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Earthworm communities in relation to tree communities in European forests

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Dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Bioscience Engineering (PhD)

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Summary

With the continuing demand for wood products, wood production is still one of the main priorities in many European forests. However, a lot of forests in Europe and North-America suffer from soil degradation and soil compaction. In the absence of a healthy soil, a forest will quickly decrease in vitality and stability, which ultimately results in a deterioration of the wood yield of a forest. Next to wood, other goods and services that forests on a healthy soil can deliver us such as carbon storage, fresh water supply, food and recreational space will also diminish or even disappear. One of the key elements in the realization and maintenance of a healthy forest soil in the majority of European forests are earthworms. These soil engineers modify the physical, chemical and biological soil properties simply by the way they feed, poop and dig tunnels in the soil. They are industrious and irreplaceable in that they tirelessly contribute to the fragmentation of litter, decomposition of soil organic matter, nutrient cycling, topsoil formation and primary production.

The objective of this research was to study earthworm communities in European forests and their relation with tree communities. The study focused on the different possible influencing factors of earthworm communities in forests at a continental scale, with special attention to tree leaf litter assumed as a major food components of the earthworm diet. Based on field data, gathered in six mature forest landscapes across Europe, we first evaluated the relative importance of a set of abiotic and biotic factors in explaining earthworm occurrence and abundance in European forests. We continued by focusing specifically on the influence of tree diversity and identity on earthworm species richness and total biomass. The gained knowledge was finally used to investigate how tree litter characteristics and functional diversity influence a hypothesised feedback loop between earthworm total biomass, humus quality and soil pH. By investigating the relationships between aboveground ecosystem engineers (i.e. trees), with the belowground ecosystem engineers (i.e. earthworms), this study tries to create deeper insights into the functioning of the forest ecosystem in Europe, for the benefit of an integrated forest soil restoration and management.

By investigating a large set of abiotic and biotic factors we could get a firm idea on what drives the occurrence of earthworms on a European scale and if earthworms were present, which aspect were the main determinants explaining total earthworm density and biomass. In general, we found that litter- and soil-related variables best explained earthworm incidence and biomass in European forests, leaving only a minor role to climate related variables. Among the litter related variables, understory vegetation played an important role in explaining earthworm incidence and abundance. Our results suggested that threshold values for soil C:N ratio, forest floor pH and understory plant biomass and plant nutrient concentrations have to be attained before earthworms can occur. Beyond these threshold values, variables like soil C:N ratio, tree litter C:P ratio and forest floor mass further explain total earthworm density and biomass.

Using the facilities of a Europe-wide forest research platform we found that the size and diversity of an earthworm community usually decreases with an increasing share of evergreen leaf litter in the forest. This negative effect of evergreen leaf litter on earthworms can be explained by its poor litter quality that is less preferred by earthworms. The location of the forest region along the European North-South axis is, however, important here, as litter quality effects differed clearly with latitudinal position. In north and central Europe, total earthworm biomass and species richness clearly increased with increasing litter nutrient concentrations, whereas this influence of litter nutrients was absent or even reversed in southern Europe. In addition, although earthworms were unaffected by the number of tree species, tree diversity positively affected total earthworm biomass at the continental scale through functional diversity of the leaf litter. By focusing on tree leaf litter traits, we advanced our understanding of the mechanisms driving tree identity effects and supported previous findings that litter quality, as a proxy of tree identity, was a stronger driver of earthworm species richness and biomass than tree diversity.

Finally, we tried to further explore the influence that trees can have on the black box of interactions belowground. With data and knowledge that we gathered throughout our research, we tried, with a statistical analysis technique, to make a reconstruction of the presumed mechanism of a self-reinforcing soil process between total earthworm biomass, humus quality and soil pH. This reconstruction confirmed, once more, the importance of leaf litter traits driving belowground dynamics. Our feedback loop hypothesis could not be confirmed because the postulted positive influence of soil pH on total earthworm biomass could not be confirmed. However, additional model results suggested that such feedback loops could exist, but that the relation between soil pH and total earthworm biomass shows a threshold behaviour rather than a linear relationship and therefore differs, depending on the soil pH range of the plots under study. Because of this threshold behaviour we assume that the mechanisms behind this earthworm-soil loop will be most visible in soils near to this pH threshold.

We conclude that the size and diversity of an earthworm community in a forest can be increased by planting tree species that increase the diversity of leaf litter characteristics and on the other hand contain nutrient rich litter. Such measures increase the vitality of a forest soil and guarantee a better and more sustainable functioning forest ecosystem to the forest managers.

Samenvatting

Met de aanhoudende vraag naar houtproducten is houtproductie nog steeds een van de belangrijkste prioriteiten in veel Europese bossen. Veel bossen in Europa en Noord-Amerika lijden echter onder bodemdegradatie en bodemverdichting. In afwezigheid van een gezonde bodem zal een bos snel in vitaliteit en stabiliteit afnemen, wat uiteindelijk resulteert in een achteruitgang van de houtopbrengst van een bos. Naast hout, zullen andere goederen en diensten die bossen op een gezonde bodem ons kunnen leveren zoals koolstofopslag, drinkbaar water, voedsel en recreatieruimte ook verminderen of zelfs verdwijnen. Een zeer belangrijke factor in de realisatie en het onderhoud van een gezonde bosbodem in de meeste Europese bossen zijn regenwormen. Deze bodemingenieurs veranderen de fysieke, chemische en biologische bodemeigenschappen door de manier waarop ze zich voeden, uitwerpselen achterlaten en voortbewegen in de bodem. Ze dragen bij aan de fragmentatie van strooisel, afbraak van organische stof in de bodem, nutriëntenkringlopen, bodemvorming en primaire productie.

Het doel van dit onderzoek was om regenwormgemeenschappen in Europese bossen en hun relatie met boomgemeenschappen te bestuderen. De studie concentreerde zich op de verschillende mogelijke factoren die regenwormengemeenschappen beïnvloeden, op een continentale schaal, met speciale aandacht voor bladstrooisel van bomen dat als een belangrijke voedselcomponenten van het regenwormdieet wordt beschouwd. Op basis van veldgegevens, verzameld in zes mature bossen in Europa, hebben we eerst het relatieve belang geëvalueerd van een reeks abiotische en biotische factoren in het verklaren van het voorkomen en de hoeveelheid regenwormen in Europese bossen. We zoomden vervolgens sterker in op de invloed van boomdiversiteit en identiteit op de diversiteit en biomassa van regenwormgemeenschappen. De opgedane kennis werd uiteindelijk gebruikt om te onderzoeken hoe de bladstrooiseleigenschappen van bomen en de functionele diversiteit van bomen van invloed zijn op een veronderstelde kettingreactie tussen regenwormbiomassa, humuskwaliteit en bodem-pH. Door het onderzoeken van de relaties tussen bovengrondse ecosysteemingenieurs (bomen) met de ecosysteemingenieurs in de bodem (regenwormen), proberen we in deze studie diepere inzichten te verkrijgen in het functioneren van het bosecosysteem in Europa, ten behoeve van een geïntegreerd herstel en beheer van bosbodems.

Door een groot aantal abiotische en biotische factoren te onderzoeken, konden we een goed idee krijgen van wat de aanwezigheid van regenwormen op Europese schaal beïnvloed en eenmaal regenwormen kunnen voorkomen, welk aspect de belangrijkste determinanten waren voor het verklaren van regenwormaantallen en -biomassa. In het algemeen hebben we gevonden dat strooisel- en bodemgerelateerde variabelen het best de aanwezigheid van regenwormen en hun totale biomassa in Europese bossen kunnen verklaren, en dat klimaatgerelateerde variabelen minder van belang zijn. Van de strooiselgerelateerde variabelen speelde de kruidvegetatie een belangrijke rol bij het verklaren van het voorkomen en de hoeveelheid regenwormen. Onze resultaten

suggereerden dat drempelwaarden voor de C:N verhouding van de bodem, bosbodem-pH, en biomassa en nutriëntenconcentratie van de kruidvegetatie moeten bereikt worden alvorens regenwormen kunnen voorkomen. Voorbij deze drempelwaarden zijn het variabelen zoals de C:N verhouding van bodem, de C:P verhouding van het bladstrooisel van bomen en de massa van de humuslaag, die de regenwormaantallen en –biomassa in de bosbodem verklaren.

Met behulp van de faciliteiten van een Europees bosonderzoeksplatform ontdekten we dat de omvang en diversiteit van een regenwormgemeenschap in de meeste gevallen afneemt met een toenemend aandeel bladstrooisel van groenblijvende boomsoorten in het bos. Dit negatieve effect van groenblijvende bladstrooisel op regenwormen is te verklaren doordat regenwormen minder de voorkeur geven aan dergelijk bladstrooisel van slechte kwaliteit. De locatie van het bosgebied langs de Europese Noord-Zuid as is hier echter belangrijk, aangezien de effecten van strooiselkwaliteit duidelijk verschilden met de positie langsheen de breedtegraad. In Noord- en Midden-Europa name regenwormbiomassa en soortenrijkdom duidelijk toe met toenemend nutriëntenconcentratie van het strooisel, terwijl deze invloed van de nutriëntenconcentratie van het strooisel afwezig of zelfs omgekeerd was in Zuid-Europa. De functionele diversiteit van het bladstrooisel van de bomen had een positieve invloed op de regenwormbiomassa op continentale schaal. Door ons op de eigenschappen van het bladstrooisel van bomen te richten, hebben we het inzicht in de mechanismen die de identiteitseffecten van bomen aansturen verbeterd en konden we eerdere bevindingen, dat strooiselkwaliteit een grotere invloed heeft op de regenwormdiversiteit en hun totale biomassa dan boomsoort diversiteit, ondersteunen.

Ten slotte hebben we geprobeerd om de invloed van bomen op ondergrondse interacties verder te onderzoeken. Aan de hand van gegevens en kennis dat we doorheen het onderzoek verzamelden hebben we getracht, met een statistische analysetechniek, een reconstructie te maken van de vermoedelijke werking van een zelfversterkend bodemproces tussen regenwormen, humuskwaliteit en bodem-pH. Onze positieve spiraal-hypothese kon echter niet worden bekrachtigd omdat de vooropgestelde positieve invloed van bodem-pH op totale regenwormbiomassa niet werd bevestigd. Aanvullende modelresultaten suggereerden echter dat een dergelijke positieve spiraal zou kunnen bestaan, maar dat de relatie tussen bodem-pH en totale regenwormbiomassa een drempelgedrag vertoont en daarom verschilt, afhankelijk van het bodem-pH bereik van de onderzochte percelen. Vanwege dit drempelgedrag gaan we ervan uit dat de werking van deze spiraal het meest zichtbaar zal zijn in bodems onder en dichtbij deze pH-drempel.

We besluiten dat de grootte en diversiteit van een regenwormgemeenschap in een bosbestand kan verhoogd worden door boomsoorten aan te planten die enerzijds de diversiteit aan bladkarakteristieken verhogen en anderzijds nutriëntrijk bladstrooisel bevatten. Dergelijke maatregelen verhogen de vitaliteit van een bosbodem en garanderen een beter en duurzamer functionerend bosecosysteem aan de bosbeheerders.

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List of abbreviations

AIC	Akaike information criterion
AICc	Akaike information criterion small-sample-size corrected version
AUC	Area under the receiver-operating characteristic curve
BEF	Biodiversity and ecosystem functioning
BRT	Boosted regression trees
C:N	Carbon:nitrogen ratio
C:P	Carbon:phosphorus ratio
Ca	Calcium
CI	Confidence intervals
Corr	Correlation score
COS	Cosine
CWM	Community weighted means
D^2	Percentage deviance explained
df	Degrees of freedom
EEA	European Environment Agency
FDis	Functional dispersion
HLI	Heat load index
ISO	International Organization for Standardization
K	Potassium
K	Lowest number of estimable parameters
LAI	Leaf area index
MAM	Minimum adequate model
MAP	Mean annual precipitation
MAT	Mean annual temperature
Max	Maximum
Mg	Magnesium
Min	Minimum
N	Nitrogen
n	Number of sites
NA	Not available
P	Phosphorus
PCA	Principle component analysis
PCoA	Principle coordinate analysis
R^2	Coefficient of determination
REML	Restricted maximum likelihood estimation
RMSE	Root mean squared error

r_p	Pearson's correlation coefficient
rRMSE	Relative root mean squared error
r_s	Spearman's correlation coefficient
SD	Standard deviation
SEM	Structural equation model
SIN	Sine
SLA	Specific leaf area index
Trunc	Truncate
VIF	Variance inflation factor
WHC	Water holding capacity

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Chapter 1

Introduction

1. Why earthworm communities in forests are worth studying

In the light of global warming, the heat island effect in urbanised areas, human health issues and an increasing demand for renewable resources, green spaces, including forest, are experiencing a higher level of appreciation and consequently also the demand for forests increases. Indeed, forests provide a multitude of benefits in terms of climate regulation, human health, recreation, refuges, food, wood and fresh water supply. Forests help to regulate the climate by sequestering carbon (Bonan 2008), they reduce air pollution (Donovan et al. 2013), minimize the urban heat island effect (Huang et al. 1990, Kurn et al. 1994, Wang and Akbari 2016), provide recreational area (Bell et al. 2007, Pröbstl et al. 2010), give access to berries (Miina et al. 2009), mushrooms (Mosquera-Losada et al. 2009) and game (Boman et al. 2011), they contribute to fresh water supply (Anderson et al. 1976) and even increase property values (Visser and Dam 2006). Next to that, they are home to a significant amount of the world's biodiversity (Aerts and Honnay 2011).

To guaranty forest ecosystem functioning and the aforementioned services it is of paramount importance to conserve a good forest soil health (Schoenholtz et al. 2000, Amacher et al. 2007, Chazdon 2008). Soil health is considered in this manuscript as a property that reflects the continued capacity of soil to respond to forest management by maintaining both the wood production and the provision of other ecosystem services (Box 1). Important functions of a healthy soil in a forest context are its capacity to provide physical support, water and essential nutrients required for plant growth, to regulate the flow of water in the environment, and to eliminate the harmful effects of contaminants by means of physical, chemical and biological processes (Laishram et al. 2012).

Earthworms are indispensable in the realization of these functions and are therefore a vital player in conserving a good forest soil health (Lavelle and Spain 2001). They play a crucial role in the bioturbation of the soil, nutrient cycling and the decomposition of soil organic matter. Earthworms are therefore also called ecosystem engineers, modifying the physical, chemical and biological soil properties (Blouin et al. 2013). As they feed and dig tunnels in the soil, they structure the habitat for other biota (Thompson et al. 1993, Baker et al. 2006), they mediate soil aggregates (Six et al. 2004) and change soil porosity (Blanchart et al. 1997). By fragmenting and mixing litter and mineral soil, they accelerate microbial processes such as decomposition of organic matter and nutrient mineralisation, and consequently also indirectly influence topsoil formation and primary production (Knollenberg et al. 1985, Scheu and Wolters 1991, Lavelle and Spain 2001, Scheu 2003, van Groenigen et al. 2014).

Box 1: Soil quality and soil health

Soil quality is recently redefined in a new ISO (11074) standard as "all current positive or negative properties with regard to soil utilization and soil functions"(FAO 2015). This indicates that currently the concept 'soil quality' is primarily focused on the dynamic soil properties that can be strongly influenced by management and are mainly monitored in the surface horizon (0-30 cm) of the soil (Karlen et al. 2003). Soil quality is basically 'how well soil does what we want it to do', and since soils can be used for a large variety of purposes (Nortcliff 2002), soil quality is dependent on the context and the goals related with them (Bünemann et al. 2018). In agro-ecosystems for example, the main though not exclusive service is productivity. For natural ecosystems, major aims are maintenance of environmental quality and biodiversity conservation. However, these services are often of much lower priority in an urban context when installing sewage systems and building roads. In this manuscript, soil quality is seen in the context of mature forest ecosystems and assessed with respect to the capacity to sustain an abundant and diverse earthworm community. The same reasoning holds for the term 'litter quality', also used in this manuscript. Consideration of soil as a finite and living resource, further led to the concept of soil health. Soil health can be defined as the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain or enhance the quality of air and water, and promote plant, animal and human health (Doran and Zeiss 2000). Consequently, soil health presents the soil as a finite non-renewable and dynamic living resource (Laishram et al. 2012).

2. Fundamentals of earthworm biology

2.1 Earthworms are ecosystem engineers

Earthworms are considered important and extremely useful as they play a crucial role in the bioturbation of the soil, nutrient cycling and the decomposition of soil organic matter. Earthworms are also known as ecosystem engineers modifying the physical, chemical and biological soil properties (Blouin et al. 2013). As they feed and dig tunnels in the soil, they structure the habitat for other biota (Thompson et al. 1993, Baker et al. 2006), they mediate soil aggregates (Six et al. 2004) and change soil porosity (Blanchart et al. 1997). By fragmenting and mixing litter and mineral soil, they accelerate microbial processes such as decomposition of organic matter and nutrient mineralisation, and consequently also indirectly influence topsoil formation and

primary production (Knollenberg et al. 1985, Scheu and Wolters 1991, Lavelle and Spain 2001, Scheu 2003, van Groenigen et al. 2014).

2.2 Earthworm phylogeny

Earthworms often represent a significant, if not the dominant part of the soil living biomass (Lavelle and Spain 2001), making them an important factor in the functioning of the soil. Earthworm population densities and biomass may range from less than 10 individuals and 1 g/m² to more than 1000 individuals and 200 g/m² under favourable conditions (Curry 2004). In multispecies studies conducted on soils, they are generally categorised as macrofauna (Fig. 1.1). Phylogenetically speaking they belong together with the leeches to the phylum Annelida, and together with small aquatic worms, potworms and the Asian moniligastrid earthworms, they form the class Oligochaeta. However, Weigert and Bleidorn (2016) demonstrated with modern sequencing technologies and phylogenomic analyses of huge data matrices that the phylogeny of the annelids needs to be revisited.

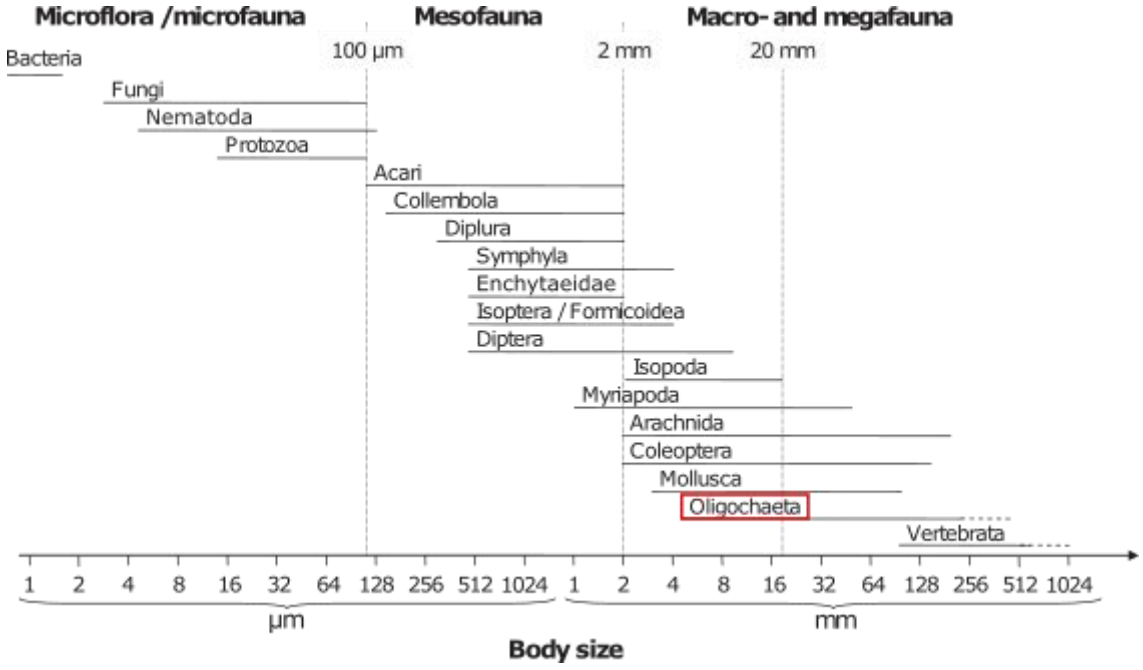


Fig. 1.1. Overview of the main taxonomic groups of soil organisms on a body-size basis with, in red, an indication of the class of interest in this manuscript (Modified after Decaëns, (2010)). Body size on the x-axis is on a log2 scale.

Earthworms are extensively studied in most soil types worldwide and recent efforts are made to group knowledge on online databases to facilitate future synthesis studies on earthworms (Cameron et al. 2016). Drilobase, one of these databases with worldwide data coverage currently reports 23 described families and more than 5000 validated

species (DriloBASE earthworms.info). Currently, several of these earthworm species are reported as invasive species in different parts of the world. European earthworm species for example are invasive in North America. They are reported to clearly modify the forest ecosystem by reducing the forest floor layer (Hale et al. 2006, Maerz et al. 2009, Sackett et al. 2013, Drouin et al. 2016).

2.3 Earthworm anatomy

Earthworms have a tube-within-a-tube body plan; they consist of a digestive tract housed within a thick cylindrical muscular tube that forms the body. Earthworms are segmented worms with a bilaterally symmetric body plan (identical right and left sides) and a hydrostatic skeleton. The first segment encloses the mouth and has a fleshly muscular, sensory lobe on the top (prostomium). It can be pulled in to seal the mouth, or extended forward to probe the immediate surroundings. The last segment at the tail encloses the anus. All segments, except the first, have eight retractable bristles that help the earthworm to grip surfaces as it moves. An earthworm moves by using the anchor function of its bristles and exerting alternating movements of the circular and longitudinal muscles to lengthen and shorten its body segments. As earthworms breath through their skin they need a moist environment to live in. Earthworms are hermaphrodites; consequentially they both possess male and female reproductive organs, as well as a clitellum. The clitellum produces egg cases (cocoons) where the eggs of the worm are fertilised with interchanged sperm.

2.4 Habitat and feeding ecology

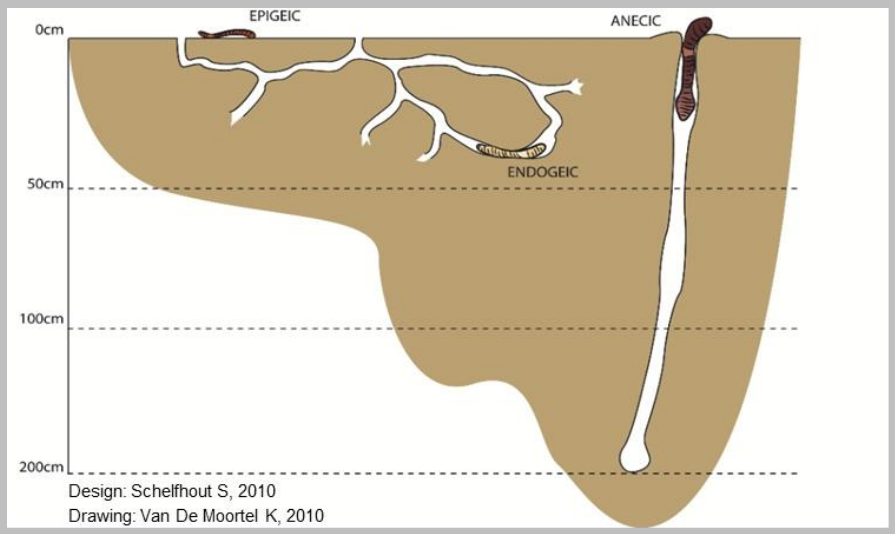
Based on their feeding habits earthworms can be classified into detritivores that feed at or near the soil surface on plant litter and dung, and geophages that feed deeper into the soil on soil organic matter and dead roots ingested with large quantities of mineral soil (Lee 1985). Various subdivisions within these two categories have been proposed. The currently most used classification of Bouché (1977), distinguishes three ecological categories based on morphology, behaviour and physiological characteristics. He divides the detritivores into two groups, the epigeic and anecic and refers to the geophages as endogeic earthworm species. The endogeic species were later further subdivided into polyhumics, mesohumics and oligohumics based on the quality of organic matter they ingest (Lavelle 1980). All these functional groups have a different behaviour, and thus affect ecosystem functioning differently (Lavelle and Spain, 2001). As a result, the simultaneous occurrence of species of several different functional groups can have significant changes in the ecosystem services they provide (Sheehan et al. 2008).

Epigeic earthworms, also called litter dwellers are generally small sized, bright red or reddy-brown pigmented species. These species tend not to make burrows but live within the litter layer or within the uppermost part of the mineral soil. They feed on plant litter and associated bacteria, microfauna and micro-organisms (Dungait et al. 2008, Monroy et al. 2008).

Endogeic earthworms, also called soil dwellers, are intermediate sized, pale coloured earthworms (pale pink, grey or green), that live and feed in a network of semi-permanent sub-horizontal burrows in the mineral soil. They feed on organic material, preferentially colonised by free living soil Protozoa (Bonkowski and Schaefer 1997) and fungi (Moody et al. 1995). Recent research hereby indicated that a large proportion of their diet would consist of organic material from roots (Pollierer et al. 2007) and old carbon resources enclosed in organo-mineral complexes of small particle size fractions (Marhan et al. 2007, Hyodo et al. 2008, Ferlian et al. 2014).

Anecic earthworms, also called deep burrowers are large earthworm species with a dark red or brown-pigmented head end and a pale tail. They live in semi-permanents vertical burrows of one to two meters depth in the mineral soil and feed on leaf litter on the soil surface that they drag into their burrows at night. They cast on the surface and make middens (piles of casts and litter fragments) around the entrance to their burrows (Grigoropoulou and Butt 2010).

Box 2: Ecological categories of earthworms: schematic representation of their microhabitat, species example per category (from left to right: *Dendrobaena octaedra* (Savigny, 1826), *Aporrectodea caliginosa* (Savigny, 1826), *Lumbricus terrestris* (Linnaeus, 1758), list of main characteristics per ecological category of European Lumbricidae (Modified after Valckx 2011).









Characteristic	Epigeic species	Endogeic species	Anecic species
Adult size	Small	Medium	Large
Pigmentation	Heavy, usually both ventrally and dorsally (red or reddy-brown)	Unpigmented or lightly pigmented (pale pink, grey or green)	Medium-heavy, usually only dorsally
Longevity	Relatively short-lived	Intermediate	Relatively long-lived
Growth rate	Fast	Intermediate	Slow
Drought survival	Cocoons	Diapause	Quiescence
Predation	Very high	Low	High
Microhabitat	Litter, bark, dung, organic matter accumulations	Mineral soil (top 10-15 cm of soil profile)	Burrows in the mineral soil (to a depth of 1-3 m)
Burrows	None	Discontinuous (sub)horizontal transient burrows	(Sub)vertical semi-permanent burrows
Food	Decaying litter on the soil surface	Organic matter dispersed in mineral soil	Decaying litter pulled from soil surface into burrows; partially mixed with soil

Most species can be situated in one of the three functional groups, others are somewhere in between having characteristics of more than one functional group (Bouché 1977). In addition, ecological categories and feeding strategies of earthworms have been shown to change during ontogeny (Schmidt et al. 1997, Briones and Schmidt 2004). For instance, many authors (e.g. Bolton and Phillipson 1976, Scheu 1987, Boström 1988, Curry et al. 1995) reviewed by Curry and Schmidt (2007) demonstrated that juvenile earthworms have higher relative soil consumption rates than adults, which reflects their higher energy requirements. At the time of defining these three main ecological categories (epigeic, endogeic and anecic, sensu Bouché (1977)), feeding strategies were mainly based on the material the animals ingest rather than what they assimilate. However, more recent research, making use of stable isotope analysis, generally confirm the conventional ecological classification system (Schmidt et al. 1997, Briones and Schmidt 2004, Ferlian et al. 2014).
In addition to aboveground plant derived material earthworms are known to feed on roots and ingest living micro-organisms, fungi, microfauna, mesofauna and their dead remain (Ferlian et al. 2014). Most earthworm species also ingest mineral soil and seem to prefer mineral-organic mixtures over pure organic sources (Doube et al. 1997). The presence of small sized sand particles in particular may hereby facilitate the assimilation of nutrients from organic material In the case of litter feeding earthworms (Marhan and Scheu 2005) as it probably contributes to the grinding of organic material in the muscular gizzard (Schulmann and Tiunov 1999). Resent research on the assimilation of organic material by earthworm species indicate that earthworms not only ingest but also really utilise bacteria, fungi and protozoa as food resources (Dungait et al. 2008, Xing et al. 2014, Larsen et al. 2016, Steffan et al. 2017); differences in the use of these resources between earthworm functional groups is suggested (Larsen et al. 2016). Regardless of the functional group, all earthworm species seem to like easy accessible food resources such as glucose and make use of mutualistic gut microbial populations to pre-digest organic compounds or as direct food source (Lattaud et al. 1998, Tiunov and Scheu 2004, Sampedro et al. 2006, Curry and Schmidt 2007).

3. Earthworm ecology of European forests

3.1 Environmental factors affecting earthworm communities

3.1.1 *Climate*

Climate affects earthworms both directly, by influencing their physiology and behaviour, as well as indirectly through its influence on their habitat and food supply (see section 2.1.2 and 2.1.3). Temperature is very important, since it determines the

individual earthworm metabolic rate and therefore has a major impact on their activity and distribution. The upper lethal temperature for most earthworms lays between 25-35 °C and the optimal temperature for cool temperate species ranges between 10-20°C (Edwards and Bohlen 1996). Most species cannot tolerate temperatures below 0°C, but some species are reported to have behavioural or physiological adaptations that enables them to survive periods with temperatures below 0°C (Holmstrup and Overgaard 2007).

Temperature is also considered a primary factor influencing earthworm community composition and structure (Lavelle 1983). Endogeic earthworm species are favoured with increasing temperatures, since they can utilize resources of lower quality through more efficient digestive processes involving mutualistic interactions with ingested soil microflora (Lavelle et al. 1995). Hence, in the Mediterranean region large oligohumic earthworm species that are able to feed on soils poor in organic matter are found deep in the soil profile (Bouché 1972, Sbordoni et al. 1992). Furthermore, higher temperatures in a European context can be associated with moisture shortage, and seasonal earthworm mortality in temperate soils has usually been attributed to moisture stress rather than to temperature extremes (Gerard 1967). The optimal soil moisture content varies for different species, even within species there seem to be a capacity to adapt to different moisture regimes (e.g. *Allolobophora chlorotica* (Bouché 1972)). In general earthworms are most active in soils at field capacity (~10kPa), and their activity declines quickly as the moisture tension exceeds 100 kPa and ceases for most species at moisture tensions below the permanent wilting point (1500 kPa) (Curry 2004). Earthworms loose body fluid in periods of drought. But they can sustain extensive water losses, up to 60% of their body weight (Grant 1955), presumably through the production of alanine that helps them cope with the osmotic stress (Holmstrup et al. 2016). Despite this physiological adaptation and the fact that earthworms in strongly seasonal climates have behavioural strategies to cope with drought periods (Bouché 1972), they still suffer heavy mortality. Especially among epigeic species and juveniles unable to escape desiccation by moving deeper into the soil profile (Rundgren 1975). Litter feeding epigeic and anecic species therefore seem to be less abundant in Mediterranean soils compared to temperate soils (Lavelle et al. 1995, Rutgers et al. 2015, De Wandeler et al. 2016).

3.1.2 *Soil properties*

The soil variables that appear to be the most related to earthworm distribution and abundance are soil moisture, texture, pH, depth and organic matter content.

Medium to fine textured soils seem to support higher earthworm abundances than sandy or heavy clay soils (Guild 1948). Within a range of 0-25% clay content, (Nordström and Rundgren 1974) reported a positive significant relationship between

total abundance of earthworms and clay content. They found this relationship for *Aporrectodea caliginosa*, *Aporrectodea rosea* (Savigny, 1826), *Aporrectodea longa* (Ude, 1885) and *Lumbricus terrestris*. Epigeic earthworms, except for *Lumbricus castaneus* (Savigny, 1826), were less influenced by soil texture as they mainly inhabit the organic horizon. Also Cotton and Curry (1980) found higher earthworm densities (max. 516 ind/m²) in loam soils in Co. Wexford Ireland, compared to similarly managed sandy and silty coastal sites (max. 73 ind/m²). Even though soil texture could have a direct effect on earthworm activity in the case of abrasive gravelly soils, its indirect effect on soil moisture and nutrient retention capacity is probably more important (Curry 2004). Heavy clay soils may become anaerobic in areas of high rainfall, and light sandy soils are prone to droughts. Soils with a smaller grain size improve water retention and tend to have a higher cation exchange capacity, which allows a higher retention of cations. In Flemish forests, soils high in clay content therefore generally lead to mull humus formation with a high earthworm diversity and biomass (Muys and Lust 1992).

In general, earthworms are absent in very acidic soils ($pH_{H2O} < 3.5$) and scarce in soils with a pH_{H2O} lower than 4.5. The majority of the earthworm species in temperate forests occurs between pH_{H2O} 5.5 and pH_{H2O} 7.4 (Bouché 1972). The pH tolerance of earthworm species seems to be species specific, but a distinction between functional groups can be made (Fig. 1.2). Epigeic species are in general more acidotolerant, whereas anecic earthworm species prefer generally higher pH ranges. In the endogeic category, most species require a pH_{H2O} well above 4. Overall, we observe a higher earthworm diversity with increasing soil pH (Curry 2004, Ammer et al. 2006, Cesarz et al. 2007, Mueller et al. 2016). Soil pH may also have its influence on earthworm biomass, as low pH values and often associated higher Al^{3+} concentrations can inhibit earthworm growth and cocoon production (van Gestel and Hoogerwerf 2001). The latter is confirmed by Nordström and Rundgren (1974) who found a higher average weight per specimen when pH increased.

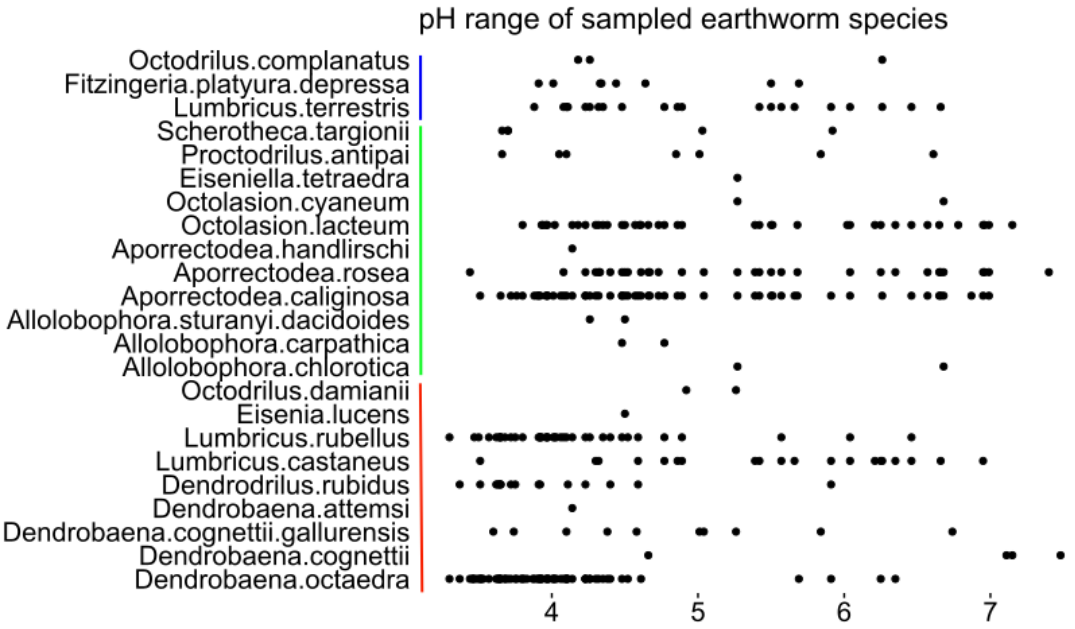


Fig. 1.2. pH ($CaCl_2$) range of the 24 species observed during fieldwork in the framework of this manuscript. Epigeic, endogeic and anecic earthworms are represented in red, green and blue, respectively. Every dot represents an observation, made in one of the 209 plots of the FunDivEUROPE exploratory platform.

Other soil chemical properties that have been linked to earthworm diversity and distributions includes calcium (Mueller et al. 2016), magnesium (Mueller et al. 2016) and nitrogen concentration (Fragoso and Lavelle 1992, Muys and Lust 1992, Resner et al. 2015, Mueller et al. 2016).

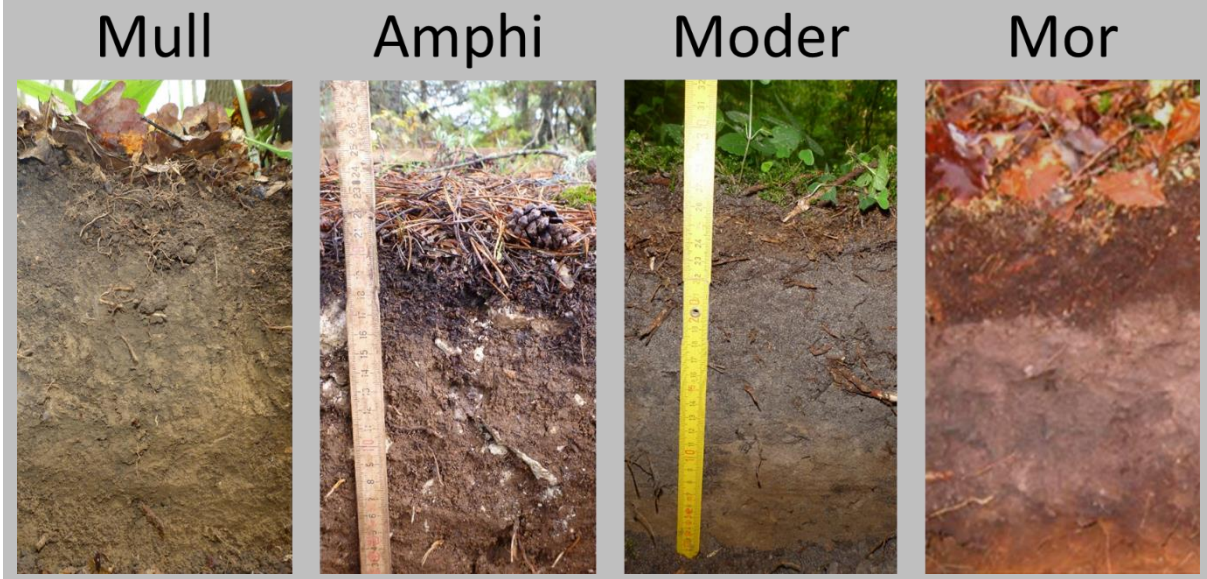
Few is known on earthworm survival and behaviour in water saturated soil. Even though earthworms are known to survive long periods in aerated water (Roots 1956), flooding generally has a negative impact on earthworms (Plum and Filser 2005, Zorn et al. 2005, Ivask et al. 2007). Different earthworm species, however, react differently towards these flooding dynamics (Zorn et al. 2005).

Soil depth is another significant factor influencing earthworm distribution. Shallow soils contain less soil volume per square meter, which result in a smaller living space. Moreover, thin soils promote strong and rapid variations in soil water content and soil temperature. This favours the presence of epigeic earthworm species (r-strategists) over deep burrowing species (K-strategists) (Guenat et al. 1999). Anecic species are consequently related to deeper soils (>40cm) (Guenat et al. 1999, Salomé et al. 2011).

The nature and quality of the soil organic matter are in general largely determined by the litter input from the vegetation (Berg and McClaugherty 2008). Litter from grasses, herbs and deciduous trees on base rich fertile soil has generally a high quality, whereas vegetation on impoverished acidic soils produces tough litter low in nutrients and is

unfavourable for earthworms (Edwards 2004). The soil organic matter is an important factor driving earthworm distributions and abundances as it is one of the major food sources for earthworms (Curry and Schmidt 2007). Soil organic carbon can sometimes be a good predictor of earthworm abundance (Hendrix et al. 1992, Ernst and Emmerling 2009), but is generally more informative when the quality of the organic material is considered (Scheu and Schaefer 1998, Cesarz et al. 2016).
The interplay of both climate, soil texture, soil depth, organic matter quality and earthworm diversity determines which type of humus form will be formed (Muys and Lust 1992, Zanella et al. 2011). The humus form in turn, has certain characteristics that might further influence the earthworm diversity and biomass in European forest floors. Four humus forms can be distinguished in the European forests under study: mull, amphi, moder and mor (Box 3).

Box 3. Representation of the four humus forms that were observed in the forest regions under study. Each humus form can be further subdivided in three to four categories (a second level classification). Subdivisions are made based on the presence and characteristics of the different organic and organo-mineral diagnostic horizons and the abruptness of the transition between organic and organo-mineral horizon. Further details can be found in Zanella et al. (2011).



3.1.3 *Food*

As introduced in paragraph 2.4, earthworms can show different food preferences and feed on different food resources. So there is little doubt that population growth and earthworm diversity are often limited by the nature of the available food resources in

the forest. It is believed that earthworms primarily feed on organic material in and on the soil surface. A significant proportion of this organic material originates from tree and understory plant leaf litter. Current research on earthworm biomass or behaviour in relation to their food therefore mainly focused on plant leaf litter (e.g. Hendriksen 1990, Hobbie et al. 2006, Holdsworth et al. 2012, Rajapaksha et al. 2013, Lincoln 2014, Liebeke et al. 2015, Józefowska et al. 2016, Yatso and Lilleskov 2016).

Many different techniques and experiments were applied to reveal which food related aspects limit earthworm growth and fecundity, and determine palatability: gut content analysis, choice chambers, palatability tests, bait removal methods, and litterbags.

Litter particle size is known to greatly influence the growth rate of *Allolobophora chlorotica* and *Lumbricus terrestris*, presumably because finer material is more readily ingested and assimilated than coarse (Lowe and Butt 2003, Sizmur et al. 2017).

Leaf litter high in nutrient concentration is preferred and promotes earthworm abundance and diversity. Generally, higher N and Ca content and a lower C:N ratio have been associated with increased palatability of leaf litter to earthworms (Rajapaksha et al. 2013, Ashwood et al. 2017b). However, the differences in palatability cannot be explained by leaf nutrient content alone. Factors such as carbohydrate content, lignin concentration and the concentration of phenolic compounds (e.g. tannins) are also reported to reduce litter palatability. The litter structural resistance component, lignin, is generally considered hard to decompose (Berg and McClaugherty 2008), which may result in selective earthworm feeding habits (Crow et al. 2009) and a negative impact on the earthworm growth rate (Kasurinen et al. 2007). However support for a negative relation between leaf litter lignin and earthworm growth rate is inconsistent (Hartenstein 1982, Meehan et al. 2010, Yatso and Lilleskov 2016). Leaf litter chemical defence components like polyphenols are reported to correlate negatively with earthworm growth rate (Kasurinen et al. 2007) and are considered as feeding deterrents for litter feeding earthworms (Satchell and Lowe 1967, Hendriksen 1990, Hättenschwiler and Vitousek 2000). Generally speaking, leaf litter quality matters, but inconsistency between study results might indicate that the sensitivity towards litter quality differs between earthworm species and more in particular, earthworm functional groups. In a litterbag experiment Hendriksen (1990) demonstrated that earthworms prefer certain litter types over others and that detritivores' litter preference was correlated with C:N ratio and the final polyphenol concentration of the litter. In contrast, the geophages did not react to the palatability parameters (C, N, C:N, lignin and polyphenol concentration).

Because of this sensitivity to litter quality variables, fresh litter is usually not preferred by earthworms but must undergo a period of weathering before it is consumed. Factors involved in leaf litter weathering are leaching of feeding inhibitors, softening

of hard tissue and microbial degradation. Microbial flora enhances litter palatability by degrading insoluble components (e.g. cellulose, hemicellulose, and lignin) and thereby simultaneously increase the available nutrient concentrations (Berg and McClaugherty 2008). The mechanisms for this increase in nutrient concentration are still food for research, Innangi et al. (2015) suggests that the increase in N during litter decomposition is due to translocation of this element mainly by fungi from the soil and/or from the more decomposed litter layers. Earthworm feeding behaviour is a complex system and as a consequence still the subject of intense research. Regardless of the capacity of earthworms to protect themselves from the harmful effects of ingested polyphenols by means of surface-active metabolites in their gut (Liebeke et al. 2015), they still seem to be sensitive towards litter components like polyphenols (Hättenschwiler and Vitousek 2000, Kasurinen et al. 2007). Next to that, earthworm preference for litter colonised by specific micro-flora and fauna has been observed (Cooke 1983, Bonkowski and Schaefer 1997, Bonkowski et al. 2000). Litter degradation by micro-flora is complex and varies depending on the environmental conditions (Innangi et al. 2015). It is not sure however what the exact reason is for earthworms to select some fungi over others, but Bonkowski et al. (2000) states that even though fungi can be partly digested by earthworms they function primarily as indicators of food quality. Finally, research by Pollierer et al. (2007) indicate that tree root litter and root exudates seem to be a significant portion of the endogeic earthworm diet.

3.2 Earthworm species distribution

Environmental factors influencing earthworm presence are scale dependent (Schröder 2008), therefore their community composition and distribution can be studied at different spatial scales e.g. local, landscape or continental scale. At the local scale, earthworm incidence and abundance in forests is influenced by soil moisture conditions (Whalen and Costa 2003), soil texture (Fragoso and Lavelle 1987), soil pH (Ma 1984), litter quantity (Jordan et al. 2000, Nachtergale et al. 2002), and litter type (Peterson et al. 2001). At the landscape and continental scale, soil pH (Ammer et al. 2006, Moore et al. 2013) and litter type (Muys and Lust 1992, Vahder and Irmler 2012) remain influential, but variables such as climate (Rutgers et al. 2015), land use history (Räty and Huhta 2004) and dispersal possibilities (Räty and Huhta 2004, Suárez et al. 2006, Shartell et al. 2013) become more important (Lavelle and Spain 2001). Earthworm distribution at local scales has been extensively studied in most terrestrial ecosystems and bioclimatic regions worldwide (e.g. Fragoso and Lavelle 1987, Peterson et al. 2001, Whalen and Costa 2003, Suárez et al. 2006). During the last decade, a next generation of larger-scale studies came about, stimulated by the

availability of new sampling and species identification techniques, more advanced statistical tools and geographical information systems (Decaëns 2010, Birkhofer et al. 2012, Shartell et al. 2013, Pansu et al. 2015).

Earthworm species distribution in Europe is shaped by Quaternary glaciations and subsequent dispersal and redistribution by men (Bouché 1983, Mathieu and Jonathan Davies 2014). The region of the former Last Glacial Maximum refugia, south of the Quaternary ice sheet lines, is characterised by a high earthworm diversity and earthworm species with a very heterogeneous distribution. Several species even have such a narrow range and low dispersal capability that they only occur under very specific environmental conditions and therefore occur on very specific locations like *Allolobophora carpathica* (Cognetti de Martiis, 1927) in the Northern and North-Eastern Carpathians and *Octodrilus aporus* (Pop, 1989) on the limestone patches of the Southern Apuseni Mountains (Csuzdi et al. 2011). In the glaciated regions of north and central Europe, only peregrine species are found. Such species have large ranges, even spanning across multiple continents (Fernández et al. 2016). Their ubiquitous distribution is associated with human activities like agriculture and commerce. Their diversity is much lower than in the south of Europe, (Sims and Gerard 1999) estimated that only 5% of the Lumbricidae species in Europe have spread into northern areas. Examples of such peregrine species are *Lumbricus terrestris*, *L. rubellus* (Hoffmeister 1843) and *Aporrectodea caliginosa*.

The earthworm species composition in a forest is shaped by the local climate, soil features and food supply (Chapter 3.1). Cold conditions, nutrient poor litter and nutrient poor parent material lead to the formation of acidic soils with high forest floor build-up (mor humus). Only a selection of acidotolerant epigeic species are able to live in such an environment (e.g. Dendrobaena octaedra). Nutrient rich vegetation and nutrient rich parent material in a temperate environment lead to a fast organic matter turnover and a thin forest floor (mull humus). Earthworms of all three categories can occur here. Xeric moisture regimes on nutrient rich soil in southern Europe create harsh top soil conditions leading to amphi humus formation with a considerable build-up of organic material. Only few epigeic species can survive in the organic horizon. These amphi soils are characterised predominantly by soil dwelling endogeic species and some large deep burrowing anecic species.
Apart from climate, soil and food supply, biotic interactions between earthworm species and predation on earthworm species will have its influence on the occurrence and abundance of earthworm species (Granval and Muys 1996, Palm et al. 2013).

3.3 Tree species effect on earthworms

Tree species can directly and indirectly influence earthworm communities. Directly by their influence on the food and indirectly by influencing the soil characteristics (pH and nutrient availability, organic matter content and moisture content) and habitat availability. As a result, tree species identity and diversity both directly and indirectly influence earthworm communities.

3.3.1 *Tree species identity effect*

The basis of scientific research on the influence of tree species on the soil characteristics was laid in the nineteen fifties (Muys 1993). In parallel, the first studies describing the relation between earthworms and tree species were published (Wittich and Mascher 1953). After about 70 years of research, we can conclude that tree species identity has an important effect on earthworms. At tree species level, common garden studies using monocultures demonstrated that topsoils under spruce, beech and oak are more acid and can only support a low earthworm abundances and species poor earthworm population compared to topsoils under birch, maple, cherry, lime and ash (Pigott 1989, Muys et al. 1992, Neirynck et al. 2000, Reich et al. 2005, Schelfhout et al. 2017).

To date, these tree species identity effects on decomposers are primarily linked to leaf litter characteristics. Leaf litter can either directly affect earthworms by its nutritional quality, physical toughness or chemical defence compounds (Hendriksen 1990, Rajapaksha et al. 2013, and Paragraph 2.1.3) or indirectly by its influence on soil characteristics such as pH and habitat availability in the forest floor (Aubert et al. 2003, Aubert et al. 2006a), its influence on soil moisture (Christiansen et al. 2010, Augusto et al. 2015) and on C and N stocks (Vesterdal et al. 2008). Tree species with high litter nutrient concentrations and calcium concentration in particular, positively affect earthworm abundance and diversity (Lavelle et al. 1995, Reich et al. 2005, Cesarz et al. 2016), with the exception of N_2-fixing tree species causing high nitrification rates like *Alnus glutinosa* (L.) Gaertn.. Such a high nitrification rates can acidify the topsoil, despite the high nutrient content of alder litter (De Schrijver et al. 2012). Tree species with recalcitrant leaf litter, high in structural compounds like lignin and cellulose, are generally negatively related to earthworm abundance and diversity, they slow down leaf litter decomposition and subsequently acidify the forest floor (De Schrijver et al. 2012, Schelfhout et al. 2017). Planting spruce on mull-soils in southern Sweden for example resulted into a build-up of forest floor material, a decrease in soil pH and a lower earthworm species diversity and abundance, characterised by surface living species (Nordström and Rundgren 1974).

3.3.2 *Tree species diversity effect*

As plant species vary in their litter traits, and in the way that they occupy space and use resources, plant species are likely to have a different influence on the decomposer community when they occur in mixed assemblages. Several mechanisms have been proposed by which decomposers respond to differences in plant species diversity. Plant species diversity can affect the earthworm community by influencing the available plant biomass (resource quantity effect; Zaller and Arnone 1999, Spehn et al. 2000) or the quality of food resources (resource quality effect; Spehn et al. 2000, Milcu et al. 2006). Plant species diversity can also influence the litter layer structure and consequently the microclimate conditions and microhabitat abundance and variety (Hättenschwiler et al. 2005), which is known to increase the abundance and diversity of soil fauna (Hansen and Coleman 1998, Kaneko and Salamanca 1999).

Despite the increasing understanding of tree diversity effects on aboveground biota, research results on the ecological linkage between tree diversity and belowground biota stay inconclusive (Korboulewsky et al. 2016). On top of that, though the number of studies on the effects of mixed forest stands on earthworm communities increases, very few of them were designed to investigate true tree species diversity effects (Korboulewsky et al. 2016). Many reports on diversity effects were from unbalanced studies, such as dilution series of one tree species: dilution of beech (Aubert et al. 2003, Cesarz et al. 2007, Jacob et al. 2009), dilution of Scots pine (Ammer et al. 2006) or dilution of spruce (Elmer et al. 2004, Salamon et al. 2008). In such studies, it is unfortunately not possible to separate diversity from identity effects (Nadrowski et al. 2010). True diversity effects are seldom reported. (Chamagne et al. 2016) indicate positive tree species diversity effects on earthworm species richness, while others found no relation with the earthworm community (Scheu et al. 2003, Schwarz et al. 2015a).

Given that earthworm abundances and species diversity can be influenced by plant species identity and plant functional traits, we may assume that in tree species-rich forests, earthworm abundance and species diversity may be affected by the dominant leaf litter trait values within a tree species community. This hypothesis is related to the 'mass-ratio hypothesis' (Grime 1998) stating that ecosystem processes are mainly determined by the functional identity of the dominant species. The functional identity of the dominant species can be quantified by the community-weighted means (CWM, Garnier et al. (2004)) of tree leaf litter trait values.
Next to that, we could postulate that the common origin of mechanisms behind tree diversity effects stem from the chemical or structural difference among leaves in the

litter mixture rather than from the taxonomic identity of tree species. Therefore, tree species mixtures composed of species having more strongly contrasting chemical or structural differences that affect earthworms (i.e. mixtures having greater functional diversity) may be more likely to provide food provision throughout the year and could lead to spatial heterogeneity in the soil (Aubert et al. 2006b). This spatial heterogeneity may facilitate the co-existence of potentially competing earthworm species and provides suitable conditions for a greater variety of species (Anderson 1978, Ettema and Wardle 2002). This hypothesis is related to the food web theory that predicts increased diversity of higher trophic levels with increased producer diversity. The producer diversity in the context of this study was quantified by the functional dispersion of the tree species community (FDis), as proposed by Laliberté and Legendre (2010), and refers to the diversity of litter types serving different ecological functions in terms of food and structural habitat. Furthermore, we may expect that the relationships of the earthworm abundance and species diversity with the different leaf litter traits could change with environmental conditions, as previously found for the relationship of moisture availability with mites and nematodes (Sylvain et al. 2014).

4. Forests as a laboratory for natural experiments

Ecosystem functions and stability have been shown to strongly benefit from biodiversity (Hector and Bagchi 2007, Morin et al. 2011, Cardinale et al. 2012, Zhang et al. 2012, Isbell et al. 2015). But in forests, research on relationships between biodiversity and ecosystem properties and functioning has for a long time been un(der)explored due to methodological difficulties (Nadrowski et al. 2010). This challenge has been taken up recently with tree species diversity experiments (Verheyen et al. 2016), exploratory platforms in mature forests representing tree species diversity gradients (Baeten et al. 2013, Chamagne et al. 2016) and processing of large forest inventory data sets (Gamfeldt et al. 2013, Vilà et al. 2013, Ruiz-Benito et al. 2014, Liang et al. 2016, Ratcliffe et al. 2016).

Michael Scherer-Lorenzen gave a detailed overview and reasoning on the why, where, when and how of tree diversity research both in experimental as well as in observational platforms (Scherer-Lorenzen et al. 2005, Scherer-Lorenzen 2013). In this section, I used this documentation as a backbone to introduce you in this matter.

4.1 Experimental studies on tree diversity

The first real manipulative tree diversity experiments were initiated about two decades ago. But the question of whether tree diversity influences forest properties already dates back to the beginning of the eighteenth century and found its origin in the

pursuit for sustainable wood production (Pretzsch 2005). It took 150 more years, at the end of the nineteenth century, before the first diversity experiments with one- and two-species mixtures were established (Scherer-Lorenzen 2013). Most of these experiments however, were with commercially important tree species and they consequentially strongly differed from natural forests in structure and function. Later, during the late twentieth century, concerns about the ecological sustainability of monocultures and their resistance and resilience to perturbations raised interest in mixed tree communities in forestry (Scherer-Lorenzen 2013). This debate, further stimulated by policy processes and the successful diversity experiments with grassland species, and terrestrial and aquatic microcosms (Loreau et al. 2001) paved the way and reasoned the establishment of the first manipulative tree diversity experiments (Scherer-Lorenzen et al. 2005). In the course of the last 17 years many more initiatives followed resulting in the largest network of biodiversity experiments worldwide (Verheyen et al. 2016). The TreeDivNet currently counts 25 experiments distributed across major forest types around the world (TreeDivNet www.treedivnet.ugent.be). These experiments allow the study of biodiversity on ecosystem properties and functioning in a forest context. Moreover, the design details of those experiments differ which allows that several different tree diversity aspects can be studied: species richness, functional diversity, genetic diversity, phylogenetic diversity and evenness.

The strength of these manipulative tree diversity experiments is that they allow to test effects of tree diversity per se and for causality or underlying mechanisms of biodiversity-ecosystem functioning relationships, because the experimental design of such experiments allows to control for the levels and range of tree diversity and allows accounting for potentially confounding differences in land use history and local environmental conditions. Furthermore, also the management of the experimental site can be fully controlled. However, most of these experiments are on soils with a non-forest land-use history and are still too young limiting their representativeness for mature stands. Recent research on abandoned arable land, by Morriën et al. (2017), support this hypothesis by proving that the efficiency of carbon uptake by fungi increases in time. Therefore, observational platforms on tree diversity, providing real-world reference data, were realised.

4.2 Observational studies on tree diversity

Observational studies on tree diversity compare natural forest stands differing in tree diversity. Two different approaches of observational studies along longer gradients of tree diversity exists: (1) sample surveys with a random or grid-based selection of plots across a specific region (e.g. forest inventories), and (2) comparative studies where a

similar number of mature stands per diversity level are deliberately chosen with comparable environmental conditions and management regimes.
Studies on both approaches are fairly recent and date from the beginning of the twenty-first century (Scherer-Lorenzen 2013), even though many permanent monitoring plots included in inventory studies exist already much longer (Tomppo et al. 2010). The advantage of observational studies is that they can use mature, relatively undisturbed forest with a near-natural canopy and root system architecture, intact food web structure and quasi steady-state carbon and nutrient stocks in soil and biomass. The disadvantages of such studies are the practical difficulty of finding suitable tree species combinations to fulfil all criteria and the potential occurrence of several confounding factors such as land use history, past management, soil type and hydrological conditions. This implies that observational studies only have a correlative nature and that building models is challenging because of the many known and unknown confounding variables.
To minimize these issues in observational studies on forest diversity, (i) maximization of tree important criteria is strived for in the design of the observational platform: orthogonality, comprehensiveness, and representativeness (Nadrowski et al. 2010). (ii) Confounding variability is accounted for in the modelling by explicitly including site conditions as covariates. Examples of large-scale observational studies on inventory data are Enquist and Niklas (2001), Paquette and Messier (2011) and Gamfeldt et al. (2013). Examples of diversity studies on comparative forest research platforms are (Leuschner et al. 2009) in central European beech forest, (Brassard et al. 2011, Brassard et al. 2013) in boreal forest, (Brezzi et al. 2017, Wang et al. 2017) in Chinese subtropical forest. In 2011, a European continental-scale forest research platform (FunDivEUROPE) was established (Baeten et al. 2013). The research in this manuscript results from data that is collected in this European research platform.

5. About this thesis

5.1 Problem statement

Due to the important influence of earthworms on the structure, composition and functioning of forest ecosystems, earthworm distribution at local scales has been studied in depth in most terrestrial ecosystems and bioclimatic regions worldwide (e.g. Fragoso and Lavelle 1987, Peterson et al. 2001, Whalen and Costa 2003, Suárez et al. 2006). However, little is known on the factors controlling their distribution across larger geographical ranges. Recently, optimised sampling techniques, as well as more advanced statistical tools and geographical information systems have facilitated studies at the landscape scale (Decaëns 2010, Birkhofer et al. 2012, Shartell et al. 2013, Pansu

et al. 2015). These, and even larger-scale studies, however, are scarce because standardised sampling executed over a large scale is costly, logistically, and often physically, demanding. An alternative solution for large-scale studies could be the compilation of datasets from different studies. However compiling existing databases is often difficult due to data limitations, taxonomic inconsistencies and practical issues with linking and transferring databases (Cameron et al. 2016).

At present the influence of tree species identity and diversity on forest ecosystem functioning is widely accepted. About 70 years of research led to the general acceptance that tree species identity greatly influences forest floor quality. More recently, tree species diversity is proven to support forest ecosystem functions, like primary production (Morin et al. 2011, Paquette and Messier 2011, Liang et al. 2016), stability of wood production (Jucker et al. 2014), resistance to biotic and abiotic disturbances (Pretzsch et al. 2013, Jactel et al. 2017) and nutrient cycling (Richards et al. 2010, Handa et al. 2014). Next to these aboveground biotic factors, also earthworms can play a key role in forest ecosystem functioning. Consequently, it is important to understand the influence of tree species identity and diversity on earthworm abundance and composition. However, up until now the mechanisms driving tree species identity effects on belowground biota are not sufficiently understood and most studies investigating tree species diversity effects failed to disentangle the confounding effect of tree species identity from tree species diversity effects on belowground biota.
Finally, earthworms are called ecosystem engineers because they can make state changes in biotic or abiotic material in the soil system (Jones et al. 1994, Lemtiri et al. 2014), and these changes in turn may feedback to the ecosystem engineer. With the growing literature on these individual cause-effect relationships between earthworms and soil physics or soil chemistry, there is an emerging opportunity towards an evaluation of the existence and mechanisms of an earthworm-soil feedback loop. To date however, only few studies have made suggestions towards the existence and mechanisms of an earthworm-soil feedback loop, or have studied the full loop of interlinked cause-effect relationships between soil ecosystem engineers, soil physics and soil chemistry, let alone investigated how external factors could influence this loop. Furthermore quantifying the relative significance of the different relationships in this complex network of aboveground-belowground interactions is of paramount importance for present and future land management.

5.2 Aim and objectives

The goal of this thesis was to study earthworm communities in European forests and their relation with tree communities. I aimed at relating aboveground ecosystem engineers (i.e. trees), which create the forest microclimate, regulate subcanopy light dynamics, energy, temperature, moisture, and produce large amounts of detritus, with the belowground ecosystem engineers (i.e. earthworms), who decompose and mix this detritus into soil humus.

Within this research project, I identified and resolved existing knowledge gaps by:

- identifying and evaluating the relative importance of a set of abiotic and biotic variables in explaining earthworm occurrence and abundance in European forests (Chapter 2);
- assessing the influence of tree species diversity and identity on earthworm abundance and diversity (Chapter 3);
- investigating how tree litter characteristics and functional diversity influence a hypothesised earthworm-soil feedback loop between earthworm biomass, humus quality and soil pH (Chapter 4).

5.3 Study area

Earthworm communities were studied using a European continental-scale forest research platform (FunDivEUROPE) spanning six major forest types. The six studied regions span most of the European bioclimatic gradient and represent major European forest types including boreal forest (North Karelia, Finland), temperate mixed coniferous and broadleaf forest (Białowieża, Poland), temperate deciduous forest (Hainich, Germany), mountainous deciduous forest (Râsça, Romania), thermophilous deciduous forest (Colline Metallifere, Italy) and Mediterranean mixed forest (Alto Tajo, Spain) (Fig. 1.3). The research platform was specially designed to test biodiversity-ecosystem function relationships along a tree species richness gradient (Baeten et al. 2013). Each studied region includes between 28 and 43 selected plots (30 x 30 m) with different combinations of a fixed set of locally dominant tree species (the so-called target species). The established plots ranged in tree species richness from one to five species per plot (Table 1.1).

More details, including an environmental description, of each studied forest region is given in the following subsections. All information originates from FunDivEUROPE datasheets and reports.

Even though this research platform was specially designed to research diversity effects, it also lends itself to study related research questions. Being part of this large research project on the functional significance of diversity in European forests allowed the integration of detailed local information on several aspects of the forest ecosystem, collected by fellow scientists during the same period. Information that would not be able to be collected with the budget and within the timeframe of a single PhD study.

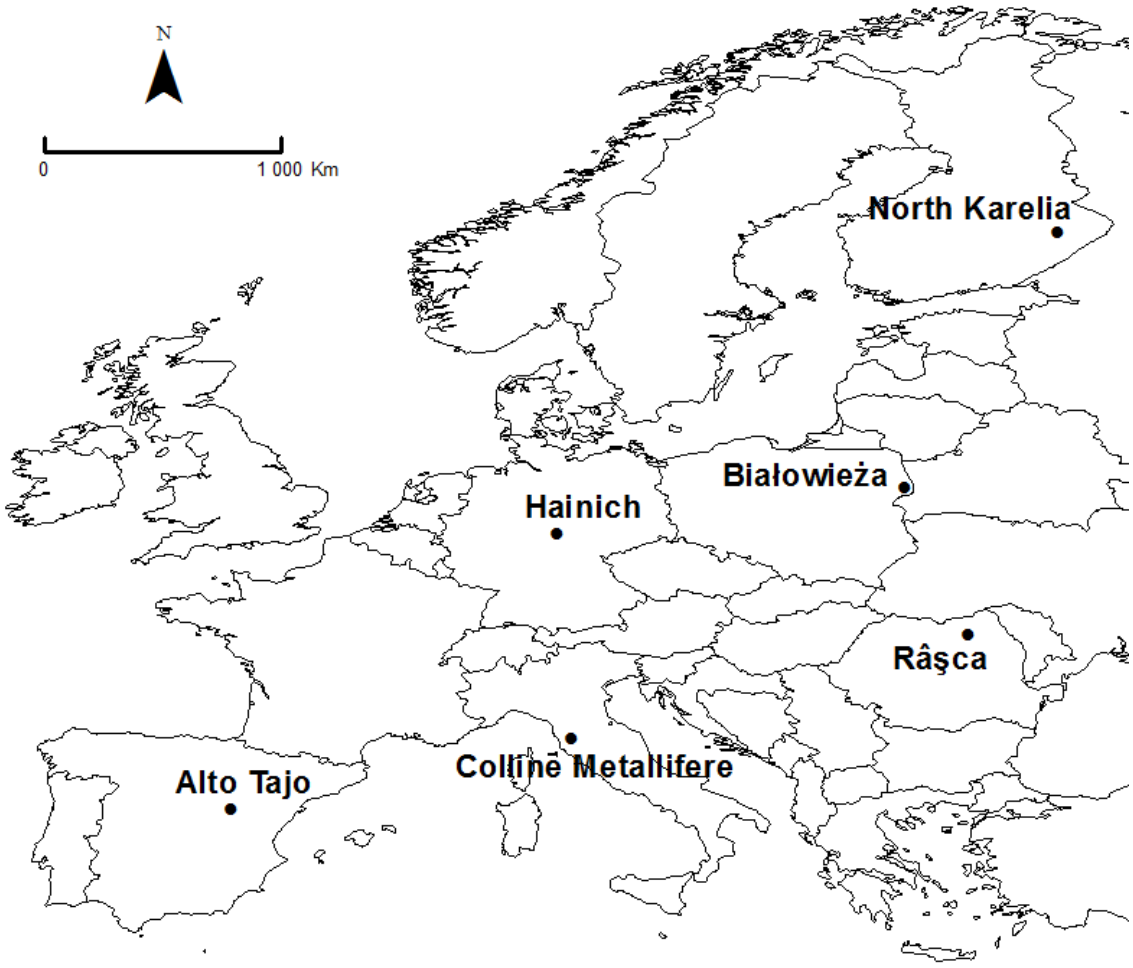


Fig. 1.3. Location of all six sampled forest regions.

Table 1.1. Description of the six exploratory regions and region-specific design, including the pool of species used to create a gradient in species diversity (Modified from Baeten et al. (2013)).

	Finland	Poland	Germany	Romania	Italy	Spain
(a) Site description						
Location	North Karelia	Białowieża	Hainich	Râșca	Colline Metallifere	Alto Tajo
Latitude/longitude (degree)	62.6, 29.9	52.7, 23.9	51.1, 10.5	47.3, 26.0	43.2, 11.2	40.7, -1.9
*Forest type	Boreal	Hemiboreal, nemoral coniferous, mixed broadleaved-coniferous	Beech	Mountainous beech	Thermophilous deciduous	Mediterranean mixed
†MAT, MAP	2.1 °C, 700 mm	6.9 °C, 627 mm	6.8 °C, 775 mm	6.8 °C, 800 mm	13 °C, 850 mm	10.2 °C, 499 mm
‡Topography, altitude	Flat, 80-200 m	Flat, 135-185 m	Mainly flat, 500-600 m	Medium-steep slopes, 600-1000 m	Medium-steep slopes, 260-525 m	Flat-medium slopes, 960-1400 m
Study area size (km ×km)	150 ×150	30 ×40	15 ×10	5 ×5	50 ×50	50 ×50
Responsible site manager	L. Finér	B. Jaroszewicz	H. Bruelheide	O. Bouriaud	F. Bussotti	F. Valladares
¶Stand developmental stage						
(1) Mid/late stem exclusion	x	x		x	x	x
(2) Understory reinitiation	x	x	x	x		x
(3) Old growth			x			
Tree cohorts	Single	Multiple	Multiple	Single	Multiple	Multiple
(b) Platform design						
Species richness levels	3	5	4	4	5	4
Number of plots	28	43	38	28	36	36
Plots per richness level	11/14/3	6/11/13/11/2	6/14/14/4	8/10/8/2	9/10/9/7/1	11/18/4/3
Species pool:						
(1) Coniferous						
Abies alba						
Picea abies	×	×	×	×	×	×
Pinus nigra						
Pinus sylvestris	×	×				×
(2) Broadleaved						
Acer pseudoplatanus			×	×		
Betula pendula/pubescens						
Carpinus betulus		×				
Castanea sativa						
Fagus sylvatica			×	×		
Fraxinus excelsior			×			
Ostrya carpinifolia					×	
Quercus robur/petraea					×	
Quercus cerris					×	
Quercus faginea						×
Quercus ilex (evergreen)						×

*Categories of the European Environment Agency (EEA, 2007); †MAT: mean annual temperature, MAP: mean annual precipitation; ‡Altitude in meters above sea level; ¶ Stand developmental stages according to Oliver and Larson (1996).

5.3.1 *North Karelia, Finland*

Fact sheet – studied forest region

Forest type	Boreal forest
Species richness levels:	3
Total number of plots:	28
Study area size:	ca 150 km x 150 km
Total number of trees measured:	2092
Bedrock type	Mica schists, granites and granodiorites, quartzites
Soil type	Podzol (partly poorly developed)
Species pool:	*Picea abies, Pinus sylvestris, Betula pendula/pubescens*

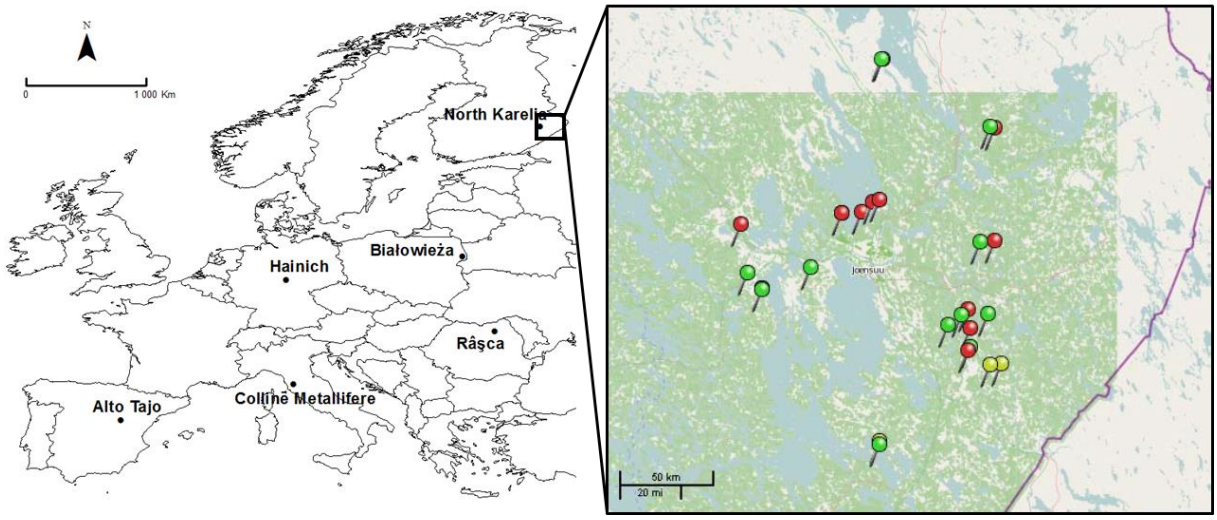


Fig. 1.4. Distribution of the 28 exploratory plots in Finland. Colours indicate different species richness levels: monoculture (red pin), 2 species mixture (green pin), 3 species (yellow pin). © OpenStreetMap contributors, CC-BY-SA.



Fig. 1.5. Impression of some exploratory plots in Finland (left: plot FIN05 - Two tree species diversity plot of *Pinus sylvestris* and *Betula pendula*, right: plot FIN09 – monoculture *Pinus sylvestris*).

5.3.2 *Białowieża, Poland*

Fact sheet – studied forest region

Forest type	
Species richness levels:	5
Total number of plots:	43
Study area size:	ca 30 km x 40 km
Total number of trees measured:	1771
Bedrock type	Fluvioglacial (sands, clays, loams)
Soil type	Cambisols and Luvisols
Species pool:	*Betula pendula, Carpinus betulus, Picea abies, Pinus sylvestris, Quercus robur*

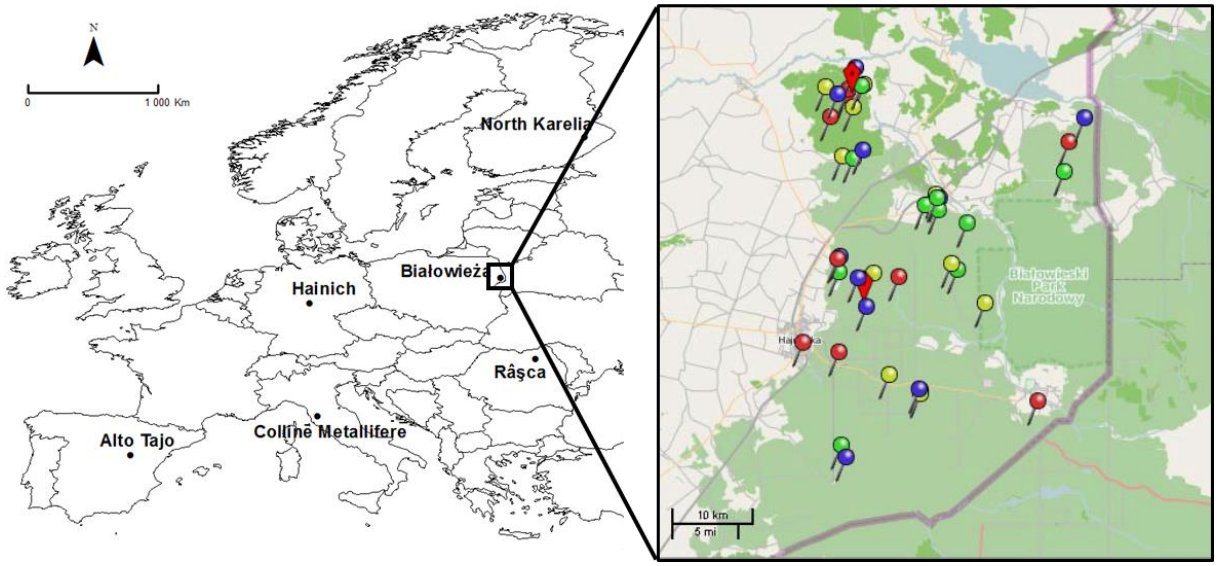


Fig. 1.6. Distribution of the 43 exploratory plots in Poland. Colours indicate different species richness levels: monoculture (red pin), 2 species mixture (green pin), 3 species (yellow pin), 4 species (blue pin), 5 species (red rhomb). © OpenStreetMap contributors, CC-BY-SA.



Fig. 1.7. Impression of some exploratory plots in Poland (left: plot POL03 - monoculture *Picea abies*, right: plot POL05 – Two tree species diversity plot of *Carpinus betulus* and *Quercus robur*).

5.3.3 *Hainich, Germany*

Fact sheet – studied forest region

Species richness levels:	5
Total number of plots:	38
Study area size:	ca 15 km x 10 km
Total number of trees measured:	1934
Bedrock type	Muschelkalk
Soil type	Luvisols
Species pool:	*Fagus sylvatica*, *Fraxinus excelsior*, *Quercus species* (mainly *petraea*) ,*Picea abies*, *Acer pseudoplatanus*

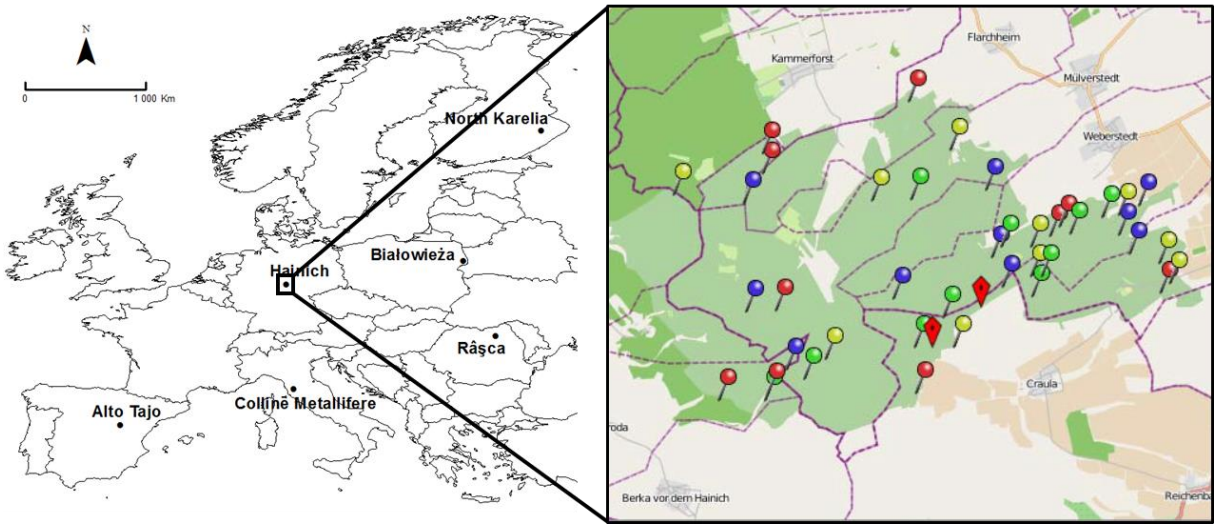


Fig. 1.8. Distribution of the 38 exploratory plots in Germany. Colours indicate different species richness levels:monoculture (red pin), 2 species mixture (green pin), 3 species (yellow pin), 4 species (blue pin), 5 species (red rhomb). © OpenStreetMap contributors, CC-BY-SA.



Fig. 1.9. Impression of some exploratory plots in Germany (left: plot GER13 – Three species diversity plot *Fraxinus excelsior*, *Quercus petraea* and *Acer pseudoplatanus*, right: plot GER35 – Two tree species diversity plot of *Fagus sylvatica* and *Quercus petraea*).

5.3.4 *Râsça, Romania*

Fact sheet – studied forest region

Species richness levels:	4
Total number of plots:	28
Study area size:	ca 5 km x 5 km
Total number of trees measured:	1343
Bedrock type	sandstone (+marl)
Soil type	Eutric cambisol
Species pool:	*Picea abies, Abies alba, Fagus sylvatica, Acer pseudoplatanus*

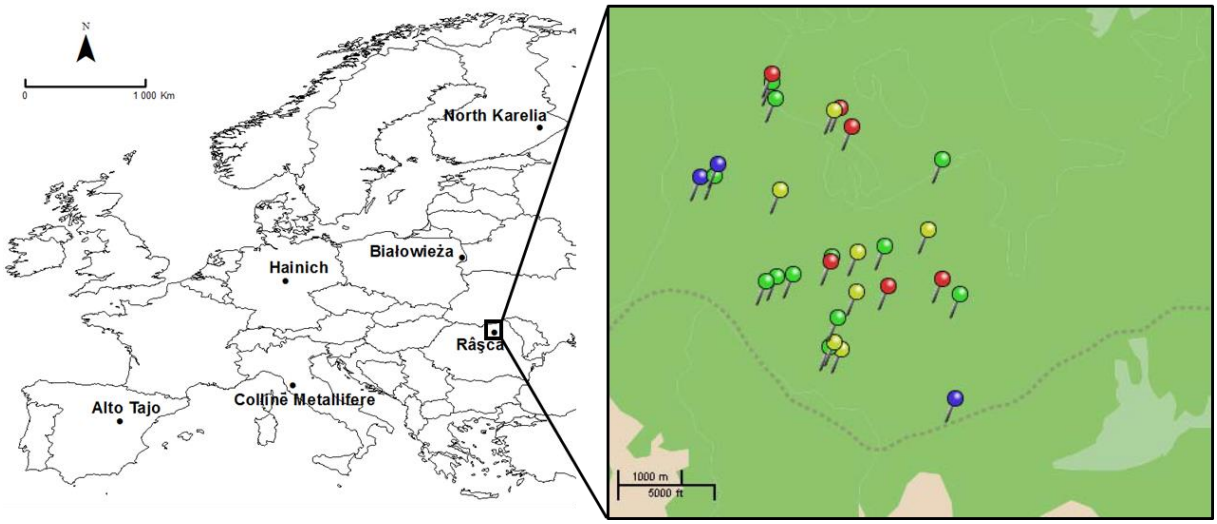


Fig. 1.10. Distribution of the 28 exploratory plots in Romania. Colours indicate different species richness levels: monoculture (red pin), 2 species mixture (green pin), 3 species (yellow pin), 4 species (blue pin). © OpenStreetMap contributors, CC-BY-SA.



Fig. 1.11. Impression of some exploratory plots in Romania (left: plot ROM28 – Two tree species diversity plot *Abies alba* and *Fagus sylvatica*, right: plot ROM08 – Four tree species diversity plot of *Picea abies*, *Abies alba, Fagus sylvatica* and *Acer pseudoplatanus*).

5.3.5 *Colline Metallifere, Italy*

Fact sheet – studied forest region

Species richness levels:	5
Total number of plots:	36
Study area size:	ca 50 km x 50 km
Total number of trees measured:	2364
Bedrock type	quartzite-anagenite (verrucano), sandstone conglomerate
Soil type	Cambisol
Species pool:	*Quercus cerris, Quercus ilex, Castanea sativa, Quercus petraea, Ostrya carpinifolia*

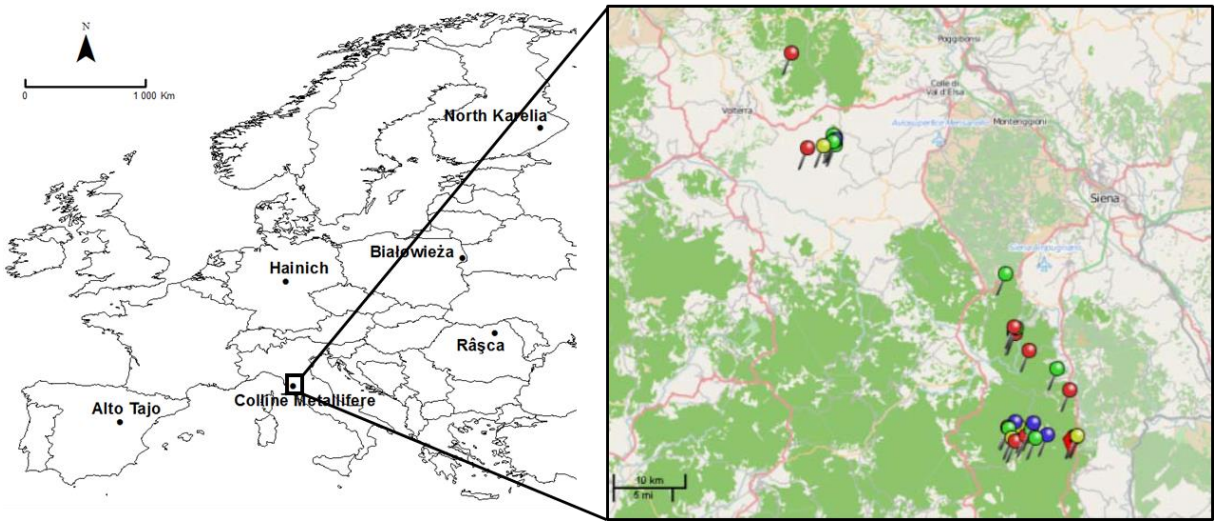


Fig. 1.12. Distribution of the 36 exploratory plots in Italy. Colours indicate different species richness levels: monoculture (red pin), 2 species mixture (green pin), 3 species (yellow pin), 4 species (blue pin), 5 species (red rhomb). © OpenStreetMap contributors, CC-BY-SA.



Fig. 1.13. Impression of some exploratory plots in Italy (left: plot ITA25 - Three tree species diversity plot of *Quercus ilex*, *Castanea sativa and Ostrya carpinifolia*, right: plot ITA35 – monoculture *Quercus cerris*).

5.3.6 *Alto Tajo, Spain*

Fact sheet – studied forest region

Species richness levels:	4
Total number of plots:	36
Study area size:	ca 50 km x 50 km
Total number of trees measured:	3028
Bedrock type	quartzite-anagenite (verrucano), sandstone conglomerate
Soil type	Cambisol
Species pool:	*Pinus sylvestris, Pinus nigra, Quercus faginea, Quercus ilex*

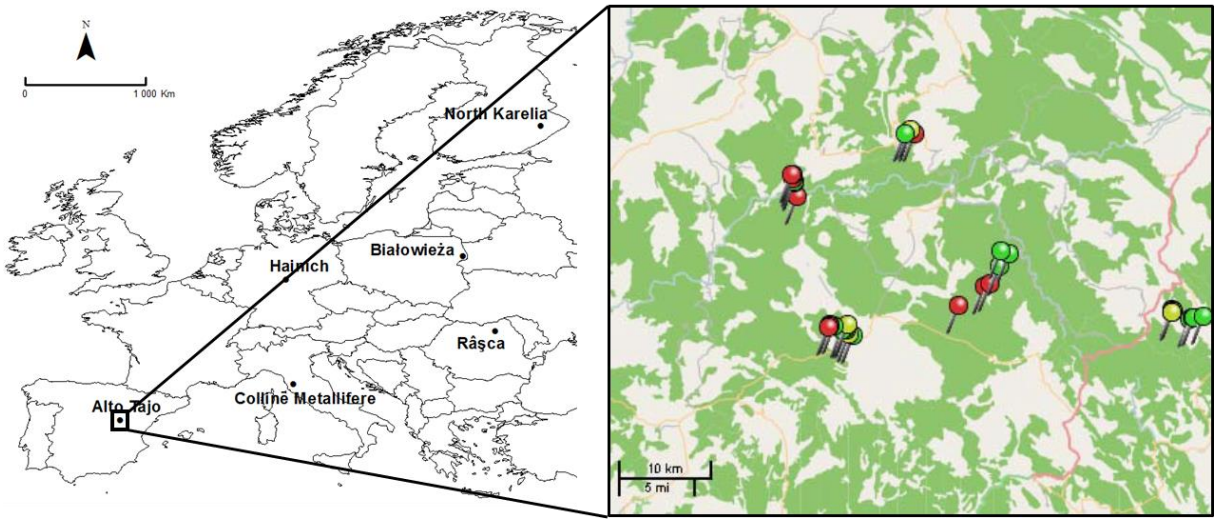


Fig. 1.14. Distribution of the 36 exploratory plots in Spain. Colours indicate different species richness levels: monoculture (red pin), 2 species mixture (green pin), 3 species (yellow pin), 4 species (blue pin). © OpenStreetMap contributors, CC-BY-SA.



Fig. 1.15. Impression of some exploratory plots in Spain (left: plot SPA11 - monoculture *Quercus faginea*, right: plot SPA14 – Two tree species diversity plot of *Pinus nigra* and *Quercus ilex*).

5.4 Outline

Chapter 2 identifies and evaluates the relative importance of a set of abiotic and biotic environmental factors in explaining earthworm incidence and abundance in European forests. I evaluated both the incidence and abundance of earthworm communities and the different functional groups of earthworms (epigeic, endogeic and anecic). By collecting the earthworm data in a single study in the same standardized way, I was able to conduct this research on a continental scale, which is to date still very seldom in soil fauna research. As our sampling locations were spread along a latitudinal gradient from the boreal to the Mediterranean region in Europe, covering distinct climate types, we hypothesised that earthworm community characteristics are primarily driven by climatic factors.

As Chapter 2 evaluates the main environmental factors that drive earthworm abundance at a continental scale, **chapter 3** extends on chapter 2 by evaluating the response and relative importance of different factors of tree diversity and tree species identity on earthworm biomass and diversity. This study takes advantage of the FunDivEUROPE exploratory platform, which is specially designed to explore the relationship between biodiversity and ecosystem functioning. I investigated how tree diversity and proportion of evergreen leaf litter influences earthworm abundance and diversity. Furthermore, I analysed which tree leaf litter traits affect earthworm abundance and diversity and whether their relative importance changes along a latitudinal gradient.

Chapter 4 investigated how tree litter characteristics and tree functional diversity influence a hypothesised 'earthworm-soil feedback loop'. We hypothesised that by influencing the humus form quality and subsequently the soil acidity, changes in soil pH may feedback to earthworm activity. This chapter further extends on chapter 2 and 3 by quantifying the influence of tree litter characteristics and functional diversity on earthworm driven belowground dynamics. This plant-soil relationship is hypothesised to occur independently from forest biome type and is therefore analysed on a continental scale across all six forest regions that were included in the FunDivEUROPE observational platform.

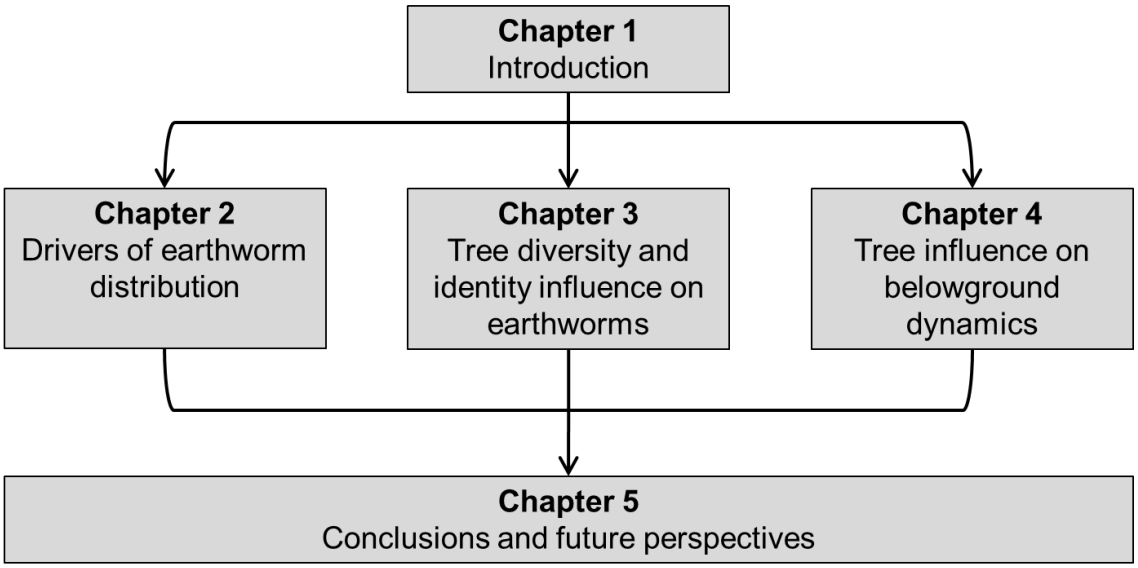


Fig. 1.16. General overview of the structure of the PhD dissertation.

5.5 Earthworm community sampling

Earthworm community sampling for scientific research is generally done by hand sorting of a litter sample and a soil monolith, combined with an ethological method, such as formalin or mustard extraction (ISO 23611-1 standard, see Römbke et al. 2006, Jeffery et al. 2010, Valckx et al. 2011). Despite the existence of an official standardized earthworm sampling method (ISO 23611-1) variation in sampling techniques exist, depending on: the research budget, the sampling objective, sampling effort, site conditions and the research team background and experience. The earthworm data gathered for this thesis are sampled by means of a method developed for forest research in the framework of the FunDivEUROPE project and is based on literature and past field experience.

Earthworms were sampled in tree steps (Fig. 1.17). First, the litter dwelling earthworms were collected by hand sorting a litter sample over an area of 25 x 25 cm. Second, litter was removed over a larger area of 100 x 50 cm in order to effectively apply an ethological extraction of earthworms using a mustard suspension to focus on burrowing earthworms. Thirdly, hand sorting of a soil sample of 25 x 25 cm and 20 cm depth was performed in the middle of the 100 x 50 cm area to sample remaining burrowing earthworms that were not caught with the ethological extraction.

I

Litter sampling



Litter sample
(25 x 25 cm)

Hand sorting



Hand sorting of
litter sample

II

Mustard extraction



Sprinkle mustard suspension
(100 x 50 cm)



Capture earthworms
after each mustard sprinkling

III

Soil sampling



Soil sample
(25 x 25 x 20 cm)

Hand sorting



Hand sorting of
soil sample

Fig. 1.17. Earthworm sampling procedure

Earthworms were preserved in ethanol (70%) for two weeks, transferred to a 5% formaldehyde solution for fixation (until they reached a constant weight), after which they were transferred back to ethanol (70%) for further preservation. More information can be found in the FunDivEUROPE earthworm sampling field protocol (Muys et al. 2013).

Chapter 2

Drivers of earthworm incidence and abundance across European forests

Adapted from: Hans De Wandeler, Rita Sousa-Silva, Evy Ampoorter, Helge Bruelheide, Monique Carnol, Seid M. Dawud, Gabriel Dănilă, Leena Finér, Stephan Hättenschwiler, Martin Hermy, Bogdan Jaroszewicz, François-Xavier Joly, Sandra Müller, Martina Pollastrini, Sophia Ratcliffe, Karsten Raulund-Rasmussen, Federico Selvi, Fernando Valladares, Koenraad Van Meerbeek, Kris Verheyen, Lars Vesterdal, Bart Muys. Drivers of earthworm incidence and abundance across European forests. Soil Biology and Biochemistry, 99, 167-178

1. Introduction

Earthworms are known as ecosystem engineers modifying the physical, chemical and biological soil properties (Blouin et al. 2013). They contribute to ecosystem functioning by changing soil porosity, by controlling the rate of organic matter decomposition and nutrient release, and consequently also indirectly influence primary production (Scheu and Wolters 1991, Lavelle and Spain 2001, Scheu 2003, Edwards 2004). Based on their ecology, Bouché (1977) classified earthworm species into three ecological groups, namely epigeic, endogeic and anecic earthworms. These functional groups have a different behaviour, and thus affect ecosystem functioning differently (Lavelle and Spain 2001). Epigeic earthworms are rather small-sized species that live within the litter layer on the soil surface or within the uppermost part of the mineral soil and they feed on plant litter. Endogeic earthworms are geophagous species of generally intermediate size that live in a network of subhorizontal burrows in the mineral soil. Anecic earthworms finally, are large earthworm species that live in deep (semi)permanent vertical burrows in the mineral soil and feed on leaf litter that they drag into their burrows. Representing the greatest animal biomass component in most European forest soils (Lavelle and Spain 2001), earthworm community composition and activity can have major consequences for the structure, composition and functioning of forest ecosystems (Hale et al. 2006, Lukac and Godbold 2011). It is therefore highly relevant to know how environmental variables affect the incidence and abundance of earthworms (Schröder 2008).

The composition of earthworm communities and their distribution can be studied at different spatial scales, e.g. local, landscape or continental scale. It is likely that factors influencing earthworm presence are scale-dependent (Schröder 2008). At the local scale, earthworm incidence and abundance in forests has been shown to be influenced by soil moisture conditions (Whalen and Costa 2003), soil texture (Fragoso and Lavelle 1987), soil pH (Ma 1984), litter quantity (Jordan et al. 2000, Nachtergale et al. 2002), and litter type (Peterson et al. 2001). At the landscape and continental scales, soil pH (Ammer et al. 2006, Moore et al. 2013) and litter type (Muys and Lust 1992, Vahder and Irmler 2012) remain influential, but variables such as climate (Rutgers et al. 2015), land use history (Räty and Huhta 2004) and dispersal possibilities (Räty and Huhta 2004, Suárez et al. 2006, Shartell et al. 2013) become more important (Lavelle and Spain 2001). Earthworm distribution at local scales has been studied in depth in most terrestrial ecosystems and bioclimatic regions worldwide (e.g. Fragoso and Lavelle 1987, Peterson et al. 2001, Whalen and Costa 2003, Suárez et al. 2006). More recently, a next generation of larger-scale studies has been stimulated by the availability of new sampling and species identification techniques, more advanced statistical tools and geographical information systems (Decaëns 2010, Birkhofer et al. 2012, Shartell et

al. 2013, Pansu et al. 2015). Despite these recent advancements and the importance for synthetic data analysis at larger spatial scales in soil ecology, only a few studies on earthworm distribution have exceeded the landscape scale (Lindahl et al. 2009, Decaëns 2010, Rutgers et al. 2015). Studies at larger scales can provide new insights about the drivers of the abundance and composition of soil macrofauna communities, but these are challenging for a number of reasons (Schröder 2008, Cameron et al. in press). A standardised sampling executed over a large scale is costly, logistically, and often physically, demanding. Compiling datasets from different studies could therefore be an alternative solution. However, as Cameron et al. (in press) summarise, compiled datasets often suffer from data limitations due to differing sampling techniques, taxonomic inconsistencies and practical issues with linking and transferring databases.

In this paper, we present the results of a continental-scale field-based study on earthworm incidence and abundance in mature forests. The objective of this research was to identify and evaluate the relative importance of a set of abiotic environmental variables (climate and soil parameters) and biotic variables such as vegetation composition and forest stand characteristics in explaining earthworm incidence and abundance in European forests. We evaluated the incidence and abundance of earthworm communities and the different functional groups of earthworms (epigeic, endogeic and anecic). As our sampling locations were spread along a latitudinal gradient from boreal to Mediterranean regions in Europe, covering distinct climate types (Peel et al. 2007), we hypothesised that earthworm community characteristics are primarily driven by climatic factors. Furthermore, since the three earthworm functional groups occupy different niches in the forest soil system and have distinct feeding behaviours (Bouché 1977, Sheehan et al. 2008, Ferlian et al. 2014), we expected that the relative importance of the different control factors would differ between the three functional groups. We hypothesised that the incidence and abundance of endogeic species would primarily be explained by soil quality related factors, while that of epigeic and anecic species would be more influenced by litter quality parameters. This study extends on previous research at the landscape scale (Palm et al. 2013, Shartell et al. 2013, Marchán et al. 2015) by investigating earthworm community characteristics in mature forests at a continental scale. This research overcomes most of the aforementioned problems with large-scale data sets, as all data were collected within a single study in the same standardised way, species were identified to the same taxonomic level and compiled into one single database.

2. Material and methods

2.1 Study sites

Sampling took place in the exploratory platform of the FunDivEUROPE project (Baeten et al. 2013). This platform was designed to assess biodiversity-ecosystem function relationships along a tree species richness gradient in mature forests. The six studied forest landscapes (hereafter called sites) in this platform span most of the European bioclimatic gradient and represent major European forest types including the boreal forest (North Karelia, Finland), hemiboreal forest (Białowieża, Poland), temperate beech forest (Hainich, Germany), mountain beech forest (Râsça, Romania), thermophilous deciduous forest (Colline Metallifere, Italy) and Mediterranean-mixed forest (Alto Tajo, Spain) (Appendix A2, Fig. S1).

Each study site included between 28 and 43 selected plots (30 x 30 m) with different combinations of a fixed set of locally dominant tree species. The established plots ranged in tree species diversity from one to five species per plot. Tree diversity however had no relevant effect on any of the earthworm variables and is therefore not further discussed. In total, the platform consisted of 209 plots with 16 target tree species, some of them occurring in multiple sites. The species pool comprised of conifers, deciduous broadleaves and evergreen broadleaves. For more details consult Baeten et al. (2013).

2.2 Field sampling

Earthworm sampling was carried out in spring 2012 for the sites in Italy, Germany and Finland, and in autumn 2012 for the sites in Poland, Romania and Spain. As advised in literature, we targeted our earthworm sampling in spring or autumn because of the humid soil conditions and positive temperatures (Berry and Jordan 2001, Holmstrup 2001, Baker and Whitby 2003, Eggleton et al. 2009). In spite of this principle, our sampling campaign in Romania and Italy was characterised by extended drought periods prior to sampling, which influenced the sampling success. Plum and Filser (2005) estimated that it takes about half a year for an earthworm population to recover after a disturbance. Consequently, it could be that sites affected by recent droughts had not fully recovered yet, resulting in a lower earthworm abundance. Moreover, earthworms still in diapause (curled up 60-120 cm deep in soil (Sims and Gerard 1999)) do not react to mustard suspension (Plum and Filser 2005) and are out of reach for the 20 cm deep soil sampling. This could have resulted in an undersampling of anecic and endogeic earthworms.

Plots were divided in nine (10 x 10 m) subplots. In each plot, one earthworm sample was taken in the central subplot. Sampling close to tree stems was avoided and

whenever possible performed in between different tree species. Earthworms were sampled by means of a combined method. First, litter was hand sorted over an area of 25 x 25 cm. Then, litter was removed over a larger area of 1 x 0.5 m in order to effectively apply an ethological extraction of earthworms using a mustard suspension (Valckx et al. 2011). Finally, hand sorting of a soil sample of 25 × 25 cm and 20 cm depth was performed in the middle of the 1 x 0.5 m area. Collected earthworms were preserved in ethanol (70%) for two weeks, transferred to a 5% formaldehyde solution for fixation (until constant weight), after which they were transferred back to ethanol (70%) for preservation of at least one month. Upon identification, all earthworms were individually weighed, including gut content, and classified into three functional groups, namely epigeic, endogeic and anecic species. Since preservation experiments showed that the weight of *Lumbricus rubellus* stabilises after two days of ethanol preservation (De Wandeler et al., unpublished results) we assumed the effect of a longer preservation period on weight was negligible. Results per unit area of the three sampling techniques were summed to determine the earthworm presence/absence, density and biomass per m^2. For biomass recordings all earthworm biomass, even worm fragments, were considered. For density (=number of earthworms per m^2) and presence/absence recordings, earthworm fragments were only considered if they had a head. By counting only heads, we prevented different fragments of the same earthworm being identified as multiple individuals.

For all 209 plots, a set of 35 explanatory variables were determined (Table 2.1). See supplementary material for details (Appendix A1). The explanatory variables used in this study described climate, soil, forest floor, understory, canopy and stand characteristics in the forest plots. Tree litter traits were calculated as community weighted means (CWM), both per plot (plot scale) and for a circular area with a 10 m radius surrounding the earthworm sampling location (local neighbourhood scale) and were represented by tree litter C:N ratio, C:P ratio, specific leaf area index, cellulose, hemicellulose and polyphenol content. Plot scale litter trait CWMs are used in all reported models in the manuscript. In this study the specific leaf area index of tree litter (SLA) was found to be positively correlated with (i) litter nutritional quality variables (availability of base cations, readily available dissolved organic carbon and total dissolved nitrogen: Spearman's correlation coefficient, r_s resp. = 0.77, 0.61, 0.74; $P < 0.001$, respectively) and (ii) the regulation of forest floor microclimatic conditions (the capacity of litter to retain water: $r_s = 0.70$; $P < 0.001$). Consequently, SLA will be interpreted as such. The non-woody understory vegetation was determined and will be simply called understory vegetation in the remainder of the text.

2.3 Data analysis

We used Boosted Regression Trees (BRT) to identify the environmental variables most relevant in explaining the incidence and abundance of earthworms in mature European forests. BRT is a regression technique than can fit complex nonlinear relationships with threshold patterns, able to handle different types of explanatory variables, and it has a straightforward interpretable output (Elith et al. 2008, Aertsen et al. 2010). In addition, BRT has already proven to be a useful tool to model species distribution patterns (Leathwick et al. 2006, Elith et al. 2008, Cappelle et al. 2010, Palm et al. 2013).

Across all sampled forest plots, 24 different earthworm species were found. The majority of these species (67%) only occurred in one particular site and the most common species were found in 4 out of 6 sampled sites (Appendix A3, Table S1). Because of the high species turnover, we decided not to investigate species-specific responses, but instead we used the total earthworm incidence, density and biomass and the incidence, density and biomass of each functional group (i.e. epigeic, endogeic, anecic) as response variables in the BRT analyses. Explanatory variables are summarised in Table 2.1.

Data exploration indicated that the earthworm data were zero-inflated and had strong positively skewed distribution. As a high proportion of zero observations in abundance models may impact model parameter estimates and consequently the response variable estimates (Lo et al. 1992), a two-step model approach called Delta approach or Hurdle model (Zuur et al. 2009) was used: first we modelled the probability of presence/absence with binomial BRTs (incidence models) and subsequently modelled the density and biomass using only non-zero observations with BRTs assuming a Gaussian error distribution (density and biomass models). In order to meet the requirements of homogeneity and normality of residuals, earthworm biomass was log10 transformed prior to modelling.

To prevent our models from being affected by outliers or false negative values, some plots were removed from the analysis. Outliers and false negative values can arise from design and observer errors (Zuur et al. 2009). Design errors occurred in two plots where intense sheep grazing and defecation resulted in unusually high earthworm biomass. Observer errors occurred presumably due to inaccurate sampling practices in two plots or when the observer was unable to identify the sampled earthworm individual. This happened occasionally when small juveniles or damaged individuals could not be identified to species or functional group level. When the unidentified individuals contributed to 33% or more of total earthworm density or biomass, the plot was considered as an outlier with subsequent omission from the analysis, which was the case for only one plot in Italy. Since earthworm fragments were normally only

considered as an existing individual if they had a head, false negative plots were created where only unidentified earthworm fragments without heads were present. To minimise these false negatives and to maximise the number of plots used in the total incidence models, plots with only earthworm fragments were nevertheless recorded as containing earthworms. In our incidence models, up to 4 outliers and 4 false negative plots were recorded and removed. Up to 5 outliers were recorded and removed from the density and biomass models.

Table 2.1. Summary of the climate, soil and vegetation variables used in Boosted Regression Trees (BRT) analyses to explain the presence of earthworms in mature European forests. Mean (standard deviation), minimum and maximum for continuous variables; Median, minimum and maximum for rank variables; left blank in case of nominal variable. (See Appendix A1 for details)

Variable name	Description	Mean (SD)	Min.	Max.
CLIMATE				
Heat load index	Heat load index	0.82 (0.1)	0.52	1.02
Annual precipitation	Annual precipitation (mm)	641 (77)	484	819
Annual temperature	Annual mean temperature (°C)	7.7 (3.4)	1.3	14.0
Aridity	Aridity index	0.86 (0.21)	0.48	1.30
SOIL				
Slope	Slope of the plot (classes)	1	1	3
Bedrock	Bedrock weathering type (classes)			
Calcareous bedrock	Presence of calcareous bedrock (classes)			
Sand	Sand fraction (classes)	1	1	3
Silt	Silt fraction (classes)	2	1	3
Clay	Clay fraction (classes)	2	1	3
Soil drainage	Soil drainage condition (classes)	2	1	3
Soil Depth	Soil Depth (classes)	3	1	3
Soil type	acidic or buffered substrate (classes)			
Soil pH	pH of mineral soil at 10-20 cm ($CaCl_2$)	4.62 (0.95)	3.39	7.31
Soil C:N	Soil C:N ratio at 10-20 cm of soil	16.2 (5.2)	6.8	35.6
Soil moisture	Soil volumetric water content (%)	31.64 (6.9)	17.10	46.87
FOREST FLOOR				
Humus form	Main humus forms (classes)			
Forest floor pH	pH of ectorganic layer ($CaCl_2$)	5.08 (0.82)	2.95	6.76
Forest floor mass	Dry mass of ectorganic layer (kg/m^2)	2.102 (1.227)	0.404	9.851
Management	Current forest management (classes)			
Forest structure	Current forest structure (classes)			
LAI	Leaf area index (m^2/m^2)	4.38 (1.97)	0	9.10
Tree age distribution	Age distribution of tree canopy (classes)			
Understory biomass	Non-woody understory biomass (g/m^2)	17.25 (20.54)	0	136.96
Understory C:N	C:N ratio of non-woody understory biomass	19.4 (7.6)	8.9	38.8
Understory C:P	C:P ratio of non-woody understory biomass	267.1 (164.1)	64.8	811.1
Tree richness	Number of tree species per plot	3 (1.1)	1	5
Tree evenness	Tree species evenness per plot	0.702 (0.275)	0	1
Litterfall biomass	Biomass of tree leaf litter (g/m^2)	231.4 (112.95)	9.42	645.4
Litterfall C:N	CWM C:N of tree leaf litter	65.7 (35.8)	24.7	174.7
Litterfall C:P	CWM C:P of tree leaf litter	1131.0 (936.8)	195.9	4818.0
Litterfall cellulose	CWM cellulose of tree leaf litter (%)	18.52 (2.25)	12.61	26.26
Litterfall hemicellulose	CWM hemicellulose of tree leaf litter (%)	16.82 (1.48)	13.77	22.40
Litterfall polyphenols	CWM total phenolics of tree leaf litter (%)	39.9 (21.55)	9.7	109.5
Litterfall SLA	CWM SLA of tree leaf litter (cm^2/g)	12.7 (6.1)	3.4	31.8

CWM = community weighted mean; SLA = specific leaf area

All analyses were performed in R (R Development Core Team 2014 version 3.1.1), using 'gbm' package version 2.1 (Ridgeway 2006) and its extensions developed by Elith et al. (2008). BRT model parameters were set to constrain overfitting and optimise the number of regression trees for each model (mostly 2000-6000 trees), with a learning rate of 0.005-0.0005 and a bag fraction of 0.75-0.85. Tree complexity, i.e. the number of nodes in a tree, was set to 3 as recommended by Elith et al. (2008) for small datasets. Model fit and predictive performance after 10-fold cross validation of the biomass and density models were assessed by the percentage deviance explained (D^2) (Littke et al. 2014), the correlation between observed and predicted values and the relative Root Mean Squared Error (rRMSE) (Aertsen et al. 2010). The incidence models were, in addition to the D^2 and the correlation coefficient, assessed by the calculated area under the receiver-operating characteristic curve (AUC). The rRMSE was calculated by dividing the RMSE by the range of the response data [RMSE/(Max(response)-Min(response))]*100. D^2 is a measure for model fit (Buston and Elith 2011), the RMSE is a measure of model accuracy (Aertsen et al. 2012) and the AUC is a measure of model discrimination (Reineking and Schröder 2006). Higher D^2, correlation and AUC scores, and lower rRMSE values indicate better performing models.

We minimised multicollinearity between explanatory variables in all models by the elimination of highly correlated variables that resulted in high variance inflation factors (i.e. individual (VIF) < 5 and median VIF < 3). In this selection process priority was given to retain variables that were, based on biological knowledge, assumed to be most directly linked to the response variable (Zuur et al. 2010) and to climate variables as in our continental-scale study, we wanted to test their importance as potential explanatory variable next to soil and forest floor variables. If both correlated variables were assumed to be equally closely related to the response variables, we dropped the explanatory variable with the lowest correlation with the response variable in order to maximise the explanatory power of the final models. As a consequence, the following explanatory variables were nearly always removed: tree leaf litter polyphenol content (r_s= - 0.76 with aridity; $P < 0.001$), aromaticity of dissolved organic carbon (r_s= - 0. 78 with tree leaf litter C:P; $P < 0.001$), tree litter cellulose content (r_s= - 0.7 with SLA; $P < 0.001$), tree litter C:N ratio (r_s=0.67 with tree litter C:P ratio; $P < 0.001$), and understory C:N ratio (r_s=0.72 with understory C:P ratio; $P < 0.001$). Collinearity between climate variables, namely between aridity and both annual mean temperature and annual precipitation, was handled differently to get a detailed understanding of the effects of different climate aspects. Final models were run twice with two different sets of climate variables: 1) aridity, heat load index and annual mean temperature; and 2) annual precipitation and heat load index. As the main results of these models were

similar we opted to only show the 'aridity models' and considered observed differences with the 'annual mean temperature models' in the discussion. In addition, the total incidence and biomass models were reanalysed with diversity and litter trait variables calculated at the local neighbourhood scale to test possible changes in relative importance of diversity and litter trait variables, but no consequential differences in relative importance were found (Appendix A2, Fig. S2).

In order to structure variable selection, two separate submodels with explanatory variables related to vertically distinct zones in the forest were developed. The first one contained forest floor, vegetation and stand characteristic variables and the second submodel contained all variables related with and situated below the ectorganic horizon, hereafter called: (i) forest floor submodel and (ii) soil submodel, respectively. These submodels were fitted with the *gbm.step* function and further simplified in a backwards stepwise manner by using the *gbm.simplify* function. Next we used a selection of explanatory variables from these simplified submodels whose relative importance exceeded 5%, together with the climate variables, to construct a final ecosystem model. In turn, final models were further simplified with the *gbm.simplify* function.

In the case of the density and biomass models for anecic earthworms we could not use the simplify function because of the small sample size (31 observations). However, we experienced from the other models that the relative importance of the most important variables hardly changed between different model runs, even after model simplification. Therefore the total anecic density and biomass models were built with the variables that had a relative importance of 5% or greater in the submodels. Since Kohavi (1995) reported that the cross-validation estimates of model predictive performance from small datasets can fluctuate, an average of five model runs was taken. From this model set, the model with the highest model performance estimates was retained to report model performance and partial dependence plots. We verified whether density models could be used as an indicator of earthworm biomass in case biomass values are not available. We realised that, despite the capabilities of BRT to limit overfitting, some could still occur in our partial dependence plots. To avoid evaluating potential spurious results we therefore only discussed the main trends in the partial dependence plots.

All reported final models had an acceptable level of collinearity between explanatory variables (i.e. Spearman correlations between variables < 0.7, individual variance inflation factor (VIF) < 0.5 and median VIF < 0.3). All final models were checked for residual spatial autocorrelation by calculating Moran's I and spline correlograms. Moran's I was calculated using the 'ape' package (Paradis et al. 2015), after 'Vincenty'

ellipsoid great circle distances between sampling points had been calculated with the 'geosphere' package (Hijmans et al. 2015). Spline correlograms with 95% pointwise bootstrap confidence intervals were calculated with the 'ncf' package (Bjornstad 2013). Lastly, we checked whether the results of the density models could be used as an indicator of earthworm biomass in the case that biomass values are not available in earthworm studies. The observed earthworm biomass was therefore plotted against predicted earthworm density.

3. Results

3.1 Earthworm incidence and abundance

In the 209 sampled plots earthworm density ranged from 0 to 548 individuals per m^2, with a biomass range from 0 to 204 g/m^2. Average earthworm density and biomass were 90 earthworms/m^2 and 24 g/m^2, respectively (Fig. 2.1). Earthworms were found in 81% of the plots. Endogeic species occurred in 57% of all sample plots, epigeic species in 50%, and anecic earthworms in only 16% of all sample plots (Fig. 2.2). Two functional groups were recorded in 33% of the plots and all three functional groups were recorded in 9% of the plots.

In mid-latitude forests (Germany and Poland) earthworms were found in all plots, in contrast with the northern and southern sites. In four of the six sampling sites endogeic earthworm species dominated (Fig. 2.2). However, in the forests of Finland and Poland, the epigeic species were more frequent than the other two functional groups. Anecic earthworms were generally less frequent, and they were completely absent in the forests of Spain.

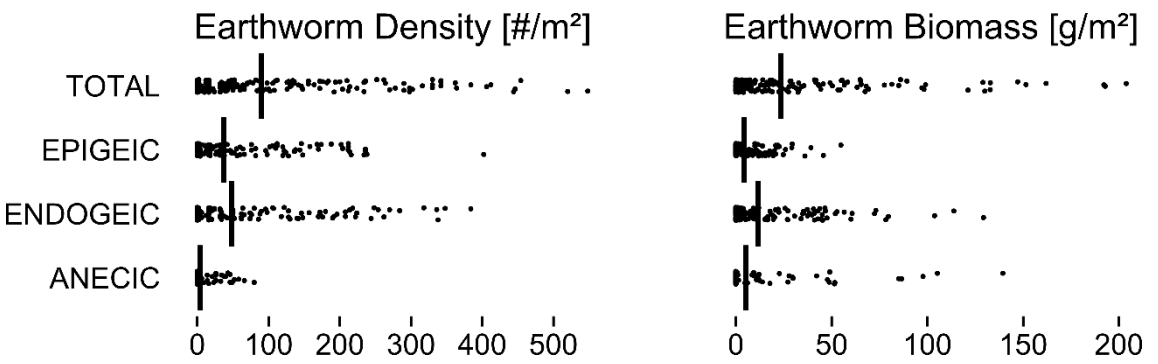


Fig. 2.1. Descriptive statistics of earthworm density (number/m²) and earthworm biomass (g/m²) across all individual sample plots. Each black dot indicates one of the 209 sampled plots distributed among six forest types across Europe. Black vertical bars indicate the respective mean values. To better visualise the data points, individual points are slightly vertically shifted (with the 'Jitter' function in R) to prevent superposition.

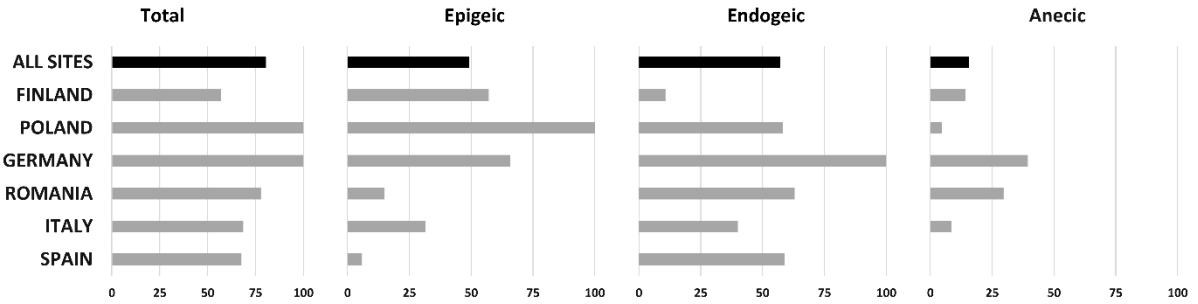


Fig. 2.2. Earthworm incidence (%) in total (pooled across functional groups) (black), and for each functional group separately in the different forest sites (grey).

3.2 Relationship between environmental variables and earthworm incidence, biomass and density

The BRT model fit statistics for the incidence and biomass models ranged from 0.89 to 0.74 for the correlation score and from 0.77 to 0.48 for the percentage deviance explained (D^2). These statistics, together with the statistics after the 10-fold cross validation, are summarised in Table 2.2 and indicate confidence in the modelled results. Nevertheless, slight under- and overpredictions were observed for small and large biomass values, respectively (Appendix A2, Fig. S3). The weakest model statistics were found in the anecic biomass model. The final models did not show any significant residual spatial autocorrelation.

Table 2.2. Model performance indicators of the final boosted regression tree models , grouped by used response variable: area under the receiver-operating characteristic curve (AUC), percentage deviance explained (D^2), correlation score (Corr) and relative Root Mean Squared Error (rRMSE) for model fit and after 10-fold cross-validation.

	Fit				Cross-validation			
	AUC	D^2	Corr	rRMSE	AUC	D^2	Corr	rRMSE
INCIDENCE MODELS								
Total	0.95	0.48	0.74		0.84	0.23	0.47	
Epigeic	0.97	0.60	0.84		0.89	0.40	0.68	
Endogeic	0.98	0.60	0.86		0.87	0.32	0.63	
Anecic	0.96	0.53	0.77		0.86	0.27	0.54	
BIOMASS MODELS								
Total		0.69	0.85	12.18		0.47	0.70	16.01
Epigeic		0.77	0.89	10.38		0.52	0.74	14.92
Endogeic		0.65	0.83	12.01		0.34	0.58	16.65
Anecic		0.67	0.86	13.61		0.13	0.31	22.16

The total earthworm incidence was most strongly related to the C:N ratio and mass of the non-woody understory vegetation (35 and 32%, Fig. 2.3). Since C:N and C:P ratios of the understory vegetation were strongly positively correlated (r_s= 0.72; P < 0.001), earthworms appeared to prefer sites with an understory vegetation with a low C:N and C:P ratio (< 20 and < 300, respectively). Their presence was also most likely in forest floors with a relatively high pH (pH_{CaCl2} 5-6) and soils with a low C:N ratio (< 20). When earthworms were present, their biomass was primarily related to soil C:N and tree litter C:P ratio, with higher earthworm biomass when the ratios were lower than 15 and 1000, respectively. Furthermore, a low forest floor mass and understory C:P ratio, and a high specific tree leaf area and aridity index, indicated a higher earthworm biomass.

Both the incidence and biomass of epigeic earthworms were primarily related to the C:P ratio of the tree litter and non-woody understory vegetation, with a clearly higher probability of earthworm presence and also a higher earthworm biomass below C:P ratios of 200 and 1000, respectively (Fig. 2.4). Not only favourable litter quality, but also deep soils and sites with a low heat load index (N-NE facing slopes) promoted epigeic earthworm incidence.

In contrast, endogeic earthworm incidence was mainly related to humus form (29%), with a higher probability of earthworm presence in Amphi and Mull humus forms (Fig. 2.5). Where endogeic earthworms occurred, their biomass was highest in plots with a low forest floor mass and a soil pH_{CaCl2} greater than 4. In addition, their incidence and biomass were higher at low soil C:N and both low litter C:N and C:P ratios. The epigeic and endogeic incidence models showed an opposite relation with

soil C:N ratio around a common threshold of 12. The probability of the presence of epigeic earthworms was much higher in soils with a C:N ratio higher than 12, whereas the opposite was observed for the endogeic probability of presence (Fig. 2.6).
Anecic earthworm incidence was most related to the mass of the forest floor (63%), indicating higher probability of earthworm presence below 2 kg/m² dry matter and in more humid sites with an aridity index larger than 0.8 (Fig. 2.7). Anecics were most common in sites with a soil pH_{CaCl2} range of 4.5 to 5.5, where the tree litter cellulose content was low (< 17%) and the specific tree leaf area larger than 16 cm²/g.
Since annual mean temperature and annual precipitation were respectively strongly negatively and positively correlated with aridity, they were included in separate models. No remarkable differences between 'aridity models' and 'annual mean temperature models' were observed. However, the probability of total earthworm presence and total earthworm biomass were highest within a range of annual mean temperature of 6 - 9 °C (Appendix A2, Fig. S7).

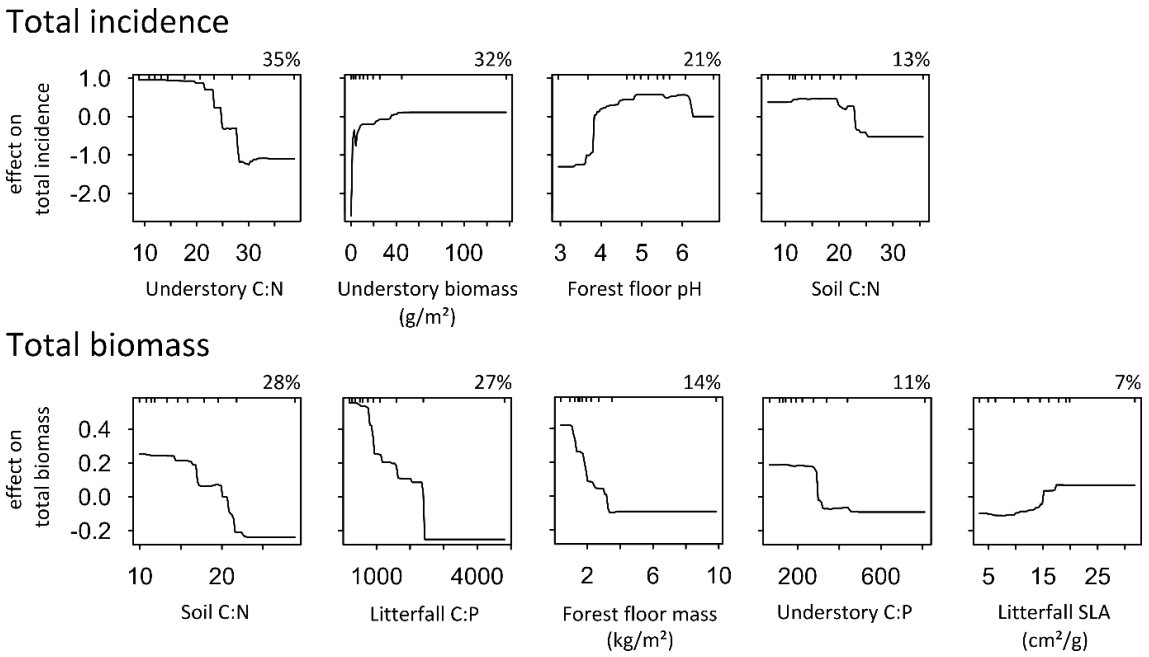


Fig. 2.3. Partial dependence plots of the five most influential variables that explain the total earthworm incidence (above) and total earthworm biomass (below) in European forests . The relative influence (%) of each explanatory variable is indicated above each graph. Each graph illustrates how the response variable (y-axis) is affected by a certain explanatory variable, after accounting for the average effects of all other variables in the model. The fitted functions are centred around the mean response value and plotted on a common scale (earthworm incidence on logit scale and the earthworm biomass on log scale). Hash marks along the top of the plots show the distribution of each explanatory variable in deciles.

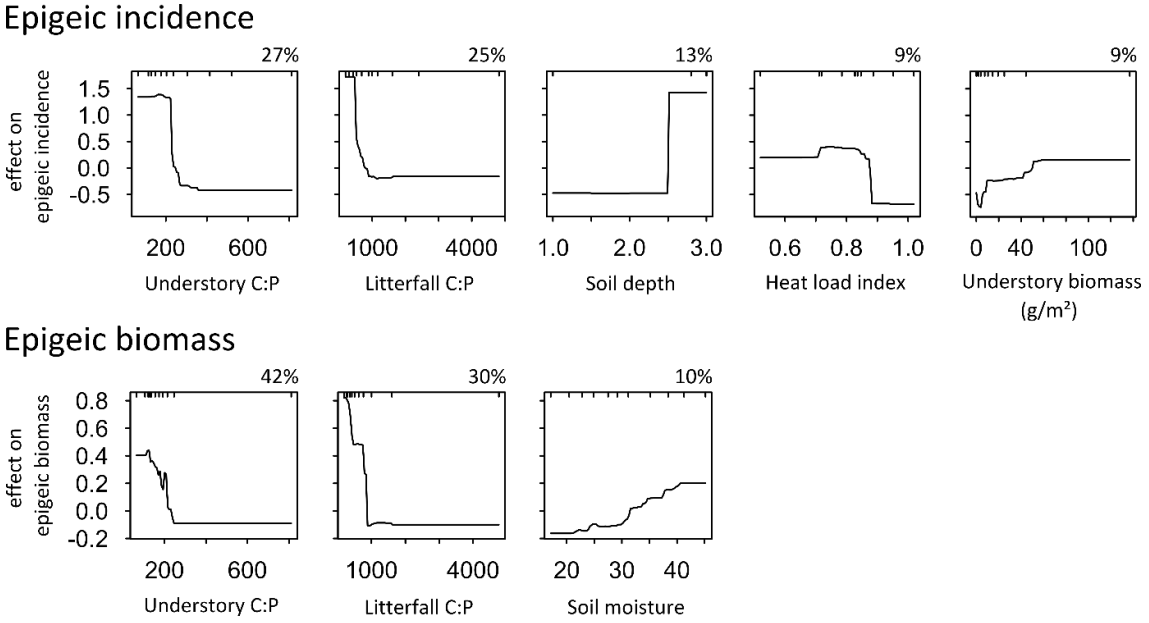


Fig. 2.4. Partial dependence plots of the five most influential variables that explain the epigeic earthworm incidence (above) and epigeic earthworm biomass (below) in European forests . The relative influence (%) of each explanatory variable is indicated above each graph. (cf. Fig. 2.3 for details)

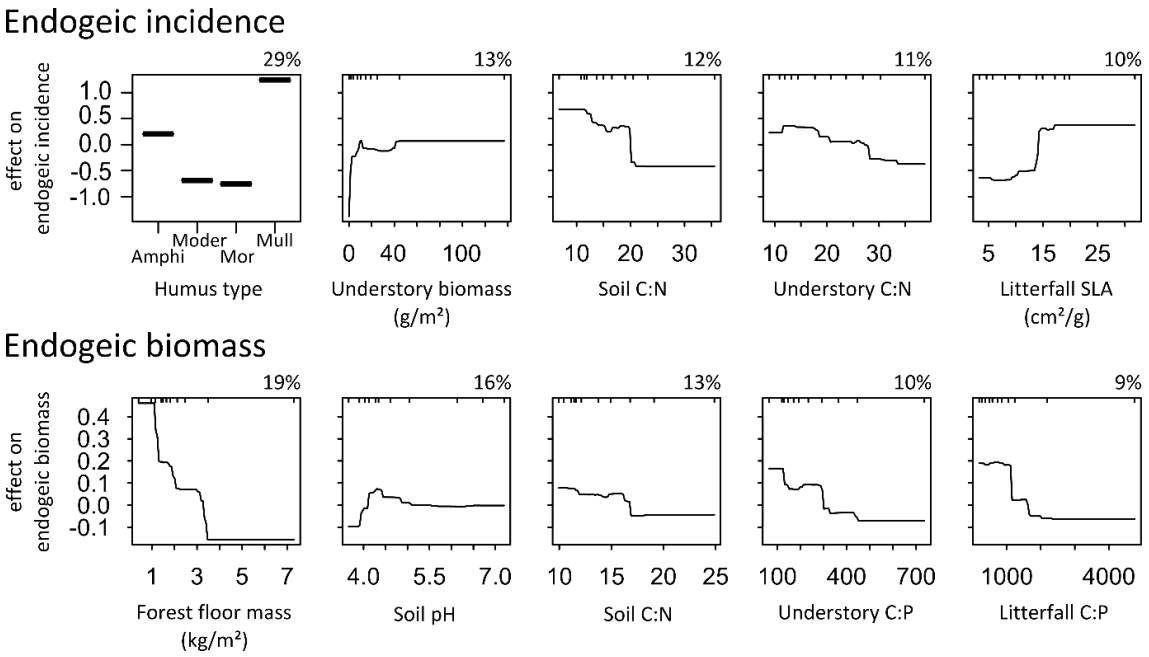


Fig. 2.5. Partial dependence plots of the five most influential variables that explain the endogeic earthworm incidence (above) and endogeic earthworm biomass (below) in European forests . The relative influence (%) of each explanatory variable is indicated above each graph. (cf. Fig. 2.3 for details)

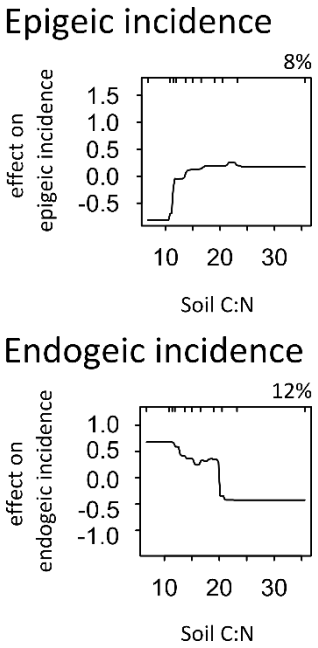


Fig. 2.6. Partial dependence plots of the epigeic and endogeic earthworm presence probability for soil C:N ratio . The relative influence (%) of each explanatory variable is indicated above each graph. (cf. Fig. 2.3 for details)

Additionally, we identified and evaluated the environmental factors mostly related to earthworm density measures (total earthworm density and epigeic, endogeic and anecic

earthworm density). The BRT model fit statistics for the density models indicate that we may have confidence in the modelled results (Appendix A3, Table S3). When comparing these density model results with biomass model results, we observed fairly similar response curves (Appendix A2, Fig. S4 and S5). Next to that, relatively strong positive relationships between predicted earthworm densities and observed earthworm biomass were observed (Appendix A2, Fig. S6). However in spite of these relatively high correlations, the relative importance (%) of the environmental variables between density and biomass models differed considerably.

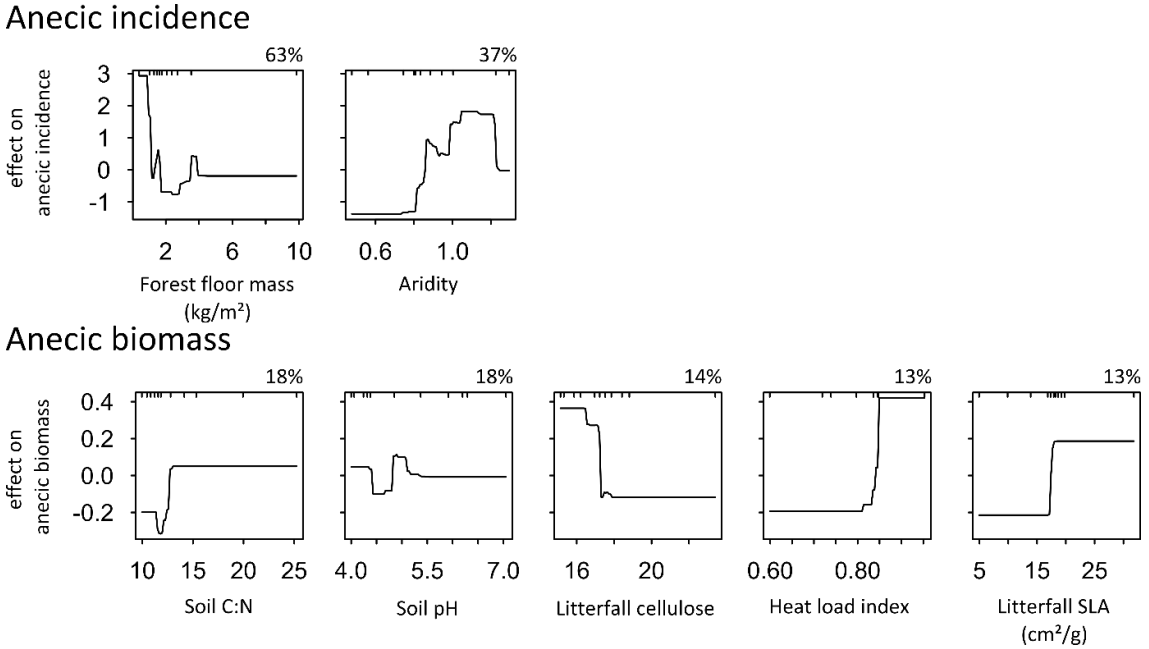


Fig. 2.7. Partial dependence plots of the five most influential variables that explain the anecic earthworm incidence (above) and anecic earthworm biomass (below) in European forests . The relative influence (%) of each explanatory variable is indicated above each graph. (cf. Fig. 2.3 for details)

4. Discussion and conclusion

4.1 Environmental factors related to earthworm incidence and biomass in European forests

We identified the following explanatory variables as most important across the different models: forest floor mass, soil and forest floor pH, and soil and leaf litter nutrient concentrations. These results are consistent with studies at local (Hendriksen 1990, Neirynck et al. 2000, Laganière et al. 2009) and landscape scales (Muys and Lust 1992, Ammer et al. 2006, Shartell et al. 2013). The model results demonstrated that Boosted Regression Tree modelling is a useful technique to identify the relative importance of a set of different explanatory variables and how they influence

earthworm incidence and abundance across different forests. Our models showed good explanatory power, though they could still be improved with additional sampling or eventually by merging our dataset with previously collected data. Doing so, it may become possible to create powerful distribution models to estimate predictive maps of total earthworm density, biomass and the three functional groups we distinguished across European forests. Our results may have been influenced by somewhat untypically low earthworm abundances in Italy and Romania resulting from unpredictable drought events during the respective sampling periods. Such stochastic effects of climate variability on the results could only be evaluated by repeated sampling campaigns over several years, which was beyond the scope of this study.

4.1.1 *Climate*

According to our model results, climatic factors play only a minor role in explaining earthworm incidence and biomass across European forests. Apart from a higher probability of earthworm presence and higher earthworm biomass within a range of annual mean temperatures between 6 and 9° C (Appendix A2, Fig. S7), there were only weak effects of climatic factors. In particular, the low predicted probability of the presence of anecic earthworms in arid conditions was unexpected, given the fact that many of these species show diapause behaviour as an adaptive strategy to drought (Lavelle 1988). We may have missed the anecic earthworms in the drier sites, because large species occur at lower densities and because the sampling period may not have been optimal. The low probability of the presence of anecics in humid and cold conditions on the other hand confirms previous observations by Terhivuo (1989). The low probability of anecic earthworm presence in forests of northern Europe is likely to be related to the unfavourable environmental conditions. Boreal forests are generally conifer-dominated forests on nutrient-poor podzolic soils (Barbati et al. 2007) with a low topsoil pH, which creates a toxic environment for anecic earthworms (Räty and Huhta, 2004).

4.1.2 *Soil properties*

Soil C:N ratio was the most important soil property in our models related to total earthworm incidence as well as biomass. The entire earthworm community reached a biomass of 100 to 200 g/m² in fertile forest soils with soil C:N ratios less than 15. Such forest plots typically have Mull-like topsoils. The probability of earthworm presence decreased considerably above a C:N threshold value of 22 at 10-20cm depth, which corresponds to Mor-like topsoils. Similar observations were made by Muys and Lust (1992) in Flemish forests.

As could be assumed based on the feeding behaviour of endogeic earthworms (Ferlian et al. 2014), soil variables had a prominent role in predicting their distribution (Fig.

2.5), much more than in predicting the incidence and biomass of epigeic and to some extent also the anecic earthworms. This corroborates our hypothesis that endogeic species would primarily be influenced by soil properties. Furthermore, the different ecology of the epigeic and endogeic earthworm groups was emphasised by their opposite relationships with soil C:N ratio. High topsoil C:N ratios were attributed to poorly decomposable litter and consequent accumulation of incompletely humified organic material (Zanella et al. 2011) providing a habitat for epigeic earthworms. Low soil C:N ratios, on the other hand, are related to Mull-like topsoils with high bioturbation activity of endogeic and anecic earthworms, leading to a rapidly disappearing litter layer, and thus, to fewer habitat opportunities and food for epigeic earthworms (Butt 1998, Capowiez and Belzunces 2001, Palm et al. 2013). It is striking that climatic variables had only weak effects in our models, but it also shows that, in relative terms, local humus form and the soil as a buffering system are of critical importance to earthworms (Edwards and Bohlen 1996, Bonkowski et al. 1998). The term 'soil type' used in this study was a binomial variable indicating whether a soil was acidic or buffered. This somewhat narrow approach of determining soil type is not ideal, however it was the only viable option considering the available data.

In this context of predictive modelling it is important to understand that the choice of whether a variable is used as an explanatory or as a response variable is unrelated to ecological cause and effect relationships, and is rather driven by the objective to identify the factors that can explain earthworm presence and abundance in European forests. In this study for example, humus form is used as an explanatory variable for earthworm presence and abundance, but obviously earthworms themselves are a key driver in humus form formation.

4.1.3 *Vegetation and soil-vegetation interactions*

As hypothesised, leaf litter quality was an important factor in explaining earthworm incidence and biomass in European forests, i.e. tree leaf litter C:P, C:N and SLA (Hendriksen 1990, Cortez 1998, Holdsworth et al. 2012). Besides tree leaf litter, however, the quality of the non-woody understory vegetation (C:P and C:N ratio) was also important, but is commonly less considered. It is known, that low understory biomass C:N and C:P ratios support high soil microbial activity and growth (Bardgett 2005b) which in turn provides earthworms with more nutrients (Sampedro et al. 2006, Dungait et al. 2008, Ferlian et al. 2014). This suggests that understory vegetation may locally stimulate earthworm incidence, density and biomass via increased nutrient availability. This is supported by Kourtev et al. (1999) who reported that two exotic species in understory vegetation stimulated earthworm densities by increasing soil pH and nitrogen availability. It seems that non-woody understory vegetation can significantly contribute to an increased food quality by decreasing the C:P ratio below

a certain threshold (< 200). It appears that epigeic earthworms can only occur below this threshold. Once this threshold is reached, epigeic earthworm biomass increases gradually with decreasing understory C:P ratio (Fig. 2.4). Similarly, we found a relatively high importance of tree leaf litter C:P ratio in the models for the total earthworm biomass and epigeic incidence and biomass. As phosphorus availability is generally a problem, it may limit microbial decomposer abundance and consequently the recycling of other important nutrients (Gosz et al. 1973, Tessier and Raynal 2003, Blanes et al. 2012), which in turn could limit the nutrient availability for other organisms like earthworms.

While less important than leaf litter C:nutrient ratios, the SLA of the tree litter contributed significantly to 60% of our models with a relative importance ranging from 6 to 16% (Appendix A3, Table S2). In models where SLA had an impact, we identified a threshold value of 15 cm^2/g (10 cm^2/g for epigeics), with a higher probability of earthworm presence and higher biomass values above this threshold value. High SLA is typically positively correlated with high leaf nutrient concentrations (Poorter and De Jong 1999, Hoffmann et al. 2005), which we also observed in our study. Accordingly, high nutrient concentrations associated with high SLA values may be the ultimate driver stimulating earthworm incidence and abundance (Muys and Lust 1992, Reich et al. 2005, De Schrijver et al. 2012).

Forest floor mass was an important explanatory factor in the total earthworm biomass model (Fig. 2.3). The negative relationship between forest floor mass and earthworm biomass illustrates that high earthworm abundance typically leads to a rapid disappearance of the forest floor (Cortez and Bouché 1998). Furthermore, forest floor mass correlated well with total earthworm biomass, but not with earthworm incidence. Therefore forest floor mass does not allow us to predict earthworm incidence, however where earthworms are present, the forest floor mass is negatively correlated to earthworm biomass due to earthworm decomposition and bioturbation activity (Bohlen et al. 2004, Drouin et al. 2016).

It would have been interesting to also consider plant root related parameters, such as root biomass, root exudates and root nutrient concentrations (C:N, C:P). In forest ecosystems root biomass typically makes up a significant fraction of the total litter production (Vogt et al. 1986, Saugier et al. 2001, Leppälammi-Kujansuu et al. 2014), and seems to play an often overlooked, but important role (Pollierer et al. 2007). Even though root and foliar N and P are often correlated across species on a global scale, their relation within sites is not always clear (Hobbie 2015). Further exploration of their importance, in addition to the aboveground predictors, is therefore important.

4.2 Different factors explaining earthworm incidence and biomass

We observed that influential variables specifically explaining earthworm incidence often differed from factors explaining earthworm biomass. Similar observations were reported by Palm et al. (2013), where predictor contributions changed between incidence and density models. We believe that these differences are indicative of threshold values that must be reached before earthworms can occur (Curry 2004).

The incidence of earthworms was generally high when the soil C:N ratio was lower than 23, where sufficient non-woody understory vegetation was present with a low C:N ratio (< 25), and also when the forest floor pH_{CaCl2} was higher than 4. Once these prerequisites for earthworm incidence were met, earthworm biomass could be more precisely explained by soil C:N, tree litter C:P and mass of the forest floor (Fig. 2.3). The epigeic functional group was atypical in this case, as the influential explanatory factors for incidence and biomass were the same. This may indicate that earthworm presence and biomass for epigeics are influenced by similar covariates whereas presence and biomass of the other groups were explained by different factors.

4.3 Earthworm density as proxy for biomass

Our results indicate that earthworm density might be a good proxy for earthworm biomass in European forests. Nevertheless considerable differences in the relative importance of explanatory variables existed between density and biomass models. The largest differences between the density and biomass model results were observed between the endogeic and anecic groups (Fig. 2.5, Fig. 2.7and Fig. S4). This could be explained by the distortion in the linear relation between density and biomass for these functional groups. There is a greater range in earthworm biomass in plots with only few individuals (Appendix A2, Fig. S8). This range is enlarged by the presence of large K-selected endogeic and anecic earthworm species (Bouché 1977, Dash 2001) that occur in low densities primarily in Italy, but also in Spain and Romania. A direct confirmation of this inference can be observed in the endogeic and anecic density models that described a decreasing number of earthworms in more arid environments. Incorporation of either density or preferentially biomass models along with incidence models will provide a better understanding of earthworm distributions (Meynard and Quinn 2007).

Chapter 3

Tree identity rather than tree diversity drives earthworm communities in European forests

Adapted from: Hans De Wandeler, Helge Bruelheide, Seid M. Dawud, Gabriel Dănilă, Timo Domisch, Leena Finér, Martin Hermy, Bogdan Jaroszewicz, François-Xavier Joly, Sandra Müller, Sophia Ratcliff, Karsten Raulund-Rasmussen, Emilia Rota, Koenraad Van Meerbeek, Lars Vesterdal, Bart Muys. Tree identity rather than tree diversity drives earthworm communities in European forests. Pedobiologia, 67, 16 - 25

1. Introduction

Among all soil biota, earthworms are considered key ecosystem engineers given their role in litter decomposition (Bonkowski et al. 1998, Holdsworth et al. 2012), bioturbation (Meysman et al. 2006, Bityutskii et al. 2016), water regulation (Blouin et al. 2013), nutrient cycling (Resner et al. 2015, Yang et al. 2015), soil carbon stocks and vertical distribution (Frouz et al. 2013, Vesterdal et al. 2013) and even seed germination (Forey et al. 2011). We studied the effect of tree species identity and diversity on the abundance and richness of these ecosystem engineers. Research on the aboveground-belowground relationship is highly valuable because of the importance of earthworms as ecosystem engineers in forest ecosystem functioning. They are important contributors to litter fragmentation and burial (Scheu 1987, Staaf 1987, Hättenschwiler and Gasser 2005, Holdsworth et al. 2012) and have a fundamental influence on forest ecosystem dynamics (Satchell 1983, Zicsi 1983, Parkinson et al. 2004).

Earthworm biomass was reported to vary with tree species identity (Neirynck et al. 2000, González et al. 2003, Sarlo 2006) but also with plant functional groups: earthworm biomass and density was reported to increase with increased incidence of legumes in grassland communities (Milcu et al. 2008) and also with a conversion from conifer into mixed stands with broadleaved trees (Ammer et al. 2006, Salamon et al. 2008). One of the reasons for these relationships might be the difference in leaf litter quality, with higher leaf litter quality stimulating earthworm density and biomass. This litter quality effect has been demonstrated by common garden studies using tree monocultures that attributed differences in earthworm community structure to leaf litter quality (Muys et al. 1992, Neirynck et al. 2000, Reich et al. 2005). Leaf litter can either directly affect earthworms by its nutrient concentration and carbon quality (Hendriksen 1990, Rajapaksha et al. 2013) or indirectly by its influence on humus characteristics such as pH and habitat availability in the forest floor (Aubert et al. 2003, Aubert et al. 2006b). Similar observations have been made in tropical agriculture where earthworm densities were negatively correlated with the lignin:N ratio of plant residues (Tian et al. 1993) and in grassland experiments where species with nitrogen-rich litter stimulated earthworm biomass and density (Milcu et al. 2008) and affected earthworm species composition (Eisenhauer et al. 2009, Piotrowska et al. 2013).

As plant species vary in their litter traits, and in the way they occupy space and use resources, mixed assemblages are likely to have a different influence on the decomposer community compared to systems dominated by a single plant species. Several mechanisms have been proposed by which decomposers respond to

differences in plant species diversity. Plant species diversity can affect the earthworm community by influencing the available plant biomass (resource quantity effect (Zaller and Arnone 1999, Spehn et al. 2000)) or the quality of food resources (resource quality effect (Spehn et al. 2000, Milcu et al. 2006)). Plant species diversity can also influence the litter layer structure and consequently the microclimate conditions and microhabitat abundance and variety (Hättenschwiler et al. 2005), which is known to increase the abundance and diversity of soil fauna (Hansen and Coleman 1998, Kaneko and Salamanca 1999).

Since the presence of belowground biota can have important effects on forest ecosystem functioning (Lavelle et al. 2006, Bardgett and Wardle 2010) it is important to have a greater understanding of the factors that influence their abundance and composition (De Wandeler et al. 2016), including different aspects of tree species diversity. Even though the number of studies on the effects of mixed forest stands on earthworm communities is increasing, very few studies were designed to investigate true tree species diversity effects (Korboulewsky et al. 2016). Many reports on diversity effects were from unbalanced studies, such as dilution series of one tree species: dilution of beech (Aubert et al. 2003, Cesarz et al. 2007, Jacob et al. 2009), dilution of Scots pine (Ammer et al. 2006) or dilution of spruce (Elmer et al. 2004, Salamon et al. 2008). In such studies, it is not possible to separate diversity from identity effects (Nadrowski et al. 2010). True diversity effects are more rarely reported and indicate positive tree species diversity effects on earthworm species richness (Chamagne et al. 2016), while others found no relationship with the earthworm community (Scheu et al. 2003, Schwarz et al. 2015b).

In a previous study based on partly the same dataset (De Wandeler et al. 2016), we found that litter- and soil-related variables were more important for explaining earthworm incidence and biomass than climatic variables. Here we extended the study to investigate the importance of different aspects of tree diversity on earthworm biomass and diversity. In this study we investigated: (i) how tree species diversity and proportion of evergreen leaf litter influences earthworm abundance and diversity, and (ii) which tree leaf litter traits affect earthworm abundance and diversity and whether their relative importance changes along a latitudinal gradient.
Given that earthworm abundances and species diversity can be influenced by plant species identity, plant functional traits and plant diversity-related mechanisms, we hypothesise that in mixed forests, earthworm abundance and species diversity may be affected by (1) the functional diversity of the tree species community rather than tree species richness or true Shannon diversity and (2) the dominant leaf litter trait values

within a tree species community. The first hypothesis is related to the food web theory that predicts increased diversity of higher trophic levels with increased producer diversity (Knops et al. 1999, M. Haddad et al. 2001). The producer diversity in the context of this study was quantified by the functional dispersion of the tree species community (FDis), as proposed by Laliberté and Legendre (2010), and refers to the diversity of litter types serving different ecological functions in terms of food and structural habitat. The second hypothesis is related to the 'mass-ratio hypothesis' (Grime 1998) stating that ecosystem processes are mainly determined by the functional identity of the dominant species. The functional identity of the dominant species can be quantified by the community-weighted mean of tree leaf litter trait values (CWM, Garnier et al. (2004)). Furthermore, we expected context dependency (Fridley 2003, Tedersoo et al. 2016, Eisenhauer and Powell 2017); the relationships between earthworm abundance and species diversity with the different leaf litter traits could change with environmental conditions, as previously found for the relationship of moisture availability with mites and nematodes (Sylvain et al. 2014).

The link between above- and belowground communities was studied in a European network of mature forest plots that was especially designed to explore the relationship between biodiversity and ecosystem functioning (Baeten et al. 2013) and allowed us to assess the effects of tree species diversity (species richness, True Shannon diversity index, functional diversity) while controlling for the effects of tree species identity and abiotic environmental variables. Unlike most research on tree species identity effects, we went beyond tree species identity as an explanatory variable and focused on the role of tree leaf litter traits, further described as 'litter quality'.

2. Material and Methods

2.1 Study area

The six studied regions span most of the European bioclimatic gradient and represent major European forest types including boreal forest (North Karelia, Finland), temperate mixed coniferous and broadleaf forest (Białowieża, Poland), temperate deciduous forest (Hainich, Germany), mountainous deciduous forest (Râsça, Romania), thermophilous deciduous forest (Colline Metallifere, Italy) and Mediterranean mixed forest (Alto Tajo, Spain) (see FunDivEUROPE research platform in Baeten et al. (2013); Appendix B1, Fig. S1). These six regions are the basis for the FunDivEUROPE exploratory platform, which was specifically designed to assess biodiversity-ecosystem function relationships along tree species richness gradients in mature forests. Each studied region includes between 28 and 43 selected plots (30 x 30 m) with different combinations of a fixed set of locally dominant tree

species (the so called target species). The established plots ranged in tree species richness from one to five species per plot. Three important criteria were applied in designing this platform. First, to ensure evenness in the tree species composition of the plots, a lower limit of 60% of maximum evenness based on basal area was set. Second, the research platform was designed in such a way that the admixture of non-target species was minimised. The basal area of the admixed species was generally kept below 5% of the total basal area, with a maximum of ca. 10%. Third, plots within a region were selected to minimise differences in soil related conditions, such as bedrock type, soil type, texture and depth. In total, the platform consists of 209 plots with 16 target tree species, some of them occurring in multiple regions. The species pool comprised conifers, deciduous broadleaved and evergreen broadleaved trees. For more details on the design of the platform consult Appendix B2 (Table S1) and Baeten et al. (2013).

2.2 Data collection

2.3 Assessment of earthworm community properties

Earthworm sampling was carried out in spring 2012 in Italy, Germany and Finland, and in autumn 2012 in Poland, Romania and Spain. We scheduled earthworm sampling in spring or autumn because of their requirement for humid soil conditions and positive temperatures (Berry and Jordan 2001, Holmstrup 2001, Baker and Whitby 2003, Eggleton et al. 2009). In spite of this principle, our sampling campaign in Romania and Italy was characterized by extended drought periods prior to sampling, which negatively influenced the sampling success. Plum and Filser (2005) estimated that it takes about half a year for an earthworm population to recover after a disturbance. Consequently, it could be that regions affected by recent drought had not fully recovered yet, resulting in lower earthworm abundance. Therefore our results may have been influenced by unusually low earthworm abundances in Italy and Romania resulting from unpredictable drought events preceding the respective sampling periods. However, such stochastic effects of climate variability on the results could only be evaluated by repeated sampling campaigns over several years, which was impossible within the context of this study. All 209 plots of the FunDivEUROPE exploratory platform were sampled once.

Plots were divided in nine (10 x 10 m) subplots. In each plot, one earthworm sample was taken in the central subplot (Appendix B1, Fig. S2). Sampling close to tree stems was avoided and in mixed stands performed in the inter-space between different tree species. Earthworms were sampled by means of a combined method. First, litter (OL

and OF horizon, Zanella et al. (2011)) was hand sorted over an area of 25 x 25 cm to focus on epigeic earthworm species. Second, litter was removed over a larger area of 100 x 50 cm in order to effectively apply an ethological extraction of earthworms using a mustard suspension to focus on anecic species (Valckx et al. 2011). Third, hand sorting of a soil sample from an area of 25 × 25 cm and 20 cm depth was performed in the middle of the 100 x 50 cm area to focus on endogeic species. Collected earthworms were preserved in ethanol (70%) for two weeks, transferred to a 5% formaldehyde solution for fixation (until constant weight), after which they were transferred back to ethanol (70%) for preservation for at least one month. Upon identification, all earthworms were individually weighed, including gut content, and identified to species level with the use of different identification keys (Bouché 1972, Sims and Gerard 1999, Csuzdi and Zicsi 2003, Pop et al. 2012) or primary literature. Earthworm individuals that could only be identified at the level of the ecological group (0.5 %) or genus level (64%), which were mostly juveniles, were pro rata assigned to species based on their biomass. The earthworm biomass data obtained from the three sampling techniques were converted to 1 m^2 and then summed to obtain the earthworm biomass in gram per m^2 (total biomass and biomass of the three functional groups) (Appendix B1, Fig. S3). Earthworm individuals were appointed to one of the three functional groups defined by Bouché (1977): epigeic, endogeic or anecic. Plot-level earthworm species richness was calculated as the total number of earthworm species obtained from the three collection methods (Appendix B2, Table S2).

2.3.1 *Tree diversity, evergreen proportion and litter quality variables*

In all 209 plots, tree leaf litter of all target tree species was collected with litter traps to estimate tree leaf litter biomass per species per plot (De Wandeler et al. 2016). The litter was then used to determine tree species specific leaf litter C:N ratio and calcium concentration per plot (Appendix B3). Fourteen additional leaf litter traits (Appendix B4) were determined from freshly fallen leaf litter of each species at regional level at several locations around the plots (Joly et al. 2015).

For each plot we calculated three diversity indices, an evergreen proportion metric, and five leaf litter quality indices. We estimated the variables based on leaf litter mass abundance, rather than tree species basal area, because earthworms are directly and indirectly dependent on leaf litter for food and habitat opportunity (Sims and Gerard 1999, Curry 2004, Edwards 2004). The basal area of tree species within plots was highly correlated with their corresponding litter mass (Pearson's correlation coefficient r_p=0.96; $P < 0.001$). The diversity indices were: 1) tree species richness (the number of target trees); 2) True Shannon index (exponent of Shannon diversity index; (Jost 2006)); and 3) functional dispersion, calculated as a proxy of tree functional diversity.

Functional dispersion measures the distance between tree species in a multivariate functional trait space (Laliberté and Legendre 2010), with high values indicating that the tree species are more functionally dissimilar from each other. It was calculated in R with the *dbFD* function from the FD package (Laliberté et al. 2014), based on five relevant tree litter quality traits (C:N, C:P, calcium and lignin concentration and litter water holding capacity), weighted by the species' leaf litter mass. Traits were selected out of a pool of 18 measured litter traits by means of PCA and pairwise correlations to select five traits that optimally represented the litter trait space and are minimally correlated (see Appendix B4 for details). The evergreen proportion metric represents the proportion of evergreen leaf litter in the total litterfall of a plot. In five of the six regions, evergreen tree species were coniferous, while *Quercus ilex*, an evergreen angiosperm, occurred in Italy and Spain. As leaf litter quality indicators, the community weighted means (CWM) of the same five litter traits were calculated per plot. These CWM traits quantify the dominant trait values within a community by summarising the functional composition of single traits (Ricotta and Moretti 2011). More details about the leaf litter mass collection can be found in De Wandeler et al. (2016).

2.3.2 *Abiotic variables*

Four abiotic variables were recorded in each plot: soil pH (0-10 cm horizon), soil depth, stoniness and a heat load index. Stoniness of the soil is assumed to directly affect the earthworm community's living space and soil pH, soil depth and the heat load index were identified in Chapter 2 as important factors explaining earthworm incidence and biomass in European forest soils. A composite sample of nine subsamples was analysed to determine the soil pH of the 0-10 cm layer (cf. Dawud et al. 2017). Soil pH ($CaCl_2$) of the mineral soil was determined with 0.01 M $CaCl_2$ solution at a ratio of 1:2.5, using 827 pH lab (Metrohm AG, Herisau, Switzerland) after the soil was dried to constant weight (55°C) and sieved through a 2 mm diameter mesh. Soil depth to bedrock (cm) was measured in each plot using a soil auger or taken from literature (Guckland et al. 2009). Stone content of the soil was estimated with the "iron-rod" method by Viro (1952) and the empirical equation presented by Tamminen and Starr (1994). Heat load index, as a proxy for climate variation within regions, was calculated according to McCune and Keon (2002) where plot specific values for latitude, slope and aspect were used to feed and calculate following equation 3: Heat load index = 0.339 + 0.808*COS(latitude)*COS(slope) - 0.196*SIN(latitude)*SIN(slope) - 0.482*COS(aspect)*SIN(slope). To prevent correlation with latitude, plot-level heat load index values were scaled by the region maximum. The heat load index reflects the heat load that a particular location receives

due to annual direct incidence radiation. The higher the value, the greater the heat load and the warmer that location will be.

2.4 Statistical analysis

2.4.1 *Earthworm response variables*

The earthworm community was represented by five different response variables: total earthworm biomass, biomass of epigeic, endogeic and anecic species and community species richness. Biomass data (g/m^2) were [log10(xij + d) – c] transformed, where c = Trunc(log10(Min(x)) and d = 10^(c) following McCune et al. (2002) to meet the requirements of homogeneity and normality of residuals. This specific transformation tends to preserve the original order of magnitude in data and was preferred above the more commonly used log10(x+1) since our lowest non-zero value of x differs from one by more than an order of magnitude, which would distort the relationship between zeros and other values in our dataset. As in De Wandeler et al. (2016) up to eight plots of the 209 were removed to prevent outliers and probable recording errors unduly influencing the results. In addition, we used a rarefaction procedure (rarefy function in the vegan package (version 2.4-3) (Oksanen et al. 2007)) to investigate the potential confounding effect of earthworm abundance on earthworm species richness estimations. Given that the rarefied earthworm richness values where highly correlated with the original species richness variable (Pearson's correlation coefficient r_p=0.96; P < 0.001) we chose to use to the widely used and easy to understand earthworm species richness values.

2.4.2 *Tree diversity, evergreen proportion and litter quality effects*

In order to investigate tree diversity, evergreen proportion and litter quality effects we applied information-theory based analyses (Burnham et al. 2011). These analyses use different approaches to data analysis and inference compared to the traditionally used null hypothesis testing of the frequentist approach (Anderson et al. 2001). The potential effect of tree diversity indices and proportion of evergreen leaf litter on the earthworm community was tested using mixed-effects models for all regions together (continental scale) and each region separately (regional scale). The covariates (soil pH, soil depth, stoniness and heat load index) and random effects of tree species composition and region were included in all models. Tree species composition was included as random effect and was a categorical variable with 90 levels at the continental scale (one level for each tree species combination in this study). In each region, every tree species composition was represented 2 – 4 times, except the two- and three-species mixtures in the regions with a pool of five species. The species composition random effect term accounted for the non-independence of plots with

the same species composition. Composition was nested within region to account for regional phenotypic plasticity of the litter quality. For the analysis within the separate regions, 'region' was deleted from the random structure. Our null model contained only the covariates and the random error structure whilst the full model contained two additional explanatory variables: a single diversity index and the proportion of evergreen leaf litter. Before analysis, all explanatory variables were standardised to zero mean and standard deviation of 0.5 (Schielzeth 2010). The three explanatory tree diversity indices (species richness, True Shannon Index and functional dispersion) were strongly correlated (variance inflation factor (VIF) > 5 and all pairwise correlations $r_p > 0.65$). Therefore separate models were constructed for each diversity index: [R-syntax of the full model: y ~ Diversity index + Evergreen proportion + Covariates , random = ~1 | Region/Composition]. We used an information theory approach to select the best model based on Akaike's information criterion by removing each predictor variable (diversity index and proportion of evergreen leaf litter) in turn from the model. We corrected for small sample sizes (AICc) using the *SelMod* function in the pgrimess package (version 1.6.4) (Giraudoux 2015). Among the best fitting models, the minimum adequate model (MAM) was that with the lowest number of estimable parameters (K) within 2 AICc units of the model with the lowest AICc. Differences in AICc scores (Δ_i) > 2 can be interpreted as indicating strong support for the MAM compared to poorer models (Burnham and Anderson 2002). All four covariates were kept in the continental models, but to minimise over-parameterisation in the regional scale models, the number of covariates per model was reduced to the two that explained the most variation in the response variable.

To prevent over-parameterised models the tree litter quality analysis was carried out separately, using a different modelling approach. At both the continental and regional scale a global model was defined for each earthworm response variable. We used a mixed-effects model with leaf litter CWM traits, covariates and a random term: [R-syntax: y ~ $Trait_1$ + $Trait_2$ + … + $Trait_p$ + Covariates + (1 | Region/Composition)]. At the regional scale however, multicollinearity between several litter trait variables occurred. A variance inflation factor (VIF) analysis was therefore performed to drop variables with a too high VIF score so that all individual VIF < 5 and median VIF < 3 (Zuur et al. 2010). By means of the *dredge* function in the MuMIn package (version 1.15.6) (Bartoń 2015) all possible combinations of candidate models that can be built with litter trait variables from the global model were built with maximum-likelihood estimation and ranked using AICc. To account for model uncertainty, we performed full model averaging of parameter estimates across all models with ΔAICc < 2 (Burnham and Anderson 2002), after which model estimates and confidence intervals

were calculated (Grueber et al. 2011). Relative variable importance values (RI) per variable were calculated as the sum of Akaike weights over all models from the top model set including the variable. To minimise over-parameterisation in the regional scale models (Grueber et al. 2011), only two covariates and two litter trait variables were included per candidate model. In order to be able to compare diversity, evergreen proportion and litter quality effects the same two covariates were used per region.

To model the two response variables of this study, earthworm biomass and species richness, we used linear and generalised linear mixed-effects models, respectively (*lme* in the nlme and *glmer* in lme4 package (Bates et al. 2016, Pinheiro et al. 2016)). Model assumptions of normality and homogeneity of residuals were checked and improved by either changing the model type (linear instead of generalised linear mixed-effects modelling) or by adding a variance function (Zuur et al. 2009, Cleasby and Nakagawa 2011) if necessary. To examine whether litter quality variables were stronger drivers of earthworm biomass and richness than tree diversity, model R^2 values were calculated as likelihood ratio-based R^2 (Magee 1990) using the *r.squaredLR* function in the MuMin package.

At the continental scale, anecic earthworm biomass could not be analysed due to the small number of plots where anecic species were found. In addition, due to very low sample sizes, the Romanian and Spanish regions were removed from the epigeic biomass model and the Finnish region was removed from the endogeic biomass model. Several regional scale models could not be fitted due to low sample sizes (earthworm individuals present in <40% of the plots), we therefore decided to analyse the endogeic earthworm biomass for the German, Italian, Polish, Romanian and Spanish region and the biomass of each individual earthworm functional group for the German region only.

Since earthworm distribution can change over smaller spatial scales than the selected research plots of 30 x 30 m (Valckx et al. 2009), tree diversity, evergreen proportion and leaf litter quality effects were also investigated at the neighbourhood level (5 m radius surrounding the earthworm sampling locations). In general, the model results were similar to the results at plot level. Details about the neighbourhood analysis and results can be found in Appendix B5.

3. Results

3.1 Tree diversity and evergreen proportion effects

At the continental scale, across the six regions, tree functional diversity (based on functional dispersion) had a positive effect on the total earthworm biomass (Fig. 3.1a). However, the magnitude of the effect was very small and explained only 1% of the total variation in earthworm biomass. A positive effect of tree functional diversity was also visible in the other earthworm response variables (species richness, and epigeic and endogeic biomass), but was not well supported by our model selection results (Appendix B2, Table S4-S5). There was little support for a taxonomic tree diversity effect, as the null model was always better than the models including the tree richness or True Shannon index (Appendix B2, Table S3-S5). Compared to the diversity indices, the proportion of evergreen leaf litter in the forest stands had a stronger effect on the earthworm response variables and negatively affected both total earthworm biomass, species richness as well as their functional group biomass (epigeic and endogeic) (Fig. 3.1b and Appendix B2, Table S3-S5). In most cases the support was strong (ΔAICc > 2 compared to the null model), but it still explained only 1.7% to 3.6% of the variation in the models (Appendix B2, Table S3-S5). When the evergreen leaf litter and tree functional diversity variables were tested together in one model we found an additional effect of tree functional diversity on top of the evergreen proportion effect on total earthworm biomass, together they explained 4.2% of the total variation (Appendix B2, Table S3).

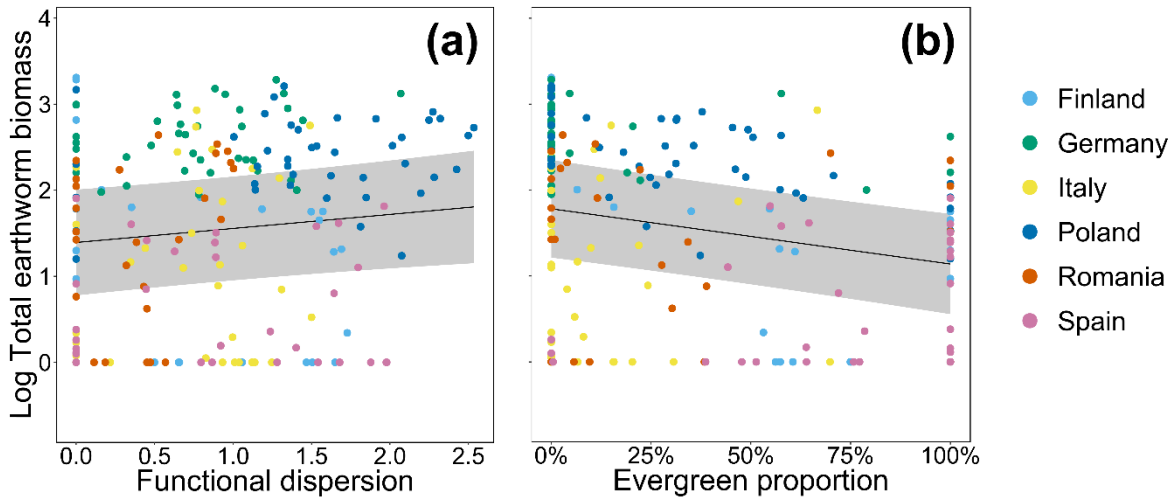


Fig. 3.1. Effect of (a) functional dispersion of tree litter and (b) proportion of evergreen leaf litterfall on the total earthworm biomass. Lines and shaded areas represent predicted responses and corresponding 95% confidence intervals (CI) based on the respective models with respectively functional dispersion and proportion of evergreen leaf litterfall as fixed effect, tree species

composition nested within region as random effect, and all other covariates set to their mean value across all the plots. Grey shaded areas represent 95% CIs of the predictions pooled across regions. Biomass data (g/m²) are [log10(x_{ij} + d) – c] transformed, where c = Trunc(log10(Min(x)) and d = 10^(c). Colours of the circles represent the different regions (light blue = Finland, green = Germany, yellow = Italy, dark blue = Poland, Red = Romania, pink = Spain).

At the regional scale, there was little support for the importance of the tree diversity indices (species richness, True Shannon Index and functional dispersion) in explaining variation in the earthworm response variables (total biomass, species richness and functional groups of earthworms). After model selection, either the null model was the best model or models including a diversity variable had the smallest AICc but were within two AICc units of the null model. We found a weak negative effect of tree species taxonomic diversity in the Polish region, suggesting a decreasing total earthworm biomass as tree species diversity increased (Appendix B2, Table S3). In the German region, evidence of a weak positive effect of the True Shannon's tree diversity index and a tree functional diversity effect were identified for epigeic and anecic biomass, but not for the endogeic biomass. Whereas only 1 % of the total variation was explained by tree diversity variables in the endogeic models, up to 7% and 10% were explained in the epigeic and anecic models, respectively (Appendix B2, Table S6). In general, endogeic biomass was not related to tree diversity variables in any of the studied regions (Appendix B2, Table S7).
Consistent with the diversity variables, we found little support for the importance of the proportion of evergreen leaf litter at regional scale. The exception was the Finnish region where the proportion of evergreen leaf litter explained 28% and 9% of the variation in the total earthworm biomass and species richness, respectively. The total earthworm biomass and species richness in the Finnish region decreased with increasing proportion of evergreen leaf litter (Appendix B2, Table S3-S4).

3.2 Tree litter quality effects

At the continental scale there was strong evidence for a positive effect of the water holding capacity (WHC) of the litter and a negative effect of the litter C:P ratio on the total earthworm biomass and species richness (Table 3.1, Fig. 3.2 and Fig. 3.3). In addition we found significant effects of litter C:N ratio, calcium and lignin on the total earthworm biomass, however their relative importance was low (Appendix B2, Table S8).

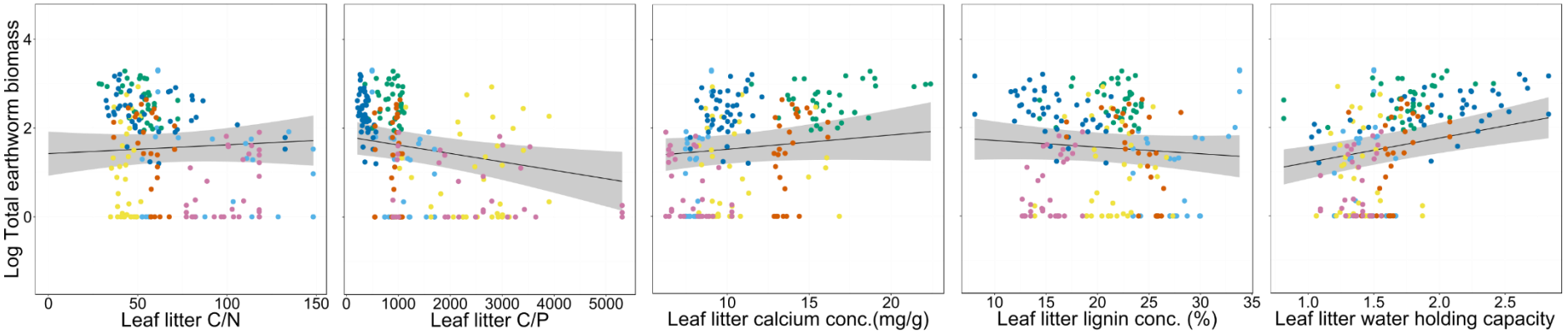


Fig. 3.2. Effect of tree leaf litter traits on the total earthworm biomass . Lines and shaded areas represent predicted responses and corresponding 95% confidence intervals (CI) based on the respective models with the respective litter trait as fixed effect, tree species composition nested within region as random effect, and all other litter traits and covariates set to their mean value across all the plots. Grey shaded areas represent 95% CIs of the predictions pooled across region. Biomass data (g/m²) are [log10(x_{ij} + d) – c] transformed, where c = Trunc(log10(Min(x)) and d = 10^(c). Colours of the circles represent the different regions (light blue = Finland, green = Germany, yellow = Italy, dark blue = Poland, Red = Romania, pink = Spain).

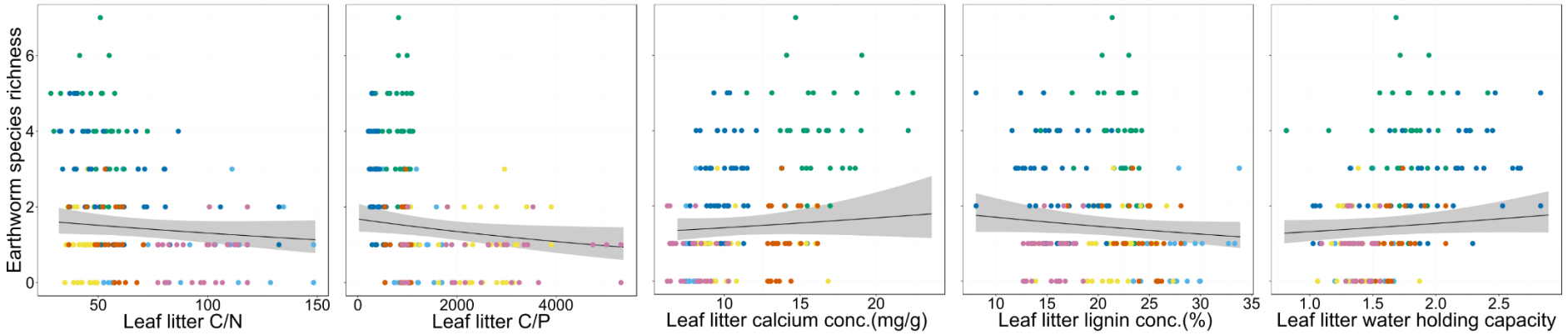


Fig. 3.3. Effect of tree leaf litter traits on the earthworm species richness . Lines and shaded areas represent predicted responses and corresponding 95% confidence intervals (CI) based on the respective models with the respective litter trait as fixed effect, region as random effect, and all other litter traits and covariates set to their mean value across all the plots. Grey shaded areas represent 95% CIs of the predictions pooled across regions. Colours of the circles represent the different regions (light blue = Finland, green = Germany, yellow = Italy, dark blue = Poland, Red = Romania, pink = Spain).

Table 3.1. The effect of litter quality traits on a) total earthworm biomass and b) earthworm species richness for the continental and six regional scale models, demonstrated by a simplified reproduction of the model parameter estimates and their relative importance. The arrows represent the direction of the averaged parameter estimates of the best candidate models and the values right of the arrows represent their relative importance values. Detailed results of the parameter estimates can be found in Appendix B2, Table S8-S12. Parameters that were retained in the best candidate models are represented with a black arrow. Parameters that were not included in the analysis due to multicollinearity issues, but that showed high correlations with variables that were retained in the best candidate models have grey arrows (Appendix B2, Table S15). Parameters that were included in the model, but not retained in the best candidate models are indicated with a hyphen. Parameters that were highly correlated with parameters that were not retained in the best candidate models are left blank.

Model parameters	**CONTINENTAL**		**FINLAND**		**GERMANY**		**ITALY**	**POLAND**		**ROMANIA**		**SPAIN**	
a) Total earthworm biomass													
C:N ratio	↗	0.13			↘	1	-	-		↘		↗	
C:P ratio	↘	0.80	↘		↘	0.33		↘		-		-	
Calcium	↗	0.16	↗	1	-		-	↗	0.31	-		↘	0.67
Lignin	↘	0.16	↗		-			-		↘	1	↗	
Water holding capacity	↗	1	-		↗		-			↗		↗	0.22
b) Earthworm species richness													
C:N ratio	↘	0.49	↘		↘	0.98	-	↘	0.30	↘			
C:P ratio	↘	0.21	↘		↘	0.31		↘		-		-	
Calcium	↗	0.09	↗	0.69	-		-	-		↗	0.23	-	
Lignin	↘	0.09	↗		↘	0.16		-		↘	0.21		
Water holding capacity	↗	0.54	↘	0.27	↗		-			↗		-	

↗ : Earthworm biomass/species richness increases with increasing model parameter value

↘ : Earthworm biomass/species richness decreases with increasing model parameter value

Table 3.2. The effect of litter quality traits on earthworm functional group biomass (epigeic, endogeic and anecic) for continental scale models , demonstrated by a simplified reproduction of the model parameter estimates and their relative importance. Anecic models could not be fitted due to a too low amount of plots where anecic species were present. (cf. Table 3.1 for details)

Model parameters	Epigeic		Endogeic		Anecic	
C:N ratio	-		-		NA	NA
C:P ratio	↘	0.20	↘	0.55	NA	NA
Calcium	-		-		NA	NA
Lignin	↗	0.32	↘	0.45	NA	NA
Water holding capacity	↗	1	↗	1	NA	NA

↗ : Earthworm biomass increases with increasing model parameter value

↘ : Earthworm biomass decreases with increasing model parameter value

Table 3.3. The effect of litter quality traits on endogeic earthworm biomass for the continental and regional scale models that had sufficient endogeic earthworms, demonstrated by a simplified reproduction of the model parameter estimates and their relative importance. (cf. Table 3.1 for details)

Model parameters	CONTINENTAL		FINLAND		GERMANY		ITALY	POLAND		ROMANIA	SPAIN	
C:N ratio	-		NA	NA	↘	1	-	-			↗	
C:P ratio	↘	0.20	NA	NA	-					-	-	
Calcium	-		NA	NA	-		-	-		-	↘	0.63
Lignin	↘	0.32	NA	NA	-			↘	0.40	-	↗	
Water holding capacity	↗	1	NA	NA	↗		-	↗			-	

↗ : Earthworm biomass increases with increasing model parameter value

↘ : Earthworm biomass decreases with increasing model parameter value

Table 3.4. The effect of litter quality traits on earthworm functional group biomass (epigeic, endogeic and anecic) for the German region, demonstrated by a simplified reproduction of the model parameter estimates and their relative importance. (cf. Table 3.1 for details)

Model parameters	Epigeic		Endogeic		Anecic	
C:N ratio	↘	1	↘	1	↘	0.42
C:P ratio	↘	1	-		↘	0.13
Calcium	-		-		↗	0.42
Lignin	-		-		↘	0.13
Water holding capacity	↗		↗		↗	

↗ : Earthworm biomass increases with increasing model parameter value

↘ : Earthworm biomass decreases with increasing model parameter value

Table 3.5. Likelihood ratio R^2 of the total earthworm biomass null models (Biomass ~ 1 + Covariates + Random effect) and respective diversity (Biomass ~ Diversity index + Covariates + Random effect), proportion of evergreen leaf litter (Biomass ~ Evergreen proportion + Covariates + Random effect) and litter quality models (Biomass ~ Litter quality traits + Covariates + Random effect). The litter quality models are the best models after model selection (see section 2.4.2 for details). The last column (Litter quality traits) describes which traits were included in the best litter quality model. All models were fitted with restricted maximum likelihood estimation.

		Diversity			Identity		
Model scale	**Null model**	**Richness**	**Shannon**	**FDis**	**Evergreen**	**Litter quality**	**Litter quality traits**
Continental	0.575	0.575	0.576	0.585	0.611	0.624	C:P ratio + WHC
Finland	0.289	0.284	0.304	0.297	0.565	0.715	Calcium
Germany	0.049	0.054	0.128	0.105	0.077	0.269	C:N ratio
Italy	0.092	0.177	0.176	0.151	0.094	0.092	-
Poland	0.568	0.612	0.59	0.573	0.573	0.568	-
Romania	0.24	0.281	0.267	0.305	0.286	0.494	Lignin
Spain	0.04	0.059	0.089	0.041	0.079	0.139	Calcium

At the continental scale epigeic and endogeic earthworm biomass were positively correlated with litter water holding capacity (Table 3.2). In addition, both groups showed a negative correlation with C:P ratio but opposite relationships with litter lignin concentration, with epigeic biomass increasing and endogeic biomass decreasing with increasing lignin concentration.

At the regional scale we observed similar responses for total earthworm biomass in the Finnish, German and Polish regions. However, in the Spanish region relationships with litter nutrient traits were reversed (Table 3.1a), total earthworm biomass was higher in plots with higher litter C:N ratio and lower in plots with a higher litter calcium concentration. In contrast, water holding capacity was positively related to earthworm biomass in Spain as in Germany and Romania. In general, all litter quality traits that were retained in the averaged top model sets at the regional scale, showed similar directional responses for both total earthworm biomass and species richness (Table 3.1). Moreover, there were strong correlations between earthworm biomass and species richness (r_p = 0.88 - 0.51). Nevertheless, we found differences in the relative importance of the explanatory variables (Appendix B2, Table S8-S9). In contrast to total earthworm biomass where no such relationships were detected, earthworm species richness decreased with increasing litter water holding capacity in the Finnish region, increasing lignin concentration in the German region and increasing C:N ratio in the Polish region (Table 3.1b). We found little support for an effect of litter quality on endogeic biomass (Table 3.3). However, litter quality appeared to have opposite effects on endogeic earthworm biomass in the Spanish region and the temperate forest regions in Germany and Poland.

When we compared earthworm functional group models in the German region, we found support in all functional groups for a negative relationship between earthworm biomass and litter C:N ratio and an increase in earthworm biomass in forest plots with litter with a higher water holding capacity (r_p = -0.8; WHC~C:N) (Table 3.4). Lignin and litter calcium concentration were only related to anecic earthworm biomass in the German forest region.

3.3 Comparing importance of species diversity, evergreen proportion and litter quality effects

Litter quality traits explained a larger proportion of the variation in total earthworm biomass than the proportion of evergreen leaf litter and any of the tree diversity indices. This is clearly indicated in the continental and most regional models (Table 3.5) by higher R^2 values for the litter quality models in comparison with the evergreen

proportion and diversity models. Similarly, the variation in the biomass of earthworm functional groups was generally better explained by tree litter quality traits than by the proportion of evergreen leaf litter or tree diversity (Appendix B2, Table S14). The results of the earthworm species richness models were less consistent: At the continental scale and in the Finnish region the proportion of evergreen leaf litter explained the highest amount of variation in species richness. However, in the German region litter quality was the best predictor, while in the other regions R^2 values were much lower and their differences between predictor variables small (Appendix B2, Table S13).

4. Discussion

4.1 Tree diversity and evergreen proportion effects

We found little support for the importance of tree diversity on earthworm biomass and species richness, at either the regional or continental scale. Nevertheless, functional dispersion as a proxy for tree functional diversity contributed to explain total earthworm biomass at the continental scale. Our findings are largely consistent with the findings of Schwarz et al. (2015b) who found no significant relationships between tree diversity indices and earthworm abundance and species richness in young tree diversity experiments (8-10 years old). These forest experiments, however, might have been too young to allow the trees to create changes in soil properties that could facilitate earthworm abundance or species richness. In contrast, Chamagne et al. (2016) reported a significant positive True Shannon diversity effect on earthworm species richness in mature forests in the Czech Republic. They reasoned that earthworm species might be specialized on certain tree species, which results in higher earthworm species richness when these tree species occur together in a mixture. In addition, the effect of litter functional diversity on earthworm biomass observed in this study echoes results from another study from the FunDivEUROPE platform, wherein the effect of the overall decomposer community on decomposition was partly driven by the litter functional diversity (Joly et al. 2017). It is not clear why functional diversity was only significant at the continental scale. In this case there can be several reasons why different conclusions can be drawn from effects acting on different scales. (i) Environmental and earthworm biomass gradients might be longer at the continental scale compared to the regional scale. (ii) A larger sample of the continental model can also result in higher statistical power. (iii) Factors other than functional diversity are more important at the regional scale, such as differences in soil characteristics that were not covered by our covariates. Only after accounting for such

hidden differences in the random factor "region", functional diversity signals emerged at the continental scale.

Tree diversity (both True Shannon index and functional diversity) was more important for detritivore earthworm biomass (epigeic and anecic species) than for geophagous biomass (endogeic species), in the German region. This is consistent with the different feeding behaviour of the two groups (Hendriksen 1990): detritivore earthworm species mainly feed on surface litter, while geophagous species feed on organic matter in the organo-mineral horizon.

The negative trends in the tree diversity-total earthworm biomass relationships in Poland and Finland are most probably caused by litter quality or tree species identity effects, where one or two tree species with leaf litter rich in N, P and several base cations have a decisive impact on the soil (Cesarz et al. 2016) and thus could diversify the soil ecosystem in these poorly buffered sandy soils (Reich et al. 2005). A certain proportion of good quality litter seems to be necessary in this poorly buffered system to bring the soil nutrient conditions to a certain level that earthworm biomass can increase significantly. This mechanism is similar to the threshold effect observed by De Wandeler et al. (2016) where a threshold value for several litter and soil variables had to be attained before earthworms can occur. It can also be interpreted as a selection effect of tree diversity: if only a few species with good litter quality occur in the species pool, the proportion of low quality litter increases as the tree diversity increases.

In this study the evergreen proportion effect was a more important factor in explaining earthworm biomass and species richness than tree diversity. At the continental scale we observed a negative effect of the proportion of evergreen leaf litter on the earthworm community and hereby confirm results of previous studies in Europe and North America (González et al. 2003, Scheu et al. 2003, Elmer et al. 2004, Ammer et al. 2006). The mechanism behind this negative relationship is most probably a litter quality effect. Evergreen leaf litter is generally lower quality litter in terms of low nutrient content and high lignin content and therefore less preferred by soil organisms (Korboulewsky et al. 2016), which in turn slows down litter decomposition and promotes soil acidification (Augusto et al. 2002, De Schrijver et al. 2012). Even though this negative effect of the proportion of evergreen leaf litter on the earthworm community was observed in northern Europe, our results indicate that it might not hold true in southern Europe where the more arid climate and the

presence of broadleaved angiosperm trees in the evergreen functional group (*Q. Ilex*) may alter this effect (section 4.3. and Appendix B2, Table S3-S4).

4.2 Litter quality effects

Several litter trait effects were similar for total earthworm biomass and richness, both at the continental and regional scale. Earthworm biomass and species richness both increased with decreasing C:N and C:P ratios and increasing water holding capacity and calcium concentration in all but the southern (Italian and Spanish) regions. These effects of tree litter calcium concentration and C:N and C:P ratios on earthworm biomass are in concordance with earlier research by De Wandeler et al. (2016) at a continental scale and with many other local scale studies (Reich et al. 2005, Holdsworth et al. 2012, Ott et al. 2014, Cesarz et al. 2016, Yatso and Lilleskov 2016). A high litter calcium concentration, high water holding capacity and low C:N and C:P ratios create favourable conditions that stimulate microbial decomposer abundance and consequently the recycling of other important nutrients, resulting in an increased nutrient availability (Gosz et al. 1973, Bardgett 2005a, Manzoni et al. 2012). Nevertheless, the mechanisms behind these relationships could be diverse: Litter calcium can influence soil pH (Reich et al. 2005) and as a consequence indirectly increase earthworm richness and biomass by allowing acidophobic species to occur. This is especially important in a sandy soil with low pH values, consistent with those recorded in the Finnish and Polish plots (Ammer et al. 2006). More calcium-rich litter generally means more easily digestible food with high macronutrient concentrations, which can also more directly support a higher earthworm biomass (Lavelle et al. 1995, Reich et al. 2005, Cesarz et al. 2016). In addition, it is known that some species, mainly litter feeders (Piearce 1972), need calcium to supply their well-developed calciferous glands, that produce calcium carbonate (Canti and Piearce 2003). $CaCO_3$ production in these earthworms is probably related to pH regulation of blood and tissue fluids when soil CO_2 levels are high (Versteegh et al. 2014). The fact that litter calcium was an important variable explaining variation only in anecic earthworm biomass in the German region could be related to their stronger dependence on litter as a food source and, at the same time, their need for calcium to regulate pH levels in the blood.

As expected we found a decrease in total earthworm biomass and species richness with increasing litter lignin concentration and decreasing water-holding capacity. In contrast, we found the opposite patterns in the Finnish region. Increasing earthworm biomass and species richness with increasing litter lignin concentration and decreasing water-holding capacity might seem counterintuitive, but in the Finnish forest region lignin was highly positively correlated with litter calcium concentration across the three

tree species (r_p=0.98) and negatively with C:N and C:P ratio (respectively r_p= -0.73 and r_p= -0.95, Appendix B2, Table S15). It confirms earlier observations that as soon as a sufficient amount of macronutrients are present (threshold effect, see De Wandeler et al. (2016)), other litter traits like lignin and WHC become less important (Rajapaksha et al. 2013). The opposite relationships of the epigeic and endogeic earthworm biomass with the litter lignin concentration across regions was against our expectations as leaf litter high in lignin is generally considered hard to decompose (Hobbie et al. 2006, Berg and McClaugherty 2008) and high-lignin substrate is not preferred by earthworms (Kasurinen et al. 2007). However, the relative importance of litter lignin concentration for epigeic earthworm biomass was small (Appendix B2, Table S10), and this positive relationship with lignin disappeared when the Italian plots were excluded from the analysis. Drought earlier in the season, in the Italian region might have contributed to this rather unexpected result at a continental scale.

4.3 Latitudinal variations in earthworm biomass response to litter quality

A latitudinal variation in trait specific responses was observed for total and endogeic earthworm biomass, but also for earthworm species richness. Consistent with Reich et al. (2005), who associated increased earthworm abundance and diversity to calcium-rich litter in Poland, we observed increasing earthworm biomass and species richness with increasing litter nutrient concentrations (low C:N ratio and high calcium concentration; Table 3.1) in north and central Europe (Finland, Poland, Germany, Romania). In southern Europe, earthworms seemed less dependent on nutrient-rich litter. We found endogeic earthworm biomass to even decrease with increasing leaf litter nutrient concentration in the Spanish forest region (Table 3.3). This is inconsistent with the well-accepted hypothesis that earthworms prefer to ingest litter with high nutrient concentrations (Hendriksen 1990, Rajapaksha et al. 2013). This contrasting relationship of endogeic earthworm biomass with leaf litter nutrient concentration in Spain could have several reasons. On the one hand, litter nutrient concentrations are less important to endogeic earthworms in nutrient rich soil (Cesarz et al. 2016). On the other hand, the higher temperatures in the Italian and Spanish regions may facilitate the mutual digestion system of the earthworm species (Lavelle et al. 1995). However, the most important explanation as to why endogeic earthworm biomass decreases with increasing leaf litter nutrient concentration in Spain is probably the correlation of nutrient rich litter with very dry soil conditions that negatively impact the earthworm community. This reasoning is based on our assumption that calcium-rich litter in Spain indicates shallow soils on calcareous bedrock that results in temporally very dry conditions and sparse vegetation.

4.4 Importance of tree diversity versus tree identity effects for earthworms

We found that litter quality was a stronger driver of earthworm biomass than tree diversity, as reported by Schwarz et al. (2015b). At the continental scale and in the Finnish and German region, tree identity (evergreen proportion and litter quality) rather than tree diversity was driving earthworm species richness. Herewith, our results contradict findings of Chamagne et al. (2016) who found that tree species richness rather than differences in litter quality affected the earthworm diversity. However, the range of litter traits of the four tree species studied by Chamagne et al. (2016) is smaller than in our study and might not have been large enough to detect litter quality effects in the studied forest soil.

5. Conclusion

Overall, our study showed that covering the large geographic area and the multitude of factors considered advances our understanding of tree diversity and identity effects on earthworm communities. By focusing on tree leaf litter traits, this study provided greater insight into the mechanisms driving tree identity effects. We specifically found a latitudinal variation in earthworm biomass response to tree leaf litter quality. Furthermore, by making use of a research platform that allowed unconfounded tree species diversity tests, this study supports previous findings that litter quality, as a proxy of tree identity, was a stronger driver of earthworm biomass than tree species diversity. In addition, of the three diversity indices tested (richness, True Shannon diversity index, functional diversity), tree functional diversity positively affected earthworm biomass at the continental scale. This study was however limited to the diversity and litter quality effects of trees as they were assumed to be the main players in the forest ecosystem in terms of litter production, although the ground was often covered with herbaceous plants or shrubs in most of the studied regions. Previous research highlighted the link between earthworm distribution and the presence, biomass and nutrient quality of understory vegetation, and we therefore encourage future tree diversity research to incorporate understory vegetation in their study.

Chapter 4

Tree litter effects on an earthworm-soil feedback loop in European forests

Adapted from: Hans De Wandeler, Koenraad Van Meerbeek, Martin Hermy, Bart Muys. Tree litter effects on an earthworm-soil feedback loop in European forests. In Preparation

1. Introduction

Plant-soil interactions have been a major subject of study in the past decades (van der Putten et al. 2013). By now it is well accepted that plant community composition and litter traits are strong drivers of litter decomposition and subsequent nutrient mineralization (Hättenschwiler 2005, Cornwell et al. 2008, Joly et al. 2017), and the soil biota associated with this process (Sauvadet et al. , Scheu 2005, Aponte et al. 2013, Ott et al. 2014). In many soil types, including forest soils, earthworms represent a significant part of the soil biomass and are important actors of litter decomposition and soil transformation (Satchell 1983, Lavelle and Spain 2001).

Earthworms are called ecosystem engineers because they can make state changes in biotic or abiotic material in the soil system (Jones et al. 1994, Lemtiri et al. 2014), which makes them important drivers of several soil functions and ecosystem services (Blouin et al. 2013). Therefore, evaluation and quantification of factors and mechanisms that relate to earthworm activity can have major implications for present and future land management.

As described by Jones et al. (2010) the physical engineering by ecosystem engineers, like earthworms, can be described by several cause-effect relationships: (1) They cause structural changes in the soil system - earthworms have been shown to decrease the forest floor mass by feeding on fresh litter or partly decomposed organic material (Satchell 1983, Haimi and Huhta 1990, Scheu and Wolters 1991, Fahey et al. 2013). Furthermore, they alter the soil structure by their burrowing activities, fragmentation and burial of litter and their contribution in soil aggregation (Satchell 1983, Jones et al. 1994, Blanchart et al. 1997, Ketterings et al. 1997). (2) These structural changes may cause abiotic changes - a decreased forest floor mass and incorporation of organic matter in an A horizon can result in reduced acidity of the topsoil (Haimi and Huhta 1990, Räty 2004), a decrease in exchangeable soil aluminium (Schelfhout 2010) and associated increased macro-nutrient availability (Reich et al. 2005). (3) Structural and abiotic changes in turn may feedback to the ecosystem engineer - as spatial resource availability influence epigeic earthworm densities (Aubert et al. 2003), high exchangeable Al^{3+} concentrations inhibit earthworm growth and cocoon production (van Gestel and Hoogerwerf 2001), low soil nutrient availability is often reported to negatively influence earthworm biomass (Cesarz et al. 2016) and soil pH is influencing earthworm species occurrence (Bouché 1972, Curry 2004, Ammer et al. 2006, Cesarz et al. 2007, Mueller et al. 2016).

Until now, these individual cause-effect relationships between soil ecosystem engineers and soil physics or soil chemistry have been observed in controlled experiments as well as in the field. Next to studies that confirm one or several of these aforementioned cause-effect relationships, and that speculate in their discussion on the

consequences of the patterns that they found, we need to make and test more elaborate models to fully understand the complex aboveground-belowground interactions and its consequences on the belowground system. However, only few studies have made suggestions towards the existence and mechanisms of an earthworm-soil feedback loop, or have studied the full loop of interlinked cause-effect relationships between soil ecosystem engineers, soil physics and soil chemistry, let alone investigated how external factors could influence this loop.

In this chapter we investigated how tree litter characteristics and tree functional diversity influence a hypothesised earthworm-soil feedback loop. The hypothesis of this positive feedback loop consists in the idea that earthworm activity influences the humus quality and subsequently the soil acidity, where changes in soil pH may feedback to earthworm activity. The earthworm-soil feedback loop was studied in a European network of mature forests comprising different forest biomes spread across the continent.
Previous studies by De Wandeler et al. (2016, 2018) already showed that tree litter functional dispersion (a proxy of tree functional diversity) and especially tree litter identity are drivers of earthworm biomass at a European continental scale. We now extend these studies by quantifying the influence of tree litter characteristics and functional dispersion on an earthworm-soil feedback loop. We acknowledge, however, that earthworm-induced structural and abiotic changes in the soil can also cause biotic changes to other soil organisms, like fungi and soil bacteria (Dempsey et al. 2013) and that feedbacks may exist to plant community structure, composition, diversity and litter nutrient quality (Wurst et al. 2003, Scheu 2005, Holdsworth et al. 2007, Ponge 2013). However integrating these effects into our causal model is beyond the scope of this study.

2. Material and methods

2.1 Study area

All samples were collected in the exploratory platform of the FunDivEUROPE project (Baeten et al. 2013). This platform was designed to assess biodiversity-ecosystem function relationships along a tree species richness gradient in mature forests. The six studied forest landscapes (hereafter called regions) in this platform span most of the European bioclimatic gradient and represent major European forest types including the boreal forest (North Karelia, Finland), hemiboreal forest (Białowieża, Poland), temperate beech forest (Hainich, Germany), mountain beech forest (Râsça, Romania), thermophilous deciduous forest (Colline Metallifere, Italy) and Mediterranean-mixed forest (Alto Tajo, Spain) (Appendix C1, Fig.. S1).

Each region included between 28 and 43 selected plots (30 x 30 m) with different combinations of a fixed set of locally dominant tree species. The established plots ranged in tree species diversity from one to five species per plot. Because De Wandeler et al (2018) found only a significant positive effect of tree species functional dispersion on total earthworm biomass, other tree diversity mechanisms are not further discussed. In total, the platform consisted of 209 plots with 16 target tree species, some of them occurring in multiple regions. The species pool comprised evergreen conifers, deciduous broadleaves and evergreen broadleaves. For more details consult Baeten et al. (2013).

2.2 Data collection

2.2.1 *Earthworm biomass*

Earthworm sampling was carried out in spring 2012 for the regions in Italy, Germany and Finland, and in autumn 2012 for the regions in Poland, Romania and Spain. As advised in literature, we targeted our earthworm sampling in spring or autumn because of the humid soil conditions and positive temperatures (Berry and Jordan 2001, Holmstrup 2001, Baker and Whitby 2003, Eggleton et al. 2009).

Plots were divided in nine (10 x 10 m) subplots. In each plot, one earthworm sample was taken in the central subplot. Earthworms were sampled by means of a combined method. First, litter was hand sorted over an area of 25 x 25 cm. Then, litter was removed over a larger area of 1 x 0.5 m in order to effectively apply an ethological extraction of earthworms using a mustard suspension (Valckx et al. 2011). Finally, hand sorting of a soil sample of 25 × 25 cm and 20 cm depth was performed in the middle of the 1 x 0.5 m area. Collected earthworms were preserved in ethanol (70%) for two weeks, transferred to a 5% formaldehyde solution for fixation (until constant weight), after which they were transferred back to ethanol (70%) for preservation of at least one month. Upon identification, all earthworms were individually weighed, including gut content. Results per unit area of the three sampling techniques were summed to determine the earthworm biomass per m^2. For biomass recordings all earthworm biomass, even worm fragments, were considered. For more details consult (De Wandeler et al. 2016).

2.2.2 *Tree leaf litter traits*

In all 209 plots, tree leaf litter of all studied tree species was collected with litter traps (De Wandeler et al. 2016). The litter was used to determine tree species specific leaf litter C:N ratio and calcium concentration per plot (Appendix C3). Fourteen additional leaf litter traits (Appendix C2, Table S3) were determined from freshly fallen leaf litter of each species at regional level at several locations around the plots (Joly et al. 2015).

Then, tree basal area-based community weighted means (CWM) of these eighteen leaf litter traits were calculated per plot (De Wandeler et al. 2016).
We used principal components analysis (PCA) to reduce the number of litter variables and extract the most important gradients in litter trait space, in order to reduce the complexity of the structural equation model. The first PCA axis explained 39% of the variation in the litter trait space and was highly negatively correlated with leaf litter C:N ratio and positively with the concentration of several leaf litter base cations (Fig. 4.1; Appendix C2, Table S1). Consequently the scores of this axis were used as a single indicator of leaf litter nutrient quality. The second PCA axis explained 25% of the variation and was highly positively correlated with lignin concentration and negatively with polyphenol content (total phenolics) of the leaf litter (Fig. 4.1; Appendix C2, Table S1). The scores of this second axis were used as an indicator of structural resistance.

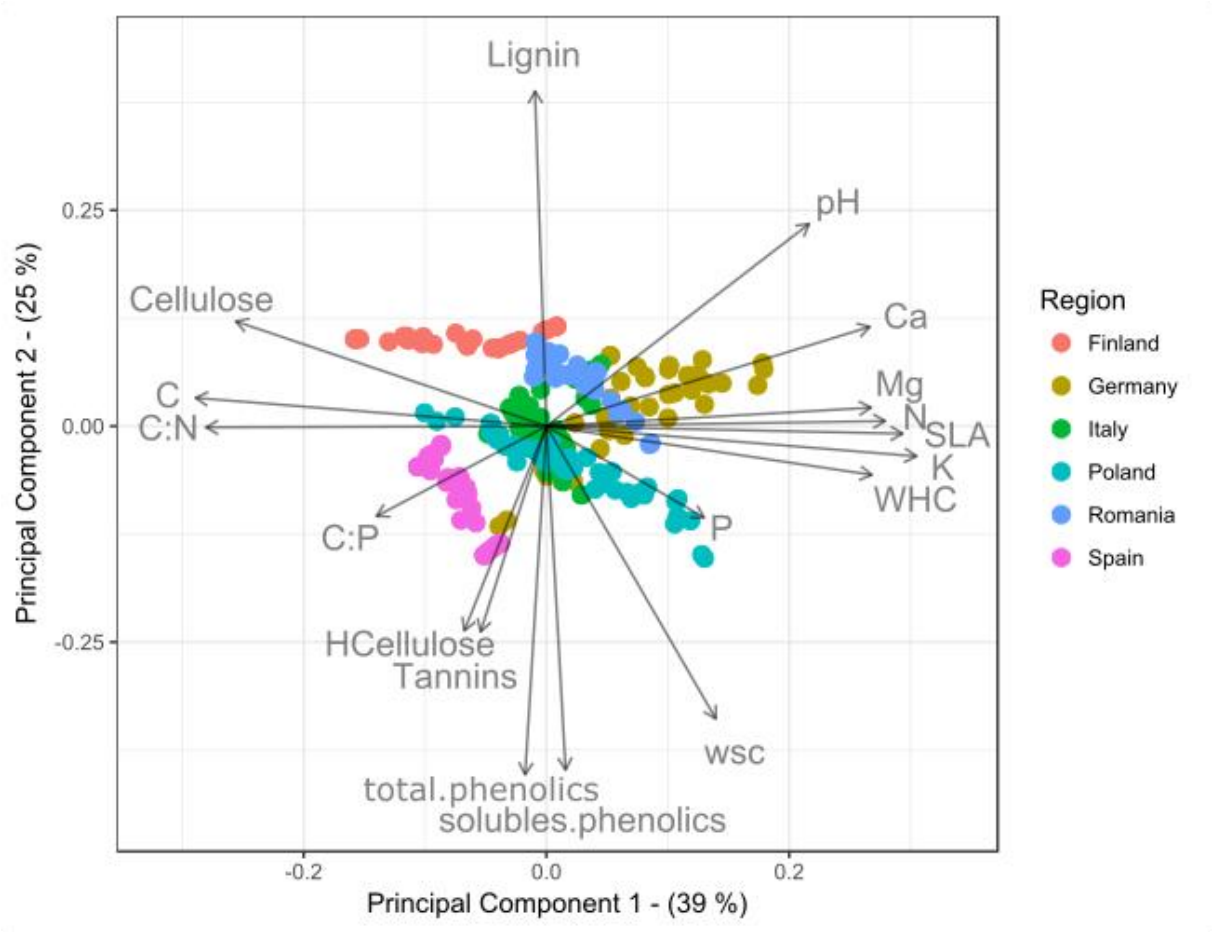


Fig. 4.1. Principal component analysis (PCA) of 18 tree leaf litter CWM traits . Further info on the 18 litter traits can be found in Appendix C2, Table S3.

2.2.3 *Humus quality*

In the central subplot of each of the 209 plots, humus form was assessed based on a European reference base for humus forms. We performed a detailed description of the humus forms up to the second level of humus form classification (Box 3 and Zanella et al. 2010). The A horizon type (biomacrostructured, biomesostructured, biomicrostructured, single grain, massive) and the incidence of an OH horizon (presence vs. absence) were visually assessed in the field. The forest floor thickness (cm) was measured and additional samples were taken to determine the dry mass (kg/m^2) and C:N ratio of the forest floor. The forest floor in this manuscript refers to the ectorganic layer and comprises three organic horizons: the OL, OF and OH horizon as described by (Zanella et al. 2011). For more details on the assessment of the forest floor thickness, biomass and C:N ratio see Appendix C3. We used a principal coordinate analysis (PCoA) to reduce the number of humus variables and extract the most important gradients, in order to reduce the complexity of the structural equation model. In contrast to the leaf litter trait ordination we used PCoA instead of PCA, because several variables where qualitative or discrete. PCoA is a distance based method that is based on a similarity matrix of the used variables rather than the initial raw data matrix of qualitative variables which is used in a PCA ordination (McCune et al. 2002). The first PCoA axis explains 60% of the variation in these humus variables and is strongly correlated with nearly each of them (Fig. 4.2; Appendix C2, Table S2). The scores of this axis were used as a single non-categorical indicator for humus quality.

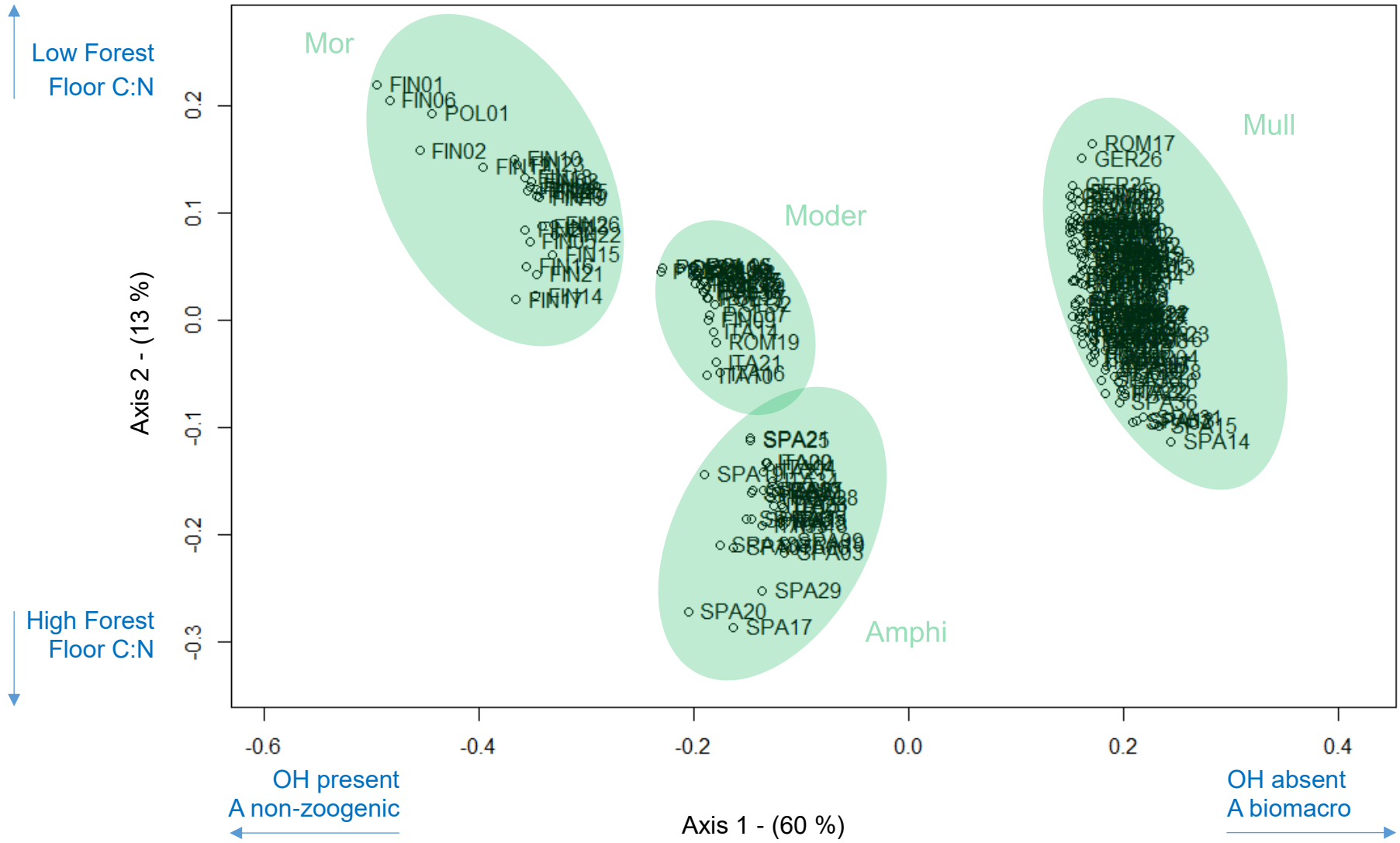


Fig. 4.2. Principal Coordinate analysis (PCoA) biplot of the 203 sampled forest plots based on humus form characteristics. The clustering of the plots according to the four basic humus forms is indicated in green. Range information of the variables that showed the highest correlation with the first two PCoA axes (OH horizon, A horizon and Forest Floor C:N) are shown in blue. More details on the humus form characteristics and the correlation coefficients can be found in Appendix C2 (Table S3 and S2, respectively).

2.2.4 *Soil properties*

In each plot, soil pH of the upper 10cm of the mineral soil (0-10 cm) was determined on a composite sample of 9 subsamples (Dawud et al. 2016). Soil pH ($CaCl_2$) of the mineral soil was determined with 0.01 M $CaCl_2$ solution at a ratio of 1:2.5, using 827 pH lab (Metrohm AG, Herisau, Switzerland) after the soil was dried to constant weight (55°C) and sieved through a 2 mm diameter mesh. Clay fraction of the mineral soil was estimated per plot by palpation. Measurements were done on an ordinal scale, with values ranging from 1 (absent) to 3 (very common). Next to soil pH and clay, volumetric stone content of the upper 30 cm of mineral soil was estimated as an indicator of biotope space. Stone content of the soil was estimated with the "iron-rod" method by Viro (1952) and the empirical equation presented by Tamminen and Starr (1994). Stone content was chosen above a soil depth measure on ordinal scale, as it was considered a more accurate measure.

2.2.5 *Microclimate*

As indicators of forest floor microclimate conditions both heat load index and leaf area index were measured. The heat load index (HLI) reflects the heat load that a particular location gets because of annual direct incidence radiation, the higher the value the larger the heat load and the warmer that location will be (McCune and Keon 2002). Leaf area index (LAI, total one-side area of leaf tissue per unit ground surface area, m^2m^{-2}, Watson, (1947)) was measured on five locations within each plot and was assessed as a proxy for light incidence underneath the canopy. Further details on both measurements can be found in Appendix C3.

2.3 Statistical analysis

We used structural equation modelling (SEM; Grace 2006) to quantify the relationships between tree leaf litter, earthworm biomass, humus quality and mineral soil pH. These relationships are investigated on a continental scale across the six sampled regions. Structural equation modelling is a multivariate statistical analysis technique that is used to evaluate a set of structural relationships in a single network. It can be used to represent and test mediation and direct (X → Y) versus indirect (X → Z → Y) relationships and the partial contributions of correlated explanatory variables (Eisenhauer et al. 2015). It also allows pooling effects of multiple variables into composite variables (Grace and Bollen 2008). With structural equation modelling, one can develop causal understanding from observational data by testing mechanistic hypotheses based on ecological knowledge derived from earlier (experimental) research (Shipley 2000). The most important feature in the context of this study, next to the already mentioned functionalities, is the ability of structural equation models to

model feedback loops (Grace 2006, Grace et al. 2016). Structural equation modelling is commonly used in ecological literature to develop causal understanding from observational data (e.g., Barrufol et al. (2013), De Mazancourt et al. (2013), Matteson et al. (2013), Tedersoo et al. (2014), Lefcheck and Duffy (2015), Chamagne et al. (2016)). Nevertheless, it is still an underused tool in soil ecology (Eisenhauer et al. 2015). It can help us shining light into the black box of interactions belowground and make us understand the relations at the aboveground-belowground interface.

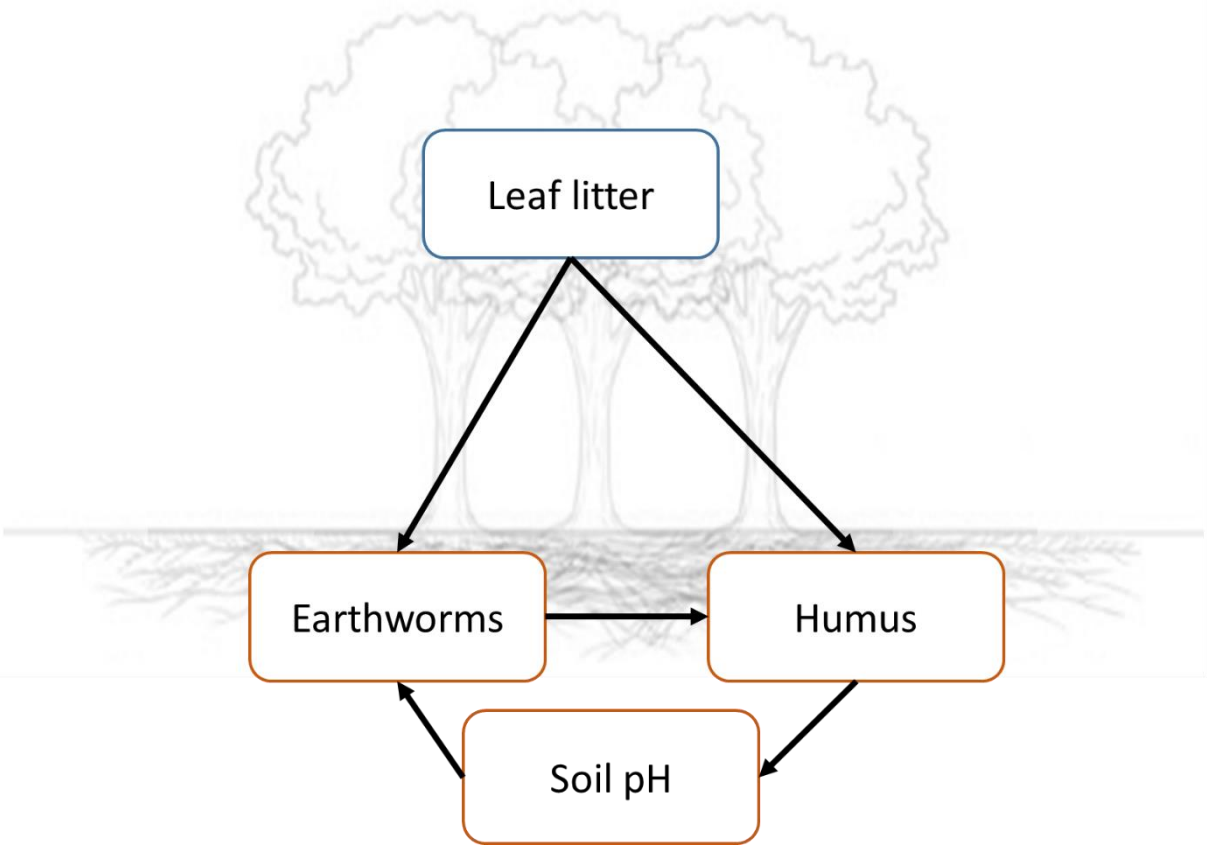


Fig. 4.3. The hypothesised meta-model . Black arrows indicate causal relations and brown squares indicate the elements of the hypothesised earthworm-soil feedback loop. Different leaf litter trait variables drive a belowground earthworm-soil feedback loop comprising earthworm biomass, humus quality variables and soil pH.

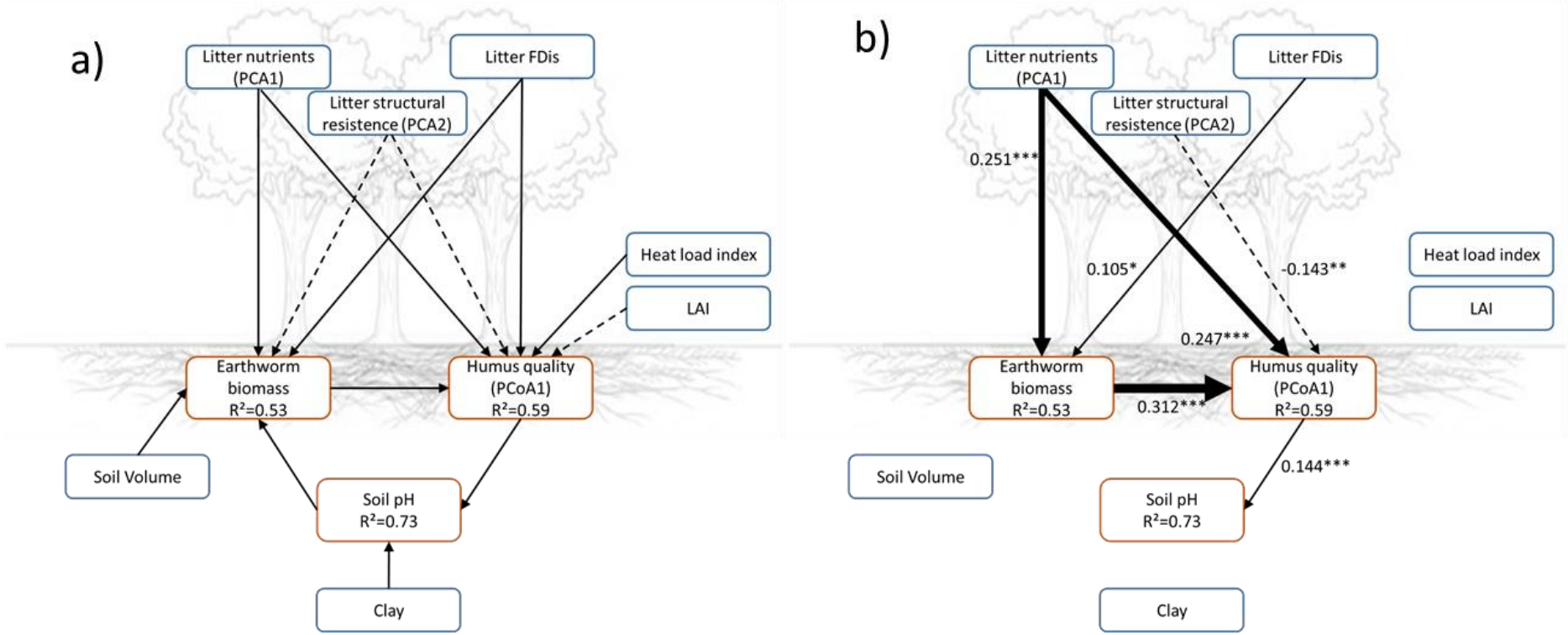


Fig. 4.4. a) The a priori causal model. Solid arrows represent positive effects, dashed arrows represent negative effects. The variables of the hypothesised loop are marked in brown. The relationships between the variables are explained in detail in Appendix C4. b) The final continental model (Chi-square test statistic = 22.5, df = 17, P = 0.165). Numbers next to arrows give standardized path coefficients with their significance indicated as ***P < 0.001, **P < 0.01, *P < 0.05. Solid arrows represent positive effects, dashed arrows represent negative effects, non-significant relations are not displayed. Arrow width reflects path coefficient magnitude. The variables of the hypothesised loop are marked in brown. Numbers below these variables indicate the percentage variation explained (adjusted R^2).

Based on our general hypothesis a meta-model was constructed (Fig. 4.3). Subsequently we built an a priori causal model by populating each component of our meta-model with our measured variables (Fig. 4.4a). This a priori model captured hypothesised causal relationships between the variables that are explained in detail in Appendix C4. In order to meet the requirements of homogeneity and normality of residuals, earthworm biomass data (g/m^2) were [log10(xij + d) – c] transformed, where c = Trunc(log10(Min(x)) and d = 10^(c) following McCune et al. (2002). Model-data consistency was then evaluated by investigating whether there were missing linkages in the initial model. Testing for missing linkages allows alternative mechanisms to explain variation in the response variables (Eisenhauer et al. 2015). All missing paths with an mi > 2 were considered. The modification index (mi) suggests missing links to add to your model structure, its value represents the estimated reduction in model chi-square you would presumably obtain by including a specific missing path (Whittaker 2012). Significance and goodness-of fit of the final model were assessed using a combination of model fit indices: (i) a chi-square test (a non-significant chi-square statistic indicates that the observed data is not-significantly different from the proposed model), (ii) the Tucker-Lewis Index (TLI) where a value of >0.97 indicates a good model fit and a value between 0.95 to 0.97 an acceptable model fit, (iii) and the Root Mean Square Error of Approximation (RMSEA) where a value ≤ 0.05 can be considered as a good fit, values between 0.05 and 0.08 as an adequate fit, and values between 0.08 and 0.1 as a mediocre fit (Schermelleh-Engel et al. 2003). Finally each link in the final model was evaluated for significant contribution to the model and standardized coefficients and the explained variance per response variable were calculated.

The hierarchical structure in the data of the model is accounted for by including a categorical representation of the sampled regions in the model. This is done by constructing a composite variable with the different sites (n-1, with n=number of sites) as dummy variables (Grace and Bollen 2008). We choose for this composite approach as our data did not allow for a multilevel or multigroup structural equation modelling. The number of clusters (6 sites) was too low for multilevel modelling (McNeish and Stapleton 2016) and the number of parameters was too high in relation to the number of observations to allow for an accurate multigroup SEM model fit. In addition, aspects like earthworm diversity and understory plants nutrient quality could not be incorporated into the SEM due to methodological constraints. The number of observations in relation to the number of parameters was too low to have more parameters in our model. We encourage further research however to incorporate them especially because De Wandeler et al. (2016) identified understory plant C:N and C:P ratio as an important predictor of both earthworm incidence and biomass.

To prevent our models from being affected by outliers, four plots were removed from the analysis (cf. De Wandeler et al. 2016) together with two other plots because of missing values.

Since a linear pattern between soil pH and earthworm total biomass could not be confirmed, a threshold pattern was expected in this relationship (De Wandeler et al. 2016). Therefore, in addition to structural equation modelling, generalised additive mixed modelling (GAMM) was used to examine the relationships between soil pH and earthworm total biomass more closely: [R-syntax of the full model: Earthworm Biomass ~ s(soil pH) + s(Covariates) + random=list(Site=~1)]. Model assumptions of normality and homogeneity of residuals were checked (Appendix C1, Fig.S2b).
All analyses were performed in the R software platform (R Development Core Team 2014 version 3.3.3), using the 'lavaan' package version 0.6-1.1141 for our structural equation model analysis and the 'mgcv' package version 1.8-22 for the generalised additive mixed modelling.

3. Results

The first two gradients in the litter trait space were a litter nutrient (PC1) and a litter structural resistance axis (PC2) explaining respectively 39% and 25% of the variation in the litter trait space (Fig. 4.1). The Polish and German region showed the longest range in litter nutrient concentration. In general the German region had tree leaf litter with the highest nutrient quality and the Spanish and Finnish region the lowest quality. Next to rather nutrient poor litter, the litter in the Finnish region also had the highest lignin concentration.
Across the investigated 203 forest plots the four basic humus forms were found (Fig. 4.2). The first axis of the forest plot ordination based on humus form characteristics clearly indicated a humus quality gradient from mor-humus soils with a clear OH horizon and a poorly structured A horizon with no or hardly any animal activity, to mull-humus soils without OH horizon and a well-structured A horizon created by zoological agents. Poor mor-humus soils were mainly present in the Finnish region, richer moder-humus soil in Poland and amphi-humus soils in Italy and Spain, and the German and Romanian sites consisted primarily out of rich mull-humus soils. The second humus-PCoA axis clearly illustrated the difference between the humus forms in cold and temperate Europe and amphi-humus soils in southern-Europe. In mor- and moder-humus forms the forest floor is a product of decomposition, while in an amphi-humus form the forest floor is the undecomposed rest fraction after decomposition, explaining its higher C:N ratio.

The final structural equation model fitted the data well: Chi-square test statistic = 22.5, df = 17, P = 0.165, TLI = 0.982 and RMSEA = 0.04. Our feedback loop hypothesis could not be confirmed by the continental scale model (Fig. 4.4b), as the direct causal link between soil pH and earthworm biomass could not be confirmed. However, the positive causal effect between earthworm biomass and humus quality and humus quality and soil pH were confirmed by the model. In addition, litter nutrient quality was found to be an important predictor both stimulating a higher earthworm biomass as well as improving the humus quality.
In order to test model-data consistency and potentially optimise the models, the initial model was tested for missing links allowing alternative mechanisms to explain variation in the response variables. In doing so, also possible feedback mechanisms to litter functional dispersion and litter nutrient quality were tested. Inclusion of missing links in the continental model however did not significantly improve the continental model fit (ΔAICc < 2; Burnham and Anderson 2002).

Additional generalized additive mixed modelling confirmed our expectations of an increasing earthworm total biomass with increasing soil pH, however only in soils with a low soil pH. This increasing relation approaches a threshold and levels off as soon as a soil pH_{CaCl2} of 4.1 is reached (Fig. 4.5). Next to that these model results indicate a non-linear relation between earthworm biomass and the litter structural resistance axis (Appendix C1, Fig. S2a).

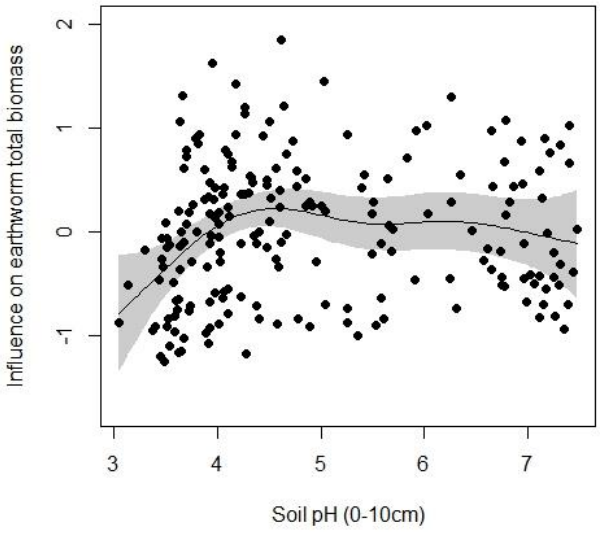


Fig. 4.5. Smoothing curve of soil pH_{CaCl} obtained from the GAMM model explaining earthworm total biomass. This graph shows the relationship between earthworm total biomass (Log transformed) and soil pH taking into account the influence of the other predictor variables in the model. More details on the model see M&M. The shaded parts are the 95% point-wise confidence bands and the little vertical lines along the x-axis indicate the pH values of the observations.

4. Discussion

4.1 Effects on earthworm biomass

4.1.1 *Litter quality effects*

On a continental scale leaf litter nutrient concentration had a positive effect on earthworm biomass. This is in accordance with previous results of chapter 2 and 3 and many studies worldwide who recorded an increase in earthworm biomass with increasing leaf litter nutrient concentrations (Muys et al. 1992, Zou 1993, Reich et al. 2005, Holdsworth et al. 2012, Sigurdsson and Gudleifsson 2013, Ott et al. 2014, Yatso and Lilleskov 2016). In our case this relation means that an increase in macronutrients (Ca, Mg, K) and a decrease in leaf litter C:N ratio causes an increase in total earthworm biomass.

Even though the litter structural resistance axis was strongly correlated with lignin, which was proven to negatively influence earthworm biomass at a European scale (De Wandeler et al. 2016), no significant negative linear relation with this structural resistance axis was found in our model. The generalised additive mixed model however suggests a more complicated relation with this axis. Separate models with individual components of the structural resistance axis (e.g. lignin and polyphenol content) might have assisted here, but were not performed in the current study. Unlike its potential direct influence on earthworm biomass leaf litter structural resistance negatively affected the humus quality. This is in accordance with Cornwell et al. (2008) who summarised 66 decomposition experiments and found that litter structural resistance was decreasing litter decomposition rate, and additional findings by De Schrijver et al. (2012) who observed transitions in humus forms and forest floor build-up as a consequence of slow litter decomposition in a chronosequence of broadleaved deciduous forests in Belgium.

The positive relationship of leaf litter functional dispersion (FDis) with earthworm biomass in De Wandeler et al. (2016) was confirmed by our model as a direct significant causal effect. This is a confirmation of the food web theory that predicts increased biomass of higher trophic levels with increased producer diversity (Cardinale et al. 2011). The diversity of litter types possibly serve different functions in terms of food and structural habitat.

4.1.2 *Soil pH effects*

Contrary to our expectation our model did not confirm the hypothesised direct causal effect of soil pH on earthworm biomass. The fact that the relationship between soil pH and earthworm biomass was not supported by our continental model might indicate that it is a threshold rather than a linear relation with pH (Shartell et al. 2013,

De Wandeler et al. 2016). This assumption was confirmed by a separate generalised additive mixed model that only focused on the relation between earthworm total biomass and soil pH (Fig. 4.5). In most sampled regions, soil pH_{CaCl2} was generally higher than 4 and possibly not a limiting factor for earthworm survival because soil pH effects can primarily be expected around the tipping point of pH_{CaCl2} 3-4. Below a soil pH_{CaCl2} of 4, mostly only small epigeic earthworm species can survive. From a soil pH of 4 onwards, also larger bodied endogeic and anecic species can occur (Bouché 1972, Shartell et al. 2013), and probably other factors like the amount of food supply come into play. This is consistent with a study from Sackett et al. (2012) that showed no effect of soil pH on earthworm abundance in areas where the soil pH_{CaCl2} exceeds 4.1. Furthermore, after controlling for the elemental stoichiometry of leaf litter, also Ott et al. (2014) did not find a significant relation with pH. In this way they demonstrated that soil pH was not directly affecting the abundance of soil organisms. Even though soil pH could have direct effects on acidity or alkalinity, soil pH also drives chemical processes contributing to soil nutrient availability. For instance, acidic soils can cause an increased concentration of exchangeable soil aluminium which is toxic to earthworms (van Gestel and Hoogerwerf 2001) and a low soil pH stimulates Al and Fe to form insoluble compounds with phosphorous, making it no longer bioavailable (Hinsinger 2001).

The slightly decreasing earthworm biomass as soon as soil pH values exceed a pH_{CaCl2} of 6.5-7 is probably due neither to a direct nor an indirect soil pH effect, but most probably indicates an aridity effect in the calcareous soils in Spain, characterized by amphi humus forms, rather than mulls. In such soils, the superficial, periodically very dry organic horizon is generally not inhabited by earthworms, restricting their presence only to the deeper soil horizons (De Nicola et al. 2014).

4.2 Effects on the forest soil

In our model, humus quality is both directly as well as indirectly affected by leaf litter nutrient concentrations. Directly, leaf litter nutrient concentrations affect humus quality by its chemical composition and by mechanisms involving arthropods, bacteria and fungi, which are not specified in this manuscript but extensively described in Berg and McClaugherty (2008). And indirectly, leaf litter nutrient concentrations have a positive effect on earthworm biomass which in turn improved humus quality by stimulating litter fragmentation, decomposition and incorporation of organic material in the soil (Muys et al. 1992, Reich et al. 2005). The relationship between earthworms and humus forms in temperate biomes is one of the relations for which a general agreement is found in literature (Zanella et al. 2011, Ponge 2013). The fact that these relations are among the strongest relations in our model (Fig. 4.4b), only confirms this

conclusion. Furthermore, an indirect significant effect of earthworm biomass, via humus quality on soil pH highlights the importance of soil fauna mediated organic matter decomposition on soil pH (Haimi and Huhta 1990, De Schrijver et al. 2012, Schelfhout et al. 2017).

In literature support exists for the influence of soil temperature on organic matter decomposition. Hobbie et al. (2006) observed a positive correlation between mean annual soil temperature and decomposition rates of tree leaf litter. However, our model did not support this.

4.3 Conclusion

This study confirms the importance of leaf litter traits driving belowground dynamics. Despite the existence of the individual causal relations within the hypothesised earthworm-soil feedback loop, our continental-scale path model could not confirm our general hypothesis that there is a feedback loop between earthworm biomass, humus quality and soil pH, because the hypothesised positive relationship between soil pH and earthworm biomass was not significant. However, additional model results suggested that such feedback loops could exist, but that the relation between soil pH and earthworm biomass shows a threshold behaviour rather than a linear relationship and therefore differs, depending on the soil pH range of the plots under study. Because of this threshold behaviour we assume that the mechanisms behind this earthworm-soil loop will be most visible in soils under and near to this pH threshold.

Chapter 5

Conclusions and perspectives

1. General conclusions

The overall aim of this study was to research earthworm communities and their relation with the present tree communities in European forests, with a special focus on tree diversity and identity effects. This chapter addresses our most important results, implications for forest management, the limitations of our research and suggestions for future research.

We started our research with an explorative study and investigated which environmental factors were important in explaining earthworm incidence, density and biomass in forests at a European scale (Chapter 2). As expected, we found that the most important environmental factors explaining earthworm incidence, density and biomass differed among earthworm functional groups. These results make sense, given that these earthworm functional groups occupy different niches in the forest soil system and have distinct feeding behaviours. Furthermore, our subsequent hypothesis, that the incidence and abundance of endogeic species would primarily be explained by soil quality related factors, while that of epigeic and anecic species would be more influenced by litter quality parameters, could only partly be confirmed. Soil variables had a prominent role in predicting the distribution of endogeic earthworms, much more than in predicting the incidence and biomass of epigeic and to some extent also the anecic earthworms. Litter quality parameters, in turn, were important predictors of epigeic earthworm incidence and biomass; however, they were not the principle predictors of the incidence and biomass of anecic earthworms. Regardless of the functional group, litter and soil-related variables best explained earthworm presence, leaving only a minor role to climate related variables. Trees have their influence on the microclimate underneath the canopy, consequently forest canopies might temper climate related effects on species living on the soil surface and in the soil. Among the litter related variables, understory vegetation played an important role in explaining earthworm incidence and abundance. Our results suggested that threshold values for soil C:N ratio (C:N < 23), forest floor pH (pH_{CaCl2} > 4) and understory plant nutrient concentration (C:N < 25) have to be attained before earthworms can occur. Beyond these threshold values, variables like soil C:N ratio, tree litter C:P ratio and forest floor mass further explain earthworm biomass. By combining a European wide standardised earthworm sampling and advanced statistical tools, this study emphasises the relative importance of litter and soil-related variables explaining earthworm incidence (presence/absence) and abundance (density and biomass) in European forests.

Next, an in depth analysis of different aspects of tree diversity and tree litter trait effects on earthworm species richness and biomass in European forests was

performed (Chapter 3). We hypothesised that in mixed forests, earthworm abundance and species diversity may be affected by the functional diversity of the tree species community rather than tree species richness or true Shannon diversity. These expectations were confirmed by our study. Our results indicated that earthworms in mature forests are indifferent to tree species richness. In contrast to the number of tree species present, the functional diversity of the tree community and the proportion of evergreen tree species in the forest influenced the earthworm community. At a continental scale, tree leaf litter functional diversity promoted earthworm total biomass and an increasing proportion of evergreen tree leaf litter negatively influences total earthworm biomass and species richness, as well as their biomass per functional group. Furthermore, as hypothesised, there were clear leaf litter quality effects with a latitudinal variation in trait-specific responses. In north and central Europe, earthworm biomass and species richness clearly increased with increasing litter nutrient concentrations (decreasing C:N ratio and increasing calcium concentration), whereas this influence of litter nutrients was absent or even reversed in southern Europe. Soil moisture is most probably more important than litter quality in southern Europe. In addition, litter with high nutrient concentrations in these regions is associated with shallow soils on calcareous bedrock that results in temporally very dry soil conditions and sparse vegetation. By focusing on tree leaf litter traits, this study advanced our understanding of the mechanisms driving tree identity effects and supported previous findings that litter quality, as a proxy of tree identity, was a stronger driver of earthworm species richness and biomass than tree diversity.

Chapter 4 is dedicated to plant-soil interactions and earthworm mediated belowground dynamics. We investigated how tree litter characteristics and tree functional diversity influence a hypothesised earthworm-soil feedback loop. A feedback loop where earthworm activity influences the humus quality and subsequently the soil acidity, changes in soil pH could in turn feedback to earthworm activity. When integrated in a complex network of causal relationships, leaf litter traits stay important drivers of earthworm biomass, which confirms earlier observations in Chapter 2 and 3. More specifically, on a continental scale leaf litter nutrient concentration stimulated a higher earthworm biomass and improved the forest humus quality. The litter - humus form relation is enhanced by an increasing earthworm biomass, which promotes further development of the humus quality. Contrary to our expectation, our causal path model did not confirm the hypothesised direct causal effect of soil pH on earthworm biomass. However, additional model results suggested that a causal effect of soil pH on earthworm biomass could exist, but that the relation between soil pH and earthworm biomass shows a threshold behaviour rather than a linear relationship and

therefore differs, depending on the soil pH range of the plots under study. From chapter 2 and 3 we learned which environmental factors shape earthworm communities in forests and how. With this knowledge in mind, we shifted focus from describing patterns to developing causal understanding of the aboveground-belowground interface and the black box of interactions belowground. As a result, we found in this chapter 4 that earthworms can modify the forest soil system to their own advantage. We could not proof, however, that they hereby also indirectly stimulate the growth of their own population.

2. Implications for forest management

2.1 Towards a rich and abundant earthworm population

Of the various aspects of tree species diversity that we tested, only tree functional diversity had a significant effect on the earthworm biomass at the continental scale. The significance of this result, however, should not be underestimated. It indicates that tree species richness does not influence earthworm communities at both regional and continental scale. Consequently, this implies that, in general, a variation in tree species richness does not negatively influence the present earthworm community. As tree species richness promotes forest ecosystem performance and resistance to insect pests and diseases (Jucker et al. 2014, Guyot et al. 2016, Jactel et al. 2017), it is good to know that promoting tree species rich forests generally does not have a negative impact on the earthworm community. In the framework of sustainable forest management, more attention for the relation between tree species diversity and soil biodiversity, and their consequences towards forest ecosystem functioning is, however, necessary (SoilForEurope www.biodiversa.org/977).

Diversifying the forest stand with different tree species will usually not harm the earthworm population, but is no guaranty for a better functioning earthworm community and soil quality improvement. When one wants to support the earthworm community in a forest, we recommend planting tree species that differ in leaf litter trait values among the regional tree species pool. This increases the diversity of litter types serving different ecological functions in terms of food and structural habitat. This study demonstrates that a stand of tree species with a dissimilar set of leaf litter characteristics promotes earthworm total biomass. Furthermore, it shows that the presence of broadleaved trees and tree species with nutrient rich leaf litter can both stimulate earthworm diversity and earthworm biomass. Moreover, this study shows that the selection of targeted tree species may affect earthworm communities more than solely increasing the tree species functional diversity. This implies that a forest

management targeting a rich and abundant earthworm community asks for a functional diversification of the forest stand by including tree species with good quality leaf litter.

In a NW European context this means planting rich-litter species next to the currently commercially interesting tree species like Common beech (Fagus sylvatica), Sessile oak (Quercus petraea), Pedunculate oak (Quercus robur), American Red oak (Quercus rubra), and Scots pine (Pinus sylvestris). In general, pioneer tree species (Fraxinus excelsior, Populus tremula, Betula spp. and Alnus glutinosa) have a rich litter and are known to ameliorate the forest soil conditions. Also other rich-litter species have a positive effect on the stand conditions: Prunus avium, Ulmus spp., Tilia platyphyllos, Tilia cordata, Carpinus betulus and Acer pseudoplatanus (Reich et al. 2005, De Schrijver et al. 2012, Verstraeten et al. 2018). Planting tree species that differ in leaf litter trait values in practice means, combining tree species that differ in leaf litter morphology and chemical composition. Advising tree species that fulfill these requirements is not straightforward and highly dependent on the site conditions and the tree species already present. Examples of forests stands with a high tree species functional diversity encountered in the researched forest stands in Poland are stands with a more or less equal proportion of: P. sylvestris – Betula sp. – C. betulus, Q. petraea – P. sylvestris – C. betulus, Q. petraea – Picea abies – P. sylvestris – Betula sp.. Currently more and more foresters target tree species rich forest, however, this is often in contexts where ecological aspects and recreation are prioritized. In the commercial wood production sector in NW Europe, there is currently too little demand for quality wood from rich-litter species, which makes it economically not very interesting to invest in planting, harvesting and processing such types of wood.

We have shown that a forest stand with leaf litter rich in nutrients and with a high water holding capacity, in general, stimulates total earthworm biomass and earthworm species diversity. Next to that, a high leaf litter functional diversity stimulates total earthworm biomass. But this must by no means be a plea to immediately transform all forests into functionally diverse stands with high quality litter. In many cases, this biotic homogenization would be a good thing for the stability of the forest ecosystem and the long-term productivity at the local scale, but there are also many unique forest types, often poor in tree species richness, with very specific soil conditions. These biotopes should not disappear, because they harbor an often very exceptional flora and fauna. For example, some epigeic earthworm species are specialized in living in very acidic topsoil conditions in coniferous forests. In addition to creating high tree diversity on a plot scale, to promote high local earthworm diversity and all the positive

consequences that may follow, we must therefore value these unique forest biotopes and conserve landscape-scale tree biodiversity (van der Plas et al. 2016b).

2.2 Earthworm community management as a tool for sustainable wood production

With the continuing demand for wood products, wood production is still one of the main priorities in many European forests. However, a lot of forests in Europe and North-America suffer from soil degradation (Drohan and Sharpe 1997, Hovmand and Bille-Hansen 1999, Jönsson et al. 2003, De Schrijver et al. 2006, Janowiak and Webster 2010) and soil compaction (Greacen and Sands 1980, Ampoorter et al. 2010, Hansson et al. 2018). To halt or reverse this process several actions were investigated in the past: mechanical loosening, mineral amendments, earthworm introduction and introduction of shrubs and tree species with nutrient rich litter (Muys 1993, Deleporte and Tillier 1999, Ampoorter et al. 2011, De Schrijver et al. 2012, Van Nevel et al. 2014). We strongly believe that these interventions can only be sustainable if they contribute to the stability of the forest ecosystem. Mechanical loosening and nitrogenous amendment may have a negative effect on the soil fauna (Muys 1993, Deleporte and Tillier 1999) and fertilisation with ash (e.g. from wood burning) may have detrimental impacts on moss and lichen communities (Pitman 2006). These interventions should therefore be discouraged.

Our research confirms that earthworm activity and/or the presence of tree species with high leaf litter quality can improve humus form quality. A certain forest soil quality (soil C:N < 23 and forest floor $pH_{CaCl2} > 4$), however, has to be reached before earthworms can occur. Once earthworms occur they have the potential to modify the forest soil and improve soil quality, but poor mineral soil and/or poor litter quality can impede or even counteract this. In extreme cases, poor litter quality can even cause a shift in soil process domains (Verstraeten et al. 2018), resulting in soils with high loads of exchangeable aluminium that are toxic to soil species and reduce plant vitality (van Gestel and Hoogerwerf 2001, de Wit et al. 2010). These different elements underline the importance of an integrated forest soil restoration and management approach, where several factors like leaf litter quality, mineral amendments and earthworm introduction need to be in balance to reach a stable and productive forest ecosystem (Muys et al. 2003, Ashwood et al. 2017a). A targeted intervention that supports earthworm population establishment has for example the potential to, avoid the difficult reversible shift in soil process domain on acidifying clay-rich forest soils (Verstraeten et al. 2018) and to promote soil development in land reclamation projects (Józefowska et al. 2016, Ashwood et al. 2017a). If a soil quality treatment succeeds in supporting the development of an earthworm population, earthworms will contribute

to soil nutrient cycling and the development of an aerated well-structured topsoil through the way they feed and move in the soil.

Our research showed a positive effect of water holding capacity and nutrient concentration of tree leaf litter on the total earthworm biomass and species richness at a European scale. Despite this general continental trend, we found that the relationships between earthworm abundance and species diversity with the different leaf litter nutrient traits could change with environmental conditions. This context dependency turns trees with good quality litter into earthworm supporting tree species in north and central Europe (Finland, Poland, Germany, and Romania). However, it makes them less important to earthworm communities in calcareous soils in southern Europe (Italy and Spain).
Context dependent effects of tree species on earthworm communities underline the importance of region specific forest soil management decisions, but do not exclude clear management recommendations at a European scale. Assuring a good soil nutrient quality and stabilized forest stand health over time (prevent pests and diseases outbreaks) stay the most important challenges for the future. In European forests, it will therefore generally be more sustainable to strive towards a diverse tree species stand with a high leaf litter functional diversity, including a significant proportion of tree species with good quality litter.

3. Future research challenges

In this study, we looked at environmental factors like climate, soil and flora related factors to understand earthworm communities in European forests and their relation with tree communities. To further increase our understanding and improve model accuracy, I suggest exploring the following aspects: interspecific tree leaf litter trait variation in a stand, context dependency of earthworm responses, tree root related variables, earthworm functional diversity and biotic interactions with forest fauna and soil microorganisms. Given the complexity to study this matter within a forest context, it was not feasible to include them in the current study.

We found that litter trait functional dispersion was related to total earthworm biomass (Chapter 3). Some litter traits, however, will contribute more to the functional dispersion value of a stand. It would be useful to know whether the traits contributing the most to stand level litter functional dispersion are the same as the most influential tree species litter traits affecting earthworm communities. Next to that, identifying the importance of single leaf litter trait variation versus their community weighted mean

value, and the mechanisms behind potential effects of trait variability will contribute significantly to understand the relation of earthworms with tree communities.

Our results seem to be context dependent (e.g. influenced by soil quality), which is in accordance with results of Tedersoo et al. (2015) and Cesarz et al. (2016). So to better understand the context dependency one need: (i) more earthworm species and soil specific experimental research on components in their diet that would inhibit or stimulate their growth and activity along a soil quality gradient (cf. Cesarz et al. (2016)), or (ii) a larger regional study in southern Europe with more observational forest stands to allow more complex mechanistically oriented modelling. A large number of forest plots is necessary to accurately model soil quality, litter nutrient quality and a tree composition driven topsoil microclimate.

In forest ecosystems root biomass typically makes up a significant fraction of the total litter production (Vogt et al. 1986, Saugier et al. 2001, Leppälammi-Kujansuu et al. 2014) and endogeic earthworms obtain significant amounts of their carbon from root exudates and root litter (Pollierer et al. 2007). Further exploration of the importance of root related parameters, in addition to the aboveground predictors, is therefore important.

We used an earthworm taxonomic diversity index and a priori defined functional groups (epigeic, endogeic and anecic, sensu Bouché (1977)) to assess the impact of tree species diversity and identity on earthworm communities. This index and these functional groups are informative, commonly used tools that allow comparison of results with other research. Nevertheless, some drawbacks are associated with these tools: (i) Their usefulness is limited in environments with low earthworm taxonomic diversity. (ii) Using a priori defined functional groups implies, discrete functional differences between taxa which are usually continuous, and varying results that depend on the definition of the functional groups (Pelosi et al. 2016). We therefore encourage to use earthworm functional diversity metrics in the future, which can be based on clearly defined earthworm functional traits (e.g. body length, diameter, weight, epithelium type, pigmentation, pH range, C/N (soil) preference, reproductive strategy, cast type, burrowing behaviour). Such a trait-based approach can complement the conclusions based on taxonomic diversity indices (Pey et al. 2014b). For example if earthworm taxonomic diversity does not change, but earthworm functional diversity increases with increasing tree species richness, it means that the earthworm species composition changes towards a community with more functionally dissimilar species. A trait-based diversity index is a promising, but fairly new approach were a lot of

challenges still have to be tackled (Pey et al. 2014b). Recently published experiences with such approaches for several soil invertebrates and growing databases with earthworm functional traits will facilitate this approach in the future (Hedde et al. 2012, Pelosi et al. 2014, Pey et al. 2014a, Abgrall et al. 2017, Marichal et al. 2017, Briones and Álvarez-Otero 2018).

The next step towards a comprehensive analysis of earthworm communities in the forest ecosystem would be to include biotic interactions between earthworm species, predation and parasitism on earthworms and earthworm-microorganism relationships. Laboratory and field microcosm experiments documented both inter- and intra-specific competition between earthworm species (Butt 1998, Lowe and Butt 2002, Uvarov 2017) as well as commensalism between endogeic and anecic earthworm species. Many birds, and several mammals have earthworms as one of their main food sources (Granval and Muys 1996), their behaviour could consequently influence earthworm species coexistence (Saleem et al. 2012). Microorganisms are known to be very important for earthworms. They facilitate the digestion for earthworm (Lavelle et al. 1995), they serve as food source (Curry and Schmidt 2007), but could also exert biotic resistance to earthworm colonization (Simmons et al.). First steps towards assessing the importance of competition between earthworms and other environmental variables in earthworm distribution modelling are made by Palm et al. (2013). They identified the biotic interaction between earthworm functional groups as an important predictor of earthworm functional group occurrence in agro-ecosystems, next to management practices, topography and soil conditions.

When undertaking large-scale earthworm samplings across countries and biomes, chances are high that you find rare endemic earthworm species. To identify these rare species, detailed documentation and identification guides are necessary. Such identification guides, if available, often only exist in the local language and additional support from local specialized taxonomists is necessary to identify rare species. The decreasing number of specialised taxonomists in Europe emphasise the need for detailed (English) identification guides and well documented databanks with morphologic and genetic reference material.

The available data for this study is used to the fullest to answer our research questions. Like all ecological data, also our dataset had limitations. To get stronger model results we need either more observations or different techniques that are able to deal with 'small' sample sizes. The data used in this research, however, is the result of 3-4 years scientific work of more than 10 people, a rarity in soil ecological research. To reach

higher precision in continental scale earthworm ecology research, one has either to invest in more fieldwork and larger projects, more data sharing opportunities, or statistical knowledge and approaches to deal with this type of ecological data. An additional limitation in this study was that all our research sites could only be visited once, which increases the risk that unusual events affect the patterns to be observed normally. Stochastic effects of climate variability on the results, like observed in the Italian and Romanian research sites, may have influenced the results. Such stochastic effects of climate variability on the results, however, could only be evaluated by repeated sampling campaigns over several years, which was beyond the scope of this study. In this research I used, next to the earthworm data, detailed data on different layers of the forest ecosystem: forest canopy, understory, forest floor, topsoil and subsoil. There is, however, a slight imbalance between aboveground and belowground data. We have accurate field measurements of the aboveground system, but where obliged to extract some of the subsoil variables from literature. To understand the observed patterns in this research to the fullest, more detailed soil related data like soil management history and soil type is necessary.

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Appendices

Appendix A. Supplementary material to Chapter 2

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Appendix A1. Data collection and derivation of model explanatory variables

Climate

For every sampling plot, annual Precipitation (mm) and annual Mean Temperature (°C) were selected from WorldClim (http://www.worldclim.org/) and the aridity index was taken from the Global Aridity and Potential Evapo-Transpiration Database (http://www.cgiar-csi.org/). Aridity index = Mean Annual Precipitation/Mean Annual Potential Evapotranspiration (Zomer et al. 2008). The higher the index, the wetter the location. Heat load index, as a proxy for local climate variation, was calculated conform McCune and Keon (2002) where plot specific values for latitude, slope and aspect were used to feed and calculate 'equation 3': Heat load index = 0.339 + 0.808*COS(latitude)*COS(slope) - 0.196*SIN(latitude)*SIN(slope) - 0.482*COS(aspect)*SIN(slope). To prevent correlation with latitude, these heat load index values were relativised by site maximum. The heat load index reflects the heat load that a particular location gets because of annual direct incidence radiation, the higher the value the larger the heat load and the warmer that location will be.

Soil

Soil texture was estimated using expert assessment as the abundance of sand (size), silt (size) and clay (size) content. Measurements were done on an ordinal scale, with values ranging from 1 (absent) to 3 (very common).

The **soil descriptors**: Slope, Bedrock, Calcareous bedrock, Soil drainage, Soil Depth and Soil type originate from forest management plans and soil maps, complemented with additional field visits within the FunDivEUROPE project. Slope has been assessed as a category: flat (<5%), medium slope (<50%), steep slope (>50%). Bedrock type was redesigned into a standardised variable expressing the kind of material that is released after weathering of the bedrock (Lime-clay, Sand, Sand-clay and Sand-lime). Calcareous bedrock was a binomial variable indicating whether the

bedrock was calcareous or not. Soil drainage classes were: dry, fresh, moist, wet. Soil depth (cm depth to bedrock) was measured in each plot using a soil auger or taken from literature (Guckland et al. 2009) and afterwards translated into an ordinal scale variable (Shallow (1) ≤30cm, Medium (2) 30-60cm, Deep (3) ≥ 60cm). Soil type variable (IUSS Working Group WRB, 2014) was reclassified into a two level nominal variable (Buffered soil, Acidic soil).

In order to measure the pH ($CaCl_2$) and determine the C:N ratio of the **mineral soil** at 10-20cm depth per plot, each plot was subdivided in nine subplots of 10 m x 10 m. In every subplot, one mineral soil core sample 10-20 cm depth was taken given the stoniness of the site. No data was available for Spain because the soil was mostly too shallow. We used 10-20 cm depth soil instead of topsoil to prevent the influence of tree leaf litter on the soil parameter values as much as possible. By doing so, we also kept the correlation coefficient between variables low and minimised multicollinearity between explanatory variables. Cores were sampled with a long corer (inner diameter 3.56 cm, length 50 cm). The nine replicate samples serve to represent the soil conditions across the core plot. Analyses of C, N and pH were done on these composite samples. The mineral soil samples were dried at 55 °C to constant weight and sieved with 2 mm diameter mesh and pH was determined at a suspension ratio of 1:2.5 in 0.01M $CaCl_2$ solution using a 827 pH lab meter (Metrohm AG, Herisau, Switzerland). For C and N analysis, the soil samples were further ground with Planetary Ball Mill PM 400-Retsch for six minutes at 280 rpm (Retsch, Germany). The soil C and N concentrations were then determined with a Thermo Scientific FLASH 2000 soil CN analyzer, based on the dry combustion method (Matejovic 1993).

Soil volumetric **water content** (%) was measured within one meter from the earthworm sampling location by means of a TRIME-FM3 field measurement device with a 'P3' probe at the moment of sampling.

Forest floor

Main **humus type** was recorded in the field nearby to the earthworm sampling location according to the European humus form reference base (Zanella et al. 2011).

In order to determine the pH ($CaCl_2$) and dry mass of the **ectorganic layer** per plot, each plot was subdivided in nine subplots of 10 m x 10 m. In every subplot, the forest floor was sampled from 25 x 25 cm plots using a wooden frame. The forest floor was removed by hand and/or knife, after all living plant parts within the frame were clipped off. The nine replicate samples serve to represent the soil conditions across the

core plot. Weight determination and analyses of C, N and pH were done on these composite samples. After drying at 55 °C to constant weight, the forest floor was ground with Heavy-duty Model SM 2000-Retsch cutting mill (Retsch, Germany). Subsamples of this coarsely ground fraction were further ground into finer particles with Planetary Ball Mill PM 400-Retsch for six minutes at 280rpm (Retsch, Germany). The forest floor pH was determined at a suspension ratio of 1:10 with 0.01M $CaCl_2$ solution using 827 pH labs Metrohm AG, Herisau, Switzerland. The C and N concentrations of the forest floor were determined with a Thermo Scientific FLASH 2000 soil CN analyzer, based on the dry combustion method (Matejovic 1993).

The understory was surveyed in all 209 plots between May and August 2012. Each plot was subdivided into nine subplots of 10 m x 10 m. In the central subplot, close to the earthworm sampling location, a frame of 0.5 m x 0.5 m was placed at a location where **understory vegetation** was representative for the whole subplot. Within that frame, all understorey vegetation was clipped. We divided the biomass sample into a woody (woody juveniles and plants) and a non-woody subsample (young seedlings, 'green' herbs). Both subsamples were then dried for 24 to 48h at 70 °C and weighed, resulting in values for the understory biomass (g/m^2).

Next, the subsamples were ground using a sieve of 1 mm. The ground material was partly used for P-analysis and partly for simultaneous C and N-analysis. Before analysis of P-content, the ground material was digested with HNO3/HClO4, diluted with demineralised water and filtered according to the method of Varian Instruments at work, AA-24 (McKenzie 1982). Then the extract was mixed with metholsulphite, ammoniumheptamolybdate and sodiumacetate solution. Finally, the total P-content (mg/kg) was measured with a spectrophotometer type Varian Cary 50 at 700nm (Cottenie et al. 1982). C and N-analysis, analysis was performed using an Elementar analyzer, type Vario Macro Cube in configuration CNS, with Argon as carrier gas. Catalytic combustion of the sample (1.5g) was carried out at a permanent temperature of up to 1200 °C. This was followed by reduction of the combustion gasses on hot copper in the reduction tube. The formed gases N2, CO2, H2O, SO2 were separated via purge and trap chromatography and afterwards detected on a thermal conductivity detector (TCD). A connected PC computed the element concentration from the detector signal, and the sample weight on the basis of stored calibration curves, which resulted in the C and N content (%) per subsample.

For this study only the results on the non-woody subsample were used.

Tree litter was collected from autumn 2012 till autumn 2013, using five litter traps that were placed systematically on all 209 plots: one in the central subplot, and one in each of the four subplots in the cardinal directions or along and perpendicular to the slope. Each litter trap had an opening area of 0.5 m^2. The litter from the traps was air dried or dried in an oven at 38 °C and pooled per plot. Samples were then sorted into the different fractions for dry weighing.

The following fractions were separated:

- Foliar litter (leaves or needles), per target species
- Foliar litter (leaves or needles), all other species
- Woody litter (twigs, branches, bark parts)
- Reproductive litter (flowers, cones, fruits, seeds, fruit capsules etc.)
- Other (e.g. bud scales, indefinable or small parts)

Target species refer to the main tree species a plot is composed of and according to which the plot was classified into specific richness level (see Baeten et al. (2013) for details on species composition and plot selection). Foliar litter of non-target species could be litter from understorey shrubs or from admixed species that have a low occurrence (that means they contributed generally less than 5% to the total basal area of a plot).
After sorting, all fractions were weighted. A subsample of each fraction was dried at 65 °C to constant weight. The ratio between dry mass at 65 °C and the air-dried samples was used to calculate constant dried weight for all litter samples.

For this study only the total foliar litter weight of the target tree species was used.

For all **tree litter traits**, a community weighted mean was calculated for each plot (plot scale) and for a circular area with a 10 m radius surrounding the earthworm sampling location (local neighbourhood scale). To this end, relative abundance was computed, based on basal area of the focal tree species both in each plot and in each local neighbourhood, and species-specific litter traits values were measured for each focal tree species of each site. Relative abundance estimates based on amounts of collected litter would be more accurate and therefore the preferred choice. However, since these data were not available at the appropriate spatial scale, we believe that the basal area-based CWMs are the best alternative in our particular case. In the absence of suitable litter mass data, basal area is actually a good proxy for tree leaf litter mass/area, given that the basal area reflects the sapwood area needed to support the

sapflow of a given leaf area (Vertessy et al. 1995). To measure species-specific litter traits for each focal tree species of each site, freshly fallen leaf litter of each species was collected at the site level at several locations around the plots, at peak leaf litterfall between October 2011 and November 2012, depending on species-specific leaf fall. Except for the Finnish site, where freshly fallen leaf litter was collected from the forest floor, litter was collected using suspended litter traps that were fortnightly harvested. For each species of each site, three litter subsamples were drawn from a common litter pool and analysed separately. Specific leaf area (SLA) was determined by measuring leaf area with an area meter (Delta-T Devices, model MK2, Cambridge, UK) and subsequent drying at 65 °C for 48 hours to obtain dry mass. Water holding capacity (WHC) was measured by placing intact leaves in non-hermetically lidded large plastic containers and spraying them three times a day with deionised water. The amount of water retained by the leaves was then measured after 48 h and compared to the 65 °C litter dry mass. To measure concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN), intact air-dried leaf litter was placed into deionised water (ratio of 1 g of litter dry-weight for 20 ml of water), and shaken on a rotator spinning at 8 rpm for 24 h. Water extracts were then filtered through a 0.45 µm cellulose nitrate filters. DOC and TDN concentrations were then measured in litter leachates with a TOC analyser equipped with a supplementary module for N (CSH E200V, Shimadzu, Kyoto, Japan). All other chemical analyses were performed using subsamples of litter ground to obtain a uniform particle size of 1 mm (Cyclotec Sample Mill, Tecator, Höganäs, Sweden). Total carbon and nitrogen concentrations were measured with a flash CHN Elemental Analyser (Flash EA 1112 Series, ThermoFinnigan, Milan, Italy). Phosphorus (P) concentration was measured colorimetrically after a mineralization step with an autoanalyzer (SmartChem 200, Alliance Instruments, Roma, Italy). Cellulose and hemicellulose fractions were determined using a fiber analyser (Fibersac 24, Ankom, Macedon, NJ, USA) according to the Van Soest extraction protocol (Van Soest and Wine 1967). Calcium (Ca), Magnesium (Mg) and Potassium (K) concentrations were measured using an atom absorption spectrometer (AAS, iCE 3000 series, ThermoScientific, China). In this paper, Ca, Mg and K were summed to represent the total availability of base cations in tree leaf litter. To measure the concentration of polyphenols (= total phenolics), 0.5 g of ground litter was soaked in 30 ml of 50% methanol, shaken for 2 h, filtered (filter number 112, Durieux, Torcy, France), and the extracts were analysed spectrophotometrically using the Folin-Ciocalteau reagent using gallic acid as a standard. The 'Polyphenols' described in this study include every compound that contains a phenol unit, including, among other compounds, soluble phenolics, non-soluble phenolics, condensed tannins and hydrolysable tannins.

Tree diversity indices were calculated based on a total set of 16 focal tree species that are regionally common and/or important from a silvicultural point of view (Baeten et al. 2013). The tree species richness variable used in our study represents the number of regional occurring focal tree species in a plot, also known as the N_0 of Hill (1973). The tree species evenness was calculated as the True Shannon index based on focal tree basal areas divided by the tree species richness (N_1/N_0, (Hill 1973)).

The **stand characteristics** in each plot: forest management, forest structure and free age distribution originate from forest management plans and were complemented with additional field visits within the FunDivEUROPE project. Forest management is reported as a nominal variable that reflects the type of disturbance in the plots made by management interventions (coppicing, high intensity thinning, low intensity thinning, and minimal intervention). The forest structure variable reports the horizontal structure of the canopy in a plot (double, multiple, single). Tree age distribution is characterised as a nominal variable (even, uneven).

Leaf Area Index (total one-side area of leaf tissue per unit ground surface area, m^2m^{-2}, Watson, 1947) was measured in each plot (five measurements) early in the morning (shortly after sunrise) or late in the evening (shortly before sunset) in order to work in the presence of diffuse solar radiation. LAI measurements were carried out in summer 2012 (in Italy, Germany and Finland) and in 2013 (in Spain, Romania and Poland), using a Plant Canopy Analyser LAI-2000 (LI-Cor Inc., Nebraska, USA). With the LAI-2000, the incident light above the canopy and the light transmission below the canopy were measured using one sensor with five fisheye light sensors (lenses), with central zenith angle of 7°, 23°, 38°, 53° and 68° (LAI-2000 manual, LI-Cor (1991)). The protocol used in each plot consisted of five measurements within the plots (light transmission below the canopy), and five measurements outside the forest (as proxy of the light incidence above the canopy), in an open space that was in close proximity to the sampled plots. LAI data was processed using LI-Cor's FV2200 software (LI-COR Biogeosciences, Inc. 2010). The light transmittance measurements of the fifth ring were removed to minimise the boundary effects on LAI. The LAI value per plot was the mean value of the five measurements for each plot.

Appendix A2. Additional figures

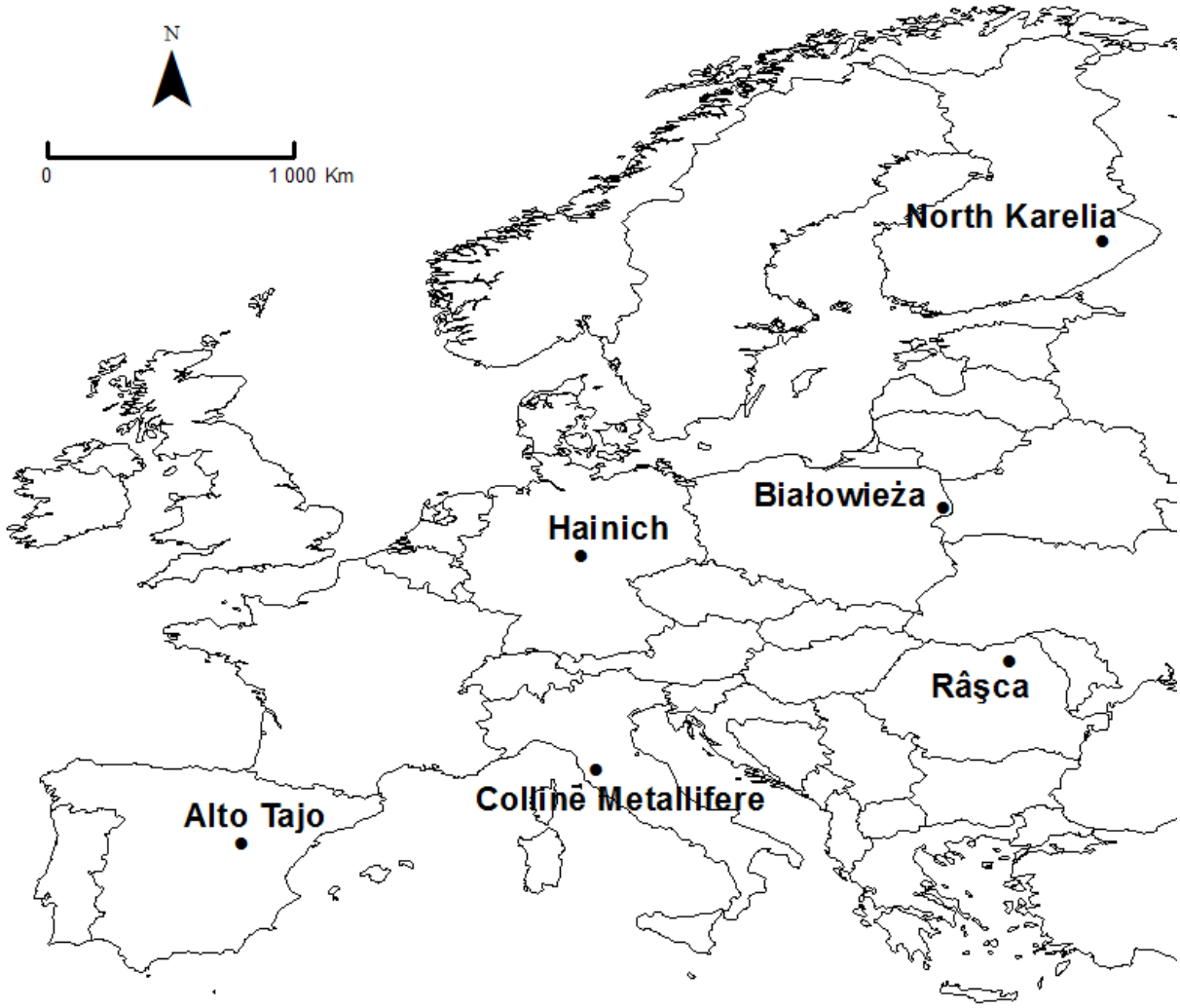


Fig. S1. Map of Europe with the locations of all sites.

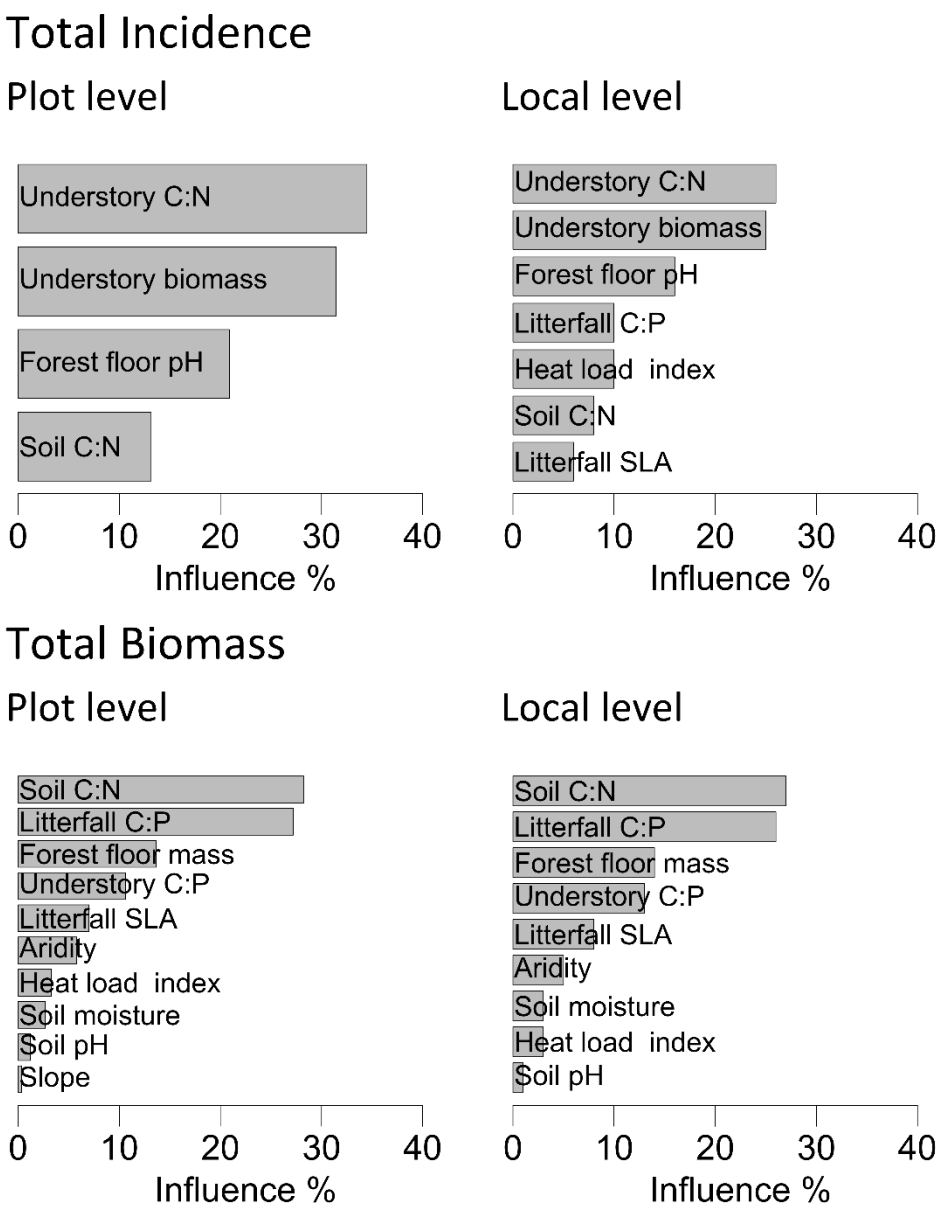


Fig. S2. Most influential variables that predict the total earthworm incidence and biomass in European forests ranked according to their modeled relative influence (%) of the explanatory variables on the total earthworm incidence (above) and biomass (below). 'Plot level' and 'local level' models differ in the scale on which diversity and tree litter trait parameters are calculated. In the 'Plot level' models, these parameters are calculated at plot scale, whereas in the 'Local level' models these diversity and tree litter traits are calculated at local neighbourhood scale (10 m radius around the earthworm sampling point).

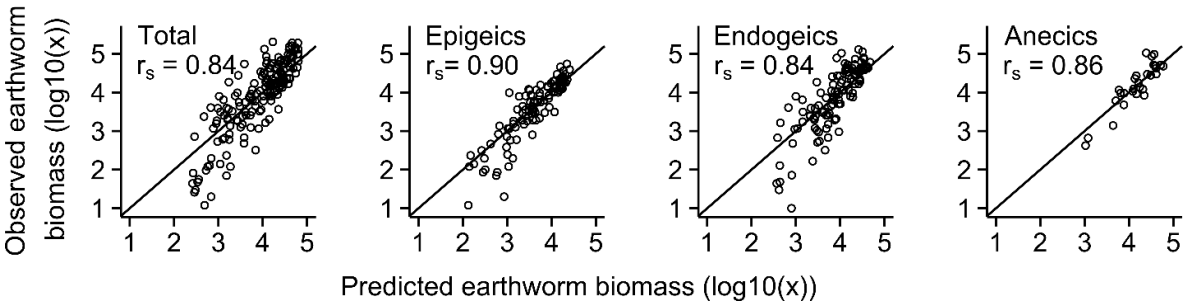


Fig. S3. Relationship between predicted and observed earthworm biomass (log10(x)) for total, epigeic, endogeic and anecic biomass. rs = Spearman correlation coefficient

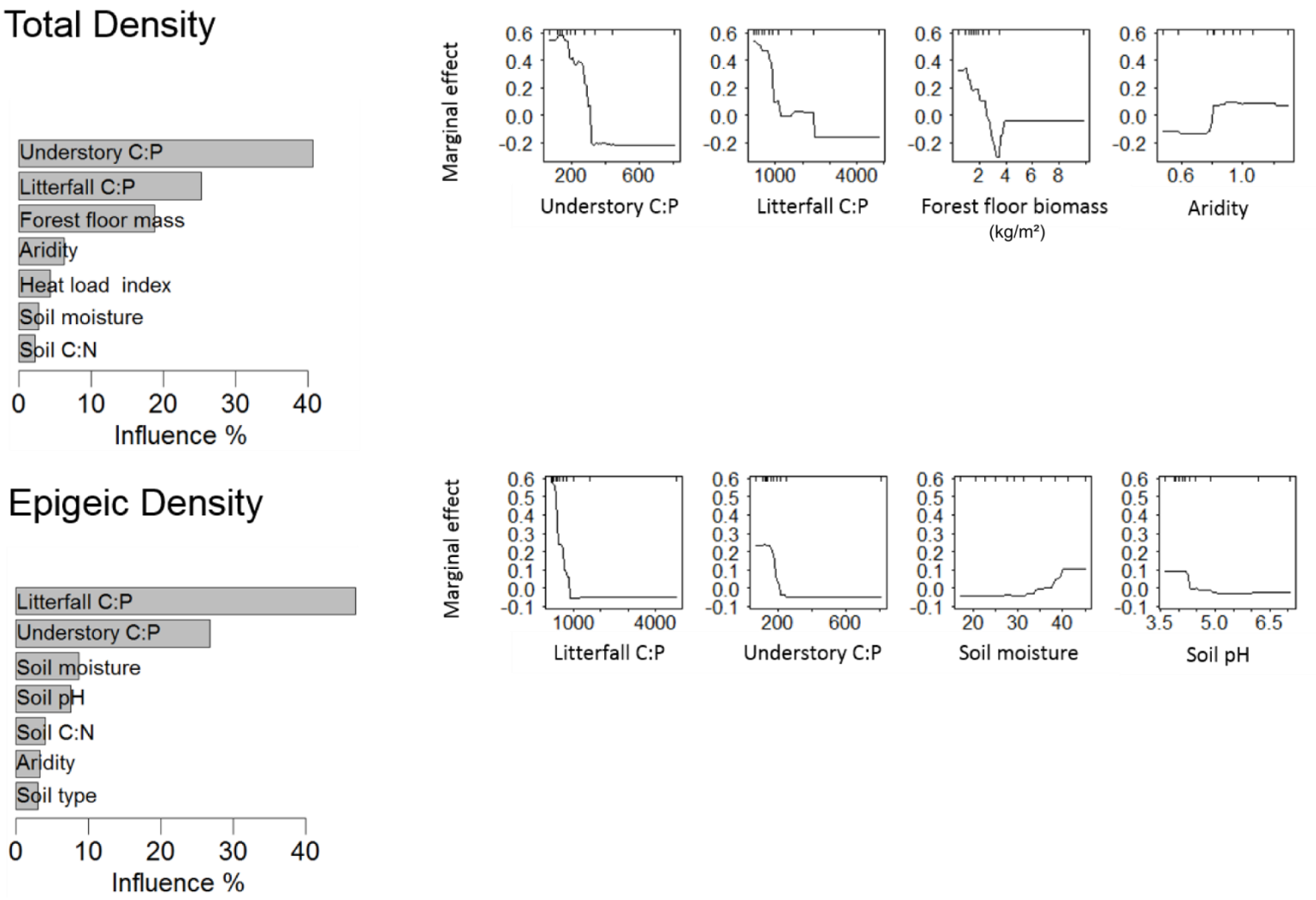


Fig. S4. Most influential variables that predict the total earthworm density and epigeic earthworm density in European forests. Left: Relative influence (%) of the explanatory variables on the total (above) and epigeic (below) earthworm density. Right: Corresponding partial dependence plots where each graph illustrates the marginal effect on the earthworm occurrence probability and the earthworm biomass respectively (y-axis) along an explanatory variable range (x-axis), while keeping all other explanatory variables average. The fitted functions are centred around the mean response value and plotted on a common scale. Hash marks along the top of the plots show the range of each explanatory variable in deciles.

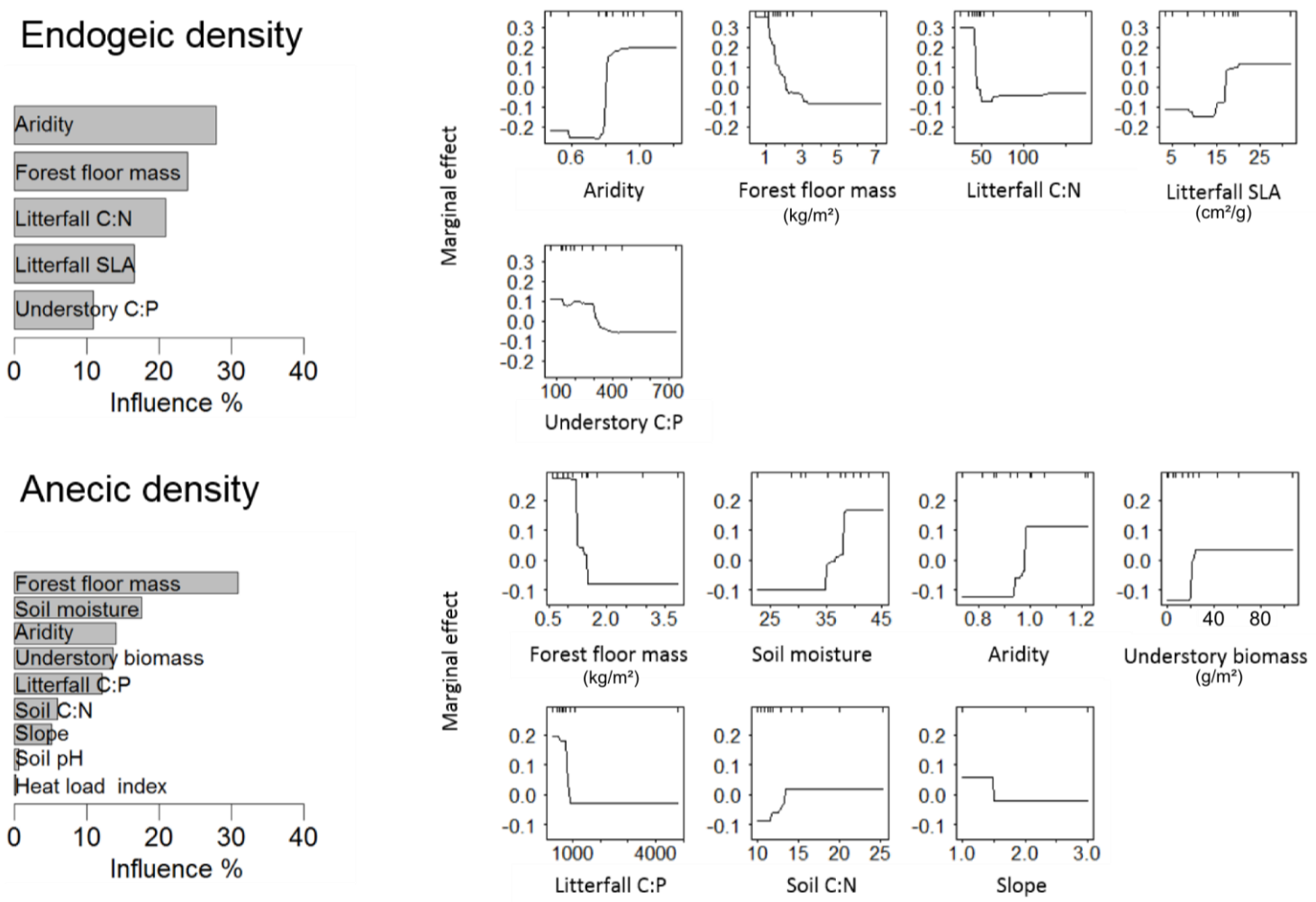


Fig. S5. Most influential variables that predict the endogeic earthworm density and anecic earthworm density in European forests. Left: Relative influence (%) of the explanatory variables on the endogeic (above) and anecic (below) earthworm density. Right: Corresponding partial dependence plots where each graph illustrates the marginal effect on the earthworm occurrence probability and the earthworm biomass respectively (y-axis) along an explanatory variable range (x-axis), while keeping all other explanatory variables average. The fitted functions are centred around the mean response value and plotted on a common scale. Hash marks along the top of the plots show the range of each explanatory variable in deciles.

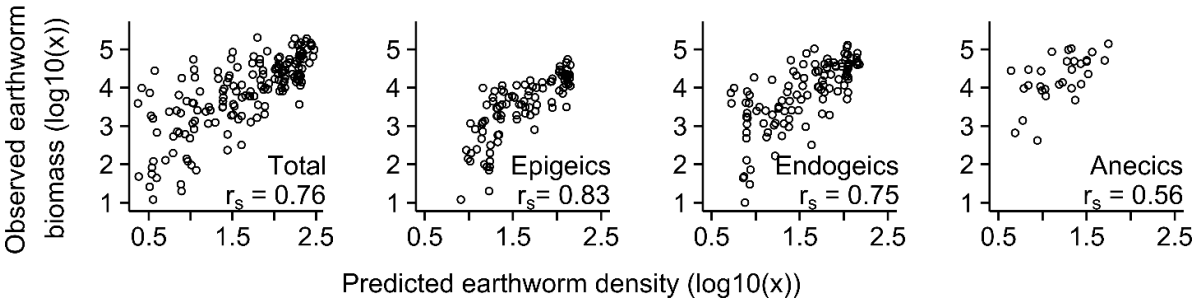


Fig. S6. Relationship between predicted earthworm density and observed earthworm biomass (log10(x)) for total, epigeic, endogeic and anecic biomass. rs = Spearman correlation coefficient

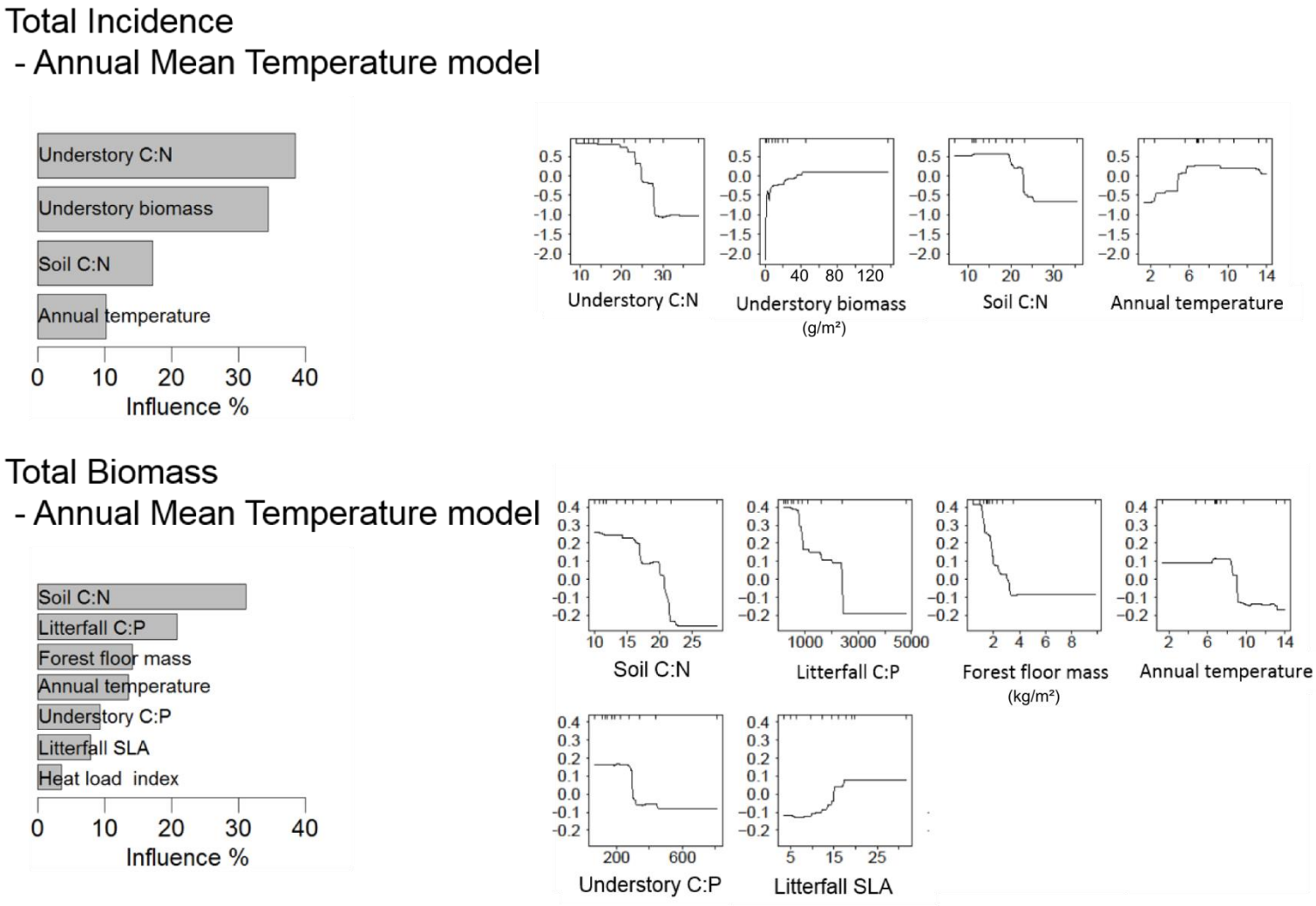


Fig. S7. Most influential variables that predict the total earthworm incidence and total earthworm biomass in European forests (Mean Annual temperature models). Left: Relative influence (%) of the explanatory variables on the endogeic (above) and anecic (below) earthworm density. Right: Corresponding partial dependence plots where each graph illustrates the marginal effect on the earthworm occurrence probability and the earthworm biomass respectively (y-axis) along an explanatory variable range (x-axis), while keeping all other explanatory variables average. The fitted functions are centred around the mean response value and plotted on a common scale. Hash marks along the top of the plots show the range of each explanatory variable in deciles.

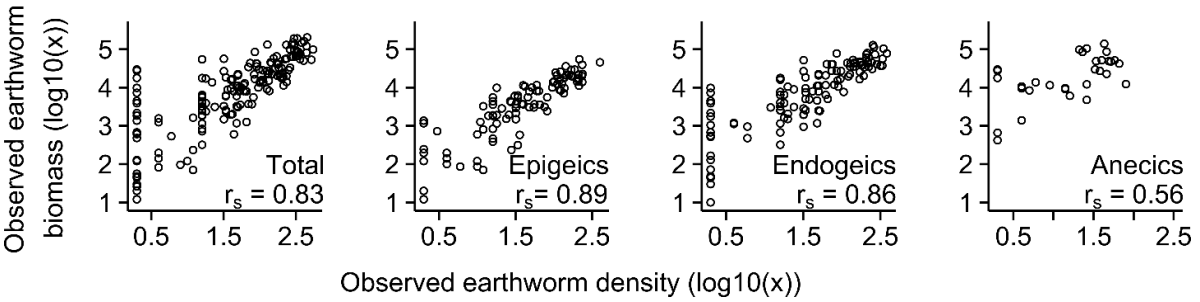


Fig. S8. Relationship between log10 transformed observed earthworm densities and log10 transformed observed earthworm biomass for total, epigeic, endogeic and anecic earthworms.

Appendix A3. Additional tables

Table S1. List of all identified earthworm species per sampling site and their incidence across the six sampling sites.

	Functional group	Finland	Germany	Italy	Poland	Romania	Spain	Incidence
Dendrobaena attemsi	epigeic					1		1
Dendrobaena cognettii	epigeic		1				1	2
Dendrobaena cognettii gallurensis	epigeic			1				1
Dendrobaena octaedra	epigeic	1	1		1	1		4
Dendrobaena species 5	epigeic			1				1
Dendrodrilus rubidus	epigeic	1	1		1			3
Eisenia lucens	epigeic					1		1
Lumbricus castaneus	epigeic		1					1
Lumbricus rubellus	epigeic		1		1			2
Octodrilus damianii	epigeic			1				1
Allolobophora carpathica	endogeic					1		1
Allolobophora chlorotica	endogeic						1	1
Allolobophora sturanyi dacidoides	endogeic					1		1
Aporrectodea caliginosa	endogeic	1	1		1		1	4
Aporrectodea handlirschi	endogeic					1		1
Aporrectodea rosea	endogeic		1		1	1	1	4
Eiseniella tetraedra	endogeic						1	1
Octolasion cyaneum	endogeic						1	1
Octolasion lacteum	endogeic		1		1	1	1	4
Proctodrilus antipai	endogeic			1				1
Scherotheca targionii	endogeic			1				1
Fitzingeria platyura depressa	anecic					1		1
Lumbricus terrestris	anecic	1	1		1			3
Octodrilus complanatus	anecic			1				1

Table S2. Relative influence (%) of explanatory variables for the incidence and biomass models.

	Incidence models				Biomass models			
	Total	Epigeic	Endogeic	Anecic	Total	Epigeic	Endogeic	Anecic
CLIMATE								
Heat load index	-	9	-	-	3	2	-	13
Annual precipitation	-	-	-	-	-	-	-	-
Annual temperature	-	-	-	-	-	-	-	-
Aridity	-	3	8	37	6	4	6	0
SOIL								
Slope	-	-	-	-	0	4	-	-
Bedrock	-	-	-	-	-	2	-	-
Calcareous bedrock	-	-	-	-	-	-	-	-
Sand	-	-	-	-	-	-	-	-
Silt	-	-	-	-	-	-	-	-
Clay	-	-	7	-	-	-	-	-
Soil drainage	-	-	-	-	-	-	-	-
Soil Depth	-	13	-	-	-	-	-	-
Soil type	-	-	-	-	-	1	-	-
Soil pH	-	-	3	-	1	3	16	15
Soil C:N	13	8	12	-	28	1	13	13
Soil moisture	-	-	4	-	3	10	5	2
FOREST FLOOR								
Humus type	-	-	29	-	-	-	-	-
Forest floor pH	21	-	-	-	-	-	9	-
Forest floor mass	-	-	-	63	14	-	19	7
Management	-	-	-	-	-	-	-	-
Forest structure	-	-	-	-	-	-	-	-
LAI	-	-	-	-	-	-	-	-
Tree age distribution	-	-	-	-	-	-	-	-
Understory biomass	31	9	13	-	-	-	-	6
Understory C:N	34	-	11	-	-	-	-	-
Understory C:P	-	27	-	-	11	42	10	5
Tree richness	-	-	-	-	-	-	-	-
Tree evenness	-	-	-	-	-	-	-	-
Litterfall biomass	-	-	-	-	-	-	-	-
Litterfall C:N	-	-	-	-	-	-	5	-
Litterfall C:P	-	25	-	-	27	30	9	-
Litterfall cellulose	-	-	-	-	-	-	-	18
Litterfall hemicellulose	-	-	3	-	-	-	-	5
Litterfall polyphenols	-	-	-	-	-	-	-	-
Litterfall SLA	-	6	10	-	7	-	8	16

CWM = community weighted mean; SLA = specific leaf area

Table S3. Model performance indicators of the final boosted regression tree density models, grouped by used response variable: area under the receiver-operating characteristic curve (AUC), percentage deviance explained (D^2), correlation score (Corr) and relative Root Mean Squared Error (rRMSE) for model fit and after 10-fold cross-validation.

	Fit				Cross-validation			
	AUC	D^2	Corr	rRMSE	AUC	D^2	Corr	rRMSE
DENSITY MODELS								
Total		0.82	0.91	12.78		0.57	0.76	19.64
Epigeic		0.68	0.85	14.62		0.48	0.72	18.74
Endogeic		0.63	0.82	18.35		0.38	0.61	23.86
Anecic		0.65	0.84	19.55		0.09	0.43	31.40

Appendix B. Supplementary material to Chapter 3

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Appendix B1. Additional figures

Fig. S1. Map of Europe with the locations of all six regions.

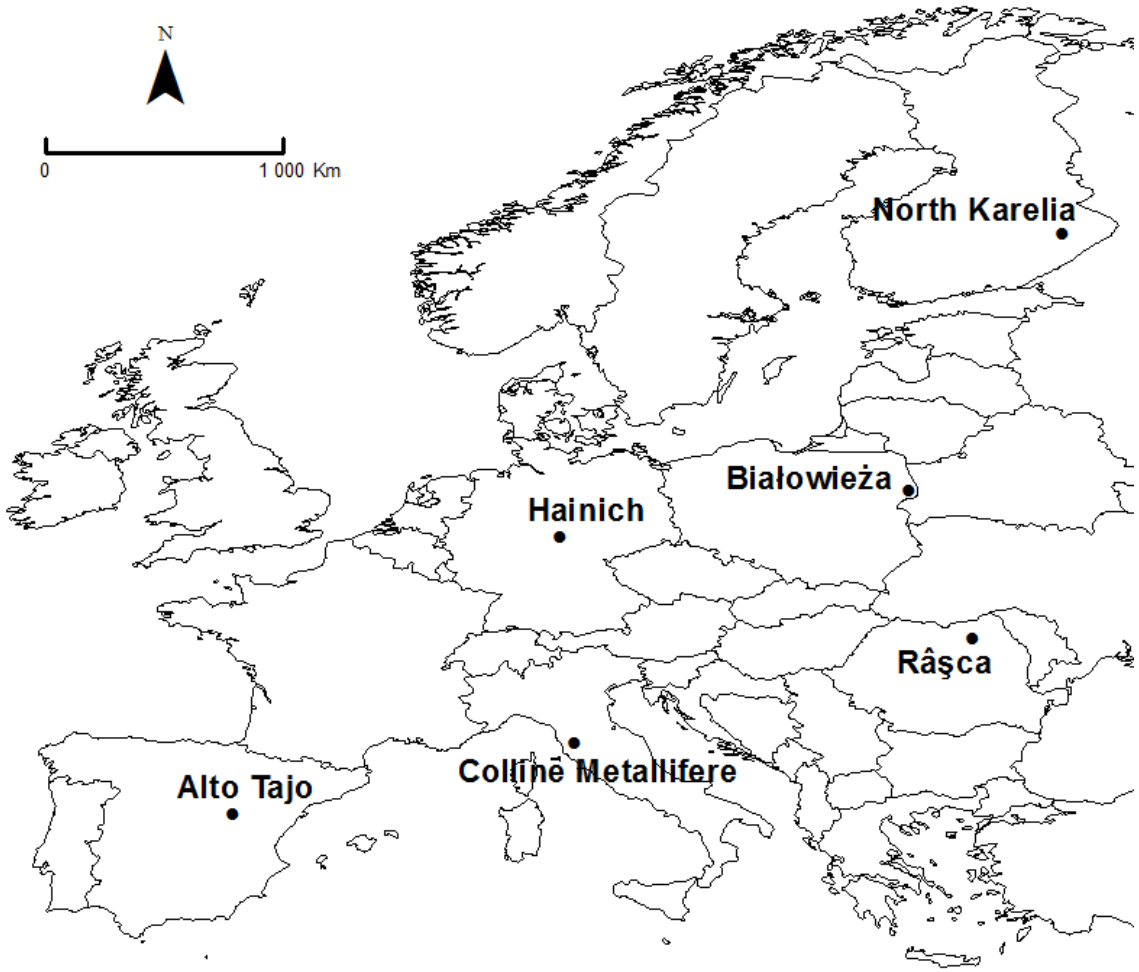


Fig. S2. Schematic representation of the plot sampling design. Dots represent trees, with dot sizes proportional to the tree diameter at breast height. Shapes with different colours indicate the various locations for sampling and setting up experiments. The brown square in the centre subplot indicates the earthworm sampling location and the red circles with black rim indicate the litter traps.

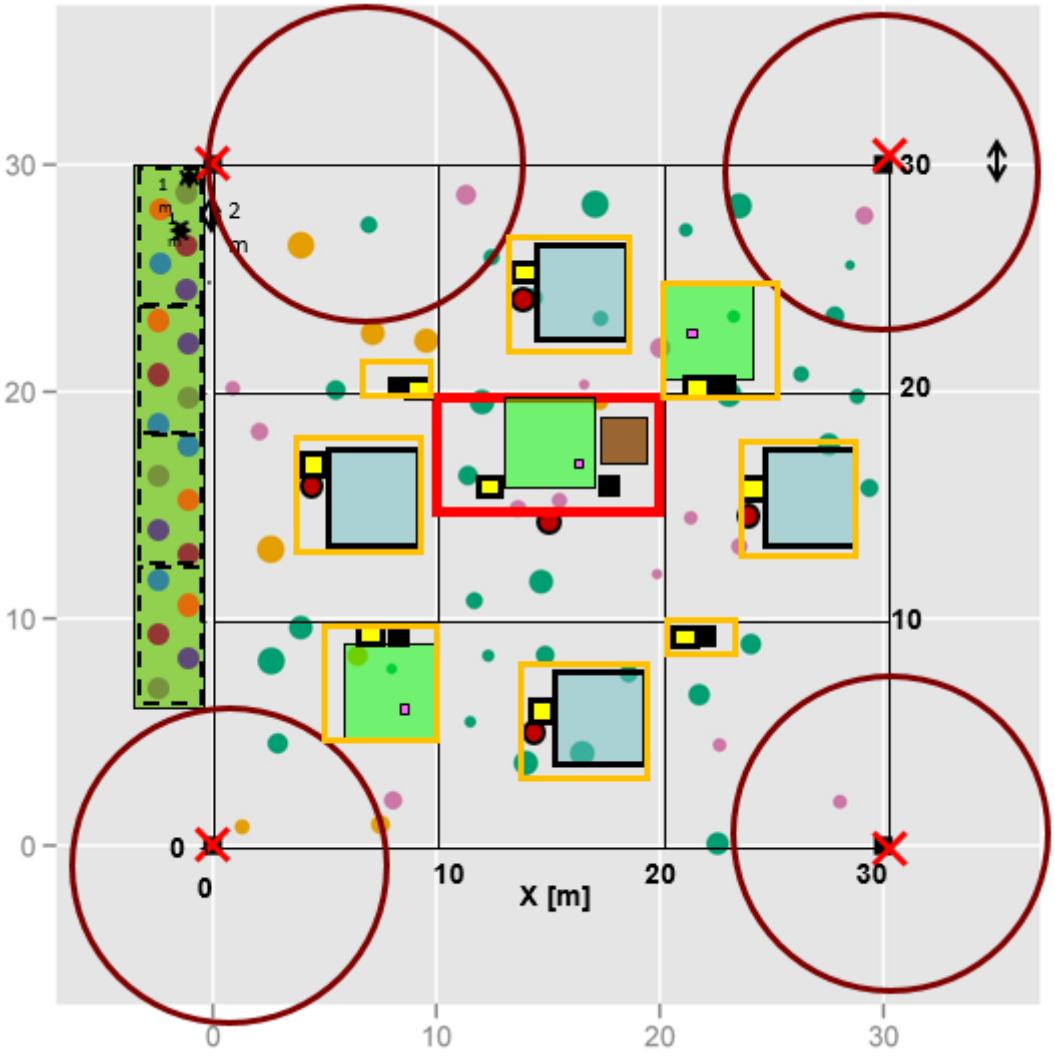
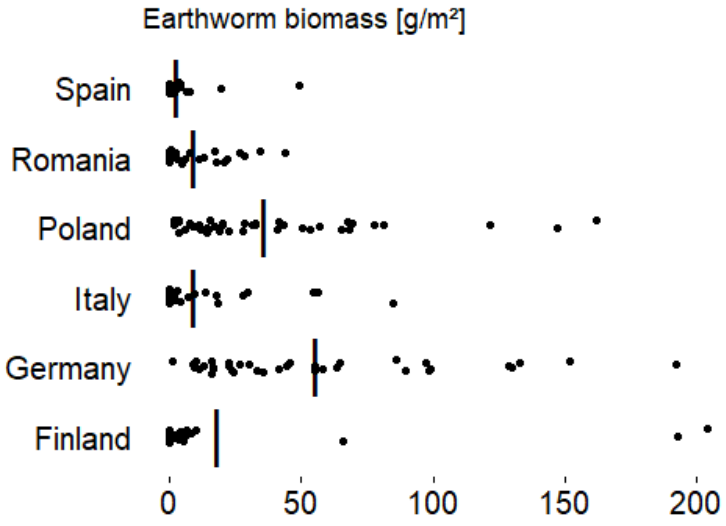


Fig. S3. Descriptive statistics of earthworm biomass (g/m2) across all individual sample plots. Each black dot indicates one of the 209 sampled plots distributed among six forest types across Europe. Black vertical bars indicate the respective mean values. To better visualise the data points, individual points are slightly vertically shifted (with the 'Jitter' function in R) to prevent superposition.



Appendix B2. Additional tables

Table S1. Description of the six exploratory regions and region-specific design, including the pool of species used to create a gradient in species diversity.

	Finland	Poland	Germany	Romania	Italy	Spain
(a) Site description						
Location	North Karelia	Białowieża	Hainich	Râșca	Colline Metallifere	Alto Tajo
Latitude/longitude (degree)	62.6, 29.9	52.7, 23.9	51.1, 10.5	47.3, 26.0	43.2, 11.2	40.7, -1.9
*Forest type	Boreal	Hemiboreal, nemoral coniferous, mixed broadleaved-coniferous	Beech	Mountainous beech	Thermophilous deciduous	Mediterranean mixed
†MAT, MAP	2.1 °C, 700 mm	6.9 °C, 627 mm	6.8 °C, 775 mm	6.8 °C, 800 mm	13 °C, 850 mm	10.2 °C, 499 mm
‡Topography, altitude	Flat, 80-200 m	Flat, 135-185 m	Mainly flat, 500-600 m	Medium-steep slopes, 600-1000 m	Medium-steep slopes, 260-525 m	Flat-medium slopes, 960-1400 m
Study area size (km ×km)	150 ×150	30 ×40	15 ×10	5 ×5	50 ×50	50 ×50
Responsible site manager	L. Finér	B. Jaroszewicz	H. Bruelheide	O. Bouriaud	F. Bussotti	F. Valladares
¶Stand developmental stage						
(1) Mid/late stem exclusion	x	x		x	x	x
(2) Understory reinitiation	x	x	x	x		x
(3) Old growth			x			
Tree cohorts	Single	Multiple	Multiple	Single	Multiple	Multiple
(b) Platform design						
Species richness levels	3	5	4	4	5	4
Number of plots	28	43	38	28	36	36
Plots per richness level	11/14/3	6/11/13/11/2	6/14/14/4	8/10/8/2	9/10/9/7/1	11/18/4/3
Species pool:						
(1) Coniferous						
Abies alba						
Picea abies	×	×	×	×	×	×
Pinus nigra						
Pinus sylvestris	×	×				×
(2) Broadleaved						
Acer pseudoplatanus			×	×		
Betula pendula/pubescens						
Carpinus betulus		×				
Castanea sativa						
Fagus sylvatica			×	×		
Fraxinus excelsior			×			
Ostrya carpinifolia					×	
Quercus robur/petraea					×	
Quercus cerris					×	
Quercus faginea						×
Quercus ilex (evergreen)						×

*Categories of the European Environment Agency (EEA, 2007); †MAT: mean annual temperature, MAP: mean annual precipitation; ‡Altitude in meters above sea level; ¶ Stand developmental stages according to Oliver and Larson (1996).

Table S2. List of all identified earthworm species and number of species found per region.

	Functional group	Finland	Germany	Italy	Poland	Romania	Spain
Dendrobaena attemsi	epigeic					1	
Dendrobaena cognettii	epigeic		1				1
Dendrobaena cognettii gallurensis	epigeic			1			
Dendrobaena octaedra	epigeic	1	1		1	1	
Dendrobaena species 2	epigeic			1			
Dendrodrilus rubidus	epigeic	1	1		1		
Eisenia lucens	epigeic					1	
Lumbricus castaneus	epigeic		1				
Lumbricus rubellus	epigeic		1		1		
Octodrilus damianii	epigeic			1			
Allolobophora carpathica	endogeic					1	
Allolobophora chlorotica	endogeic						1
Allolobophora sturanyi dacidoides	endogeic					1	
Aporrectodea caliginosa	endogeic	1	1		1		1
Aporrectodea handlirschi	endogeic					1	
Aporrectodea rosea	endogeic		1		1	1	1
Eiseniella tetraedra	endogeic						1
Octolasion sp.	endogeic			1			
Octolasion cyaneum	endogeic						1
Octolasion lacteum	endogeic		1		1	1	1
Proctodrilus antipai	endogeic			1			
Scherotheca targionii	endogeic			1			
Fitzingeria platyura depressa	anecic					1	
Lumbricus terrestris	anecic	1	1		1		
Octodrilus complanatus	anecic			1			
Regional earthworm species diversity		4	9	7	7	9	7

Table S3. Results of model selection for the analyses of total earthworm biomass in relation to tree diversity and proportion of evergreen leaf litterfall. Models are ordered by best model using Akaike's information criterion, corrected for small sample sizes (AICc). Sum (WH) of the Akaike weights (Wi) and the likelihood ratio R^2 of the model without (R^2fixed) and with random effect (R^2all) are also given. The right part of the tables represent the parameter estimates of Tree richness, True Shannon, Functional dispersion (FDis) and Proportion of evergreen litter.

Total earthworm biomass - Continental scale

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
FDis + Prop evergreen	15	442.75	0	0.48	0.126	0.617		NA	NA	0.170	-0.476
Prop evergreen	14	443.75	1	0.29	0.112	0.611	0.99	NA	NA	NA	-0.504
Richness + Prop evergreen	15	445.37	2.62	0.13	0.115	0.612		-0.083	NA	NA	-0.532
True Shannon + Prop evergreen	15	446.07	3.32	0.09	0.112	0.611		NA	-0.011	NA	-0.507
FDis	14	456.82	14.07	0	0.053	0.585	0.48	NA	NA	0.225	NA
Null model	13	459.41	16.66	0	0.03	0.575		NA	NA	NA	NA
True Shannon	14	461.03	18.28	0	0.033	0.576	0.09	NA	0.080	NA	NA
Richness	14	461.32	18.57	0	0.032	0.575	0.13	0.065	NA	NA	NA

Total earthworm biomass - Finland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	6	75.14	0	0.57	0.509	0.565	1	NA	NA	NA	-1.425
True Shannon + Prop evergreen	7	77.5	2.36	0.17	0.526	0.58		NA	-0.370	NA	-1.445
Richness + Prop evergreen	7	77.96	2.83	0.14	0.516	0.571		-0.325	NA	NA	-1.513
FDis + Prop evergreen	7	78.33	3.19	0.12	0.509	0.565		NA	NA	-0.292	-1.495
Null model	5	85.82	10.68	0	0.197	0.289		NA	NA	NA	NA
True Shannon	6	88.32	13.18	0	0.215	0.304	0.17	NA	-0.524	NA	NA
FDis	6	88.55	13.41	0	0.206	0.297	0.12	NA	NA	-0.474	NA
Richness	6	89.03	13.89	0	0.192	0.284	0.14	-0.168	NA	NA	NA

Total earthworm biomass - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
True Shannon	6	43.29	0	0.28	0.128	0.128	0.35	NA	0.224	NA	NA
Null model	5	43.63	0.33	0.24	0.049	0.049		NA	NA	NA	NA
FDis	6	44.25	0.95	0.17	0.105	0.105	0.23	NA	NA	0.188	NA
Prop evergreen	6	45.4	2.11	0.1	0.077	0.077	0.25	NA	NA	NA	-0.129
True Shannon + Prop evergreen	7	46.05	2.76	0.07	0.135	0.135		NA	0.203	NA	-0.069
FDis + Prop evergreen	7	46.26	2.97	0.06	0.13	0.13		NA	NA	0.183	-0.123
Richness	6	46.31	3.01	0.06	0.054	0.054	0.08	0.054	NA	NA	NA
Richness + Prop evergreen	7	48.47	5.17	0.02	0.077	0.077		0.003	NA	NA	-0.128

Total earthworm biomass - Italy

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Richness	6	109.74	0	0.25	0.172	0.177	0.3	0.715	NA	NA	NA
True Shannon	6	109.8	0.06	0.24	0.171	0.176	0.29	NA	0.692	NA	NA
Null model	5	110.41	0.67	0.18	0.087	0.092		NA	NA	NA	NA
FDis	6	110.89	1.15	0.14	0.145	0.151	0.17	NA	NA	0.549	NA
True Shannon + Prop evergreen	7	112.8	3.06	0.05	0.172	0.177		NA	0.689	NA	-0.095
Richness + Prop evergreen	7	112.81	3.07	0.05	0.172	0.177		0.711	NA	NA	-0.050
Prop evergreen	6	113.23	3.49	0.04	0.088	0.094	0.17	NA	NA	NA	-0.097
FDis + Prop evergreen	7	113.9	4.16	0.03	0.146	0.151		NA	NA	0.543	-0.090

Total earthworm biomass - Poland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Richness	6	31.57	0	0.36	0.503	0.612	0.57	-0.257	NA	NA	NA
Richness + Prop evergreen	7	32.66	1.09	0.21	0.523	0.628		-0.288	NA	NA	-0.204
Null model	5	33.37	1.8	0.15	0.447	0.568		NA	NA	NA	NA
True Shannon	6	33.86	2.29	0.12	0.475	0.59	0.18	NA	-0.178	NA	NA
True Shannon + Prop evergreen	7	35.26	3.69	0.06	0.493	0.604		NA	-0.212	NA	-0.190
Prop evergreen	6	35.62	4.04	0.05	0.453	0.573	0.06	NA	NA	NA	-0.115
FDis	6	35.65	4.08	0.05	0.453	0.573	0.06	NA	NA	0.084	NA
FDis + Prop evergreen	7	38.06	6.49	0.01	0.459	0.577		NA	NA	0.082	-0.112

Total earthworm biomass - Romania

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	77.4	0	0.31	0.062	0.24		NA	NA	NA	NA
FDis	6	78.29	0.89	0.2	0.142	0.305	0.25	NA	NA	0.612	NA
Prop evergreen	6	78.99	1.58	0.14	0.119	0.286	0.25	NA	NA	NA	-0.601
Richness	6	79.21	1.8	0.13	0.112	0.281	0.16	0.556	NA	NA	NA
True Shannon	6	79.76	2.36	0.1	0.094	0.267	0.13	NA	0.408	NA	NA
FDis + Prop evergreen	7	81.04	3.64	0.05	0.169	0.327		NA	NA	0.507	-0.431
Richness + Prop evergreen	7	81.87	4.46	0.03	0.142	0.305		0.421	NA	NA	-0.458
True Shannon + Prop evergreen	7	81.98	4.58	0.03	0.14	0.303		NA	0.341	NA	-0.540

Total earthworm biomass - Spain

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	78.09	0	0.34	0.04	0.04		NA	NA	NA	NA
True Shannon	6	79.35	1.26	0.18	0.089	0.089	0.25	NA	0.318	NA	NA
Prop evergreen	6	79.71	1.62	0.15	0.079	0.079	0.29	NA	NA	NA	0.283
Richness	6	80.43	2.34	0.11	0.059	0.059	0.15	0.199	NA	NA	NA
FDis	6	81.04	2.95	0.08	0.041	0.041	0.11	NA	NA	0.057	NA
True Shannon + Prop evergreen	7	81.29	3.2	0.07	0.125	0.125		NA	0.305	NA	0.268
Richness + Prop evergreen	7	82.26	4.17	0.04	0.099	0.099		0.200	NA	NA	0.284
FDis + Prop evergreen	7	82.73	4.64	0.03	0.086	0.086		NA	NA	0.111	0.304

Table S4. Results of model selection for the analyses of earthworm species richness in relation to tree diversity and proportion of evergreen leaf litterfall. Models are ordered by best model using Akaike's information criterion, corrected for small sample sizes (AICc). Sum (WH) of the Akaike weights (Wi) and the likelihood ratio R^2 of the model without (R^2fixed) and with random effect (R^2all) are also given. The right part of the tables represent the parameter estimates of Tree richness, True Shannon, Functional dispersion (FDis) and Proportion of evergreen litter.

Earthworm species richness - Continental scale

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	8	591.61	0	0.45	0.06	0.514	0.98	NA	NA	NA	-0.427
True Shannon + Prop evergreen	9	593.37	1.76	0.19	0.062	0.515		NA	0.075	NA	-0.421
FDis + Prop evergreen	9	593.45	1.83	0.18	0.062	0.514		NA	NA	0.075	-0.428
Richness + Prop evergreen	9	593.73	2.11	0.16	0.06	0.514		0.031	NA	NA	-0.421
Null model	7	598.91	7.3	0.01	0.015	0.49		NA	NA	NA	NA
True Shannon	8	600.09	8.48	0.01	0.02	0.493	0.2	NA	0.112	NA	NA
Richness	8	600.33	8.71	0.01	0.019	0.492	0.17	0.096	NA	NA	NA
FDis	8	600.59	8.97	0.01	0.017	0.492	0.19	NA	NA	0.085	NA

Earthworm species richness - Finland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	5	67.27	0	0.39	0.38	0.452	0.65	NA	NA	NA	-0.800
Null model	4	68.44	1.18	0.21	0.281	0.364		NA	NA	NA	NA
FDis + Prop evergreen	6	70.02	2.75	0.1	0.392	0.462		NA	NA	0.312	-0.906
Richness + Prop evergreen	6	70.42	3.15	0.08	0.383	0.454		0.165	NA	NA	-0.820
True Shannon + Prop evergreen	6	70.53	3.26	0.08	0.38	0.452		NA	0.043	NA	-0.809
Richness	5	71.38	4.11	0.05	0.282	0.365	0.13	0.102	NA	NA	NA
FDis	5	71.39	4.12	0.05	0.282	0.365	0.15	NA	NA	0.080	NA
True Shannon	5	71.4	4.13	0.05	0.282	0.365	0.13	NA	-0.074	NA	NA

Earthworm species richness - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
True Shannon	6	126.08	0	0.45	0.186	0.186	0.56	NA	0.803	NA	NA
Null model	5	127.53	1.45	0.22	0.086	0.086		NA	NA	NA	NA
True Shannon + Prop evergreen	7	128.89	2.81	0.11	0.192	0.192		NA	0.863	NA	0.191
FDis	6	129.41	3.33	0.08	0.11	0.11	0.1	NA	NA	0.390	NA
Richness	6	130.16	4.08	0.06	0.092	0.092	0.07	0.189	NA	NA	NA
Prop evergreen	6	130.37	4.29	0.05	0.087	0.087	0.19	NA	NA	NA	-0.066
FDis + Prop evergreen	7	132.45	6.37	0.02	0.11	0.11		NA	NA	0.388	-0.053
Richness + Prop evergreen	7	133.22	7.14	0.01	0.092	0.092		0.192	NA	NA	0.010

Earthworm species richness - Italy

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	89.89	0	0.39	0.119	0.119		NA	NA	NA	NA
Prop evergreen	5	91.72	1.83	0.16	0.141	0.141	0.29	NA	NA	NA	-0.476
Richness	5	92.28	2.39	0.12	0.127	0.127	0.17	0.211	NA	NA	NA
True Shannon	5	92.44	2.55	0.11	0.123	0.123	0.15	NA	0.148	NA	NA
FDis	5	92.57	2.68	0.1	0.12	0.12	0.14	NA	NA	0.069	NA
Richness + Prop evergreen	6	94.12	4.23	0.05	0.152	0.152		0.267	NA	NA	-0.553
True Shannon + Prop evergreen	6	94.3	4.41	0.04	0.148	0.148		NA	0.211	NA	-0.543
FDis + Prop evergreen	6	94.54	4.65	0.04	0.142	0.142		NA	NA	0.109	-0.500

Earthworm species richness - Poland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	142.44	0	0.4	0.129	0.129		NA	NA	NA	NA
Prop evergreen	5	144.62	2.18	0.14	0.137	0.137	0.25	NA	NA	NA	-0.184
True Shannon	5	144.8	2.37	0.12	0.134	0.134	0.16	NA	-0.094	NA	NA
Richness	5	144.87	2.44	0.12	0.132	0.132	0.16	-0.077	NA	NA	NA
FDis	5	145.02	2.58	0.11	0.129	0.129	0.14	NA	NA	-0.007	NA
True Shannon + Prop evergreen	6	147.11	4.67	0.04	0.142	0.142		NA	-0.099	NA	-0.186
Richness + Prop evergreen	6	147.19	4.75	0.04	0.141	0.141		-0.081	NA	NA	-0.185
FDis + Prop evergreen	6	147.35	4.91	0.03	0.138	0.138		NA	NA	0.012	-0.186

Earthworm species richness - Romania

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	76.62	0	0.44	0.006	0.006		NA	NA	NA	NA
Richness	5	79.03	2.41	0.13	0.029	0.029	0.16	0.327	NA	NA	NA
True Shannon	5	79.08	2.45	0.13	0.027	0.027	0.16	NA	0.323	NA	NA
FDis	5	79.29	2.66	0.11	0.02	0.02	0.13	NA	NA	0.259	NA
Prop evergreen	5	79.34	2.71	0.11	0.018	0.018	0.19	NA	NA	NA	-0.231
True Shannon + Prop evergreen	6	82.14	5.52	0.03	0.037	0.037		NA	0.310	NA	-0.219
Richness + Prop evergreen	6	82.21	5.58	0.03	0.035	0.035		0.288	NA	NA	-0.174
FDis + Prop evergreen	6	82.42	5.79	0.02	0.027	0.027		NA	NA	0.220	-0.193

Earthworm species richness - Spain

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	76.69	0	0.44	0.023	0.023		NA	NA	NA	NA
FDis	5	78.89	2.19	0.15	0.041	0.041	0.18	NA	NA	-0.381	NA
Prop evergreen	5	79.4	2.7	0.11	0.026	0.026	0.2	NA	NA	NA	0.126
True Shannon	5	79.47	2.78	0.11	0.024	0.024	0.14	NA	0.055	NA	NA
Richness	5	79.47	2.78	0.11	0.024	0.024	0.14	-0.050	NA	NA	NA
FDis + Prop evergreen	6	81.83	5.14	0.03	0.043	0.043		NA	NA	-0.377	0.099
Richness + Prop evergreen	6	82.39	5.7	0.03	0.026	0.026		-0.051	NA	NA	0.126
True Shannon + Prop evergreen	6	82.4	5.7	0.03	0.026	0.026		NA	0.045	NA	0.123

Table S5. Results of model selection for the continental scale analyses of earthworm functional group biomass in relation to tree diversity and proportion of evergreen leaf litterfall. Models are ordered by best model using Akaike's information criterion, corrected for small sample sizes (AICc). Sum (WH) of the Akaike weights (Wi) and the likelihood ratio R^2 of the model without (R^2fixed) and with random effect (R^2all) are also given. The right part of the tables represent the parameter estimates of Tree richness, True Shannon, Functional dispersion (FDis) and Proportion of evergreen litter. Due to very low sample sizes, the Romanian and Spanish regions were removed from the epigeic biomass model and the Finnish region was removed from the endogeic biomass model.

Epigeic earthworm biomass - Continental scale

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	12	259.21	0	0.46	0.09	0.664	0.93	NA	NA	NA	-0.324
FDis + Prop evergreen	13	261.11	1.9	0.18	0.093	0.665		NA	NA	0.068	-0.317
Richness + Prop evergreen	13	261.51	2.3	0.15	0.091	0.664		-0.039	NA	NA	-0.336
True Shannon + Prop evergreen	13	261.63	2.42	0.14	0.09	0.664		NA	-0.009	NA	-0.326
Null model	11	264.36	5.14	0.04	0.04	0.646		NA	NA	NA	NA
FDis	12	265.93	6.72	0.02	0.046	0.648	0.2	NA	NA	0.090	NA
True Shannon	12	266.57	7.35	0.01	0.042	0.646	0.15	NA	0.036	NA	NA
Richness	12	266.61	7.4	0.01	0.041	0.646	0.16	0.031	NA	NA	NA

Endogeic earthworm biomass - Continental scale

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	13	543.08	0	0.22	0.021	0.48	0.52	NA	NA	NA	-0.275
Null model	12	543.71	0.63	0.16	0.004	0.471		NA	NA	NA	NA
FDis + Prop evergreen	14	544.08	1	0.13	0.029	0.485		NA	NA	0.193	-0.258
FDis	13	544.27	1.19	0.12	0.014	0.477	0.25	NA	NA	0.218	NA
True Shannon	13	544.34	1.26	0.12	0.014	0.477	0.22	NA	0.192	NA	NA
True Shannon + Prop evergreen	14	544.66	1.58	0.1	0.026	0.483		NA	0.136	NA	-0.236
Richness	13	545.07	1.99	0.08	0.01	0.474	0.15	0.133	NA	NA	NA
Richness + Prop evergreen	14	545.27	2.19	0.07	0.022	0.481		0.063	NA	NA	-0.250

Table S6. Results of model selection for the analyses of earthworm functional group biomass in relation to tree diversity and proportion of evergreen leaf litterfall in the German region. Models are ordered by best model using Akaike's information criterion, corrected for small sample sizes (AICc). Sum (WH) of the Akaike weights (Wi) and the likelihood ratio R^2 of the model without (R^2fixed) and with random effect (R^2all) are also given. The right part of the tables represent the parameter estimates of Tree richness, True Shannon, Functional dispersion (FDis) and Proportion of evergreen litter.

Epigeic earthworm biomass - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
True Shannon	6	93.61	0	0.29	0.129	0.226	0.36	NA	0.466	NA	NA
Null model	5	93.92	0.31	0.25	0.051	0.156		NA	NA	NA	NA
FDis	6	95.02	1.41	0.14	0.095	0.195	0.19	NA	NA	0.361	NA
Prop evergreen	6	95.87	2.26	0.09	0.074	0.176	0.23	NA	NA	NA	-0.318
Richness	6	96.17	2.56	0.08	0.066	0.17	0.1	0.247	NA	NA	NA
True Shannon + Prop evergreen	7	96.43	2.81	0.07	0.134	0.23		NA	0.420	NA	-0.171
FDis + Prop evergreen	7	97.35	3.73	0.05	0.112	0.21		NA	NA	0.330	-0.276
Richness + Prop evergreen	7	98.7	5.09	0.02	0.078	0.18		0.154	NA	NA	-0.266

Endogeic earthworm biomass - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	47.53	0	0.39	0.008	0.008		NA	NA	NA	NA
Prop evergreen	6	49.03	1.5	0.18	0.044	0.044	0.3	NA	NA	NA	-0.153
True Shannon	6	49.93	2.4	0.12	0.02	0.02	0.16	NA	0.092	NA	NA
Richness	6	50.27	2.73	0.1	0.011	0.011	0.14	0.048	NA	NA	NA
FDis	6	50.32	2.79	0.1	0.01	0.01	0.14	NA	NA	0.037	NA
True Shannon + Prop evergreen	7	51.97	4.44	0.04	0.047	0.047		NA	0.048	NA	-0.138
FDis + Prop evergreen	7	52.03	4.5	0.04	0.045	0.045		NA	NA	0.032	-0.152
Richness + Prop evergreen	7	52.08	4.55	0.04	0.044	0.044		-0.016	NA	NA	-0.159

Anecic earthworm biomass - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
FDis	6	94.31	0	0.37	0.185	0.185	0.45	NA	NA	0.517	NA
True Shannon	6	95.49	1.18	0.21	0.158	0.158	0.26	NA	0.447	NA	NA
Null model	5	95.73	1.41	0.18	0.085	0.085		NA	NA	NA	NA
FDis + Prop evergreen	7	97.37	3.06	0.08	0.185	0.185		NA	NA	0.517	0.014
Richness	6	98.15	3.84	0.05	0.096	0.096	0.06	0.168	NA	NA	NA
True Shannon + Prop evergreen	7	98.23	3.92	0.05	0.166	0.166		NA	0.491	NA	0.143
Prop evergreen	6	98.59	4.28	0.04	0.085	0.085	0.18	NA	NA	NA	-0.003
Richness + Prop evergreen	7	101.14	6.83	0.01	0.097	0.097		0.198	NA	NA	0.075

Table S7. Results of model selection for the regional scale analyses of earthworm endogeic biomass in relation to tree diversity and proportion of evergreen leaf litterfall. Models are ordered by best model using Akaike's information criterion, corrected for small sample sizes (AICc). Sum (WH) of the Akaike weights (Wi) and the likelihood ratio R^2 of the model without (R^2fixed) and with random effect (R^2all) are also given. The right part of the tables represent the parameter estimates of Tree richness, True Shannon, Functional dispersion (FDis) and Proportion of evergreen litter.

Endogeic earthworm biomass - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	47.53	0	0.39	0.008	0.008		NA	NA	NA	NA
Prop evergreen	6	49.03	1.5	0.18	0.044	0.044	0.3	NA	NA	NA	-0.153
True Shannon	6	49.93	2.4	0.12	0.02	0.02	0.16	NA	0.092	NA	NA
Richness	6	50.27	2.73	0.1	0.011	0.011	0.14	0.048	NA	NA	NA
FDis	6	50.32	2.79	0.1	0.01	0.01	0.14	NA	NA	0.037	NA
True Shannon + Prop evergreen	7	51.97	4.44	0.04	0.047	0.047		NA	0.048	NA	-0.138
FDis + Prop evergreen	7	52.03	4.5	0.04	0.045	0.045		NA	NA	0.032	-0.152
Richness + Prop evergreen	7	52.08	4.55	0.04	0.044	0.044		-0.016	NA	NA	-0.159

Endogeic earthworm biomass - Italy

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	125.28	0	0.42	0.126	0.141		NA	NA	NA	NA
True Shannon	6	127.54	2.26	0.14	0.142	0.157	0.17	NA	0.374	NA	NA
FDis	6	127.61	2.32	0.13	0.14	0.155	0.16	NA	NA	0.338	NA
Richness	6	127.8	2.52	0.12	0.135	0.15	0.15	0.287	NA	NA	NA
Prop evergreen	6	128.02	2.74	0.11	0.129	0.145	0.2	NA	NA	NA	-0.176
True Shannon + Prop evergreen	7	130.57	5.29	0.03	0.143	0.158		NA	0.359	NA	-0.104
FDis + Prop evergreen	7	130.59	5.31	0.03	0.142	0.157		NA	NA	0.324	-0.130
Richness + Prop evergreen	7	130.83	5.55	0.03	0.136	0.151		0.266	NA	NA	-0.105

Endogeic earthworm biomass - Poland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	147.19	0	0.31	0.396	0.424		NA	NA	NA	NA
Richness	6	148.39	1.19	0.17	0.418	0.445	0.22	-0.482	NA	NA	NA
True Shannon	6	148.65	1.45	0.15	0.415	0.441	0.2	NA	-0.435	NA	NA
FDis	6	148.94	1.75	0.13	0.41	0.437	0.17	NA	NA	0.423	NA
Prop evergreen	6	149.64	2.44	0.09	0.4	0.428	0.23	NA	NA	NA	-0.288
Richness + Prop evergreen	7	150.76	3.57	0.05	0.426	0.452		-0.518	NA	NA	-0.407
True Shannon + Prop evergreen	7	151.02	3.83	0.05	0.422	0.448		NA	-0.472	NA	-0.407
FDis + Prop evergreen	7	151.54	4.35	0.04	0.414	0.44		NA	NA	0.425	-0.277

Endogeic earthworm biomass - Romania

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	94.05	0	0.45	0.111	0.315		NA	NA	NA	NA
FDis	6	96.03	1.98	0.17	0.159	0.352	0.2	NA	NA	0.856	NA
Richness	6	96.45	2.4	0.14	0.145	0.341	0.16	0.790	NA	NA	NA
True Shannon	6	97.15	3.1	0.1	0.122	0.323	0.12	NA	0.405	NA	NA
Prop evergreen	6	97.37	3.32	0.09	0.114	0.317	0.16	NA	NA	NA	0.225
FDis + Prop evergreen	7	99.55	5.5	0.03	0.167	0.358		NA	NA	0.874	0.343
Richness + Prop evergreen	7	99.89	5.84	0.02	0.155	0.349		0.903	NA	NA	0.425
True Shannon + Prop evergreen	7	100.83	6.78	0.02	0.125	0.326		NA	0.380	NA	0.231

Endogeic earthworm biomass - Spain

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	104.92	0	0.35	0.043	0.043		NA	NA	NA	NA
Prop evergreen	6	106.25	1.33	0.18	0.096	0.096	0.34	NA	NA	NA	0.537
True Shannon	6	106.55	1.63	0.15	0.087	0.087	0.23	NA	0.501	NA	NA
Richness	6	107.57	2.65	0.09	0.057	0.057	0.13	0.279	NA	NA	NA
True Shannon + Prop evergreen	7	107.94	3.02	0.08	0.144	0.144		NA	0.519	NA	0.554
FDis	6	107.98	3.06	0.07	0.044	0.044	0.11	NA	NA	0.077	NA
Richness + Prop evergreen	7	109.09	4.17	0.04	0.112	0.112		0.297	NA	NA	0.546
FDis + Prop evergreen	7	109.31	4.39	0.04	0.105	0.105		NA	NA	0.222	0.594

Table S8. Results of the model selection and model averaging for the best candidate models relating total earthworm biomass to litter quality variables (CWMs). Parameter estimates and relative importance values (RI) result from model averaging across all models with Δi < 2. Values of litter traits that were not included in the analysis due to multicollinearity issues were left blank. Values of parameters that were included in the model, but not retained in the best candidate models were indicated with a hyphen. Bold and italic, confidence intervals do not include zero. If only one model within 2 Δi units, relative importance value of the model predictors are given the value of one.

	All Sites		**Finland**		**Germany**		**Italy**		**Poland**		**Romania**		**Spain**	
Model parameters	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI
C:N ratio	0.013	0.13			***-0.375***	***1***	-	-	-	-				
C:P ratio	-0.287	0.8			-0.047	0.33					-	-	-	-
Calcium	0.026	0.16	***1.849***	***1***	-	-	-	-	0.039	0.31	-	-	-0.301	0.67
Lignin	-0.02	0.16			-	-			-	-	***-0.931***	***1***		
Water holding capacity	***0.416***	***1***	-	-			-	-					0.066	0.22

Table S9. Results of the model selection and model averaging for the best candidate models relating earthworm richness to litter quality variables (CWMs). Parameter estimates and relative importance values (RI) result from model averaging across all models with Δi < 2. Values of litter traits that were not included in the analysis due to multicollinearity issues were left blank. Values of parameters that were included in the model, but not retained in the best candidate models were indicated with a hyphen. Bold and italic, confidence intervals do not include zero. If only one model within 2 Δi units, relative importance value of the model predictors are given the value of one.

	All Sites		**Finland**		**Germany**		**Italy**		**Poland**		**Romania**		**Spain**	
Model parameters	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI
C:N ratio	*-0.142*	*0.49*			***-0.357***	***0.98***	-	-	-0.068	0.3				
C:P ratio	-0.046	0.21			-0.047	0.31					-	-	-	-
Calcium	0.009	0.09	0.658	0.69	-	-	-	-	-	-	0.108	0.23	-	-
Lignin	-0.014	0.09			-0.015	0.16			-	-	-0.086	0.21		
Water holding capacity	*0.114*	*0.54*	-0.318	0.27			-	-					-	-

Table S10. Results of the model selection and model averaging for the best candidate models relating earthworm functional group (epigeic and endogeic) biomass to litter quality variables (CWMs). Parameter estimates and relative importance values (RI) result from model averaging across all models with Δi < 2. Values of litter traits that were not included in the analysis due to multicollinearity issues were left blank. Values of parameters that were included in the model, but not retained in the best candidate models were indicated with a hyphen. Bold and italic, confidence intervals do not include zero. If only one model within 2 Δi units, relative importance value of the model predictors are given the value of one. Anecic models could not be fitted due to the small number of plots where anecic species were found.

	Epigeic		**Endogeic**		**Anecic**	
Model parameters	Estimate	RI	Estimate	RI	Estimate	RI
C:N ratio	-	-	-	-	NA	NA
C:P ratio	-0.033	0.2	-0.258	0.55	NA	NA
Calcium	-	-	-	-	NA	NA
Lignin	0.073	0.32	-0.136	0.45	NA	NA
Water holding capacity	***0.261***	***1***	***0.518***	***1***	NA	NA

Table S11. Results of the model selection and model averaging for the best candidate models relating earthworm endogeic biomass to litter quality variables (CWMs) for the regional models that had sufficient endogeic earthworms. Parameter estimates and relative importance values (RI) result from model averaging across all models with Δi < 2. Values of litter traits that were not included in the analysis due to multicollinearity issues were left blank. Values of parameters that were included in the model, but not retained in the best candidate models were indicated with a hyphen. Bold and italic, confidence intervals do not include zero. If only one model within 2 Δi units, relative importance value of the model predictors are given the value of one.

	Finland		**Germany**		**Italy**		**Poland**		**Romania**		**Spain**	
Model parameters	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI	Estimate	RI
C:N ratio	NA	NA	***-0.309***	***1***	-	-	-	-			-	-
C:P ratio	NA	NA	-	-					-	-	-	-
Calcium	NA	NA	-	-	-	-	-	-	-	-	-0.503	0.63
Lignin	NA	NA	-	-			-0.243	0.4	-	-	-	-
Water holding capacity	NA	NA			-	-					-	-

Table S12. Results of the model selection and model averaging for the best candidate models relating earthworm functional group (epigeic, endogeic and anecic) biomass to litter quality variables (CWMs) in the German forest site. Parameter estimates and relative importance values (RI) result from model averaging across all models with Δi < 2. Values of litter traits that were not included in the analysis due to multicollinearity issues were left blank. Values of parameters that were included in the model, but not retained in the best candidate models were indicated with a hyphen. Bold and italic, confidence intervals do not include zero. If only one model within 2 Δi units, relative importance value of the model predictors are given the value of one.

	Epigeic		**Endogeic**		**Anecic**	
Model parameters	Estimate	RI	Estimate	RI	Estimate	RI
C:N ratio	-0.835	1	***-0.309***	***1***	-0.211	0.42
C:P ratio	-0.634	1	-	-	-0.038	0.13
Calcium	-	-	-	-	0.22	0.42
Lignin	-	-	-	-	-0.036	0.13
Water holding capacity						

Table S13. Likelihood ratio R^2 of the earthworm species richness null models (Worm Richness ~ 1 + Covariates + Random effect) and respective diversity (Worm Richness ~ Diversity index + Covariates + Random effect), proportion of evergreen litter (Worm Richness ~ Evergreen proportion + Covariates + Random effect) and identity models (Worm Richness ~ Litter quality traits + Covariates + Random effect). The litter quality models are the best models after model selection (see section 2.4 for details). The last column (litter quality traits) describes which traits were included in the best litter quality model. All models were fitted with maximum likelihood estimation.

		Diversity			**Identity**		
Model scale	**Null model**	**Richness**	**Shannon**	**FDis**	**Evergreen**	**Litter quality**	**Litter quality traits**
Continental	0.49	0.492	0.493	0.492	0.514	0.501	WHC
Finland	0.364	0.365	0.365	0.365	0.452	0.441	Ca_CWM_Maria_res
Germany	0.086	0.092	0.186	0.11	0.087	0.322	CN + Lignin
Italy	0.119	0.127	0.123	0.12	0.141	0.119	
Poland	0.129	0.132	0.134	0.129	0.137	0.129	
Romania	0.006	0.029	0.027	0.02	0.018	0.006	
Spain	0.023	0.024	0.024	0.041	0.026	0.023	

Table S14. Likelihood ratio R^2 of the earthworm biomass of the different functional group null models (Biomass ~ 1 + Covariates + Random effect) and respective diversity (Biomass ~ Diversity index + Covariates + Random effect), proportion of evergreen litter (Biomass ~ Evergreen proportion + Covariates + Random effect) and identity models (Biomass ~ Litter quality traits + Covariates + Random effect). The litter quality models are the best models after model selection (see section 2.4 for details). The first column indicates the response variable of the model. The last column (litter quality traits) describes which traits were included in the best litter quality model. All models were fitted with restricted maximum likelihood estimation.

			Diversity			Identity		
Response variable	**Model scale**	**Null model**	**Richness**	**Shannon**	**FDis**	**Evergreen**	**Litter quality**	**Litter quality traits**
Epigeic	Continental	0.646	0.646	0.646	0.648	0.664	0.659	WHC
Endogeic	Continental	0.471	0.474	0.477	0.477	0.480	0.508	C:P + WHC
Epigeic	Germany	0.156	0.170	0.226	0.195	0.176	0.392	C:N + C:P
Anecic	Germany	0.085	0.158	0.158	0.185	0.085	0.18	Calcium
Endogeic	Germany	0.008	0.011	0.020	0.010	0.044	0.156	C:N
Endogeic	Italy	0.141	0.150	0.157	0.155	0.145	0.141	
Endogeic	Poland	0.424	0.445	0.441	0.437	0.428	0.424	
Endogeic	Romania	0.315	0.341	0.323	0.352	0.317	0.315	
Endogeic	Spain	0.043	0.057	0.087	0.044	0.096	0.162	Calcium

Table S15. Pearson correlation diagram of the litter quality traits per region.

FINLAND	C:N ratio	C:P ratio	Calcium	Lignin	Water holding capacity
C:N ratio	1	0.91	-0.73	-0.59	-0.97
C:P ratio	0.91	1	-0.95	-0.87	-0.78
Calcium	-0.73	-0.95	1	0.98	0.54
Lignin	-0.59	-0.87	0.98	1	0.37
Water holding capacity	-0.97	-0.78	0.54	0.37	1
GERMANY					
C:N ratio	1	-0.22	-0.17	-0.64	-0.8
C:P ratio	-0.22	1	0.67	0.38	0.66
Calcium	-0.17	0.67	1	-0.12	0.67
Lignin	-0.64	0.38	-0.12	1	0.56
Water holding capacity	-0.8	0.66	0.67	0.56	1
ITALY					
C:N ratio	1	0.81	-0.4	-0.69	-0.07
C:P ratio	0.81	1	-0.32	-0.8	0.06
Calcium	-0.4	-0.32	1	0.57	-0.72
Lignin	-0.69	-0.8	0.57	1	-0.04
Water holding capacity	-0.07	0.06	-0.72	-0.04	1
POLAND					
C:N ratio	1	0.81	-0.4	-0.69	-0.07
C:P ratio	0.81	1	-0.32	-0.8	0.06
Calcium	-0.4	-0.32	1	0.57	-0.72
Lignin	-0.69	-0.8	0.57	1	-0.04
Water holding capacity	-0.07	0.06	-0.72	-0.04	1
ROMANIA					
C:N ratio	1	-0.5	0.64	-0.7	-0.78
C:P ratio	-0.5	1	-0.13	-0.27	0.54
Calcium	0.64	-0.13	1	-0.56	-0.9
Lignin	-0.7	-0.27	-0.56	1	0.39
Water holding capacity	-0.78	0.54	-0.9	0.39	1
SPAIN					
C:N ratio	1	-0.56	0.8	-0.96	-0.19
C:P ratio	-0.56	1	-0.51	0.76	-0.15
Calcium	0.8	-0.51	1	-0.77	0.43
Lignin	-0.96	0.76	-0.77	1	0.13
Water holding capacity	-0.19	-0.15	0.43	0.13	1

Appendix B3. Determination of Leaf litter C:N ratio and calcium concentration

Litter samples were analysed by Near Infra-Red Spectroscopy (NIRS), as described by Niederberger et al. (2015). In order to calibrate the NIRS spectra and validate the calibration for the determination of (a) Ca concentration, a subset of samples were mineralized and analysed with an atom absorption spectrometer (AAS, iCE 3000 series, ThermoScientific, China), (b) N concentration, a subset of samples were analysed with a flash CHN Elemental Analyser (Flash EA1112 Series, ThermoFinnigan, Milan, Italy). The procedure of spectra calibration and NIRS application is described by Pollastrini et al. (2016).

Appendix B4. Variable reduction procedure to optimally represent the litter trait space

Correlation matrix and ordination is constructed with a 'tree species x litter trait' matrix composed of 26 tree species and 18 tree litter traits (Table 1). Some tree species occur in multiple forest regions (e.g. Pinus sylvestris) but they are counted as different species (e.g. FIN_Pisy & SPA_Pisy). Only four litter traits are plot specific (C, N, C/N, Ca), therefore average litter trait values were calculated per site for every tree species.

Matrix example:

	C	N	C:N	P	C:P	Lignin	Cellulose	....
FIN_Pisy	*52.008*	0.356	148.288	0.031	1695.861	23.446	26.263	...
GER_Acps	*45.546*	1.304	35.880	0.035	1291.889	14.365	15.716	...
FIN_Bepe	*50.808*	0.855	61.284	0.103	494.719	33.794	15.156	...
ITA_Osca	*48.490*	1.480	34.784	0.052	935.323	27.318	15.469	...
...	...	...	...	...	...	...	...	...

Overview of traits used:

Plot specific	**Site specific**	
C	P	lignin
N	C:P	cellulose
C:N	Mg	hemicellulose
Calcium	K	tannins
	water holding capacity (WHC)	soluble phenolics
	specific leaf area (SLA)	total phenolics
	pH	water soluble compounds

Both Spearman and Pearson correlation are conducted with these variables. When Spearman > Pearson it is an indication that the relation between 2 variables are monotonic, but not linear. Just 11% of the pairwise correlations have a Spearman correlation coefficient which is more than 10% higher than the Pearson ones and only 2% (3 cases) more than 15% higher. This indicates that most relations are close to linear. Based on this, we decide to report Pearson correlations for environmental variables and use PCA as ordination technique, since it is often used, easy to apply and straightforward to interpret.

Data reduction and selection: We retained five tree litter trait variables for further analyses that optimally represent the tree litter multidimensional trait space (C:N, Calcium, Lignin, C:P, WHC). Litter traits were selected based on prior knowledge and by minimizing the correlation between the traits (Table 2) and maximizing the correlation with the PCA axes (Fig. 1 & Table 3).

Table 1. Average tree species litter trait value per forest region. Litter trait values are expressed in percentages, all but Ca, Mg and K who are expressed in mg/g and SLA which is expressed in cm²/g. Tree species name codes are the first two letters of the Latin genus and species name (e.g. Bepe = Betula pendula).

Tree species	C	N	C:N	P	C:P	Lignin	Cellulose	Hemi-cellulose	wsc	Tannins	Soluble phenolics	Poly-phenols	Ca	Mg	K	pH	WHC	SLA
FIN_Bepe	50.81	0.86	61.28	0.10	494.72	33.79	15.16	15.97	35.08	4.35	6.94	12.77	9.03	4.08	2.81	5.58	1.50	18.31
FIN_Piab	48.82	0.95	52.49	0.05	923.57	27.03	19.43	16.16	37.39	3.79	5.62	13.35	7.71	1.73	1.50	5.22	1.67	4.57
FIN_Pisy	52.01	0.36	148.29	0.03	1695.86	23.45	26.26	16.44	33.85	0.87	2.62	9.74	7.44	1.72	0.47	4.49	1.20	4.50
GER_Acps	45.55	1.30	35.88	0.04	1291.89	14.37	15.72	13.91	56.01	1.54	39.78	11.20	20.72	7.33	7.23	6.12	1.66	19.62
GER_Fasy	47.31	0.81	59.31	0.05	1056.57	24.25	20.44	15.35	39.97	8.29	17.13	25.48	15.46	3.66	5.22	6.40	1.80	20.69
GER_Frex	46.04	1.73	28.38	0.06	783.50	22.00	14.88	13.62	49.51	0.53	14.88	13.47	22.42	6.78	7.33	6.47	2.41	18.93
GER_Piab	48.86	0.75	72.40	0.12	421.76	14.38	18.72	18.97	47.93	36.25	54.90	81.88	15.22	1.54	3.68	4.57	0.82	3.82
GER_Qupe	48.87	1.09	46.62	0.14	355.71	20.56	15.71	15.46	48.27	13.77	46.71	52.29	11.48	3.33	4.42	5.15	1.56	17.09
ITA_Casa	49.60	1.35	38.72	0.03	1627.86	13.90	16.67	18.83	50.60	2.32	55.54	61.49	10.29	4.19	2.11	4.43	1.87	19.34
ITA_Osca	48.49	1.48	34.78	0.05	935.32	27.32	15.47	17.55	39.66	5.13	12.81	13.27	16.84	3.94	2.18	5.37	1.31	20.98
ITA_Quce	50.34	1.26	41.36	0.02	2905.04	23.27	16.76	15.53	44.45	2.94	20.31	24.17	8.26	2.65	2.51	4.74	1.24	12.32
ITA_Quil	50.06	1.04	49.43	0.02	2244.13	21.05	20.07	16.21	42.67	10.94	46.04	59.64	9.09	1.79	3.63	5.20	1.06	6.72
ITA_Qupe	49.03	1.01	51.74	0.01	3901.96	19.43	17.54	17.32	45.71	6.95	39.96	40.83	9.08	2.30	2.94	4.57	1.55	14.60
POL_Bepe	49.09	1.16	43.11	0.20	242.66	21.80	11.80	19.69	46.70	16.75	39.24	45.45	12.93	4.05	6.29	5.25	2.37	18.27
POL_Cabe	46.54	1.28	37.01	0.17	280.82	8.07	15.06	18.79	58.07	2.05	76.45	77.11	10.19	3.85	6.95	4.46	2.83	33.33
POL_Piab	47.39	0.78	61.78	0.08	568.38	20.82	20.19	17.80	41.20	11.60	20.25	39.96	11.27	1.53	2.77	4.44	1.08	4.00
POL_Pisy	50.47	0.41	132.50	0.10	514.89	17.17	25.58	17.49	39.76	6.91	20.04	28.16	7.74	1.43	2.94	4.70	1.03	4.67
POL_Quro	48.56	1.68	30.79	0.26	187.72	18.94	16.17	16.24	48.66	13.75	53.34	57.53	7.47	2.86	3.96	5.22	1.97	17.07
ROM_Abal	49.19	0.89	58.01	0.09	542.85	28.10	18.49	17.28	36.14	4.27	9.70	16.84	15.05	2.68	3.12	5.65	1.31	5.85
ROM_Acps	46.53	1.33	36.55	0.05	888.09	16.99	17.75	15.92	49.35	5.62	53.89	44.13	16.14	4.35	5.83	5.03	2.08	16.93
ROM_Fasy	47.99	0.84	57.39	0.05	1074.91	25.69	19.28	18.20	36.83	9.43	23.21	25.66	12.86	3.14	6.36	6.20	1.65	23.15
ROM_Piab	48.09	0.70	70.62	0.09	551.50	22.71	19.74	16.08	41.48	6.33	11.08	23.37	13.18	2.71	3.70	5.25	1.59	5.28
SPA_Pini	51.80	0.47	118.10	0.05	1007.09	18.54	19.41	14.71	47.34	19.88	35.32	41.28	6.24	2.69	2.07	4.79	1.56	3.36
SPA_Pisy	51.95	0.45	118.03	0.02	2632.78	14.67	19.89	15.33	50.12	22.95	34.72	43.48	6.59	3.11	1.64	4.87	1.09	4.06
SPA_Qufa	47.44	0.63	77.21	0.01	5309.02	13.51	18.71	22.40	45.38	8.47	97.41	82.89	10.28	3.38	2.46	4.44	1.42	9.66
SPA_Quil	48.91	0.61	82.21	0.06	895.20	12.68	19.17	16.61	51.54	12.06	95.71	109.53	8.48	2.78	3.26	4.90	1.35	5.76

Table 2. Correlation matrix (Pearson's r) for tree litter trait variables

	C	N	C:N	P	C:P	lignin	cellulose	hemi-cellulose	wsc	tannins	soluble phenolics	total phenolics	Ca	Mg	K	pH	WHC	SLA
C	1.00																	
N	-0.55	1.00																
C:N	0.67	-0.89	1.00															
P	-0.12	0.31	-0.25	1.00														
C:P	-0.08	0.22	-0.25	-0.61	1.00													
lignin	0.22	0.02	-0.09	-0.03	-0.12	1.00												
cellulose	0.41	-0.74	0.80	-0.38	-0.13	-0.01	1.00											
hemicellulose	-0.05	-0.16	0.01	0.14	0.14	-0.25	-0.03	1.00										
wsc	-0.39	0.39	-0.32	0.18	0.13	-0.83	-0.47	-0.05	1.00									
tannins	0.28	-0.33	0.26	0.25	-0.28	-0.26	0.02	0.17	0.18	1.00								
soluble phenolics	-0.25	0.06	-0.13	0.11	0.21	-0.80	-0.22	0.45	0.70	0.27	1.00							
total phenolics	-0.06	-0.09	-0.01	0.19	0.04	-0.72	-0.10	0.49	0.56	0.48	0.92	1.00						
Ca	-0.74	0.50	-0.52	-0.03	-0.01	0.08	-0.39	-0.18	0.17	-0.18	-0.14	-0.28	1.00					
Mg	-0.56	0.59	-0.50	-0.02	0.13	-0.10	-0.62	-0.31	0.48	-0.37	0.08	-0.21	0.70	1.00				
K	-0.75	0.52	-0.56	0.32	-0.13	-0.20	-0.51	-0.13	0.47	-0.11	0.18	0.02	0.67	0.66	1.00			
pH	-0.40	0.33	-0.37	0.01	-0.15	0.48	-0.26	-0.49	-0.16	-0.24	-0.40	-0.51	0.64	0.58	0.57	1.00		
WHC	-0.52	0.60	-0.53	0.41	-0.09	-0.17	-0.60	-0.02	0.45	-0.36	0.18	0.02	0.29	0.59	0.67	0.28	1.00	
SLA	-0.53	0.67	-0.65	0.27	0.09	-0.05	-0.63	0.06	0.34	-0.38	0.15	-0.04	0.39	0.63	0.66	0.36	0.74	1.00

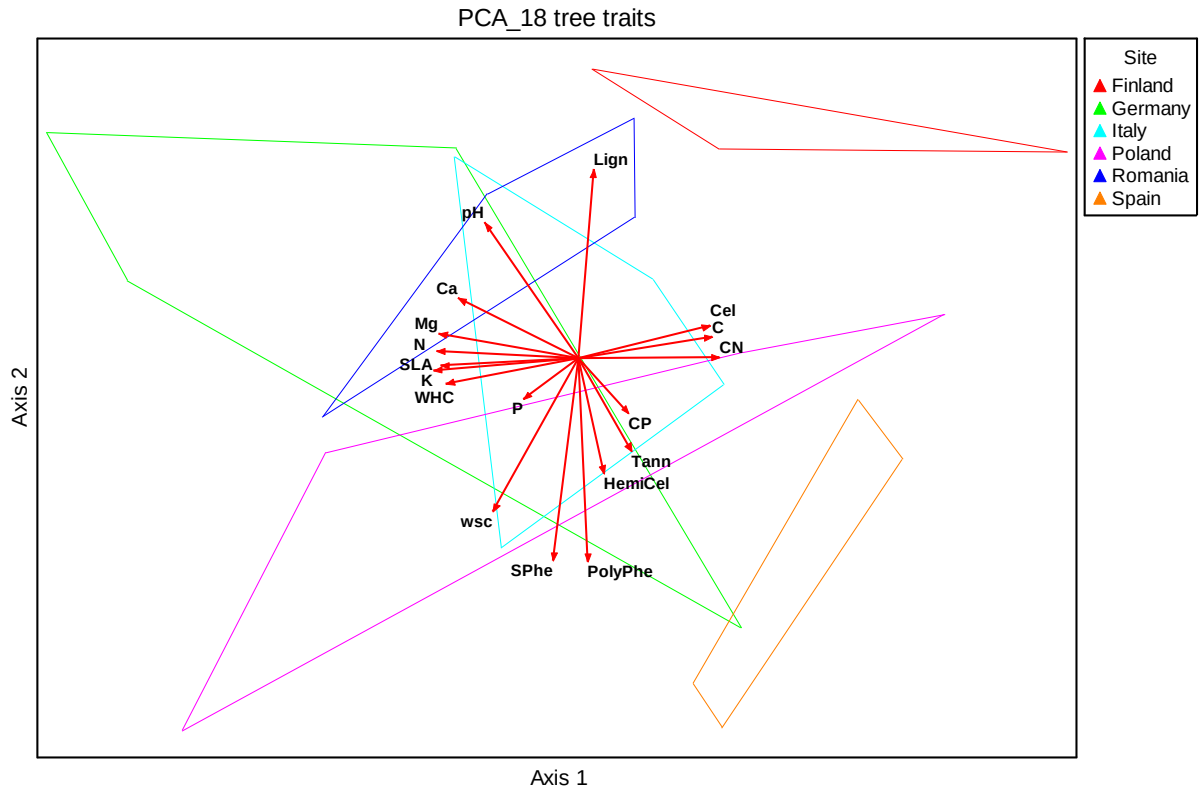


Figure 1. PCA diagrams of the tree litter trait variables, showing their correlation with the two first PCA axes.

Table 3. Correlations (Pearson's r) between tree litter traits and the first three axes of the PCA. They explain respectively 37%, 23% and 10% of the variation in the data (70% in total). The third PC axis however has a p>0.05 and as a consequence can't be trusted.

litter variables	Axis.1		Axis.2		Axis.3	
C	0.78		0.10	(n.s.)	-0.20	(n.s.)
N	-0.83		0.03	(n.s.)	-0.03	(n.s.)
C:N	0.82		0.01	(n.s.)	0.02	(n.s.)
P	-0.32	(n.s.)	-0.19	(n.s.)	-0.86	
C:P	0.29	(n.s.)	-0.25	(n.s.)	0.77	
lignin	0.09	(n.s.)	0.87		-0.19	(n.s.)
cellulose	0.77		0.15	(n.s.)	0.16	(n.s.)
hemicellulose	0.15	(n.s.)	-0.53		-0.02	(n.s.)
wsc	-0.51		-0.70		0.10	(n.s.)
tannins	0.31	(n.s.)	-0.43		-0.45	
sol.phenolics	-0.15	(n.s.)	-0.93		0.14	(n.s.)
polyphenols (total phenolics)	0.05	(n.s.)	-0.93		-0.09	(n.s.)
Ca	-0.70		0.28	(n.s.)	0.19	(n.s.)
Mg	-0.82		0.11	(n.s.)	0.32	(n.s.)
K	-0.85		-0.06	(n.s.)	-0.07	(n.s.)
pH	-0.55		0.62		0.02	(n.s.)
WHC	-0.78		-0.12	(n.s.)	-0.11	(n.s.)
SLA	-0.81		-0.03	(n.s.)	0.02	(n.s.)

Appendix B5. Analysis and results at neighbourhood level

Material and methods

Study area

Since earthworm distribution can change over smaller spatial scales than the selected research plots of 30 x 30 m (Valckx et al. 2009), tree species diversity, proportion of evergreen leaf litterfall and litter quality analyses are also performed on neighbourhood level. One neighbourhood of 5m radius surrounding the earthworm sampling location was defined per original sample plot of 30 x 30 m.

Diversity and identity predictors

At neighbourhood level there was no leaf litter mass data available, so both the diversity indices as well as the litter trait CWMs were calculated based on relative tree basal areas of the target tree species in the neighbourhood radius. To define the tree basal area of the tree species that where within the neighbourhood radius of 5m we used the following approach: First, we calculated the basal area (m^2) of all tree species whose canopies overlapped the neighbourhood radius in a plot. The basal area of each of those trees was then multiplied by the proportion of its canopy that overlapped the local neighbourhood area.

As on plot level, neighbourhoods with a non-target tree admixture up to 10% are tolerated, but they are not taken into account in any of the index or trait calculations. Neighbourhoods where the canopy cover of the non-target trees was more than 10% of the summed canopy cover of the tree species in the neighbourhood radius were excluded from the analyses (10% of the plots).

Tables with results

Table S1. Model selection results for the analyses of total earthworm biomass in relation to tree diversity and proportion of evergreen leaf litterfall on neighbourhood level. Models are ordered by best model using Akaike's information criterion, corrected for small sample sizes (AICc). Sum (WH) of the Akaike weights (Wi) and the likelihood ratio R^2 of the model without (R^2fixed) and with random effect (R^2all) are also given. The right part of the tables represent the parameter estimates of Tree richness, True Shannon, Functional dispersion (FDis) and Proportion of evergreen litter.

Total earthworm biomass - Continental scale

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	14	403.81	0	0.43	0.096	0.58	0.99	NA	NA	NA	-0.405
Richness + Prop evergreen	15	405.01	1.2	0.24	0.101	0.582		0.096	NA	NA	-0.410
FDis + Prop evergreen	15	405.64	1.84	0.17	0.098	0.581		NA	NA	0.069	-0.402
True Shannon + Prop evergreen	15	405.91	2.1	0.15	0.097	0.58		NA	0.046	NA	-0.403
Null model	13	413	9.19	0	0.037	0.552		NA	NA	NA	NA
Richness	14	414.34	10.53	0	0.042	0.555	0.24	0.089	NA	NA	NA
FDis	14	414.48	10.67	0	0.041	0.554	0.17	NA	NA	0.091	NA
True Shannon	14	414.68	10.87	0	0.04	0.554	0.15	NA	0.070	NA	NA

Total earthworm biomass - Finland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	6	70.4	0	0.49	0.587	0.634	1	NA	NA	NA	-1.408
FDis + Prop evergreen	7	71.59	1.2	0.27	0.615	0.659		NA	NA	0.416	-1.513
True Shannon + Prop evergreen	7	73.01	2.62	0.13	0.598	0.644		NA	0.280	NA	-1.435
Richness + Prop evergreen	7	73.31	2.91	0.11	0.594	0.64		0.257	NA	NA	-1.410
Null model	5	85.82	15.43	0	0.197	0.289		NA	NA	NA	NA
Richness	6	87.74	17.34	0	0.234	0.321	0.11	0.632	NA	NA	NA
True Shannon	6	88.69	18.29	0	0.206	0.297	0.13	NA	0.322	NA	NA
FDis	6	89.09	18.69	0	0.193	0.285	0.27	NA	NA	0.057	NA

Total earthworm biomass - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	39.42	0	0.38	0.07	0.07		NA	NA	NA	NA
Richness	6	40.7	1.28	0.2	0.117	0.117	0.24	0.169	NA	NA	NA
True Shannon	6	41.52	2.1	0.13	0.095	0.095	0.16	NA	0.123	NA	NA
FDis	6	42.18	2.77	0.1	0.077	0.077	0.12	NA	NA	-0.064	NA
Prop evergreen	6	42.22	2.8	0.09	0.076	0.076	0.18	NA	NA	NA	-0.060
Richness + Prop evergreen	7	43.92	4.5	0.04	0.118	0.118		0.164	NA	NA	-0.021
True Shannon + Prop evergreen	7	44.71	5.3	0.03	0.096	0.096		NA	0.115	NA	-0.032
FDis + Prop evergreen	7	45.18	5.76	0.02	0.084	0.084		NA	NA	-0.069	-0.066

Total earthworm biomass - Italy

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	83.41	0	0.33	0.054	0.054		NA	NA	NA	NA
Richness	6	84.29	0.88	0.21	0.142	0.142	0.25	0.586	NA	NA	NA
FDis	6	84.41	0.99	0.2	0.138	0.138	0.23	NA	NA	0.577	NA
True Shannon	6	85.71	2.3	0.1	0.094	0.094	0.12	NA	0.420	NA	NA
Prop evergreen	6	86.54	3.13	0.07	0.064	0.064	0.16	NA	NA	NA	-0.218
Richness + Prop evergreen	7	87.75	4.34	0.04	0.153	0.153		0.588	NA	NA	-0.225
FDis + Prop evergreen	7	88.05	4.64	0.03	0.143	0.143		NA	NA	0.562	-0.155
True Shannon + Prop evergreen	7	89.15	5.74	0.02	0.106	0.106		NA	0.430	NA	-0.237

Total earthworm biomass - Poland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	5	34.71	0	0.31	0.412	0.532		NA	NA	NA	NA
True Shannon	6	35.7	0.98	0.19	0.438	0.552	0.24	NA	-0.160	NA	NA
Richness	6	35.89	1.18	0.17	0.435	0.55	0.23	-0.150	NA	NA	NA
Prop evergreen	6	36.93	2.21	0.1	0.421	0.538	0.24	NA	NA	NA	0.117
FDis	6	37.11	2.4	0.09	0.418	0.536	0.12	NA	NA	-0.076	NA
Richness + Prop evergreen	7	38.07	3.35	0.06	0.446	0.559		-0.159	NA	NA	0.136
True Shannon + Prop evergreen	7	38.39	3.68	0.05	0.442	0.555		NA	-0.147	NA	0.085
FDis + Prop evergreen	7	39.48	4.77	0.03	0.426	0.543		NA	NA	-0.078	0.119

Total earthworm biomass - Romania

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	6	72.32	0	0.52	0.29	0.403	0.84	NA	NA	NA	-1.092
Richness + Prop evergreen	7	74.7	2.38	0.16	0.33	0.436		-0.401	NA	NA	-1.012
FDis + Prop evergreen	7	76.09	3.78	0.08	0.29	0.403		NA	NA	-0.141	-1.135
True Shannon + Prop evergreen	7	76.12	3.8	0.08	0.289	0.402		NA	-0.081	NA	-1.092
Null model	5	76.23	3.92	0.07	0.062	0.211		NA	NA	NA	NA
Richness	6	76.78	4.46	0.06	0.161	0.294	0.22	-0.570	NA	NA	NA
FDis	6	79.5	7.18	0.01	0.067	0.215	0.09	NA	NA	0.126	NA
True Shannon	6	79.56	7.24	0.01	0.065	0.214	0.09	NA	-0.174	NA	NA

Total earthworm biomass - Spain

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Richness	6	70.98	0	0.4	0.204	0.204	0.48	0.556	NA	NA	NA
True Shannon	6	72.52	1.54	0.19	0.162	0.162	0.23	NA	0.464	NA	NA
Null model	5	72.62	1.65	0.18	0.066	0.066		NA	NA	NA	NA
Richness + Prop evergreen	7	74.26	3.28	0.08	0.208	0.208		0.543	NA	NA	0.096
FDis	6	74.97	3.99	0.05	0.09	0.09	0.06	NA	NA	0.222	NA
Prop evergreen	6	75.35	4.38	0.05	0.079	0.079	0.18	NA	NA	NA	0.171
True Shannon + Prop evergreen	7	75.69	4.71	0.04	0.169	0.169		NA	0.452	NA	0.129
FDis + Prop evergreen	7	77.79	6.82	0.01	0.109	0.109		NA	NA	0.248	0.206

Table S2. Results of model selection for the analyses of earthworm species richness in relation to tree diversity and proportion of evergreen leaf litterfall on neighbourhood level. Models are ordered by best model using Akaike's information criterion, corrected for small sample sizes (AICc). Sum (WH) of the Akaike weights (Wi) and the likelihood ratio R^2 of the model without (R^2fixed) and with random effect (R^2all) are also given. The right part of the tables represent the parameter estimates of Tree richness, True Shannon, Functional dispersion (FDis) and Proportion of evergreen litter.

Earthworm species richness - Continental scale

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	8	540.28	0	0.41	0.06	0.483	0.95	NA	NA	NA	-0.383
Richness + Prop evergreen	9	541.85	1.58	0.19	0.064	0.485		0.089	NA	NA	-0.388
True Shannon + Prop evergreen	9	541.94	1.66	0.18	0.063	0.484		NA	0.082	NA	-0.382
FDis + Prop evergreen	9	542.03	1.76	0.17	0.063	0.484		NA	NA	0.081	-0.388
Null model	7	546.22	5.94	0.02	0.018	0.459		NA	NA	NA	NA
True Shannon	8	547.63	7.36	0.01	0.022	0.462	0.19	NA	0.097	NA	NA
Richness	8	547.81	7.54	0.01	0.021	0.461	0.2	0.086	NA	NA	NA
FDis	8	548.02	7.74	0.01	0.02	0.461	0.18	NA	NA	0.073	NA

Earthworm species richness - Finland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Prop evergreen	5	67.4	0	0.35	0.377	0.45	0.64	NA	NA	NA	-0.738
Null model	4	68.44	1.05	0.21	0.281	0.364		NA	NA	NA	NA
FDis + Prop evergreen	6	69.54	2.14	0.12	0.402	0.471		NA	NA	0.554	-0.924
True Shannon + Prop evergreen	6	70.17	2.77	0.09	0.388	0.459		NA	0.317	NA	-0.793
Richness + Prop evergreen	6	70.28	2.89	0.08	0.386	0.457		0.268	NA	NA	-0.765
Richness	5	71.21	3.82	0.05	0.286	0.369	0.13	0.207	NA	NA	NA
True Shannon	5	71.24	3.84	0.05	0.286	0.369	0.14	NA	0.194	NA	NA
FDis	5	71.32	3.93	0.05	0.284	0.367	0.17	NA	NA	0.157	NA

Earthworm species richness - Germany

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
True Shannon	6	114.91	0	0.42	0.219	0.219	0.57	NA	0.931	NA	NA
True Shannon + Prop evergreen	7	116.97	2.06	0.15	0.247	0.247		NA	1.034	NA	0.439
Null model	5	117.15	2.25	0.14	0.084	0.084		NA	NA	NA	NA
Richness	6	117.28	2.38	0.13	0.161	0.161	0.17	0.702	NA	NA	NA
FDis	6	118.34	3.43	0.08	0.134	0.134	0.1	NA	NA	0.562	NA
Richness + Prop evergreen	7	119.71	4.81	0.04	0.181	0.181		0.790	NA	NA	0.379
Prop evergreen	6	119.96	5.06	0.03	0.09	0.09	0.24	NA	NA	NA	0.190
FDis + Prop evergreen	7	121.25	6.34	0.02	0.142	0.142		NA	NA	0.583	0.242

Earthworm species richness - Italy

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	70.13	0	0.39	0.054	0.054		NA	NA	NA	NA
Prop evergreen	5	71.47	1.34	0.2	0.116	0.116	0.33	NA	NA	NA	-0.681
Richness	5	72.84	2.71	0.1	0.068	0.068	0.15	0.247	NA	NA	NA
FDis	5	73.07	2.93	0.09	0.06	0.06	0.13	NA	NA	0.159	NA
True Shannon	5	73.14	3.01	0.09	0.057	0.057	0.13	NA	0.122	NA	NA
Richness + Prop evergreen	6	74.32	4.19	0.05	0.135	0.135		0.301	NA	NA	-0.738
True Shannon + Prop evergreen	6	74.65	4.52	0.04	0.124	0.124		NA	0.215	NA	-0.740
FDis + Prop evergreen	6	74.73	4.6	0.04	0.121	0.121		NA	NA	0.157	-0.696

Earthworm species richness - Poland

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	135.35	0	0.4	0.14	0.14		NA	NA	NA	NA
Richness	5	137.47	2.12	0.14	0.151	0.151	0.18	-0.143	NA	NA	NA
True Shannon	5	137.76	2.4	0.12	0.145	0.145	0.15	NA	-0.097	NA	NA
Prop evergreen	5	137.78	2.42	0.12	0.145	0.145	0.22	NA	NA	NA	-0.119
FDis	5	137.83	2.47	0.12	0.144	0.144	0.15	NA	NA	-0.080	NA
Richness + Prop evergreen	6	140.19	4.83	0.04	0.153	0.153		-0.127	NA	NA	-0.070
True Shannon + Prop evergreen	6	140.36	5	0.03	0.149	0.149		NA	-0.092	NA	-0.112
FDis + Prop evergreen	6	140.47	5.12	0.03	0.147	0.147		NA	NA	-0.061	-0.100

Earthworm species richness - Romania

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	74.59	0	0.42	0.01	0.01		NA	NA	NA	NA
Prop evergreen	5	76.46	1.86	0.16	0.056	0.056	0.26	NA	NA	NA	-0.435
FDis	5	77.1	2.51	0.12	0.032	0.032	0.15	NA	NA	0.288	NA
True Shannon	5	77.26	2.67	0.11	0.026	0.026	0.15	NA	0.254	NA	NA
Richness	5	77.68	3.09	0.09	0.011	0.011	0.12	-0.033	NA	NA	NA
True Shannon + Prop evergreen	6	79.49	4.9	0.04	0.07	0.07		NA	0.244	NA	-0.434
FDis + Prop evergreen	6	79.65	5.06	0.03	0.064	0.064		NA	NA	0.182	-0.385
Richness + Prop evergreen	6	79.87	5.28	0.03	0.056	0.056		0.020	NA	NA	-0.438

Earthworm species richness - Spain

Model	K	AICc	Δi	Wi	R^2fixed	R^2all	W_H	Richness	True Shannon	FDis	Prop evergreen
Null model	4	71.8	0	0.47	0.022	0.022		NA	NA	NA	NA
FDis	5	74.53	2.72	0.12	0.028	0.028	0.15	NA	NA	-0.188	NA
Prop evergreen	5	74.6	2.79	0.12	0.025	0.025	0.19	NA	NA	NA	0.138
True Shannon	5	74.62	2.82	0.11	0.025	0.025	0.13	NA	0.126	NA	NA
Richness	5	74.66	2.86	0.11	0.023	0.023	0.13	0.091	NA	NA	NA
FDis + Prop evergreen	6	77.59	5.79	0.03	0.031	0.031		NA	NA	-0.182	0.123
True Shannon + Prop evergreen	6	77.68	5.88	0.02	0.028	0.028		NA	0.112	NA	0.127
Richness + Prop evergreen	6	77.72	5.92	0.02	0.026	0.026		0.075	NA	NA	0.130

Table S3. Simplified reproduction of the model parameter estimates demonstrating the effect of litter quality traits on total earthworm biomass at neighbourhood level. The arrows represent the direction of the averaged parameter estimates of the best candidate models. Parameters that were retained in the best candidate models are represented with a black arrow. Parameters that were not included in the analysis due to multicollinearity issues, but that showed high correlations with variables that were retained in the best candidate models have grey arrows. Parameters that were included in the model, but not retained in the best candidate models are indicated with a hyphen. Parameters that were highly correlated with parameters that were not retained in the best candidate models are left blank.

Model parameters	CONTINENTAL	FINLAND	GERMANY	ITALY	POLAND	ROMANIA	SPAIN
C:N ratio	-		-	-	-		↗
C:P ratio	↘	↘	-			↗	-
Calcium	-	↗	-	-	-	-	↘
Lignin	-	↗	-		-	↘	↗
Water holding capacity	↗	-		-		↗	-

↗ : worm biomass increases with increasing predictor value

↘ : worm biomass decreases with increasing predictor value

Table S4. Simplified reproduction of the model parameter estimates demonstrating the effect of litter quality traits on earthworm species richness at neighbourhood level. (cf. S3 for details)

Model parameters	CONTINENTAL	FINLAND	GERMANY	ITALY	POLAND	ROMANIA	SPAIN
C:N ratio	↘	↘	↘	-	-		
C:P ratio	↘		-			-	-
Calcium	↗	↗	-	-	-	-	-
Lignin	↘	↗	↘		-	-	
Water holding capacity	↗	↘	↘	-			-

↗ : worm species richness increases with increasing predictor value

↘ : worm species richness decreases with increasing predictor value

Appendix C. Supplementary material to Chapter 4

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Appendix C1. Additional figures

Fig. S1. Map of Europe with the locations of all six regions.

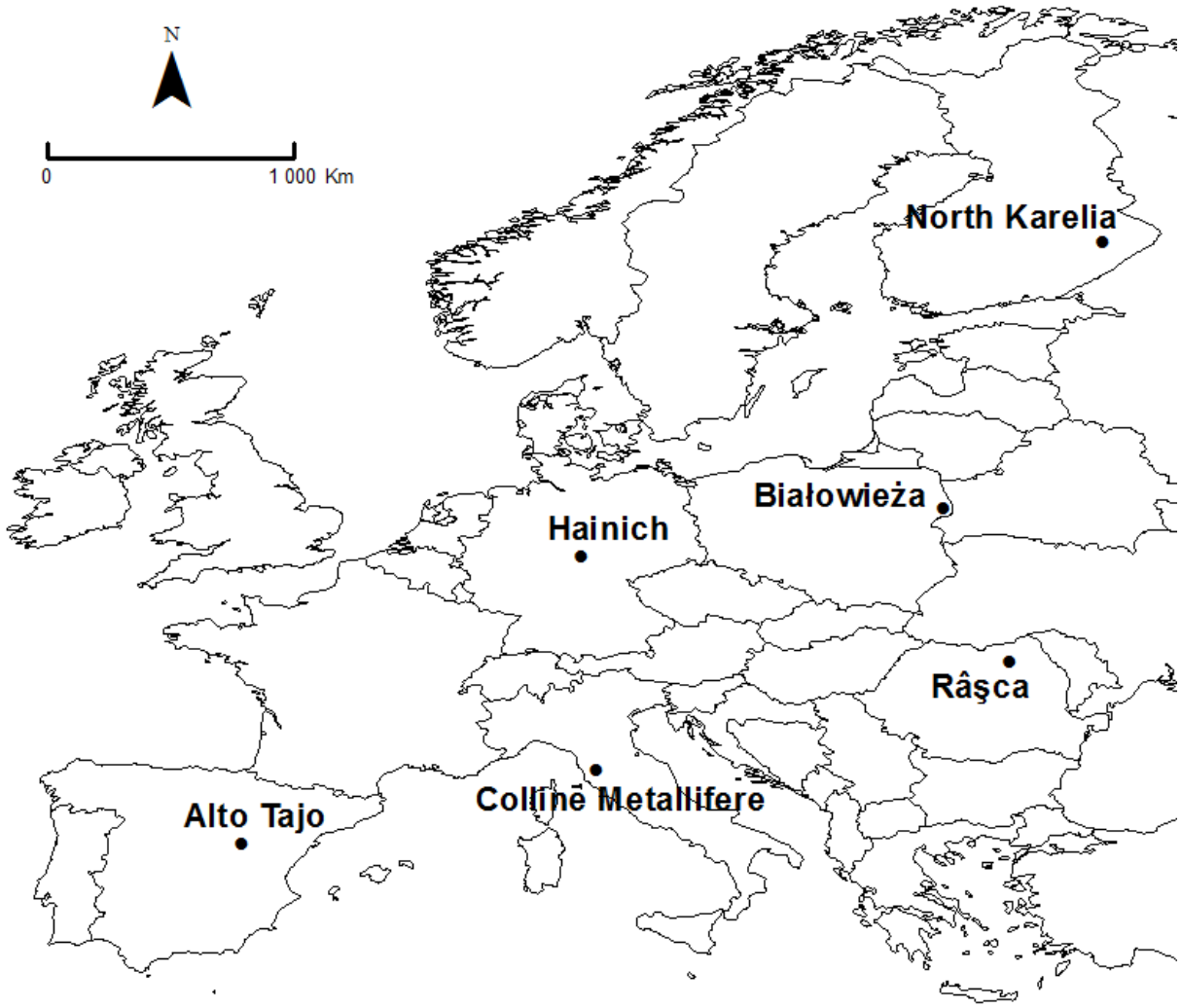
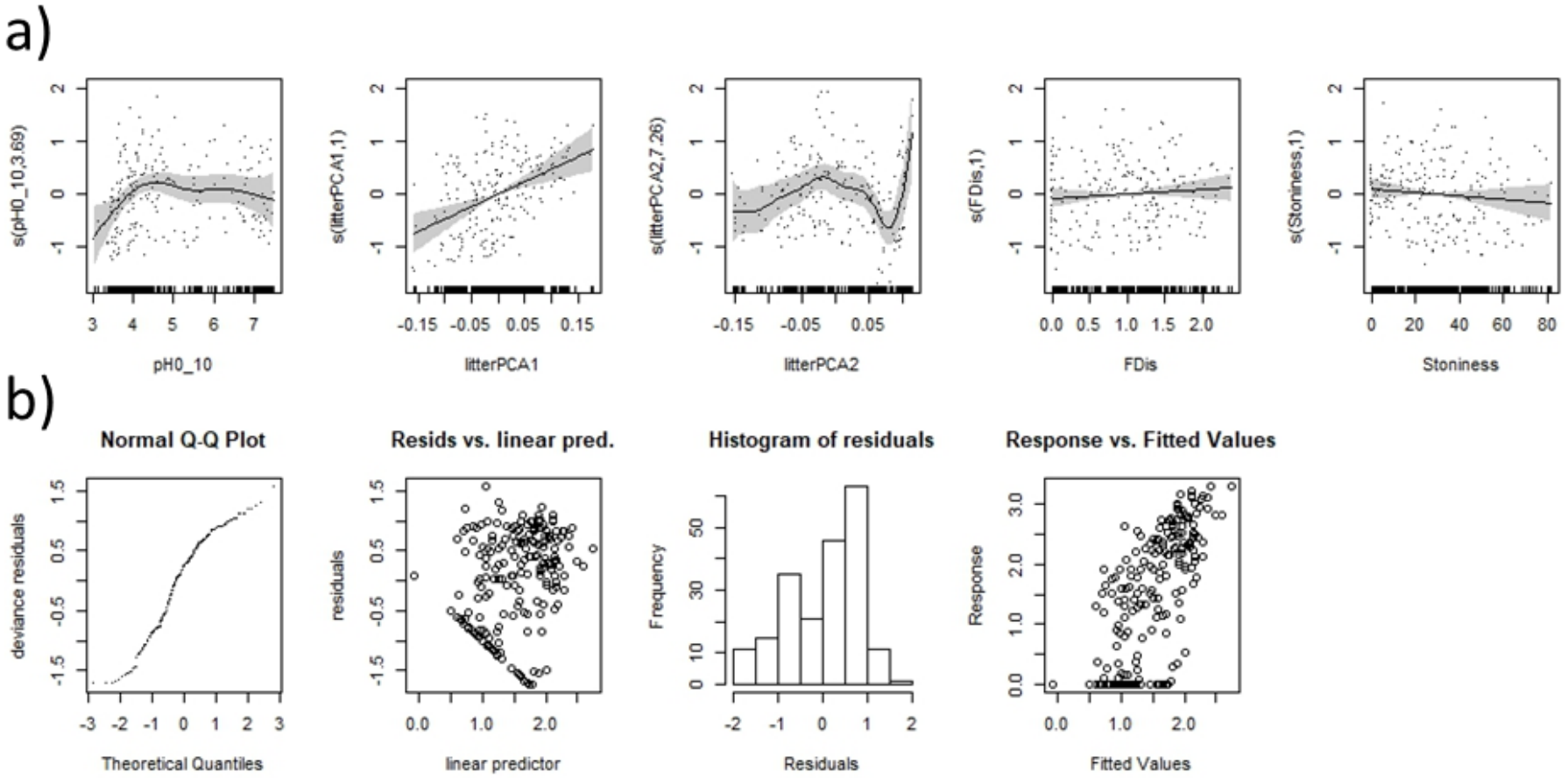


Fig. S2. Generalised additive mixed modelling result of five variables explaining earthworm total biomass: a) Smoothing curves of the five variables explaining earthworm total biomass. This graph shows the relationship between earthworm total biomass (Log transformed) and each predictor variable, taking into account the influence of the other predictor variables in the model. More details on the model see M&M. The shaded parts are the 95% point-wise confidence bands and the little vertical lines along the x-axis indicate the pH values of the observations. b) Validation tools for the model GAMM model. The QQ-plot and the histogram are used to assess normality, and the residuals versus fitted values homogeneity. The response versus fitted values should ideally show a straight line.



Appendix C2. Additional tables

Table S1. Correlation matrix of leaf litter variables with the first two PCA axes. The darker the cell in the table (red=negative, blue=positive) the larger the correlation.

	litter PCA1	litter PCA2
C	-0.85	0.08
N	0.82	0.01
C:N	-0.82	0.00
P	0.38	-0.25
C:P	-0.41	-0.25
Lignin	-0.03	0.92
Cellulose	-0.75	0.29
HCellulose	-0.16	-0.56
wsc	0.41	-0.80
Tannins	-0.20	-0.56
Soluble.Phenolics	0.05	-0.94
Total.Phenolics	-0.05	-0.95
Ca	0.78	0.27
Mg	0.78	0.05
K	0.89	-0.08
pH	0.63	0.55
WHC	0.79	-0.13
SLA	0.86	-0.02

Table S2. Correlation matrix of humus variables with the first PCoA axis. The darker the cell in the table (red=negative, blue=positive) the larger the correlation.

	HumusPCoA1	HumusPCoA2
Humus form	NA	NA
OH horizon	-0.86	-0.14
A horizon	-0.77	0.44
Forest floor C:N	0.24	-0.76
Forest floor mass	-0.53	0.24
Forest floor thickness	-0.55	0.48

Table S3. Overview of all the model variables

Variable name	description	Unit	Used for
Site	Name of the sampled site (Finland, Poland, Germany, Romania, Italy, Spain)		
Worm biomass	Earthworm total biomass	g/m²	
Clay	Clay fraction	classes (1,2,3)	
Soil volume	Stoniness of the soil	%	
LAI	Leaf area index value (= leaf area/ground area)	m2/m2	
Heat Load Index	Standardised Heat Load Index (combination of: aspect, slope and latitude)		
Humus form	Humus form according to the European Humus form reference base 2011 (Zanella et al. 2011)		Humus PCoA
A horizon	A horizon type (1= A biomacro and biomeso; 2= predominantly A biomicro/non-zoogenic; 3= predominantly A non-Zoogenic)	Ordinal	Humus PCoA
OH horizon	Presence/absence of OH horizon (1 = present, 0 = absent)	Binary	Humus PCoA
FF mass	Dry mass of ectorganic layer, composite sample of 9 (25 x 25 cm) subplots	kg/m²	Humus PCoA
FF thickness	Thickness of the ectorganic layer	cm	Humus PCoA
FF CN	C:N ratio of the forest floor		Humus PCoA
CN 0_10	C:N ratio of mineral soil at 0-10 cm		
pH 0_10	pH of mineral soil at 0-10 cm ($CaCl_2$)		
C	CWM C of the tree leaf litter (weighed by the BA of the focal tree species on plot level)	%	Tree litter PCA
N	CWM N of the tree leaf litter	%	Tree litter PCA
C:N	CWM C:N ratio of the tree leaf litter		Tree litter PCA
P	CWM P of the tree leaf litter	%	Tree litter PCA
C:P	CWM C:P ratio of the tree leaf litter		Tree litter PCA
Lignin	CWM lignin of the tree leaf litter	%	Tree litter PCA
Cellulose	CWM cellulose of the tree leaf litter	%	Tree litter PCA
HCellulose	CWM hemicellulose of the tree leaf litter	%	Tree litter PCA
Wsc	CWM water soluble compounds of the tree leaf litter	%	Tree litter PCA
Tannins	CWM tannins of the tree leaf litter	%	Tree litter PCA
Soluble.Phenolics	CWM soluble phenols of the tree leaf litter	%	Tree litter PCA
Polyphenols	CWM total phenolics of the tree leaf litter	%	Tree litter PCA

Variable name	description	Unit	Used for
Ca	CWM calcium of the tree leaf litter	mg/g	Tree litter PCA
Mg	CWM Mg of the tree leaf litter	mg/g	Tree litter PCA
K	CWM K of the tree leaf litter	mg/g	Tree litter PCA
pH	CWM pH of the tree leaf litter		Tree litter PCA
WHC	CWM water holding capacity of the tree leaf litter		Tree litter PCA
SLA	CWM specific leaf area of the tree litter	$cm^2\ g^{-1}$	Tree litter PCA
FDis	Functional dispersion of focal tree species (based on five relevant tree litter quality traits (C:N, C:P, calcium, lignin and litter water holding capacity) of the tree species and the BA of the focal tree species on plot level)		

Appendix C3. Data collection and derivation of model explanatory variables

Forest floor mass, thickness, biomass and C:N ratio

In order to determine the dry mass of the ectorganic layer per plot, each plot was subdivided in nine subplots of 10 m x 10 m. In every subplot, the forest floor thickness was measured (cm) and the forest floor was sampled from 25 x 25 cm plots using a wooden frame. The forest floor was removed by hand and/or knife, after all living plant parts within the frame were clipped off. The nine replicate samples serve to represent the soil conditions across the core plot. Weight determination and analyses of C and N were done on these composite samples. After drying at 55 °C to constant weight, the forest floor was ground with Heavy-duty Model SM 2000-Retsch cutting mill (Retsch, Germany). Subsamples of this coarsely ground fraction were further ground into finer particles with Planetary Ball Mill PM 400-Retsch for six minutes at 280rpm (Retsch, Germany). The C and N concentrations of the forest floor were determined with a Thermo Scientific FLASH 2000 soil CN analyser, based on the dry combustion method (Matejovic 1993).

Determination of leaf litter C:N ratio and calcium concentration

Tree leaf litter was collected from autumn 2012 till autumn 2013, using five litter traps that were placed systematically on all 209 plots: one in the central subplot, and one in each of the four subplots in the cardinal directions or along and perpendicular to the slope. Each litter trap had an opening area of 0.5 m^2. The litter from the traps was air dried or dried in an oven at 38 °C and pooled per plot.

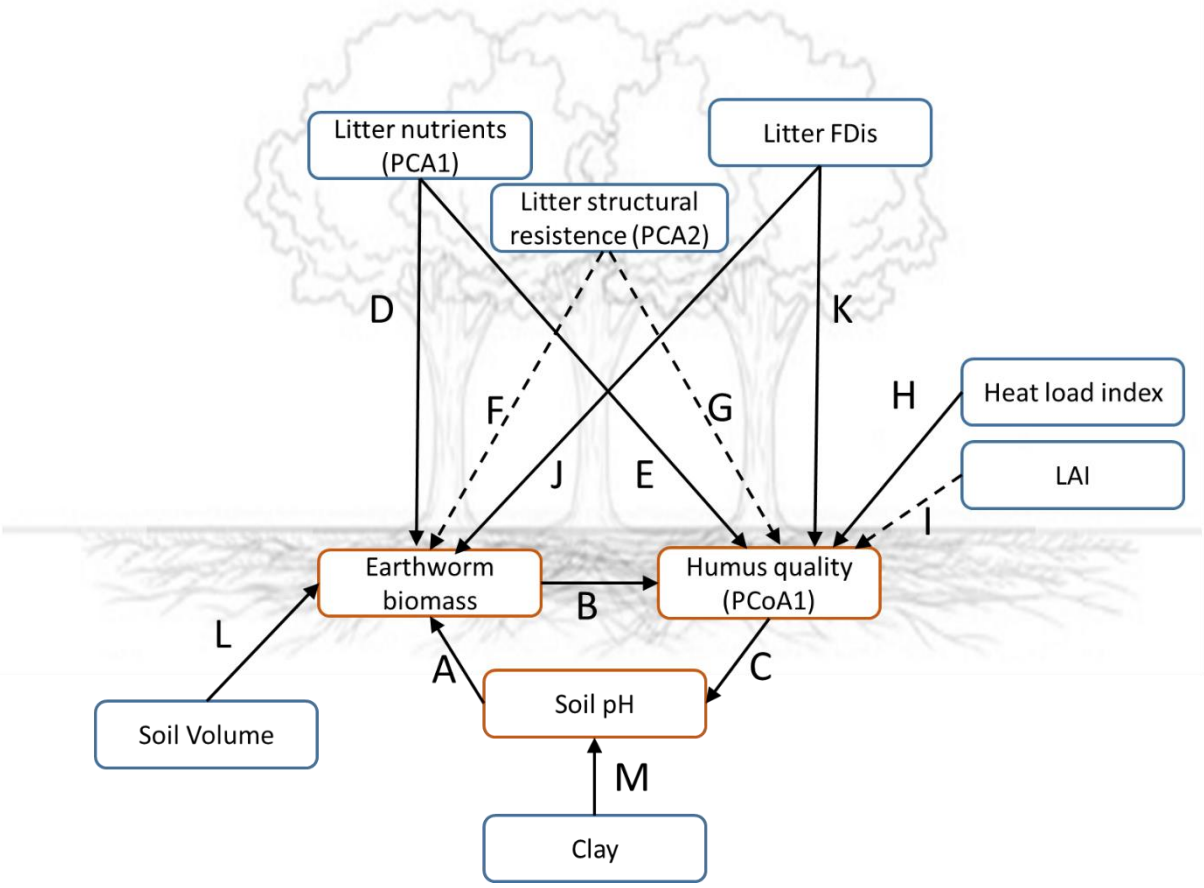
Litter samples were analysed by Near Infra-Red Spectroscopy (NIRS), as described by Niederberger et al. (2015). In order to calibrate the NIRS spectra and validate the calibration for the determination of (a) Ca concentration, a subset of samples were mineralized and analysed with an atom absorption spectrometer (AAS, iCE 3000 series, ThermoScientific, China), (b) N concentration, a subset of samples were analysed with a flash CHN Elemental Analyser (Flash EA1112 Series, ThermoFinnigan, Milan, Italy). The procedure of spectra calibration and NIRS application is described by Pollastrini et al. (2016).

Microclimate

Heat load index, as a proxy for local climate variation, was calculated conform McCune and Keon (2002) where plot specific values for latitude, slope and aspect were used to feed and calculate 'equation 3': Heat load index = 0.339 + 0.808*COS(latitude)*COS(slope) - 0.196*SIN(latitude)*SIN(slope) - 0.482*COS(aspect)*SIN(slope). To prevent correlation with latitude, these heat load index values were relativised by site maximum. The heat load index reflects the heat

load that a particular location gets because of annual direct incidence radiation, the higher the value the larger the heat load and the warmer that location will be.
Leaf Area Index (total one-side area of leaf tissue per unit ground surface area, m^2m^{-2}, Watson, 1947) was measured in each plot (five measurements) early in the morning (shortly after sunrise) or late in the evening (shortly before sunset) in order to work in the presence of diffuse solar radiation. LAI measurements were carried out in summer 2012 (in Italy, Germany and Finland) and in 2013 (in Spain, Romania and Poland), using a Plant Canopy Analyser LAI-2000 (LI-Cor Inc., Nebraska, USA). With the LAI-2000, the incident light above the canopy and the light transmission below the canopy were measured using one sensor with five fisheye light sensors (lenses), with central zenith angle of 7°, 23°, 38°, 53° and 68° (LAI-2000 manual, LI-Cor (1991)). The protocol used in each plot consisted of five measurements within the plots (light transmission below the canopy), and five measurements outside the forest (as proxy of the light incidence above the canopy), in an open space that was in close proximity to the sampled plots. LAI data was processed using LI-Cor's FV2200 software (LI-COR Biogeosciences, Inc. 2010). The light transmittance measurements of the fifth ring were removed to minimise the boundary effects on LAI. The LAI value per plot was the mean value of the five measurements for each plot.

Appendix C4. Detailed description of the SEM model and justification for the included paths.



To start off with/we created a conceptual meta-model (Fig. 4.3) where we hypothesise that there exist a feedback loop between earthworms, humus form and soil pH: Earthworms contribute to a changing humus form (decreasing forest floor mass, well-structured A-horizon) that in turn improves pH of the topsoil which then allows a higher earthworm biomass.

We filled this meta-model with the variables measured in the field, to end up with an a priori causal model. The arrows in these diagram represent a hypothesized direct causal effect of the variable at the end of the arrow on the variable at the tip of the arrow. Black arrows indicate positive relationships, red arrows indicate negative relationships.

In what follows we describe the rationale behind every path.

A) **Soil pH** is expected to influence **earthworm biomass** directly since high exchangeable Al_{3+} concentrations and low pH values inhibit earthworm growth and cocoon production (van Gestel and Hoogerwerf 2001). On the other hand, soil pH also influence earthworm biomass indirectly, as an increased soil pH is

observed to harbour a higher earthworm richness (Curry 2004, Ammer et al. 2006, Cesarz et al. 2007, Mueller et al. 2016). Endogeic and anecic species are not able to survive in acidic soils because (i) of toxic Al^{3+} concentrations (van Gestel and Hoogerwerf 2001) and (ii) a low soil pH prevents microbial decomposition and decreases nutrient availability in the soil (Ott et al. 2014).

B) **Earthworms** contribute to a changing **humus form**. Earthworms are predicted to decrease the forest floor mass as they feed on fresh litter or partly decomposed organic material. In addition, they mix the topsoil layer by their burrowing activity and consequently contribute to a well-structured A-horizon (Zanella et al. 2011). More earthworm biomass is therefore predicted to increase humus quality. A lower earthworm biomass is predicted to decrease humus quality; Forest floor build-up, due to retarded leaf litter decomposition, acidifies the forest floor (De Schrijver et al. 2012) which results in the presence of an OH horizon and transition from a biomacrostructured A horizon to a non-zoogenic A horizon.

C) Finally, the change in **humus form** is expected to influence the **soil pH**. Changes towards a biologically more active humus form will lead to higher pH of the mineral soil (Haimi and Huhta 1990, Räty 2004, Zanella et al. 2011).

D) We expect that **tree leaf litter nutrient concentration** positively stimulates **earthworm biomass** (Hendriksen 1990, Reich et al. 2005, Milcu et al. 2008, Rajapaksha et al. 2013, Cesarz et al. 2016).

E) Independent from earthworm activity, high **leaf litter nutrient concentration** is also expected to increase leaf litter decomposition (Hättenschwiler 2005) , which results in a decreasing mass of the ectorganic layer and consequently an increase in **humus quality**.

F) **Leaf litter structural resistance** on the other hand is expected to have a negative influence on **earthworm biomass**, since recalcitrant litter is disliked (Hendriksen 1990), hard to digest by earthworms (Ponge 2013) and proven to have a negative impact on their growth (Kasurinen et al. 2007).

G) As **lignin-rich** litter is hard to digest by most soil organisms (Ponge 2013) it is expected to decompose slowly, which may cause forest floor build-up and a decrease in **humus quality**.

Both LAI and relative heat load index are microclimate predictors and can be a proxy for soil temperature. Warm understory air and topsoil potentially increase the energy availability for decomposers, stimulating litter decomposition (Hobbie et al. 2006).

H) We expect that a higher **relative heat load index** leads to an increasing litter decomposition and consequently an increase in **humus quality**.

I) Also a lower **LAI**, so a higher light availability in the understory, is expected to increase the **humus quality** by stimulating litter decomposition. In addition, the light availability on the forest floor may also influence the amount and chemical composition of understory plant-derived inputs to the forest floor (Thomaes et al. 2013).

J) We hypothesise that **tree species functional dispersion** (to what extend their litter characteristics differ) influences **earthworm biomass** since it increases the diversity of litter types serving different ecological functions in terms of food and habitat availability for earthworm species (De Wandeler et al. under review).

K) But the **functional dispersion index** of tree species litter is also expected to influence the **humus quality** independent from earthworms as an increasing leaf litter functional diversity also increases the diversity in food types and habitat availability of other litter dwellers and decomposers (Hättenschwiler et al. 2005).

L) An increasing **soil volume** is expected to increase the **earthworm biomass** as it increases the habitat availability of endogeic and anecic earthworm species.

M) A higher **clay** content in the mineral soil increases its CEC and subsequently its potential towards an increasing base saturation and higher **soil pH.**

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