Impacts of earthworm invasion on soils of the Canadian boreal forest

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Soil Science

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Abstract

Exotic species of earthworms are invading North American forests, where native earthworms were extirpated by the last glaciation. The invasion of these ecosystem engineers can alter soil organic matter (SOM) dynamics, carbon (C) persistence, and ecosystem functioning. While the topic has been widely studied in temperate forests, the understanding of the consequences of this invasion in boreal forests is still insufficient. I aimed to determine how invasive earthworms are affecting boreal forest soils, their C stocks, SOM dynamics, and associated microbial communities. To address these objectives, I selected sites to encompass the three most common soil types across the Canadian boreal forest habitable by earthworms: Luvisols, Podzols, and Brunisols. Within each site, sampling zones were delimited to only differ by invasion status while keeping other environmental and pedological factors as similar as possible. I described soil morphological features and estimated the C stocks for the forest floors and mineral soils of invaded and non-invaded soils. I then compared their bacterial and fungal communities using phospholipid fatty acid (PLFA) analysis and metabarcoding of the 16S rRNA gene and ITS2 region. Finally, I estimated the labile (i.e. mineralizable) C using laboratory incubations, characterized the chemical composition of SOM by pyrolysis-gas chromatography-mass spectrometry, and determined C distribution in different density and size fractions of the mineral soil.

The presence of invasive geoengineering earthworms resulted in the thinning of forest floors and the development of novel Ahu horizons, enriched in C and clay. For the forest floors, there was a net loss of C stocks, although the proportion of labile C remained unchanged. While fungal communities of forest floors were unaffected by earthworm invasion, their bacterial communities did shift, notably with increased Gram(+):Gram(-) bacteria ratios, suggesting decreased C availability.

In the novel Ahu horizons, the proportion of labile C was higher than in non-invaded mineral soils and C content was significantly higher across all < 2 mm soil fractions, with a greater proportion of C found in the occluded light fraction. The proportion of root-derived SOM, identified from suberin markers, decreased after earthworm invasion, while that of microbially degraded SOM increased. Increased microbial decomposition was further supported by higher C oxidation states observed in earthworm-invaded soils, and increased C content of the silt and clay-sized fractions, predominantly containing microbially degraded C. In mineral soils, earthworm invasion favoured fungi over bacteria, mainly by an increased relative abundance of ectomycorrhizal fungi at the expense of saprotrophic fungi. For bacterial communities, increased Proteobacteria:Acidobacteriota and decreased Gram(+):Gram(-) bacteria ratios indicated higher nutrient availability in earthworm-invaded mineral soils. These findings show that earthworm invasion significantly affects C dynamics and microbial communities in boreal forest soils, notably through a faster degradation of fresh SOM. This could alter C sequestration and other ecosystem services of boreal forests in the long term.

Preface

This thesis is an original work by Justine Lejoly.

Chapter 2 of this thesis has been published as Lejoly, J., Quideau, S., Laganière, J., 2021. Invasive earthworms affect soil morphological features and carbon stocks in boreal forests. Geoderma 404, 1–13. doi:10.1016/j.geoderma.2021.115262. J. Lejoly and S. Quideau developed the idea. J. Lejoly, S. Quideau, and J. Laganière designed and executed the study. J. Lejoly analyzed the data, J. Lejoly and S. Quideau wrote the manuscript, and J. Laganière contributed to revisions.

A version of **Chapter 3** of this thesis has been submitted for publication to Soil Biology and Biochemistry as Lejoly, J., Quideau, S., Laganière, J., Karst, J., Martineau, C., Swallow, M., Norris, C., Samad, A: Invasive earthworms alter fungal and bacterial communities of Canadian boreal forest soils. J. Lejoly, S. Quideau, J. Laganière, and C. Martineau developed the idea and designed and executed the study. J. Lejoly and A. Samad analyzed the data, C. Martineau, C. Norris, J. Karst, and M. Swallow helped for data interpretation. J. Lejoly, S. Quideau, and J. Karst wrote the manuscript, and C. Martineau, C. Norris, A. Samad, J. Laganière, and M. Swallow contributed to revisions.

A version of **Chapter 4** of this thesis is in preparation for submission for publication to Global Change Biology as Lejoly, J., Quideau, S., Laganière, J.: Invasive earthworms increase litter decomposition and soil labile carbon in boreal forests. J. Lejoly and S. Quideau developed the idea. J. Lejoly, S. Quideau, and J. Laganière designed and executed the study. J. Lejoly analyzed the data, J. Lejoly and S. Quideau wrote the manuscript, and J. Laganière contributed to revisions.

Acknowledgments

I would like to start by thanking my supervisor, Dr. Sylvie Quideau, for giving me the opportunity to pursue this PhD research. Thank you for trusting me and giving me the space to become the researcher that I am today. I will be forever grateful for these 4 ½ years working under your guidance. Your advice, patience, and expertise taught me a lot and I could not have dreamed of a better PhD supervisor.

I also want to thank Drs. Justine Karst, Mathew Swallow, and Nadir Erbilgin for the valuable support and constructive feedback throughout my PhD. This project was also an extensive collaboration with Dr. Jérôme Laganière. Thank you for your help in the development of this project, the logistic support in Québec, the always interesting discussions, and your presence in many other aspects of my PhD.

This research would not have been possible without the financial support of the Natural Science and Engineering Research Council (NSERC), the Faculty of Graduate Studies and Research (FGSR) at the University of Alberta, the Forest and Climate Change Program of the Canadian Forest Service, the Alberta Conservation Association, UAlberta North, the Dr. Ian G W Corns Memorial Graduate Scholarship, the Henry Kroeger Memorial Graduate Scholarship, and the Alberta Graduate Excellence Scholarship.

I would also like to thank the Biogeochemistry group, all the summer students and interns who have helped in my project, and especially Jela Burkus for your support and expertise in the lab.

My appreciation also goes out to my family and friends for their encouragements. My parents, who have been my biggest supporters since day one, raised me to believe that I could do anything. I would not be the person I am today if it was not for their constant and unwavering support. *Vous m'avez donné des racines pour que j'aie des ailes.* I would also like to thank my Edmonton friends for making my life there about so much more that just my PhD. Edmonton will always be home for me. A special thanks to my life partner Antonio Torres Cuenca, for always being incredibly caring.

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Chapter 1 : General introduction

The boreal forest

Covering 1.89 billion of hectares, the boreal zone can be defined as the bioclimatic region of high northern latitudes principally covered by cold-tolerant tree species in the form of forests and wooded land, but also including water bodies and wetlands (Brandt et al., 2013; Brandt, 2009). 28% of the global boreal zone is found in Canada and half of the country's boreal zone (270 millions of hectares) is covered by forests, representing 21-27% of the global boreal forest area across the globe (Brandt et al., 2013; Brandt, 2009).

For the circumpolar region, Brunisols (Cambisols) and Podzols are globally the major soil types found in upland forest (DeLuca and Boisvenue, 2012). In the upland Canadian boreal forest, in addition to these two soil types, Luvisols also cover an important area (Lavkulich and Arocena, 2011). Podzols typically develop on non-calcareous, coarse-textured parent materials under wet climate and coniferous vegetation (Sanborn et al., 2011), while Luvisols develop in well-drained forest soils on calcareous parent materials such as till and lacustrine deposits (Lavkulich and Arocena, 2011). Brunisols are less-developed soils found on various parent materials, forming a continuum of development with either Luvisolic or Podzolic soils (Smith et al., 2011).

The typical vegetation of the boreal forest varies across the boreal zone, as soil type and climatic conditions play a major role in vegetation development. Coniferous forests dominate the boreal forest in eastern Canada, including *Abies, Pinus,* and *Picea* species (Canada's National Forest Inventory, 2021; Gauthier et al., 2015). Mixed-wood stands, which often include trembling aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* (Moench) Voss), are common in the boreal forest of western Canada. Across the Canadian boreal forest, the importance of

trembling aspen stands is increasing as they replace coniferous-dominated stands, a process triggered by changes in the frequency and size of disturbances associated with climate change and forest management (Aubin et al., 2018; Schneider et al., 2009; Soja et al., 2007). Maple (*Acer*) species are also often found in eastern Canada at the southern limit of the boreal zone (Goldblum and Rigg, 2005).

Recent estimations of carbon (C) storage in the boreal forest are as high as 1095 Pg C, making the boreal forest the largest terrestrial C reservoir (Bradshaw and Warkentin, 2015). Most of this C is found belowground and is distributed between the litter (24%), soil organic matter (SOM, 40%) and belowground biomass (5%) pools (Kurz et al., 2013). Boreal forests are characterized by a large slow-cycling carbon pool, whose decomposition is mainly limited by low annual mean temperatures as well as the abundance of recalcitrant compounds (e.g.; tannins) in boreal vegetation litter (De Deyn et al. 2008).

Soil organic matter and carbon dynamics

Soil organic matter (SOM), predominantly made of C, mainly originates from surficial litterfall, decaying roots, root exudates, and dead microorganisms, hereafter referred to as microbial necromass (Post and Kwon 2000). Understanding SOM dynamics is important to determine the fate of the major terrestrial C reservoir that is the boreal forest. Multiple approaches to partition SOM into meaningful and useful pools have been proposed, from the outdated chemical separation into humic and fulvic substances found to be a laboratory artifact (Piccolo, 2001) to physical fractionation based on size and/or density (von Lützow et al., 2007). Physical fractionation of soil yields different aggregate sizes, typically obtained by wet or dry sieving and slacking (von Lützow et al., 2007). From these fractions, different pools of SOM are obtained, from the silt and clay-sized fraction (< 53 µm) to aggregates of different sizes (53–250 µm, 250 µm–2 mm, 2–8 mm; Fig. 1-1). The SOM

found in microaggregates (< 250 μm) is typically considered more decomposed and older than that of macroaggregates (> 250 μm; von Lützow et al., 2007). Those macroaggregates can be further fractionated into occluded light fraction (250 μm–2 mm), occluded microaggregates (53–250 μm), and occluded silt and clay-sized fraction (< 53 μm). In an effort to decrease the number of fractions to the most relevant for SOM dynamics, a more recent approach consists of dividing SOM into the particulate organic matter (POM) and the mineral-associated organic matter (MAOM) fraction (Cotrufo et al., 2019). The classic physical fractionation and the POM-MAOM framework are both based on stabilization mechanisms and resulting persistence in soils (Lavallee et al., 2020). The POM pool contains fragmented plant residues, only partially processed, and its only protection mechanism is occlusion within aggregates while the MAOM pool is stabilized by mineral associations, contains smaller, more processed compounds, and has a longer mean residence time (Lavallee et al., 2020; von Lützow et al., 2006). While POM mainly originates from vegetation, MAOM largely contains microbial-derived compounds, as well as some labile fresh litter compounds (Cotrufo et al., 2019).

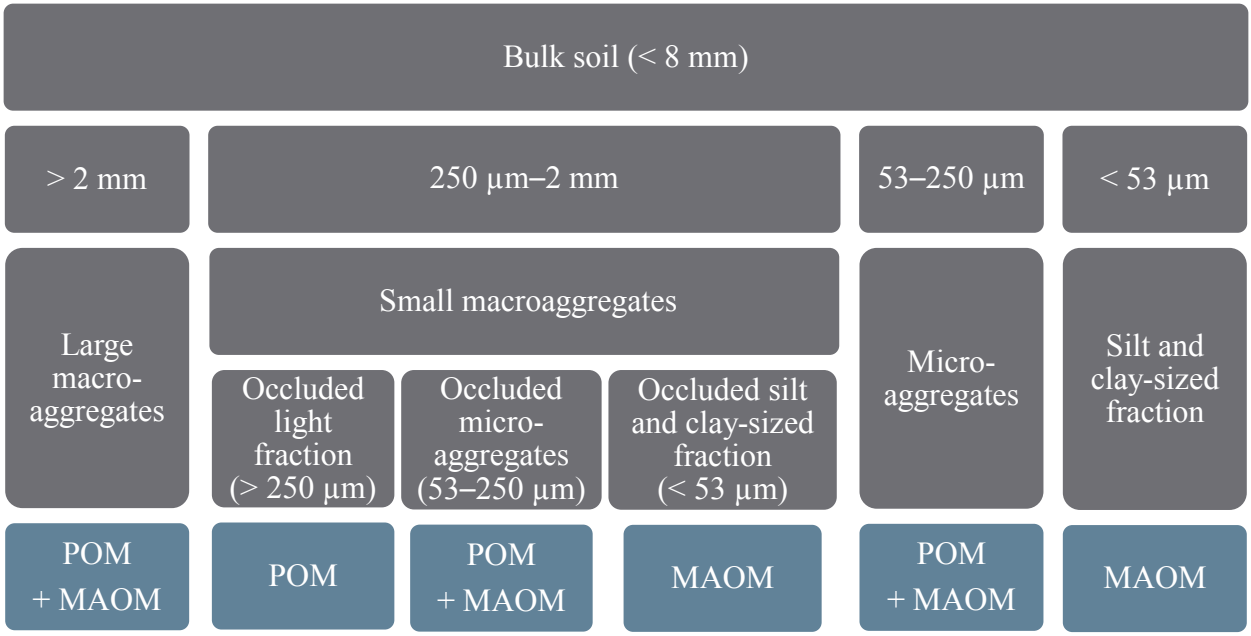


Figure 1-1. Comparison between the aggregate fractionation method and the particulate organic matter (POM) and mineral-associated organic matter (MAOM) framework, adapted from Lavallee et al. (2020).

Stabilization of SOM is also linked to its structure and composition (Kögel-Knabner, 2000). Plant residues are highly complex organic compounds, often divided into above (leaves and shoots) and belowground (roots) inputs, and represent the main contributors to SOM (Kögel-Knabner, 2002). Root-derived residues are typically more abundant than leaf-derived residues in the mineral soil, the latter supposedly degrading faster, although the mechanisms behind are still uncertain (Mueller et al., 2013). Some of those plant-derived residues, such as lipids, are partly preserved in soils and therefore represent a substantial part of SOM (Kögel-Knabner, 2000). Cutin and suberin, lipids found in leaves and roots respectively, have been used as biomarkers to differentiate above from belowground inputs to SOM (Hamer et al., 2012; Otto et al., 2005). Among many methods, analytical pyrolysis, a form of thermal degradation, gives a broad overview of SOM compounds, compared to more selective chemical extractions (Derenne and Quéné, 2015). In addition to the above-mentioned plant-derived lipids, pyrolysis, coupled with gas chromatography and mass

spectrometry (Py-GC-MS), also provides information on other aliphatic as well as aromatic compounds (for example, Vancampenhout et al., 2009). An important part of the stable SOM pool is obtained from the decomposition of labile plant inputs by microorganisms (Cotrufo et al., 2013). However, in forested ecosystems where conditions are less favorable for microbial growth, the proportion of plant residues in stabilized SOM is higher than in more fertile soils (Angst et al., 2021; B. Wang et al., 2021). Estimating the relative abundance of plant *versus* microbial-derived SOM is therefore important for understanding SOM dynamics (Angst et al., 2021).

Regardless of chemical composition and physical distribution, the stability of soil C can also be estimated by incubation at high temperatures (McLauchlan and Hobbie, 2004). This empirical technique is based on the concept that high temperature (between 20 and 35 ºC) will maximize C mineralization by microorganisms (Laganière et al., 2013; Paré et al., 2006). After one year of incubation, the cumulative CO_2 typically reaches a plateau, and the data can be fitted on an exponential model to estimate the amount of C potentially mineralizable by microorganisms (Laganière et al., 2013; Maillard et al., 2010). This C pool is therefore considered biologically active, as it is potentially mineralizable by microorganisms.

Soil microbial communities

Soil microbial communities play a major role in litter decomposition (Prescott 2010) and nutrient cycling (Uroz et al., 2016). The forest microbiome typically contains archaea, bacteria, fungi, and protists but most of the literature focuses on bacterial and fungal communities (Uroz et al., 2016). Many techniques have been used for quantifying and/or characterizing soil microbial communities, from direct cell counting and culture to analysis of cell wall or membrane derived compounds (chitin, phospholipid fatty acids) and DNA-based approaches (Strickland and Rousk, 2010). The relative abundance of fungi compared to bacteria is dependent on environmental conditions,

including physical disturbances and nutrient availability (Strickland and Rousk, 2010). Notably, the fungi:bacteria ratio is often positively correlated with soil C:N (Waring et al., 2013).

Fungi, typically decomposing more recalcitrant organic compounds (Treseder et al., 2016), can be free-living decomposers (saprotrophs) or root-associated symbionts (Lladó et al., 2017). Except for maple (*Acer*) species which form symbiosis with arbuscular mycorrhizae (AM), most boreal tree species are associated with ectomycorrhizal (EcM) fungi (Brundrett et al., 1990). In most cases, microbial necromass is predominantly derived from root-associated fungi, accounting for 50 to 70% of the C stored in the boreal forest soils together with roots (Clemmensen et al., 2013). For this reason, many studies have focussed on fungal rather than bacterial communities of forest soils (Lladó et al., 2017). Bacteria are however involved in a wider range of biogeochemical processes, such as N fixation (Lladó et al., 2017), of particular interest in boreal forest soils where N fixation is limited (DeLuca et al., 2002).

Earthworm ecology

Charles Darwin was the first scientist to publish a book on earthworms in 1881, named "The formation of vegetable mould through the action of worms with observations on their habits", after 40 years of observations (Feller et al., 2003). Since then, many scientists have studied earthworm ecology and impacts on soil properties. Earthworms have long been defined as "ecosystem engineers", meaning that "modulate the availability of resources to other species" (Jones et al. 1994). Their positive impact on soil fertility has been largely recognized in agricultural systems (Lavelle et al., 1998; Van Groenigen et al., 2019).

Bouché (1977) defined three ecological categories for earthworms based on their feeding behaviour and soil habitat: epigeic, endogeic, and anecic species. Epigeic species inhabit the forest floor layer and feed on litter, while endogeic species inhabit the mineral soil, which they also feed on.

Anecic species share traits with both epigeic and endogeic species, as they burrow deep galleries, feed on litter, and bring soil material to the surface. Rather than a strict classification, these three categories represent three poles of a triangular plot, within which all earthworm species are found (Capowiez et al., 2022). While some species are only associated with one category, such as the epigeic *Dendrobaena octaedra* Savigny, other species fall between two categories, such as the epi-anecic *Lumbricus rubellus* Hoffmeister. Earthworms dwelling into the mineral soil - corresponding to endogeic and anecic species - are also referred to as geoengineering earthworms (Blume-Werry et al., 2020; Wackett et al., 2018). Some authors have also made the distinction between de-compacting and compacting earthworms, based on their effect on soil porosity and aggregation (Lavelle et al., 2006). Regardless of the classification used, the way that earthworms impact soil physicochemical properties might vary among species (Lavelle et al. 2006).

Through their feeding behaviour, earthworms greatly impact soil aggregation and enhance C stabilization within aggregates (Bossuyt et al. 2004, 2005; Zhang et al. 2013). On the other hand, by mixing the forest floor into the mineral soil, they also enhance microbial decomposition by increasing OM accessibility. In a review, Lubbers et al. (2013) concluded that earthworms increase CO_2 emissions during an initial phase of one to two weeks, but the magnitude of this increase becomes insignificant after 200 days of incubation. There is however no consensus as to whether earthworms increase or decrease C storage once established for more than a few years (Angst et al., 2019; Lubbers et al., 2017), although they seem to increase soil greenhouse gas emissions (Lubbers et al., 2013). This concept is referred to as the *earthworm dilemma* as earthworms accelerate SOM decomposition, thus increasing CO_2 emissions, while also increasing its stability, by occluding SOM within aggregates and/or chemically altering it to microbial necromass (Angst et al., 2019; Lubbers et al., 2013). The impacts of earthworms on C sequestration have mainly been

indirectly calculated, as changes in C stocks occur over the long-term, from years to decades and even centuries (von Lützow et al. 2006; Zhang et al. 2013; Lubbers et al. 2017). Angst et al. (2017) suggested that C stabilization by earthworms occurs mainly by physical protection within aggregates, as no changes in SOM chemical composition were reported, even after more than two years of incubation. However, Vidal et al. (2019) observed a clear shift from POM to MAOM-dominated C pool within earthworm casts, after one year of incubation, which shows that SOM composition changes as casts age. Overall, these observations suggest that changes in chemical composition could take longer than a few years to be detectable in the bulk soil.

Earthworm invasion in North American forests

The Wisconsin glaciation, which ended about 12,000 years BP, completely extirpated native earthworms from most of North America (Hendrix 2006). As a result, northern ecosystems, including their soils, vegetation, and C pools have developed in the absence of earthworms (Addison 2009). Since the European settlement, exotic earthworms have been introduced and are invading North America (Scheu and McLean 1993). Their invasion in the boreal forest is relatively recent, as environmental conditions are more hostile and human activities are less developed when compared to temperate forests. Although earthworms can withstand relatively cold temperatures (Addison, 2009), the expected 4–5 ºC increase in the Canadian boreal zone by the end of the 21st century (Price et al., 2013) is likely going to facilitate their establishment in a greater part of the boreal forest. In addition, the rapid growth of recreational and industrial activities in the boreal further multiplies the opportunities for invasion (Cameron et al. 2007). Waterways and roads are thought to be the main vectors for earthworm dispersal at a local scale (Cameron and Bayne, 2015). In other parts of the boreal biome – even in Eurasia –, geoengineering European earthworms are also considered invasive and greatly impact soil morphological properties (Wackett et al., 2018).

More recently, a second wave of invasion by Asian earthworm species has been reported in North America (Chang et al., 2021). These jumping worms represent an additional threat for northern temperate and boreal forests, yet the competition with other earthworm species is poorly understood. To this date, Asian earthworms have only been observed in southern Canada, and have not reached the boreal forest yet (Reynolds, 2018).

Effects of invasive earthworms in forested ecosystems

Although earthworms are generally seen as beneficial in agricultural soils, their effects on forest soils might be very different (Addison, 2009; Frelich et al., 2019). Studies have reported negative effects on soil bulk density (Frelich et al., 2006), above and belowground arthropods (Cameron et al., 2013; Jochum et al., 2021), and native plant communities (Dobson and Blossey, 2015; Eisenhauer et al., 2009; Hale et al., 2008). The invasion of the American continent by non-native earthworms can be seen as a large-scale experiment, where non-invaded zones act as control sites (Blouin et al. 2013). It therefore represents a unique – yet unfortunate – opportunity to monitor the changes in soil physicochemical properties associated with earthworm activity. Visible changes in soil morphological features have also been noticed in temperate forests of New Brunswick (Langmaid 1964) and Wisconsin (Nielsen and Hole 1964). Three years after earthworm invasion, the Ae horizon and some of the B horizon had been completely transformed into a thick dark grey loam mainly consisting of earthworm casts. Earthworms lead to a relocation of carbon from the forest floor to the upper mineral horizon, increasing its thickness and OM content, whereas the forest floor thickness decreases compared to earthworm-free soils (Hale et al. 2005). The total loss of forest floor has been observed in many cases (Bohlen et al. 2004; Wironen and Moore 2006; Fahey et al. 2013; Groffman et al. 2015). The consequences of these changes for SOM dynamics and C sequestration are, however, still uncertain (Lubbers et al., 2013). While the decrease in forest floor thickness and C stock is often reported, the fate of the C found in the mineral soil is still debated in temperate forests (Bohlen et al., 2004a; Fahey et al., 2013; Scheu and Parkinson, 1994). Similar studies are lacking for the boreal biome.

Besides direct effects on C dynamics, earthworms also greatly affect the belowground diversity, including enchytraeid communities (Schlaghamersky et al., 2014) and microarthropods (Cameron

et al., 2013). Changes in soil microbial communities and their activity have also been observed in contrasting environments, including: a decrease in microbial respiration (Eisenhauer et al. 2007), an increase in the bacterial:fungal ratio (Scheu and Parkinson 1994), an increase in Gram-negative bacteria (Clapperton et al. 2001; Enami et al. 2001), or a reduction in total bacterial PLFAs as well as fungal biomarkers (Butenschoen et al. 2007). The passage of soil through earthworm gut seems to strongly influence microbial communities by inhibiting and activating different groups of microorganisms (Clapperton et al. 2001; Aira and Domínguez 2014). The type of organic residues ingested by earthworms was also found to highly affect microbial communities (Tiunov and Scheu 2000; Egert et al. 2004). Considering the microbial-mediated SOM stabilization mechanisms, earthworms are likely altering the dynamics of SOM formation and accumulation by modifying the spatial availability of plant materials for decomposition.

Earthworms play a key role in plant residue incorporation and degradation. They can alter the litter decomposition by selectively feeding on lignin-poor residues such as leaves (Filley et al., 2008), resulting in a POM pool enriched in lignin (Crow et al., 2009). They preferentially accumulate root over shoot litter, the latter decomposing at a higher rate in casts (Vidal et al., 2016). As earthworm casts age, mineral-associated and microbially derived OM prevails and aliphatic moieties become more abundant (Vidal et al., 2019). It is suggested that by decomposing plant-derived OM and transforming it into microbial necromass stabilized within aggregates, earthworms potentially increase C resilience against disturbances (Angst et al., 2019).

Knowledge gap for the boreal forest

While earthworm invasion has been extensively studied in temperate forests, research efforts started more recently in boreal forests, which remain understudied. To support this statement, I made a query in the Web of Science using "earthworm", "forest", "soil", "inva*", and "temperate" or "boreal" and obtained only 40 results for the boreal forest, compared to 169 for the temperate forest (Fig. 1-2).

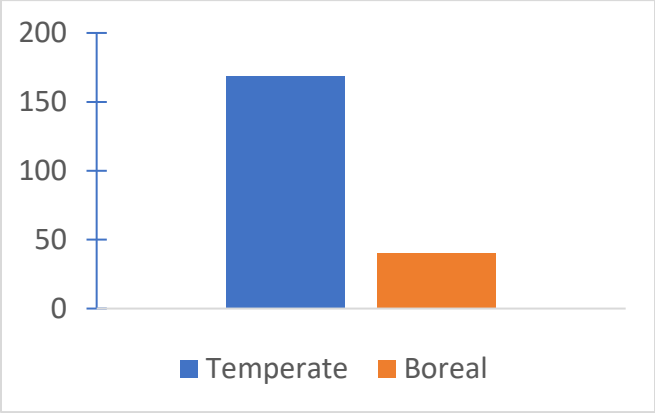


Figure 1-2. Number of papers on earthworm invasion and boreal or temperate forest soils. Results of a query in Web of Science on April 3, 2022.

While one might expect to observe similar effects of invasive earthworms on those two northern biomes, boreal and temperate forests also greatly differ. Boreal forests are characterized by colder annual mean temperatures and longer winters compared to temperate forests. The importance of fungal communities is greater in boreal forest soils (Chen et al., 2020), which store more than twice as much C per unit of area compared to temperate forests (Lal, 2005). Any change in the C balance has therefore greater consequences for this ecosystem, already threatened by climate change (Gauthier et al., 2015). Considering that earthworm invasion is ongoing and expected to affect at least 50% of the boreal forest (Cameron and Bayne, 2009; Saltmarsh et al., 2016), there is an urgent need to better understand its dynamics and implications for C storage.

Objectives and outline

The main objective of this doctoral thesis is to understand the impacts of earthworm invasion on soils of the Canadian boreal forest, by comparing soils that have been affected by earthworms with soils that are not invaded. The sites were selected in the provinces of Alberta and Québec to represent the major upland soil types found across the boreal forest of Canada.

My first research chapter (Chapter 2) gives a general overview of the soil morphological changes associated with earthworm invasion. I compared basic soil properties (pH, texture, TOC) between invaded and non-invaded soil pedons and estimated C stocks for the forest floor and surface mineral soil. For this chapter, the objectives were to:

1. Describe soil morphological features in the presence and absence of invasive earthworms.
2. Identify any significant changes in morphological features of forest floors and mineral soils associated with invasive earthworms.
3. Determine if soil C stocks are affected by earthworm invasion.

In my second research chapter (Chapter 3), I focussed on the potential changes observed in soil bacterial and fungal communities, using a combined approach of PLFA analysis and metabarcoding of the 16S rRNA and ITS genes. I described the taxonomical shifts associated with earthworm invasion and linked them with potential functional changes, considering environmental conditions for microbial activity. The objectives of this second research chapter were to:

1. Characterize the microbial communities of forest floors and mineral soils in the presence and absence of invasive earthworms.
2. Identify microbial taxonomic groups positively and negatively affected by earthworm invasion.

3. Link the taxonomic changes observed in fungal and bacterial communities with changes in soil environment conditions.

For my final research chapter (Chapter 4), I characterized SOM using a range of analyses, including physical aggregation, chemical composition, and biological persistence. The objectives of this chapter were to:

1. Determine whether earthworm invasion affects soil C persistence.
2. Describe changes in the physical distribution of soil C associated with earthworm invasion.
3. Determine whether earthworm invasion affects the chemical composition of SOM, including the relative abundance of above *versus* belowground plant residues and of microbial residues.

In Chapter 5, I summarized the results of the three research chapters, discussed the significance of these findings, and proposed direction for future research.

Chapter 2 : Invasive earthworms affect soil morphological features and carbon stocks in boreal forests

Abstract

Non-native earthworms have been invading North America since European settlement. Compared to temperate forests, their presence in the boreal forest is much more recent and thus remains understudied, despite the potential threat they represent for soil carbon (C) stocks. Here we compared earthworm-invaded and earthworm-free zones in soil types representative of the boreal forest, including Luvisols, Podzols, and Brunisols (Cambisols). We observed that the forest floor (surface organic layer, or LFH) decreased in thickness after invasion in most cases and developed into a Vermimull, with the loss of the most humified layer (humic or H horizon). Simultaneously, the surface mineral horizon was reworked by earthworms into a novel Ahu horizon, characterized by higher organic matter and enriched in earthworm casts. Forest floor C stocks decreased by 94 % and 59 % for Luvisols and Brunisols respectively, while those of Podzols remained apparently unaffected. Mineral soil C stocks in Brunisols increased after invasion, while no changes were observed in Luvisols and Podzols. Our results demonstrated the substantial impact that invading earthworms are having on soil morphological features and C stocks in boreal forests. Effects were similar to what has been reported for temperate forests, although the degree of impact depended on soil type. Although C stocks were less affected in the mineral soil compared to the forest floor, the development of a novel surface horizon reworked by earthworms could alter microbial dynamics and impact mineral C persistence. Further research is needed to quantify long-term implications of earthworm presence for boreal soil C stocks.

Highlights

- Earthworm impacts on morphological features varied among studied soil types.
- Development of a carbon-rich top mineral horizon and vermimull humus forms.
- Forest floor carbon stocks decreased for Luvisols and Brunisols.
- Surface mineral soil carbon stocks increased for Brunisols only.
- Minor effects on carbon stocks for Podzols.

Introduction

The Wisconsin glaciation, which ended about 12,000 years BP, completely extirpated native earthworms from most of North America (Hendrix, 2006). As a result, northern ecosystems, including their soils, vegetation and carbon (C) pools, have developed in the absence of earthworms (Addison, 2009). Since European settlement, exotic earthworms have been introduced and have been invading North America (Scheu and McLean, 1993). Despite limiting factors such as long cold winters and acidic soil conditions, earthworms have been observed in boreal forests (Addison, 2009), where the rapid growth of recreational and industrial activities is facilitating their invasion (Cameron et al., 2007). The boreal forest is the largest terrestrial C reservoir on Earth, and any change in its C dynamics can potentially have major implications for global C pools (Frelich et al., 2019; Kurz et al., 2013). With an expected 4–5 ºC increase in temperature by the end of the 21st century (Boulanger et al., 2017), a greater part of the boreal forest will become hospitable to earthworms (Booysen et al., 2018). Moreover, liming has been commonly used to restore sugar maple forests in Eastern Canada, alleviating the acidic conditions found in those soils (Moore et al., 2013). Earthworms have already been observed in boreal forests of Alaska (Saltmarsh et al., 2016), Western Canada (Cameron et al., 2007) and Eastern Canada (Moore and Reynolds, 2003). The concerns about this ongoing invasion are not limited to North America, as the presence of European geoengineering (endogeic and anecic, see below) earthworm species have been recorded in various parts of the Arctic biome such as Russia and Fennoscandia, where they are considered exotic and invasive (Blume-Werry et al., 2020; Wackett et al., 2018).

Although mostly seen as beneficial in agricultural soils and for post-mining reclamation, earthworms may not be desirable in forest soils since they mainly feed on organic matter (OM) found in the forest floor or mineral soil (Blouin et al., 2013; Frouz et al., 2009). Some exotic earthworm

species can fundamentally change soil properties in a few years for temperate forests, even more so in soils devoid of endemic species by occupying a vacant ecological niche (Hendrix and Bohlen, 2002; Langmaid, 1964). In temperate forests of North America, earthworms have been shown to substantially increase C losses from the forest floor, which even disappeared in extreme cases, thereby decreasing overall soil C stocks and increasing CO_2 emissions, at least transitionally (Blouin et al., 2013; Bohlen et al., 2004a; Hale et al., 2005b). However, some of the C lost from the forest floor is transferred to the upper mineral horizon by bioturbation and is potentially stabilized in aggregates formed by earthworms (Bossuyt et al., 2004). The loss of forest floor C following earthworm invasion is probably also occurring in boreal forests, albeit at a slower pace, but there are insufficient data and understanding of net effects (Kurz et al., 2013). When compared to agroecosystems, earthworm effects on net CO_2 emissions in natural ecosystems such as temperate forests are smaller because of larger C pools, as was reviewed by Lubbers et al. (2013). Boreal forests are characterized by lower decomposition rates and thicker forest floors compared to temperate forests (DeLuca and Boisvenue, 2012). For these reasons, the conclusion of Lubbers et al. (2013) for temperate forests may not hold true for the boreal biome. Although a few studies showed that litter decomposition rate was increased in the presence of earthworms in some boreal forest soils (Laganiere et al., 2010), it is still unclear whether these organisms can affect the vertical distribution of C and the overall C balance in these soils.

It is challenging to study long term effects of earthworms on soil properties (Clements et al., 1991). Under laboratory conditions, studies span from 21 days to 2.4 years (Bossuyt et al., 2005; Frouz et al., 2014; Hale et al., 2008). Under field conditions, studies also typically focus on quantifying short term effects of earthworms over a maximum of 3 years (Bohlen et al., 2004a; Fisk et al., 2004; Groffman et al., 2004). As an alternative, simultaneously studying earthworm-invaded and

earthworm-free zones in otherwise similar ecosystems can be considered as a long term experiment, where non-invaded zones act as control sites (Blouin et al., 2013; Lubbers et al., 2013). This 'space-for-time' approach is commonly used in community ecology (Damgaard, 2019; Sax et al., 2005; Thomaz et al., 2012) and has been applied in southern Alberta to compare soil chemistry and microbial activity along a transect corresponding to different invasion stages (Eisenhauer et al., 2007). Although for many sites it is nearly impossible to determine when the invasion started, historical knowledge of road construction, fishing activities, and/or agriculture should give valuable hints for potential invasion vectors (Cameron et al., 2007).

Most invasions involve multiple earthworm species, with different ecological behaviours. Bouché (1977) defined three categories based on their feeding behaviour and habitat: epigeic, endogeic and anecic earthworms. By feeding on plant litter, epigeic and anecic species accelerate litter disappearance from the soil surface, the latter having the strongest effect because of higher body mass (Hale et al., 2005a; Huang et al., 2020). As endogeic species inhabit the mineral soil and do not feed on plant litter, they have little impact on the litter layer but have the strongest effect on the structure and aggregation of the underlying mineral horizons (Bossuyt et al., 2006; Knowles et al., 2016). The association of at least two functional groups can yield to a greater cumulative effect on litter decomposition and soil organic carbon (SOC) stabilization and/or persistence (Huang et al., 2020). In temperate forests, Hale et al. (2005a) described a succession of earthworm species along the leading edge of invasion, which was defined as the point where forest floor thickness had decreased to zero. Epigeic species were observed 20 m or more in advance of the leading edge, endogeic species were found right behind the leading edge, and anecic species thrived 20–30 m behind. This gradient found at the stand scale by Hale et al. (2005a) was also described along a 125 km north-south transect in temperate forests of south-central Ontario by Choi et al. (2017),

with the most northern site being the least invaded. At a global scale, Phillips et al. (2019) showed that earthworm diversity increases with latitude, and is mostly correlated with climate variables. But in these invaded regions, the reported earthworm density is higher than for most of the globe, as those exotic species access a large, otherwise unused pool of resources.

Understanding the factors and processes of soil formation is a prerequisite for the description of soil horizons and morphological features (Jenny, 1941). In addition, the use of a soil classification system – such as the Canadian System for Soil Classification in the case of the present study – is fundamental to organize knowledge in a defined frame, allowing for comparisons among soil types (Soil Classification Working Group, 1998). In temperate forests, effects of invasive earthworms on soil morphology have been described in Podzols (Langmaid, 1964; Nielsen and Hole, 1964) and Luvisols (Alban and Berry, 1994; Eisenhauer et al., 2007). In all cases, authors described development of an organic-rich A horizon mainly comprised of earthworm casts. In boreal forests, the main soil types potentially hospitable to earthworms are Podzols, Brunisols (corresponding to Cambisols in the World Reference Base classification by FAO (2014)), and Luvisols (DeLuca and Boisvenue, 2012). Podzolic and Luvisolic soils, respectively covering 14.3 and 8.8 % of Canada's land area, develop on drastically different parent materials: acidic coarse-textured for the former and calcareous fine-textured for the latter (Lavkulich and Arocena, 2011; Maynard et al., 2014; Sanborn et al., 2011). As for Brunisolic soils, covering about 14 % of land surface in Canada, they typically co-exist with both Podzolic and Luvisolic soils but lack the same level of horizon development (Smith et al., 2011).

This study aimed to describe soil morphological features and estimate organic C stocks for the forest floor and top mineral soil in earthworm-invaded and earthworm-free zones for contrasting soil types and boreal ecozones present within the Canadian boreal forest: Luvisolic soils typical of

the Boreal Plains in Western Canada and Podzolic and Brunisolic soils found in the Boreal Shield of Eastern Canada. We hypothesized that effects of invasive earthworms in the boreal forest would be similar to those in temperate forests, with zones invaded by earthworms associated with thinner forest floors and cast-rich uppermost mineral soils, and that more pronounced changes would be evident in the presence of at least two earthworm functional groups. In terms of C stocks, we expected a decrease in the forest floor and an increase in the mineral soil resulting from a transfer of organic matter from the forest floor to the mineral soil after earthworm invasion. Furthermore, we expected to see a variable degree of earthworm effects related to soil type, considering the fundamental differences between the three studied soil types. Luvisols under trembling aspen (*Populus tremuloides* Michx.) canopy would show the largest changes after earthworm invasion, while acidic Podzols would be the least affected. Brunisols would be intermediate as they developed on acidic parent material but under deciduous canopy.

Materials and methods

Study sites

The study was conducted in the Boreal Plains and the Boreal Shield ecozones of Canada's forest, respectively in Alberta and Québec (Table 2-1; Fig. 2-1). The different sites were selected based on existing research data as well as the likelihood to find both earthworm-invaded and earthworm-free zones within the same site or in similar environmental conditions. The selected sites represent some of the most common scenarios in the boreal: Podzols (WRB: Podzols; USDA: Spodosols) under coniferous canopy, Luvisols (WRB: Luvisols; USDA: Alfisols) under aspen and Brunisols (WRB: Cambisols; USDA: Inceptisols) under other broadleaves (FAO, 2014; Soil Survey Staff, 1999). In Québec, four sites were selected: Lac Piché in Montmorency Forest (referred to as Montmorency), Lac Laroche in Parc National des Grands Jardins (referred to as Grands Jardins), Canadian Forces Base Valcartier (referred to as Valcartier), and Golf Castor (referred to as Golf). Montmorency and Grands Jardins have a mixed coniferous dominant canopy, respectively balsam fir (*Abies balsamea* (L.) Mill.) and black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenburg), and Podzolic soils. Valcartier and Golf have sugar maple (*Acer saccharum* Marshall) as dominant canopy and Brunisolic soils (Table 2-1). The three Alberta sites have similar Luvisolic soils and trembling aspen dominated canopy: the Ecosystem Management Emulating Natural Disturbance (referred to as EMEND), Wolf Lake Provincial Recreation Area (referred to as Wolf Lake), and the Breton plots (referred to as Breton). For five of these seven sites (EMEND, Montmorency, Grands Jardins, Valcartier, and Golf), we were able to define earthworm-invaded and earthworm-free zones within a couple hundred meters from one another, providing the opportunity for a site-level paired comparison between earthworm-invaded and earthworm-free soils. By

comparing physicochemical properties of deeper soil horizons, less likely to be affected by earthworms, we ensured a baseline as similar as possible for site-level comparisons (Table 2-2).

In addition, different stages of invasion were identified in Alberta along a latitudinal gradient similar to the one described by Choi et al. (2017): the most northern site (EMEND) was only partly invaded by epigeic earthworms while the most southern site (Breton) was invaded by epigeic, endogeic, and anecic earthworms; the intermediate site (Wolf Lake) being invaded by epigeic and endogeic species. By selecting three sites with similar overstory aspen vegetation and comparable glacial till parent material, we minimized variations in environmental soil forming factors as defined by Jenny (1941) (Table 2-1). Understory vegetation was also similar for all sites, including mooseberry (*Viburnum edule* (Michx.) Raf.), Canadian bunchberry (*Cornus canadensis* L.) and prickly wild rose (*Rosa acicularis* Lindl.). To validate our space-for-time approach, we conducted preliminary analyses, which confirmed that all sites had comparable texture, although that of Wolf Lake was slightly coarser (Table 2-2). In addition to the site-level paired comparison mentioned in the previous paragraph, this experimental design was an opportunity to compare soil morphological features associated with different levels of earthworm invasion for a similar soil type (Luvisol) and vegetation cover (trembling aspen).

Table 2-1. Main site characteristics. Climate data were obtained from Alberta Climate Information Service (2020) for Alberta (AB) sites and Environment Canada (2010) for Québec (QC) sites. For understory vegetation, only the three most abundant species are mentioned.

		Soil type	Dominant tree canopy	Understory vegetation	Coordinates		Mean annual temperature [ºC]	Mean annual precipitation [mm]
					Latitude (N)	Longitude (W)		
AB	*EMEND*	Luvisol	*Populus tremuloides* Michx.	*Viburnum edule* (Michx.) Raf. *Cornus canadensis* L. *Rosa acicularis* Lindl.	56º46’	118º22’	1.4[1]	413[a]
	Wolf Lake				54º40’	110º58’	1.3[2]	462[b]
	Breton				53°05'	114°26'	3.1[3]	552[c]
QC	*Golf Valcartier*	Brunisol	*Acer saccharum* Marshall	*Maianthemum canadense* Desf. *Cornus canadensis* L. *Ulmus americana* L.	46°55’ 46°55’	71°27’ 71°36’	1.6[4]	1460[d]
	Montmorency	Podzol	*Abies balsamea* (L.) Mill.	*Sphagnum* spp. *Dryopteris* spp. *Cornus canadensis* L.	47°19’	71°09’	0.5[5]	1583[e]
	Grands Jardins		*Picea mariana* (Mill.) Britton, Sterns & Poggenburg	*Sphagnum* spp. *Oxalis* spp. *Cornus canadensis* L.	47°40’	70°50’	3.7[6]	930[f]

[1] Obtained from Fairview station, 80 km South of the site
[2] Obtained from Sand River station, situated within the park limit
[3] Obtained on site
[4] Obtained from Riviere Verte Ouest station, 20 km North-West of the site
[5] Obtained on site
[6] Obtained from Saint-Urbain station, 26 km South of the site

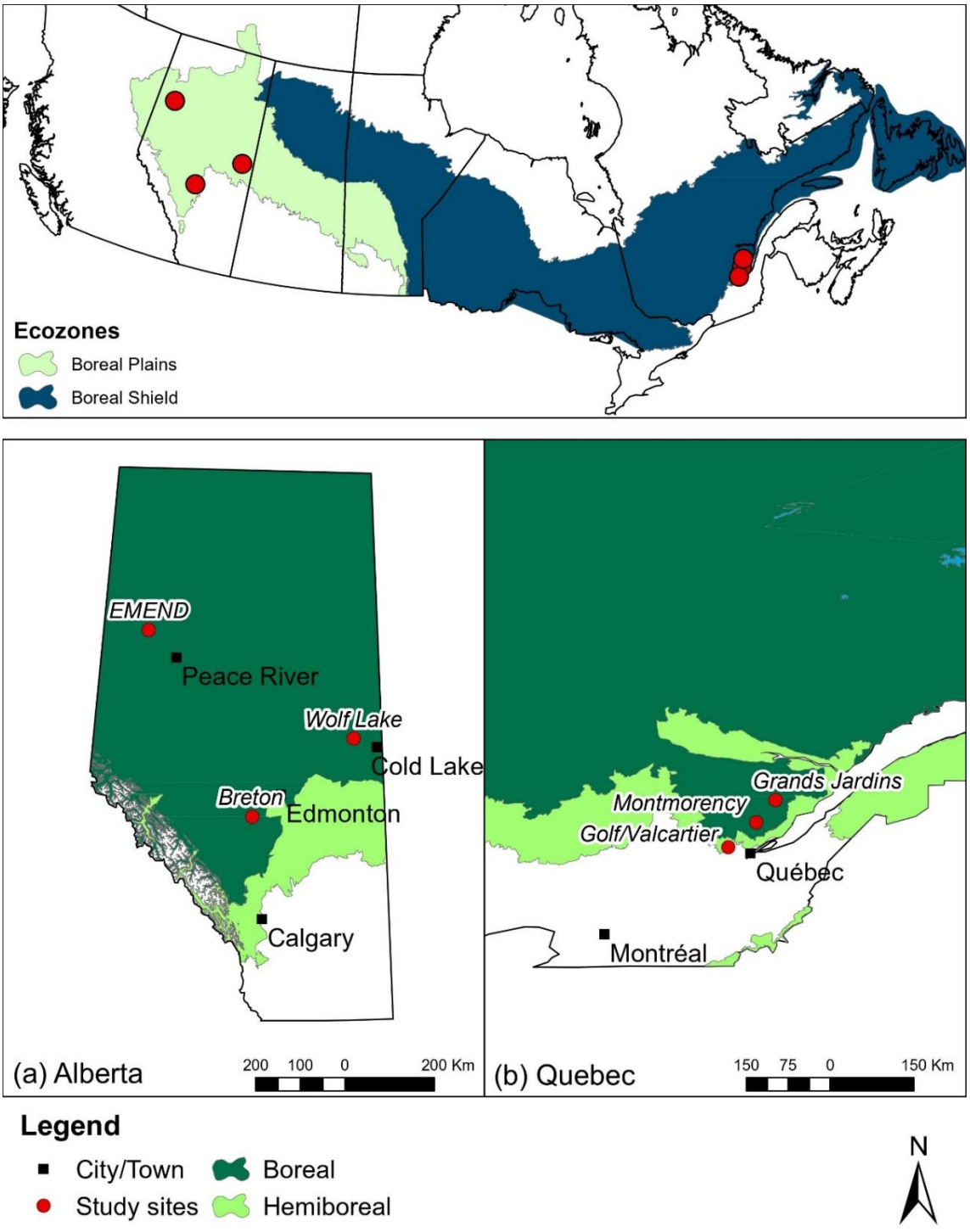


Figure 2-1. Site location within Canada. Luvisols (EMEND, Wolf Lake, and Breton) are found in the Boreal Plains ecozone in Alberta. Brunisols (Golf and Valcartier) and Podzols (Montmorency and Grands Jardins) are situated in the Boreal Shield ecozone in Québec.

Table 2-2. Key soil properties. Mean values and standard errors (in parentheses) are displayed (n=2–6; n=1 if no standard error displayed). For the forest floor layers, different letters indicate significant differences among invasion stages (Luvisols) or between control and invaded soils (Brunisols and Podzols; p-value < 0.05).

	Soil type	Site	Invasion	Horizon	$CaCl_2$ pH	TOC [%]	Thickness [cm]	Textural class	% Clay
AB	Luvisol	EMEND	Control	LFH	5.6 (0.3) *a*	48.53 (1.64) *a*	10.7 (0.5) *a*		
				Ahe	5.7	1.86 (0.01)	3.5 (1.4)	Loam	13
				Ae	4.7 (0.4)	1.32 (0.28)	10.3 (3.4)	Loam	24 (7)
				Bt	4.5 (0.1)	1.46 (0.10)	-	Clay loam	40 (9)
			1st stage	LFH	5.7 (0.1) *a*	37.83 (5.88) *a*	7.7 (0.9) *ab*		
				Ah	5.4 (0.1)	4.83 (0.65)	4.3 (0.2)	Loam	21 (1)
				Ae	5.3 (0.1)	1.46 (0.09)	4.7 (0.9)	Loam	24 (4)
				Bt	5.2 (0.1)	1.51 (0.11)	-	Clay loam	30 (2)
		Wolf Lake	2nd stage	LFH	5.7 (0.2) *a*	29.66 (6.96) *a*	7.8 (1.4) *ab*		
				Ae	4.3 (0.1)	0.47 (0.07)	9.6 (1.7)	Sandy loam	11 (2)
				Bt	4.3 (0.2)	0.56 (0.12)	-	Sandy loam	19 (5)
		Breton	3rd stage	LFH	6.3 (0.2) *a*	27.93 (4.34) *a*	4.2 (1.0) *b*		
				Ahu	5.8 (0.3)	7.87 (1.06)	8.5 (0.6)	Clay loam	30
				Ae	nd	1.51 (0.26)	1.8 (0.4)	Nd	Nd
				Bt	4.8 (0.2)	1.20 (0.11)	-	Clay loam	34
QC	Brunisol	Golf	Control	LFH	3.8 (0.1) *b*	50.23 (1.07) *a*	8.5 (0.5) *a*		
				Ae	3.2	2.52	3.0 (1.0)	Nd	
				Bm	4.0 (0.3)	11.19 (1.54)	-	Loam	15 (3)
			Invaded	LFH	4.6 (0.2) *a*	34.71 (1.51) *b*	2.0 (0.6) *b*		
				Ahu	4.3 (0.3)	11.82 (0.61)	11.0 (1.0)	Silt loam	11 (1)
				Bm	4.4 (0.1)	9.58 (2.16)	-	Loam	15 (3)
		Valcartier	Control	LFH	3.2 (0.2) *b*	48.49 (2.41) *a*	7.8 (0.9) *a*		
				Ah	3.1 (0.3)	8.35 (1.71)	4.7 (2.8)	Loam	12
				Ae	3.1	2.66	7	Sandy loam	12
				Bm	4.0 (0.2)	7.24 (0.44)	11.3 (0.7)	Loam	22 (5)
				C	4.0	5.18	-	Nd	
			Invaded	LFH	4.8 (0.3) *a*	37.19 (3.43) *b*	3.1 (0.9) *b*		
				Ahu	4.3 (0.2)	9.79 (2.36)	5 (0.8)	Clay loam	25 (4)
				Bm	4.6 (0.1)	5.18 (0.64)	11.7 (1.9)	Loam	26 (6)
				C	4.6 (0.2)	4.38 (1.87)	-	Loam	19 (6)
	Podzol	Montmorency	Control	LFH	3.3 (0.6) *a*	51.24 (1.72) *a*	10.3 (0.4) *a*		
				Ae	3.6 (0.3)	1.17 (0.08)	7.3 (0.9)	Sandy loam	6 (2)
				Bhf	3.8 (0.2)	11.06 (2.43)	13.3 (3.4)	Sandy loam	10 (1)
				C	4.2	5.37	-	Sandy loam	8
			Invaded	LFH	3.4 (0.2) *a*	43.45 (4.59) *b*	9.7 (0.9) *a*		
				Ae	3.4 (0.1)	1.95 (0.11)	6.7 (1.7)	Sandy loam	6 (2)
				Bhf	3.9 (0.2)	14.59 (2.75)	11.7 (1.7)	Sandy loam	9 (1)
				C	4.3	3.40	-	Loam	10
		Grands Jardins	Control	LFH	3.0 (0.5) *a*	54.09 (1.53) *a*	10.5 (0.3) *a*		
				Ah	3.4 (0.1)	8.18 (0.92)	3.3 (0.9)	Nd	Nd
				Ae	3.9 (0.1)	3.30 (1.87)	6.5 (2.8)	Sandy loam	14 (4)
				Bf	5.0 (0.3)	3.57 (0.84)	13.0 (1.8)	Loamy sand	9 (4)
				C	5.6 (0.2)	0.89 (0.11)	-	Nd	Nd
			Invaded	LFH	3.7 (0.1) *a*	46.58 (8.03) *a*	5.3 (0.9) *b*		
				Ahu	4.6	4.76 (1.65)	8.7 (1.7)	Sandy clay loam	23 (3)
				Ae	5.0 (0.5)	1.09 (0.33)	6 (2.7)	Loamy sand	5 (1)
				Bf	6.5 (0.8)	3.46 (1.51)	14.5 (2.8)	Loamy sand	7 (2)
				C	5.8	0.67	-	Sand	2

Field sampling

Sampling was conducted in June-August 2018 and 2019. For each zone of similar invasion level, three sampling points were randomly chosen in a mid-slope position to be representative of the zone. For each sampling point, a pit was dug for soil description and sampling of each horizon was conducted to an approximate depth of 50 cm when possible (Supplementary Figure 2.B-1).

Soil profiles were described using the Canadian System of Soil Classification (Soil Classification Working Group, 1998) and the forest floor (surface organic layer or LFH) using the third approximation for humus classification (Klinka et al., 1997). Organic horizon nomenclature is described in Supplementary Table 2.A-1. Horizon boundaries were determined in the field by a visible difference in color, texture, structure, and/or root density. Each horizon, including the forest floor, was sampled for key soil properties. Bulk densities of the forest floor and mineral soil were determined with two replicates at each sampling location (n=6 for each homogeneous zone at each site). For the organic layer, a 20x20 cm^2 frame was used to cut out the entire organic layer and the depth was calculated as the average of the four corner depths. For mineral soil, bulk density was determined using a 7.2 cm diameter metallic core to a depth of 15 cm. Samples for C stocks were stored at 4 ºC prior to analysis.

Earthworm sampling and identification

Earthworm specimens were obtained by hand-sorting of the litter and hot mustard application to the mineral soil surface (0.0625 m^2) within two meters of the pit used for soil description (Lawrence and Bowers, 2002). Each mature specimen was identified to the species level according to Reynolds (1977) and functional groups of earthworms were determined following Bouché (1977). After measuring the length of every collected specimen, ash-free dry earthworm biomass was estimated using the allometric equations developed by Hale et al. (2004). In the absence of

earthworms and signs of activity such as casts and visible mixing of organic and mineral soil layers, the zones were considered non-affected by earthworms and acted as controls for result interpretation, including for the site-level paired comparison. If a species was not found during the sampling described above but was observed in the soil pit, the biomass density was estimated based on the total surface covered by the pit in addition to the quadrat used for earthworm sampling (0.3125 m^2).

Laboratory analyses

To estimate C stocks, organic and mineral samples were oven-dried at 105 °C for 24 hours (Maynard and Curran, 2008) and then weighed to determine bulk density. For mineral soils from Québec, bulk density was estimated according to the organic density concept developed for Brunisols and Podzols found in the boreal forest in Québec (Périé and Ouimet, 2008):

$$BD = \frac{D_{MIN} - D_{ORG}}{(SOM * D_{MIN}) + (1 - SOM) * D_{ORG}}$$

where D_{MIN} and D_{ORG}, which represent the density of pure mineral and organic fractions, were calibrated for our dataset (Supplementary Table 2.A-2). Soil organic matter content (SOM) content was estimated by loss-on-ignition on sieved samples (2 and 4 mm for mineral soil and forest floor, respectively) by loss-on-ignition at 375 ºC for 16 hours after gradually increasing temperature to 225 ºC for 40 minutes and 300 ºC for 15 minutes (Konen et al., 2002). SOM content was then converted into total organic carbon (TOC) using 0.58 as the conversion factor, also known as the Van Bemmelen factor, for both the forest floor and mineral soil (Kalra and Maynard, 1991). The C stocks were calculated based on bulk density and TOC content, after correction for coarse fragments, using the following equation:

$$Stock_{Carbon}[Mg\ C.ha^{-1}] = TOC[\%] * BD * depth[cm]$$

The depth corresponded to the measured depth of forest floor and to 15 cm for the mineral soil of Brunisols and Podzols, and 10 cm for that of Luvisols, because of sampling limitations. Total soil organic C stocks (SOC) were calculating by summing the forest floor and mineral soil C stocks, to a depth of 10 or 15 cm depending on soil type.

Soil samples for key soil properties were air-dried and sieved (2 and 4 mm for mineral soil and forest floor, respectively) prior to analysis. The soil pH was measured in a 0.01 M $CaCl_2$ solution on an Accumet XL200 (ThermoFisher Scientific, Waltham, MA, USA), using a soil to solution ratio of 1:2 for mineral soil samples and 1:4 for forest floor samples following the ISO 10390 protocol (ISO, 2005). Total organic carbon (TOC) was estimated by loss-on-ignition as described above for each horizon (Konen et al., 2002). Particle size analysis was performed following the hydrometer method with 5 % sodium hexametaphosphate solution (Gee and Bauder, 1986). Soil textural class was determined using the textural triangle from the Canadian System of Soil Classification (Soil Classification Working Group, 1998).

Statistical analyses

Bulk density and C stock data for forest floors and mineral soils were assessed separately by analysis of covariance (ANCOVA) with earthworm biomass as a covariable. Data analysis was carried out for Luvisols with invasion stage as the only factor, while Podzols and Brunisols data were analyzed separately by two-way ANCOVAs (Invasion stage and site as factors). Each ANCOVA was followed by a pair-wise comparison using posthoc honestly significant different (HSD) test from the agricolae package (de Mendiburu, 2020). For key forest floor properties (TOC, pH and thickness), significant differences among invasion stages for Luvisols, or between control and invaded soils for Brunisols and Podzols (p-value < 0.05) were determined by ANCOVA followed by pairwise comparison using posthoc HSD test from the agricolae package (de Mendiburu, 2020).

For all statistical analyses, data were transformed using Tukey's ladder of powers from the rcompanion package when necessary to ensure normality and the Levene's test from the rstatix package was performed to check homogeneity of variance (Kassambara, 2020; Mangiafico, 2021). All statistical analyses were performed on two separate datasets based on differences in sampling design (Alberta with Luvisols; Québec with Podzols and Brunisols) using R version 6.3.2 (R Core Team, 2021).

Results and discussion

Earthworm biomass and functional groups

The epigeic species *Dendrobaena octaedra* has been shown to be the most abundant and first invader in North American forests (Cameron et al., 2007; Hale et al., 2005a). Our results match this observation as the species was found in all sites, except Grands Jardins, and was at its highest density at EMEND and Wolf Lake, considered to be the earlier stages of invasion (Table 2-3). However, the density of *D. octaedra* (432 ind.m^{-2}; Supplementary Table 2.A-3) observed at EMEND was drastically lower than previously reported for early invasion in similar aspen stands of southwestern Alberta (3,000 ind.m^{-2}; Dymond et al., 1997). On the contrary, lower densities of *D. octaedra* were previously observed at Wolf Lake by Cameron et al. (2007) and McAdams (2017), respectively 5 and 17 ad.m^{-2}, compared to 68 ad.m^{-2} in this study. Although biomasses observed for Alberta sites (0.4–1.4 g.m^{-2}) were higher than those of Québec sites (0.1–0.2 g.m^{-2}), they were overall in the same range as previous observations in northern hardwood forests (Hale et al., 2005a; Lyttle et al., 2014) and aspen forests (Eisenhauer et al., 2007). At Grands Jardins, the epigeic species *Dendrodrilus rubidus,* also referred to as *Bimastos rubidus,* was observed for the first time (Csuzdi et al., 2017). Both species are known to survive in very acidic environments (Moore et al., 2009).

Table 2-3. Earthworm biomass density (g.m^{-2}) of each species identified. Mean values and standard errors (in parentheses) are displayed (n=3–4). When specimens of a given species were observed on site but not during earthworm sampling, the standard error is absent. The cell is left blank when the species was not observed at the site.

		AB			QC			
		Luvisols			Brunisols		Podzols	
Functional group	Species	EMEND	Wolf Lake	Breton	Golf	Valcartier	Montmorency	Grands Jardins
Epigeic	*Dendrobaena octaedra* Savigny	1.3 (0.3)	1.4 (0.9)	0.4 (0.2)	0.1	0.2 (0.2)	0.1 (0.1)	-
	Dendrodrilus rubidus Savigny[7]	-	-	-	-	-	-	0.1
Endogeic	*Aporrectodea* spp.	-	1.4 (1.4) [8]	-	0.1	0.2 (0.2) [9]	0.1 (0.1)	0.1
	Lumbricus rubellus Hoffmeister	-	-	-	-	1.8 (31.6)	0.9 (0.6)	-
	Octolasion spp.	-	-	21.8 (19.7)[10]	-	-	-	0.1[11]
Anecic	*Lumbricus terrestris* L.	-	-	-	-	-	-	0.4
	Lumbricus sp.	-	-	0.6 (0.2)	0.3 (0.3)	1.2	0.1	-
TOTAL		1.3 (0.3)	1.4 (0.8)	7.6 (6.8)	0.3 (0.3)	0.7 (0.6)	0.4 (0.3)	0.1

[7] Now considered to be *Bimastos rubidus* (Csuzdi et al., 2017)
[8] *Aporrectodea tuberculata* Eisen
[9] *Aporrectodea turgida* Eisen
[10] *Octolasion cyaneum* Savigny
[11] *Octolasion tyrtaeum* Savigny

The endogeic *Aporrectodea* spp. were observed at Wolf Lake, Golf, Valcartier, and Montmorency. The density observed at Wolf Lake (*A. tuberculata,* 4 ad.m^{-2}) was lower than the 29 ad.m^{-2} previously reported (McAdams, 2017). The biomass of *Aporrectodea* spp. ranged from 0.1 to 1.4 g.m^{-2}, similar to previous observations in invaded northern forests (Hale et al., 2005a). The endogeic *Octolasion cyaneum* was only observed at Breton and for the first time. The average biomass in this study (21.8 g.m^{-2}; Table 2-3) is particularly high compared to values previously reported for *Octolasion* spp.: double the average biomass observed in aspen stands in southwestern Alberta (Eisenhauer et al., 2007) and ten times higher than the highest biomass reported by Hale et al. (2005a) in northern hardwood forests of Minnesota. The endogeic *Lumbricus rubellus* was observed at Valcartier and Montmorency. Overall, endogeic earthworms were the dominant contributors to total earthworm biomass, in line with previous findings (Costello et al., 2011; Lyttle et al., 2014).

Anecic *Lumbricus* spp. were observed at Breton and all Québec sites, at a rather low biomass density (0.1–0.6 g.m^{-2}; Table 2-3) compared to previous mentions in northern forests: 15.9 g.m^{-2} on average in southwestern Alberta (Eisenhauer et al., 2007) and reaching 7 g.m^{-2} in Minnesota (Hale et al., 2005a). In Luvisols, the average total earthworm biomass was higher for the most invaded site (Breton, 7.6 g.m^{-2}) compared to the sites invaded by epigeic and/or endogeic species (EMEND, 1.3 g.m^{-2} and Wolf Lake, 1.4 g.m^{-2}). Wackett et al. (2018) reported density numbers in the same range for birch forests, although reaching a maximum biomass of approximately 16 g.m^{-2}.

All identified species had been previously mentioned in the corresponding provinces of Canada (Addison, 2009). While their presence had previously been reported at Montmorency and Grands Jardins (Moore et al., 2009), Wolf Lake (Cameron et al., 2007), and Breton (Jackson et al., 2017),

this paper is to our knowledge the first mention of exotic European species in the literature for Valcartier, Golf, and EMEND. Although earthworms were never mentioned at Valcartier and Golf prior to this study, they were observed 6 km away, at Duchesnay station (Moore et al., 2009). *A. tuberculata, L. rubellus,* and *L. terrestris* are commonly used as fishing bait (Cameron et al., 2007) and were only found in sites with a lake in proximity (Wolf Lake, Montmorency, Grands Jardins, Golf, and Valcartier), suggesting that their presence was mainly linked to fishing activities. Species identified in this study had been mentioned and considered invasive in other boreal and arctic regions: all species were also found in Alaska (Booysen et al., 2018; Costello et al., 2011), and *Lumbricus* spp. and *Aporrectodea* spp. in Fennoscandia (Wackett et al., 2018). For the latter, *D. octaedra* was observed as well but it is not considered invasive as it was found in remote forests devoid of evident human-mediated sources of dispersal.

Effects of earthworms on forest floor morphology

In Alberta, the non-invaded forest floor (EMEND, control) classified as Resimor, was composed of a thin litter (L) layer, thick fermented (F) layer dominated by fungal hyphae and root residues (Frm/Fm) and thin recalcitrant humic (H) layer (Supplementary Tables 2.A-1 & 4). At the first stage of invasion (EMEND, invaded by the epigeic *D. octaedra*), the forest floor was thinner, classified as Mullmoder and dominated by faunal droppings, with a structure similar to that of control pits (Fig. 2-2). At the second stage of invasion (Wolf Lake, epigeic and endogeic species), the forest floor was also dominated by small faunal droppings and root residues, classified as Mor-moder or Leptomoder, respectively, in the presence or absence of mycogenous material. At the last invasion stage (Breton, all three functional groups), the forest floor was significantly thinner (p-value < 0.05, Table 2-2) when compared to the EMEND control, had a simpler structure, lacking

an H horizon (Fig. 2-2), and was dominated by larger faunal droppings, therefore classified as Vermimull.

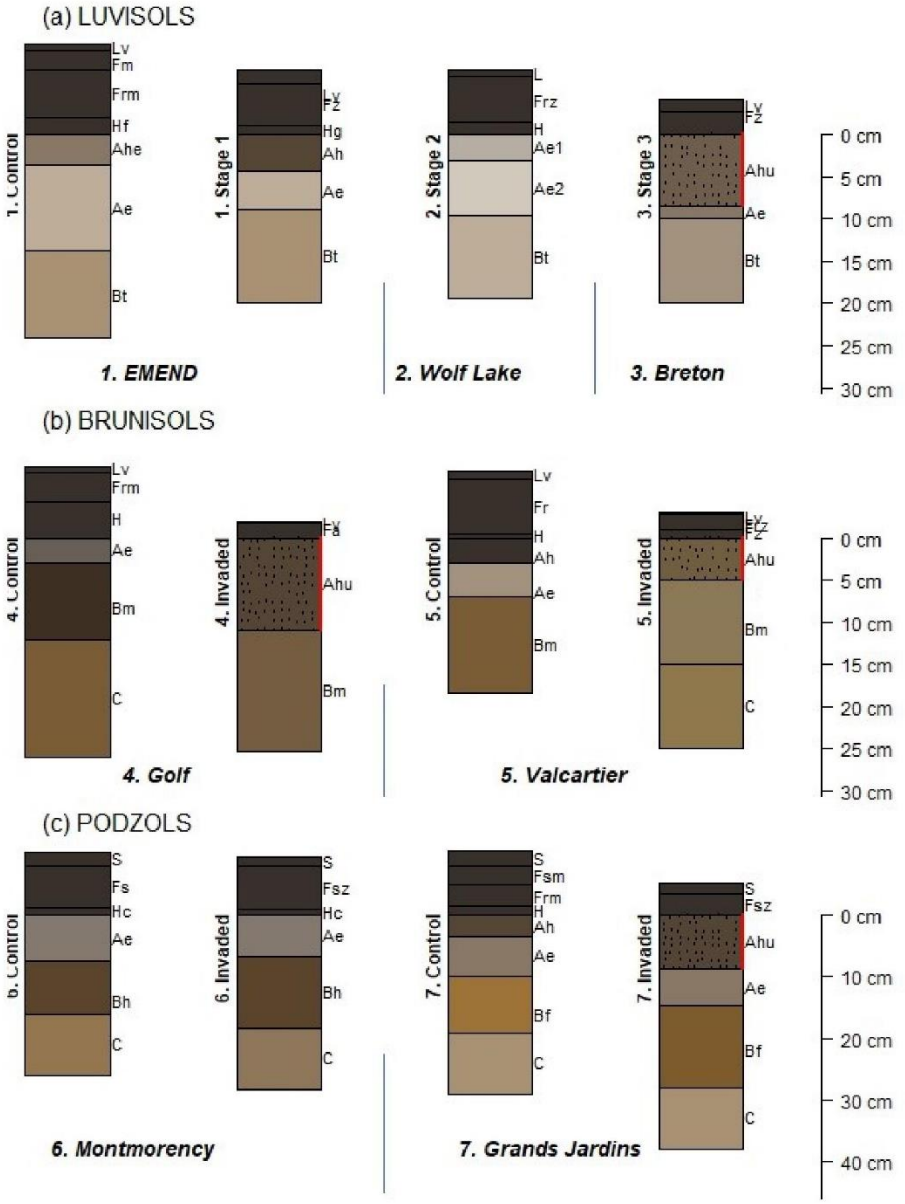


Figure 2-2. Representative profiles from Luvisols (a), Brunisols (b), and Podzols (c). Colors represent Munsell soil colors determined on air-dried soils. The presence of a new Ahu horizon is highlighted in red. Graphic computed with the Algorithms for Quantitative Pedology (AQP) package using R (Beaudette et al., 2020).

For the Québec sites with Podzols under coniferous canopy (Montmorency and Grands Jardins), the forest floor of non-invaded zones, classified as Sphagnomor, was composed of a thin bryophyte layer (S), thick fermented layer consisting of disintegrated bryophyte tissues and in some cases mycogenous tissues (Fs/Fsm) and thin humic layer (H). In the earthworm-invaded zones, the forest floor structure differed in both sites (Fig. 2-2). For Montmorency, the forest floor was similar to that of the earthworm-free zones except for the fermented layer dominated by faunal droppings (Fsz), therefore classified as Sphagnomoder. For Grands Jardins, the forest floor was significantly thinner when compared to the control zone (p-value < 0.05, Table 2-2), with the disappearance of the humic layer in the earthworm-invaded zone and the fermented layer dominated with earthworm casts (Fsz), therefore classified as Vermimull.

For the Québec sites with Brunisols under sugar maple canopy (Golf and Valcartier), the forest floor in non-invaded zones consisted of a thin litter horizon (Lv), thick root residue-rich and sometimes mycogenous fermented horizon (Fr(m)) and thin humic horizon (H), therefore classified as Resimor (Fig. 2-2). In the presence of earthworms, the forest floor was significantly thinner (p-value < 0.05, Table 2-2), with a thin Lv horizon and thin to absent Frz/Fa (dominated by fungal material and faunal droppings), classified as Vermimull.

The thinner forest floor observed in zones invaded by anecic earthworms was associated with lower TOC concentrations compared to non-invaded zones, and significantly so for Luvisols and Brunisols (p-value < 0.05, Table 2-2). Decreases in TOC were likely due to the incorporation of mineral particles into the forest floor, which we observed during morphological description for several of the invaded sites. Burtelow et al. (1998) also reported a 36 % decrease of OM content in the forest floor (O horizon). In all cases, invaded sites were systematically characterized by a numerically higher $CaCl_2$ pH than control sites for the forest floor, increasing by 0.7 in Luvisols,

0.8 to 1.6 in Brunisols, and 0.1 to 0.7 in Podzols, although only significant for Brunisols (p-value < 0.05, Table 2-2). This pH increase could be partly explained by bioturbation and/or the production of $CaCO_3$ granules by earthworms, which has been recorded for pH as low as 5.1 (measured in water, roughly equal to a $CaCl_2$ pH of 4.4; Lambkin et al., 2011).

The decrease in forest floor thickness associated with earthworm activity has been observed in numerous field and laboratory studies (Bohlen et al., 2004b; Fahey et al., 2013; Groffman et al., 2015; Hale et al., 2005b; Wironen and Moore, 2006). The disappearance of the humic horizon, also described by Eisenhauer et al. (2007), and the decrease in forest floor thickness suggests a faster incorporation of organic matter into the underlying mineral soil (Haydu-Houdeshell et al., 2017). Reverse effects have also been observed by Beyer et al. (1991) in a European beech and oak forest after a decrease in earthworm density associated with decreased pH. In that case, the soil, originally classified as a Luvisol, showed incipient podzolization, associated with an increase in fulvic acid translocation, and the forest floor evolved from mull to moder. These observations suggest that effects could be reversible if environmental conditions become less hospitable to earthworms.

Effects of earthworms on surface mineral soil morphology

In Alberta, the mineral horizons followed the common pattern for Orthic Gray Luvisols, with thin to absent Ah(e) and the presence of distinct Ae and Bt horizons for all sites, even in the presence of earthworms (epigeic and/or endogeic species), except Breton (Fig. 2-2). For the latter, the uppermost part of the profile was replaced by a 6–9 cm dark A horizon, named Ahu, mainly composed of earthworm casts and with higher clay content relative to the other A horizons (Table 2-2; Supplementary Table 2.A-4). This soil was therefore classified as a Dark Gray Luvisol because of the OM-rich A horizon, although this classification is not accounting for bioturbation. In Québec

sites with Podzols, the mineral horizons followed the classic pattern of Orthic Humic Podzols (WRB: Humic Podzol) at Montmorency (Ae, Bh, C) and Orthic Humo-ferric Podzols at Grands Jardins (Ah, (Ahe,) Ae, Bhf/Bf, C). For the Québec sites with Brunisols (Valcartier and Golf), the mineral profiles consisted of Ah, (Ae,) Bm, and C horizons in the control zone, and were therefore classified as Eluviated Dystric Brunisols. The main difference observed in earthworm-invaded zones was the development of an Ahu horizon, dominated by earthworm casts, for all sites except Montmorency (Fig. 2-2). This novel horizon had been described by Langmaid (1964), classified as Ap in New Brunswick Podzols three years after invasion, and by Nielsen and Hole (1964), classified as coprogenous A1 in Wisconsin Gray-Brown Podzols. Eisenhauer et al. (2007) referred to this newly formed horizon as Ah in an Orthic Gray Luvisol. For most of our sites, this Ahu horizon had higher clay content than that of underlying B and C horizons and/or corresponding earthworm-free A horizon (Table 2-2). Van Groenigen et al. (2019) reported from a meta-analysis of 405 observations that fresh earthworm casts contain more clay than bulk mineral soil. As clay particles are usually transferred to deeper horizons by lessivage, the clay-enriched uppermost horizon found in the presence of earthworms is a clear sign of bioturbation (Phillips, 2007). Clay enrichment in surface horizons was also observed and attributed to earthworms in a lysimeter study by Graham and Wood (1991). At EMEND, the Ah horizon was thicker and had a higher TOC and clay content in the epigeic-invaded zone compared to the control (Table 2-2). This suggests a vertical transfer of OM from the forest floor, which resulted in higher mineral soil C stocks. McLean and Parkinson (1997) had also described the development of a thin Ah horizon in a mesocosm study six months after adding *D. octaedra*.

The observations described above can be attributed to bioturbation, which is the main physical process associated with earthworm activity. By using a space-for-time approach, it is virtually

impossible to determine if earthworms were absent from the non-invaded zones because they had not reached it yet, or because the environmental conditions were unsuitable. However, as the pH and clay content of deeper soil horizons (B and C) were similar for our paired sites, we can confidently assume that soil physicochemical properties were similar prior invasion (Table 2-2).

Earthworm bioturbation is denoted differently depending on the soil classification system used. In the Canadian System for Soil Classification (CSSC), used during this study, the lowercase suffix *u* can be used to describe a mineral horizon disrupted by faunal processes and burrowing animals (Soil Classification Working Group, 1998). Melanic Brunisols and Gray Brown Luvisols are the only two great groups that take into account earthworm activity in uncultivated soils (Soil Classification Working Group, 1998). In the World Reference Base classification, the qualifier *vermic* is used to describe a soil with more than 50 % of the volume occupied by worm holes and casts in the upper 100 cm and is found in Chernozems, Kastanozems, Phaeozems, and Regosols (FAO, 2014). None of these soil orders correspond to our studied soils, again highlighting the limitations of the classification system. The American System of Soil Classification also uses the *vermic* subgroup when referring to soils with various degrees of bioturbation. When earthworm activity is the dominant trait, soils can be classified within the Vermudoll or Vermustoll great groups (Soil Survey Staff, 1999). The limited mentions of bioturbation by earthworms within the CSSC shows that earthworm activity has been understudied and/or has not been recognized in most Canadian soils, as they likely were not part of the originally surveyed soils. To illustrate this, the Alberta sites are all classified as Gray Luvisols because the mean annual soil temperature (MAST) is below 8 ºC, one of the criteria for this great group in the CSSC system. Instead, the Breton soils, which include the novel forest mull Ahu, would be better classified as Gray Brown

Luvisols, similarly to the Luvisolic soils typically found in southern Ontario (Lavkulich and Arocena, 2011).

Effects of earthworms on C stocks and implications

Forest floor C stocks were significantly affected by earthworm invasion in Luvisols and Brunisols but did not change in Podzols (Fig. 2-3). For Luvisols in Alberta, the forest floor C stocks were similar in controls and earlier stages of invasion (EMEND and Wolf Lake, ranging from 28–34 Mg $C.ha^{-1}$) whereas they were drastically lower at the latest stage of invasion (Breton), with 2 Mg $C.ha^{-1}$ (p-value < 0.01, Table 2-4). For Brunisols, the forest floor C stocks were significantly lower in invaded zones (10 Mg $C.ha^{-1}$, p-value < 0.01) compared to controls (24 Mg $C.ha^{-1}$). Hence, our original hypothesis was verified for the forest floors, in that Luvisols underwent the largest decrease in C stocks after earthworm invasion, while it was smaller in Brunisols and not detected in Podzols. As mentioned above, many studies have reported a decrease of forest floor C stocks as a result of thinner forest floor and lower TOC content (Kurz et al., 2013). The differences observed between soil types might be explained by contrasting litter quality, the C:N ratio being the lowest for Luvisols, intermediate for Brunisols and highest for Podzols (Supplementary Table 2.A-2). Earthworms have been shown to prefer protein and carbohydrate-rich litters, corresponding to lower C:N ratio, although this may vary greatly among species (Curry and Schmidt, 2007).

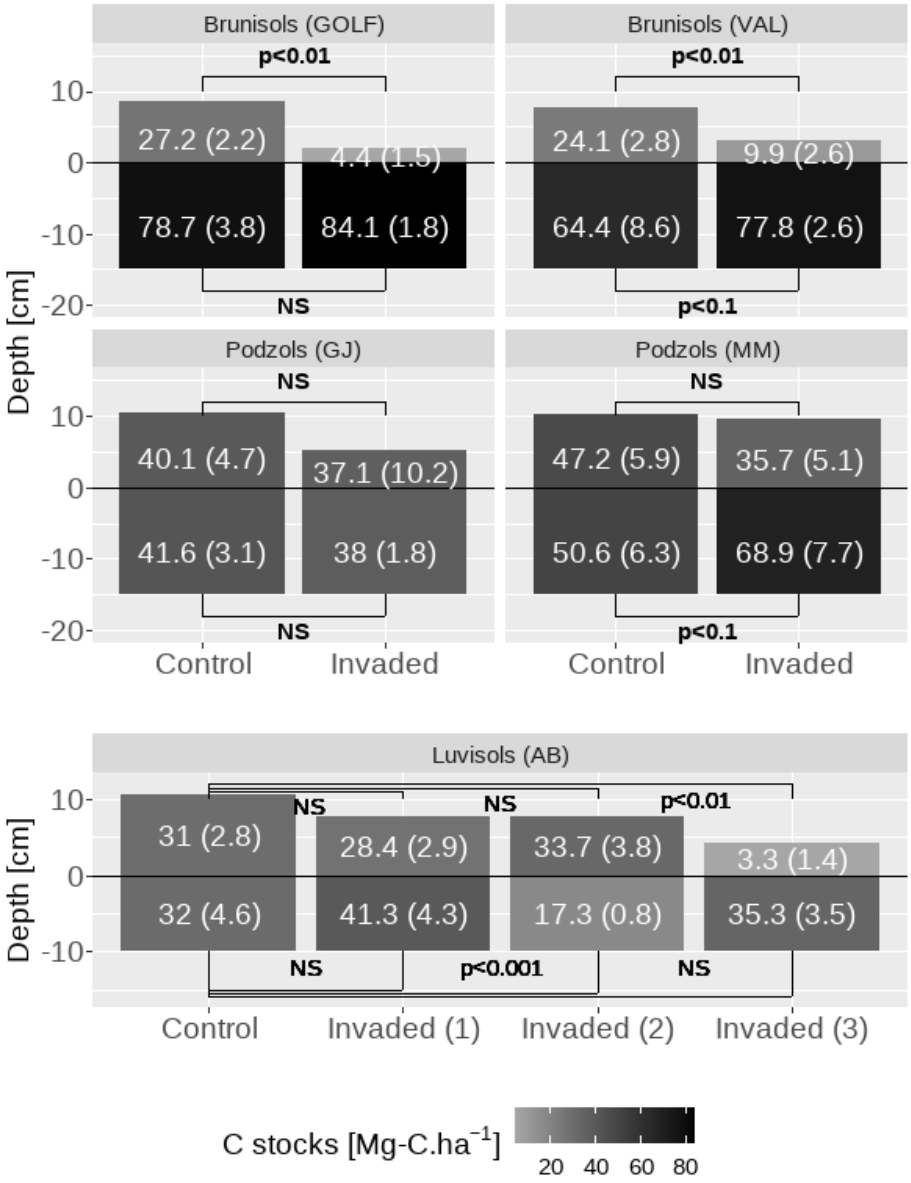


Figure 2-3. Carbon stocks in Mg-C.ha^{-1} for forest floor (above 0 cm) and surface mineral soil (below 0 cm) are presented with a gray gradient, with darker colors corresponding to higher values. Mineral soil carbon stocks were estimated to a depth of 10 cm for Luvisols and 15 cm for Podzols and Brunisols. Forest floor thickness is displayed on the y-axis. The average and standard error (in parentheses) for carbon stocks are indicated on the corresponding bar. Significant differences between invasion stages for each site were determined by ANCOVA for forest floor and mineral carbon stocks. For Luvisols, Invaded (1), (2), and (3) respectively correspond to invasion by epigeic (EMEND), epigeic and endogeic (Wolf Lake), and epigeic, endogeic, and anecic (Breton) earthworms.

Table 2-4. Analysis of covariance (ANCOVA) of C stocks for all sites with earthworm biomass as a covariable. Significant p-values are displayed in bold.

	SOIL TYPES		FACTOR	DF	SUM SQ	MEAN SQ	F VALUE	P-VALUE
QC	Brunisols	LFH	Site	1	0.1	0.1	0.01	0.9
			Invasion	1	90.4	90.4	15.9	**< 0.01**
			Earthworm biomass	1	4.6	4.6	0.8	0.4
			Residuals	16	90.9	5.7		
		Mineral (0–15 cm)	Site	1	$3.2*10^{12}$	$3.2*10^{12}$	2.0	0.2
			Invasion	1	$7.7*10^{12}$	$7.7*10^{12}$	4.7	**< 0.05**
			Earthworm biomass	1	$4.3*10^{11}$	$4.3*10^{11}$	0.3	0.6
			Residuals	16	$2.6*10^{13}$	$1.6*10^{12}$		
		Total	Site	1	178.0	178.0	178.0	0.3
			Invasion	1	157.3	157.3	157.3	0.3
			Earthworm biomass	1	206.5	206.5	206.5	0.3
			Residuals	10	1403.3	1403.3	140.3	
	Podzols	LFH	Site	1	3.2	3.2	0.3	0.6
			Invasion	1	17.4	17.4	1.4	0.2
			Earthworm biomass	1	2.5	2.5	0.2	0.7
			Residuals	20	239.9	12.0		
		Mineral (0–15 cm)	Site	1	$1.2*10^{13}$	$1.2*10^{13}$	6.1	**< 0.05**
			Invasion	1	$6.5*10^{12}$	$6.5*10^{12}$	3.2	**< 0.1**
			Earthworm biomass	1	$4.3*10^{12}$	$4.3*10^{12}$	2.2	0.2
			Residuals	13	$2.6*10^{13}$	$2.0*10^{12}$		
		Total	Site	1	1452.6	1452.6	3.6	**< 0.1**
			Invasion	1	2.9	2.9	0.01	0.9
			Earthworm biomass	1	172.8	172.8	0.4	0.5
			Residuals	8	3242.5	405.3		
AB	Luvisols	LFH	Invasion	3	9529.9	3176.6	6.6	**< 0.01**
			Residuals	25	12105.9	484.2		
		Mineral (0–10 cm)[1]	Invasion	3	0.03	0.01	17.2	**< 0.001**
			Residuals	25	0.02	0.000006		
		Total	Invasion	3	2223.3	741.1	4.9	**< 0.05**
			Residuals	12	1819.3	151.6		

Carbon stocks of the surface mineral soil were differently affected by earthworm invasion based on soil type (Table 2-4 & Fig. 2-3). For Luvisols and Podzols, the mineral C stocks were not significantly affected by earthworm invasion while for Brunisols they increased significantly from 71.6 to 81.0 Mg C.ha^{-1}. For Luvisols, the decrease in forest floor C stocks was not compensated

[1] Mineral C stocks were only determined to a depth of 10 cm in Alberta because of high clay content.

by an increase in mineral soil C, while it was partly compensated for Brunisols. Our results suggest that Luvisols are the most affected soil types by this ongoing invasion, as most C stored in forest floors was not transferred to the mineral soils. Brunisols showed a partial compensation of forest floor C loss by an increase in mineral C stocks while Podzols were unaffected. Our hypothesis is partially confirmed, with an increase in mineral C stocks only observed for Brunisols. Although mineral C stocks were largely unaffected by earthworm invasion, the development of a novel Ahu horizon with a drastically different structure could influence C persistence. The incorporation of labile organic compounds from the litter into the Ahu horizon could potentially boost microbial activity (Li et al., 2002) as earthworms have been shown to cause a priming effect, making older C more available for microbial decomposition (Brown et al., 2000). Similarly, Lubbers et al. (2013) did not find any evidence that earthworms affected SOC stocks; yet earthworms increased CO_2 emissions. The results of their review, however, were mainly based on laboratory incubations and agricultural soils (Huang et al., 2020). In contrast, Alban and Berry (1994) found an average decrease of 0.6 $Mg.ha^{-1}$ yr^{-1} for soil C stocks to a depth of 50 cm, mainly due to a decrease in forest floor C, in a forest of northern Minnesota over 14 years as the density of epi-endogeic earthworms (*A. tuberculata* and *L. rubellus*) increased. Huang et al. (2020) also reported a decrease in SOC stocks in the presence of at least two of the three functional groups of earthworms. These inconsistent results suggest that the mechanisms of C sequestration linked to earthworm activity are not fully understood yet and that they are greatly dependent on biotic and environmental factors (Frelich et al., 2019). Our study did not integrate deeper mineral layers for C stock calculations, which can account for up to 50 % of total soil C stocks (Rumpel and Kögel-Knabner, 2011) and could potentially impact the present results. As a matter of fact, Jennings and Watmough (2016) found significantly lower SOC stocks in the presence of all three functional groups of earthworms

(*D. octaedra, Aporrectodea* spp.*,* and *L. terrestris*) in southern Ontario, only based on deeper mineral layers (20-30 cm depth), with no change above.

Huang et al. (2010) developed a model to predict changes in soil C stocks after earthworm invasion for temperate forests. His modeling efforts showed a decrease in C stocks for the forest floor, similarly to what we observed in our study. In the boreal forest of Western Canada, fire is one of the main factors driving boreal forest dynamics (Weber and Flannigan, 1997), and will continue to be of major importance in future successional dynamics (de Groot et al., 2003). The forest floor is one of the main fuels for fire (de Groot et al., 2013). Therefore, the partial or complete removal of this layer by earthworms is likely to change fire dynamics as it would reduce fuel availability. Cameron et al. (2015) estimated a decrease of 0.1 $Mg.ha^{-1}.yr^{-1}$ for forest floor C stocks when simultaneously modelling invasion by epigeic and endogeic earthworms and wildfires for aspen-dominated boreal forest in northeastern Alberta. These predictions together with results of our study suggest that the boreal forest soil C stocks might be in jeopardy, because of direct earthworm effects such as forest floor consumption, as well as indirect effects on vegetation, fire dynamics and microbial decomposition.

Conclusion

This study highlights changes occurring in boreal soils following earthworm invasion. Luvisols, Brunisols, and Podzols invaded by at least two functional groups of earthworms developed a similar dark A horizon enriched in TOC and earthworm casts associated with a Vermimull. As hypothesized, we observed changes linked to earthworm invasion similar to what has been observed in temperate forests, namely a decrease in forest floor thickness and the development of a reworked surface mineral horizon. Cameron et al. (2015) projected that 50–94 % of the C stocks contained in Alberta forest floors would be lost after 125 years with the simultaneous action of fires and

earthworm invasion. Our results suggest that the higher end of this estimation is more accurate as 94 % of the forest floor C stocks in Luvisols were lost after invasion by all three functional groups of earthworms when compared to our control zone, without compensation by higher mineral C stocks. For Brunisols, forest floor C stocks decreased by 59 % on average, partially compensated by higher mineral C stocks, while no changes were observed for Podzols. The contrasting results we observed for C stocks and soil morphological features confirm the importance of including different soil types when studying earthworm impacts as these are dependant on environmental conditions such as soil pH, climate, and vegetation. Although we only found European earthworm species at our sites, the presence of Asian earthworm species has been documented recently in forests of northern US and they are likely to spread into Canadian forests as well (Moore et al., 2018). Assuming that those newly invading earthworms could reach and survive in boreal forests, they have the potential to further increase the changes already observed. Further research is required to document this ongoing earthworm invasion and to understand its long-term implications on the overall boreal C balance. Our study provides important information regarding the amount of C present in invaded soils but information is still lacking regarding the persistence of this C. We also recommend further study of the interactive effects of earthworm invasion with other factors driving C dynamics in the boreal forest, including fire regime, vegetation changes and other climate change-related topics.

Chapter 3 : Invasive earthworms alter fungal and bacterial communities of Canadian boreal forest soils

Abstract

Earthworm invasion in North American forests has the potential to greatly impact soil microbial communities by altering soil physicochemical properties, including structure, pH, nutrient availability, and soil organic matter (SOM) dynamics. While most research on the topic has been carried out in northern temperate forests, little is known on the impact of invasive earthworms on soil microbial communities in the boreal forest, a region characterized by a slower decay of organic matter (OM). Earthworm activities can increase OM mineralization, altering nutrient cycling and biological activity in a biome where low carbon (C) and nitrogen (N) availability is typically limiting microbial and plant growth. Here, we characterized and compared microbial communities of earthworm-invaded and non-invaded soils across three major soil types found in the Canadian boreal forest. Microbial communities of forest floors and surface mineral soils were characterized using phospholipid fatty acid (PLFA) analysis and metabarcoding of 16S rRNA gene and ITS2 region of bacteria (including archaea) and fungi, respectively. Earthworm-invaded forest floors showed decreased ectomycorrhizal fungal richness and shifts for specific bacterial taxa, notably an increase in Gram(+):Gram(-) ratios. In mineral soil horizons, earthworm invasion resulted in an increase in fungal relative to bacterial PLFAs and a decrease in Gram(+):Gram(-) ratios. Additionally, fungal communities shifted from saprotrophic to ectomycorrhizal-dominated, the Proteobacteria:Acidobacteriota ratio increased, and species diversity and richness increased for both fungi and bacteria. Changes observed in mineral soils could be linked to higher nutrient status and pH, as well as increased C availability in earthworm-invaded soils. Considering the important role of

microbes for ecosystem functioning, such earthworm-induced shifts in soil microbial community composition are likely to impact vegetation development and forest productivity at a large scale as the invasion progresses in these boreal systems.

Introduction

Microorganisms play a key role in soils, regulating the decomposition of litter and soil organic matter (SOM) and resulting nutrient availability. By ingesting litter and mixing it with mineral soil, earthworms provide a better access to C sources for microbes, speeding up decomposition and nutrient cycling (Blouin et al., 2013; Curry and Schmidt, 2007; Edwards, 2004). In North American forests, where they are invasive, earthworms can therefore alter existing SOM dynamics and associated fungal and bacterial communities (Frelich et al., 2019). As forest floor and mineral soil have intrinsically different physicochemical properties and harbour distinct microbial communities (Prescott and Grayston, 2013), earthworm invasion is likely differentially affecting these two horizons (Ferlian et al., 2018).

Changes in microbial community composition and diversity have been linked to the presence of earthworms under controlled laboratory conditions (Butenschoen et al., 2007; Chang et al., 2016; de Menezes et al., 2018; Gómez-Brandón et al., 2010) and under field conditions (Dempsey et al., 2013; Groffman et al., 2015; McLean et al., 2006; McLean and Parkinson, 1997; Price-Christenson et al., 2020), although most studies have focussed on temperate forests. Comparable studies remain marginal for the boreal biome despite reports of earthworm invasion occurring throughout North American boreal forests (Cameron et al., 2007; Moore et al., 2009).

In an extensive survey of the Great Lakes region of the United States, invasive earthworms were found in more than 80 % of suitable habitats (Fisichelli et al., 2013; Frelich et al., 2019). In higher latitudes, 50 % of the low human-impact sites sampled in Alaska by Saltmarsh et al. (2016) were invaded and Cameron and Bayne (2009) estimated that 49 % of the boreal forest of north-eastern Alberta will be invaded by 2059. Impacts of invasive earthworms on soil morphology are similar in temperate and boreal forests (Bohlen et al., 2004b), and include: (1) thinning of the forest floors,

accompanied by decreased OC content and increased pH, and (2) development of novel Ahu surface mineral horizons, characterized by higher clay content, pH, and OC content (Chapter 2, published as Lejoly et al., 2021; Lyttle et al., 2011). However, as N limitations for microbial activity are greater in boreal forests (Högberg et al., 2017), the action of invasive earthworms on microbial communities could be much larger than in temperate forests.

Fungi, including free-living decomposers and root-associated symbionts such as mycorrhizal fungi (Lladó et al., 2017), make up a large fraction of biomass in boreal forest soils (Clemmensen et al., 2015). Fungi can be ingested by earthworms (Curry and Schmidt, 2007), but many survive gut transit (Tiunov and Scheu, 2000). Most boreal tree species form symbiotic associations with ectomycorrhizal (EcM) fungi, with the exception of maple (*Acer* spp.) stands where arbuscular mycorrhizal (AM) fungi dominate (Brundrett et al., 1990). In those maple forests, earthworms can increase AM fungi abundance (Dempsey et al., 2013; Drouin et al., 2016) but, to our knowledge, the response of EcM fungi to earthworm invasion has never been studied in the field (Addison, 2009; Cameron et al., 2012). Like plants, EcM fungal growth is limited by N (Högberg et al., 2021). With earthworm invasion and reported associated increases in N availability (e.g. Alban and Berry, 1994; Blume-Werry et al., 2020; Wironen and Moore, 2006), two alternative scenarios may emerge: (1) an increased abundance of EcM fungi as N limitation is lessened, or (2) a decreased abundance as a result of decreased tree belowground C allocation to EcM fungi in accordance with the plant C allocation theory (Averill and Hawkes, 2016; Högberg et al., 2017; Nilsson et al., 2005; Yarwood et al., 2009). Saprotrophs – or free-living decomposers – play a greater role in SOM decomposition, and are able to decompose a wide range of organic compounds, including lignin and cellulose, with the help of extracellular enzymes (Lebreton et al., 2021). Unlike EcM fungi, their growth directly depends on C and N soil availability (Högberg et al., 2017). Crowther et al.

(2013) reported that increased grazing by soil fauna (isopods) reduced the importance of the dominant saprotrophic cord-forming basidiomycetes but increased fungal diversity as competition with other saprotrophic taxa decreased. However, the impacts of earthworm invasion on soil fungal diversity and functions are still not well known.

Bacteria and archaea decompose plant and soil organic macromolecules, but they are also involved in a wider range of ecosystem processes than fungi, including N fixation and transformations (Lladó et al., 2017). Compared to fungi, vegetation composition is a less important driver for bacterial community composition (Baldrian, 2017) while pH plays a key role (Fierer and Jackson, 2006; Lladó et al., 2017). Earthworm gut transit alters bacterial communities both structurally and functionally, although a high percentage of bacteria survives (Medina-Sauza et al., 2019; Pedersen and Hendriksen, 1993; N. Wang et al., 2021). Because of an overall increase in nutrient availability following earthworm invasion, bacteria would be favoured over fungi, the latter being characterized by slower growth and typically decomposing more complex organic compounds (McLean et al., 2006; Soares and Rousk, 2019). Decreased fungi:bacteria ratios has been reported following earthworm invasion in temperate forests (Dempsey et al., 2011), but not systematically (Chang et al., 2017), and this remains to be investigated for boreal forests.

Soil microbial communities are commonly characterized using either DNA metabarcoding or PLFA analysis. These techniques are considered complementary as they show different sensitivities to land use and other environmental changes (Orwin et al. 2018). Both methods have been successfully used to study the impacts of endemic and exotic earthworms on soil microbial communities (Butenschoen et al., 2007; Chang et al., 2016; de Menezes et al., 2018; Dempsey et al., 2013; Gómez-Brandón et al., 2010; Price-Christenson et al., 2020). Although the DNA and PLFA methods used in combination may provide more robustness to the analysis and conclusions of a

study, this approach has been rarely used in past studies assessing earthworm-induced changes on soil bacterial and fungal communities.

Here, we characterized soil microbial communities of the major boreal forest types of North America, invaded and non-invaded by earthworms, using both metabarcoding and PLFA analysis. Our objective was to describe the potential shifts in forest floor and mineral soil microbial communities associated with earthworm invasion. We combined two molecular methods (DNA and PLFA analyses) to detect potential differences in the relative abundance of both functional and taxonomical groups and used PLFA data for quantitative analysis. We hypothesized that earthworm invasion would significantly shift microbial community composition in both mineral soils and forest floors. Despite differences in soil type and vegetation among sites, we expected shifts to be similar in all sites and to be indicative of increased nutrient availability and pH, including lower fungi:bacteria ratios.

Materials and methods

Sample collection

Sites were selected from the most common soil types and vegetation covers of the Canadian boreal forest, including Brunisols (Soil Classification Working Group, 1998) under sugar maple (*Acer saccharum* Marshall; one site: Valcartier), Podzols under black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenburg; one site: Grands Jardins), and Luvisols under trembling aspen (*Populus tremuloides* Mich.; two sites: EMEND and Breton; Fig. 3-1 & Suppl. Table 3.A-1). The selected sites are a subset of the sites presented in Chapter 2 (published as Lejoly et al., 2021), where an extensive site description is presented, and correspond to those with lower anthropogenic impacts, similar level of earthworm invasion (presence of all three types of earthworms – epigeic, endogeic,

and anecic; except for EMEND, which is the control Luvisol site) and similar morphological changes (development of an Ahu horizon).

Earthworm invasion status was determined by hand-sorting of the litter and hot mustard extraction from the mineral soil surface (Lawrence and Bowers, 2002). Furthermore, clear signs of earthworm activity such as the presence of surface casts, extensive bioturbation, or abnormally thin forest floor were also used to assess the presence of earthworms. For three of the four locations, it was possible to identify earthworm-invaded and earthworm-free patches within the same site, while the whole site appeared to be invaded in Breton (Suppl. Fig. 3.B-1). At each site, three to four samples were collected for each level of invasion (earthworm-free and earthworm-invaded for EMEND and Grands Jardins; earthworm-free, low-density earthworm-invaded, and high-density earthworm-invaded for Valcartier; only earthworm-invaded for Breton) over the months of June and July 2019. As recommended by Ferlian et al. (2018), we separately tested the impact of earthworm invasion on the forest floor and the bulk mineral soil (0–10 cm), and thus took separate samples of each. All samples were stored at -20 ºC for transportation and then at -80 ºC prior to freeze-drying.

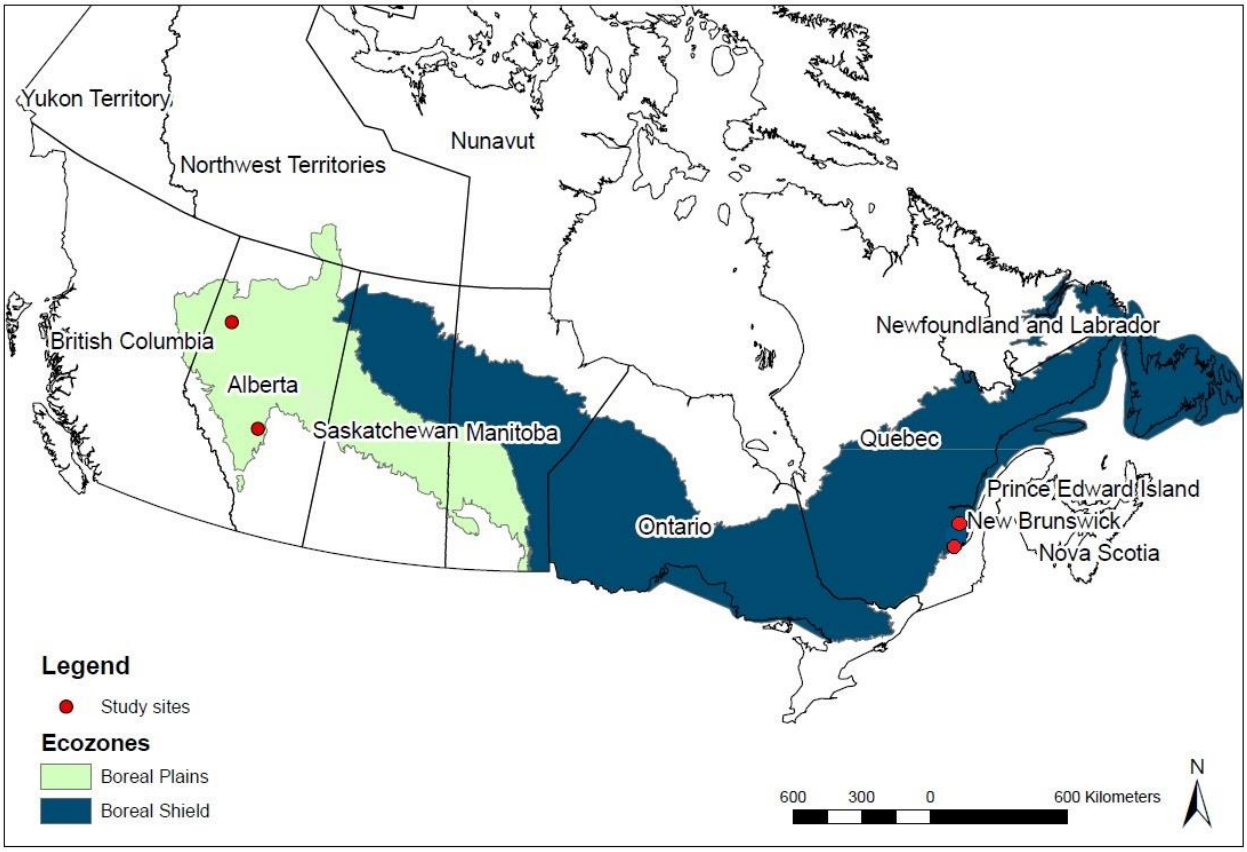


Figure 3-1. Map of the different sites selected across the Canadian boreal forest.

DNA extraction and sequencing

Freeze-dried soil samples were ground on a TissueLyser II (Qiagen, Hilden, Germany). Approximately 250 mg of ground sample were used for DNA extraction using the DNeasy PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany). Because of its stable nature, the extracted DNA also includes non-viable microorganisms (Frostegård et al., 2011). For bacteria and archaea, primers 515F-Y (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R (5'- CCGYCAATTYMTTTRAGTTT-3'; Parada et al., 2016; Rivers, 2016) were used for polymerase chain reaction (PCR) amplification of the V4-V5 regions of the 16S rRNA gene, while for fungi, primers ITS9F (5'-GAACGCAGCRAAIIGYGA-3') and ITS4R (5'- TCCTCCGCTTATTGATATGC-3'; Rivers, 2016; White et al., 1990) were used for PCR amplification of the internal transcriber spacer 2 (ITS2)

region (Integrated DNA Technologies, Coralville, IA, USA). Amplification was carried out following the Platinum SuperFi Green PCR Master Mix protocol (Invitrogen, Carlsbad, CA, USA), with 30 PCR cycles and an annealing temperature of 60 ºC for ITS2 and 55 ºC for 16S. PCR products were purified using Sera-Mag Select magnetic beads (Cytiva, Marlborough, MA. USA). In preparation for sequencing, a second index PCR was performed with oligonucleotides containing the Illumina specific overhang adapters. PCR products were again purified as described above. All purified PCR products from both amplified regions were then pooled using 3–6 µl of each sample to build the amplicon library. DNA concentration and average size were determined using the Qubit dsDNA HS assay kit on a Qubit fluorometer (Life Technologies, Carlsbad, CA, USA) prior to running a HS DNA BioAnalyzer (Agilent Technologies, Wilmington, DE, USA) to normalize the pooled library to 4 nM before sequencing. The pooled libraries were sequenced on an Illumina MiSeq platform using a MiSeq Reagent v3 600 cycles Kit (Illumina Inc., San Diego, CA, USA) at the University of Alberta Molecular Biological Sciences Unit.

Bioinformatic analyses

All bioinformatic analyses were performed in 'Quantitative insights into microbial ecology 2' (QIIME2 version 2021.4 software; Bolyen et al., 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin followed by denoising with DADA2 (Callahan et al., 2016). After trimming off the first 20 bp to remove primers, sequence reads were truncated where the average quality score dropped below 34 (at 285 and 215 base pairs (bp) for forward and reverse 16S reads; at 260 and 225 bp for forward and reverse ITS reads), and dereplicated with paired-end setting to generate amplicon sequence variants (ASVs) tables containing read counts. Using ASVs over operational taxonomic units (OTUs) results in a finer resolution, to single nucleotide differences between sequences (Callahan et al., 2017). However, this approach tends to

inflate diversity measurements, 1.3 and 2.1 times higher for fungal and bacterial richness, respectively (Glassman and Martiny, 2018). Mean amplicon size was 316 ± 37 bp ranging from 240 to 433 bp and 372 ± 2 bp ranging from 350 to 426 bp for ITS and 16S ASVs, respectively. After training the naïve Bayes classifiers (q2-feature-classifier), taxonomy was assigned to ASVs using classify-sklearn (Bokulich et al., 2018) with SILVA 138 SSURef NR99 full-length (99 %) and UNITE 8.3 (97 %) databases for 16S and ITS2, respectively (Abarenkov et al., 2010; Quast et al., 2013). Only prokaryotic (bacterial and archaeal) or fungal ASVs were kept and the ASVs tables generated by the QIIME2 were imported into R (version 4.0.5; R Core Team, 2020) for further analysis. Based on rarefaction curves, a total of 4,437 and 2,832 sequences, for bacterial (including archaea) and fungal ASVs respectively, were randomly selected for analysis of alpha diversity.

For fungal ASVs, functional guilds were determined with FUNGuild database using the FUNGuildR R package (Nguyen et al., 2016; http://github.com/brendanf/FUNGuildR). Taxa associated with a unique guild ranked probable or highly probable were selected and grouped into three categories: ectomycorrhiza, pathogens (including plant pathogens, parasites), and saprotrophs. Taxa associated with more than one guild were manually checked and added to the above categories, when suitable, after carefully reviewing the literature, as recommended by Tedersoo et al. (2022). As general ITS primers are not suitable for AM fungi, they were not included in functional guild analysis (Stockinger et al., 2010). The proportion of each fungal guild was calculated as the frequency of reads assigned to a particular guild divided by the total reads counted across all three guilds. Sequence data were archived at National Center for Biotechnology Information Sequence Read Archive (BioProject PRJNA850095).

Phospholipid fatty acid (PLFA) analysis

Polar lipids were extracted from freeze-dried samples (3 g of mineral soil and 0.25 to 0.5 g of forest floor) following a modified Bligh and Dyer extraction method (Quideau et al., 2016). Prior to extraction, the PC (19:0/19:0) nonadecanoate surrogate standard (Avanti® Plar Lipids Inc., Alabaster, AL, USA) was added to determine the final recovery. Extracted phospholipids were purified on solid-phase extraction columns (Agilent Technologies, Wilmington, DE, USA) and methylated in mild alkaline environment to obtain fatty acid methyl esters (FAMEs). FAMEs were analyzed on an Agilent 6890 Series capillary GC (Agilent Technologies, Wilmington, DE, USA) equipped with a 25 m Ultra 2 (5 %-phenyl)-methylpolysiloxane column, a flame ionization detector (HewlettPackard, Santa Clara, CA, USA), and He as the carrier gas. PLFA concentrations were determined against the internal standard methyl decanoate Me10:0 (Aldrich, St. Louis, MO, USA). Results were calculated as a concentration per gram of soil ($nmol.g^{-1}$) using the surrogate standard. PLFA identification was performed using the Sherlock Microbial Identification System version 4.5 software (MIDI, Inc., Newrak, NJ, USA), following standard nomenclature (Maxfield and Evershed, 2014). All unsaturated PLFAs were in cis configuration. Non-microbial PLFAs, with < 14 and > 20 C chain length, were removed before statistical analysis.

The following PLFAs were assigned to the following microbial functional groups: i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, i18:0, i19:0, i20:0, i15:1ω6, 10Me16:0, 10Me17:0, and 10Me18:0 to Gram-positive(+) bacteria; 14:1ω5, 14:1ω7, 14:1ω9, 15:1ω6, 16:1ω6, 17:1ω8, 18:1ω5, 18:1ω6, 18:1ω7, 19:1ω6, 19:1ω8, 20:1ω4, 20:1ω6, 20:1ω8, 20:1ω9, cy17:0ω7, cy19:0ω7, and cy17:0ω9 to Gram-negative(-) bacteria; 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0 to general bacteria; and 18:2ω6, 18:3ω6, 19:3ω3, 19:3ω3, 20:2ω6, and 20:3ω6 to general fungi.

Microbial ratio calculations

Caution must be used when interpretating specific PLFAs as indicators of specific microbial groups (Frostegård et al., 2011). For this reason, we decided to focus the use of PLFA data on selected ratios that correspond to changes in broad taxa: the fungi:bacteria ratio as the sum of fungal PLFAs divided by the sum of Gram(+), Gram(-), and general bacterial PLFAs, and the Gram(+):Gram(-) ratio as the sum of Gram-positive PLFAs divided by the sum of Gram-negative bacteria, the latter ratio being negatively correlated with carbon availability (Fanin et al., 2019). In addition, we examined potential changes in PLFAs that have been reported to vary in response to environmental stress as a change in membrane composition for microorganisms (Watzinger, 2015). The cyclo ratio was calculated as the sum of cy19:0ω9 and cy19:0ω7 divided by 18:1ω7, an increase in cyclo-PLFA indicating a response to pH, osmotic, and/or thermal stresses (Guillot et al., 2000; Mykytczuk et al., 2010; Yang et al., 2015). The 10-methyl (10Me) ratio was obtained by dividing 10Me16:0 by 16:0, considering that an increase in 10-methyl branching is linked to an increase in membrane fluidity, which is associated with lower temperatures (Poger et al., 2014).

For bacterial ASVs, the Proteobacteria:Acidobacteriota ratio was calculated as the ratio of the number of reads associated with those two phyla, higher values corresponding to higher nutrient status (Orwin et al., 2018).

Statistical analyses

All statistical analyses were performed in R version 4.0.5 (R Core Team, 2021). Threshold for significance was set at alpha = 0.1 to account for the higher probability of type two error associated with the low sample size, recognizing that regional studies such as ours necessarily have low replication. Samples (forest floor and mineral soil) were divided into two categories: invaded and non-invaded, corresponding to the factor “Invasion”.

Relative abundances of fungal and bacteria taxa at different taxonomical levels were calculated as the number of reads of the target category divided by the total number of reads. Two indices were calculated for alpha diversity based on rarefied ASV tables: species richness and effective number of species, corresponding to a transformed Shannon diversity index (Jost, 2006). For fungal ASVs, those indices were calculated for each functional guild, while for bacteria, they were based on all ASVs.

To determine whether earthworm invasion affected microbial communities functionally and taxonomically, two-way (factors: invasion and site) analyses of variance (ANOVAs) were performed separately for forest floor and mineral soil samples on fungal and bacterial taxa, diversity indices, and microbial – bacteria and PLFA – ratios presented in the previous section. For fungi, the ANOVAs were run on fungal guilds while for bacteria, the focus was on phyla, and families present in at least 60 % of the samples and accounting for > 0.5 % of relative abundance on average. When necessary, data were first transformed with Tukey's Ladder of Powers using transformTukey from the rcompanion package to ensure normality of residuals (Mangiafico, 2021). Homogeneity of variance was checked with Bartlett test. To account for the unbalanced design for both invasion and site, the type II sum of squares was used for the ANOVAs using the car R package (Fox, 2016). We ran indicator species analyses on Hellinger transformed data, for the forest floor and the mineral soil separately, to identify specific taxa associated with invaded or control samples using the multipatt function from the indicspecies R package (De Caceres and Legendre, 2009).

To determine whether earthworm invasion altered soil microbial community composition, permutational analyses of variance (PERMANOVA) were performed on all three datasets (PLFA, 16S, and ITS) using Bray-Curtis distance matrices. The adonis function from the vegan package was used on Hellinger transformed individual ASV and PLFA relative abundances, for forest floors

and mineral soils separately, followed by posthoc tests for pairwise comparisons (Oksanen et al., 2019).

Results

Earthworm invasion effects on fungi

A total of 3,236,759 reads was obtained for fungal ASVs, ranging from 70 to 136,741 reads with an average of 31,123 per sample. After quality control and trimming, we obtained a total of 2,820,523 reads associated with 10,761 ASVs (1,867 unique ASVs). On average, 80 % of ASVs belonged to at least one fungal guild. The following numbers of ASVs were identified for each guild: Ectomycorrhizal (EcM; 208), Saprotrophic (450), Pathogenic (126). After rarefaction, eight out of 58 samples were dropped for diversity indices because of low read counts: three invaded forest floors, three invaded mineral soils and two control mineral soils.

For fungal ASVs, both invasion and soil horizon significantly affected community structure according to the PERMANOVA results (Table 3-1 & Suppl. Fig. 3.B-2). The posthoc pairwise test showed that earthworm invasion significantly altered fungal community composition in the mineral soil, but not in the forest floor. The relative abundance of fungal phyla, classes, orders, and families can be found in Suppl. Fig. 3.B-3 to 6.

Table 3-1. Permutational analysis of variance (PERMANOVA) performed on Hellinger-transformed data for fungal and bacterial amplicon sequence variants (ASVs) and phospholipid fatty acids (PLFAs) using Bray-Curtis distance matrix for invaded (EW) and non-invaded (CONT) forest floors (LFH) and mineral soils (MIN). Df stands for degrees of freedom and Sum sq for sum of squares.

		Df	Sum sq	F	R^2	p-value
Fungi	Invasion	1	0.45	1.31	0.02	0.081
	Soil horizon	1	1.34	3.88	0.06	<0.001
	Interaction	1	0.31	0.91	0.02	0.64
	Residuals	54	18.66		0.90	
	Total	57	20.77		1.00	
Pairwise comparisons						
MIN-EW vs LFH-EW		1	0.91	2.61	0.06	<0.001
MIN-EW vs LFH-CONT		1	0.82	2.43	0.08	<0.001
MIN-EW vs MIN-CONT		1	0.45	1.37	0.05	<0.05
LFH-EW vs LFH-CONT		1	0.32	0.87	0.03	0.63
LFH-EW vs MIN-CONT		1	0.89	2.52	0.09	<0.001
LFH-CONT vs MIN-CONT		1	0.74	2.22	0.14	<0.001
Bacteria	Invasion	1	0.61	1.86	0.03	0.025
	Soil horizon	1	2.65	8.03	0.12	<0.001
	Interaction	1	0.34	1.04	0.02	0.38
	Residuals	54	17.84		0.83	
	Total	57	21.45		1.00	
Pairwise comparisons						
MIN-EW vs LFH-EW		1	1.80	5.36	0.12	<0.001
MIN-EW vs LFH-CONT		1	1.30	3.91	0.13	<0.001
MIN-EW vs MIN-CONT		1	0.60	1.85	0.06	<0.05
LFH-EW vs LFH-CONT		1	0.35	1.03	0.04	0.37
LFH-EW vs MIN-CONT		1	1.68	5.13	0.16	<0.001
LFH-CONT vs MIN-CONT		1	1.19	3.82	0.21	<0.001
PLFA	Invasion	1	0.02	1.37	0.02	0.23
	Soil horizon	1	0.37	24.92	0.31	<0.001
	Interaction	1	0.01	0.45	0.01	0.79
	Residuals	54	0.80		0.67	
	Total	57	1.20		1.00	
Pairwise comparisons						
MIN-EW vs LFH-EW		1	0.25	16.03	0.29	<0.001
MIN-EW vs LFH-CONT		1	0.11	8.43	0.23	<0.001
MIN-EW vs MIN-CONT		1	0.02	1.77	0.06	0.11
LFH-EW vs LFH-CONT		1	0.01	0.40	0.02	0.82
LFH-EW vs MIN-CONT		1	0.23	14.16	0.35	<0.001
LFH-CONT vs MIN-CONT		1	0.13	9.67	0.39	<0.001

In the forest floor, the relative abundance of the different fungal guilds (EcM, pathogenic, and saprotrophic) was affected by site but not by earthworm invasion (Fig. 3-2 & Suppl. Table 3.A-2). However, the indicator species analysis identified several genera associated with control forest floors: the EcM genus *Lactarius* and the saprotrophic genera *Umbelopsis* and *Syzygospora* (Table 3-2). In the forest floor, species richness was only negatively affected by earthworm invasion for EcM fungi (Table 3-3).

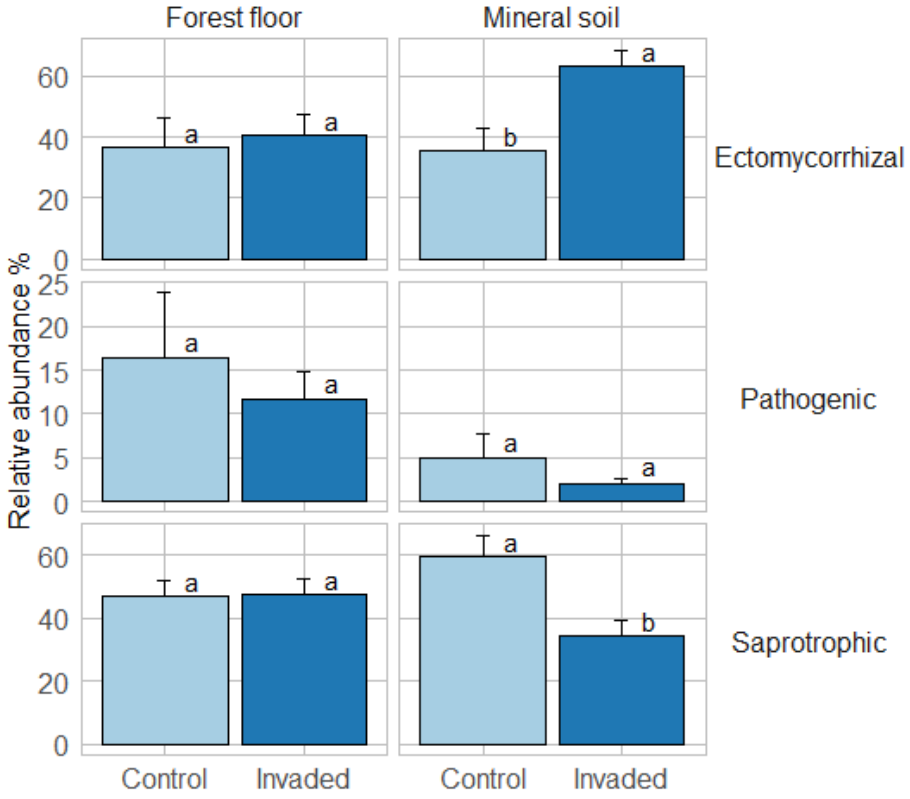


Figure 3-2. Average relative abundances of fungal guilds (± 1SE). Different letters indicate significant differences (p-value < 0.1) between control and invaded forest floors or mineral soils and were obtained with the posthoc HSD Tukey test after two-way analysis of variance (ANOVA) for all soil types combined (n=7–22).

Table 3-2. Indicator species for ectomycorrhizal and saprotrophic fungi, as well as bacteria, associated with control or invaded forest floors and mineral soils. Indicator species were obtained using the multipatt function in the indicspecies R package on Hellinger-transformed data. The amplicon sequence variants (ASVs) were grouped at the finest identified taxonomic level between order and genus, taxa only identified to the class level were excluded. The stat value is a combination of specificity (A=1 when only found in the one group) and fidelity (B=1 when found in all samples of the one group). Relevant indicators were selected with B > 0.5 and relative abundance > 0.05 % in the taxonomical group for which they were selected as indicator. No indicator species were found for pathogenic fungi.

			Invasion	Taxonomical group	A	B	Stat	p-value
Fungi	Ectomycorrhizal	Forest floor	Control	*Lactarius*	0.88	0.60	0.73	0.03
		Mineral soil	Invaded	*Tomentella*	0.94	0.71	0.82	0.010
				Amphinema	0.95	0.65	0.79	0.010
	Saprotrophic	Forest floor	Control	*Syzygospora*	0.67	1.00	0.82	0.013
				Umbelopsis	0.63	1.00	0.79	0.026
Bacteria		Forest floor	Invaded	*Parafilimonas*	1.00	0.65	0.81	0.008
				Uncultured vadinHA49	0.89	0.65	0.76	0.038
				Zavarzinella	0.86	0.65	0.75	0.045
		Mineral soil	Control	Uncultured 1921-2 and 1921-3	0.72	1.00	0.85	0.003
				Acidipila	0.71	0.89	0.80	0.010
				Uncultured B12-WMSP1	0.72	0.67	0.70	0.045
				Xanthobacteraceae	0.70	0.67	0.68	0.042
			Invaded	*Haliangium*	0.92	0.73	0.82	0.003
				Pirellula	0.86	0.77	0.82	0.004
				Fimbriiglobus	0.91	0.73	0.81	0.004
				Uncultured OM190	0.86	0.68	0.77	0.014
				Latescibacterota	0.89	0.55	0.67	0.030

In the mineral soil, the relative abundance of EcM and saprotrophic fungi was affected by both invasion and site, but the interaction of these two factors was not significant (Fig. 3-2 & Suppl. Table 3.A-2). The relative abundance of saprotrophic fungi decreased from 60 % to 34 % after earthworm invasion, while that of EcM fungi increased from 35 % to 64 %. As a result, the fungal communities shifted from saprotroph- to EcM-dominated. The indicator species analysis recognized the EcM genera *Amphinema* and *Tomentella* as indicators of invasion in the mineral soil (Table 3-2). Fungal species richness increased after invasion for EcM (+104 %), saprotrophic (+56 %), and pathogenic (+136 %) fungi while species diversity increased for saprotrophic (+45 %) and pathogenic (+136 %) fungi only (Table 3-3 & Suppl. Table 3.A-2).

Table 3-3. Averages and standard errors (in parentheses) of fungal and bacterial species richness and diversity (as the effective number of species) indices (n=7–20 and 7–22, respectively). Different letters represent significant differences between control and invaded soils (p-value < 0.1) and are presented separately for the forest floors and mineral soils.

	Fungi						**Bacteria**	
	Ectomycorrhizal		Pathogenic		Saprotrophic		Global	
	Richness	Diversity	Richness	Diversity	Richness	Diversity	Richness	Diversity
Forest floor								
Control	20.7 (3.8) a	7.4 (1.3) a	9.1 (2.2) a	4.6 (1.0) a	36.9 (5.3) a	16.5 (2.9) a	480 (45) a	293 (43) a
Invaded	16.5 (1.8) b	8.4 (1.0) a	7.1 (1.0) a	4.2 (0.6) a	33.9 (3.1) a	14.9 (2.2) a	577 (35) a	376 (27) a
Mineral soil								
Control	8.4 (1.3) b	4.5 (0.8) a	1.4 (0.5) b	1.1 (0.1) b	17.7 (1.6) b	10.0 (1.6) b	518 (27) b	297 (24) b
Invaded	17.1 (0.8) a	6.1 (0.6) a	3.3 (0.6) a	2.6 (0.5) a	27.6 (1.2) a	14.5 (1.2) a	573 (13) a	337 (11) a

Earthworm invasion effects on bacteria and archaea

For bacterial ASVs, a total of 4,570,297 reads was obtained with an average of 43,945 per sample, ranging from 28,049 to 60,698 reads. After quality control and trimming, we obtained a total of 1,376,959 reads associated with 17,086 ASVs. While the term ‘bacterial communities’ is used here, the archaea were not excluded. The overall composition of bacterial communities was significantly affected by invasion and soil horizon, as indicated by the PERMANOVA results (Table

3-1 & Suppl. Fig. 3.B-7). The shift following earthworm invasion was significant in the mineral soil (p-value < 0.05) but not in the forest floor, according to the posthoc test. The relative abundance of bacterial phyla, classes, orders, and families can be found in Suppl. Fig. 3.B-8 to 11.

In the forest floor, the relative abundance of Actinobacteriota was significantly lower in invaded samples (24 %) compared to the control samples (32 %; Fig. 3-3 and Suppl. Table 3.A-3). Within this phylum, the decrease was significant for the Micromonosporaceae and Solirubrobacteraceae families, while the Ilumatobacteraceae family increased. The Proteobacteria were not affected by earthworm invasion at the phylum level, but a significant decrease was noted for the Beijerinckiaceae family, while the Reyranellaceae family increased (Fig. 3-3). Verrucomicrobiota increased significantly in earthworm-invaded forest floors, reaching 3 % of relative abundance, mainly due to the increase in Chthoniobacteraceae family. The Chitinophagaceae family from the Bacteroidota phylum was positively affected by earthworm invasion, with *Parafilimonas* selected as indicator of invasion in the forest floor (Fig. 3-3 & Table 3-2).

In the mineral soil, the relative abundance of Acidobacteriota significantly decreased from 27 % to 21 % following earthworm invasion, resulting in a higher Proteobacteria:Acidobacteriota ratio (Fig. 3-3 & Table 3-4). Within the Acidobacteriota phylum, the response to earthworm invasion varied among taxonomic groups, notably with an increase in the Acidobacteriales order, Acidobacteriaea subgroup 2, and Thermoanaerobaculaceae family but a decrease in Solibacteraceae family (Fig. 3-3 & Suppl. Table 3.A-3). While Actinobacteriota were not affected by earthworm invasion at the phylum level, the Acidothermaceae family responded negatively and the Gaiellaceae family, positively. As in the forest floor, Reyranellaceae and Chitinophagaceae families were positively affected by earthworm invasion in the mineral soil, the latter mirroring the significant increase in Bacteroidota (Fig. 3-3). Although their relative abundance was much lower (< 10 % at

the phylum level), it is still worth noting that the Gemmatimonadaceae family was positively affected by earthworm invasion in the mineral soil while Chloroflexi, including the candidate phylum AD3, were negatively affected (Fig. 3-3). Within the Chloroflexi, the uncultured bacteria 1921-2, 1921-3, and B12WMSP1 were indicators of non-invaded mineral soils. The Hyphomicrobiaceae and Nitrosomonadaceae families were positively affected by earthworm invasion. Similarly, the relative abundance of Pirellulaceae was significantly higher in earthworm-invaded mineral soils, of which *Pirellula* was selected as indicator together with two other Planctomycetota: *Fimbriiglobus* and the uncultured bacterium OM190 (Table 3-2). Those shifts were also associated with an increase in species richness and diversity in the mineral soil (Table 3-3).

Table 3-4. Averages and standard errors (in parentheses) of selected phospholipid fatty acid (PLFA; n=8–22) and bacterial (n=7–22) ratios. Different letters represent significant differences between control and invaded soils (p-value < 0.1) and are presented separately for the forest floors and mineral soils. The 10Me ratio was obtained by dividing the PLFA 10Me16:0 by 16:0; the Cyclo ratio was calculated by dividing the sum of cy19:0ω9 and cy19:0ω7 by 18:1ω7.

	Bacteria	**PLFA**			
	Proteobacteria: Acidobacteriota	Fungi:Bacteria	Gram(+):Gram(-)	10Me ratio	Cyclo ratio
Forest floor					
Control	2.15 (0.21) a	0.27 (0.04) a	0.96 (0.08) b	0.18 (0.05) a	0.44 (0.10) a
Invaded	2.55 (0.27) a	0.30 (0.03) a	1.14 (0.08) a	0.16 (0.04) a	0.35 (0.05) a
Mineral soil					
Control	0.64 (0.05) b	0.07 (0.02) b	1.31 (0.03) a	0.67 (0.05) a	0.52 (0.0.7) a
Invaded	0.97 (0.08) a	0.09 (0.02) a	1.16 (0.04) b	0.48 (0.06) b	0.41 (0.03) b

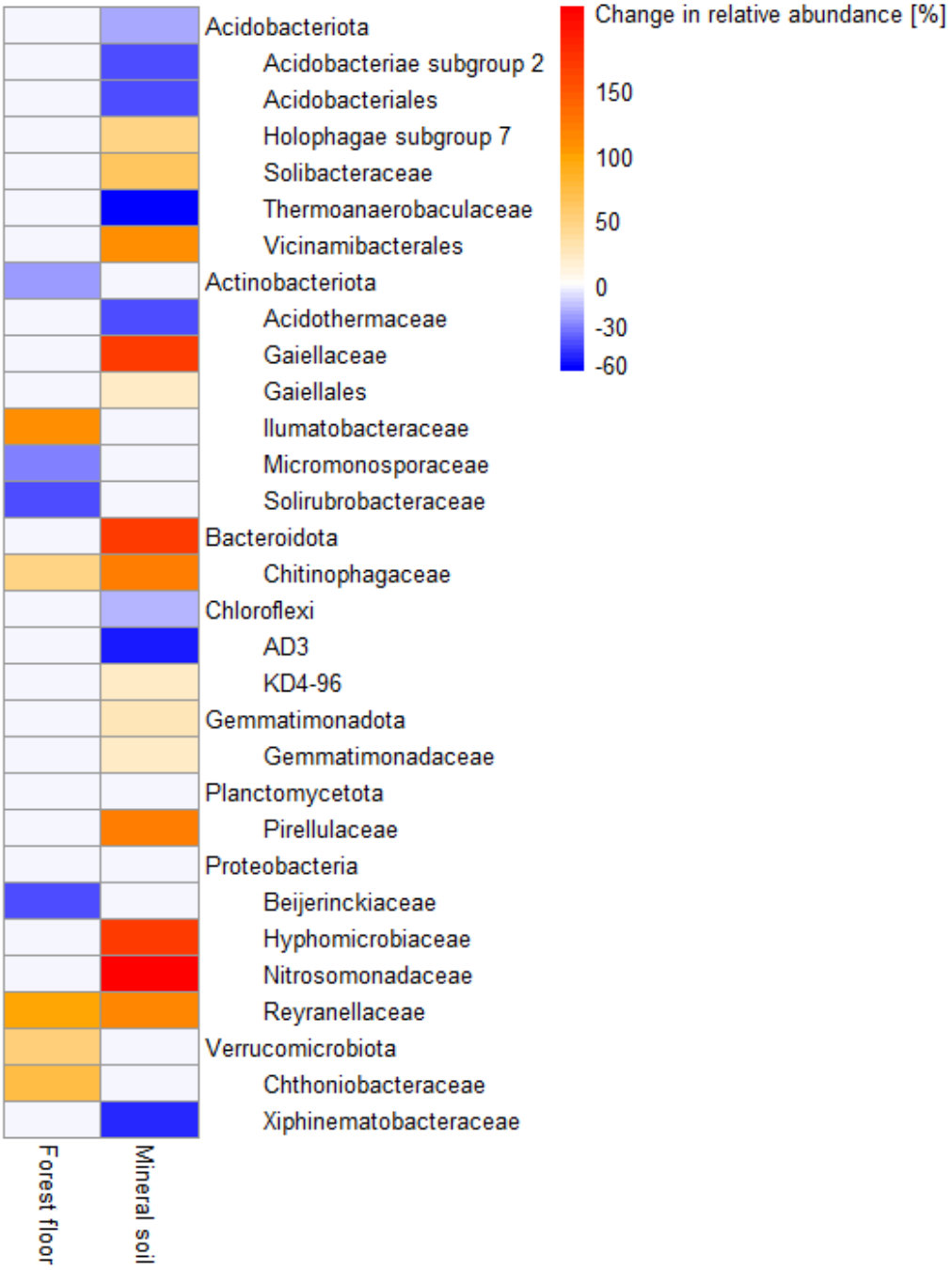


Figure 3-3. Heatmap of changes in relative abundance of bacterial phyla and families (or associated level). Only significant differences (p-value < 0.1), determined by two-way analyses of variance (ANOVAs) followed by posthoc HSD Tukey test, are displayed (n=7–22). Orange/red colour represents an increase and blue colour, a decrease. Results of the two-way ANOVAs associated with the significant differences can be found in Suppl. Table 3.A-3.

Earthworm invasion effects on microbial PLFAs

Results of the permutational analysis of variance (PERMANOVA) showed that invasion did not significantly affect the overall PLFA composition (Table 3-1 & Suppl. Fig. 3.B-12). While the total PLFA biomass was not significantly affected by earthworm invasion, fungal PLFAs were significantly higher in earthworm-invaded mineral soils, with a concentration almost twice as high as in the control soils (Table 3-5). In the forest floor, the Gram(+):Gram(-) ratio was significantly higher after earthworm invasion (Table 3-4 & Suppl. Table 3.A-4). On the contrary, the same ratio shifted in the opposite direction in the mineral soil, with a significantly lower value after invasion. The fungi:bacteria ratio was slightly but significantly higher in earthworm-invaded mineral soils (0.09) compared to the controls (0.07). The 10Me and cyclo ratios, indicative of environmental stress, were both significantly lower in invaded mineral soils compared to controls but were not affected by earthworm invasion in the forest floor. For all ratios, the interaction between invasion and site was non-significant (Suppl. Table 3.A-4).

Table 3-5. Average concentrations (nmol.g-soil^{-1}) in total, Gram(+), Gram(-), and fungal phospholipid fatty acids (PLFAs), with standard errors in parentheses (n=8–22). Different letters represent significant differences between control and invaded soils (p-value < 0.1) and are presented separately for the forest floors and mineral soils.

		Total PLFA	Gram(+) bacteria	Gram(-) bacteria	Fungi
Forest floor	Control	4909 (277) a	889 (96) a	924 (67) a	772 (91) a
	Invaded	5680 (305) a	1029 (88) a	943 (81) a	910 (77) a
Mineral soil	Control	1317 (351) a	408 (110) a	320 (93) a	55 (16) b
	Invaded	1684 (302) a	477 (96) a	397 (65) a	93 (22) a

Discussion

Earthworm invasion and shifts in microbial community composition

Using a combined PLFA and metabarcoding approach, our study presents the first evidence of microbial community composition shifts associated with earthworm invasion in boreal forest soils. Both fungal and bacterial communities were affected, but the observed changes greatly varied between forest floor and mineral soil microbial communities. This confirms the pertinence of our approach to run separate analyses for the forest floors and mineral soils. As one could expect, considering the intrinsic differences among soil types, the factor 'site' was, in most cases, significant and explained most of the variability observed. The interaction term between invasion and site was, however, non-significant, indicating that despite potentially variable amplitudes, the effects of earthworm invasion on boreal forest microbial communities were similar across the selected sites. The less pronounced changes observed in the forest floors compared to the underlying mineral soils might be linked to their higher microbial biomass, as estimated from total PLFA concentrations. Considering the persistence of DNA in soils, significant shifts would be more difficult to detect in the forest floor where high levels of relic DNA are present, compared to the mineral soil, which contains lower biomass (Carini et al., 2016).

Our results corroborate previously reported changes in bacterial communities associated with earthworm activity. De Menezes et al. (2018) also reported an increase in Verrucomicrobiota, and Gong et al. (2018) an increase in Gemmatimonadota after incubation with earthworms, respectively in pasture and arable soils. Chitinophagaceae, which increased after invasion in the current study, were also previously positively correlated with earthworm presence and are involved in the degradation of chitin, an important constituent of fungal cell walls (Bernard et al., 2012; de Menezes et al., 2018), and of other complex sugars such as cellulose and hemicelluloses

(Rosenberg, 2014) that could have become more available in the mineral layer of soil with earthworm bioturbation.

Although it has been hypothesized that earthworm bioturbation could decrease fungal abundance by disrupting fungal networks (Hansson et al., 2013), we measured an increase in fungal biomass, estimated by PLFA analysis, following invasion in the mineral soil, where EcM fungal abundance also increased while that of saprotrophic fungi decreased. Positive effects of earthworms on AM fungi in the mineral soil have been observed by Dempsey et al. (2013) and Drouin et al. (2016) in temperate forests. In comparison, Cameron et al. (2012) did not find that the presence or density of invasive earthworms affected EcM fungal community composition or colonization of white spruce roots in a mesocosm experiment. However, in the study by Cameron et al. (2012), only a subset of EcM fungi able to withstand disturbance was likely present in the pots and the seedlings were grown in conditions not conducive to growth of fine roots, a required process for changes in EcM fungal colonization to occur. Thus, the conditions of this experiment may have underestimated the effects of earthworms. In contrast, in our study, earthworm invasion negatively affected EcM fungal richness in the forest floor, in line with previous findings showing reduced microfungal community richness and diversity after earthworm invasion of forest floors in southern Alberta (McLean and Parkinson, 2000). Clearly, earthworm invasion differentially affected the forest floor and mineral soil fungal communities, suggesting divergent direct impacts.

Contradicting results have been documented in terms of the response of the fungi:bacteria and Gram(+):Gram(-) ratios to the presence of earthworms. In line with our findings, Butenschoen et al. (2007) found that endogeic earthworms favoured Gram(-) over Gram(+) bacteria in arable soils. On the other hand, Dempsey et al. (2013) saw a decrease in Gram(-) and an increase in Gram(+) bacteria in the surface mineral soil after earthworm invasion in a northern hardwood forest. Other

studies found variable responses of the relative abundance of Gram(-) and Gram(+) bacteria among earthworm species (Chang et al., 2016, 2017). For the fungi:bacteria ratio, Dempsey et al. (2011) found a decrease in a temperate hardwood forest on an area basis (m^2), but not per gram of soil – which is how we calculated our ratio. Similarly, in an incubation with arable soil, the fungal biomass decreased in the presence of earthworms (Butenschoen et al., 2007). Chang et al. (2017) observed an increase in fungi:bacteria only in the presence of multiple earthworm species in deciduous temperate forests, consistent with our findings as most of our sites were also invaded by multiple earthworm species. Because increased fungi:bacteria ratio was coupled with a decrease in AM fungi, Chang et al. (2017) deduced that saprotrophic and EcM fungi must have increased. Our results partly confirm this hypothesis, as the positive impact on fungal communities appears to be limited to EcM fungi in boreal forests while the relative abundance of saprotrophic fungi decreased, likely because of a decrease in fresh OM available for decomposition (Högberg et al., 2017). Compared to temperate forests, the fungi:bacteria ratio is typically higher in boreal forests, where fungal communities, especially EcM fungi, play a major role in C sequestration (Chen et al., 2020; Clemmensen et al., 2013; Francisco et al., 2016). The absence of consensus among studies about the impact of earthworms on the fungi:bacteria and Gram(+):Gram(-) ratios shows that the relationships are complex and not well understood. There is however evidence that N addition, pH, and C availability, among other factors, can alter the relative abundance of those large microbial groups (Fanin et al., 2019; Rousk et al., 2010; Zhou et al., 2017).

Microbial functions and environmental changes

The current invasion of North American forests by exotic earthworms can have cascading effects on ecosystem functioning (Frelich et al., 2019). Given that soil microbial communities play a central role in nutrient cycling and SOM dynamics (Simpson et al., 2007), the shifts observed in the

forest floor and the mineral soil are likely to have consequences for nutrient availability and ecosystem functioning.

Only specific fungal taxa were negatively affected by earthworm invasion in the forest floor. Notably, the EcM fungal genus *Lactarius,* identified as indicator of absence of earthworms in the forest floor, is often classified as nitrophilic (Lilleskov et al., 2011, 2002). Rodriguez-Ramos et al. (2020) also found the same taxon to be negatively affected by wildfire and salvage-logging. Both of these disturbances, together with earthworms, alter the integrity of the forest floor. Multiple taxa of the phylum Actinobacteriota, which decreased after invasion, play an important role in lignocellulose degradation (Větrovský et al., 2014). The functional role of Verrucomicrobiota is still poorly understood and its abundance in soils is thought to be underestimated (Bergmann et al., 2011). However, Verrucomicrobiota, which increased in invaded forest floors, have been negatively correlated with soil fertility in tropical environments (Navarrete et al., 2015) and are often considered oligotrophic (Hu et al., 2022; Orwin et al., 2018). Within the Verrucomicrobiota phylum, Chthoniobacteraceae, which also responded positively to earthworm invasion, were positively correlated with more oligotrophic conditions – including decrease in labile substrates – in a restoration chronosequence (Sun et al., 2017). If these observations hold true in boreal forest floors as well, the observed increase in Verrucomicrobiota and Chthoniobacteraceae could be linked to lower fertility. Further, the Gram(+):Gram(-) ratio response suggests that earthworms also decrease C availability in the forest floor (Fanin et al., 2019). As earthworms preferentially feed on more palatable substrates, more recalcitrant C compounds can progressively accumulate in the forest floor (Curry and Schmidt, 2007). Changes in microbial-driven forest floor functions could therefore include lower nutrient availability, consistent with the lower TOC and TN content

observed in the forest floor after invasion (Suppl. Table 3.A-1; Chapter 2, published as Lejoly et al., 2021).

In the mineral soil, the response of the PLFA ratios suggests that earthworm invasion affected environmental conditions for microbial activity. The decrease observed for the cyclo ratio (i.e. the sum of PLFAs cy19:0ω9 and cy19:0ω7 divided by 18:1ω7) has been previously associated with a decrease in environmental stress of osmotic or acidic origin (Guillot et al., 2000; Mykytczuk et al., 2010; Yang et al., 2015). This is consistent with the higher pH found in earthworm-invaded mineral soils (Suppl. Table 3.A-1; Chapter 2, published as Lejoly et al., 2021), which can also explain their higher bacterial diversity and richness (Fierer and Jackson, 2006) and the decrease in Gram(+):Gram(-) ratio (Frostegård et al., 1993). While 10Me PLFAs are often associated with Actinobacteriota (Chowdhury and Dick, 2012; Dungait et al., 2011), the concentration of 10Me16:0 (data not presented), used to calculated the 10Me ratio, and the relative abundance of Actinobacteriota, as estimated from 16S metabarcoding, were not affected by earthworm invasion. We can therefore confidently conclude that the lower 10Me ratio observed in invaded mineral soils is indicative of a decrease in membrane fluidity, linked to higher temperatures (Kieft et al., 1994; Poger et al., 2014; Zhang and Rock, 2008). The thinning of the forest floor layer associated with earthworm invasion could increase soil temperature during the growing season similarly to the mechanical removal of forest floor in managed forests (Tan et al., 2005).

There are multiple indications of increased C and N availability in the mineral soil, including the decrease in Gram(+):Gram(-) bacteria and Acidobacteriota as well as the increase in Bacteroidota, respectively, all associated with increased C mineralization and labile C (Fanin et al., 2019; Fierer et al., 2007). Numerous studies have shown that earthworms increase C availability, through the incorporation of fresh litter into the soil, priming microbial communities with labile C and thus

enhancing the decomposition of more recalcitrant OM (Bohlen et al., 2002; De Graaff et al., 2010; Fontaine et al., 2004; Medina-Sauza et al., 2019). Similarly, there is indication of increased N availability, such as the increase in Bacteroidota after invasion, as the phylum is also positively correlated with high N additions in boreal forests (Högberg et al., 2014). This observation is in line with the numerically higher TN content in the mineral soil after invasion (Suppl. Table 3.A-1). In low N supply soils, increased N deposition can result in increased fungal biomass and richness, in line with our findings (Moore et al., 2021). We suggest that the most likely explanation for the observed increase in EcM fungi relative abundance is therefore an increase in N availability associated with earthworm invasion (scenario 1 presented in the introduction). Earthworms have also been associated with both enhanced nitrification and denitrification (Burtelow et al., 1998; Medina-Sauza et al., 2019). Nitrosomonodaceae and some Hyphomicrobiaceae taxa, which both increased after invasion, are also involved in the N cycle, with respectively ammonia oxidation (Prosser et al., 2013) and denitrification and nitrogen fixation (Kloos et al., 1995) capacities. Chitinophagaceae, which increased in the mineral soil after invasion, are favoured in high N supply conditions (Högberg et al., 2014). In arctic ecosystems, Blume-Werry et al. (2020) found earthworm casts greatly enriched in NH_4^+ compared to the bulk soil and concluded that invasive earthworms could lift plant N limitations. All these findings from various research efforts suggest that invasive earthworms have the potential to accelerate N cycling in high latitudes.

Similarly, the increase in Proteobacteria:Acidobateriota ratio mentioned above is indicative of higher nutrient status (Smit et al., 2001) and has been previously associated with earthworm activity in arable soils (Gong et al., 2018). Acidobacteriales and Acidobacteria subgroup 2, which have been suggested as bioindicators of P mining, both decreased after earthworm invasion; their abundance similarly declined with higher P in temperate forests (Mason et al., 2021). The EcM fungal

genera *Aphimena* and *Tomentella*, indicator of earthworm invasion in the mineral soil, have been positively correlated with soil fertility and pH in boreal forests (Haas et al., 2018; Sterkenburg et al., 2015). Although the fungi:bacteria ratio should be negatively affected by an increase in pH (Rousk et al., 2010) and N addition (Zhou et al., 2017), we observed the opposite response. Similarly, the Gram(+):Gram(-) ratio is expected to increase following N addition (Zhou et al., 2017), but is also negatively correlated with C availability (Fanin et al., 2019). Additionally, in the Luvisolic and Brunisolic sites, Lejoly et al. (2021, Chapter 2) observed a decreased bulk density in the mineral soil following earthworm invasion. Such changes in soil physical properties could also affect microbial community size and composition. These observations suggest that the mechanisms by which invasive earthworms affect microbial communities are complex and that the interactions of multiple factors, such as increases in pH and N availability, need to be considered.

Soil C and N both determine microbial activity. Fungi typically have slower growth and lower nutrient requirements compared to bacteria, and their dominance increases with lower C:N ratios (Soares and Rousk, 2019). While it is commonly accepted that EcM fungal growth is limited by N availability (Morrison et al., 2016), it appears that C limitation might also be an important controlling factor when N supply is higher (Högberg et al., 2021). The hypothetical increase in C and N availability could therefore lift both limitations. As the ability of soils to store C is dependent on N availability (Cotrufo et al., 2019; Van Groenigen et al., 2017), earthworm invasion could have the potential to decrease soil C storage (Blume-Werry et al., 2020). However, EcM fungi can also slow down C cycling, increasing its storage in soils (Averill and Hawkes, 2016). Whether earthworm invasion will increase or decrease soil C storage is therefore a complex question to answer. Moreover, as boreal forests contain more than twice as much SOC as temperate forests per unit

area (Lal, 2005), even small changes in C storage can have important implications for climate change mitigation efforts.

Our key findings are conceptualized in Fig. 3-4. While most of our observations align with previous findings for earthworm invasion in temperate forests, there are some interesting divergences with previous studies, including the relative overall increase in fungi compared to bacteria and the decrease in Gram(+):Gram(-) ratio discussed above. These results suggest that boreal forests are affected by invasive earthworms differently than temperate forests and confirm the importance to implement additional studies in the boreal biome. We acknowledge that most of our observations of functional and nutrient status changes are inferred from the shifts in microbial community composition and indirect, as our study was conducted in natural systems without controlled addition/exclusion of earthworms. We therefore cannot determine whether they constitute a direct consequence of earthworm invasion. Future studies should include enzyme analyses and/or RNA techniques to target active microbial communities. Our sampling design did not allow us to differentiate between earthworm species or soil types, which could explain some of the variability observed. There is growing concern regarding the more recent invasion of Asian earthworm species in North America, although they have not been observed in the boreal forest yet (Chang et al., 2021). The findings of Chang et al. (2016) suggest that the impacts of Asian species such as *Amynthas hilgendorf* on soil microbial communities will be greater than that of similar European species, for deciduous temperate forests. Further research should include both European and Asian earthworm species.

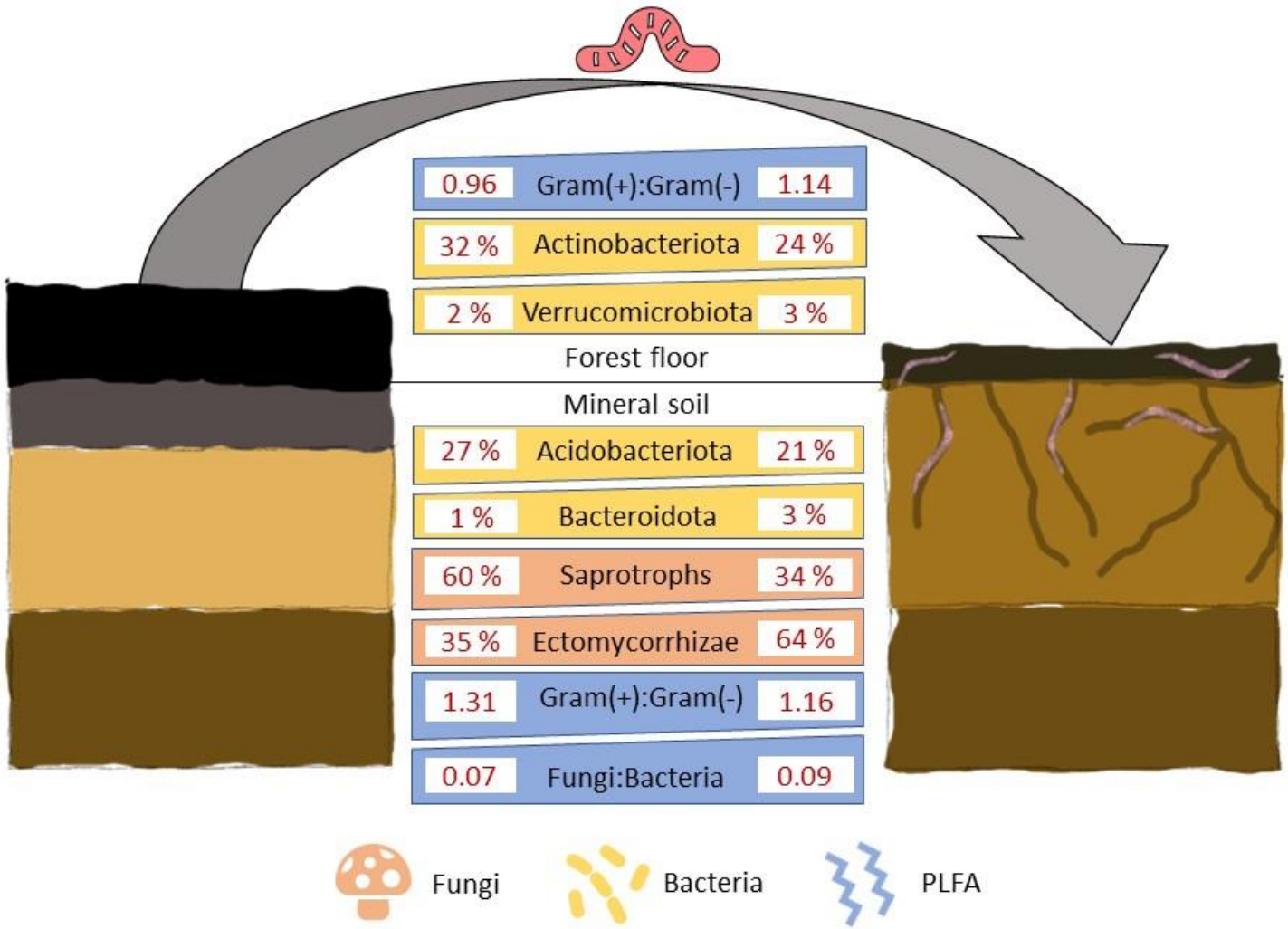


Figure 3-4. Conceptual diagram of key findings

Chapter 4 : Invasive earthworms increase litter decomposition and soil labile carbon in boreal forests

Abstract

Invasive earthworms are reshaping the soils of the boreal forest, drastically decreasing the forest floor carbon (C) stocks and reworking surface minerals soil through bioturbation. Given that the boreal forest represents the largest terrestrial C reservoir, changes in its dynamics induced by earthworm invasion could have drastic effects on its ability to store C. Here we compared physical and chemical characteristics of the C found in soils invaded by earthworms with non-invaded soils to determine whether earthworm invasion was affecting C persistence, as well as its distribution among soil physical fractions and chemical composition. We selected three sites, representing three major soil types found in the Canadian boreal forest: Luvisol, Podzol, and Brunisol (Cambisol). We estimated the amount of labile C contained in forest floors and surface mineral soils after a one-year laboratory incubation at high temperature (24 ºC) to maximize microbial mineralization. Additionally, for mineral soil samples, we quantified C physical distribution using aggregate fractionation and C chemical composition using pyrolysis-gas chromatography-mass spectrometry. We found a higher proportion of labile C in earthworm invaded soils, mainly in the mineral soil. We also found that the increase in C content was significant for all < 2 mm fractions and that the proportion of C contained in the occluded light fraction (250 µm–2 mm) was higher in earthworm-invaded soils. Earthworm invasion affected the chemical composition of soil organic matter (SOM), with lower suberin content, higher relative abundance of compounds commonly associated with microbial decomposition, and higher C oxidation state. Our results constitute evidence

that earthworm invasion is affecting SOM dynamics in boreal forests. Although labile C increased in the short term, total soil C and its persistence may increase in the long term.

Introduction

The introduction of exotic earthworm species following European settlement has been shown to negatively affect carbon (C) stocks in temperate forests, primarily by drastically decreasing forest floor thickness (Blouin et al., 2013; Bohlen et al., 2004a; Hale et al., 2005b). The presence of these invasive earthworms in boreal forests, which is the largest terrestrial C reservoir on Earth (Frelich et al., 2019; Kurz et al., 2013), has been documented more recently and remains understudied compared to temperate forests (Cameron et al., 2007; Moore and Reynolds, 2003). Decomposition rates are lower in boreal than in temperate forests and litter typically contains more phenol-rich substrates, recalcitrant to decomposition (DeLuca and Boisvenue, 2012). How these different conditions may affect the response of boreal forest soils to earthworm invasion is unknown. The study of multiple soil types within the boreal previously showed that earthworms in most cases decrease forest floor thickness and C stocks, similarly to what is observed in temperate forests, and rework the mineral soil, creating a carbon-enriched surface horizon, although total soil C stocks may not change (Chapter 2, published as Lejoly et al., 2021). In mineral soil horizons, earthworms can affect soil organic matter (SOM) dynamics with two counteracting main mechanisms: (1) increase of SOM mineralization by enhancing soil microbial activity and (2) stabilization of SOM by physical protection within aggregates (casts), leading to inaccessibility for microbial decomposition (Lubbers et al., 2017). However, the net effect of earthworm activity on soil C balance is still debated (Lubbers et al., 2013).

Plant litter, either from below (roots) or above (leaves and shoots) ground residues, is the main contributor to SOM (Kögel-Knabner, 2002). Cutin and suberin, biopolymers found in leaves and roots respectively and partly preserved in soils (Kögel-Knabner, 2000), have been used as biomarkers to differentiate above from belowground inputs to SOM (Hamer et al., 2012; Otto et al.,

2005). In the mineral soil, root-derived residues are often more abundant than leaf-derived residues, aboveground inputs typically decomposing at a faster rate (Mueller et al., 2013), supposedly because of their lower lignin content (Angst et al., 2016). However, since there is no evidence that suberin is more resistant to microbial decomposition than cutin (Angst et al., 2016), Mueller et al. (2013) suggested that the preferential consumption of leaf litter by soil biota could partially explain the greater abundance of suberin. Indeed, recent findings show that earthworms alter C dynamics by favouring the accumulation of root over leaf-derived SOM (Angst et al., 2020). It is believed that earthworms preferentially feed on leaves because of more palatable properties while accidentally ingesting roots (Curry and Schmidt, 2007). Bioturbation is a limited process in North American forests that have developed in the absence of earthworms since the last glaciation (Addison, 2009; Hendrix, 2006). The introduction of invasive earthworms could therefore drastically alter the equilibrium between above and belowground inputs by increased mixing of the forest floor with the mineral soil (Feller et al., 2003).

Some earthworm species preferentially feed on leaf body, leaving lignin-rich petioles and veins behind (Suárez et al., 2006). This emphasizes the need to consider SOM dynamics at a molecular level when studying the effects of earthworms (Angst et al., 2020; Vidal et al., 2019, 2016). Earthworms have been shown to alter the chemical composition of litter (Filley et al., 2008) and particulate organic matter (POM), the SOM pool of the mineral soil most similar to surface litter (Crow et al., 2009). These shifts may be reflected on bulk SOM chemistry as well (Kögel-Knabner, 2002), but there is limited literature on the topic.

Soil C stocks result from the balance between OM inputs and their decomposition rate. While it makes sense for ecosystems that developed with earthworm activity – such as European temperate forests – to be at equilibrium, a shift in SOM dynamics is probably occurring in recently invaded

North American forests through earthworm bioturbation and casting activities (Bohlen et al., 2004a). Earthworm invasion has been shown to affect soil microbial communities in boreal forests, notably resulting in a decrease in Gram(+) to Gram(-) bacteria (see Chapter 3) indicating higher C availability (Fanin et al., 2019). Incorporation of fresh litter from the forest floor into the mineral soil would explain this increase in C availability and could also increase the decomposition of older SOM through the so-called priming effect (Liu et al., 2017).

Here we characterized the carbon found in earthworm-free and earthworm-invaded soils using three complementary approaches. First, C persistence in the forest floor and mineral soil was determined by monitoring CO_2 production over a one-year laboratory incubation. The fast and active C pools were estimated as the cumulative CO_2 emitted in the first three months and over the entire year, respectively, according to Laganière et al. (2013). The mineral soils were further characterized by determining C physical distribution using aggregate fractionation and C chemical composition using pyrolysis – gas chromatography – mass spectrometry. The comparison of three major soil types (Luvisol, Brunisol and Podzol) found across the Canadian boreal forest revealed whether there was a similar shift in C persistence, which could be explained by changes in its chemical composition and/or its physical distribution, regardless of pedological conditions. We hypothesized that forest floor C stability would increase in all soils, as a result of the preferential feeding of earthworms on more palatable plant residues leaving behind lignin-enriched compounds. In the mineral soil, we expected to find an increased proportion of C protected in aggregates and a higher proportion of root-derived compared to leaf-derived residues and higher labile C proportion. At the same time, we hypothesized that earthworms would stimulate microbial decomposition of SOM and the synthesis of microbial-derived products. Soil C persistence will depend on the net balance between SOM protection and mineralization.

Materials and methods

Site selection and sampling

The study was conducted in two different ecozones of Canada's boreal forest: the Boreal Plains in Alberta and the Boreal Shield in Québec. In Alberta, a site with trembling aspen as the dominant canopy and Luvisolic soil was selected: the Ecosystem Management Emulating Natural Disturbance (EMEND: 56°46' N, 118°22' W). In Québec, two sites are considered: Parc National des Grands Jardins (Grands Jardins: 47°40' N, 70°50' W) with Podzolic soils under black spruce canopy and Canadian Forces Base Valcartier (Valcartier: 46°55' N, 71°36' W) with Brunisolic soils under sugar maple canopy. More information on these sites can be found in Chapter 2 (published as Lejoly et al., 2021). They were selected to encompass the three dominant soil types of the Canadian boreal forest, while limiting direct human influence.

The status of earthworm invasion was determined by hand-sorting of the litter and hot mustard application on the mineral soil surface (Lawrence and Bowers, 2002). For EMEND and Grands Jardins, earthworm-invaded and control (not invaded) zones were defined (2 levels: control and invaded), while for Valcartier, the invaded zone was further divided into two levels of invasion based on forest floor thickness, used as a proxy for earthworm density (3 levels: control, low, and high earthworm density). At EMEND, the invasion was still limited to forest floor-inhabiting (epigeic) earthworms, with little morphological changes (Chapter 2, published as Lejoly et al., 2021). For each homogeneous zone of each site, three sampling points were randomly chosen for sampling of the forest floor and the mineral soil (0–10 cm, corresponding to the A horizon).

Laboratory incubation

All samples were sieved at 2 and 4 mm, for mineral soil and forest floor respectively, and homogenized. For each homogenous zone, samples from the three different sampling points were

combined by horizon to create composite samples. The moisture content corresponding to field capacity was determined for each composite sample beforehand using pressure plates, at a water tension of -10 kPa.

For each forest floor composite sample, four replicates equivalent to 6 g of dry weight were placed in a PVC tube on a thin mesh allowing gas exchanges. For each mineral soil (A horizon) composite sample, four replicates equivalent to 40 g of dry weight were placed in small beakers. These composite samples were incubated for a year in the dark at constant moisture content (-10 kPa) and temperature (29 ºC for the first two months, decreased to 24 ºC for the remaining time of incubation after a technical issue led to an increase in temperature in the incubation chamber, reaching 45 ºC, on day 56) in 1 L Mason jars with lids fitted with a Luer Lock valve to allow for gas sampling with a 20 ml syringe. After each sampling, the jars were aerated for 20 minutes, and sealed again until the next gas sampling. Accumulated $^{12}CO_2$ (ppm) and $\delta^{13}C$ (‰) were measured using a Picarro G2201-i isotopic analyzer (Picarro Inc., Sunnyvale, CA, USA) calibrated using primary standards with a $\delta^{13}C$ range from -40.1 to 398 ‰, and CO_2 concentrations ranging from 0.4 to 200 mmol.mol^{-1} (UN1956, Airgas®, Radnor Township, PA, USA). Delta ^{13}C is reported against the Vienna Pee Dee Belemnite standard (Isotopic ratio: RVPDB =0.0111802). The concentration of $^{13}CO2$ was calculated as:

$$^{13}CO_2[ppm] = \left(\frac{\delta^{13}C}{1000} + 1\right) * R_{VPDB} * {}^{12}CO_2[ppm]$$

The accumulated CO_2 was transformed from ppm to mg C using the ideal gas law with pressure fixed at 101.3 kPa, after subtracting the baseline CO_2 concentration in the room when the jars were sealed. Aberrant values, such as very high delta values, were removed. The headspace volume considered was the jar volume, from which the volume occupied by the soil and the beaker were subtracted. The cumulative respiration, sum of $^{12}CO2$ and $^{13}CO2$, was expressed per gram of soil.

The cumulative carbon emitted as CO_2 over the first 100 days of incubation was considered as fast carbon (Laganière et al., 2013). If the sampling was not done on day 100, the value was integrated over the two samplings before and after day 100.

The pool of active carbon (C_{active}) was estimated after fitting the cumulative respiration using the following model (Maillard et al., 2010):

$$CO_2 - cumulative = C_{active} * (1 - e^{-k*Day})$$

Physical fractionation

For each homogenous zone, the air-dried and < 8 mm mineral soil samples from each sampling point were fractionated into large macroaggregates (2–8 mm), small macroaggregates (250 µm–2 mm), microaggregates (53–250 µm), and silt and clay-sized fraction (< 53 µm) by wet sieving following Six et al. (1998). A schematic of the procedure can be found in Suppl. Fig. 4.B-1. Small macroaggregates were further fractionated to collect the occluded microaggregates (53–250 µm) and occluded light fraction (Six et al., 2000). All aggregates were considered water stable as per the followed procedure. Total carbon content (TOC) of each fraction was then estimated by loss-on-ignition at 375 ºC for 16 h (Konen et al., 2002) using the Van Bemmelen factor (0.58) to convert from OM to OC (Kalra and Maynard, 1991). The TOC of the bulk soil was also determined following the same procedure. The weight-based relative proportion of total TOC found in every fraction was then estimated.

Pyrolysis-gas chromatography-mass spectrometry

After freeze-drying and pulverization, the chemical composition of pre- and post-incubation soil samples was analyzed by pyrolysis – gas chromatography – mass spectrometry (Py-GC-MS). After addition of 10 µL of 25 % tetramethylammonium hydroxide (TMAH) in methanol solution and 3 µL (corresponding to 1.5 µg) of the internal standard C17:0 methyl heptadecanoate, 10 mg of soil

sample were loaded on the AS-1020E auto shot sampler connected to SS-1010E selective sampler (Frontier Laboratories, Koriyama, Japan) before running single shot pyrolysis at 650 ºC on an EGA/Py-3030D multi-shot pyrolizer unit (Frontier Laboratories, Koriyama, Japan) with the interface and GC inlet at 300 ºC. The pyrolysis unit was coupled with a GC-7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, California, USA), whose temperature was increased from 50 ºC to 320 ºC by 3 ºC.min^{-1} after 10 min at 50 ºC then held for 15 min at 320 ºC, and a 5977A MSD mass spectrometer (Agilent Technologies Inc., Santa Clara, California, USA), running in EI mode at 70eV ionization energy using an Agilent HP-5MS UI (60 m, 0.25 mm, 0.25 µm, Agilent Technologies Inc., Santa Clara, California, USA) analytical column and He as carrier gas. Data were processed using the Agilent Mass Hunter Qualitative Analysis B.07.00 software, filtering peaks with relative area ≥ 2 % of the largest peak. In case of co-elution, only the main peak was used for identification.

Specific compounds were identified as plant (cutin, suberin, cutin or suberin, and lignin) or microbial markers. In the presence of TMAH, the SOM originating molecules were also derivatized through methylation and this method therefore cannot differentiate free hydroxyl from pre-existing methoxyl groups (Derenne and Quéné, 2015). Considering that this can result in some compounds (tannins, demethylated compounds) wrongly associated with lignin (Derenne and Quéné, 2015), we chose a conservative approach in selecting lignin markers. The lignin markers included guaiacyl, syringyl, and *p*-hydroxyphenyl derived compounds, as well as *p*-coumaric acid and ferulic acid and their derivatives (Amelung et al., 2008; Buurman et al., 2007b; Chefetz et al., 2000; del Río et al., 2007; Grote et al., 2000; Kögel-Knabner, 2002; Quénéa et al., 2006a).

With TMAH, the C chain of aliphatic molecules is not altered, but the carboxyl and hydroxyl groups are derivatized in methyl esters and ether groups (Derenne and Quéné, 2015). As a result,

hydroxy acids can be detected in both hydroxy and methoxy forms. The cutin markers included mi-chain hydroxy C14, 15, or 17 acids and C16 mono- and dihydroxy acids and diacids and suberin markers were ω-hydroxy acids C20-32 and α,ω-diacids C20-32 (Otto and Simpson, 2006). Markers that could not be unequivocally associated with cutin or suberin were identified as cutin or suberin markers: ω-hydroxy acids C16 and C18, C18 di- and trihydroxy acids, and α,ω-diacids C16 and C18 (Otto and Simpson, 2006). Substituted alkanoic acids, n-alkanoic acids, and n-alkenoic acids with 14 to 18C chain length were considered as microbial markers (Barré et al., 2018; Chefetz et al., 2000; Vidal et al., 2016). Furans can derive from the microbial degradation of cellulose and polysaccharides (Buurman et al., 2007a; Derenne and Quéné, 2015) but they can also be products of the pyrolysis of carbohydrates (Kögel-Knabner, 2002). However, as we are comparing soils from the same sites, we assumed that the proportion of furans originating from pyrolysis would not diffe between invaded and non-invaded soils, ensuring a similar baseline, and decided to consider furans as microbially altered compounds. Pyrroles, pyridines, and pyrrolidines are N-containing compounds often associated with microbial activity (Barré et al., 2018; Buurman et al., 2007a; Chen et al., 2018). Relative abundances of compound classes and markers wer calculated as area counts of the compound class divided by total area count. Because of the low sample size, all laboratory replicates were kept for statistical analysis.

The cutin:suberin ratio was calculated to determine the relative contribution of above and below ground plant residues as followed (Otto and Simpson, 2006):

$$Cutin{:}\,suberin = \frac{\sum cutin\ markers + \sum cutin\ or\ suberin\ markers}{\sum suberin\ markers + \sum cutin\ or\ suberin\ markers}$$

The microbial:plant ratio was calculated as the sum of compounds of microbial origin (microbial markers, microbial N compounds, and furans) divided by the sum of the plant markers. This ratio

was used as an estimation of the relative abundance of microbial-degraded residues compared to non-degraded plant residues.

The atomic numbers of H, C, N, and O ($n_{x\text{-}total}$) were calculated as a weighted sum of the numbers of atoms ($n_{x\text{-}compound}$) in each pyrolysis compound (pyC) using the following equation:

$$n_{x-total} = \sum \frac{n_{x-compound}}{pyC_{molecular\ weight}} * pyC_{relative\ abundance}$$

With x representing H, C, N, or O. These numbers were then used to obtain the H/C, O/C, and N/C ratios as well as the C oxidation (C_{ox}) state as $C_{ox} = 2 * n_O - n_H$ (Hockaday et al., 2009; Masiello et al., 2008; Yan et al., 2021).

Statistical analysis

All statistical analyses were performed on R version 4.1.2 (R Core Team, 2021). Prior to performing analyses of variance (ANOVA), data were checked for homogeneity of variance using Bartlett's test as well as normality of residuals and, when necessary, Tukey-transformed using transformTukey from the rcompanion R package (Mangiafico, 2021).

For both the forest floor and the mineral soil, significant differences in fast and active C pools (as concentration of mg-C.g-soil^{-1} and percentage) between earthworm-free and earthworm-invaded soils were determined with one-way ANOVAs (factor: invasion). The ANOVAs were performed on each site separately as preliminary analysis revealed a significant interaction between site and invasion.

For physical fractionation data, two-way ANOVAs (factors: site and invasion) were performed on the TOC content and relative proportion of total C of each fraction to determine whether earthworm invasion was a significant factor. For Py-GC-MS, the selected ratios and compound classes were analyzed with the same two-way ANOVA models to determine whether earthworm invasion significantly affected SOM chemical composition. The pre- and post-incubation Py-GC-MS data

were combined as preliminary statistical analysis did not show any significant difference in SOM composition between the two. The three sites were kept together for these analyses since the interaction term was not significant. For Valcartier, the two levels of invasion were combined into a single 'invaded' level to facilitate the comparison with the two other sites, which only have one level of invasion. A permutational analysis of variance (PERMANOVA) was performed on Hellinger-transformed Py-GC-MS data to determine whether earthworm invasion significantly affected SOM composition using the adonis function from the vegan R package (Oksanen et al., 2019).

For all ANOVAs, posthoc tests for pairwise comparisons were performed with HSDTukey from the agricolae R package (de Mendiburu, 2020). When necessary, type II sum of squares was selected to account for unbalanced design.

Results

Laboratory incubation

In the forest floor, the concentration of fast and active C ranged from 14 to 35 mg-C.g-soil^{-1} and from 16 to 38 mg-C.g-soil^{-1}, respectively (Fig. 4-1). It was not significantly affected by earthworm invasion in the Luvisol (Suppl. Table 4.A-1 & 2). In the Brunisol, the low earthworm density forest floors had significantly smaller fast C concentration compared to the high earthworm density soils (Fig. 4-1; p-value < 0.05), but the difference became marginally significant (p-value = 0.09) for the active C concentration. The same observations held true for the fast and active C pools as a proportion of total C in the Brunisol (Fig. 4-2 & Suppl. Table 4.A-2). Although not statistically different according to the posthoc test, the fast and active C concentrations were higher in the high earthworm density forest floors compared to the controls and represented a higher proportion of the total C in the Brunisol (Fig. 4-1 & 4-2; Suppl. Table 4.A-1 & 2). In the Podzol, while the

concentration of fast and active C was not significantly affected by earthworm invasion, they represented a marginally significantly (p-value < 0.1) higher proportion of total C, increasing from 2.9 and 3.3 % in controls to 4.0 and 5.0 % in earthworm-invaded forest floors, for fast and active C, respectively (Suppl. Table 4.A-2).

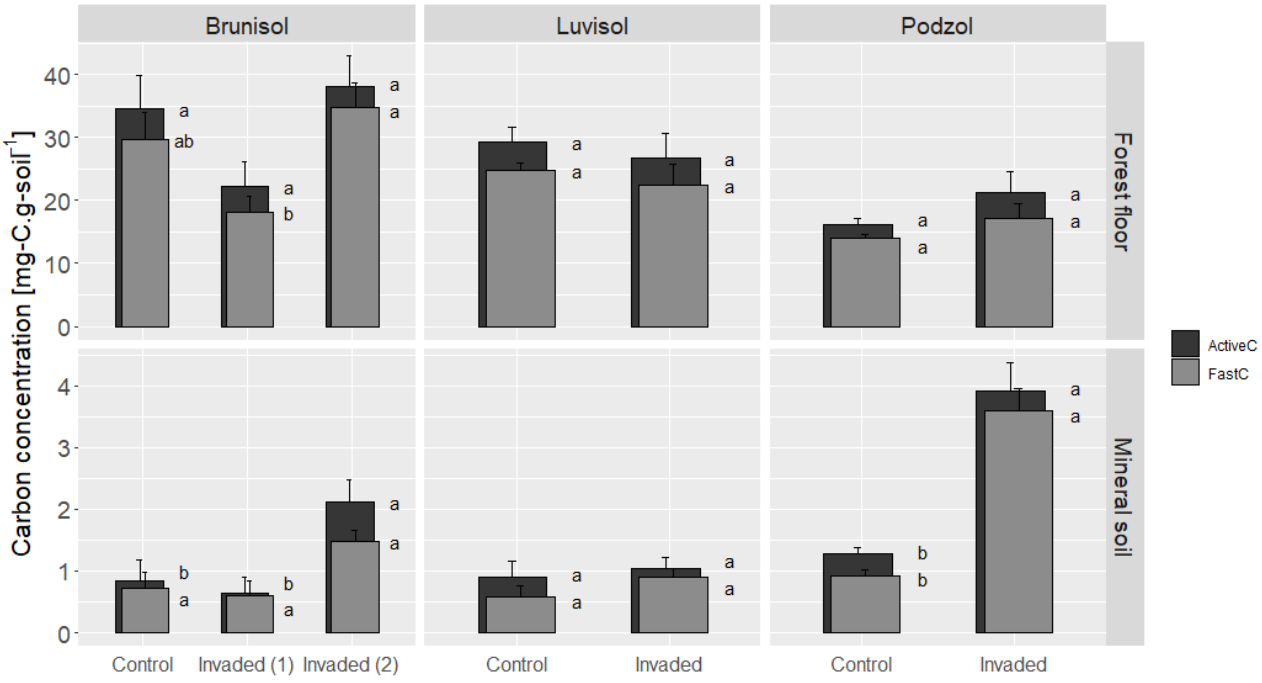


Figure 4-1. Average fast and active C concentrations as mg of C per gram of soil for each site (Brunisol: Valcartier; Luvisol: EMEND; Podzol: Grands Jardins). The error bars represent one standard error (n=4–8). Different letters denote significant differences among invasion stages, for each site and horizon separately.

In the mineral soil, the concentration of fast and active C was significantly higher in earthworm-invaded soils for two out of three sites (Fig. 4-1). In the Brunisol, the proportion of fast and active C pools reached 1.5 and 2.1 mg-C.g-soil^{-1} in the high earthworm density samples, corresponding to more than twice and three times that of the control and low earthworm density samples (Suppl.

Table 4.A-1). This also corresponded to an increase in the proportion of fast and active C, reaching 2.4 and 3.5 % compared to 0.9 and 1.1 % in the controls (Fig. 4-2 & Suppl. Table 4.A-2). In the Podzol, the proportion of fast C was 2.7 times higher while that of active C was 1.8 times higher, compared to the controls. Fast and active C represented 5.9 and 6.4 % of the total C in earthworm-invaded soils, while it accounted for only 2.2 and 3.1 % in the controls. In the Luvisol, the fast and active C pools were not affected by earthworm invasion and ranged from 0.6 to 0.9 mg-C.g-soil^{-1} and from 0.6 to 0.8 mg-C.g-soil^{-1}.

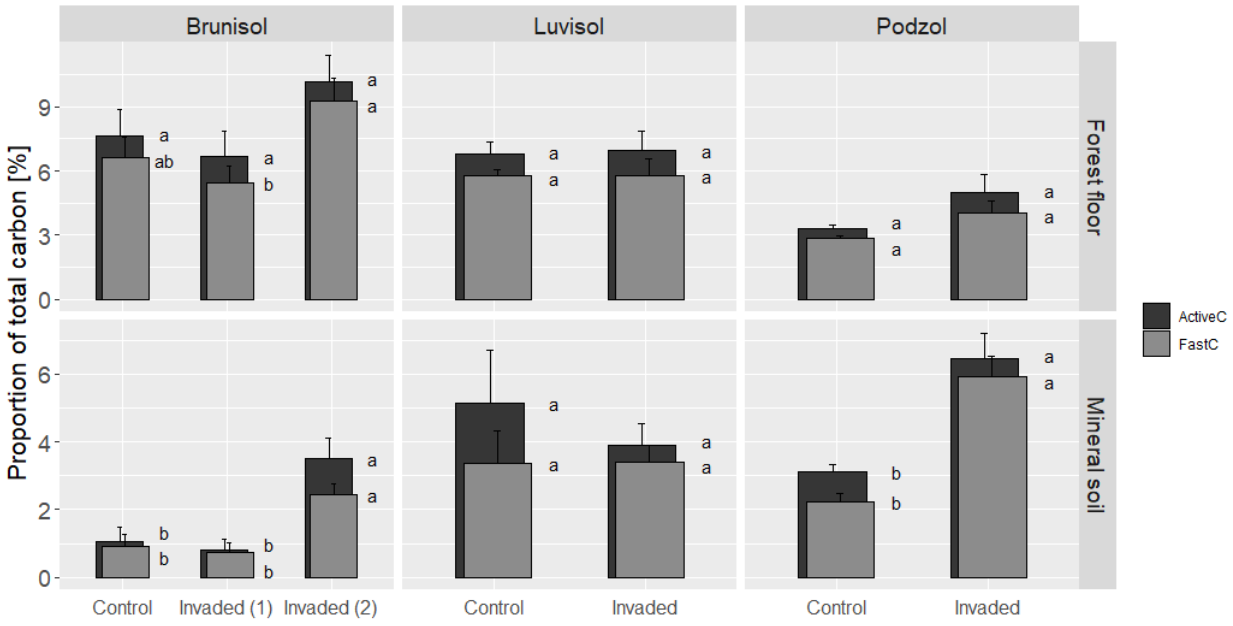


Figure 4-2. Average fast and active C pools as a proportion of initial organic carbon [%] for each site (Brunisol: Valcartier; Luvisol: EMEND; Podzol: Grands Jardins). The error bars represent one standard error (n=4–8). Different letters denote significant differences among invasion stages, for each site and horizon separately.

Physical fractionation

Sample recovery after aggregate fractionation reached 95.4 ± 1.7 %. Carbon distribution in soil physical fractions differed among sites (Fig. 4-3 & Suppl. Table 4.A-3). While large macroaggregates contained 27–28 % of the total C in the non-invaded Luvisol and Brunisol, they contained less than 1 % in the Podzol. Most of the C was found in the small macroaggregate fraction for the Podzol and the Brunisol and in the silt and clay-sized fraction for the Luvisol. Within the small macroaggregates, the occluded light fraction made up 5 % of the total C in the Brunisol, 8 % in the Luvisol, and 21 % in the Podzol in non-invaded soils.

The TOC content (%wt) of bulk soil was systematically higher in earthworm-invaded soils, compared to their controls, although not statistically significant (Table 4-1 & Suppl. Table 4.A-3). The TOC content of free microaggregates and silt and clay-size fractions increased significantly in all earthworm-invaded soils. In the Brunisolic and Luvisolic soils, the TOC content of small macroaggregates was higher in earthworm-invaded soils. The fractionation of small macroaggregates revealed that the increase was mainly due to an increase of TOC in the occluded light fraction while the increase in TOC of occluded microaggregates was not statistically significant. For the Podzol, no changes were observed in the small macroaggregate fraction. The percentage of total C found in the small macroaggregates (250 µm to 2 mm) was higher in earthworm-invaded soils for the Luvisol and the Podzol (p-value=0.07; Fig. 4-3 & Suppl. Table 4.A-3). It was associated with a significant increase in the percentage of total C found in the occluded light fraction for all sites, by 280 % for the Brunisol, 30 % for the Luvisol, and 60 % for the Podzol (p-value < 0.01; Fig. 4-3 & Suppl. Table 4.A-3).

Table 4-1. Average total organic carbon (TOC, %wt) contents of bulk soil and different soil fractions, with standard errors in parentheses (n=3). Different letters indicate significant differences between invaded and control soils within a site (Brunisol: Valcartier; Luvisol: EMEND; Podzol: Grands Jardins).

Site	Invasion	Bulk	Large macroaggregates	Free microaggregates	Free silt and clay-sized fraction	Small macroaggregates	Within small macroaggregates	
							Occluded microaggregates	Occluded light fraction
Brunisol	Control	5.6 (3.2) a	5.74 (2.28) a	8.63 (2.55) b	4.82 (1.78) b	5.74 (1.66) b	8.07 (2.45) a	1.75 (0.64) b
	Invaded	8.3 (1.9) a	8.81 (1.12) a	9.08 (1.02) a	6.93 (0.86) a	7.51 (1.06) a	8.29 (0.90) a	6.56 (1.34) a
Luvisol	Control	1.3 (0.5) a	3.97 (1.99) a	1.00 (0.26) b	0.78 (0.17) b	1.63 (0.31) b	2.32 (0.37) a	1.67 (0.15) b
	Invaded	3.2 (0.6) a	3.60 (0.07) a	2.54 (0.20) a	1.52 (0.13) a	3.63 (0.37) a	4.80 (0.59) a	3.59 (0.76) a
Podzol	Control	3.7 (3.0) a	0.37 (0.13) a	0.34 (0.01) b	0.44 (0.12) b	0.47 (0.16) a	0.52 (0.41) a	0.23 (0.06) a
	Invaded	4.6 (3.0) a	0.46 (0.30) a	0.54 (0.13) a	1.19 (0.55) a	0.50 (0.16) a	0.68 (0.52) a	0.21 (0.04) a

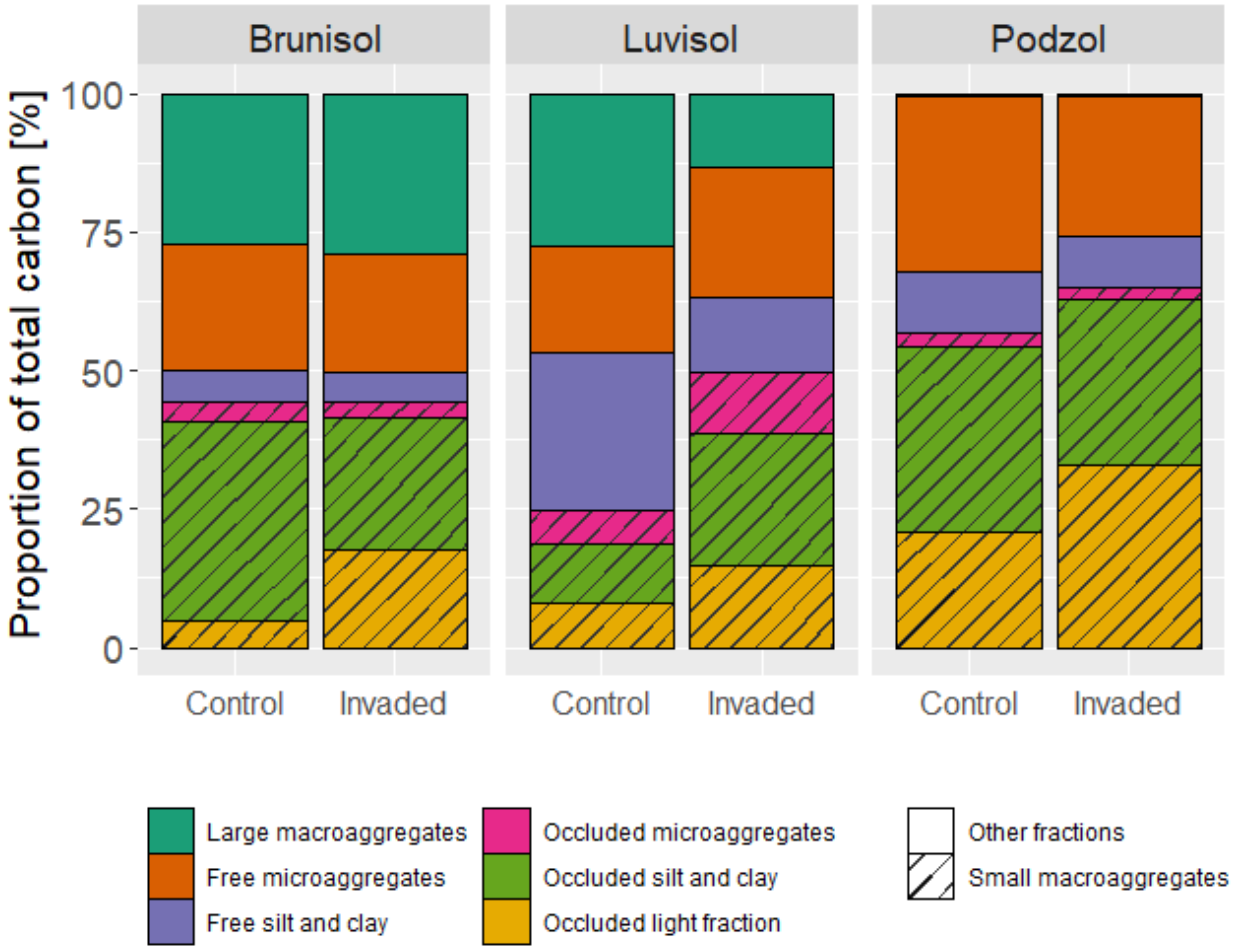


Figure 4-3. Carbon distribution in different soil fractions as a percentage of total TOC [%]: in large macroaggregates (2–8 mm), free and occluded microaggregates (53–250 µm), occluded light fraction (250 µm–2 mm), and free and occluded silt and clay-sized fraction (< 53µm). Occluded light fraction, microaggregates, and silt and clay-sized fraction correspond to small macroaggregates (250 µm–2 mm) prior to fractionation (n=3). The percentage of total TOC found in the occluded silt and clay fraction was calculated by difference.

Chemical composition of soil organic matter

With our custom-built library, we were able to identify 99.3 ± 0.1 % of products obtained from the pyrolysis-gas chromatography-mass spectrometry spectra, corresponding to a total of 259 compounds (Suppl. Table 4.A-4). The identified compounds were grouped according to their chemical structure and probable origin into fatty acids, plant phenolic compounds (lignin and tannin), N-containing compounds, polysaccharides, other aliphatics, phenols, other aromatics, and polyaromatics (Table 4-2 and Suppl. Table 4.A-4). The overall composition of SOM differed between earthworm-invaded and non-invaded soils according to the two-way PERMANOVA results (Table 4-3). This shift was also associated with changes in specific compound classes.

Table 4-2. Average relative abundances (%) of the main compound classes identified from the pyrolysis products obtained from pyrolysis-gas chromatography-mass spectrometry, with standard errors in parentheses (n=2–8), for each site (Brunisol: Valcartier; Luvisol: EMEND; Podzol: Grands Jardins).

	Brunisol		Luvisol		Podzol	
	Control	Invaded	Control	Invaded	Control	Invaded
Fatty acids (FA)	21.5 (1.7)	17.9 (0.8)	16.1 (5.0)	15.2 (2.7)	29.3 (3.0)	29.3 (3.6)
Lignin markers (Lg)	3.6 (0.2)	3.4 (0.2)	3.3 (0.3)	3.3 (0.5)	6.0 (0.4)	5.5 (0.3)
N-compounds (N)	36.3 (6.9)	33.5 (1.7)	53.9 (4.7)	53.2 (5.0)	41.3 (1.8)	35.6 (3.4)
Other aliphatics (Al)	2.8 (0.2)	2.4 (0.2)	0.2 (0.2)	0.4 (0.2)	2.0 (0.1)	1.6 (0.1)
Other aromatics (Ar)	9.9 (1.6)	11.2 (0.6)	5.2 (0.1)	5.6 (0.5)	5.8 (0.2)	8.2 (0.4)
Polyaromatics (PAH)	3.2 (0.6)	3.8 (0.5)	0.9 (1.0)	0.6 (0.4)	0.6 (0.3)	1.8 (0.4)
Phenols (Ph)	6.9 (1.7)	10.3 (0.6)	6.8 (0.2)	6.5 (0.6)	4.4 (1.0)	5.3 (0.7)
Polysaccharides (Ps)	6.4 (0.9)	7.3 (0.2)	5.3 (0.3)	5.7 (0.4)	6.9 (0.3)	6.2 (0.3)

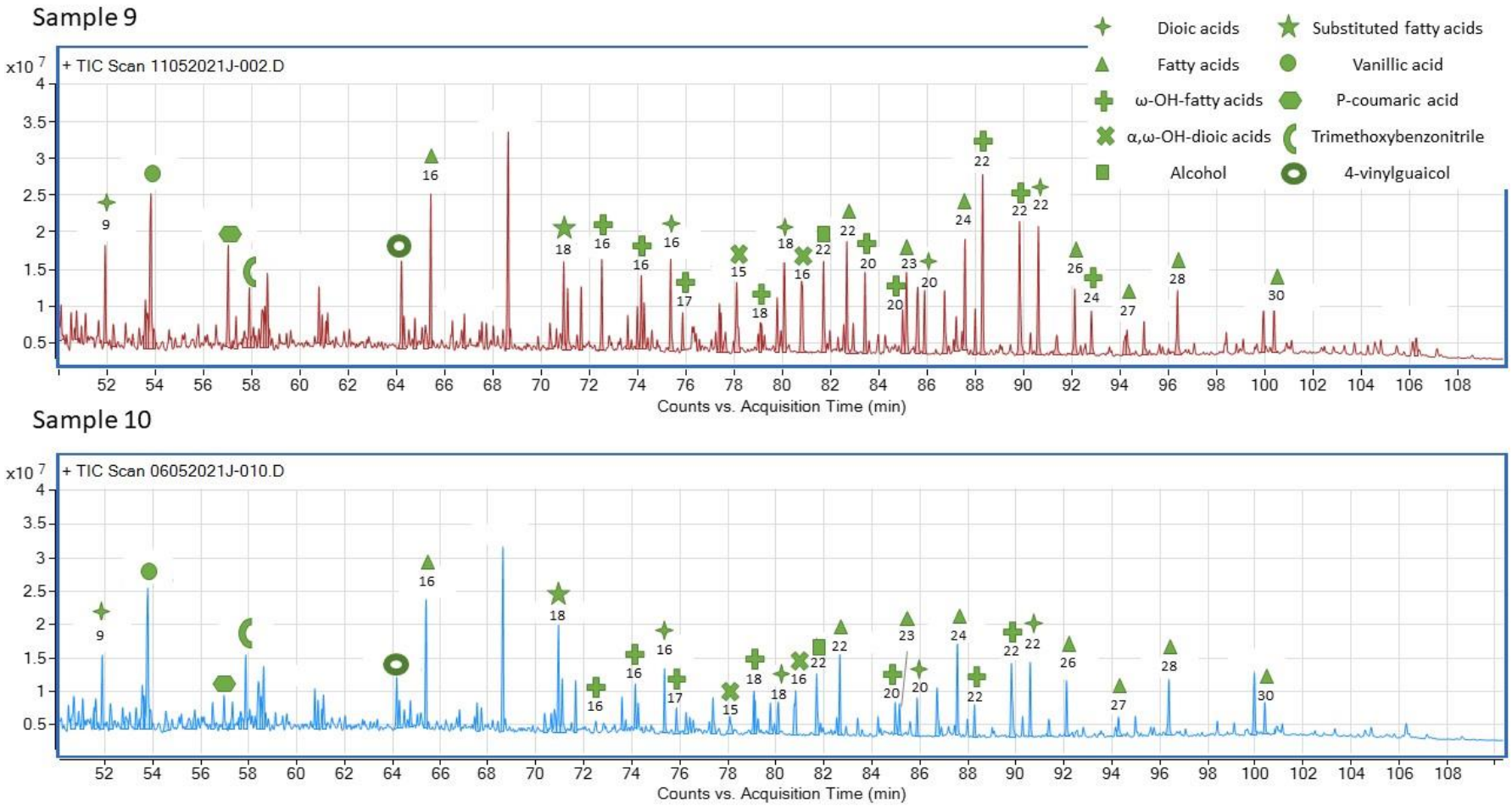


Figure 4-4. Total Ion Chromatograph (TIC) trace for soil organic matter (SOM) composition of a control and an earthworm-invaded soil sample from the same site (Valcartier, Québec) prior incubation. Numbers represent the number of C for aliphatic chains.

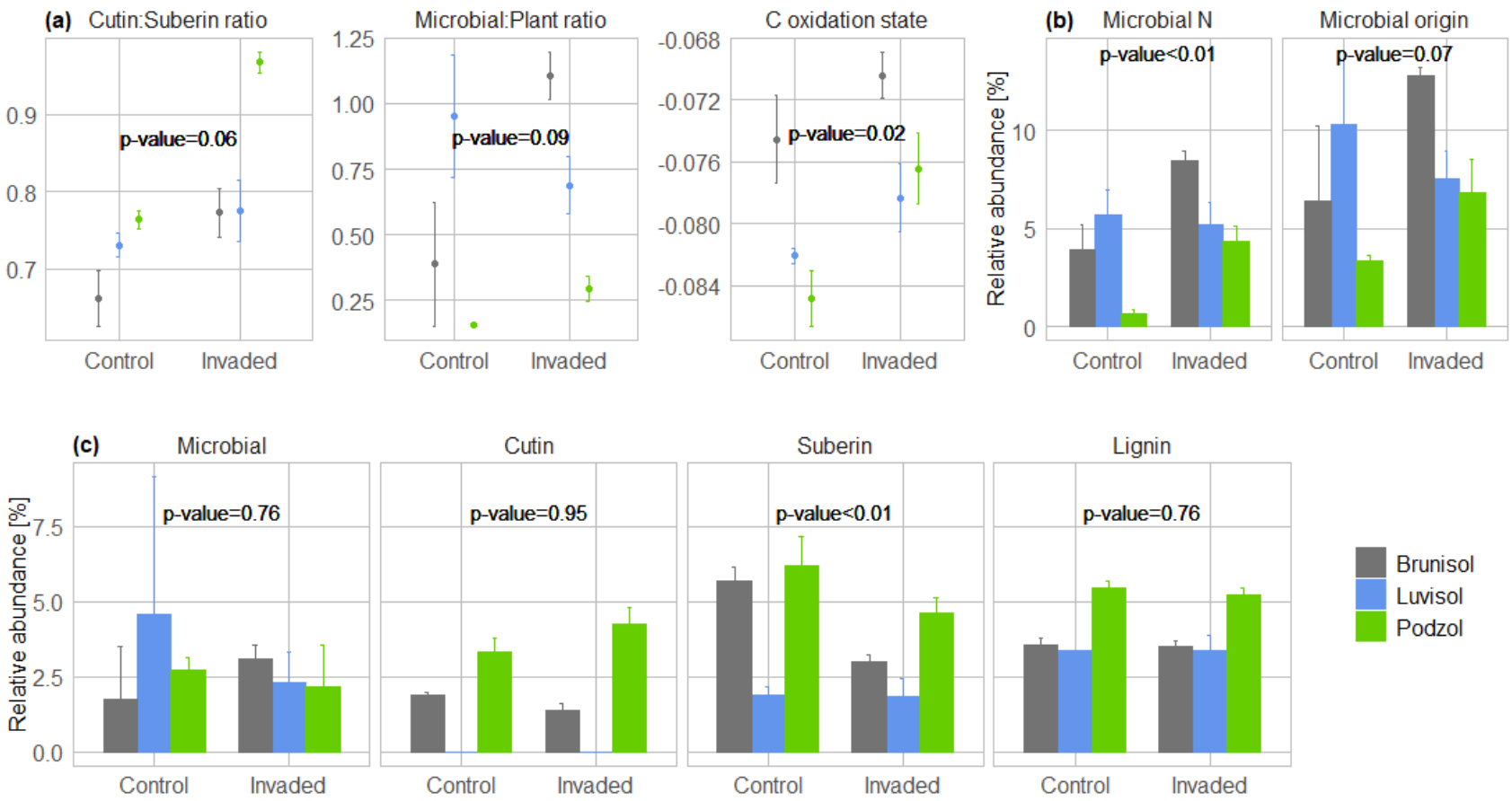


Figure 4-5. Averages of (a) the cutin:suberin marker ratios, the plant:microbial ratios, and the C oxidation states of SOM, (b) the relative abundances of N-containing microbial compounds and compounds of microbial origin, and (c) the relative abundances of microbial and plant (cutin, suberin, and lignin) markers identified by pyrolysis-gas chromatography-mass spectrometry, for earthworm-invaded and control soils. The error bars represent one standard error (n=2–8). The p-value obtained from the analysis of variance is displayed on the graph.

Table 4-3. Results of the permutational analysis of variance (PERMANOVA) of the pyrolysis products obtained from pyrolysis-gas chromatography-mass spectrometry. Df stands for degrees of freedom and Sum sq for sum of squares.

	Df	Sum sq	F	R^2	p-value
Invasion	1	0.14	3.33	0.07	0.02
Site	2	1.02	12.60	0.54	0.001
Interaction	2	0.14	1.72	0.07	0.12
Residuals	15	0.61		0.32	
Total	20	1.90		1.00	

Because of the presence of TMAH, all lignin-originating acids were detected as methyl esters and lignin monomers were derivatized into their methoxy form (Suppl. Table 4.A-4). The dominant lignin markers were 3-(4-methoxyphenyl)-2-propenoic acid, methyl ester (methoxylated *p*-coumaric acid), 3-(3,4-dimethoxyphenyl)-2-propenoic acid methyl ester (methoxylated ferulic acid), 3,4-dimethoxy-benzoic acid methyl ester (methoxylated vanillic acid), and 4-methoxybenzoic acid methyl ester, derived from *p*-hydroxyphenyl type of lignin (Fig. 4-4; Amelung et al., 2008; Grote et al., 2000; Turcotte and Quideau, 2012). Other lignin markers were found in lower concentrations and only in some samples, including: 3,4-dimethoxy-benzaldehyde (methoxylated vanillin), 4-methoxy-benzaldehyde (methoxylated *p*-hydroxybenzaldehyde), and 1-(3,4-dimethoxyphenyl)- and 1-(4-methoxyphenyl)-ethanone, both corresponding to methoxylated acetovanillone (Amelung et al., 2008; Buurman et al., 2007a; Chefetz et al., 2000; Turcotte and Quideau, 2012). Although 3,4,5-trimethoxy benzoic acid methyl ester can be a marker for lignin (Nierop, 2001), it was not selected as it was only found as a co-elute with an amine. The relative abundance of lignin markers did not differ between invaded and control soils (Fig. 4-5 & Suppl. Table 4.A-5).

A total of 61 fatty acids were detected, including α,ω-dioic acids, α,ω-(di)hydroxy dioic acids, ω-(tri)hydroxy acids, n-alkanoic and n-alkenoic acids (Suppl. Table 4.A-4). The main cutin markers identified in the Brunisol and the Podzol were: 9/10,16-dihydroxy hexadecanoic acid methyl ester,

9/10-monohydroxy-1,15-pentadecane dioic acid dimethyl ester, and 7/8/9/10-monohydroxy-1,16 hexadecanedioate. Cutin or suberin markers were identified in all samples and included ω-hydroxy acids (also methoxy form), α,ω-diacids, and di- and trihydroxy acids with 16 and 18 C chain length. The suberin markers, present in all samples, were ω-hydroxy alkanoic acids (also methoxy form) and α,ω-alkanoic dioic acids with 20, 22, and 24 C chain length (Quénéa et al., 2006b). The absence of odd numbered α,ω-diacids in our samples confirm that these compounds originate from relatively fresh suberin. The relative abundance of suberin markers was lower in earthworm-invaded soils (p-value=0.07), although the difference was only significant for the Podzol and the Brunisol (Fig. 4-5). All sites combined, suberin markers accounted for 4.6 % of all identified compounds in control soils compared to 3.0 % in earthworm-invaded soils. Earthworm invasion led to an increase in the relative abundance of cutin markers in the Podzol, but a decrease in the Brunisol (Fig. 4-5). Despite the variable response of cutin markers, the cutin:suberin ratio was significantly higher in the earthworm-invaded Brunisolic and Podzolic soils (Fig. 4-5 & Suppl. Table 4.A-5).

The microbial markers identified in our samples included n-alkenoic (16 and 18C) acids, 14C n-alkanoic acid, and substituted alkanoic (15 and 17C, both anteiso and iso) acids (Barré et al., 2018; Chefetz et al., 2000; Vidal et al., 2016), all detected in their methyl ester form. They were not affected by earthworm invasion (Fig. 4-5 & Suppl. Table 4.A-5). Most n-alkanoic acids are ubiquitous and were therefore not included as specific markers.

A total of 57 N-containing compounds were detected, including amides, amines, indoles, purines, pyridines, pyridinones, pyrroles, pyrrolidines, and pyrrolidones (Suppl. Table 4.A-4). Microbial-derived N containing compounds included pyrrole, 1-H-pyrroles with one to four methyl groups, pyridine with zero to two methyl groups, and pyrrolidine with methyl or butenyl group. Other microbial-derived compounds corresponded to furans, originating from the degradation of

polysaccharides. The other identified polysaccharides (levoglucosan, propanoic and acetic acids, and 2-cyclopenten-1-ones) are mainly pyrolysis products of carbohydrates, including cellulose for the latter (Buurman et al., 2007a; Derenne and Quéné, 2015; Page et al., 2002), and were not included as microbial-originating compounds. The relative abundance of microbial-derived N containing compounds and of all microbial-originating compounds (sum of N-containing + furans + microbial markers) was significantly higher in earthworm-invaded soils (p-value of 0.02 and 0.05, respectively), although the difference was only apparent for the Podzol and Brunisol soils (Fig. 4-5 & Suppl. Table 4.A-5).

The microbial:plant ratio, calculated as the sum of microbial-originating compounds divided by the plant markers, was also affected by earthworm invasion, with a higher ratio in earthworm-invaded Podzol and Brunisol (Fig. 4-5 & Suppl. Table 4.A-5). And lastly, the C oxidation state of bulk SOM was slightly but significantly higher in earthworm-invaded soils for all three sites, mirroring the decrease in the H:C atomic ratio (Fig. 4-5 & Table 4-4).

Table 4-4. Average atomic ratios for the bulk soil organic matter (SOM), with standard errors in parentheses (n=2–8), for each site (Brunisol: Valcartier; Luvisol: EMEND; Podzol: Grands Jardins). Different letters indicate significant differences between earthworm-invaded and control soils.

Site	Invasion	H:C atomic ratio	O:C atomic ratio	N:C atomic ratio
Brunisol	Control	1.72 (0.09) a	0.13 (0.01) a	0.15 (0.04) a
	Invaded	1.59 (0.04) b	0.13 (0.01) a	0.12 (0.01) a
Luvisol	Control	1.93 (0.01) a	0.11 (0.01) a	0.26 (0.03) a
	Invaded	1.92 (0.06) b	0.12 (0.01) a	0.26 (0.04) a
Podzol	Control	1.94 (0.02) a	0.15 (0.01) a	0.19 (0.02) a
	Invaded	1.76 (0.02) b	0.15 (0.01) a	0.15 (0.02) a

Discussion

Impacts of earthworm invasion on forest floor C persistence

Our incubation results showed that, contrary to our hypothesis, the proportion of labile (fast and active) C in the forest floor did not decrease in earthworm-invaded soils, but that it even slightly increased in the Brunisolic and Podzolic soils. In our previous study, we observed a decrease in the C stocks of these forest floors following earthworm invasion, resulting from a decrease in forest floor thickness and TOC content (Chapter 2, published as Lejoly et al., 2021). Results from the current study suggest that not only is an important fraction of the forest floor C lost due to earthworm activities, but also that the stability of the remaining C is decreased.

In the Brunisol and Podzol, we previously observed that the structure of the forest floor changed following earthworm invasion, with the most humified (H) layer disappearing and the fermented (F) layer – typically the thickest layer of the forest floor – decreasing in thickness (Chapter 2, published as Lejoly et al., 2021). It is commonly assumed that earthworms feed on more palatable – with lower C:N ratio – plant material, leaving in the forest floor more recalcitrant, lignin-rich compounds (Bohlen et al., 2004a; Curry and Schmidt, 2007). However, the C:N ratio obtained by Lejoly et al (2021, Chapter 2) was slightly lower in earthworm-invaded forest floors (22.6 for the Brunisol and 34.9 for the Podzol) compared to the controls (24.2 for the Brunisol and 32.4 for the Podzol). This lower C:N ratio could facilitate SOM decomposition and explain the increase in labile C (Zhang et al., 2008).

Forest floors also contained a substantial portion of earthworm casts (Chapter 2, published as Lejoly et al., 2021). As a result, numbers presented for the forest floors (labile C, as well as C:N) reflect the entirety of the remaining forest floor, including earthworm casts, which are hotspots of

microbial activity and SOM mineralization when fresh (Blume-Werry et al., 2020; Scheu, 1987), and can contain up to seven times as much C as the mineral soil (Bossuyt et al., 2004).

Impacts of earthworm invasion on mineral soil C persistence.

For three major soil types found in the Canadian boreal forest, we present evidence that invasive earthworms alter C dynamics in the surface mineral soils both physically and chemically. Further, for two of the soils (Brunisol and Podzol), we saw an increase in the labile C pool.

Our incubation results suggest that the carbon found in the novel Ahu was more labile than in earthworm-free soils, where the fast and active C proportions were lower. This labile C represents 0.6 and 2.5 Mg-C.ha^{-1} for the Brunisol and the Podzol respectively, calculated using the bulk density previously reported for those sites (Chapter 2, published as Lejoly et al., 2021). Additionally, the analysis of soil fractions revealed that TOC increased in free microaggregates (53–250 μm) and silt and clay-sized fractions (< 53 μm) for all sites, as well as in the small macroaggregates (250 μm–2 mm) and their occluded light fraction (53–250 μm) for the Brunisol and the Luvisol. Because the soils were sieved and homogenized prior to incubation, the coarser (> 2 mm) structure was lost. However, as mentioned above, the large macroaggregate (> 2 mm) fraction was not affected by earthworm invasion and all significant changes occurred in smaller, unaffected soil fractions.

The increase in labile C could be partly explained by the observed increase in the proportion of C found in the occluded light fraction. This fraction, also referred to as coarse non-protected POM, is considered easily decomposable and mainly contains plant-derived compounds (Lavallee et al., 2020; Six et al., 2002). However, there is no increase in the occluded light fraction for the Podzol, which suggests that (1) this fraction does not play a key role for C stability, or (2) the mechanisms are different in the Podzol and the Brunisol. In both the Podzol and the Brunisol, an increase in

TOC also occurred in the silt and clay fraction, which contains mineral-associated organic matter (MAOM), made up of low molecular weight compounds of microbial and plant origin, and dissolved organic matter (DOM), considered labile (Lavallee et al., 2020). The increase in MAOM aligns with the higher clay percentage recorded in the novel Ahu horizons developing in earthworm-invaded soils (Lavallee et al., 2020; Chapter 2, published as Lejoly et al., 2021).

Pyrolysis in the presence of TMAH allows to differentiate above ground from belowground vegetation origin (Derenne and Quéné, 2015). The observed increase in cutin:suberin ratios could indicate: (1) an increase in aboveground (cutin) vegetation inputs, (2) a decrease in root (suberin) inputs, and/or (3) the preferential decomposition of suberin in earthworm-invaded soils. Without significant bioturbation, the forest floor contains a greater proportion of aboveground litter compared to the mineral soil. In theory, earthworm bioturbation should increase the aboveground vegetation inputs into the mineral soil (1st scenario), as they incorporate OM from the forest floor (Addison, 2009). However, the relative abundance of cutin markers did not change in earthworm-invaded soils. The suberin markers decreased by 30 % compared to non-invaded soils, which could indicate a decrease in root inputs (2nd scenario). Fisk et al. (2004) found that earthworm-invaded soils had a lower fine root biomass compared to earthworm-free soils. Crow et al. (2009) observed lower concentrations of both cutin and suberin with higher earthworm biomass. Earthworms have been shown to accelerate the decomposition of plant-derived SOM (Angst et al., 2019) and especially the more recalcitrant portion which encompasses cutin and suberin (Fox et al., 2006), notably through production of serine proteases with esterase activity (Nakajima et al., 2005). Angst et al. (2020) found that earthworms degrade more easily leaf-derived compared to root-derived macromolecules, with 37 % of the cutin left at the end of a 33-week incubation compared to 86 % of the suberin. Vidal et al. (2016) also observed a higher decomposition rate for shoot compared to root

litter in earthworm casts. According to these findings, we should expect a higher degradation of cutin compared to suberin, which goes against the 3rd scenario.

The primary production shoot to root ratio in the mineral soil is typically lower for boreal forests compared to temperate forests, as aboveground litter inputs decrease with increasing latitude (Kögel-Knabner, 2002). As we observed an increase in the cutin:suberin ratio (comparable to the shoot:root ratio), we conclude that invasive earthworms shift the soil C dynamics closer to that of temperate forests. While it is not possible to determine with certainty which of the scenario(s) presented above is/are responsible for this shift, our observations suggest that a decrease in root inputs (2nd scenario) is most likely. Alternatively, it could also be a combined increase in cutin over suberin inputs coupled with increased decomposition of both.

The absence of a significant change in microbial biomass, estimated with microbial markers, is in line with previous findings for the same sites using phospholipid fatty acid total concentrations (see Chapter 3). Our results however indicate an increase in microbial-derived compounds such as furans, pyrroles, pyridines, and pyrrolidines, and thus suggest an increase in microbial activity, further supported by the increase in labile C. Similarly, Angst et al. (2019) showed that earthworms increased the concentration in microbial necromass through conversion of plant-derived compounds. The higher microbial:plant ratio shows that there are more microbial-derived compounds compared to un-degraded plant residues in the presence of earthworms, consistent with the increase in MAOM (silt and clay-sized fraction). Moreover, the higher C oxidation state in earthworm-invaded soils indicates that SOM has already undergone a higher degree of degradation (Hockaday et al., 2009; Yan et al., 2021). The C oxidation state ranged from -0.07 to -0.09 in our soils, which is higher than the findings of Hockaday et al. (2009) for boreal forest soils (-0.4 to -0.1), but very close to that of deciduous leaves (-0.083; Masiello et al., 2008).

Angst et al. (2019) suggest that by converting plant-derived SOM into microbial necromass, earthworms would increase C resilience, based on the findings that earthworms did not affect C mineralization in their incubation. However, the plant residues were uniformly mixed with the soil before starting the incubation (Angst et al., 2019), while in forests the fresh litter is physically separated from the mineral soil. In a multiyear incubation with multiple residue surface applications – more similar to field conditions, Lubbers et al. (2017) reported an increase in CO_2 emissions in the presence of earthworms. Although our incubation did not include earthworms but rather compared earthworm-affected and non-affected soils, we observed a significant increase in labile C for two of the three sites, indicating that C resilience decreased. Given that our sites had already established earthworm populations, the apparent increase in labile C might not be just a transient effect, although it is virtually impossible to determine whether the SOM dynamics have reached a new equilibrium or if the transition is still ongoing. The slow decomposition rate characteristic of boreal forests could be unlocked by earthworm invasion. The OM previously accumulated in the forest floor because of the lack of bioturbation is now intrinsically mixed with mineral soil and its microbial communities, which could prime the decomposition of older SOM (Liu et al., 2017). This would explain the increase in microbial products such as furans and N compounds, and the higher degree of SOM oxidation. However, the increase in labile C (1–3 mg-C.g-soil^{-1}) is one order of magnitude smaller than the significant increases in TOC observed in different fractions (1–5 g-C.g-soil^{-1} or %wt), suggesting that the stabilization of the freshly added litter C surpasses its decomposition.

Considering that we observed significant changes in both the occluded light fraction (part of POM) and C associated the silt and clay-sized fraction (part of MAOM), we expect that fractionating C into the two POM and MAOM pools would also yield similar conclusions while simplifying the

laboratory methods. In line with previous findings, the study of SOM molecular composition showed that earthworms play a key role in root and leaf incorporation and decomposition (Angst et al., 2020; Vidal et al., 2016). To decipher the temporal dynamics of earthworm invasion, we recommend future studies to use labelled plant residues and characterize the SOM composition of different fractions instead of bulk soil.

Chapter 5 : General conclusions and perspectives

Summary of findings

The objective of this PhD thesis was to study the invasion of exotic earthworms in boreal forests of Canada and their impacts on soil carbon dynamics.

In Chapter 2, I determined the status of earthworm invasion of three Luvisol sites in Alberta, two Brunisol sites in Québec, and two Podzol sites in Québec. I then described the morphological features of those soils in earthworm-invaded and earthworm-free zones and estimated the C stocks of the forest floors and surface mineral soils. Regardless of soil type, earthworm-invaded soils were associated with a decrease in forest floor thickness and C stocks. Forest floors were thinner in the presence of earthworms and developed into vermimull humus forms, with the most humified (H) layer disappearing and the fermented (F) layer characterized by the presence of faunal droppings, mainly earthworm casts. In the presence of geoengineering earthworms, the surface mineral soil was reworked by earthworm bioturbation, incorporating OM from the forest floor, and met the criteria for classification as an Ahu horizon although its thickness varied across sites. The C stocks of the mineral soil, however, did not show obvious trends across sites. While such observations have been previously described in temperate forest soils (Alban and Berry, 1994; Bohlen et al., 2004a; Hale et al., 2005b; Langmaid, 1964), these results constitute the first of its kind for boreal forest soils. This research is based on the 'space-for-time' approach, assuming that these sites were similar before earthworm invasion (Damgaard, 2019; Sax et al., 2005; Thomaz et al., 2012). The integration of multiple sites from the three major soil types found across the Canadian boreal forest makes me confident to say that these observed morphological differences are indeed induced by the action of invasive earthworms.

Based on the assessment of earthworm invasion on soil morphology from Chapter 2, and using the same study sites, Chapter 3 focussed on soil microbial communities, which play a key role in soil organic matter decomposition and carbon cycling. My results show that earthworm invasion alters microbial community composition and functioning without significantly affecting microbial biomass. In the forest floor, invasive earthworms led to a decrease in ectomycorrhizal fungal richness and in the relative abundance of Actinobacteria, as well as to an increase in the Gram(+):Gram(-) ratio. In the reworked mineral soil, all observed changes in bacterial communities could be linked to changing environmental conditions, including an increase in pH and nutrient availability favouring Proteobacteria over Acidobacteriota and Gram(-) over Gram(+) bacteria. Earthworm invasion also favoured ectomycorrhizal over saprotrophic fungi and led to an increase in the relative abundance of fungi compared to bacteria. Additionally, invaded mineral soils harboured higher bacterial and fungal species diversity and richness.

With Chapter 4, I quantified the labile C found in forest floors and surface mineral soils, with an incubation of earthworm-invaded and earthworm-free soil samples. Geoengineering earthworms affected C persistence, by increasing the proportion of labile C in both the mineral soil and the forest floor, although the difference was only marginally significant in the latter. Further investigation of the mineral soil C revealed that its chemical composition had shifted with invasive earthworms, towards lower suberin relative abundance. There are multiple indications of a higher degree of SOM decomposition with earthworm invasion, including higher C oxidation state, higher relative abundance of microbially degraded compounds, and increased silt and clay-associated TOC. Additionally, a higher proportion of TOC was found in the occluded light fraction, which could be linked to the incorporation of fresh litter by earthworm bioturbation, and could have a priming effect on older SOM.

Significance of these findings

Combining the results of these three research chapters, I conclude that invasive earthworms – especially geoengineering species – change SOM dynamics primarily by incorporation of the forest floor in the surface mineral soil. As a result, C stocks of the forest floor decrease, as well as C persistence. The combined findings of chapters 2, 3, and 4 suggest that, in the mineral soil, earthworm invasion resulted in:

- greater incorporation of forest floor litter, as indicated by a decrease in forest floor thickness coupled with a reworked (Ahu) surface mineral horizon, as well as an increase in the proportion of C found in the occluded light fraction and an increase in the cutin:suberin ratio.
- higher C availability, indicated by a greater labile C pool, a higher Gram(+):Gram(-) ratio, and an increase in relative abundance of Proteobacteria compared to Acidobacteriota.
- greater SOM decomposition, indicated by a higher C oxidation state, a lower relative abundance of suberin, and an increase in microbially degraded compounds.
- greater stabilization of SOM, as indicated by an increase in C content of the silt and clay fraction, corresponding to mineral-associated OM (MAOM fraction), as well as an increase in C associated with aggregates of different sizes, where C is physically protected from microbial decomposition; and lastly, an increase in the fungal:bacterial ratio.

Invasive earthworms alter C and nutrient dynamics under field conditions, suggesting long lasting effects. While it is not possible at this stage to determine which shifts occurred first, there is clear evidence that multiple aspects of SOM dynamics are affected by earthworm invasion, including microbial communities and SOM composition and distribution. The shifts in microbial community composition are likely to affect nutrient availability for plant uptake, with consequences for vegetation development and ecosystem functioning (Frelich et al., 2019).

Direction for future research

The findings of Chapter 3 suggest that microbial communities associated with the N cycle are greatly affected by earthworm invasion. However, as N was not the focus of this thesis, I was not able to draw definite conclusions. Since the C and N cycles are intrinsically linked, I recommend that future research on the topic includes measures of N availability and quantifies potential changes in nitrification and denitrification fluxes using molecular techniques.

Considering the limited number of sites, it was not possible to statistically test for the impact of earthworm species other than the distinction between epigeic and geoengineering earthworms. This would require a larger scale study and could be facilitated by the use of environmental DNA (eDNA) methods (Jackson et al., 2017), although the current methods do not allow for identification of earthworms to the species level. Additionally, eDNA analysis can be used on archival samples, which could give valuable insights of the temporal characteristics of earthworm invasion and even determine when the invasion started at a given site.

The sampling design used for this thesis gave a valuable snapshot of SOC, its distribution and chemical composition, as well as associated microbial communities. However, I could not determine if the changes in plant residue abundance (suberin relative abundance and cutin:suberin ratio) were due to a change in their relative input and/or an increase in their degradation. For a better understanding of the SOM dynamic changes associated with earthworm invasion, I recommend future research to consider using stable isotope probing to trace the fate of aboveground versus belowground litter, including their incorporation in the mineral soil and their degradation. In addition, the ability to determine when the invasion started or to track it from the start would greatly advance our understanding of long-term implications. It would also help differentiate transient from permanent effects (Hale et al., 2008).

The ongoing earthworm invasion in Canadian boreal forests is happening in a changing environment. Ecosystem disturbances can be of natural and/or anthropogenic origin and include, among others, wildfire, drought, insect outbreaks, windthrow, and forest management (Maynard et al., 2014). With global warming and anthropogenic activities, disturbance regimes are shifting from their natural historical trajectories, with consequences for forest successions (de Groot et al., 2003; Weber and Flannigan, 1997). As earthworms decrease forest floor C and affect C persistence in the mineral soil, the combined action of earthworm invasion and other disturbances will likely differ from their individual actions. For example, Cameron et al. (2015) found that the presence of earthworms and fire led to increased loss of forest floor C compared to either disturbance alone, using a 125 year long modelling. To my knowledge, this study is the only one looking at interactive effects of invasive earthworms with another disturbance. The effects of invasive earthworms might have been underestimated, as they have rarely been studied simultaneously with other major disturbances (Rillig et al., 2019). I therefore recommend future research to consider the many ongoing changes and disturbances affecting boreal forests when studying earthworm invasion.

Bibliography

Abarenkov, K., Nilsson, R.H., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vrålstad, T., Liimatainen, K., Peintner, U., Kõljalg, U., 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytologist 186, 281–285. doi:10.1111/j.1469-8137.2009.03160.x

Addison, J.A., 2009. Distribution and impacts of invasive earthworms in Canadian forest ecosystems. Biol Invasions 11, 59–79. doi:10.1007/s10530-008-9320-4

Aira, M., Domínguez, J., 2014. Changes in nutrient pools, microbial biomass and microbial activity in soils after transit through the gut of three endogeic earthworm species of the genus *Postandrilus* Qui and Bouché, 1998. Journal of Soils and Sediments 14, 1335–1340. doi:10.1007/s11368-014-0889-1

Alban, D.H., Berry, E.C., 1994. Effects of earthworm invasion on morphology, carbon, and nitrogen of a forest soil. Applied Soil Ecology 1, 243–249. doi:10.1016/0929-1393(94)90015-9

Alberta Climate Information Service, 2020. Alberta Weather Station Data [WWW Document]. URL http://agriculture.alberta.ca/acis/weather-data-viewer.jsp (accessed 5.25.20).

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., Bol, R., 2008. Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter, in: Sparks, D.L. (Ed.), Advances in Agronomy. Elsevier Inc., pp. 155–250. doi:10.1016/S0065-2113(08)00606-8

Angst, G., Angst, Š., Frouz, J., Peterse, F., Nierop, K.G.J., 2020. Preferential degradation of leaf- vs. root-derived organic carbon in earthworm-affected soil. Geoderma 372, 2019–2021. doi:10.1016/j.geoderma.2020.114391

Angst, G., Heinrich, L., Kögel-Knabner, I., Mueller, C.W., 2016. The fate of cutin and suberin of decaying leaves, needles and roots - Inferences from the initial decomposition of bound fatty acids. Organic Geochemistry 95, 81–92. doi:10.1016/j.orggeochem.2016.02.006

Angst, G., Mueller, C.W., Prater, I., Angst, Š., Peterse, F., Nierop, K.G.J., 2019. Earthworms act as biochemical reactors to convert labile plant compounds into stabilized soil microbial necromass. Communications Biology 2, 1–7. doi:10.1038/s42003-019-0684-z

Angst, G., Mueller, K.E., Nierop, K.G.J., Simpson, M.J., 2021. Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter. Soil Biology and Biochemistry 156, 108189. doi:10.1016/j.soilbio.2021.108189

Angst, Š., Mueller, C.W., Angst, G., Lhotáková, Z., Bartu, M., Alexandra, Š., Frouz, J., 2017. Stabilization of soil organic matter by earthworms is connected with physical protection rather than with chemical changes of organic matter. Geoderma 289, 29–35. doi:10.1016/j.geoderma.2016.11.017

Aubin, I., Boisvert-Marsh, L., Kebli, H., McKenney, D., Pedlar, J., Lawrence, K., Hogg, E.H., Boulanger, Y., Gauthier, S., Ste-Marie, C., 2018. Tree vulnerability to climate change: Improving exposure-based assessments using traits as indicators of sensitivity. Ecosphere 9, 1–24. doi:10.1002/ecs2.2108

Averill, C., Hawkes, C. V., 2016. Ectomycorrhizal fungi slow soil carbon cycling. Ecology Letters 19, 937–947. doi:10.1111/ele.12631

Baldrian, P., 2017. Forest microbiome: Diversity, complexity and dynamics. FEMS Microbiology Reviews 41, 109–130. doi:10.1093/femsre/fuw040

Barré, P., Quénéa, K., Vidal, A., Cécillon, L., Christensen, B.T., Kätterer, T., Macdonald, A., Petit, L., Plante, A.F., van Oort, F., Chenu, C., 2018. Microbial and plant-derived compounds both contribute to persistent soil organic carbon in temperate soils. Biogeochemistry 140, 81–92. doi:10.1007/s10533-018-0475-5

Beaudette, D.E., Roudier, P., Brown, A., 2020. Algorithms for quantitative pedology: A toolkit for soil scientists. Computers and Geosciences 52, 258–268. doi:10.1016/j.cageo.2012.10.020

Bergmann, G.T., Bates, S.T., Eilers, K.G., Lauber, C.L., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. Soil Biology and Biochemistry 43, 1450–1455. doi:10.1016/j.soilbio.2011.03.012

Bernard, L., Chapuis-Lardy, L., Razafimbelo, T., Razafindrakoto, M., Pablo, A.L., Legname, E., Poulain, J., Brüls, T., O'Donohue, M., Brauman, A., Chotte, J.L., Blanchart, E., 2012. Endogeic earthworms shape bacterial functional communities and affect organic matter mineralization in a tropical soil. ISME Journal 6, 213–222. doi:10.1038/ismej.2011.87

Beyer, L., Blume, H.P., Irmler, U., 1991. The humus of a "Parabraunerde'' (Orthic Luvisol) under *Fagus sylvatica* L and *Quercus robur* L and its modification in 25 years." Annales Des Sciences Forestieres 48, 267–278. doi:10.1051/forest:19910303

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.J., 2013. A review of earthworm impact on soil function and ecosystem services. European Journal of Soil Science 64, 161–182. doi:10.1111/ejss.12025

Blume-Werry, G., Krab, E.J., Olofsson, J., Sundqvist, M.K., Väisänen, M., Klaminder, J., 2020. Invasive earthworms unlock arctic plant nitrogen limitation. Nature Communications 11, 1–10. doi:10.1038/s41467-020-15568-3

Bohlen, P.J., Edwards, C.A., Zhang, Q., Parmelee, R.W., Allen, M., 2002. Indirect effects of earthworms on microbial assimilation of labile carbon. Applied Soil Ecology 20, 255–261. doi:10.1016/S0929-1393(02)00027-6

Bohlen, P.J., Pelletier, D.M., Groffman, P.M., Fahey, T.J., Fisk, M.C., 2004a. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7, 13–27. doi:10.1007/s10021-003-0127-y

Bohlen, P.J., Scheu, S., Hale, C.M., Mclean, M.A., Migge, S., Groffman, P.M., Parkinson, D., 2004b. Non-native invasive earthworms as agents of change in northern temperate forests. Ecology and the Environment 2, 427–435. doi:https://doi.org/10.1890/1540-9295(2004)002[0427:NIEAAO]2.0.CO;2

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with

QIIME 2's q2-feature-classifier plugin. Microbiome 6, 1–17. doi:10.1186/s40168-018-0470-z

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A. V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37, 852–857. doi:10.1038/s41587-019-0209-9

Booysen, M., Sikes, D., Bowser, M.L., Andrews, R., 2018. Earthworms (Oligochaeta: Lumbricidae) of Interior Alaska. Biodiversity Data Journal 6, 1–13. doi:10.3897/BDJ.6.e27427

Bossuyt, H., Six, J., Hendrix, P.F., 2006. Interactive effects of functionally different earthworm species on aggregation and incorporation and decomposition of newly added residue carbon. Geoderma 130, 14–25. doi:10.1016/j.geoderma.2005.01.005

Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within earthworm casts. Soil Biology and Biochemistry 37, 251–258. doi:10.1016/j.soilbio.2004.07.035

Bossuyt, H., Six, J., Hendrix, P.F., 2004. Rapid incorporation of carbon from fresh residues into newly formed stable microaggregates within earthworm casts. European Journal of Soil Science 55, 393–399. doi:10.1111/j.1351-0754.2004.00603.x

Bouché, M.B., 1977. Strategies lombriciennes. Ecological Bulletins 25, 122–132. doi:www.jstor.org/stable/20112572

Boulanger, Y., Taylor, A.R., Price, D.T., Cyr, D., McGarrigle, E., Rammer, W., Sainte-Marie, G., Beaudoin, A., Guindon, L., Mansuym Nicolas, 2017. Climate change impacts on forest landscapes along the Canadian southern boreal forest transition zone. Landscape Ecol 32, 1415–1431. doi:10.1007/s10980-016-0421-7

Bradshaw, C.J.A., Warkentin, I.G., 2015. Global estimates of boreal forest carbon stocks and flux. Global and Planetary Change 128, 24–30. doi:10.1016/j.gloplacha.2015.02.004

Brandt, J., Flannigan, M.D., Maynard, D., Thompson, I., Volney, W., 2013. An introduction to Canada's boreal zone: ecosystem processes, health, sustainability, and environmental issues. Environ. Rev 21, 207–226. doi:10.1139/er-2013-0040

Brandt, J.P., 2009. The extent of the North American boreal zone. Environmental Reviews 17, 101–161. doi:10.1139/A09-004

Brown, G.G., Barois, I., Lavelle, P., 2000. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. Eur. J. Soil Biol. 36, 177–198. doi:10.1016/S1164-5563(00)01062-1

Brundrett, M., Murase, G., Kendrick, B., 1990. Comparative anatomy of roots and mycorrhizae of common Ontario trees. Canadian Journal of Botany 68, 551–578. doi:10.1139/b90-076

Burtelow, A.E., Bohlen, P.J., Groffman, P.M., 1998. Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Applied Soil Ecology 9, 197–202. doi:10.1016/S0929-1393(98)00075-4

Butenschoen, O., Poll, C., Langel, R., Kandeler, E., Marhan, S., Scheu, S., 2007. Endogeic earthworms alter carbon translocation by fungi at the soil-litter interface. Soil Biology and Biochemistry 39,

2854–2864. doi:10.1016/j.soilbio.2007.05.028

Buurman, P., Peterse, F., Almendros Martin, G., 2007a. Soil organic matter chemistry in allophanic soils: A pyrolysis-GC/MS study of a Costa Rican Andosol catena. European Journal of Soil Science 58, 1330–1347. doi:10.1111/j.1365-2389.2007.00925.x

Buurman, P., Schellekens, J., Fritze, H., Nierop, K.G.J., 2007b. Selective depletion of organic matter in mottled podzol horizons. Soil Biology and Biochemistry 39, 607–621. doi:10.1016/j.soilbio.2006.09.012

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME Journal 11, 2639–2643. doi:10.1038/ismej.2017.119

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13, 581–583. doi:doi:10.1038/nmeth.3869.

Cameron, E.K., Bayne, E.M., 2015. Spatial patterns and spread of exotic earthworms at local scales. Canadian Journal of Zoology 93, 721–726. doi:10.1139/cjz-2014-0197

Cameron, E.K., Bayne, E.M., 2009. Road age and its importance in earthworm invasion of northern boreal forests. Journal of Applied Ecology 46, 28–36. doi:10.1111/j.1365-2664.2008.01535.x

Cameron, E.K., Bayne, E.M., Jill, M., 2007. Human-facilitated invasion of exotic earthworms into northern boreal forests. Ecoscience 14, 482–490. doi:10.2980/1195-6860(2007)14[482:HIOEEI]2.0.CO;2

Cameron, E.K., Knysh, K.M., Proctor, H.C., Bayne, E.M., 2013. Influence of two exotic earthworm species with different foraging strategies on abundance and composition of boreal microarthropods. Soil Biology & Biochemistry 57, 334–340. doi:10.1016/j.soilbio.2012.07.010

Cameron, E.K., Shaw, C.H., Bayne, E.M., Kurz, W.A., Kull, S.J., 2015. Modelling interacting effects of invasive earthworms and wildfire on forest floor carbon storage in the boreal forest. Soil Biology and Biochemistry 88, 189–196. doi:10.1016/J.SOILBIO.2015.05.020

Cameron, E.K., Zabrodski, M.W., Karst, J., Bayne, E.M., 2012. Non-native earthworm influences on ectomycorrhizal colonization and growth of white spruce. Écoscience 19, 29–37. doi:10.2980/19-1-3478

Canada's National Forest Inventory [WWW Document], 2021. URL https://nfi.nfis.org/en/ (accessed 3.21.22).

Capowiez, Y., Hedde, M., Marsden, C., Jouquet, P., Marchan, D.F., Nahmani, J., Pelosi, C., 2022. Faut-il continuer à utiliser les catégories écologiques de vers de terre définies par Marcel Bouché il y a 50 ans ? Une vision historique et critique. Etude et Gestion Des Sols 29, 51–58.

Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nature Microbiology 2, 1–6. doi:10.1038/nmicrobiol.2016.242

Carter, M.R., Gregorich, E.G. (Eds.), 2006. Soil sampling and methods of analysis, CRC Press. ed. Boca Raton, FL.

Chang, C.-H., Bartz, M.L.C., Brown, G., Callaham, M.A., Cameron, E.K., Dávalos, A., Dobson, A., Görres, J.H., Herrick, B.M., Ikeda, H., James, S.W., Johnston, M.R., McCay, T.S., McHugh, D., Minamiya, Y., Nouri-Aiin, M., Novo, M., Ortiz-Pachar, J., Pinder, R.A., Ransom, T., Richardson, J.B., Snyder, B.A., Szlavecz, K., 2021. The second wave of earthworm invasions in North America: biology, environmental impacts, management and control of invasive jumping worms. Biological Invasions 23, 3291–3322. doi:10.1007/s10530-021-02598-1

Chang, C.-H., Szlavecz, K., Buyer, J.S., 2016. Species-specific effects of earthworms on microbial communities and the fate of litter-derived carbon. Soil Biol. Biochem 100, 129–139. doi:10.1016/j.soilbio.2016.06.004

Chang, C.H., Szlavecz, K., Buyer, J.S., 2017. *Amynthas agrestis* invasion increases microbial biomass in Mid-Atlantic deciduous forests. Soil Biology and Biochemistry 114, 189–199. doi:10.1016/j.soilbio.2017.07.018

Chefetz, B., Chen, Y., Clapp, C.E., Hatcher, P.G., 2000. Characterization of organic matter in soils by

thermochemolysis using tetramethylammonium hydroxide (TMAH). Soil Science Society of America Journal 64, 583–589. doi:10.2136/sssaj2000.642583x

Chen, G., Ma, S., Tian, D., Xiao, W., Jiang, L., Xing, A., Zou, A., Zhou, L., Shen, H., Zheng, C., Ji, C., He, H., Zhu, B., Liu, L., Fang, J., 2020. Patterns and determinants of soil microbial residues from tropical to boreal forests. Soil Biology and Biochemistry 151, 1–9. doi:10.1016/j.soilbio.2020.108059

Chen, H., Blosser, G.D., Majidzadeh, H., Liu, X., Conner, W.H., Chow, A.T., 2018. Integration of an automated identification-quantification pipeline and statistical techniques for pyrolysis GC/MS tracking of the molecular fingerprints of natural organic matter. Journal of Analytical and Applied Pyrolysis 134, 371–380. doi:10.1016/j.jaap.2018.07.002

Choi, A., Sackett, T.E., Smith, S.M., Bellocq, M.I., 2017. Exotic earthworm (Oligochaeta: Lumbricidae) assemblages on a landscape scale in central Canadian woodlands: importance of region and vegetation type. Can. J. For. Res 47, 935–945. doi:10.1139/cjfr-2016-0337

Chowdhury, T.R., Dick, R.P., 2012. Standardizing methylation method during phospholipid fatty acid analysis to profile soil microbial communities. Journal of Microbiological Methods 88, 285–291. doi:10.1016/j.mimet.2011.12.008

Clapperton, M.J., Lee, N.O., Binet, F., Conner, R.L., 2001. Earthworms indirectly reduce the effects of take-all (*Gaeumannomyces graminis var. tritici*) on soft white spring wheat (*Triticum aestivum cv.* Fielder). Soil Biology and Biochemistry 33, 1531–1538. doi:10.1016/S0038-0717(01)00071-2

Clements, R.O., Murray, P.J., Sturdy, R.G., 1991. The impact of 20 years' absence of earthworms and three levels of N fertilizer on a grassland soil environment. Agriculture, Ecosystems and Environment 36, 75–85. doi:10.1016/0167-8809(91)90037-X

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Sentlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1619. doi:10.7551/mitpress/8876.003.0036

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A., Lindahl, B.D., 2015. Carbon

sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytologist 205, 1525–1536. doi:10.1111/nph.13208

Costello, D.M., Tiegs, S.D., Lamberti, G.A., 2011. Do non-native earthworms in Southeast Alaska use streams as invasional corridors in watersheds harvested for timber? Biological Invasions 13, 177–187. doi:10.1007/s10530-010-9800-1

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989–994. doi:10.1038/s41561-019-0484-6

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995. doi:10.1111/gcb.12113

Crow, S.E., Filley, T.R., McCormick, M., Szlávecz, K., Stott, D.E., Gamblin, D., Conyers, G., 2009. Earthworms, stand age, and species composition interact to influence particulate organic matter chemistry during forest succession. Biogeochemistry 92, 61–82. doi:10.1007/s10533-008-9260-1

Crowther, T.W., Stanton, D.W.G., Thomas, S.M., A'Bear, A.D., Hiscox, J., Jones, T.H., Voříšková, J., Baldrian, P., Boddy, L., 2013. Top-down control of soil fungal community composition by a globally distributed keystone consumer. Ecology 94, 2518–2528. doi:10.1890/13-0197.1

Csuzdi, C., Chang, C.H., Pavlícek, T., Szederjesi, T., Esopi, D., Szlávecz, K., 2017. Molecular phylogeny and systematics of native North American lumbricid earthworms (Clitellata: Megadrili), PLoS ONE. doi:10.1371/journal.pone.0181504

Curry, J.P., Schmidt, O., 2007. The feeding ecology of earthworms – A review. Pedobiologia 50, 463–477. doi:10.1016/j.pedobi.2006.09.001

Damgaard, C., 2019. A critique of the space-for-time substitution practice in community ecology. Trends in Ecology and Evolution 34, 416–421. doi:10.1016/j.tree.2019.01.013

De Caceres, M., Legendre, P., 2009. Associations between species and groups of sites: indices and

statistical inference. Ecology 90, 3566–3574. doi:10.1890/08-1823.1

De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters 11, 516–531. doi:10.1111/j.1461-0248.2008.01164.x

De Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist 188, 1055–1064. doi:10.1111/j.1469-8137.2010.03427.x

de Groot, W.J., Bothwell, P.M., Carlsson, D.H., Logan, K.A., 2003. Simulating the effects of future fire regimes on western Canadian boreal forests. Journal of Vegetation Science 14, 355–364. doi:https://doi.org/10.1111/j.1654-1103.2003.tb02161.x

de Groot, W.J., Flannigan, M.D., Cantin, A.S., 2013. Climate change impacts on future boreal fire regimes. Forest Ecology and Management 294, 35–44. doi:10.1016/j.foreco.2012.09.027

de Mendiburu, F., 2020. Package "agricolae" [WWW Document]. R Package Version 1.3-2. URL http://tarwi.lamolina.edu.pe/~fmendiburu

de Menezes, A.B., Prendergast-Miller, M.T., Macdonald, L.M., Toscas, P., Baker, G., Farrell, M., Wark, T., Richardson, A.E., Thrall, P.H., 2018. Earthworm-induced shifts in microbial diversity in soils with rare versus established invasive earthworm populations. FEMS Microbiology Ecology 94, 1–14. doi:10.1093/femsec/fiy051

del Río, J.C., Gutiérrez, A., Rodríguez, I.M., Ibarra, D., Martínez, Á.T., 2007. Composition of non-woody plant lignins and cinnamic acids by Py-GC/MS, Py/TMAH and FT-IR. Journal of Analytical and Applied Pyrolysis 79, 39–46. doi:10.1016/j.jaap.2006.09.003

DeLuca, T.H., Boisvenue, C., 2012. Boreal forest soil carbon: Distribution, function and modelling. Forestry. doi:10.1093/forestry/cps003

DeLuca, T.H., Zackrisson, O., Nilsson, M.C., Sellstedt, A., 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419, 917–920. doi:10.1038/nature01051

Dempsey, M.A., Fisk, M.C., Fahey, T.J., 2011. Earthworms increase the ratio of bacteria to fungi in

northern hardwood forest soils, primarily by eliminating the organic horizon. Soil Biology and Biochemistry 43, 2135–2141. doi:10.1016/j.soilbio.2011.06.017

Dempsey, M.A., Fisk, M.C., Yavitt, J.B., Fahey, T.J., Balser, T.C., 2013. Exotic earthworms alter soil microbial community composition and function. Soil Biology and Biochemistry 67, 263–270. doi:10.1016/j.soilbio.2013.09.009

Derenne, S., Quéné, K., 2015. Analytical pyrolysis as a tool to probe soil organic matter. Journal of Analytical and Applied Pyrolysis 111, 108–120. doi:10.1016/j.jaap.2014.12.001

Dobson, A., Blossey, B., 2015. Earthworm invasion, white-tailed deer and seedling establishment in deciduous forests of north-eastern North America. Journal of Ecology 103, 153–164. doi:10.1111/1365-2745.12350

Drouin, M., Bradley, R., Lapointe, L., 2016. Linkage between exotic earthworms, understory vegetation and soil properties in sugar maple forests. Forest Ecology and Management 364, 113–121. doi:10.1016/j.foreco.2016.01.010

Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, Q., Brookes, P.C., Evershed, R.P., 2011. Variable responses of the soil microbial biomass to trace concentrations of ^{13}C-labelled glucose, using ^{13}C-PLFA analysis. European Journal of Soil Science 62, 117–126. doi:10.1111/j.1365-2389.2010.01321.x

Dymond, P., Scheu, S., Parkinson, D., 1997. Density and distribution of *Dendrobaena octaedra* (Lumbricidae) in aspen and pine forests in Canadian Rocky Mountains (Alberta). Soil Biol. Biochem. 29, 265–273. doi:10.1016/S0038-0717(96)00052-1

Edwards, C.A., 2004. Earthworm ecology. CRC Press, Boca Raton, FL.

Egert, M., Marhan, S., Wagner, B., Scheu, S., Friedrich, M.W., 2004. Molecular profiling of 16S rRNA genes reveals diet-related differences of microbial communities in soil, gut, and casts of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae). FEMS Microbiology Ecology 48, 187–197. doi:10.1016/j.femsec.2004.01.007

Eisenhauer, N., Partsch, S., Parkinson, D., Scheu, S., 2007. Invasion of a deciduous forest by earthworms:

Changes in soil chemistry, microflora, microarthropods and vegetation. Soil Biology and Biochemistry 39, 1099–1110. doi:10.1016/J.SOILBIO.2006.12.019

Eisenhauer, N., Straube, D., Johnson, E.A., Parkinson, D., Scheu, S., 2009. Exotic ecosystem engineers change the emergence of plants from the seed bank of a deciduous forest. Ecosystems 12, 1008–1016. doi:10.1007/s10021-009-9275-z

Enami, Y., Okano, S., Yada, H., Nakamura, Y., 2001. Influence of earthworm activity and rice straw application on the soil microbial community structure analyzed by PLFA pattern. European Journal of Soil Biology 37, 269–272. doi:10.1016/S1164-5563(01)01096-2

Environment Canada, 2010. 1981-2010 Climate normals and averages [WWW Document]. URL https://climate.weather.gc.ca/climate_normals/index_e.html

Fahey, T.J., Yavitt, J.B., Sherman, R.E., Maerz, J.C., Groffman, P.M., Fisk, M.C., Bohlen, P.J., 2013. Earthworms, litter and soil carbon in a northern hardwood forest. Biogeochemistry 114, 269–280. doi:10.1007/s10533-012-9808-y

Fanin, N., Kardol, P., Farrell, M., Nilsson, M.C., Gundale, M.J., Wardle, D.A., 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biology and Biochemistry 128, 111–114. doi:10.1016/j.soilbio.2018.10.010

FAO, 2014. World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106. doi:10.1017/S0014479706394902

Feller, C., Brown, G.G., Blanchart, E., Deleporte, P., Chernyanskii, S.S., 2003. Charles Darwin, earthworms and the natural sciences: Various lessons from past to future. Agriculture, Ecosystems and Environment 99, 29–49. doi:10.1016/S0167-8809(03)00143-9

Ferlian, O., Eisenhauer, N., Aguirrebengoa, M., Camara, M., Ramirez-Rojas, I., Santos, F., Tanalgo, K., Thakur, M.P., 2018. Invasive earthworms erode soil biodiversity: A meta-analysis. Journal of Animal Ecology 87, 162–172. doi:10.1111/1365-2656.12746

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria.

Ecology 88, 1354–1364. doi:10.1890/05-1839

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. PNAS 103, 626–631. doi:10.1073/pnas.0507535103

Filley, T.R., McCormick, M.K., Crow, S.E., Szlavecz, K., Whigham, D.F., Johnston, C.T., van den Heuvel, R.N., 2008. Comparison of the chemical alteration trajectory of *Liriodendron tulipifera* L. leaf litter among forests with different earthworm abundance. Journal of Geophysical Research: Biogeosciences 113, 1–14. doi:10.1029/2007JG000542

Fisichelli, N.A., Frelich, L.E., Reich, P.B., Eisenhauer, N., 2013. Linking direct and indirect pathways mediating earthworms, deer, and understory composition in Great Lakes forests. Biological Invasions 15, 1057–1066. doi:10.1007/s10530-012-0350-6

Fisk, M.C., Fahey, T.J., Groffman, P.M., Bohlen, P.J., 2004. Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests. Ecosystems 7, 55–62. doi:10.1007/s10021-003-0130-3

Fontaine, S.S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters 7, 314–320. doi:10.1111/j.1461-0248.2004.00579.x

Fox, J., 2016. Package "car" [WWW Document]. R Package Version 2.1-4. URL https://cran.r-project.org/package=car,

Fox, O., Vetter, S., Ekschmitt, K., Wolters, V., 2006. Soil fauna modifies the recalcitrance-persistence relationship of soil carbon pools. Soil Biology and Biochemistry 38, 1353–1363. doi:10.1016/j.soilbio.2005.10.014

Francisco, R., Stone, D., Creamer, R.E., Sousa, J.P., Morais, P.V., 2016. European scale analysis of phospholipid fatty acid composition of soils to establish operating ranges. Applied Soil Ecology 97, 49–60. doi:10.1016/j.apsoil.2015.09.001

Frelich, L.E., Blossey, B., Cameron, E.K., Dávalos, A., Eisenhauer, N., Fahey, T., Ferlian, O., Groffman, P.M., Larson, E., Loss, S.R., Maerz, J.C., Nuzzo, V., Yoo, K., Reich, P.B., 2019. Side-swiped: ecological cascades emanating from earthworm invasions. Frontiers in Ecology and the

Environment 17, 502–510. doi:10.1002/fee.2099

Frelich, L.E., Hale, C.M., Scheu, S., Holdsworth, A.R., Heneghan, L., Bohlen, P.J., Reich, A.P.B., 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biol Invasions 8, 1235–1245. doi:10.1007/s10530-006-9019-3

Frostegård, Å., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730. doi:10.1016/0038-0717(93)90113-P

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621–1625. doi:10.1016/j.soilbio.2010.11.021

Frouz, J., Pižl, V., Cienciala, E., Kalčík, J., 2009. Carbon storage in post-mining forest soil, the role of tree biomass and soil bioturbation. Biogeochemistry 94, 111–121. doi:10.1007/s10533-009-9313-0

Frouz, J., Spaldonova, A., Fricova, K., Bartuska, M., 2014. The effect of earthworms (*Lumbricus rubellus*) and simulated tillage on soil organic carbon in a long-term microcosm experiment. Soil Biol. Biochem. 78, 58–64. doi:10.1016/j.soilbio.2014.07.011

Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A.Z., Schepaschenko, D.G., 2015. Boreal forest health and global change. Science 349, 819–22. doi:10.1126/science.aaa9092

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis, in: Dane, J.H., Clarke, G.T. (Eds.), Methods of Soil Analysis: Part 1— Physical and Mineralogical Methods. pp. 901–926. doi:10.2136/sssabookser5.1.2ed.c36

Glassman, S.I., Martiny, J.B.H., 2018. Broadscale ecological patterns are robust to use of exact sequence variants versus operational taxonomic units. MSphere 3, 1–5. doi:10.1128/MSPHERE.00148-18

Goldblum, D., Rigg, L.S., 2005. Tree growth response to climate change at the deciduous-boreal forest ecotone, Ontario, Canada. Canadian Journal of Forest Research 35, 2709–2718. doi:10.1139/x05-185

Gómez-Brandón, M., Lazcano, C., Lores, M., Domínguez, J., 2010. Detritivorous earthworms modify microbial community structure and accelerate plant residue decomposition. Applied Soil Ecology

44, 237–244. doi:10.1016/j.apsoil.2009.12.010

Gong, X., Jiang, Y., Zheng, Y., Chen, X., Li, H., Hu, F., Liu, M., Scheu, S., 2018. Earthworms differentially modify the microbiome of arable soils varying in residue management. Soil Biology and Biochemistry 121, 120–129. doi:10.1016/j.soilbio.2018.03.011

Graham, R.C., Wood, H.B., 1991. Morphologic development and clay redistribution in lysimeter soils under chaparral and pine. Soil Science Society of America Journal 55, 1638–1646. doi:10.2136/sssaj1991.03615995005500060023x

Groffman, P.M., Bohlen, P.J., Fisk, M.C., Fahey, T.J., 2004. Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7, 45–54. doi:10.1007/s10021-003-0129-9

Groffman, P.M., Fahey, T.J., Fisk, M.C., Yavitt, J.B., Sherman, R.E., Bohlen, P.J., Maerz, J.C., 2015. Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests. Soil Biol. Biochem. 87, 51–58. doi:10.1016/j.soilbio.2015.03.025

Grote, M., Klinnert, S., Bechmann, W., 2000. Comparison of degradation state and stability of different humic acids by means of chemolysis with tetramethylammonium hydroxide. Journal of Environmental Monitoring 2, 165–169. doi:10.1039/a909253f

Guillot, A., Obis, D., Mistou, M.Y., 2000. Fatty acid membrane composition and activation of glycine-betaine transport in *Lactococcus lactis* subjected to osmotic stress. International Journal of Food Microbiology 55, 47–51. doi:10.1016/S0168-1605(00)00193-8

Haas, J.C., Street, N.R., Sjödin, A., Lee, N.M., Högberg, M.N., Näsholm, T., Hurry, V., 2018. Microbial community response to growing season and plant nutrient optimisation in a boreal Norway spruce forest. Soil Biology and Biochemistry 125, 197–209. doi:10.1016/j.soilbio.2018.07.005

Hale, C.M., Frelich, L.E., Reich, P.B., 2005a. Exotic European earthworm invasion dynamics in northern hardwood forests of Minnesota, USA. Ecological Applications 15, 848–860. doi:10.1007/s10021-005-0066-x

Hale, C.M., Frelich, L.E., Reich, P.B., Pastor, J., 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. Oecologia 155, 509–518.

doi:10.1007/s00442-007-0925-6

Hale, C.M., Frelich, L.E., Reich, P.B., Pastor, J., 2005b. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, USA. Ecosystems 8, 911–927. doi:10.1007/s10021-005-0066-x

Hale, C.M., Reich, P.B., Frelich, L.E., 2004. Allometric equations for estimation of ash-free dry mass from length measurements for selected European earthworm species (Lumbricidae) in the Western Great Lakes Region. American Midland Naturalist 151, 179–185. doi:10.1674/0003-0031(2004)151[0179:AEFEOA]2.0.CO;2

Hamer, U., Rumpel, C., Dignac, M.F., 2012. Cutin and suberin biomarkers as tracers for the turnover of shoot and root derived organic matter along a chronosequence of Ecuadorian pasture soils. European Journal of Soil Science 63, 808–819. doi:10.1111/j.1365-2389.2012.01476.x

Hansson, K., Fröberg, M., Helmisaari, H.-S.H.-S., Kleja, D.B., Olsson, B.A., Olsson, M., Persson, T., Froberg, M., Helmisaari, H.-S.H.-S., Kleja, D.B., Olsson, B.A., Olsson, M., Persson, T., 2013. Carbon and nitrogen pools and fluxes above and below ground in spruce, pine and birch stands in southern Sweden. Forest Ecology and Management 309, 28–35. doi:10.1016/j.foreco.2013.05.029

Haydu-Houdeshell, C.-A., Graham, R.C., Peterson, A.C., Hendrix, P.F., Quideau, S.A., 2017. Morphology and genesis of humus profiles under chaparral shrubs in Southern California. Soil Science Society of America Journal 81, 369–379. doi:10.2136/sssaj2016.09.0290

Hendrix, P.F., 2006. Biological invasions belowground - Earthworms as invasive species, Biological Invasions. doi:10.1007/s10530-006-9048-y

Hendrix, P.F., Bohlen, P.J., 2002. Exotic earthworm invasions in North America: ecological and policy implications. BioScience 52, 801–811. doi:10.1641/0006-3568(2002)052[0801:eeiina]2.0.co;2

Hockaday, W.C., Masiello, C.A., Randerson, J.T., Smernik, R.J., Baldock, J.A., Chadwick, O.A., Harden, J.W., 2009. Measurement of soil carbon oxidation state and oxidative ratio by ^{13}C nuclear magnetic resonance. Journal of Geophysical Research: Biogeosciences 114, 1–14. doi:10.1029/2008JG000803

Högberg, M.N., Högberg, P., Wallander, H., Nilsson, L.O., 2021. Carbon–nitrogen relations of ectomycorrhizal mycelium across a natural nitrogen supply gradient in boreal forest. New Phytologist 232, 1839–1848. doi:10.1111/nph.17701

Högberg, M.N., Yarwood, S.A., Myrold, D.D., 2014. Fungal but not bacterial soil communities recover after termination of decadal nitrogen additions to boreal forest. Soil Biology and Biochemistry 72, 35–43. doi:10.1016/j.soilbio.2014.01.014

Högberg, P., Näsholm, T., Franklin, O., Högberg, M.N., 2017. Tamm Review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. Forest Ecology and Management 403, 161–185. doi:10.1016/j.foreco.2017.04.045

Hu, X., Gu, H., Liu, J., Wei, D., Zhu, P., Zhou, B., Chen, X., Jin, J., Liu, X., Wang, G., 2022. Metagenomics reveals divergent functional profiles of soil carbon and nitrogen cycling under long-term addition of chemical and organic fertilizers in the black soil region. Geoderma 418, 1–10. doi:10.1016/j.geoderma.2022.115846

Huang, C.-Y., Hendrix, P.F., Fahey, T.J., Bohlen, P.J., Groffman, P.M., 2010. A simulation model to evaluate the impacts of invasive earthworms on soil carbon dynamics. Ecological Modelling 221, 2447–2457. doi:10.1016/j.ecolmodel.2010.06.023

Huang, W., González, G., Zou, X., 2020. Earthworm abundance and functional group diversity regulate plant litter decay and soil organic carbon level: A global meta-analysis. Applied Soil Ecology 150, 1–8. doi:10.1016/j.apsoil.2019.103473

ISO, 2005. ISO 10390:2005 Soil quality — determination of pH, Internatio. ed. Geneva, Switzerland.

Jackson, M., Myrholm, C., Shaw, C., Ramsfield, T., 2017. Using nested PCR to improve detection of earthworm eDNA in Canada. Soil Biology & Biochemistry 113, 215–218. doi:10.1016/j.soilbio.2017.06.009

Jennings, B.W., Watmough, S.A., 2016. The impact of invasive earthworms on soil respiration and soil carbon within temperate hardwood forests. Ecosystems 19, 942–954. doi:10.1007/s10021-016-9977-y

Jenny, H., 1941. Factors of soil formation: a system of quantitative pedology. doi:10.1017/CBO9780511535802.014

Jochum, Malte, Thouvenot, L., Ferlian, O., Zeiss, R., Klarner, B., Pruschitzki, U., Johnson, E.A., Eisenhauer, N., Jochum, M, 2021. Aboveground impacts of a belowground invader: how invasive earthworms alter aboveground arthropod communities in a northern North American forest. Biology Letters 18, 1–7. doi:10.1098/rsbl.2021.0636

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69, 373–386. doi:10.2307/3545850

Jost, L., 2006. Entropy and diversity. Oikos 113, 363–375. doi:10.1111/j.2006.0030-1299.14714.x

Kalra, Y.P., Maynard, D.C., 1991. Methods manual for forest soil and plant analysis.

Kassambara, A., 2020. Package "rstatix" [WWW Document]. R Package Version 0.6.0. URL https://rpkgs.datanovia.com/rstatix/

Kelly, R.P., Shelton, A.O., Gallego, R., 2019. Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. Scientific Reports 9, 1–14. doi:10.1038/s41598-019-48546-x

Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Applied and Environmental Microbiology 60, 3292–3299. doi:0099-2240t94/$04.00+0

Klinka, K., Fons, J., Krestov, P., 1997. Towards a taxonomic classification of humus forms: Third approximation. Forest Science Monograph 29, 1–48. doi:10.1111/j.1365-2389.1964.tb02231.x

Kloos, K., Fesefeldt, A., Gliesche, C.G., Bothe, H., 1995. DNA-probing indicates the occurrence of denitrification and nitrogen fixation genes in *Hyphomicrobium*. Distribution of denitrifying and nitrogen fixing isolates of *Hyphomicrobium* in a sewage treatment plant. FEMS Microbiology Ecology 18, 205–213. doi:10.1016/0168-6496(95)00060-2

Knowles, M.E., Ross, D.S., Görres, J.H., 2016. Effect of the endogeic earthworm *Aporrectodea tuberculata* on aggregation and carbon redistribution in uninvaded forest soil columns. Soil Biol.

Biochem. 100, 192–200. doi:10.1016/J.SOILBIO.2016.06.016

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry 34, 139–162. doi:10.1016/j.soilbio.2016.08.011

Kögel-Knabner, I., 2000. Analytical approaches for characterizing soil organic matter. Organic Geochemistry 31, 609–625. doi:10.1016/S0146-6380(00)00042-5

Konen, M.E., Jacobs, P.M., Lee Burras, C., Talaga, B.J., Mason, J.A., 2002. Equations for predicting soil organic carbon using loss-on-ignition for North Central U.S. soils. Soil Science Society of America Journal 66, 1878–1881. doi:10.2136/sssaj2002.1878

Kurz, W. a, Shaw, C.H., Boisvenue, C., Stinson, G., Metsaranta, J., Leckie, D., Dyk, A., Smyth, C., Neilson, E.T., 2013. Carbon in Canada's boreal forest — A synthesis. Environ. Rev. 292, 260–292. doi:10.1139/er-2013-0041

Laganière, J., Paré, D., Bergeron, Y., Chen, H.Y.H., Brassard, B.W., Cavard, X., 2013. Stability of soil carbon stocks varies with forest composition in the Canadian boreal biome. Ecosystems 16, 852–865. doi:10.1007/s10021-013-9658-z

Laganiere, J., Paré, D., Bradley, R.L., 2010. How does a tree species influence litter decomposition? Separating the relative contribution of litter quality, litter mixing, and forest floor conditions. Can. J. For. Res. 40, 465–475. doi:10.1139/X09-208

Lal, R., 2005. Forest soils and carbon sequestration. Forest Ecology and Management 220, 242–258. doi:10.1016/j.foreco.2005.08.015

Lambkin, D.C., Gwilliam, K.H., Layton, C., Canti, M.G., Piearce, T.G., Hodson, M.E., 2011. Soil pH governs production rate of calcium carbonate secreted by the earthworm *Lumbricus terrestris*. Applied Geochemistry 26, S64–S66. doi:10.1016/j.apgeochem.2011.03.032

Langmaid, K.K., 1964. Some effects of earthwom invasion in virgin Podzols. Can. J. Soil Sci. 44, 34–37. doi:10.4141/cjss64-005

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and

mineral-associated forms to address global change in the 21st century. Global Change Biology 26, 261–273. doi:10.1111/gcb.14859

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.P., 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology 42, S3–S15. doi:10.1016/j.ejsobi.2006.10.002

Lavelle, P., Pashanasi, B., Charpentier, F., Gilot, C., Rossi, J.-P., Derouard, L., Andre, J., Ponge, J.-F., Bernier, N., 1998. Large-scale effects of earthworms on soil organic matter and nutrient dynamics, in: Edwards, C.A. (Ed.), Earthworm Ecology. pp. 103–122.

Lavkulich, L.M., Arocena, J.M., 2011. Luvisolic soils of Canada: Genesis, distribution, and classification. Canadian Journal of Soil Science 91, 781–806. doi:10.4141/cjss2011-014

Lawrence, A.P., Bowers, M.A., 2002. A test of the "hot" mustard extraction method of sampling earthworms. Soil Biology & Biochemistry 34, 549–552. doi:10.1016/S0038-0717(01)00211-5

Lebreton, A., Zeng, Q., Miyauchi, S., Kohler, A., Dai, Y.-C., Martin, F.M., 2021. Evolution of the mode of nutrition in symbiotic and saprotrophic fungi in forest ecosystems. Annual Review of Ecology, Evolution, and Systematics 52, 385–404. doi:10.1146/annurev-ecolsys-012021-114902

Lejoly, J., Quideau, S., Laganière, J., 2021. Invasive earthworms affect soil morphological features and carbon stocks in boreal forests. Geoderma 404, 1–13. doi:10.1016/j.geoderma.2021.115262

Li, J., Ramirez, G.H., Kiani, M., Quideau, S., Smith, E., Janzen, H., Larney, F., Puurveen, D., 2018. Soil organic matter dynamics in long-term temperate agroecosystems: Rotation and nutrient addition effects. Canadian Journal of Soil Science 98, 232–245. doi:10.1139/cjss-2017-0127

Li, X., Fisk, M.C., Fahey, T.J., Bohlen, P.J., 2002. Influence of earthworm invasion on soil microbial biomass and activity in a northern hardwood forest. Soil Biology and Biochemistry 34, 1929–1937. doi:10.1016/S0038-0717(02)00210-9

Lilleskov, E.A., Fahey, T.J., Horton, T.R., Lovett, G.M., 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83, 104–115. doi:10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2

Lilleskov, E.A., Hobbie, E.A., Horton, T.R., 2011. Conservation of ectomycorrhizal fungi: Exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecology 4, 174–183. doi:10.1016/j.funeco.2010.09.008

Liu, X.-J.A., Sun, J., Mau, R.L., Finley, B.K., Compson, Z.G., van Gestel, N., Brown, J.R., Schwartz, E., Dijkstra, P., Hungate, B.A., 2017. Labile carbon input determines the direction and magnitude of the priming effect. Applied Soil Ecology 109, 7–13. doi:10.1016/J.APSOIL.2016.10.002

Lladó, S., López-Mondéjar, R., Baldrian, P., 2017. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. Microbiology and Molecular Biology Reviews 81, 1–27. doi:10.1128/mmbr.00063-16

Lubbers, I.M., Jan van Groenigen, K., Fonte, S.J., Six, J., Brussaard, L., Willem van Groenigen, J., 2013. Greenhouse-gas emissions from soils increased by earthworms. Nature Climate Change 3, 187–194. doi:10.1038/NCLIMATE1692

Lubbers, I.M., Pulleman, M.M., Van Groenigen, J.W., 2017. Can earthworms simultaneously enhance decomposition and stabilization of plant residue carbon? Soil Biol. Biochem 105, 12–24. doi:10.1016/j.soilbio.2016.11.008

Lyttle, A., Yoo, K., Hale, C., Aufdenkampe, A., Sebestyen, S., 2011. Carbon-mineral interactions along an earthworm invasion gradient at a sugar maple forest in Northern Minnesota. Applied Geochemistry 26, S85–S88. doi:10.1016/j.apgeochem.2011.03.037

Lyttle, A., Yoo, K., Hale, C., Aufdenkampe, A., Sebestyen, S.D., Resner, K., Blum, A., 2014. Impact of exotic earthworms on organic carbon sorption on nineral surfaces and soil carbon inventories in a northern hardwood forest. Ecosystems 18, 16–29. doi:10.1007/s10021-014-9809-x

Maillard, É., Paré, D., Munson, A.D., 2010. Soil carbon stocks and carbon stability in a twenty-year-old temperate plantation. Soil Science Society of America Journal 74, 1775–1785. doi:10.2136/sssaj2009.0273

Mangiafico, S., 2021. Package “rcompanion” [WWW Document]. R Package Version 2.3.27. URL http://rcompanion.org/

Masiello, C.A., Gallagher, M.E., Randerson, J.T., Deco, R.M., Chadwick, O.A., 2008. Evaluating two experimental approaches for measuring ecosystem carbon oxidation state and oxidative ratio. Journal of Geophysical Research: Biogeosciences 113, 1–9. doi:10.1029/2007JG000534

Mason, L.M., Eagar, A., Patel, P., Blackwood, C.B., DeForest, J.L., 2021. Potential microbial bioindicators of phosphorus mining in a temperate deciduous forest. Journal of Applied Microbiology 130, 109–122. doi:10.1111/jam.14761

Maxfield, P.J., Evershed, R.P., 2014. Phospholipid fatty acid stable isotope probing techniques in microbial ecology, in: Murrell, J.C., Whiteley, A.S. (Eds.), Stable isotope probing and related technologies. ASM Press, Washington, DC, pp. 37–71. doi:10.1128/9781555816896.ch3

Maynard, D.G., Curran, M.P., 2008. Bulk density measurement in forest soils, in: Carter, M.R., Gregorich, E.G. (Eds.), Soil sampling and methods of analysis, Second Edition. pp. 863–869.

Maynard, D.G., Paré, D., Thiffault, E., Lafleur, B., Hogg, K.E., Kishchuk, B., 2014. How do natural disturbances and human activities affect soils and tree nutrition and growth in the Canadian boreal forest? Environ. Rev 22, 161–178. doi:10.1139/er-2013-0057

McAdams, B.N., 2017. Oribatid mite communities after ecosystem disturbance in Alberta. University of Alberta.

McLauchlan, K.K., Hobbie, S.E., 2004. Comparison of labile soil organic matter fractionation techniques. Soil Science Society of America Journal 68, 1616–1625. doi:10.2136/sssaj2004.1616

McLean, M.A., Migge-Kleian, S., Parkinson, D., 2006. Earthworm invasions of ecosystems devoid of earthworms: Effects on soil microbes. Biological Invasions 8, 1257–1273. doi:10.1007/978-1-4020-5429-7_7

McLean, M.A., Parkinson, D., 2000. Field evidence of the effects of the epigeic earthworm *Dendrobaena octaedra* on the microfungal community in pine forest floor. Soil Biology and Biochemistry 32, 351–360. doi:10.1016/S0038-0717(99)00161-3

McLean, M.A., Parkinson, D., 1997. Changes in structure, organic matter and microbial activity in pine forest soil following the introduction of *Dendrobaena Octaedra* (Oligochaeta, Lumbricidae). Soil

Biol. Biochem. 29, 537–540. doi:10.1016/S0038-0717(96)00178-2

Medina-Sauza, R.M., Álvarez-Jiménez, M., Delhal, A., Reverchon, F., Blouin, M., Guerrero-Analco, J.A., Cerdán, C.R., Guevara, R., Villain, L., Barois, I., 2019. Earthworms building up soil microbiota, a review. Frontiers in Environmental Science 7, 1–20. doi:10.3389/fenvs.2019.00081

Moore, J.-D., Ouimet, R., Bohlen, P.J., 2013. Effects of liming on survival and reproduction of two potentially invasive earthworm species in a northern forest Podzol. Soil Biology and Biochemistry 64, 174–180. doi:10.1016/j.soilbio.2013.04.013

Moore, J.-D., Ouimet, R., Reynolds, J.W., 2009. Premières mentions de vers de terre dans trois écosystèmes forestiers du Bouclier canadien. Le Naturaliste Canadien 133, 31–37.

Moore, J.-D., Reynolds, J.W., 2003. Premières mentions de vers de terre dans deux écosystèmes forestiers de la rive nord du Saint-Laurent, Québec. Megadrilogica 9, 53–60.

Moore, J.A.M., Anthony, M.A., Pec, G.J., Trocha, L.K., Trzebny, A., Geyer, K.M., van Diepen, L.T.A., Frey, S.D., 2021. Fungal community structure and function shifts with atmospheric nitrogen deposition. Global Change Biology 27, 1349–1364. doi:10.1111/gcb.15444

Moore, J.D., Görres, J.H., Reynolds, J.W., 2018. Exotic Asian pheretimoid earthworms (*Amynthas* spp., *Metaphire* spp.): Potential for colonisation of south-eastern Canada and effects on forest ecosystems. Environmental Reviews 26, 113–120. doi:10.1139/er-2017-0066

Morrison, E.W., Frey, S.D., Sadowsky, J.J., van Diepen, L.T.A., Thomas, W.K., Pringle, A., 2016. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. Fungal Ecology 23, 48–57. doi:10.1016/j.funeco.2016.05.011

Mueller, K.E., Eissenstat, D.M., Müller, C.W., Oleksyn, J., Reich, P.B., Freeman, K.H., 2013. What controls the concentration of various aliphatic lipids in soil? Soil Biology and Biochemistry 63, 14–17. doi:10.1016/j.soilbio.2013.03.021

Mykytczuk, N.C.S., Trevors, J.T., Ferroni, G.D., Leduc, L.G., 2010. Cytoplasmic membrane fluidity and fatty acid composition of *Acidithiobacillus ferrooxidans* in response to pH stress. Extremophiles 14, 427–441. doi:10.1007/s00792-010-0319-2

Nakajima, N., Sugimoto, M., Tsuboi, S., Tsuji, H., Ishihara, K., 2005. An isozyme of earthworm serine proteases acts on hydrolysis of triacylglycerol. Bioscience, Biotechnology and Biochemistry 69, 2009–2011. doi:10.1271/bbb.69.2009

Navarrete, A.A., Soares, T., Rossetto, R., van Veen, J.A., Tsai, S.M., Kuramae, E.E., 2015. Verrucomicrobial community structure and abundance as indicators for changes in chemical factors linked to soil fertility. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 108, 741–752. doi:10.1007/s10482-015-0530-3

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248. doi:10.1016/j.funeco.2015.06.006

Nielsen, G., Hole, F., 1964. Earthworms and the development of coprogenous A1 horizons in forest soils of Wisconsin. Soil Science Society of America Journal 54, 426–430. doi:10.2136/sssaj1964.03615995002800030037x

Nierop, K.G.J., 2001. Temporal and vertical organic matter differentiation along a vegetation succession as revealed by pyrolysis and thermally assisted hydrolysis and methylation. Journal of Analytical and Applied Pyrolysis 61, 111–132. doi:10.1016/S0165-2370(01)00132-2

Nilsson, L.O., Giesler, R., Bååth, E., Wallander, H., 2005. Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. New Phytologist 165, 613–622. doi:10.1111/j.1469-8137.2004.01223.x

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2019. Package "vegan" [WWW Document]. R Package Version 2.5-6. URL https://cran.r-project.org/web/packages/vegan/vegan.pdf

Orwin, K.H., Dickie, I.A., Holdaway, R., Wood, J.R., 2018. A comparison of the ability of PLFA and 16S rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions. Soil Biol. Biochem. 117, 27–35. doi:10.1016/j.soilbio.2017.10.036

Otto, A., Shunthirasingham, C., Simpson, M.J., 2005. A comparison of plant and microbial biomarkers in grassland soils from the Prairie Ecozone of Canada. Organic Geochemistry 36, 425–448. doi:10.1016/j.orggeochem.2004.09.008

Otto, A., Simpson, M.J., 2006. Sources and composition of hydrolysable aliphatic lipids and phenols in soils from western Canada. Organic Geochemistry 37, 385–407. doi:10.1016/j.orggeochem.2005.12.011

Page, D.W., Van Leeuwen, J.A., Spark, K.M., Mulcahy, D.E., 2002. Pyrolysis characterisation of plant, humus and soil extracts from Australian catchments. Journal of Analytical and Applied Pyrolysis 65, 269–285. doi:10.1016/S0165-2370(02)00005-0

Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18, 1403–1414. doi:10.1111/1462-2920.13023

Paré, D., Boutin, R., Larocque, G.R., Raulier, F., 2006. Effect of temperature on soil organic matter decomposition in three forest biomes of eastern Canada. Canadian Journal of Soil Science 86, 248–256. doi:10.4141/S05-084

Pedersen, J.C., Hendriksen, N.B., 1993. Effect of passage through the intestinal tract of detritivore earthworms (*Lumbricus* spp.) on the number of selected Gram-negative and total bacteria. Biology and Fertility of Soils 16, 227–232. doi:10.1007/BF00361413

Périé, C., Ouimet, R., 2008. Organic carbon, organic matter and bulk density relationships in boreal forest soils. Canadian Journal of Soil Science 88, 315–325. doi:10.4141/CJSS06008

Phillips, H.R.P., Guerra, C.A., Bartz, M.L.C., Briones, M.J.I., Brown, G., Crowther, T.W., Ferlian, O., Gongalsky, K.B., Van den Hoogen, J., Krebs, J., Orgiazzi, A., Routh, D., Schwarz, B., Bach, E.M., Bennett, J., Brose, U., Decaëns, T., König-Ries, B., Loreau, M., Mathieu, J., Mulder, C., Van der Putten, W.H., Ramirez, K.S., Rillig, M.C., Russell, D., Rutgers, M., Thakur, M.P., De Vries, F.T., Wall, D.H., Wardle, D.A., Arai, M., Ayuke, F.O., Baker, G.H., Beauséjour, R., Bedano, J.C., Birkhofer, K., Blanchart, E., Blossey, B., Bolger, T., Bradley, R.L., Callaham, M.A., Capowiez, Y.,

Caulfield, M.E., Choi, A., Crotty, F. V, Dávalos, A., Cosin, D.J.D., Dominguez, A., Duhour, A.E., van Eekeren, N., Emmerling, C., Falco, L.B., Fernández, R., Fonte, S.J., Fragoso, C., Franco, A.L.C., Fugère, M., Fusilero, A.T., Gholami, S., Gundale, M.J., López, M.G., Hackenberger, D.K., Hernández, L.M., Hishi, T., Holdsworth, A.R., Holmstrup, M., Hopfensperger, K.N., Lwanga, E.H., Huhta, V., Hurisso, T.T., Iannone, B. V, Iordache, M., Joschko, M., Kaneko, N., Kanianska, R., Keith, A.M., Kelly, C.A., Kernecker, M.L., Klaminder, J., Koné, A.W., Kooch, Y., Kukkonen, S.T., Lalthanzara, H., Lammel, D.R., Lebedev, I.M., Li, Y., Lidon, J.B.J., Lincoln, N.K., Loss, S.R., Marichal, R., Matula, R., Moos, J.H., Moreno, G., Morón-Ríos, A., Muys, B., Neirynck, J., Norgrove, L., Novo, M., Nuutinen, V., Nuzzo, V., Rahman P, M., Pansu, J., Paudel, S., Pérès, G., Pérez-Camacho, L., Piñeiro, R., Ponge, J.-F., Rashid, M.I., Rebollo, S., Rodeiro-Iglesias, J., Rodríguez, M.Á., Roth, A.M., Rousseau, G.X., Rozen, A., Sayad, E., van Schaik, L., Scharenbroch, B.C., Schirrmann, M., Schmidt, O., Schröder, B., Seeber, J., Shashkov, M.P., Singh, J., Smith, S.M., Steinwandter, M., Talavera, J.A., Trigo, D., Tsukamoto, J., de Valença, A.W., Vanek, S.J., Virto, I., Wackett, A.A., Warren, M.W., Wehr, N.H., Whalen, J.K., Wironen, M.B., Wolters, V., Zenkova, I. V, Zhang, W., Cameron, E.K., Eisenhauer, N., 2019. Global distribution of earthworm diversity. Science 366, 480–485. doi:10.1126/science.aax4851

Phillips, J.D., 2007. Development of texture contrast soils by a combination of bioturbation and translocation. Catena 70, 92–104. doi:10.1016/j.catena.2006.08.002

Piccolo, A., 2001. The supramolecular structure of humic substances. Soil Science 166, 810–832. doi:10.1097/00010694-200111000-00007

Podrebarac, F.A., Billings, S.A., Edwards, K.A., Laganière, J., Norwood, M.J., Ziegler, S.E., 2021. Soil profile connectivity can impact microbial substrate use, affecting how soil CO_2 effluxes are controlled by temperature. Biogeosciences 18, 4755–4772. doi:10.5194/bg-18-4755-2021

Poger, D., Caron, B., Mark, A.E., 2014. Effect of methyl-branched fatty acids on the structure of lipid bilayers. Journal of Physical Chemistry B 118, 13838–13848. doi:10.1021/jp503910r

Post, W.M., Kwon, K.C., 2000. Soil carbon sequestration and land-use change: processes and potential.

Global Change Biology 6, 317–327. doi:10.1046/j.1365-2486.2000.00308.x

Prescott, C.E., 2010. Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101, 133–149. doi:10.1007/s10533-010-9439-0

Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. Forest Ecology and Management 309, 19–27. doi:10.1016/j.foreco.2013.02.034

Price-Christenson, G.J., Johnston, M.R., Herrick, B.M., Yannarell, A.C., 2020. Influence of invasive earthworms (*Amynthas* spp.) on Wisconsin forest soil microbial communities and soil chemistry. Soil Biology and Biochemistry 149, 1–11. doi:10.1016/j.soilbio.2020.107955

Price, D.T., Alfaro, R.I., Brown, K.J., Flannigan, M.D., Fleming, R.A., Hogg, E.H., Girardin, M.P., Lakusta, T., Johnston, M., McKenney, D.W., Pedlar, J.H., Stratton, T., Sturrock, R.N., Thompson, I.D., Trofymow, J.A., Venier, L.A., 2013. Anticipating the consequences of climate change for Canada's boreal forest ecosystems1. Environmental Reviews 21, 322–365. doi:10.1139/er-2013-0042

Prosser, J.I., Head, I.M., Stein, L.Y., 2013. The family Nitrosomonadaceae, in: Rosenberg, E. (Ed.), The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. pp. 901–918. doi:10.1007/978-3-642-30197-1_372

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research 41, 590–596. doi:10.1093/nar/gks1219

Quénéa, K., Derenne, S., González-Vila, F.J., González-Pérez, J.A., Mariotti, A., Largeau, C., 2006a. Double-shot pyrolysis of the non-hydrolysable organic fraction isolated from a sandy forest soil (Landes de Gascogne, South-West France): Comparison with classical Curie point pyrolysis. Journal of Analytical and Applied Pyrolysis 76, 271–279. doi:10.1016/j.jaap.2005.12.007

Quénéa, K., Largeau, C., Derenne, S., Spaccini, R., Bardoux, G., Mariotti, A., 2006b. Molecular and isotopic study of lipids in particle size fractions of a sandy cultivated soil (Cestas cultivation

sequence, southwest France): Sources, degradation, and comparison with Cestas forest soil. Organic Geochemistry 37, 20–44. doi:10.1016/j.orggeochem.2005.08.021

Quideau, S.A., McIntosh, A.C.S., Norris, C.E., Lloret, E., Swallow, M.J.B., Hannam, K., 2016. Extraction and analysis of microbial phospholipid fatty acids in soils. Journal of Visualized Experiments 114, 1–9. doi:10.3791/54360

R Core Team, 2021. R: The R project for statistical computing.

Reynolds, J.W., 2018. First earthworm (Annelida: Oligochaeta) species' collections in Canada and the continental United States. Megadrilogica 23, 1–50. doi:10.3109/10717544.2013.779332

Reynolds, J.W., 1977. The earthworms (Lumbricidae and Sparganophilidae) of Ontario, Royal Ontario Museum Life Sciences Miscellaneous Publications.

Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. Science 366, 886–890. doi:10.1126/science.aay2832

Rivers, A.R., 2016. iTag amplicon sequencing for taxonomic identification at JGI. doi:10.1111/1462-2920.13023.Price

Rodriguez-Ramos, J.C., Cale, J.A., Cahill, J.F., Simard, S.W., Karst, J., Erbilgin, N., 2021. Changes in soil fungal community composition depend on functional group and forest disturbance type. New Phytologist 229, 1105–1117. doi:10.1111/nph.16749

Rosenberg, E., 2014. The family Chitinophagaceae, in: Rosenberg, E (Ed.), The prokaryotes: Other major lineages of bacteria and the archaea. pp. 493–495. doi:10.1007/978-3-642-38954-2

Rousk, J., Brookes, P.C., Bååth, E., 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology and Biochemistry 42, 926–934. doi:10.1016/j.soilbio.2010.02.009

Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant Soil 338, 143–158. doi:10.1007/s11104-010-0391-5

Saltmarsh, D.M., Bowser, M.L., Morton, J.M., Lang, S., Shain, D., Dial, R., 2016. Distribution and

abundance of exotic earthworms within a boreal forest system in southcentral Alaska. NeoBiota 28, 67–86. doi:10.3897/neobiota.28.5503

Sanborn, P., Lamontagne, L., Hendershot, W., 2011. Podzolic soils of Canada: Genesis, distribution, and classification. Canadian Journal of Soil Science 91, 843–880. doi:10.4141/cjss10024

Sax, D.F., Kinlan, B.P., Smith, K.F., 2005. A conceptual framework for comparing species assemblages in native and exotic habitats. Oikos 108, 457–464. doi:10.1111/j.0030-1299.2005.13402.x

Scheu, S., 1987. Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). Biol Fertil Soils 5, 230–234. doi:10.1007/BF00256906

Scheu, S., McLean, M.-A., 1993. The earthworm (Lumbricidae) distribution in Alberta (Canada). Megadrilogica 4, 175–185.

Scheu, S., Parkinson, D., 1994. Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains laboratory studies. Applied Soil Ecology 1, 113–125. doi:https://doi.org/10.1016/0929-1393(94)90031-0

Schlaghamersky, J., Eisenhauer, N., Frelich, L.E., 2014. Earthworm invasion alters enchytraeid community composition and individual biomass in northern hardwood forests of North America. Applied Soil Ecology 83, 159–169. doi:10.1016/j.apsoil.2013.09.005

Schneider, R.R., Hamann, A., Farr, D., Wang, X., Boutin, S., 2009. Potential effects of climate change on ecosystem distribution in Alberta. Can. J. For. Res. 39, 1001–1010. doi:10.1139/X09-033

Simpson, A., Simpson, M.J., Smith, E., Kelleher, B.P., 2007. Microbially derived inputs to soil organic matter: Are current estimates too low? Environmental Science and Technology 41, 8070–8076. doi:10.1021/es8000932

Six, J., Conant, R.T., Paul, E. a, Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155–176. doi:10.1023/A:1016125726789

Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry 32, 2099–2103. doi:10.1016/S0038-0717(00)00179-6

Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367–1377. doi:10.2136/sssaj1998.03615995006200050032x

Smit, E., Leeflang, P., Gommans, S., Van Den Broek, J., Van Mil, S., Wernars, K., 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Applied and Environmental Microbiology 67, 2284–2291. doi:10.1128/AEM.67.5.2284-2291.2001

Smith, C.A.S., Webb, K.T., Kenney, E., Anderson, A., Kroetsch, D., 2011. Brunisolic soils of Canada: Genesis, distribution, and classification. Canadian Journal of Soil Science 91, 695–717. doi:10.4141/cjss10058

Soares, M., Rousk, J., 2019. Microbial growth and carbon use efficiency in soil: Links to fungal-bacterial dominance, SOC-quality and stoichiometry. Soil Biology and Biochemistry 131, 195–205. doi:10.1016/j.soilbio.2019.01.010

Soil Classification Working Group, 1998. The Canadian System of Soil Classification, 3rd edition. Agriculture and Agri-Food Canada, Ottawa, ON.

Soil Survey Staff, 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys, Natural Re. ed. Natural Resources Conservation Service, Washington DC.

Soja, A.J., Tchebakova, N.M., French, N.H.F., Flannigan, M.D., Shugart, H.H., Stocks, B.J., Sukhinin, A.I., Parfenova, E.I., Chapin, F.S.I., Stackhouse, P.W., 2007. Climate-induced boreal forest change: Predictions versus current observations. Global and Planetary Change 56, 274–296. doi:10.1016/j.gloplacha.2006.07.028

Sterkenburg, E., Bahr, A., Brandström Durling, M., Clemmensen, K.E., Lindahl, B.D., 2015. Changes in fungal communities along a boreal forest soil fertility gradient. New Phytologist 207, 1145–1158. doi:10.1111/nph.13426

Stockinger, H., Krüger, M., Schüßler, A., 2010. DNA barcoding of arbuscular mycorrhizal fungi. New Phytologist 187, 461–474. doi:10.1111/j.1469-8137.2010.03262.x

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42, 1385–1395. doi:10.1016/j.soilbio.2010.05.007

Suárez, E.R., Fahey, T.J., Yavitt, J.B., Groffman, P.M., Bohlen, P.J., 2006. Patterns of litter disappearance in a northern hardwood forest invaded by exotic earthworms. Ecological Applications 16, 154–165. doi:10.1890/04-0788

Sun, S., Li, S., Avera, B.N., Strahm, B.D., Badgley, B.D., 2017. Soil bacterial and fungal communities show distinct recovery patterns during forest ecosystem restoration. Applied and Environmental Microbiology 83, 1–14. doi:10.1128/AEM.00966-17

Tan, X., Chang, S.X., Kabzems, R., 2005. Effects of soil compaction and forest floor removal on soil microbial properties and N transformations in a boreal forest long-term soil productivity study. Forest Ecology and Management 217, 158–170. doi:10.1016/j.foreco.2005.05.061

Tedersoo, L., Bahram, M., Zinger, L., Nilsson, R.H., Kennedy, P.G., Yang, T., Anslan, S., Mikryukov, V., 2022. Best practices in metabarcoding of fungi: from experimental design to results. Molecular Ecology 00, 1–27. doi:10.1111/mec.16460

Thomaz, S.M., Agostinho, A.A., Gomes, L.C., Silveira, M.J., Rejmánek, M., Aslan, C.E., Chow, E., 2012. Using space-for-time substitution and time sequence approaches in invasion ecology. Freshwater Biology 57, 2401–2410. doi:10.1111/fwb.12005

Tiunov, A. V., Scheu, S., 2000. Microfungal communities in soil, litter and casts of *Lumbricus terrestris* L. (Lumbricidae): A laboratory experiment. Applied Soil Ecology 14, 17–26. doi:10.1016/S0929-1393(99)00050-5

Treseder, K.K., Marusenko, Y., Romero-Olivares, A.L., Maltz, M.R., 2016. Experimental warming alters potential function of the fungal community in boreal forest. Global Change Biology 22, 3395–3404. doi:10.1111/gcb.13238

Turcotte, I., Quideau, S.A., 2012. Phenolic profiles in natural and reconstructed soils from the oil sands region of Alberta. Canadian Journal of Soil Science 92, 153–164. doi:10.4141/CJSS2010-028

Uroz, S., Buée, M., Deveau, A., Mieszkin, S., Martin, F., 2016. Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. Soil Biology and Biochemistry 103, 471–488. doi:10.1016/j.soilbio.2016.09.006

Van Groenigen, J.W., Van Groenigen, K.J., Koopmans, G.F., Stokkermans, L., Vos, H.M.J., Lubbers, I.M., 2019. How fertile are earthworm casts? A meta-analysis. Geoderma 338, 525–535. doi:10.1016/j.geoderma.2018.11.001

Van Groenigen, J.W., Van Kessel, C., Hungate, B.A., Oenema, O., Powlson, D.S., Van Groenigen, K.J., 2017. Sequestering soil organic carbon: A nitrogen dilemma. Environmental Science and Technology 51, 4738–4739. doi:10.1021/acs.est.7b01427

Vancampenhout, K., Wouters, K., De Vos, B., Buurman, P., Swennen, R., Deckers, J., 2009. Differences in chemical composition of soil organic matter in natural ecosystems from different climatic regions - A pyrolysis-GC/MS study. Soil Biology and Biochemistry 41, 568–579. doi:10.1016/j.soilbio.2008.12.023

Větrovský, T., Steffen, K.T., Baldrian, P., 2014. Potential of cometabolic transformation of polysaccharides and lignin in lignocellulose by soil Actinobacteria. PLoS ONE 9, 1–9. doi:10.1371/journal.pone.0089108

Vidal, A., Quenea, K., Alexis, M., Derenne, S., 2016. Molecular fate of root and shoot litter on incorporation and decomposition in earthworm casts. Organic Geochemistry 101, 1–10. doi:10.1016/j.orggeochem.2016.08.003

Vidal, A., Watteau, F., Remusat, L., Mueller, C.W., Nguyen Tu, T.T., Buegger, F., Derenne, S., Quenea, K., 2019. Earthworm cast formation and development: A shift from plant litter to mineral associated organic matter. Frontiers in Environmental Science 7, 1–15. doi:10.3389/fenvs.2019.00055

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183–2207. doi:10.1016/j.soilbio.2007.03.007

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B.,

Flessa, H., 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions - A review. European Journal of Soil Science 57, 426–445. doi:10.1111/j.1365-2389.2006.00809.x

Wackett, A.A., Yoo, K., Olofsson, J., Klaminder, J., 2018. Human-mediated introduction of geoengineering earthworms in the Fennoscandian arctic. Biological Invasions 20, 1377–1386. doi:10.1007/s10530-017-1642-7

Wang, B., An, S., Liang, C., Liu, Y., Kuzyakov, Y., 2021. Microbial necromass as the source of soil organic carbon in global ecosystems. Soil Biology and Biochemistry 162, 108422. doi:10.1016/j.soilbio.2021.108422

Wang, N., Wang, W., Jiang, Y., Dai, W., Li, P., Yao, D., Wang, J., Shi, Y., Cui, Z., Cao, H., Dong, Y., Wang, H., 2021. Variations in bacterial taxonomic profiles and potential functions in response to the gut transit of earthworms (*Eisenia fetida*) feeding on cow manure. Science of the Total Environment 787, 1–10. doi:10.1016/j.scitotenv.2021.147392

Waring, B.G., Averill, C., Hawkes, C. V., 2013. Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: Insights from meta-analysis and theoretical models. Ecology Letters 16, 887–894. doi:10.1111/ele.12125

Watson, K., 2009. Soils illustrated : field descriptions. International Remote Sensing Surveys, Kamloops, BC.

Watzinger, A., 2015. Microbial phospholipid biomarkers and stable isotope methods help reveal soil functions. Soil Biology and Biochemistry 86, 98–107. doi:10.1016/J.SOILBIO.2015.03.019

Weber, M.G., Flannigan, M.D., 1997. Canadian boreal forest ecosystem structure and function in a changing climate: impact on fire regimes. Environ. Rev. 5, 145–166. doi:https://doi.org/10.1139/a97-008

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic, in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, Thomas J (Eds.), PCR Protocols: A guide to methods and applications. Academic Press, Inc., pp. 315–322.

doi:10.1016/B978-0-12-372180-8.50042-1

Wironen, M., Moore, T.R., 2006. Exotic earthworm invasion increases soil carbon and nitrogen in an old-growth forest in southern Quebec. Can. J. For. Res. 36, 845–854. doi:10.1139/X06-016

Yan, M., Zhang, X., Liu, K., Lou, Y., Wang, Y., 2021. Particle size primarily shifts chemical composition of organic matter under long-term fertilization in paddy soil. European Journal of Soil Science 73, 1–14. doi:10.1111/ejss.13170

Yang, X., Hang, X., Zhang, M., Liu, X., Yang, H., 2015. Relationship between acid tolerance and cell membrane in Bifidobacterium, revealed by comparative analysis of acid-resistant derivatives and their parental strains grown in medium with and without Tween 80. Applied Microbiology and Biotechnology 99, 5227–5236. doi:10.1007/s00253-015-6447-y

Yarwood, S.A., Myrold, D.D., Högberg, M.N., 2009. Termination of belowground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. FEMS Microbiology Ecology 70, 151–162. doi:10.1111/j.1574-6941.2009.00733.x

Zhang, D., Hui, D., Luo, Y., Zhou, G., 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology 1, 85–93. doi:10.1093/jpe/rtn002

Zhang, W., Hendrix, P.F., Dame, L.E., Burke, R.A., Wu, J., Neher, D.A., Li, J., Shao, Y., Fu, S., 2013. Earthworms facilitate carbon sequestration through unequal amplification of carbon stabilization compared with mineralization. Nature Communications 4, 1–9. doi:10.1038/ncomms3576

Zhang, Y.M., Rock, C.O., 2008. Membrane lipid homeostasis in bacteria. Nature Reviews Microbiology 6, 222–233. doi:10.1038/nrmicro1839

Zhou, Z., Wang, C., Zheng, M., Jiang, L., Luo, Y., 2017. Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. Soil Biology and Biochemistry 115, 433–441. doi:10.1016/j.soilbio.2017.09.015

Appendices

Chapter 2

Appendix 2.A. Supplementary tables

Supplementary Table 2.A-1. Description of organic horizons used in this study according to Klinka et al (1997). Faunal droppings do not always correspond to earthworm casts.

Master horizon		Subordinate horizon		Description
L	**Litter**			Surface horizon consisting of relatively fresh plant residues readily identifiable as to their origin.
		Lv	Variative	L horizon consisting of plant residues showing initial decay and strong discoloration.
S	**Bryophytes**			Surface horizon consisting of tissues of living bryophytes (*Sphagnum* spp.) intermixed to a minor extent with litter.
F	**Fermented**			Organic horizon in which partly decomposed plant residues predominate; the partial structures of plant residues are macroscopically discernible.
		Fm	mycogenous	Plant residues are aggregates in a compact-matted, banded fabric interwoven by fugal hyphae with a tenacious consistence. If present, faunal droppings are very infrequent and localized.
		Fs	bryophytes	Party disintegrated tissues from *Sphagnum* spp. dominate; residues are weakly aggregated, fungal mycelia absent or very infrequent and localized.
		Fz	zoogenous	Residues weakly aggregated in a loos or friable consistency as result fragmentation and comminution by soil faunal; faunal droppings are numerous, fungal mycelia are infrequent and localized.
		Fr	root residues	Fine root residues constitute nearly all fabric; fungal mycelia are infrequent and localized.
		Fa	amphi	Characteristics of both Fm and Fz; plant residues aggregated into a weak to moderate, non-compact-matted fabric.
		Fsa	bryophytes + amphi	Both fungal mycelia and faunal droppings, but rarely as common and frequent as in Fa.
		Fsz	bryophytes + zoogenous	Faunal droppings but rarely as common and frequent as in Fz.
		Fsm	bryophytes + mycogenous	Fungal mycelia but rarely as common and frequent as in Fm.
		Frm	root residues + mycogenous	Fine roots residues constitute nearly all fabric; fungal mycelia are common and frequently abundant.
H	**Humic**			Organic horizon in which well decomposed plant residues (fine substances) predominate; the original plant structures are not macroscopically discernible.
		Hc	recalcitrant	Contains macroscopically recognizable plant residues (roots, bark and/or wood), imposing yellow, brown or particularly red colours; fine substances predominate and the material is slightly greasy but does stain fingers when rubbed
		Hf	fine	Very fine granular structure; very small faunal droppings predominate in the fabric.
		Hg	granular	Fine to medium granular structure; small faunal droppings predominate in the fabric.
Ahu				Mineral horizon (< 17 % of OC) enriched in organic matter and mainly consisting of earthworm casts, giving a crumble-like structure

Supplementary Table 2.A-2. Bulk densities [g.cm^3] and carbon:nitrogen (C:N) ratios for forest floors and surface mineral horizons (0-10 cm for Luvisols, 0-15 cm for Brunisols and Podzols). Mean values and standard errors (in parentheses) are displayed (n=3-6). Total organic carbon and nitrogen were analyzed by the dry combustion method on a ThermoScientific Flash 2000 Organic Elemental Analysis instrument (ThermoFisher Scientific, Waltham, MA, USA).

	Soil type	Site	Invasion	Bulk density		C:N	
				Forest floor	Mineral soil	Forest floor	Mineral soil
AB	LUVISOLS	EMEND	Control	0.06 (0.01) *b*	0.96 (0.06) *b*	18.8	12.4
			1st stage invasion	0.05 (0.01) *b*	0.97 (0.05) *b*	20.7	13.0
		Wolf Lake	2nd stage invasion	0.11 (0.01) *a*	1.23 (0.07) *a*	19.3	15.6
		Breton	3rd stage invasion	0.04 (0.02) *b*	0.51 (0.09) *c*	nd[1]	nd
QC	BRUNISOLS	Valcartier	Control	0.06 (0.02) *a*	0.70 (0.09) *a*	22.6	16.9
			Invaded	0.06 (0.02) *a*	0.56 (0.03) *a*	24.2	14.8
		Golf	Control	nd[2]	0.53 (0.06) *a*	nd	nd
			Invaded		0.49 (0.02) *a*		
	PODZOLS	Montmorency	Control	0.07 (0.01) *a*	0.84 (0.07) *a*	35.2	26.9
			Invaded	0.09 (0.01) *a*	0.65 (0.08) *a*	29.8	24.2
		Grands Jardins	Control	0.07 (0.01) *a*	0.93 (0.04) *a*	34.9	27.0
			Invaded	0.08 (0.02) *a*	0.96 (0.02) *a*	32.4	22.3

[1] C:N was not determined for Breton and Golf
[2] Bulk density of LFH was not determined for Golf

Supplementary Table 2.A-3. Densities (individuals per m^2) of earthworm species identified at each invaded site. Mean values and standard errors (in parentheses) are displayed (n=3-4). Species that were identified but for which density was not estimated are reported as ID.

			Luvisols			**Brunisols**		**Podzols**	
Functional group	Species		EMEND	Wolf Lake	Breton	Golf	Valcartier	Montmorency	Grands Jardins
Epigeic	*Dendrobaena octaedra* Savigny	Juveniles	**384 (138)**	**52 (33)**	**32 (25)**	**ID**	**54 (47)**	**21 (15)**	N.O.
		Adults	**48 (10)**	**68 (42)**	**53 (30)**		**8 (8)**	**5 (6)**	
	Dendrodrilus rubidus Savigny	Adults	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.	**ID**
Endogeic	*Aporrectodea* spp.	Juveniles	N.O.[1]	**4 (8)**[2]	N.O.	**ID**	N.O.	**5 (6)**	N.O.
		Adults		**4 (8)**[b]			**13 (14)**[3]	N.O.	
	Lumbricus rubellus Hoffmeister	Juveniles	N.O.	N.O.	N.O.	N.O.	**19 (13)**	**27 (20)**	N.O.
	Octolasion cyaneum Savigny	Juveniles	N.O.	N.O.	**224 (98)**	N.O.	N.O.	N.O.	N.O.
		Adults			**85 (46)**				
Anecic	*Lumbricus terrestris* L.	Adults	N.O.	N.O.	N.O.	N.O.	N.O.	N.O.	**ID**
	Non-identified	Juveniles	N.O.	N.O.	**27 (11)**	**5 (6)**	**ID**	**ID**	**ID**

[1] N.O. = not observed

[2] *Aporrectodea tuberculata* Eisen

[3] *Aporrectodea turgida* Eisen

Supplementary Table 2.A-4. Soil profile description for all sampled soil pits following Watson (2009).

Site	Invasion	Pit	Horizon	Depth [cm]	Color (dry)	Textural class [18]	Sand [%]	Silt [%]	Clay [%]	Structure[19]	Boundaries[20]	Mottles[21]	Roots[22]	Coarse fragments[23]
EMEND	Control	1	Lv	11.5 – 11	*Resimor*						Nd[24]	NA	PV, PF, FM, PC (R)	NA[25]
			Fm	11 – 6										
			Frm	6 – 1										
			Hf	1 – 0							AS			
			Ahe	0 – 1	nd	Nd				SGR	CW	N.O.[26]	VF (R)	N.O.
			Ae	1 – 18	10YR7/2	L	34	41	25	SGR	AS	N.O.	VF (R)	1% GS
			Bt	18 –	10YR6/3	C	23	32	45	MGR	Nd	N.O.	FV, FF (R)	1% GS
		2	Lv	10 – 9	*Resimor*						AW	NA	AV, AF, FC (H)	NA
			Fm	9 – 7										
			Frm	7 – 2										
			Hf	2 – 0										
			Ahe	0 – 4	10YR5/2	L	45	43	13	MGR	AS	N.O.	PF, PM (H+V)	N.O.
			Ae	4 – 12	10YR6/3	CL	35	30	35	FGR	AS	N.O.	FV, FF (H)	N.O.
			Bt	12 –	10YR6/3	CL	22	26	52	CSBK	Nd	I: FF	FV, FF, FC (H)	1% GS
		3	Lv	10.5 – 10	*Resimor*						CS	NA	PV, PF, FM, FC (H)	NA
			F	10 – 8										
			Frm	8 – 3										
			Hf/Hc	3 – 0										
			Ahe	0 – 5.5	10YR5/3	Nd				FGR	AB	N.O.	PV, PF (H)	N.O.
			Ae	5.5 – 11.5	10YR7/2	SiL	35	53	13	MGR	CS	N.O.	FM (O)	N.O.
			Bt	11.5 –	10YR7/2	L	36	41	23	CSBK	Nd	N.O.	FV, FF, FC (H)	N.O.
	Epigeic	1	Lv	8 – 7.5	*Mullmoder*						CS	NA	AV, AF, PM, VC (H)	NA
			F	7.5 – 5.5										
			Fa	5.5 – 2										
			Hg	2 – 1										
			Hc	1 – 0										
			Ah	0 – 4	10YR 4/3	L	45	35	20	MGR	CB	BC: CF	N.O.	5% GS

[18] SL=Sandy loam; LS=Loamy sand; L=Loam; SiL=Silt loam; CL=Clay loam; C=Clay; SCL=Sandy clay loam; S=Sand
[19] SGR=single grain; MGR=medium granular; FGR= fine granular; CGR= coarse granular, CSBK= coarse sub-angular blocky.
[20] 1st letter: A=Abrupt, C=Clear, G=Gradual; 2d letter: S=Smooth, W=Wavy, B=Broken
[21] I=Iron, BC=Black Carbon, G=Gley; 1st letter (abundance): F=Few (< 2 %), C=common (2–20 %); 2d letter (size): F=Fine (< 5 mm), M=Medium (5–15mm), C=Coarse (> 15 mm)
[22] 1st letter (abundance): V=Very few (< 1/ Unit Area), F=Few (2–3),P=Plentiful (4–14), A=Abundant (> 14); 2d letter (size): V=Very fine (< 0.5mm), F=Fine (0.5–2 mm), M=Medium (2–5 mm), C=Coarse (5–10 mm); in parenthesis: H=horizontal, V=vertical, O=oblique, R=random
[23] G=gravel, C=cobbles, S= sub-rounded/sub-angular
[24] Nd= not determined
[25] NA=not applicable
[26] N.O.= not observed

			Ae	4 – 7	10YR 6/3	CL	37	34	29	MGR	AS	N.O.	VV, VF, VC (H)	5% GS
			Btj	7 – 20	10YR 6/3	CL	39	28	33	MGR	GS	BC: CF; I: FF	VV, VF. PC (H)	5% GS, 10% CS
			BC	20 –	Nd	Nd				MGR	Nd	BC: CF; I: FF	Nd	5% GS, 10% CS
		2	Lv	7 – 6.5	*Mullmoder*					Nd	CS	NA	AV, AF, PM, VC (R)	NA
			S	6.5 – 6										
			Fz	6 – 4										
			Fa	4 – 1										
			Frm	1 – 0.5										
			Hf	0.5 – 0										
			Ah	0 – 4.5	10YR 3/2	L	47	32	21	MGR	CW	BC: CF	AV, AF (R)	5% GS
			Ae	4.5 – 9.5	10YR 6/3	L	49	33	18	MGR	AS	BC: CF	FM (R)	10% GS
			Bt	9.5 –	10YR 6/3	SCL	47	26	27	CSBK	Nd	BC: CF; I: CF	VF, FF, FM, FC (R)	10% GS
		3	Lv	5.5 – 4.5	*Mullmoder*					Nd	Nd	NA	AV, AF, FC (H)	NA
			S	4.5 – 4							AB			
			Fa/Fsa	4 – 1							Nd			
			Hf	1 – 0							AS			
			Ah	0 – 4.5	10YR 3/2	L	45	33	23	MGR	AW	BC: CM	AV, AF, FM (R)	N.O.
			Ae	4.5 – 10.5	10YR 6/3	L	42	30	25	MGR	AS	N.O.	FM (R)	N.O.
			Btj	10.5 –	10YR 6/3	CL	40	31	29	CSBK	Nd	BC: CM	FM, FC (R)	10% GCS
Wolf Lake	Epigeic + endogeic	1	L	10 – 9	*Mormoder*	Nd	CS	NA	Nd	NA				
			Fa	9 – 7										
			Fz	7 – 2										
			H	2 – 0										
			Ae1	0 – 11	10YR 7/1	SL	62	24	14	SGR	CS	N.O.	AC (R)	1% GS
			Ae2	5 – 20	10YR 8/1					SGR	AS	N.O.	AC (R)	1% GS
			Bt	20 – 40	10YR 7/2	SL	54	18	28	CGR	GS	N.O.	AF (R)	N.O.
		2	L	15 – 12	*Mormoder*					Nd	CS	NA	Nd	NA
			Fa	12 – 8										
			Fz	8 – 2										
			H	2 – 0										
			Ae1	0 – 2	10YR 7/1	SL	60	32	8	SGR	BS	N.O.	AC (R)	5% GS
			Ae2	2 – 7						SGR	GS	N.O.	AC (R)	5% GS
			Bt	7 –	10YR 7/1	SL	60	25	15	CGR	Nd	N.O.	AF (R)	N.O.
		3	L/S	5 – 4	*Leptomoder*					Nd	Nd	NA	Nd	NA
			Fz	4 – 0							CS			
			Ae1	0 – 3	10YR 7/1	SL	52	37	11	SGR	AS	N.O.	AC (R)	5% GS
			Ae2	3 – 9						SGR	CS	N.O.	AC (R)	5% GS
			Bt	9 –	10YR 8/1	SL	67	24	9	CGR	Nd	N.O.	AF (R)	1% GS
		4	L	10 – 9	*Leptomoder*					Nd	Nd	NA	Nd	NA
			Fz	9 – 2										
			H	2 – 0							CS			
			Ae1	0 – 2.5	10YR 8/1	SL	64	28	8	SGR	BS	N.O.	AC (R)	1% GS
			Ae2	2.5 – 10.5						SGR	GS	N.O.	AC (R)	1% GS

			Bt	10.5 –	10YR 8/2	SCL	56	20	24	CGR	nd	N.O.	AF (R)	5% GS
Breton	Epigeic + endogeic + anecic	1	Lv	6 – 5	*Vermimull*	Nd	Nd	N.O.	Nd	NA				
			Fz	5 – 0							CW			
			Ahu	0 – 9	10YR 4/2	CL	38	30	32	SGR/MGR	AS	N.O.	AF, AV, FM (R)	N.O.
			Bt	9 –	10YR 6/2	CL	32	34	34	CGR	Nd	BC: C-F	VF, VM (V/O)	N.O.
		2	Lv	7 – 5	*Vermimull*					Nd	Nd	N.O.	Nd	NA
			Fz	5 – 0							CS			
			Ahu	0 – 6	10YR 4/2	CL				FGR	AW	N.O.	FM, AF, AV (H)	N.O.
			Ae	6 – 8	10YR 5/2	Nd				SGR	AB	N.O.	VF (R)	N.O.
			Bt	8 –	10YR 6/2	CL				FGR	Nd	BC: F	VF (R)	N.O.
		3	Lv	5 – 4	*Vermimull*					Nd	Nd	N.O.	Nd	NA
			Fz	4 – 0							AS			
			Ahu	0 – 8	10YR 4/2	CL				FGR	AW	N.O.	AV, AF, AM (R)	N.O.
			Ae	8 – 11	10YR 5/2	Nd				SGR	AB	N.O.	FF, FM (R)	N.O.
			Bt	11 –	10YR 6/2	CL				MGR	Nd	N.O.	FM (R)	N.O.
Golf	Control	1	Lv	9 – 8.5	*Resimor*						AS	NA	AV, AF, AM (R)	NA
			Frm	8.5 – 4.5										
			H	4.5 – 0										
			Ae	0 – 2	10YR4/1	Nd				SGR	AW	N.O.	AV, AF, AM (O)	10% GS
			Bm1	2 – 6	10YR2/2	Nd				FGR	CW	N.O.	AV, AF, AM (H)	N.O.
			Bm2	6 – 16	10YR4/4	L	49	41	10	FGR	GS	N.O.	AV, AF, AM (O)	3% GS
		2	Lv	8 – 7	*Resimor*						AS	NA	AV, AF, AM (R)	NA
			Frm	7 – 4										
			H	4 – 0										
			Ae	0 – 4	10YR2/1	Nd				SGR	CW	N.O.	AV, AF, AM, AC (O)	N.O.
			Bm	4 –	10YR3/4	SiL	32	58	10	FGR	Nd	N.O.	AV, AF, AM, AC (O)	N.O.
	Invaded	1	Lv	3 – 2	*Vermimull*						CS	NA	AV, AF, AM (R)	NA
			Fa	2 – 0										
			Ahu	0 – 13	10YR3/3	L	41	47	12	FGR	AS	N.O.	AV, AF, AM (O	N.O.
			Bgj	13 – 17	10YR4/3	Nd				FGR	AB	N.O.	AV, AF, AM (O)	N.O.
			Bm	17 – 34	10YR3/2	L	41	47	12	MGR	AS	N.O.	AV, AF, AM (O)	N.O.
			C	34 –	Nd	Nd				SGR	Nd	N.O.	AV, AF, AM (V)	N.O.
		2	Lv	1 – 0	*Vermimull*						AB	NA	AV, AF (O)	NA
			Ahu	0 – 10	10YR3/1	Nd				SBK	AW	G: CF	AV, AF (O)	20% GS
			Bm	10 –	10YR3/3	L	37	49	14	FGR	Nd	N.O.	AV, AF (H)	20% GS
		3	Lv	2 – 1.5	*Vermimull*						AS	NA	VV, AF, AM (R)	NA
			Fa	1.5 – 0										
			Ahu	0 – 10	10YR3/2	SiL	33	57	10	SBK	CS	N.O.	FV, AF (O)	N.O.
			Bm	10 – 22	10YR4/3	L	35	45	20	SBK	CS	N.O.	FV, AF (V)	N.O.

			C	22 –	Nd	Nd				SGR	Nd	N.O.	N.O.	N.O.
Valcartier	Control	1	Lv	9.5 – 8.5	*Resimor*						AS	NA	AF, AM (R)	NA
			Frm	8.5 – 0.5										
			H	0.5 – 0										
			Ah	0 – 3	10YR2/1	Nd				FGR	AW	N.O.	AF, AM (O)	N.O.
			Ae	3 – 16	10YR4/1	SL	55	33	12	FGR	CW	N.O.	FF, FM (V)	N.O.
			Bm	16 – 22	10YR2/2	SL	62	22	16	FGR	CS	C: CF	AF (V)	20% GS
			C	22 –	10YR3/4	Nd				SBK	Nd	N.O.	N.O.	50% GS
		2	Lv	6.5 – 5.5	*Resimor*						CS	NA	AV, AF (O)	NA
			Fr	5.5 – 0.5										
			H	0.5 – 0										
			Ah	0 – 10	10YR2/1	L	52	36	12	FGR	CS	N.O.	AF, AM, AC (H)	30% GCS
			Bm	10 –	10YR4/4	Nd				MGR	Nd	N.O.	FV, FF (O)	30% GCS
		3	Lv	7.5 – 7	*Resimor*						CS	NA	AF, AM, AC (O)	NA
			Fr	7 – 2										
			H	2 – 0										
			Ah	0 – 1	2.5Y5/2	nd				SBK	GS	N.O.	FF, FM, FC (H)	N.O.
			Bm	1 –	10YR4/4	CL	27	45	28	MGR	Nd	N.O.	AF, AM, AC (O)	N.O.
	Invaded	1	Lv	6 – 4	*Mullmoder*						AB	NA	AF, AM (O)	NA
			Frz	4 – 0							AS			
			Ahu	0 – 3	10YR4/2	L	38	44	18	FGR	CS	N.O.	AF, AM (H)	N.O.
			Bm	3 – 15	10YR5/3	L	39	45	16	SBK	CS	N.O.	FF, FM (H)	1% GS
			C	15 –	10YR4/4	L	39	45	16	Nd	Nd	N.O.	N.O.	N.O.
		2	Lv	5 – 3	*Vermimull*						AB	NA	AV, AF, AM (R)	NA
			Frz	3 – 0							CS			
			Ahu	0 – 4	2.5Y4/4	CL	24	48	28	MGR	AS	N.O.	FV, FF, FM (O)	N.O.
			Bm	4 – 11	10YR5/3	L	28	50	22	SBK	CS	N.O.	FV, FF, FM (H)	N.O.
			C	11 –	2.5Y5/4	SiL	27	55	18	SBK	Nd	BC: CF	N.O.	10% GS
		3	Lv	3 – 2	*Vermimull*						AS	NA	AV, AF (R)	NA
			F	2 – 0.5										
			H	0.5 – 0										
			Ahu	0 – 6	2.5Y4/4	SiL	27	47	26	SGR	GS	N.O.	AV, AF, AM (H0	1% GS
			Bm	6 – 26	2.5Y5/3	SiL	23	51	26	SBK	GS	N.O.	FV, FF, FM (O)	2% GS
		4	Lv	0.5 – 0	*Vermimull*						AB	NA	N.O.	NA
			Ahu	0 – 5	2.5Y4/3	SiL	14	52	34	MGR	GS	N.O.	AF, AC (H)	N.O.
			Bm	5 – 16	2.5Y5/3	C	17	39	44	CGR	GS	I: CF; BC: CM	FF, FM, FC (H)	N.O.
			C	16 –	2.5Y6/3	CL	35	33	32	SBK	Nd	N.O.	Nd	N.O.
		5	Lv	2 – 1	*Vermimull*						AS	NA	N.O.	NA
			Frz	1 – 0										
			Ahu	0 – 4	2.5Y3/2	Nd				SGR	GB	N.O.	AV, AF, AM (O)	N.O.
			Bm	4 –	10YR4/4	CL	24	46	30	SGR	Nd	N.O.	AV, AF, AM (O)	N.O.

		6	Lv	2 – 1	*Vermimull*						AB	NA	N.O.	NA
			Fz	1 – 0										
			Ahu1	0 – 2	Nd		Nd			Nd	AB	N.O.	AV, AF, AM (H)	N.O.
			Ahu2	2 – 8	2.5Y4/4	SC	52	28	20	FGR	GS	N.O.	AV, AF, AM (O)	N.O.
			Bm	8 – 18	2.5Y4/3	L	47	35	18	FGR	AS	N.O.	AV, AF, AM (O)	N.O.
			C	18 –	2.5Y4/4	SL	67	23	10	SGR	Nd	N.O.	AV, AF, AM (O)	20% GS
Montmorency	Control	1	S	10 – 7	*Sphagnomor*						AW	NA	AV, AF, PM, PC (R)	NA
			Fs	7 – 1										
			Hc	1 – 0										
			Ae	0 – 6	10YR6/1	SL	71	21	8	SGR	AW	N.O.	FM (O)	5% GS
			Bh	6 – 16	10YR3/4	SL	75	15	10	CSBK	GW	N.O.	VV, VF, PM, PC (H)	20% GS
			C	16 –	10YR5/4	SL	76	16	8	Nd	Nd	N.O.	Nd	20% GS
		2	S	11 – 9	*Sphagnomor*						AS	NA	PV, PF, VM, VC (R)	NA
			Fs	9 – 2										
			Hc	2 – 0										
			Ae	0 – 7	10YR6/2	SL	67	29	4	SGR	CW	N.O.	N.O.	3% GS
			Bh	7 – 17	10YR3/4	SL	71	19	10	MGR	AW	N.O.	FV, FF, AM, VC (H)	2% GS
			Bhf	17 –	10YR4/6		Nd			CSBK	Nd	N.O.	VV, FF, PM, FC (H)	10% GS
		3	S	10 – 8	*Sphagnomor*						AW	NA	AV, AF, VM, VC (R)	NA
			Fsm	8 – 1										
			Hc	1 – 0										
			Ae	0 – 9	10YR5/1	SL	74	20	6	SGR	AW	N.O.	N.O.	3% GS
			Bh	9 –	10YR3/3	SL	77	11	12	CSBK	Nd	N.O.	FV, FF, FM (R)	10% GS
	Invaded	1	S	10 - 8	*Sphagnomor*						AS	NA	PV, AF, AM, AC (R)	NA
			Fsz	8 – 0.5										
			Hc	0.5 – 0										
			Ae	0 – 5	10YR5/1	LS	77	19	4	SGR	CW	N.O.	AM, AC (O)	N.O.
			Bh	5 –	10YR3/3	SL	67	23	10	CSBK	Nd	N.O.	FV, FF (O)	5% GS
		2	S	11 – 10	*Sphagnomor*						AS	NA	AV, AF, AM (R)	NA
			Fsz	10 – 2										
			Hc	2 – 0										
			Ae	0 – 5	10YR5/2	SL	65	29	6	SGR	AS	N.O.	N.O.	5% GS
			Bh	5 –	10YR3/3		Nd			CSBK	Nd	N.O.	FV, FF, FM (H)	20% GS
		3	S	8 – 6	*Sphagnomoder*						AS	NA	AV, AF, AM (R)	NA
			Fsz	6 – 1										
			Hc	1 – 0										
			Ae	0 – 9.5	10YR5/1	SL	56	36	8	SGR	CW	N.O.	N.O.	5% GS
			Bh1	9.5 – 24.5	10YR3/3	SL	67	25	8	MSBK	GS	N.O.	FF, FM (O)	5% GS
			Bh2		10YR4/4	SL	58	32	10	CSBK	GS	N.O.	FF, FM (O)	N.O.
			C	24.5 –	10YR5/3	L	52	38	10	CSBK	Nd	N.O.	N.O.	5% GS
Grands Jardins	Control	1	S	11 – 8.5	*Sphagnomor*						AW	NA	Nd	NA
			Fsm	8.5 – 5							AW		PV, PF (O)	
			Frm	5 – 0							AI		PV, FF, FM (O)	
			Ah	0 – 2.5	10YR2/1		Nd			FGR	AS	N.O.	PV, PF (O)	N.O.

			Ae	2.5 – 6	10YR5/2	SL	82	4	14	SGR	AW	N.O.	N.O.	N.O.
			Bf	6 – 16	10YR5/6	LS	83	13	4	MSBK	AW	N.O.	VF, VC (O)	N.O.
			BC	16 –	10YR5/6	LS	79	17	4	CSBK	Nd	N.O.	N.O.	50% GS
		2	S	10.5 – 8.5	*Sphagnomor*						AS	NA	Nd	NA
			Fsm	8.5 – 6							AS		VF, PM (R)	
			Fr	6 – 1							AS		VF, PM (R)	
			H	1 – 0							AW		VF (R)	
			Ah	0 – 3	10YR4/2	SL	67	25	8	FGR	AS	N.O.	PV, PF, PM (H)	N.O.
			Hb	3 – 5	10YR3/3					FGR	AS	N.O.	VV, FF (H)	N.O.
			Ae	5 – 17	10YR5/2	SCL	73	6	21	SGR	AW	N.O.	VV, VF (O)	50% GS
			Bhf	17 –	10YR3/6	SL	79	5	16	MSBK	Nd	N.O.	Nd	60% GS
		3	S	10 – 7	*Sphagnomor*						SA	NA	N.O.	NA
			Fs	7 – 4							SA		FF (R)	
			Fr	4 – 2							CS		PF, PV, PM (R)	
			H	2 – 0							SA		FC (R)	
			Ah	0 – 2.5	10YR3/2	Nd				FGR	WC	N.O.	FF, FV (O)	N.O.
			Ae /burnt	2.5 – 6.5	10YR4/1	Nd				SGR	WA	N.O.	FM (H)	N.O.
			Bf	6.5 – 12.5	10YR5/3	LS	79	15	6	MSBK	BA	N.O.	FF (O)	N.O.
			Bhf	12.5 –	10YR5/8	SL	74	18	8	MSBK	GW	N.O.	FF (O)	N.O.
	Invaded	1	S	4.5 – 2.5	*Vermimull*						AS	NA	AF, AV (O)	NA
			Fsz	2.5 – 0							CS			
			Ahu	0 – 12	10YR4/2	SCL	54	20	26	MGR	AS	N.O.	PF, PV, FC (O)	10% GS
			Ae	12 – 14	10YR5/2 (Burnt: 10YR2/1)	Nd				SGR	AB	N.O.	N.O.	N.O.
			Bf	14 – 28	10YR5/6	SL	61	27	12	MSBK	C	N.O.	FC, FF (H)	20% CS
			C	28 –	10YR6/3	S	87	11	2	SGR	Nd	N.O.	N.O.	N.O.
		2	S	4 – 2.5	*Vermimull*						Nd	NA	PF, PV (H)	NA
			Fsz	2.5 – 0							AS			
			Ahu	0 – 7	10YR3/2	SL	76	4	20	MGR	AS	N.O.	AF, AV, AM, AC (O)	N.O.
			Ae1	7 – 12	10YR4/2	Nd				SGR	AB	N.O.	FC, FF, FV (O)	N.O.
			Bf1	12 – 16	10YR4/3	S	89	7	4	MSBK	AS	N.O.	N.O.	N.O.
			Ae2	16 – 21.5	10YR5/4	S	85	11	4	SGR	AW	N.O.	FF, FV, FM (O)	N.O.
			Bf2	21.5 –	10YR5/6	LS	84	10	6	MSBK	Nd	N.O.	N.O.	50% CS
		3	L	7 – 6	*Vermimull*						AS	NA	N.O.	NA
			Fsz	6 – 0							CS		AF, AV (R)	
			Ahu	0 – 7	10YR3/2	Nd				MGR	AS	N.O.	FV, FF, FM (H)	N.O.
			Hb	7 – 8	Burnt	Nd				FGR	CS	N.O.	N.O.	N.O.
			Ae	8 – 18	10YR6/3	SL	73	21	6	SGR	CW	N.O.	FC (O)	50% GS
			Bhf	18 –	10YR3/6	Nd				MSBK	Nd	N.O.	N.O.	80% CS

Appendix 2.B. Supplementary figures

Supplementary Figure 2.B-1. Representative pedons for each site control zone, with the exception of Wolf Lake and Breton where only invaded soils could be found and described.

Chapter 3

Appendix 3.A. Supplementary tables

Supplementary Table 3.A-1. Main site characteristics and key soil properties. Climate data were obtained from Alberta Climate Information Service (2020) for Alberta (AB) and from Environment Canada (2010) for Québec (QC). Total nitrogen (TN) and total organic carbon (TC) were analyzed by the dry combustion method on a ThermoScientific Flash 2000 Organic Elemental Analysis instrument (ThermoFisher Scientific, Waltham, MA, USA).

Site	Site name	Parent material	MAT [ºC]	MAP [mm]	Coordinates		Textural class		Forest floor thickness [cm]		$CaCl_2$ pH		TN [%]		TC [%]	
	AB						Control	Invaded	Control	Invaded	Control	Invaded	Control	Invaded	Control	Invaded
Luvisol	EMEND	Glacial till	1.4	413	56º46'N	Forest floor			10.7	7.7	5.6	5.7	2.29	1.86	43.0	38.7
					118º22'W	Mineral soil	Loam	Loam			5.2	5.4	0.14	0.20	1.7	2.6
	Breton	Glacial till	3.1	552	53º05'N	Forest floor			NA	4.2	NA	6.3	NA	-	NA	27.9[1]
					114º26'W	Mineral soil	NA	Clay loam			NA	5.8	NA	0.21[2]	NA	7.9[1]
	QC															
Brunisol	Valcartier	Fluvial deposit	1.6	1460	46º55'N	Forest floor			7.8	3.1	3.2	4.8	1.99	1.48	45.1	35.4
					71º36'W	Mineral soil	Sandy loam	Clay loam			3.1	4.3	0.46	0.49	7.8	7.7
Podzol	Grands Jardins	Sandy glacial till	3.7	930	47º40'N	Forest floor			10.5	5.3	3.0	3.7	1.40	1.31	48.9	42.3
					70º50'W	Mineral soil	Sandy loam	Sandy clay loam			3.4	4.6	0.15	0.27	4.1	6.1

[1] Analysis by loss on ignition
[2] Data from Li et al. (2018)

Supplementary Table 3.A-2. Two-way analyses of variance (factors: invasion and site) of fungal guild relative abundances and diversity indices, for forest floors and mineral soils separately. Df stands for degrees of freedom and Sum sq for sum of squares.

	FOREST FLOOR				MINERAL SOIL			
	Df	Sum Sq	F value	p-value	Df	Sum Sq	F value	p-value
Relative abundance								
Ectomycorrhizae								
Invasion	1	<0.01	<0.01	0.96	1	0.49	15.13	<0.001
Site	3	1.36	13.80	<0.001	3	0.83	8.46	<0.001
Interaction	2	0.06	0.98	0.39	2	0.02	0.33	0.72
Residuals	20	0.66			24	0.78		
Pathogens								
Invasion	1	0.02	0.57	0.46	1	<0.01	0.18	0.68
Site	3	0.57	5.86	<0.01	3	0.17	3.24	0.04
Interaction	2	0.02	0.30	0.75	2	<0.01	0.02	0.98
Residuals	20	0.65			24	0.43		
Saprotrophs								
Invasion	1	0.01	0.46	0.51	1	0.38	12.83	<0.01
Site	3	0.48	6.23	<0.01	3	0.68	7.64	<0.001
Interaction	2	0.05	1.06	0.37	2	0.04	0.74	0.49
Residuals	20	0.51			24	0.71		
Species richness								
Ectomycorrhizae								
Invasion	1	105.29	3.08	<0.1	1	381.80	20.21	<0.001
Site	3	2776.89	27.04	<0.001	3	107.39	1.90	0.16
Interaction	2	64.91	0.95	0.41	2	53.52	1.42	0.27
Residuals	17	581.87			20	377.76		
Pathogens								
Invasion	1	11.41	1.11	0.31	1	0.89	3.57	0.07
Site	3	203.01	6.59	<0.01	3	1.60	2.14	0.13
Interaction	2	78.98	3.84	0.04	2	0.48	0.97	0.40
Residuals	17	174.63			20	4.98		
Saprotrophs								
Invasion	1	18.67	0.16	0.70	1	0.01	7.74	0.01
Site	3	1380.28	3.88	0.03	3	0.01	3.06	0.05
Interaction	2	229.38	0.97	0.40	2	<0.01	0.34	0.72
Residuals	17	2018.13			20	0.01		
Species diversity								
Ectomycorrhizae								
Invasion	1	4.98	0.43	0.52	1	28.33	2.83	0.11
Site	3	441.61	12.68	<0.001	3	38.35	1.28	0.31
Interaction	2	11.33	0.49	0.62	2	21.30	1.06	0.36
Residuals	17	197.34			20	200.05		
Pathogens								
Invasion	1	0.65	0.15	0.70	1	0.53	9.05	<0.01
Site	3	43.75	3.30	0.05	3	0.28	1.58	0.23

Interaction	2	8.17	0.92	0.42	2	0.05	0.43	0.66
Residuals	17	75.22			20	1.16		
Saprotrophs								
Invasion	1	2.40	0.07	0.79	1	122.51	5.69	0.03
Site	3	990.74	9.69	<0.001	3	178.75	2.77	0.07
Interaction	2	17.36	0.25	0.78	2	20.96	0.49	0.62
Residuals	17	579.25			20	430.32		

Supplementary Table 3.A-3. Two-way analyses of variance (factors: invasion and site) of relative abundance of bacterial phyla and family levels, and global diversity indices, for forest floors and mineral soils separately. At the family-level, taxa present in >60% of the samples and representing >0.5% of average relative abundance were selected for ANOVA and only groups significant for forest floor and/or mineral soil are displayed. Missing groups for either the forest floor or the mineral soil correspond to those that did not meet the selection criteria. Df stands for degrees of freedom and Sum sq for sum of squares.

	FOREST FLOOR				MINERAL SOIL			
	Df	Sum Sq	F value	p-value	Df	Sum Sq	F value	p-value
Relative abundance								
Phylum level								
Actinobacteriota								
Invasion	1	0.02	3.41	0.08	1	0.00	0.00	1.00
Site	3	0.03	2.01	0.15	3	0.06	12.43	<0.001
Interaction	2	0.01	0.87	0.43	2	0.01	0.21	0.82
Residuals	20	0.11			24	0.04		
Acidobacteriota								
Invasion	1	0.00	0.00	0.98	1	0.01	3.65	0.07
Site	3	0.01	1.56	0.23	3	0.04	5.90	<0.01
Interaction	2	0.01	1.54	0.24	2	0.01	0.39	0.68
Residuals	20	0.03			24	0.05		
Bacteroidota								
Invasion	1	0.01	0.82	0.38	1	1.26	10.39	<0.01
Site	3	0.05	12.11	<0.001	3	4.10	11.27	<0.001
Interaction	2	0.01	0.06	0.95	2	0.34	1.40	0.27
Residuals	20	0.03			24	2.91		
Chloroflexi								
Invasion	1	0.00	0.21	0.65	1	0.01	3.92	0.06
Site	3	0.01	6.99	<0.01	3	0.03	16.17	<0.001
Interaction	2	0.00	0.99	0.39	2	0.01	1.79	0.19
Residuals	20	0.01			24	0.01		
Gemmatimonadota								
Invasion	1	0.00	0.05	0.83	1	0.00	3.03	0.09
Site	3	0.00	25.60	<0.001	3	0.00	12.79	<0.001
Interaction	2	0.00	0.06	0.95	2	0.00	0.59	0.56

Residuals	20	0.00			24	0.00		
Planctomycetota								
Invasion	1	0.01	0.14	0.71	1	0.01	0.30	0.59
Site	3	0.01	0.07	0.97	3	0.03	7.24	<0.01
Interaction	2	0.01	0.09	0.91	2	0.01	0.72	0.50
Residuals	20	0.10			24	0.03		
Proteobacteria								
Invasion	1	0.01	0.90	0.36	1	0.01	6.90	0.01
Site	3	0.04	3.15	0.05	3	0.01	10.05	<0.001
Interaction	2	0.01	0.40	0.67	2	0.01	7.84	<0.01
Residuals	20	0.08			24	0.01		
Verrucomicrobiota								
Invasion	1	0.01	5.95	0.02	1	0.01	0.79	0.38
Site	3	0.01	24.44	<0.001	3	0.01	5.42	<0.01
Interaction	2	0.00	0.85	0.44	2	0.01	0.15	0.86
Residuals	20	0.01			24	0.01		
Relative abundance								
Family or associated level								
Acidobacteriae – subgroup 2								
Invasion	1	0.04	0.36	0.56	1	65.43	7.91	<0.01
Site	3	1.09	3.21	<0.05	3	180.83	7.29	<0.01
Interaction	2	0.37	1.64	0.22	2	34.32	2.08	0.15
Residuals	20	2.28			24	198.45		
Acidobacteriales								
Invasion	1	0.03	0.18	0.67	1	85.35	9.21	<0.01
Site	3	1.07	2.15	0.13	3	160.74	5.78	<0.01
Interaction	2	0.04	0.13	0.88	2	72.08	3.89	<0.05
Residuals	20	3.33			24	222.43		
Acidothermaceae								
Invasion	1	0.13	2.38	0.14	1	1.56	4.00	0.06
Site	3	4.40	26.66	<0.001	3	7.71	6.61	<0.01
Interaction	2	0.15	1.34	0.29	2	1..46	1.88	0.17
Residuals	20	1.10			24	9.34		
AD3								
Invasion					1	8.28	9.72	<0.01
Site					3	19.27	7.54	<0.01
Interaction					2	10.88	6.39	<0.01
Residuals					24	20.44		
Beijerinckiaceae								
Invasion	1	0.09	3.31	0.08	1	0.22	2.57	0.12
Site	3	3.10	36.05	<0.001	3	1.70	6.52	<0.01
Interaction	2	0.26	4.60	0.02	2	1.17	6.74	<0.01
Residuals	20	0.57			24	2.09		
Chitinophagaceae								
Invasion	1	12.03	3.23	0.09	1	0.00	5.40	0.03
Site	3	213.54	19.14	<0.001	3	0.01	11.35	<0.001
Interaction	2	4.30	0.58	0.57	2	0.00	0.60	0.56
Residuals	20	74.39			24	0.01		
Chthoniobacteraceae								

Invasion	1	0.41	9.77	<0.01	1	7.92	2.52	0.13
Site	3	4.26	33.61	<0.001	3	180.20	19.13	<0.001
Interaction	2	0.04	0.46	0.64	2	10.46	1.67	0.21
Residuals	20	0.84			24	7537		
Gaiellaceae								
Invasion					1	0.80	11.73	<0.01
Site					3	2.29	11.26	<0.001
Interaction					2	0.10	0.70	0.50
Residuals					24	1.63		
Gaiellales								
Invasion	1	0.04	0.05	0.82	1	0.31	7.18	0.01
Site	3	14.63	6.53	<0.01	3	3.26	25.27	<0.001
Interaction	2	1.29	0.86	0.44	2	0.16	1.83	0.18
Residuals	20	14.95			24	1.03		
Gemmatimonadaceae								
Invasion					1	0.09	8.57	<0.01
Site					3	0.58	18.05	<0.001
Interaction					2	0.07	3.36	0.05
Residuals					24	0.26		
Holophagae Subgroup 7								
Invasion					1	0.74	3.04	0.09
Site					3	2.69	3.68	0.03
Interaction					2	0.21	0.43	0.65
Residuals					24	5.83		
Hyphomicrobiaceae								
Invasion					1	0.02	9.76	<0.01
Site					3	0.03	3.34	0.01
Interaction					2	0.01	2.75	0.08
Residuals					24	0.05		
Ilumatobacteraceae								
Invasion	1	0.15	8.68	<0.01				
Site	3	3.41	6.76	<0.001				
Interaction	2	0.17	4.80	0.02				
Residuals	20	0.35						
KD4-96								
Invasion					1	0.42	3.24	0.08
Site					3	6.88	17.57	<0.001
Interaction					2	0.64	2.44	0.11
Residuals					24	3.13		
Micromonosporaceae								
Invasion	1	0.60	3.00	0.10				
Site	3	13.30	22.00	<0.001				
Interaction	2	0.85	2.12	0.15				
Residuals	20	4.03						
Nitrosomonadaceae								
Invasion					1	0.47	5.88	0.02
Site					3	0.47	1.97	0.15
Interaction					2	0.26	1.65	0.21
Residuals					24	1.93		

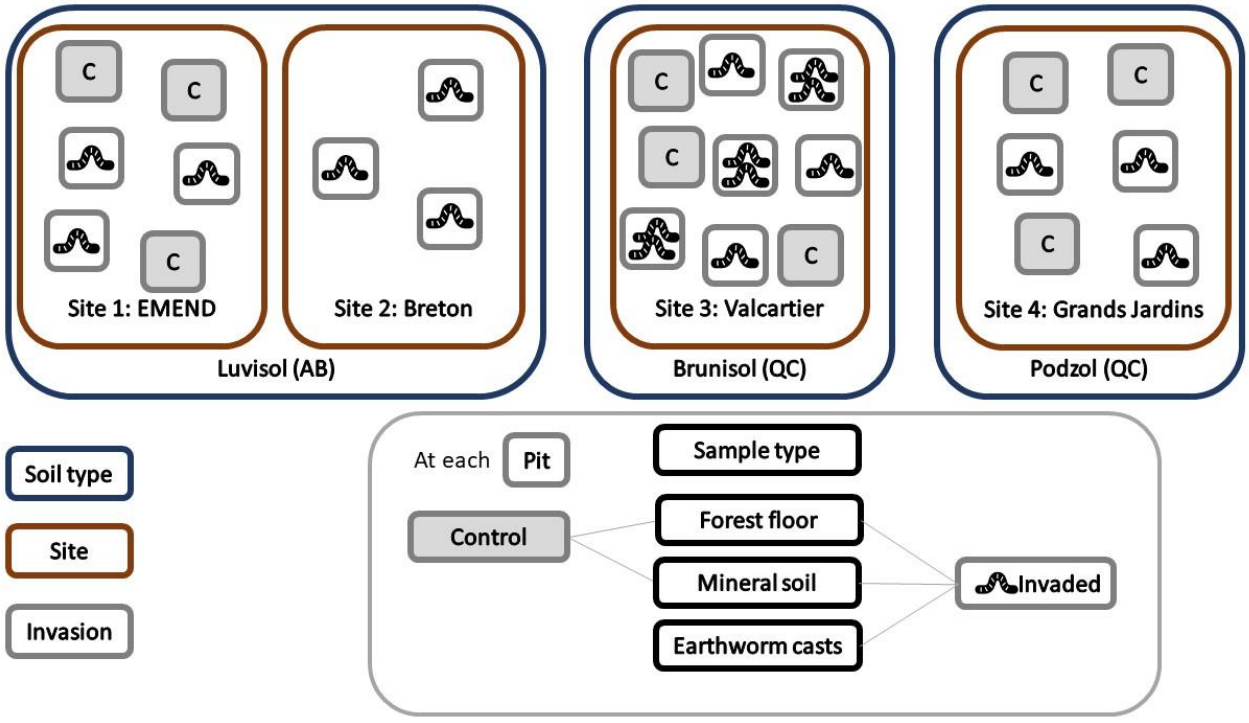
Pirellulaceae								
Invasion	1	0.00	0.00	0.96	1	17.28	11.20	<0.01
Site	3	115.69	19.70	<0.001	3	41.74	9.01	<0.001
Interaction	2	1.76	0.45	0.64	2	3.47	1.13	0.34
Residuals	20	39.15			24	37.03		
Reyranellaceae								
Invasion	1	0.36	3.20	0.09	1	0.60	8.56	<0.01
Site	3	3.34	9.78	<0.001	3	3.09	14.79	<0.001
Interaction	2	0.02	0.11	0.90	2	0.18	1.27	0.30
Residuals	20	2.27			24	1.67		
Solibacteraceae								
Invasion					1	0.53	3.14	0.09
Site					3	1.28	2.61	0.08
Interaction					2	0.27	0.79	0.46
Residuals					24	4.08		
Solirubrobacteraceae								
Invasion	1	0.28	7.83	0.01	1	0.09	0.10	0.76
Site	3	0.67	6.24	<0.01	3	6.26	2.30	0.10
Interaction	2	0.07	1.00	0.38	2	6.48	3.58	0.04
Residuals	20	0.71			24	21.74		
Thermoanaerobaculaceae								
Invasion					1	0.50	5.30	0.03
Site					3	0.90	3.20	0.04
Interaction					2	1.07	5.73	<0.001
Residuals					24	2.25		
Vicinamibacterales								
Invasion	1	2.56	0.98	0.33	1	0.15	9.06	<0.01
Site	3	90.47	11.56	<0.001	3	0.20	3.89	0.02
Interaction	2	0.75	0.14	0.87	2	0.08	2.35	0.12
Residuals	20	52.19			24	0.40		
Xiphinematobacteraceae								
Invasion					1	0.53	11.09	<0.01
Site					3	1.12	7.89	<0.001
Interaction					2	0.67	7.06	<0.01
Residuals					24	1.14		
Species richness								
Invasion	1	39462	2.00	0.17	1	23984	5.18	0.03
Site	4	276632	3.50	0.02	4	25386	1.37	0.27
Interaction	2	1588	0.04	0.96	2	5137	0.55	0.58
Residuals	22	435112			28	129653		
Species diversity								
Invasion	1	14631	1.31	0.26	1	10337	3.31	0.08
Site	4	261426	5.86	<0.01	4	20331	1.63	0.20
Interaction	2	3100	0.14	0.87	2	13601	2.18	0.13
Residuals	22	245407			28	87469		

Supplementary Table 3.A-4. Two-way analyses of variance (factors: invasion and site) of microbial ratios and PLFA concentration (total, Gram(+) and Gram(-) bacteria, and fungi), for forest floors and mineral soils separately. The 10Me ratio was obtained by dividing the phospholipid fatty acid (PLFA) 10Me16:0 by 16:0 and the Cyclo ratio by dividing the sum of cy19:0ω9 and cy19:0ω7 divided by 18:0. The only non-PLFA ratio is the Proteobacteria:Acidobacteriota (Proteo:Acido). Df stands for degrees of freedom and Sum sq for sum of squares.

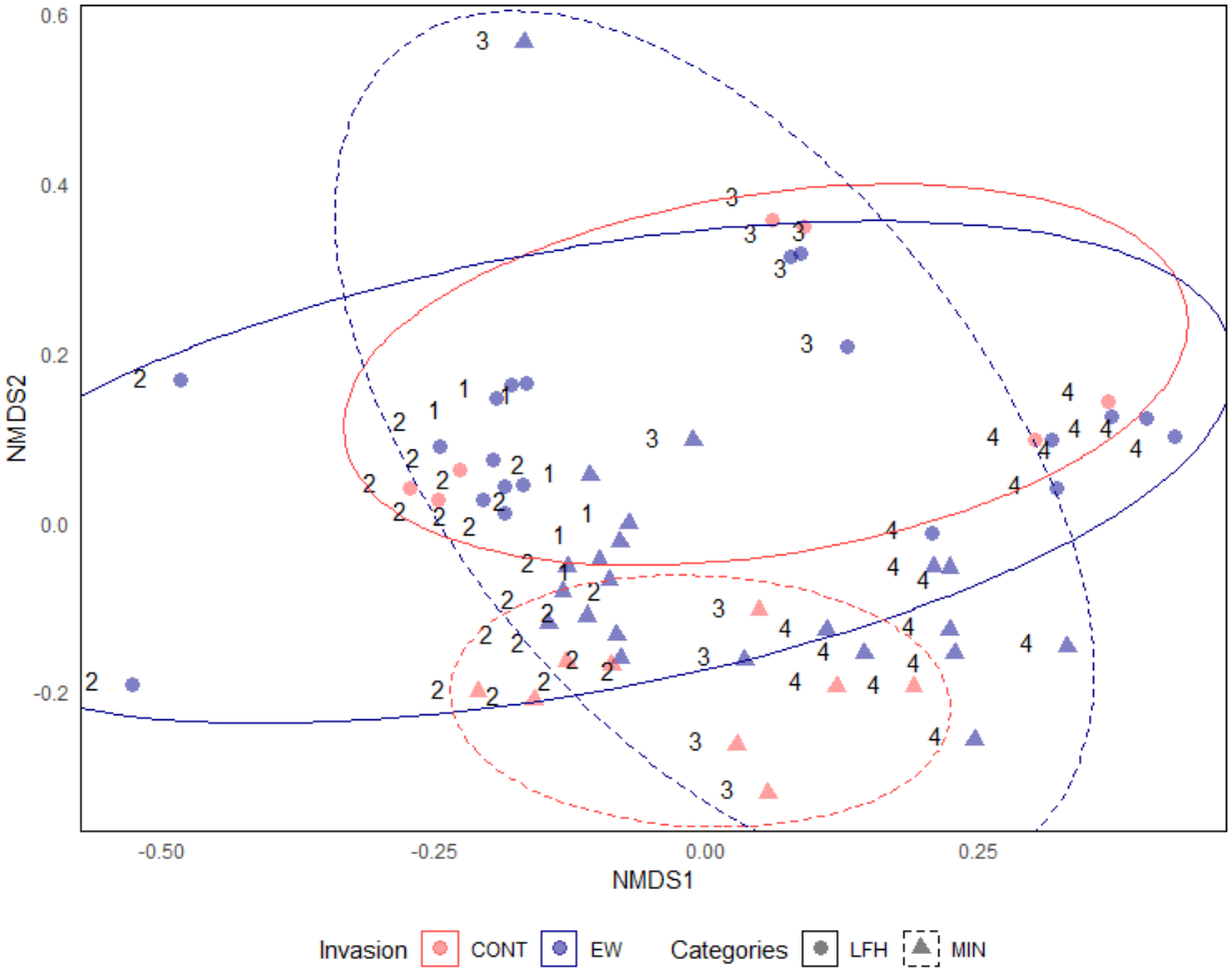
	FOREST FLOOR				MINERAL SOIL			
	Df	Sum Sq	F value	p-value	Df	Sum Sq	F value	p-value
Proteo:Acido								
Invasion	1	0.44	0.38	0.54	1	0.27	8.74	<0.01
Site	3	3.38	0.98	0.42	3	0.48	5.18	<0.01
Interaction	2	2.32	1.01	0.38	2	0.03	0.47	0.63
Residuals	20	22.93			24	0.74		
PLFA total								
Invasion	1	3999847	2.83	0.11	1	<0.01	1.83	0.19
Site	3	2784271	0.66	0.59	3	<0.01	2.46	0.09
Interaction	2	5020883	1.78	0.20	2	<0.01	1.13	0.34
Residuals	20	28282594			24	<0.01		
Gram(+) bacteria								
Invasion	1	76.45	1.57	0.22	1	<0.01	0.57	0.46
Site	3	248.11	1.70	0.20	3	<0.01	4.18	0.02
Interaction	2	37.75	0.39	0.68	2	<0.01	1.10	0.35
Residuals	20	973.43			24	<0.01		
Gram(-) bacteria								
Invasion	1	176110	0.35	0.56	1	<0.01	1.18	0.29
Site	3	883530	0.58	0.63	3	0.01	2.83	0.06
Interaction	2	569931	0.56	0.58	2	<0.01	0.90	0.42
Residuals	20	10089530			24	0.02		
Fungi								
Invasion	1	74965	0.69	0.42	1	0.01	7.40	0.01
Site	3	24812	0.08	0.97	3	0.02	6.70	<0.01
Interaction	2	257837	1.18	0.33	2	<0.01	2.04	0.15
Residuals	20	2187967			24	0.02		
Gram(+):Gram(-)								
Invasion	1	0.08	3.30	0.08	1	1.17	4.11	0.05
Site	3	0.14	2.07	0.14	3	3.00	3.53	0.03
Interaction	2	0.03	0.72	0.50	2	0.05	0.10	0.91
Residuals	20	0.46			24	6.81		
Fungi:Bacteria								
Invasion	1	0.00	0.00	0.97	1	0.18	3.26	0.08
Site	3	0.01	0.60	0.62	3	3.97	23.75	<0.001
Interaction	2	0.01	0.21	0.82	2	0.16	1.44	0.26
Residuals	20	0.01			24	1.34		
10Me ratio								
Invasion	1	0.00	0.09	0.76	1	0.14	9.87	<0.01

Site	3	0.12	2.39	<0.1	3	1.06	25.65	<0.001
Interaction	2	0.01	0.38	0.87	2	0.02	0.56	0.58
Residuals	20	0.32			24	0.33		
Cyclo ratio								
Invasion	1	0.21	1.05	0.31	1	<0.01	8.10	<0.01
Site	3	12.50	20.60	<0.001	3	<0.01	9.89	<0.001
Interaction	2	0.19	0.47	0.63	2	<0.01	0.21	0.81
Residuals	20	3.64			24	<0.01		

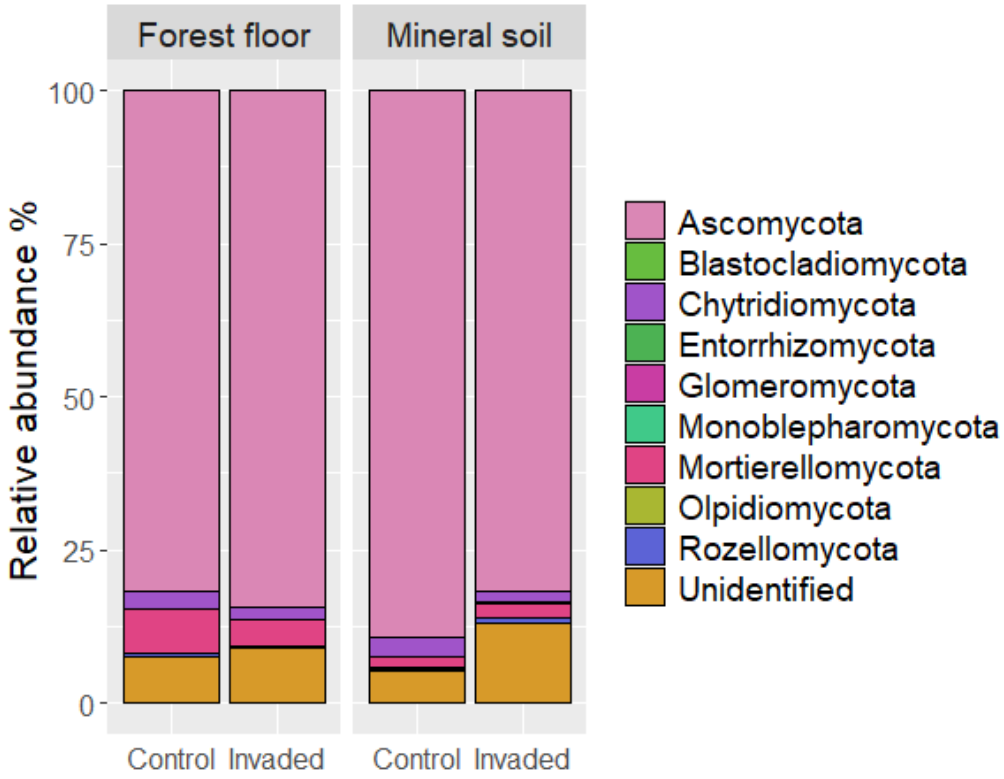
Appendix 3.B. Supplementary figures



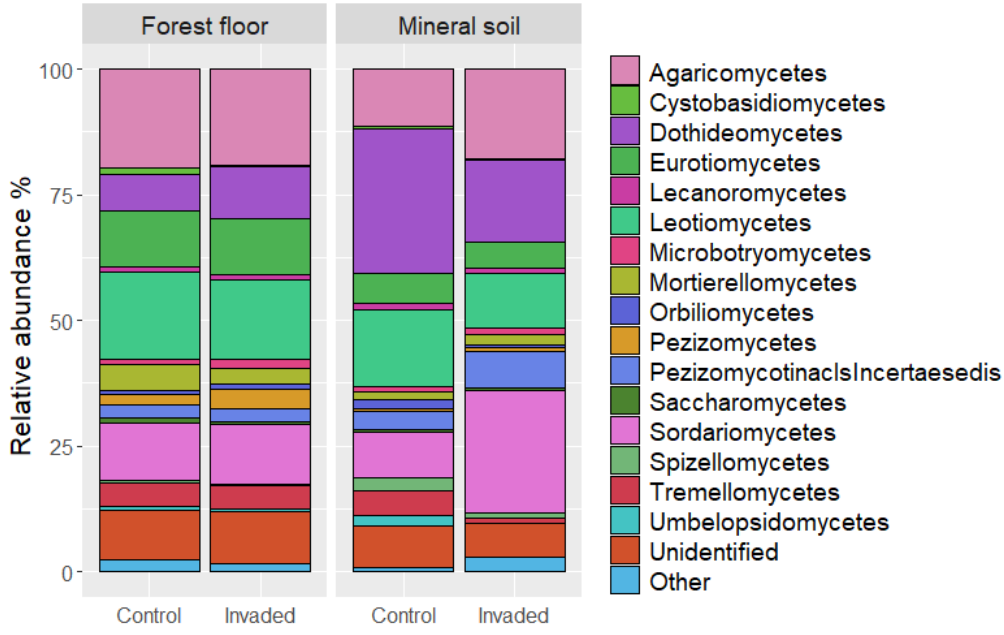
Supplementary Figure 3.B-1. Schematic of the sampling design.



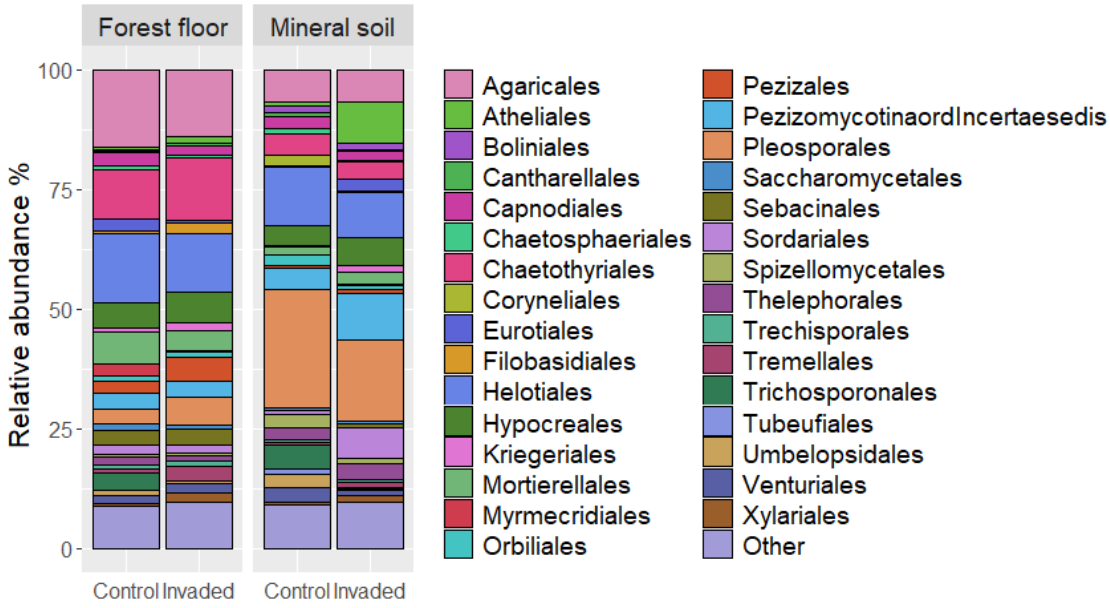
Supplementary Figure 3.B-2. Non-metric multidimensional scaling of fungal amplicon sequence variants (ASVs) using metaMDS function from the vegan package on Bray-Curtis distance matrices after Hellinger transformation of individual ASV abundance (Anderson and Willis, 2003; Legendre and Gallagher, 2001; Oksanen et al., 2019). The final stress was 0.17, non-metric fit R^2=0.97 and linear fit R^2=0.86. Numbers correspond to sites: Breton (1), EMEND (2), Grands Jardins (3) and Valcartier (4).



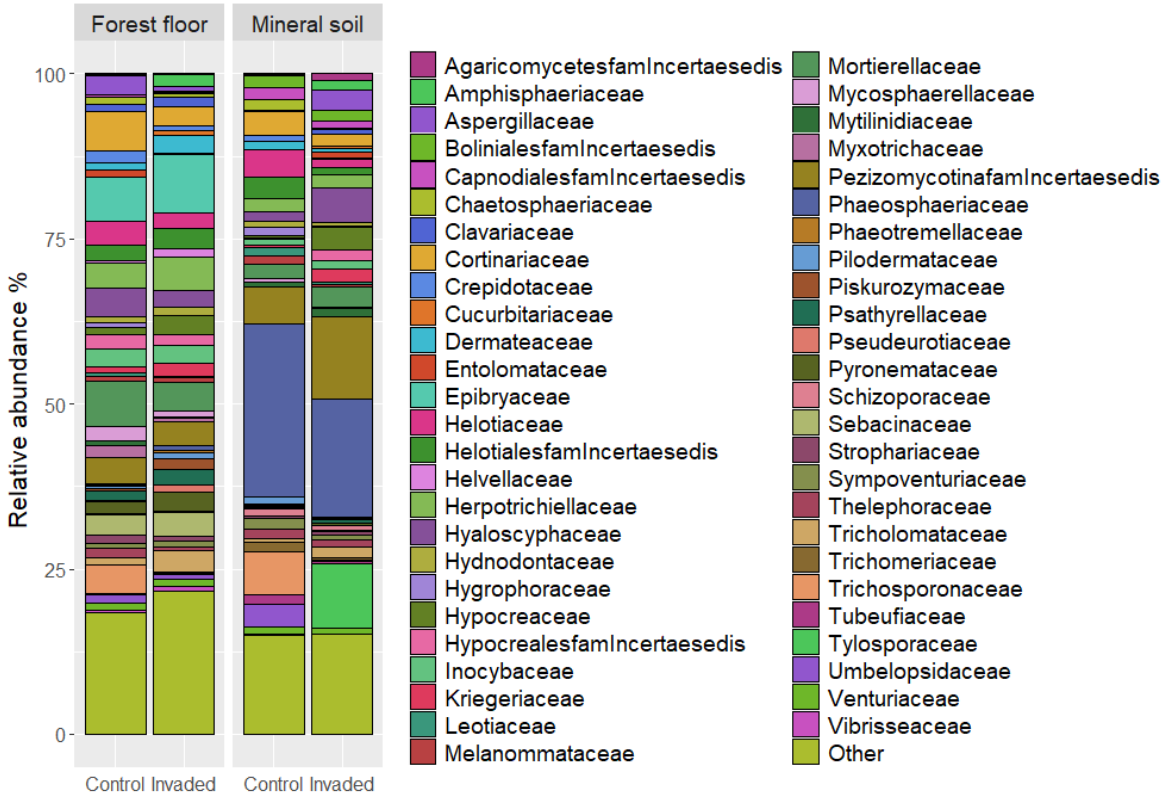
Supplementary Figure 3.B-3. Average relative abundances of fungal phyla representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



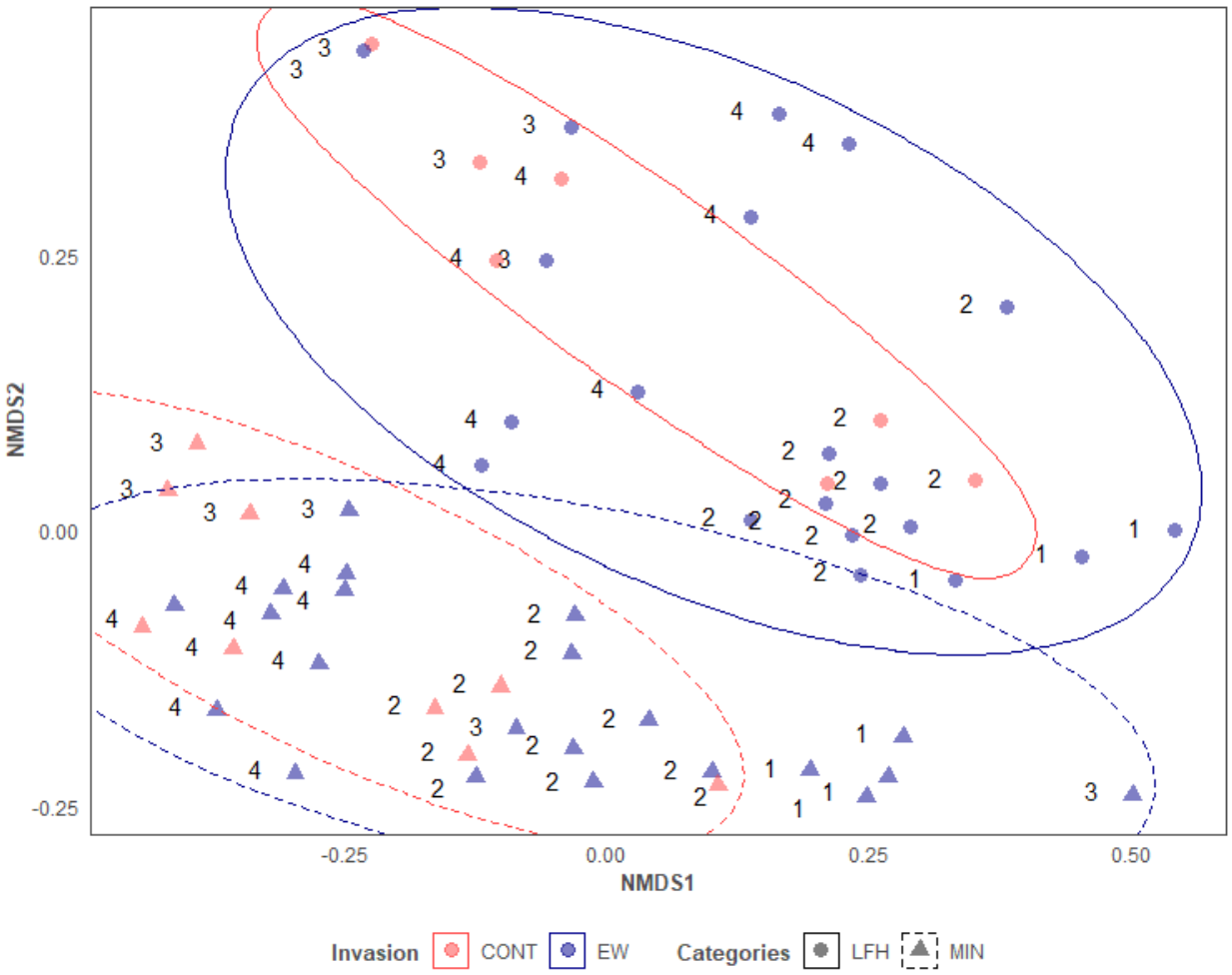
Supplementary Figure 3.B-4. Average relative abundances of fungal classes representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



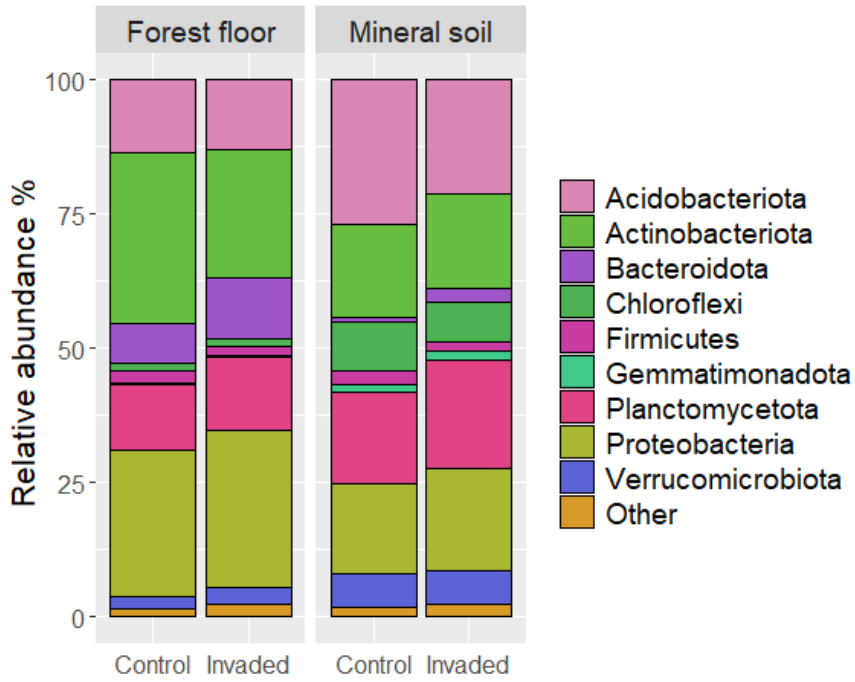
Supplementary Figure 3.B-5. Average relative abundances of fungal orders representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



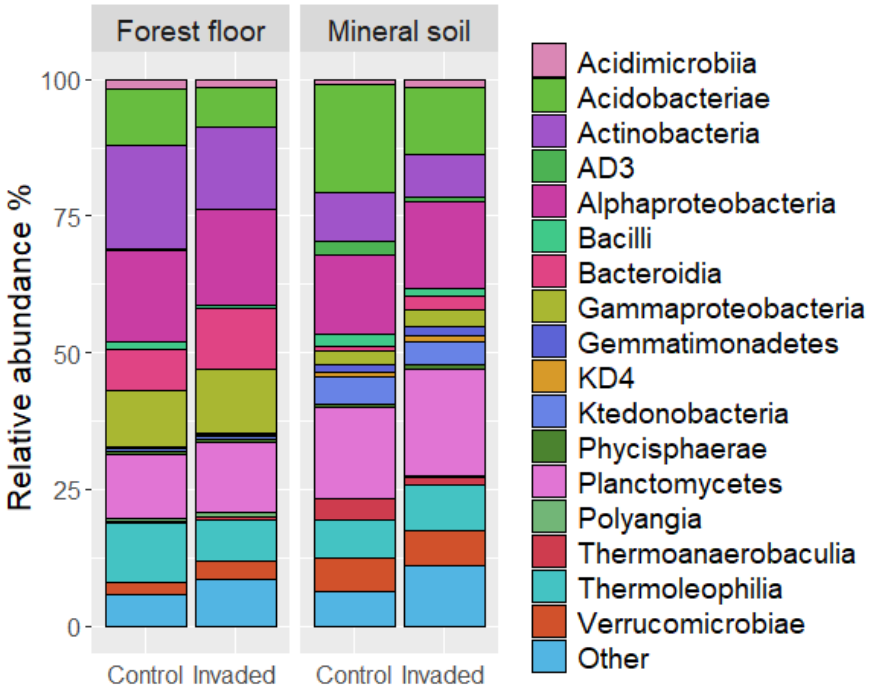
Supplementary Figure 3.B-6. Average relative abundances of fungal families representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



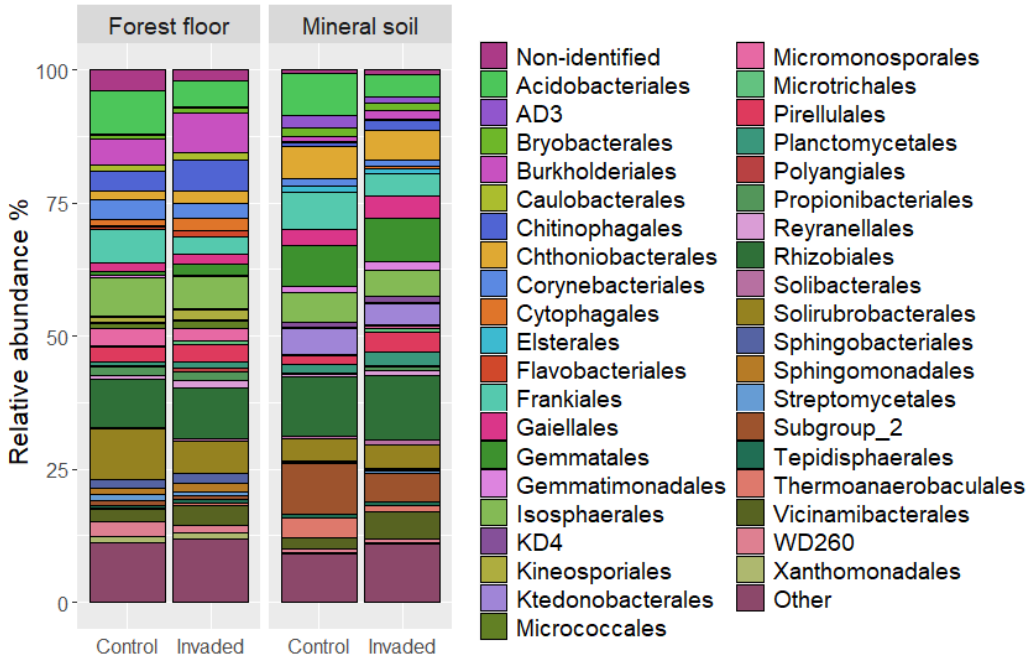
Supplementary Figure 3.B-7. Non-metric multidimensional scaling of bacterial amplicon sequence variants (ASVs) using metaMDS function from the vegan package on Bray-Curtis distance matrices after Hellinger transformation of individual ASV abundance (Anderson and Willis, 2003; Legendre and Gallagher, 2001; Oksanen et al., 2019). The final stress was 0.13, non-metric fit R^2=0.98 and linear fit R^2=0.92. Numbers correspond to sites: Breton (1), EMEND (2), Grands Jardins (3) and Valcartier (4).



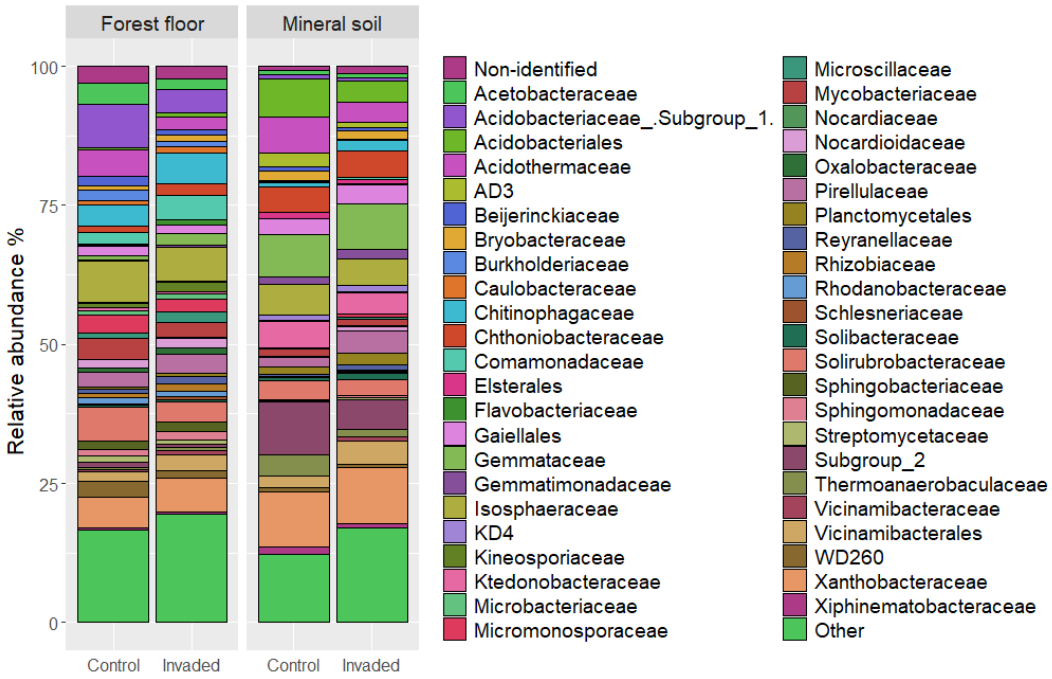
Supplementary Figure 3.B-8. Average relative abundances of bacterial phyla representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



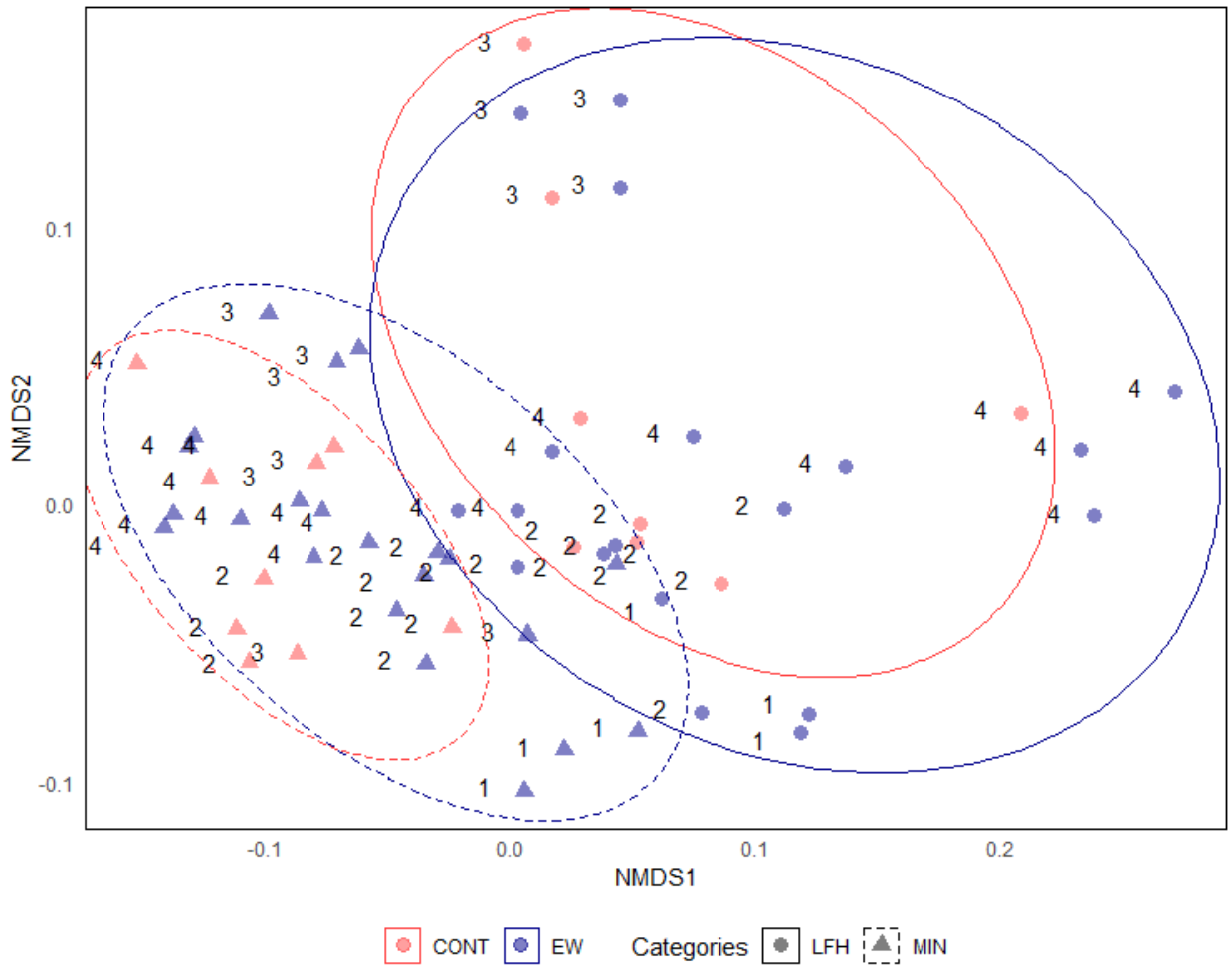
Supplementary Figure 3.B-9. Average relative abundances of bacterial classes representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



Supplementary Figure 3.B-10. Average relative abundances of bacterial orders representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



Supplementary Figure 3.B-11. Average relative abundances of bacterial families representing > 1% of the number of reads in control and earthworm-invaded forest floors and mineral soils.



Supplementary Figure 3.B-12. Non-metric multidimensional scaling of phospholipid fatty acids (PLFAs) using metaMDS function from the vegan package on Bray-Curtis distance matrices after Hellinger transformation of individual PLFA abundance (Anderson and Willis, 2003; Legendre and Gallagher, 2001; Oksanen et al., 2019). The final stress was 0.10, non-metric fit R^2=0.99 and linear fit R^2=0.96. Numbers correspond to sites: Breton (1), EMEND (2), Grands Jardins (3) and Valcartier (4).

Appendix 3.C. Supplementary data and analyses

1. Alternate statistical analysis of DNA data

An index of read-count proportions (referred to as "eDNA index") was calculated for the three main fungal guilds and the bacterial phyla following Kelly et al. (2019):

$$eDNA\ Index_{ij} = \frac{\frac{C_{ij}}{\sum_i C_i}}{max_j(\frac{C_{ij}}{\sum_i C_i})}$$

It corresponds to the relative abundance within a sample ($\frac{C_{ij}}{\sum_i C_i}$) divided by the maximum relative abundance found across samples ($max_j\left(\frac{C_{ij}}{\sum_i C_i}\right)$), resulting in a standardized index ranging from 0 to 1. Compared to relative abundance, the eDNA index showed better correlation with actual biomass changes and is therefore considered a more robust quantitative approach (Kelly et al., 2019). However, as preliminary analyses showed similar trends between relative abundance and eDNA index, I decided to keep relative abundance in Chapter 3 for better readability. In support of this statement, I am reporting the results of the eDNA index analyses of variance in the present appendix.

Supplementary Table 3.C-1. Average eDNA index (± 1 standard error) of the three major fungal guilds (ectomycorrhizal, pathogenic, and saprotrophic) identified with FUNGuild using ITS2 amplicon size variants. Different letters indicate significant differences between control and earthworm-invaded soils, for each horizon.

	Forest floor		Mineral soil	
	Control	Invaded	Control	Invaded
Ectomycorrhizal fungi	0.38 (0.11) a	0.43 (0.07) a	0.37 (0.08) b	0.66 (0.06) a
Pathogenic fungi	0.27 (0.13) a	0.19 (0.06) a	0.08 (0.05) a	0.03 (0.01) a
Saprotrophic fungi	0.49 (0.06) a	0.50 (0.06) a	0.63 (0.08) a	0.36 (0.06) b

Supplementary Table 3.C-2. Two-way analyses of variance using eDNA index data from the three major fungal guilds (ectomycorrhizal, pathogenic, and saprotrophic) identified with FUNGuild using ITS2 amplicon size variants.

			Df	Sum Sq	F value	p-value
Forest floor	Ectomycorrhizal fungi	Invasion	1	<0.01	<0.01	0.99
		Site	3	1.40	12.87	<0.001
		Interaction	2	0.07	0.92	0.42
		Residuals	20	0.72		
	Pathogenic fungi	Invasion	1	0.03	0.57	0.46
		Site	3	0.79	5.86	<0.01
		Interaction	2	0.03	0.30	0.75
		Residuals	20	0.90		
	Saprotrophic fungi	Invasion	1	0.01	0.30	0.59
		Site	3	0.45	6.10	0.01
		Interaction	2	0.05	1.01	0.38
		Residuals	20	0.49		
Mineral soil	Ectomycorrhizal fungi	Invasion	1	0.55	15.55	<0.001
		Site	3	0.90	8.46	<0.001
		Interaction	2	0.02	0.33	0.72
		Residuals	24	0.85		
	Pathogenic fungi	Invasion	1	<0.01	0.18	0.68
		Site	3	0.24	3.24	0.04
		Interaction	2	<0.01	0.02	0.98
		Residuals	24	0.58		
	Saprotrophic fungi	Invasion	1	0.42	12.83	<0.01
		Site	3	0.76	7.64	<0.001
		Interaction	2	0.05	0.74	0.49
		Residuals	24	0.79		

Supplementary Table 3.C-3. Average eDNA index (±1 standard error) of the major bacterial phyla obtained from 16S amplicon size variants. Different letters indicate significant differences between control and earthworm-invaded soils, for each horizon.

	Forest floor		Mineral soil	
	Control	Invaded	Control	Invaded
Acidobacteriota	0.38 (0.06) a	0.36 (0.03) a	0.76 (0.05) a	0.59 (0.04) b
Actinobacteriota	0.68 (0.07) a	0.52 (0.04) b	0.37 (0.05) a	0.38 (0.03) a
Bacteroidota	0.31 (0.08) a	0.47 (0.06) a	0.04 (0.02) b	0.11 (0.03) a
Chloroflexi	0.10 (0.03) a	0.11 (0.02) a	0.63 (0.07) a	0.51 (0.07) b
Firmicutes	0.13 (0.09) a	0.09 (0.05) a	0.14 (0.05) a	0.09 (0.02) a
Gemmatimonadota	0.05 (0.02) a	0.06 (0.02) a	0.24 (0.09) b	0.29 (0.06) a
Planctomycetota	0.36 (0.07) a	0.40 (0.05) a	0.51 (0.04) a	0.60 (0.04) a
Proteobacteria	0.57 (0.05) a	0.61 (0.04) a	0.35 (0.02) b	0.39 (0.02) a
Verrucomicrobiota	0.18 (0.03) b	0.28 (0.03) a	0.52 (0.10) a	0.53 (0.05) a

Supplementary Table 3.C-4. Two-way analyses of variance using eDNA index data from the major bacterial phyla obtained from 16S amplicon size variants.

			Df	Sum Sq	F value	p-value
Forest floor	Acidobacteriota	Invasion	1	<0.01	<0.01	0.98
		Site	3	0.06	1.56	0.23
		Interaction	2	0.04	1.54	0.24
		Residuals	20	0.28		
	Actinobacteriota	Invasion	1	0.08	3.41	0.08
		Site	3	0.15	2.01	0.15
		Interaction	2	0.04	0.87	0.43
		Residuals	20	0.50		
	Bacteroidota	Invasion	1	0.02	0.82	0.38
		Site	3	0.93	12.11	<0.001
		Interaction	2	<0.01	0.06	0.95
		Residuals	20	0.51		
	Chloroflexi	Invasion	1	<0.01	0.21	0.65
		Site	3	0.05	6.99	<0.01
		Interaction	2	<0.01	0.99	0.39
		Residuals	20	0.04		
	Firmicutes	Invasion	1	<0.01	0.06	0.81
		Site	3	0.34	4.32	0.02
		Interaction	2	0.05	0.91	0.42
		Residuals	20	0.52		
	Gemmatimonadota	Invasion	1	<0.01	0.05	0.83
		Site	3	0.04	25.60	<0.001
		Interaction	2	<0.01	0.06	0.95
		Residuals	20	0.01		
	Planctomycetota	Invasion	1	0.01	0.14	0.71

		Site	3	0.01	0.07	0.97
		Interaction	2	0.01	0.09	0.91
		Residuals	20	0.84		
	Proteobacteria	Invasion	1	0.02	0.90	0.36
		Site	3	0.17	3.15	<0.05
		Interaction	2	0.01	0.40	0.67
		Residuals	20	0.35		
	Verrucomicrobiota	Invasion	1	0.02	5.95	0.02
		Site	3	0.28	24.44	<0.001
		Interaction	2	0.01	0.85	0.44
		Residuals	20	0.08		
Mineral soil	Acidobacteriota	Invasion	1	0.06	3.65	0.07
		Site	3	0.28	5.90	<0.01
		Interaction	2	0.01	0.39	0.68
		Residuals	24	0.38		
	Actinobacteriota	Invasion	1	0.00	0.00	0.99
		Site	3	0.29	12.43	<0.001
		Interaction	2	0.00	0.21	0.82
		Residuals	24	0.19		
	Bacteroidota	Invasion	1	0.66	10.39	<0.01
		Site	3	2.16	11.27	<0.001
		Interaction	2	0.18	1.40	0.27
		Residuals	24	1.53		
	Chloroflexi	Invasion	1	0.11	3.92	0.06
		Site	3	1.30	16.17	<0.001
		Interaction	2	0.10	1.79	0.19
		Residuals	24	0.65		
	Firmicutes	Invasion	1	0.01	0.92	0.35
		Site	3	0.03	1.22	0.32
		Interaction	2	0.05	3.39	0.05
		Residuals	24	0.19		
	Gemmatimonadota	Invasion	1	0.05	9.02	<0.01
		Site	3	0.31	17.86	<0.001
		Interaction	2	0.04	3.77	0.04
		Residuals	24	0.14		
	Planctomycetota	Invasion	1	<0.01	0.30	0.59
		Site	3	0.27	7.24	<0.01
		Interaction	2	0.02	0.72	0.50
		Residuals	24	0.30		
	Proteobacteria	Invasion	1	0.01	6.90	0.01
		Site	3	0.05	10.05	<0.001
		Interaction	2	0.03	7.84	<0.01
		Residuals	24	0.04		
	Verrucomicrobiota	Invasion	1	0.03	0.79	0.38
		Site	3	0.65	5.42	<0.01
		Interaction	2	0.01	0.15	0.86
		Residuals	24	0.97		

2. Comparing microbial communities of earthworm casts and bulk soils

Using the same combined PLFA and DNA metabarcoding approach, the microbial community composition of earthworm casts was also analyzed and then compared with that of bulk earthworm-invaded soils (forest floor and mineral soil).

Variables were analyzed by two-way analyses of variance (factors: sample type and site), followed by HSD Tukey posthoc test, or Schreirer-Ray-Hare test (factors: sample type and site) when normality assumption was violated, followed by Dunn posthoc test.

a. Fungal ASVs

Supplementary Table 3.C-5. Average relative abundances of fungal guilds (n=20-22) with standard errors in parentheses. Different letters denote significant differences (p-value < 0.1) among sample types, as determined by two-way analyses of variance (factors: sample type and site) followed by posthoc HSD Tukey test.

Sample type	Ectomycorrhizal fungi	Pathogenic fungi	Saprotrophic fungi
Earthworm casts	17.9 (4.5) c	13.4 (3.3) a	68.8 (4.9) a
Forest floor	40.8 (6.7) b	11.7 (3.1) a	47.4 (5.0) b
Mineral soil	63.5 (5.2) a	2.1 (0.5) b	34.4 (5.0) c

Supplementary Table 3.C-6. Two-way analyses of variance (factors: sample type and site) of fungal guild relative abundances. Df stands for degrees of freedom and Sum sq for sum of squares.

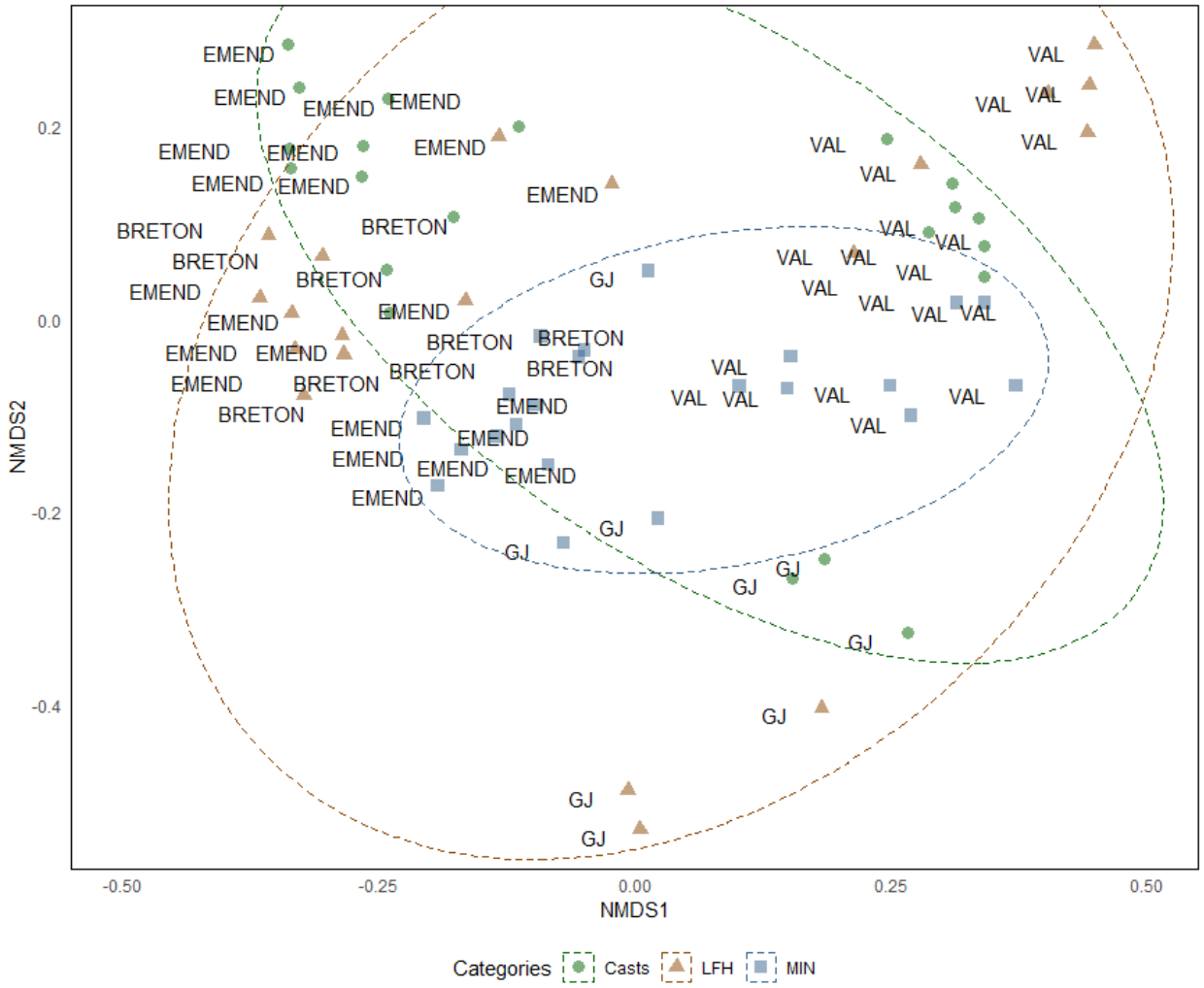
		Df	Sum Sq	F value	p-value
Ectomycorrhizal fungi	Sample type	2	2.31	44.25	<0.001
	Site	3	2.17	27.70	<0.001
	Interaction	6	0.30	1.93	0.09
	Residuals	51	1.33		
Pathogenic fungi	Sample type	2	0.51	11.37	<0.001
	Site	3	0.42	6.12	<0.01
	Interaction	6	0.21	1.54	0.18
	Residuals	51	1.15		
Saprotrophic fungi	Sample type	2	1.28	20.30	<0.001
	Site	3	1.16	12.25	<0.001
	Interaction	6	0.29	1.53	0.19
	Residuals	51	1.61		

Supplementary Table 3.C-7. Average species richness and diversity of fungal guilds (n=20-22) with standard errors in parentheses. Different letters denote significant differences (p-value < 0.1) among sample types, as determined by two-way analyses of variance (factors: sample type and site) followed by HSD Tukey posthoc test.

	Ectomycorrhizal		Pathogenic		Saprotrophic	
	Richness	Diversity	Richness	Diversity	Richness	Diversity
Casts	8.3 (1.8) b	3.4 (0.6) c	8.3 (0.7) a	4.3 (0.5) a	27.6 (1.9) a	8.2 (1.2) b
Forest floor	16.5 (2.8) a	8.4 (1.4) a	7.1 (1.0) a	4.2 (0.6) a	33.9 (3.1) a	14.9 (2.2) a
Mineral soil	17.1 (1.1) a	6.1 (0.8) b	3.3 (0.6) b	2.6 (0.5) b	27.6 (2.4) a	14.5 (1.2) a

Supplementary Table 3.C-8. Two-way analyses of variance (factors: sample type and site) of fungal guild species richness and diversity. Df stands for degrees of freedom and Sum sq for sum of squares.

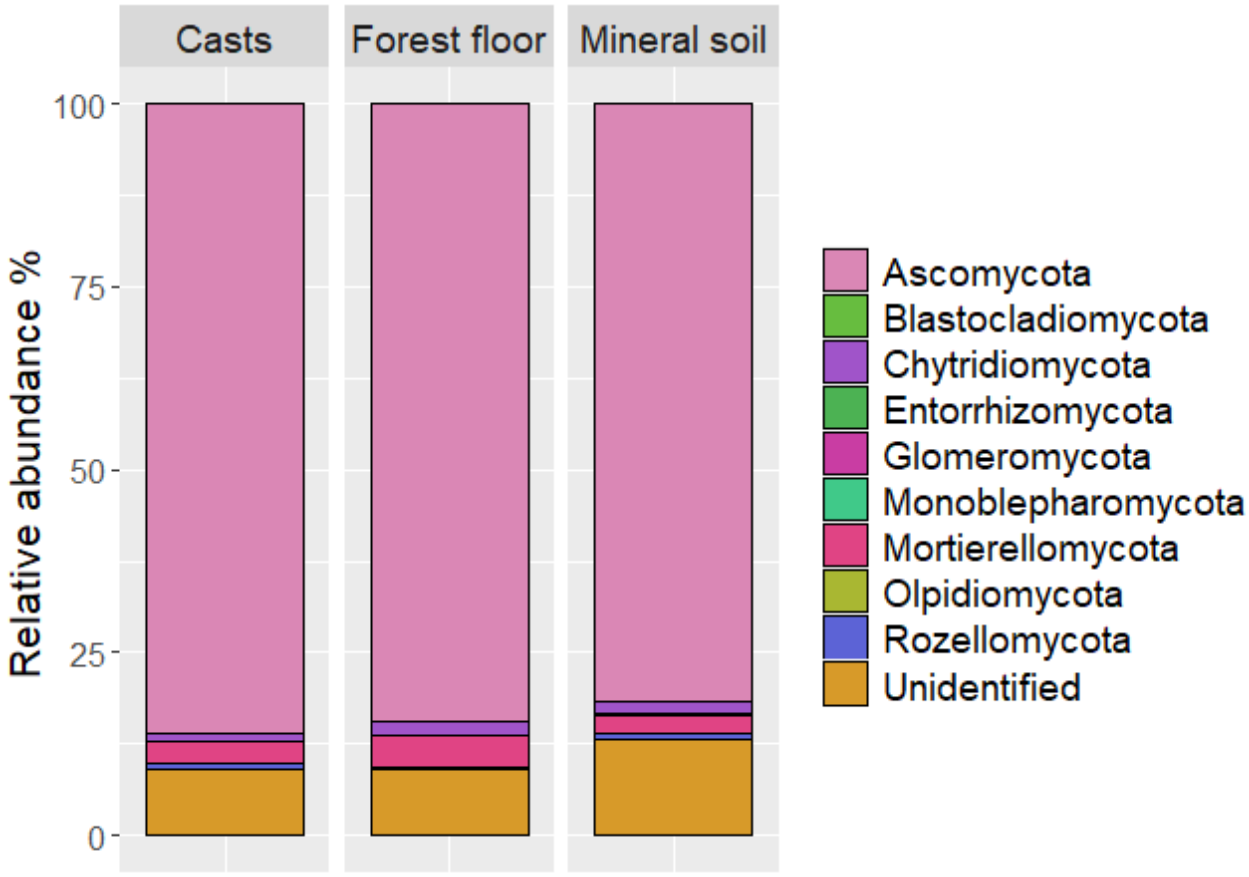
			Df	Sum sq	F	p
Ectomycorrhizal	Richness	Sample type	2	793.96	15.86	<0.001
		Site	3	1607.30	21.41	<0.001
		Interaction	6	719.75	4.79	<0.001
		Residuals	43	1076.13		
	Diversity	Sample type	2	211.46	11.36	<0.001
		Site	3	181.94	6.52	<0.001
		Interaction	6	270.77	4.85	<0.001
		Residuals	43	400.23		
Saprotrophic	Richness	Sample type	2	438.5	2.54	0.09
		Site	3	360.6	1.39	0.26
		Interaction	6	1506.9	2.91	0.02
		Residuals	43	3714.7		
	Diversity	Sample type	2	488.11	9.02	<0.001
		Site	3	523.22	6.45	<0.01
		Interaction	6	489.90	3.02	0.01
		Residuals	43	1163.06		
Pathogenic	Richness	Sample type	2	238.84	16.09	<0.001
		Site	3	148.14	6.65	<0.001
		Interaction	6	31.02	0.70	0.65
		Residuals	43	319.15		
	Diversity	Sample type	2	0.47	5.29	<0.01
		Site	3	0.48	3.54	0.02
		Interaction	6	0.09	0.35	0.91
		Residuals	43	1.93		



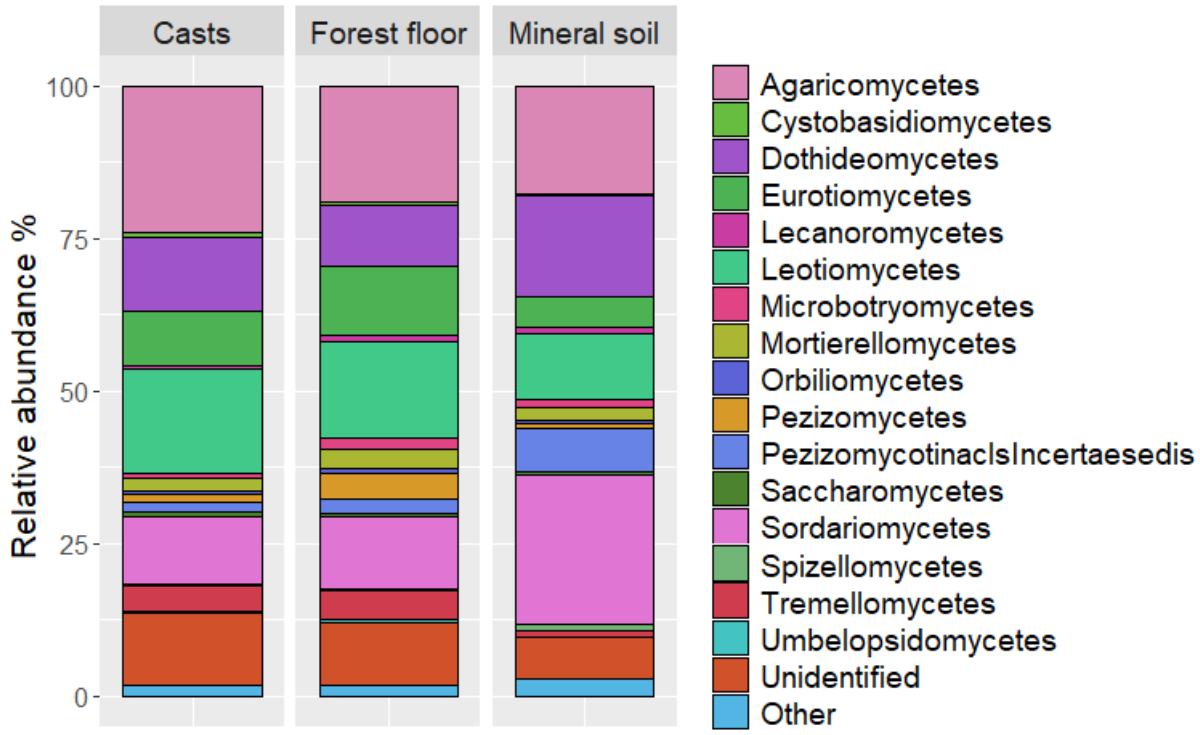
Supplementary Figure 3.C-1. Non-metric multidimensional scaling of fungal amplicon sequence variants (ASVs) using metaMDS function from the vegan package on Bray-Curtis distance matrices after Hellinger transformation of individual ASV abundance using earthworm casts, invaded forest floors and invaded mineral soils (Anderson and Willis, 2003; Legendre and Gallagher, 2001; Oksanen et al., 2019). The final stress was 0.17, non-metric fit R^2=0.97 and linear fit R^2=0.87. The following sites were included: Breton, EMEND, Grands Jardins (GJ) and Valcartier (VAL).

Supplementary Table 3.C-9. Permutational analysis of variance (PERMANOVA) performed on Hellinger-transformed data for fungal amplicon sequence variants (ASVs) using Bray-Curtis distance matrix. Df stands for degrees of freedom and Sum sq for sum of squares.

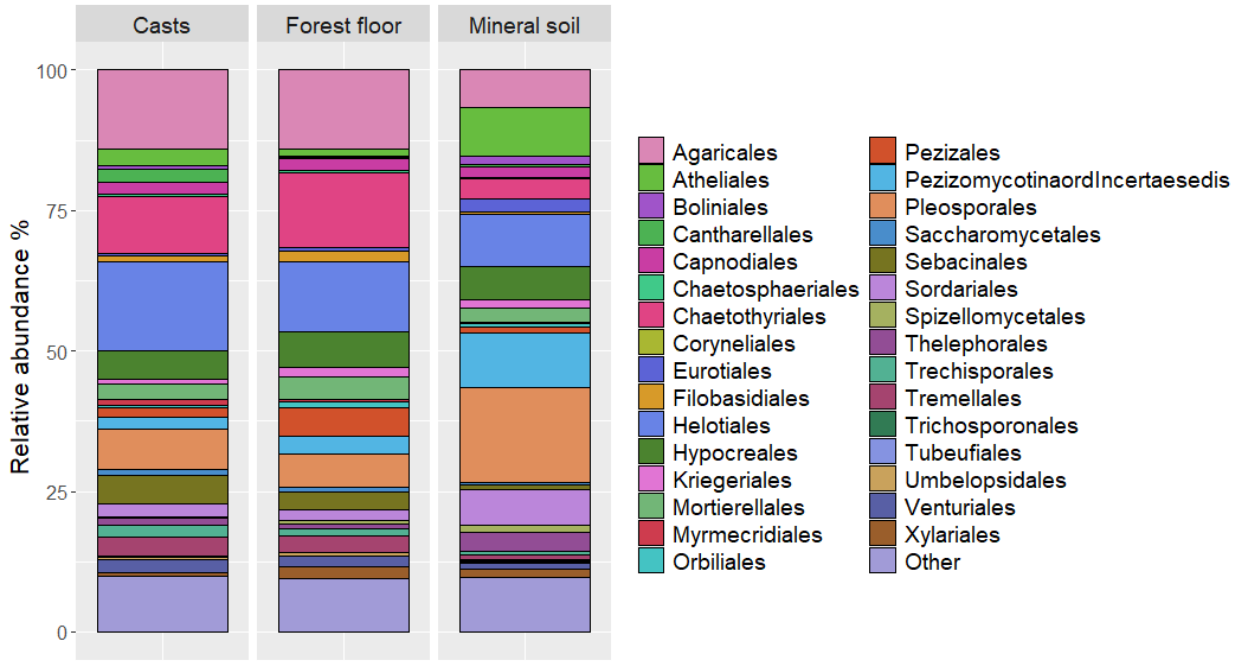
	Df	Sum sq	F model	R^2	p-value
Sample type	2	1.76	3.31	0.08	<0.001
Site	3	4.33	5.42	0.19	<0.001
Interaction	6	3.14	1.96	0.14	<0.001
Residuals	51	13.58		0.60	
Total	62	22.81		1.00	



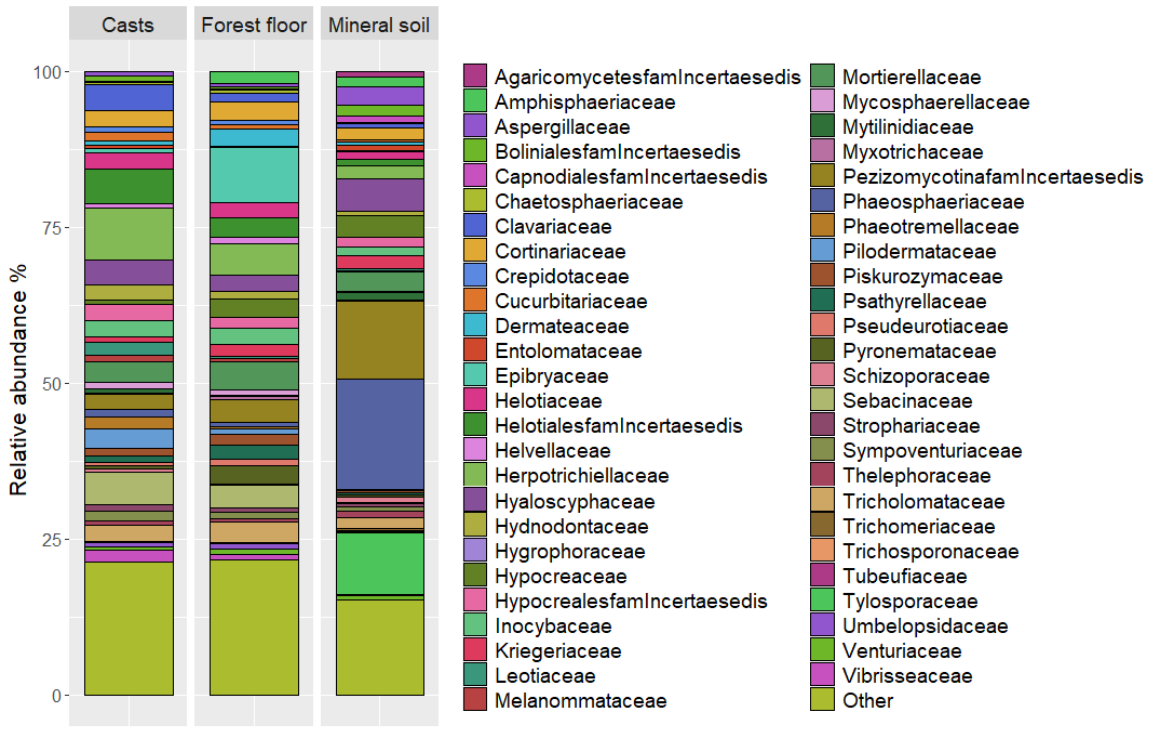
Supplementary Figure 3.C-2. Average relative abundances of fungal phyla representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.



Supplementary Figure 3.C-3. Average relative abundances of fungal classes representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.



Supplementary Figure 3.C-4. Average relative abundances of fungal orders representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.



Supplementary Figure 3.C-5. Average relative abundances of fungal families representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.

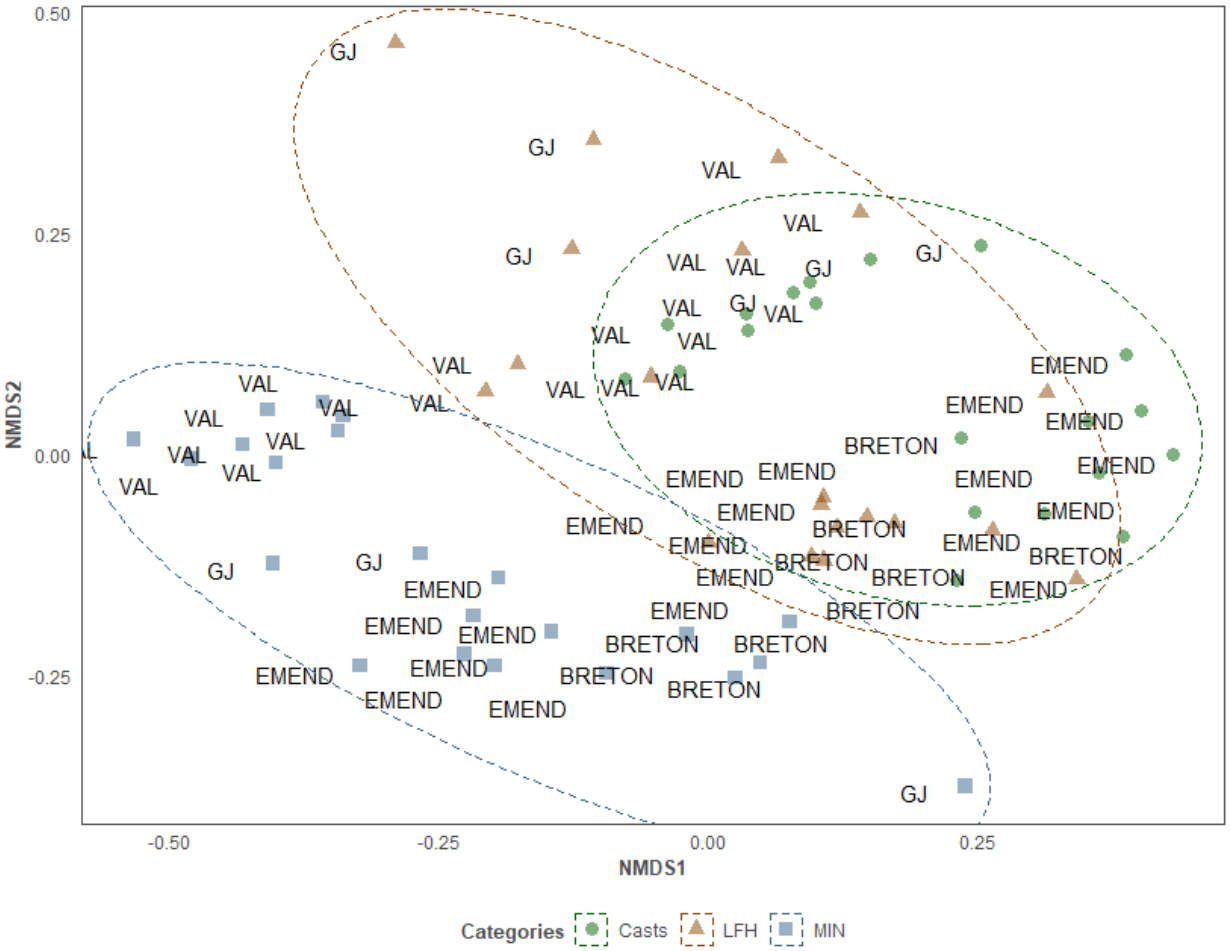
b. *Bacterial ASVs*

Supplementary Table 3.C-10. Average relative abundance of the bacterial phyla, with standard errors in parentheses (n = 20–22). Different letters denote significant differences (p-value < 0.1) among sample types, as determined by two-way analyses of variance (factors: sample type and site) followed by HSD Tukey posthoc test. Only phyla accounting for > 1 % of relative abundance are displayed.

	Earthworm casts	Forest floor	Mineral soil
Acidobacteriota	7.8 (0.7) c	12.9 (1.0) b	21.1 (1.2) a
Actinobacteriota	18.4 (1.6) b	24.1 (1.8) a	17.7 (1.4) b
Bacteroidota	17.5 (1.2) a	11.2 (1.4) b	2.7 (0.6) c
Chloroflexi	1.6 (0.2) b	1.5 (0.2) b	7.4 (0.9) a
Firmicutes	0.5 (0.2) b	1.6 (0.9) ab	1.6 (0.4) a
Gemmatimonadota	0.4 (0.1) b	0.3 (0.1) c	1.7 (0.4) a
Planctomycetota	19.3 (1.9) a	13.7 (1.5) b	20.3 (1.2) a
Proteobacteria	27.8 (0.7) a	29.1 (1.7) a	18.9 (0.7) b
Verrucomicrobiota	3.1 (0.3) b	3.3 (0.4) b	6.3 (0.6) a
Species richness	596 (25) a	587 (40) a	567 (33) a
Diversity	381 (18) a	367 (33) a	335 (13) a

Supplementary Table 3.C-11. Two-way analyses of variance of bacterial phylum relative abundances. Df stands for degrees of freedom, Sum sq for sum of squares.

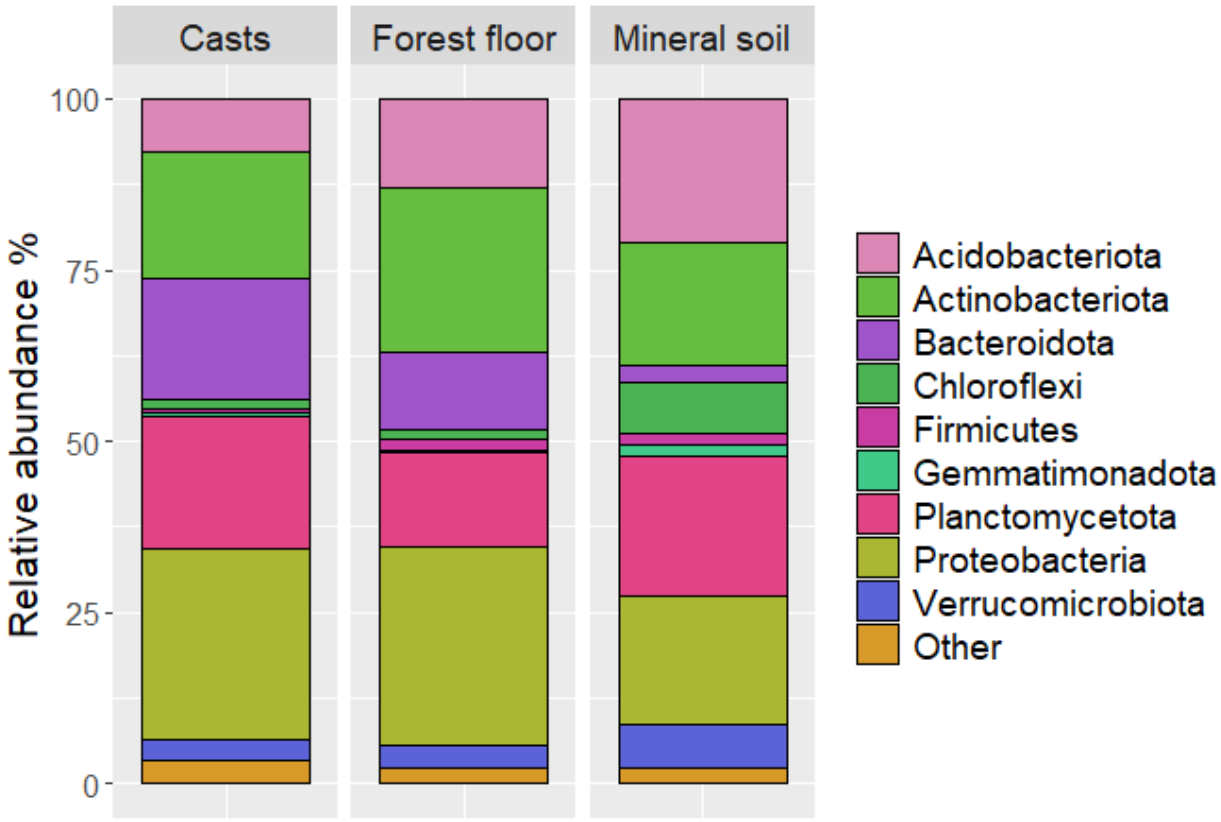
		Df	Sum Sq	F value	p-value
Acidobacteriota	Sample type	2	0.20	71.15	<0.001
	Site	3	0.02	4.86	<0.01
	Interaction	6	0.02	2.49	0.03
	Residuals	51	0.07		
Actinobacteriota	Sample type	2	0.04	6.90	<0.01
	Site	3	0.08	8.32	<0.001
	Interaction	6	0.06	3.20	<0.01
	Residuals	51	0.16		
Bacteroidota	Sample type	2	0.24	109.71	<0.001
	Site	3	0.06	16.97	<0.001
	Interaction	6	0.02	3.63	<0.01
	Residuals	51	0.06		
Chloroflexi	Sample type	2	0.34	82.12	<0.001
	Site	3	0.05	8.19	<0.001
	Interaction	6	0.10	7.88	<0.001
	Residuals	51	0.10		
Firmicutes	Sample type	2	0.08	5.93	<0.01
	Site	3	0.18	9.32	<0.001
	Interaction	6	0.12	3.16	0.01
	Residuals	51	0.33		
Gemmatimonadota	Sample type	2	0.17	48.25	<0.001
	Site	3	0.05	10.19	<0.001
	Interaction	6	0.07	6.21	<0.001
	Residuals	51	0.09		
Planctomycetota	Sample type	2	0.05	9.52	<0.001
	Site	3	0.08	10.93	<0.001
	Interaction	6	0.08	5.34	<0.001
	Residuals	51	0.12		
Proteobacteria	Sample type	2	2.39	54.28	<0.001
	Site	3	0.34	5.14	<0.01
	Interaction	6	0.46	3.50	<0.01
	Residuals	51	1.12		
Verrucomicrobiota	Sample type	2	<0.01	30.64	<0.001
	Site	3	<0.01	13.05	<0.001
	Interaction	6	<0.01	4.54	<0.001
	Residuals	51	<0.01		
Species richness	Sample type	2	<0.01	0.48	0.62
	Site	3	<0.01	2.56	0.07
	Interaction	6	<0.01	2.50	0.03
	Residuals	51	<0.01		
Diversity	Sample type	2	15.11	1.59	0.21
	Site	3	63.39	4.45	<0.01
	Interaction	6	106.74	3.75	<0.01
	Residuals	51	242.05		



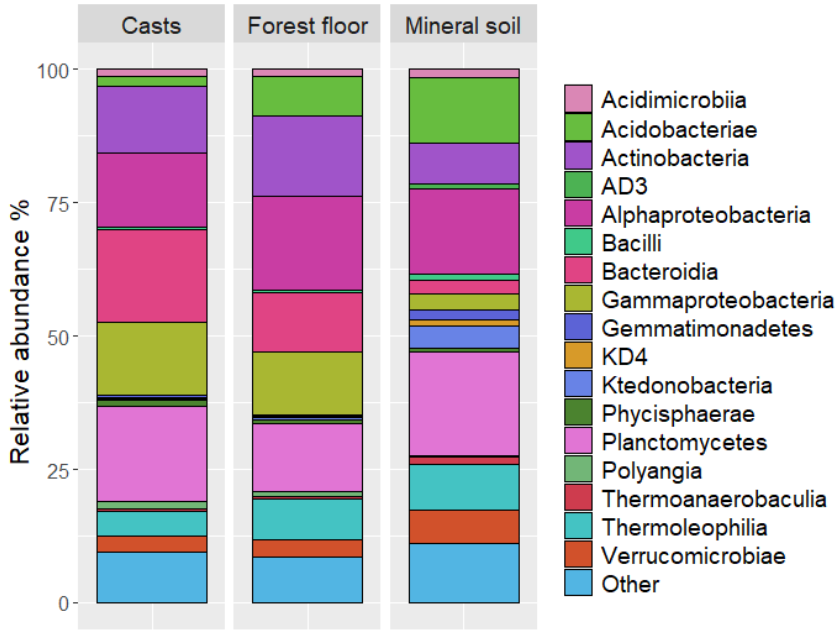
Supplementary Figure 3.C-6. Non-metric multidimensional scaling of bacterial amplicon sequence variants (ASVs) using metaMDS function from the vegan package on Bray-Curtis distance matrices after Hellinger transformation of individual ASV abundance using earthworm casts, invaded forest floors and invaded mineral soils (Anderson and Willis, 2003; Legendre and Gallagher, 2001; Oksanen et al., 2019). The final stress was 0.15, non-metric fit R^2=0.98 and linear fit R^2=0.89. The following sites were included: Breton, EMEND, Grands Jardins (GJ) and Valcartier (VAL).

Supplementary Table 3.C-12. Permutational analysis of variance (PERMANOVA) performed on Hellinger-transformed data for bacterial amplicon sequence variants (ASVs) using Bray-Curtis distance matrix. Df stands for degrees of freedom and Sum sq for sum of squares.

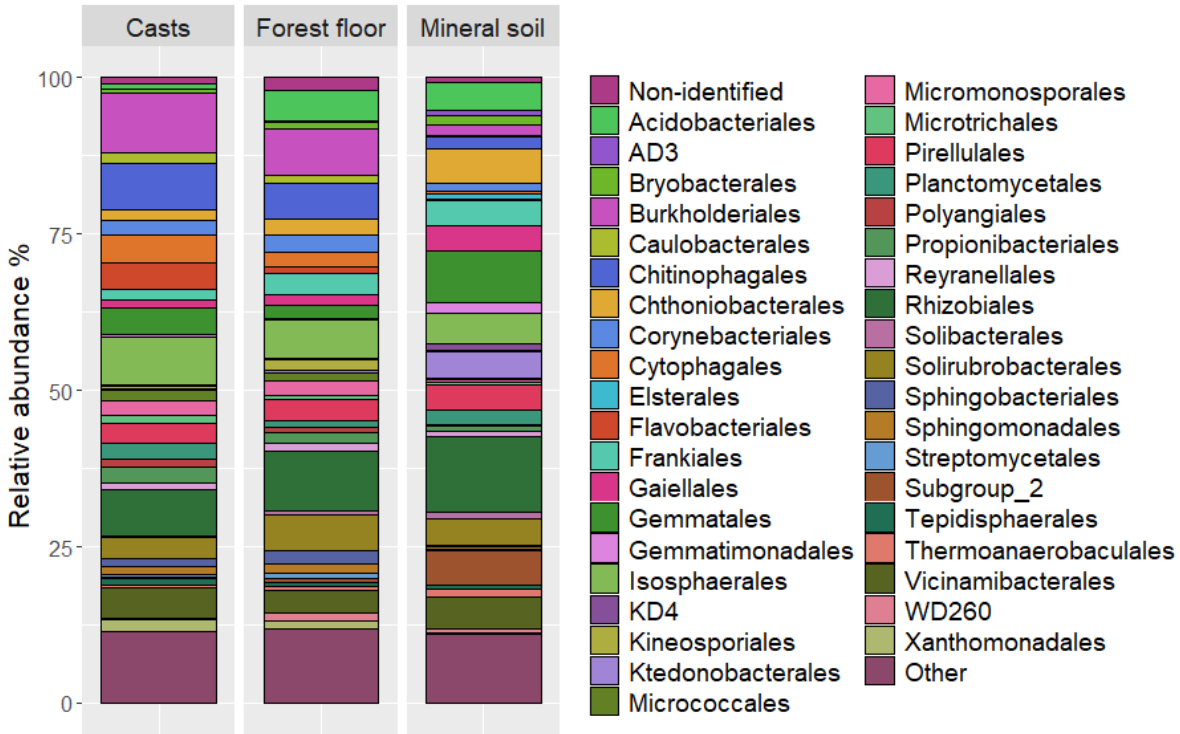
	Df	Sum sq	F model	R^2	p-value
Sample type	2	3.69	9.52	0.16	<0.001
Site	3	5.15	8.69	0.22	<0.001
Interaction	6	4.61	3.96	0.20	<0.001
Residuals	50	9.69		0.42	
Total	61	23.05		1.00	



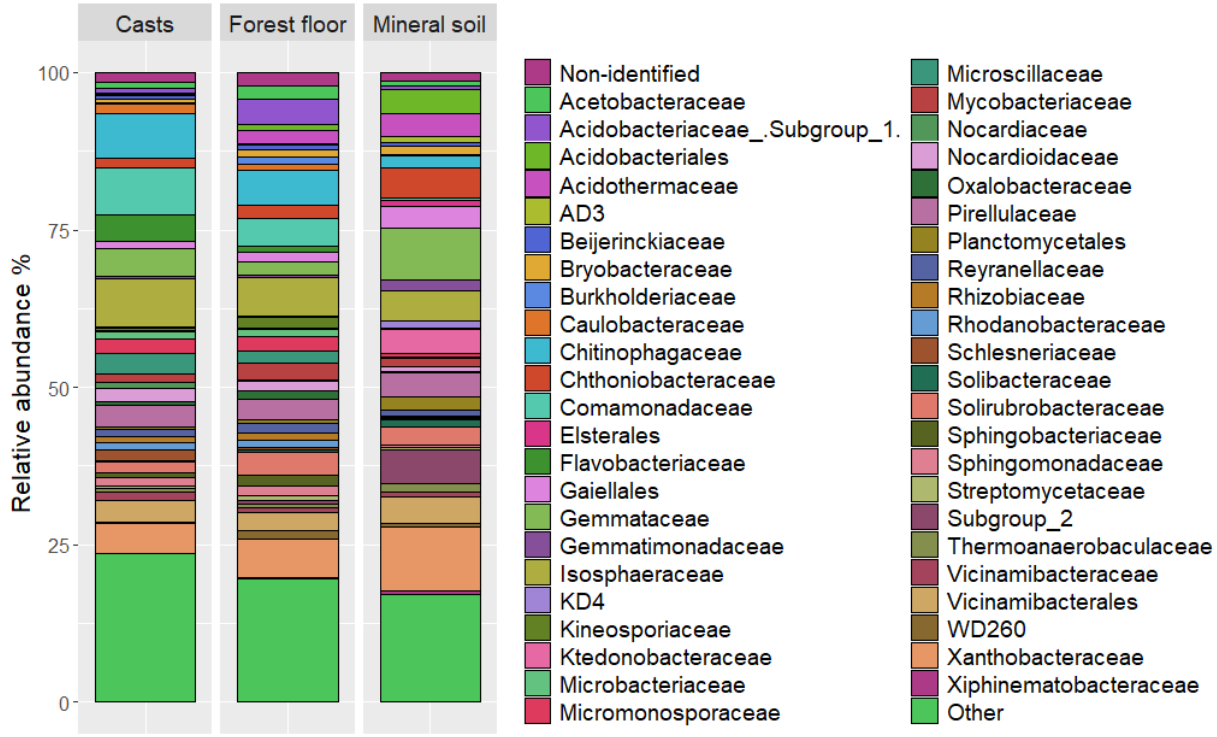
Supplementary Figure 3.C-7. Average relative abundances of bacterial phyla representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.



Supplementary Figure 3.C-8. Average relative abundances of bacterial classes representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.



Supplementary Figure 3.C-9. Average relative abundances of bacterial orders representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.



Supplementary Figure 3.C-10. Average relative abundances of bacterial families representing > 1% of the number of reads in earthworm casts, invaded forest floors, and invaded mineral soils.

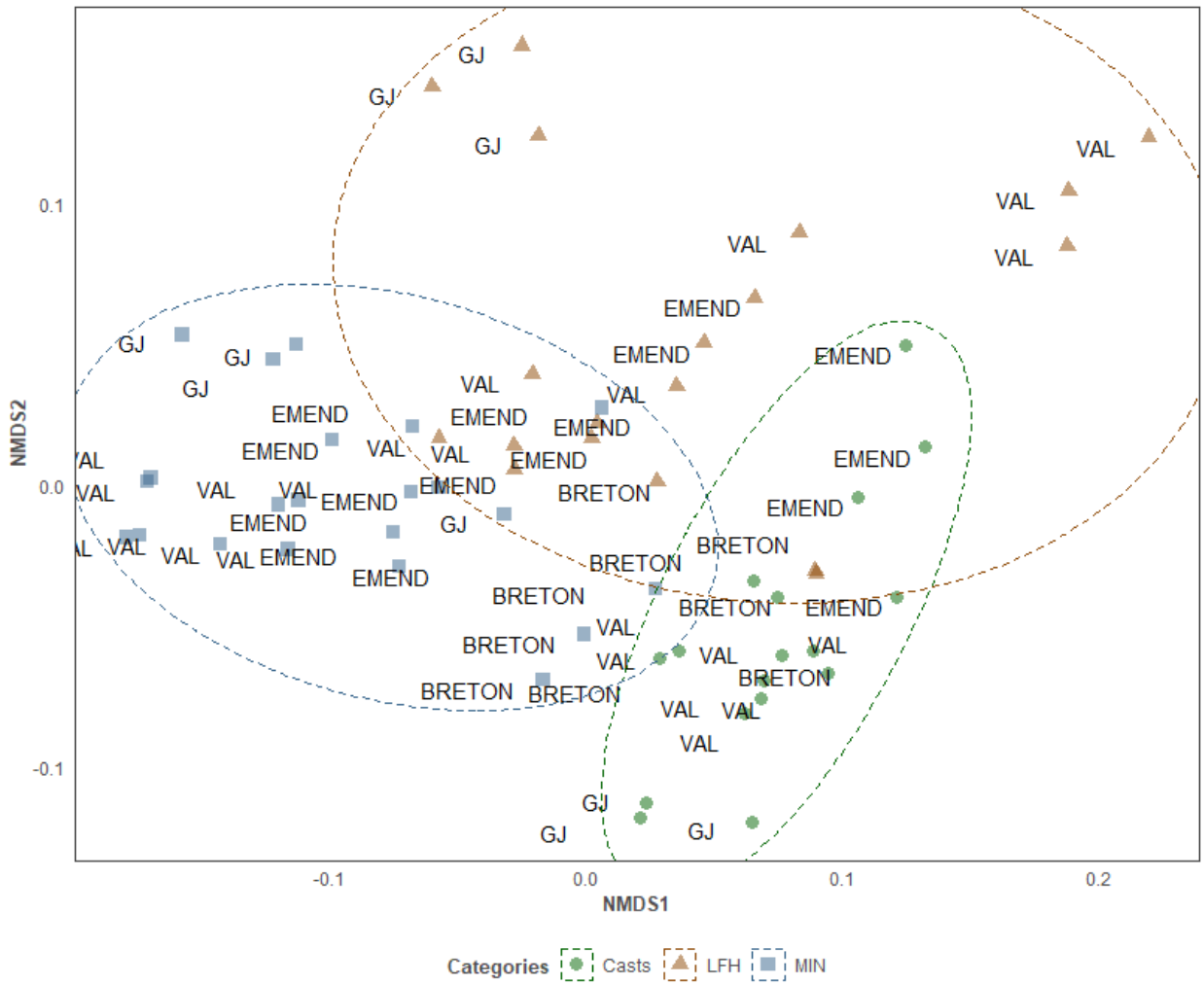
c. *Phospholipid fatty acids (PLFAs)*

Supplementary Table 3.C-13. Average concentrations (nmol.g-soil^{-1}) of total, Gram(+) and (-) bacteria, and fungal phospholipid fatty acids (PLFAs) and PLFA ratios, with standard errors in parentheses (n = 17–22). Different letters denote significant differences (p-value < 0.1) among sample types, as determined by the Schreirer-Ray-Hare test (factors: sample type and site) followed by Dunn posthoc test.

		Earthworm casts	Forest floor	Mineral soil
Concentration	Total PLFA	5195 (494) a	5700 (305) a	1684 (302) b
	Gram(+) bacteria	1006 (105) a	1029 (88) a	477 (96) b
	Gram(-) bacteria	857 (91) a	943 (81) a	397 (65) b
	Fungi	544 (71) b	910 (77) a	93 (22) c
Ratios	Gram(+):Gram(-)	1.19 (0.05) a	1.14 (0.08) a	1.16 (0.04) a
	Fungi:Bacteria	0.18 (0.03) b	0.30 (0.03) a	0.09 (0.02) c
	10Me ratio	0.02 (0.02) c	0.16 (0.04) b	0.48 (0.06) a
	Cyclo ratio	0.17 (0.01) b	0.35 (0.05) a	0.41 (0.03) a

Supplementary Table 3.C-14. Results of the two-way analysis of variance (ANOVA, when normality of residues is obtained) or the nonparametric Scheirer–Ray–Hare Test (when data does not meet normality of residues criteria) for PLFA concentrations and ratios. Df stands for degrees of freedom and Sum sq for sum of squares. H and F values are obtained from Scheirer-Ray-Hare test or ANOVA, respectively.

			Df	Sum Sq	H/F value	p-value
Scheirer-Ray-Hare	Total PLFA	Sample type	2	9785	34.31	<0.001
		Site	3	583	2.04	0.56
		Interaction	6	1816	6.37	0.38
		Residuals	46	3996		
	Gram(+) bacteria	Sample type	2	7539	26.44	<0.001
		Site	3	665	2.33	0.51
		Interaction	6	2459	8.62	0.20
		Residuals	46	5565		
	Gram(-) bacteria	Sample type	2	6866	24.08	<0.001
		Site	3	913	3.20	0.36
		Interaction	6	2152	7.54	0.27
		Residuals	46	6229		
	Fungi	Sample type	2	12038	42.22	<0.001
		Site	3	915	3.21	0.36
		Interaction	6	1191	4.18	0.65
		Residuals	46	2349		
	Gram(+):Gram(-)	Sample type	2	612	2.15	0.35
		Site	3	2205	7.73	0.05
		Interaction	6	3341	11.72	0.07
		Residuals	46	10059		
	Fungi:Bacteria	Sample type	2	8405	52.73	<0.001
		Site	3	2823	10.03	0.02
		Interaction	6	1693	4.52	0.43
		Residuals	46	3541		
	10Me ratio	Sample type	2	7834	29.30	<0.001
		Site	3	2042	7.64	0.05
		Interaction	6	2391	8.94	0.18
		Residuals	46	2587		
ANOVA	Cyclo ratio	Sample type	2	0.60	46.78	<0.001
		Site	3	0.28	14.89	<0.001
		Interaction	6	0.28	7.28	<0.001
		Residuals	44	0.28		



Supplementary Figure 3.C-11. Non-metric multidimensional scaling of phospholipid fatty acids (PLFAs) using metaMDS function from the vegan package on Bray-Curtis distance matrices after Hellinger transformation of individual ASV abundance using earthworm casts, invaded forest floors and invaded mineral soils (Anderson and Willis, 2003; Legendre and Gallagher, 2001; Oksanen et al., 2019). The final stress was 0.11, non-metric fit R^2=0.99 and linear fit R^2=0.94. The following sites were included: Breton, EMEND, Grands Jardins (GJ) and Valcartier (VAL).

Supplementary Table 3.C-15. Permutational analysis of variance (PERMANOVA) performed on Hellinger-transformed data for phospholipid fatty acids (PLFAs) using Bray-Curtis distance matrix. Df stands for degrees of freedom and Sum sq for sum of squares.

	Df	Sum sq	F model	R^2	p-value
Sample type	2	0.51	37.52	0.40	<0.001
Site	3	0.24	11.81	0.19	<0.001
Interaction	6	0.20	4.91	0.16	<0.001
Residuals	46	0.32		0.25	
Total	57	1.27		1.00	

d. Results

For most of the variables tested by two-way analysis of variance (ANOVA), the interaction between sample type and site was significant, indicating that the differences among sample types might vary across sites (Suppl. Table 3.C-8, 11, 14). Similarly, the permutational analysis of variance (PERMANOVA) results showed that the fungal, bacterial, and PLFA community compositions were significantly affected by both site and sample type, as well as their interaction (Suppl. Tables 3.C-9, 12, 15; Suppl. Fig. 3.C-1, 6, 11).

The analysis of fungal communities using metabarcoding of ITS2 region revealed that earthworm casts had the lowest relative abundance of EcM fungi (18 %) and the highest of saprotrophic fungi (69 %), compared to forest floors and mineral soils (Suppl. Table 3.C-5 & 6). In earthworm casts, EcM fungi species richness and diversity were at least twice as low as in the forest floors and mineral soils (Suppl. Table 3.C-7). However, despite harbouring higher relative abundance of saprotrophic fungi, their species richness was not higher in earthworm casts, compared to bulk soils, while their diversity was significantly lower (Suppl. Table 3.C-8). At the class, order, and family levels, the general pattern of fungal relative abundances was more similar between earthworm casts and forest floors (Suppl. Fig. 3.C-3 to 5).

Bacterial communities of earthworm casts were more similar to those of forest floors than those of mineral soils. At the phylum level, earthworm casts and forest floors were dominated by Proteobacteria (28–29 %), while mineral soils were dominated by Acidobacteriota (21 %). Forest floors and earthworm casts also had similar relative abundances of Chloroflexi, Firmicutes, and Gemmatimonadota. However, mineral soils and earthworm casts had similar relative abundances of Actinobacteriota (18 %) and Planctomycetota (19 – 20 %). Compared to forest floors and mineral soils, the relative abundances of Acidobacteriota and Bacteroidota were respectively lower and higher in earthworm casts. Bacterial species richness and diversity were similar across sample types (Suppl. Table 3.C-10 & 11).

The microbial biomass, calculated as the total PLFA concentration, was similar in earthworm casts and forest floors and significantly lower in mineral soils (Suppl. Table 3.C-13 & 14). Earthworm casts harboured significantly lower fungal PLFA concentration, compared to the forest floors. While the Gram(+):Gram(-) ratio was similar across sample types, the fungi:bacteria ratio was highest in forest floors, intermediate in earthworm casts, and lowest in mineral soils. The 10Me and cyclo ratios were significantly lower in earthworm casts.

e. Key findings

Microbial communities of earthworm casts appeared to be more similar to those of forest floors compared to mineral soils, in terms of microbial biomass, bacterial community composition, and fungal guild composition. As casts were primarily sampled at the soil surface (i.e. in the forest floor), these results are not surprising.

Compared to bulk soils, the higher relative abundance of Bacteroidota found in earthworm casts could be associated with higher N supply, while lower cyclo ratios would indicate lower environmental stress.

Chapter 4

Appendix 4.A. Supplementary tables

Supplementary Table 4.A-1. Results of the one-way analysis of variance (ANOVA) for fast and active C concentration (mg of C/g of soil). Df stands for degrees of freedom and Sum sq for sum of squares.

				Fast C pool			Active C pool		
		Factor	Df	Sum Sq	F-value	p-value	Sum Sq	F-value	p-value
Brunisol	Forest floor	Invasion	2	584.2	5.36	<0.05	556.6	3.13	0.09
		Residuals	9	490.8			800.3		
	Mineral soil	Invasion	2	1.77	4.09	0.05	5.1	6.18	<0.05
		Residuals	9	1.95			3.7		
Luvisol	Forest floor	Invasion	1	14.1	0.23	0.65	15.3	0.17	0.69
		Residuals	10	626.9			929.2		
	Mineral soil	Invasion	1	0.3	2.03	0.19	0.2	0.23	0.64
		Residuals	10	1.6			6.4		
Podzol	Forest floor	Invasion	1	19.0	1.51	0.26	51.0	2.07	0.20
		Residuals	6	75.3			147.9		
	Mineral soil	Invasion	1	14.4	50.43	<0.001	13.8	30.23	<0.01
		Residuals	6	1.7			2.7		

Supplementary Table 4.A-2. Results of the one-way analysis of variance (ANOVA) for fast and active C pool proportions (%). Df stands for degrees of freedom and Sum sq for sum of squares.

				Fast C pool			Active C pool		
		Factor	Df	Sum Sq	F-value	p-value	Sum Sq	F-value	p-value
Brunisol	Forest floor	Invasion	2	3050.0	4.35	<0.05	2555.3	2.18	0.17
		Residuals	9	3158.3			5283.8		
	Mineral soil	Invasion	2	687.5	8.58	<0.01	1772.9	10.31	<0.01
		Residuals	9	360.5			773.8		
Luvisol	Forest floor	Invasion	1	0.1	<0.01	0.99	4.8	0.01	0.93
		Residuals	10	3304.5			5453.1		
	Mineral soil	Invasion	1	1.2	0.01	0.95	41.5	0.82	0.39
		Residuals	10	2424.1			5008.9		
Podzol	Forest floor	Invasion	1	277.0	4.01	0.09	585.6	4.33	0.08
		Residuals	6	414.7			812.0		
	Mineral soil	Invasion	1	2735.7	32.09	<0.01	2205.5	17.05	<0.01
		Residuals	6	511.4			776.3		

Supplementary Table 4.A-3. Results of the two-way analyses of variance (ANOVAs) for TOC [%wt] of every soil fraction and its corresponding proportion of total C. Df stands for degrees of freedom and Sum sq for sum of squares.

			TOC [%wt]			Proportion of total C [%]		
		Df	Sum Sq	F value	p-value	Sum Sq	F value	p-value
Bulk soil	Invasion	1	0.17	1.72	0.21			
	Site	2	1.82	9.06	<0.01			
	Interaction	2	0.45	2.23	0.15			
	Residuals	14	1.40					
Large macroaggregates	Invasion	1	0.19	1.14	0.30	0.01	0.19	0.67
	Site	2	9.47	28.35	<0.001	0.84	17.00	<0.001
	Interaction	2	0.27	0.80	0.47	0.01	0.17	0.84
	Residuals	14	2.34			0.35		
Small macroaggregates	Invasion	1	0.24	5.02	<0.05	0.05	3.96	0.07
	Site	2	5.38	56.28	<0.001	0.18	7.18	<0.01
	Interaction	2	0.13	1.32	0.30	0.05	2.04	0.17
	Residuals	14	0.67			0.18		
Free microaggregates	Invasion	1	<0.01	7.85	<0.05	<0.01	0.07	0.80
	Site	2	0.08	108.98	<0.001	0.02	0.88	0.44
	Interaction	2	<0.01	2.10	0.16	0.01	0.40	0.68
	Residuals	14	<0.01			0.15		
Free silt and clay	Invasion	1	0.02	8.59	<0.05	<0.01	1.20	0.29
	Site	2	0.15	33.74	<0.001	0.07	9.25	<0.01
	Interaction	2	<0.01	0.38	0.69	<0.01	0.46	0.64
	Residuals	14	0.03			0.06		
Occluded microaggregates	Invasion	1	0.60	1.61	0.23	<0.01	1.67	0.22
	Site	2	27.63	37.20	<0.001	0.01	14.14	<0.001
	Interaction	2	0.67	0.90	0.43	<0.01	2.81	0.09
	Residuals	14	5.20			0.01		
Occluded light fraction	Invasion	1	0.62	12.57	<0.01	0.05	11.08	<0.01
	Site	2	2.36	23.92	<0.001	0.08	8.92	<0.01
	Interaction	2	0.33	3.31	0.07	<0.01	0.40	0.68
	Residuals	14	0.64			0.06		

Supplementary Table 4.A-4. List of compounds identified by pyrolysis-gas chromatography-mass spectrometry and their class (N: N-containing compound; Ps: polysaccharides; FA: fatty acids; Ph: phenols; Ar: other aromatic compounds; Al: other alipathic compounds; T: tannin markers; Lg: lignin markers). Average retention time (RT) is showed in minutes.

Pyrolysis product name		RT	Marker
1-methyl-pyrrolidine	N1	7.9	
2,5-dimethyl-furan	Ps1	8.2	
butanoic acid, methyl ester = methyl butanoate	FA1	8.7	
1-nitroso-pyrrolidine	N2	9.1	
1-methyl-1h-pyrrole	N3	9.6	
pyridine	N4	9.8	
dimethylamino acetonitrile	N5	10.0	
pyrrole	N6	10.3	
acetic acid, methoxy-, methyl ester	Ps2	10.6	
toluene	Ar1	10.9	
propanoic acid, 2-methoxy-, methyl ester	Ps3	12.2	
formamide, n,n-dimethyl-	N7	12.4	
n-dimethyl-n-formylamine	N8	12.6	
acetamide	N9	12.7	
3-furaldehyde	Ps4	13.5	
2-propanol, 1-(dimethylamino)-	N10	13.8	
2-methyl-pyridine	N11	13.9	
2 or 3-methyl-pyridine	N12	14.1	
4-methyl-pyridine	N13	14.2	
furfural	Ps5	14.8	
2-cyclopenten-1-one	Ps6	14.9	
1h-pyrrole, 2,5-dimethyl-	N14	15.0	
2,3-dimethyl-cyclohexa-1,3-diene	Al1	15.5	
acetamide, n-methyl-	N15	15.5	
1h-pyrrole, 2-methyl-	N16	15.5	
1h-pyrrole, 3-methyl-	N17	15.9	
ethylbenzene	Ar2	16.6	
benzenamine	Ar3	16.8	
1,3-cyclohexadiene, 5,6-dimethyl-	Al2	16.9	
m- or p-xylene (=1,3 or 4-dimethylbenzene)	Ar4	17.5	
glycine, n,n-dimethyl-, methyl ester	N18	17.6	
butanoic acid, 2-oxo-, methyl ester	Ps7	17.7	
n,n-dimethylacetamide	N19	17.8	
2-propanol, 1,3-dimethoxy-	Al3	17.9	
(r)-3-hydroxybutyric acid, methyl ether, methyl ester	FA2	18.2	
o -xylene (= 1,2-dimethylbenzene)	Ar5	18.3	

styrene = ethenylbenzene	Ar6	18.5	
2-cyclopenten-1-one, 2-methyl-	Ps8	19.6	
ethanone, 1-(2-furanyl)-	Ps9	19.9	
2,3-dimethoxypropan-1-ol	Al4	20.1	
anisole = methoxybenzene	Ar7	20.3	
1h-pyrrole, 2-ethyl-4-methyl-	N20	21.1	
pyridine, 2,4-dimethyl-	N21	21.4	
pyridine, 3,5-dimethyl-	N22	21.4	
2-cyclopenten-1-one, 3,4-dimethyl-	Ps10	21.7	
2-furancarboxaldehyde, 5-methyl-	Ps11	22.5	
benzene, propyl-	Ar8	22.6	
1h-pyrrole-2-carboxaldehyde, 1-methyl-	N23	22.6	
4-hydroxypiperidine	N24	22.7	
2(1h)-pyridinone, 1-methyl-	N25	22.8	
benzaldehyde	Ar9	23.0	
benzene, 1-ethyl-3-methyl-	Ar10	23.1	
benzene, 1-ethyl-4-methyl-	Ar11	23.2	
1,2,4-triazine, hexahydro-3,5-dione	N26	23.2	
2,4-dimethylfuran	Ps12	23.5	
2-cyclopenten-1-one, 2,3-dimethyl-	Ps13	23.5	
1h-pyrrole, 2,3,5-trimethyl-	N27	23.6	
3,4-dimethylfuran	Ps14	23.6	
2-cyclopenten-1-one, 3-methyl-	Ps15	23.6	
3-furancarboxylic acid, methyl ester	Ps16	24.0	
benzene, 1-ethyl-2-methyl-	Ar12	24.3	
1,2,5-trimethylpyrrole	N28	24.8	
1,3,5-triazine, hexahydro-1,3,5-trimethyl-	N29	24.8	
phenol	Ph1	25.2	
benzene, 1-ethenyl-4-methyl-	Ar13	25.4	
benzene, 1-ethenyl-2-methyl-	Ar14	25.5	
benzene, 2-propenyl-	Ar15	25.7	
2,3,4-trimethylpyrrole	N30	25.9	
benzene, 1,2,3-trimethyl-	Ar16	26.3	
benzene, 1-methoxy-4-methyl-	Ar17	26.7	
mesitylene = benzene, 1,3,5-trimethyl	Ar18	26.9	
but-2-enedioic acid, dimethyl ester	FA3	26.9	
1h-indene, 2,3-dihydro-	Ar19	27.1	
phenol, 4-amino-	Ph2	27.3	
butanedioic acid, dimethyl ester	FA4	27.5	
benzene, 1,2,4-trimethyl-	Ar20	27.8	
indene	Ar21	28.2	
2-pyrrolidinone, 1-methyl-	N31	28.3	
1,2-ethanediamine, n,n'-dibutyl-	N32	28.9	
o-cresol = 2-methyl phenol	Ph3	29.2	

butanedioic acid, methyl-, dimethyl ester	FA5	29.4	
m-cresol = 3-methyl phenol	Ph4	29.5	
p-cresol = 4-methyl phenol	Ph5	30.4	
2,5-pyrrolidinedione, 1-methyl	N33	30.9	
benzoic acid, methyl ester	Ar22	31.1	
2(1h)-pyridinone, 1,3-dimethyl-	N34	31.2	
2(1h)-pyridinone, 3,6-dimethyl-	N35	31.2	
benzofuran, 2-methyl-	Ar23	31.5	
benzene, 1-ethenyl-3,5-dimethyl-	Ar24	31.7	
benzene, 2-ethenyl-1,4-dimethyl-	Ar25	31.7	
benzene, 1-ethynyl-4-methoxy-	Ar26	31.7	
1h-pyrrole, 2,3,4,5-tetramethyl-	N36	32.0	
phenol, 4-ethyl-2-methyl-	Ph6	32.1	
phenol, 2,6-dimethyl-	Ph7	32.2	
methyl 1-methylpyrrole-2-carboxylate	N37	32.6	
phenol, 2,5-dimethyl-	Ph8	33.2	
2-cyclopenten-1-one, 4-methoxy-	Ps17	33.2	
phenol, 3-methoxy-	Lg1	33.3	Lignin
2,4-dimethylstyrene	Ar27	33.5	
benzene, 1,2-dimethoxy-	Ar28	33.8	
2,3-dimethylhydroquinone	Ph9	33.8	
phenol, 3,5-dimethyl-	Ph10	33.9	
benzofuran, 2,3-dihydro-2-methyl-	Ar29	34.2	
1h-indene, 1-methyl-	Ar30	34.2	
1h-indene, 3-methyl-	Ar31	34.4	
naphthalene, 1,2-dihydro-	PAH1	34.5	
benzene, 1,4-dimethoxy-	Ar32	34.7	
phenol, 2,4-dimethyl-	Ph11	35.0	
phenol, 3-methoxy-2-methyl-	Ph12	35.3	
phenol, 2,3-dimethyl-	Ph13	35.3	
benzeneacetic acid, methyl ester	Ar33	35.5	
naphthalene	PAH2	36.0	
phenol, 3,4-dimethyl-	Ph14	36.0	
benzenamine, 3-ethoxy-	Ar34	36.2	
phenol, 2-ethyl-4-methyl-	Ph15	36.5	
phenol, 3-ethyl-5-methyl-	Ph16	36.6	
phenol, 3-(dimethylamino)-	Ph17	37.1	
benzofuran, 4,7-dimethyl-	Ar35	37.4	
2,4(1h,3h)-pyrimidinedione, 1,3-dimethyl-	N38	37.4	
2,4-imidazolidinedione, 3,5,5-trimethyl-	N39	37.6	
phenol, 2,3,6-trimethyl-	Ph18	37.6	
pyrrolidine, 1-(1-butenyl)-	N40	37.7	
phenol, 2,3,5-trimethyl-	Ph19	37.7	
4-amino-2,3-xylenol	Ph20	38.2	

benzaldehyde, 4-methoxy-	Lg2	39.4	Lignin
1h-indene, 1,3-dimethyl-	Ar36	39.7	
1h-indene, 2,3-dimethyl-	Ar37	39.7	
naphthalene, 1,2-dihydro-6-methyl-	PAH3	39.8	
phenol, 2,4,6-trimethyl-	Ph21	39.8	
naphthalene, 1,2-dihydro-3-methyl-	PAH4	39.9	
phenol, 2,4,5-trimethyl-	Ph22	40.0	
1h-indole, 1-methyl-	N41	40.2	
1h-indole, 2-methyl-	N42	40.2	
1h-indene-1,2-diol, 2,3-dihydro-	Ar38	40.7	
1h-inden-1-ol, 2,3-dihydro-2-methyl-	Ar39	40.9	
1h-indene, 1,1-dimethyl-	Ar40	41.2	
naphthalene, 2-methyl-	PAH5	41.4	
1h-inden-1-one, 2,3-dihydro-3-methyl-	Ar41	41.9	
naphthalene, 1-methyl-	PAH6	42.0	
1h-indole-2,3-dione, 1-methyl-, 3-hydrazone	N43	42.2	
2-naphthalenol, 1,2-dihydro-, acetate	PAH7	42.9	
benzoic acid, 3-methoxy-, methyl ester	Lg3	43.3	Lignin
2-pyrrolidone-5-carboxylic acid, n-methyl, methyl ester	N44	43.4	
phenol, 5-methyl-2-(1-methylethyl)-	Ph23	44.0	
acetophenone, 4'-methoxy-	Lg4	44.3	Lignin
1h-indole, 1,4-dimethyl-	N45	44.4	
phenol, 2-methoxy-6-(1-propenyl)-	Ph24	44.4	
1h-indole, 1,3-dimethyl-	N46	44.6	
1,2,4-trimethoxybenzene	T1	44.6	Tannin
benzoic acid, 4-methoxy-, methyl ester	Lg5	44.9	Lignin
1,3,5-triazine-2,4,6(1h,3h,5h)-trione, 1,3,5-trimethyl-	N47	45.3	
phenol, 2,3,5,6-tetramethyl-	Ph25	45.4	
fructofuranose, 2,6-anhydro-1,3,4-tri-o-methyl-, omega-d-	Ps18	45.4	
2,3,4-trimethyllevoglucosan	Ps19	45.9	
naphthalene, 1,2-dimethyl-	PAH8	46.0	
benzene, 1,3,5-trimethoxy-	T2	46.3	Tannin
naphthalene, 1,3-dimethyl-	PAH9	46.5	
naphthalene, 2,7-dimethyl-	PAH10	46.9	
1h-isoindole-1,3(2h)-dione, 2-methyl-	N48	47.1	
1,4-benzenedicarboxylic acid, methyl ester	Un1	47.3	
phenol, 2,3,4,6-tetramethyl-	Ph26	47.4	
phenol, 2-methyl-5-(1-methylethyl)-	Ph27	47.5	
octanedioic acid, dimethyl ester	FA6	47.7	
1h-indole, 5-methoxy-2-methyl-	N49	48.0	
2,4(1h,3h)-pyrimidinedione, 1,3,5-trimethyl-	N50	48.3	
quinoline, 3-ethyl-	N51	48.5	
naphthalene, 1,4-dimethyl-	PAH11	48.5	
naphthalene, 1,2-dihydro-1,5,8-trimethyl-	PAH12	48.7	

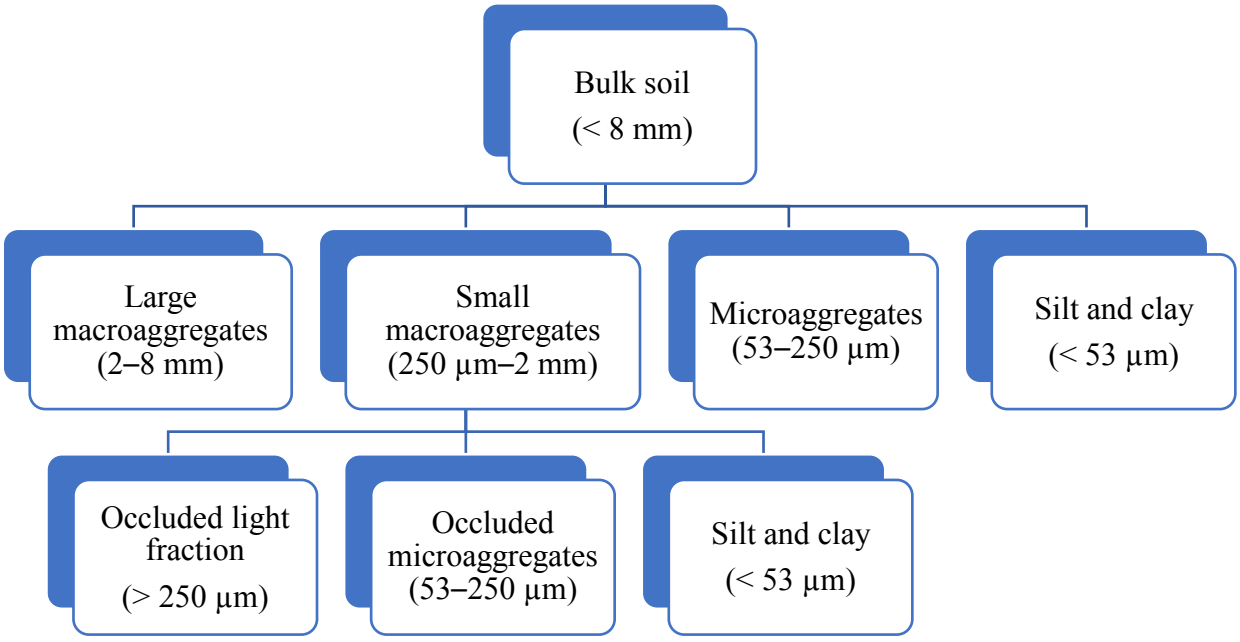
benzaldehyde, 3,4-dimethoxy-	Lg6	49.3	Lignin
2,4,6-trimethoxytoluene	T3	49.6	Tannin
2,4,5,6,7-pentamethoxyheptanoic acid, methyl ester	FA7	49.7	
1h-indole, 1,2-dimethyl-	N52	49.9	
t-butylhydroquinone	Ph28	50.5	
dodecanoic acid, methyl ester = methyl dodecanoate	FA8	50.9	
dibenzofuran	Ar42	51.1	
1h-indole, 1,2,3-trimethyl-	N53	51.6	
nonanedioic acid, dimethyl ester	FA9	51.9	
ethanone, 1-(3,4-dimethoxyphenyl)-	Lg7	52.7	Lignin
benzeneacetic acid, 3,4-dimethoxy-	Ar43	53.3	
benzoic acid, 3,4-dimethoxy-, methyl ester	Lg8	53.8	Lignin
1h-indole, 5,6,7-trimethyl-	N54	54.5	
phenol, 3-methoxy-2,5,6-trimethyl-	Ph29	54.5	
durohydroquinone	Ph30	54.5	
benzeneacetic acid, 3,4-dimethoxy-, methyl ester	Ar44	55.1	
3,3'-dimethylbiphenyl	PAH13	55.8	
4,4'-dimethylbiphenyl	PAH14	55.8	
2-propenoic acid, 3-(4-methoxyphenyl)-, methyl ester	Lg9	57.0	Lignin
9h-purin-6-amine,n,9-dimethyl-	N55	57.3	
2,4,6-trimethoxybenzonitrile	N56	57.9	
9h-fluorene, 9-methyl-	PAH15	58.0	
tetradecanoic acid, methyl ester = methyl tetradecanoate	FA10	58.5	Microbial
benzoic acid, 3,4,5-trimethoxy-, methyl ester+ 9h-purin-6-amine, n,n,9-trimethyl-coelutes	Ar45	58.6	
9h-purin-6-amine, n,n,9-trimethyl-	N57	58.6	
tetradecanoic acid, 12-methyl-, methyl ester	FA11	60.9	Microbial
phenanthrene	PAH16	61.1	
2-propenoic acid, 3-(3,4-dimethoxyphenyl)-, methyl ester	Lg10	64.2	Lignin
1,1'-biphenyl, 3,4-diethyl-	PAH17	64.3	
3,5,3',5'-tetramethylbiphenyl	PAH18	64.3	
9-hexadecenoic acid, methyl ester, (z)-	FA12	64.7	Microbial
9-hexadecenoic acid, methyl ester	FA13	65.0	Microbial
hexadecanoic acid, methyl ester = methyl hexadecanoate	FA14	65.4	
methyl 14 metoxytetradecanoate	FA15	66.3	
hexadecanoic acid, 15-methyl-, methyl ester	FA16	66.8	Microbial
hexadecanoic acid, 14-methyl-, methyl ester	FA17	67.7	Microbial
methyl 14-hydroxytetradecanoate	FA18	68.0	
pyrene	PAH19	70.3	
9-octadecenoic acid (z or e)-, methyl ester	FA19	70.9	Microbial
16-octadecenoic acid, methyl ester	FA20	71.1	Microbial
octadecanoic acid, methyl ester = methyl stearate	FA21	71.6	
methyl 16-metoxy hexadecanoate	FA22	72.5	Cutin or suberin
retene	PAH20	73.6	
methyl 17-metoxy heptadecanoate	FA23	74.0	

methyl 16-hydroxy-hexadecanoate	FA24	74.1	Cutin or suberin
eicosyl methyl ether = 1-methoxyeicosane	Al5	74.6	
hexadecanedioic acid, dimethyl ester	FA25	75.3	Cutin or suberin
methyl 17-hydroxyheptadecanoate	FA26	75.8	
1-eicosanol	Al6	76.2	
pyrene, 1-methyl-	PAH21	76.2	
mix of 9,16 & 10,16-dimethoxy hexadecanoic acid, methyl esters	FA27	76.3	Cutin
eicosanoic acid, methyl ester	FA28	77.4	
methyl 18-hydroxyoctadec-9-enoic acid methyl ester, methyl ether	FA29	77.5	Cutin or suberin
methyl 9,10,16-trimethoxyhexadecanoate	FA30	78.0	
mix of 9 &10-monohydroxy-1,15-pentadecanedioic acid, dimethyl esters	FA31	78.1	Cutin
octadecanoic acid, 18-methoxy-, methyl ester	FA32	78.2	Cutin or suberin
mix of 7 & 8 - methoxy hexadecanedioic acid-, dimethyl esters	FA33	79.0	Cutin
methyl 18-hydroxy-octadec-9-enoate should be here	FA34	79.1	Cutin or suberin
pyrene, 1,3-dimethyl-	PAH22	79.1	
heneicosanoic acid, methyl ester	FA35	79.3	
mix of 9,16 & 10,16-dihydroxy hexadecanoic acid-, methyl esters	FA36	79.8	Cutin
methyl octadec-9-ene-1,18 dioate	FA37	80.1	Cutin or suberin
7 or 8 or 9 or 10-monohydroxy-1,16 hexadecanedioate	FA38	80.8	Cutin
octadecanedioic acid, dimethyl ester	FA39	80.8	Cutin or suberin
1-docosanol	Al7	81.7	
docosanoic acid, methyl ester	FA40	82.7	
methyl 20-metoxy eicosanoate	FA41	83.4	Suberin
9,10 or 8,9-dimethoxy octadecanoic acid, methyl ester	FA42	84.0	Cutin or suberin
methyl 20-hydroxyeicosanoate	FA43	85.0	Suberin
tricosanoic acid, methyl ester	FA44	85.1	
9,10,18-trihydroxy octadecanoic acid methyl ester, dimethyl ether	FA45	85.6	Cutin or suberin
eicosanebioic acid, dimethyl ester	FA46	85.9	Suberin
1-tetracosanol	Al8	86.7	
9,10-dimethoxy octadecanedioic acid, dimethyl ester	FA47	87.2	Cutin or suberin
tetracosanoic acid, methyl ester	FA48	87.6	
9,10-dihydroxy-1,18-octadecanedioic acid, dimethyl ester	FA49	88.0	Cutin or suberin
methyl 22-metoxy docosanoate	FA50	88.3	Suberin
methyl 22-hydroxydocosanoate	FA51	89.8	Suberin
docosanedioic acid, dimethyl ester	FA52	90.6	Suberin
hexacosanoic acid, methyl ester	FA53	92.1	
methyl 24-metoxy tetracosanoate	FA54	92.8	Suberin
heptacosanoic acid, methyl ester	FA55	94.3	
tetracosanedioic acid, dimethyl ester	FA56	95.0	Suberin
octacosanoic acid, methyl ester	FA57	96.4	
nonacosanoic acid, methyl ester	FA58	98.4	
omega-sitosterol methyl ether	Un2	99.7	
triacontanoic acid, methyl ester	FA59	100.4	
dotriacontanoic acid, methyl ester	FA60	104.8	

Supplementary Table 4.A-5. Results of the two-way analyses of variance (ANOVAs) for microbial and plant-derived compounds. Df stands for degrees of freedom and Sum sq for sum of squares.

		Df	Sum Sq	F value	p-value
Cutin:suberin	Invasion	1	3.68	10.49	<0.01
	Site	2	4.75	6.77	<0.01
	Interaction	2	0.74	1.05	0.38
	Residuals	15	5.27		
Plant marker:microbial origin	Invasion	1	0.23	3.97	0.06
	Site	2	1.51	13.28	<0.001
	Interaction	2	0.72	6.31	0.01
	Residuals	15	0.85		
Microbial markers [%]	Invasion	1	<0.01	0.09	0.76
	Site	2	<0.01	0.13	0.88
	Interaction	2	<0.01	0.84	0.45
	Residuals	15	0.01		
Microbial N compounds [%]	Invasion	1	<0.01	10.85	<0.01
	Site	2	0.01	10.39	<0.01
	Interaction	2	<0.01	3.80	0.05
	Residuals	15	<0.01		
Microbial origin [%]	Invasion	1	<0.01	3.91	0.07
	Site	2	0.01	7.35	<0.01
	Interaction	2	<0.01	4.44	0.03
	Residuals	15	0.01		
Cutin markers [%]	Invasion	1	<0.01	<0.01	0.95
	Site	2	0.02	89.18	<0.001
	Interaction	2	<0.01	1.98	0.17
	Residuals	15	<0.01		
Suberin markers [%]	Invasion	1	<0.01	11.99	<0.01
	Site	2	<0.01	19.31	<0.001
	Interaction	2	<0.01	3.16	0.07
	Residuals	15	<0.01		
Lignin markers [%]	Invasion	1	<0.01	0.10	0.76
	Site	1	<0.01	17.07	<0.001
	Interaction	1	<0.01	0.05	0.95
	Residuals	17	<0.01		
C oxidation state	Invasion	1	<0.01	7.45	0.02
	Site	2	<0.01	9.12	<0.01
	Interaction	2	<0.01	0.53	0.60
	Residuals	15	<0.01		
H:C atomic ratio	Invasion	1	0.04	6.57	0.02
	Site	2	0.33	24.74	<0.001
	Interaction	2	0.02	1.36	0.29
	Residuals	15	0.10		

Appendix 4.B. Supplementary figures



Supplementary Figure 4.B-1. Schematic of the aggregate fractionation procedure.

Appendix 4.C. Supplementary data and analyses

1. Supplementary laboratory analyses

a. Total organic carbon and nitrogen

Total organic carbon (TOC) and nitrogen (TN) were determined on pre- and post-incubation samples (air-dried, sieved < 4 mm for LFH and < 2 mm for mineral soil, and ground) using the dry combustion method on a ThermoScientific Flash 2000 Organic Elemental Analysis instrument (ThermoFisher Scientific, Waltham, MA, USA). Additionally, the elemental analyzer was coupled with a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Scientific Inc., Bremen, Germany) to determine the $\delta^{13}C$ against VPDB and $\delta^{15}N$ against air. Data can be found in Suppl. Table 4.C-1.

b. Isotopic composition of respired CO_2

The average isotopic composition of emitted CO_2 was estimated as a weighed average for each gas sampling interval using the $\delta^{13}C$ value obtained from the Picarro G2201-i isotopic analyzer (Picarro Inc., Sunnyvale, CA, USA) as followed (Podrebarac et al., 2021):

$$\delta^{13}C_{emitted\ CO_2} = \sum_{n=1}^{final}\left(\frac{CO_2-cumulative_n - CO_2-cumulative_{n-1}}{CO_2-cumulative_{final}}\right) * \delta^{13}C_n$$

Data can be found in Supp. Table 4.C-2.

c. Light fraction

The light fraction was extracted from bulk mineral soils prior incubation by density fractionation using sodium iodide (NaI, density of 1.7 $g.cm^{-3}$) after mechanical shaking (Carter and Gregorich, 2006). TOC and TN of the extracted light fraction were determined following the procedure above. Data can be found in Suppl. Table 4.C-3.

2. Additional tables and figures

The composition of soil fractions, as a weight-based percentage of bulk soil, was also obtained from aggregate fractionation and can be found in Suppl. Table 4.C-4. The cumulative respiration data can be found in Suppl. Fig. 4.C-1 to 3.

3. Additional sites (raw data)

In addition to the sites presented in Chapter 4, samples from other sites (Montmorency, Golf, Wolf Lake, and Breton) were also analyzed by aggregate fractionation (Montmorency, Golf, Wolf Lake, and Breton) and laboratory incubation (Montmorency and Wolf Lake). For detailed site description, please refer to Chapter 2. Cumulative respiration data can be found in Suppl. Fig. 4.C-4 & 5. Aggregate fractionation data can be found in Suppl. Tables 4.C-4 to 6. Additionally, isotopic data are available in Suppl. Tables 4.C-1 & 3.

Supplementary Table 4.C-1. Average total carbon and total nitrogen of bulk soil and their isotopic composition (n=2-8), with the standard error in parentheses.

Site	Invasion	Forest floor				Mineral soil			
		TOC [%]	$\delta^{13}C$ [‰]	TN [%]	$\delta^{15}N$ [‰]	TOC [%]	$\delta^{13}C$ [‰]	TN [%]	$\delta^{15}N$ [‰]
EMEND	Control	44.3 (1.4)	-28.3 (0.2)	2.5 (0.3)	-0.5 (0.3)	1.6 (0.2)	-26.0 (0.7)	0.2 (0.1)	4.0 (0.5)
	Invaded	38.1 (3.4)	-28.3 (0.2)	1.9 (0.2)	0.0 (0.4)	2.5 (0.2)	-25.9 (0.3)	0.2 (0.1)	4.4 (0.2)
Wolf Lake	Invaded	38.11 (2.6)	-28.7 (0.3)	2.0 (0.2)	-0.4 (0.3)	0.9 (0.1)	-26.3 (0.4)	0.1 (0.1)	4.5 (0.3)
Valcartier	Control	45.5 (0.5)	-29.0 (0.2)	2.3 (0.4)	-1.0 (1.1)	7.2 (0.6)	-26.4 (0.2)	0.4 (0.1)	5.7 (0.7)
	Lightly invaded	34.2 (1.0)	-28.4 (0.1)	1.9 (0.3)	-0.4 (0.3)	8.1 (0.1)	-26.4 (0.1)	0.5 (0.1)	5.4 (0.1)
	Heavily invaded	37.2 (0.5)	-29.4 (0.2)	1.6 (0.3)	-1.8 (0.3)	5.9 (0.2)	-26.6 (0.1)	0.4 (0.1)	4.8 (0.4)
Grands Jardins	Control	49.2 (0.4)	-27.7 (0.1)	1.4 (0.1)	-0.3 (0.5)	4.4 (0.4)	-26.3 (0.1)	0.2 (0.1)	3.6 (0.5)
	Invaded	45.6 (3.4)	-28.2 (0.3)	1.2 (0.1)	-0.8 (0.2)	6.7 (0.7)	-26.4 (0.1)	0.3 (0.1)	2.4 (0.1)
Montmorency	Control	47.6 (0.1)	-29.2 (0.1)	1.5 (0.2)	-0.6 (0.1)	2.8 (0.4)	-26.0 (0.1)	0.1 (0.1)	3.8 (0.5)
	Invaded	28.5 (4.4)	-28.3 (0.2)	1.1 (0.3)	-0.2 (0.3)	4.7 (1.1)	-26.4 (0.2)	0.2 (0.1)	3.0 (0.3)

Supplementary Table 4.C-2. Average isotopic composition $\delta^{13}C$ [‰] of the emitted CO_2 with the standard error in parentheses (n=4-8).

Site	Invasion	Forest floor	Mineral soil
EMEND	Control	-31.6 (0.2)	-29.9 (0.9)
	Invaded	-31.4 (0.5)	-30.1 (0.2)
Wolf Lake	Invaded	-30.4 (0.9)	-28.2 (0.4)
Valcartier	Control	-30.9 (0.7)	-29.1 (0.5)
	Lightly invaded	-30.0 (0.3)	-28.7 (0.3)
	Heavily invaded	-32.3 (1.0)	-29.9 (0.2)
Grands Jardins	Control	-30.4 (0.5)	-28.5 (0.2)
	Invaded	-30.6 (0.6)	-28.1 (0.7)
Montmorency	Control	-30.6 (0.5)	-29.2 (0.5)
	Invaded	-30.0 (0.2)	-29.3 (0.3)

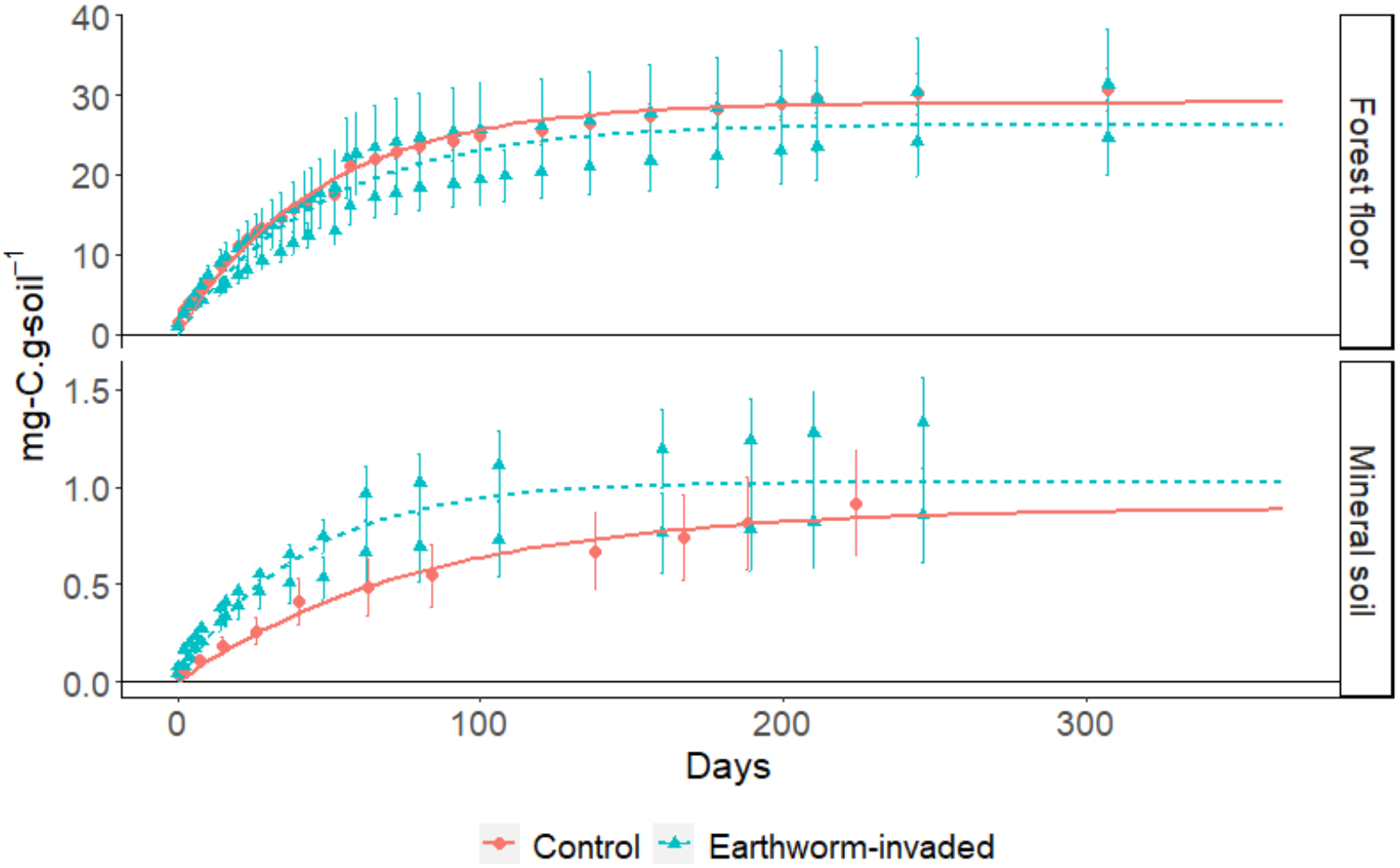
Supplementary Table 4.C-3. Total nitrogen (TN) and total carbon (TC) content of the light fraction, as well as the weight-based percentage of bulk soil it represents.

		TN [%]	TC [%]	% of bulk soil [%wt]
Valcartier	Control	1.1	24.9	0.7
	Low density	1.0	32.1	1.3
	High density	1.0	30.9	0.5
EMEND	Control	1.0	31.2	0.9
	Invaded	1.0	32.1	1.6
Grands Jardins	Control	1.0	39.7	2.3
	Invaded	1.1	32.1	6.5

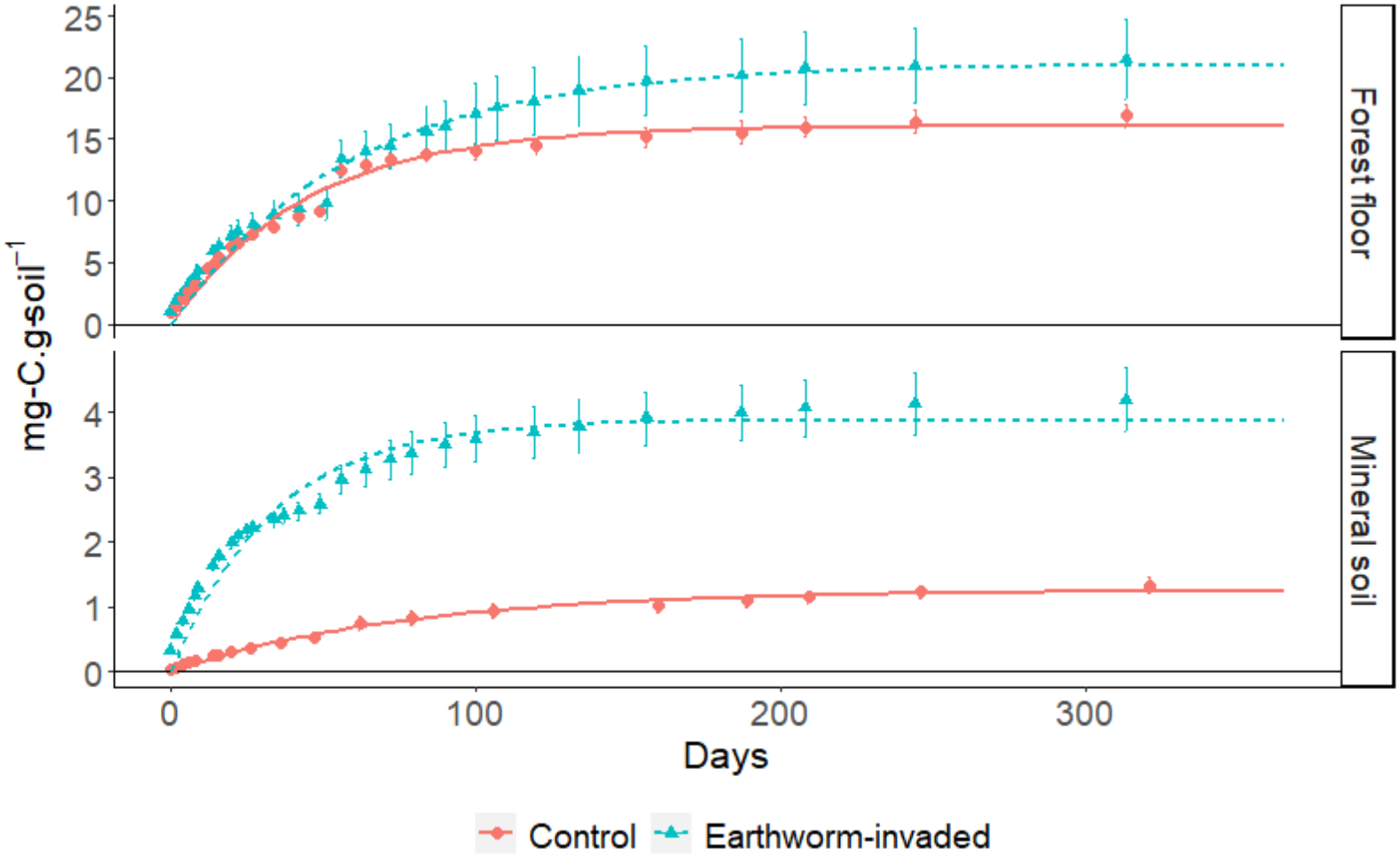
Supplementary Table 4.C-4. Average (± 1SE) aggregate composition as a percentage of sand-free soil.

Site	Invasion	Large macroaggregates	Small macroaggregates	Microaggregates	Silt and clay	Protected microaggregates[29]	Protected light fraction[30]
EMEND	Control	23.9 (12.7)	12.7 (3.9)	13.9 (2.5)	28.3 (11.3)	31.5 (6.2)	34.0 (14.0)
	Invaded	17.1 (5.0)	28.5 (2.8)	14.9 (4.1)	13.4 (0.4)	40.1 (1.7)	30.3 (4.2)
Wolf Lake	Invaded	2.6 (0.9)	8.9 (1.0)	17.2 (4.7)	45.9 (5.9)	9.5 (1.9)	82.5 (2.6)
Breton	Invaded	46.1 (4.2)	18.2 (3.0)	10.7 (3.2)	8.1 (1.4)	44.4 (1.5)	24.6 (4.7)
Golf	Control	52.4 (15.6)	36.8 (13.1)	3.8 (2.0)	2.6 (0.1)	24.6 (11.4)	66.2 (12.0)
	Invaded	50.6 (4.4)	29.6 (8.7)	7.9 (1.4)	4.0 (1.2)	54.5 (6.8)	40.0 (3.0)
Valcartier	Control	45.3 (14.0)	33.4 (6.0)	7.2 (2.8)	5.5 (2.2)	46.4 (5.8)	39.2 (8.3)
	Invaded	46.3 (13.9)	36.8 (9.1)	4.9 (1.7)	5.1 (1.5)	39.1 (6.1)	49.8 (6.8)
Grands Jardins	Control	2.2 (1.0)	25.5 (7.7)	24.8 (4.2)	10.9 (3.2)	21.0 (6.2)	72.0 (8.7)
	Invaded	3.8 (1.4)	30.5 (4.1)	26.4 (3.6)	9.5 (0.9)	12.1 (4.2)	83.3 (5.0)
Montmorency	Control	34.7 (1.3)	46.3 (4.7)	3.0 (1.1)	6.7 (1.3)	18.3 (7.3)	71.6 (3.4)
	Invaded	28.4 (6.9)	40.6 (3.1)	7.6 (2.7)	6.3 (1.7)	27.6 (3.2)	67.6 (4.3)

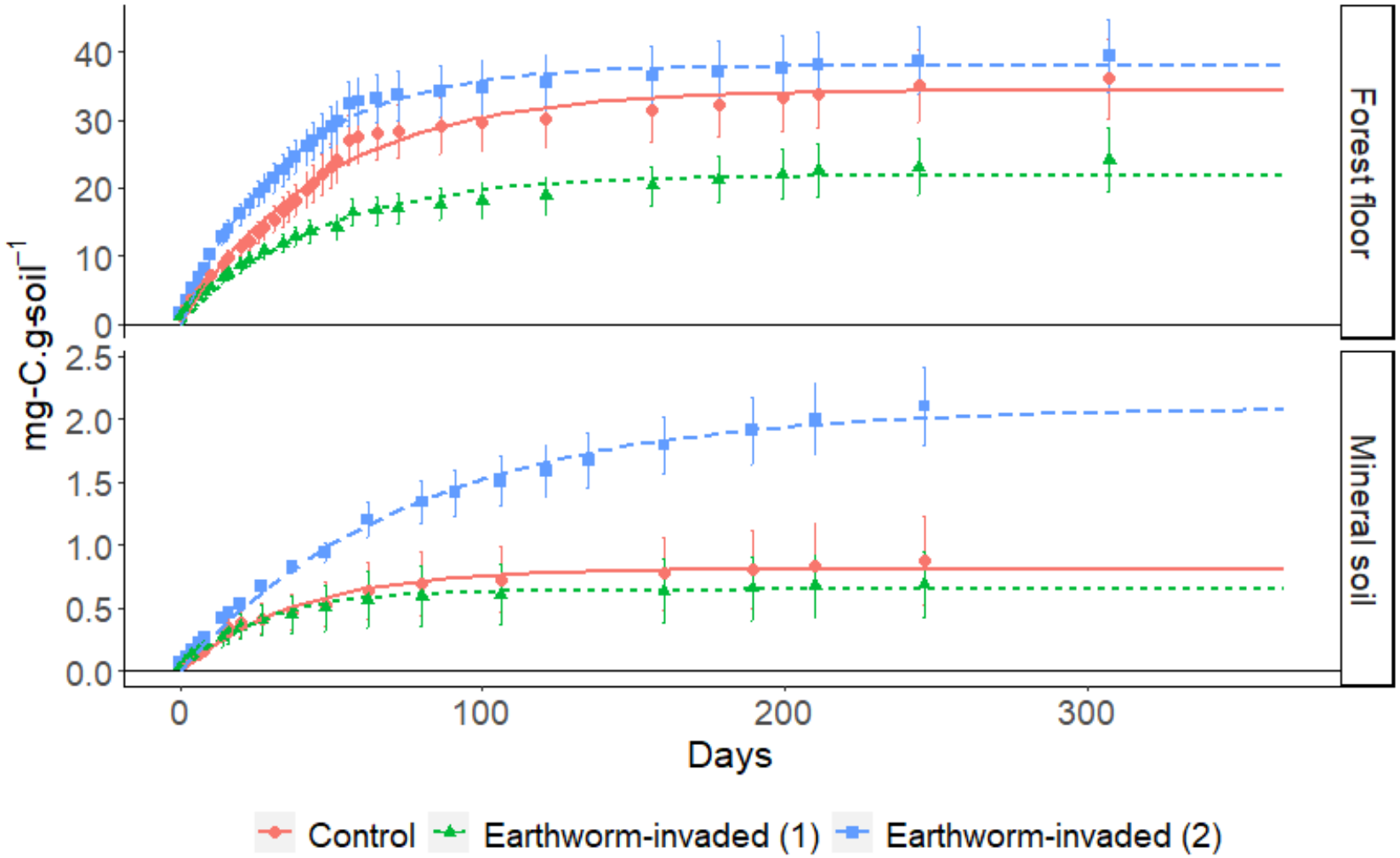
[29] As a percentage of small macroaggregates
[30] As a percentage of small macroaggregates



Supplementary Figure 4.C-1. Cumulative respiration as mg of C per gram of soil for the Luvisol samples (EMEND). Points represent the average from the raw data, with error bars representing one standard error. The data was fitted to a first order kinetic exponential model with one compartment (Maillard et al, 2010), displayed as lines.



Supplementary Figure 4.C-2. Cumulative respiration as mg of C per gram of soil for the Podzol samples (Grands Jardins). Points represent the average from the raw data, with error bars representing one standard error. The data was fitted to a first order kinetic exponential model with one compartment (Maillard et al, 2010), displayed as lines.



Supplementary Figure 4.C-3. Cumulative respiration as mg of C per gram of soil for the Brunisol samples (Valcartier). Points represent the average from the raw data, with error bars representing one standard error. The data was fitted to a first order kinetic exponential model with one compartment (Maillard et al, 2010), displayed as lines.

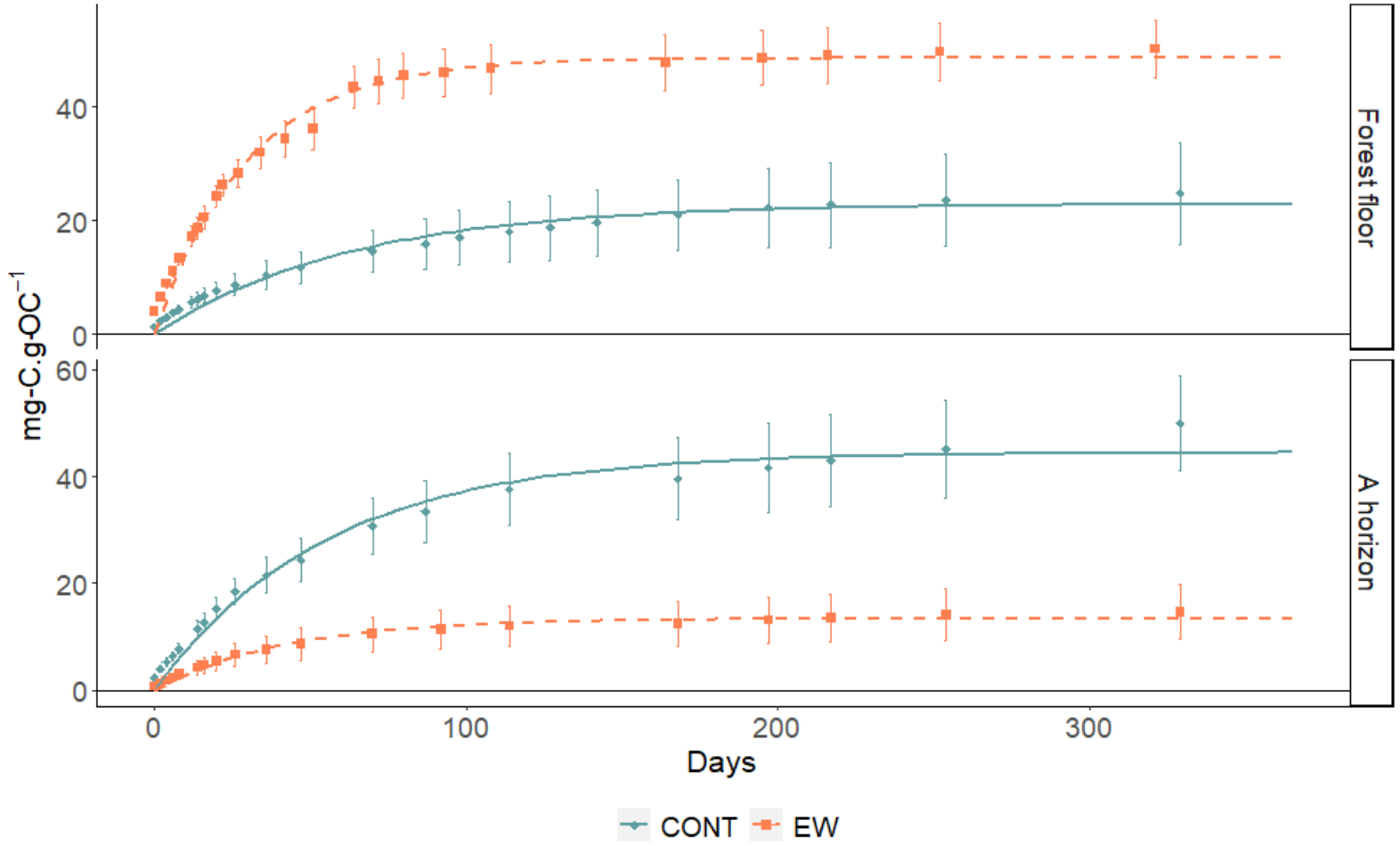
Supplementary Table 4.C-5. Average (± 1SE) TOC contents [% wt] of each soil fraction for additional sites.

Site	Invasion	Large macroaggregates	Small macroaggregates	Microaggregates	Silt and clay	Protected microaggregates[31]	Protected light fraction[32]
Wolf Lake	Invaded	1.4 (0.2)	0.5 (0.2)	0.3 (0.1)	0.6 (0.1)	2.0 (0.5)	0.3 (0.1)
Breton	Invaded	7.6 (1.0)	7.2 (0.4)	4.7 (0.8)	3.1 (0.5)	10.4 (0.2)	7.7 (0.5)
Golf	Control	30.5 (5.9)	27.8 (3.9)	18.9 (2.8)	14.1 (2.4)	24.0 (1.4)	28.7 (2.6)
	Invaded	12.1 (0.3)	13.2 (1.1)	8.7 (0.5)	8.3 (3.5)	12.5 (0.4)	16.1 (3.9)
Montmorency	Control	0.9 (0.1)	1.1 (0.2)	0.9 (0.1)	1.5 (0.3)	1.7 (0.4)	0.6 (0.2)
	Invaded	2.2 (0.4)	2.5 (0.5)	1.5 (0.2)	1.9 (0.4)	3.6 (0.3)	0.6 (0.1)

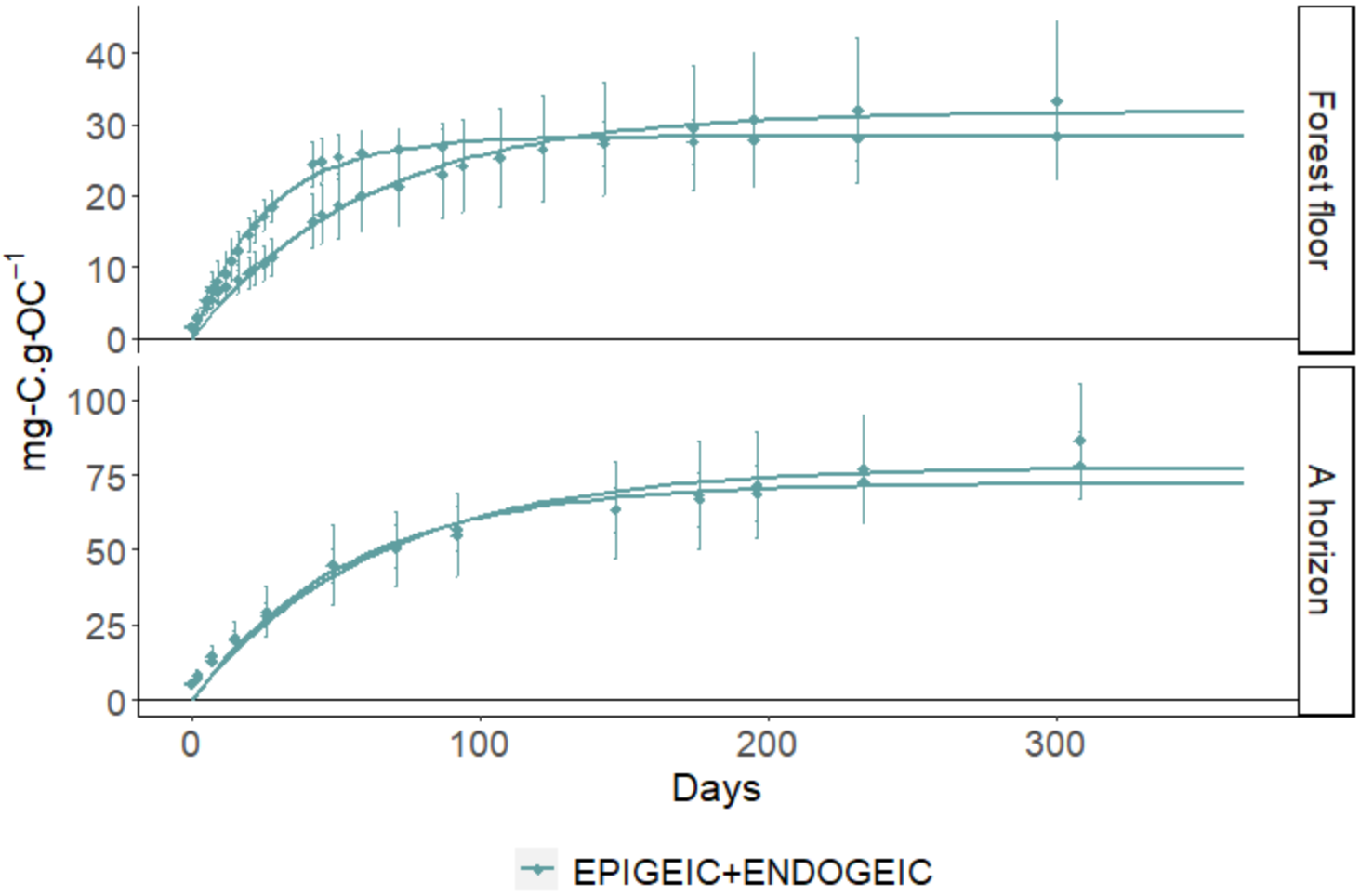
Supplementary Table 4.C-6. Average (± 1SE) percentages of the total C found in each soil fraction for additional sites.

Site	Invasion	Large macroaggregates	Small macroaggregates	Microaggregates	Silt and clay	Protected microaggregates[33]	Protected light fraction[34]
Wolf Lake	Invaded	3.6 (1.0)	28.7 (4.6)	21.8 (2.7)	45.9 (5.9)	1.9 (0.9)	14.4 (3.8)
Breton	Invaded	39.1 (5.3)	39.1 (1.3)	16.7 (3.7)	8.1 (1.4)	4.8 (1.2)	10.8 (3.2)
Golf	Control	19.5 (0.8)	56.6 (0.3)	21.3 (1.2)	2.6 (0.1)	8.0 (5.6)	34.9 (4.1)
	Invaded	34.8 (5.6)	38.4 (6.2)	22.9 (0.7)	4.0 (1.2)	2.3 (0.6)	17.9 (2.9)
Montmorency	Control	10.0 (2.7)	65.5 (5.1)	17.8 (1.9)	6.7 (1.3)	1.3 (0.2)	28.7 (7.9)
	Invaded	8.9 (1.3)	67.7 (6.6)	17.2 (4.3)	6.3 (1.7)	1.6 (0.3)	11.4 (2.4)

[31] As a percentage of small macroaggregates
[32] As a percentage of small macroaggregates
[33] As a percentage of small macroaggregates
[34] As a percentage of small macroaggregates



Supplementary Figure 4.C-4. Cumulative respiration as mg of C per gram of OC for Montmorency. Points represent the average from the raw data, with error bars representing one standard error. The data was fitted to a first order kinetic exponential model with one compartment (Maillard et al, 2010), displayed as lines.



Supplementary Figure 4.C-5. Cumulative respiration as mg of C per gram of OC for Wolf Lake. Points represent the average from the raw data, with error bars representing one standard error. The data was fitted to a first order kinetic exponential model with one compartment (Maillard et al, 2010), displayed as lines.