm), was continuously measured by TMS4 sensors (Fraiture 2023) in all considered plots. To create the regression graphs examining the relationship between earthworm density or biomass and microclimatic variables, the mean biomass and density per plot were used. For each microclimatic variable, the mean values of the sampling months were considered, in order to reflect the environmental conditions at the time of sampling.

In addition to the regression graphs, a Pearson correlation coefficient (r) was calculated between biomass and each tested variable to assess the strength and direction of linear relationships. The significance of each correlation was evaluated using the associated p-value (p). Values of r close to +1 or -1 indicated strong positive or negative linear relationships, respectively, while values near 0 suggested weak or no linear association.

5.5. PCA analyses and linear mixed models

A principal component analysis (PCA) was conducted separately for soil variables (between 0 and 5 cm deep), leaf litterfall traits, and plot characteristics, in order to reduce dimensionality, limit multicollinearity, and extract key environmental gradients for further analysis. Before visualization, all variables were standardized (mean-centered and scaled to unit variance).

To further explore the factors influencing earthworm communities, linear mixed models were implemented using the `lme4` (Bates et al. 2015) package. These models incorporated the environmental variables identified as relevant in previous principal component analyses

(PCA). It also included a random term (1/Plot) to model plot-specific variability. This term is essential, as it takes into account unobserved or unmeasured plot-specific effects that can influence biomass, independently of other explanatory variables. It allows to be statistically correct as it considers the hierarchical structure of the data. By incorporating this variability, the accuracy of fixed factor estimates was improved, while taking into account the particularities of each site.

This approach provides a better understanding of the relationships between environmental conditions and earthworm biomass, while controlling for random effects linked to sampling. The equation of the first model, considering all PCA results, is as follows:

Equation 7:

$$Biomass \sim PC1_{soil} + PC1_{plot} + PC1_{litterfall} + PC2_{soil} + PC2_{plot} + PC2_{litterfall} + \left(\frac{1}{Plot}\right)$$

The significance codes associated with p values are based on the following convention: results are very highly significant for p ≤ 0.001 (***), highly significant for p ≤ 0.01 (**), significant for p ≤ 0.05 (*), marginally significant for p ≤ 0.1 (.), and considered insignificant above this threshold ( ).

The next model only considered the soil characteristics to be explanatory variables of the biomass.

Equation 8:

$$Biomass \sim PC1_{soil} + PC2_{soil} + \left(\frac{1}{Plot}\right)$$

For the next model, the first principal component (PC1) of the PCA for plot, leaf litterfall, and soil characteristics was considered.

Equation 9:

$$Biomass \sim PC1_{soil} + PC1_{plot} + PC1_{litterfall} + \left(\frac{1}{Plot}\right)$$

In order to explore in greater detail, the mechanisms underlying variation in earthworm biomass, a linear mixed model incorporating an interaction between the principal components of soil ($PC1_{soil}$) and leaf litterfall ($PC1_{litterfall}$) was tested. The addition of this interaction term enables to check whether the effect of soil on biomass varies according to leaf litterfall characteristics.

Equation 10:

$$Biomass \sim PC1_{soil} \times PC1_{litterfall} + \left(\frac{1}{Plot}\right)$$

Finally, a last model, based on the next equation was tested, using the second principal components as explanatory variables.

Equation 11:

$$Biomass \sim PC2_{soil} + PC2_{plot} + PC2_{litterfall} + \left(\frac{1}{Plot}\right)$$

Although there is no single p-value associated with the model as a whole, overall significance was assessed by comparing the full model with a null model (containing only the intercept) using a likelihood ratio test. This test determines whether the addition of explanatory variables significantly improves model fit. The degrees of freedom and associated p-value are reported to interpret the overall contribution of the explanatory variables.

To compare the performance of the different models tested, the Akaike Information Criterion (AIC) was used. This criterion assesses the relative quality of a statistical model, while penalizing overly complex models. It is based on a compromise between fit to data and model parsimony.

Results

1. Description of earthworm communities in the arboretum

This section presents an overview of the collected earthworm data (species, density and biomass), including totals, averages, and extreme values, prior to in-depth statistical analysis.

Overall, a total of 2,904 earthworms were collected during the sampling, including 614 adults and 2,290 juveniles. In all plots, juveniles accounted for the majority of earthworms except in the Manchurian walnut (Plot 52), where more adults were found (~70%). In the Japanese larch (Plot 51), not a single adult was found, only juvenile individuals. On average, 77.32% of the earthworms found in one plot were juveniles (median = 78.01%).

As explained in detail in a previous section (*Materials and Methods*), all earthworms were collected by combining two sampling methods: mustard extraction and hand sorting. Table 3 reports, for each plot, the average earthworm density (expressed in individuals/m²) and the average biomass (in g/m²), calculated for each method averaging the three pseudo-replicates per plot then for each plot. The standard deviations are listed as well.

The minimum average density was observed under *Juglans mandshurica* (Plot 53) with 25 individuals/m², while the maximum average density reached 515 individuals/m² under *Quercus robur* (Plot 29). In terms of biomass, extreme values were recorded in other plots: the minimum average biomass was 1.17 g/m² under *Larix kaempferi* (Plot 51), while the maximum average biomass, 37.38 g/m², was recorded under *Calocedrus decurrens* (Plot 7). These results underline the high variability between plots, in terms of both density and average biomass.

In addition to the plot-level averages reported above, individual extreme values (i.e., the minimum and maximum values observed per pseudo-replicate, independently of the plot mean) were examined. The lowest recorded earthworm density was 8 individuals/m², observed in plot 52 (*Juglans mandshurica*). The maximum density was 712 individuals/m², measured in plot 29 (*Quercus robur*). Regarding earthworm biomass, the extreme values are not associated with the same plots as for density: the minimum biomass observed was 0.38 g/m² in plot 51 (*Larix kaempferi*), while the maximum value was 49.9 g/m² in plot 7 (*Calocedrus decurrens*). These observations confirm that plots associated with individual extreme values are the same as those with the lowest or highest averages. These values are listed in the Appendix 2.

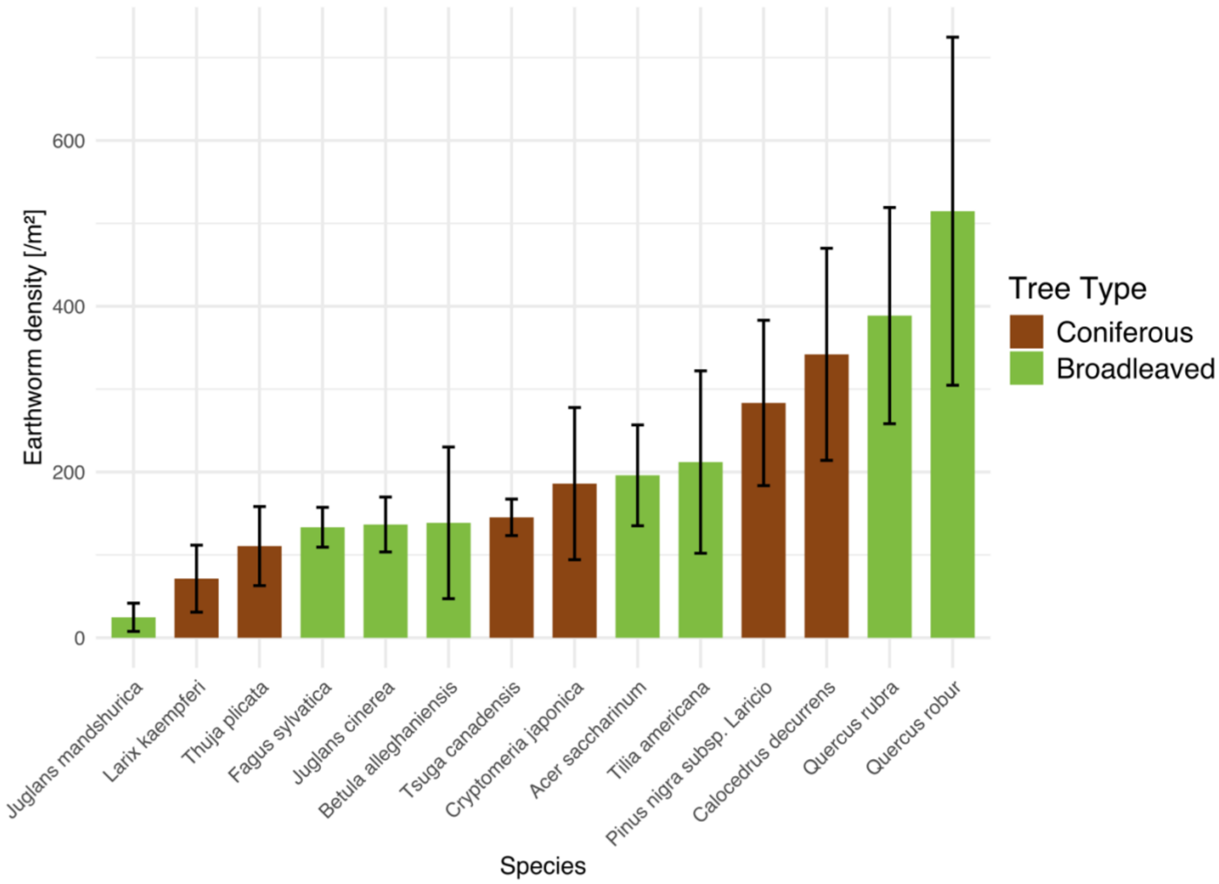
Table 3: Plot id, Tree species, Earthworms/m^2 for each method (obtained by averaging the 3 pseudo-replicates), Mean density per plot (values obtained using the equation 1) and associated standard deviation, Mean earthworm biomass per plot and associated standard deviation.

Plot id	Tree species	Earthworms/m^2 (mustard method)	Earthworms/m^2 (hand sorting method)	Earthworms/m^2	Biomass (g/m^2)
5	*Thuja plicata*	100	11	111 ± 48	9.660 ± 5.17
7	*Calocedrus decurrens*	209	133	342 ± 128	37.380 ± 11.06
15	*Juglans cinerea*	115	22	137 ± 33	9.773 ± 2.48
17	*Tsuga canadensis*	145	0	145 ± 22	2.287 ± 0.41
19	*Betula alleghaniensis*	133	5	138 ± 91	4.440 ± 3.16
20	*Acer saccharinum*	79	117	196 ± 61	22.607 ± 10.94
25	*Quercus rubra*	341	48	389 ± 130	11.273 ± 6.01
26	*Tilia americana*	164	48	212 ± 110	19.200 ± 7.99
29	*Quercus robur*	104	411	515 ± 210	12.513 ± 3.93
36	*Pinus nigra subsp. laricio*	81	202	283 ± 100	7.200 ± 0.92
38	*Fagus sylvatica*	112	21	133 ± 24	6.187 ± 1.45
49	*Cryptomeria japonica*	133	53	186 ± 92	5.080 ± 3.61
51	*Larix kaempferi*	71	0	71 ± 40	1.167 ± 1.07
52	*Juglans mandshurica*	14	11	25 ± 17	20.033 ± 14.89

The variability inside each plot (intra-plot variability) is represented in Figure 8 through bar plots, showing mean values and standard deviations. Figure 8a shows earthworm density (individuals/m^2) measured under different tree species, while Figure 8b presents the corresponding biomass (g/m^2). Marked variability was observed between species, both in terms of density and biomass. Some species exhibited a wide range of values, such as the earthworm biomass for *Juglans mandshurica* and density for *Quercus robur,* suggesting notable heterogeneity of observations between the pseudo-replicates within these stands.

Data on earthworm biomass was available only for each plot, replicate, and method but not for each earthworm species. Therefore, all results presented below in this section are based on the earthworm density.

a.



b.

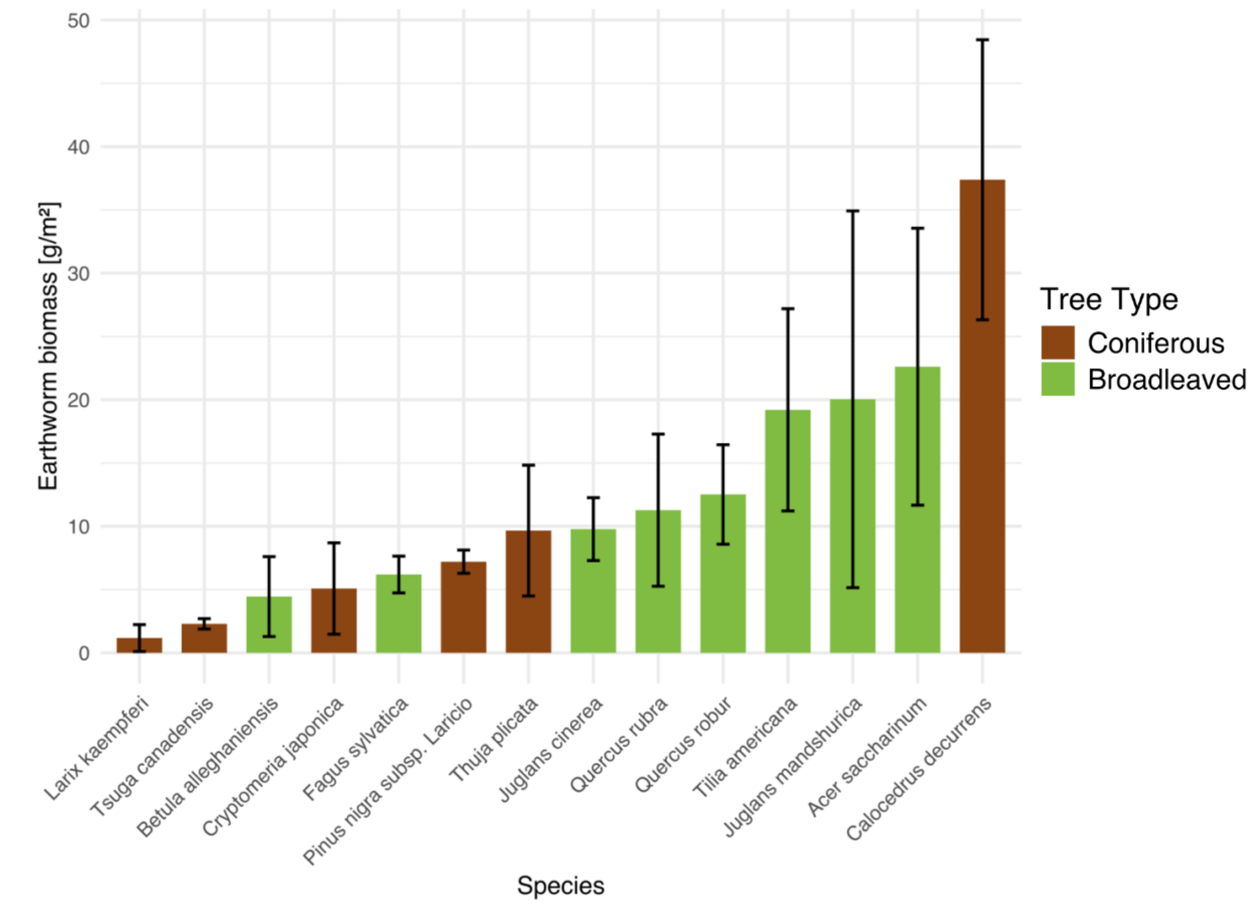


Figure 8: (a) Bar plot representing the mean earthworm density (earthworms/m²) and error bars representing

the standard deviation for each plot (b) Bar plot representing the mean earthworm biomass (g/m^2) and error bars representing the standard deviation for each plot.

In total, eight different earthworm species were identified across all sampled plots: *Aporrectodea rosea, Dendrobaena octaedra, Dendrobaena pygmaea, Dendrodrilus rubidus sp., Lumbricus castaneus, Lumbricus sp* (*L. castaneus* or *L. rubellus*)*, Lumbricus terrestris, Octolasion cyaneum.* Table 4 presents the number of earthworm species identified per plot. The lowest species richness was recorded in plot 51 (*Larix kaempferi*), where only one species, *Dendrobaena octaedra,* was observed*.*

Figure 9 shows the relative proportions of earthworm species identified across all plots. This pie chart illustrates the relative frequency of occurrence of the eight earthworm species identified in all samples. Each sector represents the proportion of individuals belonging to a given species in the total sample. The species are also distributed into their ecological categories, indicated by color. *Octolasion cyaneum* was the most abundant species. This endogeic earthworm, also known as the blue-grey worm, is widely distributed in Europe (Sims & Gerard, 1999). It was then followed by *Dendrobaena octaedra*, an epigeic species also commonly found in Europe (Sims & Gerard, 1999). In terms of ecological categories, epigeic species dominated (64.3%), followed by endogeic species (34.1%). Anecic species were poorly represented, accounting for only 1.6% of the total.

Species composition by plot is shown in Figure 10. This stacked bar graph represents the density (expressed in individuals/m^2) of different earthworm species in each plot. Each bar corresponds to a plot, and the colors identify the relative contribution of each species to the total density per plot. These bars represent the mean values for the three pseudo-replicates. The species *Dendrobaena octaedra* was found in every plot while *Lumbricus castaneus,* an epigeic species, and *Aporrectodea rosea,* an endogeic species, were each found in only one plot. It is important to note that a substantial proportion (64,5% of the density) of the juveniles found were not identified. The grey area represents the proportion of undetermined juvenile individuals for each plot, with strong differences observed between plots.

As presented in Figure 11, anecic species were found in two plots, epigeic in all plots and endogeic in eleven plots. Epigeic earthworms were the dominant ecological category most plots, except under Silver maple (Plot 20) and European beech (Plot 38), where endogeic species dominated. In contrast, under Manchurian walnut (Plot 52), anecic species were most abundant. The grey area again represents the proportion of undetermined individuals in each plot.

Table 4: Number of earthworm species per plot, list of earthworm species found in each plot or genus if the species couldn't be determined. TP = *Thuja plicata*, CD = *Calocedrus decurrens*, JC = *Juglans cinerea*, TC = *Tsuga canadensis*, BA = *Betula alleghaniensis*, AS = *Acer saccharinum*, QRu = *Quercus rubra*, TA = *Tilia americana*, QRo = *Quercus robur*, PN = *Pinus nigra subsp. laricio*, FS = *Fagus sylvatica*, CJ = *Cryptomeria japonica*, LK = *Larix kaempferi*, JM = *Juglans manschurica*

Id	Number of species	Earthworm species
5_TP	3	*Lumbricus sp., Dendrodrilus rubidus sp., Dendrobaena octaedra*
7_CD	5	*Lumbricus terrestris, Octolasion cyaneum, Lumbricus sp., Dendrodrilus rubidus sp., Dendrobaena octaedra*
15_JC	3	*Octolasion cyaneum, Dendrodrilus rubidus sp., Lumbricus sp*
17_TC	1	*Dendrobaena octaedra*
19_BA	3	*Dendrodrilus rubidus sp., Octolasion cyaneum, Dendrobaena octaedra*
20_AS	5	*Lumbricus sp., Dendrobaena octaedra, Dendrodrilus rubidus sp., Octolasion cyaneum, Aporrectodea rosea,*
25_QRu	4	*Dendrodrilus rubidus sp., Dendrobaena octaedra, Octolasion cyaneum, Lumbricus sp.*
26_TA	4	*Dendrobaena octaedra, Dendrodrilus rubidus sp., Lumbricus castaneus, Octolasion cyaneum*
29_QRo	4	*Dendrobaena octaedra, Dendrobaena pygmaea, Dendrodrilus rubidus sp., Octolasion cyaneum*
36_PN	3	*Dendrodrilus rubidus sp., Dendrobaena octaedra, Octolasion cyaneum*
38_FS	3	*Dendrodrilus rubidus sp., Dendrobaena octaedra, Octolasion cyaneum*
49_CJ	5	*Dendrobaena pygmaea, Lumbricus sp., Dendrodrilus rubidus sp., Dendrobaena octaedra, Octolasion cyaneum*
51_LK	1	*Dendrobaena octaedra*
52_JM	4	*Lumbricus terrestris, Dendrobaena octaedra, Lumbricus sp., Octolasion cyaneum*

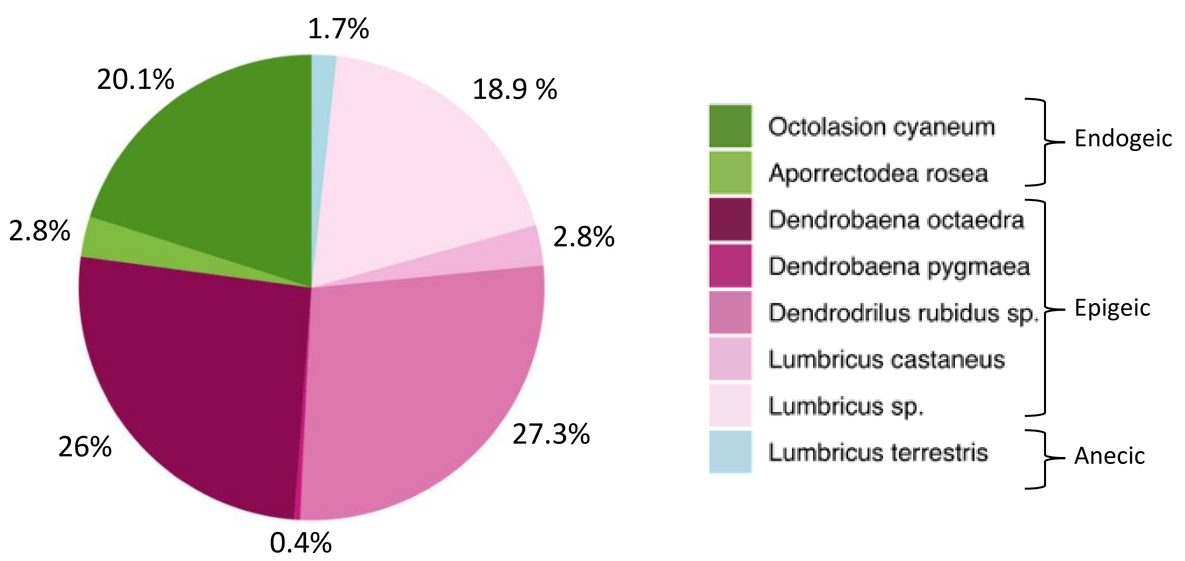


Figure 9: Pie chart representing the earthworm species found when all plots are combined with the percentage of occurrence of each species (based on the earthworm density in earthworms/m²). The species are distributed into the ecological categories according to the colors.

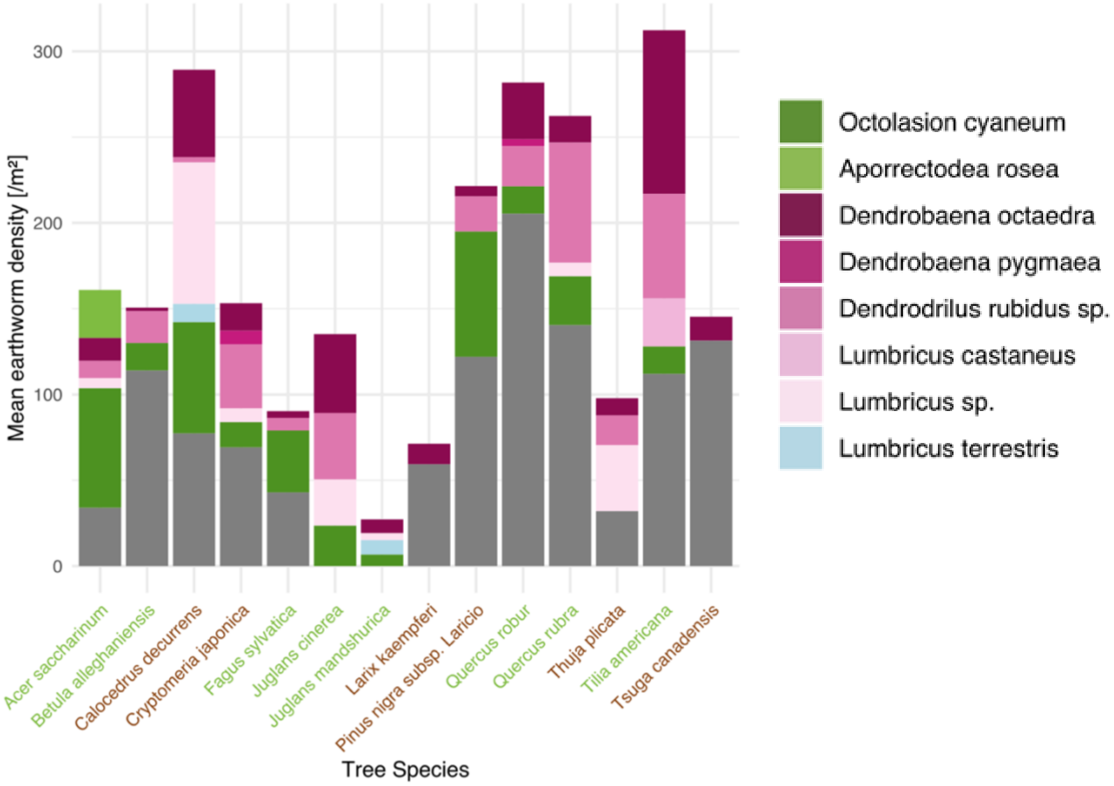


Figure 10: Bar plot of the number of earthworms/m² belonging to each earthworm species for every plot (based on the mean earthworm density calculated from the three replicates). The undetermined juvenile individuals for each plot are represented by the grey areas.

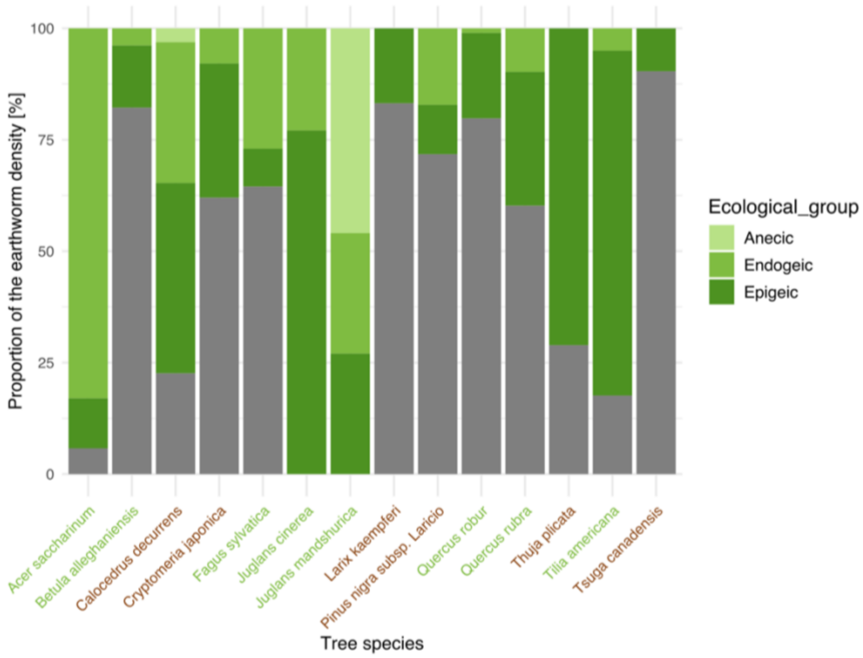


Figure 11: Plot representing the proportion of each ecological category (anecic, endogeic and epigeic) in every plot (in percentage of the earthworm density in earthworms/m²). The % of undetermined individuals for each plot are represented by the grey areas.

2. Differences between angiosperms and gymnosperms

To evaluate the first research hypothesis, broadleaved stands support a higher earthworm density, biomass and species diversity than coniferous stands, several statistical analyses were conducted, and the results are presented in this section.

2.1. Descriptive analyses

As a general overview, the mean eartworm density under broadleaved tree species was 218 ± 174 individuals/m² (median = 163) compared to 190 ± 119 individuals/m² (median = 162) under coniferous species, however this differences is not statistically significant (p-value = 0.7112). In terms of earthworm biomass, the average for broadleaved trees was 13.3 g/m^2 ± 9.06 (median = 11.1) which is higher than 10.5 g/m^2 ± 13.5 (median = 6.34) for coniferous species. The p-value = 0.1812, also indicates a non-significant difference. The median biomass and density values for the coniferous trees was substantially lower than the mean, suggesting skewed distributions, likely influenced by outliers. This is also illustrated in Figure 8b, where the biomass under *Calocedrus decurrens* was the highest recorded value, coniferous and broadleaved species combined.

Figure 12 represents the occurrence frequency (%) of each earthworm species identified in coniferous and broadleaved stands. This percentage is based on the earthworm density. It shows that more species were found under broadleaved trees (8) than under coniferous trees (6). In both type of trees, the most abundand species was *Octolasion cyaneum* followed by *Dendrodrilus rubidus sp.*, under broadleaved trees and by *Lumbricus sp*., under coniferous trees. Figure 12 also shows the distribution of earthworms into their ecological category in broadleaved and coniferous stands. The distribution is relatively similar between the two types of stand, with a clear dominance of epigeic species (in pink), followed by endogeic species (in green). This suggests that the stands, regardless of tree type, do not favor deep burrowing species (in blue). Some earthworm species were present in both tree types: *Dendrobaena octaedra, Dendrobaena pygmaea, Dendrodrilus rubidus sp, Lumbricus sp., Octolasion cyaneum* and *Lumbricus terrestris.* Two species, on the other hand, were found exclusively in broadleaved stands: *Aporrectodea rosea* and *Lumbricus castaneus*.

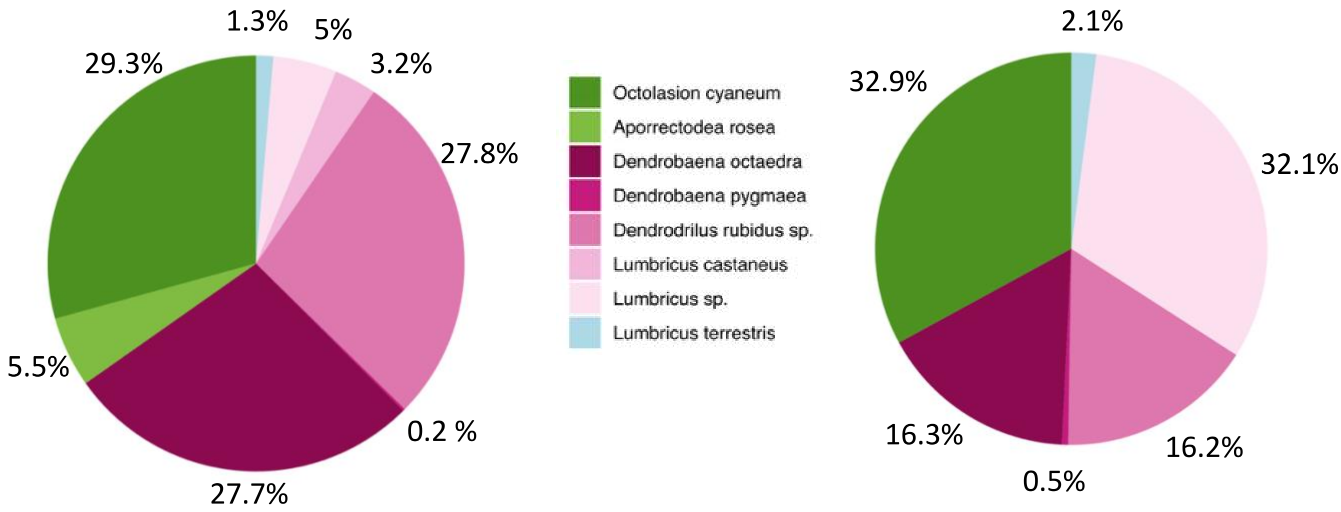


Figure 12: Pie charts representing the proportion of each earthworm species for both tree types, left combined for broadleaved trees, right the coniferous trees with the percentage of occurrence of each species (based on the earthworm density).

2.2. Diversity indexes

The Shannon index was calculated to test for any differences in earthworm diversity between the two types of trees. With a p-value of 0.4009, it indicated no significant difference in terms of diversity between coniferous and broadleaved stands. The Simpson index was also calculated and yielded a p-value of 0.272, indicating no significant difference between the two types of trees. The mean, standard deviation, and standard error values for both indices are shown in Tables 5 and 6.

Table 5: Mean, standard deviation and standard error values for both tree types using the Shannon diversity index

	Mean	**Standard deviation**	**Standard error**
Broadleaved	1.194562	0.259496	0.0917457
Coniferous	1.00786	0.5164094	0.2108232

Table 6: Mean, standard deviation and standard error values for both tree types using the Simpson diversity index

	Mean	**Standard deviation**	**Standard error**
Broadleaved	0.624908	0.1349058	0.04769641
Coniferous	0.5340905	0.2477689	0.10115122

Extended evenness was slightly higher in coniferous stands (0.80) than in broadleaved stands (0.75). This suggests a more homogeneous community structure in coniferous stands, with no single species dominating strongly. However, this difference does not appear to be marked, and complements the results obtained with the Shannon and Simpson indices, which also showed no significant difference in diversity between the two stand types.

In Figure 13, each point represents a plot, and the colors correspond to the type of stand (angiosperm or gymnosperm). Visually, the plots do not form clear groupings according to tree type: the points are partially mixed, suggesting a certain similarity in species composition between the two stand types. This observation was confirmed statistically by a PERMANOVA test (adonis), which gave a p-value of 0.279. This indicates that there was no significant difference in community composition between broadleaved and coniferous stands at the scale of the 14 plots analyzed.

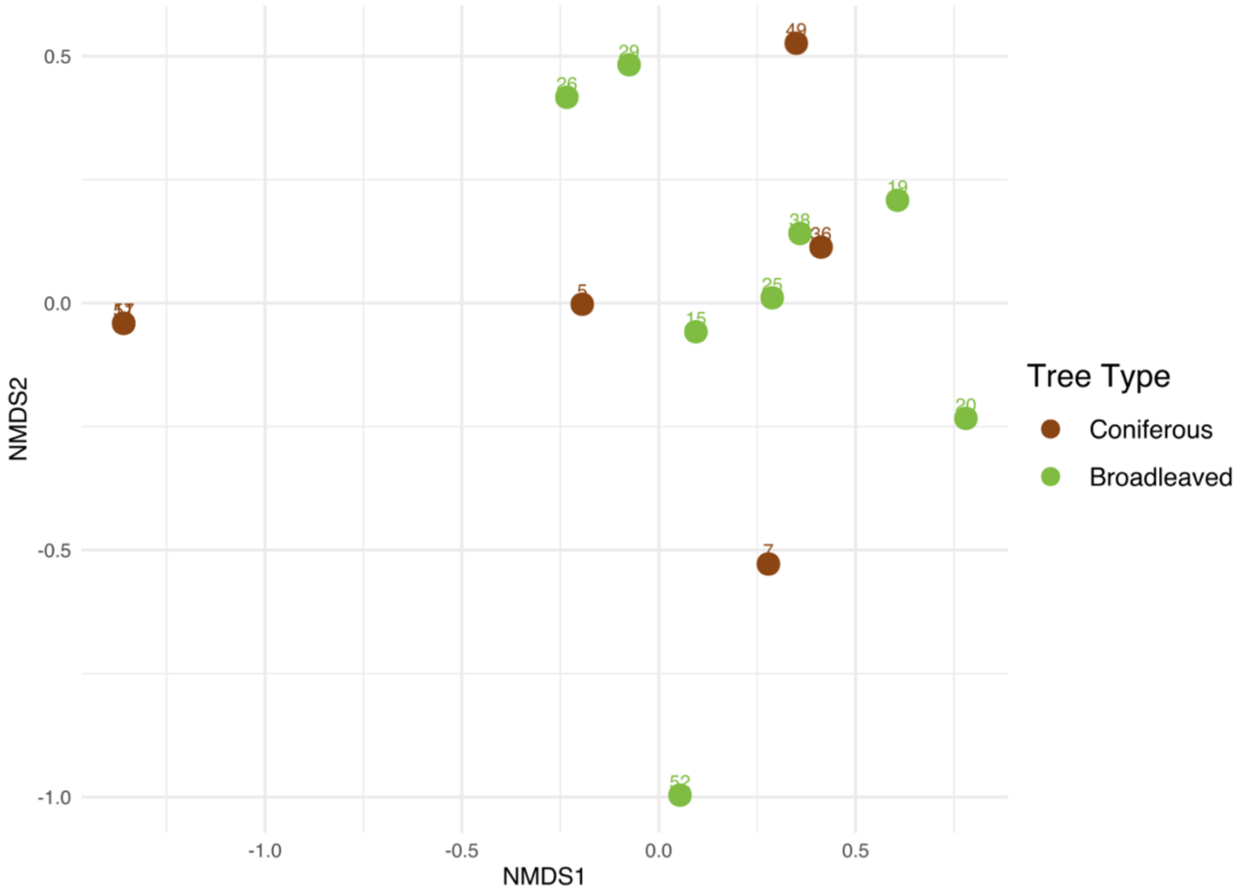


Figure 13: NMDS (Non-metric Multidimensional Scaling) ordination based on Bray-Curtis dissimilarity between earthworm communities according to tree species type (broadleaved vs. coniferous). Each point represents a plot (the plot IDs refer to Table 3), and the distance between points reflects the difference in specific composition of earthworm communities. Symbols are colored according to the dominant species (broadleaved species are represented in light green and coniferous species are represented in brown). Coordinates have been reduced to two dimensions (NMDS1 and NMDS2 axes) to visualize composition gradients between stands.

2.3. Differences in microclimate between coniferous and broadleaved stands

Figure 14 illustrates the annual variation and the variation in September and October in soil moisture and temperature in broadleaved and coniferous stands. Although there were slightly different trends between the two types of stand during the year, soil moisture differences remained relatively low during the sampling months. However, soil moisture was generally higher in broadleaved stands. On the contrary, soil temperature tend to be lower in these same stands during the sampling period.

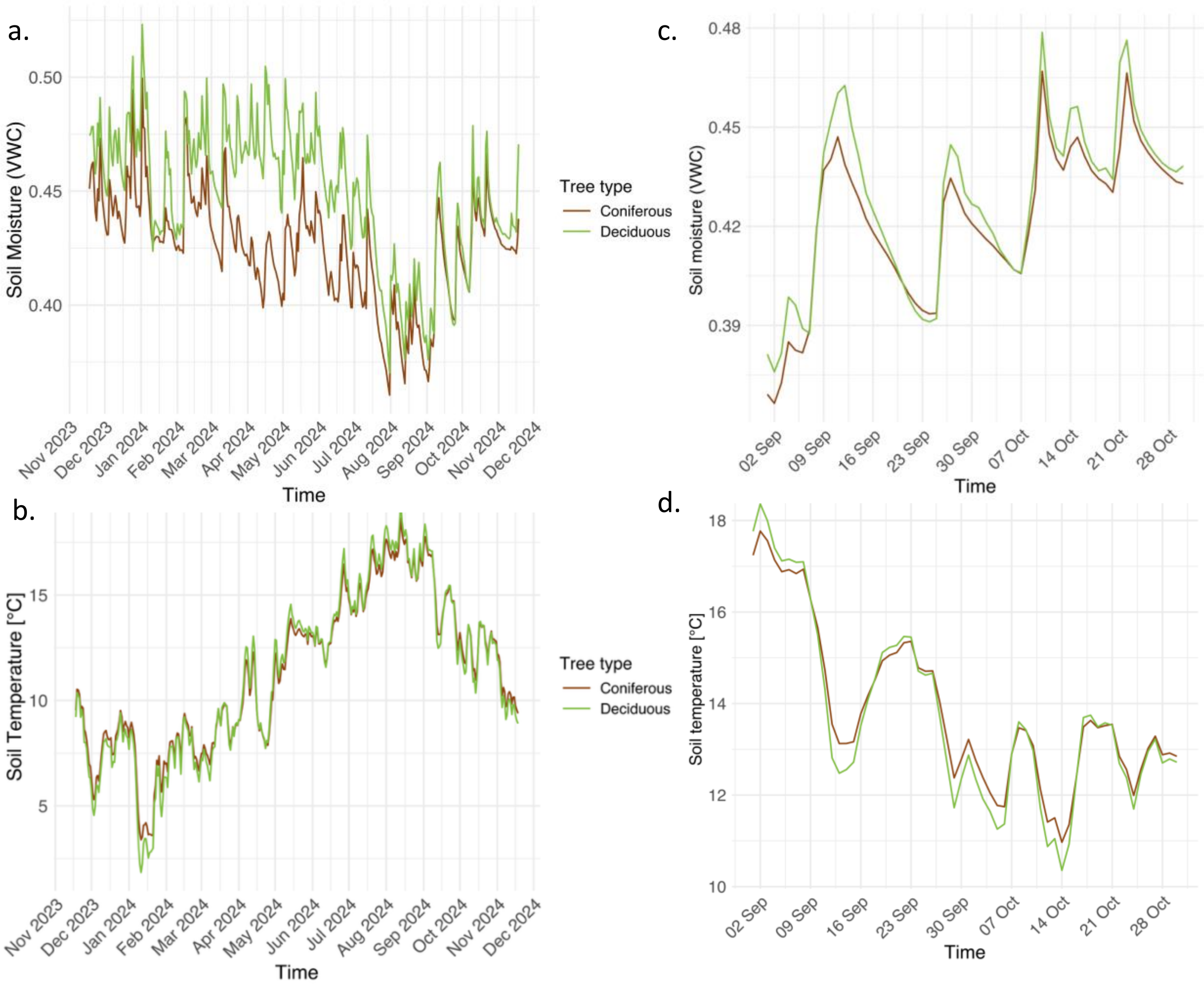


Figure 14: (a) Annual variation of soil moisture (m^3/m^3) between November 2023 and December 2024, (b) Annual variation of soil temperature between November 2023 and December 2024, (c) Variation of soil moisture (m^3/m^3) between September and October 2024, (d) Variation of soil temperature between September and October 2024

2.4. Analysis excluding *Calocedrus decurrens*

According to previous results, plot 7, a monoculture of *Calocedrus decurrens,* showed higher values of biomass and density when compared to the other coniferous species. It has therefore been decided to remove this plot to see if the results would be more homogeneous inside the coniferous category of trees.

After excluding the *Calocedrus decurrens* plot from the data set, the mean earthworm density was 159 ± 94.4 earthworms/m^2 and the biomass was 5.08 ± 4.05 g/m^2. This shows that the *Calocedrus decurrens* mostly had an impact on the mean biomass, reducing it by more than half. The difference in the density between the two groups, broadleaves and conifers, was still not statistically different (p-value = 0.6216). However, the mean values became statistically different for the biomass with a p-value = 0.02953.

Figure 15 shows the total density (a) and biomass (b) of earthworms in the two groups: gymnosperms (conifers) and gymnosperms without *Calocedrus decurrens*. In graph a, the average earthworm density is slightly higher when *Calocedrus decurrens* is included in the dataset, although variability is important in both groups, as indicated by the large error bars. In graph b, earthworm biomass and the variability inside the category are lower when *Calocedrus decurrens* is removed from the gymnosperm category.

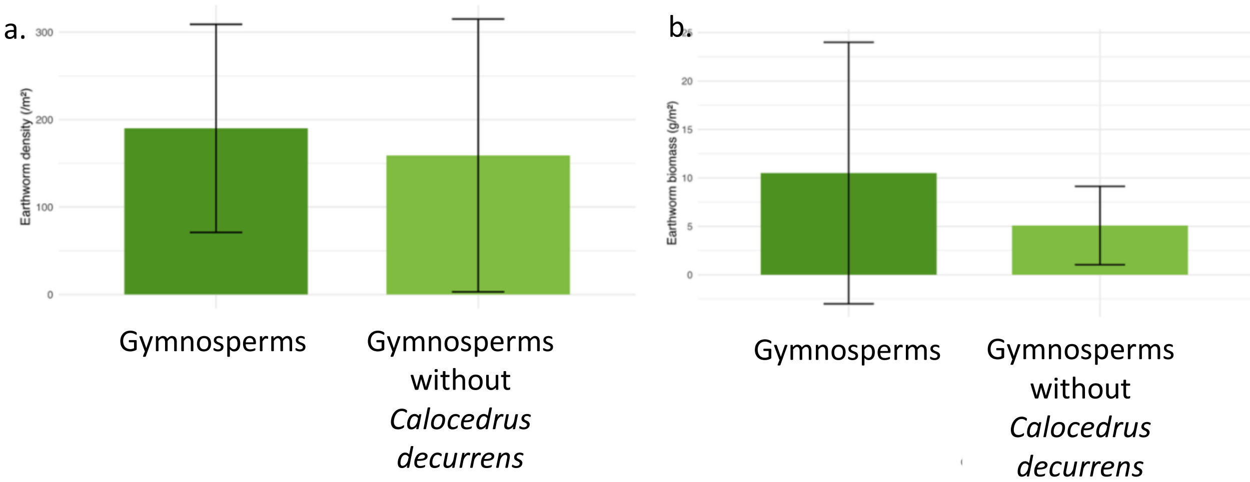


Figure 15 : (a) Bar plot showing the comparison of the earthworm density (earthworms/m^2) between the Gymnosperms (dark green) and the Gymnosperms without *Calocedrus decurrens* (light green). The bars represent the mean density, and the error bars show the standard deviation based on the individual measurements across all plots. (b) Bar plot showing the comparison of the earthworm biomass (g/m^2) between the Gymnosperms (dark green) and the Gymnosperms without *Calocedrus decurrens* (light green). The bars represent the mean biomass, and the error bars show the standard deviation based on the individual measurements across all plots.

3. Influence of trees trough soil characteristics, litter traits and microclimate on earthworm communities

This section explores the relationships between earthworm communities and environmental factors potentially influenced by the presence and characteristics of trees. More specifically, the aim is to address the second hypothesis and assess the extent to which soil composition, plot characteristics, microclimate and leaf litterfall, all four shaped by trees, can explain the variation observed in earthworm abundance, biomass and species diversity.

3.1. Soil characteristics

Figure 16 shows a regression illustrating the relationship between earthworm biomass and soil pH measured in $BaCl_2$ solution at a depth of 0 to 5 cm. A strong and highly significant positive correlation was found between soil pH and earthworm biomass ($r = 0.626$, $p < 0.001$), suggesting that earthworm biomass tends to increase in less acidic soils. The same figure was created for the density and is shown in Appendix 7.

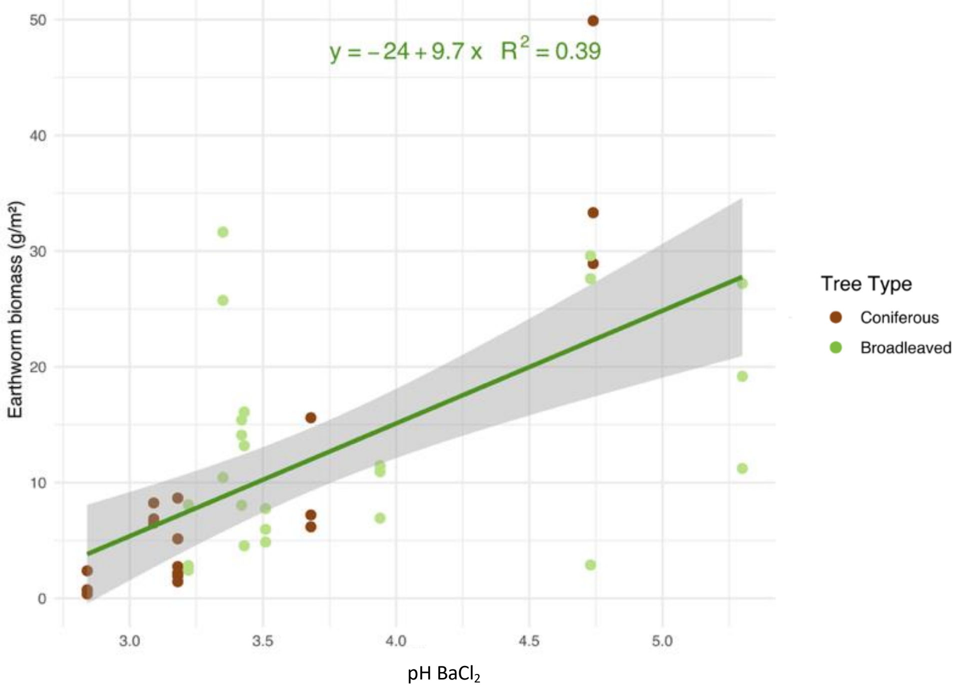


Figure 16: Relationship between earthworm biomass (g/m²) and soil pH-$BaCl_2$ (0-5 cm), showing a fitted linear regression (green) and the associated 95% confidence interval (grey area).

In Figure 17, the linear relationship between the earthworm biomass and base cation content in the soil (cmolc/kg) is presented. The earthworm biomass increases strongly with an increase in the base cation content and a strong and highly significant positive correlation was found between the two variables ($r = 0.782$, $p < 0.001$), suggesting that higher base cation content is associated with greater earthworm biomass across the study plots.

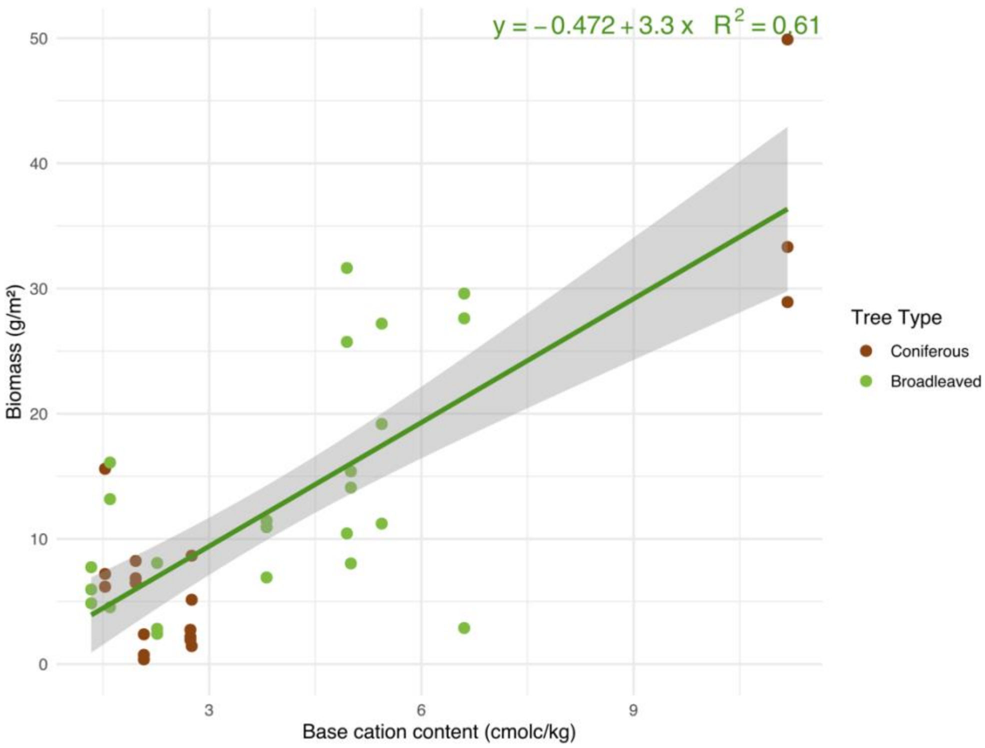


Figure 17: Relationship between earthworm biomass (g/m²) and soil base cation content (cmolc/kg), showing a fitted linear regression (green) and the associated 95% confidence interval (grey area)

The results of the PCA performed for soil characteristics are shown in figure 18. Axis 1, which explained nearly 50% of the variance, mainly reflected a gradient of chemical richness, with elements such as carbon, nitrogen, and aluminum. Axis 2, on the other hand, was more influenced by physical properties, such as bulk density. A strong correlation between carbon, nitrogen, and hydrogen can be observed. In contrast, elements like calcium and magnesium are positioned opposite aluminum and iron, likely reflecting differences in pH and base saturation.

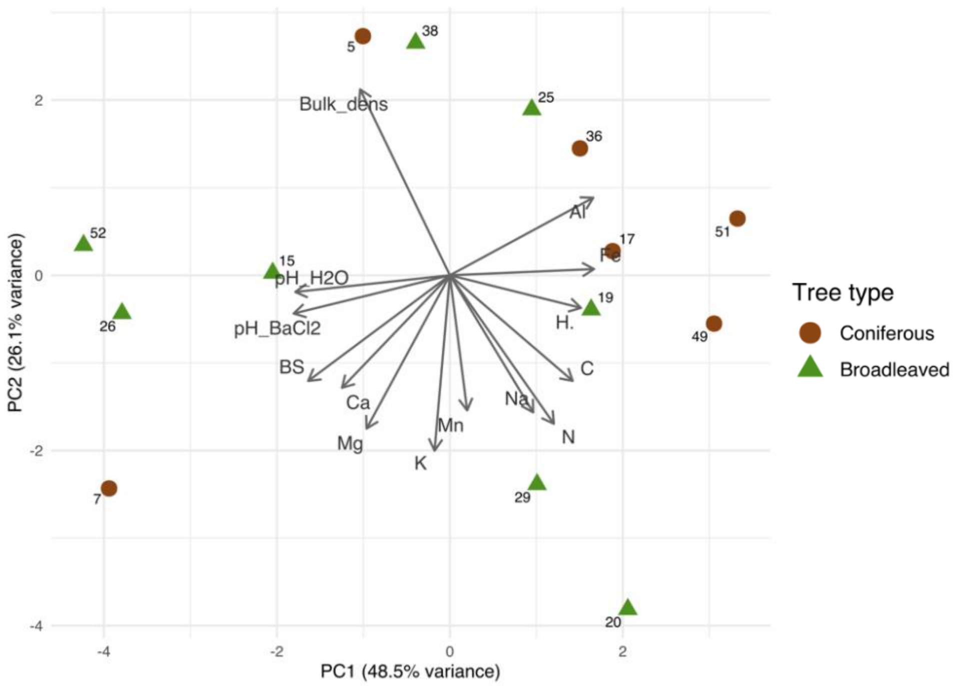


Figure 18: Results of principal component analysis (PCA) of soil physico-chemical characteristics (between 0 and 5cm). The arrows show the contribution of variables to the first two principal dimensions (Dim1: 48.5 % of variance; Dim2: 26.1%). The direction and length of the arrows indicate the weight and correlation of each variable with the axes. Coniferous species are represented with the brown circles and broadleaved species with green triangles.

Not only soil characteristics shape earthworm communities, these communities are also able to influence soil characteristics as carbon stocks. The next figure explores the relationship between the carbon stock in the forest floor and the earthworm biomass. The carbon stock decreases significantly with a higher earthworm biomass ($r = -0.446$, $p = 0.003$). This suggests that plots with higher earthworm biomass tend to have lower carbon stocks.

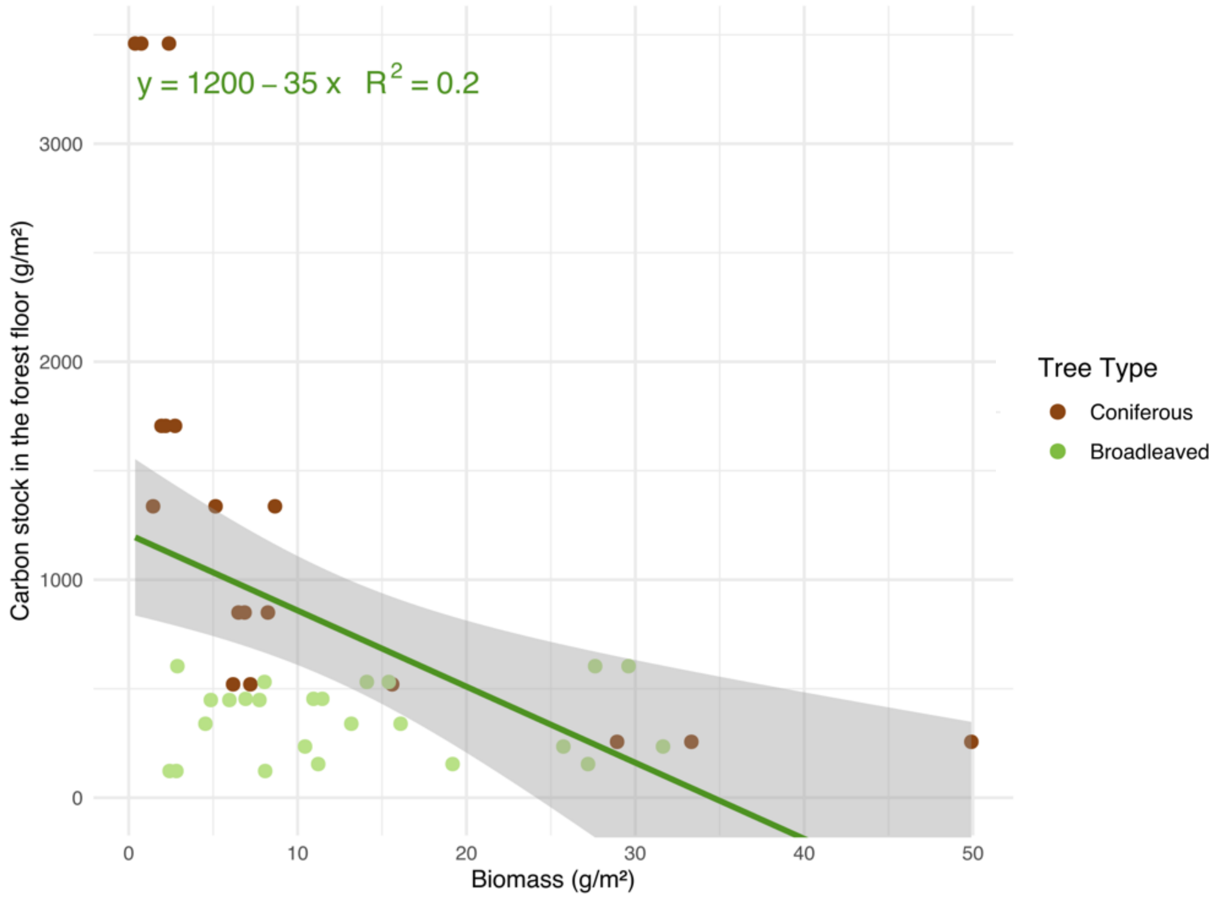


Figure 19: Relationship between forest floor carbon stock (g/m²) and earthworm biomass (g/m²), showing a fitted linear regression (green) and the associated 95% confidence interval (grey area).

3.2. Plot characteristics

Figure 20 presents how earthworm biomass evolve with leaf litter turnover rate. Earthworm biomass shows a positive response as it increases with an increase in leaf litter turnover rate. A moderate and significant positive correlation was found between leaf turnover rate and earthworm biomass ($r = 0.385$, $p = 0.012$), indicating that species with faster litter input, and therefore lower forest floor mass, may enhance earthworm activity and abundance.

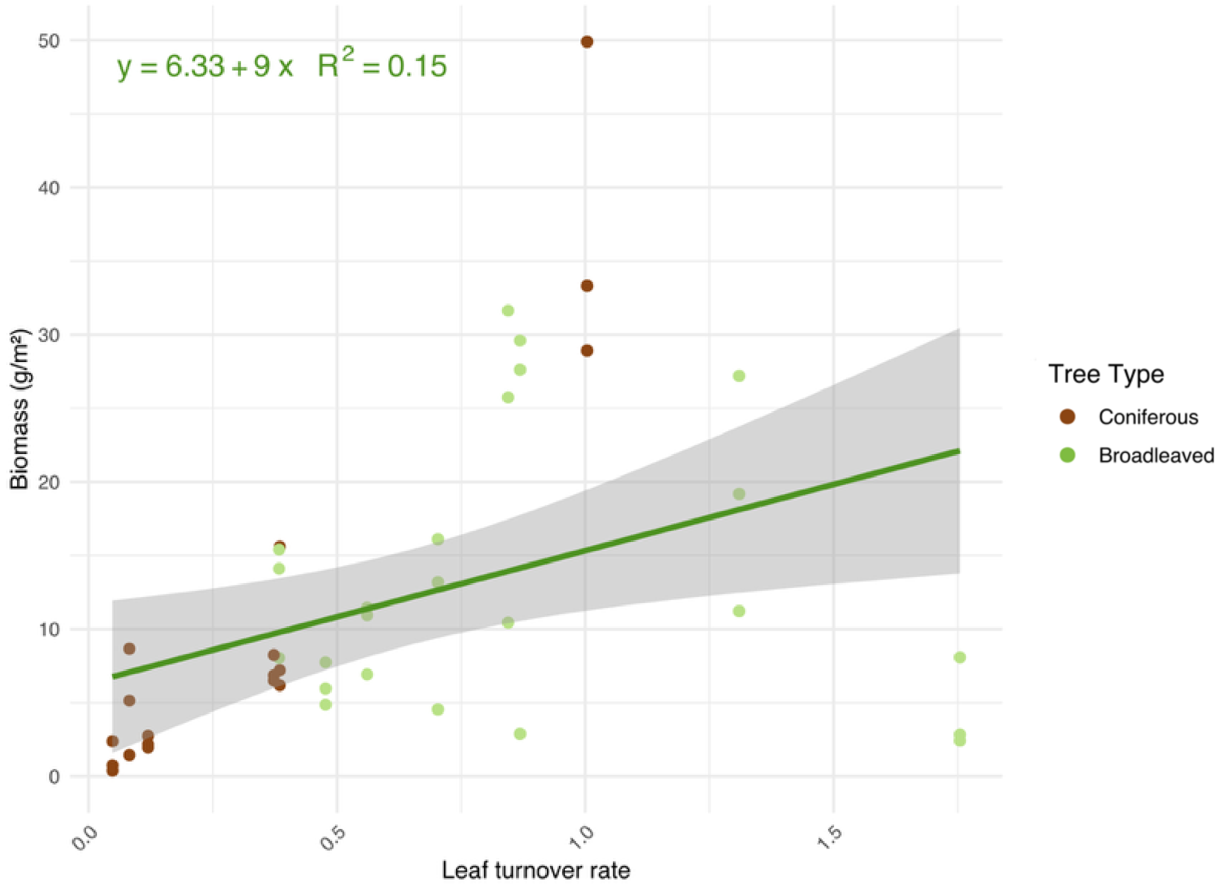


Figure 20: Earthworm biomass (g/m²) as a function of the leaf turnover rate represented by a trend curve (green) obtained by a linear regression with a 95% confidence interval (grey area).

The results of the PCA on soil characteristics are shown in the Appendix 8. A clear distinction can be made between coniferous and broadleaved stands. In fact, coniferous stands are mostly located on the left side of the graph, highlighting lower leaf turnover rates and canopy cover, and thicker organic layers.

3.3. Leaf litterfall characteristics

The linear regressions regarding the relationships between biomass and leaf litterfall characteristics, C:N, C:P, and N:P ratios, and base cation content, showed weak effects and non-significant correlations. Al and Ca contents were tested and are presented later, when the third hypothesis is addressed.

This results of the PCA showed in the next figures clearly highlights a distinction between broadleaved and coniferous species regarding leaf litterfall quality. The conifers, located on the left part of the graph, tend to produce nutrient-poor litter with higher C:N ratio and C content.

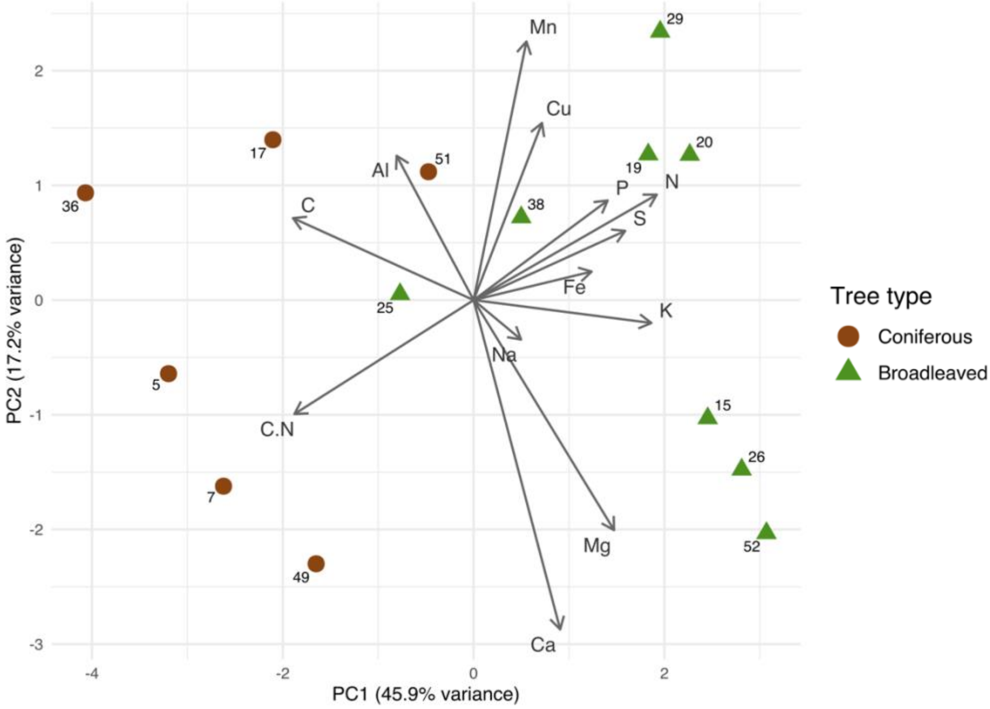


Figure 21: Results of principal component analysis (PCA) of leaf litterfall characteristics (between 0 and 5cm). The arrows show the contribution of variables to the first two principal dimensions (Dim1: 45.9 % of variance; Dim2: 17.2 %). The direction and length of the arrows indicate the weight and correlation of each variable with the axes. Coniferous species are represented with the brown circles and broadleaved species with green triangles.

3.4. Influence of microclimate on earthworm communities

Figure 22 illustrates the relationships between soil moisture and temperature with earthworm biomass. Even though Figure 22b illustrates a positive trend between mean soil moisture and mean biomass, this result is not statistically significant ($p = 0.1558$). The results show a negative non-significant relationship with soil temperature, with a p-value of 0.3066.

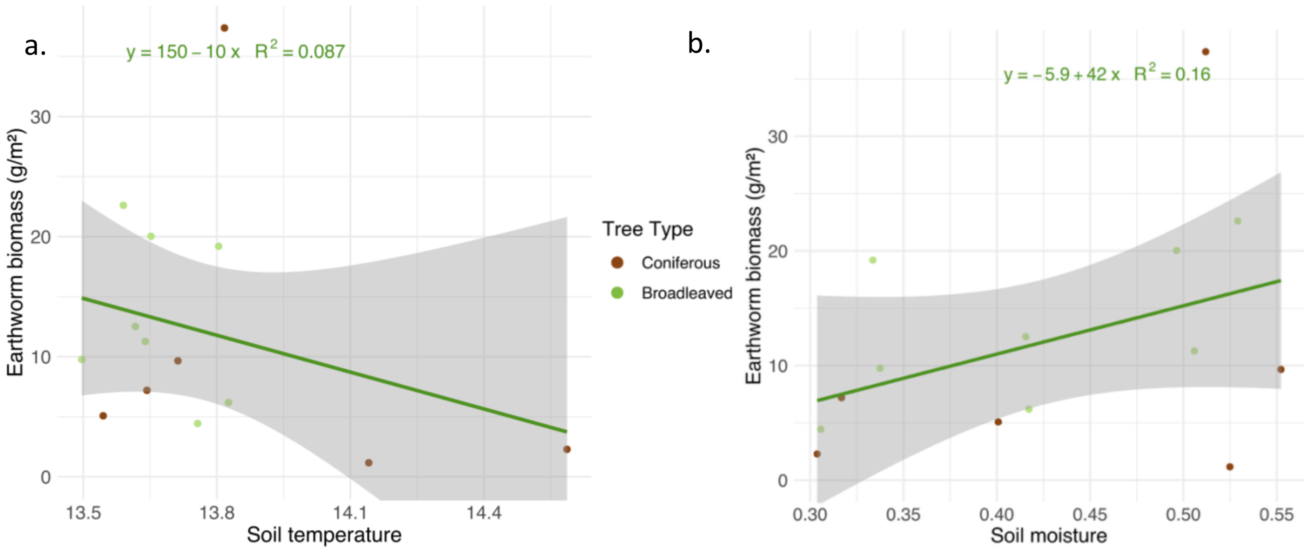


Figure 22: Earthworm biomass as a function of microclimatic values: (a) soil temperature (°C), (b) soil moisture (m^3/m^3), represented by a trend curve (green) obtained by a linear regression with a 95% confidence interval (grey area).

3.5. Identifying key drivers of earthworm biomass variability

All the models presented below were fitted to predict the earthworm biomass. A parameter that was influenced, unlike density, by the previous explanatory variables, as shown by the correlations and linear regressions.

The table below illustrates the results of the first linear mixed model, considering all PCA components. The significant parameter the second component of the soil PCA, representing mainly the bulk density and negatively affecting the earthworm biomass. The fact that R^2c is greater than R^2m means that variability between plots explains a significant proportion of biomass variation, in addition to fixed variables.

Table 7. Fixed-effect estimates, standard errors, t-values and p-values for the intercept, PC1, and PC2 components (litterfall, plot, and soil) in the linear mixed model explaining earthworm biomass (random effect: Plot).

	Estimate	**Standard Error**	**t value**	**p-value**
(Intercept)	12.0571	1.2478	9.663	2.68e-05 ***
$PC1_{Plot}$	1.4984	1.2620	1.187	0.27382
$PC2_{Plot}$	0.3622	1.1836	0.306	0.76851
$PC1_{soil}$	-1.9697	1.0710	-1.839	0.10847
$PC2_{soil}$	-3.4063	0.7694	-4.427	0.00306 **
$PC1_{Leaf}$	-1.5137	0.7154	-2.116	0.07215 .
$PC2_{Leaf}$	-0.9048	1.4028	-0.645	0.53948
		Marginal R^2	**Conditional R^2**	**p-value**
		0.6075	0.6569	6.002e-05 ***

The next model exclusively considered the soil characteristics, the first component of the PCA representing a nutrient gradient and the second mainly representing bulk density. Both variables, associated with lower pH conditions and higher bulk density, seemed to negatively influence earthworm biomass.

Table 8. Fixed-effect estimates, standard errors, t-values and p-values for the intercept, PC1soil, and PC2soil components (litterfall, plot, and soil) in the linear mixed model explaining earthworm biomass (random effect: Plot).

	Estimate	**Std. Error**	**t value**	**p-value**
(Intercept)	12.0571	1.3665	8.823	2.54e-06 ***
$PC1_{soil}$	- 2.5558	0.5443	- 4.695	0.000655 ***
$PC2_{soil}$	- 2.8770	0.7412	- 3.881	0.002557 **
		Marginal R^2	**Conditional R^2**	**p-value**
		0.5564	0.6420	3.267e-05 ***

The next linear mixed model is based on the first components of the PCA (for plot, soil and leaf litterfall characteristics). Among the fixed effects, the nutrient gradient ($PC1_{Soil}$) was significant at the 0.1 threshold. This suggests that an increase in the overall soil nutrients, here positively linked with Al concentrations and negatively linked with Ca and Mg concentrations, is associated with a decrease in total earthworm biomass. The effects of plot ($PC1_{plot}$) and leaf litterfall ($PC1_{litterfall}$) characteristics were not statistically significant.

Table 9. Fixed-effect estimates, standard errors, t-values and p-values for the intercept and PC1 components (litterfall, plot, and soil) in the linear mixed model explaining earthworm biomass (random effect: Plot).

	Estimate	**Std. Error**	**t value**	**p-value**
(Intercept)	12.0571	2.1510	5.605	0.000226 ***
$PC1_{Plot}$	1.0781	1.5737	0.685	0.508864
$PC1_{Soil}$	- 2.2149	1.0606	-2.088	0.063313 .
$PC1_{Leaf}$	- 0.3816	0.9777	-0.390	0.704469
		Marginal R^2	**Conditional R^2**	**p-value**
		0.3192	0.6735	0.02566 *

The following model indicates a significant and negative main effect of the soil fertility gradient ($PC1_{Soil}$), as well as a non-significant effect of leaf litterfall quality ($PC1_{Leaf}$). However, a positive and significant interaction between these two variables was found ($PC1_{Soil} \times PC1_{Leaf}$). These results suggest that the negative effect of soil properties on earthworm biomass is modulated by litter characteristics: as litter quality increases, this negative effect tends to attenuate or even become positive.

Table 10: Fixed-effect estimates, standard errors, t-values and p-values from the linear mixed model examining the interaction between soil and litterfall principal components (PC1) on earthworm total biomass (random effect: Plot).

Parameter	**Estimate**	**Std. Error**	**t value**	**p-value**
(Intercept)	13.2139	1.8381	7.189	2.97e-05 ***

$PC1_{soil}$	- 2.8205	0.7379	-3.823	0.00336 **
$PC1_{Leaf}$	0.3322	0.8135	0.408	0.69163
$PC1_{soil} : PC1_{Leaf}$	0.7229	0.3094	2.336	0.04161 *
		Marginal R^2	**Conditional R^2**	**p-value**
		0.4464	0.6598	0.002047 **

When the second principal components of the environmental variables are used, the model reveals that total earthworm biomass is negatively influenced by the bulk density. This suggests that specific soil properties, as captured by PC2, may limit the presence or activity of earthworms. The effect of the concentrations of Mn, Al and Cu in the leaf litterfall ($PC2_{Leaf}$) and understory vegetation cover ($PC2_{Plot}$) remain non-significant.

Table 11. Fixed-effect estimates, standard errors, t-values and p-values for the intercept and PC2 components (litterfall, plot, and soil) in the linear mixed model explaining earthworm biomass (random effect: Plot).

Parameter	**Estimate**	**Std. Error**	**t value**	**p-value**
(Intercept)	12.057	2.1218	5.683	0.000203 ***
$PC2_{Plot}$	0.2568	1.4248	0.180	0.860542
$PC2_{soil}$	- 3.0443	1.2578	- 2.420	0.036045 *
$PC2_{Leaf}$	- 2.8419	1.4796	- 1.921	0.083704 .
		Marginal R^2	**Conditional R^2**	**p-value**
		0.3294	0.6724	0.02156 *

The table below shows the AICs of different models, which help to identify those offering a balance between complexity and performance. The model including the first two principal axes of soil, stand structure, and leaf litterfall variables had the lowest AIC (285.06), suggesting it might better explain the variation observed compared to simpler models including fewer variables. However, the difference in AIC values with some simpler models was relatively small (less than 5 units), indicating that simpler models might also be competitive. Moreover, several parameters in the full model were not statistically significant, whereas parameters in some simpler models, the second one for instance, showed significance, suggesting that model parsimony and interpretability should also be considered alongside AIC.

Table 12 : Summary of model comparisons showing the degrees of freedom (df) and corresponding Akaike Information Criterion (AIC) values.

	df	**AIC**
$PC1_{soil} + PC1_{plot} + PC1_{litterfall} + PC2_{soil} + PC2_{plot} + PC2_{litterfall} + \left(\frac{1}{Plot}\right)$	9	285.060
$PC1_{soil} + PC2_{soil} + \left(\frac{1}{Plot}\right)$	5	289.939
$PC1_{soil} + PC1_{plot} + PC1_{litterfall} + \left(\frac{1}{Plot}\right)$	6	298.592

$PC1_{soil} * PC1_{litterfall} + \left(\frac{1}{Plot}\right)$	6	297.558
$PC2_{soil} + PC2_{plot} + PC2_{litterfall} + \left(\frac{1}{Plot}\right)$	6	296.952

4. Influence of Al and Ca concentrations

Regarding the third hypothesis, calcium and aluminum were examined separately from other soil and litter characteristics because of their specific and well-documented physiological effects on earthworms. Calcium is said to have a key role in buffering soil acidity and in earthworm metabolism, while aluminum is known to be toxic in acidic environments.

4.1. Soil exchangeable Al and Ca

Figure 23 illustrates the relationship between soil exchangeable aluminum content and earthworm biomass. Biomass tends to decrease with increasing Al content and a strong and highly significant negative correlation was observed ($r = -0.637$, $p < 0.001$), indicating that aluminum toxicity may be a limiting factor for earthworm biomass in acidic soils.

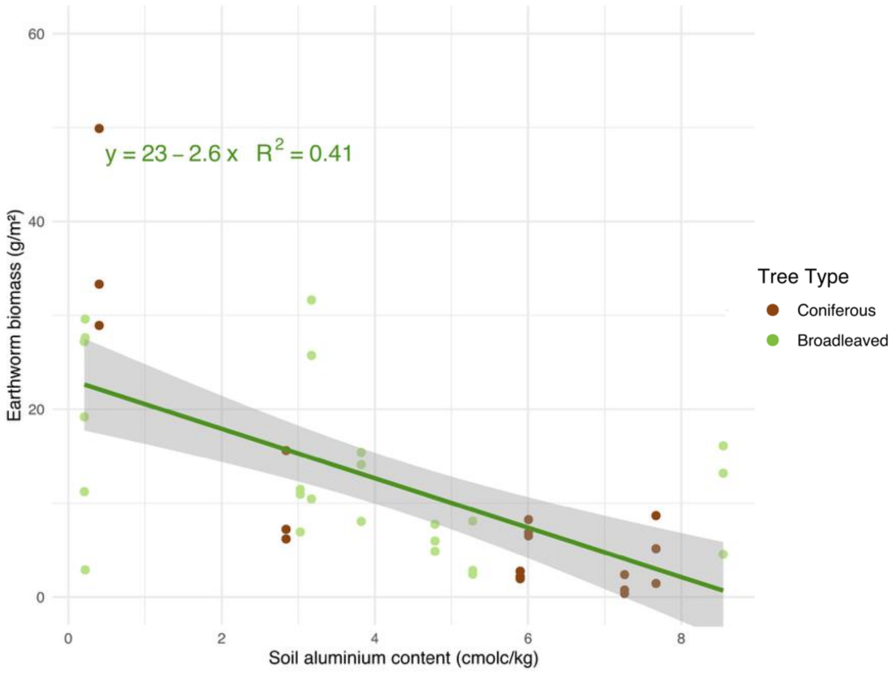


Figure 23: Earthworm biomass as a function of exchangeable soil Al content represented by a trend curve (green) obtained by a linear regression with a 95% confidence interval (grey area).

Figure 24 shows the relationship between soil exchangeable calcium concentration and earthworm biomass. A clear positive trend and a strong and highly significant positive are observed ($r = 0.743$, $p < 0.001$), indicating that higher soil calcium levels were generally associated with heavier earthworm populations.

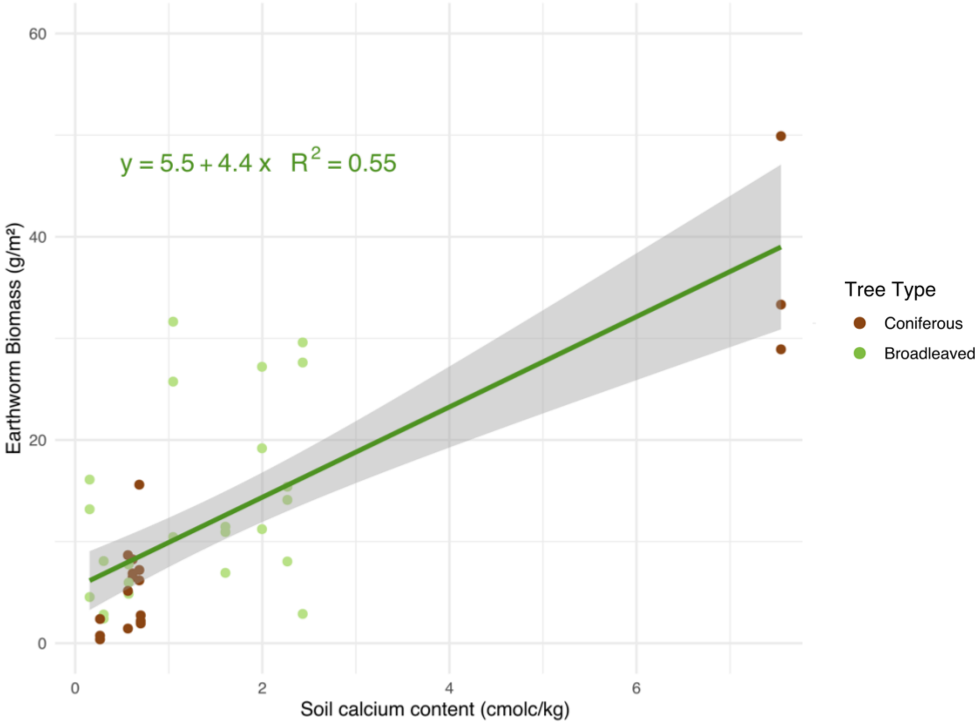


Figure 23: Earthworm biomass as a function of soil Ca content represented by a trend curve (green) obtained by a linear regression with a 95% confidence interval (grey area).

4.2. Ca and Al in leaf litter

Figure 25 illustrates the relationship between calcium concentration in leaf litterfall and biomass. A slight upward trend and a weak but significant positive correlation were observed between calcium concentration in leaf litterfall and earthworm biomass ($r = 0.347$, $p = 0.024$), suggesting that higher calcium content in leaf litterfall may contribute to increased biomass.

As represented in Figure 26, a general decreasing trend is observed between leaf litterfall aluminum content and earthworm biomass. A weak but significant negative correlation was detected between the aluminum concentration and earthworm biomass ($r = -0.316$, $p = 0.042$). This suggests that the potential toxicity of aluminum in litter may limit biomass, although this effect is less marked than for aluminum in mineral soil.

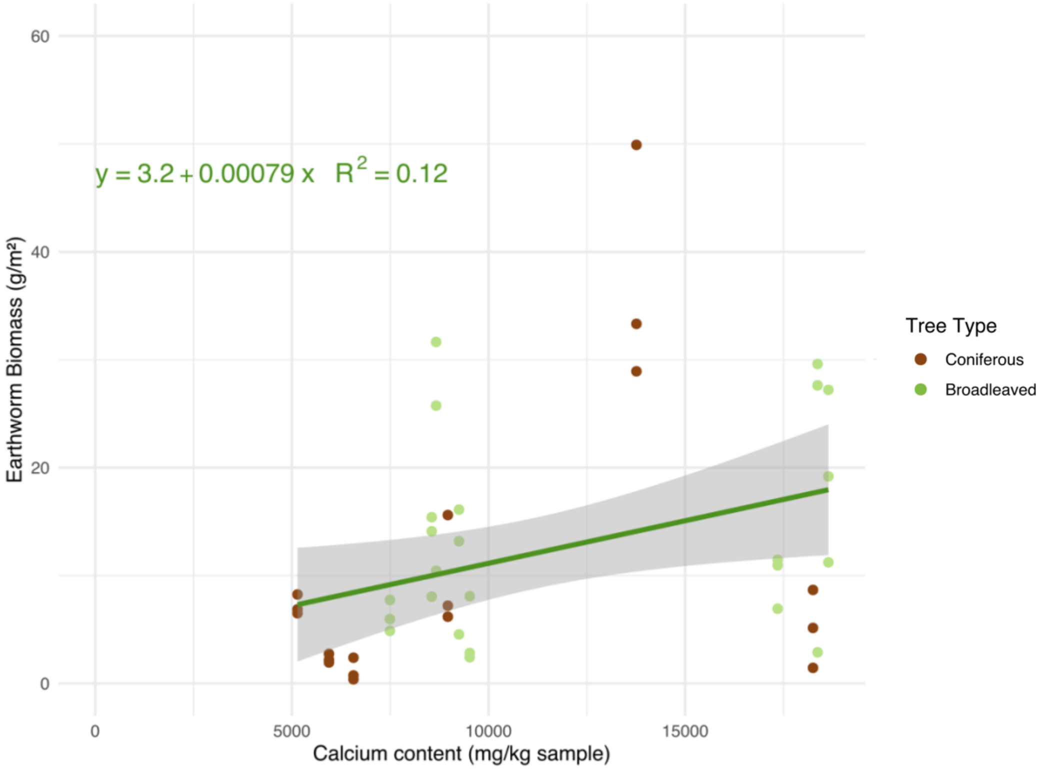


Figure 25: Earthworm biomass as a function of leaf litterfall Ca content represented by a trend curve (green) obtained by a linear regression with a 95% confidence interval (grey area).

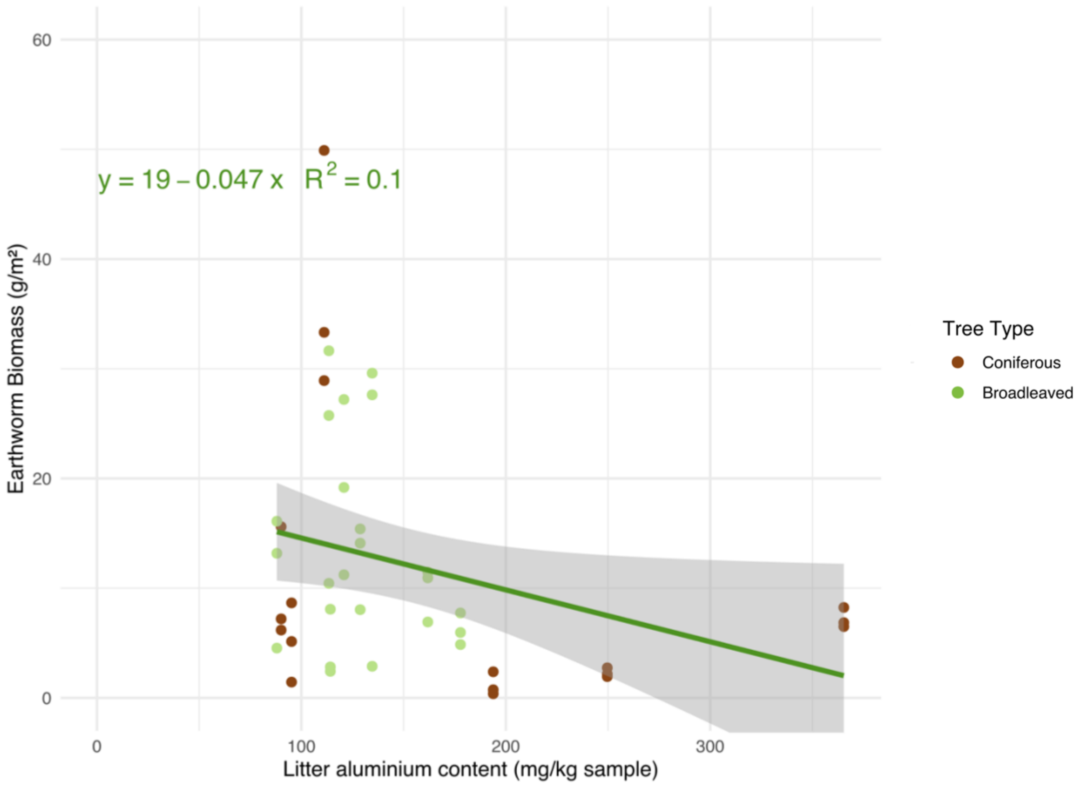


Figure 26: Earthworm biomass as a function of leaf litterfall Al content represented by a trend curve (green) obtained by local smoothing with a 95% confidence interval (grey area).

Discussion

1. Structure and characteristics of the earthworm communities

1.1. Proportion of juveniles and adults

As mentioned in the beginning of the results section, a huge part of the sampled earthworms were juveniles, they represented a total of 79% when all plots were considered and the distribution varied between individual plots. Schelfhout et al. (2017), who also performed a common garden experiment, found lower values with about half the earthworms being juveniles and Szlavecz et al. (2018) found this proportion to be 68%. One possible explanation for this particularly high proportion of juvenile individuals is that the reproduction rate may be relatively high for some species. For instance, epigeic species typically show high reproduction rates and tend to grow rapidly (Edwards & Arancon 2022). This may suggest that environmental conditions, such as food availability, soil moisture and temperature, were favorable for reproduction, this matter will be address later, in the discussion of the second hypothesis. However, this remains unconfirmed due to the absence of reproduction rates measurements.

According to Edwards & Arancon (2022), the proportions of juveniles and adult earthworms vary seasonally. They reported that juvenile densities peaked at the end of April and again at the end of October, which coincides with the sampling period of this research. Furthermore, they reported that the highest densities of adults were seen during the end of August and April. This could partly account for the low number of adult individuals and the high proportion of juveniles observed in this research. Sampling at a different time of the year would be necessary to test this hypothesis. Edwards & Arancon (2022) also stated that the year repartition depends on the earthworm species, explaining why some species have shown higher numbers of juveniles than others in this research.

However, these proportions may not accurately reflect the actual structure of earthworm communities. Several studies suggested that adult or near-mature individuals of certain European lumbricid species tend to inhabit deeper soil layers than juveniles. Satchell (1955) was the first to observe this trend, later confirmed by Gerard (1967) in British pastures, where adult worms were found to retreat deeper into the soil before entering the resting phase. Similarly, Piearce (1983) found that juveniles generally stayed closer to the surface than adults. This trend might partly explain the higher proportion of juveniles collected during sampling: being closer to the surface, they could have reacted more quickly to the mustard extraction method, whereas adults, located deeper down, may not have been affected or came after the second 10-minute interval. This vertical distribution pattern is generally explained by the depth at which cocoons are laid, which means that juveniles hatch closer to the surface, and by the limited burrowing capacity of small juveniles compared to adults (Jiménez & Decaëns 2000).

Finally, a last plausible explanation of the life stages distribution could be the use of the mustard method. Juveniles and adults of the same species answer differently to the mustard method (Singh et al. 2016). In contrast, the hand sorting method involves physically searching through soil layers to a depth of 20 cm, which allows for the detection of deeper-dwelling adults that may not be stimulated to surface by the mustard. Therefore, hand sorting should have captured a higher proportion of adults compared to the mustard method but this is not what was observed here.

1.2. Ecological categories

Regarding the ecological categories, the majority of earthworms collected were classified as epigeic both across individual tree types and when combined (Appendix 6. Figure a.). This pattern may be influenced by the sampling method as different collection techniques vary in their efficiency for capturing specific ecological groups (Singh et al. 2016).

The mustard extraction method is generally considered to be more effective for collecting anecic (deep-burrowing) and epigeic (litter-dwelling) earthworm species (Singh et al. 2016). As more epigeic than other categories were collected, it could mean that they are more present, but it could also be explained by the method used. Surprisingly, anecic species represented the smallest proportion of the collected individuals, despite the use of the mustard method, which is typically effective to collect this ecological group. Finally, few numbers of endogeic were found. This could be explained because they tend to move horizontally instead of vertically, when the mustard solution touches their membranes. Then, they could have been out of reach as they would have been outside the wooden frame (Singh et al. 2016).

The use of a specific sampling method is not the only explanation for the higher proportions of certain ecological categories and the influence of ecological processes should not be overlooked. The key factors influencing earthworm presence also depend on the ecological group to which the earthworms belong (Schelfhout et al. 2017).

Anecic species were shown to be greatly influenced by soil pH (Schelfhout et al. 2017), their presence is negatively affected by soil acidity (Muys & Granval 1992). Only one anecic species, *Lumbricus terrestris*, was found but only in two plots (*Calocedrus decurrens* and *Juglans mandschurica*) (Figure 11) where soil pH was higher (> 5) than in most plots. Another important factor that was proven to drive anecic presence, is the composition of leaf litterfall (Schelfhout et al. 2017). The soil in the *Calocedrus decurrens* stand showed the highest value of base cation content with especially high values of exchangeable Ca content even though leaf litterfall characteristics didn't seem to be particularly favorable. Therefore, the soil characteristics are probably the main driver of anecic earthworm presence in this plot. Regarding *Juglans mandschurica*, the presence of this ecological category seems to be mainly driven by leaf litterfall characteristics. In fact, this plot showed the highest values of base cation content in the leaves with a high Ca content and one of the lowest C:N ratio while soil characteristics were also favorable with a low Al content and high Ca content. These observations suggest that the presence of anecic species is the result of an interaction between soil and litter characteristics, with their relative importance varying between tree species.

Endogeic species are also influenced by soil characteristics and according to Schelfhout et al. (2017), they are sensitive to soil acidification. However, their presence has also been linked to leaf litter quality (Schelfhout et al., 2017). In low quality soil, with lower pH values and nutrient concentrations, Cesarz et al. (2016) found that the presence of endogeic species was dependent on leaf litter quality (leaf litter N, P, Ca, and Mg).

In this research, the highest densities of endogeic earthworms (individuals/m²) were observed under *Acer saccharinum* and *Pinus nigra subsp. laricio* (Figure 11). In contrast, no endogeic individuals were found under *Thuja plicata*, *Larix kaempferi*, and *Tsuga canadensis*.

Across all these stands, soil pH values were below 5 and base cation contents were under 5 cmolc/kg, indicating rather unfavorable soil conditions for endogeic species. Moreover, these conditions were similar both in plots with high and with no endogeic densities. Other soil characteristics also failed to explain the observed differences: exchangeable aluminum concentrations were not particularly low, and calcium concentratinos were not particularly high in the plots with more endogeic individuals. Overall, these soil conditions are not particularly favorable for endogeic earthworms, which aligns with their absence in some plots, but not with their presence in high numbers in others.

Looking at leaf litterfall characteristics, *Acer saccharinum*, where endogeic densities were the highest, had a litter rich in base cations (notably magnesium and potassium) and a low C:N ratio. In contrast, *Pinus nigra*, which also showed high endogeic densities, had the lowest base cation content and calcium concentration, combined with a high C:N ratio and the highest aluminum content in the litter, characteristics generally considered unfavorable for earthworm activity.

Thus, no consistent pattern emerged to explain the presence or absence of endogeic individuals solely based on soil or litter properties. In particular, no clear trait combination could be identified for the tree species under which no endogeic individuals were found. These findings highlight that soil chemistry and leaf litterfall quality alone cannot fully explain endogeic earthworm distribution. While *Acer saccharinum* suggests that high litter quality can favor their presence despite poor soils, *Pinus nigra* indicates that other factors may also contribute as the ones cited were not favorable.

1.3. Species distribution

The results regarding the earthworm species must be analyzed with caution has an important proportion of the juveniles weren't identified to the species or genus level. This proportion is different between the plots. Therefore, other species were probably present in some plots but weren't identified as only juveniles were observed. This uncertainty may lead to an underestimation of species richness or a misallocation of occurrences in certain plots. As the proportion of unidentified juveniles varies between plots, this may also influence the comparison of communities between plots, and between stand types. In particular, some stands might appear less diverse simply because a greater proportion of individuals were juvenile and unidentifiable

1.4. Intra plot variability

Figure 8 revealed notable variability within the plots, showing a high intra-plot variability. As the arboretum is set up as a forest ecosystem, the sampled stands were sometimes located close to each other and if the replicate was too close to it, the results could have been influenced by other tree species than the one considered. Furthermore, it was not always possible to be far enough from tree stems as some stands show high values of stem density and basal area.

Even within small forest plots, soil conditions show significant microenvironmental heterogeneity. Earthworm community studies consistently report patchy distributions and variability within sites. This spatial distribution of earthworms can be linked to soil properties and vegetation but also to seasonality as the position of patches was shown to vary during the year. Consequently, earthworm distribution in soils is influenced by both the time of year and local soil conditions, following a distinct spatiotemporal pattern (Nuutinen et al. 1998; Whalen & Costa 2003).

2. Discussion of hypotheses

The aim of this section is to compare the working hypotheses formulated prior to the study, in the objective section, with the results obtained, in order to assess the extent to which they are confirmed, invalidated or need to be qualified.

2.1. Differences in earthworm communities between broadleaved and coniferous stands

The first working hypothesis stated that broadleaved stands host a larger and more diverse earthworm community. Meaning that higher earthworm density, biomass and species richness should be found under broadleaves compared to conifers.

The first graphic results on earthworm species richness (Figure 12) indicated differences in earthworm species diversity, suggesting that broadleaved stands hosted more earthworm species, and therefore showed a more important diversity than coniferous stands. However, further statistical analyses (see *Results*, Section 2.2) on the diversity showed that those differences weren't statistically significant, meaning that a difference of diversity between the stands couldn't be confirmed.

Earthworm biomass and density were on average higher in broadleaved stands. However, statistical tests revealed no significant difference between the two stand types. This is unexpected as several studies found that broadleaved species created favorable living conditions, leaf litterfall, soil, and microclimate characteristics, for earthworms compared to coniferous species (Augusto et al. 2015). The principal component analysis (PCA) performed on leaf litterfall characteristics (Figure 21) highlighted that coniferous species presented a leaf litter that is, for most conifers, poorer in nutrients and with higher C:N ratios. This chemical composition indicates a less decomposable litter, corroborating the observations of Frouz et al. (2013) and Augusto et al. (2015).

This finding, that coniferous species could decompose more slowly, is also supported by values of leaf turnover rates, which are lower overall in conifers. This low turnover rate contributes to the accumulation of organic matter in the forest floor. Augusto et al. (2015) also stated that the forest floors were thicker under coniferous stands. However, there are a few exceptions to this general trend: *Fagus sylvatica*, although broadleaf, had the lowest leaf turnover rate of all the species studied. Conversely, a coniferous species, *Calocedrus decurrens*, showed particularly high leaf turnover rate values, clearly distinguishing itself from other conifers. This dynamic, characterized by less decomposable litter and slower leaf turnover in conifers, also explains the higher carbon stocks observed under these stands, both in the forest floor and in the first five centimeters of soil (Di Francesco 2023). Indeed, the highest soil carbon values were recorded in coniferous forests.

Regarding soil pH and base cation content, coniferous species also showed the lowest values even though a general trend could not be defined. In general, other studies found that pH under coniferous stands to be lower as they tend to acidify the topsoil (Augusto et al. 2015).

Finally, another factor that supposedly favors earthworm communities is the soil moisture, and annual variation of the soil moisture in the coniferous stands showed lower values, the average soil water content was lower under conifers compared to broadleaves. This is linked with other studies that stated that drier soils were caused by conifers through a permanent canopy cover and high leaf area index all year. These factors cause higher evaporation and rainfall interception and lead to drier soils (Ganault et al. 2020; Augusto et al. 2015). An ideal range of moisture is difficult to determine for earthworms as moisture requirements differ between species and within the species in different regions (Edwards & Arancon 2022). However, a study conducted in another temperate forest in Ohio, USA, reported that the largest numbers of earthworms occurred in soils containing between 12% and 30% moisture (Edwards & Arancon 2022).

This hypothesis is therefore at first not fully supported by the results. The results were not statistically significant for the biomass, density and species richness (see *Results*, section 2.). These first findings are unexpected as microclimatic conditions and leaf litterfall analyses indicated more favorable conditions for earthworm populations under broadleaved species. However, the relatively small sample size, eight broadleaved species and six coniferous species, may have limited the statistical power of the analyses, increasing the risk of a Type II error.

The results also showed that the mean biomass was greatly affected by the removal of one tree species, *Calocedrus decurrens*. Even though excluding the *Calocedrus decurrens* plot from the dataset affected the density, the difference between the two tree categories was still not significant. However, the difference in the biomass became significant and confirmed that a lower earthworm biomass was found under coniferous species.

This more marked difference than for density could indicate a smaller average worm mass in these stands and the presence of the earthworm species *Lumbricus terrestris,* found only in this coniferous stand and another broadleaved stand. This anecic species is one of the biggest in Europe and its mass and size are way higher than the other species found. So, although the number of worms is similar, their mass seems to differ greatly.

Although a foliar C:N ratio of 37, for *Calocerus decurrens*, typically indicates low leaf litterfall quality and slower decomposition, the observed leaf turnover rate of approximately 1.00 suggests that the persistent ectorganic layer is nonetheless renewed annually. This rate is higher than for any other conifer in this research. Furthermore, the Ca content in the litterfall is one of the highest but the leaf litterfall quality (nutrient content and C:N ratio) is not particularly high compared to other conifers. The soil of this plot showed different characteristics than the soils of the other coniferous species sampled here. The soil pH in the first soil layers is the highest, it has one of the lowest soil exchangeable Al contents, it showed the highest Ca and base cation content in the soil, and previous analyses showed the lowest total C stock in the coniferous category (Di Francesco 2023). These specific litter and soil characteristics likely explain the unusually high earthworm biomass observed under *Calocedrus decurrens*, suggesting that local edaphic conditions can override broader functional group trends and leaf litter characteristics. In fact, no other scientific studies have focused on this tree species and reported similar results.

Models showed that the biomass was mainly influenced by soil characteristics and that the influences of leaf litterfall and plot characteristic weren't significant. In all the models, including soil properties, the effect was significant for at least one soil PCA axis and both axes were affecting negatively the earthworm biomass (Tables 7,8,9,10,11,12). However, the effect of first axis of the soil PCA, a nutrient gradient with the axis evolving negatively with Ca concentrations and positively with Al concentrations in the soil, was greater when leaf litterfall was taken into account (Table 10). This could mean that the effects on earthworm biomass vary according to the type of litter present and don't depend on the soil characteristics solely. For example, when leaf litter quality increases by one unit, the negative effect of the fertility gradient in the soil on biomass is reduced by 0.72 units.

So even though the initial hypothesis suggested a clear advantage for broadleaved stands in hosting richer and more abundant earthworm communities, the results show a more complex reality. While broadleaved stands tend to host a greater diversity and biomass, these differences are not always statistically significant. Moreover, the case of *Calocedrus decurrens* highlights that soil conditions can override leaf litter quality for some coniferous species, challenging the general distinction between conifers and broadleaves. This suggests that tree species identity, and not just tree functional group, plays a key role in shaping earthworm communities.

2.2. Influence of trees trough soil characteristics, litter traits and microclimate on earthworm communities

The second working hypothesis mentioned that trees influence soil characteristics, litter traits and microclimate which then have a direct impact on the earthworm communities.

2.2.1. Soil characteristics

As expected, the highest values of the earthworm biomass were located in higher pH values (pH-$BaCl_2$). The regression curve showed that an increase in the pH lead to an increase in the biomass (Figure 16). In accordance with these results, previous studies have demonstrated the same trends: Jänsch et al. 2013; De Wandeler et al. 2016; Jozefowska et al. 2016, Van Gestel & Hoogerwerf 2001. Surprisingly, an effect of the pH on the earthworm density was not detected.

The results showed a marked positive correlation between earthworm biomass and base cation content (Figure 17), with higher biomass values in soils with higher base cation content. This relationship can be explained by the greater availability of essential exchangeable cations such as calcium and magnesium, which help improve soil fertility, mainly related to soil physical structuration and nutrient availability (Lavelle et al. 1993), and create favorable conditions for earthworm activity and survival. High base cation content is also generally associated with a more neutral pH, providing a more suitable environment for the majority of earthworm species, compared to acidic soils (Desie et al. 2020).

The soil carbon stocks in the forest floor were shown, to be decreasing with earthworm biomass (Figure 19). This finding is consistent with the one of Reich et al. (2005) who stated that lower organic horizon carbon content was associated with high earthworm biomass.

2.2.2. Leaf litterfall characteristics

Several litter quality measures were tested to see if a correlation was existing with the earthworm biomass: C:N ratio, C:P ratio, N:P ratio and base content. However, none of them was shown to have a significant impact on the earthworm biomass. The other litter components, Ca and Al concentrations, will be discussed later, in the discussion of the third hypothesis.

Another environmental variable related to leaf litterfall, leaf turnover rate, was also found to be positively associated with earthworm biomass (Figure 20), while no effect was observed on earthworm density. This relationship can be interpreted in both directions: on the one hand, a high biomass of earthworms, particularly of epigeic and anecic species, may accelerate litter decomposition, thus contributing to a higher turnover rate. As ecosystem engineers, earthworms have the ability to modify their environment. An important process they are key actors in is litter decomposition, and their impact on it can be seen through the leaf turnover rate. This result is in line with the observations of Hobbie et al. (2006), who highlighted the role of earthworms in accelerating litter decomposition, thus directly influencing the leaf turnover rate. Similarly, Reich et al. (2005) reported that plots with high earthworm biomass had lower ground litter mass and higher leaf turnover rates.

On the other hand, rapid litter turnover rate can provide favorable conditions for these species, ensuring a continuous supply of fresh organic matter. Schelfhout et al. (2017) identified litter turnover rates as significant predictors of the presence of epigeic species, reinforcing the hypothesis that these species respond particularly to the availability and turnover of above-ground organic matter.

2.2.3. Microclimate

The analyses showed that the trends observed for microclimatic factors, soil moisture and temperature, on earthworm communities were not statistically significant. Although earthworm biomass appeared to increase with higher soil moisture, this tendency was not supported by the statistical results. Several authors have documented positive effects of soil moisture on earthworm abundance and biomass, particularly under extreme dry or wet conditions (Eggleton et al. 2009). Other studies, reported in Edwards & Arancon (2022) found a positive correlation between earthworm biomass and soil moisture. These contrasting findings suggest that while soil moisture and temperature can influence earthworm dynamics, their effects may be context-dependent and less pronounced under moderate environmental variation.

When environmental conditions become unfavorable for earthworms, their responses vary depending on the severity of the stress and the species involved. Initially, earthworms can react directly to environmental changes, such as drought or extreme temperatures ; by entering a state of quiescence, becoming inactive but ready to resume activity as soon as conditions improve. Under more adverse or prolonged stress, they may enter a state of facultative diapause. In this state, even when favorable conditions return, earthworms do not immediately become active; instead, there is a delay in their response (Edwards & Arancon, 2022).

2.3. Influence of Al and Ca concentrations on earthworm communities

The last hypothesis stated that the presence, density and biomass of earthworms were positively influenced by high concentrations of calcium (Ca) and negatively influenced by high concentrations of aluminum (Al) in their environment. These Ca and Al influences can come from two compartments: the soil (through the exchangeable form of the elements) or the litter (their food source). The effect of these two cations was therefore analyzed separately according to their origin, in order to better understand their respective roles.

First, the hypothesis was tested for the soil compartment. The results revealed clear trends for earthworm biomass: it increased sharply with calcium concentration and decreased with aluminum concentration. Moreover, the relatively high Pearson correlation results suggested a positive significant correlation. In contrast, earthworm density did not appear to be strongly influenced by these factors, and the associated R^2 were low, indicating limited explanatory power. Reich et al. (2005) found high earthworm biomass to be associated with exchangeable soil Ca. The decrease with exchangeable soil Al content was also reported by Schelfhout et al. (2017).

These findings are consistent with the previously reported results concerning soil pH and base cation content. Calcium is a base cation and therefore plays a major role in buffering soil acidity (Bowman et al. 2008; Desie et al. 2020). High levels of exchangeable Ca contribute to a higher base saturation and an increase in pH, both of which create more favorable conditions for earthworms. Conversely, elevated concentrations of exchangeable Al are typically associated with acidic soils (pH < 5) where base cation availability is low, and where aluminum toxicity can directly negatively affect earthworms (Schelfhout et al., 2017). Therefore, the observed positive relationship between earthworm biomass and Ca, and the negative relationship with Al, likely reflect the broader effect of soil chemical fertility, particularly the balance between acidifying elements and base cations such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}.

Tree leaf litterfall Ca concentrations can influence the exchangeable Ca pool and interactions in the soil. Therefore, the exchangeable soil Ca concentrations can be linked to leaf litterfall (Reich et al, 2005). However, the Ca-rich leaf litterfall could be induced by carbonate rich loam located deeper in the soil. In fact, trees are able, through deep roots, to access to this Ca in the deeper soil layers and translocate it to aerial parts such as leaves. A proportion of this Ca can return to the soil afterwards, through dead plant tissues and relying on the efficiency of recycling of nutrients, but in the litter layer (Jobbágy & Jackson 2001; Prescott 2002).

The effect of these elements was then tested in the leaf litterfall. It was found that earthworm biomass also increased with increasing leaf litter calcium concentration and decreased with increasing leaf litter aluminum concentration. This result is consistent with those of Reich et al. (2005), who observed a higher abundance of earthworms in calcium-rich leaf litter plots than in calcium-poor ones. Hobbie et al. (2006) also showed a strong correlation between litter Ca concentration and earthworm abundance. These authors suggested that calcium played a role in the effect of plant species on earthworm litter consumption.

Schelfhout et al. (2017) further suggested that calcium-poor litter contributes to the absence of burrowing worm communities, which slows the decomposition of organic matter, promotes litter accumulation and leads to high concentrations of exchangeable aluminum in the soil - an unfavorable factor for earthworm communities. Similarly, Ponge et al (1999) indicated that the calcium requirements of earthworms can be met either by consuming litter, or by ingesting soil locally enriched in calcium as a result of litter decomposition.

These results support the initial hypothesis that earthworm biomass is positively influenced by calcium concentrations and negatively influenced by aluminum concentrations. This effect was particularly clear in the case of soil and litter biomass. While earthworm density showed no strong correlations with either element, biomass reacted significantly, particularly to soil concentrations. The results suggest that litter chemistry and soil base content play a key role in the structure and functioning of earthworm communities, and that calcium-rich environments favor more productive earthworm communities, while aluminum-rich conditions may act as limiting factors.

3. Research limitations

3.1. Sampling scheme

Several methodological and contextual factors limit the scope of the results obtained in this study. Firstly, the small sample size is a major constraint. With a limited number of plots (14) and only one survey period per tree species, the statistical power of the tests is weakened, increasing the risk of type II errors. In other words, some real effects may not have been detected. This low representativeness also limits the generalizability of conclusions to other forests or stand types. However, the sampling and identification of species were labor intensive and time-consuming stages of this thesis, with nearly 3000 earthworms found, stored and identified one by one. A time efficient alternative would be welcomed if a larger sample is considered.

Another potential source of bias is the time of year during which the samples were collected. Earthworms are particularly sensitive to microclimatic conditions, especially soil moisture and temperature and there is a seasonal pattern in their populations. As showed in Figure 14, soil moisture and temperature vary across the year. The month of October registered low values of soil moisture compared to winter and the beginning of spring. Soil temperature was also lower than during summer months. The sampling was mostly done during the early morning, when temperatures are lower, this may not be well represented in the daily mean soil temperature.

Edge effects could also influence the results. The plots studied are not isolated from their environment, and their proximity to other stands and frequented paths could affect soil characteristics, microfauna and edaphic dynamics.

Finally, it should be reminded that only one plot per tree species was sampled, with three pseudo replicates per plot. This makes it difficult to distinguish between species-specific effects and other local factors (soil conditions, topography, water sources) even though most of them (climate, soil formation processes and management history) were homogeneous inside the arboretum.

3.2. Methods

Another methodological limitation concerns the detection rate of individuals, which is directly influenced by the sampling methods used. The mustard extraction method can be biased by external factors such as light conditions and the visibility of worms on the soil surface. It tends to favor the detection of larger individuals, potentially underestimating the presence of smaller or deeper earthworms. Moreover, it has been proven to be more effective for some ecological categories, but not for all of them.

Similarly, the hand sorting method, although complementary, is very laborious and time-consuming. It is also sensitive to field conditions such as poor lighting or damp soil, which can make small individuals difficult to detect manually. Furthermore, this method can cut earthworms and isn't suitable for collecting fast moving species.

The relative efficiency of the two methods in this study highlights this imbalance: around 93% of earthworms (based on density) were recovered by mustard extraction, and only 7% by manual sorting. This suggests that manual sorting may not have been applied thoroughly enough, or that the protocol could be refined to increase its contribution. However, mustard extraction could have collected all the present earthworms, being particularly efficient to a depth of at least 20 cm, and not leaving earthworms to find for the hand sorting. Meaning that two applications might be sufficient to collect all the earthworms.

3.3. Laboratory dentification

A major limitation of this research concerns the identification of earthworms. A very high proportion of the individuals collected were juveniles (~79%), which prevented the specific identification for some of them due to the absence of clitellum. This limits the analysis of the specific composition of the communities and prevents any detailed interpretation of the functional or ecological diversity of the species present in the different stands. The proportion of unidentified species were different between each plot and therefore influenced greatly the results.

In addition, biomass measurements may be subject to certain inaccuracies. Weighing errors are possible if individuals are not sufficiently dehydrated before weighing, which can lead to an overestimation of biomass. Similarly, imperfect scale calibration can introduce bias, particularly in the case of very small individuals. Counting errors can also occur which can affect the quality of the data obtained.

4. Further research perspectives

A first avenue for further research would be to explore the effects of mixed stands. It would be relevant to compare earthworm communities between monocultures and mixed stands, in order to better understand whether tree diversity influences the diversity or abundance of soil fauna. Ganault et al (2020) found a more diverse and abundant earthworm community under mixed stands. A conclusion also made by Cesarz et al. (2007) and Jacob et al. (2009), who each observed a positive correlation between earthworm abundance and tree diversity. However, in a review about the effects of mixed stands on soil fauna, Korboulewskya, Perez & Chauvat (2016), stated that non-significant, positive and negative effects have all been reported in studies before even though the majority found a positive correlation.

The study could then be extended to all the arboretum's plots. Focusing only on a small selection of stands limits the scope and representativeness of the results. More exhaustive sampling would provide a more complete picture of earthworm distribution in relation to the diversity of tree species present.

Another possible extension would be to include a temporal dimension. As earthworms are sensitive to seasonal variations in soil temperature and humidity, it would be interesting to carry out sampling campaigns at different times of the year. This would enable us to better identify the potential influence of trees according to the season, to better understand the dynamics of earthworm activity, and to assess the spatiotemporal variability of these communities. Due to changes in soil moisture and temperature, earthworm abundance and biomass vary across the year with a different peak between the species (Edwards & Arancon 2022; Eisenhauer et al. 2009).

Furthermore, although earthworms are considered bioindicators, they represent only a fraction of soil fauna. Integrating other groups such as microorganisms or fungi would provide a more global view of the health and functioning of forest soils.

Finally, multi-year monitoring would enable us to observe the evolution of earthworm populations over time. This would pave the way for a more detailed understanding of long-term trends, particularly in relation to stand ageing or climate change (Eisenhauer et al. 2009).

Conclusion

In the context of this master thesis, the aim was the characterize the earthworm communities, species, abundance, and biomass, under a range of tree species and assess the differences in these communities according to two types of trees: broadleaves and conifers.

This research highlighted the complexity of the relationships between earthworms and their forest environment, revealing that the influence of trees on these communities goes far beyond the simple classification into broadleaved or coniferous trees. The partial confirmation of the biomass hypothesis, obtained after excluding *Calocedrus decurrens*, underlines the importance of considering the specificities of individual tree species rather than generic groups.

This research also confirmed differences in the soil, plot and leaf litterfall characteristics between broadleaves and conifers. However, range of values was not always showing a marked difference between the tree types but, here again, more of a tree species effect, independent of the tree type. These results show that earthworms, key players in the quality and fertility of forest soils, are sensitive to precise chemical gradients, notably calcium and aluminum content, and that these factors are modulated at the scale of tree species.

However, the scope of this study remains limited by the sample size and collection period, which do not capture the spatial and temporal variability of earthworm communities. Future research would benefit from broader sampling with more tree species, including mixed stands and multiple seasons, to deepen understanding of spatiotemporal dynamics.

Bibliography

Aerts, R. (1997). Climate, Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial Ecosystems : A Triangular Relationship. Oikos, 79(3), 439-449. https://doi.org/10.2307/3546886

Aitken, S. N., Yeaman, S., Holliday, J. A., Wang, T., & Curtis-McLane, S. (2008). Adaptation, migration or extirpation : Climate change outcomes for tree populations. Evolutionary Applications, 1(1), 95-111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Aphalo P (2024). _ggpmisc: Miscellaneous Extensions to 'ggplot2'_. R package version 0.6.1, https://CRAN.R-project.org/package=ggpmisc.

Aubin, I., C.M. Garbe, S. Colombo, C.R. Drever, D.W. Mckenney, C. Messier, J. Pedlar, et Al. (2011). Why we disagree about assisted migration: Ethical implications of a key debate regarding the future of Canada’s forests. For. Chron. 87:755–765.

Augusto, L., De Schrijver, A., Vesterdal, L., Smolander, A., Prescott, C., & Ranger, J. (2015). Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. Biological Reviews, 90(2), 444-466. https://doi.org/10.1111/brv.12119

Baker, G. H., & Whitby, W. A. (2003a). Soil pH preferences and the influences of soil type and temperature on the survival and growth of Aporrectodea longa (Lumbricidae): The 7th inter- national symposium on earthworm ecology· Cardiff· Wales· 2002. Pedobiologia, 47(5–6), 745–753.

Baker, G. H., & Whitby, W. A. (2003b). Soil pH preferences and the influences of soil type and temperature on the survival and growth of Aporrectodea longa (Lumbricidae). Pedobiologia, 47, 745–753.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67, 1-48. https://doi.org/10.18637/jss.v067.i01

Berend, K., Haynes, K., & MacKenzie, C. M. (2019). Common Garden Experiments as a Dynamic Tool for Ecological Studies of Alpine Plants and Communities in Northeastern North America. Rhodora, 121(987), 174-212.

Berendse, F. (1998). Effects of dominant plant species on soil during succession in nutrient-poor ecosystems. Biogeochemistry, 42, 73–88.

Berry, E. C., & Jordan, D. (2001). Temperature and soil moisture content effects on the growth of Lumbricus terrestris (Oligochaeta: Lumbricidae) under laboratory conditions. Soil Biology and Biochemistry, 33(1), 133–136.

Binkley, D. (1995). The influence of tree species on forest soils: processes and patterns. In Trees and Soil Workshop (Volume 10) (eds D. J. Mead and I. S. Cornforth), pp. 1–33. Lincoln University Press, Canterbury.

Bohlen, P. J., & Edwards, C. A. (1995). Earthworm effects on N dynamics and soil respiration in microcosms receiving organic and inorganic nutrients. Soil Biology and Biochemistry, 27(3), 341–348. https://doi.org/10.1016/0038-0717(94)00184 -3

Bottinelli, N., Hedde, M., Jouquet, P., & Capowiez, Y. (2020). An explicit definition of earthworm ecological categories – Marcel Bouché's triangle revisited. *Geoderma*, *372*, 114361. https://doi.org/10.1016/j.geoderma.2020.114361

Bouché M. (1977). - Stratégies lombriciennes. In: Lohm U, Persson T (Eds) Soil organisms as components of ecosystems, vol. 25, Ecology Bulletin, Stockholm, pp. 122-132.

Boucher-Lalonde, V., Morin, A. & Currie, D. J. (2012). How are tree species distributed in climatic space? A simple and general pattern. Global Ecology and Biogeography 21, 1157–1166.

Bowman, W.D.; Cleveland, C.C.; Halada, L.; Hresko, J. (2008). Negative impact of nitrogen deposition on soil buffering capacity. Nat. Geosci., 1, 767–770 http://dx.doi.org/10.1038/ngeo339

Bradford, M. A., Berg, B., Maynard, D. S., Wieder, W. R., & Wood, S. A. (2016). Understanding the dominant controls on litter decomposition. Journal of Ecology, 104(1), 229-238. https://doi.org/10.1111/1365-2745.12507

Capowiez, Y., Decaëns, T., Hedde, M., Marsden, C., Jouquet, P., Marchán, D. F., Nahmani, J., Pelosi, C., & Bottinelli, N. (2022). Faut-il continuer à utiliser les catégories écologiques de ver de terre définies par Marcel Bouché il y a 50 ans ? Une vision historique et critique. *Étude et Gestion des Sols*, *29*, 51-58.

Castellano, M., Mueller, K., Olk, D., Sawyer, J., & Six, J. (2015). Integrating Plant Litter Quality, Soil Organic Matter Stabilization and the Carbon Saturation Concept. Global Change Biology, 21. https://doi.org/10.1111/gcb.12982

Cesarz, S., Fahrenholz, N., Migge-Kleian, S., Platner, C., & Schaefer, M. (2007). Earthworm communities in relation to tree diversity in a deciduous forest. European Journal of Soil Biology, 43, S61-S67. https://doi.org/10.1016/j.ejsobi.2007.08.003

Cesarz S, Craven D, Dietrich C, Eisenhauer N (2016) Effects of soil and leaf litter quality on the biomass of two endogeic earthworm species. Eur J Soil Biol 77:9–16

Ciais, P., Reichstein, M., Viovy, N. et al. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529–533. https://doi.org/10.1038/nature03972

Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., Hobbie, S. E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H. M., Santiago, L. S., Wardle, D. A., Wright, I. J., Aerts, R., Allison, S. D., van Bodegom, P., Brovkin, V., Chatain, A., … Westoby, M. (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters, 11(10), 1065-1071. https://doi.org/10.1111/j.1461-0248.2008.01219.x

Coûteaux, M.-M., Bottner, P., & Berg, B. (1995). Litter decomposition, climate and liter quality. Trends in Ecology & Evolution, 10(2), 63-66. https://doi.org/10.1016/S0169-5347(00)88978-8

Cramer, W., Bondeau, A., Woodward, F. I., Prentice, I. C., Betts, R. A., Brovkin, V., Cox, P. M., Fisher, V., Foley, J. A., Friend, A. D., Kucharik, C., Lomas, M. R., Ramankutty, N., Sitch, S., Smith, B., White, A. & Young-Molling, C. (2001). Global response of terrestrial ecosystem structure and function to CO_2 and climate change: results from six dynamic global vegetation models. Global Change Biology 7, 357–373.

Curry, J.P. (2004). Factors affecting the abundance of earthworms in soils. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, LLC, Boca Raton, pp. 263–286.

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature, 440(7081), 165-173. https://doi.org/10.1038/nature04514

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. Science (New York, N.Y.), 292(5517), 673-679. https://doi.org/10.1126/science.292.5517.673

De Frenne, P. et al. (2013) Microclimate moderates plant responses to macroclimate warming. Proc. Natl. Acad. Sci. U.S.A. 110, 18561-18565

De Frenne, P., Zellweger, F., Rodríguez-Sánchez, F., Scheffers, B. R., Hylander, K., Luoto, M., Vellend, M., Verheyen, K., & Lenoir, J. (2019). Global buffering of temperatures under forest canopies. Nature Ecology & Evolution, 3(5), 744-749. https://doi.org/10.1038/s41559-019-0842-1

De Schrijver, A., de Frenne, P., Staelens, J., Verstraeten, G., Muys, B., Vesterdal, L., Wuyts, K., van Nevel, L., Schelfhout, S., de Neve, S. & Verheyen, K. (2012). Tree species traits cause divergence in soil acidification during four decades of postagricultural forest development. Global Change Biology 18, 1127–1140.

De Wandeler, H., Sousa-Silva, R., Ampoorter, E., Bruelheide, H., Carnol, M., Dawud, S. M., Dănilă, G., Finer, L., Hättenschwiler, S., Hermy, M., Jaroszewicz, B., Joly, F.-X., Müller, S., Pollastrini, M., Ratcliffe, S., Raulund-Rasmussen, K., Selvi, F., Valladares, F., Van Meerbeek, K., … Muys, B. (2016). Drivers of earthworm incidence and abundance across European forests. Soil Biology and Biochemistry, 99, 167-178. https://doi.org/10.1016/j.soilbio.2016.05.003

Demir, G., Guswa, A. J., Filipzik, J., Metzger, J. C., Römermann, C., & Hildebrandt, A. (2024). Root water uptake patterns are controlled by tree species interactions and soil water variability. Hydrology and Earth System Sciences, 28(6), 1441-1461. https://doi.org/10.5194/hess-28-1441-2024

Desie, E., Van Meerbeek, K., De Wandeler, H., Bruelheide, H., Domisch, T., Jaroszewicz, B., Joly, F.-X., Vancampenhout, K., Vesterdal, L., & Muys, B. (2020). Positive feedback loop between earthworms, humus form and soil pH reinforces earthworm abundance in European forests. Functional Ecology, 34(12), 2598-2610. https://doi.org/10.1111/1365-2435.13668

Di Francesco E. (2023). Tree species effects on soil organic carbon stocks and chemical properties of the topsoil in the arboretum of Tervuren. Master thesis. UCLouvain.

Earthworm Biology | Earthworm Society of Britain. (s. d.). Consulté 17 mars 2025, à l’adresse https://www.earthwormsoc.org.uk/earthworm-biology

Edwards, C. A., & Arancon, N. Q. (2022). Biology and Ecology of Earthworms. Springer US. https://doi.org/10.1007/978-0-387-74943-3

Eggleton P., Inward K., Smith J., Jones D. T., Sherlock E. (2009) A six year study of earthworm (Lumbricidae) populations in pasture woodland in southern England shows their responses to soil temperature and soil moisture, Soil Biology and Biochemistry, Volume 41, Issue 9, Pp. 1857-1865, ISSN 0038-0717, https://doi.org/10.1016/j.soilbio.2009.06.007.

Eisenhauer, N., Milcu, A., Sabais, A. C. W., Bessler, H., Weigelt, A., Engels, C., & Scheu, S. (2009). Plant community impacts on the structure of earthworm communities depend on season and change with time. Soil Biology and Biochemistry, 41(12), 2430-2443. https://doi.org/10.1016/j.soilbio.2009.09.001

Facelli, J. M., & Pickett, S. T. A. (1991). Plant litter : Its dynamics and effects on plant community structure. *The Botanical Review*, *57*(1), 1-32. https://doi.org/10.1007/BF02858763

FAO. (2020). The State of the World’s Forests 2020. https://openknowledge.fao.org/items/d0f20c1c-7760-4d94-86c3-d1e770a17db0

Fourcade, Y., & Vercauteren, M. (2022). Predicted changes in the functional structure of earthworm assemblages in France driven by climate change. Diversity and Distributions, 28(5), 1050-1066. https://doi.org/10.1111/ddi.13505

Fraiture J. (2023). Impact de la structure forestière et de la composition spécifique sur le microclimat dans l’Arboretum de Tervuren. Master thesis. UCLouvain

Frey, S.J.K. et al. (2016). Spatial models reveal the microclimatic buffering capacity of old-growth forests. Science Adv. 2, e1501392

Frouz, J., Livečková, M., Albrechtová, J., Chroňáková, A., Cajthaml, T., Pižl, V., Háněl, L., Starý, J., Baldrian, P., Lhotáková, Z., Šimáčková, H., & Cepáková, Š. (2013). Is the effect of trees on soil properties mediated by soil fauna? A case study from post-mining sites. *Forest Ecology and Management*, *309*, 87-95. https://doi.org/10.1016/j.foreco.2013.02.013

Ganault, P., Nahmani, J., Hättenschwiler, S., Gillespie, L. M., David, J.-F., Henneron, L., Iorio, E., Mazzia, C., Muys, B., Pasquet, A., Prada-Salcedo, L. D., Wambsganss, J., & Decaëns, T. (2021). Relative importance of tree species richness, tree functional type, and microenvironment for soil macrofauna communities in European forests. *Oecologia*, *196*(2), 455-468. https://doi.org/10.1007/s00442-021-04931-w

Geiger R. , Aron R. H.,Todhunter P. (2003). The climate near the ground (Rowman and Littlefield, Oxford).

Gerard, B. M. (1967). Factors affecting earthworms in pastures. The Journal of Animal Ecology, 36, 235–252.

Gonzalez, G., T.R. Seastedt and Z. Donato, (2003). Earthworms, arthropods and plant litter

decomposition in aspen and lodge pole pine forests in Colorado, USA. Pedobiologia, 47: 863-869.
Grant, W. C. J. I. (1955). Studies on moisture relationships in earthworms. Ecology, 36(3), 400–407.
Gray, L. K., & Hamann, A. (2013). Tracking suitable habitat for tree populations under climate change in western North America. Climatic Change, 117(1), 289-303. https://doi.org/10.1007/s10584-012-0548-8
Hansen, K., Vesterdal, L., Schmidt, I. K., Gundersen, P., Sevel, L., Bastrup-Birk, A., Pedersen, L. B. & Bille-Hansen, J. (2009). Litterfall and nutrient return in five tree species in a common garden experiment. Forest Ecology and Management 257, 2133–2144.
Heal, O.W., Anderson, J.M., and Swift, M.J. (1997). Plant litter quality and decomposition: an historical overview. In Driven by nature: plant litter quality and decomposition. Edited by G.Cadisch and K.E. Giller. CAB International, Wallingford, U.K. pp. 3–32.
Hobbie, S. E., Reich, P. B., Oleksyn, J., Ogdahl, M., Zytkowiak, R., Hale, C., & Karolewski, P. (2006). Tree Species Effects on Decomposition and Forest Floor Dynamics in a Common Garden. Ecology, 87(9), 2288-2297. https://doi.org/10.1890/0012-9658(2006)87[2288:TSEODA]2.0.CO;2
imearth-adm. (2023). L'arboretum de Tervuren. IMEARTH. https://www.imearth.tv/decouvrir/nature/arboretum-de-tervuren/
IRM - Climat dans votre commune. (2020). meteo.be. https://www.meteo.be/fr/climat/climat-de-la-belgique/climat-dans-votre-commune
Jacob, M., Weland, N., Platner, C., Schaefer, M., Leuschner, C., & Thomas, F. M. (2009). Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity. Soil Biology and Biochemistry, 41(10), 2122-2130. https://doi.org/10.1016/j.soilbio.2009.07.024
Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., Johnson, D. W., Minkkinen, K., & Byrne, K. A. (2007). How strongly can forest management influence soil carbon sequestration? Geoderma, 137(3), 253-268. https://doi.org/10.1016/j.geoderma.2006.09.003
Jänsch, S., Steffens, L., Höfer, H., Horak, F., Roß-Nickoll, M., Russell, D., Burkhardt, U., Toschki, A., & Römbke, J. (2013). State of knowledge of earthworm communities in German soils as a basis for biological soil quality. Soil Organisms, 85(3), Article 3.f Ecol. Appl. 10, 423–436a
Jiménez, J. J., & Decaëns, T. (2000). Vertical distribution of earthworms in grassland soils of the Colombian Llanos. *Biology and Fertility of Soils*, *32*(6), 463-473. https://doi.org/10.1007/s003740000277
Jobbágy, E. G., & Jackson, R. B. (2000). The Vertical Distribution of Soil Organic Carbon and Its Relation to Climate and Vegetation. Ecological Applications, 10(2), 423-436. https://doi.org/10.2307/2641104
Jobbágy, E.G., Jackson, R.B. (2001). The distribution of soil nutrients with depth: Global

patterns and the imprint of plants. Biogeochemistry **53**, 51–77 https://doi.org/10.1023/A:1010760720215

Józefowska A., Woś B., Pietrzykowski M.(2016). Tree species and soil substrate effects on soil biota during early soil forming stages at afforested mine sites, Applied Soil Ecology, Volume 102, Pp. 70-79, ISSN 0929-1393, https://doi.org/10.1016/j.apsoil.2016.02.012.

Jucker T. et al. (2018). Canopy structure and topography jointly constrain the microclimate of human-modified tropical landscapes. Glob. Chang. Biol. 24, 5243–5258

Kögel-Knabner, I. (2002). The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry, 34(2), 139-162. https://doi.org/10.1016/S0038-0717(01)00158-4

Korboulewsky, N., Perez, G., & Chauvat, M. (2016). How tree diversity affects soil fauna diversity : A review. Soil Biology and Biochemistry, 94, 94-106. https://doi.org/10.1016/j.soilbio.2015.11.024

Krishna, M. P., & Mohan, M. (2017). Litter decomposition in forest ecosystems : A review. Energy, Ecology and Environment, 2(4), 236-249. https://doi.org/10.1007/s40974-017-0064-9

Lal, R. (2005). Forest soils and carbon sequestration. For. Ecol. Manage. 220, 242–258. https://doi.org/10.1016/j.foreco.2005.08.015.

Lange, B., Lüescher, P., & Germann, P. F. (2009). Significance of tree roots for preferential infiltration in stagnic soils. Hydrology and Earth System Sciences, 13(10), 1809-1821. https://doi.org/10.5194/hess-13-1809-2009

Lavelle, P., Blanchart, E., Martin, A., Martin, S., & Spain, A. (1993). A Hierarchical Model for Decomposition in Terrestrial Ecosystems : Application to Soils of the Humid Tropics. Biotropica, 25(2), 130-150. https://doi.org/10.2307/2389178

Lavelle, P., D. Bignell & M. Lepage (1997). Soil function in a changing world: the role of invertebrate ecosystem engineers. – European Journal of Soil Biology 33: 159–193.

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR. (2009). Elevated CO2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60: 2859–2876.

Lenoir, J., Gegout, J. C., Marquet, P. A., de Ruffray, P. & Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. Science 320, 1768–1771.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M. J., & Marchetti, M. (2010). Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecology and Management, 259(4), 698-709. https://doi.org/10.1016/j.foreco.2009.09.023

Millar, C. S. (1974). 4—Decomposition of Coniferous Leaf Litter. In C. H. Dickinson & G. J. F.

Pugh (Éds.), Biology of Plant Litter Decomposition (p. 105-128). Academic Press. https://doi.org/10.1016/B978-0-12-215001-2.50010-6

Mina, M., Messier, C., Duveneck, M. J., Fortin, M.-J., & Aquilué, N. (2022). Managing for the unexpected : Building resilient forest landscapes to cope with global change. Global Change Biology, 28(14), 4323-4341. https://doi.org/10.1111/gcb.16197

Mueller, K. E., Eissenstat, D. M., Hobbie, S. E., Oleksyn, J., Jagodzinski, A. M., Reich, P. B., Chadwick, O. A., & Chorover, J. (2012). Tree species effects on coupled cycles of carbon, nitrogen, and acidity in mineral soils at a common garden experiment. Biogeochemistry, 111(1), 601-614. https://doi.org/10.1007/s10533-011-9695-7

Muys, B., Lust, N. & Granval, P.H. (1992). Effects of grassland afforestation with different tree species on earthworm communities, litter decomposition, and nutrient status. Soil Biol. Biochem., 24, 1459–1466.

Muys, B., Ampoorter, E., Hermy, M., Valckx, J., De Wandeler, H. (2013). Earthworm sampling. Functional significance of forest biodiversity in Europe. K.U.Leuven

Nuutinen, V., Pitkänen, J., Kuusela, E., Widbom, T., & Lohilahti, H. (1998). Spatial variation of an earthworm community related to soil properties and yield in a grass–clover field. Applied Soil Ecology, 8(1), 85-94. https://doi.org/10.1016/S0929-1393(97)00063-2

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J, Borman T (2025). _vegan: Community Ecology Package_. R package version 2.6-10 https://CRAN.R-project.org/package=vegan>.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, et al. (2011). A large and persistent carbon sink in the world’s forests. Science.2011;333(6045):988–93 https://doi.org/10.1126/science.1201609

Paquette, A., Hector, A., Castagneyrol, B., Vanhellemont, M., Koricheva, J., Scherer-Lorenzen, M., & Verheyen, K. (2018). A million and more trees for science. Nature Ecology & Evolution, 2(5), 763-766. https://doi.org/10.1038/s41559-018-0544-0

Pastor, J., Dewey, B., Naiman, R.J., McInnes, P.F. & Cohen, Y. (1993). Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. Ecology, 74, 467–480.

Paul, K. I., Polglase, P. J., Smethurst, P. J., O’Connell, A. M., Carlyle, C. J., & Khanna, P. K. (2004). Soil temperature under forests : A simple model for predicting soil temperature under a range of forest types. Agricultural and Forest Meteorology, 121(3), 167-182. https://doi.org/10.1016/j.agrformet.2003.08.030

Peng, Y., Holmstrup, M., Schmidt, I. K., De Schrijver, A., Schelfhout, S., Heděnec, P., Zheng, H., Bachega, L. R., Yue, K., & Vesterdal, L. (2022). Litter quality, mycorrhizal association, and soil properties regulate effects of tree species on the soil fauna community. Geoderma, 407, 115570.

https://doi.org/10.1016/j.geoderma.2021.115570
Piearce, T. G. (1983). Functional morphology of lumbricid earthworms, with special reference to locomotion. Journal of Natural History, 17(1), 95–111.
Ponge, J.-F., Patzel, N., Delhaye, L., Devigne, E., Levieux, C., Beros, P., & Wittebroodt, R. (1999). Interactions between earthworms, litter and trees in an old-growth beech forest. Biology and Fertility of Soils, 29(4), 360-370. https://doi.org/10.1007/s003740050566
Prescott, C.E. (2002). The influence of the forest canopy on nutrient cycling. Tree Physiol. 22:1193–1200.
Prescott, C. E. (2010). Litter decomposition : What controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry, 101(1), 133-149. https://doi.org/10.1007/s10533-010-9439-0
Prescott, C. E., & Vesterdal, L. (2021). Decomposition and transformations along the continuum from litter to soil organic matter in forest soils. Forest Ecology and Management, 498, 119522. https://doi.org/10.1016/j.foreco.2021.119522
Quideau, S. A., Graham, R. C., Chadwick, O. A. & Wood, H. B. (1998). Organic carbon sequestration under chaparral and pine after four decades of soil development. Geoderma 83, 227–242.
Reid, W. V. (2005). *Millennium Ecosystem Assessment : Ecosystems and Human Well-being*. https://www.wri.org/research/millennium-ecosystem-assessment-ecosystems-and-human-well-being
Roots, B. I. (1956). The water relations of earthworms: II. Resistance to desiccation and immer- sion, and behaviour when submerged and when allowed a choice of environment. Journal of Experimental Biology, 33(1), 29–44.
Royal Trust. (2020). « Arboretum van Tervuren ». 2020. https://arboretum-tervuren.be/fr/home- fr/
Saintonge, F.-X., Gillette, M., Blaser, S., Queloz, V., & Leroy, Q. (2021). Situation et gestion de la crise liée aux scolytes de l'Épicéa commun fin 2021 dans l'est de la France, en Suisse et en Wallonie. Revue forestière française, 73(6), Article 6. https://doi.org/10.20870/revforfr.2021.7201
Satchell, J. E. (1955). Some Aspects of Earthworm Ecology. – In: McE. Kevan, D.K. (ed.): Soil Zoology. – Butterwoth, London. UK: 180–201.
Scheffers, B.R. et al. (2013). Microhabitats reduce animal's exposure to climate extremes. Glob. Change Biol. 20, 495–503
Schelfhout, S., Mertens, J., Verheyen, K., Vesterdal, L., Baeten, L., Muys, B., & De Schrijver, A. (2017). Tree Species Identity Shapes Earthworm Communities. Forests, 8(3), Article 3. https://doi.org/10.3390/f8030085
Schröter, D., Cramer, W., Leemans, R., Prentice, I.C., Araujo, M.B., Arnell, N.W.,Bondeau, A., Bugmann, H., Carter, T.R., Gracia, C.A., De La Vega-Leinert, A.C., Erhard, M., Ewert, F., Glendining, M., House, J.I., Kankaanpää, S., Klein, R.J.T., Lavorel, S., Lindner, M.,

Metzger, M.J., Meyer, J., Mitchell, T.D., Reginster, I., Rounsevell, M., Sabate, S., Sitch, S., Smith, B., Smith, J., Smith, P., Sykes, M.T., Thonicke, K., Thuiller, W., Tuck, G., Zaehle, S., Zierl, B. (2005). Ecosystem service supply and vulnerability to global change in Europe. Science 310, 1333– 1337.

Schwartz, M.W., J.J. Hellmann, J.M. Mclachlan, D.F. SAX, J.O. Borevitz, J. Brennan, A.E. Camacho, et Al. (2012). Managed relocation: Integrating the scientific, regulatory, and ethical challenges. BioScience 62: 732–743.

Senior R.A. et al. (2018). Tropical forests are thermally buffered despite intensive selective logging. Glob. Change Biol. 24, 1267-1278

Sims, R. W., & Gerard, B. M. (1999). Earthworms. Notes for the identification of British species. Synopses of the British Fauna (New Series), 31, 1–169.

Singh, J., Singh, S., & Vig, A. P. (2016). Extraction of earthworm from soil by different sampling methods : A review. Environment, Development and Sustainability, 18(6), 1521-1539. https://doi.org/10.1007/s10668-015-9703-5

Singh, J., Schädler, M., Demetrio, W., Brown, G. G., & Eisenhauer, N. (2019). Climate change effects on earthworms—A review. Soil Organisms, 91(3), Article 3. https://doi.org/10.25674/so91iss3pp114

Ste-Marie, C., E.A. Nelson, A. Dabros, AND M. Bonneau (2011). Assisted migration: Introduction to a multifaceted concept. For. Chron. 87:724 –730.

Swift, M.J., Heal, O.W., and Anderson, J.M. (1979). Decomposition in terrestrial ecosystems. University of California Press, Berkeley and Los Angeles.

Szlavecz, K., Chang, C.-H., Bernard, M. J., Pitz, S. L., Xia, L., Ma, Y., McCormick, M. K., Filley, T., Yarwood, S. A., Yesilonis, I. D., & Csuzdi, C. (2018). Litter quality, dispersal and invasion drive earthworm community dynamics and forest soil development. *Oecologia*, *188*(1), 237-250. https://doi.org/10.1007/s00442-018-4205-4

Tao, J., Zuo, J., He, Z., Wang, Y., Liu, J., Liu, W., & Cornelissen, J. H. C. (2019). Traits including leaf dry matter content and leaf pH dominate over forest soil pH as drivers of litter decomposition among 60 species. Functional Ecology, 33(9), 1798-1810. https://doi.org/10.1111/1365-2435.13413

TEEB (2010), The Economics of Ecosystems and Biodiversity Ecological and Economic Foundations. Edited by Pushpam Kumar. Earthscan: London and Washington.

Torremans A. (2022). Impact de la composition spécifique du peuplement sur la décomposition de litières standardisées dans l'Arboretum de Tervuren. Master thesis. UCLouvain

Van Gestel C.A.M., Hoogerwerf G. (2001). Influence of soil pH on the toxicity of aluminium for Eisenia andrei (Oligochaeta: Lumbricidae) in an artificial soil substrate, Pedobiologia, Volume 45, Issue 5, Pp. 385-395, ISSN 0031-4056, https://doi.org/10.1078/0031-4056-00094.

Vesterdal, L., Clarke, N., Sigurdsson, B. D. & Gundersen, P. (2013). Do tree species influence soil carbon stocks in temperate and boreal forests? Forest Ecology and

Management 309, 4–18.
Vitt, P., Havens, K., Kramer, A. T., Sollenberger, D., & Yates, E. (2010). Assisted migration of plants : Changes in latitudes, changes in attitudes. Biological Conservation, 143(1), 18-27. https://doi.org/10.1016/j.biocon.2009.08.015
Volney, W. J. A., & Fleming, R. A. (2000). Climate change and impacts of boreal forest insects. Agriculture, Ecosystems and Environment, 82(1-3), 283-294. Scopus. https://doi.org/10.1016/S0167-8809(00)00232-2
von Arx, G., Graf Pannatier, E., Thimonier, A., & Rebetez, M. (2013). Microclimate in forests with varying leaf area index and soil moisture : Potential implications for seedling establishment in a changing climate. Journal of Ecology, 101(5), 1201-1213. https://doi.org/10.1111/1365-2745.12121
Watson, G.W., V. Heywood & W. Crowley. (1993). North American botanic gardens. Hort. Rev. 15(1):1-62.
Whalen, J. K., & Costa, C. (2003). Linking spatio-temporal dynamics of earthworm populations to nutrient cycling in temperate agricultural and forest ecosystems : The 7th international symposium on earthworm ecology · Cardiff · Wales · 2002. Pedobiologia, 47(5), 801-806. https://doi.org/10.1078/0031-4056-00262
Wickham H, François R, Henry L, Müller K, Vaughan D (2023). _dplyr: A Grammar of Data Manipulation_. R package version 1.1.4, https://CRAN.R-project.org/package=dplyr.
Winder, R., E.A. Nelson, AND T. Beardmore (2011). Ecological implications for assisted migration in Canadian forests. For. Chron. 87:731–744.
World Bank Climate Change Knowledge Portal. (s. d.). Consulté 22 avril 2025, à l'adresse https://climateknowledgeportal.worldbank.org/
Zellweger F. et al., (2019). Seasonal drivers of understorey temperature buffering in temperate deciduous forests across Europe. Glob. Ecol. Biogeogr. 28, 1774–1786
Zhu, K., Woodall, C. W., & Clark, J. S. (2012). Failure to migrate : Lack of tree range expansion in response to climate change. Global Change Biology, 18(3), 1042-1052. https://doi.org/10.1111/j.1365-2486.2011.02571.x
Zhu P, Zhuang Q, Ciais P, Welp L, Li W, Xin Q. (2017). Elevated atmospheric CO_2 negatively impacts photosynthesis through radiative forcing and physiology-mediated climate feedback. Geophysical Research Letters 44: 1956–1963.

Effects of stand-specific composition on earthworm communities in the Arboretum of Tervuren

Abstract

As temperate forests face the growing pressures of climate change, their resilience is becoming a major issue. In this context, understanding the interactions between vegetation, soil, and soil fauna, particularly earthworms, is crucial. As ecosystem engineers, earthworms influence the fertility and structure of forest soils, thus affecting their capacity to store carbon. Previous studies have already focused on the effects of tree species and soil parameters on earthworms. However, only a few have explored the differences between conifers and broadleaves, and the number of tree species considered was often low. Furthermore, the unique set-up of the Arboretum of Tervuren allows for comparisons between a broad range of tree species, native and exotic, growing under similar site conditions. The aim was to assess how different tree species, broadleaves and conifers, influence the composition, abundance, and biomass of earthworm communities via their effects on leaf litterfall, microclimate, and soil properties.
Even though the results showed no statistical difference between the two groups, they revealed that tree species identity seemed to matter more than broad classification. Species-specific traits, particularly those influencing calcium and aluminum availability, appeared to drive earthworm responses. The removal of a coniferous species, *Calocedrus decurrens*, leading to a statistical difference in the biomass, and the variability of litter and soil properties across species highlight the complexity of these interactions. Due to limitations in sampling size and period, future studies should include more tree species, mixed stands, and extended sampling periods to capture temporal and spatial dynamics.

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