The reassembly of soil food web structure and functioning in restored urban forests across Aotearoa - New Zealand

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With special dedication

to

my mother, late Narayani Devi Rai

and

my father, Late Hem Kumar Rai

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Abstract

Soil food webs play a central role in the successful restoration of critical ecosystem processes. Yet, we lack an understanding of how these highly diverse and functionally complex belowground communities reassemble in forests undergoing restoration, and the factors responsible for varying trajectories of soil community reassembly. Even more poorly understood is the reassembly of soil invertebrates across multiple trophic levels after disturbance. Moreover, any potential effect of ecological succession on food web structural properties (connectance and maximum trophic level) and its implications on soil invertebrate trophic functions remains poorly understood. In this PhD thesis, I investigate the effect of restoration age and biotic and abiotic characteristics of restored forests on the reassembly of soil invertebrate communities across different trophic groups. I also explore the effect of the aforementioned predictors on soil food web structure and associated ecosystem functioning.

I sampled soil invertebrate communities from 70 urban restored forests, as well as three unrestored and three remnant urban forests. All forest sites were distributed across eight cities in Aotearoa, New Zealand and were historically occupied by native vegetation until cleared for either agriculture or urban development. The sites were then planted with native woody species between six and 60 years prior to this research, forming a chronosequence of forest succession. In addition to soil invertebrates, vegetation data (e.g., mean tree diameter, tree species richness) and site characteristics (e.g., litter depth, soil temperature) were also collected from these forests. I identified the soil invertebrates into major size-based functional groups (i.e., microfauna, mesofauna and macrofauna) and further into trophic levels: detritivores, fungivores, herbivores, omnivores and predators. Additionally, I quantified the abundance, biomass and mean body mass of all soil invertebrates. Finally, I constructed 70 local food webs across the restored forest sites and quantified the flux of energy between different feeding groups and their food sources to estimate five important ecosystem functions in soil food webs: detritivory, bacterivory, fungivory, herbivory and predation.

I analysed the relative importance of restored forest age and environmental variables as predictors of variation in soil invertebrate communities. I found that restoration age, in itself, had no discernible effect on the abundance, biomass or mean body size of soil invertebrate communities. Instead, site characteristics like mean tree diameter, tree species richness, litter depth and soil temperature were the main drivers of variability in soil communities. Notably,

these factors influenced larger macrofauna and mesofauna more than nematodes. However, when I modelled the effects of restoration age on soil communities in a structural equation modelling framework, taking into account bottom-up effects from plant communities to invertebrate predators, restoration age was shown to have indirect effects on the biomass of mesofauna decomposers, omnivores and nematode predators. Finally, by investigating shifts in food web structure and resulting changes in energy fluxes across trophic levels, I found that forest restoration age did not directly influence either the structure (connectance, maximum trophic level) or the five examined trophic functions of the soil food webs. Instead, effects of forest restoration age occurred through changes in vegetation characteristics as forest succession progresses.

My results suggests that forest age is not a direct major driver of the reassembly of soil invertebrate communities. Instead, mean tree diameter, tree species richness, litter depth, and soil temperature play important roles in the process. My results show the significance of planting trees that can grow large, promoting species diversity and allowing leaf litter to accumulate naturally to support soil invertebrate community of different trophic functions. Through my results, I also show how the aforementioned vegetation characteristics can contribute to flow of energy in the soil food web. In addition to demonstrating how crucial food web structural properties are to energy flow via trophic interactions such as detritivory, fungivory and predation, this research also highlights the interconnectedness of above and belowground ecosystems. Taken together, my findings suggest that urban restoration efforts should focus on promoting these beneficial vegetation characteristics, alongside considering food web complexity, to optimize trophic functions performed by soil food webs.

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Chapter 1 . Introduction

1.1 Research topic

Ecosystems have an inherent quality of resilience which allows them to absorb effects of disturbance up to a certain level without compromising on their functional properties, but beyond which, the effects of disturbance can become irreversible (Wiens & Hobbs 2015). Such disturbance can be both natural (e.g., volcanic eruptions) and anthropogenic (e.g. land-use) (Corlett 2016). Urbanization, one of the most prominent examples of land use change (Vitousek *et al.* 1997), has become a modern driver of environmental change in a relatively short amount of time (Lambin *et al.* 2001; Montgomery 2008; Leblois *et al.* 2017) and is one of the largest demographic events to occur with 55 % of the world population being urban dwellers and an additional 13 % expected to follow that trend by the middle of the century (United Nations 2018). Introduction of non-native species and the urban heat island effect are some examples of how urbanisation can affect functioning of ecosystems (Niemelä 1999). Furthermore, urbanization affects ecosystem processes through natural habitat destruction and fragmentation (Lambin *et al.* 2001). Such destruction of natural habitat and invasions by alien species have led to biodiversity loss and degraded ecosystems, along with the associated ecosystem services (e.g. regulation of air, water and soil quality) that they provide to humanity (Butchart *et al.* 2010).

Over the last four decades, there has been a major decrease in global forest cover (Aerts & Honnay 2011) and invertebrate populations (van Klink *et al.* 2020). Since biodiversity has the potential to support ecosystem functions and services of value to humans (Soliveres *et al.* 2016; van der Plas 2019), loss of species (at any trophic level) can seriously affect functioning of ecosystems by altering processes such as nutrient cycling and productivity (Cardinale *et al.* 2012). For example, consumption of endolithic lichens, one of the primary producers in the Negev desert, Israel, by snails, *Euchondrus albulus* and *Euchondrus desertorum* is an important agent for weathering of rocks and soil formation in the desert. Hence removal of these consumers from the desert will have major impacts on such ecosystem processes (Shachak *et al.* 1987). Species interactions, in particular, give rise to ecosystem processes (Barnes *et al.* 2018); interactions such as predation, herbivory, and competition can directly modify energy and material flow pathways (De Ruiter *et al.* 1995). In addition, such trophic interactions can also influence energy and material fluxes indirectly via influences on species abundances (Chapin III *et al.* 2000). The significance of trophic interactions on ecosystem processes can be illustrated by the effect of predation on bacteria by protozoan grazers for enhancing nitrogen cycling and availability to plants (Clarholm

1985; Chapin III *et al.* 2000). Since there is variability in how different species respond to biotic and abiotic factors in an ecosystem, species diversity supports ecosystem functioning and resilience of the system to external perturbations (McNaughton 1977; Tilman *et al.* 1997; Chapin III *et al.* 2000). For example, a study involving primary producers, herbivores, predators, detritivores, plant symbionts and bacterivores found increased diversity across multiple trophic levels better supported multiple ecosystem processes (e.g., litter decomposition, biomass production, pest control) in comparison to individual trophic groups (Soliveres *et al.* 2016). Hence, consideration of multi-trophic diversity and abundance is needed to fully understand the relationship between biodiversity and ecosystem functioning (Clarholm 1985; Soliveres *et al.* 2016).

Ecological restoration, in its simplest sense, is an attempt to return an ecosystem back to the way it was before the occurrence of disturbance (Clewell & Aronson 2006; Corlett 2016). The society for ecological restoration (SER) defines ecological restoration as ‘assisting the recovery of an ecosystem that has been degraded, damaged or destroyed’ (Society for Ecological Restoration International Science & Policy Working group 2004). It follows the concept of succession, a more or less ordered and predictable sequence where species and their interactions with each other recover over the course of time (Young *et al.* 2001). The practice of ecological restoration generally involves the process of attempting to restore the composition, structure and functioning of a degraded ecosystem to represent a historical reference system (Corlett 2016). Hence, the ultimate objective of ecological restoration should be to reinstate a stable, resilient ecosystem (Clewell & Aronson 2006; Barabás et al. 2017) where the structure of trophic interactions can quickly return to pre-disturbance conditions in the event of any perturbation (Cairns 2004). Therefore, to restore resilient ecosystems, entire communities and interactions among constituent species needs to be restored (Palmer *et al.* 1997), since such interactions determine the flux of energy to living organisms, which underlies ecosystem processes such as nutrient cycling and productivity (Barnes *et al.* 2018). Moreover, the distribution and strength of trophic interactions influences ecological stability (De Ruiter *et al.* 1995; Neutel *et al.* 2002), as the balance between top-down and bottom-up effects on consumers governs the resilience of food webs to perturbations (Schwarz *et al.* 2017). Soil food webs that have been degraded by multiple stressors, often especially prevalent in cities (e.g., warming, drought, land-use intensification), suffer changes in energetic structure that can lead to losses in ecosystem functioning (Schwarz *et al.* 2017). Ecological restoration, however, may facilitate the recovery of the energetic structure of soil food webs in restored forest as different trophic groups respond to associated changes such as shifting organic inputs from the new plant community (Kardol *et al.* 2005; Kardol & Wardle 2010; Martucci do Couto *et al.* 2016; Yan *et al.* 2018). This should then alter the relative top-down and bottom-up forces that act on consumers in soil food webs (Hunter & Price 1992; Ponsard *et al.* 2000), which modulate food web stability and ecosystem process rates such as nutrient uptake and decomposition (Rosemond *et al.* 2001; Moore *et al.* 2004).

Since the late 20th century, restoration ecology has generated increasing scientific interest (Young 2000). Yet, little is known about how planted forest ecosystems reassemble in urban areas over decadal time scales, or about the co-determining biotic and abiotic factors of urban forest restoration (Wallace *et al.* 2017). Further, natural spaces in urban areas are subject to chronic disturbance, resulting in ecosystems that are at different stages of ecological succession (e.g., from freshly mown lawns to mature forest in a park) (Niemelä 1999). Such constant disturbance means that more is required than only planting activities to achieve restoration objectives in urban areas (Morimoto 2011). Moreover, little is known about the succession of soil fauna assemblages in relation to aboveground restoration (Perring *et al.* 2015; Morriën *et al.* 2017; Nielsen 2019) as most previous studies have been limited to aboveground plant communities (Kardol & Wardle 2010; Adl *et al.* 2011; Corlett 2016). Hence, to address the unexplored link between forest soil food webs and restoration of vegetation, I investigated aspects such as community reassembly, effects of restoration age across trophic levels, fluxes of energy in soil food webs and associated ecosystem functions in relation to forest succession.

1.2 Soil community reassembly during succession

Changes in plant community composition and diversity along successional gradients can alter soil chemical and physical properties (Maharning *et al.* 2009), root exudates, litter (Wardle *et al.* 2004b) and canopy cover (Koivula *et al.* 2002), consequently influencing soil invertebrate diversity (Siemann *et al.* 1998). Studies have found functional shifts in invertebrate communities that track changes in resource availability during succession. For example, a grassland experiment showed that the number of species planted had a significant impact on richness of herbivore, predator and parasite invertebrate species (Siemann *et al.* 1998). In another study, the quality and quantity of litter and humus layer dependent on the vegetation were found to influence diversity of diplopods, isopods and oribatid mite communities

(Scheu & Schulz 1996). In a similar study looking at carabid assemblages along a successional gradient, Koivula *et al.* (2002) found that canopy cover played a significant role in the distribution of carabid beetles in forests of different ages. Further evidence suggests that older ecosystems also tend to support higher fungal biomass (Klein *et al.* 1995; Bokhorst *et al.* 2017) as compared to bacterial biomass, which has been shown to remain fairly similar irrespective of forest age (i.e. the successional status) (Williamson *et al.* 2005; Van Der Wal *et al.* 2006; Maharning *et al.* 2009). This is likely due to declining soil fertility with decreased soil organic matter, nitrogen and phosphorous concentrations towards later stages of succession, which favours fungi since they are generally better suited to nutrient-limited conditions than most bacteria (Ingham *et al.* 1989; Ingham & Thies 1996; Ohtonen *et al.* 1999; Bokhorst *et al.* 2017). In another study, soil fauna assemblages changed from dominance of live root feeders to those who survive on decaying root tissue (nematodes and springtails) as there was succession from young healthy roots to later root senescence, disease and death (Wallwork 1976). These examples, all support the idea that forest succession does not only result in changes in species presence, but also changes in their relative abundances.

While there are few empirical studies that investigate the speed and trajectory of soil community reassembly in active forest restoration sites, some evidence suggests that aboveground restoration efforts may indeed yield benefits for belowground biodiversity. A restoration study in Western Australia found that the species richness of ants, springtails, mites and termites were proportional to the richness and diversity of plant species, suggesting plantations of diverse large woody trees will positively influence soil invertebrates (Majer *et al.* 2007). Furthermore, actively increasing habitat heterogeneity through manipulating tree species, planting density and species composition results in higher arthropod abundance, taxonomic richness and functional group richness compared to sites undergoing natural regeneration (Cole *et al.* 2016). Some of the practices carried out in an effort to recover aboveground vegetation, such as improving soil pH, C and nutrient concentrations can also assist native soil fauna recovery (Nielsen 2019). For example, active restoration (3-21 years) of native oak-dominated woodlands located in the Chicago metropolitan region was found to increase density (number captured per trap-per day) of epigeic (surface-active) arthropod communities to closely resemble densities found in the target reference sites (McCary *et al.* 2015). However, recovery of soil fauna also depends upon the ability of species to colonise and re-establish in a restoration site. For example, earthworms, isopods and diplopods were observed to colonise abandoned arable land faster than oribatid mites throughout secondary succession, likely owing to the differences in their mobility and phoretic behaviour (Scheu & Schulz 1996).

In addition to variability in vegetation, variation in abiotic site characteristics also play important roles in belowground community structure (Gibert 2019). Temperature is a significant factor affecting both life history (reproduction, development) and foraging traits of organisms (attack rates) (Amarasekare 2015). Temperature sensitive processes such as metabolic and physiological rates increase with temperature, consequently increasing the productivity of plants, microbes and arthropods (Gillooly *et al.* 2001; Ehnes *et al.* 2011). Soil respiration via microbial activity, an essential process for nutrient cycling and decomposition of dead organic matter, is also strongly influenced by soil temperature. For example, a comparative study in plots without any disturbance and plots with recultivated grass mixture sown after tilling found a notable increase in respiration rates (i.e., mineralization rate) each year in both treatments in response to temperature change (Mathes & Schriefer 1985). Temperature can have direct effects on physiology of organisms (growth and mortality), thereby affecting feeding interactions, resulting in changes in connectance and the number of trophic levels in food webs (Petchey *et al.* 2010; Brose *et al.* 2012). To test these effects, a microcosm study manipulated temperature and dryness and found they both increased rates of consumption i.e., an increase in top-down control of springtails by top predators (mites and centipedes) (Lang *et al.* 2014). Additionally, the study found increased litter decomposition by fungi at higher temperatures. This suggests that changes associated with succession such as canopy openness (Lu *et al.* 2014) and light availability (Matsuo *et al.* 2021), which have been found to influence surface temperature (Lebrija-Trejos *et al.* 2011), could have a notable influence on soil invertebrate food webs in forests undergoing restoration.

Soil moisture also plays a significant role in ecosystem processes such as decomposition and mineralisation (Swift *et al.* 1979), both of which influence the distribution, physiology and phonology of plant species (Bakkenes *et al.* 2002; Thuiller *et al.* 2005) and in turn affect the microbial community (Cornwell *et al.* 2008). In the field, low moisture in dry summers has been found to hinder soil organism activity causing a decrease in decomposition rates (Sulkava *et al.* 1996). Similarly, soil drying can reduce bacterial-feeding nematode abundance, which can be significantly increased again by rainfall (A'Bear *et al.* 2013). Precipitation has also been shown to support mesofauna densities and fungal feeders such as springtail and enchytraeid abundance in soil, though this can interact negatively with mean annual temperature (Blankinship *et al.* 2011). As precipitation and temperature have been widely shown to play a strong role in structuring soil food webs, this raises the question of the importance of such environmental factors for moderating the rate and trajectory of ecological succession in soil food webs undergoing restoration.

1.3 <u>Effects of forest restoration on ecosystem functions provided by soil food webs</u>

Given the importance of soil biodiversity for ecosystem functioning (Bardgett & Van Der Putten 2014), restoration should focus on restoring trophic interactions (Stanturf *et al.* 2014) in soil food webs to help support associated ecosystem functioning, such as organic matter decomposition and soil nutrient cycling (Palmer *et al.* 1997; Aerts & Honnay 2011). Microcosm studies of soil have revealed how trophic interactions between organisms in the food web contribute to essential ecosystem functions such as decomposition and nutrient mineralization, and consequently regulating plant productivity (Clarholm 1985; Ingham *et al.* 1985). For example, community evenness of arbuscular mycorrhizal fungi was found to have a significant positive effect on phosphorus-use efficiency in a series of replicated tropical forests (Lovelock & Ewel 2005). The important roles microbial soil communities play in organic matter processing, such as decomposition and plant nutrient uptake, are typically highly disturbed in degraded sites. Therefore, consideration of belowground biodiversity should be an essential part of any restoration process (Harris 2009).

Active restoration programs aim to facilitate or accelerate secondary succession (Young *et al.* 2005). There is often a gradual decrease in species richness during mid-secondary to late stage succession (Bonet & Pausas 2004; Amici *et al.* 2013). Such changes in vegetation characteristics leads to gradual increases in vegetation biomass (Brown & Lugo 1990; Wardle *et al.* 2004b; Chazdon *et al.* 2007), tree basal area, canopy cover (Lebrija-Trejos *et al.* 2010; Lu *et al.* 2014; Matsuo *et al.* 2021) and a steady decline in understorey light availability at later stages of secondary succession (Denslow & Guzman G. 2000; Matsuo *et al.* 2021). Since both aboveground and belowground components of an ecosystem interact with each other through trophic interactions, competitive, mutualistic and pathogenic interactions or via soil biogeochemical cycles (Van Der Putten *et al.* 2001; Wardle *et al.* 2004a; Kardol & Wardle 2010), vegetational changes during succession can strongly influence the structure of soil invertebrate biodiversity through changes in food and habitat resource availability (Scheu & Schulz 1996; Viketoft *et al.* 2009).

The effects of successional change on soil invertebrates can be both direct and indirect (Jackson *et al.* 2000; Wolters *et al.* 2000; Kardol & Wardle 2010). For example, the functional characteristics of the vegetation community and nutrient resources released by them can shift soil energy channels (i.e., transfer of energy between trophic levels within a food web) from bacterial dominated to fungal dominated with succession (Holtkamp *et al.* 2008; Bokhorst *et al.* 2017). Such a shift will then result in increases in fungal-feeding nematodes compared to bacterial-feeding nematodes (Holtkamp *et al.* 2008; Bokhorst *et al.* 2017) and finally cascade to invertebrates such as mites and springtails at higher trophic levels (Bokhorst *et al.* 2017). Similarly, Wardle *et al.* (1999) found that changes in vegetation had a direct effect on microbial community structure, which affected microbivorous nematodes and eventually cascaded to predacious nematodes. Indirect interactions have been observed in ecosystems from agricultural fields, soils and forests to lakes, streams and ocean as indirect interactions are not restricted by ecosystem type, diversity, habitat complexity, types of top predators or the trophic mode of consumers (Pace *et al.* 1999). Indirect effects can also occur through changing basal resources and habitat structure i.e., bottom-up cascades (Barnes *et al.* 2017). These effects can be demonstrated by both simple Lotka-Volterra models (including only primary producers, herbivores and predators) and more complex models (which also include detritus), which show that producers can affect consumers at higher trophic levels through their direct influence on primary consumers (Moore *et al.* 1993, 2003).

The indirect effect of vegetation on soil invertebrates generally decreases in strength from lower to higher trophic levels (Scherber *et al.* 2010). Using a plant biodiversity experiment Scherber *et al.* (2010) found weakening effects of plant species richness on invertebrate abundance and species richness with increasing trophic level. Similarly, other studies have shown that plant species composition significantly influences the biomass and abundance of soil invertebrates at primary (microbes, herbivorous nematodes) and secondary (microbivorous nematodes, enchytraeids) trophic levels, but this influence diminishes at higher trophic levels (i.e., predatory nematodes) (Wardle *et al.* 2003; De Deyn *et al.* 2004; Balvanera *et al.* 2006).

1.4 Effect of succession on ecological networks and processes in soil

Food webs describe trophic interactions between organisms along which energy flows from the base of the food web, i.e., producers to top predators (Pimm *et al.* 1991). Parameters commonly used to describe the structure of food webs include connectance (Erdos & Rényi 1960) and maximum trophic level (Dunne *et al.* 2002a, 2004; Riede *et al.* 2010). Connectance measures the proportion of possible connections within a food web that are actually realized by feeding interactions observed within the food web (Gardner & Ashby 1970; Pimm *et al.* 1991). Food chain length is the number of trophic links required for nutrients and energy to reach a consumer from its resources (Williams & Martinez 2004). Maximum trophic level of a food web is the measurement of the breadth of the vertical dimension of these links (Wang & Brose 2018). These parameters offer a powerful tool to describe and compare ecosystems based on trophic interactions within them, regardless of specific species involved (Dunne *et al.* 2002a). Furthermore, these parameters have also been revealed by model analyses to play critical roles in food web stability and functioning (Dunne *et al.* 2002b; Montoya *et al.* 2006; Tylianakis *et al.* 2010; Wang & Brose 2018). Hence these parameters can also be used to predict the sensitivity of food webs to environmental change (Digel *et al.* 2014).

While aquatic and terrestrial aboveground food webs have been extensively studied, belowground food webs have been somewhat neglected (Scheu & Setälä 2001). But belowground food webs can be very different to aboveground and aquatic food webs because of the unique features of soil ecosystems as a habitat, such as its porous structure (Kalinkat *et al.* 2013) and resultant evolutionary adaptation pressure on soil invertebrates to limit their body size (Scheu & Setälä 2001; Digel *et al.* 2014). Hence, soil food webs exhibit strong compartmentalisation into size classes including small basal microfauna (nematodes and protists), mesofauna (body size < 2 mm such as microarthropods and enchytraeids) and macrofauna (body size > 2 mm such as beetles, millipedes and earthworms), the trophic interactions among which can determine network parameters (Brose 2010). Such body size heterogeneity also results in greater species diversity, high number of sit-and-wait and random encounters between predator and prey, which consequently leads to more omnivorous, generalist intra-guild predators and less specialists in soil food webs (Digel *et al.* 2014).

Changes in biotic and abiotic environmental characteristics alter food web structure through their effects on trophic interactions between constituent species (Digel 2014). Hence successional change in such characteristics during restoration is also expected to affect food web structure (Kaiser-Bunbury *et al.* 2017; Moreno-Mateos *et al.* 2020). Shifting resource availability results in variation in assembly of trophic groups during succession with some groups better at exploiting available resources than others throughout different stages of succession (Wall *et al.* 2002). For example, Wall *et al.* (2002) found omnivores to be more dominant followed by bacterivores, while herbivores and predators were much scarcer in abundance with increasing successional stage in coastal sand dune system. However, the responses of herbivores and decomposers to succession can strongly depend on availability of different plant species, compared to higher trophic levels i.e., predators and omnivores (De Deyn *et al.* 2004). Furthermore, a significant decrease in microbial biomass per unit soil carbon with increasing age of the system has been found to be associated with a decline in the quality of organic matter entering the soil (Williamson *et al.* 2005), with a negative effect of declining resource quality with succession also observed for microbivore nematodes and enchytraeids, as well as omnivorous and predatory nematodes (Williamson *et al.* 2005). The changes in relative abundances of different trophic levels because of changes in biotic and abiotic characteristics could alter interaction frequencies, ultimately leading to shifts in food web structure.

Biodiversity loss because of disturbance can not only result in species extinctions but also the extinction of ecological interactions carried out by the species involved (Tylianakis *et al.* 2008). Hence, ecological disturbances can affect the trophic interactions within communities (Tylianakis *et al.* 2007) when the abundance of species decreases down to a threshold below which the species does not interact anymore (Moreno-Mateos *et al.* 2020). It is well established that loss of predatory species can have effects in food webs that cascade as far as to producers, thereby affecting a range of ecosystem processes (Pace *et al.* 1999; Duffy *et al.* 2007). In addition, bottom-up forces (e.g., via changes in availability of detritus), fundamental to ecological processes such as nutrient recycling can also influence the strength of top down control (Kalinkat *et al.* 2013). For example, an increase in litter density was found to provide more refugia for springtails, resulting in a decrease in their capture rates by centipedes (Kalinkat *et al.* 2013). Litter density can also influence microclimatic conditions such as humidity and moisture, affecting predator performance since predators such as centipedes are extremely sensitive to dry conditions (Kalinkat *et al.* 2013). These trophic interactions between resources and consumers are what gives structure to food webs, which when disturbed, can affect ecosystem functioning (Pimm 1979). This is because disruption in the flow of energy and interaction strengths can have negative impact on ecosystem functioning and stability (Rooney *et al.* 2006; Barnes *et al.* 2018).

Succession likely causes changes in food web properties. For example, increasing productivity during succession has often been found to cause an increase in length of food chains (Lindeman 1942; Oksanen *et al.* 1981; Kaunzinger & Morin 1998)(but see Post *et al.* 2000). An increase in food chain length has been shown by Neutel *et al.* (2007) in a successional study of belowground food webs in sandy dune soils, revealing non-linear changes in species composition with different trophic groups appearing and disappearing at different stages of succession. Nevertheless, the number of trophic groups, maximum food chain length, and connectance, all were found to increase during succession (Neutel *et al.* 2007). The resultant increase or decrease in food chain length influenced by productivity can then affect population density at several trophic levels and, consequently, the presence and absence of trophic links between the constituent trophic groups (Kaunzinger & Morin 1998). In addition, the strength of the trophic links in the food web can also vary across different stages of succession (Neutel *et al.* 2007) as shifts in resource availability results in a shift in trophic interactions between resource and consumer groups (Wardle *et al.* 1995). In contrast, neither connectance, nor maximum trophic level (an indicator of food chain length) have been found to differ significantly across different successional stages of post-mining land abandonment (Frouz *et al.* 2013). This could be due to the slow developmental process of soil structure and horizon development after the abandonment of mining activity. This suggests that successional trajectories may be context dependent, and are not always a gradual transition between different stages as expected, but could rather oscillate back and forth between different stages (Kaufmann 2001; Frouz *et al.* 2013).

1.5 Thesis objectives

The main objective of my PhD research is to shed light on the influence of forest restoration on trophic interactions in soil food webs. For this purpose, I collected data from permanent plots established in 70 urban restored forests in eight cities in Aotearoa-New Zealand that span approximately 9° of latitude and encompass most of the country’s climate range (Clarkson & Kirby 2016). Importantly, the restoration sites cover a chronosequence from 6 to 60 years since initial restoration forest plantings occurred. By utilising this unique network set of urban restoration sites with recently developed techniques in quantifying ecosystem functioning in multi-trophic systems (Barnes *et al.* 2014b, 2018; Gauzens *et al.* 2019), I analysed how soil food webs reassemble over decades of forest restoration and the resulting implications for the functioning of urban forest ecosystems. I also collected data from three unrestored, and three remnant urban forest sites. This helped me compare data collected from each restoration forest against both extremes of restoration trajectories; i.e., against the onset of restoration and against old-growth urban forests. This allowed me to distinguish the effects of aboveground restoration on belowground soil food webs. Data collected from unrestored sites showed how disturbed forest soils respond to an absence of aboveground restoration. Remnant forest data represent a reference and a target ecosystem which allows for the determination of whether the direction of recovery of restored forest soil food webs is developing along the target trajectory towards the stat of remnant-forests and their associated forest soil food webs (Fig. 1).

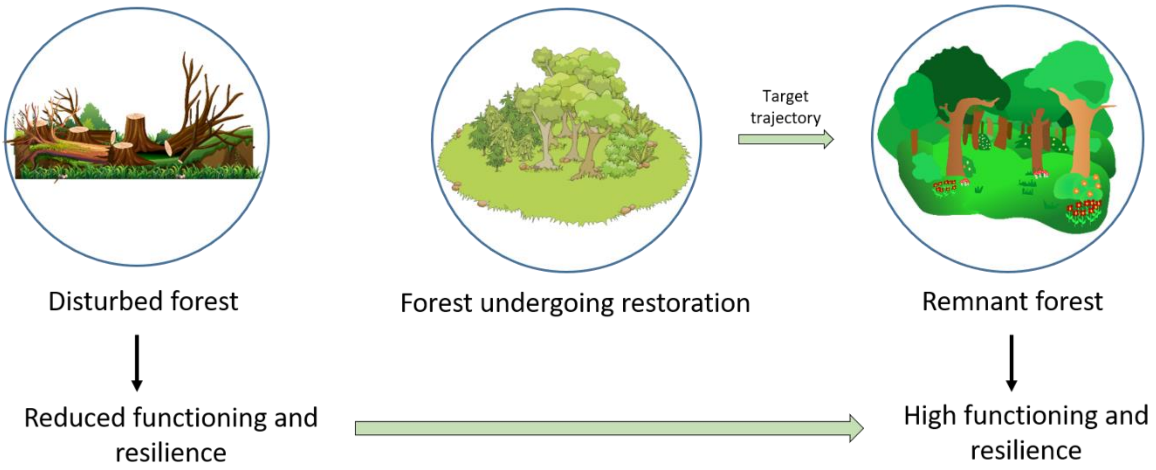

Figure 1. Illustration of three classes of urban ecosystems used in this study. From left to right: deforested ecosystem after disturbance (unrestored), forests ranging from 6-60 years since initial restoration planting, and remnant urban forest with no history of clear felling. I compared the data from disturbed forest with restored forest to understand how soil food web respond to restoration after disturbance. Remnant forest was used as a reference ecosystem to determine if soil food webs undergoing restoration are developing in the intended direction towards those in undisturbed forests.

In the following chapters, I first investigate how soil invertebrate communities reassemble following restoration planting of native vegetation and how different biotic and abiotic factors influence their reassembly trajectories. For the second chapter, I test for direct and indirect effects of time since restoration (forest succession) on soil invertebrates at different trophic levels to determine the role of bottom-up effects in mediating responses of soil food webs to restoration planting. And finally, in chapter 3, I disentangle the effects of restoration age on food web structure and associated ecosystem functions by quantifying energy fluxes in reassembling soil food webs. By doing so, this thesis addresses the unexplored link between soil food web structure and ecosystem functioning during the reassembly of forest soil food webs in urban ecosystems. Hence, this research plays a significant role in global efforts to expand the understanding of restoration ecology. In particular, my work contributes to greater knowledge about the interaction between above- and belowground dynamics in restored forest ecosystems.

1.6 <u>Ethical approval statement</u>

Under the Code of Ethical Conduct for the ‘Use of Animals for Teaching & Research’, arthropods are invertebrates and are not considered in the animal ethics requirements. The invertebrate species and soil biota that will be collected and studied in this study will not affect any rare or endangered species including the ecosystem itself. Hence there is no requirement to submit an application for ethics approval to the faculty of ethics committee. Furthermore, landowner consent or permission from respective authority (e.g. each city council) has already been sought and granted in order to gain access to (and collect samples from) each of the sampling sites.

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Chapter 2 . Vegetation and soil temperature determine the restoration trajectory of belowground forest invertebrate communities

2.1 Abstract

Understanding the structure of soil food webs can be important for determining the trajectory of ecosystem restoration due to its role in maintaining critical ecosystem processes. However, reassembly of belowground invertebrates across multiple trophic levels is poorly understood, and very little is known about the factors that influence restoration trajectories of belowground fauna. To investigate how forest restoration interventions in urban environments influence the reassembly of abundance, biomass and body size structure of belowground invertebrate communities, I sampled 76 sites including unrestored, restored, and remnant urban forests across eight cities in Aotearoa New Zealand. Mean annual temperature of the sites ranged from 10 to 15 ºC. The restored forests formed a chronosequence in age since planting (6-60 years) and were established in spaces where forests had been removed and later restored by active re-planting with native saplings. I quantified the abundance, biomass and mean body mass of major trophic groups in soil food webs and analysed the relative importance of restored forest age compared to various environmental variables as predictors of variation in soil food webs in restored versus remnant forests. Contrary to my hypotheses, I found very little difference in invertebrate communities between unrestored sites, restored forests, and remnant forests. Restored forest age also did not significantly affect the relative difference in abundance, biomass or mean body mass between restored and remnant forests for any invertebrate trophic group. Instead, litter depth was the most common predictor influencing the invertebrate community at all trophic levels, alongside other significant predictors: average tree size, tree species richness, and soil temperature. Furthermore, I found that macrofauna and mesofauna trophic groups were more strongly influenced by changing environmental variables than nematodes of any trophic level. My results demonstrate that the age of restored forests, alone, is a relatively weak driver of soil food web trophic groups. Instead, the recovery of belowground invertebrate communities is strongly reliant on a range of forest characteristics, which I suggest should be the focus of efforts to restore belowground biodiversity and ecosystem functioning.

2.2 Introduction

Ecological restoration is the process of assisting in the recovery of an ecosystem that has been degraded, damaged, or destroyed (Gann *et al.* 2019). This often entails steering an ecosystem towards some semblance of its pre-disturbance state, structurally and functionally (Palmer *et al*. 1997; Clewell & Aronson 2006; Corlett 2016; Barabás *et al*. 2017). Therefore such practice needs to incorporate the restoration of entire communities and interactions among constituent species (Palmer *et al.* 1997). However, studies on the subject have mostly focused on aboveground vegetation with a lack of consideration of soil invertebrate reassembly in relation to aboveground restoration actions (Perring *et al.* 2015; Morriën *et al.* 2017; Nielsen 2019a). Furthermore, studies have typically been conducted at small spatial scales and over relatively short timescales—e.g., less than 10 years (Piechnik *et al*. 2008)—or with very few time steps—e.g., restored versus degraded (Kaiser-Bunbury et al. 2017) or degraded, partially restored and restored (Morriën *et al*. 2017). Additionally, there is little knowledge of the effects of restoration efforts in urban environments, which are often subject to pollution and high levels of invasive species. Therefore, studies on decadal time scales and at large spatial scales are needed to understand temporal dynamics (Wallace *et al*. 2017) and to identify environmental influences on restoration trajectories, especially of multi-trophic responses to aboveground succession (Palmer *et al*. 1997; Maharning *et al*. 2009).

Previous studies have shown that secondary succession can strongly influence the structure of soil communities (Scheu & Schulz 1996). For example, studies have shown that large detritivores in the litter layer, such as isopods and millipedes, re-colonise before soil-dwelling organisms like endogeic earthworms and insect larvae (Bastow 2012). Additionally, past studies have found shifts from bacterivorous to fungivorous nematodes (Georgieva *et al*. 2005), and from fungivorous to detritivorous Collembola, which drive the fate of soil organic matter as succession progresses (Bastow 2012). Predatory nematodes are expected to appear later in the forest succession when their prey become more available (Wardle *et al*. 1995). On the contrary, herbivorous nematodes and Collembola are thought to appear early in succession followed by those that survive on decaying root tissue as the forest ages (Wallwork 1976). This supports the idea that forest succession not only results in changes in species' presence but also changes in the relative abundances of different functional groups. Similarly, a study comparing ant communities in mature undisturbed forest against abandoned regrowth forests found that older regrowth forest had more similar ant abundance and species composition to mature forest (Vasconcelos 1999). This suggests that if secondary succession is allowed to continue, soil invertebrate communities of disturbed forests might be able to recover to resemble those of old mature forests, depending upon the type and intensity of disturbance (Vasconcelos 1999; Dunn 2004; Bihn *et al*. 2008). Furthermore, variation in site characteristics such as canopy cover and vegetation are likely to be influential in the relative recovery of different taxa and functional groups (Koivula *et al*. 2002; Riley & Browne 2011).

In addition to aboveground vegetation, abiotic variables also influence belowground community structure (Gibert 2019) and could therefore influence the restoration trajectory of soil food webs at large spatial scales. Metabolic rates increase with temperature, consequently increasing the productivity of arthropods (Gillooly *et al*. 2001; Ehnes *et al*. 2011; Brose *et al*. 2012). Temperature can also affect trophic interactions, resulting in changes in food web structure (Petchey *et al.* 2010). For example, a microcosm study investigating the effects of soil temperature and soil water content on trophic interactions found that an increase in temperature and dryness increased consumption rate and, therefore, top-down control of Collembola by predators such as mites and centipedes (Lang *et al*. 2014). Moreover, a study on stream banks along geothermal regions showed that an increase in soil temperature significantly increased the total abundance, but it significantly decreased the mean body mass of the invertebrate community (Robinson *et al*. 2018). This follows the general pattern in relationships between body size and abundance of organisms (Roeder *et al.* 2022). Litter characteristics have also been shown to strongly influence the biomass and diversity of soil invertebrates across various functional groups (Ott *et al*. 2014; Jochum *et al.* 2017). The quantity and chemical composition of litter produced is in turn driven by the species composition of aboveground vegetation (Kaspari & Yanoviak 2008; Donoso *et al*. 2010; Ganault *et al*. 2021). Despite the demonstrated importance of environmental variation for the abundance, biomass and body size structure of soil food webs, little is known about how these factors influence the restoration trajectory of soil food webs following active replanting interventions.

To explicitly test the relative influence of environmental gradients requires standardised sampling of restoration sites at large spatial scales that capture substantial biotic and abiotic variation. I collected soil samples from an urban forest restoration chronosequence comprising 76 sites spread over nearly 9 degrees of latitude in Aotearoa New Zealand, and investigated how soil food webs reassemble over multiple decades of forest development. Using this unprecedented network of urban forest restoration sites, we tested for the relative roles of plant community structure, environmental variables, and forest planting age in shaping invertebrate community assembly. I first hypothesised that soil food webs would differ significantly between unrestored, restored, and remnant forest sites, whereby soil invertebrate communities in restored sites would more closely resemble those of remnant forests than unrestored sites (H1). I hypothesized an increase in abundance, biomass and mean body mass of soil invertebrates with age to more closely resemble the belowground invertebrate communities found in remnant sites (H2), due to increased resource availability. I also expected environmental variables, such as tree species richness, soil temperature and litter depth, to have positive effects on abundance, biomass, and mean body size of soil invertebrates across all trophic groups (H3).

2.3 Methods

2.3.1 Study Location

This study was carried out in eight cities across the North Island and South Island of Aotearoa New Zealand during the summer of November 2019 – February 2020. Sampling of soil invertebrate communities took place within urban restored forests (n = 70), remnant forests (n = 3), and unrestored sites adjacent to restored forests (n = 3). The study sites were distributed across a latitudinal gradient of approximately 9° and spanning a mean annual temperature of 10.1 °C in Invercargill (Invercargill climate 2022) to 14.8 °C in Tauranga (NIWA 2019). I studied 8–11 sites per city, all of which were on slopes of less than 15º. The restored urban forests were planted with native tree and shrub species in a single cohort, on retired farmland or mowed parkland. The restored forest sites were selected to represent a chronosequence spanning 6–60 years elapsed since their initial restoration planting.

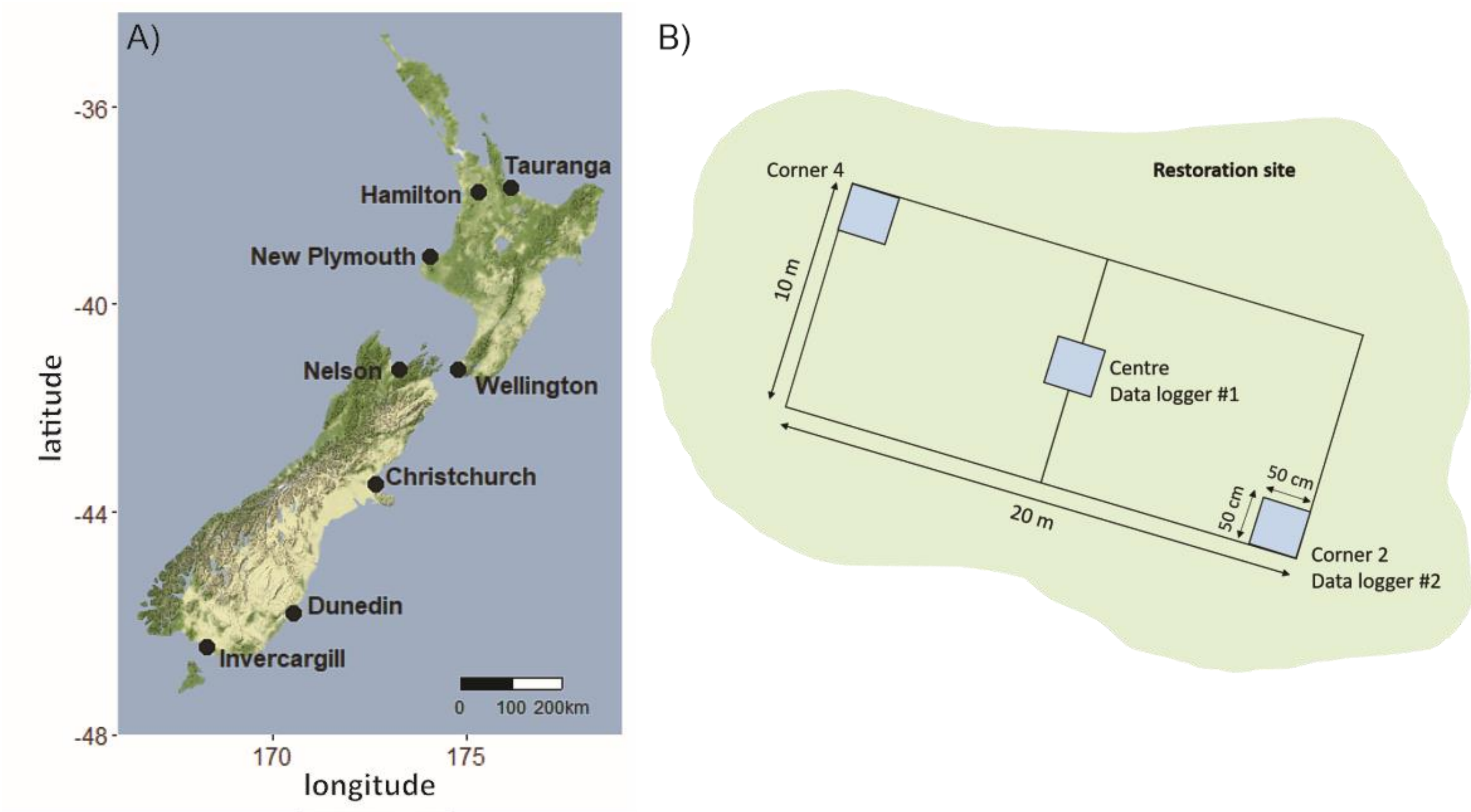

Figure 1. A) Map of Aotearoa, New Zealand showing the eight cities where soil samples were collected. B) The 76 urban sites distributed across the eight cities (including unrestored sites, restored forests, and remnant forests) were 20 m * 10 m, with three sub-plots (indicated by blue squares) located at the centre, corner two, and corner four of each site where belowground invertebrates were sampled. Temperature data loggers were buried at the corner two of the restoration sites.

2.3.2 Experimental Design and Sampling

At each site, I established 20 m × 10 m experimental plots with three subplots of 50 cm × 50 cm (Fig. 1B). The subplots were placed diagonally across the plot, located at the centre point, corner two, and corner four. Within each subplot, I collected one 22 cm diameter soil core and one 5 cm diameter soil core to sample macrofauna and mesofauna, respectively, and five 2.7 cm diameter soil cores to sample nematodes. To capture spatial heterogeneity within each subplot, all soil cores were collected at random points and with a distance of ca. 22 cm between coring locations within each subplot. All the soil cores were collected to a depth of 10 cm from the surface of the organic horizon. To sample the food web as completely as possible, soil cores were taken with the litter layer left in place, but the litter layer did not count towards the 10 cm coring depth.

Temperature data were collected from the centre point and corner 2 via iButton data loggers deployed at 10 cm depth that logged temperature data every 4 hours from 24 March 2018 to 24 June 2018. Canopy openness (sky visible through the forest canopy) was estimated using a convex densiometer (Convex model A; Forestry Suppliers, Jackson, Mississippi, USA) once at all four corners and the centre of each plot. The presence or absence of canopy cover was recorded at each of the points in a grid engraved on the surface that reflected the image of the forest canopy above. The average of these five densiometer measurements was then calculated. Leaf litter depth was measured using a ruler at five points in each subplot (at each of the four corners and centre) from which I calculated an average depth. Each measurement was taken from the surface of the organic horizon to the maximum height occupied by the litter layer.

Additionally, I identified each adult tree (diameter at breast height ≥ 2.5 cm), both natives and exotics, in the plots to species level to calculate plot-level tree species richness. Each stem larger than 2.5 cm in diameter at breast height was measured to then calculate the basal area per stem, which was used to calculate the mean quadratic diameter of all trees in the plots. Seedling density within the plot area was recorded as the total number of woody species under a height of 135 cm within ten circular subplots of a 1.5 m radius. These subplots, together, covered a total of 70.7 m^2 of the plot and were later scaled up to approximate seedling density per 200 m^2.

2.3.3 Extraction of Soil Invertebrates

Soil macrofauna (body width ≥ 2 mm), such as millipedes, centipedes, and beetles, were extracted from the largest soil cores using modified 'Kempson' high-gradient heat extractors (Edwards 1991). Soil mesofauna (body width 0.1 mm–2 mm), such as mites and springtails, were collected following a similar approach using a Macfadyen high-gradient heat extractor (Edwards 1991). Extractions were run for at least 11 days using a programmed temperature ramp starting at 20 °C on day one and reaching 55 °C in the last two days of the extraction. To extract free-living soil nematodes, soil from the five cores per subplot was sieved using a 2-mm mesh and homogenised. Nematodes were then extracted from 25 g of sieved soil using an active 'Baermann funnel' method (Coleman & Wall 2015; Cesarz *et al.* 2019b). Extracted nematodes were collected and stored in 4% formalin. I then calculated the dry weight of the soil samples to obtain nematode abundance per 100 g of dry soil (Cesarz *et al*. 2019).

2.3.4 Soil Invertebrate Identification and Measurements

Macrofauna (including adults and larvae) and mesofauna samples were sorted and identified in the lab according to Naumann (1991) and various online resources (CSIRO, 2012 & VanDyk et al. 2020). Specimens were identified to a level that was sufficient to determine their general feeding behaviour (typically to the order or family level) based on Potapov et al. 2022 and Potapov 2022. Trophic groups were further assigned to all macrofauna (detritivores, fungivores, herbivores, omnivores & predators) and mesofauna (detritivores, fungivore-herbivores combined fungivores and herbivores within the mesofauna group, herbivores-predators (combined herbivores and predators within the mesofauna group), omnivores, and predators). Nematodes were identified to the genus level, such that the first hundred nematodes encountered per sample were identified, according to "De Nematoden van Nederland" (Bongers 1994). All nematodes were also further assigned to trophic groups (bacterivores, fungivores, herbivores, omnivores, and predators). Adults were not distinguished from juveniles for all invertebrate groups.

Following identification, the body length (mm) of each macro- and mesofauna individual was measured using a stereo microscope with an ocular micrometre. I then calculated the fresh body mass (mg) of each individual using published length-mass scaling relationships for meso- and macrofauna (Mercer *et al.* 2001; Barnes *et al.* 2014b; Sohlström *et al.* 2018). Gastropods and leeches were excluded from analyses due to extremely low numbers in the samples (63 and 1, respectively, out of 15,831 observations). The body masses of Coleopteran, Dipteran, and Lepidopteran larvae were estimated using the Coleoptera larvae-specific formula in Sohlström et al. (2018), because no other taxa-specific formula was available for Diptera and Lepidoptera. Furthermore, for some groups, there was no regression formula available in the literature. Therefore, for Diplura and Symphyla, I used a scaling relationship published for Dermaptera and Chilopoda, respectively (Barnes et al. 2014; Sohlstrom et al. 2018), and for nematodes, I collected genus-specific information on body mass using the online platform Nemaplex ("Nemaplex Main Menu" 2022), which was based on female adults (but see Klusmann et al. (2022) for potential caveats). These body masses were then assigned to all associated genera of nematode individuals in my dataset. Invertebrate abundance was calculated as the total number of individuals collected from each trophic level, and of whole macrofauna, mesofauna and nematode communities within each plot. I further calculated total biomass by summing all individual body masses for each group at each forest plot. Additionally, average body mass was calculated by taking the mean of fresh body mass for each invertebrate group at each forest plot.

2.3.5 Data Analysis

I constructed Generalised Linear Mixed Effects Models (GLMM) to test for differences in soil invertebrate communities between unrestored, restored, and remnant forests. 'Forest type' was included as a single fixed effect (i.e., if the site was 'unrestored', 'restored' or 'remnant'), and 'city' was included as a random effect to account for the hierarchical grouping of multiple sites within cities. I modelled abundance and biomass responses using a Tweedie compound Poisson distribution to accommodate zero-inflated, fully continuous data that approximated a Poisson distribution (Delong *et al*. 2021). Mean body mass of soil invertebrates was modelled on a Gaussian distribution after it was log-transformed to meet assumptions of normality and heterogeneity of variance. Individual models were constructed for whole communities of each invertebrate size class (macrofauna, mesofauna, and nematodes), as well as for each trophic group within each size class across the three response variables (abundance, biomass, and mean body mass), yielding a total of 54 models.

Secondly, I tested for the relative importance of forest age (years since initial planting) and other biotic and abiotic variables as determinants of the similarity of restoration sites versus urban forest remnants (i.e., restoration trajectories relative to remnant forest communities). To do so, I first calculated the log response ratio of each soil invertebrate response (abundance, biomass, and mean body size for each size class and trophic group) between restored versus remnant forest sites, using the formula

$$log\ response\ ratio = \frac{\log Y_{restored\ forest}}{\log \overline{Y}_{remnant\ forests}}$$

where Y is the soil invertebrate response variable of interest (e.g., abundance, biomass, or mean body mass of a given trophic group or community). All models were checked for assumptions of normality and homogeneity of variance, and each response variable, as well as mean tree diameter, were log-transformed to enhance model fitting. Models were then simplified using the 'dredge' function in the MuMIn package in R (Burnham & Anderson 2002) to first identify all possible model combinations. This was done by first constructing maximal models with the predictor variables restoration age, litter depth, tree species richness, mean tree diameter, mean soil temperature, mean canopy openness and woody seedling density (< 15 cm in height), with 'city' specified as a random effect. These models were then ranked based on delta AICc (AIC correct for small sample sizes), from which we chose the minimum adequate model with the smallest AICc score. Where two models were within 2 AIC units of each other, I selected the model that had the fewest predictors.

2.4 Results

With data from 76 soil invertebrate communities across a 60-year restoration chronosequence spanning most of the latitudinal extent of Aotearoa New Zealand's two main islands, I found a total of 15 major trophic groups present across almost all studied soil food webs (Fig. 2). In general, my data shows declining abundance (Fig. 3A-C) and biomass (Fig. 3D-F) with increasing trophic level, and widely varying mean body mass across trophic groups (Fig. 3G-I). Furthermore, I found wide variation in these three measured community attributes among restored sites, particularly in comparison to variation observed within the unrestored and remnant sites.

2.4.1 *Variation in Soil Food Webs Among Unrestored, Restored and Remnant Urban Forests*

There was generally little evidence that the abundances of soil invertebrate trophic groups were affected by restoration status (i.e., unrestored, restored, and remnant forest sites; Table S1; Fig. 3A-C). Across the macrofauna, mesofauna, and nematodes, abundance of all trophic groups did not differ significantly with restoration status ($p > 0.05$; Table S1). However, macrofauna omnivore abundance in unrestored sites was marginally higher ($p = 0.07$) than in remnant forest sites (Fig. 3A; Table S1). In contrast, macrofauna detritivores were marginally more abundant ($p = 0.07$) in restored forest sites than remnant forests (Fig. 3A; Table S1).

Similar to abundance of soil invertebrates, I detected very few differences in the biomass of any trophic group between the three forest site restoration statuses (Table S2; Fig. 3F). The total community biomass of all invertebrate groups in unrestored and restored sites was similar to remnant sites. This was also true for almost all individual trophic levels, with the exception of herbivore-predator mesofauna. In particular, biomass of mesofauna herbivore-predators was significantly higher, on average, in unrestored sites than remnant forest sites (Fig. 3E; Table S2).

In contrast to the abundance and biomass data, I found that mean body mass of some invertebrate groupss differed significantly with restoration status of sites across a number of trophic groups. I found that the mean body mass of macrofauna detritivores was significantly higher in remnant forest as compared to restored forests and unrestored sites (Fig. 3G; Table S3). Furthermore, mean body mass of herbivore-predators was significantly higher in unrestored sites than in remnant sites (Fig. 3H; Table S3) and, similarly, nematode fungivore body mass was significantly higher in unrestored sites than in either restored or remnant sites (Fig. 3I; Table S3). There was also a marginally significant difference in the mean body mass of the whole mesofauna community between unrestored and remnant sites pointing to, on average, smaller mesofauna in remnant forest versus unrestored sites (Fig. 3B).

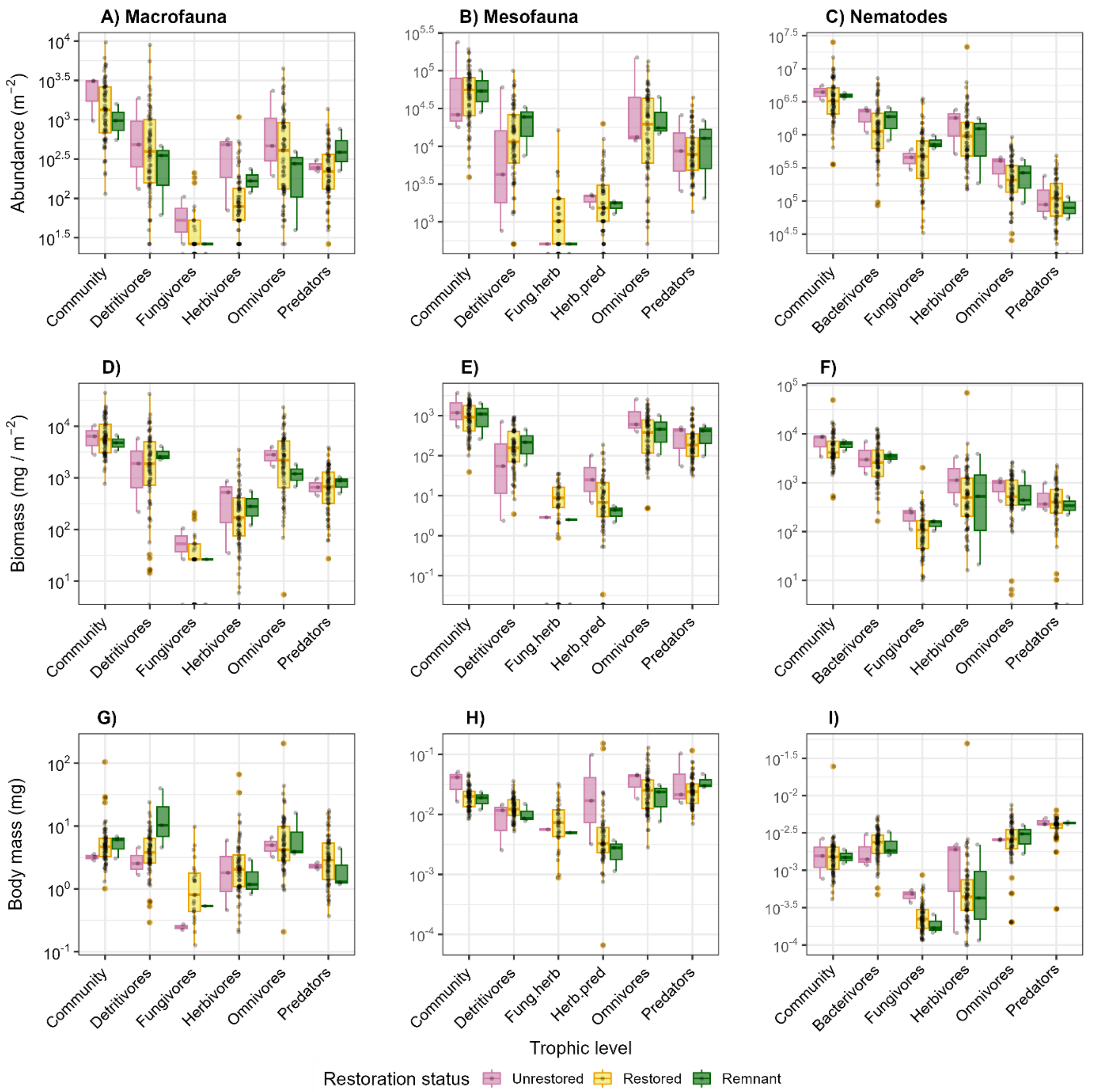

Figure 2. Abundance, biomass and mean body mass of macrofauna (A, D, G), mesofauna (B, E, H) and nematodes (C, F, I) at the whole community level (all trophic groups aggregated together) and for each trophic group across the three site restoration categories (restoration status). Mesofauna herb.pred represents herbivore-predators and fung.herb represents fungivores-herbivores. Lower and upper hinges correspond to the 25th and 75th percentiles; black points represent individual sites. There are three categories of restoration status, termed ‘restored’ (n = 70), ’unrestored’ (n = 3) and ‘remnant’ (n = 3).

2.4.2 *Influence of Environmental Drivers on the Restoration Trajectory of Soil Food Webs*

To determine how forest age and site characteristics influence the trajectory of belowground restoration compared to remnant forest sites, I analysed the log-response ratio of the three soil community response variables—abundance, biomass and mean body mass—between restored and remnant forest sites. Litter depth was the most common significant predictor of invertebrate abundance in all invertebrate groups, at all trophic levels (Table 1; Fig. 4). The abundance of macrofauna and mesofauna omnivores, mesofauna predators, and total abundance of the mesofauna community all increased with litter depth (Fig. 4A, B, E and F), whereby intermediate depths of leaf litter in restored forests seemed to harbour abundances of mesofauna soil invertebrate groups that were most similar to remnant forest soil communities. The abundance of the whole mesofauna community in restored forests also increased and became progressively more similar to remnant sites with increasing mean tree diameter (Fig. 4C). In contrast, increasing soil temperature led to significantly lower abundances of mesofauna herbivore-predators in restored sites compared to remnant sites. Neither forest age nor any of the other measured site characteristics significantly explained similarity in nematode abundance between restored and remnant forests ($p > 0.05$) for any of the trophic groups or of the whole nematode community.

Mean tree diameter and litter depth were also a common predictor of the biomass of soil invertebrates, with soil temperature and tree species richness also having some predictive power (Table 2; Fig. 5). Total mesofauna community and omnivore biomass in restored forests increased with depth of litter, with mesofauna in intermediate litter depths from the restoration sites converging on the average biomass recorded at remnant forest sites. A positive trend was also found for the mesofauna detritivore and predator trophic groups in response to mean tree diameter, whereby those restored forests that had the largest trees harboured a biomass of predators and detritivores similar to levels found in remnant forests (Table 2; Fig. 5C and 5F). At the lowest levels of tree species richness in restored forests, macrofauna herbivore biomass was most similar to that of the remnant forests, but it increased significantly relative to remnant forest communities with increasing tree species richness (Table 2; Fig. 5A). In contrast, herbivore-predator biomass decreased significantly with increasing soil temperature, converging on biomass values most similar to those of remnant forest communities at sites with mean soil temperatures of approximately 13 °C. Similar to abundance, neither forest age nor any site characteristics significantly explained absence of variation in nematode biomass ($p > 0.05$) in restored versus remnant forest soil food webs.

Litter depth and tree species richness were the only two predictor variables identified from my models of soil invertebrate mean body mass (Table 3), that had significant effects on invertebrate mean body mass across all trophic levels (Table 3; Fig. 6). In particular, the average body mass of the whole macrofauna community in restored forest sites was significantly higher than in remnant forest sites at low values of litter depth, but significantly lower than in remnant forest sites where litter depth was deeper (Fig. 6A). Interestingly, I found the opposite effect of litter depth on mesofauna omnivore body mass, which were smallest at low litter depths and this increased significantly with increasing depth of leaf litter in the restored forest sites. Similarly, the mean body mass of macrofauna herbivores was lower in the restored versus remnant forest sites when tree species richness of restored forest sites was low, but herbivore body mass became increasingly higher than remnant forest communities at high levels of restored forest tree species richness (Fig. 6B). Neither the forest age nor any of the site characteristics could significantly explain variation in nematode body mass ($p > 0.05$) across any of the trophic groups or at the community level.

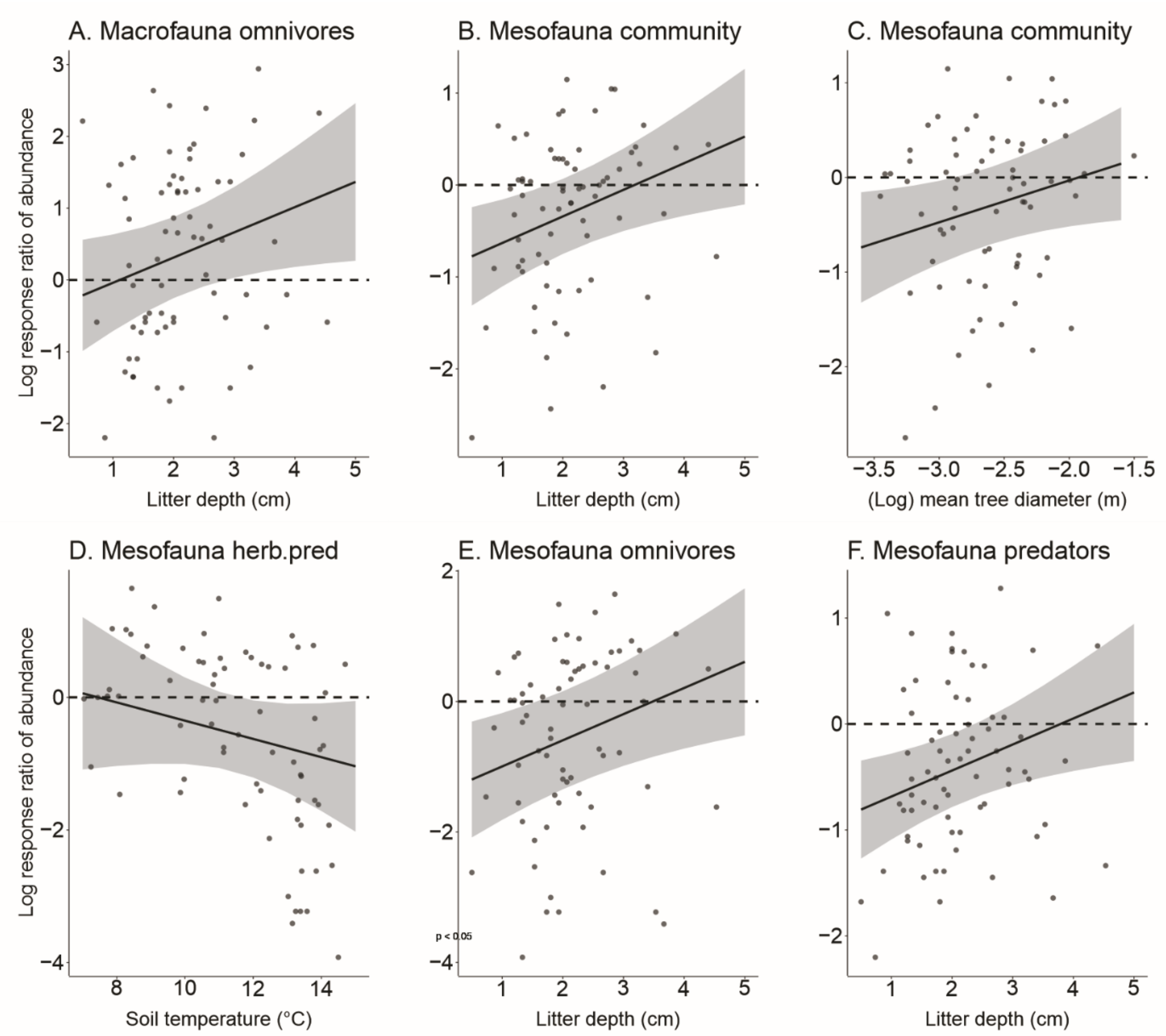

Figure 3. The significant partial effects of restoration site characteristics on the log response ratios between restored and remnant forests for macrofauna (A) and mesofauna (B-F) abundance from the generalised linear mixed effects models. Significant predictors shown are litter depth (A, B and E), mean tree diameter (C), and soil temperature (D). The horizontal dashed line indicates a zero difference from the remnant forest sites. The shaded region is the 95% confidence interval.

Table 1. Results from linear mixed-effects models testing the effects of environmental variables on the log response ratios of invertebrate abundance across trophic groups and the whole community between restored versus remnant forests (intercept). Predictor variables shown are from the best fit linear mixed-effects models as selected by AIC. Statistically significant *p*-values are indicated in bold font.

Trophic levels	Predictor variable	Macrofauna abundance			Mesofauna abundance			Nematode abundance		
		Estimate	SE	*p*	Estimate	SE	*p*	Estimate	SE	*p*
Community	Intercept	0.190	0.160	0.250	0.240	0.680	0.730	-0.210	0.150	0.170
	Litter depth				0.290	0.110	**0.010**			
	Mean tree diameter				0.440	0.210	**0.040**			
Predators	Intercept	-0.800	0.100	0.000	-0.930	0.270	0.000	1.490	3.400	0.660
	Litter depth				0.250	0.100	**0.010**	1.090	1.280	0.400
Omnivores	Intercept	-0.390	0.450	0.390	-1.40	0.490	0.010	-0.420	0.200	0.040
	Litter depth	0.350	0.160	**0.040**	0.400	0.150	**0.010**			
Herbivores-predators	Intercept				7.790	3.240	0.020			
	Mean tree diameter				1.220	0.820	0.140			
	Soil temperature				-0.500	0.170	**0.010**			
Herbivores	Intercept	-0.830	0.150	0.000				-0.130	0.170	0.440
Detritivores	Intercept	2.200	1.060	0.040	0.930	0.870	0.290			
	Mean tree diameter	0.770	0.390	0.060	0.600	0.320	0.070			
Fungivores	Intercept	-0.930	0.280	0.000				-0.810	0.360	0.030
Fungivores-herbivores	Intercept				-1.700	0.600	0.010			
Bacterivores	Intercept							-0.460	0.190	0.020

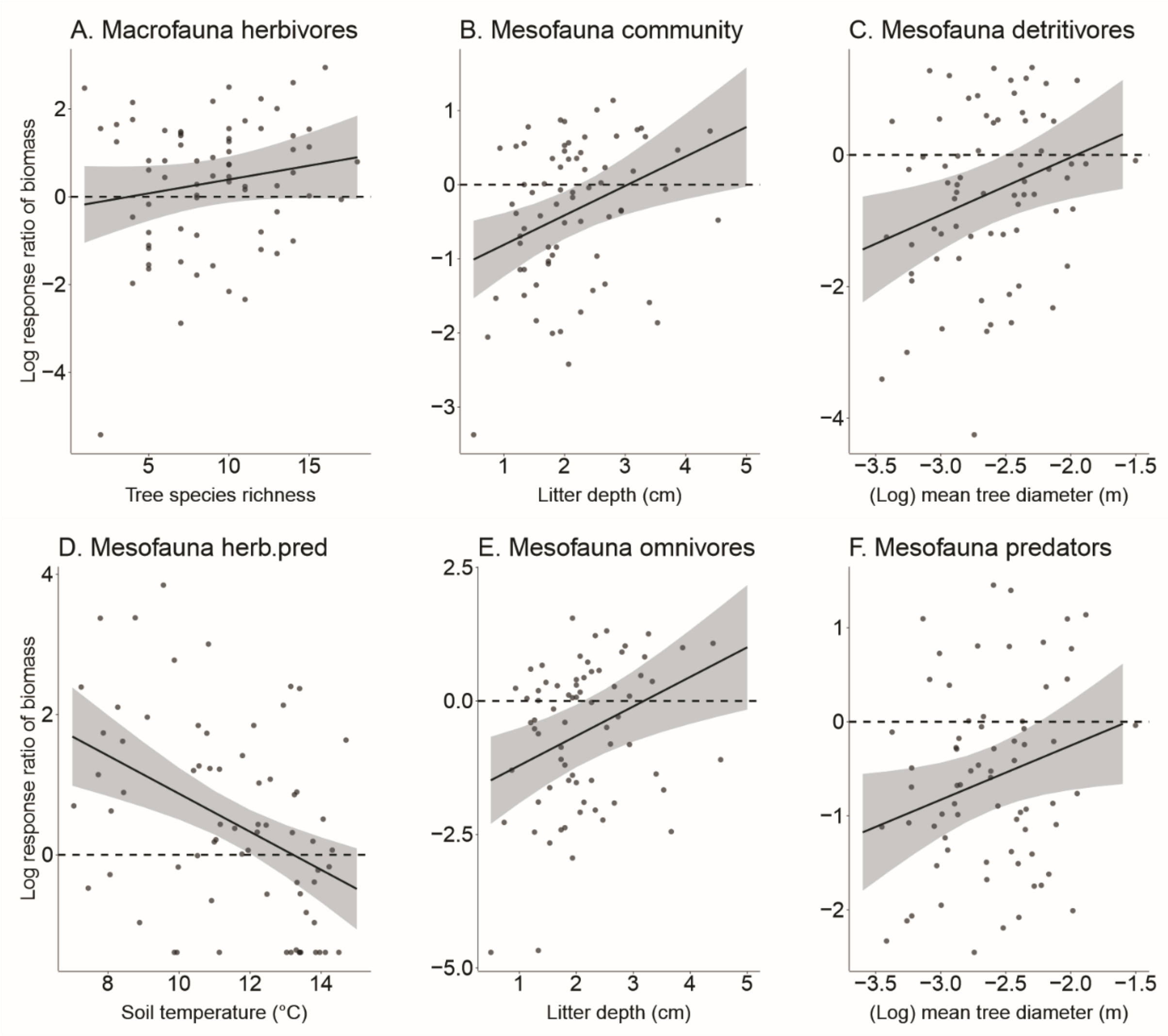

Figure 4. The significant partial effects of restoration site characteristics on the log response ratios between restored and remnant forests for macrofauna (A) and mesofauna (B-F) biomass from the generalised linear mixed effects models. Significant predictors shown are tree species richness (A), litter depth (B and E), mean tree diameter (C and F), and soil temperature (D). The horizontal dashed line indicates a zero difference from the remnant forest sites. The shaded region is the 95% confidence interval.

Table 2. Results from linear mixed-effects models testing the effects of environmental variables on the log-response ratios of invertebrate biomass across trophic groups and the whole community between restored versus remnant forests (intercept). Predictor variables shown are from the best-fit linear mixed-effects models as selected by AIC. Statistically significant p-values are indicated in bold font.

Trophic levels	Predictor variable	Macrofauna biomass			Mesofauna biomass			Nematode biomass		
		Estimate	SE	*p*	Estimate	SE	*p*	Estimate	SE	*p*
Community	Intercept	0.200	0.130	0.140	-1.210	0.320	0.000	-0.270	0.150	0.080
	Litter depth				0.400	0.130	**0.000**			
Predators	Intercept	-0.330	0.170	0.060	0.900	0.720	0.210	2.000	3.950	0.620
	Mean tree diameter				0.580	0.270	**0.030**	1.480	1.490	0.320
Omnivores	Intercept	0.330	0.270	0.230	-1.830	0.460	0.000	-0.740	0.250	0.000
	Litter depth				0.590	0.170	**0.000**			
Herbivores-predators	Intercept				3.580	0.820	0.000			
	Soil temperature				-0.270	0.070	**0.000**			
Herbivores	Intercept	-2.210	0.420	0.000				-1.190	0.210	0.000
	Tree species richness	0.160	0.040	**0.000**						
Detritivores	Intercept	1.290	1.390	0.360	1.710	0.900	0.060			
	Mean tree diameter	0.820	0.510	0.110	0.870	0.330	**0.010**			
Fungivores	Intercept	-0.320	0.310	0.300				-0.580	0.290	0.050
Fungivores-herbivores	Intercept				1.260	0.220	0.000			
Bacterivores	Intercept							-0.320	0.190	0.090

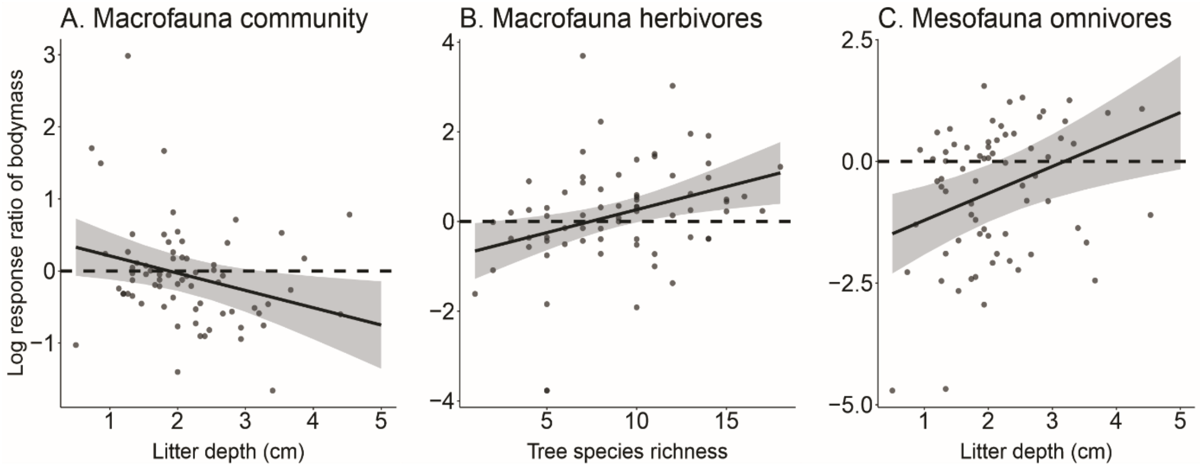

Figure 5. The significant partial effects of restoration site characteristics on the log response ratios between restored and remnant forests for macrofauna (A and B) and mesofauna (C) mean body mass from the generalised linear mixed effects models. Significant predictors shown are litter depth (A and C) and tree species richness (B). The horizontal dashed line indicates a zero difference from the remnant forest sites. The shaded region is the 95% confidence interval.

Table 3. Results from linear mixed-effects models testing the effects of environmental variables on the log-response ratios of invertebrate mean body mass across trophic groups and the whole community between restored versus remnant forests (intercept). Predictor variables shown are from the best fit linear mixed-effects models as selected by AIC. Statistically significant p-values are indicated in bold font.

Trophic levels	Predictor variable	Macrofauna body mass			Mesofauna body mass			Nematode body mass		
		Estimate	SE	*p*	Estimate	SE	*p*	Estimate	SE	*p*
Community	Intercept	0.450	0.240	0.060	-0.040	0.070	0.550	-0.050	0.070	0.410
	Litter depth	-0.240	0.100	**0.020**						
Predators	Intercept	0.210	0.160	0.210	-0.240	0.100	0.020	0.050	0.100	0.640
Omnivores	Intercept	-0.420	0.230	0.070	-0.460	0.250	0.070	0.030	0.090	0.780
	Litter depth				0.280	0.100	**0.010**			
Herbivores-predators	Intercept				6.220	0.000	0.000			
Herbivores	Intercept	-0.760	0.340	0.030						
	Tree species richness	0.100	0.040	**0.000**				-0.630	0.110	0.000
Detritivores	Intercept	-1.690	0.170	0.000	0.230	0.100	0.020			
Fungivores	Intercept	-0.320	0.310	0.300				1.460	0.030	0.000
Fungivores-herbivores	Intercept				6.220	0.000	0.000			
Bacterivores	Intercept							0.240	0.040	0.000

2.5 Discussion

Overall, my results demonstrate how restoration plantings can lead to widely varying outcomes for belowground biodiversity depending on the vegetation structure and microclimatic characteristics of restoration sites. Although I did find some significant differences between restored forest sites versus unrestored reference sites and remnant forests, my results suggest that the variation across urban forest restoration sites can be so great as to obscure effects of restoration on belowground invertebrate communities. Interestingly, I found that the age of restored forests since the initiation of restoration planting could not explain the observed variation in soil invertebrate communities between unrestored and remnant forest sites, suggesting that other factors must be driving successional trajectories of soil invertebrate communities. Instead, my results suggest that the large variation in litter depth, average tree size, tree species richness, and mean soil temperature across the 70 restoration sites jointly influenced the reassembly of soil invertebrate abundance, biomass, and mean body mass. These findings have important implications for setting management priorities in urban forest restoration projects that are looking to simultaneously restore soil biodiversity. However, any conclusions drawn from comparisons between restored sites, remnant forest, and unrestored sites in my study must be made with caution due to the highly unbalanced study design (i.e., only three remnant and unrestored sites, compared with 70 restored sites).

2.5.1 Variation in Soil Food Webs Among Unrestored, Restored and Remnant Urban Forests

In contrast to my first hypothesis, I found virtually no effects of restoration status on abundance or biomass of any invertebrate group. According to my results, the abundance of all invertebrate groups across all trophic levels did not differ significantly between restored forest communities versus unrestored and remnant forest communities, and I only found a significant difference between unrestored and remnant forests in herbivore-predator macrofauna biomass (Table S1). These unexpected findings are likely due to the wide variation observed among restoration sites as compared to unrestored and remnant forests. Previous studies have found land-use history is an important predictor in defining the recovery of arthropods in successional forests (Vasconcelos 1999; Dunn 2004). However, most of the restoration sites in my study were initially under agricultural pasture and was later invaded by grassland and shrubland before the establishment of native forest through planting. This indicates past land-use type is unlikely to be the main cause of this large variation in invertebrate communities among restoration sites. Vegetation during early secondary succession tends to grow quickly, thereby initiating the formation of suitable habitat conditions for soil invertebrate through leaf litter fall and shade, which can help in the recovery of soil invertebrates (de Paula *et al*. 2016). Likewise, the more degraded restoration sites in my study may have also supported appropriate and sufficient resources for soil invertebrates, such as those remaining from nutrient-rich pastures, and high herb and grass layer biomass resulting in the high abundance observed in both unrestored sites and young restoration sites (Parkhurst *et al*. 2022). Such dense herb and grass layers may partially compensate for the lack of tree species by providing food and habitat resources, particularly for the herbivorous and detritivorous invertebrates, subsequently favouring high predator abundance (Ng *et al.* 2017).

In contrast to soil invertebrate abundance and biomass, I did detect a significant difference in average detritivore body size between restored and remnant forests (Table S3). Notably, macrofauna detritivores at both unrestored and restored sites were smaller on average than those at remnant sites, suggesting longer time frames may be needed for the largest macrofauna to recolonise forests undergoing restoration in urban settings. This could be because old forests are expected to accumulate more litter (Lawrence 2005), including coarse woody debris that provides abundant habitat and food resources for detritivores *(*Wagner *et al.* 2003). In addition, abiotic stability such as reduced temperature fluctuations in remnant forests (Retana & Cerdá 2000), can provide more favourable conditions for invertebrate growth through reduced environmental stress (Horne *et al.* 2015). While I did not find any other significant differences in mean body mass between restored forests and remnant sites, the average body mass of mesofauna herbivore-predators and nematode fungivores was significantly higher in unrestored sites (Table S3). The larger average body mass of mesofauna herbivore-predators in unrestored sites could be due to a high availability of the nutrient-rich herb and grass layer (Parkhurst *et al*. 2022), providing both food and habitat resources for soil invertebrates. Growth of invertebrates can also be promoted by warm temperatures (Chown & Gaston 2010), which might have influenced the average body mass of mesofauna herbivore-predators in the unrestored sites where they have open and longer exposure to sun radiation.

The increased average body mass of fungivorous nematodes in unrestored sites follows earlier studies which have found unfertilised grasslands to have higher fungal biomass

(Yeates *et al*. 1997; Bardgett & McAlister 1999). This should theoretically result in higher food availability for fungivores, which could lead to subsequent increases in their body mass due to reduced energetic constraints. Moreover, all of my unrestored sites were grasslands, which typically produce large amounts of CO_2 (De Vries *et al*. 2013), thereby supporting high fungal biomass (De Vries *et al*. 2013). Consequently, this could favour larger-bodied fungivorous nematodes. Additionally, the fungal energy channel tends to be more stable under disturbance owing to the slow growth of the dominant organisms and weak interactions (de Vries & Wallenstein 2017), which could further support large fungivorous nematodes at the unrestored sites in my study.

2.5.2 Influence of Environmental Drivers on the Restoration Trajectory of Soil Food Webs

Although previous studies have suggested that age should have positive effects on abundance, biomass and mean body size (e.g., Wallwork 1976; Wardle *et al*. 1995; Georgieva et al. 2005; Bastow 2012), I found no support for this in my study from across 70 urban restoration forest sites. More specifically, there was no evidence that time elapsed since initial restoration planting increased the similarity of soil invertebrate abundance, biomass, or average body size to invertebrate communities in the remnant forest sites. One potential reason could be that restoration sites can differ greatly in how they have been planted and managed e.g., planting density, which could cause large variation in site characteristics like vegetation structure and diversity, litter depth and soil temperature, that do not always correlate directly with site age (Fig. S1). In turn, this could lead to such environmental factors masking any effects I would expect to see from age since restoration planting. Furthermore, it could also be that my sample size for remnant sites was too small (n = 3) to accurately represent typical late-successional forest environments. However, this is unlikely to be a major determining factor because the remnant sites in my study were distributed across the full extent of the geographic range of my restoration chronosequence in order to capture as much biogeographic and environmental variation as possible. Despite these sites being so spatially disparate, I still found proportionally very minimal variation among the remnant sites in any of the measured soil community variables (Fig. 3).

Though I did not find any significant effects of restoration forest age on soil invertebrate communities, I did find that a number of local biotic and abiotic factors influenced invertebrate community restoration trajectories. Litter depth was consistently a common predictor of invertebrate abundance, biomass and body mass across all trophic groups. Mean tree diameter, soil temperature and tree species richness were also shown to influence invertebrate communities. This is in accordance with earlier studies (Wagner *et al*. 2003; Müller *et al*. 2008; Schuldt *et al*. 2010; Ebeling *et al*. 2014), which have highlighted all of these environmental and structural variables as important factors influencing soil invertebrate communities. The increase in the abundance of macrofauna detritivores, mesofauna omnivores and mesofauna predators with increasing mean tree diameter can likely be attributed to the provision of more food and habitat resources for detritivores and omnivores by larger trees (Müller *et al.* 2008; Bässler & Müller 2010) and consequently more prey available for the predators. This is also likely why I found a positive relationship between the biomass of mesofauna detritivores and mean tree diameter. Furthermore, larger trees not only provide more coarse woody debris through senescence, but they also provide canopy cover that influences other environmental variables such as litter depth, litter decomposition, and soil temperature (Müller *et al*. 2008).

Leaf litter depth had a positive influence on the abundance of macrofauna omnivores and on the abundance, biomass and body mass of mesofauna omnivores. Leaf litter provides a critical source of energy for soil food webs. Deeper litter can also provide a greater number of microhabitats and create varying microclimate within the litter layer, that affect soil moisture and relative humidity (Wagner *et al*. 2003). Such diversity in resources can support invertebrates with different habitat requirements, with a resulting positive influence on their abundance and biomass production. However, the overall mean body mass of the macrofauna community was found to decrease, relative to the average body mass of remnant forest communities, with increasing litter depth, potentially because it is difficult for larger invertebrates to navigate around the litter if it is too dense and complex (Wagner *et al*. 2003). Furthermore, increasing litter depth could also influence other abiotic factors such as soil temperature (Paul *et al*. 2004; Sayer 2006; Fekete *et al.* 2016), although I did not find any evidence of this correlation in my study (Fig. S1). According to my results, increasing soil temperature significantly reduced the abundance and biomass of mesofauna herbivores-predators in restored sites compared to remnant sites. This finding is consistent with predictions of negative temperature–consumer abundance relationships because of increased metabolic demands at higher temperatures (O'Connor *et al*. 2011), but declining ingestion efficiency (Lemoine & Burkepile 2012). Remnant sites potentially exhibit more stable temperatures than restored sites through their higher canopy cover (Retana & Cerdá 2000), thus providing a more suitable habitat for mesofauna herbivores-predators.

Tree species richness was also shown to increase the biomass and body mass of macrofauna herbivores in restored forests relative to remnant forest sites. This is in accordance with past studies (Schuldt *et al.* 2010; Ebeling *et al*. 2014), which have found positive effects of aboveground vegetation on herbivore invertebrates. Such increases in tree species richness provide herbivores with a diverse variety of food resources, thereby supporting community-level increases in herbivore biomass and body mass. Furthermore, the presence of different plant species for feeding also helps with toxin dilution as well as availability of specific required nutrients (Unsicker *et al*. 2008; Schuldt *et al*. 2010).

It is not clear why abundance, biomass and body mass of nematodes at all trophic levels were unrelated to restoration age and to site characteristics (Table1; Table 2; Table3; Table S1; Table S2; Table S3). Previous studies have reported relationships of nematode communities with several factors including soil nutrients, root hairs and depth (Wardle & Yeates 1993; Briar et al. 2012). Since I did not analyse any of these factors, further studies are needed that take these into account before drawing broader conclusions about the response of the nematode communities to forest restoration.

2.6 Conclusions

In this study, I found no significant influence of age of forest on the abundance, biomass and body mass of soil macrofauna, mesofauna and nematodes. Instead, mean tree diameter, litter depth and soil temperature were major factors that influenced the restoration trajectories of soil invertebrate abundance, biomass, and body size. Potential effects of age may have been obscured by wide inter-site variation in other variables such as soil fertility, planting density and species composition. Therefore, future efforts to restore belowground biodiversity across the wide range of functional groups present in soil food webs should target management efforts at these forest characteristics; in particular, maximising average tree size, species richness, and litter depth, while maintaining lower soil temperatures. In doing so, this will assist in the recovery of soil food webs and thereby support the restoration of disturbed urban sites to more closely resemble mature forests.

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Appendix

Table S1. Mean invertebrate abundance comparisons between restored, unrestored and remnant forest (intercept) sites for community level and trophic level group analyses. Coefficients tables are from linear mixed effect models, with 'city' specified as a random effect. SE is the standard error of the coefficient estimate. The remnant forest site category was selected as the intercept by the model.

Invertebrate group	Restoration status	Macrofauna abundance			Mesofauna abundance			Nematode abundance		
		Estimate	SE	*p*	Estimate	SE	*p*	Estimate	SE	*p*
Community	Intercept	6.90	0.50	0.00	11.03	0.42	0.00	15.20	0.40	0.00
	Restored	0.50	0.50	0.26	-0.08	0.42	0.84	0.01	0.40	0.98
	Unrestored	0.80	0.60	0.17	0.19	0.56	0.74	0.13	0.54	0.81
Predators	Intercept	6.13	0.42	0.00	9.48	0.41	0.00	10.80	0.64	0.00
	Restored	-0.49	0.43	0.26	-0.26	0.41	0.52	0.79	0.64	0.22
	Unrestored	-0.58	0.59	0.34	-0.26	0.55	0.63	0.81	0.77	0.30
Omnivores	Intercept	5.96	0.63	0.00	10.42	0.59	0.00	12.48	0.39	0.00
	Restored	0.40	0.61	0.51	-0.33	0.58	0.57	0.01	0.40	0.98
	Unrestored	1.53	0.82	0.07	0.33	0.78	0.67	0.26	0.54	0.63
Herbivores-predators	Intercept				7.33	0.62	0.00			
	Restored				0.26	0.59	0.66			
	Unrestored				0.20	0.78	0.80			
Herbivores	Intercept	5.14	0.48	0.00				13.69	0.53	0.00
	Restored	-0.38	0.48	0.43				0.44	0.52	0.40
	Unrestored	0.70	0.61	0.26				0.60	0.70	0.39
Detritivores	Intercept	5.27	0.69	0.00	9.93	0.50	0.00			
	Restored	1.18	0.65	0.07	-0.08	0.51	0.88			
	Unrestored	1.03	0.84	0.23	-0.04	0.70	0.96			

Fungivores	Intercept	1.93	1.30	0.14				13.52	0.50	0.00
	Restored	0.88	1.31	0.50				-0.15	0.51	0.77
	Unrestored	1.65	1.44	0.25				-0.50	0.72	0.49
Fungivores-herbivores	Intercept				4.75	1.40	0.00			
	Restored				1.77	1.39	0.21			
	Unrestored				0.00	1.93	1.00			
Bacterivore	Intercept							14.51	0.46	0.00
	Restored							-0.27	0.45	0.54
	Unrestored							0.09	0.61	0.89

Table S2. Mean invertebrate biomass comparisons between restored, unrestored, and remnant forest (intercept) sites for community-level and trophic group analyses. Coefficients tables are from linear mixed effect models, with 'city' specified as a random effect. SE is the standard error of the coefficient estimate. The remnant forest site category was selected as the intercept by the model.

Invertebrate group	Restoration Status	Macrofauna biomass			Mesofauna biomass			Nematode biomass		
		Estimate	SE	*p*	Estimate	SE	*p*	Estimate	SE	*p*
Community	Intercept	8.54	0.42	0.00	7.04	0.43	0.00	8.79	0.41	0.00
	Restored	0.42	0.42	0.32	-0.01	0.44	0.98	-0.12	0.40	0.77
	Unrestored	0.26	0.57	0.66	0.47	0.59	0.43	0.11	0.54	0.84
Predators	Intercept	6.64	0.49	0.00	5.86	0.52	0.00	5.27	0.69	0.00
	Restored	0.09	0.49	0.85	-0.15	0.52	0.78	0.79	0.69	0.25
	Unrestored	-0.20	0.66	0.76	-0.10	0.72	0.89	0.80	0.85	0.35
Omnivores	Intercept	7.29	0.64	0.00	6.27	0.60	0.00	6.75	0.45	0.00
	Restored	0.85	0.64	0.19	0.05	0.61	0.93	-0.21	0.44	0.63
	Unrestored	0.82	0.87	0.35	0.81	0.84	0.34	0.06	0.60	0.92
Herbivores-predators	Intercept				1.61	0.92	0.09			
	Restored				0.86	0.90	0.34			
	Unrestored				2.23	1.05	**0.04**			
Herbivores	Intercept	5.73	0.67	0.00				7.14	0.76	0.00
	Restored	0.10	0.67	0.88				-0.28	0.71	0.69
	Unrestored	0.38	0.90	0.68				0.22	0.95	0.82
Detritivores	Intercept	7.76	0.70	0.00	5.36	0.61	0.00			
	Restored	0.17	0.64	0.79	0.14	0.62	0.83			
	Unrestored	-0.31	0.87	0.73	0.03	0.85	0.97			
Fungivores	Intercept	1.93	1.30	0.14				4.96	0.56	0.00
	Restored	0.88	1.31	0.50				0.12	0.57	0.83

	Unrestored	1.65	1.44	0.25				0.41	0.77	0.60
Fungivores-herbivores	Intercept				-0.58	1.62	0.72			
	Restored				2.07	1.61	0.20			
	Unrestored				0.12	2.21	0.96			
Bacterivores	Intercept							8.20	0.43	0.00
	Restored							-0.07	0.42	0.87
	Unrestored							0.10	0.57	0.86

Table S3. Mean invertebrate body mass comparisons between restored, unrestored, and remnant forest (intercept) sites for community-level and trophic group analyses. Coefficients tables are from linear mixed effect modes, with 'city' specified as a random effect. SE is the standard error of the coefficient estimate. The remnant forest site category was selected as the intercept by the model.

Trophic levels	Restoration status	Macrofauna body mass			Mesofauna body mass			Nematode body mass		
		Estimate	SE	*p*	Estimate	SE	*p*	Estimate	SE	*p*
Community	Intercept	1.66	0.40	0.00	-4.10	0.24	0.00	-6.49	0.32	0.00
	Restored	-0.05	0.40	0.90	0.14	0.24	0.55	-0.04	0.32	0.90
	Unrestored	-0.45	0.54	0.41	0.63	0.32	0.06	-0.03	0.45	0.94
Predator	Intercept	0.55	0.51	0.28	-3.52	0.32	0.00	-5.55	0.36	0.00
	Restored	0.49	0.50	0.33	-0.22	0.31	0.49	-0.09	0.36	0.81
	Unrestored	0.20	0.68	0.77	-0.05	0.43	0.90	0.06	0.44	0.90
Omnivore	Intercept	1.90	0.55	0.00	-4.11	0.42	0.00	-5.85	0.44	0.00
	Restored	-0.27	0.52	0.61	0.34	0.42	0.42	-0.22	0.44	0.62
	Unrestored	-0.27	0.70	0.71	0.63	0.56	0.26	-0.06	0.59	0.92
Herbivores-predators	Intercept				-6.06	0.66	0.00			
	Restored				0.53	0.65	0.42			
	Unrestored				2.04	0.88	**0.02**			
Herbivore	Intercept	0.35	0.60	0.56				-7.65	0.58	0.00
	Restored	0.40	0.61	0.52				0.02	0.59	0.97
	Unrestored	0.18	0.85	0.84				0.57	0.81	0.48
Detritivore	Intercept	2.59	0.34	0.00	-4.71	0.23	0.00			
	Restored	-0.99	0.33	**0.00**	0.33	0.22	0.13			
	Unrestored	-1.29	0.45	**0.01**	-0.28	0.29	0.34			
Fungivore	Intercept	-0.63	1.06	0.56				-8.51	0.24	0.00
	Restored	0.57	1.09	0.60				0.14	0.23	0.54

	Unrestored	-0.77	1.30	0.56				0.90	0.32	**0.01**
Fungivores-herbivores	Intercept				-5.93	0.83	0.00			
	Restored				0.93	0.83	0.27			
	Unrestored				0.12	1.09	0.91			
Bacterivore	Intercept							-6.15	0.28	0.00
	Restored							0.04	0.28	0.90
	Unrestored							-0.21	0.39	0.59

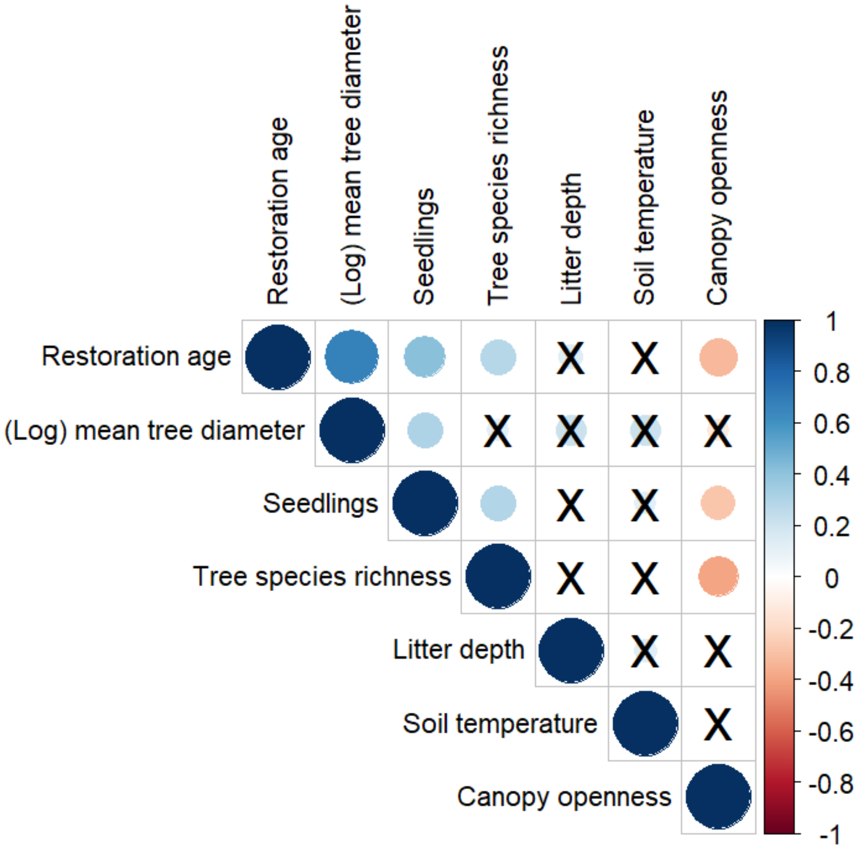

Figure S1. Pearson correlation coefficients for age of the forest i.e., restoration age and environmental variables. Positive correlations are represented in blue, while negative correlations are shown in red. The intensity of the colour and the size of the circles correspond to the strength of the correlation coefficients. On the right side, the colour legend indicates the correlation coefficients and their associated colours. 'X' denotes a statistically non-significant correlation (i.e., p-value > 0.05).

Chapter 3 . Indirect effects of restoration planting age across trophic levels of soil food webs

3.1 Abstract

Successional changes in vegetation can have indirect effects on soil invertebrate communities by impacting resource-consumer trophic interactions. However, the nature of these effects in restoration planting context is poorly understood. To better understand these effects, I examined soil invertebrate communities in 70 restored urban forests across eight cities in Aotearoa New Zealand. These sites, once occupied by native forest ecosystems, were cleared for agriculture or urban development before restoration planting with native woody species between 6 and 60 years ago, forming a chronosequence. I collected soil invertebrates from all these sites, identifying macrofauna and mesofauna to either order or family as possible, and nematodes to genus level. Then I classified them into three broad trophic levels: decomposers, omnivores and predators. I estimated the body mass of each individual using allometric scaling equations and then calculated the biomass of each trophic group. Using structural equation models, I tested for any direct and indirect effects of forest restoration age, mean tree diameter, tree species richness, and litter depth and soil temperature on biomass of soil invertebrates across the three trophic levels. I found that forest restoration age had positive and negative indirect effects on invertebrate biomass of trophic levels modulated primarily by mean tree diameter and tree species richness. The effect of forest restoration age was particularly positively strong on mean tree diameter compared to tree species richness. Mean tree diameter had a direct positive effect on the biomass of mesofauna decomposers, while tree species richness had a direct negative effect on the biomass of mesofauna omnivores and nematode predators. Mesofauna were the group most responsive to variation in forest restoration age and environmental predictors, and nematodes were the least responsive. Macrofauna decomposers were negatively affected by litter depth, whereas they responded positively to soil temperature. My results demonstrate that increasing forest restoration age does not directly influence the biomass structure of soil food webs. Instead, the effects are likely to be better observed through the indirect impacts forest restoration age creates in the wider ecosystem, e.g., via changes in vegetation characteristics resulting in bottom-up trophic cascades from decomposers to predators. These findings highlight the significance of restoring forest with specific vegetation characteristics such as encouraging growth of large trees to support restoration of the soil community.

3.2 Introduction

Vegetation changes during secondary forest succession are among the best-documented of all ecological processes. Secondary succession usually sees a decrease in tree species richness as the early successional phase comes to an end (Bonet & Pausas 2004; Amici *et al.* 2013), a difference from early stage of primary succession where there is an increase in tree species richness (Carswell *et al.* 2012). As the succession progresses to late stage, it involves slow growing large forest species communities (Lebrija-Trejos *et al*. 2010) which are more-distantly related to each other than early successional communities (Letcher 2010; Letcher *et al*. 2012). Such shift from fast growing tree species during early succession to slow growing trees results in changes in plant traits such as shift from high specific leaf area (SLA) and leaf nitrogen concentration (LNC) to low SLA and LNC at the later stages of succession (Garnier *et al.* 2004). Functional (e.g. canopy height, plant life-form) and phylogenetic (e.g., seed mass, seed production) diversity can also increase during early-mid and late-successional stages (Purschke *et al.* 2013). Changes in vegetational characteristics between successional phases typically lead to increase in vegetation biomass (Brown & Lugo 1990; Wardle *et al*. 2004b; Chazdon *et al*. 2007), tree basal area, and canopy cover (Lebrija-Trejos *et al*. 2010; Lu *et al*. 2014). As a result, light availability steadily declines during the early stages of succession (Denslow & Guzman G. 2000; Matsuo *et al*. 2021).

In contrast, the development of soil invertebrate communities during secondary succession is still a budding area of research with studies finding mixed patterns in assemblage of functional diversity and species richness. For example, a study by Kaufmann *et al*. (2002) suggests predators arrive before herbivores and decomposers, while another study found that decomposers such as oribatid mites, springtails and predators like spiders arrive at the same time (Hodkinson *et al*. 2004). Furthermore, Schlegel & Riesen (2012) found a progressive increase in carabid beetle species (predators) richness but Bokhorst *et al*. (2017) found no directional change in Acari (predator) mite species abundance or species richness. Additionally, there was a decrease in nematode diversity during succession according to Wall *et al*. (2002), but Wardle *et al*. (1995) found nematode diversity to increase as succession progresses. The disparity in these findings could be a result of complex networks of interacting trophic groups that respond differently to vegetation and other environmental characteristics (Wardle *et al*. 2004a; Wardle 2006). Trophic interactions such as competition for same resources (Wardle 2006), indirect effects of aboveground trophic interactions, change in organic matter, and resource input in the soil depending upon changes in vegetation

(Bardgett & Wardle 2003; Wardle *et al.* 2004a) as well as a combination of top-down and bottom-up forces can shape the structure of the soil community (De Ruiter *et al*. 1995; Moore *et al*. 2003).

The response of soil invertebrates to successional change in vegetation and environmental characteristics can be both direct and indirect (Jackson *et al*. 2000; Wolters *et al*. 2000; Wardle *et al*. 2004a). For example, an increase in primary productivity during the initial stages of succession can increase root biomass and soil organic matter, thereby having a direct effect on herbivorous and omnivorous nematodes (Wall *et al*. 2002; Laliberté *et al.* 2017). Additionally, soil organic matter and litter can affect the quality and quantity of resources for decomposers, thereby driving decomposer consumer species richness (Armbrecht *et al*. 2004). The increase in the decomposer community may then have indirect effects on omnivores and predators in the soil food web (Scheu & Schulz 1996; Bardgett *et al*. 2005; Williamson et al. 2005). Similarly, in a grassland biodiversity experiment, plant species richness was found to have bottom-up effects across trophic levels when belowground food webs were examined (Scherber *et al*. 2010). Additionally, an increase in the diversity of resources from vegetation and detritus can support more niches for soil invertebrates to exploit (Sinclair *et al*. 2003; Kissling *et al*. 2009). For example, a higher diversity of ant species was found when there was a greater diversity of twigs for them to build nests with (Armbrecht *et al.* 2004). Thus, increased ecosystem complexity formed during successional processes can boost resources available from vegetation biomass, litter and soil organic matter (Peltzer *et al.* 2010; Nielsen 2019b) and impact the soil invertebrate community.

Similarly, vegetation can influence consumers at higher trophic level also via soil microbes. Soil microbial biomass can affect the biomass of microbivore consumers (Wardle & Yeates 1993; Mikola & Setälä 1998). For example, Laliberté *et al*. (2017) found that an increase in bacterial biomass resulted in an increase in bacterial-feeding nematodes, and an increase in fungal biomass resulted in an increase in fungal-feeding nematodes. Furthermore, increase in the biomass of bacterial-feeding nematodes was followed by an increase in biomass of omnivorous and carnivorous nematodes, and the increase in fungal-feeding nematode biomass was followed by an increase in predatory mite biomass (Laliberté *et al*. 2017). A decrease in soil organic matter, along with a shift from a bacterial- to fungal-dominated energy channel (the latter is associated with lower nutrient availability (Williamson *et al*. 2005; Frouz *et al*. 2013)) can therefore result in a decrease in the abundance of enchytraeids (omnivores) and predatory nematodes at later successional stages (Williamson *et al.* 2005). Although the effects of vegetation succession on lower trophic level invertebrates can be strong, the strength of indirect effects typically decreases as the trophic distance increases between primary producers and consumers of higher trophic levels in the food chain (Scherber *et al*. 2010; Barnes *et al.* 2017).

An understanding of the impacts of long-term successional processes on different trophic levels of soil invertebrate communities is still lacking (Berg & Hemerik 2004; Tylianakis *et al.* 2008; Frouz et al. 2013). Most studies have been restricted to ≤ 30 years timescales — e.g., Piechnik *et al*. 2008; Morriën *et al.* 2017; Strickland *et al*. 2017, or to certain faunal groups — e.g., microflora (Tscherko *et al*. 2003), nematodes (Wall *et al*. 2002), nematodes and oribatid mites (Kardol *et al*. 2009), and Collembola (Chauvat *et al*. 2011). Furthermore, studies have highlighted the importance of linking changes in vegetation with soil invertebrate food webs (Ettema & Wardle 2002; Bardgett *et al*. 2005; De Deyn & Van Der Putten 2005; Fischer *et al*. 2019) at larger temporal and spatial scales to identify the processes that influence soil invertebrates at different trophic levels (Palmer *et al*. 1997; Maharning *et al*. 2009). Therefore, to understand how the biomass structure of soil food webs reassembles following restoration planting and ensuing succession, research is needed across longer, more continuous timescales that explicitly investigates the bottom-up effects of changing vegetation across trophic levels in soil food webs.

In this study, I apply a chronosequence approach to investigate the direct and indirect effects of forest restoration age (time since the initial restoration planting) across trophic levels in soil food webs. I collected soil samples across a restoration chronosequence of 70 urban restored forests ranging in age since planting from six to 60 years and calculated changes in the biomass of microbes and soil invertebrates (grouped by trophic level: decomposers, omnivores and predators) found in the soil samples. I hypothesised that forest restoration age would have indirect positive effects on the biomass of all trophic groups in the soil food web, which would result from the effects of forest restoration age on vegetation and other environmental variables. Furthermore, I expected that the greatest effect sizes of forest restoration age would occur at lower trophic levels (e.g., decomposers), with attenuating effect sizes at higher trophic levels (e.g., predators) (Scherber *et al.* 2010).

3.3 Methods

3.3.1 Study location

In this study I sampled soil communities across the North and South Islands of Aotearoa New Zealand during the austral summer of November 2019 – February 2020. Soils were collected in restored urban forest sites (n = 70) in eight cities distributed across a latitudinal gradient of approximately 9º, with mean annual temperatures ranging from a lowest of 10.1º C in Invercargill (Climate-data.org 2022) to highest of 14.8 ºC in Tauranga (NIWA 2019). There were 8–11 sites per city, all of which were on slopes of < 15º. The sites were all restored urban forests which were planted with native tree and shrub species in a single initial cohort on retired farmland or mowed parkland. The restored forest sites represented a chronosequence spanning 6–60 years in age since their initial restoration planting.

3.3.2 Experimental design and sampling

Data were gathered from a single 20 m × 10 m plot at each site. Soil invertebrates were sampled in three subplots of 50 cm × 50 cm placed diagonally across the plot, located at the centre, corner two, and corner four (Fig. 1B). Within each subplot, I collected one 22 cm diameter soil core and one 5 cm diameter soil core to sample macrofauna and mesofauna, respectively, and five 2.7 cm diameter soil cores to sample nematodes and the microbial community. To capture spatial heterogeneity within each subplot, all soil cores were collected at random points and with a distance of *ca.* 22 cm between coring locations. All soil cores were taken from a depth of 10 cm from the surface of the soil organic layer. To sample the food web as completely as possible, soil cores were taken with the litter layer left in place, but the litter layer did not count towards the 10 cm coring depth.

Soil temperature data were collected from the centre point and corner 2 via iButton data loggers deployed at 10 cm depth. Temperatures were logged every 4 hours from 24 March 2018 to 24 June 2018. Canopy openness (measured as sky visible through the forest canopy) was used as a proxy for light reaching the forest floor and microclimatic conditions on the forest floor. Canopy openness was estimated using a convex densiometer (Convex model A; Forestry Suppliers, Jackson, Mississippi, USA), with a measurement taken once at each corner and the centre of each plot. The average of these five densiometer measurements was then calculated and used in statistical analysis. Leaf litter depth was measured using a ruler at five points in each subplot (at each of the four corners and centre) from which I calculated an average depth which as used for analysis. Each measurement was taken from the surface of the organic horizon to the height of the litter layer at that point.

Vegetation was sampled throughout the same 200 m^2 plot where soil invertebrates were sampled. It was assessed by identifying species and measuring diameters at breast height (DBH) of all trees and shrubs $\geq$ 2.5 cm diameter at breast height in each plot and taller than 135 cm. DBH measurements were then used to calculate the mean quadratic diameter (Saud et al. 2016) of all trees in the plot. Seedling density was calculated by tallying the total number of woody species present under a height of 135 cm across 10 circular sub-plots which together covered a total of 70.7 m^2 of the plot and were later scaled up to approximate seedling density per 200 m^2.

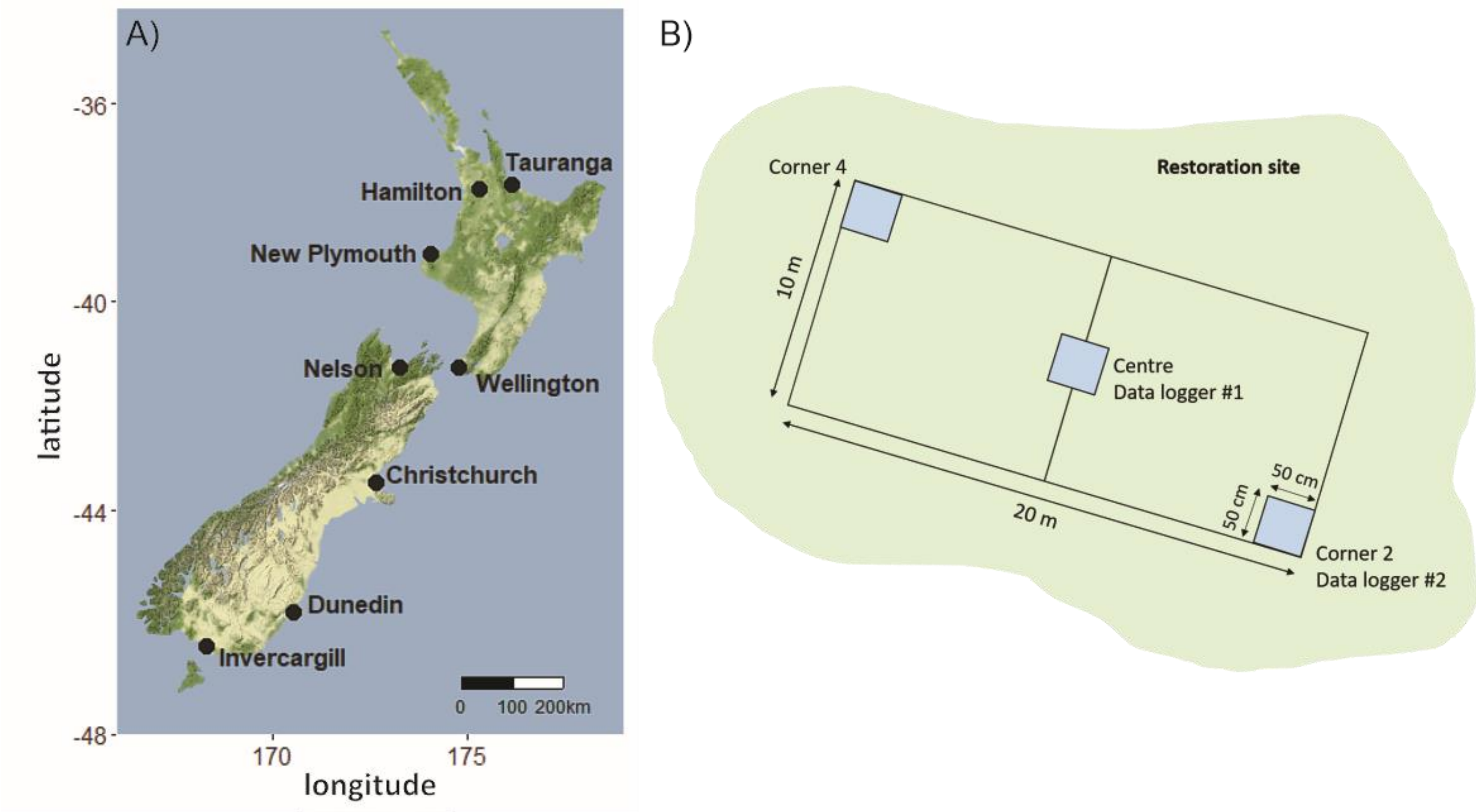

Figure 1. A) Map of Aotearoa, New Zealand showing the eight cities where soil samples were collected. B) The 76 urban sites distributed across the eight cities (including unrestored sites, restored forests, and remnant forests) were 10 m x 20 m, with three sub-plots (indicated by blue squares) located at the centre, corner two, and corner four of each site where belowground invertebrates were sampled. Temperature data loggers were buried at the centre and corner two of the restoration sites.

3.3.3 Extraction of soil invertebrates and microbes

Soil macrofauna (body width ≥ 2 mm), such as millipedes, centipedes, and beetles, were extracted from the largest soil cores using modified 'Kempson' high-gradient heat extractors (Edwards 1991). The smaller soil mesofauna (body width 0.1 mm–2 mm), such as mites and springtails, were collected following a similar approach using a Macfadyen high-gradient heat extractor (Edwards 1991). Extractions were run for at least 11 days using a programmed temperature ramp starting at 20 °C on day one and reaching 55 °C in the last two days of the extraction. To extract free-living soil nematodes, soil from the five cores per subplot was sieved using a 2-mm mesh and homogenised. Nematodes were then extracted from 25 g of this sieved, homogenised soil using an active organism 'Baermann funnel' method (Coleman & Wall 2015; Cesarz *et al.* 2019a). Extracted nematodes were collected and stored in 4% formalin. I then calculated the dry weight of the soil samples to obtain nematode abundance per 100 g of dry soil (Cesarz *et al.* 2019a).

The soil samples from the five 2.7 cm diameter cores per subplot were homogenised and a 150g subsample was taken for microbial analysis. Substrate-induced respiration (SIR) technique was used to measure microbial biomass (µg C/g soil dry mass). The SIR technique was performed on an O_2-microcompensation apparatus. The dry soil was added with 8mg/g of glucose in water solution and incubated for over 24-hours. During this incubation period, metabolically active microorganisms were detected by recording O_2 consumption.

3.3.4 Soil invertebrate identification and measurements

Determining functional roles for each species is an important approach for understanding their significance in a successional framework (Montoya *et al*. 2012). These roles can be assigned via their trophic interactions with other species in the system (Dehling *et al*. 2016). I used this approach to quantify trophic interactions, and therefore macrofauna (including adults and larvae) and mesofauna samples were sorted and identified in the lab according to Naumann (1991) and various online sources (CSIRO, 2012; VanDyk *et al.* 2020). Specimens were identified to a taxonomic level (typically to order or family) that was sufficient to determine their general feeding behaviour, which was assigned based on Potapov *et al*. (2022) and Potapov (2022). Trophic groups were further assigned to all macrofauna, mesofauna and nematodes. Adults were not distinguished from juveniles for all invertebrate groups.

Macrofauna samples were first identified as detritivores, fungivores, herbivores, omnivores and predators. Mesofauna were identified as detritivores, fungivore-herbivores (invertebrates that are both fungivores and herbivores), herbivore-predators (invertebrates that are both herbivores and predators), omnivores, and predators. Similarly, nematodes were first identified as bacterivores, fungivores, herbivores, omnivores and predators. For later inclusion in the structural equation model, I excluded herbivores (invertebrates with exclusive herbivore feeding behaviour were absent in mesofauna samples) for all these invertebrate groups and re-categorised them into three main trophic levels (Fig. 2). A general term 'decomposers' was used for detritivores, fungivores and bacterivores to accommodate all invertebrates that feed on soil organic matter and associated microbes growing on it (Potapov *et al*. 2019).

Following identification of each individual, assignment to a functional role, and grouping into trophic level, the body length (mm) of each macro- and mesofauna individual was measured

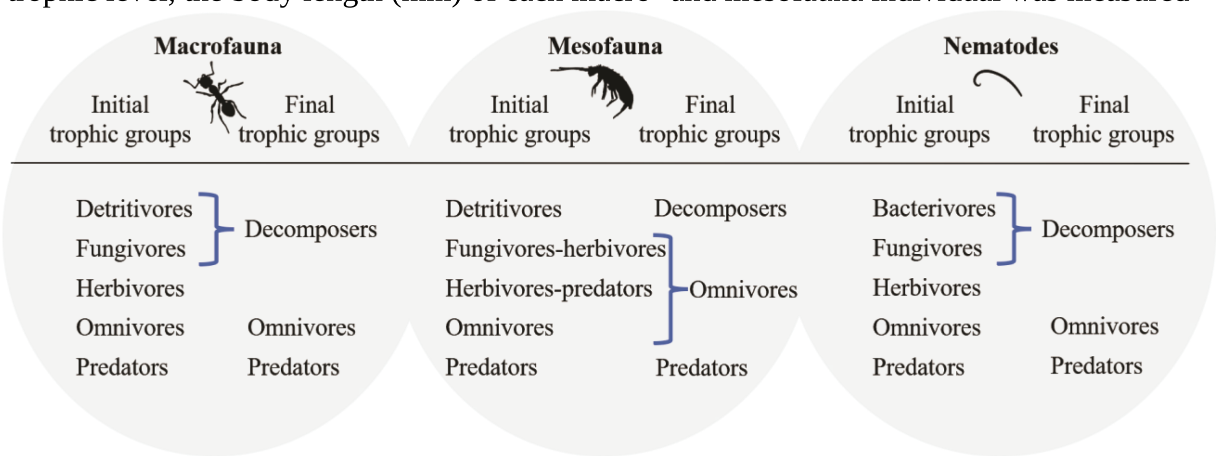

Figure 2. Attribution of final trophic levels used in the Structural Equation Model based on initial trophic groups used in soil fauna identification.

using a stereo microscope with an ocular micrometre. I then calculated the fresh body mass (mg) of each individual using published length-mass scaling relationships (Table S1 & S2) for meso- and macrofauna (Mercer *et al.* 2001; Barnes *et al.* 2014a; Sohlström *et al.* 2018). Gastropods and leeches were excluded from analyses due to extremely low numbers in the samples (63 and 1, respectively, out of a total 15,831 individuals). The body masses of Coleopteran, Dipteran, and Lepidopteran larvae were estimated using the Coleoptera larvae-specific formula in Sohlström *et al*. (2018) because no other taxa-specific formula was available for the Diptera and Lepidoptera. Furthermore, for some groups, there was no regression formula available in the literature. Therefore, for Diplura and Symphyla, I used a scaling relationship published for Dermaptera and Chilopoda, respectively (Barnes *et al.* 2014a; Sohlström *et al.* 2018).

The first hundred nematodes encountered per plot were identified to genus, according to "De Nematoden van Nederland" (Bongers 1994). Those one hundred nematodes were assigned to trophic levels (bacterivores, fungivores, herbivores, omnivores, and predators). I later collected genus-specific information on body mass using the online platform Nemaplex ("Nemaplex Main Menu" 2022), which was based on female adults (but see Klusmann et al. (2022) for potential caveats). These body masses were then assigned to all associated genera of nematode individuals in my dataset. I first measured the abundance of the invertebrates as the number of individuals present at a particular site. The average body mass for all three invertebrate groups was calculated by taking the mean of fresh body mass for each invertebrate group at each forest plot. Total biomass was then calculated by summing all individual body masses for each group at each restoration plot.

3.3.5 *Data analysis*

I constructed linear mixed-effects models using the 'nlme' package (version 3.1.157) to test for the effects of biotic and abiotic variables on the biomass of soil invertebrate communities with 'city' as a random effect. Before running models, I checked the normality and homoscedasticity of the data, and log-transformed variables to satisfy assumptions where necessary. The log transformed variables included mean tree diameter, litter depth, macrofauna, mesofauna & nematode decomposers, macrofauna & mesofauna omnivores and macrofauna & mesofauna predators. I then used piecewise structural equation modelling (R package '*piecewiseSEM'*, version 2.1.2) (Shipley 2009; Lefcheck 2016) to test for the direct and indirect effects of forest restoration age across trophic levels of the soil food web. Structural Equation Model is a framework which uses theoretical ideas to understand direct and indirect effects between variables to understand multiple processes that exist in a system (Grace *et al*. 2010; Du *et al*. 2015; Eisenhauer *et al*. 2015). While identifying these direct and indirect effects, SEMs describes multiple causal pathways between the variables, the relative strength of which can be compared, to better understand complex ecological processes (Du *et al*. 2015; Eisenhauer *et al*. 2015). For this study, forest restoration age was used as the main, exogenous predictor variable with mean tree diameter, tree species richness, litter depth, soil temperature, and microbial, macrofaunal, mesofaunal and nematode biomass as response variables. The latter three groups were further categorised as decomposers, omnivores, or predators to be able to model bottom-up indirect effects of forest restoration age on soil food web biomass structure.

To build the model, I first included all possible ecologically meaningful relationships between potential predictor and response variables in a maximal model (Fig. S1). I then iteratively removed relationships with weak effects based on the standard estimates and p-values (this constituted standard estimate values close to 0 with corresponding p-values > 0.5) and checked for changes in the global model AIC value. Relationships were only removed if the global AIC value decreased by at least two units. This allowed us to identify the final minimal adequate model (Supplementary material; Table 2) with the lowest possible AIC based on the original hypothetical model structure. Direct effect sizes were obtained from the standardised estimates of predictor effects on response variables. Indirect effect sizes were obtained by multiplying the standardised estimates of each predictor on respective response variables along pathways of effects in the final SEM. All data analyses were run in R (version 4.2.0) (R Core Team 2022).

3.4 Results

My results show that during forest succession, increasing restoration age indirectly drives bottom-up effects on the biomass of trophic groups in soil food webs. Forest restoration age was shown to have direct effects on mean tree diameter and tree species richness, with a particularly strong effect on the former (Fig. 3). These vegetation variables, in turn, had direct effects on the biomass of mesofauna decomposers and omnivores. In addition, litter depth and soil temperature also significantly affected macrofaunal and mesofaunal groups, though these effects appeared to be completely independent of forest restoration age. Most of the significant effects detected in the SEM on the biomass of trophic groups were positive. Notable exceptions to this finding were the negative effects of tree species richness on mesofauna omnivores and nematode predators, the effect of litter depth on macrofauna decomposers, and the negative effect of microbial biomass on nematode decomposers (Fig. 3). Macrofauna decomposers and mesofauna omnivores were the only trophic levels which were responsive to more than one predictor variable described in the SEM. But overall, I found that mesofauna as a trophic group was the most responsive to changes in both the vegetation and environmental variables, compared with macrofauna and nematodes. Interestingly, I did not find any significant effects of forest restoration age, mean tree diameter, tree species richness, and litter depth or soil temperature on the biomass of microbes (Fig. 3).

Forest restoration age did not have direct effects on biomass of soil invertebrates (Fig. 3). The SEM did not find any significant direct effect of forest restoration age on any trophic level of any trophic group. The only direct effect of forest restoration age was observed on macrofauna predators, which was not statistically significant (Fig. S2). Instead, all effects of forest restoration age on the soil food web resulted from shifts in mean tree size and richness during succession. The effect of forest restoration age on tree species richness, compared with mean tree diameter, was weak and only explained 10% of the total variation in tree species richness.

Mean tree diameter, which was positively influenced by forest restoration age and soil temperature, had a positive direct effect on mesofauna decomposers and positive indirect effect on mesofauna predators. A negative direct effect of tree species richness was detected on nematode predators and mesofauna omnivores, the later resulting in a negative indirect effect on mesofauna predator biomass. Litter depth had various influences, with negative effect on macrofauna decomposers which in turn had a positive effect on macrofauna predators. In contrast, litter depth had a strong positive indirect effect on mesofauna predators via mesofauna omnivores.

In addition to the positive effect of soil temperature on mean tree diameter, warmer soils were also associated with greater biomass of macrofauna decomposers, which was shown to have a positive indirect influence on macrofauna predators. Furthermore, the effect of mesofauna decomposers on mesofauna predators was stronger than the effect of macrofauna decomposers on macrofauna predators. All three trophic levels of mesofauna had a statistically non-significant effect on either macrofauna omnivores or macrofauna predators with no effect detected going the other way round (Fig. S2). As for nematodes, none of the environmental predictors, except for tree species richness, were found to have any significant effect on the three nematode trophic levels. Additionally, no significant effect of any kind was detected between nematodes and either mesofauna or macrofauna in any of the trophic levels.

Despite not detecting any significant effects of forest restoration age, vegetation, or environmental characteristics on microbial biomass, there were several significant effects of microbial biomass across all three invertebrate taxonomic groups. In particular, there was a positive effect of microbial biomass on macrofauna decomposers and mesofauna predators but a negative effect on nematode decomposers (Fig. 3). Mesofauna and omnivores of all taxonomic trophic groups were more responsive to the indirect effect of forest restoration age than any other taxonomic and trophic groups (Fig. S2).

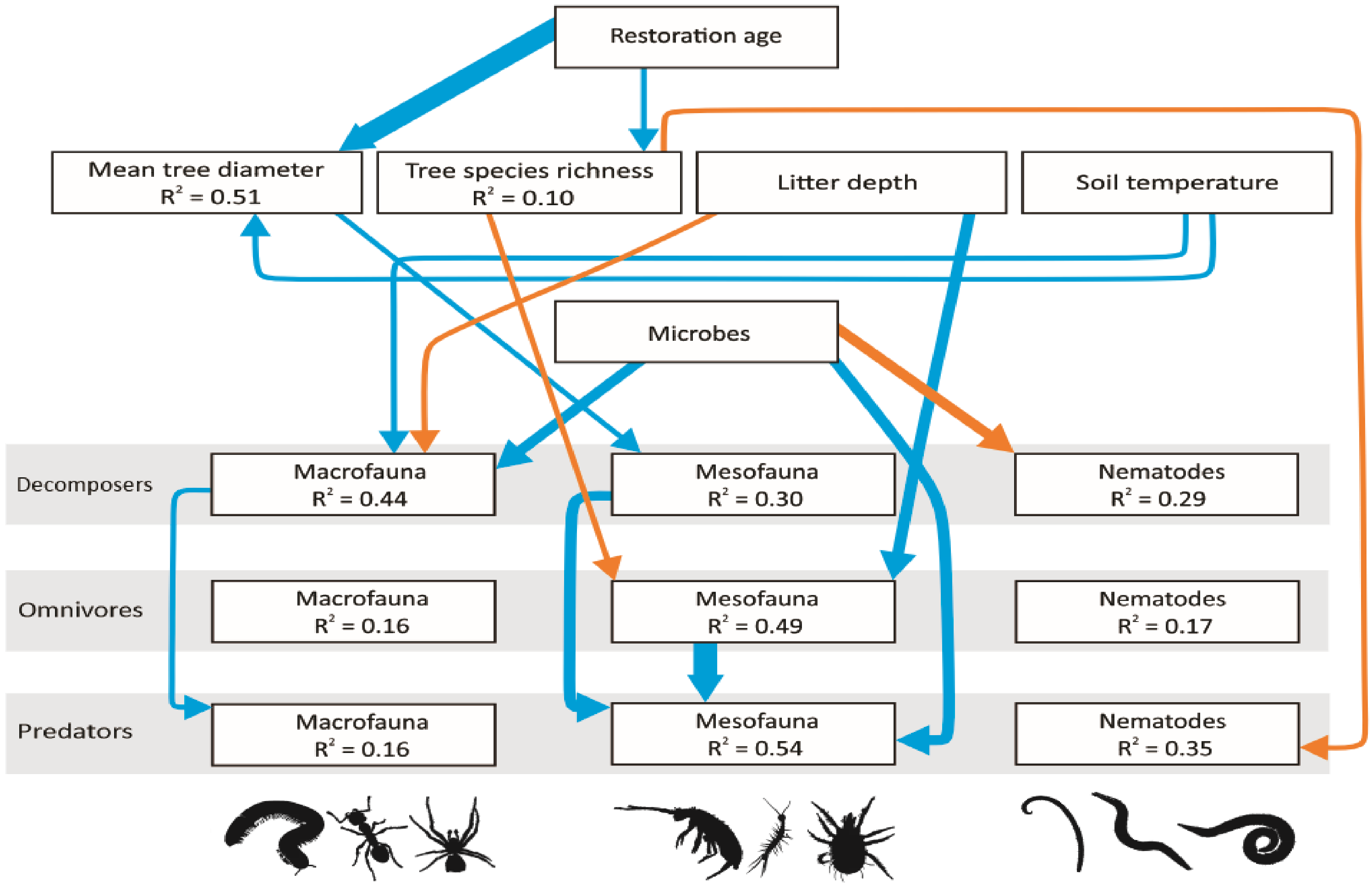

Figure 3. Minimum adequate structural equation model describing direct and indirect effects of forest restoration age on the biomass of major trophic groups in developing forest soil food webs ($\chi 2$ = 39.244, d.f. = 76, P = 1, AIC = 191.244). Blue and orange arrows denote positive and negative effects, respectively. Arrow widths are proportional to the range-standardised coefficients and indicate relative effect strengths (i.e., the wider the arrow, the stronger the effect). R^2 values indicate the proportion of variance explained in the respective response variable by the effects of all associated predictor variables (i.e., those with arrows leading to the response variable).

Overall, forest restoration age did not have any direct effects on any of the trophic groups included in the model (Fig. 4A, Table 1). Instead, it had indirect positive effects on decomposers, and negative indirect effects on omnivores and predators. Mean tree diameter had direct effects on decomposers and indirect effect on predators, both within the mesofauna group only, though it had neither direct or indirect effects on any of the three trophic levels for macrofauna and nematodes. The model did not detect any effects of tree species richness on any of the three macrofauna trophic levels, but it had direct effects on omnivorous mesofauna through which it had a positive indirect effect on predatory mesofauna. Tree species richness had a direct negative effect on predatory nematodes (Fig. 3). Litter depth and soil temperature had direct effects on decomposers which led to indirect effects on predators in the macrofauna group. Litter depth also had a indirect effect on mesofauna predators via mesofauna omnivores. Taken together, my results show that direct effects were more common for decomposers and omnivores, whereas indirect effects were more common for predators (Fig. 4, Table 1). Indirect effects were also slightly stronger than direct effects (average effect strength was 56.9% and 43.1% of the total effect for indirect and direct effect, respectively). There was more positive effect on decomposers (n = 3) than negative effects (n = 1), and more negative effect (n = 2) on omnivores than positive (n = 1), while the model showed an equal number of positive and negative effects on predators (n = 3).

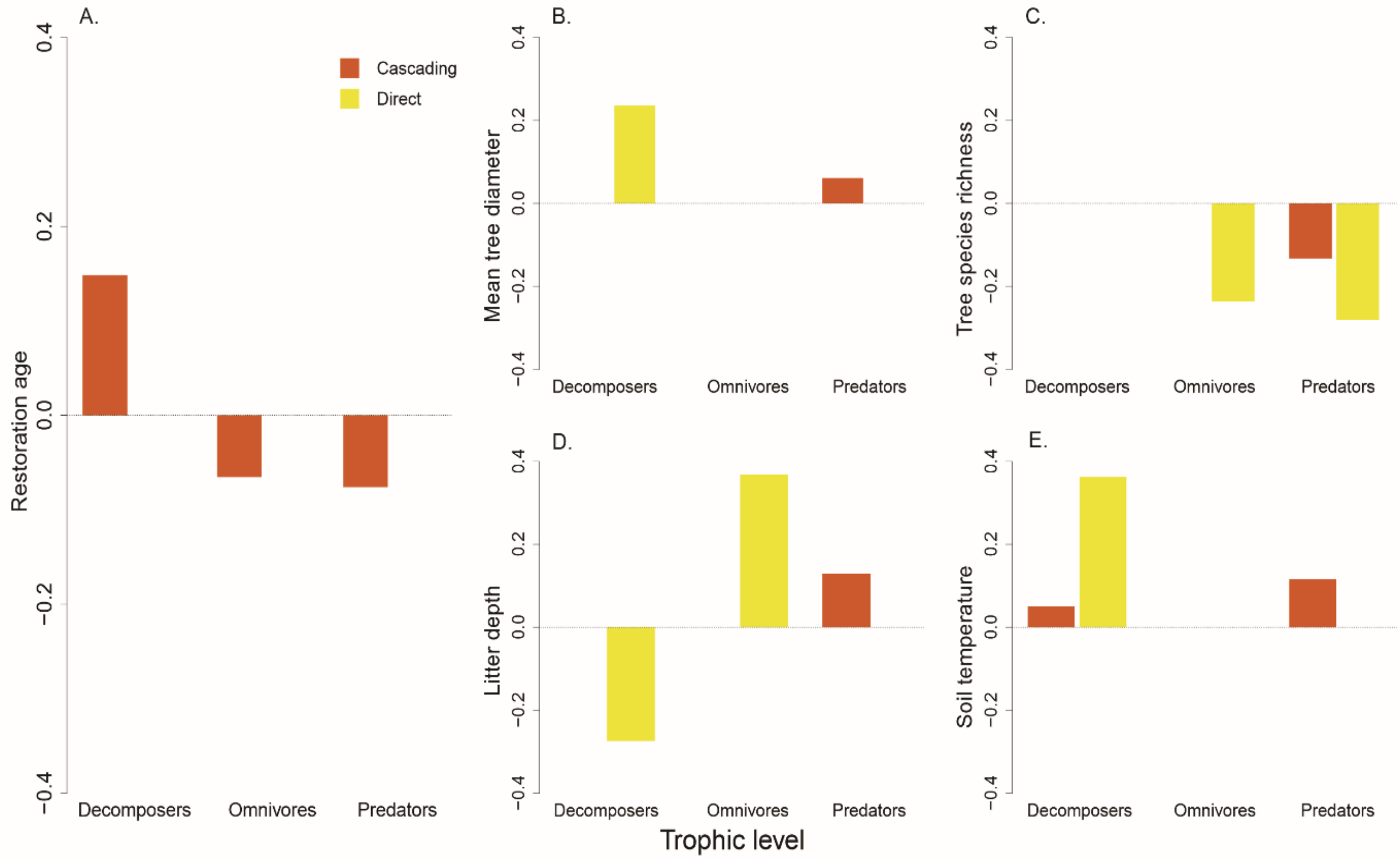

Figure 4. Mean standardised effect sizes of direct and indirect effects of restoration age, and other site characteristics on the biomass of macrofauna, mesofauna and nematodes in soil food webs. Indirect effects were estimated by multiplying standard estimates along pathways of interacting trophic groups in the path model. Red and yellow bars respectively represent indirect and direct mean standardized effect sizes across trophic levels in the soil food webs.

Table 1. Direct and indirect effects for all predictor and response variables in the structural equation model (SEM). Indirect effects were estimated by multiplying coefficients along pathways of significant effects in the SEM.

Response	Predictor	Standardised effects	
		Direct	Indirect
Mean tree diameter	Restoration age	0.630	
	Litter depth	0.100	
	Soil temperature	0.213	
Tree species richness	Restoration age	0.280	
Macrofauna decomposers	Restoration age		-0.038
	Tree species richness	-0.140	
	Litter depth	-0.274	
	Soil temperature	0.362	
Mesofauna decomposers	Restoration age		0.148
	Mean tree diameter	0.235	
	Litter depth	0.113	0.023
	Soil temperature		0.050
Nematode decomposers	Restoration age		0.095
	Mean tree diameter	0.151	
	Litter depth		0.015
	Soil temperature	-0.260	0.032
Macrofauna omnivores	Restoration age		0.016
	Mean tree diameter		-0.033
	Tree species richness	0.189	-0.053
	Litter depth		0.006
	Soil temperature		-0.052
Mesofauna omnivores	Restoration age		0.005
	Mean tree diameter	0.093	0.018
	Tree species richness	-0.237	
	Litter depth	0.368	0.023
	Soil temperature	-0.252	0.074
Nematode omnivores	Litter depth	0.156	
	Soil temperature	-0.202	
Macrofauna predators	Restoration age	0.206	-0.160
	Mean tree diameter	-0.223	-0.010
	Tree species richness		-0.048

	Litter depth		-0.048
	Soil temperature		0.056
Mesofauna predators	Restoration age		0.052
	Mean tree diameter		0.096
	Tree species richness	0.103	-0.132
	Litter depth		0.258
	Soil temperature	0.130	-0.092
Nematode predators	Restoration age		-0.146
	Mean tree diameter	-0.146	0.036
	Tree species richness	-0.281	
	Litter depth		0.020
	Soil temperature		-0.126

3.5 **Discussion**

This study demonstrates that exploring only the direct relationship between forest restoration age and soil invertebrate community assembly may be inadequate to capture the true effects of successional processes on belowground communities. I found that effects of forest restoration age on the biomass of soil invertebrates were mostly indirect and mediated by vegetation characteristics. I further showed that increases in both tree size and tree species richness in maturing forest ecosystems are major drivers of soil invertebrate community succession. The influence of these changing vegetation characteristics, however, was both positive (from mean tree size) and negative (from tree species richness) and was dependent on the trophic group of invertebrates. My results suggest that decomposers are most likely to be directly affected by both forest restoration age and environmental characteristics, which partially supports my initial hypothesis that the greatest effect sizes would occur at lower trophic levels. Despite expectations based on previous findings (Scherber *et al.* 2010), the effects of restoration age did not, however, consistently lessen in strength from lower to higher trophic levels.

My findings suggest that increasing forest restoration age has indirect bottom-up effects on the biomass of soil invertebrates, and these are mediated by changes in vegetation characteristics as succession progresses (Fig. 3). The positive effect of mean tree diameter on mesofauna decomposers probably reflects a positive effect of tree biomass on soil organic matter through increasing detritus input (Nielsen 2019b). This consequently provides more habitat structure and basal resources for decomposers (Kalif *et al*. 2001; Bihn *et al.* 2008).

Furthermore, larger trees tend to form more complex root systems producing more root exudates, which are resources for soil invertebrates (Johnson & Turner 2019; Balandier *et al*. 2022). Such increases in multiple resources via these different plant-derived energy channels, as well as effects of vegetation on habitat structure (Barantal *et al*. 2011; Joly *et al.* 2017) over time can influence the biomass production of decomposer organisms. This also potentially explains the positive effect of forest restoration age on decomposer biomass (Fig. 4A) since there is increase in mean tree diameter as succession progresses (Lebrija-Trejos *et al*. 2010). Additionally, changes in tree species richness during successional development also affects litter quality and nutrient availability in soil (Vilà *et al*. 2005; Auclerc *et al.* 2019), both of which are likely to influence soil invertebrates of lower trophic level.

Forest restoration age had an indirect negative effect on the biomass of mesofauna omnivores but indirect positive effect on mesofauna predators, both of which were mediated by tree species richness. Increases in tree species richness can result in increased niche partitioning among herbivores (Schoener 1974; Kartzinel *et al*. 2015). Since specialists (as herbivores typically are (Bernays & Graham 1988)) are better equipped to assimilate food/nutrients (vegetation) through their special functional and morphological features in comparison to generalists (omnivores) (Sanderson 1991), herbivores can exploit the available resources proficiently, thereby reducing availability of the resources for omnivores or interfere with the ability of omnivores to obtain resources (Schoener 1974). This potentially explains the negative effect of tree species richness on mesofauna omnivores that I found in my study. Increases in habitat and food resources resulting from increasing tree species richness can also support higher numbers of prey species for predators (Koricheva *et al*. 2000; Jactel *et al*. 2005; Dinnage *et al*. 2012; Staab *et al*. 2014). Consequently, increases in such prey resources, omnivores included, can have a strong positive effect on the overall biomass of predators. This reasoning is further supported by the existing ‘enemies hypothesis’ which suggests tree species mixtures can support larger predator populations than monocultures (Root 1973; Staab *et al*. 2014). However, the negative effect of restoration age on predators in general (Fig. 4A) suggests that the increase in refuge for prey species in older successional forests can make them less vulnerable to predation (Riihimäki *et al*. 2005). Moreover, additional factors such as cannibalism and competition can also be influential in keeping the predator biomass in check (Mooney et al. 2010; Digel *et al*. 2014). In other words, prey availability alone cannot always be a determining factor in predator activity (Schuldt *et al.* 2011). So, the general negative trend in predator biomass with increasing forest age suggests that when the tree species richness starts to decrease during succession, trophic interactions among predators (i.e., intraguild predation) may become increasingly important in regulating the observed patterns.

The prevalence of indirect effects compared to direct effects in the restored forests in my study (Fig. 3) suggests that bottom-up trophic cascades are a key mechanism shaping the succession of forest ecosystems. I found forest restoration age can have both positive and negative indirect effects on different trophic levels in soil invertebrates. Given that vegetational shifts (e.g. tree species richness (Amici *et al*. 2013)) occurs during succession, it is expected that detritus input and nutrient availability in soil also changes with such shifts in vegetation (Johnson & Turner 2019; Nielsen 2019b; Balandier *et al.* 2022). The effect of these shifts will eventually cascade and affect certain soil invertebrates, e.g., indirect effects of restoration age on (mesofauna) omnivores via tree species richness and on mesofauna (decomposers) via mean tree diameter (Fig. 3.). This is consistent with earlier studies which have found shifts in resource quantity and quality during succession have indirect effects on soil invertebrates at different trophic levels (Kaufmann *et al.* 2002; Williamson et al. 2005; Doblas-Miranda *et al.* 2008; Laliberté *et al.* 2017).

3.6 Conclusion

The results of this study suggest that successional development of restored forests can affect soil food web structure through changes in vegetation characteristics over time (Laliberté *et al.* 2017; Nielsen 2019b). I demonstrate that successional processes affect the biomass of soil invertebrates, particularly through increases in average tree size and tree species richness. These findings present a step towards filling the critical knowledge gap in our understanding of linkages between aboveground biotic and abiotic characteristics and soil food webs during forest succession. Therefore, restoration planting design could benefit from including a large number of tree species, which are managed to be able to grow larger, and practitioners should allow accumulating leaf litter to remain undisturbed on the forest floor. Results also show that the soil invertebrate community can develop without active intervention from practitioners, but may lag behind aboveground interventions such as the initial vegetation planting. Restoring ecosystem functioning above and belowground is critical for the success of any restoration effort (Benayas *et al*. 2009), and my study provides missing information on the indirect effects of forest restoration age across major functionally important trophic groups in soil food webs.

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Appendix

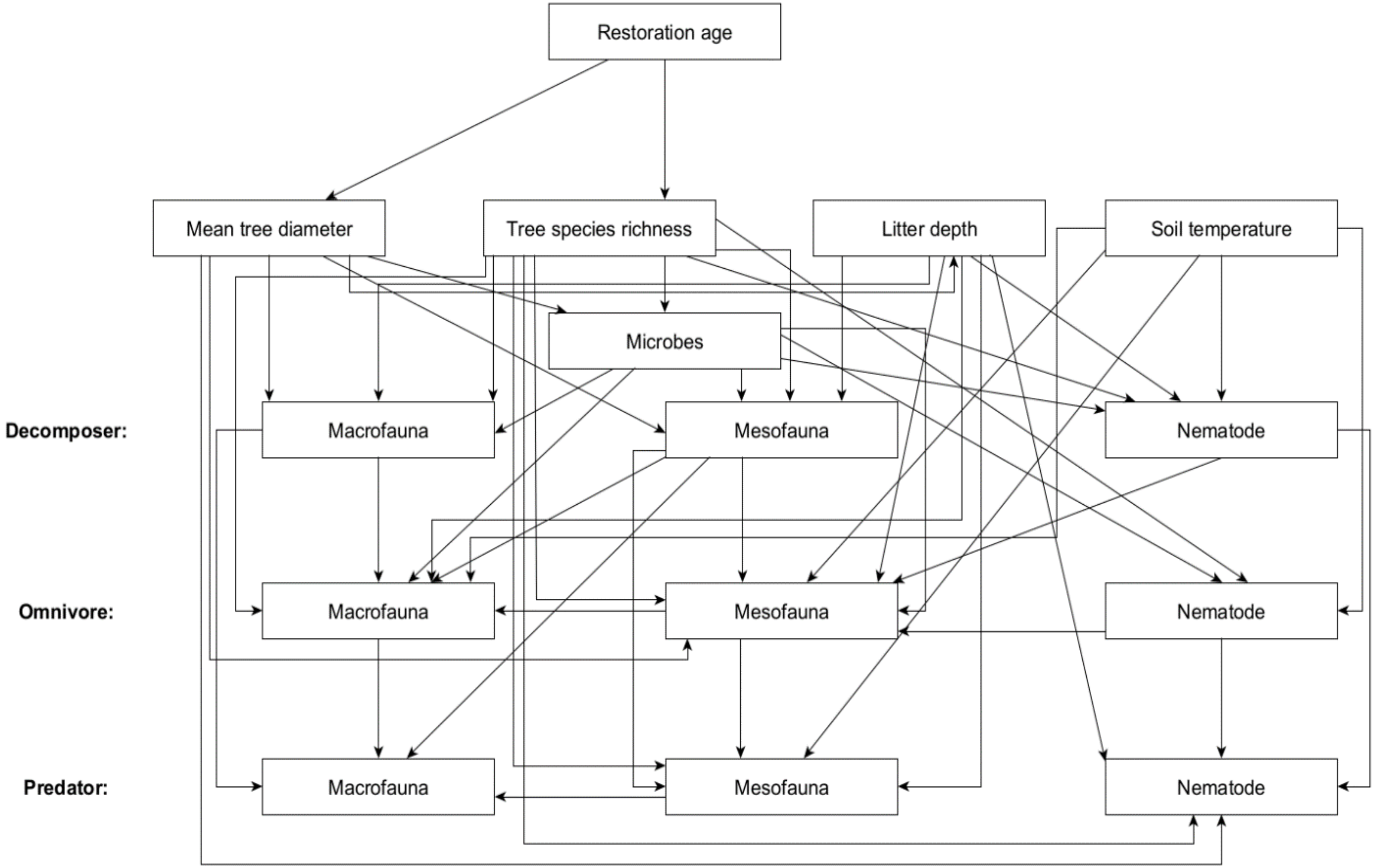

Figure S1 The hypothesised effects of restoration age on biomass of different trophic groups in soil food webs. The initial maximal Structural Equation Model was built around this conceptual diagram.

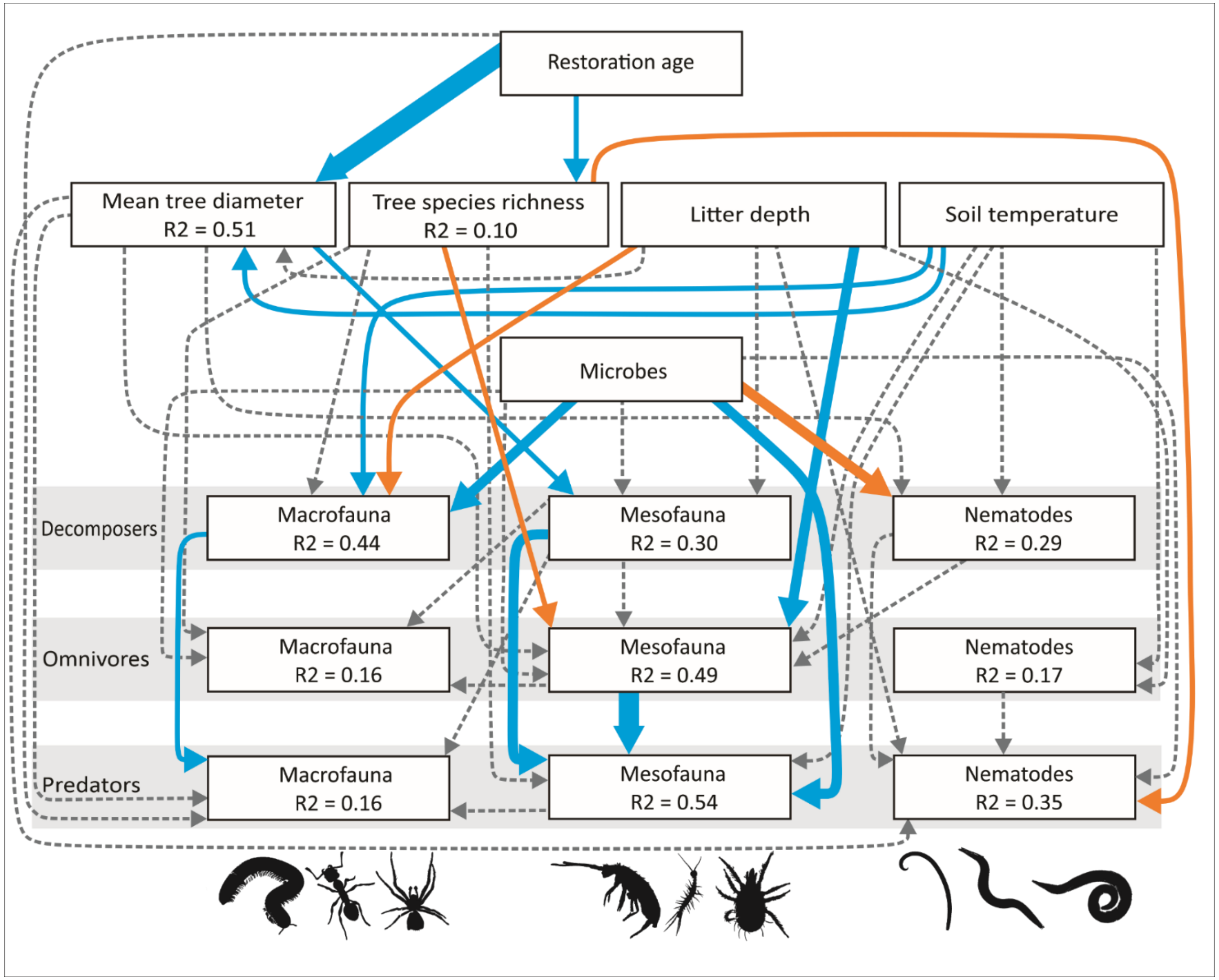

Figure S2. Minimum adequate structural equation model describing direct and indirect effects of forest restoration age on the biomass of major trophic groups in developing forest soil food webs ($\chi2$ = 39.244, d.f. = 76, P = 1, AIC = 191.244). Blue and orange arrows denote significant positive and negative effects, respectively. Arrow widths are proportional to the range-standardised coefficients and indicate relative effect strengths (i.e., the wider the arrow, the stronger the effect). Dotted arrows indicate insignificant effects. R2 values indicate the proportion of variance explained in the respective response variable by the effects of all associated predictor variables (i.e., those with arrows leading to the response variable).

Chapter 4 . Contrasting effects of food web connectance and maximum trophic level on soil faunal ecosystem functions during secondary forest succession

4.1 Abstract

Studies on forest restoration has typically focused either on plant communities or without explicit consideration of multiple trophic levels, with very limited research investigating entire food webs. To date, there is a general lack of understanding of how food web structural properties, such as connectance and maximum trophic level, change with succession and the implications for ecosystem functions in soil. In this study, I quantified the structure of soil food webs during secondary forest succession including invertebrate taxa ranging from nematodes to meso- and macrofauna and their interactions. Furthermore, I quantified energy fluxes between trophic guilds and their resources to estimate five key ecosystem functions in soil food webs: detritivory, bacterivory, fungivory, herbivory and predation. I then applied the food web energetics approach to quantify fluxes of energy among resources and their consumers by estimating ingoing fluxes that balanced known energetic losses from consumers in food webs. Using structural equation modelling, I tested for how forest restoration age and associated shift in vegetation characteristics (i.e. mean tree diameter, tree species richness, tree density and tree species evenness) affects the five ecosystem functions (estimated from energy fluxes) via changes in food web structure during secondary forest succession. According to the structural equation model (SEM), there was no direct effect of forest restoration age on either food web network properties or ecosystem function carried out by soil invertebrates in this study. Moreover, the SEM also did not detect any significant effects of restoration age on soil food web network properties. The only ecosystem function that significantly responded to age, though indirectly, was bacterivory. My results provide no evidence that restoration age *per se* influences either connectance or maximum trophic level, suggesting that forest succession is most likely to affect soil ecosystem function indirectly via vegetation characteristics. Food web network properties can, however, significantly affect ecosystem function at both lower and higher trophic levels. Hence, forest restoration practices could benefit from focusing on restoring specific vegetation characteristics to assist in the recovery of soil trophic functions. Furthermore, food web network properties should still be considered for long term stability of ecosystem functions in restored soils.

4.2 Introduction

Forest restoration research has predominantly focused on plant communities (Nielsen 2019; Meneses *et al.* 2022; Hernández & Pérez 2023) with some studies investigating other taxa and ecosystem components such as birds (Ortega-Álvarez & Lindig-Cisneros 2012; Destro *et* *al.* 2018), mammals (Derhé *et al.* 2018; Palmer *et al.* 2020), soil microbial communities (Potthoff *et al.* 2006; Bach *et al.* 2010; Farrell *et al.* 2020) and soil nutrients (Chen *et al.* 2016; Huang *et al.* 2020). Few studies, however, have investigated entire food webs, with only few existing studies on other network types, such as bipartite networks of plant-pollinator interactions (Menz *et al.* 2011; Kaiser-Bunbury *et al.* 2017). Bipartite networks are subcomponents of more complex unipartite (or even multiplex) networks, which represent interaction network with two trophic levels or two groups of species (Dormann *et al.* 2009; Simmons *et al.* 2018). But trophic interactions, together with the structural properties of whole food webs, drive stability and functioning of ecosystems (Berlow *et al.* 2004; De Vries *et al.* 2013; Yen *et al.* 2016). Furthermore, food webs depict the flow of energy from through ecosystems and also showcase an explicit link between multitrophic community structure and ecosystem processes (Barnes *et al.* 2018).

Trophic interactions between organisms at different trophic levels create the structure of food webs (Petchey *et al.* 2004). Any structural variation resulting from changes in population abundances across nodes, as well as in presence/absence of nodes, has large consequences for energy flux (King & Pimm 1983; Petchey *et al*. 2004). The direction and intensity of effects on food web structure, from changes in the relative abundance to presence/absence of species, depends on both the species' trophic position and the existing species richness at that level (Pimm 1980; Petchey *et al*. 2004). For example, a loss of a predator will have a different consequence for the food web structure compared to a loss of a herbivore (Petchey *et al*. 2004). In a marine shore, removal of a top predator led to a decrease in species diversity and the food web underwent trophic simplification because of the ability of certain species to outcompete others for available resources in the absence of their predator (Paine 1966). On the other hand, food webs with generalist herbivore predators tend to experience less impact from the loss of herbivores (Pimm 1980). However, species at higher trophic levels, still are more vulnerable to extinction because of their dependency on species at lower trophic levels (Holt *et al*. 1999). Furthermore, species at higher trophic levels also have smaller population sizes and on average larger body size, putting them at higher risk to any environmental fluctuations (Lande 1993). All these bottom-up and top-down effects occurring between resources and consumers help establish networks between species and the energy links between them (Thompson *et al.* 2012; Barnes *et al.* 2018).

Food web approaches consider trophic interactions to provide insights into the number of trophic levels, energy flow pathways or important linkages in a system (Pimm *et al.* 1991;

Thompson *et al.* 2012; Zanden *et al.* 2016). Food webs depict trophic relationships between species within an ecosystem (Dunne *et al.* 2002a). The structure of a food web can be described by network properties, such as connectance (Erdos & Rényi 1960), or by maximum trophic level (Dunne *et al.* 2002a, 2004; Riede *et al.* 2010). Connectance is the proportion of all realised (i.e., observed) interactions that occur out of all potential interactions within a food web (Gardner & Ashby 1970; Pimm *et al.* 1991). Increases in connectance can positively influence structural stability via an increase in trophic link complexity as food webs with increasing connectance display low sensitivity to removals of highly connected species (Dunne *et al.* 2002b). A trophic level can be defined as the number of links required for nutrients and energy to reach a consumer from its resources (Williams & Martinez 2004). Therefore, maximum trophic level of a food web is the measurement of the breadth of the vertical dimension of these links in a food web (Wang & Brose 2017). The shift in resource availability during succession can influence the number of trophic levels present and food web connectance, via effects on species population dynamics, composition and community structure (Jeffries *et al.* 2006; Schrama *et al.* 2013). Studies have shown that there is a decrease in organic matter, soil nutrients (Bokhorst *et al.* 2017), light availability (Matsuo *et al.* 2021), and increase in vegetation biomass (Chazdon *et al.* 2007) and canopy cover (Lebrija-Trejos *et al.* 2010) as succession progresses. The change in species population composition and structure occurs as the change in resource availability can favour different trophic groups at different periods during succession (e.g. Kaufmann 2001; Kaufmann *et al.* 2002; Chauvat *et al.* 2011; Laliberté *et al.* 2017).

Soil food webs consist of invertebrates which interact with vegetation directly (through pathogens, herbivores) or indirectly through regulation of available nutrients (i.e. decomposers) and predators that feed on these invertebrates, all of which encompass a diverse taxonomic range and differ in body size by several orders of magnitude (Kardol & Wardle 2010; Sackett *et al.* 2010). Soil food webs are an important component of terrestrial ecosystems, which can influence aboveground organisms and their interactions with each other (Birkhofer *et al.* 2008; Holtkamp *et al.* 2008; Kardol & Wardle 2010). However there is limitation in our understanding of how interactions between vegetation and soil invertebrates respond to aboveground succession (Kardol & Wardle 2010). Changes in vegetation characteristics during succession can have a significant effect on the quantity and quality of organic matter in soils (Lavelle *et al*. 1997). Such change in available resources can then influence soil invertebrate density and diversity in the forest (Bernier & Ponge 1994; Lavelle *et al.* 1997). Many studies have reported successional changes in soil invertebrate communities, such as (1) an increased relative abundance of omnivorous nematodes with succession (Wall *et al.* 2002), (2) a shift towards predator dominance during early succession and herbivore and decomposer dominance during later succession (Kaufmann 2001; Kaufmann *et al.* 2002), (3) an increased species richness of predatory carabid beetles during early succession (Schlegel & Riesen 2012), (4) a shift in influence from bacterial dominance to fungal dominance during succession (Bokhorst *et al.* 2017; Laliberté *et al.* 2017), and (5) an increased decomposer species richness with succession (Chauvat *et al.* 2011). Changes in soil invertebrate communities can eventually result in increased network connectivity and more complex biotic interactions (Morriën *et al.* 2017).

An increase in species diversity can promote flux of nutrients or energy from resource to consumer species (Cardinale *et al.* 2011, 2012). However, previous studies relating biodiversity to ecosystem functioning focused usually on either single trophic levels or single communities (e.g., plants) (Barnes *et al.* 2018; Eisenhauer *et al.* 2019). With this study, I quantified structure of soil food webs including different taxa and their interactions during the early stages of secondary forest succession. Furthermore, I quantified energy fluxes between trophic guilds to test for ecosystem trophic function (i.e., functions resulting from trophic interactions between resource and consumer species such as predation, herbivory, fungivory, bacterivory and detritivory) carried out by invertebrates across different trophic levels. I hypothesized that soil food webs will become more complex over successional development. I posited that such change is mediated by increases in two food web structural properties: connectance and maximum trophic level (Cardinale *et al.* 2006; Morriën *et al.* 2017). Lastly, I hypothesised that the underlying mechanisms such as energy flow can vary over time due to changes in food web structural properties, resulting in changes in ecosystem trophic functioning in reassembling food webs. Answering these hypotheses will help to understand how successional changes in food web structural properties affect multiple ecosystem functions carried out in soil ecosystems.

4.3 <u>Methods</u>

4.3.1 <u>Study location</u>

The study was carried out in eight cities across the North and South Islands of Aotearoa New Zealand during the austral summer of November 2019 – February 2020 (Fig. 1A). Soils with their invertebrate communities within were sampled in restored urban forest sites (n = 70) distributed across a latitudinal gradient of approximately 9º, and mean annual temperatures ranging from the lowest of 10.1 ºC in Invercargill (Climate-data.org 2022) to the highest of 14.8 ºC in Tauranga (NIWA 2019). There were 7–9 sites per city, all of which were on flat sites or slopes of < 15º. The restored urban forests were planted with native tree and shrub species in a single initial cohort, on retired farmland or mowed parkland. The restored forest sites represent a chronosequence spanning 6–60 years in age since their initial restoration planting.

4.3.2 Experimental design and sampling

Data were gathered from a single 20 m × 10 m plot at each site. Vegetation was sampled throughout this 200 m^2 plot, and soil invertebrates were sampled by taking soil cores in three subplots of 50 cm × 50 cm placed diagonally across the plot, located at the centre point, corner two, and corner four (Fig. 1B). Within each subplot, I collected one 22 cm diameter soil core and one 5 cm diameter soil core to sample macrofauna and mesofauna, respectively, and five 2.7 cm diameter soil cores to sample nematodes and the microbial community. To capture spatial heterogeneity within each subplot, all soil cores were collected at random points and with a distance of *ca*. 22 cm between coring locations within each subplot. All soil cores were collected to a depth of 10 cm from the surface of the soil organic layer. To sample the food web as completely as possible, soil cores were taken with the litter layer left in place, but the litter layer did not count towards the 10 cm coring depth.

Soil temperature data were collected from the centre point and corner 2 via iButton data loggers deployed at 10 cm depth (iButton dataloggers model DS1921G-F5; Maxim Integrated, San Jose, California, USA). Temperatures were logged every 4 hours from 24 March 2018 to 24 June 2018. Canopy openness (measured as sky visible through the forest canopy) was used as a proxy for light reaching the forest floor and microclimatic conditions on the forest floor. Canopy openness was quantified using a convex densiometer (Convex model A; Forestry Suppliers, Jackson, Mississippi, USA) measurement once at all four corners and the centre of each plot. The average of these five densiometer measurements was then calculated for use in the statistical analysis. Leaf litter depth was measured using a ruler at five points in each subplot (at each of the four corners and centre) from which we calculated an average depth. Each measurement was taken from the surface of the organic horizon to the maximum height occupied by the litter layer.

Forest structure and composition were assessed by identifying and measuring diameters at breast height of all trees and shrubs ≥ 2.5 cm diameter at breast height in each plot. Both native and non-native species were included. Diameters at breast height measurements were then used to calculate the mean quadratic diameter of all trees in the plot.

The soil samples from the five 2.7 cm diameter cores per subplot were homogenised and a 150g subsample was taken for microbial analysis. Substrate-induced respiration (SIR) technique was used to measure microbial biomass (μg C/g soil dry mass). The SIR technique was performed on an O2-microcompensation apparatus. The dry soil was added with 8mg/g of glucose in water solution and incubated for over 24-hours. During this incubation period, metabolically active microorganisms were detected by recording O2 consumption.

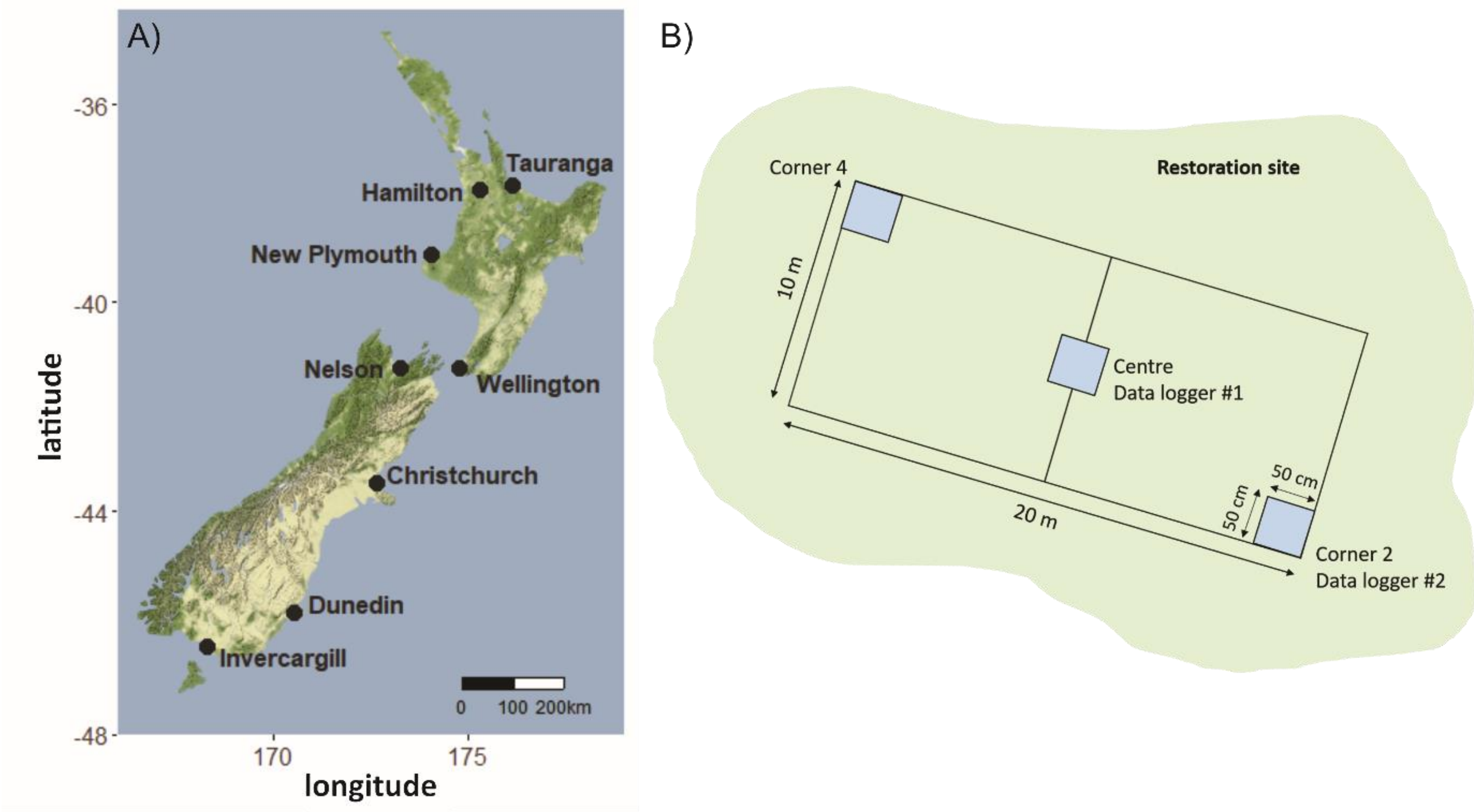

Figure 1. (A) Map of Aotearoa New Zealand showing the eight cities where soil invertebrate samples were collected (B) Layout of a single 10 m × 20 m sampling plot, containing three sub-plots (50 × 50 cm; indicated by blue squares) located at the centre, corner two, and corner four of each plot where soil cores to collect invertebrates were taken. Soil temperature data loggers were buried at the centre and corner two of each plot.

4.3.3 Invertebrate extraction from soil cores

Soil macrofauna (body width ≥ 2 mm), such as millipedes, centipedes, and beetles, were extracted from the largest soil cores using modified 'Kempson' high-gradient heat extractors (Edwards 1991). The smaller soil mesofauna (body width 0.1 mm–2 mm), such as mites and springtails, were collected following a similar approach using a Macfadyen high-gradient heat extractor (Edwards 1991). Extractions were run for at least 11 days using a programmed temperature ramp starting at 20 °C on day one and reaching 55 °C in the last two days of the extraction. To extract free-living soil nematodes, soil from the five cores per subplot was sieved using a 2-mm mesh and homogenised. Nematodes were then extracted from 25 g of this sieved, homogenised soil using an active organism 'Baermann funnel' method (Coleman & Wall 2015; Cesarz *et al*. 2019). Extracted nematodes were collected and stored in 4% formalin. I then calculated the dry weight of the soil samples to obtain nematode abundance per 100 g of dry soil (Cesarz *et al*. 2019). To calculate the individuals per m^2, first, I multiplied individuals/g of dry soil with the respective dry soil weight (g) of each sample. The resulting value, was then divided by the area of the soil core and finally multiplied by 10,000 to obtain individuals per m^2.

4.3.4 Soil invertebrate identification and measurements

Determining a functional role for each species is an important approach for understanding the significance of a species in a successional framework (Montoya *et al*. 2012). These roles can be assigned via their trophic interactions with other species in the system (Dehling *et al*. 2016). To use this approach in quantifying trophic interactions, macrofauna (including adults and larvae) and mesofauna samples were sorted and identified in the lab according to Naumann (1991) and various online sources (CSIRO, 2012; VanDyk *et al.* 2020). Specimens were identified to a taxonomic level (typically to order or family) that was sufficient to determine their general feeding behaviour, which was assigned based on Potapov *et al*. (2022) and Potapov (2022). Trophic groups were further assigned to all macrofauna, mesofauna and nematodes.

Each individual was identified, assigned to a functional role, and grouped into a trophic level, and the body length (mm) of each macro- and mesofauna individual was measured using a stereo microscope with an ocular micrometre. I then calculated the fresh body mass (mg) of each individual using published length-mass scaling relationships (Table S1 & S2) for meso- and macrofauna (Mercer *et al.* 2001; Barnes *et al.* 2014a; Sohlström *et al.* 2018). Gastropods and leeches were excluded from analyses due to extremely low numbers of these organisms in the samples (63 and 1, respectively, out of a total 15,831 individuals). The body masses of Coleopteran, Dipteran, and Lepidopteran larvae were estimated using the Coleoptera larvae-specific formula in Sohlström et al. (2018) because no other taxa-specific formula was available for the Diptera and Lepidoptera. Furthermore, because no regression formula available in the literature for Diplura and Symphyla, I used a scaling relationship published for Dermaptera and Chilopoda, respectively (Barnes *et al.* 2014a; Sohlström *et al.* 2018).

The first hundred nematodes encountered per plot were identified to genus by Tao Liu, a professional nematologist, according to "De Nematoden van Nederland" (Bongers 1994). Those one hundred nematodes were assigned to trophic levels (bacterivores, fungivores, herbivores, omnivores, and predators). I, later collected genus-specific information on body mass using the online platform Nemaplex ("Nematode Ecophysiological Parameter" 2023), which was based on female adults (see Klusmann *et al*. (2022) for potential caveats). These body masses were then assigned to all associated genera of nematode individuals in my dataset.

I first measured the abundance of the invertebrates as the total number of individuals present at a particular site. The average biomass for all three invertebrate groups was calculated by first, taking a sum of fresh body mass for each invertebrate group at each of the three-forest subplot. The sum of fresh body mass in three subplots were then added together and divided by three to get average biomass for each group at each restoration plot.

4.3.5 Food web matrix

I acquired the food web meta-matrix from Digel *et al.* (2014) and used its structure as a template to assign feeding links and construct my own food web meta-matrix (i.e., universal food web topology to be applied to all local soil communities) based on trophic groups found in my samples. The acquired food web matrix was based on beech and coniferous forests, all of which range from earlier to later-successional forests, located in southern, central and northern Germany. All the sites within these forests, where the data was collected from, represents land use of different intensities (i.e., high, intermediate and low intensity to some sites being nearly natural) (Digel *et al.* 2014). For macrofauna and mesofauna, each unique taxonomic identification was included in the matrix, but nematodes were re-classified into five groups based on their feeding behaviour (i.e., herbivores, fungivores, bacterivores, omnivores and predators) rather than grouping strictly by their taxonomy. Feeding interactions and feeding preferences were based on Digel et al. (2014) and Potapov et al. (2022). I also added fungi, bacteria, plants and detritus as basal resources known to be available at each sampled site.

4.3.6 Metabolism and assimilation efficiency

Metabolic rates for each individual organism within each order (or to family when possible) of macrofauna and mesofauna was first, calculated using the general equation

$$\exp(\ln i_o + \alpha \times \ln M - E(1/\, k \times 273.15 + T))$$

Where, k = Boltzmann constant (8.62×10^{-05}), M = fresh body mass (mg), T = temperature in C^o , and '273.15 + temp' is to convert temperature from C^o to K (Ehnes *et al.* 2011). Metabolism rate was then log transformed and summed per node (i.e., per trophic group) per plot and finally scaled to achieve metabolism per m^2. Taxa-specific model parameters were derived from fits published in Ehnes *et al.* (2011) (Table 1).

Table 1. Regression model parameters for each taxonomic group. ln i_o represents log normalisation factor, α is the allometric exponent, and E is the activation energy.

Regression group	ln i_o	α	E	Reference
Oribatida	22.02	0.68	0.7	(Ehnes *et al.* 2011)
Mesostigmata	9.67	0.69	0.38	(Ehnes *et al.* 2011)
Prostigmata	10.28	0.66	0.41	(Ehnes *et al.* 2011)
Arachnida	24.58	0.56	0.71	(Barnes *et al.* 2014b)
Coleoptera	21.42	0.74	0.64	(Barnes *et al.* 2014b)
Insecta	21.97	0.76	0.66	(Barnes *et al.* 2014b)
Hymenoptera	22.01	0.74	0.67	(Barnes *et al.* 2014b)
Isopoda	23.17	0.55	0.69	(Ehnes *et al.* 2011)
Chilopoda	28.25	0.56	0.8	(Ehnes *et al.* 2011)
Clitellata	12.44	0.8	0.44	(Ehnes *et al.* 2011)
Progoneata	22.35	0.57	0.67	(Ehnes *et al.* 2011)
General	23.05	0.69	0.69	(Barnes *et al.* 2014b)

For nematodes, metabolism was calculated using the equation

$$y = (0.71x + C) - 0.69/k\ (T + 273.15)$$

Where x = body mass (g), c is normalization constant equivalent to 17.17 (for invertebrates) (Brown *et al.* 2004). On the left side of the equation, the slope estimates the allometric exponent and the intercept estimates the normalization constants, C = ln(i0), for each group. On the right side of the equation, - 0.69/k is the slope that estimates the activation energy of metabolism where k = Boltzmann's constant and 'T + 273.15' converts temperature from degree Celsius to Kelvin. Both the slopes are calculated using ANCOVA (Brown *et al.* 2004). Later, metabolism was multiplied by density for each genus per plot and summed within trophic nodes to get metabolic rate/m^2 for each node in the food web.

Secondly, I assigned assimilation efficiency for all animals, plants, and detritus following (Lang *et al.* 2017) and for fungi and bacteria according to De Ruiter *et al.* (1993). Assimilation efficiency is the proportion of total energy consumed by consumer from its resource and assimilated for biomass production, reproduction and respiration but not egested (Barnes *et al.* 2018; Jochum *et al.* 2021). Assimilation efficiency determines the efficiency with which energy is extracted by the consumer from the food consumed and usually presented as a proportion (Lang *et al.* 2017). The assimilation efficiency was allocated to be 0.906 for all invertebrate consumers, 0.545 for plants, 0.158 for detritus (Barnes *et al.* 2020), 0.6 for bacteria and 0.5 for fungi (De Ruiter *et al.* 1993). All metabolism calculations were run in R (version 4.2.0) (R Core Team 2022).

4.3.7 Energy flux

Energy flux (joules day^{-1}), represented as F_{ij} i.e., the flux of energy from resource i to consumer j, was calculated using the equation

$$\Sigma_i (e_{ij} F_{ij}) = X_i + \Sigma_k (W_{jk} F_k)$$

where e_{ij} is the assimilation efficiency that consumer j converts the consumed energy into energy for metabolism and biomass production (Gauzens *et al.* 2019; Barnes *et al.* 2020). Energetic gains of consumers are represented by the left side of the equation while the energetic loss via metabolism X_j and from predation on consumer j by higher trophic levels is represented by the right side of the equation. The metabolism X_j for invertebrate consumers was equal to the sum of individual metabolic rates per node, per food web.

I calculated the flux of energy to each consumer as

$$F_{ij} = W_{ij} F_i$$

where F_j is the sum of ingoing fluxes to species j and W_{ij} is the proportion of F_j that is obtained from species i. Scaled consumer preferences (w_{ij}) based on different available prey was calculated as

$$W_{ij} = w_{ij} B_i \, / \, \Sigma_k w_{kj} B_k$$

where B_i is the biomass of resource i. This biomass of resource was equal to the sum of fresh body mass of all individuals per node type, per plot while all biomass of basal resources were equal to the average biomass per node type per plot (Barnes *et al.* 2020).

Furthermore, following Barnes *et al.* (2020), I set the cannibalistic preference to 0.1 to reduce the amount of energy consumed by a predator from its own biomass pool. The energy flux in each food web was calculated using the 'fluxing' function in the 'fluxweb' package (version 0.2.0) (Gauzens *et al.* 2019). All energy flux calculation were run in R (version 4.2.0) (R Core Team 2022). I finally calculated the total (sum) of outgoing fluxes from each of the detritus, bacteria, fungi, plants and all prey resources as estimates of the five main trophic functions: detritivory, bacterivory, fungivory, herbivory and predation, respectively.

4.3.8 Data Analysis

4.3.8.1 Structural equation model

I constructed linear mixed-effects models ('nlme' package in R, version 3.1.157) to test for the effect of restoration age, vegetation characteristics and food web structural properties on five major trophic functions (i.e., predation, herbivory, fungivory, bacterivory and detritivory). I constructed a hypothetical model aimed at testing how the age of forests and associated variation in vegetation following restoration planting influenced multiple trophic functions in soil food webs via changes in food web structure (Fig 2). Before running models, I checked the normality of the data and homoscedasticity, and log-transformed variables to satisfy assumptions where necessary. The log-transformed variables included mean tree diameter, tree species evenness, maximum trophic level (in this study, the measure of prey-averaged trophic level) and all five trophic functions. During this process, I removed one site from the total of 70 sites (so n = 69) due to missing data on tree species evenness on that particular site because it is mathematically impossible to log 0. I, then used piecewise structural equation modelling (SEM; R package '*piecewiseSEM'*, version 2.1.2) (Shipley 2009; Lefcheck 2016) to test for the direct and indirect effects of forest restoration age on ecosystem functions in a soil food webs. Structural equation modelling is a framework which uses hypothetical approach to understand direct and indirect effects between variables to understand multiple processes that exist in a system (Grace *et al.* 2010; Du *et al.* 2015; Eisenhauer *et al.* 2015). While identifying these direct and indirect effects, SEM describes multiple causal pathways between the variables, the relative strength of which can be compared to better understand complex ecological processes (Du *et al.* 2015; Eisenhauer *et* *al.* 2015). For this study, in the first step, forest restoration age was used as the main, exogenous predictor variable with mean tree diameter, tree species richness, tree density, and tree species evenness as response variables. In the second step, these four vegetation characteristics were used as the predictor variables while connectance and maximum trophic level were the response variables. In a final step, connectance and maximum trophic level were used as the predictor variables and five major ecosystem functions were used as the response variables in the SEM. All data analyses were run in R (version 4.2.0) (R Core Team 2022).

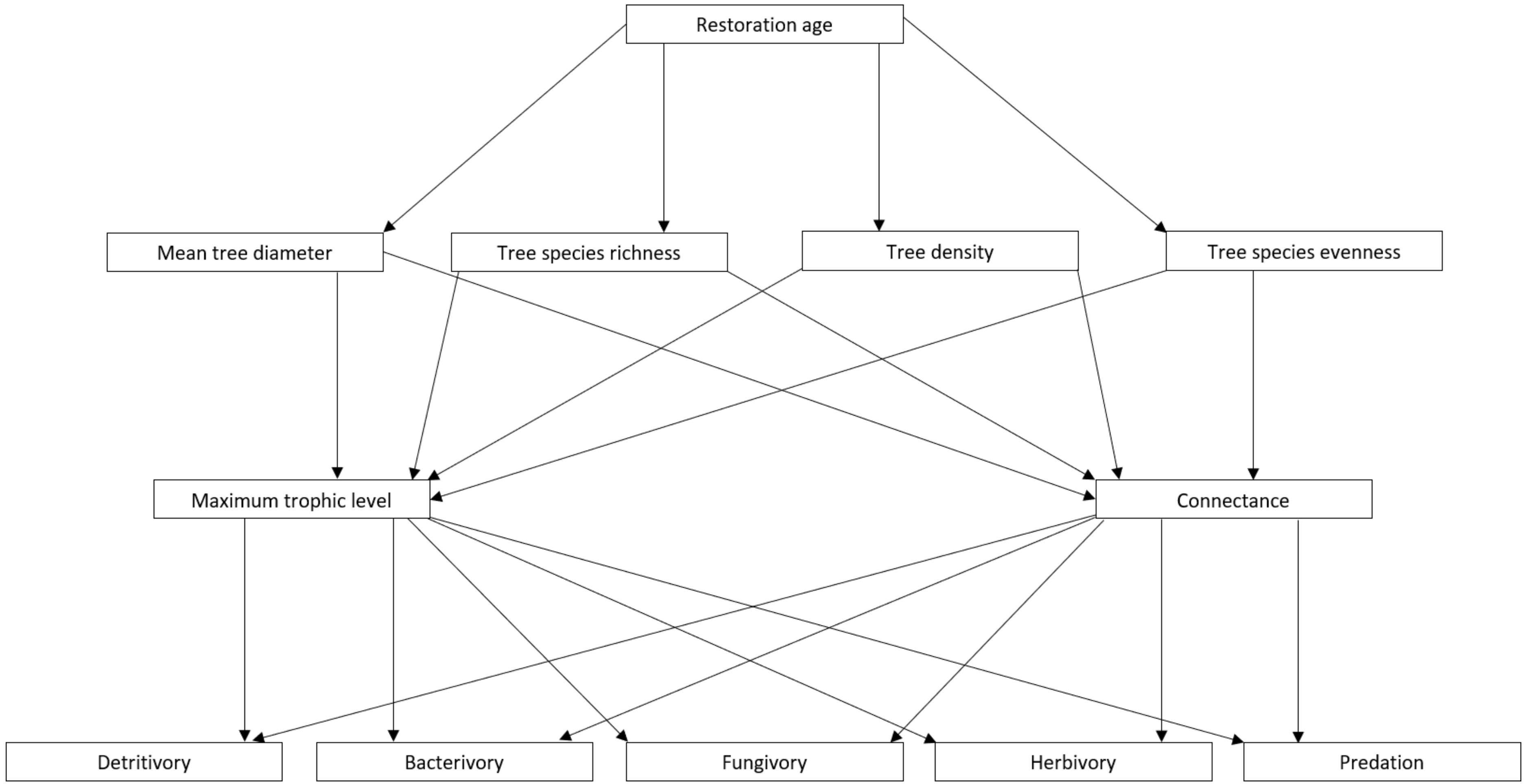

Figure 2. The hypothesised effects of restoration age on multiple ecosystem trophic functions in soil food webs. The maximal model in the Structural equation model was built around this conceptual diagram.

4.4 Results

Age of restoration plantings did not directly affect five ecosystem trophic functions among soil invertebrates (Table 2 & 3, Fig. 3). Instead, restoration age was shown to have an indirect effect only on bacterivory, mediated by variation in mean tree diameter, tree species richness, tree density and tree species evenness (Fig. 4). In addition, restoration age was shown to have neither direct nor indirect effect on either of the soil food web structural properties i.e., connectance or maximum trophic level (Fig. 4). However, the model detected significant effects of connectance and maximum trophic level on all trophic functions analysed, except bacterivory, in this study.

Table 2. Effect of forest restoration age on trophic functions of soil invertebrates.

Trophic function	Predictor	Estimate	Standard error	*p*
Predation	Intercept	11.271	0.278	0.000
	Restoration age	0.000	0.011	0.998
Herbivory	Intercept	11.590	0.231	0.000
	Restoration age	0.006	0.009	0.536
Fungivory	Intercept	12.156	0.246	0.000
	Restoration age	0.008	0.010	0.412
Bacterivory	Intercept	10.436	0.349	0.000
	Restoration age	0.003	0.012	0.821
Detritivory	Intercept	14.717	0.268	0.000
	Restoration age	0.003	0.009	0.768

Restoration age had direct positive effects on mean tree diameter and tree species richness (Fig. 4). However, no direct effects of restoration age were detected on tree density and tree species evenness. Instead, the effect of restoration age on tree density was conditionally dependent on mean tree diameter and tree species richness. Tree density, in turn, had a direct positive effect on tree species evenness (Fig. 4). In contrast, the effects of mean tree diameter on tree density and of tree density on tree species evenness were negative. The effect of restoration age on mean tree diameter was twice as strong as the effect of restoration age on tree species richness. According to the SEM, restoration age explained 50% of the variation in mean tree diameter, but only 13% of variation in tree species richness.

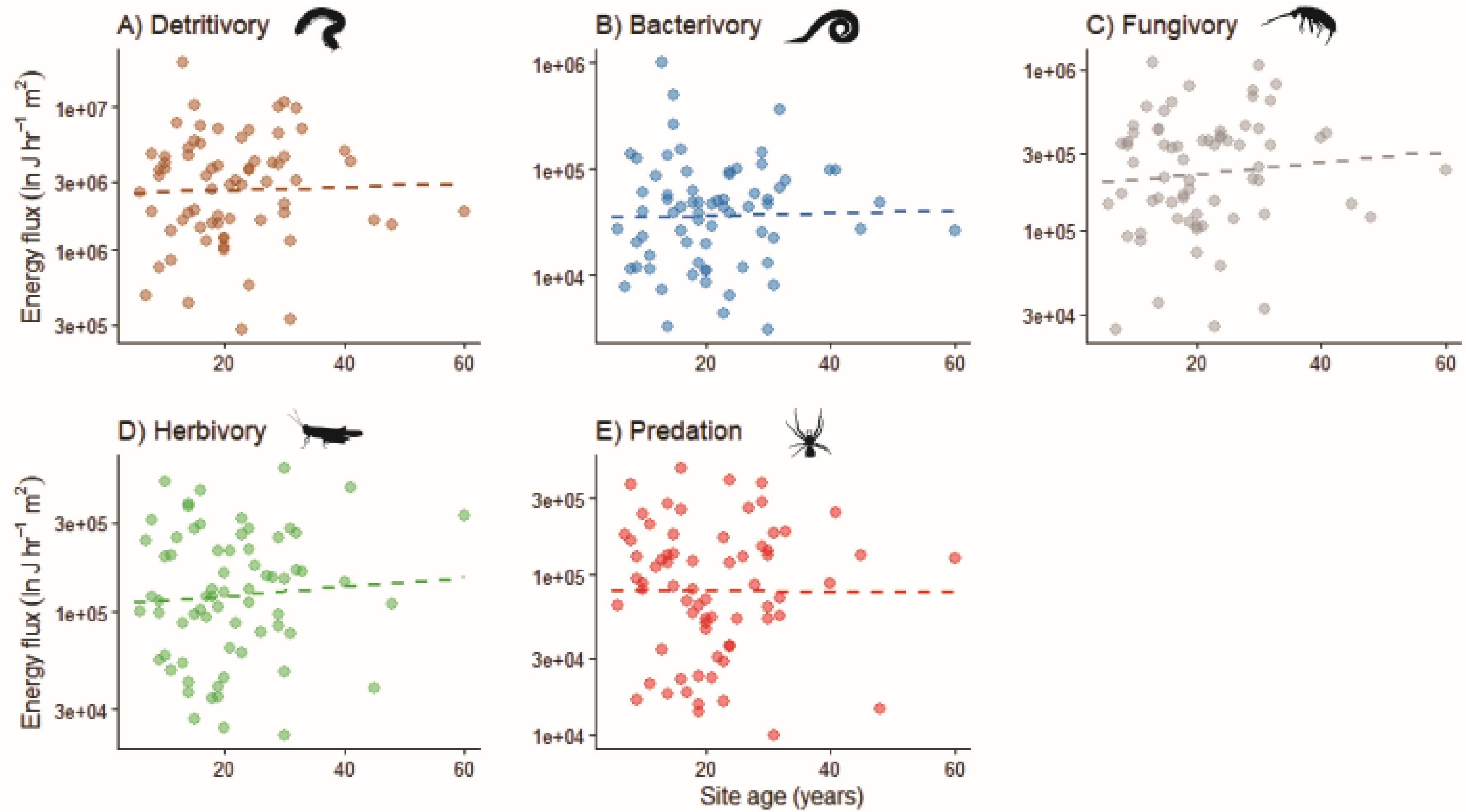

Figure 3. Estimated effects of forest restoration age on the sum of all outgoing energy fluxes from each of the five main resource types in the soil food webs: detritus (A), bacteria (B), fungi (C), vegetation (D), prey invertebrates (E). Each point represents energy flux from one sampled food web. The dashed line represents the regression fit between outgoing energy flux and forest restoration age. In this study, the relationships were not statistically significant.

The structural equation model did not detect any direct effect of forest restoration age on either food web structural properties or soil invertebrate trophic functions (Fig. 4). Moreover, significant indirect effects of restoration age on soil food web structural properties were also absent according to the model. Restoration age had an indirect effect on bacterivory, which was mediated by tree characteristics; it had no such effect on the other trophic functions (detritivory, fungivory, herbivory and predation). Tree species evenness was the only vegetation characteristic to directly affect bacterivory; mean tree diameter, tree species richness and tree density all had positive (though weak) indirect effects on bacterivory via tree species evenness.

Despite expectation of changing food web structure (i.e., connectance and maximum trophic level) with forest age, the SEM did not detect such pattern in my study (Fig. 4). Moreover, there were no significant effects of vegetation characteristics on either connectance or maximum trophic level. The model identified significant effects of both, connectance and maximum trophic level on four out of five trophic functions performed by soil invertebrates. Connectance had negative effects on both detritivory and fungivory (Fig. 4 & 5) suggesting decrease in outflux of energy from detritus and fungi with increase in proportion of realised interactions between species in the food web. Maximum trophic level, in contrast had a negative effect on herbivory and a positive effect on predation (Fig 4 & 5). This suggests that as the number of trophic level increases, there is a decrease in herbivory but increase in the outflux of energy through predation.

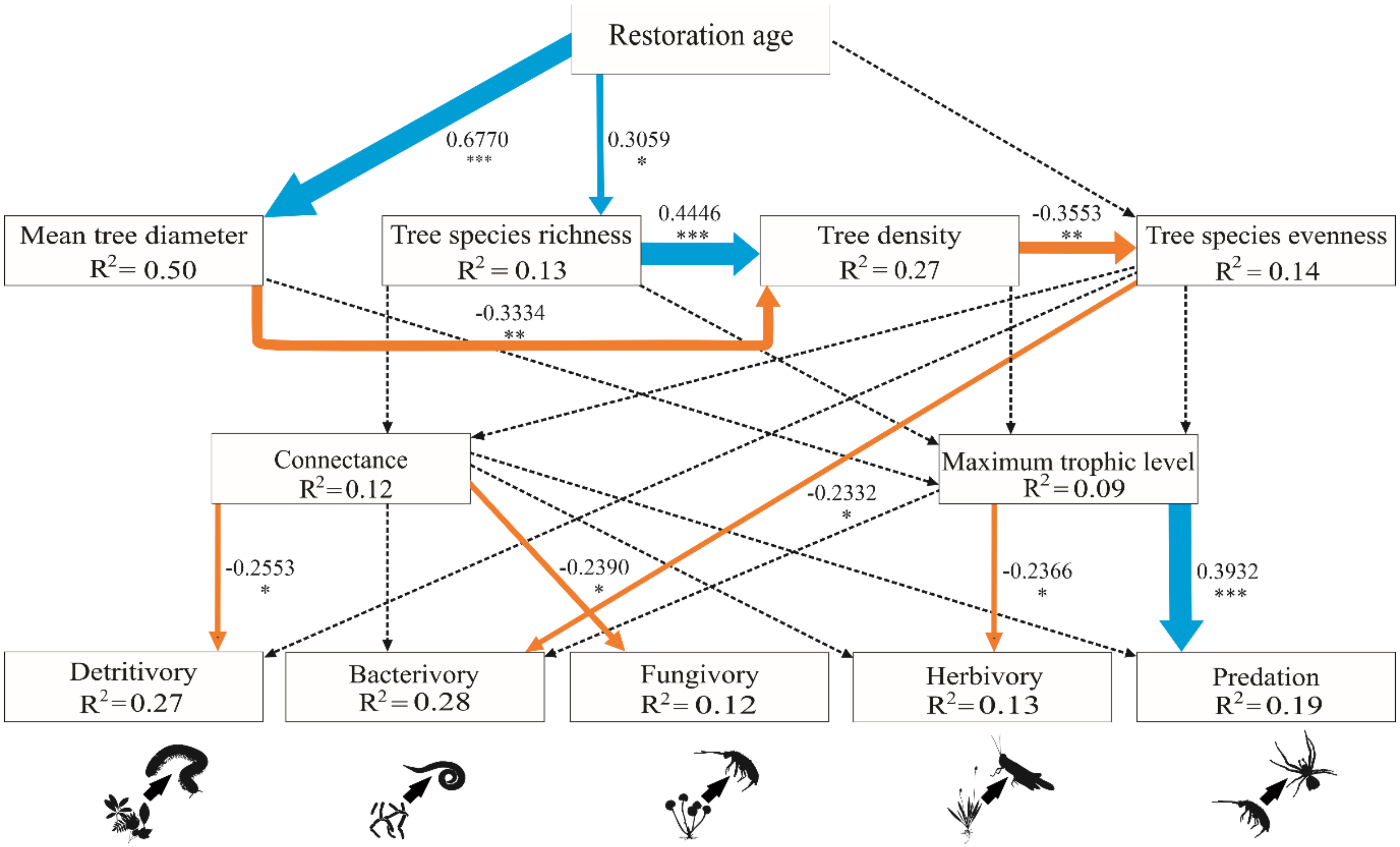

Figure 4. Structural Equation Model describing the effects of forest restoration age on the energy fluxes to each soil invertebrate trophic function (χ2 = 46.715, d.f. = 52, P = 0.681, AIC = 156.715). Blue and orange arrows denote positive and negative effects, respectively. Arrow widths are proportional to the range-standardised coefficients and indicate relative effect strengths (i.e., the wider the arrow, the stronger the effect). The numbers next to the arrow indicate Standard Estimates, the * symbol under these Standard Estimates indicates statistical significance based on *p* values (< 0.001 = '***', 0.001 = '**', 0.05 = '*'). R^2 values indicate the proportion of variance explained in the respective response variable by the effects of all associated predictor variables (i.e., those with arrows leading to the response variable).

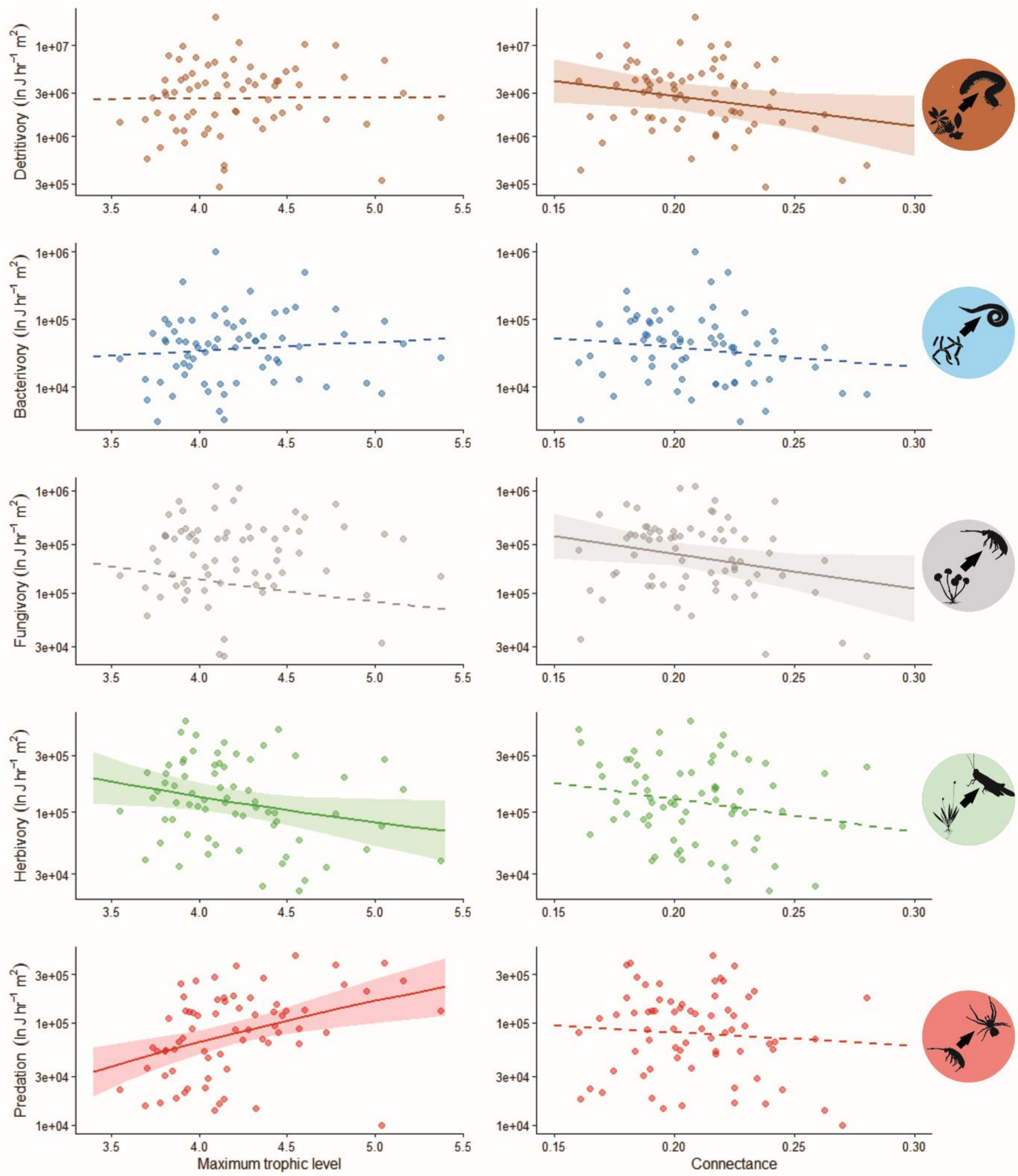

Figure 5. Estimated effects of maximum trophic level (left column) and connectance (right column) in food webs on the sum of all outgoing energy fluxes resulting from the trophic interaction between resource and consumer species. The y-axis shows five of the main resource types in the soil food webs – from top to bottom; detritus, bacteria, fungi, vegetation & prey invertebrates. Each point represents energy flux from one sampled food web. Solid lines indicate a significant relationship between outflux of energy and the respective food web property, while dashed lines indicate non-significant relationships. The 95% confidence interval is shown by the shaded area with respective colour for herbivory and predation in the right column, and detritivory and fungivory in the left column. The silhouettes in the circles represent outflux of energy from resource to consumer species.

Table 3. Summary of the minimum structural equation model testing for the effects of restoration age on ecosystem trophic function. Significant p-values are in bold. The relative strength and direction of the relationship between two variables is shown as std. estimate (i.e., standardised estimate).

Predictor	Response	Std. error	*p*	Std. estimate
Site age	Mean tree diameter (m)	0.004	**0.000**	0.677
	Tree species richness	0.043	**0.01**	0.306
	Tree species evenness	0.008	0.316	-0.115
Mean tree diameter (m)	Tree density	10.431	**0.003**	-0.333
	Maximum trophic level	0.029	0.151	-0.200
Tree species richness	Tree density	1.119	0.000	0.445
	Maximum trophic level	0.003	0.590	0.080
	Connectance	0.001	0.230	-0.143
Tree density	Tree species evenness	0.002	**0.003**	-0.355
Tree density	Maximum trophic level	0.000	0.134	-0.248
Tree species evenness	Maximum trophic level	0.017	0.165	-0.197
Tree species evenness	Connectance	0.004	0.091	-0.199
Tree species evenness	Detritivory	0.131	0.159	-0.155
Tree species evenness	Bacterivory	0.174	**0.037**	-0.233
Connectance	Detritivory	3.917	**0.031**	-0.255
Connectance	Bacterivory	5.163	0.085	-0.203
Connectance	Fungivory	3.931	**0.049**	-0.239
Connectance	Herbivory	3.657	0.099	-0.197
Connectance	Predation	4.142	0.314	-0.114
Maximum trophic level	Bacterivory	1.469	0.43	0.089
Maximum trophic level	Herbivory	1.069	**0.047**	-0.237
Maximum trophic level	Predation	1.21	**0.001**	0.393

4.5 Discussion

This study investigated the influence of restoration age on soil food web structure and functioning in successional forests. While there were no direct effects of restoration age on either the soil food web structural properties or trophic functions measured in this study (Fig. 4), my results indicate that restoration age has an indirect effect on the soil food web mediated by vegetation characteristics. Contrary to expectation, I also did not find any indirect effect of forest restoration age on the complexity of the food web i.e., connectance and maximum trophic level. Instead, changes in trophic functioning in reassembling food web was shown to be a result of changes in vegetation characteristics.

My analysis suggests that restoration age, itself may not directly influence trophic function of soil invertebrates. This supports previous observation on how soil invertebrates have reassembled without restoration age dominating the process during succession (Wall *et al.* 2002; Schlegel & Riesen 2012; Bokhorst *et al.* 2017). These studies suggest that successional age in itself is not of primary influence, but rather the changes in vegetation characteristics over time that drives the assembly of ecosystem functions in soil food webs. Food webs are complex networks of different energy channels, influenced differently by above and belowground components of ecosystems and based on different resources which, most often does not spread homogenously across space and time (Berg & Bengtsson 2007; Rooney *et al.* 2008; Schrama *et al.* 2013). Successional change in vegetation can lead to nutrient limitation and decline in soil organic matter quality during late successional stages, resulting in a shift to a fungal dominated energy channel in food webs. These changes at lower trophic levels can then result in changes on higher trophic levels within the ecosystem (Williamson *et al.* 2005). Therefore, while restoration age may be the main exogenous driver of change, the complex interplay of changes associated with restoration age is likely what directly drives the development of trophic functions in soil food webs (Williamson *et al.* 2005).

Findings of this study align with previous studies (Wall *et al.* 2002; Biederman & Boutton 2009) by demonstrating that restoration age indirectly influences bacterivory through changes in vegetation characteristics (Fig. 5). Indeed, previous research from urban restoration sites in New Zealand has shown that tree basal area and tree species richness significantly increase over 70 years of succession since restoration planting began (Wallace *et al.* 2017). This is consistent with other studies which have found a general increase in mean tree diameter and tree species richness with successional progression (Chazdon *et al.* 2007; Coomes & Allen 2007; Martin *et al.* 2013). These changes in turn, were shown to affect tree density, which in turn had a negative effect on tree species evenness. The study by Wallace *et al.* (2017) in particular, suggests that I could expect significant changes in tree communities at my sites that likely influence soil food webs and associated ecosystem processes. According to my results, tree species evenness had a negative effect on bacterivory (Fig. 5). Plants are the predominant source of organic matter input into the soil , thereby supporting energy to detritus based food webs (Wallace *et al.* 1999; Kominoski *et al.* 2007; Lecerf *et al.* 2007). Litter mixing studies have found leaf species composition to be more important than richness in terms of litter chemistry (Gartner & Cardon 2004; Swan & Palmer 2006). Hence, a decrease in tree species evenness can support litter decomposition by microbial consumers (Swan *et al.* 2009) and ultimately the invertebrates that feed on those microbes.

My finding that restoration age did not influence food web connectance or maximum trophic level is in contrast to previous findings reported by Morriën *et al.* (2017) & Neutel *et al.* (2007). Potential reasons for a lack of change in these food web properties with succession could be due to a loss of species with specific trophic function (Tylianakis *et al.* 2007). This is because, depending upon the species' trophic level position, their absence can, sometimes have minimum effect on the structure of the food web (Petchey et al. 2004). Another possible reason for an absence of influence on food web structural properties in my study could be because of a lowered ratio of consumers to resources due to increased sensitivity of species at higher trophic levels (Valladares *et al.* 2012). These potential reasons can happen when a restored forest undergoing secondary succession has not yet reached community composition comparable to that of a mature forest (Moreno-Mateos *et al.* 2020; Resch *et al.* 2021). Since all of my sites are forests undergoing secondary succession in their early and mid-successional stage, they are unlikely to be anywhere near mature forests in terms of ecosystem processes.

According to my results, predation rates increase while herbivory declines with increasing maximum trophic level in soil food webs (Fig. 6). Habitat heterogeneity in soil can support predators of different size and hunting modes (Digel *et al.* 2014). In addition, soil predators tend to exhibit generalist feeding behaviour (Scheu & Setälä 2001). The diversity in hunting techniques including increased chance of random encounters, lack of feeding preference, evolution of body morphology to movement around porous spaces and dominance of intraguild predation, all of these result in greater top-down pressure on all trophic levels (Digel *et al.* 2014). Hence, as the number of predator populations increase with increasing trophic level, predation pressure will be strong even more which leads to rise in competition for food resources. To reduce niche overlap with other species, predators, then shift their niche to be more complementary in their use of resources (or efficient use of limiting resources) (Fargione *et al.* 2007; Eisenhauer *et al.* 2019). Such niche complementarity can occur during both interspecific as well as intraspecific competition (Roscher *et al.* 2015). Consequently, increased predation pressure on herbivores will adversely affect herbivory. Decomposers were not shown to be significantly affected by the increase in maximum trophic level, probably because the intensity of top-down control via predators can vary significantly depending on the habitat. For example, in ecosystems with slow decomposition rates, top-down control by predators on decomposers are likely to be very weak (Kalinkat *et al.* 2013). This is because slowly decomposing litter, which results in increase in amount of litter, can provide more refuge for the prey species thereby reducing the frequency of their encounter with their predators (Kalinkat *et al.* 2013).

A decrease in detritivory with increasing connectance (Fig. 6) partially supports my initial hypothesis where I expected changes in food web properties to affect food web trophic functions. Boit & Gaedke (2014) found an increase in weighted connectance (i.e. food web where each link is weighed on the basis of the flux rate associated with the link) with succession. It has also been suggested that food web structure shifts towards fungal-dominated energy channels as succession progresses (Bokhorst *et al.* 2017). In this vein, it is unclear why my findings suggest a negative effect of connectance on fungivory. It is possible that food webs with higher connectance supports greater richness of fungi but less fungivores, as there is typically greater species richness at lower trophic levels compared to higher trophic levels (Sánchez-Moreno *et al.* 2011). Furthermore, species richness and abundance tend to be correlated, with the intensity of this correlation weakening progressively from lower to higher trophic levels in food webs (Sánchez-Moreno *et al.* 2011). Hence there was reduced fungivory because fungivores, as consumers, might have had less individuals and taxa than their resource nodes in the food web, i.e., fungi. In addition, an increase in connectance can also indicate less specialization from consumer species (Sánchez-Moreno *et al.* 2011), specifically fungivores and detritivores in this study. Hence, outflux of energy through realized trophic interactions is shared between different resources resulting in a decrease in fungivory and detritivory. Furthermore, the decrease in both fungivory and detritivory with increasing connectance also suggests the presence of niche overlap as the underlying mechanism affecting trophic interactions in the food web. Increase in connectance enhances species richness and hence abundance of resources available resulting in greater competition (Valdovinos *et al.* 2016). While fungivores and detritivores in my study might have been negatively affected by the increase in competition, they could also have adapted their feeding behaviour to be more specialised in their dietary preference to escape negative consequences of competition (Valdovinos *et al.* 2016). Both of these processes, increased competition and resultant switching to specialised feeding behaviour, together might have exacerbated the negative effect of increased connectance on fungivory and detritivory.

4.6 Conclusion

This study investigated how restoration age influences the ecosystem trophic functioning of soil food webs, specifically their contribution to energy flow through the food web. My results suggest that the effect is not via structural changes in soil food-web network properties, but primarily mediated by vegetation characteristics, while still emphasizing the significance of network properties on the flux of energy via detritivory, fungivory, herbivory and predation. This research reinforces the growing recognition of the interconnectedness between aboveground and belowground ecosystems. As such, even though restoration age might not directly affect soil food web structure and ecosystem functioning, the study highlights the importance of considering food web properties for understanding energy flow in belowground ecosystems.

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Chapter 5 . Synthesis

5.1 Synopsis

Despite the importance of soil food webs in maintaining critical ecosystem processes, knowledge gaps remain regarding how these belowground communities rebuild across multiple trophic levels. We also do not know how their reassembly is influenced by ecological restoration and what factors are important in understanding the trajectory of their recovery. Further, the nature of indirect effects of successional change in vegetation on resource-consumer trophic interactions remain unclear. To date, there is a general lack of understanding of how food web structural properties (connectance and maximum trophic level) changes during ecological succession and how this affects the soil invertebrate trophic function in the ecosystem. In this study, I examined how restoring urban forests impacts the reassembly of belowground invertebrate communities (their abundance, biomass and mean body size). In addition, I also quantified the structure of soil food webs during succession, examining the trophic interactions among soil organisms. By doing so, I calculated the flow of energy between trophic guilds and their resources to estimate five key ecosystem functions in soil food web; detritivory, bacterivory, fungivory, herbivory and predation.

My thesis presents an investigation of belowground invertebrate communities in urban restoration forests, exploring the effect of succession on such communities across Aotearoa, New Zealand. The first data chapter looks at the effect of forest restoration interventions on reassembly of abundance, biomass and body size structure of belowground invertebrate communities. I reveal that forest restoration age, in itself, was not a major driver of belowground invertebrate recovery. Instead, vegetation characteristics such as mean tree diameter, tree species richness and environmental factors such as litter depth and soil temperature appear to be stronger drivers of the reassembly of belowground invertebrate communities.

In data chapter two, I investigate how changes in resources explain variation in their consumers through bottom-up effects. Using structural equation models to test for the direct and indirect effects of forest restoration age on biomass of soil invertebrates across the three trophic levels, I show that while forest restoration age may not directly influence soil invertebrate biomass, it can trigger indirect effects on their biomass through changes in biotic and abiotic forest characteristics. Effects of restoration age appeared to act via changes in average tree size, which resulted in notable increases in biomass of decomposers.

In data chapter three, I progressed to investigating how forest restoration age and associated changes in vegetation characteristics affects soil food web structure and ecosystem functions by quantifying energy fluxes from resources to their consumers. Similar to my findings in preceding chapters, forest restoration age, again did not directly influence either the structure or the functioning of the soil food web. Instead, specific vegetation characteristics such as mean tree diameter and tree species evenness played a more significant role in determining rates of ecosystem functioning. Despite my results failed to show any evidence for changes in food web structure (i.e., connectance and maximum trophic level) with succession, these structural properties did strongly influence energy fluxes related to decomposition and predation.

5.2 Discussion

Urbanization, a major form of land-use change (Vitousek *et al.* 1997) disrupts ecosystems through habitat loss, fragmentation (Lambin *et al.* 2001), introduction of non-native species, and increased temperatures due to urban heat islands (Niemelä 1999). This often leads to biodiversity loss, degraded ecosystems, and a decline in the services they provide, such as clean air and water (Butchart *et al.* 2010). Ecological restoration aims to bring a degraded ecosystem back to resilient state (Clewell & Aronson 2006). This involves restoring not just the plant and animal species, but also the complex interactions between them (Palmer *et al.* 1997). These trophic interactions determine the transfer of energy through consumers at different trophic levels and, hence, are crucial for ecosystem processes such as nutrient cycling and productivity (De Ruiter *et al.* 1995; Barnes *et al.* 2018).

Succession is a process during ecological restoration which often follows a predictable path to a climax community over time following disturbance (Young *et al.* 2005). Early stages typically see a rise in plant species richness (Carswell *et al.* 2012), followed by a decline as the ecosystem matures into a late-stage community dominated by large trees (Lebrija-Trejos *et al.* 2010). These changes in vegetation characteristics – increasing biomass, canopy cover, and decreasing understory light (Denslow & Guzman G. 2000; Chazdon *et al.* 2007; Matsuo *et al.* 2021) – impact the resources and habitat available for soil invertebrates. As a result, the structure and biodiversity of soil invertebrate communities also change through the restoration process, strongly influenced by the changing plant community (Scheu & Schulz 1996). In line with the anticipated effect of biotic and abiotic characteristics during succession, the results in my first chapter suggest average tree size and litter depth to be the most common significant predictor for increased abundance and biomass of invertebrates in all trophic groups and all trophic levels. The effect of succession also cascaded through average tree size to positively affect biomass of mesofauna decomposers and through tree species richness to negatively affect mesofauna omnivore biomass. Contrary to my expectation, food web structure was not found to be affected by successional changes, probably because the sites for my study, which are primarily in mid-late stages of secondary succession, have not reached community composition comparable to that of a mature forest (Moreno-Mateos *et al.* 2020; Resch *et al.* 2021).

Studies on ecological restoration have often prioritized vegetation neglecting how restoration planting affects the reassembly of soil invertebrates (Perring *et al.* 2015; Morriën *et al.* 2017). There is also clear limitation in our understanding of how long-term successional processes affect different feeding groups of soil invertebrates (Tylianakis *et al.* 2008; Frouz *et al.* 2013). Furthermore, the multi-trophic community changes and interactions between aboveground and belowground species across spatial and temporal is still remains unknown (De Deyn & Van Der Putten 2005; Neutel *et al.* 2007). The findings of my research aimed to fill this gap by showing long-term successional effect on reassembly of different trophic community. For example, my results indicate that successional increase in average tree size can indeed positively influence abundance of mesofauna and macrofauna trophic groups. This effect is probably because of the increase in food and habitat resource availability by larger trees (Bässler & Müller 2010). Furthermore, I also highlighted the positive indirect effect of restoration age on biomass of mesofauna decomposers and predators, mediated via an increase in average tree size. This effect of increasing average tree size can be attributed to a positive effect of tree biomass on soil organic matter through increasing detritus input, providing more basal resources to decomposers (Bihn *et al.* 2008). Furthermore, larger trees also form complex root systems producing more root exudates which can act as a valuable resource for soil invertebrates (Balandier *et al.* 2022). However, in addition to different trophic groups in a soil food webs showing different responses to the resource inputs from vegetation, they are also further governed by the trophic interactions from which they are structured, which can simultaneously have both top-down and bottom-up effects on the system (De Ruiter *et al.* 1995; Wardle *et al.* 1999; Moore *et al.* 2003). I demonstrate this by showing that while basal resources such as leaf litter can positively influence predators through the pathway of decomposers and omnivores, predation pressure can also be prominent simultaneously in food webs to control prey populations.

Despite the recognized importance of complex food webs, there has been a lack of research quantifying their development during long-term ecological succession (Neutel *et al.* 2007). Ecological restoration should aim to restore not only assemblages of species but also the ecological processes carried out by mature ecosystems (Clewell & Aronson 2006). In addition to understanding the reassembly of soil invertebrates during restoration, recognizing the importance of trophic interactions within soil invertebrate communities will help us better understand and predict trajectories of soil invertebrate reassembly during long term succession (Schneider *et al.* 2004). For example, the decline in soil organic matter quality at later stages of succession will have an important feedback in the interaction between belowground and aboveground systems affecting the decomposers in the soil food web (Williamson *et al.* 2005). The analysis in my chapter four also showed negative feedback from tree species evenness on the rate of bacterivory with increase in successional stage. This is in line previous findings that suggest bacterial-based energy channels are less dominant as succession progresses (Williamson *et al.* 2005), with these effectspropagating to top predatory nematodes and contributing to greater nutrients retention. Thus, understanding how intricate food webs build over time is crucial for both ecological theory and developing sustainable practices in ecosystem conservation and restoration (Neutel *et al.* 2007).

Long term restoration of ecosystems requires a shift in focus from a more traditional focus on assessing biodiversity and ecosystem functioning at single trophic levels to a more comprehensive approach looking at food web structure, and associated ecosystem functioning and stability (Moreno-Mateos *et al.* 2020). Although my study did not detect any effect of restoration age on either connectance or maximum trophic level, these two-food web structural properties were shown to have significant effects on trophic functioning at all trophic levels. The significant effects seen on decomposition rate as well as predation indicates simultaneous occurrence of top-down and bottom-up forces, the balance between which governs the stability of soil food webs (Neutel *et al.* 2007). Furthermore, species interactions vary both in terms of time (Petanidou *et al.* 2008) and across space (Hackett *et al.* 2019) so restoration studies should adapt their assessments to accommodate the dynamic nature of communities (Moreno-Mateos *et al.* 2020). This was highlighted by my study which showed some unexpected patterns such as decreased fungivory with increasing connectance, or complete absence of any effect of restoration age on food web structural properties. Although it is difficult to explain the exact reasons behind these patterns, it is possible this was due to an increase in generalist consumers with increasing connectance

(Sánchez-Moreno *et al.* 2011) and the stage of succession in my forests sites being still too early to exhibit expected patterns of a mature forests (Moreno-Mateos *et al.* 2020; Resch *et al.* 2021). Such complex community dynamics and successional patterns demand for careful application of methods such as chronosequence (i.e. space-for-time substitutions) approach (Damgaard 2019). Nevertheless, since restoration is a centennial process requiring long-term commitment, chronosequences are a valuable method to understand the ecosystem recovery process, to predict the trajectory of succession and how it might respond to changing environmental conditions (Walker *et al.* 2010; Moreno-Mateos *et al.* 2020). I highlight this idea in my study by showing which are the significant biotic and abiotic characteristics affecting the reassembly of soil invertebrates (e.g., positive effect of litter depth on the abundance of macrofauna and mesofauna omnivores and predators), how the effect of increase in restoration age cascade to affect soil invertebrates at different trophic level (e.g., positive effect of restoration age on mesofauna decomposers mediated via average tree size) and finally how a long term restoration practice can affect (or potential absence of such effect) ecological trophic function carried out by soil invertebrates.

5.3 Conclusion

While forest age itself was not a major driver for the reassembly of soil invertebrate communities, this study revealed that time since restoration can have indirect effects on abundance, biomass and mean body mass of soil invertebrates in urban forests. My study emphasizes the importance of including restoration of specific vegetation characteristics, such as tree size, tree species richness and litter layer in restoration strategies to assist the reassembly of soil communities instead of depending on age of the forest, alone. In addition, these findings enhance our knowledge about how aboveground vegetation can influence the biomass of soil taxa communities across different size classes (microbes, nematodes, mesofauna and macrofauna) and trophic groups (decomposers, omnivores and predators). More importantly, my study also shows how forest succession can have indirect effects on ecosystem functions carried out in soil food webs. I also highlight the significance of food web complexity for the flow of energy between resource and consumers in the food web. By doing so, I conclude that it will be beneficial to consider food web complexity in restoration plans to effectively restore the structure and functioning of soil ecosystems.

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