FACULTY OF BIOSCIENCE ENGINEERING

The impact of forest management on soil biodiversity in acidified sandy soils

Promotor:
Dr. Ellen Desie
Prof. Koenraad Van Meerbeek
Department Earth and Environmental Sciences
Division Forest, Nature and Landscape (DFNL)

Dissertation presented in
fulfillment of the requirements
for the degree of
Master of Bioscience Engineering:
Agro- and Ecosystems Engineering

Julie SIMONS

June 2023

Acknowledgements

First of all, I want to thank my promotor Ellen Desie for guiding me through this thesis and challenging me along the way. Second, great thanks goes to my co-promotor Koenraad Van Meerbeek for teaching me more about the programming language R. Both nudged me in the right directions when I could not see a solution for myself.

Furthermore, great appreciation goes to Lore Fondu, Eric Van Beek, and Remi Chevalier for welcoming me in their lab and assisting me with practical matters. Thanks to Keunbae Kim as well for letting me use his Tullgren funnel setup and showing me the ropes. I want to thank Mario Driessen as well for providing initial information regarding mesofauna identification.

Moreover, great appreciation goes to Bodine, Yarissa, and Kato, whose thesis researches are, like my research, also part of a bigger project. I learned from them while going in the field together for sample collection and during data processing.

Last but not least, I want to thank friends and family for supporting me during this project. Especially my boyfriend Sander for keeping me aligned to my goals. Without them I would not have ended this project as I have done.

Summary

Soil biodiversity is often overlooked and victim of the ‘out of sight, out of mind’ principle. Nevertheless, soil ecosystems are considered one of the most diverse systems on earth. Therefore, much soil organisms are yet to be discovered. In addition, the soil and its biodiversity are the basis of multiple ecosystem services such as food production and nutrient provisioning. However, soil biodiversity in general is threatened and declining due to increasing pressure by human activities and climate change. What the main threats and their relative impact are, depends on local conditions. In the forests on sandy soils in northeast Belgium and the southern Netherlands, one of the main threats is acidification of the forest floor. At the moment, most of these forests are located on soils that are already degraded as a consequence of land use history and past and present acidifying deposition loads.

Besides governmental regulations, soil biodiversity could be restored by means of forest management as the aboveground and belowground systems are intrinsically connected. Our focus lies on the impact of tree species selection, and more specifically tree species with litter rich in base cations (rich litter species). As this litter is easier to decompose, nutrient cycling could be enhanced. In addition, other factors such as pH, base saturation and aluminium toxicity are improved in a way that the conditions are more suitable for a larger amount of soil species.

In this thesis, the main focus is the evaluation of the state of the soil biodiversity in forests on sandy soils and how this differs between contrasting forest types; i.e. pine monocultures or mixed broadleaved forests dominated by rich litter species. Our results show that the general soil biodiversity is low for both forest types in the study region due to a long history of acidification. Nevertheless, rich litter species have a positive influence on soil abiotic characteristics and earthworm abundance and diversity. The effect of rich litter species is less conclusive for mesofauna populations as these species are more tolerant to low pH’s. Therefore we can conclude that rich litter species have a positive influence on the soil yet the effect depends on local conditions. Moreover, we most likely assessed the effect of the first generation of rich litter species after transition from poor litter species. It is recommended to assess long term effects and other measures such as assisted migration of soil biota in the future.

List of abbreviations

BS	Base Saturation
CEC	Cation Exchange Capacity
CI	Confidence interval
CPA	Count per area
CPV	Count per volume
ERB	European Reference Base
FAO	Food and Agriculture Organization of the United Nations
FT	Forest type
GLMM	Generalised Linear Mixed Model
MAP	Mean Annual Precipitation
MAT	Mean Annual Temperature
OF	Organic horizon, Fragmented litter
OH	Organic horizon, Humus
OL	Organic horizon, Litter
ShD	Shannon Diversity Index
ShE	Shannon Evenness Index
SOC	Soil organic carbon
SOM	Soil organic matter
SR	Species Richness

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1 Introduction

About 75% of all land on earth is influenced by humans. Combined with increasing populations and our ecological footprint, pressure on the remaining natural land is increasing. In addition, extreme events caused by climate change put the resilience of ecosystems to the test. One of the consequences is the decrease of species biodiversity at alarming rates (Geisen et al., 2019). This negative trend did not remain unnoticed by scientists and politicians. During the Convention on Biodiversity in Rio De Janeiro in 1992, a target was agreed upon to reduce the rate of biodiversity loss by 2010. However, the target was not met (Gardi et al., 2013).

When biodiversity is mentioned, people often only think about the plant and animal species that they can see. What can be found beneath their feed is overlooked. Hence it is a victim of the ‘out of sight, out of mind’ principle (Jeffery et al., 2010). Despite soil biodiversity being one of the most diverse systems on earth, not much is known about it (Geisen et al., 2019). Nevertheless soil biodiversity is the basis for multiple ecosystem services such as food production and nutrient provisioning. In addition, a healthy soil system is crucial for climate mitigation by means of carbon storage (Simon Jeffery et al., 2010).

Like the aboveground ecosystems, soil biodiversity is threatened and declining. Which threats and how severe the impact is, depends on the local community and conditions. In European forests for example, one of the main threats is acidification of the forest floor (FAO et al., 2020). This reduces the community and the soil functions that they provide, as the majority of species is not acid tolerant. Soil functions are the basis for soil ecosystem services hence this will decrease as well.

Most of the treats to soil biodiversity need governmental regulations in order to reduce the impact. In forest ecosystems, another way to help restore the soil biodiversity is by means of forest management. Different practices such as wood harvest and thinning influence the soil as it is linked with life aboveground. For this research, we want to focus on the impact of tree species choice, and more specifically rich litter species. These species are known to have a litter nutrient composition that enhances decomposition rates. Improved cycles will then attract more soil species which further improve (additional) cycling mechanisms (den Ouden et al., 2020). Hence we believe that forest management is an important tool to restore soil biodiversity.

2 Literature study

2.1 Life in the soil

Soils are one of the key components of ecosystems. However, many mysteries remain about their functionality and their contribution to important nutrient and live cycles (Briones, 2014). The four main components of the soil are mineral matter, water, air and organic matter (White, 2006). The latter is a combination of dead matter such as plant and animal residues, and living matter composed of diverse soil organisms (White, 2006). The largest part of this soil organic matter (SOM) consists of carbon, hence, it is commonly referred to as soil organic carbon (SOC). Although only a small proportion of the soil consist of SOC, it plays an important role in the ecosystem and provides regulating and supporting services such as mitigation of climate change by capturing of CO_2, water buffering, and nutrient provisioning (Jackson et al., 2017; Minasny et al., 2017).

Soil biota are a crusial factor in order to maintain these provided services. The living micro-organisms decompose and mineralise the incoming dead matter into nutrients and compounds that can be taken up by larger organisms (Cole et al., 2004). The interdepence between dead organic matter, micro-organisms, small invertebrates and vertebrates creates a large food web (White, 2006). The result of these multiple connections is a system where carbon and nutrients constantly flow both above and belowground.

2.1.1 Soil fauna classifications

Body size

Although there is limited knowledge on these soil communities, some classifications can be made. The most used classification is based on body size as it gives a good indication of which size classes provide what soil functions and it is relatively easy to measure (Briones, 2014) (Figure 1). Microbiota are smaller than 0.1 mm and consist of bacteria and fungi. Mesofauna contains mites and springtails, which are smaller than 2 mm. Small invertebrate animals such as earthworms and ants are categorised as macrofauna (Briones, 2014; Decaëns, 2010).

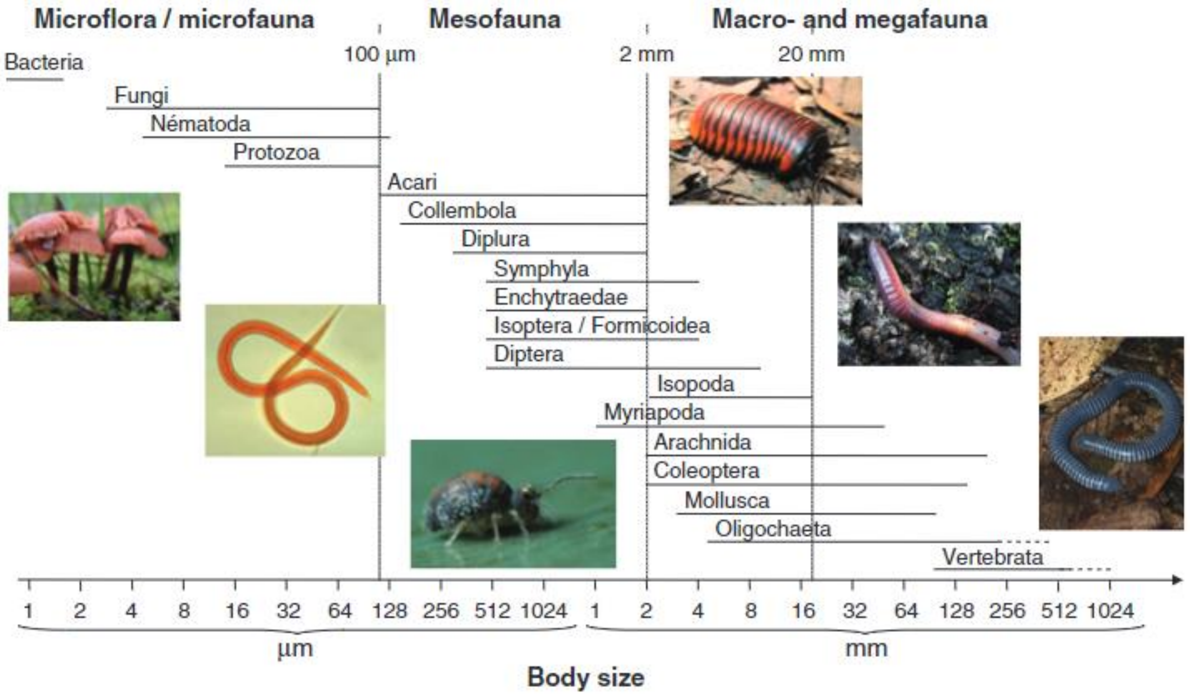


Figure 1 Classification based on body size of the main taxonomic groups of soil organisms (Decaëns, 2010).

However, it should be noted that these are not strict classes. Due to the natural species variability within a taxonomic group, some groups could be allocated into more than one class (Briones, 2014; Orgiazzi et al., 2016). Research has shown that classification based on the body width turns out to be more consistent than that based on body length. The latter is more variable between species of the same taxonomic group (Orgiazzi et al., 2016; Swift et al., 1979). This could thus be considered as one of the downsides of the classification based on body size.

A second downside of size classification is that over the years, significantly less research is done regarding the belowground biodiversity than aboveground (Decaëns, 2010). As a result, many of the taxonomic catalogues are not complete at all (Decaëns, 2010; FAO et al., 2020). A part of the problem is that studying microscopic organisms (< 100 µm) is restricted by technical limitations. However, the smaller the size, the more individual species there are (Briones, 2014).

Reproductive strategies

Other functional classifications are also created, since these coarse groups based on size do not take into account the chemo-physiological variability of different species within a class. Nor their individual relative importance on different temporal and spatial scales (Briones, 2014). Reproductive strategies for example, give an indication of a species response to current and changing environmental conditions (Brussaard, 1998; Moore et al., 1988). However, no detailed reproductive classification is yet available due to its complexity (Briones, 2014; Moore et al., 1988).

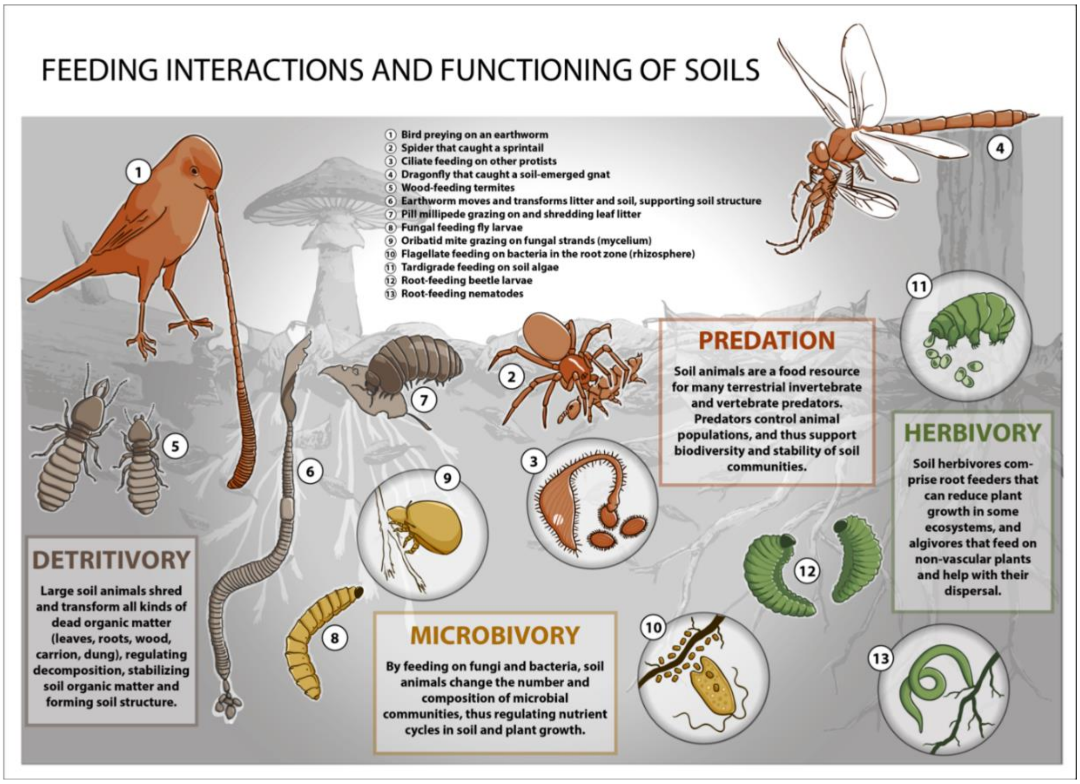
Feeding patterns and trophic levels

Another possibility is based on the feeding patterns because of the connection between the variation of feeding niches and the success of the food-webs in performing their ecosystem services (Briones, 2014; J. C. Moore & De Ruiter, 1991).

The first attempt to divide the soil community in functional groups based on feeding behaviour was done by Root (1967) as the "guild" concept was introduced. A guild is defined as "*a group of species having similar exploration patterns*" (Briones, 2014). Later, Root (1973) reviewed the definition and added a second criterium, the "*mode of feeding*" (Briones, 2014; Brussaard, 1998). These guild are useful for modelling food chains and their different levels. Nowadays, multiple classifications based on feeding patterns have been proposed and are still regularly updated (Briones, 2014). Most of these focus on a single type of organism instead of the whole soil community. Hence a point of improvement is to create and review more complete classifications (Briones, 2014).

Building on the guild concept is a "league", or "*a group of organisms, not necessarily taxonomically related, that exploit and process more than one habitat resource in a homologous manner*" (Faber, 1991). The soil profile is stratified into horizons, resulting in different microhabitats along the vertical axis. These could each be exploited by different species of the same guild resulting in a stratification of the guild (Briones, 2014; Faber, 1991). A league is then more refined since the classification is based on two axes: feeding patterns and microhabitat (Briones, 2014; Faber, 1991). A downside here is the current state of knowledge regarding the specific functional roles of the taxa to further complete the classification (Faber, 1991; Potapov et al., 2022).

Most of these guilds or leagues that were worked out by different researches are in most cases classified based on different criteria (Briones, 2014; Potapov et al., 2022). This makes them useful when looking at a specific taxonomic group. However, on a community level, it is more difficult to combine them (Potapov et al., 2022). In that case, it is useful to look at the feeding interactions (Figure 2). The connections between species reveal different hierarchical levels that emerge into a trophic classification. This network of different chains in turn provides insight in ecosystem functioning (Potapov & Schmidt, 2022).



FEEDING INTERACTIONS AND FUNCTIONING OF SOILS

① Bird preying on an earthworm
② Spider that caught a sprintail
③ Ciliate feeding on other protists
④ Dragonfly that caught a soil-emerged gnat
⑤ Wood-feeding termites
⑥ Earthworm moves and transforms litter and soil, supporting soil structure
⑦ Pill millipede grazing on and shredding leaf litter
⑧ Fungal feeding fly larvae
⑨ Oribatid mite grazing on fungal strands (mycelium)
⑩ Flagellate feeding on bacteria in the root zone (rhizosphere)
⑪ Tardigrade feeding on soil algae
⑫ Root-feeding beetle larvae
⑬ Root-feeding nematodes

PREDATION

Soil animals are a food resource for many terrestrial invertebrate and vertebrate predators. Predators control animal populations, and thus support biodiversity and stability of soil communities.

HERBIVORY

Soil herbivores comprise root feeders that can reduce plant growth in some ecosystems, and algivores that feed on non-vascular plants and help with their dispersal.

DETRITIVORY

Large soil animals shred and transform all kinds of dead organic matter (leaves, roots, wood, carrion, dung), regulating decomposition, stabilizing soil organic matter and forming soil structure.

MICROBIVORY

By feeding on fungi and bacteria, soil animals change the number and composition of microbial communities, thus regulating nutrient cycles in soil and plant growth.

Figure 2 Feeding interactions in the soil community (Potapov & Schmidt, 2022).

Life strategies

When looking the life strategies of the soil fauna, another coarse classification can be made. It is based on how many stages of life are spend in the soil, which differs among taxa (Jeffery et al., 2010). Based on their ecological functions and morphological adaptations, four groups can be separated: temporary inactive geophiles, temporary active geophiles, periodical geophiles, and geobionts (Figure 3) (Menta, 2012). The more time spent in the soil, the more functions they carry out and the larger their influence in general.

Temporary inactive geophiles are only in the soil for a small part of their lives. The soil environment create a stable and protective environment for species to overwinter or undergo metamorphosis (Jeffery et al., 2010; Menta, 2012). Besides being a possible prey, their influence on the ecosystem is weak (Menta, 2012). Secondly, temporary active geophiles stay in the soil while maturing and leave the soil as an adult (Simon Jeffery et al., 2010). These are mostly species with a larval stage in their live cycle such as many insect species. These larvae are part of the food web as detrivores, herbivores or predators (Menta, 2012). Thirdly, periodical geophiles are similar to temporary active geophiles. The difference between them is that the soil is partially a habitat for adult individuals as well for periodical geophiles (Mishra & Singh, 2020). The environment is used for activities such as feeding, laying eggs, and finding shelter (Menta, 2012; Mishra & Singh, 2020). Lastly, geobionts have adapted to a full live cycle in the soil. As a result of the adaptation, they cannot survive outside the soil (Jeffery et al., 2010). A disadvantage that comes with a higher dependence on the soil habitat, is that species are more vulnerable to soil threats as they cannot leave (Mishra & Singh, 2020).

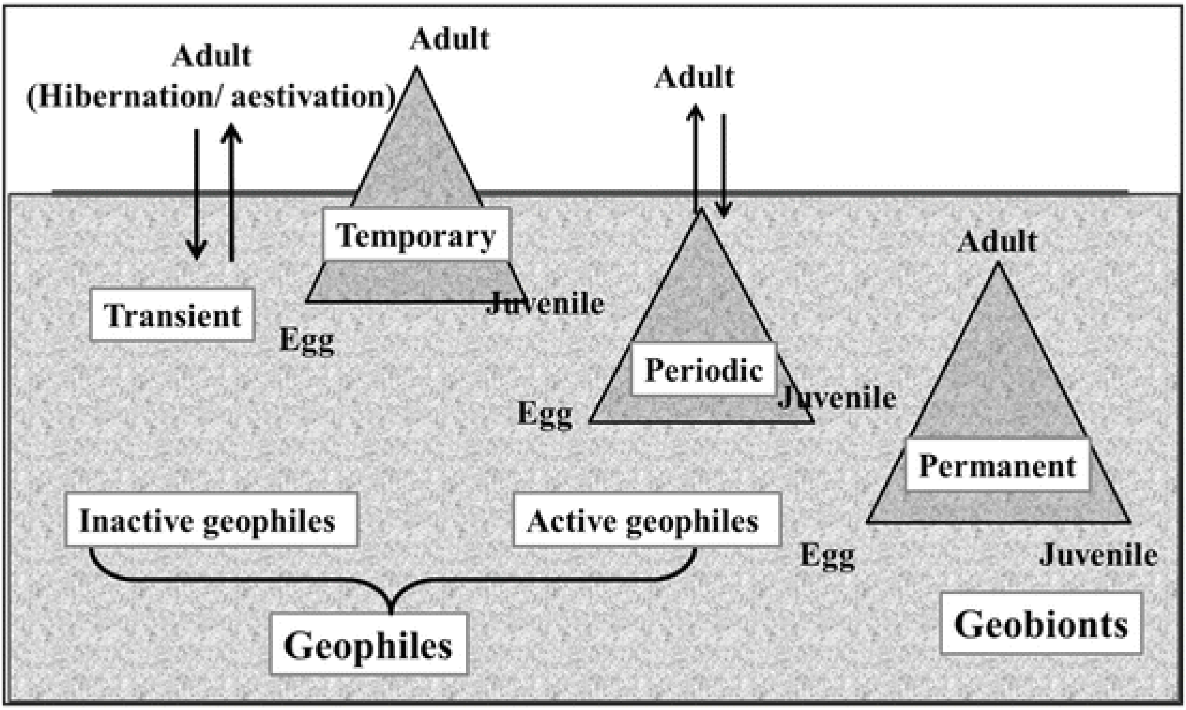


Figure 3 Classification based on how many life stages are spend in the soil (Roy et al., 2018).

Multifunctional classification system

A study by Potapov et al. (2022) proposed a multifunctional classification system after reviewing existing research. The purpose of the new system is to combine and make better use of the current information. This would then be a reference tool that could facilitate future soil food web research and reconstructions (Le Guillarme et al., 2023; Potapov et al., 2022). The system builds further on the classification based on feeding patterns and trophic interactions by adding key traits such as microhabitat (vertical stratification), body mass, and survival mechanisms (Figure 4) (Potapov, 2022; Potapov et al., 2022). Nevertheless, this multifunctional classification still needs to be developed further (Potapov et al., 2022).

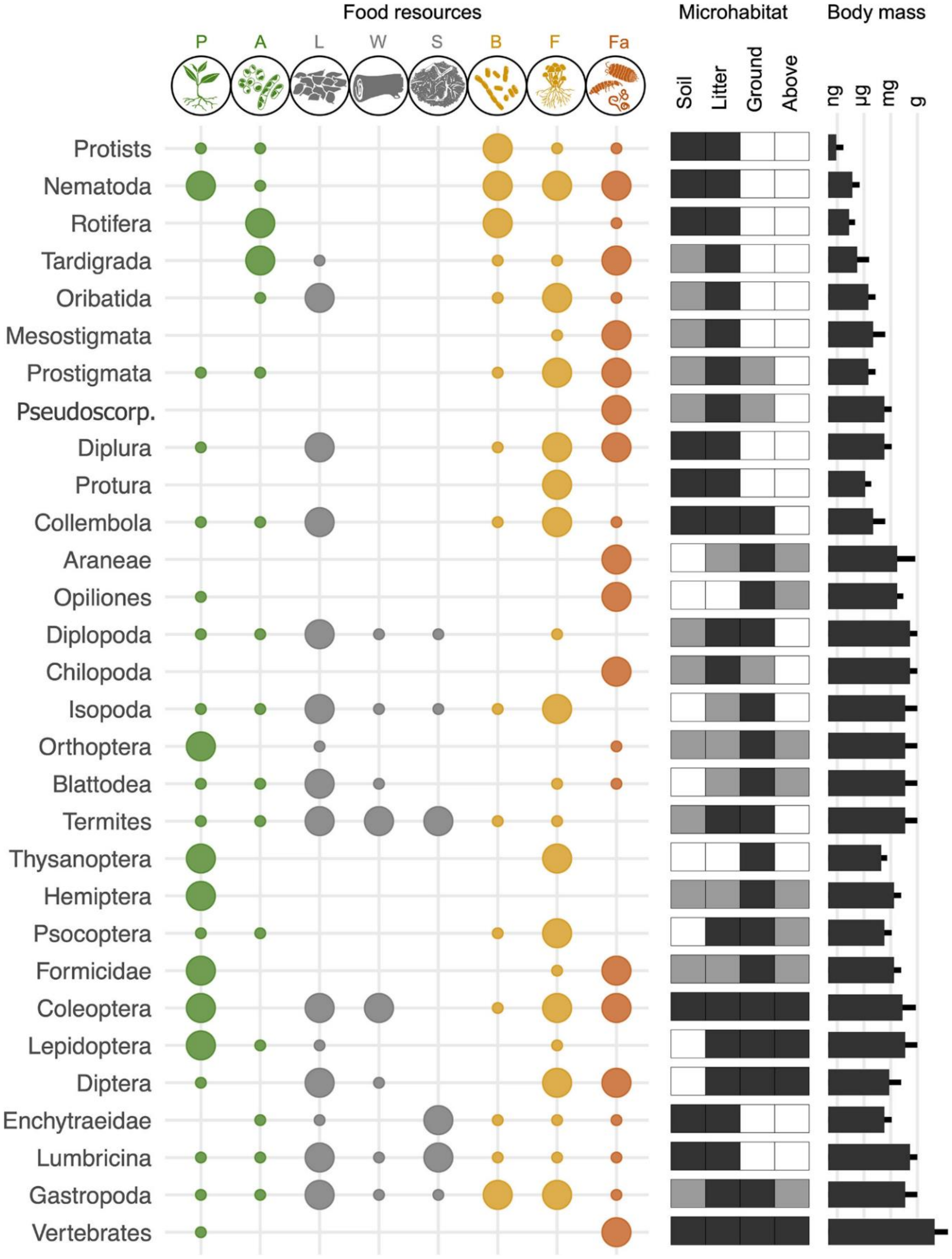


Figure 4 Summary of the food resources, distribution in the soil environment, and dominant body mass of 30 dominant taxa of soil life. The colour of the circle represent the type of resource: green for living autotrophic material (Plants and Algae), grey for dead material (Litter, dead Wood, and Soil), yellow for microbial sources (Bacteria and Fungi), and red for preys (other Fauna). The larger circles indicate the main resources and smaller circles are alternative resources. Possible microhabitats are marked in grey, where the darker shade represents preferred microhabitats. The ground is defined as on the litter layer, stones and wood surfaces. The microhabitat 'above' implies that the taxonomic group lives aboveground during certain parts of their life but at least one life stage occurs in the soil. Lastly, body mass is

presented by the mean value with its corresponding standard deviation on a logarithmic scale (Potapov et al., 2022).

2.1.2 Functionality of the soil community

As discussed previously, all species of the belowground community are intertwined in a food web with different trophic levels (Figure 5). These are the so called ‘brown’ webs where the starting point is dead organic matter. This differs from ‘green’ webs aboveground with living autotrophic species, mostly plants, at the base (Steffan & Dharampal, 2019). Although the general processes are mostly understood, the many interactions that happen, are complex and often include multiple participants (Menta, 2012).

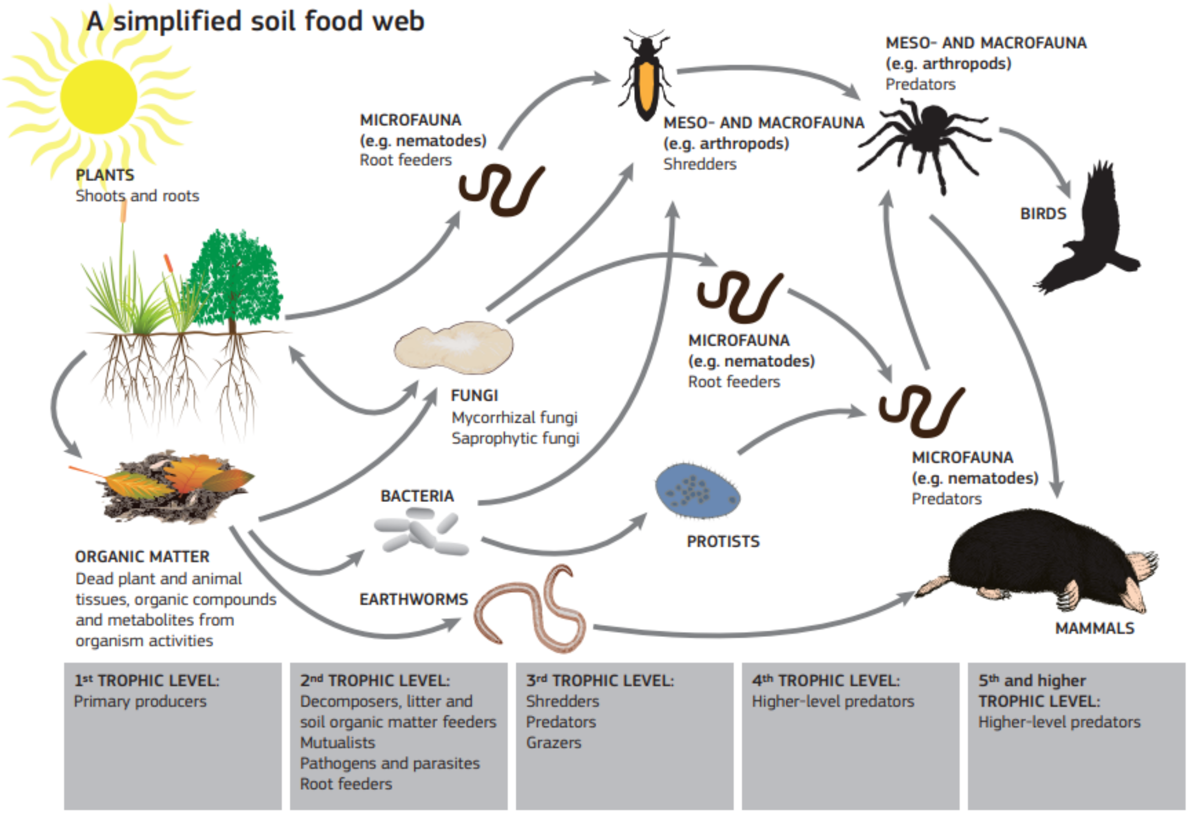


Figure 5 The soil food web (Orgiazzi et al., 2016).

There are three broad functional groups: chemical engineers, litter transformers, and ecosystem engineers (Potapov et al., 2022; Turbé et al., 2010). Chemical engineers are organisms having metabolic pathways that are part of the biogeochemical cycles (Mishra & Singh, 2020). It gives them the ability to process organic matter as cellulose and lignin that are difficult to degrade due to their molecular structure (de Schrijver et al., 2018; White, 2006). Chemical engineers, also called decomposers, are mainly bacteria and fungi but other micro-organisms are included as well (Mishra & Singh, 2020). The organic carbon is used by these organisms for growth and maintenance. The rest is released as CO_2 (White, 2006). Similarly, nutrients are mineralised and partially immobilised by the microfauna. Leftovers are taken up by roots and other species (Briones, 2014; White, 2006). Hence decomposers aid in the (re)cycling of carbon and nutrients through the ecosystem (de Schrijver et al., 2018; White, 2006). In addition, multiple symbiotic relation between microbiota exist. An example is the relationship between mycorrhiza (fungi) and root systems of plants (de Schrijver et al., 2018).

Litter transformers break down the incoming litter into smaller pieces. This promotes further breakdown by the decomposers and increases the active surface for them (de Schrijver et al., 2018; Mishra & Singh, 2020). In addition, their faeces are an important by-product (Coq et al., 2022; Joly et al., 2023). Since litter is hard to digest and nutrient-depleted, high consumption rates are required. Therefore large quantities of detrivore faeces are produced (Coq et al., 2022). During gut passage, the organic compounds are partially digested by intestinal microbiota and enriched with enzymes (Menta, 2012). Research has shown that this leads to different physiochemical characteristics which influences decomposition rates (Coq et al., 2022; Joly et al., 2023). Besides the direct effects, they affect the cycling processes indirectly as well. By grazing and predating on decomposers, population control occurs. As a result, the composition os the microbial community differs and the pace of degradation is altered (de Schrijver et al., 2018).

The third functional group, ecosystem engineers, are of a size that allows them to move through the soil matrix and alter it (Mishra & Singh, 2020). As they move through soil, they mix and redistribute the organic matter and create holes that can be filled with water or air (Orgiazzi et al., 2016). On top of that, they indirectly influence cycling processes as part of the food web and by population control.

All of these soil ecosystem functions are necessary for the provisioning of soil related ecosystem services such as secure food production, clean water, boosting our health by nutrient provisioning, etc (Delgado-Baquerizo et al., 2020; Menta, 2012). A healthy and functioning soil belowground is a basis for the ecosystems aboveground, both natural and artificial (Figure 6). In the following sections some of the important taxonomic groups of soil fauna will be discussed into more detail. Here they are divided according to body size classification. As mentioned earlier, some groups could fit in multiple classes.

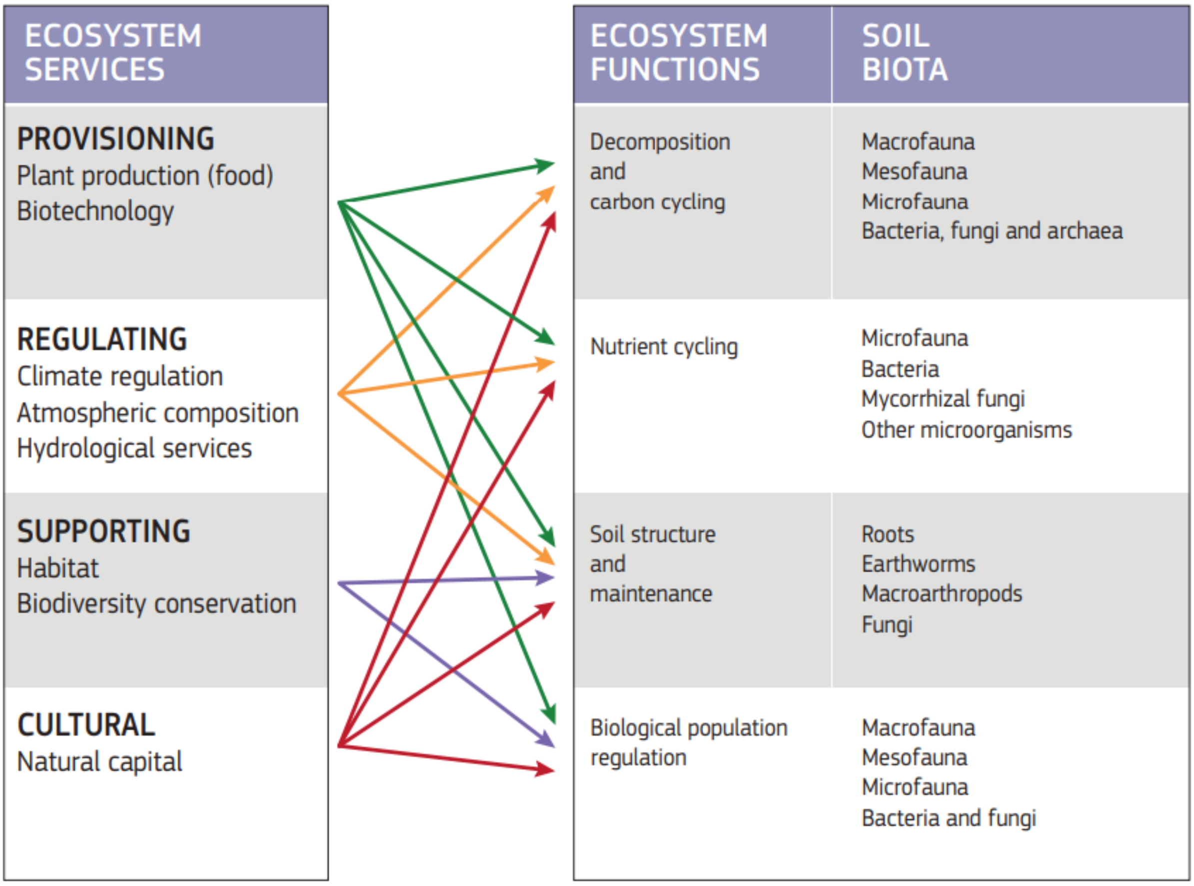


Figure 6 Ecosystem services and functions provides by the soil and its residential biota. The arrows indicate which ecosystem functions are linked to the different types of ecosystem services. Each functions is mainly provided by certain soil biota groups (Orgiazzi et al., 2016).

2.1.2.1 *Microfauna*

The focus of this thesis research mainly lies on the mesofauna groups and some of the macrofauna groups. Nevertheless microfauna provide important functions for the soil ecosystem functioning. The general services they provide, include decomposition of incoming plant matter and weathering minerals present in the soil.

Bacteria and Archaea

Bacteria and archaea appear in a range of different shapes and sizes. This in combination with their various metabolic pathways, creates a highly diverse community. Their size highly limits movement through the soil. Therefore, they are often concentration on biofilms (Turbé et al., 2010). By means of their metabolic pathways, complex organic compounds are decomposed to simple nutrients. In addition, nitrification bacteria are part of the nitrogen cycle (Mishra & Singh, 2020). Furthermore, they are one of the first links of the food chains.

Fungi

Similar to bacteria and archaea, the diversity within fungal species is very high. Their contribution to the nutrient cycling includes decomposing dead plant material. Especially lignin as fungi are the only organisms able to degrade it (Yadav et al., 2022). Through symbiotic relations they also support nitrogen fixation and phosphor mineralisation. Secondly, the soil structure is enhanced by the hyphae network (Turbé et al., 2010). Besides their direct functions, indirect biological regulation is provided since fungi are part of the food chain and some species are pathogens for others.

Nematoda

Nematodes, non-segmented and cylindrical worms, are one of the most abundant species groups within the soil (Wheater & Read, 1996). Hence they have multiple functions within the system. Research has even shown that the community composition of nematodes can be an indicator of the soil quality (Hoorman, 2011). In terms of carbon transformation, a large amount of the soil carbon is respired by nematodes due to their large quantity. As they play a role as grazers, predators and prey, they contribute to the nutrient cycling through trophic levels. In addition, the parasitic species act as a biocontrol agent (de Schrijver et al., 2018; Hoorman, 2011).

Protozoa

These single-celled and aquatic animals can be found on soil water films and within water filled pores in the soil matrix. Bacteria are their main food source but protozoa species prey on fungi, algae, and other protozoa as well (Hoorman, 2011; Jayapalan, 2017). These feeding patterns are important for the mineralisation of nutrients (Hoorman, 2011; Jayapalan, 2017). Bacteria often provide more nitrogen than is needed for the protozoa hence it is released as ammonium. The ammonium can be taken up by other soil organisms and root systems of plants (Hoorman, 2011). Additional to the nutrient cycling, grazing of microfauna is important for the regulation of the populations. It stimulates new growth which enhances the decomposition rate (Hoorman, 2011; Jayapalan, 2017).

2.1.2.2 Mesofauna

Acari (mites)

In terms of classification, acari are a subclass of the Arachnida (Tilling, 2014). This subclass is then further divided in two superorders, namely the Parasiformes and the Acariformes (FAO et al., 2020; Mullen & O'Connor, 2019). The first superorder consist mainly of ticks and other parasitic mites. The second one is a diverse group containing both parasitic and free living mites species. (Mullen & O'Connor, 2019). In general, adult mites have four pairs of legs. Their head, thorax, and abdomen seem to be fused into one when looking at an individual from above (Figure 7a**Error! Reference source not found.**). The larvae are similar looking but have only three pairs of legs (Mullen & O'Connor, 2019; Tilling, 2014).

Although Acari is a very large and abundant subclass, it can be roughly divided in two groups with similar functions in the soil system (FAO et al., 2020; Tilling, 2014). Detritivorous and microbivorous mites feed on decaying plant material and soil microfauna (Tilling, 2014). By grazing the soil microfauna, their growth is stimulated. Hence they enhance nutrient cycling, decomposition and regulation of herbivorous fauna. Additionally, depending on which species are preferred by the mites the microfauna species composition can differ (FAO et al., 2020). The most studied suborder within this group are the Oribatida, which are omnipresent in soil ecosystems (FAO et al., 2020; Tilling, 2014). The second largest group are predatory mites. By being both a predator for microfauna as a prey for macrofauna, they are important links within the food chains (FAO et al., 2020).

Collembola (springtails)

The order of the Collembola is part of the Entognatha. This class consist of ametabolous arthropods that are wingless (Janssens, 2022). Springtails can be recognized by their three pairs of legs, a furcula and a ventral tube (Figure 7b) (GEA, n.d.; Janssens, 2022; van Duinen, 2022). The furcula, a forked organ, makes it possible to jump for avoiding predators. The ventral tube is used for the uptake and release of water and osmolytes (GEA, n.d.). There are two main body types: elongated or globular (Figure 7b) (van Duinen, 2022).

There are four trophic niches, based on where springtails species reside in or above the soil: 1) on plants, tree trunks and branches, and the litter surface, 2) in the upper litter layer (OL) and fallen trunk, 3) in the (fragmented) humus layer (OF+OH), and 4) in the upper mineral soil (FAO et al., 2020). The food sources of springtails are mostly fungal hyphae and decaying vegetation in the litter layer. The species that live on the soil surface also consume living plant material (Wheater & Read, 1996). By their feeding patterns they contribute to the formation of the humus layer and the distribution and cycling of nutrients. The predation on fungi is furthermore a support for the control of fungal diseases. As part of the food web, they are prey species as well for larger soil organisms (FAO et al., 2020).

Diplura (two-pronged bristletales)

The Entognatha includes Diplura and Protura besides the Collembola. A general trait within this class is that the larvae resemble adults (Tilling, 2014). Instead of metamorphosis, they moult while maturing and keep moulting even as adults . Two-pronged bristletails can be recognized by their white or colourless, elongated and narrow body (Tilling, 2014; Wheater & Read, 1996). The eyeless head has a pair of antenna that comprises of spherical segments. The anterior pair of cerci is shaped differently for the three superfamilies: Campodeidae (long and flexible), Japygidae (pincer like), and Projapygidae (short and rigid) (Figure 7c) (Orgiazzi et al., 2016). The cerci of the Projapygidae can contain spinning glands. This super family is less common and also less studied in comparison to the other two.

Two-pronged bristletails can be found on the soil surface, under logs and stones or in the moss or litter layer, and deeper in the soil (Tilling, 2014). In the ecosystem they support the decomposition of the organic matter, since most species are herbivores and/or detrivores. Some species predate on Nematoda, Fungi and Collembola hence they as well provide population control services (Orgiazzi et al., 2016; Tilling, 2014).

Diptera (true flies)

This large groups contains a large range of species that each have a diverse appearance. However, a common trait of individuals in this order is that they have a pair of wings (Figure 7d) (Tilling, 2014). The second pair of wings that most flying insects have is modified into halteres. These are balancing structures with sensors that assist while flying (Moore, 2012). In the soil environment, mostly the larvae are found instead of adult individuals (Tilling, 2014; Wheater & Read, 1996). While maturing in the soil, the fly larvae feed on decaying organic matter (Wheater & Read, 1996). Hence they aid with the decomposing processes.

Thysanoptera (thrips)

Within this group are two suborders: Terebrantia and Tubulifera. The difference between both is visible at the anterior of the abdomen. For Terebrantia, the last abdominal segment is shaped rather short and pointed while for the Tubulifera it is longer and more tubular (Tipping, 2008). Most of the adult individuals have narrow and fringed wings which allow them to make short flights (thripping) (Figure 7e) (Tilling, 2014). Depending on the environmental conditions, individuals can have reduced or no wings. Some important factors for this, are the food quality and the seasons (Tipping, 2008). In the soil ecosystem their diet mainly consists of plant material and some fungi. Some species predate on mites and other small insects and thus contribute to the regulations of these populations (Tilling, 2014; Tipping, 2008).

Formicidae (ants)

Ants belong to the order of the Hymenoptera, which also includes bees, wasps, and sawflies. The Formicidae is a family within the suborder Apocrita. Species within the suborder have two pairs of wings (some individuals are wingless) and a petiole, a narrow constriction between the thorax and the abdomen (Figure 7f) (Tilling, 2014). The strong fliers of this group are usually not found in or close to the soil. Ants on the other hand are mostly wingless and can be encountered in large numbers in the soil (Wheater & Read, 1996). As they are one of the few socially cooperative species, they live in large and complex colonies (Wheater & Read, 1996). Depending on the species, they can be herbivores, (specialist) predators, or omnivores (Orgiazzi et al., 2016; Wheater & Read, 1996). Besides their trophic functions, they provide services such as nutrient circulation and water infiltration while digging tunnels for the colony complex. The debris that is left behind, is later decomposed by micro-organisms (Orgiazzi et al., 2016). In addition, the soil matrix is modified when building their anthill. Therefore, ants are considered ecosystem engineers as well (Menta, 2012).

Isoptera (termites)

Similar to ants, termites live in a complex social colony with a king and queen, workers, and soldiers (Orgiazzi et al., 2016). However, Isoptera is another order and thus unrelated to ants (Scheffrahn, 2008). Depending on their caste, their morphology differs depending on their function in the colony (Figure 7g). King and queen termites stay inside to reproduce. Soldiers mainly defend the colony. Workers have a range of tasks such as food collection, nest building, and caring for the larvae (Orgiazzi et al., 2016). Termites feed on plant matter which aids with decomposition processes. In addition, the soil is stabilized when these ecosystem engineers transform it for the colony structures (A. Orgiazzi et al., 2016; Scheffrahn, 2008).

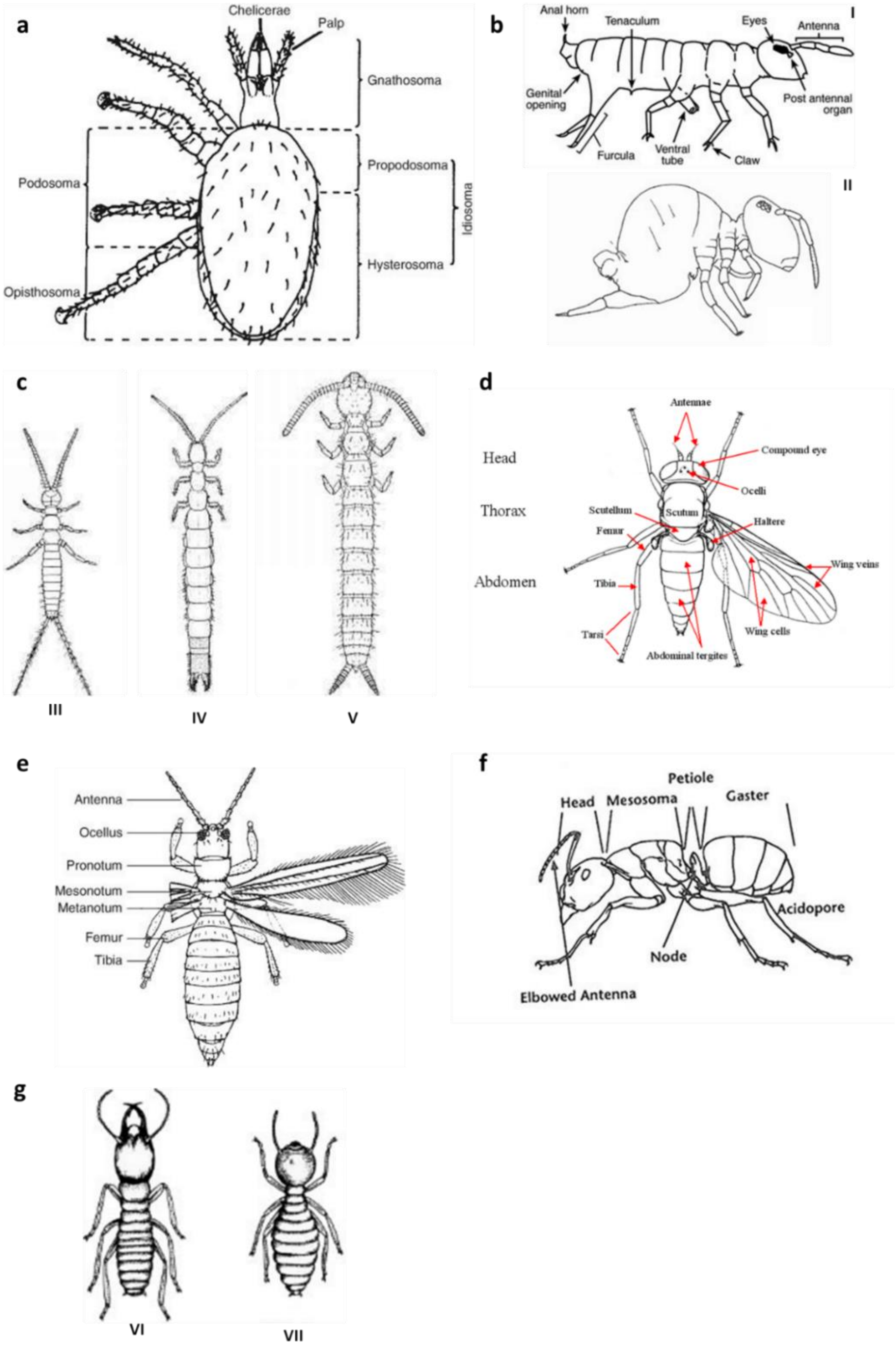


Figure 7 General external anatomy of mesofauna orders with a) Acari (Coons & Rothschild, 2008). b) elongated (I) and globular (II) Collembola (CSIRO, n.d.; GEA, n.d.). c) Diplura where the cerci vary per superfamily: (III) Campodeidae, (IV) Japygidae, and (V) Projapygidae (Imms, 1948). d) Diptera ("Fly

parts - morphology diagrams | Dipterists forum," n.d.). e) Thysanoptera (Tipping, 2008). f) Formicidae ("Carpenter Ants | Ant Pest Community of Practice," n.d.). g) Isoptera with (VI) soldier termite and (VII) worker termite ("Isoptera | General Enthomology NC State University," n.d.).

2.1.2.3 Macro- and megafauna

Chilopoda (centipedes)

Centipedes are easily recognised by their narrow and flattened body that consists of multiple segment (Figure 8a). Each segment has one pair of legs attached to it (Capinera, 2008a; Tilling, 2014). The first pair of legs are modified into poisonous claws (forcipules) that are used to capture prey, such as snails, arthropods and earthworms (Capinera, 2008a). In our study region, there are three common order of species. Geophilomorpha live in the soil or in the litter layer. These often blind species have bodies that are long and flexible with short legs (Tilling, 2014; Wheater & Read, 1996). The surface dwelling species, orders Lithobiomorpha, Scolopendromorpha, and Scutigeromorpha, often have fewer segments with longer legs. The body is more rigid which enables to move at higher speed over the surface floor (Tilling, 2014; Wheater & Read, 1996).

Diplopoda (millipedes)

Millipedes resemble centipedes in a way that they have an elongated body with more segments than typical Arthropoda (Figure 8b). Both classes can be distinguished since millipedes have two pair of body segments instead of one and the body segments are generally less flat (Capinera, 2008b; Tilling, 2014; Wheater & Read, 1996). Smaller differences are the fewer antenna segments and the absence of forcipules (Tilling, 2014). As opportunistic saprophages they feed on dead organic matter such as leaf litter, roots and dead arthropods. Hence they participate in the decomposing process while residing in the soil and litter layer (Capinera, 2008b; Wheater & Read, 1996). In addition, millipedes convert a large amount of litter into faeces. Research has shown that this changes the physiochemical characteristics of the organic material in favour of further decomposition (Coq et al., 2022; Joly et al., 2023). Important orders for this research are Chordeumatida, Julida, Glomerida, Polydesmida, Polyzoniida, and Polyxenida (Tilling, 2014). The morphology of species can slightly differ depending on their precise diet and habitat (Capinera, 2008b).

Isopoda (woodlice)

As for most insects, the woodlice body is separated into three parts (Figure 8c). First the head with the eyes, mouth and antenna. Secondly, the thorax has seven segments, with a pair of legs on each segments. Lastly six smaller and platelike segments that form the abdomen (Capinera, 2008c). On the ventral side of these segments, there are rudimental abdominal gills. These are remnants from living in an aquatic environment as most species of the Crustacae still do. Since these gills are still sensitive to water loss, most woodlice species are restricted to a humid habitat and are mostly active during the night (Tilling, 2014). In the soil ecosystems, woodlice take a role as litter fragmenters as their diet mainly consist of dead organic material, fungi and bacteria present on the dead material (Capinera, 2008c; Tilling, 2014; Wheater & Read, 1996). While searching for food, they mix the organic matter with the mineral soil as well. The produces faeces have favourable physiochemical characteristics for further decomposition (Coq et al., 2022; Joly et al., 2023). In addition and as part of the food chain, they are preyed upon by larger fauna and can be parasitized (Capinera, 2008c).

Pseudoscorpiones (false scorpions)

As the name already indicated, pseudoscorpions resemble scorpions but without the characteristic tail with the venomous stringer (Figure 8d). In addition, their length stays in the millimetre range while real scorpions are multiple centimetres (Orgiazzi et al., 2016; Tilling, 2014). When looking at the morphology, there are two main parts. The cephalothorax, the head fused with the thorax, has four pairs of legs and two pair of cephalic appendages . The chelicerae are claws used for feeding and they also contain silk glands (A. Orgiazzi et al., 2016). The pedipalps are the larger pincers with poison glands for grabbing pray and as a defence mechanism (Tilling, 2014; Wheater & Read, 1996). The second part, the abdomen has no additional appendages (Orgiazzi et al., 2016). Their habitat includes the litter layer, mosses, the top layer of the soil, and under logs and stones. There they capture prey such as springtails or lice (Tilling, 2014).

Araneae (spiders)

Similar to other arachnids, spiders have four pairs of legs (Figure 8e). They can be further distinguished by their morphology. The fused head and thorax are separated by a narrow waist, the pedicel, from the seemingly unsegmented abdomen (Tilling, 2014). The anterior of the abdomen contains silk glands that produces their characteristic web structures (Tilling, 2014). Looking at their hunting strategy on other arthropods, species can be divided in two broad groups: stalking the prey or using web to capture prey. After the capture, the prey is paralyzed and eaten (Wheater & Read, 1996). Besides hunting, other uses for the spiderwebs are creating retreats, to coat eggs with a protective layers, and sometimes for transport via air currents (Tilling, 2014; Wheater & Read, 1996). Hatched immature spiders resemble adult individuals already. By a series of moulding, they become mature (Tilling, 2014).

Coleoptera (beetles)

The order of the beetles is the largest order of all the different orders of life. When randomly selecting 5 species, one of them will be a Coleoptera species (Thomas, 2008). As a result of the size of the order, there is a range of families and in general diversity between species as well (Tilling, 2014; Wheater & Read, 1996). But even in this case, general traits can be observed. When looking at the morphology, the mouthparts are always of the biting type, with strong jaws and segmented palps (Figure 8f) (Thomas, 2008; Tilling, 2014). While most species are predators, there are also strict herbivores and/or scavengers (Tilling, 2014). Another common trait are the wings, with the first pair, the elytra, are a hardened protection case for the second pair of wings used for flying (Thomas, 2008; Tilling, 2014). Although many species can fly well, none have the real aerial lifestyle such as dragon- or butterflies (Thomas, 2008). Instead their general habitat is a combination of the upper soil layers, litter layer, logs and stones, and occasionally aquatic bodies (Tilling, 2014; Wheater & Read, 1996).

Oligochaeta (earthworms and potworms)

These invertebrate worms have a segmented body with multiple pairs of hairs on each segment (Tilling, 2014). In general they inhabit multiple parts of the soil profile, from the litter layer to the deeper soil (Valckx et al., 2009). Potworms (Enchytraeidae) are small and have a pale colour. Residing in the humus layer, they graze microfauna and consume dead organic matter (Orgiazzi et al., 2016). The family of the earthworms (Lumbricidae) can become larger and often have a pink or dark pigment (Tilling, 2014). These saprophages process dead organic matter and aid in mixing of the soil and formation of the humus layers (Wheater & Read, 1996). Based on their behaviour and microhabitat, these ecosystem engineers are divided in three ecological categories (Valckx et al., 2009).

Epigeic worms are small litter dweller that are not capable to create burrows. They feed on the incoming organic matter that is not yet decomposed. These litter dwellers are more tolerant to changes in the environment since they live on the soil surface (Valckx et al., 2009). Endogeic species are slightly larger and reside in the humus layer and upper mineral soil. While eating their way through the soil, they create small ans semi-permanent horizontal burrows (Hoeffner et al., 2018; Schelfhout et al., 2017). A large amount of soil and humus can be processed and mixed during feeding (Valckx et al., 2009). Anecic worms are the largest and remain deeper in the soil. They are strong enough to create large vertical burrows (Schelfhout et al., 2017). During nighttime the soil dwellers come to the surface to transport litter deeper in the soil where they eat it later on (Valckx et al., 2009). Some species show characteristics of multiple categories, for example epi-anecic earthworms (Hoeffner et al., 2018).

Vertebrata

Mega soil animals are generally vertebrated animals. These groups has a very diverse range and includes mammals, reptiles and amphibians. This group only includes species that use the soil matrix both as habitat and as a place to search for food. The term ‘mega’ comes from the fact that these individuals are relatively large with reference to the micro- and mesofauna. In absolute terms, they are often smaller than 10 cm (Orgiazzi et al., 2016). Because of the high species diversity, the variety in morphology is broad as well. A similar trait between species is that they are adapted in some way to living underground. Another defining trait is that other senses than sight are dominating (Orgiazzi et al., 2016). In the soil ecosystem, they are at the top of the food chain as they feed on meso- and macrofauna. Eventually they give back by producing nutrient rich faeces and by decomposing at the end of their lives. Other than that, they alter the soil structure and the community structure within it when moving through it. This makes them ecosystem engineers.

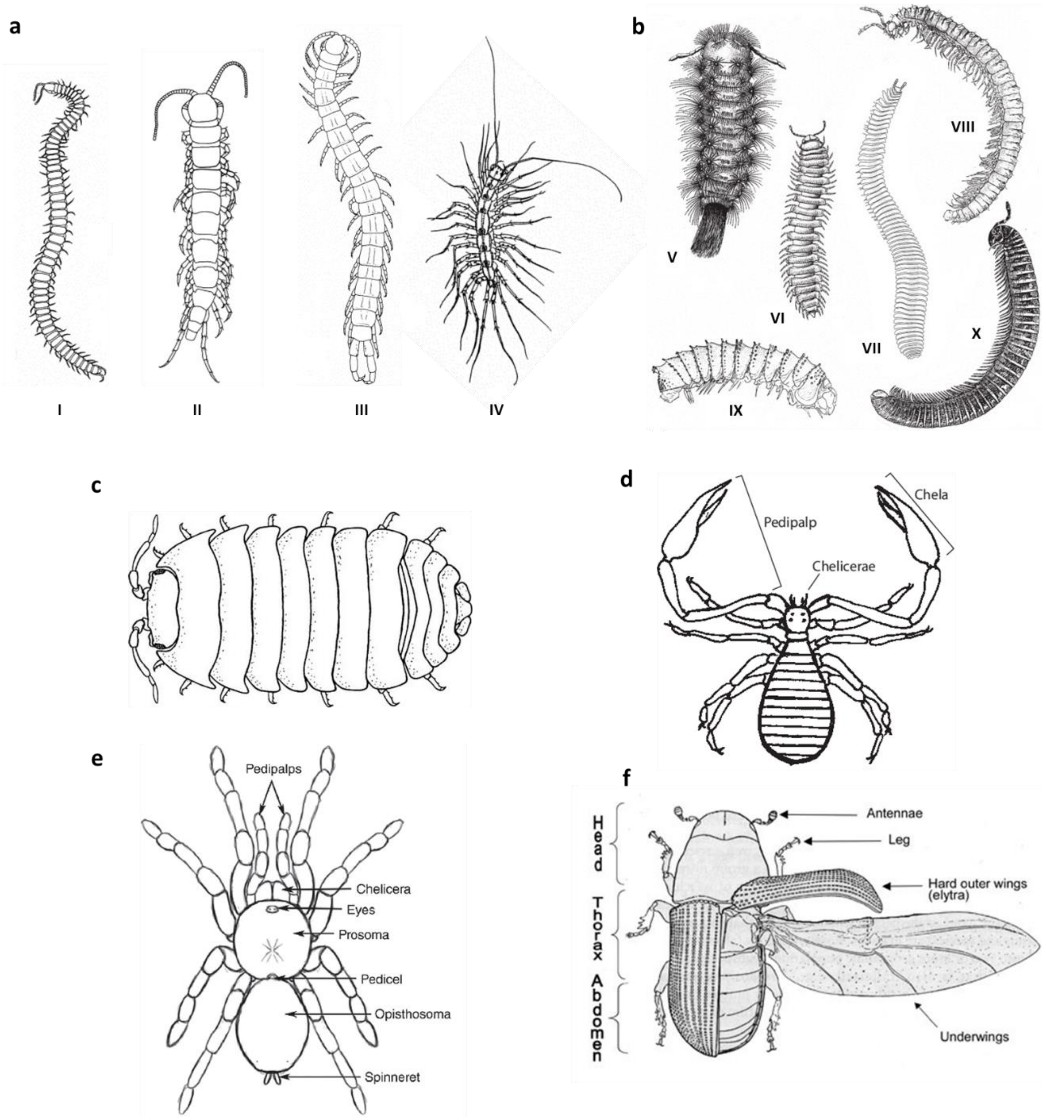


Figure 8 General external anatomy of macrofauna orders with a) the Chilopoda class with orders: Geophilomorpha (I), Lithobiomorpha (II), Scolopendromorpha (III), and Scutigeromorpha (IV) (Minelli & Golovatch, 2001). b) the Diplopoda class with orders: Polyxenida (V), Polydesmida (VI), Platydesmida (VII), Chordeumatida (VIII), Glomerida (IX), and Stemmiulida (X) (Minelli & Golovatch, 2001). c) Isopoda (Capinera, 2008c). d) Pseudoscorpiones (Watermolen, 2007). e) Araneae (Kennedy et al., n.d.). f) Coleoptera (Government of Canada, n.d.).

2.1.3 Soil biodiversity

2.1.3.1 Definition

The diversity of the life underground is also called the soil biodiversity. Giving a definition for this term is difficult, since there are multiple and it varies depending on whom is asked the question (for example a policymaker or a scientist) (Orgiazzi, 2022). The definition stated by the FAO is the following: "*The variety of life belowground, from genes and species to the communities they form, as well as the ecological complexes to which they contribute and to which they belong, from soil micro-habitats to landscapes*" (FAO et al., 2020). For this research, we emphasize soil biodiversity as the variety of life within the soil and the functions they provide. Soils are the most species-rich environments on earth, making this aspect of the definition already complex enough (Mishra & Singh, 2020). A downside of this complexity is that the soil fauna species are still largely unknown (Decaëns, 2010; Turbé et al., 2010).

2.1.3.2 The importance of soil biodiversity

As mentioned earlier, soil fauna is key to soil ecosystem functioning and the ecosystem services that stem from them. Because of this, the composition of the soil community and soil biodiversity are important. The functions that are provided depend on it (Delgado-Baquerizo et al., 2020; Turbé et al., 2010). Although the knowledge regarding biodiversity in increasing, taxonomic diversity and ecological functioning at species level remains largely unknown (Geisen et al, 2019). Three general mechanisms that connect biodiversity to soil functions are: repertoire, the potential of interactions, and functional redundancy (Jeffery et al., 2010).

Repertoire refers to species richness. Certain processes are only executed by certain species. A more diverse community increases the chance that the required species are present (Jeffery et al., 2010). Secondly, high diversity suggest a more complex network. The amount of potential interactions increases the adaptability and resilience for change and disturbance (Jeffery et al., 2010; Turbé et al., 2010). Lastly, functional diversity is not equal to taxonomic diversity as multiple species carry out similar functions (Turbé et al., 2010). This implies functional redundancy, or that a soil function can still be maintained after a loss of a species as others contribute to the same function (Bardgett & Van Der Putten, 2014; Simon Jeffery et al., 2010). In addition, soil organisms often can participate in more than one process. A species that provides a function can be redundant for another one at the same time (Turbé et al., 2010). However there are a few remarks. More unique processes such as nitrification and the breakdown of toxic chemicals can only be done by a handful of species. Once they are removed from the community, there will loss of those functions as well (Wolters, 2001). Another remark is the context dependency. Different species could perform the same function but other factors could differ such as the environmental conditions. If these are not right for the other species, the function could be lost as well (Turbé et al., 2010).

To recapitulate, soil biodiversity has an insurance effect on soil functions and creates resilience (Baumgärtner, 2007; Geisen et al., 2019). Due to the knowledge gaps, predictions regarding potential loss of biodiversity in the future are hard to make. Hence it is wise to take precautionary measurements to preserve the biodiversity (Turbé et al., 2010).

2.1.3.3 Threats for soil biodiversity

The delivery of these ecosystem services and the general soil biodiversity are however threatened. The degradation of these belowground ecosystems is largely affected by the change of natural area into human influenced biomes (Geisen et al., 2019). Intensive agricultural practices are often non-sustainable and enhance the decrease of SOC and soil biodiversity. This makes the community sensitive to pathogens (Balmford & Bond, 2005). Deforestation results in nutrient depletion, acidification of the soil, and erosion (Smith et al., 2016). Urbanisation causes soil compaction and sealing, contamination, and the introduction of invasive species (Pickett & Cadenasso, 2009). Environmental disturbance is a natural process but there are more frequent and extreme events because of climate change. The limits of resilience of the ecosystems are stretched with increases stress levels (Turbé et al., 2010).

In general, it is known that all of the above are responsible for the degradation of the soil and its soil biota. Nevertheless many questions remain regarding the exact response of the soil to these threats. Not all taxa react the same and this also depends on the exact region (FAO et al., 2020). Zooming in on the temperate broadleaf and mixed forests of Europe, one of the main threats is the acidification of the forests floors. It is a natural process for the soil pH to lower over time. This is due to processes such as weathering with slightly acidic rainwater, nitrification, and nutrient uptake by plants (de Schrijver et al., 2012). When protons (H^+) enter the soil environment, there are different buffer mechanisms that neutralise them: dissolution of carbonates (pH 8.6-6.2), silicate weathering (pH 6.2-5.0), base cations exchange (pH 5.0-4.5), and aluminium hydroxides (below pH 4.5) (Figure 9) (Bowman et al., 2008; Desie et al., 2021). Only when a mechanism is exhausted, a small increase in protons induces a large pH drop. These steep regions in the curve are also called pedogenic thresholds (Desie et al., 2021). The time to undergo the whole process depends on environmental and soil characteristics but in general this is a slow process (Yadav et al., 2020).

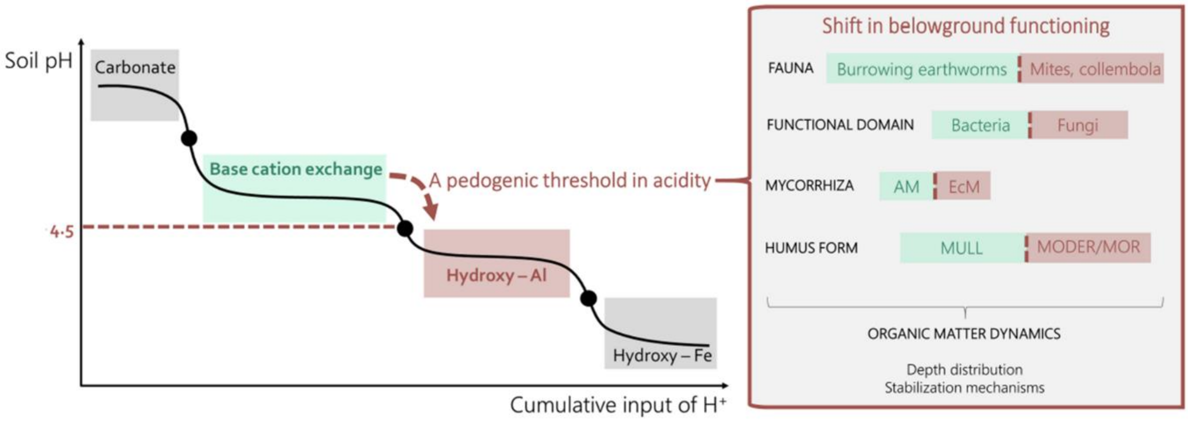


Figure 9 Left: Changes in soil pH-H_2O buffering mechanisms with increasing input of H^+. Subsequent buffering zones are connected by pedogenic thresholds. Right: Shifts in belowground functioning as a result of the pedogenic threshold between the cation exchange and aluminium zone (Desie et al., 2021).

What makes soil acidification problematic is the fact that these natural processes are accelerated by human influence. Atmospheric deposition of SO_2 and NO_2 are increased by combustion processes (industry and transport) and are two of the main sources of acidification. Nitrogen emission by agriculture contribute to the acidification as well. In addition, it increases the total nitrogen in natural systems that are nitrogen-limited (Bowman et al., 2008; de Schrijver et al., 2018; D. S. Yadav et al., 2020). These components reach natural ecosystems by (acid) rain (wet deposition) or by capture by aboveground vegetation (dry deposition) (Augusto et al., 2002).

The impact of soil acidification will cascade through the entire ecosystem, since soil is the point where biosphere, lithosphere, atmosphere and hydrosphere meet (D. S. Yadav et al., 2020). Above a pH of 4.5, base cation are leaching due to the buffering mechanisms. Below a pH of 4.5, aluminium and iron hydroxides becomes soluble. This point of acidity causes a large shift in the soil functioning (Figure 9) (de Schrijver et al., 2012). Below the surface, the composition of the soil community changes. The presence of earthworms and other burrowing species reduce, except for acid tolerant species (de Schrijver et al., 2018). The dominance of mesofauna orders such as Collembola, Acari, and Enchytraeidae increases as they are more adapted to an acid environment (Desie et al., 2021; Turbé et al., 2010). Soil mixing activities are reduced resulting in a disconnection between the organic and the mineral layer. The microbial community that mainly resides in the organic layer shifts as well (Desie et al., 2021). Fungal species dominate since they have a broader pH range. Bacterial activity reduces and specialized species such as for the nitrification process have a low performance (D. S. Yadav et al., 2020). This change in community slows down the decomposition processes what leads to the built up of the humus layer. In addition, mor and moder humus types become dominant (Desie et al., 2021). The change in soil community also influences further cascades in the food chain (de Schrijver et al., 2012).

Furthermore, the soil matrix and SOC are affected as well. Due to a decrease in biological activity and soil fertility the structural stability of the soil changes. Water holding capacity and porosity decrease which limit plant moisture uptake. Soil aggregates become less stable due to low base cation availability, the presence of soluble metal hydroxides, and burrowing activities (D. S. Yadav et al., 2020). SOC is concentrated in the organic layer and is less stable because of this and the changed chemical composition. The latter is caused by the change in microbial community (Desie et al., 2021).

Aboveground species diversity decreases on account of the decreased variability in niches, metal toxicity and nitrogen abundance. The herbaceous layer consists more of generalist that are tolerant for the high nitrogen concentration and low pH (de Schrijver et al., 2012). Tree species have a different acidification tolerance level. In general, deciduous trees species have more issues to maintain optimal growth and rejuvenation than conifer trees (de Schrijver et al., 2018). In addition to the species tolerance, plants often live in symbiosis with mycorrhiza. They increase the plant root system and aid in the absorption of nutrients (de Schrijver et al., 2012). Toxic levels of aluminium damages the root-fungi system which affect plant growth (de Schrijver et al., 2018). Older trees have vitality problems due to this as well (Olsthoorn & Wolf, 2006).

2.2 The effect of forest management on soil life

Each soil organism requires certain conditions, such as pH, temperature and soil moisture in order to be present in a habitat. Hence the community differs across forest ecosystems (de Schrijver et al., 2018). Forest management practices are a way to alter the current habitat. Each measure can thus influence the soil community in a direct or indirect way, and can be both positive or negative for a certain organism (Table 1). Tree species choice is important as each species has different characteristics such as root systems, nutrient composition in the leaves and environmental requirements (den Ouden et al., 2020). Strong thinning practices and large impact fellings (clearcut) have a negative influence on soil biota. Abrupt and major changes to the microclimate (light, temperature, and moisture) have a large impact on the community which makes it difficult to recover (de Schrijver et al., 2018; Menta, 2012). Nevertheless, the soil community could recover fast from mild interventions as each community is resilient to some level (Menta, 2012). This is also true for perturbations such as fire and floods. Often these are harmful for the community. An exception is storm damage. Dead trees are a food source for soil biota and the nutrients from it will eventually be recycled. In case of fellings, it is advised as well to leave smaller branches in the forest. This way a part of the nutrients won't be removed from the system (de Schrijver et al., 2018). Furthermore, site management practices focus more on abiotic factors instead of the trees and their composition. Problems with nutrient imbalances and pH often reduce the productivity of the soil community for example. This could be (temporarily) solved with liming and fertilisation.

For acidification problems discussed earlier, there are some interventions possible. First of all, as these acidification processes are accelerated by emissions, these sources need to be reduced by policies (de Schrijver et al., 2018). In addition, there are two management practices that can be used: liming and tree species choice.

Liming increases the buffering capacity of the soil by adding elements such as $CaCO_3$ and $MgCO_3$. For a fast reaction a powder form is used. For a more long term effect, rock dust is a combination of different particle sizes. Some will dissolve slower while others faster (de Schrijver et al., 2018). The elements neutralise the acids and base cations are released. This encourages the soil community to increases decomposition. A decrease of the humus thickness and the release of nutrients within the humus layer result from liming (den Ouden et al., 2020). However, liming should be done carefully. If pH is increased too much too fast, decomposition increases without regulations higher up in the food chain. This could lead to further loss of nutrients and carbon (de Schrijver et al., 2018; den Ouden et al., 2020).

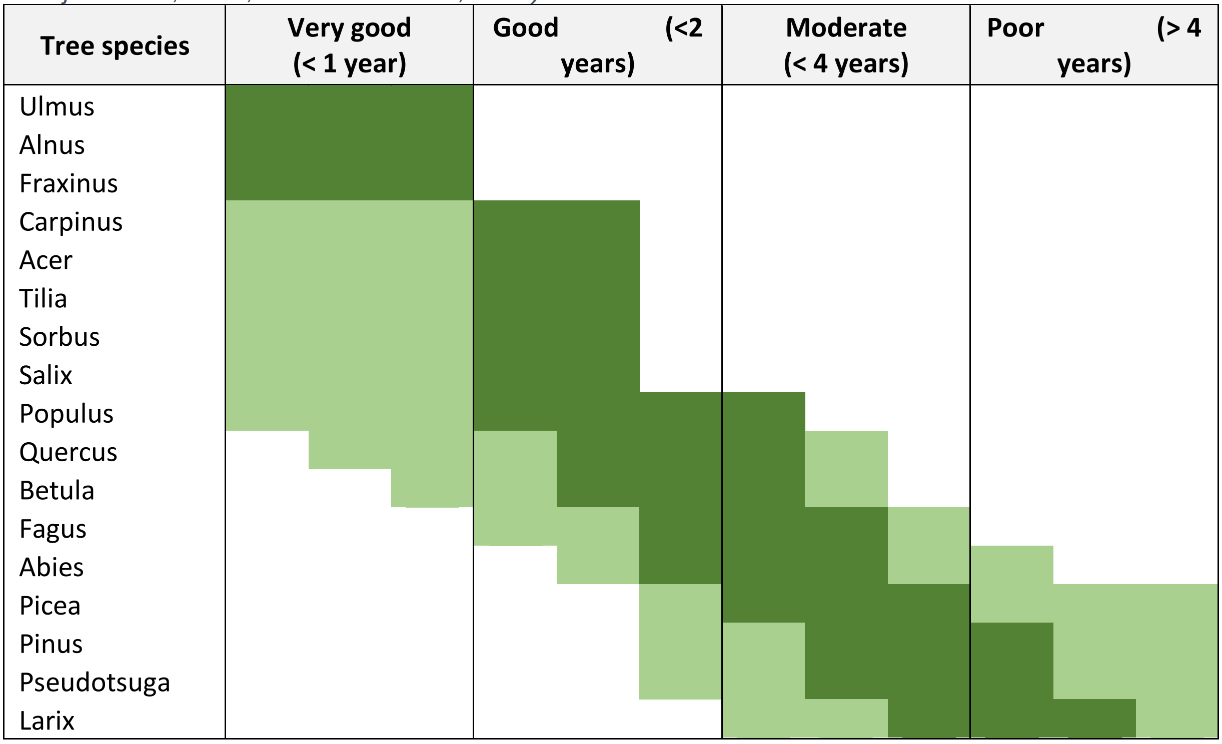
Tree species choice is a management practice that could work on the long term. Leaf litter from different tree species has a variable nutrient composition and concentration. In general litter from deciduous trees contains more base cations than conifer trees (Table 2) (den Ouden et al., 2020). The decomposition rate largely depends on the ratio of lignin (C) and nutrients. The lower this ratio, the easier breakdown and the more preferred by soil biota (den Ouden et al., 2020). In addition, forest capture a large amount of the atmospheric dry deposition than other landscape systems. The rougher and larger structure increases the take up. Comparing between forest types, this is the highest in conifer forests as they stay evergreen and have a large interception area (de Schrijver et al., 2012).

Rich litter species such as Acer, Fraxinus, Tilia, and Prunus could help restore acidified soils. Their litter stimulates increased (bacterial) decomposition and a decrease of the built up of the organic layer. The slow increase in base cations causes an increase in pH. In turn, this attracts more soil biota such as burrowing earthworms. Burrowing activities also help reduce the organic layer and mixing the organic and mineral layer. Hence this will eventually lead to a balanced soil. This sustainable strategy is aimed at the nutrient cycling processes and ecology. Liming on the other hand is mainly focussed in abiotic soil chemistry (Desie, Vancampenhout, Nyssen, et al., 2020). Note that not all rich litter tree species are tolerant to low pH. Hence it could be useful to combine adding these species with liming to increase the success rate (de Schrijver et al., 2012).

Table 1 Impact of forest management and perturbations on the biodiversity of the dominant soil biota. Positive influences are indicated by an upward arrow (↗), negative influences are denoted by an downward arrow (↘) . Arrows in bold indicate that the effect is strong. A neutral effect is shown by a dash (—) and empty cells indicate lack of scientific research (adapted from de Schrijver et al., 2018).

			Wortels		Micro-flora en fauna						Mesofauna		Macrofauna									Megafauna	
Management	**Related issues**	**Division**	**Herbaceous plants**	**Trees and shrubs**	**Bacteria & Archaea**	**Arbuscular mycorrhiza**	**Ectomycorrhiza**	**Other fungi**	**Amoebas en Protists**	**Eelworms (Nematoda)**	**Springstails (Collembola)**	**Mites (Acarina)**	**Millipedes (Diplopoda)**	**Centipedes (Chilopoda)**	**Ants (Formidicae)**	**Woodlice (Isopoda)**	**Beetles (Coleoptera)**	**True flies (Diptera)**	**Potworms (Enchytraeidae)**	**Earthwoms (Lumbricidae)**	**Snails (Gastropoda)**	**Voles (Cricetidae)**	**Moles (Talpidae)**
Forestry interventions																							
Tree species choice	Site (un)suitability	Rich litter species	↗	↗	↗			↘	↗	↗	↗	↗	↗	↗	↗		↘	↗		↗	↗	↗	↗
		Recalcitrant litter species	↘	↘	↘			**↗**		↗	—	↗			—	—	↗	↘	↗	**↘**	↘	—	↘
Regeneration	Inadequate occupation	Natural	—	↗	—		↗	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Artificial	—	↘	—			—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Thinning	Space distribution	Strong thinning	↗	↘	↗	—	—	↘	—	↗	↗	↗	↘	↘	↗	↘	↗	↗	↘	↘	↘	↗	↗
		Mild thinning	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Felling & exploitation	Soil compaction; nutrient export	Clearcut	↗	↘	↗	↘	↘	↗	↗	—	↗	↗	↘	↘	↗	↘	↗	↗	↗	↗	↘	↗	↗
		Group/strip cut	↗	↘	—	—	—	—	—	—	↗	↗	↘	↘	↗	↘	↗	↗	↗	↗	↗	↗	—
		Selection cut	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Site management																							
Liming	Acidification		↗	↗	**↗**		—	↘	↗	↗	—				↗				—	↗	↗	↗	↗
Fertilization/N deposition	Nutrient imbalance		↗	↘	**↗**	**↗**	**↘**	↘	↗	↗	—									↗	↗	↗	↗
Drainage	Desiccation		↗	↗	↗		↘	↘		↘	↘	↘	↘	↘	↗	↘	↗	↗	↘	↘	↘	↗	↗
Increasing water level	Oxygen shortage		↘	↘	↘	↗	↗	↗		↗	↗	↗	↗	↗	↘	↗	↘	↘	↗	↗	↗	↘	↘
Fencing in	Soil compaction		↗	↗	—			↗	—	—	—	—	—	—	↗	—	—	—	—	↗	—	↗	↗
Litter removal	Nutrient imbalance		↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	—	↘	↘	**↘**	—	—
Perturbations																							
Forest fire (incl ground fire)	Heat damage & mortality		↘	↘	↘	—	↘	—	↘	↘	↘	↘	↘	↘	↘	↘	↘	↘	—	—	↘	↘	—
Flood	Oxygen shortage		↘	↘	↘		↘	—	—	—	↘	↘	↘	↘	↘	↘	↘	↘	—	—	↘	↘	↘
Storm damage/ windthrow	Augmented amount necromass		↗	↘	↗			↗	—	—	—	—	↗	↗	↗	↗	↗	↗		↗	↗	↗	↗

Table 2 Relative decomposition rates of litter of different tree species. Light green represents the whole range of all observations while dark green indicates the range of most observations. Data for this hierarchy is collected in sites with similar environmental and soil characteristics (adapted from de Schrijver et al., 2018; den Ouden et al., 2020).



Tree species	**Very good (< 1 year)**			**Good (<2 years)**			**Moderate (< 4 years)**			**Poor (> 4 years)**		
Ulmus	🟩	🟩	🟩									
Alnus	🟩	🟩	🟩									
Fraxinus	🟩	🟩	🟩									
Carpinus	▨	▨	▨	🟩	🟩							
Acer	▨	▨	▨	🟩	🟩							
Tilia	▨	▨	▨	🟩	🟩							
Sorbus	▨	▨	▨	🟩	🟩							
Salix	▨	▨	▨	🟩	🟩							
Populus	▨	▨	▨	🟩	🟩	🟩	🟩					
Quercus		▨	▨	▨	🟩	🟩	🟩	▨				
Betula			▨	▨	🟩	🟩	🟩	▨				
Fagus				▨	▨	🟩	🟩	🟩	▨			
Abies					▨	🟩	🟩	🟩	▨	▨		
Picea						▨	🟩	🟩	🟩	▨	▨	▨
Pinus						▨	▨	🟩	🟩	🟩	▨	▨
Pseudotsuga						▨	▨	🟩	🟩	🟩	▨	▨
Larix							▨	▨	🟩	🟩	🟩	▨

2.3 The forests in the Southern Netherlands and Northeast Belgium

For this thesis research, the focus lies on the forests in the Netherlands and Belgium. These former heathlands were transformed into forests by humans. These initially nutrient-poor soils were even further degraded by the establishment of monocultures of pine species with poor litter quality, removal of litter, and overexploitation (Augusto et al., 2002). The acidification and imbalance of the nutrient cycles had a large impact on the diversity of the flora and fauna, both above and below the surface (Desie et al., 2021). Individual trees became less vital and the number of species decreased. In addition, the soil biodiversity downgraded as well.

Poor litter species provide litter that is slowly biodegradable and has a high C/N ratio (Desie et al., 2021; Ge et al., 2013). The lack of base cations can shift the soil to a more acidic pH, which influences the soil biota composition. As a result, there is a large reduction of the soil fauna. Acid tolerant mesofauna become more dominant over macrofauna species that mostly disappear. Fungal species, which are slow decomposers, become dominant microbiota (Desie et al., 2021). Lack of burrowing activities and slow decomposition results in the built up of the organic layer and a disconnection with the mineral soil (Desie et al., 2021).

One of the possible remediation actions that is considered, is the introduction of rich litter species and more species diversity. According to Ge et al. (2013), there is a positive correlation between soil nutrient contents and litter substrate quality. The nutrient-rich and easily-biodegradable litter would slowly complement the poor and slow-degradable litter (Desie, Vancampenhout, Nyssen, et al., 2020). The nutrient cycles would then become more balanced again (Ge et al., 2013), attracting diverse soil biota. The result would be a more diverse and vital ecosystem (Desie, Vancampenhout, Nyssen, et al., 2020).

However, there are still uncertainties on whether these remediation measures have a desirable effect, how large this effect is and what the necessary timeframe is. Hence, Bosgroep Zuid-Nederland has set up a research plan in collaboration with KU Leuven to check and evaluate the effect of the introduction of rich litter species in a first phase. In a second phase, the remediation technique can be optimised for each situation to improve different habitat types. The focus of this thesis research lies in the first phase and more specifically, to analyse the condition of the soil biodiversity in these forests in the Northern-Brabant region. In addition, this condition will then be linked to the forest management strategies in each unique location to examine which actions influence the soil biodiversity.

2.4 Research goals

The main research goals are the evaluation of the state of the soil biodiversity in forest on sandy soils and how this differs in between contrasting forest types; i.e. pine monocultures or mixed broadleaved forests dominated by species with litter rich in base cations (further: rich litter). First we want to evaluate the presence or absence of mesofauna and earthworm species in all plots. In addition, the total amount and amount per group of organisms will be compared between plots with species that have litter poor in base cations (further: poor litter species) and rich litter species. We hypothesize that the amount of earthworms will increase in rich litter plots since they are less acid tolerant. Mesofauna will decrease as their habitat, the organic layer, will decrease due to better soil functioning. Second, we want to assess the overall composition of the soil community. As a result of the acidifying conditions, a low diversity is expected. However, we hypothesize that diversity is higher in plots with rich litter species when comparing both forest types (poor litter species plots and rich litter species plots). Third, the link between the amount of soil fauna, soil and environmental characteristics are analysed. This is one of the ways to assess the influence of rich litter species admixture as a management measure. It is assumed that these characteristics will be more suitable in plots with rich litter species. Lastly, functional traits and feeding patterns collected from literature are linked with the found soil species composition. It is hypothesized that there is an imbalance in functionality as some species cannot tolerate a low pH and will not be providing its soil functions. Based on all these analyses, we will assess the effect of adding rich litter species as a management practice that can aid in the restoration of acidified soils.

3 Materials and method

3.1 Study region

The study region is located in the southern part of the Netherlands and northeast of Belgium in the provinces of Noord-Brabant, Limburg (Belgium) and Antwerp (Figure 10). This area is located within the West-European sand belt, which are Pleistocene sand deposits (Beerten et al., 2014). These sediments have a mixed origin of continental and marine sources, hence the texture ranges from coarse sand to sandy loam (Beerten et al., 2014; Kasse et al., 2007). The region has a temperate maritime climate with mean annual temperature (MAT) of 10.5°C and mean annual precipitation (MAP) of 910 mm (Royal Meteorological Institute of Belgium, n.d.).

The effect of adding rich litter species was assessed by selection of sites in the study region that had both a conifer dominated area (poor forest type) and mixed forest patches with rich litter species (rich forest type). This ensured a comparison between 2 plots with similar land-use history to avoid unwanted additional variation. All possible locations were visited beforehand to check for suitability. In the end, six sites were chosen for the field campaign (Figure 10, Table 3). The reference plots had a less severe history of degradation and rich litter species have already been present for a longer time.

From this point on and to minimise confusion, plots with a poor forest type indicate a dominance of recalcitrant (poor) litter species. A rich forest type refers to a dominance of rich litter species.

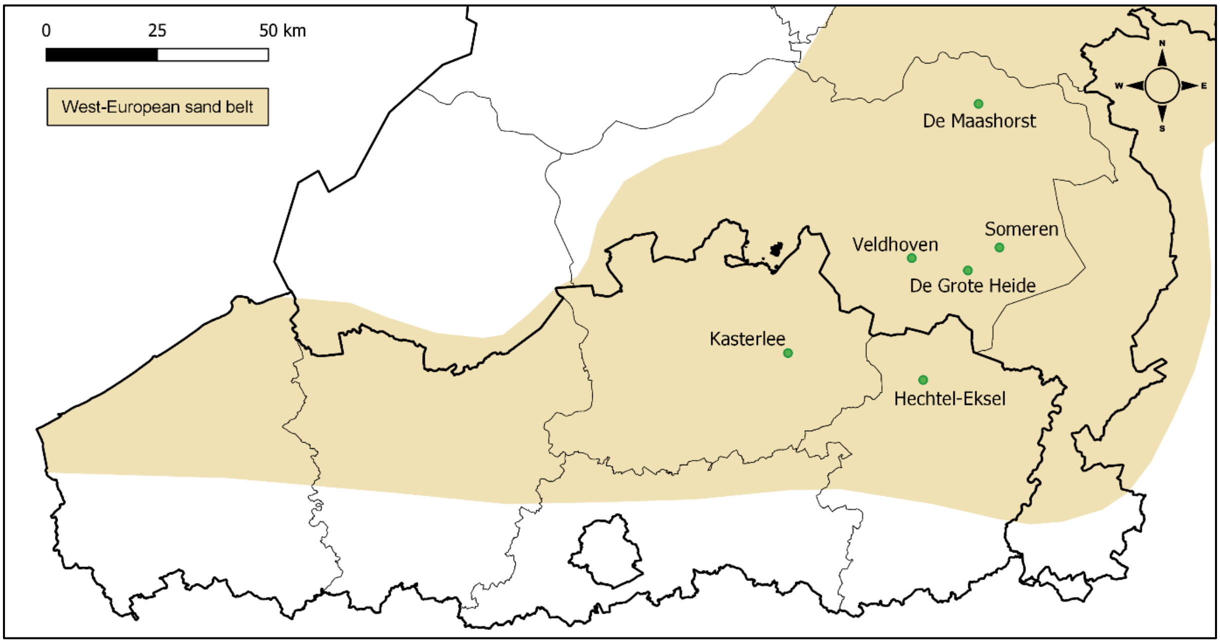


Figure 10 Overview of the study region in Belgium and the Netherlands (Agentschap Digitaal Vlaanderen, n.d.; Nationaal Georegister, n.d.). The yellow region indicated the Pleistocene sand deposition, which is called the West-European sand belt (adapted from Beerten et al., 2014; Desie et al., 2020). The sample sites are indicated by green dots.

Table 3: Overview of the sample sites.

Sample location	**Date of sampling**	**Poor plot**	**Rich plot**	**Reference plot**
Someren	26/09/2022	X	X	
Hechtel-Eksel	27/09/2022	X	X	
Kasterlee	28/09/2022	X	X	X
De Maashorst	30/09/2022	X	X	
Veldhoven	3/10/2022	X	X	X
De Grote Heide	4/10/2022	X	X	

3.2 Field campaign

For each location, the plots of 10x10m were chosen and should be representative for the species present its surroundings (Table 3). First, general information was collected: address and coordinates, meters above sea level, slope and exposition, and sketches and pictures of the plot. With the use of a spherical densiometer the tree cover density could be estimated. The total basal area was determined by the Bitterlich method. The latter was also done per tree species. For completion, land use history and the current management was looked up afterwards.

Secondly, the soil was described and sampled. The humus layer was classified based on the European Reference Base (ERB) (Zanella et al., 2011) with three repetitions per plot. The thickness of the different organic horizons (OL, OF, and OH) were measured for each repetition. Further, a drilling was made to reveal the soil profile. For each horizon the depth was measured and a sample was collected. The general soil profile was described according to the FAO guidelines for soil description (Jahn et al., 2006). Additional soil samples were taken for the soil characterisation at three different depths. For bulk density the organic layer sample (OL+OF+OH) was gathered over a surface area of 20x20 cm. The mineral layer was sampled at 0-10 cm and 10-20 cm depth by means of Kopecky rings. For the chemical and eDNA analysis, samples were taken of the organic layer (OF+OH) and the mineral layer at 0-10 cm and 10-20 cm. These larger samples were mixtures of 10 random small drillings in the plot. To prevent contamination of human DNA, gloves were used during the sampling process.

Lastly, fauna samples were collected. Three mesofauna samples were collected randomly in the plot. Each sample consisted of the organic layer (OF+OH) from a 20x20 cm area and one small drill of the 0-10 cm mineral layer. The soil samples were preserved cold until further analysis. Earthworm sampling was done according the FunDivEurope protocol on a 0.5 m^2 area (Muys, et al., 2013). For each plot the protocol was repeated three times. The captured worms were preserved in a 70% ethanol solution.

3.3 Processing in the lab

Soil characteristics

After returning from the field, the horizon samples and soil samples for bulk density and chemical analysis were dried. This could be done by airdrying or placing the samples in an oven. For the latter approach, the temperature was set to remain below 36°C to prevent potential changes in the community composition. After drying, the samples were grinded in a mill and split to get homogeneous and representative samples.

The dry samples were weighted to determine the bulk density in g/cm^3. Next, the C:N ratio was determined by grinding the samples with a mortar and taking subsamples between 3-20 mg, which depended on the organic matter content. The samples were weighed and stored in a tin casing to be analysed with a Thermofisher Scientific elemental analyser, type Flash 2000 later on. Total carbon stock in ton/ha could then be calculated by combining the bulk density and percentage carbon. The equation used, differed based on whether the samples from the organic layer or subsoil were processed. This had to do with the use of different extraction methods (Equation 1 and 2). By means of a HI 991000 pH/Temperature meter with HI 1292 from Hanna Instruments the pH was measured from the samples with a 5 mg soil/25ml demi water ratio. Lastly and according to the ISO 23470 standardised protocol, CEC was measured in $cmol_c/kg$. The base saturation could be determined, since the CEC and the amount of exchangeable cations were known (Equation 3).

$$Organic\ layer: C\ stock\ \left(\frac{ton}{ha}\right) = 0.25\,\frac{ton}{ha} * m(g) * C(\%) \tag{1}$$

$$Top\ soil\ layer: C\ stock\ \left(\frac{ton}{ha}\right) = 10\,000\ m^2 * 0.1\ m * bulk\ density\ \left(\frac{g}{cm^3}\right) * C(\%) \tag{2}$$

$$Base\ saturation\ (\%) = \frac{\sum(Ca^{2+}, Mg^{2+}, K^{+}, Na^{+})}{CEC} * 100 \tag{3}$$

Earthworms

The captured earthworms were identified to species level with the use of a microscope. The identification was based on the structure of the prostomium, the pattern of the setae rows and the colour of the furrows. For species differentiation, the placement of the male gonopore, clitellum, and tubercula pubertatis were important traits. For this process, three identification books were used: “De Nederlandse regenwormen” by Krediet (2019), “Earthworms” by Sims & Gerard (1999), and “Dieper graven naar het belang van regenwormen in duurzaam akkerbeheer” by Valckx et al. (2009). If it was not possible to reach species level, worms were identified to genus level. When it was also not possible to distinguish the family, the label of ‘juvenile earthworm’ was given. In addition, the biomass was determined by weighing the individual worms. Count and biomass for the different ecological categories, anecic, endogeic, epigeic, and epi-anecic earthworms, were calculated as well. Juvenile worms with no species or genus name were classified separately.

Mesofauna

The mesofauna organisms were extracted from the soil samples by means of the Tullgren funnel method (Figure 11). The soil samples were surrounded by a gauze and put in a funnel. The gauze had openings of 3 mm to prevent macrofauna from leaving the soil and combining with the mesofauna. The lightbulb above the funnel acted as a heat source that dries out the soil sample. This forced the fauna downwards, away from the heat. Under the funnel a beaker was placed with a 70% ethanol solution to capture the organisms that come out at the bottom of the funnel. When the soil samples were completely dried, the content in the beaker was transferred to a beaker or cup that could be closed at the top. This prevented evaporation of the alcohol solution and made longer preservation of the mesofauna possible.

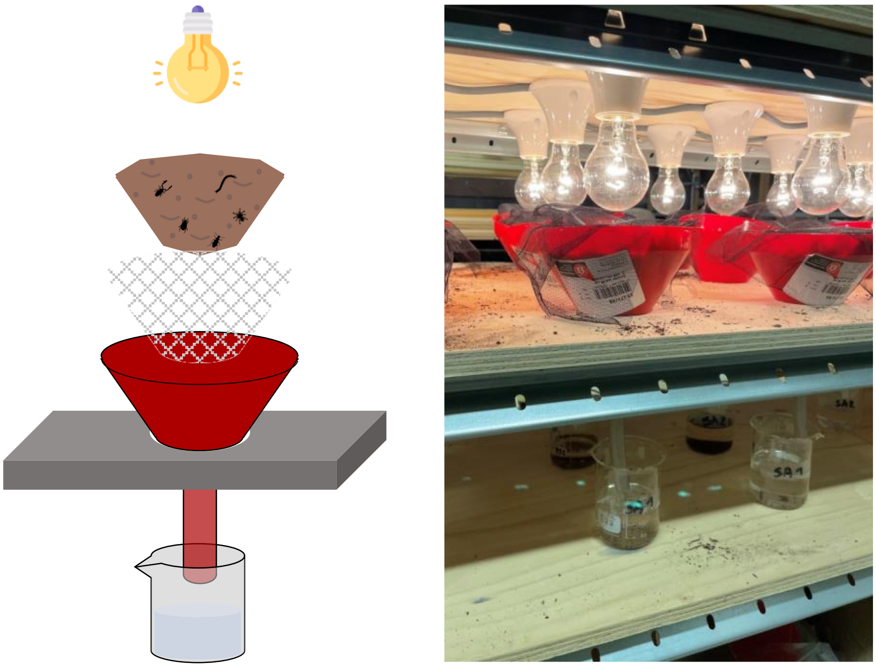


Figure 11 Tullgren funnel setup with a schematic representation on the left and a picture the used setup on the right.

The next step was the identification of the individuals. Mesofauna is very diverse hence most determination keys stop at the level of order. Due to lack of expertise, this was also the level that we chose to reach if possible. The identification keys used were “A key to the major groups of British terrestrial invertebrates” by Tilling (2014) and “Animals under logs and stones” by Wheater & Read (1996). Identification needed to be done under a microscope. A more detailed protocol regarding the mesofauna extraction and identification can be found in the Appendix (8.1 Mesofauna protocol).

3.4 Statistical analysis

All statistical analyses were done in R version 4.2.3 (R Core Team, 2023). The first step after requiring the raw data was data exploration. The humus layer, mesofauna and earthworms were visualised to check the difference between the individual plots. The focus here mainly lied on the contrast between rich and poor plots and thus the effect of rich litter species. Mesofauna count was examined both per unit of area and per unit of volume. Since the volumes of the organic layer and thus the samples in the field differed largely, this could potentially give divergent results. For the earthworms, both biomass and count (per unit of area) were considered as well. Since size and thus weight differed per species, this could also lead to variable results. For further analyses, only biomass was considered as it is a more commonly used measurement and represents ecological effects better (Peigné et al., 2017). In addition, the earthworms were classified earlier based on their ecological categories. Here we wanted to check for a possible difference between forest type as well. Then a diversity analysis was performed on the fauna data. Indices such as species richness, Shannon diversity, and Shannon evenness were calculated and compared between forest type as well.

The effect of forest type and thus the influence of tree species on the above responses (humus thickness, mesofauna count, earthworm biomass and count, and diversity indices) were tested by means of generalised linear mixed models (GLMM). In all models site was a random effect. They were created by using the packages lme4 and glmmTMB (Bates et al., 2015; Brooks et al., 2017). In case of count data, tests based on the DHARMa package were done to check for over- or underdispersion (Hartig, 2022). In addition, a normal distribution of the model residuals was checked graphically. After checking the model assumptions, estimates for the mean value and 95% confidence intervals (CI) per forest type were acquired with the ggeffects package (Lüdecke, 2018).

Relations between soil and environmental characteristics were tested similarly. Characteristics that were measured at different depths were all modelled as separate fixed effects. For the mesofauna, the most interesting region is the organic layer since that is their main habitat in the soil system (Turbé et al., 2010). For earthworms there was no preference as the different ecological categories have different niches. Here the random nested effect of the GLMM's consisted of forest type that is nested within site. The predicted values were represented graphically. Full lines indicate a significant effect while dashed lines corresponded to non-significant effects. P-values of the intercept and fixed effect of the models were given by package lmerTest which is based on the Satterthwaite's method (Kuznetsova et al., 2017). The model to evaluate the effect of rich litter influence on humus type was an exception compared to the rest. Since humus type was a categorical variable with 3 levels, multinominal logistic regression was required. The model was created using the nnet package (Venables &

Ripley, 2002). P-values were calculated from the z-values (Wald test). During calculation of some of the models, we encountered conversion problems. In this case the model would provide estimates of the effects but could not provide P-values or standard errors. We only continued with these models if these conversion problems could not be solved by looking into different model types. This could be related to the sample size of the datasets.

Lastly we wanted to evaluate the distribution of feeding strategies and soil function provisioning in the samples. Information regarding the feeding strategies and functionality of the soil fauna was collected from literature (Table 4) (Coq et al., 2022; Gapp et al., 2023; Jouquet et al., 2006; Lavelle et al., 2006; Potapov et al., 2022; Tilling, 2014; Wheater & Read, 1996). Some groups had alternative food sources which were indicated with a small asterisk (*) . Since not all groups had alternative food sources, we decided not to look into them to remain consistent. For each feeding strategy or soil function, the sum was taken of the count of all the relevant groups. Mesofauna was sampled on 400 cm^2 and the earthworms on 0.5 m^2 hence the amount of mesofauna was extrapolated to 0.5 m^2. Because previous result indicated an opposite results when counting per unit or area or volume, count per volume was also calculated. Each of the individual feeding strategies and soil functions were then tested on the influence of forest type analogue as above. Site was seen as a random effect in the GLMM’s.

Table 4: Overview of feeding strategies and functional traits of the mesofauna orders and ecological categories of earthworms. In case that multiple strategies or traits applicable for an order or category, all are indicated. For the feeding patterns a large X indicates main food sources or soil function while an asterisk (*) is used for alternative sources.

Fauna	Detrivore	Microbivore	Herbivore	Predator	Parasite	Litter fragmentation	Bioturbation	Population regulation	Gut passage
Geophylomorpha (Chilopoda)				X				X	
Scolopendromorpha (Chilopoda)				X				X	
Lithobiomorpha (Chilopoda)				X				X	
Chordeumatida (Diplopoda)	X		*			X			X
Polydesmida (Diplopoda)	X		*			X			X
Polyxenida (Diplopoda)	X		*			X			X
Symphyla	X		*			X			
Coleoptera	X	X	X	X	X	X		X	
Thysanoptera		X	X					X	
Acari	X	X	X	X	X	X			
Diplura	X	X	*	X		X		X	
Isopoda	X	X	*			X		X	X
Collembola	X	X	*			X		X	
Diptera	X	X	*	X	*	X		X	
Hemiptera			X						
Psocoptera		X	*					X	
Pseudoscorpiones				X				X	
Araneae				X				X	
Apocrita (Hymenoptera)			*	X	X			X	
Formicidae (Hymenoptera)		*	X	X			X	X	
Protura	*	X		*		X		X	
Nematoda/Nematomorpha		X	X	X	X			X	
Oligochaeta	X	*	*			X	X		
Tubellaria				X				X	
Epigeic earthworms	X					X			
Endogeic earthworms	X					X	X		X
Epi-anecic earthworms	X					X	X		X

4 Results

During sampling in the field, we observed that the rich plot in Someren was located on a plaggic Anthrosol whereas the poor litter plot was located on a Podzol. Therefore both plots cannot be used as a twinplot and it was decided to remove Someren datapoints from the datasets and continue further analyses without them.

4.1 Humus description

The average combined thickness of the OF and OH humus layer was larger for plots with a poor forest type than in rich or reference plots, with the exception for Kasterlee (Figure 12). This was also the case when looking at the OF humus layer separately. For the OH layer, this was less clear since in multiple plots the thickness was very small or even absent. When looking further into the difference between rich and poor forest type, there was a significant difference between means with or without outlier (Kasterlee Rich and Maashorst Poor) (P = 0.03) (Figure 13, Table 5).

Furthermore, the effect of rich litter on humus type is not significant (Figure 14, Table 5). However, graphically can be seen that moder and mull humus is associated with a higher rich litter influence. The probability of a certain humus type is not significantly impacted by rich litter (P = 0.25 and P = 0.15). The probability of mull humus increases with higher rich litter influence, for moder humus this is initially similar but the probability decreases when rich litter influence becomes higher whereas the probability for mor humus shows a decreasing trend in relation to rich litter influence.

*Table 5: Estimates, standard error, and P-values of the intercept and fixed effects of the GLMM for thickness of the humus layer as response and site as a random effect, and multinominal logistic regression model for humus type. * 0.01<p<0.05, ** 0.001<p<0.01, *** p<0.001.*

Response	Intercept	Fixed effect	
OF + OH layer	0.16 (±0.05)*	Forest type (rich)	0.18 (±0.08)*
$\log\left(\frac{P(type = moder)}{P(type = mor)}\right)$	-4.25 (±3.70)	Rich litter influence	15.80 (±13.75)
$\log\left(\frac{P(type = mull)}{P(type = mor)}\right)$	-7.86 (±4.65)	Rich litter influence	20.82 (±14.38)

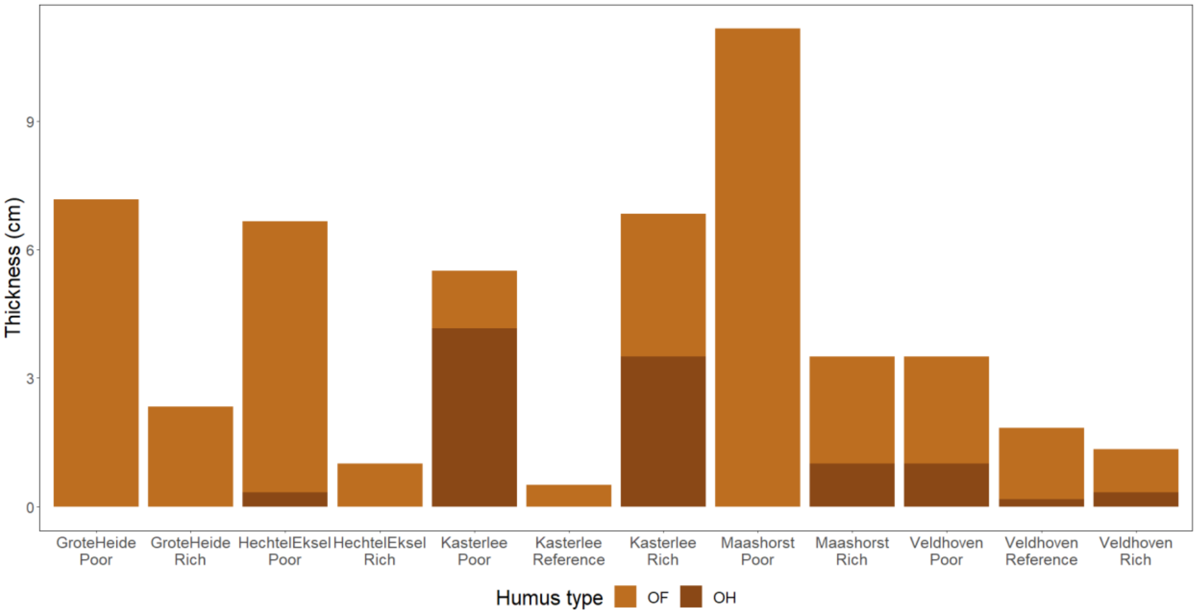


Figure 12 The average thickness (in cm) of the OF and OH humus layer of the different plots.

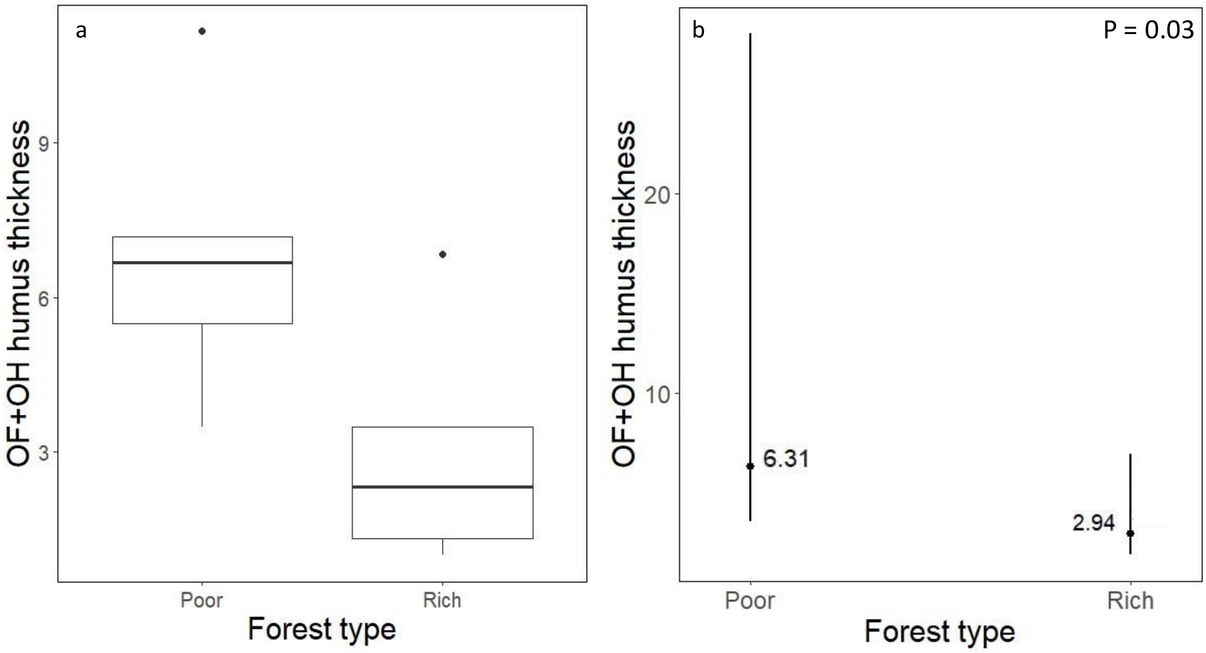


Figure 13 a) boxplot for the average OF + OH humus layer thickness for the 2 different forest types and b) the predicted values of the GLMM with site as a random effect (P = 0.03) (Table 5). The points and numbers indicate the estimated values and the adjoining line represents the 95% confidence intervals (CI poor = [3.55 ; 28.09] and CI rich = [1.87 ; 6.95]).

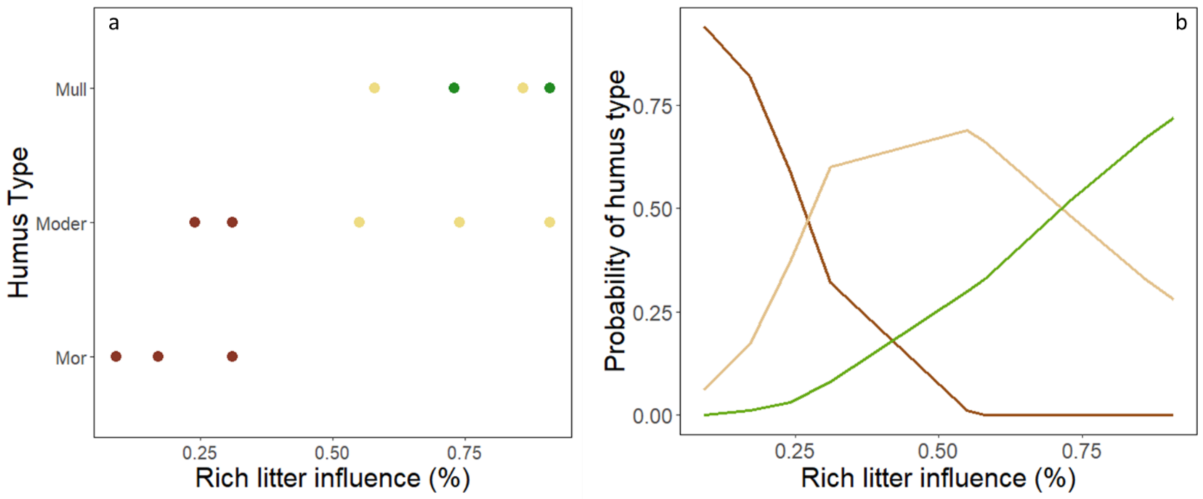


Figure 14 a) Humus type as a functions of rich litter influence. Forest type is indicated in colour: poor in red, rich in green and reference in yellow. b) The probability of occurrence of a humus type in function of rich litter influence (Table 5). Humus type is indicated in colour: mor in brown, moder in beige and mull in green.

4.2 Mesofauna

Based on the count per area, plots with poor litter quality contain more mesofauna organisms than under a rich forest type (Figure 15a). This changes when including the thickness of the OF and OH the humus layer and looking at count per volume (Figure 15b). In the latter case, rich litter quality plots have more individuals present in the humus layer. In general, both plots in Hechtel-Eksel and the reference plots have a lower count while in Grote Heide most individuals are found. Furthermore, the composition of mesofauna orders differs between plots though omnipresent orders are: *Acari, Chilopoda, Coleoptera, Diplopoda, Diptera, Formicidae*, and *Thysanoptera*. A more detailed figure with only the summarized orders can be found in the Appendix (Figure 31).

If the thickness of the humus layer is not considered (count per area), significantly more mesofauna organisms are found in the poor sites ($P < 0.001$) (Figure 16, Table 6). Even when the outlier (Grote Heide) is removed, the mean count in poor forests is still significantly larger than in rich forest types ($P < 0.001$). In case of count per volume, there were slightly more individuals on average in the rich plots and this difference is significant ($P < 0.001$). However, after removing the outlier (Grote Heide) the difference between both means is not significant ($P = 0.95$).

To further analyse the difference between poor and rich forest type, the diversity indices show a higher species richness, evenness and overall diversity in poor forest types (Figure 17, Figure 18). This is the case when count is expressed per unit of area as well as per unit of volume. However, these differences are not significant for species richness and Shannon evenness and marginally significant for the Shannon diversity for count per area ($P_{SR} = 0.21$, $P_{ShD} = 0.08$, and $P_{ShE} = 0.26$). The difference in forest type remains non-significant when removing the outliers for Shannon diversity and evenness (Veldhoven and Hechtel Eksel resp.). The P-values for the indices based on count per volume are analogue and resp. $P_{SR} = 0.56$, $P_{ShD} = 0.06$, and $P_{ShE} = 0.15$. When removing the outlier (Veldhoven), species richness is still not significantly different between forest type.

The amount of mesofauna increases significantly with increasing humus thickness ($P < 0.001$) (Figure 19a). A reverse and significant trend can be seen for the rich litter influence ($P = 0.003$) (Figure 19b). Increasing pH and base saturation seems to have a negative effect on mesofauna count, however these effects are not significant ($P_{pH,O} = 0.25$, $P_{pH,0\text{-}10} = 0.93$, $P_{pH,10\text{-}20} = 0.53$, $P_{BS,0\text{-}10} = 0.17$, $P_{BS,10\text{-}20} = 0.44$). Mesofauna count increases with carbon stock in the organic layer. And although not significant, the direction of the relation with CEC is different depending on the soil depth.

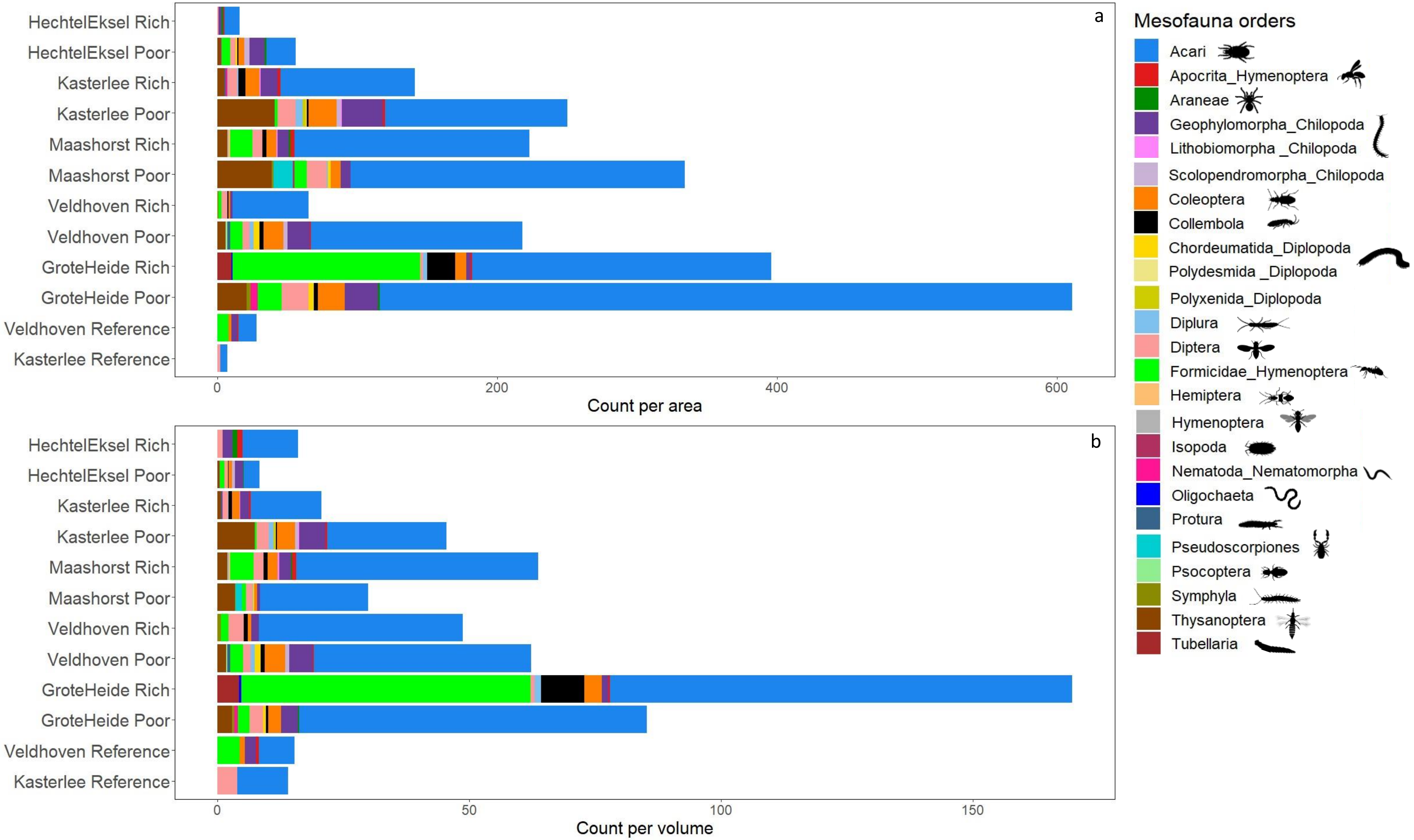


Figure 15 a) count per area and b) count per volume of mesofauna orders collected in each plot.

*Table 6 Estimates, standard error, and P-values of the intercept and fixed effects of the generalised linear mixed models with mesofauna attributes used as a response and site as random effect when forest type is a fixed effect or forest types nested in site as for other fixed effects. CPA: count per area, CPV: count per volume, SR: species richness, ShD: Shannon diversity, ShE: Shannon evenness, FT: forest type. * 0.01<p<0.05, ** 0.001<p<0.01, *** p<0.001.*

Response	**Intercept**	**Fixed effect**	
CPA	5.38 (± 0.39)***	FT (rich)	-0.56 (± 0.04)***
CPV	3.56 (± 0.35)***	FT (rich)	0.32 (± 0.09)***
SR (area)	2.47 (± 0.13)***	FT (rich)	-0.25 (± 0.20)
ShD (area)	0.75 (± 0.08)***	FT (rich)	0.21 (± 0.12)
ShE (area)	0.12 (± 0.23)*	FT (rich)	-0.22 (± 0.20)
SR(volume)	2.05 (± 0.16)***	FT (rich)	-0.14 (± 0.234)
ShD (volume)	0.80 (± 0.07)***	FT (rich)	0.19 (± 0.10)
ShE (volume)	0.60 (± 0.30)*	FT (rich)	-0.46 (± 0.32)
CPA	3.18 (± 0.36)***	OF + OH layer	0.07 (± 0.02)***
CPA	5.98 (± 0.63)***	Rich litter influence	-2.39 (± 0.81)**
CPA	6.68 (± 1.84)***	pH O layer	-0.46 (± 0.40)
CPA	5.12 (± 4.73)	pH 0-10 cm	-0.09 (± 1.00)
CPA	6.62 (± 3.15)*	pH 10-20 cm	-0.41 (± 0.65)
CPA	3.77 (± 0.62)***	C stock O layer	0.01 (± 0.004)*
CPA	4.18 (± 1.09)***	C stock 0-10 cm	0.02 (± 0.04)
CPA	4.19 (± 0.87)***	C stock 10-20 cm	0.02 (± 0.03)
CPA	4.92 (± 0.70)***	CEC 0-10 cm	-0.04 (± 0.08)
CPA	4.24 (± 0.78)***	CEC 10-20 cm	0.09 (± 0.66)
CPA	5.69 (± 0.56)***	BS 0-10 cm	-0.02 (± 0.02)
CPA	5.06 (± 0.64)***	BS 10-20 cm	-0.03 (± 0.03)

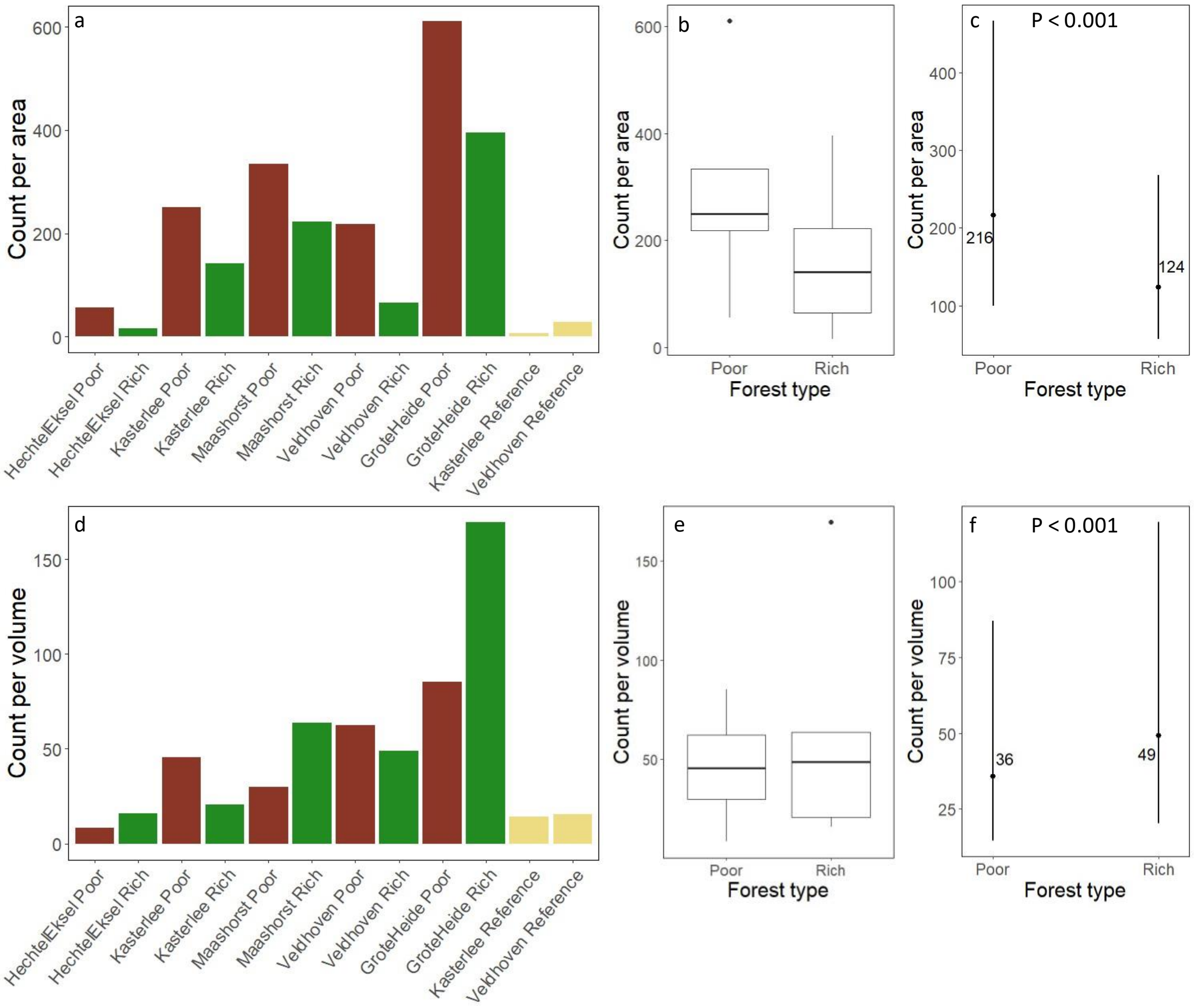


Figure 16 Overview of the total mesofauna count per area and per volume. The colours indicate the forest type with red for poor, green for rich, and yellow for reference types. The boxplots indicate possible outliers for the poor and rich datapoints. e) and f) show the predicted values of GLMM's to evaluate forest type as an significant effect for both count per area as count per volume (Table 6) ($P < 0.001$). The points and numbers indicates the estimated values and the adjoining line represents the 95% confidence intervals ($CI_{Area,\ Poor}$ = [100 ; 467], $CI_{Area,\ Rich}$ = [57 ; 268], $CI_{Volume,\ Poor}$ = [18 ; 69], $CI_{Volume,\ Rich}$ = [25 ; 95]).

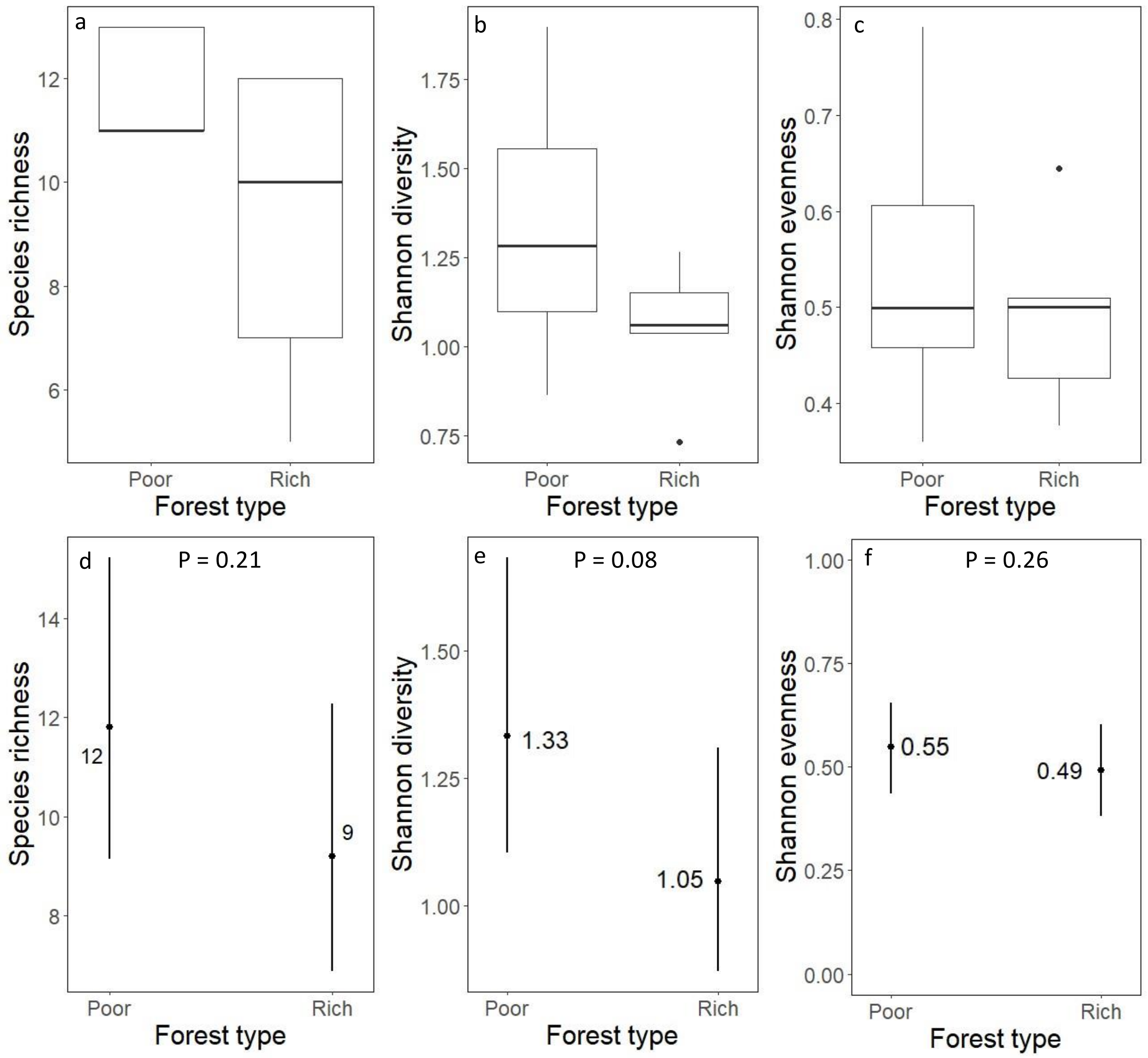


Figure 17 Boxplots of the species richness, Shannon diversity, and Shannon evenness of mesofauna count per area for poor and rich forest type. Based on GLMM's with forest type as fixed effect, predicted values were estimated for each of the indices (Table 6). P-values of the fixed effect are in the top middle. The points and numbers indicates the estimated values and the adjoining line represents the 95% confidence interval ($CI_{SR, Poor}$ = [9 ; 15], $CI_{SR, Rich}$ = [7 ; 12], $CI_{ShD, Poor}$ = [1.10 ; 1.68], $CI_{ShD, Rich}$ = [0.87 ; 1.31], $CI_{ShE, Poor}$ = [0.44 ; 0.65], $CI_{ShE, Rich}$ = [0.39 ; 0.60]).

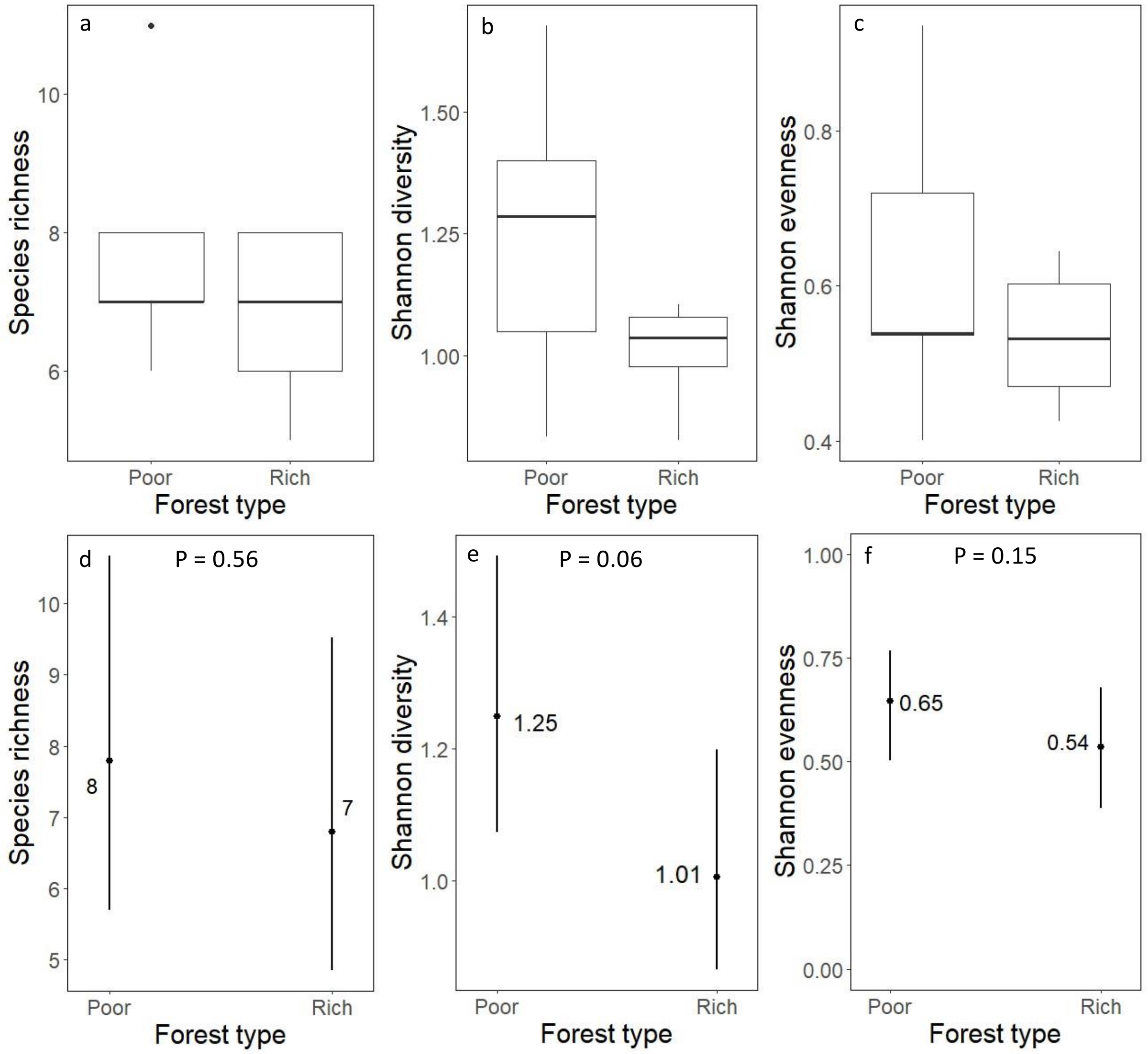


Figure 18 Boxplots of the species richness, Shannon diversity, and Shannon evenness of mesofauna count per volume for poor and rich forest type. Based on GLMM's with forest type as fixed effect, predicted values were estimated for each of the indices (Table 6). P-values of the fixed effect are in the top middle. The points and numbers indicates the estimated values and the adjoining line represents the 95% confidence interval ($CI_{SR,\ Poor}$ *= [6 ; 11],* $CI_{SR,\ Rich}$ *= [4.86 ; 9.52],* $CI_{ShD,\ Poor}$ *= [1.07 ; 1.49],* $CI_{ShD,\ Rich}$ *= [0.87 ; 1.20],* $CI_{ShE,\ Poor}$ *= [0.50 ; 0.77],* $CI_{ShE,\ Rich}$ *= [0.39 ; 0.68]).*

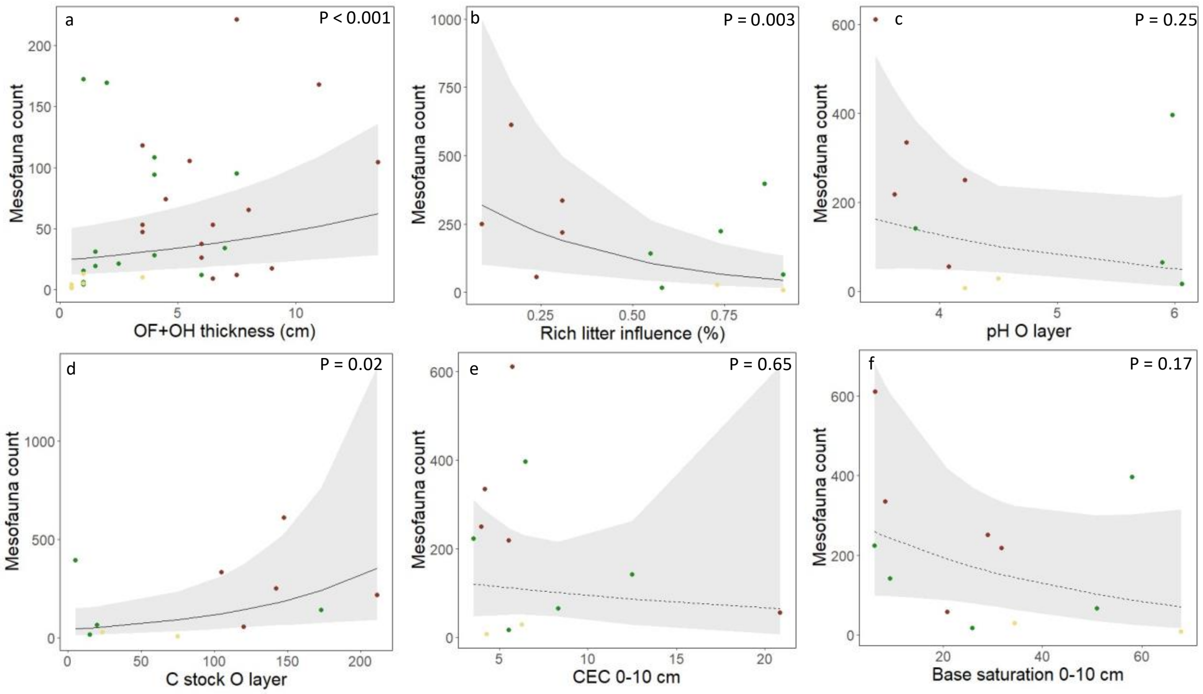


Figure 19 Mesofauna count per area as a function of a) OF+OH humus thickness (cm), b) rich litter influence (%), c) pH of the organic layer, d) carbon stock of the organic layer (ton/ha), e) CEC of 0-10 cm mineral soil (cmol_c/kg), and f) base saturation of 0-10 cm mineral soil (%). Forest type is marked by colour: red for poor, green for rich, and yellow for reference. Full lines represent a significant relation while dashed lines indicate a non-significant effect. The grey region corresponds with the 95% confidence interval. P-values of the independent variables in the upper right corner are determined in GLMM's with the independent variables as fixed effect and an additional random nested effect where forest type is nested in site (Table 6).

4.3 Earthworms

In general rich plots contain more earthworms, both in terms of biomass and count, than the poor plots (Figure 20). For biomass specifically, species from the Lumbricus genus are dominant. The largest biomass of earthworms is found in the reference plot in Kasterlee. Based on the amount of individuals in the plots, the rich plot in Hechtel Eksel has the most worms, with smaller species such as from the Dendrobaena genus becoming more dominant in numbers. Forest type is not a significant driver of earthworm biomass (Figure 21, Table 7). However, the effect becomes significant if the outlier (Hechtel Eksel Poor) is removed. The model for count indicates that forest type is a significant effect, with and without outliers (Hechtel Eksel). Here, rich litter quality increases the earthworm count.

Next, the earthworm species are divided according to their ecological categories (Figure 22). Epi-anecic species are most dominant based on biomass while for count Epigeic species are most abundant. The amount of earthworms is significantly different for all categories, with or without outliers, except for Endogeic species (Figure 23, Table 7). Plots with rich litter species contain more worms of each category. Based on weight, the difference in mean per forest type is not significant with or without outlier. The biomass of Endogeic, Epi-anecic, and juvenile species is a larger in poor plots while the reverse is true for Epigeic species. Two exceptions are Endogeic and Epi-anecic species. Forest type becomes a significant fixed effect when removing outliers ($P < 0.001$). Only a few individuals are found in two plots, hence the models for Endogeic species are not very representative. This can be a possible explanation for the conversion problems which made it not possible to provide P-values.

Forest type has a significant effect on earthworms species richness and Shannon diversity (resp. $P = 0.009$ and $P = 0.002$) (Figure 24, Table 7). We hypothesize that this will also be the case for the Shannon evenness if the outlier (Hechtel Eksel Poor) is removed. However, due to model convergence problems, there is no P-value of the model without the outlier. For all indices the models estimate a higher diversity of earthworms in the rich plots.

The earthworm biomass has a non-significant, negative effect on the thickness of the humus layer ($P = 0.01$) (Figure 25, Table 7). Rich litter has a significant influence on earthworm biomass ($P = 0.04$). pH and CEC show a weak positive effect on earthworm biomass, however this trend is not significant ($P_{pH,O} = 0.39$, $P_{pH,10\text{-}20} = 0.72$, $P_{CEC,0\text{-}10} = 0.24$, $P_{CEC,10\text{-}20} = 0.36$). C stock in the organic layer and topsoil (0-10 cm) has a weak negative, non-significant influence on earthworm biomass ($P_{C,O} = 0.27$, $P_{C,0\text{-}10} = 0.71$).

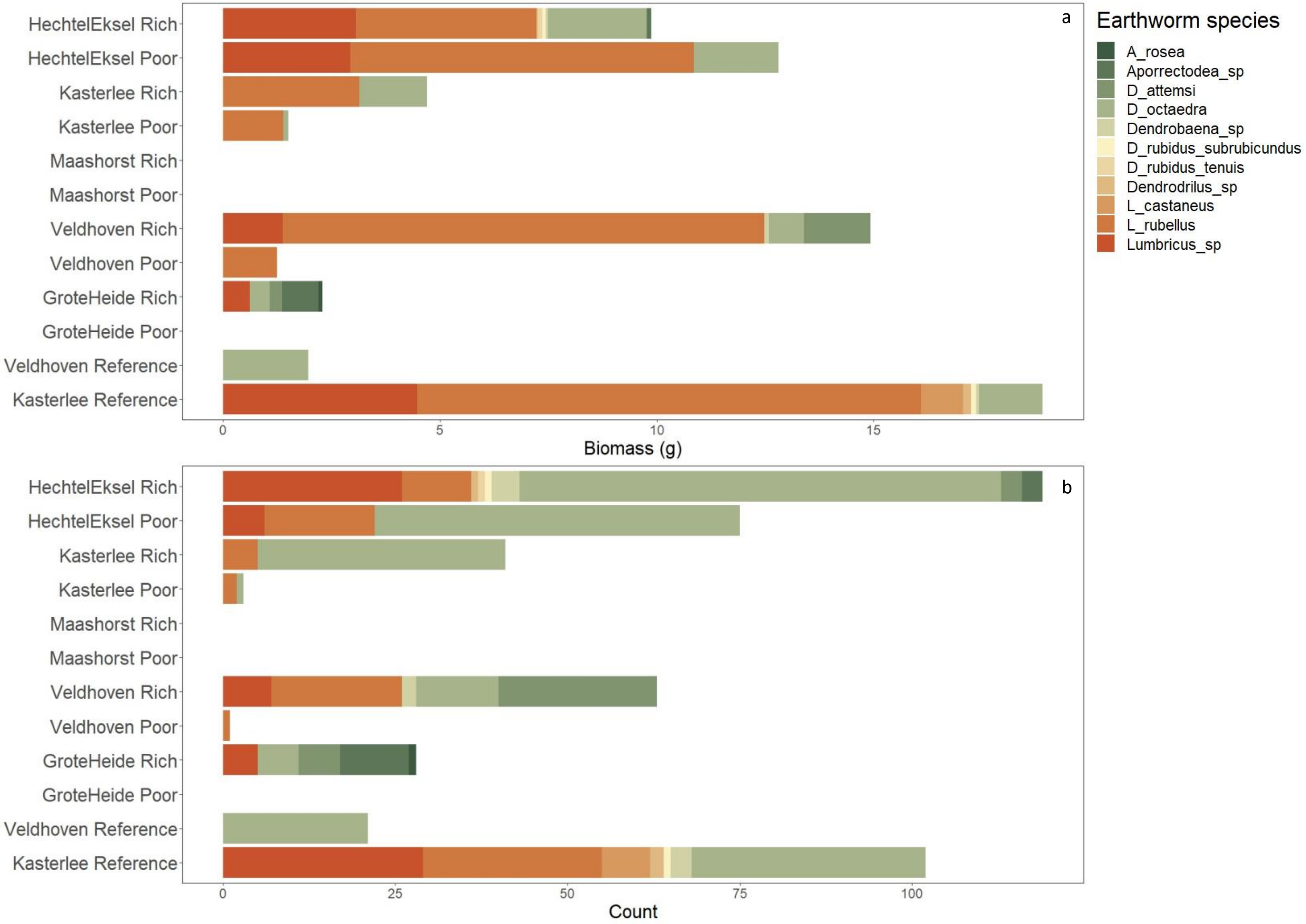


Figure 20 a) Biomass (g) and b) count of the earthworm species collected in each plot. If not possible to identify to species level, earthworms were assorted to genus level.

*Table 7 Estimates, standard error, and P-values of the intercept and fixed effects of the generalised linear mixed models with earthworm attributes as a response and site as random effect when forest type is a fixed effect or forest type nested in site as for other fixed effects. SR: species richness, ShD: Shannon diversity, ShE: Shannon evenness, FT = forest type. * 0.01<p<0.05, ** 0.001<p<0.01, *** p<0.001.*

Response	Intercept	Fixed effect	
Biomass	1.65 (± 0.48)***	FT(rich)	0.42 (± 0.64)
Count	1.84 (± 0.95)	FT(rich)	1.07 (± 0.12)***
Endogeic biomass	-0.66 (± NaN)	FT(rich)	-0.66 (± NaN)
Epigeic biomass	-0.95 (± 0.42)*	FT(rich)	0.47 (± 0.39)
Epi-anecic biomass	0.79 (± 0.31)*	FT(rich)	-0.16 (± 0.31)
Juvenile biomass	-1.22 (± 0.84)	FT(rich)	-0.02 (± 0.41)
Endogeic count	-24.70 (± 24140)	FT(rich)	22.92 (± 24140)
Epigeic count	-0.76 (± 1.19)	FT(rich)	2.54 (± 0.71)***
Epi-anecic count	-1.12 (± 1.25)	FT(rich)	0.58 (± 0.29)*
Juvenile count	-1.78 (± 1.01)	FT(rich)	1.89 (± 0.66)**
SR biomass	0.01 (± 0.52)	FT(rich)	1.20 (± 0.47)*
ShD biomass	-0.58 (± 0.25)*	FT(rich)	0.59 (± 0.19)**
ShE biomass	0.49 (± 0.56)	FT(rich)	0.52 (± 0.70)
OF+OH layer	1.46 (± 0.24)***	Biomass	-0.17 (± 0.07)*
Biomass	0.22 (± 0.83)	Rich litter influence	2.26 (± 1.11)*
Biomass	0.16 (± 1.67)	pH O layer	0.30 (± 0.35)
Biomass	0.98 (± NaN)	pH 0-10 cm	0.13 (± NaN)
Biomass	0.58 (± 2.75)	pH 10-20 cm	0.21 (± 0.57)
Biomass	2.18 (± 0.68)**	C stock O layer	-0.01 (± 0.01)
Biomass	2.04 (± 1.03)*	C stock 0-10 cm	-0.01 (± 0.04)
Biomass	1.56 (± 0.90)	C stock 10-20 cm	0.01 (± 0.03)
Biomass	0.99 (± 0.60)	CEC 0-10 cm	0.07 (± 0.06)
Biomass	1.18 (± 0.49)*	CEC 10-20 cm	0.08 (± 0.09)
Biomass	1.45 (± NaN)	BS 0-10 cm	0.02 (± NaN)
Biomass	2.71 (± NaN)	BS 10-20 cm	-0.04 (± NaN)

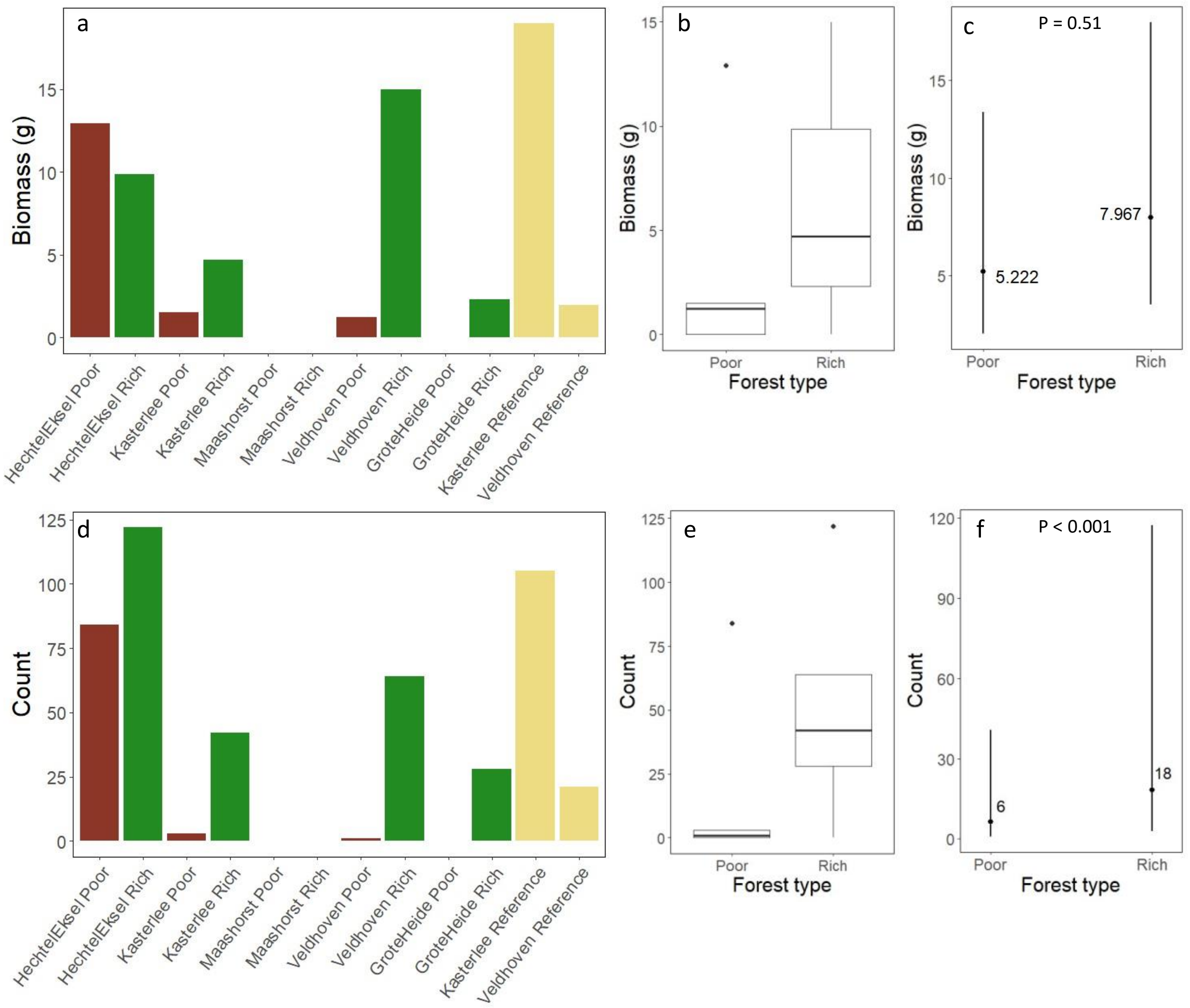


Figure 21 a) and d) barcharts of the total biomass (g) and count of earthworms for each plot. The colour or the bars indicates forest type: poor (red), rich (green), and reference (yellow). The boxplots indicate possible outliers for the poor and rich datapoints (b and e). c) and f) show the predicted values of GLMM's to evaluate forest type as an significant effect for both biomass as count (Table 7) (P = 0.50 and P < 0.001 resp.). The points and numbers indicates the estimate values and the adjoining line represents the 95% confidence intervals ($CI_{Biomass,\ Poor}$ = [2.04 ; 13.38], $CI_{Biomass,\ Rich}$ = [3.53 ; 17.99], $CI_{Count,\ Rich}$ = [1 ; 41], $CI_{Count,\ Poor}$ = [3 ; 117]).

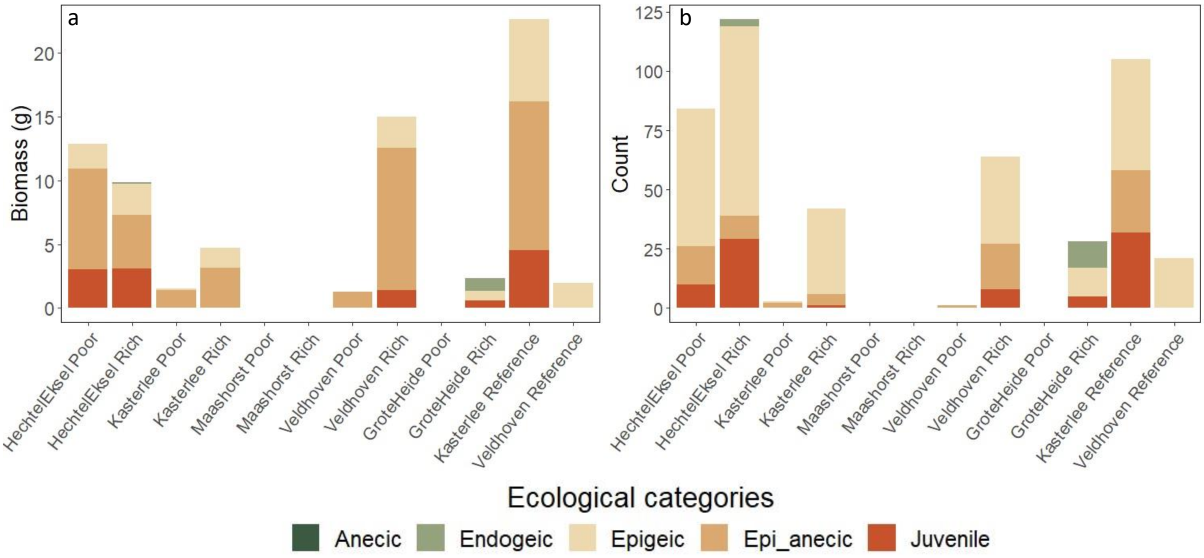


Figure 22 a) biomass (g) and b) amount of the ecological earthworm categories in each plot.

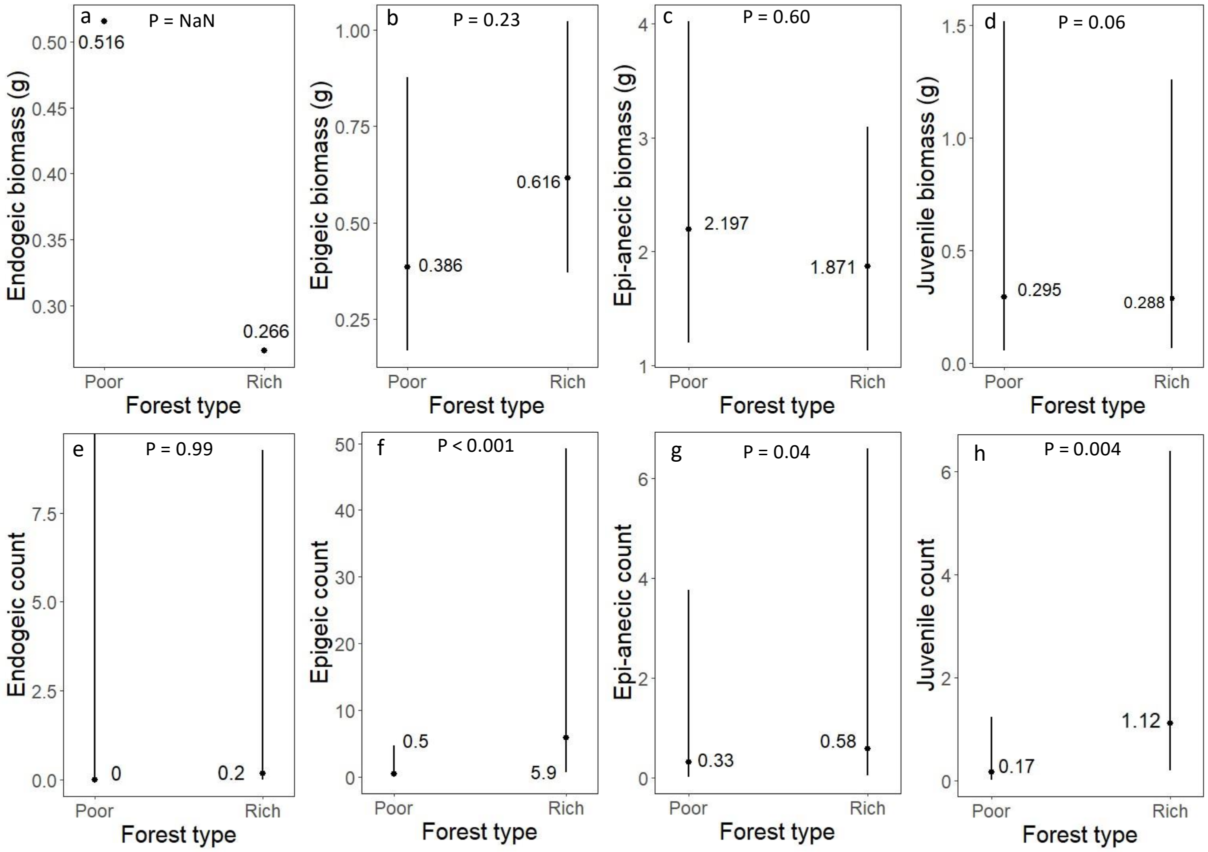


Figure 23 GLMM estimates for the mean and 95% CI of poor and rich forest type for each of the ecological earthworm categories (Table 7). The top row are the models considering biomass while the bottom row shows the models estimating count. P-value in the top middle indicates of the fixed effect NaN indicates convergence problems of the GLMM and p-values an CI could not be calculated. The points and numbers indicates the estimated values and the adjoining line represents the 95% confidence interval: $CI_{Biomass, Epi, Poor}$ = [0.17 ; 0.88], $CI_{Biomass, Epi, Rich}$ = [0.37 ; 1.02], $CI_{Biomass, Epi-an, Poor}$ = [1.20 ; 4.03], $CI_{Biomass, Epi-an, Rich}$ = [1.13 ; 3.09], $CI_{Biomass, Juv, Poor}$ = [0.06 ; 1.52], $CI_{Biomass, Juv, Rich}$ = [0.07 ; 1.26], $CI_{Count, Endo, Poor}$ = [0.00 ; inf], $CI_{Count, Endo, Rich}$ = [0.00 ; 9.27], $CI_{Count, Epi, Poor}$ = [0.05 ; 4.76], $CI_{Count, Epi, Rich}$ = [0.71 ;

49.30], CI$_{Count, Epi\text{-}an, Poor}$ = [0.03 ; 3.76], CI$_{Count, Epi\text{-}an, Rich}$ = [0.05 ; 6.60], CI$_{Count, Juv, Poor}$ = [0.02 ; 1.23], CI$_{Count, Juv, Rich}$ = [0.20 ; 6.41].

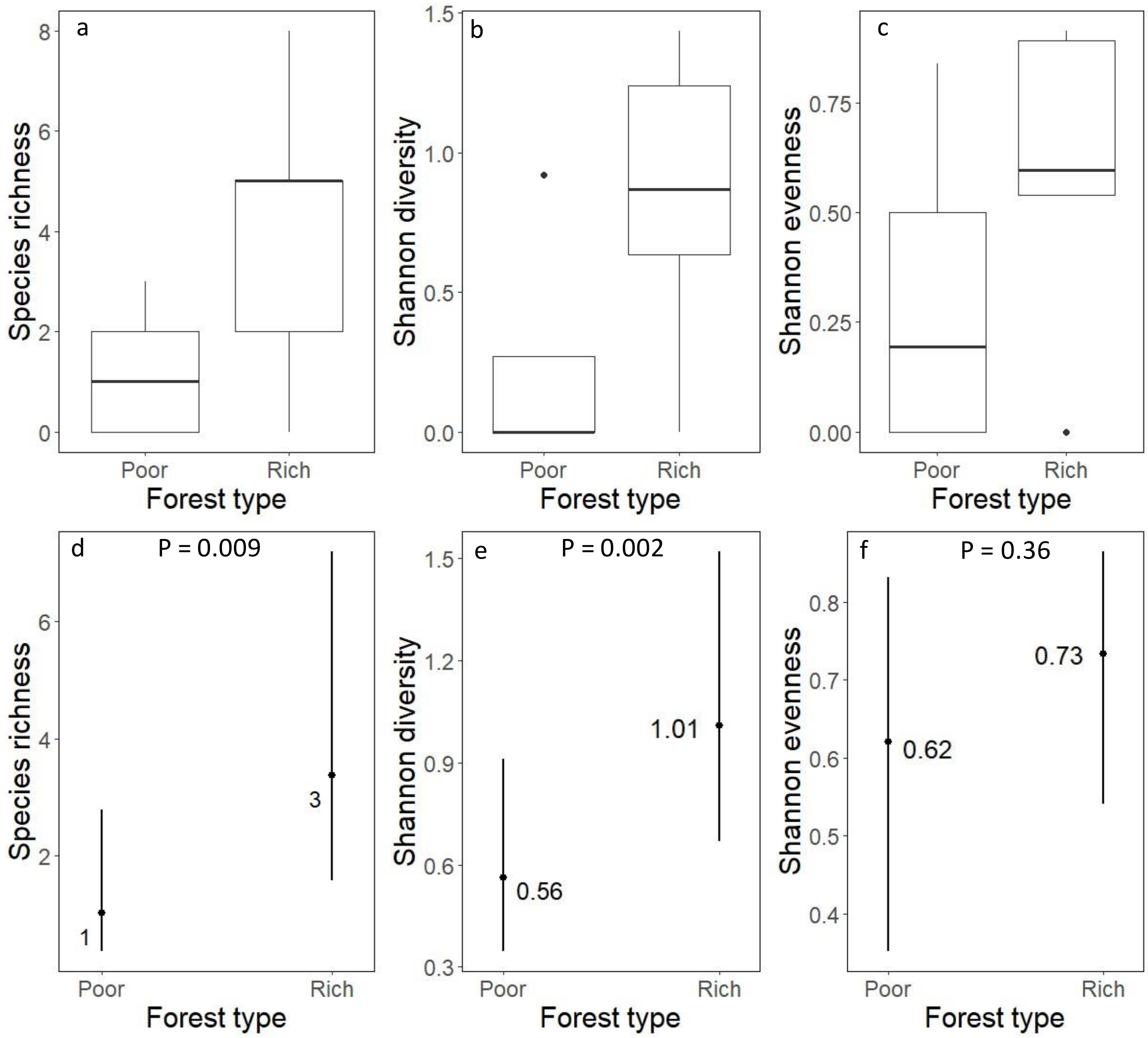


Figure 24 Boxplots of the species richness, Shannon diversity, and Shannon evenness of earthworm biomass for rich and poor forest type. Based on GLMM's with forest type as fixed effect, predicted values were estimated for each of the indices (Table 7). P-values of the fixed effect are in the top middle, NaN indicates convergence problems of the GLMM and p-values could not be calculated. The points and numbers indicates the estimated values and the adjoining line represents the 95% confidence interval (CI$_{SR, Poor}$ = [0 ; 3, CI$_{SR, Rich}$ = [2; 7], CI$_{ShD, Poor}$ = [0.35 ; 0.91], CI$_{ShD, Rich}$ = [0.67 ; 1.52], CI$_{ShE, Poor}$ = [0.35 ; 0835], CI$_{ShE, Rich}$ = [0.54 ; 0.87]).

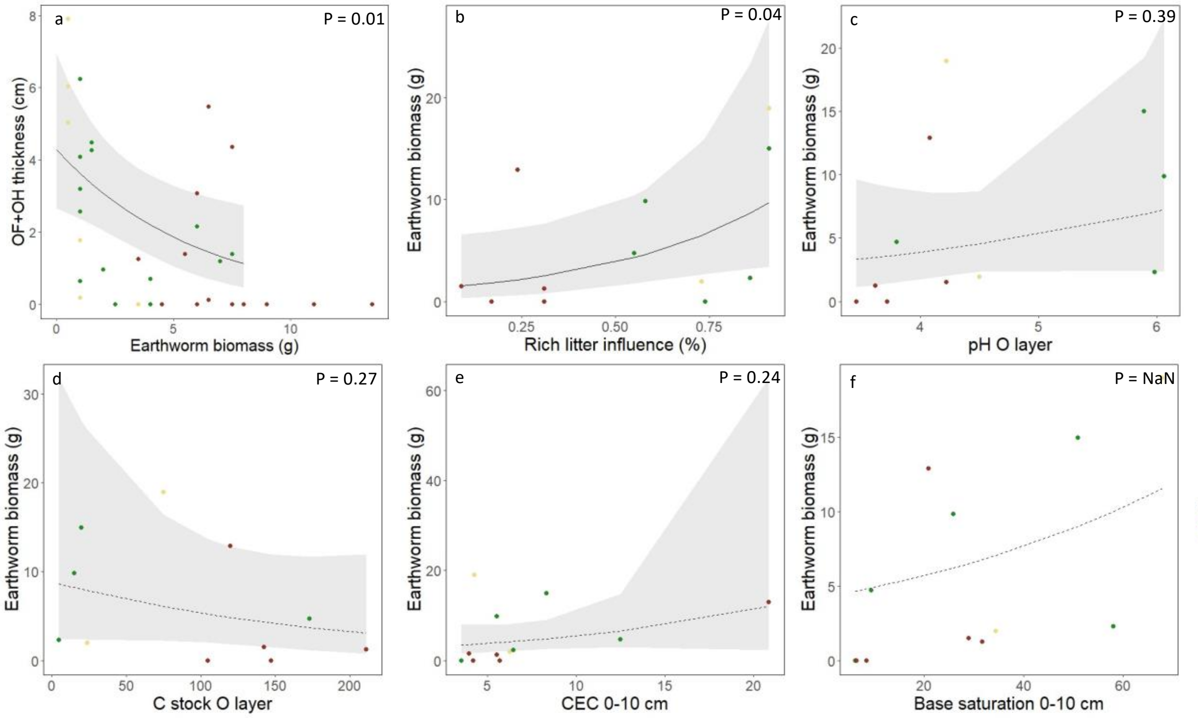


Figure 25: a) The influence of earthworm biomass (g) on thickness of the humus layer. Earthworm biomass (g) as a function of b) rich litter influence (%), c) pH of the organic layer, d) carbon stock of the organic layer (ton/ha), e) CEC of 0-10 cm mineral soil (cmol$_c$/kg), and f) base saturation of 0-10 cm mineral soil (%). Forest type is marked by colour: red for poor, green for rich, and yellow for reference. Full lines represent a significant relation while dashed lines indicate a non-significant effect. The grey region corresponds with the 95% confidence interval. P-values of the independent variables in the upper right corner are determined in GLMM's with the independent variables as fixed effect and an additional random nested effect where forest type is nested in site (Table 7). NaN P-values indicates GLMM's with convergence that could not provide P-values and CI.

4.4 Feeding patterns and functional traits

4.4.1 Feeding patterns

For both forest types and units, there is an even distribution of the different feeding strategies with subtle differences between the different strategies (Figure 26). Predation is overall the most present feeding strategy. For the amount of organisms per unit of area, more are found in the poor plots (Figure 26a and b). The reverse is seen when including the thickness of the humus layer (unit volume) (Figure 26c and d). This contradiction is also present in the GLMM's to compare the difference between poor and rich forest types for the individual feeding strategies (Figure 27, Table 8). For all feeding strategies, forest type is a significant effect.

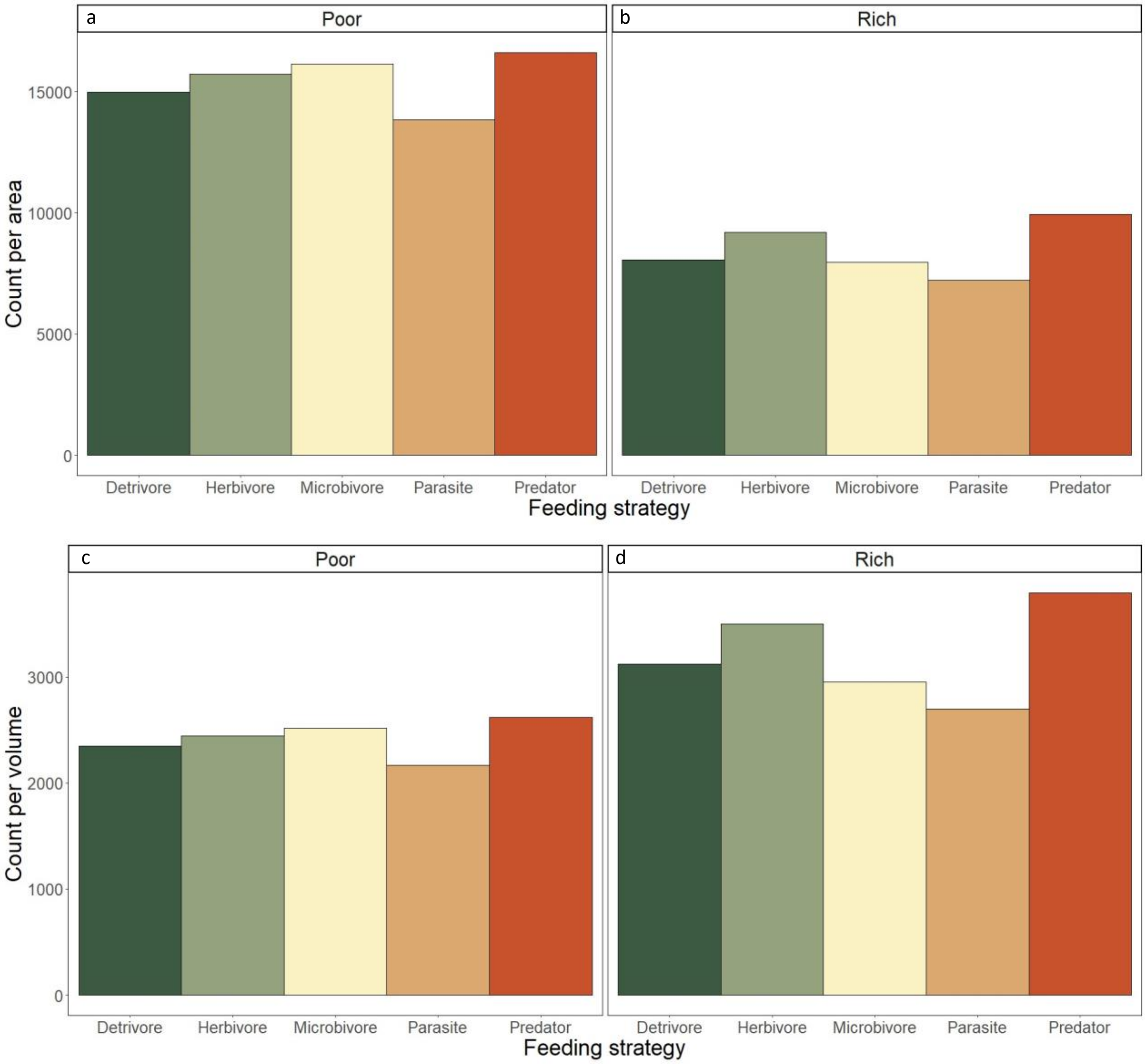


Figure 26 a) and b) count per unit of area of the different feeding strategies of the soil fauna. a) represent the poor plot data and b) the rich plots. This is analogous for d) and f) but count per volume.

*Table 8 Estimates, standard error, and P-values of the intercept and fixed effects of the generalised linear mixed models with feeding strategy count as a response and site as random effect. CPA: count per area, CPV: count per volume, FT: forest type. * 0.01<p<0.05, ** 0.001<p<0.01, *** p<0.001.*

Response	Intercept	Fixed effect	
Detrivore CPA	6.61 (± 0.38)***	FT (rich)	-0.62 (± 0.01)***
Detrivore CPV	4.86 (± 0.29)***	FT (rich)	0.28 (± 0.03)***
Herbivore CPA	6.57 (± 0.45)***	FT (rich)	-0.5 (± 0.01)***
Herbivore CPV	4.76 (± 0.39)***	FT (rich)	0.36 (± 0.03)***
Microbivore CPA	6.63 (± 0.44)***	FT (rich)	-0.71 (± 0.01)***
Microbivore CPV	4.86 (± 0.35)***	FT (rich)	0.16 (± 0.03)***
Parasite CPA	6.46 (± 0.45)***	FT (rich)	-0.65 (± 0.02)***
Parasite CPV	4.70 (± 0.36)***	FT (rich)	0.22 (± 0.03)***
Predator CPA	6.69 (± 0.39)***	FT (rich)	-0.52 (± 0.03)***
Predator CPV	4.89 (± 0.34)***	FT (rich)	0.37 (± 0.03)***

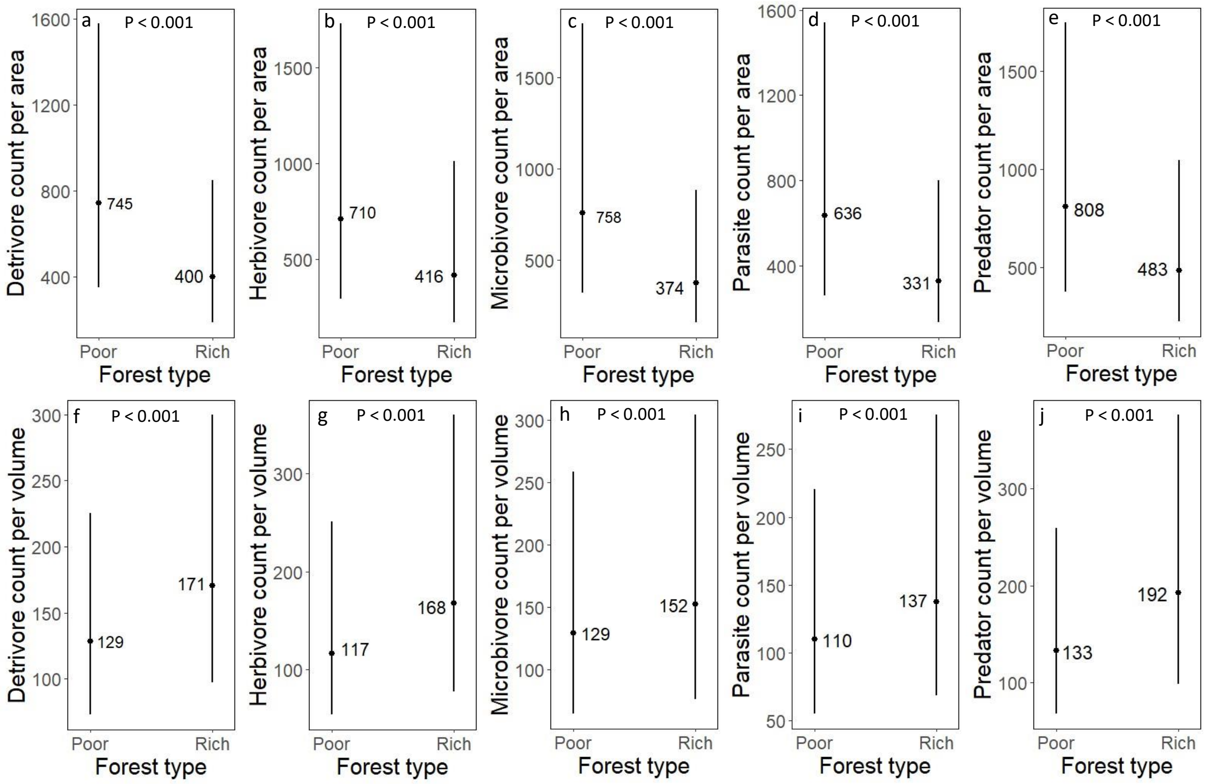


Figure 27 GLMM estimates for the mean and 95% CI of poor and rich forest type for each of the feeding strategies (Table 8). The top row are the models considering count per unit of area while the bottom row shows the models estimating per unit of volume. In the top middle indicates the P-value of the fixed effect. The points and numbers indicates the estimated values and the adjoining line represents the 95% confidence intervals ($CI_{Detrivore, Area, Poor}$ = [351 ; 1578], $CI_{Detrivore, Area, Rich}$ = [189 ; 849], $CI_{Herbivore, Area, Poor}$ = [292 ; 1730], $CI_{Herbivore, Area, Rich}$ = [171 ; 1012], $CI_{Microbivore, Area, Poor}$ = [321 ; 1794], $CI_{Microbivore, Area, Rich}$ = [158 ; 884], $CI_{Parasite, Area, Poor}$ = [263 ; 1540], $CI_{Parasite, Area, Rich}$ = [137 ; 802], $CI_{Predator, Area, Poor}$ = [374 ; 1747], $CI_{Predator, Area, Rich}$ = [223 ; 1044], $CI_{Detrivore, Volume, Poor}$ = [73 ; 226], $CI_{Detrivore, Volume, Rich}$ = [97 ; 300], $CI_{Herbivore, Volume, Poor}$ = [54 ; 251], $CI_{Herbivore, Volume, Rich}$ = [78 ; 360], $CI_{Microbivore, Volume, Poor}$ = [65 ; 258], $CI_{Microbivore, Volume, Rich}$ = [76 ; 304], $CI_{Parasite, Volume, Poor}$ = [55 ; 221], $CI_{Parasite, Volume, Rich}$ = [69 ; 275], $CI_{Predator, Volume, Poor}$ = [68 ; 259], $CI_{Predator, Volume, Rich}$ = [98 ; 376]).

4.4.2 Functional traits

The amount of organisms aiding with population regulation and litter fragmentation is clearly larger than for bioturbation and gut passage (Figure 28). For the two dominating functions, there is again a difference between area and volume. Mesofauna count per area is higher for population regulation and litter fragmentation in poor plots while the reverse is observed for unit per volume. This is mirrored in the GLMM's with the different soil functions as response and forest type as a significant fixed effect (Figure 29, Table 9). Bioturbation and gut passage are carried out more under rich forest type (Figure 28). The differences in mean between forest type is not significant for bioturbation and gut passage except when looking at the amount of mesofauna per volume contributing to gut passage. Litter fragmentation and population regulation are significantly different between rich and poor plots.

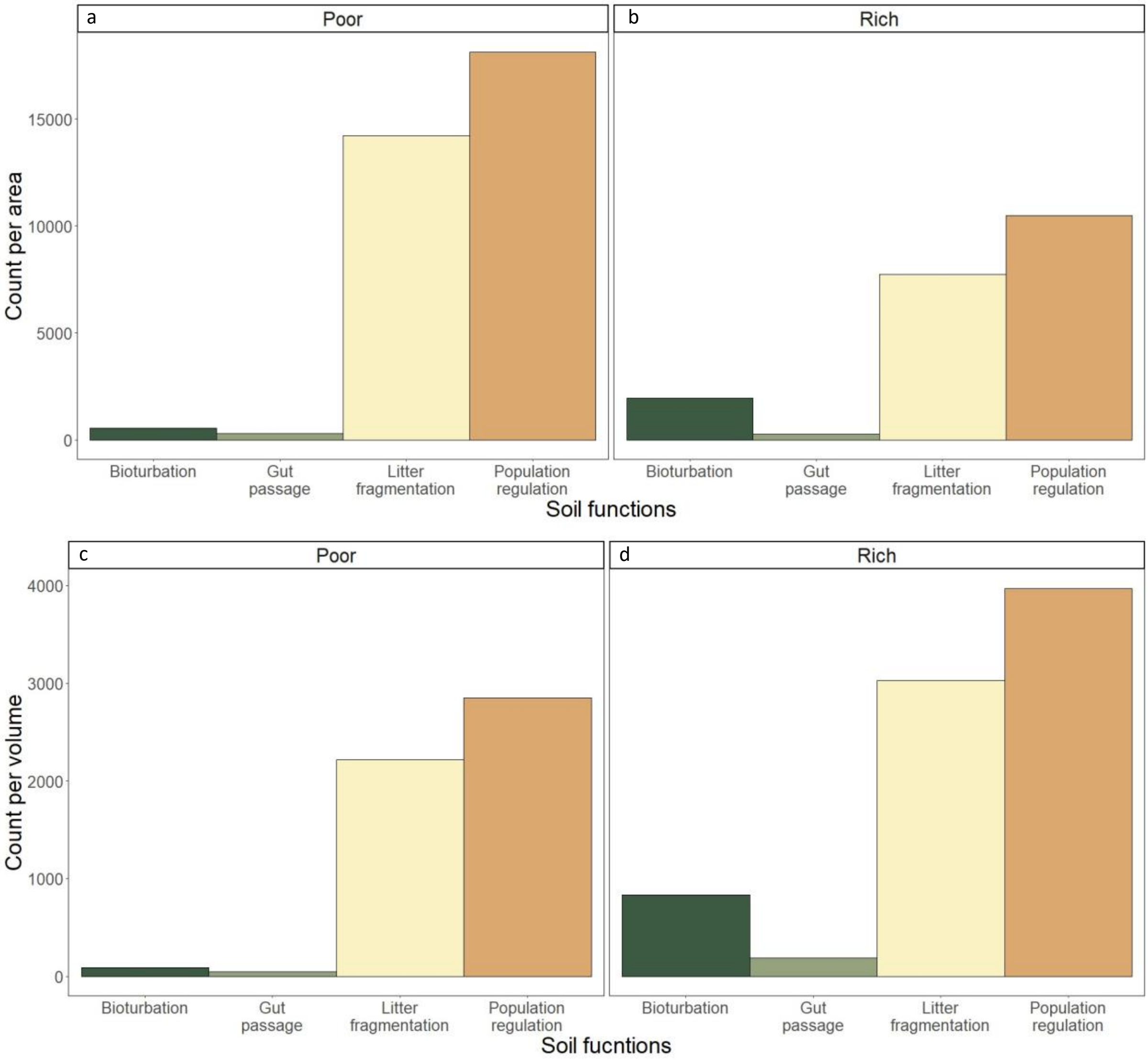


Figure 28 a) and b) count per unit of area of the different soil functions of the soil fauna. a) represent the poor plot data and b) the rich plots. This is analogous for d) and f) but count per volume.

*Table 9 Estimates, standard error, and P-values of the intercept and fixed effects of the generalised linear mixed models with the amount of mesofauna performing soil functions as a response and site as random effect. CPA: count per area, CPV: count per volume, FT: forest type. * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$.*

Response	**Intercept**	**Fixed effect**	
Bioturbation CPA	3.71 (± 0.68)***	FT (rich)	0.04 (± 0.78)
Bioturbation CPV	1.73 (± 0.71)*	FT (rich)	1.13 (± 0.71)
Gut passage CPA	2.95 (± 0.36)***	FT (rich)	-0.20 (± 0.50)
Gut passage CPV	0.31 (± 0.61)	FT (rich)	1.48 (± 0.17)***
Litter fragmentation CPA	6.55 (± 0.39)***	FT (rich)	-0.61 (± 0.01)***
Litter fragmentation CPV	4.79 (± 0.29)***	FT (rich)	0.31 (± 0.03)***
Population regulation CPA	6.79 (± 0.40)***	FT (rich)	-0.55 (± 0.01)***
Population regulation CPV	4.98 (± 0.34)***	FT (rich)	0.33 (± 0.03)***

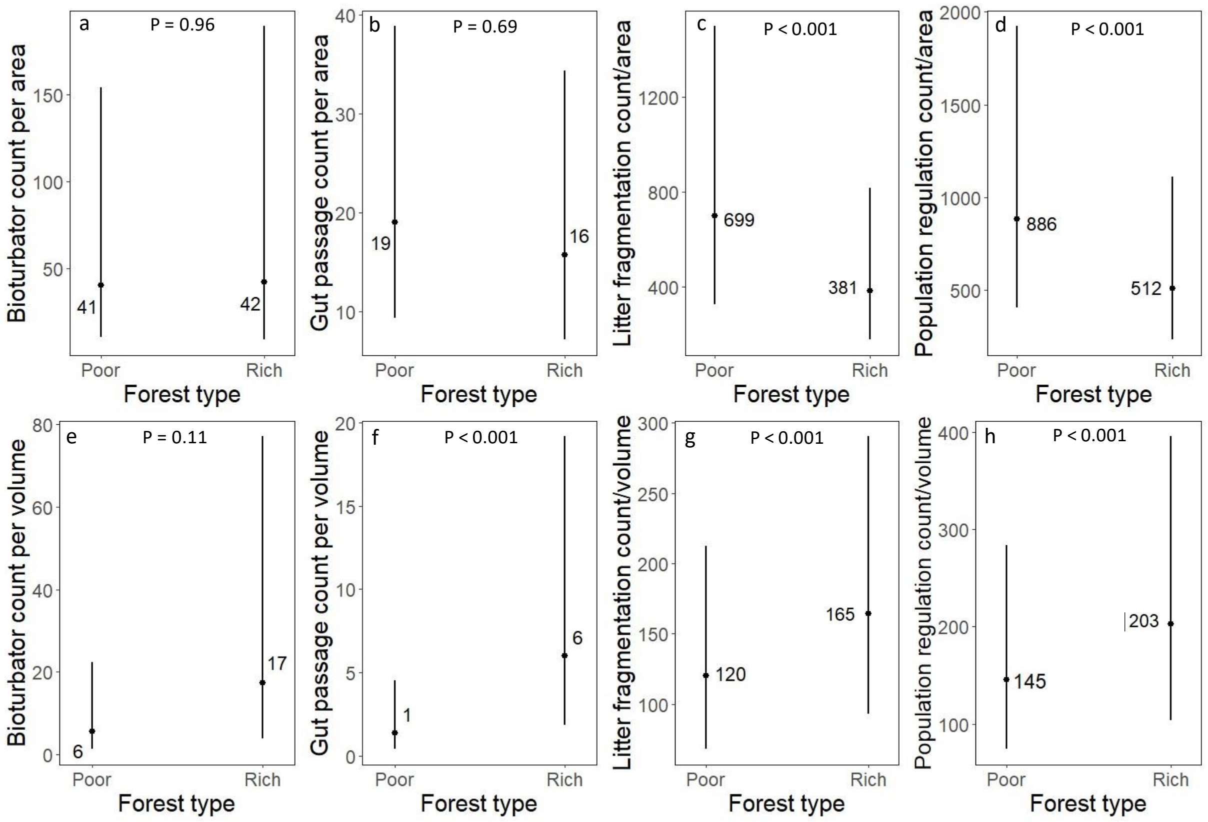


Figure 29 GLMM estimates for the mean and 95% CI of poor and rich forest type for each of the soil functions (Table 9). The top row are the models considering count per unit of area while the bottom row shows the models estimating per unit of volume. In the top middle indicates the P-value of the fixed effect. The points and numbers indicates the estimated values and the adjoining line represents the 95% confidence intervals ($CI_{\text{Bioturbation, Area, Poor}}$ = [11 ; 154], $CI_{\text{Bioturbation, Area, Rich}}$ = [10 ; 189], $CI_{\text{Gut passage, Area, Poor}}$ = [9 ; 39], $CI_{\text{Gut passage, Area, Rich}}$ = [7 ; 34], $CI_{\text{Litter fragmentation, Area, Poor}}$ = [326 ; 1497], $CI_{\text{Litter fragmentation, Area, Rich}}$ = [178 ; 816], $CI_{\text{Population regulation, Area, Poor}}$ = [408 ; 1922], $CI_{\text{Population regulation, Area, Rich}}$ = [236 ; 1110], $CI_{\text{Bioturbation, Volume, Poor}}$ = [1 ; 22], $CI_{\text{Bioturbation, Volume, Rich}}$ = [4 ; 77], $CI_{\text{Gut passage, Volume, Poor}}$ = [0 ; 5], $CI_{\text{Gut passage, Volume, Rich}}$ = [2 ; 19], $CI_{\text{Litter fragmentation, Volume, Poor}}$ = [68 ; 212], $CI_{\text{Litter fragmentation, Volume, Rich}}$ = [93 ; 290], $CI_{\text{Population regulation, Volume, Poor}}$ = [74 ; 284], $CI_{\text{Population regulation, Volume, Rich}}$ = [104 ; 396]).

5 Discussion

For this research the goal is to assess the state of the soil biodiversity in forests characterized by degraded soils due to land use history, monotonous tree species choice and acidifying deposition. According to literature, soil acidification negatively influences the soil community which is key for ecosystem functioning. As science has become more aware of the importance of soil biodiversity, the next step is to look for measures to stop further soil degradation and even restore it. Therefore our second goal is to evaluate the implementation of rich litter species as a measures to counteract soil acidifications.

5.1 Status of soil biodiversity

In temperate broadleaved forests decomposers have a dominant role as large amounts of organic material are recycled. This group is accompanied by a diverse range of mesofauna species to accelerate these decomposition processes (Orgiazzi et al., 2016). In our study, the five most counted orders in decreasing amount are: *Acari, Formicidae, Geophylomorpha, Thysanoptera,* and *Coleoptera* (Figure 15). Note that *Acari* are considered as a subclass in modern classifications, given the amount and diversity of the species that are included (Tilling, 2014). The fact that the subclass has the largest count in multiple plots can be related to the large diversity. For *Acari*, it is not possible to identify the captured mesofauna to order level due to the lack of expertise.

Mites (*Acari*) and springtails (*Collembola*) represent the majority of mesofauna in soils (up to 80%) (Jeffery et al., 2010; Menta, 2012). However, *Collembola* individuals were not found in all of our samples and the quantity is less than expected. Moreover, for most mesofauna orders, individuals were only observed in some plots with overall low numbers (<10). Nevertheless, forest soils can contain up to 140,000 mesofauna individuals per m², which is far more than we collected (Jeffery et al., 2010). Other research found more arthropods in their samples as well (Nielsen et al., 2015; Tajik et al., 2019). Therefore the overall soil biodiversity of mesofauna in our study region is low.

Furthermore, the two most occurring earthworms species are *Lumbricus rubellus* and *Dendrobaena octaedra* (Figure 20). In addition, five other species were found. Note that a part could not be identified to species levels as they did not show adult traits. In the study region there are 24 earthworm species that commonly occur (Valckx et al., 2009). Only 7 of these species were found in our samples thus also indicating a low biodiversity. Moreover, the total biomass and count of earthworms found is also low. Estimated earthworm biomass in temperate forest is around 30 gram per m² and more than 200 individuals are often found (Kooch et al., 2008; Schelfhout et al., 2017). However, sandy soils have a low CEC and BS and thus a weak buffer mechanism. As they are more prone to acidification and less fertile, we would expect lower earthworm abundance and diversity than other soil textures (Desie et al., 2021; Huang & Hartemink, 2020). Land use history and previous tree species choice (pine monocultures) have created a less fertile soil as well. In addition, we samples in the end of September and the beginning of October. Earthworms activity could still be low from the dry summer period (Valckx et al., 2009).

Low biodiversity can be seen as well in the calculated diversity indices (Figure 17, Figure 18, Figure 24). The max amount found in in all plots are 13 mesofauna orders and 7 earthworms species which is very low compared to the amount of mesofauna orders and earthworm species discovered (Sims & Gerard, 1999; Tilling, 2014; Wheater & Read, 1996). In general, the range of the Shannon Diversity Index lies between 1.5 and 3.5 in most cases (Ortiz-Burgos, 2016). Values below 2 are seen as very low diversity while high diversity is indicated by 3.5 or higher (Baliton et al., 2020). For earthworms species the Shannon Diversity Index is below 1.5, regardless of forest type, thus corresponding with a (very) low diversity. The Shannon Diversity Index for mesofauna orders is below 1.5 as well for both forest types. Identification of a higher taxonomic rank (order) requires less expertise and is less time consuming than identification of species. However, it should be a good estimator for species diversity. Few research has assessed this trade off at different taxonomic level. Nevertheless, research by Driessen & Kirkpatrick (2019) and Zou et al. (2020) concluded that family-level diversity could be a proxy for species diversity while order-level diversity is also an effective surrogate but more prone to give a poor reflection of species diversity. How well higher taxa preform as proxies is influenced by the structure of the community and taxonomic diversity (Driessen & Kirkpatrick, 2019; Zou et al., 2020). Therefore, we do not want to make a conclusion on the general diversity of the mesofauna orders based on these diversity indices. However, it is possible to assess the difference for the Shannon Diversity Index between forest types, as it is a relative comparison.

Furthermore, Shannon evenness index is higher than 0.5 for both mesofauna and earthworms, indicating that most orders/species are rather evenly distributed.

5.2 Impact of forest type

The total amount of mesofauna found in the plots depends on the unit used. Count per area is higher in the plots with a poor forest type while it is lower for count per volume (Figure 15, Figure 16). However, when comparing the total count of each mesofauna order between area and volume, for most orders this gives a similar result. Orders with the highest amount, regardless of the unit, in plots with a poor litter quality are: *Geophylomorpha, Scolomendromorpha, Chordeumatida, Polyxenida, Coleoptera, Thysanoptera, Diplura, Isopoda, Diptera, Psocoptera, Pseudoscorpoines, Protura*, and *Nematoda*. Higher count in plots with rich litter species are seen in: *Lithobiomorpha, Collembola, Hemiptera, Aranaea, Apocrita, Formicidae, Hymenoptera, Oligocheata*, and *Tubellaria*. Furthermore, unit per area or volume does make a difference for *Symphyla* and *Acari*. The count is higher in a poor forest type per unit of area and smaller per unit of volume for both.

The change between higher total count in the poor and rich plots when changing the unit, is mostly because of mites as they account for more than 50% of the organisms found in all samples. Based on the area, we expected that plots with a poor litter quality would contain more mesofauna. One of the results of the acidification processes is the built up of the humus layer due to slower decomposition rates (Olsthoorn & Wolf, 2006) (Figure 12, Figure 13). This increases the mesofauna habitat as they mostly reside in the organic layer (Turbé et al., 2010). Therefore the total amount will be higher in plots with a poor litter quality, which is in line with our hypothesis. When taking into account the thickness of the humus layer and looking at count per volume, the trend reverses. The latter is because a better functioning and healthy soil will contain relatively more soil organisms. Soil characteristics such as pH, CEC and BS will lie within a range that is suitable for more species. Processes such as acidification will also reduce the soil community besides the change in composition (Cole et al., 2004; Desie et al., 2021).

For earthworms the difference between count and biomass is small (Figure 20, Figure 21). Plots with rich litter quality have both a higher amount and biomass, except for Hechtel Eksel. The large difference between both units, is the relative contribution of species. Larger species such as from the *Lumbricus* genus have a larger relative contribution to the total biomass (Valckx et al., 2009). Smaller species such as *Dendrobaena octaedra* weigh less but are sampled more. Furthermore, plots with poor litter quality contain less epigeic and epi-anecic species (Figure 22, Figure 23). Epigeic species are more acid tolerant than other ecological categories but the reduction in pH and increased aluminium concentrations affect the abundance of the populations. Epi-anecic species are affected more by a low pH and should have a lower abundance as well (Schelfhout et al., 2017). In our results we see that the difference between poor and rich forest types is larger for epigeic species than epi-anecic species. This is not what is expected according to other research (Desie et al., 2020; Frouz, 2018; Menta, 2012; Schelfhout et al., 2017). A possible explanation is the smaller samples size of our dataset. Furthermore, endogeic species were only found in 2 plots, Kasterlee and Grote Heide rich, which makes the results less representative to derive conclusions from.

The above trends can be seen as well in the thickness and type of humus layer (Figure 12, Figure 13, Figure 14). Decreased bioturbation activities causes built-up of the humus layer and less mixing between the organic and mineral layer (Schelfhout et al., 2017).

Furthermore, species richness, Shannon diversity and Shannon evenness for mesofauna are all higher in plots with poor quality litter, regardless of the unit used (Figure 17, Figure 18). Nevertheless, the difference is not significant. We assume that the difference is small because not all orders are present in each plot. The presence or absence of one order can be counterbalanced by the absence or presence by another order. Hence the mean values of the indices do not differ much between forest types but are slightly higher in plots with poor litter quality. In addition, most mesofauna species are acid tolerant to some degree (Desie et al., 2021; Turbé et al., 2010). For earthworm biomass, all diversity indices have a higher value in plots with rich litter quality (Figure 24). Earthworms are influenced by acidification more strongly than mesofauna species and our data show a significant difference between forest types, which is in line with our hypothesis.

Note that although these indices combine information such as species richness and diversity, ecological roles of species/orders are not taken into account nor the state of the community. For the latter, the presence or absence of different groups and the sensitivity of each groups is a better indicator. More research is necessary for indicators based on fauna (Menta & Remelli, 2020).

Ecological roles can be estimated when feeding patterns and soil functions are considered (Figure 26, Figure 28). In general the five different feeding patterns are almost evenly distributed along the soil organisms for each forest type. Regardless of the unit, predators are most abundant in total. Starting from the second or third trophic level in the food webs, predators occur in each trophic level. Other feeding patterns such as detrivory, herbivory, and microbivory are often concentrated at the first trophic levels of the food chain.

The difference in count per area or count per volume is again similar as before (Figure 27). Per area the poor litter plots containes more mesofauna in absolute values. The habitat size, more specifically the humus thickness, is larger there. The amount per volume on the other hand shows relative amounts. Rich litter plots with better nutrient cycling and pH buffering should also attract more mesofauna or soil biota in general. For earthworms this is not the case. However, earthworm count is lower than all mesofauna combined. Hence the variability between both units is mostly because of the mesofauna count.

The distributions of soil functions is less balanced between the plots (Figure 29). Litter fragmentation and population regulation dominate over soil functions regarding mixing of the soil (bioturbation and gut passage). Population regulation and litter fragmentation are both functions that many species can carry out. In addition, mixing functions are performed by a smaller amount of species.

Rich litter influence

Previous research has shown that rich litter species induce and increase bacterial decomposition rates. They enhance nutrient cycling and pH buffering as well (Desie et al., 2020; van den Berg et al., 2022). Likewise, our data show a positive influence of rich litter on earthworms (Figure 25). Rich litter species found in the plots are mainly *Tilia, Prunus*, and *Acer*. The litter of *Tilia* and *Prunus* species contains larger concentration of calcium and small amount of lignin (van den Berg et al., 2022). Soil under these species thus show an improved buffer capacity and soil pH. Therefore, more endogeic and anecic earthworms are found in these soils (den Ouden et al., 2020). In our data we also see a significant, positive influence by rich litter species on total earthworm biomass.

Furthermore, the increased bioturbation activities influence the total carbon stock and CEC in the topsoil (Figure 25). Total carbon stock decreases in the organic layer while it increases in the mineral soil. In acid soils all decomposition processes are concentrated in the organic layers. The reintroduction of burrowing earthworms helps with the redistribution of carbon through the different soil layers. Higher CEC on the other hand increases the earthworm biomass. CEC is closely linked with the pH and the base cation exchange buffer mechanisms. A higher CEC means that the soil has a better pH buffer capacity and less base cations are leached out. Rich litter species improves the CEC in the organic layer and topsoil. The latter attract more earthworms than the poor litter species plots with a low pH. Models to evaluate the effect of base saturation on earthworm biomass are unreliable due to conversion problems during calculations. Hence we will not look further into the results. We suggest resampling and increasing the sample size to look further into this potential relation. Other research already found links between pH, BS and earthworm biomass (de Schrijver et al., 2018; Desie, Vancampenhout, Nyssen, et al., 2020; Schelfhout et al., 2017). Other relations between earthworm biomass and soil characteristics were non-significant so additional sampling and testing is advised.

Mesofauna per unit of area, on the other hand decreases with increasing rich litter influence (Figure 19). As nutrient cycling, pH buffering and bioturbation increases, the organic layer is slowly decomposed. In addition to the shrinking of the humus layer, the type changes as well from mor to moder or mull (Desie et al., 2020; Olsthoorn & Wolf, 2006; Schelfhout et al., 2017). The moder and mull profiles are mostly found under *Prunus* and *Acer* species (van den Berg et al., 2022). The probability of encountering these humus types in the sampled plots increases as well with increasing rich litter influence (Figure 14). To come back to the decreasing mesofauna count per area: their habitat size is decreasing thus the amounts here are smaller. The trend seen between mesofauna count per area and pH at different depths is not significant and the slope is almost flat. Mesofauna is tolerant for a larger pH range than most earthworm species (Desie et al., 2021; Turbé et al., 2010). This is similar for CEC and BS. Both have a weak influence on mesofauna count per area.

5.3 Implications for forest management

Based on our results, the implementation of rich litter species as a management practice shows an improvement in the soil conditions and the humus profile. Soil functions such as bioturbation and gut passage are carried out, mainly because of the enhanced earthworm community in soil influenced by rich litter species. As for mesofauna, the impact of tree species is less conclusive as they are tolerant to a low pH.

However, overall soil biodiversity in the study regions is very low, with or without the presence of rich litter species. In our study region, sandy soils are less fertile and more prone to acidification (Huang & Hartemink, 2020). In addition, the soils are known to have disrupted element cycles and an imbalance of nutrients due to practices as litter raking, atmospheric depositions and poor litter tree plantations (Desie et al., 2020a). Therefore, the success of rich litter species depends on local conditions. Furthermore, this raises the question if rich litter species could be used to restore soil biodiversity in the study area or other measures such as induced reintroduction of soil fauna is necessary. Nevertheless, previous research has shown that mixed forests with rich litter species show significant positive influence on soil biodiversity (Augusto et al., 2002; Desie et al., 2020a; Desie et al., 2020b; Kooch et al., 2021; Schelfhout et al., 2017).

5.4 Limitations and recommendations for future research

The sites chosen for sampling seem to be a good representation to assess the effect of rich litter species, as rich litter tree species have a significant influence on mesofauna and earthworms. The only exception is Someren, where the poor and rich plot are located on different soil profiles. Therefore a comparison between them cannot be made since we want to focus on the effect of rich litter species while keeping other factors constant within a site. In addition, a limitation of the sampling design is sample size, especially with the removal of Someren samples. The trends seen in our data show a positive influence of rich litter species. A recommendation is to increase the dataset by including more sites to see if our trends correspond with the general trend of the study area.

Furthermore, it is suggested for future research to extend the knowledge and research on mesofauna. Family-level identification is a better proxy for species diversity than order-level identification (Driessen & Kirkpatrick, 2019; Zou et al., 2020). Therefore we can better evaluate the soil biodiversity and how it is influenced by the acidification processes. In addition, soil functions can be linked with specific species and the number of functions could be extended. However, this requires expert knowledge.

Lastly, we recommend to assess the long term effect of rich litter species. The rich litter tree species in our study area are most likely the first generation of rich litter trees that were introduced after multiple generations of poor litter species. Although there is an uncertainty of how long the rich litter trees have been introduced, we probably look at a short term effect. This raises the question if the effect of rich litter trees is enhanced when extending the time frame and how much time is needed for the full effect. Based on these answers, it can be decided if the effect of rich litter species if sufficient to boost soil biodiversity. If not, more research is necessary to assess other measures, for example assisted migration of soil biota. However, increased knowledge about these measures is necessary to make an evaluation of their effects in practice.

6 Conclusion

The goals of this research are the evaluation of the state of the soil biodiversity in forests on sandy soils and how this differs between contrasting forest types; i.e. pine monocultures or mixed broadleaved forests dominated by rich litter species. In the forests on sandy soils in northeast Belgium and southern Nederland, there is a low general biodiversity, regardless of the forest types due to the land use history and long term acidification processes. Nevertheless, soil characteristics, humus profile, and earthworm populations are positively influenced by the presence of rich litter species. The effect of rich litter species is less conclusive for mesofauna populations as these species are more tolerant for pH. However, the composition of mesofauna differs between forest types.

We can conclude that rich litter species have a positive influence on the soil yet the effect depends on local conditions. Moreover, we most likely assessed the effect of the first generation of rich litter species after transition from poor litter species. It is recommended to assess long term effects and other measures such as assisted migration of soil biota in the future.

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8 Appendices

8.1 Mesofauna protocol

Tullgren funnel method

Materials:

- Funnel
- Clear plastic PVC tube
- Lighter
- Lightbulb
- Gauze (CanDo, 3 mm gaps)
- Glass beakers
- 70% ethanol solution

Setup (Figure 30):

- Cut a part of the PVC tube and attach it to the funnel with a lighter. This is to extend the end of the funnel into the beaker that is placed under it. If the funnel itself is long enough, this step can be skipped.
- Put the funnels in the wooden frame that hold them in place and under the lightbulb.
- Place a beaker under the funnel.
- Cut out pieces of gauze to surround a soil sample in the funnel and put them in the funnel.

Method:

- Put the soil samples in the funnels. To decrease drying time it is advised to fill the funnels halfway and use multiple funnels for the same sample if needed.
  - Make notes which samples are placed into which funnels.
- Close the gauze so that the sample is completely surrounded.
- Fill the beakers partially with the alcohol solution and place a label of the sample code or number on the beakers.
- Put on the light and let the samples completely dry.
  - A week is suggested but always check the samples before removing them.
- When dry, collect the beakers and transfer the content to a different cup that had a lid or cover the initial beaker. This is to prevent evaporation of the alcohol solution. The cup can then be stored until identification.
- Remove the dry soil in a bucket and clean out the funnels with a brush or a paper. The soil can be disposed of outside on grass or under trees since this is just dry soil without any chemicals

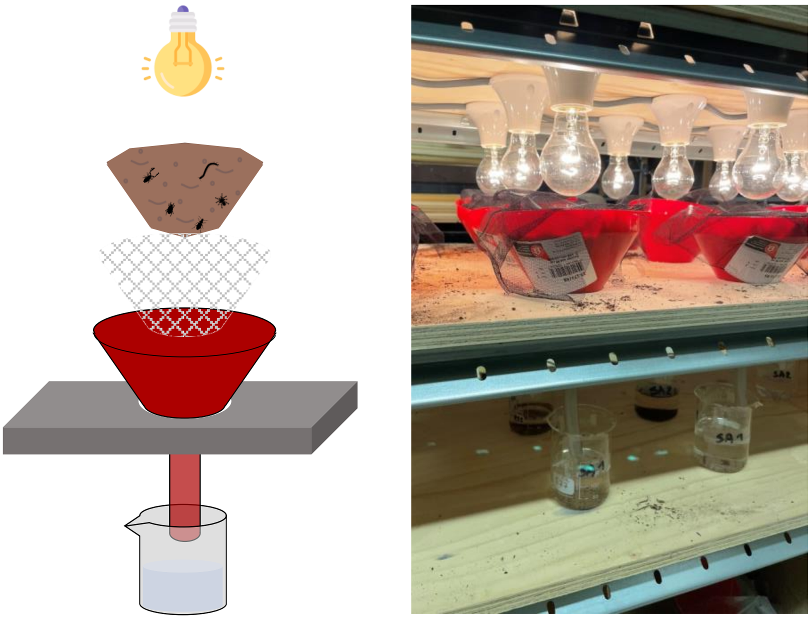

Figure 30 a) schematic example and b) real setup of the Tullgren funnel

Identification of the mesofauna

Materials:

- Microscope (400x or more)
- Petri dishes
- Eppendorf tubes
- 70% ethanol solution
- Plastic pipette
- Tweezers (bug tweezers are preferred)
- Identification key(s)

Method:

- Put the alcohol solution and mesofauna on a petri dish and place under the microscope.
- Make a rough initial sorting (mites, centipedes and millipedes, bugs and beetles, ants, larvae, …) and place them separately in an Eppendorf tube.
    - Use a pipette or (bug) tweezers.
- Take each of the Eppendorf tubes and identify and count the invertebrates in each tube with the identification key(s).
    - “A key to the major groups of British terrestrial invertebrates” by Tilling (2014)
    - “Animals under logs and stones” by Wheater & Read (1996)
- When ready with al the rough groups, place the whole sample back in the original beaker or cup with a lid in case the samples need to be preserved longer or dispose of the samples if not needed anymore.
- In case of doubt, place the unknown invertebrates in a separate Eppendorf tube (1 for each sample) and ask for a second opinion.

8.2 Supporting information

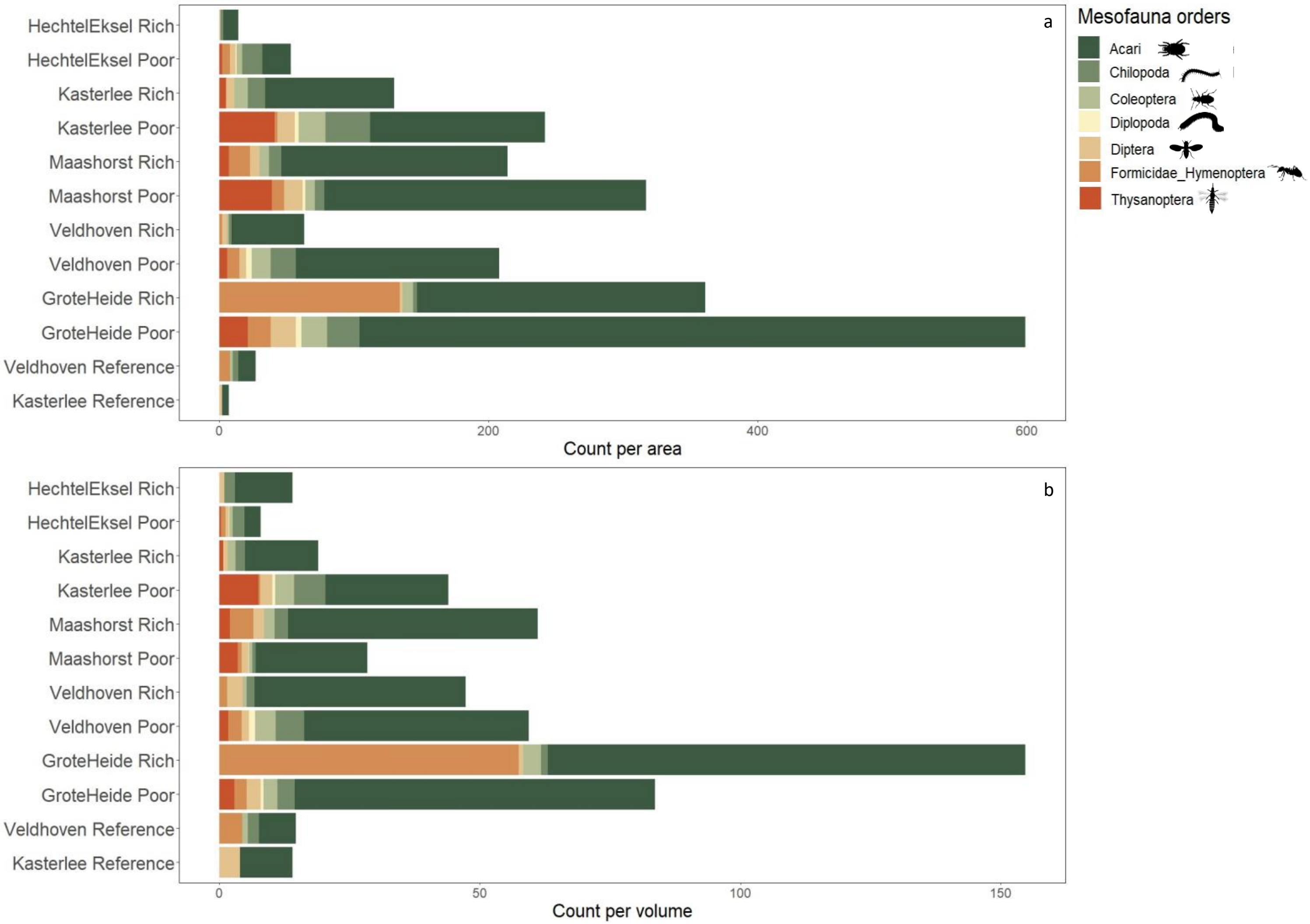

Figure 31 a) count per area and b) count per volume of the recurring mesofauna orders in each sample.

8.3 Form: use of AI assistance

Use of ChatGPT (or any other AI Writing/Coding/Visualisation Assistance)

Student name: Julie Simons
Student number: r0711837
Please indicate with "X" whether it relates to a course assignment or to the master's thesis:

X This form is related to my master's thesis.
Title master's thesis: The impact of forest management on soil biodiversity in acidified sandy soils
Promotor: Ellen Desie (promotor) and Koenraad Van Meerbeek (co-promotor)

O This form is related to a BIG-project.
Title BIG-project: …
Promoter: …

O This form is related to a course assignment.
Course name: …
Course code: …

Please indicate with "X":
X I did not use ChatGPT or any other AI Writing/Coding/Visualisation Assistance.
O **I did use** AI Writing/Coding/Visualisation Assistance. In this case **specify which one** (e.g. ChatGPT/GPT4/...): …

Please indicate with "X" (possibly multiple times) in which way you were using it:

- ○ **Assistance purely with the language of the paper** (This use is similar to using a spelling checker)
- ○ **As a search engine to learn on a particular topic (**This use is similar to e.g. a Google search or checking Wikipedia. Be aware that the output of ChatGPT evolves and may change over time)
- ○ **For literature search (**This use is comparable to e.g. a Google Scholar search. However, be aware that ChatGPT may output no or wrong references. As a student you are responsible for further checking and verifying the absence or correctness of references)
- ○ **To let generate programming code (**Correctly mention the use of ChatGPT and cite it)
- ○ **To let generate graphics (**Correctly mention the use of ChatGPT and cite it)
- ○ **To let generate new research ideas (**Further verify in this case whether the idea is novel or not. It is likely that it is related to existing work, which should be referenced then)
- ○ **To let generate blocks of text (**Inserting blocks of text without quotes from ChatGPT to your report or thesis is not allowed. According to Article 84 of the exam regulations in evaluating your work one should be able to correctly judge

on your own knowledge. In case it is really needed to insert a block of text from ChatGPT, mention it as a citation by using quotes. But this should be kept to an absolute minimum)

- **Other** (Contact the professor of the course or the promotor of the thesis or BIG-project. Motivate how you comply with article 84 of the exam regulations. Explain the use and the added value of ChatGPT or other AI tool: ….)

Further important guidelines and remarks:

- ChatGPT cannot be used related **to data or subjects under a Non-Disclosure Agreement.**
- ChatGPT cannot be used related **to sensitive or personal data due to privacy issues**.
- **Take a scientific and critical attitude** when interacting with ChatGPT and interpreting its output.
- As a student you are responsible to comply with article 84 of the exam regulations: your report or thesis should reflect your own knowledge. Be aware that plagiarism rules also apply to the use of ChatGPT or any other AI tools.
- **Exam regulations article 84**: "Every conduct individual students display with which they (partially) inhibit or attempt to inhibit a correct judgement of their own knowledge, understanding and/or skills or those of other students, is considered an irregularity which may result in a suitable penalty. A special type of irregularity is plagiarism, i.e. copying the work (ideas, texts, structures, designs, images, plans, codes, ...) of others or prior personal work in an exact or slightly modified way without adequately acknowledging the sources. Every possession of prohibited resources during an examination (see article 65) is considered an irregularity."
- **Information on citing ChatGPT as proposed by ChatGPT itself**: "Citing and referencing ChatGPT output is essential to maintain academic integrity and avoid plagiarism. Here are some guidelines on how to correctly cite and reference ChatGPT in your master's thesis:
    1. Citing ChatGPT: Whenever you use a direct quote or paraphrase from ChatGPT, you should include an in-text citation that indicates the source. For example: (ChatGPT, 2023).
    2. Referencing ChatGPT: In the reference list at the end of your thesis, you should include a full citation for ChatGPT. This should include the title of the AI language model, the year it was published or trained, the name of the institution or organization that developed it, and the URL or DOI (if available). For example: OpenAI. (2021). GPT-3 Language Model. https://openai.com/blog/gpt-3-apps/
    3. Describing the use of ChatGPT: You may also want to describe how you used ChatGPT in your research methodology section. This could include

details on how you accessed ChatGPT, the specific parameters you used, and any other relevant information related to your use of the AI language model. Remember, it is important to adhere to your institution's specific guidelines for citing and referencing sources in your master's thesis. If you are unsure about how to correctly cite and reference ChatGPT or any other source, consult with your thesis advisor or a librarian for guidance.”

9 Popularised summary

Soil biodiversity, the diversity of life belowground, is often overlooked and victim of the 'out of sight, out of mind' principle. Nevertheless, soil ecosystems are considered one of the most diverse systems on earth. Therefore, much soil organisms are yet to be discovered. In addition, the soil and its biodiversity are the basis of multiple ecosystem services such as food production and nutrient provisioning. However, soil biodiversity in general is threatened and declining due to increasing pressure by human activities and climate change. What the main threats and their relative impact are, depends on local condition. In the forests on sandy soils in northeast Belgium and the southern Netherlands, one of the main threats is acidification of the forest floor, which results in low pH's. At the moment most of these forests are located on soils that are already degraded as a consequence of land use history and past and present acidifying emissions.

Besides governmental regulations for emissions, soil biodiversity could be restored with forest management as the aboveground and belowground systems are intrinsically connected. Our focus lies on the impact of tree species selection, and more specifically tree species with leaf litter high in nutrients (rich litter species). As this leaf litter is easier to decompose, nutrient cycling could be enhanced. In addition, other factors such as pH, amount of nutrients and aluminium toxicity are improved in a way that the conditions are more suitable for a larger amount of soil species.

In this thesis, the main focus is the evaluation of the state of the soil biodiversity in forests on sandy soils and how this differs between contrasting forest types; i.e. pine monocultures or mixed broadleaved forests dominated by rich litter species. Our results show that the general soil biodiversity is low for both forest types in the study region due to a long history of acidification. Nevertheless, rich litter species have a positive influence on soil quality and earthworm abundance and diversity. The effect of rich litter species is less conclusive for mesofauna (fauna < 2 mm) populations as these species are more tolerant to low pH's. Therefore we can conclude that rich litter species have a positive influence on the soil yet the effect depends on local conditions. Moreover, we most likely looked at the effect of the first rich litter trees planted after transition from pine monocultures. It is recommended to assess long term effects and other measures such as assisted re-introduction of soil fauna in the future.