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Earthworm Presence in Northern Forests: Impact on Distribution of Soil Carbon within Aggregate Fractions

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EARTHWORM PRESENCE IN NORTHERN FORESTS: IMPACT ON DISTRIBUTION OF SOIL CARBON WITHIN AGGREGATE FRACTIONS

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ABSTRACT

Growing concerns over climate change is driving research aimed at determining ways of retaining soil carbon (C) within managed northeastern forests. Earthworms are exotic to the state of Vermont and the current extent of earthworm community presence in the state's forests, as well as the long term impact these communities will have on soil C storage, is still unknown. Current research suggests that earthworms have conflicting effects on the C cycle of soils, simultaneously enhancing mineralization through soil mixing, while protecting C through the stabilization of microaggregate (mA) structures. The mA soil fraction represents a pool of physically stable structures capable of maintaining occluded C for long periods of time. To date, studies investigating earthworm effects on mA formation and occluded C have rarely been done in undisturbed forest soils.

Earthworms were found in 10 of 18 forest sites utilized in a statewide Vermont earthworm survey, and community presence correlated with thinner forest floor depths. For 8 sites, the impact of earthworm presence on the quantity of C within water stable mA was investigated. Earthworm presence correlated with greater total C in the top 20 cm of mineral soil, highlighting the relocation of the forest floor noted in all 18 sites. A small, but significant, decrease was noted in the proportion of bulk soil mA, however through C enrichment from the forest floor, there was a significant increase in the pool of mA-associated C. A paired mesocosm study was also conducted, utilizing the endogeic earthworm species Aporrectodea tuberculata, placed in an earthworm-free, undisturbed forest soil. Findings from this study corroborated the correlations noted in the field with small, though insignificant decreases in the proportion of bulk soil mA. The larger macroaggregate fraction was increased by about 4 times under earthworm influence. The C enrichment of mA structures occluded within the macroaggregate fraction accounted for approximately 95% of the total increase in mA-associated C, and 50% of the total C integrated into the mineral soil. It can be assumed that the C preferentially occluded within the mA structures by earthworm ingestion will experience longer mean residence time relative to bulk soil C.

We conclude that, for the forest soils investigated, earthworm communities decreased the proportion of mA slightly but that the pool of physically stabilized C was increased through mA turnover. Forest soils usually experience low soil mixing and therefore typically contain high proportions of mA, though the quantity of C within these structures varies. Due to mA restructuring within the earthworm gut, it is unlikely that earthworm community expansions will alter the proportion of mA in forest soils, however the quantity of C present within these structures is likely to increase. The individual site investigated in the controlled study was particularly low in mineral soil C, and therefore the long-term presence of earthworms would likely result in an increase to mineral C storage. However, this result may not be applicable for forests with high levels of mineral soil C prior to earthworm invasion.

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COMPREHENSIVE LITERATURE REVIEW

1.1 Introduction

Concern over the global impact of increased carbon dioxide (CO_2) levels in the atmosphere has encouraged recent research aimed at enhancing understanding of the carbon (C) cycle. An intimate relationship exists between the soil and the atmosphere, with the soil acting as a potential buffer for CO_2. Most forests of the northeastern United States were impacted by the last glaciation event, receding approximately 12,000 years ago, and therefore developed without influence from native earthworms. Since the introduction of earthworms from Europe and Asia, various species have slowly started to make their way into these ecosystems, altering soil morphology, chemistry, and ecology. Forests are often actively managed, offering an opportunity to implement best practice in order to dictate whether these soils will act as a sink or source for CO_2 in the future. Understanding the impact that earthworms will have on the C dynamics of these soils is just one piece in this puzzle.

Earthworm presence in soil is currently believed to enhance C loss through an stimulation of microbial respiration, and reduce C loss through the integration of C within stabilized soil aggregates. The long term implications of this shift in C cycling on the C balance of these ecocystems is still very unclear, though it is most certainly influenced by the interactions of soil structure, microbial communities, C quality, and earthworm species.

1.2 Aggregation

A soil aggregate is a basic unit of soil structure, consisting of various organic materials and mineral particles (sand, silt and clay) binding more powerfully to themselves than to surrounding substances (Frey, 2005; Oades, 1993). Aggregates can be described based on their structure (size, shape and constituting parts), stability (ability of the structure to withstand disturbance) or resiliency (ability of structure to recover after disturbance) (Kay, 1998). The three dimensional spatial environment formed through aggregation is referred to as the soil matrix, and it dictates, and is dictated by, the interactions of water, oxygen, organic matter and soil microbial communities (Six, Bossuyt, Degryze, and Denef, 2004). This matrix is dynamic. Through the construction and deconstruction of aggregates, soil organic matter (SOM) is occluded or released, degraded anaerobically or aerobically and the residence time of its C containing compounds determined.

Aggregates may be formed and stabilized abiotically or biotically, the influence of these mechanisms varying with a soil's texture and organic matter inputs (Oades, 1993). Abiotic stabilization occurs on the micron scale, and therefore exerts more influence in soils high in clay. Abiotic aggregation occurs through freeze/thaw and wet/dry cycling. During these processes the tensile strength of water reorientates clays, placing them in close proximity to one another where they may be cemented together using inorganic, highly charged or cementing binding agents such as calcium and oxyhydroxides (Six et al., 2004). Though methods to evaluate soil structure rarely differentiate between biotic and abiotic factors (Jouquet, Zangerle, Rumpel, Brunet, Bottinelli, and Tran Duc, 2009; Oades, 1993), in all soils except those very high in clay, and low in organics, biotic processes are thought to have the primary influence over a soil's structure and stability (Baldock, 2002).

Different biological binding agents act at different scales in the process of aggregation (Oades, 1984). A representation of these scales is found in the rhizosphere, the area around a plant's root zone, where aggregates are stabilized directly and indirectly, on both large and small scales (Baldock, 2002). Free mineral particles are typically bound by small molecular binding agents such as humified organic compounds, metal complexes and cellular residues, into microaggregates (20-250 µm). In the rhizosphere, roots supply these small binding agents directly through root exudates, and indirectly through secondary metabolic products released by microbial communities utilizing the root exudates as a food source (Baldock, 2002). These microaggregates and sand sized minerals, may be bound into macroaggregates (>250 µm) by the direct binding within root hairs, or the indirect binding within the root associated fungal hyphae. This hierarchy of scale exists not just around root zones, but rather ubiquitously throughout most soil types. The smaller binding agents are ineffective at binding macroaggregates over wider pores spaces, while root hairs and fungal hyphae are often larger than the pore spaces formed within microaggregates (Six et al., 2004).

1.2.1 Aggregate Hierarchy

In the above model of aggregation a hierarchy exists in both structure and stability. Macroaggregates, which are larger and less stable, degrade into microaggregates, which are smaller and very stable, along planes of weakness (Oades and Waters, 1991; Tisdall and Oades, 1982). On the smallest scale, individual clay particles are bound together abiotically. This binding is based on the composition/concentration of electrolytes and metal oxides, as well as the make up of the soil's cation exchange complex (CEC) (Baldock, 2002). These soil qualities are relatively stable throughout time and space and consequently the aggregation of individual clays is rather ho-

mogenous throughout the soil matrix (Baldock, 2002). At the next structural level, clay complexes are further bound together biotically into microaggregates (<250 µm) utilizing organic molecules such as microbially derived polysaccharides and proteins (Oades and Waters, 1991). For larger microaggregates (20-250 µm) particulate organic matter (POM) is often found at the center of the structures. The biomolecules released by microorganisms during decomposition bind to local mineral particles and transient clays moving within the soil pore water, resulting in an encapsulation of the organic matter (Oades and Waters, 1991; Waters and Oades, 1991). The binding and stabilization of microaggregates into macroaggregates (>250 µm) is accomplished by bridging with POM capable of spanning larger pore distances, or networks of fibrous fungal hyphae or small roots.

The hierarchy of size and constituting parts is mirrored with a hierarchy of structural stability. The binding strength of water to pores (Braunack, Hewitt, and Dexter, 1979), and the effectiveness of binding agents (Kay, 1998) are inversely proportional to size within this aggregate hierarchy. Smaller aggregates have lower porosity, and greater physical contact between particles (Currie, 1966), strengthening the physiochemical bonds. Larger binding agents such as root hairs and fungal hyphae are known as "temporary" binding agents, and will decay readily, leaving structures vulnerable to disruption within shorter time frames (Tisdall and Oades, 1982). Smaller binding agents consist of "transient" or "persistent" substances, which due to their small size and association with the mineral fraction of soil, offer durability and longer residence time (Tisdall and Oades, 1982).

It was previously thought that the above hierarchy of aggregation formed sequentially, microaggregates being formed first and then afterward being bound together to form macroaggregates (Six et al., 2004; Tisdall and Oades, 1982). Oades (1984) was first to suggest that the larger binding agents, POM, roots and fungal hyphae, that

hold the macroaggregates together could form the core for the formation of microaggregates as they decomposed. The formation of microaggregates within macroaggregates has since been widely supported (Angers, Recous, and Aita, 1997; Beare, Hendrix, and Coleman, 1994; Jastrow, 1996; Six, Elliott, Paustian, and Doran, 1998), and attributed, in part, to the anaerobic environment developed at the center of macroaggregates (Elliott and Coleman, 1988; Tiedje, Sexstone, Parkin, and Revsbech, 1984). Mirroring the hierarchy of aggregation, there exists a hierarchy of poor space and water retention governing microbial communities within the soil matrix. Organic matter alone has very little influence over a soil's aggregation (Frey, 2005; Lynch and Bragg, 1985; Tisdall, 1991), and it is through the actions of microbial communities during decomposition that work in the stabilizing of soil structure.

1.2.2 Aggregation and Microbial Communities

Effect of microorganisms on aggregation

When microbial communities are abundant and active, organic materials are continually being decomposed, and microbial communities turned over. Through decomposition, organic materials, including microbial biomass, are utilized in the formation of new cellular structures and metabolites, mineralized through hetertrophic respiration, or otherwise chemically and physically altered (Baldock, 2002). Through the enmeshment of soil particles by fungal hyphae, and cementation by various microbial metabolites, the activity of microorganisms play an important roll in soil structural stability.

Fungal communities work intimately in the creation and maintenance of a soil's structure. Fungal hyphae physically alter the arrangement of mineral particles similarly to how plant root growth separate or associate adjacent particles during the

creation of macropores (Dorioz, Robert, and Chenu, 1993), however this effect is on a much smaller scale. Fungal hyphae work in the formation and stability of macroaggregates, having little affect on microaggregates (Tisdall, 1991). Fungal mycelium entangle mineral particles and cement them together through the production of polysaccharides (Oades and Waters, 1991). Fungal communities can remain active even at very low water potentials and are suited to live and grow within interpore space devoid of liquid water (Shipton, 1986). This ability makes fungi mycelium growth an ideal mechanism of enmeshing particles across relative distances and changing water potentials.

Many types of fungi exist in soil, with varying degrees of effectiveness at creating and maintaining soil structure. The stabilization of structure by fungi will typically exist only as long as the hyphal network remains intact. While saprophytic fungi die once all available substrates have been utilized, certain symbiotic species are known to persist even after the death of their host plant (Tisdall, 1991). It has been suggested that the fungi most effective at soil stabilization will produce an abundance of sticky mucilage, be capable of binding particles by many mechanisms (i.e. several mucilage types/charges and filament sizes/lengths), exert enough force to reorient soil particles, and persist for long periods of time (Tisdall, 1991). When fungal networks die, the remains are classified as particulate organic matter (POM) and these may subsequently be colonized by bacterial communities to further stabilize soil structure

Bacteria, individually or as a community, exude solutions rich in polysacharrides and proteins (Baldock, 2002), and these solutions allow bacterial cells to form intimate associations with the mineral particles and organic matter around them (Dorioz et al., 1993). As a soil dries, the amount of extracellular solution produced by most bacterial cells is increased in order to protect the organism from desiccation. Bacterial communities are closely associated with mineral surfaces and their exudates, as well

as cell debris from dead and decomposing cells, are capable of entering into very small pore spaces (<1 µm). Once inside these small pore spaces the bioavailibility of these binding agents is extremely low, prolonging their influence on structural stabilization (Dorioz et al., 1993).

Unlike fungal mycelium, bacterial derived exudate allows for soil structure to persist long after the bacterial sources have decomposed (Foster, 1994), and hollowed microaggregate structures can be observed as an example of this process (Foster, 1988). Bacterial communities alter the arrangement of fine clay particles, bringing them parallel to the cellular surface (Foster, 1988), however besides these very small changes to structure on the micro scale, bacterial communities exhibit very little influence over the formation of soil structure, primarily impacting the stability of soil structure. The mucilage and polysaccharide-rich exudate produced by bacteria only impact the formation and stabilization of microaggregation (Oades, 1993; Tisdall, 1994), being too small to exert enough force in the stabilization of larger structures. The force of these binding agents at this scale is much greater relative to the forces exerted within macroaggregate structures, allowing these small structures to withstand greater levels of physical disturbance (Kay, 1998).

Fungal and bacterial populations impact aggregation processes primarily through their production of binding agents, and in the case of fungal hyphae the entanglement of soil constituents. These microflora are at the bottom of the soil food chain and other larger organisms, such as protozoa and nematodes, also exert indirect influences on soil structure and stability (Coleman, Crossley, and Hendrix, 2004; Swift, Heal, and Anderson, 1979). Larger soil fauna (>100 µm, micro and macro arthropods) may alter nutrient cycling through predation on microflora as well as by a production of fecal pellets, which when stabilized, represent biologically sourced aggregates. While these larger organisms impact soil structure through burrowing and ingestion, it is

through their interactions with the smaller microflora, through predation or influences within the foodweb, that have an effect on soil structural stability (Baldock, 2002).

Effect of aggregation on microorganisms

Up to 60% of total soil volume is comprised of pore space found in various shapes and sizes. These pores may be interconnected or isolated from bulk soil, and filled with either air or water (Paul, 2014). This three dimensional environment influences nutrient availability, water potential, oxygen diffusion, and predation, greatly affecting the organisms living within it. Estimates range from 10^4 to 10^6 distinct species represented in each gram of soil (Curtis, Sloan, and Scannell, 2002), though most of these species are unable to be cultured (Hill, Mitkowski, and Aldrich-Wolfe, 2000). The availability and segregation of spatially and temporally diverse habitats is likely what gives rise to this large biodiversity (Schmidt, Torn, Abiven, Dittmar, Guggenberger, Janssens, Kleber, Kögel-Knabner, Lehmann, Manning, Nannipieri, Rasse, Weiner, and Trumbore, 2011). As highlighted in the previous section, the effect of this environment on microbial communities feeds back to the the soil's structure and stability. Despite this intimate relationship between microorganisms and the soil environment, much more is known about how microorganisms impact a soil's aggregation than about how the soil's aggregation and pore network impact the soil's microbial communities (Six et al., 2004).

The soil pore network influences soil processes primarily through a restriction of access and the interaction of water within the pore networks. Each aggregate, and even individual pores, may be considered its own microcosm, with highly variable environments and microbial community structures. Soil microorganisms range in size from bacteria (0.2-1 µm), to micro and macroarthropods (>100 µm) (Swift et al., 1979), with smaller organisms (bacteria and fungi) essentially restricted to existing

pore networks. While fungal hyphae are capable of moving into and out of pores, regardless of water content, bacteria and most other small soil fauna are restricted to areas saturated with water (Hattori, 1994).

Bacterial presence is rarely found in pores <0.8 µm (Ranjard and Richaume, 2001) meaning that, depending on soil texture and other factors influencing pore size distribution, about 25-50% of a soil's pore space is inaccessible to any soil organism. More than 80% of bacterial biomass, across all soil types, is found associated with the interior of pores 1-9 µm (Foster, 1988; Hassink and Bouwman, 1993). Pores of this size exclude the access of larger predatory soil organisms, that would otherwise feed on the occluded bacterial colonies (Foster, 1988; Six et al., 2004). Bacteria located in pores < 30 µm are protected from nematode predation while bacteria in pores <5 µm are also protected from predation by protozoa (van der Linden and Jeurissen, 1989).

This preferential colonization of various bacterial communities inside small pores (Ranjard and Richaume, 2001) may be explained by predation, or through the interactions of water within small pores. Water circulates freely in the pores between microaggregates (>10 µm), while capillary forces retain water tightly in the internal micoraggregate pores (<10 µm). This ability to hold onto water, even during bulk soil desiccation, provides a stable environment for bacterial communities that may be sensitive to the fluctuating moisture content of larger pores (Ranjard and Richaume, 2001). The restricted movement of water within micropores limits the diffusion of oxygen and nutrients into and out of these poor spaces and so these environments quickly development and maintain an anaerobic environment preferential to denitrifying bacteria (Lensi, Clays-Josserand, and Jocteur Monrozier, 1995). This micropore-rich fraction of the soil accounts for 85% of total soil denitrifying activity (Lensi et al., 1995). The limited diffusion of nutrients also means that bacterial communities tend to be patchy (Ranjard and Richaume, 2001).

Soil aggregation is dynamic, and as soil goes through wet-dry and freeze-thaw cycles, and as larger soil fauna such as earthworms and termites move through the soil, aggregates are destroyed and reformed, and microbial communities are shifted around within the soil matrix. This shifting may release or occlude communities within aggregates, destroy fungal hyphal networks, or place communities in contact with nutrient sources or predation. It is therefore not just soil structure that influences these microbial communities, but also the rate and severity of structural turnover. Through its influence on soil microbial communities, aggregate turnover also has a direct and profound influence over the cycling of soil organic carbon (SOC) on small and large scales.

1.3 Soil Carbon and Aggregate Turnover

1.3.1 Soils and the Carbon Cycle

Soils contain more carbon (C) (1,500 Gt organic, 950 Gt inorganic) than in all terrestrial biomass (560 Gt) and the atmosphere (720 Gt) combined (Birdsey, 1992), making soil a key player in the attempt to offset anthropogenic C emissions. An intimate relationship exists between the soil and the atmosphere, with soil respiration accounting for roughly 20% of total CO_2 emissions (Rastogi, Singh, and Pathak, 2002), which is far greater than the emissions attributed to human activities. Soils represent a large stock of potentially volatilizable C, and land management is widely known to have huge impacts on a soil C stores (Lal, 2005). Deforestation and conversion to agriculture has been shown to reduce SOC by 20-50% due to reduced C inputs, increased decomposition, and decreased aggregation from frequent tillage (Post and Kwon, 2000). As global attempts are made in reducing anthropogenic CO_2 emissions, efforts are also needed to develop best management practices for the terrestrial

ecosystems (Rastogi et al., 2002). Soils have the potential to act as a buffer against rising CO_2 levels, and the role of a soil as a C sink or source is dependent on the balance between soil respiration, photosynthesis, and the stabilization of C within soils.

1.3.1.1 Soil Organic Matter

Baldock and Nelson (2000) summarized the various components of soil organic matter (SOM) as follows, ranging from living to highly decomposed materials. The living component of SOM includes plant roots, microbial biomass and soil fauna, as well as various exudates and enzymes used during the decomposition of other SOM fractions. This living SOM fraction represents the primary source of SOC inputs in the soil and, as described above, is extremely dynamic in its influence on, and being influenced by, the physical soil environment. The non-living component is often distinguished operationally by size and chemical properties. Particulate organic matter (POM) maintains a recognizable cellular structure (dead roots, plant litter, faunal skeletons), and represents the earliest stage in decomposition. POM therefore exhibits a diverse range of chemical properties and assumed residence time in the soil. Dissolved organic matter (DOM) is composed of various organic materials that remain in soil solution and move within the soil pore water. Due to its mobility within the soil matrix, DOM can often be the only source of nutrients for isolated biotic communities, and therefore plays an important role in many soil processes despite its relatively small proportion of the total SOM pool. Humus refers to insoluble organic materials that are no longer recognizable from their source POM. This fraction may originate from the decomposition and alteration of POM and cellular debris, or from the mucilage and exudate of decomposer communities. This fraction consists of a mixture of unaltered (sugars, proteins, lipids, etc.) and altered bio-molecules. Bio-molecules altered chemically or

through enzyme activity are referred to as humic substances due to their inability to be placed into any other discrete chemical category.

The above categories of SOM may be further defined by the C stabilization they exhibit in the soil matrix. Definitions for these pools of C are typically operationally defined through the methods used to measure them. This practice of definition through measurement has resulted in many different models for organic matter turnover, with varying degrees of functionality within the wide range of soil characteristics (Six, Conant, Paul, and Paustian, 2002). New technology is continually being developed, and the resolution of data collected on organic compounds and their ages (Riley, Maggi, Kleber, Torn, Tang, Dwivedi, and Guerry, 2014), as well as the microbial communities utilizing them (You, Wang, Huang, Tang, Liu, and Sun, 2014), will undoubtedly improve in the future, with new models and definitions developed also. In one of the simpler current models of C stabilization, Six et al. (2002) defined the C pools as unprotected, chemically protected, biochemically protected, and/or physically protected.

Unprotected C pool Any plant and animal residues not associated with the mineral portion of the soil constitutes an "unprotected" fraction according to Six et al. (2002). Operationally, these pools would be identified as free POM and the light fraction (LF). POM is typically measured based on size. The LF is composed of non-complexed decomposing plant and animal tissues and is separated from bulk soil based on density (Evans, Fernandez, Rustad, and Norton, 2001). It is assumed that during the humification process, recalcitrant SOM (biochemical stabilization) becomes intimately associated with mineral portions of the soil (chemical stabilization) (Barrios, Buresh, and Sprent, 1996). Operationally, any fraction having a density less than that of the mineral fraction, which is not occluded within microaggregation (physical

stabilization), is assumed to be free LF, and more bio-available. Though some of the LF is likely composed of biochemically stabilized materials not associated with the mineral fraction, this fraction, as well as free POM, has been shown to be easily decomposable (Cambardella and Elliott, 1992) and therefore a good indicator of the labile fraction of SOM (Janzen, Campbell, Brandt, Lafond, and Townley-Smith, 1992). This unprotected pool is comprised largely of plant derived SOM, however it also contains significant amounts of microbial debris, fungal hyphae, seeds and spores, representing a diverse mix of chemical compounds (Oades, Vassallo, Waters, and Wilson, 1987). This fraction is especially sensitive to climate, land-use, and disturbance, with its turnover governed by seasonally regenerated plant residues and the recycling of biomass due to microbial decomposition and proliferation (Six et al., 2002). The unprotected C pool is continuously cycled with other stabilized pools through aggregate turnover, incorporation within microbial biomass, adsorption and desorption with the mineral fraction, and transformation into biochemically recalcitrant compounds.

Biochemical stabilization Biochemical stabilization occurs when an organic compound is inherently difficult for most decomposer organisms to utilize as a substrate (von Luetzow, Kögel-Knabner, Kogel-Knabner, Ekschmitt, Flessa, Guggenberger, Matzner, and Marschner, 2007). The residue quality of fresh plant material may indicate the presence of complex molecules (lignin, waxes, lipids etc.) that contain bonds thermodynamically resistant to degradation (Kleber, 2010). Additionally, during humification organics may condense, limiting the access of degrading enzymes, or they may become complexed with metals. These processes change the structure of molecules into those of humic substances unrecognizable to most decomposer organisms (Jenkinson and Rayner, 1977; Paustian, Parton, and Persson, 1992).

Labile compounds, such as proteins and simple carbohydrates, have an energeti-

cally better payoff for the effort required to break the bonds, and it is assumed that, if present, these labile compounds will be preferentially utilized, resulting in an accumulation of recalcitrant compounds with time (von Luetzow et al., 2007). Though extremely old examples of biochemically stabilized compounds are often found (Bol, Huang, Meridith, Eglinton, Harkness, and Ineson, 1996), supposedly easily degradable metabolic compounds have also been found stabilized for millennia (Paustian et al., 1992). Degradation of any organic compound is highly dependent on the microbial communities present, as well as environmental factors such as pH and temperature (Kleber, 2010). It is widely held that, within any naturally occurring soil, the diversity of microoganisms and their enzymes contain the capacity to degrade any substance no matter how complex (Dungait, Hopkins, Gregory, and Whitmore, 2012). The situational dependence of biochemical recalcitrance has been shown empirically through the rapid degradation of complex molecules such as lignin (Thevenot, Dignac, and Rumpel, 2010), waxes (Wiesenberg, Schwarzbauer, Schmidt, and Schwark, 2004) and humic substances (Stevenson, 1982), relative to labile compounds, when environmental conditions are altered. While the molecular structure and chemical complexity of organic molecules obviously has an effect over their decomposition, it is generally accepted that this effect is only influential on short time scales (i.e. seasonally) (Amelung, Brodowski, Sandhage-Hofmann, and Bol, 2008; Stockmann, Adams, Crawford, Field, Henakaarchchi, Jenkins, Minasny, McBratney, Courcelles, Singh, Wheeler, Abbott, Angers, Baldock, Bird, Brookes, Chenu, Jastrow, Lal, Lehmann, O'Donnell, Parton, Whitehead, and Zimmermann, 2013) and that it is biological and environmental conditions that exert primary control over a compound's long term residence time in the soil (Schmidt et al., 2011; Stockmann et al., 2013).

1.3.1.2 Physical Protection of Carbon

Chemical stabilization Chemical stabilization occurs when organic compounds, either biochemically labile or complex (Sorenson, 1972), form intimate associations with mineral particles through cation bridging, hydrogen bonding, or van der Waals forces (Jastrow and Miller, 1997). When stabilized, the affinity of organics to the charged mineral surface exceeds that of enzymatic active sites, rendering the materials unavailable to microbial degradation, even when in close spatial proximity (Dungait et al., 2012).

Due to the charges present on the extensive surfaces represented by the silt and clay fraction, fine textured soils accumulate more C than sandy soils with similar organic matter inputs (Hassink, 1997). Its association with texture means that this fraction of stabilized C is highly dependent on the physical properties of the soil. Total surface area, pH, CEC, clay and metal varieties, as well as the chemistry of the organic matter inputs, all greatly impact the affinity of organics in the formation of these organo-mineral complexes (Plante and Conant, 2006). This form of stabilization is generally accepted as the primary mechanism whereby C is stored for millennia (Dungait et al., 2012), however due to its reliance on available surface area, there exists a limit to the quantity of C that can accumulate within this fraction (Hassink, 1997; Stewart, Paustian, Conant, Plante, and Six, 2007).

Physical protection with microaggregates Occlusion of SOM within aggregation works with adsorption to silt and clay surfaces in the physical preservation of SOM (Dungait et al., 2012). While adsorption makes substrates unavailable to organisms, even when in close proximity, physical protection of SOM within pore spaces restricts the proximity of substrates from the microbial communities which would otherwise be able to use them (Kuka, Franko, and Rühlmann, 2007). This separation is

accomplished by the compartmentalization of microbial communities and substrates in small pore spaces, or large pore spaces with narrow pore openings (pore exclusion) (Killham, Amato, and Ladd, 1993). Additionally, the reduced diffusion of oxygen, nutrients and enzymes within small pores limits microbial metabolism and proliferation (Sexstone, Revsbech, Parkin, and Tiedje, 1985). If all SOM is theoretically degradable (see above), the constraints on microbial decomposition would be limited by the co-occurance of water, substrate, microbe, and possibly oxygen (Kuka et al., 2007).

There exists a reduced level of biotic activity at the center of aggregates (Sollins, Homann, and Caldwell, 1996), and a substantial proportion of SOM is found within aggregation rather than free within the soil matrix (Elliott and Coleman, 1988; Golchin, Oades, Skjemstad, and Clarke, 1994). Occlusion within aggregates seems to change the quality of SOM, occluded SOM having a higher carbon to nitrogen ratio (Golchin et al., 1994), suggesting that nitrogen rich labile compounds may be preferentially decomposed at the center of aggregation, leaving behind more recalcitrant C compounds.

The physical protection of C from degradation varies with aggregate size. Macroaggregates (250-2000 µm) are not very stable and therefore occluded C is more likely to be re-exposed to decomposer communities. Additionally, the pore spaces within macroaggregates are large enough to allow for water and microbial movement as well as oxygen and nutrient diffusion. Conversely microaggregates (53-250 µm) are extremely stable, protecting the occluded C from re-exposure. Microaggregate pores are small enough to effectively limit the accessibility of microorganisms and hold water with enough attraction to limit oxygen and nutrient diffusion (Frey, 2005).

The variation in C protection within different aggregate sizes is supported in the literature. When macroaggregates were crushed, the increase in mineralization ac-

counted for only about 1-2% of the total C content of the structures (Beare et al., 1994; Elliott, 1986), meaning the biochemical availability of occluded C was not that much different than if the C had not been occluded. Conversely, when free microaggregates were crushed they demonstrated three to four times the mineralization of crushed macroaggregates of the same soil (Bossuyt, Six, and Hendrix, 2002). Using ^{13}C abundance Jastrow, Miller, and Boutton (1996) found that C associated with free microaggregates was biochemically more recalcitrant than that of macroaggregates, and free microaggregates had an average turnover time of 412 years, while macroaggregate C turnover time was only 140 years. While research supports the theory that macroaggregates exert minimal influence over SOM protection, macroaggregates are crucial in the formation of microaggregates, and macroaggregate turnover is a major contributor in governing long term C stabilization in soils (Six, Elliott, and Paustian, 2000; Six et al., 1998; Six, Schultz, Jastrow, and Merckx, 1999).

1.3.2 Aggregate Dynamics and Carbon Stabilization

Carbon sequestration is often discussed as the capture and long term storage of atmospheric CO_2 (Lal, 2008), however this definition can be misleading when discussing sequestration in soils. Soil C containing compounds are not stable, but rather they are a dynamic component in the decomposition cycle. C may be mineralized immediately through microbial metabolism, or be cycled through microbial biomass several times before becoming mineralized (Dungait et al., 2012). The time between a C compound entering a soil environment, and being mineralized back into the atmosphere, is determined by the probability in time and space of being physically available to an appropriate organism (Ekschmitt, Liu, Vetter, Fox, and Wolters, 2005). The goal of soil C sequestration is therefore shifting the cycle of decomposition to one that maintains more C for longer periods of time, not as an indefinite storage mechanism,

but rather as a down shifting the rate of a cycling system.

Physical protection of C within aggregation reduces the probability of that C encountering an appropriate soil organism and is a viable way that the rate of C mineralization may be slowed (Dungait et al., 2012; Ekschmitt et al., 2005; Lavelle, 1997; Lutzow, Kogel-Knabner, Ekschmitt, Matzner, Guggenberger, Marschner, and Flessa, 2006). A paradox exists, however in that the preservation of a soil's structure, and therefore the preservation of the occluded C, depends on the continual biological contributions of SOM decomposition by microorganisms (Baldock, 2002; Dungait et al., 2012; Watts, Whalley, Brookes, Devonshire, and Whitmore, 2005). The agents holding all biotically sourced aggregates together are subject to biotic and abiotic degradation, and once all POM is fully degraded there is no longer a substrate provided for microorganisms to utilize and the production of aggregating agents is reduced, reducing structural stability (Baldock, 2002). The natural turnover of aggregation is more rapid within large aggregate classes due to the temporary nature of the binding agents (Tisdall, 1991). Microaggregation is very stable due to the intimate associations between clays and organic matter (Tisdall and Oades, 1982), and its turnover is much slower than that of macroaggregation, aiding in the protection of its occluded C.

The formation of microaggregates within macroaggregates has been widely demonstrated (Angers et al., 1997; Beare et al., 1994; Golchin et al., 1994; Jastrow, 1996; Oades, 1984), and based on this research Six et al. (1998) developed a model to explain the influence of disturbance, in this case agricultural tillage, on the stabilization of C within microaggregates. In his model, frequent disturbance inhibits the formation of microaggregates within macroaggregtes. If time is not allowed for microbial bio-products to be produced, and the organo-mineral associations to form, microaggregates will not stabilize and will offer limited protection to the associated organic

matter. While tillage has been demonstrated to negatively affect the accumulation of microaggregate associated organic matter (Yoo and Wander, 2008), some mixing is required in order to put organics into contact with mineral surfaces in the first place. It is likely a combination of contact time and soil mixing that determines the rate of microaggregate and organo-mineral complex formation (Yoo, Ji, Aufdenkampe, and Klaminder, 2011).

To investigate the effect of tillage frequency on the incorporation of fresh POM into microaggregate structures Plante and McGill (2002) conducted a lab study. They found that simulated tillage did not have the hypothesized effect of increased soil respiration. It was hypothesized that the frequent exposure of substrate to microbial communities would increase the net microbial activity. It was found that added POM increased soil respiration shortly after addition, regardless of treatment, but by the end of 8 weeks, total CO_2 emissions were significantly higher in the non-disturbed treatments. Results suggested that the labile portion of added POM was rapidly decomposed regardless of treatment, and that while no-till samples had higher aggregation at the beginning of the experiment, this effect declined steadily with time. Those soils that underwent tillage had lower, but more dynamic aggregation, with aggregates rapidly recovering after each tillage event. These results led Plante and McGill (2002) to hypothesize that 3 thresholds of aggregate turnover likely exist dictating whether organic matter is released or protected, and that different tillage frequencies likely impact old and new SOM differently. If a soil is already highly aggregated, and the turnover of that aggregation is slow, than incoming organics will be rapidly mineralized before aggregation can form to protect it. At this rate of turnover (R1), any disturbance will likely result in the occlusion of fresh organic matter, reducing the net mineralization from the soil. As the rate of turnover increases, a level will be reached (R2) where fresh occluded SOM will be re-exposed before stable microaggre-

gates and organo-mineral complexes can form, and at that point net mineralization will begin to increase. A third threshold (R3) is proposed where old and previously protected organic matter is exposed so frequently that aggregation no longer provides any physical protection. The turnover rates for each of these thresholds will obviously differ among soil types, clay content, and quality and quantity of SOM inputs, as well as the mechanism of turnover.

Most studies investigating aggregate turnover have looked at the impacts of various agricultural tillage systems (Beare et al., 1994; Bossuyt et al., 2002; Paustian, Six, and Elliott, 1999; Plante and McGill, 2002; Six et al., 2000). Much less study has been done investigating the aggregate dynamics within temperate forests , ecosystems that are widely considered important as potential sinks for atmospheric CO_2 (Lal, 2005). In forested systems aggregate turnover is naturally slower, limited to freeze/thaw and wet/dry cycles, wind throw, and bioturbation (Currie, Yanai, Piatek, Prescott, and Goodale, 2002). Many of the forests of the northeast currently lack the efficient soil mixing provided by various earthworm species. Forests not influenced by the bioturbation of earthworms are likely to be considered native ecosystems and therefore may reside near R1 on the threshold scale proposed by Plante and McGill (2002). It is likely that earthworms will enter into these ecosystems in the future (Hale, Frelich, Reich, and Pastor, 2005), increasing the aggregate turnover of the soils, and yet the impact of their particular type of bioturbation on the C dynamics of forest soils is still largely unknown.

1.4 Earthworms as Ecosystem Engineers

Oligochaeta contains approximately 8000 species from 800 genera (Edwards, 2004) inhabiting both aquatic and terrestrial ecosystems and ranging in size from a few

millimeters to several meters long. The occurrence and abundance of earthworms in terrestrial soils is based on soil moisture, temperature, acidity and organic matter content, as well as the presence of predation or the co-occurrence of other earthworm species (Lee, 1985).

Earthworm populations can be subjectively judged as either beneficial or detrimental, depending on where thay are located. Many earthworm species occupy more than one ecological niche, and with extensive diversity in reproduction and feeding preference among species, the specific effect of any one earthworm community may be viewed as unique to that community in that environment. However, many commonalities exist between species, especially with those inhabiting similar ecological roles, and so these roles are helpful in conceptualizing how earthworm presence may influence soil properties.

Earthworms are placed into three distinct groups (Bouche, 1975), each inhabiting a specific ecological role. Anecic species break down fresh litter from the surface, pulling it into the soil surrounding their deep permanent burrows. Epigeic species live and feed on the litter at the surface, rarely burrowing into the mineral soil. Endogeic species live and burrow in the upper areas of the mineral soil, feeding on mineral-associated organic matter. Many species may occupy more than one role, depending on where they live and feed, altering their soil impact beyond the above mentioned groupings. The different classification of earthworms, working as a community and individually, drastically alter the chemical, physical, and microbial environments in the soils they inhabit. Endogeic species can ingest 5 to 30 times their body weight of mineral soil per day (Lavelle, Bignell, and Lepage, 1997). Depending on a population's density the drilosphere, defined as any area in the soil directly effected by earthworm manipulation, can encompass the majority of upper mineral soil volume (Brown, Barois, and Lavelle, 2000).

Earthworms have been termed "ecosystem engineers" due to this wide reaching impact on soil structure and ecology (Doube and Brown, 1998). Earthworms have long been revered in agriculture for the positive effect they have on soil health parameters. The reorganization of mineral and organic materials within their gut increases soil porosity and subsequently soil water retention and aeration. They also increase available soil nutrient concentrations by breaking down complex organic materials into microbially accessible food sources, while distributing them throughout the soil profile and making them more available for root uptake (Bohlen, Pelletier, Groffman, Fahey, and Fisk, 2004). Increased nutrient levels, aeration and water retention are positive factors for plant growth, and desirable in agricultural settings.

1.4.1 The Earthworm Invasion

The distribution of earthworm species throughout the globe is widespread, with earthworms found in almost any area inhabited by humans. Earthworm species are in constant movement, and exotic species are continually moving into areas devoid of earthworms or coming into contact with native earthworm populations (James and Hendrix, 2004). Many earthworm species can demonstrate invasive qualities, such as prolific breeding behaviors, resistance to disturbance, or the ability to become dormant in response in unfavorable conditions (Lee, 1985). However some species may simply be inadvertently transported by human activities, placed into a new habitable environment where they do not proliferate. Without inadvertent long distance transport due to human activities it is unlikely that the variety of earthworm species colonizing such a large area would be seen (Parkinson, McLean, and Scheu, 2004). Either scenario of establishment, population movement or human transport, has wide reaching impacts on the ecosystems earthworms newly inhabit, and the extent of these interactions is complex and still in need of investigation.

Earthworm species inhabit many ecosystems and display a wide range of behavioral, physiological, and morphological adaptations to changing environmental conditions (Lee, 1985). Even in supposedly unsuitable environments such as deserts and urban settings, earthworms may inhabit local micro-sites where favorable conditions exist (Curry, 2004). During the last glaciation, ending approximately 10,000 years ago, earthworms were eradicated from the current temperate areas of North America (latitudes of 45-60^{o} north). It has only been within the last 300 years, with the introduction of the European and Asian earthworms, that these soils have been exposed to earthworm influence (Frelich, Hale, Scheu, Holdsworth, Heneghan, Bohlen, and Reich, 2006).

Earthworms have long been revered for the beneficial effects they have on plant growth, and therefore they are often a welcome site in gardens and other agricultural settings. However, when put in forested ecosystems earthworms are often considered invasive, and they are capable of having long lasting and wide reaching ecological impacts. The movement of European and Asian species of earthworms into forests from horticultural settings has been the subject of considerable study (Fahey, Yavitt, Sherman, Maerz, Groffman, Fisk, and Bohlen, 2013; Groffman, Bohlen, Fisk, and Fahey, 2004; Hale, Frelich, and Reich, 2005; Hopfensperger and Leighton, 2011; Sackett, Smith, and Basiliko, 2013), with the understanding that earthworm presence will have long term implications for forest health and soil C dynamics.

Colonization of forests has been shown to begin at roads, and other areas of frequent travel, and progress into forests in a wavelike manner (Dymond, Scheu, and Parkinson, 1997; Hale et al., 2005). Hale et al. (2005) described a dynamic of earthworm invasion in the hardwood forests of Minnesota where epigeic and epi-endogeic species were established first, integrating the forest floor into the mineral soils. This action allowed for the establishment of endogeic species, which live and feed

on C enriched mineral soils. Hale et al. (2005) hypothesized that it was this presence of endogeic species, in concert with anecic establishment, that made it difficult for the forest floor to recover, and this represented the furthest stage of invasion. The colonization of new habitats by earthworm movement is usually slow, moving at about 10 meters per year (Marinissen and van den Bosch, 1992), though this rate is dependent on the populations colonizing and the habitability of the new environment. The population size of earthworms usually reaches maximum levels at the front of invasion, numbers decreasing with the reduction of usable food sources and the co-habitation of many species (Dymond et al., 1997; Hale et al., 2005)

1.4.2 Earthworms and Carbon in Northeastern Forests

1.4.2.1 Removal of the Forest Floor

As earthworms have migrated from agriculture systems into forests, or as forests have re-established on soils with active earthworm populations, the same processes which made earthworms beneficial for agricultural settings have had negative impacts on forested ecosystems. This negative impact has occurred primarily through the manipulation of the forest floor. Numerous studies have noted that over the course of a few years the forest floor is significantly decreased, if not completely absent, in newly earthworm invaded forests (Bohlen et al., 2004; Holdsworth, Frelich, and Reich, 2012).

The forest floor is an accumulation of litter, in various stages of decay, located above mineral soil horizons. Due to a lack of disturbance and mixing the forest floor represents a progression of decomposition consisting of relatively unaltered leaf litter on top (Oi), fragmented and darkening litter (Oe) below, and amorphous humified substances (Oa) above mineral soil (Currie et al., 2002). When this organic matter

accumulation is defined from the sub mineral horizons it is classified as a *mor* soil, and when the delineation between the organic accumulation and mineral soil is unclear, it is classified as a *mull* soil (Müller and Tuxen, 1878). The forest floor is a fully functional part of the forest ecosystem and plays a dynamic role in the regulation of soil temperature, pH, water retention and infiltration, nutrient management and C storage. These functions are part of a complex ecological system which is strongly linked to forest production, regeneration, and nutrient cycling through dynamic feedback systems. Small changes in the forest floor have the potential for large consequences on the forest community (Currie et al., 2002).

The forest floor acts as a necessary seed bed for many native herbaceous plants and trees. Through earthworm bioturbation forest soils are shifted from a *mor* to *mull* classification and the environment is shifted from what is appropriate for native forest species, to one favorable for plants adapted to germinating in bare mineral soils. Earthworm invasion has been shown to change an understory diverse in native herbaceous plants and tree seedlings into one dominated by grasses (*Carex sp.*) (Hale, Frelich, and Reich, 2006) and invasive plants (Nuzzo, Maerz, and Blossey, 2009). It has also been documented that some rare plant species have been eradicated from forested areas as a direct result of earthworm integration of the forest floor (Gundale, 2002). The shifting in understory has an affect on the entire above ground forest ecosystem, likely impacting the future productivity of a forest.

The relocation of the C-rich forest floor also has implications for a forests roll as either a sink or source of atmospheric C. The role that the forest floor plays in the C cycle is associated with its ecological importance. Although mineral soil accounts for the majority of C in forest soils, most of mineral soil C originates from the forest floor (Currie et al., 2002), moving downward in the soil profile by soil mixing and DOC percolation. Other mineral C sources include microbial biomass

and roots. The redistribution of this organic-rich layer into deeper mineral horizons can have long term impacts on the total C storage of these soils. While most research strongly favors the hypothesis that earthworm presence results in a net loss of C from forest soil systems (Alban and Berry, 1994; Bohlen et al., 2004; Frelich et al., 2006; Lyttle, Yoo, Hale, Aufdenkampe, and Sebestyen, 2011), there is conflicting research that finds the opposite when looking at different depths (Wironen and Moore, 2006), or different time scales (Alban and Berry, 1994; Zhang, Hendrix, Dame, Burke, Wu, Neher, Li, Shao, and Fu, 2013). This reduction in total soil C is accomplished through a relocation, and enhanced decomposition of the forest floor, however as C is moved lower into the profile gathering an accurate summation of total C stores becomes more difficult. There is also a growing body of evidence that in many circumstances earthworms may be capable of stabilizing C for long periods of time within their burrow walls (Don, Steinberg, Schöning, Pritsch, Joschko, Gleixner, and Schulze, 2008) and castings (Bossuyt, Six, and Hendrix, 2004,0; Shan, Liu, Wang, Yan, Guo, Li, and Ji, 2013; Zhang et al., 2013).

1.4.2.2 Earthworm Enhancement of Bacteria Dominated Respiration

How soil microorganisms are affected by passage through the earthworm gut is controversial. There are many different ways to measure microbial activity, many of which may result in conflicting conclusions (Insam, 2001). Variations among species are also often generalized for entire earthworm groupings (Butenschoen, Marhan, Langel, and Scheu, 2009), which may not neccessarily be true. External variables such as soil texture (Butenschoen et al., 2009), quality of food sources (Tiunov and Scheu, 2000), and interactions between species may also drastically alter findings. Additionally, it has been found consistently that the microbial community structure of freshly excreted castings is different than those that have been aged (Scheu, 1987; Tiunov and

Scheu, 1999), meaning that time of analysis will impact the conclusions drawn from passage through the earthworm gut.

Microbial biomass is believed to increase, especially in lower mineral horizons (McLean, Migge-Kleian, and Parkinson, 2006), through the enhancement of organic C chemical and physical availability (Li, Fisk, Fahey, and Bohlen, 2002). However, the inconsistency of this effect suggests that it is likely dependent on other variables, such as whether an earthworm species uses a microbial communities as a secondary food source (Zhang, Li, Shen, Wang, and Sun, 2000). Despite inconsistent proof of their impact on microbial communities, through their influence over substrate availability and soil physical characteristics, earthworms are still believed to be a major indirect contributor to greenhouse gas emissions from the soils they inhabit (Lubbers, van Groenigen, Fonte, Six, Brussaard, and van Groenigen, 2013).

The earthworm gut as a microenvironment From a microbial perspective the conditions within the earthworm gut are markedly different than those of the ingested soil (Drake and Horn, 2007). Ingested microorganisms would immediately encounter an anoxic environment, rich in easily accessible organic molecules derived from both locally ingested SOM, and mucous rich in polysaccharides and proteins secreted by the earthworm to aid in the passage of materials (Drake and Horn, 2007). This environment would theoretically favor anaerobic and facultative aerobic organisms, and the ingested and egested soils would be expected to differ in community structure due to this transient environment. This effect is very difficult to study, due to the limited number of microbial species capable of being cultured (Hill et al., 2000), however it appears that the primary difference in ingested and egested materials is due to a quantitative enhancement of the ingested anaerobic communities relative to aerobic communities, and not a reduction of aerobic communities (Karsten and

Drake, 1995). Additionally, the difference an anaerobic communities appears to be strictly quantitative, excreted community diversity being similar to that of bulk soil (Ihssen, Horn, Matthies, Gossner, Schramm, and Drake, 2003), although it is still likely that excreted soils are inoculated with gut-specific microbes (Shipitalo and Bayon, 2004). In general it may be concluded that earthworm ingestion appears to stimulate microbial communities in the soil (Drake and Horn, 2007), however the specific changes appear to vary with earthworm species, substrate quality, soil texture, and the co-occurance of other soil fauna.

Priming effect Due to the limited availability of substrates, most bacterial cells in the soil are in a state of dormancy (Morita, 1993). De Nobili, Contin, Mondini, and Brookes (2001) proposed the "trigger molecule hypothesis" a theory which explains the survival of soil microorganisms physically separated from a usable substrate the majority of the time. It is hypothesized that most microorganisms are capable of maintaining a "metabolically alert" state when not in the proximity of a usable substrate, or when that substrate would cost more energy to metabolize than it would provide the organism (Stenstrom, Svensson, and Johansson, 2001). When low molecular weight molecules are detected, the organism can switch to a "metabolically active" state which would allow them to access not only the low molecular weight substrates but also the previously unattainable ones. This hypothesis would explain the well understood "priming effect" (Kuzyakov, Friedel, and Stahr, 2000), which occurs when small additions of organic matter result in an disproportionate response in respiration (Blagodatskaya and Kuzyakov, 2008). Because of the combining of substrate and microbial biomass, this priming effect has been attributed to passage through the earthworm gut (Lavelle et al., 1997).

Through a disruption of hyphal networks, and the potential to preferentially utilize

fungal cells as a substrate, many earthworm species are thought to reduce the ratio of fungi to bacteria in soils (Dempsey, Fisk, and Fahey, 2011). This change in the ratio of fungi to bacteria may alter C stabilization in soils indirectly by shifting the degradation of organic compounds from a system dominated by fungi to one dominated by bacteria. The degradation of lignin in soil it primarily fungal driven (Thevenot et al., 2010), and bacteria are less efficient than fungi at assimilating C and therefore respire a higher ratio of CO_2 than fungi (Adu and Oades, 1978). Additionally, the chemistry of the microbial biomass would change C turnover, fungal biomass being constructed of more complex biomolecules (chitin, melanin etc.), which are not typically found in bacterial biomass (Jastrow, Amonette, and Bailey, 2006).

1.4.2.3 Earthworm Protection of C Through Aggregate Formation

Earthworms are the most studied of all soil fauna, especially in relation to soil aggregation (Six et al., 2004). Shipitalo and Protz (1989) were the first to propose a model for the formation of microaggregates by earthworms. As earthworms move through the soil they ingest both organic material and mineral soil particles, which are then mixed within the earthworm gut and excreted as castings. Different earthworm species expose ingested soils to a variety of pressures and gut transit times (Shipitalo and Bayon, 2004). The pressures in the earthworm gut, together with additions of large amounts of watery mucus (Barois, 1992), disrupt the cation bridges binding the smallest and most stable of soil aggregates, mobilizing individual clay particles (Marinissen, Nijhuis, and van Breemen, 1996). Clay particles are then brought into intimate contact with new binding agents, which are rich within the earthworm gut, forming the basis for new microaggregate structures once excreted (Shipitalo and Protz, 1989). It can therefore be assumed that during passage through the gut of most soil dwelling earthworm species, the majority of all soil structure is destroyed,

left to reform upon excretion. Additionally, as earthworms move through the soil, they exert pressure on their burrow walls (Edwards and Bohlen, 1996). This pressure works with the addition of external mucus to orient clays along burrow walls, forming stabilized structure (Don et al., 2008; Lee, 1985). Of the three ecological groups, endogeic and anecic species are considered the most influential in soil aggregation properties (Lavelle and Spain, 2001).

While most earthworm castings have a defined physical structure, the structure is initially unstable (Barois, 1992; Marinissen et al., 1996; Shipitalo and Bayon, 2004). Castings appear to typically stabilize with aging, however the level of stabilization depends on all variables regarding soil and individual earthworm qualities, as well as the methodology used to measure stabilization (Shipitalo and Bayon, 2004). Physical stabilization of castings may occur because of the intimate association of clays within the earthworm gut, however this stabilization happens relatively quickly, failing to explain the increased stabilization noted over the long times frames of days to weeks (Marinissen et al., 1996). The effectiveness of various biologically sourced binding agents (microbially sourced polysaccharide, earthworm mucus, etc.) in cast stabilization remains controversial and situationally based (Shipitalo and Bayon, 2004). Many earthworms are known to secrete amorphous calcium carbonate (Edwards and Bohlen, 1996), which is possibly a very effective binding agent (Zhang and Schrader, 1993). In addition to its effect on aggregation, this calcium carbonate excretion has been suggested as an alternative way that some species are capable of immobilizing large quantities of C within the soil system (Briones, Ostle, and Piearce, 2008).

A decline in microbial activity is usually detected in casts shortly after they are excreted, relative to bulk soil. When investigating the aged castings of *Lumbricus terrestris,* Tiunov and Scheu (2000) found that while basal respiration was 30-130% higher than expected after excretion, by day 10 both respiration was significantly

less than expected. When investigating the casts of *Aporrectodea calingnosa,* Scheu (1987) noted a significant decline in microbial activity with time, however these values never reached a level that was less than the bulk soil. These findings, among many other conflicting studies (McLean et al., 2006), highlight that the effect earthworms will have on microbial dynamics is situationally based, and will depend highly on the method and time of measurements.

In recent years, many studies have been conducted supporting the theory of earthworm enhanced microaggregation in soils. (Bossuyt et al., 2004,0) found that earthworms increased large macroaggregates ($>$ 2000 µm) by 3.6 times, increased microaggregates within macroaggregates by 4 times, and that 22% of the C within these occluded microaggregates was from newly added C sources. This supports earthworms ability of rapidly incorporating new residues into physically protected microaggregates. The majority of studies investigating earthworm influenced microaggregation have been conducted within agricultural soils (Don et al., 2008; Pulleman, Six, Uyl, Marinissen, and Jongmans, 2005), or have used homogenized soils (Bossuyt et al., 2004), leaving a gap in the understanding of the effect of earthworms on the aggregation within non-invaded forest soils.

Six, Callewart, Lenders, Morris, and Paul (2002) found that the protection of C within microaggregates accounted for 20% of the increased C levels seen in forest soils compared to similar agricultural systems. Microaggregates play a crucial role in a forest's potential to sequester C, and it is a buildup of C witin these structures that may be the key in mitigating anthropogenic C through forest management practices. While it has been demonstrated that earthworm invasion into forests generally results in a loss of C from the system in the short term (Lubbers et al., 2013), and that earthworms are capable of creating a physically protected C source through increased microaggregation (Bossuyt et al., 2005), the influence these soil animals have on the

microstructure already present in forests has not yet been thouroughly investigated.

1.5 Conclusion

There exist a large number of publications that show conflicting findings as to the net effect of earthworms in forests (Brown et al., 2000), and as highlighted in the above summary the processes are complex and often feedback on one another. Organisms and structural properties likely demonstrate opposing effects on SOM dynamics at different spatial or temporal scales. The net effect of earthworms on any one system will likely involve a concurrent acceleration of decomposition and accumulation of physically stabilized C fractions, the net loss or capture dependent on the myriad of variables present in any given soil ecosystem. Microbial communities and their functions are still very poorly understood with some researchers estimating that more than 90% of the soil's microbial community is unable to be cultured with current technology (Hill et al., 2000). Microbial aspects are therefore considered a "black box" in the realm of SOM dynamics and turnover (Tiedje, Asuming-Brempong, Nusslein, Marsh, and Flynn, 1999), and likely the missing link in truly understanding the relationships between earthworms and C turnover. In the meantime however, microaggregate creation and turnover is a viable proxy for analyzing changes in the C turnover dynamics under earthworm influences because of its known relationship with soil microbial communities.

1.6 Research Objectives

Earthworms are likely to impact many of the forests in Vermont in the future, and yet very little is known of the long term implications they will have on the C retention of the forest soils. The bioturbation by earthworms is very different than what would

occur during conversion to agriculture, and therefore the net loss of C should not be expected to be comparable. Very little is still known about how this particular type of aggregate turnover will impact the quality and quantity of the microaggregate pools already present in many forest soils. The objectives of this research were to

1. Conduct a statewide survey to increase our understanding of where earthworm communities are currently located in the forests of Vermont and gather baseline community data on the impacts they may be having.

2. Correlate earthworm community presence with the quantity and quality of microaggregates in the soil.

3. Conduct a controlled paired study investigating the effect of one earthworm species on the quantity and quality of microaggregates in an undisturbed forest soil.

1.6.1 Rational for Methods

Most soil C in surface soils is found within aggregates, and 20-40% of total SOM is found inside microaggregates (Carter, 1996). Using ^{13}C natural abundance, many studies have demonstrated that the turnover time of C occluded in macroaggregates (>250 µm) is substantially shorter than that which is occluded within microaggregates (Angers et al., 1997; Carter, 1996; Six et al., 2002; von Luetzow et al., 2007). The C stabilization in microaggregates is increased if the SOM demonstrates both physical protection and chemical recalcitrance, and if both stabilization mechanisms are present SOM associated with microaggregates may have a residence times of several thousand years (Carter, 1996).

The isolation and analysis of these structures represents a pool of C having an assumed mean residence time (MRT) that is greater than that of the bulk soil (Lutzow

et al., 2006). However, because the SOM present within these structures has formed, and become stabilized, by simultaneously acting mechanisms (humification, organo-mineral complexion, physical protection) the quantification of these structures can not represent a functionally homogeneous fraction useful for modeling or predictions. By analyzing the soil microaggregate pool, what is actually being investigated is the C pool associated with micro-pore spaces that undergo more C-stabilizing biological and physio-chemical processes than the bulk soil. Therefore the operational definition of "microaggregate", and the methods used to obtain these structures, has an inseparable influence on how various resulting values are interpreted.

To fully gain insight into a soil's pool of micropores, the free microaggregates as well as the occluded microaggregates must be investigated. Six et al. (2000) was the first to suggest a method of analyzing not only the free microaggregate structures, but also the microaggregates occluded within stable larger aggregate structures. This is done by first separating the soil into its water stable constituents, the theory being that if a structure is not capable of withstanding the disruption of rapid wetting than it is unlikely to be to maintained within the soil under natural conditions. After the free water stable microaggregates are obtained, the larger aggregate classes are re-wet and gently shaken with beads to release the the occluded microaggregates. These microaggregates are quickly collected in a series of sieves so as to avoid further disruption. Though this method of analyzing occluded microaggregates my cause disruption that is greater than what would be noted in nature, the values obtained from this method represent a minimum number of defined microaggregate structures found in the soil at the time of analysis, and may still be beneficial for comparison analysis. During the above process only the microaggregates 53-250 µm are collected and analyzed, though depending on soil properties there may be a significant proportion of clay bound into micro-structures $<$53 µm that also have an abundance of microp-

ores. Electron microscopy has confirmed that many of these structures are capable of withstanding even the best attempts at full dissociation (Chenu and Plante, 2006), and no consistent way of isolating and analyzing the C occluded within these clay microstructures exists (Chenu and Plante, 2006), depending heavily on clay minerology and the presence of inorganic binding agents.

The proportion of soil microaggregates is determined strictly by the defined sieve size used, and the disruptive forces placed on the aggregate structures. The size cutoff between macro and microaggregates for many methods is 250 µm, and while consistent between many studies this number is a relatively arbitrary cutoff when investigating the distribution of pore sizes within a structure. The disruptive forces placed on the structures is certainly highly variable between studies, with different people and equipment being used. The methods used are required to be highly calibrated between individuals, in order to make comparisons valid within the study.

CHAPTER 2

EARTHWORM PRESENCE IN VERMONT FORESTS: IMPACT ON DISTRIBUTION OF SOIL CARBON WITHIN AGGREGATE FRACTIONS

2.1 Abstract

The impact of earthworm presence on the soil carbon (C) dynamics of previously uninhabited northeastern forests is still largely unknown. Currently, earthworm presence is understood to both enhance soil respiration, and create stable microaggregates, processes assumed to have conflicting effects on long-term C storage. In addition to affecting a forest's ability to sequester C in the long term, earthworm presence is also known to have extensive impacts on a forest's native vegetation and regeneration. The state of Vermont is 75% forested and with the full extent of earthworm communities unknown the management of these forests in the context of increasing earthworm populations is difficult. The purpose of this study was to gather earthworm community data on 18 previously established forest monitoring sites, in which only earthworm absence or presence had previously been noted. For eight northern hardwood sites, earthworm data was correlated with the quantity and quality of mineral soil microaggregates (53-250 µm), a pool of C assumed to be physically protected. For all sites, earthworms had previously been noted, all ecological functional groups were found. Single species were noted in 3 sites that did not have earthworm presence during initial plot establishment, suggesting new communities. Of the species found, *Octalasion cynaeum* is new to the state of Vermont, having never been noted in previous studies. Number of species, or species richness, was used as the metric for earthworm impact in any one sampling location. Species richness showed a strong relationship with a reduction in forest floor depth. Species richness was negatively correlated with the to-

tal proportion of soil dry mass composed of microaggregates. Sampling plots with no earthworm species had approximately 5% less soil mass comprised of microaggregates than those plots with the highest species richness (4-6 species) ($P<0.001$). Species richness was positively correlated with the total C associated within microaggregates, and plots with high species richness contained almost twice the quantity of C within microaggregates than plots with no species noted ($P<0.001$). Earthworms correlated with an increased proportion of macroaggregates (>250 µm) within forest soils, however the proportion of microaggregates within these macroaggregates was reduced. The differences between plots with no earthworms and the plots with few earthworm species (1-3) was rarely significant for any variable measured. The shift from 1-3 species to 4-6 species was dominated by an increase in endogeic species, suggesting that it is primarily this functional group affecting soil aggregation properties.

2.2 Introduction

Earthworms have been termed "ecosystem engineers" due to the significant impact they have on the morphology, nutrient cycling and microbial communities of the soils they inhabit (Lavelle et al., 1997). Earthworms are exotic to previously glaciated areas of North America, and prior to European settlement these soils were devoid of earthworm communities (Gates, 1976). The movement of European and Asian species of earthworms into forests from horticultural settings, has been the subject of considerable study (Fahey et al., 2013; Groffman et al., 2004; Hale et al., 2005; Hopfensperger and Leighton, 2011; Sackett et al., 2013), with the understanding that earthworm presence will have long term implications for forest health and soil carbon (C) dynamics.

Soils contain more C (1,500 Gt) than in all terrestrial biomass (560 Gt) and the

atmosphere (720 Gt) combined (Birdsey, 1992), making them a key player in the attempt to offset anthropogenic C emissions. An intimate relationship exists between the soil and the atmosphere, with soil respiration accounting for roughly 20% of total CO_2 emissions (Rastogi et al., 2002). Northern forests are important C sinks, however sensitivity to environmental changes means earthworm bioturbation has the potential to alter C cycling in these ecosystems (Bohlen and Scheu, 2004).

The state of Vermont is 75% forested, with many forested areas owned and managed by individuals and families (Sinclair, 2013). The primary vectors of earthworm invasion are likely disposed fishing bait, horticultural plant material, and the transport of soils and fill throughout the state (Hendrix and Bohlen, 2002), however the full extent of current earthworm presence in Vermont forests is unknown, making management difficult. In a state where forestry represents a large portion of the economy, and many of the states forests are actively managed, understanding where earthworm species are, and the precise effects they are having on the forests, would help in developing best practices for the future.

Earthworms are typically placed into three groups, each inhabiting a specific ecological role and influencing C turnover differently (Bouche, 1975). Epigeic species live and feed on the litter at the surface, anecic species traverse the litter and mineral soils, pulling fresh litter into their deep, permanent burrows, and endogeic species live and burrow in the upper areas of the mineral soil, feeding on mineral-associated organic matter (Doube and Brown, 1998). Earthworm presence impacts forests negatively through the same actions that make them beneficial in agricultural settings. Different species, working individually or as a community, incorporate nutrient rich surface organics (agricultural debris or the forest floor depending on the setting) downward in the soil profile, resulting in increased nutrient cycling, soil structure, and water infiltration, effects which are beneficial for root growth and nutrient absorption. The

forest floor, composed of litter in various stages of decay, performs many functions within the forest ecosystem, acting as a necessary seed bed for native plants while also regulating the soils moisture, temperature, and nutrient cycling (Currie et al., 2002). The translocation and mixing of the C-rich forest floor into the mineral soil has implications for a forest's role as either a sink or source of atmospheric C.

Most studies investigating the *in situ* impact of earthworm invasion into native northern forests have found a reduction of the forest floor, however there have been conflicting findings on the effect this has on total soil C storage. In a 14-year field study, Alban and Berry (1994) reported that earthworms decreased total soil C by 600 kg per ha per year, however Zhang et al. (2013) notes that reduction was only seen for the first 2 years, before C levels were maintained at a new equilibrium. Bohlen et al. (2004) found that earthworm invasion decreased soil C storage by 28% in the upper 12 cm of a sugar maple dominated forest in New York, while Wironen and Moore (2006), investigating a similar forest type in Quebec Canada, had findings that suggested earthworm presence increased total soil C to a depth of 30 cm. There is little doubt that invading earthworms are increasing the mineralization of C in the short term (Lubbers et al., 2013), however inconsistencies among field findings highlight that there are secondary earthworm processes which have conflicting impacts on C retention depending on situational spatial and temporal circumstances, as well as the methods used during investigation.

There are likely many ways C is stabilized within soils, however the mechanisms of stabilization, as well as methods of measurement, are still being debated. While chemical properties will dictate, to some degree, how resistant soil C may be to microbial attack (Bol et al., 1996; Lutzow et al., 2006), the currently held belief is that it is the accessibility of C compounds to degradation, rather than a compound's intrinsic chemical quality, which dictates its residence time within soils (Dungait et al.,

2012; Kleber, 2010; Marschner, Brodowski, Dreves, Gleixner, Gude, Grootes, Hamer, Heim, Jandl, Ji, Kaiser, Kalbitz, Kramer, Leinweber, Rethemeyer, Schäffer, Schmidt, Schwark, and Wiesenberg, 2008). One frequently cited stabilization mechanism is the physical segregation of bacterial communities, and their enzymes, from C occluded within a soil microaggregates (mA, 250 µm-53 µm) (Adu and Oades, 1978; Dungait et al., 2012; Elliott and Coleman, 1988; Sanchez-de Leon, Lugo-Perez, Wise, Jastrow, and Gonzalez-Meler, 2014; Schmidt et al., 2011; Six et al., 2002; Stewart et al., 2007). The majority of pore space located within microaggregates is limiting to bacterial communities and their of enzymes (Lutzow et al., 2006). Due to the nature of their binding agents and size, microaggregates are extremely stable, and therefore the pore system, and its exclusion properties, are maintained for long periods of time (Oades, 1993). Presuming that microaggregate occluded C does, in fact, represent a pool of stabilized C within the soil, the ability to operationally isolate these structures, as outlined in Six et al. (2000), allows for one mechanism of C stabilization to be analyzed.

During passage through the gut of soil dwelling earthworm species, existing microaggregates are destroyed by grinding within the gizzard and peristalsis through the gut. Through these actions organic debris, polysaccharides and mineral particles come into intimate contact with each other, forming the nuclei for newly formed microaggregates (Shipitalo and Protz, 1989). This process of microaggregation has been observed in homogenized (Bossuyt et al., 2005; Sanchez-de Leon et al., 2014) and undisturbed agricultural soils (Fonte, Kong, van Kessel, Hendrix, and Six, 2007; Pulleman et al., 2005), utilizing various earthworm species and methods of measurement. It has been proposed through these studies that earthworm facilitated microaggregation, and the C enrichment of microaggregates, is a mechanism by which earthworms may stabilize C in the long term, offsetting their affect on increased soil respiration.

As earthworms move from cultivated soils into less disturbed, forest soils it is unclear how the quality and quantity of soil microaggregates may change.

In 2008 and 2009, 18 forest reference plots were established throughout Vermont; the initial purpose being to investigate the effects of forest harvesting on a soil's C storage. During plot establishment it was noted that some sites contained earthworm species, while others did not. It was speculated that, in addition to harvesting effects, land-use history and earthworm presence likely play an important role in the C dynamics of these forest soils. In 2012 and 2013, the 18 reference plots were surveyed and earthworm communities quantified. Objectives were to determine if earthworm metrics correlated with forest floor depth, mineral soil C, and microaggregate associated C.

2.3 Methods

2.3.1 Site Characteristics

The 18 reference sites were established across the state of Vermont using protocol modified from the United States Department of Agriculture Forest Service Forest Inventory Analysis (USDA FS FIA). These sites were funded through the Northern States Research Cooperative (NSRC) and were originally established to investigate the effects of timber harvesting on forest soil carbon (C) dynamics. Site establishment was based on biophysical region and schedule of harvesting as well as forest and soil types (Juillerat, 2011). For locations and site characteristics see Fig. 2.1 and Table 2.1.

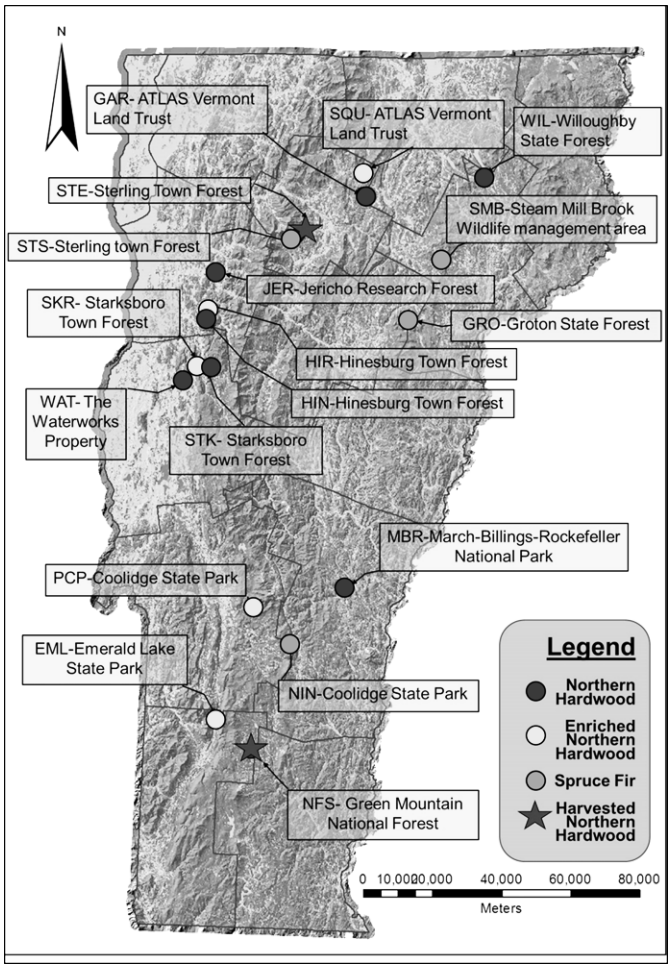

Figure 2.1: Map of Vermont sampling sites. "Harvested" sites are those which had recently undergone forest harvesting prior to the earthworm survey, resulting in visual ground disturbance and possible impacts on other metrics.

2.3.2 Survey Methods

Earthworm surveys were carried out in 9 sites (5 Northern Hardwood, 3 Enriched Northern Hardwood, 1 Spruce-Fir) in 2012, and 9 sites (4 Northern Hardwood, 2 Enriched Northern Hardwood, 3 Spruce-Fir) in 2013. Six 50 x 50 cm survey pits were added to the pre-existing FIA-type plots (Fig. 2.2). Pits were placed equal distance at 30^o, 90^o, 150^o, 210^o, 270 and 330^o magnetic north, 32 m from plot center. Soil moisture (volumetric), and temperature (^{o}C) were read from the upper mineral immediately

Code	Site name	Elevation (m)	Tree Species (common name)	% basal area	Soil Series	Coordinates	Possible Land-Use History	Approximate Time of Abandonment
EML*	Emerald Lake State Park (ENH)	299	Eastern Hemlock Sugar Maple	57.6 25.4	Galway variant	73°0'28.927" (W) 43°17'4.206" (N)	Pasture Woodlot	1980's
GAR	Atlas Partnership Garfield" (NH)	488	Sugar Maple W. Ash	61.3 28.8	Tunbridge, Lyman	72°28'29.873" (W) 44°39'11.231" (N)	Continuous Woodland	N/A
GRO	Groton State Forest (SF)	425	Red Spruce/Red Maple Balsam Fir	44.4/31.9 17.1	Colonel, Bouldery Cabot	72°19'4.231"(W) 44°19'3.589" (N)	Farmland Tilled	1950's
HIN*	Hinesburg Town Forest "poor" (NH)	403	Sugar Maple/Red Maple Paper Birch/White Ash	33.0/17.7 21.6/20.7	Marlow, Peru	73°2'17.603" (W) 44°19'45.78" (N)	Pasture Possible Tillage	1920's
HIR*	Hinesburg Town Forest "rich" (ENH)	370	Paper Birch/Sugar Maple White Ash	36.9/34.5 21	Berkshire, Peru, Shelburne	73°2'40.093" (W) 44°19'29.455" (N)	Pasture Woodland	1900's
JER*	Jericho Research Forest (UVM) (NH)	154	Sugar Maple American Beech	62.5 20.4	Adams	73°0'21.47" (W) 44°26'51.152" (N)	Farmland Pasture	1940's
MBR*	Marsh-Billings-Rockefeller National Park (NH)	397	Sugar Maple White Ash	91.1 8	Vershire deep variant	72°32'42.04" (W) 43°37'52.736" (N)	Pasture No Tillage	1940's
NFS	Green Mountain National Forest (NH)	493	Sugar Maple/Red Maple Paper Birch/Red Spruce	42.7/21.6 17.1/16.1	Marlow, Peru	72°52'40.931" (W) 43°11'49.402" (N)	Pasture Possible Tillage	1960's
NIN	Coolidge State Forest "Ninevah" (SF)	552	Balsam Fir Red Maple	47.3 37.3	Peru	72°44'54.471" (W) 43°28'45.42" (N)	Pasture Never Tilled	1980's
PCB	Coolidge State Forest "PCB" (ENH)	651	Sugar Maple White Ash/ American Beech	53.6 20.4/17.3	Peru, Colonel	72°51'41.004" (W) 43°34'14.486" (N)	Continuous Woodlot	N/A
SKR*	Starksboro Town Forest "rich" (ENH)	349	Red Oak/Black Cherry Sugar Maple/Red Maple	21.6/19.4 18.7/15.4	Dummerston, Taconic	73°2'23.205" (W) 44°12'7.962" (N)	Pasture Woodland	1940's
SMB*	Steam Mill Brook Wildlife Management Area	649	Balsm Fir/Red Spruce Red Maple	53.3/12.0 27.6	Dixfield, Tunbridge	72°11'37.917" (W) 44°28'37.023" (N)	Pasture Never Tilled	1970's
SQU	Atlas Partnership Square" (ENH)	589	Sugar Maple Yellow Birch	96.5 1.9	Buckland, Shelburne	72°28'17.679" (W) 44°42'19.337" (N)	Continuous Woodland	N/A
STE	Sterling Town Forest "hardwoods" (NH)	528	Sugar Maple Yellow Birch	87.5 8.1	Colonel, Fullum, Peru	72°42'49.114" (W) 44°32'42.08" (N)	Pasture	1930's
STK*	Starksboro Town Forest "poor" (NH)	333	Sugar Maple/Red Maple White Ash/Red Oak	26.1/12.8 22.6/21	Dummerston	73°2'19.301" (W) 44°11'51.844" (N)	Tilled Intense Pasture	1980's
STS	Sterling Town Forest "spruce-fir" (SF)	524	Sugar Maple/Red Maple Balsam Fir	22.4/44.3 14.9	Buckland, Fullum	72°43'37.373" (W) 44°32'7.553" (N)	Pasture Never Tilled	1960's
WAT*	Waterworks Property Vergennes" (NH)	237	Sugar Maple/Red Maple Aspen/American Beech	22.1/47.6 14.8/10.5	Marlow non-spodic variant	73°7'58.68" (W) 44°9'48.142" (N)	Pasture	1930's
WIL	Willoughby State Forest (NH)	465	Sugar Maple Balsam Fir/Yellow Birch	53.2 21.3/11.4	Dixfield	72°2'12.481" (W) 44°41'48.231" (N)	Continuous Woodlot	N/A

Table 2.1: Characteristics of original forest plots. Site Codes with a (*) indicate sites where earthworms were noted in the 2008/2009 plot establishment. All other data and be found in Juillerat (2011). Land use history compiled based on personal communications with Charlie Cogbill as well as Juillerat (2011).

below the Oe horizon using a W.E.T. sensor (Delta T Devices, Cambridge, UK). Bulk density cores were taken from areas immediately around the pits, 3 cores from 0-10 cm (including Oa, if present) and 3 cores from the 10-20 cm. Depths of litter (Oi, Oe, Oa) were measured, and the complete Oi and Oe horizons collected separately to be dried and weighed in the lab. Soil from 0-10 cm (including the Oa horizon), followed by 10-20 cm, was removed and hand sorted for earthworms. Earthworms were identified and enumerated on site and than discarded. A representative sample of undisturbed soil was collected from the pit's face at 10-20 cm, followed by 0-10 cm, and transported in rigid containers back to the lab for aggregation and C analysis. For these samples the Oa horizon was not included, the purpose of the study being the investigation of mineral soil aggregation, and so samples represented a maximum of 10 cm, and less if an Oa was present.

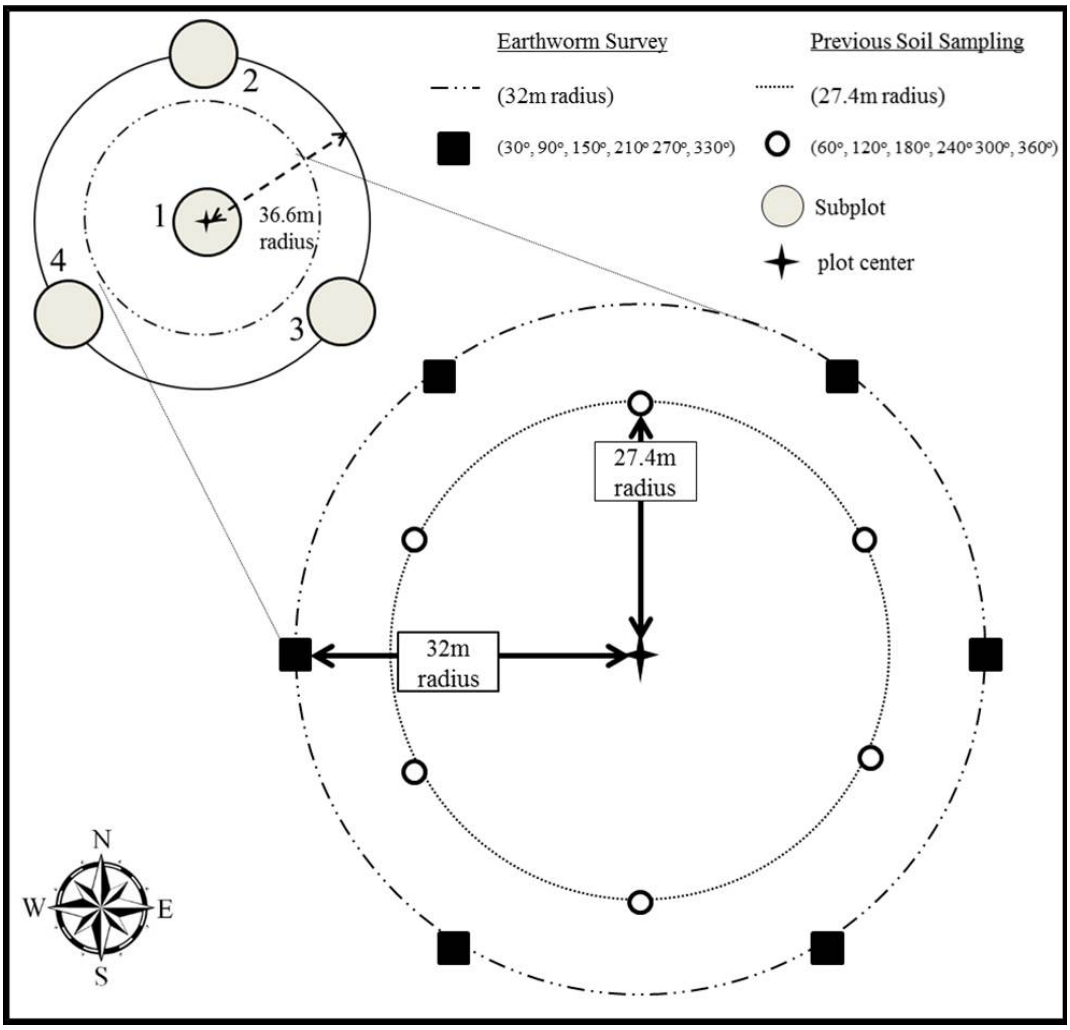

Figure 2.2: Location of the earthworm survey plots within the previously established FIA modeled plots. Placement of the survey was designed to avoid areas which were likely disturbed by previous sampling.

2.3.3 Water Stable Aggregate Fractionation

From each sample collected in 2012 (9 sites x 6 plots/site x 2 depths/plot = 108 samples) a representative 50 g subsample was wet sieved in duplicate according to the methods found in Six et al. (2002), modified from Elliott (1986). Briefly, 50 g of air dried soil was submerged in reverse osmosis (RO) water on top of a 2000 µm

sieve for 5 minutes to induce slaking. The sieve was moved in and out of the water, in approximate 3cm circular motions, 50 times over the course of 2 minutes, and the material that remained on the sieve was back washed into a clean container with RO water. Any floating organic matter greater than 2000 µm was not considered to be soil, and was therefore decanted and discarded. What remained after decanting was the large macroaggregate fraction (lgMA, >2000 µm), with coarse POM and coarse sand. The particles that passed through the 2000 µm sieve were transferred over a 250 µm sieve and the 2 minutes (50 motions up and down) was repeated. What was retained on the 250 µm sieve, the small macroaggregate fraction (smMA, 250-2000 µm), with POM and sand of the same size, was back washed into a clean container with RO water. The particles that passed through the 250 µm sieve were transferred over a 53 µm sieve and the process repeated. What was retained on the 53 µm sieve, the free microaggregate fraction (fmA , 53-250 µm), with POM and sand of the same size, was back washed into a clean container with RO water. The silt and clay fraction (< 53 µm), was discarded. The lgMA, smMA, and mA fractions were all back washed through coffee filters (modification, *Home 360 Hannaford Brand #2 cone filters*) which were then placed in 65^{o}C for 18-24 hours. Once dry, the fractions were weighed and carefully brushed away from the coffee filters to be stored in plastic bags until further processing.

2.3.4 Microaggregate Isolation

The below method for releasing the occluded microaggregates from the larger aggregate fractions was conducted following the process outlined in Six et al. (2000) for both the lgMA and smMA fractions.

From the above fractionation method duplicates, each macroaggregate fraction was combined into 8 g samples. These samples were slaked in RO water on top of a

250 µm sieve for 20 minutes. The submerged 250 µm sieve was then shaken vigorously by hand with 50 stainless steel bearings (4mm diameter) while a continuous flow of RO water passed over the apparatus. This was done in order to wash the smaller material through the sieve quickly, and avoid the further breakup of the microaggregates. After 4 minutes of shaking, the larger aggregates remaining on the sieve were gently prodded with a soft rubber stopper. The prodding, combined with shaking and water flow, continued until all but coarse sand and POM (lgPOM, >250 µm or smPOM, 250-2000 µm, dependent on starting fraction) had passed through the sieve. Material which passed through the 250 µm sieve was collected on a 53 µm sieve and wet sieved for 2 minutes (see Water Stable Aggregate Fractionation above), resulting in the stable materials occluded within the large macroaggregates (mAlg, 250-53 µm) or small macroaggregates (mAsm, 250-53 µm), depending on the starting material. The material which passed through the 53 µm sieve was the lgMA and smMA occluded silt and clay fraction (< 53 µm), and was discarded. All retained fractions were back washed into clean containers and then filtered through coffee filters before being dried and weighed.

2.3.5 Density Fractionation and Dispersion

2.3.5.1 Density Fractionation

The light fraction (LF) is composed of non-complexed decomposing plant and animal tissues, believed to be more labile, i.e. having a rapid turnover (Evans et al., 2001). The density fractionation procedure assumes that, during the humification process, the more recalcitrant SOM becomes intimately associated with mineral portions of the soil (Barrios et al., 1996). Therefore, any fraction having a density less than the mineral fraction, which is not occluded within microaggregation, is assumed to be

free LF, and more bio-available. In order to get a proper assessment of the amount of protected C found within the microaggregates (fmA, mAsm, mAlg), this LF must be removed prior to C analysis. The method for this process is outlined in Six et al. (1998) which was modified from Elliott and Cambardella (1991).

The microaggregate fractions were oven dried at 70°C for 18-24 hours. After cooling to room temperature in a desiccator, the samples were weighed and added to a 50-mL graduated conical centrifuge tube already filled with 25 mL of 1.85 g/cm^3 (+/- 0.01 g/cm3) sodium polytungstate (SPT). This mixture was then slowly inverted 10 times, bringing the sample into suspension without disruption of the microaggregate structures, the goal being to remove only the LF outside of any microaggregate. The material remaining on the cap and sides of the centrifuge tube was rinsed into the suspension with an additional 10 mL SPT, and after 20 min at equilibrium the samples were centrifuged at 2500 rpm for 60 min. The samples sat at room temperature for 18-24 hours in order to allow materials to settle completely before the floating material (free LF), as well as most of the SPT, was aspirated onto a 10 µm nylon mesh, rinsed thoroughly with RO water to remove any remaining SPT, and transferred to a small aluminum pan. Samples were dried at 60°C for 18-24 hours, cooled to room temperature in a desiccator, and weighed.

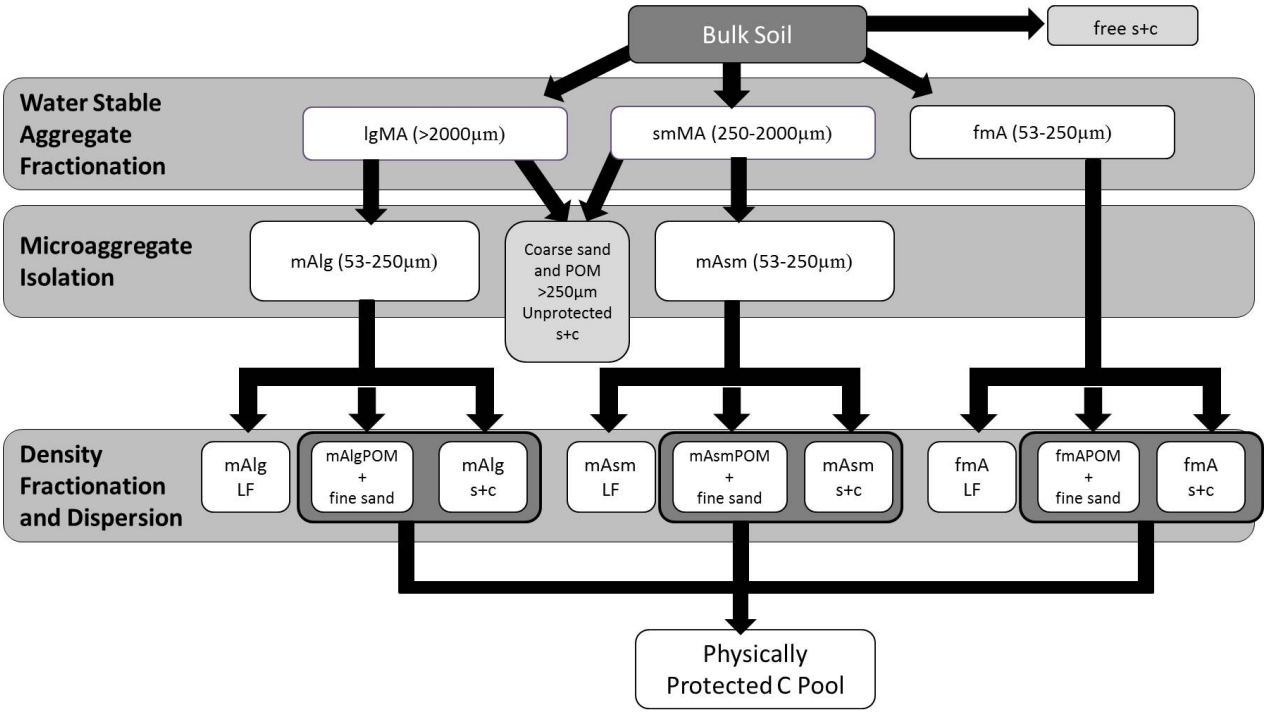

Figure 2.3: Diagram representing processing steps and functional soil fractions obtained from each. s+c: silt and clay fraction <53 µm, LF: organics (<1.85 g/cm3) between mA fractions, POM: particulate organic matter within mA fractions.

2.3.5.2 Dispersion

The heavy fraction (HF) remaining on the bottom of the conical tube after aspiration was rinsed twice with 50 mL of RO water in order to clean away any remaining SPT. The sample was mixed with 35 mL of 0.5% hexametaphosphate and dispersed by shaking on a reciprocal shaker for 18 hours. The dispersed HF was then passed through a 53 µm sieve, rinsed with RO water, and wet sieved for 2 min. The material remaining on the sieve was quantified as the intra-microaggregate POM (fmAPOM, mAlgPOM, mAsmPOM), and fine sand. This fraction was transferred to a small aluminum pan and dried 18-24 hrs at 60°C. The material passing through the sieve (fmAs+c, mAlgs+c, mAsms+c) was discarded and the C values determined by mass balance.

2.3.6 Carbon Analysis

Total carbon analysis was conducted at the University of Vermont on a Flash EA 1112 NC Analyzer (CE Elantech). The bulk soil was ground by hand to pass through a 250μm sieve, with coarse rocks and twigs >2000μm removed. All fractions were oven dried to a constant weight at 60^oC prior to analysis. Duplicate 20-80 mg of mineral fractions were weighed into tin capsules. Analyzer calibration and quality control (QC) soils were obtained from the North American Proficiency Testing program.

Any QC sample with greater than 10% error had samples immediately proceeding and following it re-run, along with any samples in which duplication had greater than 10% error. A QC run was included at the end of all sample processing for which 10% of all samples were randomly chosen and re-run.

2.3.7 Calculations and Statistical Analysis

2.3.7.1 Survey Analysis

Due to the "snapshot" nature of the above survey, metrics less affected by short term, seasonal, influences were used. The number of individual specimens seen (Curry and Schmidt, 2007), as well as the depth of the Oi horizon, would both be dependent on the time of year. Species richness was used as the metric for earthworm presence and was calculated simply by the number of species noted in one survey plot (6 plots per site). For forest floor depth measurements, only the Oe/Oa horizon data were included. The earthworm survey was intentionally conducted in the late spring or early fall, when soils were cool and earthworm activity was at its highest. In the spring, recent snow melt leaves behind a compacted Oi, while in the fall fresh litter results in a deep, loose Oi. The Oe/Oa horizons are less impacted seasonally and represent a more accurate indicator for long term earthworm effects. A linear

regression was conducted in which the forest floor depth and species richness for each of a site's 6 plots was averaged, yielding a single value for each of the 18 sites.

2.3.7.2 Calculations

Due to our assessment of lgMA (>2000 µm), the non-soil fraction (coarse fragments and free POM >2000 µm) was calculated and subtracted from the total soil starting weight for all calculations. Sand has a low likelihood of being incorporated within similar sized aggregation (Six et al., 2000) and therefore weights for all aggregate sizes were corrected for sand content of the same size class. All silt and clay fractions were discarded; values for these fractions were calculated by mass balance.

Due to time restrictions only the bulk soil and microaggregate fractions (fmA, mAsm, mAlg) were analyzed for C (see above). The weight of the corresponding LF obtained from the dispersion step (see section 2.3.5.2 above) was assumed to be composed of $28\% \pm 2.3\%$ C, an average calculated from over 100 LF samples run in duplicate over the course of two other studies that followed the same procedure as outlined above (unpublished data). This calculated LF C value was then subtracted from the whole microaggregate C value in order to obtain the assumed protected C value.

2.3.7.3 Statistical Methods

All statistical calculations were performed using JMP Pro 11.0 (SAS Institute Inc., 2013). Categorical data analysis conducted with Tukey-Kramer HSD. Categorical analysis was done analyzing difference in sites, as well as differences in individual plots. Plot depths were averaged and then analyzed with the assumption that plots, are independent of one another, the variation within sites often being more than between sites. Plots were categorized as "none", "1-3", and "4-6", based on the number

of species noted in the plots, having 19, 18, and 11 plots in each category, respectively.

2.4 Results

2.4.1 Species Compositions

During initial site establishment (2008/2009) earthworms were noted at EML, HIN, HIR, JER, MBR, STK, SKR, SMB, and WAT (Table 2.1). During the recent earthworm survey, species were confirmed in all sites except SMB, and WAT, where no earthworms were noted. Earthworms were newly noted at STE, SQU, and PCB. Regarding these three new sites, they each contained only one functional group, epigeic at STE, and endogeic at both SQU and PCB. All other sites noted with earthworms, contained species representative of all functional groups.

Site Code	Survey Date	Average Volumetric (%) Moisture Content (±SE)	Average Mineral Soil Temp. (°C) (±SE)	Average Forest Floor (Oe/Oa) Depth (cm)	Average pH*	Average bulk density 0-10cm (g/cm^3) (±SE)	Primary Horizon 0-10cm	Average bulk density 10-20cm (g/cm^3) (±SE)	Primary Horizon 10-20cm
EML	18-Jun-13	22.9±2.2	13.6±0.1	1.85	6.3	0.66±0.12	Oa/A	0.92±0.22	A/B
GAR	6-Jun-13	30.2±2.5	12.5±.03	3.00	4.0	0.57±0.11	Oa/A	0.77±0.14	E/B
GRO	15-Aug-13	NA	NA	10.50	4.6	0.60±0.23	Oa/E/Bg	1.17±0.35	E/Bg
HIN	3-May-12	47.7±3.1	14.3±2.8	0.17	4.5	0.69±0.15	A	0.78±0.17	A/B
HIR	31-May-12	27.6±2.4	15.2±0.8	1.92	4.4	0.57±0.11	A/B	0.80±0.14	B/C
JER	6-Jun-12	30.4±2.2	16.3±0.8	2.67	3.9	0.75±0.22	Oa/E/B	1.11±0.12	B
MBR	4-Jun-13	20.2±1.9	16.3±0.8	0.67	5.7	0.63±0.17	A	0.81±0.23	B
NFS	19-Jun-13	29.9±1.3	13.7±0.2	4.00	3.9	0.64±0.17	Oa/A	0.93±0.13	B
NIN	20-Aug-13	17.6±2.2	17.5±0.3	7.92	3.9	0.73±0.21	Oa/A/B	0.99±0.12	B
PCB	19-Aug-13	34.7±4.8	17.0±0.7	3.83	4.6	0.48±0.15	Oa/A	0.80±0.32	A/B
SKR	13-Jun-12	34.3±2.3	16.7±.04	0.17	4.4	0.63±0.15	A	0.80±0.15	B
SMB	2-Aug-13	38.5±3.6	15.7±0.9	7.75	3.3	0.50±0.29	Oa/E/B	0.85±0.15	B
SQU	20-Sep-12	25.1±2.1	14.0±0.3	2.83	4.8	0.61±0.17	Oa/A	0.88±0.16	B
STE	3-Jul-12	28.5±3.9	18.5±0.2	1.92	4.9	0.82±0.17	A/E	0.93±0.15	B
STK	19-Jun-12	37.1±4.8	19.1±0.7	0.08	5.2	0.63±0.17	A	0.68±0.24	B
STS	31-Aug-12	23.1±2.4	18.8±0.4	3.83	4.3	0.51±0.26	Oa/E	0.86±0.17	B
WAT	11-Oct-12	16.1±1.5	10.7±0.2	1.92	4.1	0.86±0.14	A/B	1.00±0.13	B
WIL	12-Oct-13	NA	NA	4.25	3.7	NA	A/E/B	NA	B

Table 2.2: Site measurements taken at time of survey. *pH measurements taken during initial site establishment from the B horizon (Juillerat, 2011). Depth of forest floor includes Oe/Oa only. Oi is not represented due to wide changes based on time of year the survey was conducted. SE represented for volumetric moisture, mineral soil temperature and bulk density (n=18), however not for forest floor depth (n=6), due to the common absence of any forest floor.

Earthworm Species	Sites Found
Epigeic	
Dendrobaena octaedra	EML, JER, MBR
Dendrobaena rubida	HIN, HIR, SKR
Endogeic	
Aporrectodea turgida	HIN, HIR, SKR
Aporrectodea rosea	EML, HIN, STK
Aporrectodea tuberculate	HIN, HIR, JER, MBR SKR, SQU, STK
Aporrectodea trapazoides	JER, SKR, STK
Octolasion cyaneum	HIN
Octolasion tyrtaeum	EML, HIN, SKR
Epi-Endogeic	
Lumbricus rubellus	JER, SKR, STK
Amynthas agrestis	JER
Anecic	
Lumbricus terrestris	SKR
Juveniles	
Pigmented	EML, HIN, HIR MBR, SKR, STK
Unpigmented	EML, HIN, HIR, JER MBR, PCB, SKR, STK, SQU

Table 2.3: Earthworm species identified and the specific sites in which they were found.

2.4.2 Forest Floor Depth and Other Forest Measurements

2.4.2.1 Moisture

There was a trend in the soil moisture content with earthworm presence within the sites from 2012 (9 sites total); the plots without earthworms having almost half the volumetric moisture content as those with 4-6 species (26.2±1.8% (None), 45.0±2.0% (4-6) $P<0.001$, Table 2.6). This trend was not seen when analyzing all 18 sites (Table 2.2), the moisture content appearing to be random and primarily determined by site rather than earthworm presence. This could be explained by the high variability of

Site Code	Survey Date	Average Epigeic Worms/m^2	Average Endogeic Worms/m^2	Average Epi-Endogeic Worms/m^2	Average Anecic Worms/m^2	Average Total Worms/m^2	Number of species noted	Number of plots worms found	Disturbance Classification *
EML	18-Jun-13	2	15	1	0	17	3	4 of 6	3
GAR	6-Jun-13	0	0	0	0	0	0	0 of 6	1
GRO	15-Aug-13	0	0	0	0	0	0	0 of 6	1
HIN	3-May-12	10	130	5	13	153	6	6 of 6	6
HIR	31-May-12	4	4	7	1	16	4	5 of 6	4
JER	6-Jun-12	2	31	4	0	37	5	3 of 6	3
MBR	4-Jun-13	118	1	2	4	121	3	6 0f 6	3
NFS	19-Jun-13	0	0	0	0	0	0	0 of 6	1
NIN	20-Aug-13	0	0	0	0	0	0	0 of 6	1
PCB	19-Aug-13	0	1	0	0	1	1	1 of 6	3
SKR	13-Jun-12	29	49	23	4	103	6	6 of 6	3
SMB	2-Aug-13	0	0	0	0	0	0	0 of 6	1
SQU	20-Sep-12	0	12	0	0	12	1	2 of 6	3
STE	3-Jul-12	2	0	0	0	2	1	1 of 6	2
STK	19-Jun-12	23	226	66	3	319	4	6 of 6	5
STS	31-Aug-12	0	0	0	0	0	0	0 of 6	1
WAT	11-Oct-12	0	0	0	0	0	0	0 of 6	1
WIL	12-Oct-13	0	0	0	0	0	0	0 of 6	1

Table 2.4: Worms/m^2 represent the mean of the 6 plots. Anecic species determined by visual identification as well as presence of middens (data not shown). *Disturbance class modified from rapid assessment outlined in Loss, Hueffmeier, and Hale (2013) 1-no earthworms impact. 2- Oi intact with Oe and Oa horizons intact or present in patches. Epigeic earthworms found in forest floor. No middens. 3- Oi intact with Oe and Oa present in patches. Epigiec earthworms found in the forest floor. Endogiec species found in the mineral soil, though soil horizons are intact. No middens. 4- Oi horizon is present though reduced. Oe horizons essentially gone with no fine roots or fungi present. Small to large epigiec, epi-endogiec, and endogeic species present, however no large surface castings or middens present. 5-Oi horizon is present, though reduced. Oe and Oa horizons are absent as are fine roots and fungi. Casts at the surface are abundant but Middens are absent or rare ($<$ 2 per m^2). 6- Oi is present at beginning of growing season but generally diminished by end of fall, leaving large patches of mineral soil. Oe and Oa absent and more than 2 middens per m^2 present.

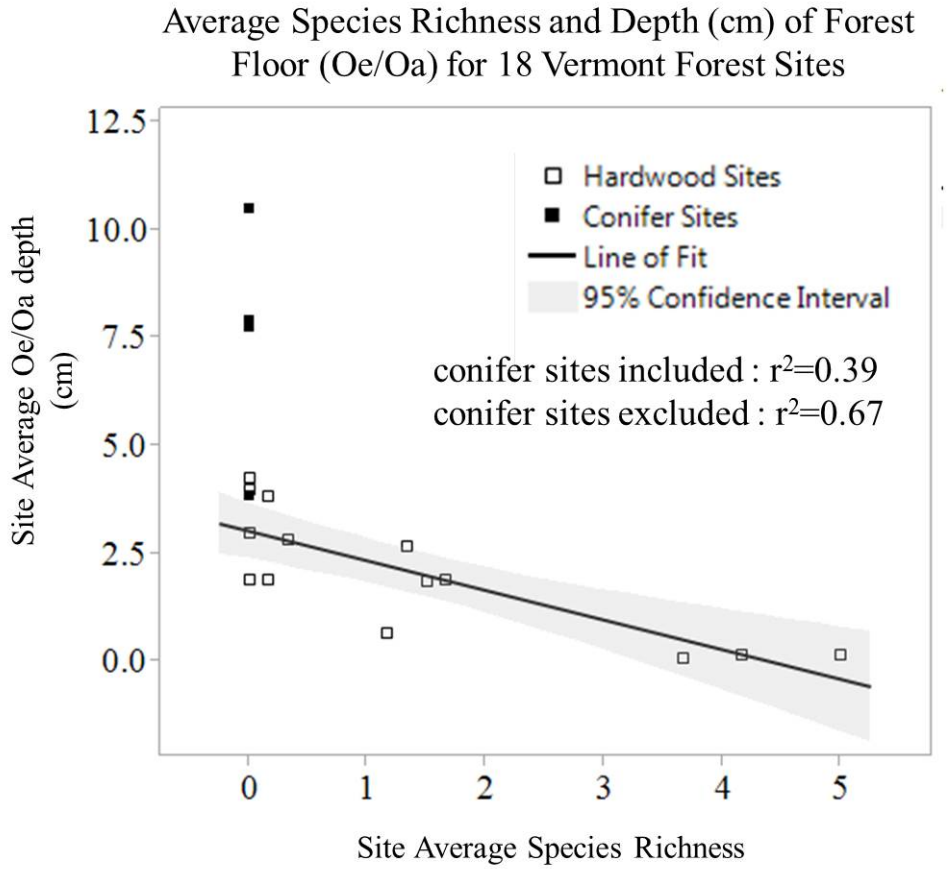

Figure 2.4: Linear regression of average forest floor (Oe/Oa) depth (cm) and average number of earthworm species found (species richness). ($P<0.001$). Line of fit takes conifer sites into account ($r^2=0.39$), though the relationship is higher when they are removed ($r^2=0.67$)

soil moisture content in sites without earthworms. Additionally, the most pronounced difference is noted in the 4-6 category, and most of these plots are represented in the 8 sites analyzed for C, the sites sampled in 2012 not being an accurate representation of the full 18 sites.

2.4.2.2 Forest Floor and Total Soil Carbon

There was a significant relationship ($r^2=0.39$, $P<0.001$) between species richness and the depth of the forest floor (Oe/Oa) when all 18 sites were analyzed. Notably, when the conifer sites are removed the strength of this relationship almost doubles

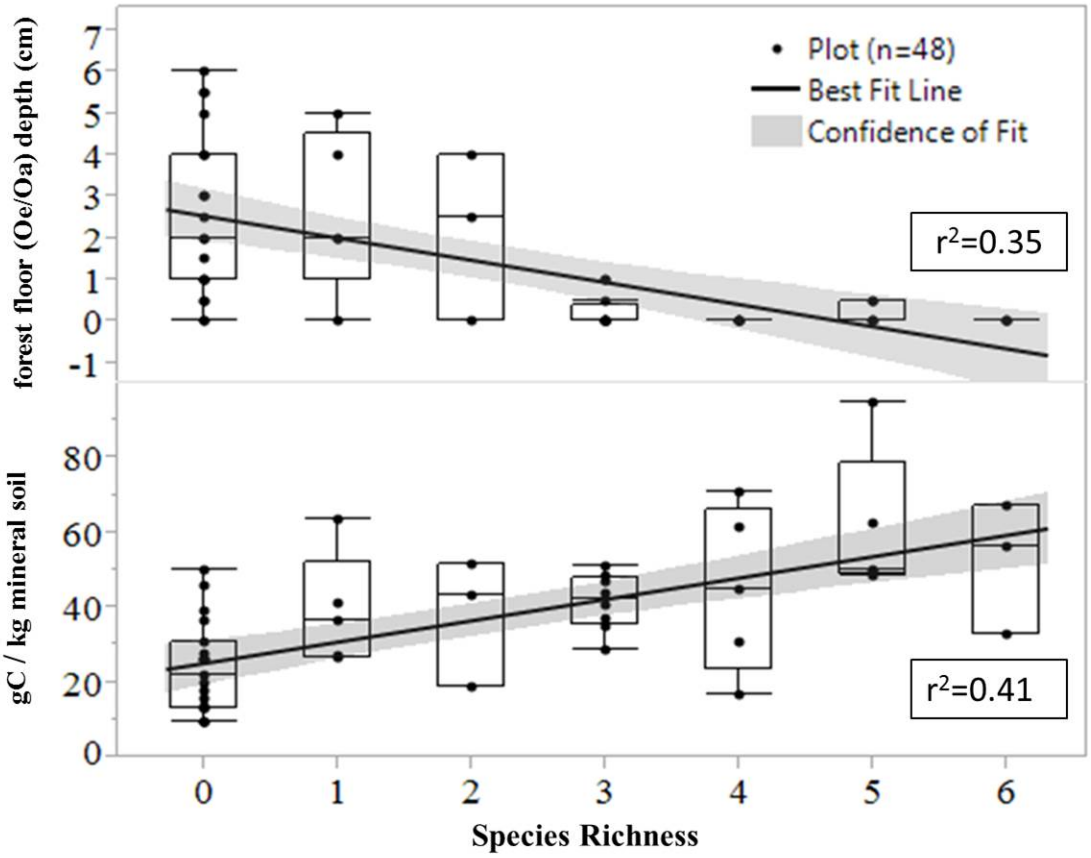

Figure 2.5: Forest Floor (Oe/Oa) depth and total mineral soil C (0-20 cm depth) for increasing earthworm presence. n=48 (8 sites, 6 plots/site) Number of plots in each species richness category varied: 0 (n=19), 1 (n=5), 2 (n=3), 3 (n=8), 4 (n=5), 5 (n=5), 6 (n=3). Box plots represent mean, minimum and maximum values for each species richness category. Earthworm species count represents identified adults as well as juveniles of different functional groups. Line of fit represents linear regression for all data and is significant ($P<0.001$) for both forest floor depth and gC/kg bulk soil.

(r^2=0.67, P<0.001) (2.4). There were no correlations with earthworm presence and soil moisture or bulk density in either depths (0-10 cm, 10-20 cm).

Of the 9 sites which underwent aggregate analysis, STS was the only conifer site. The differences between this site and all others was enough to justify its removal from analysis. This resulted in 8 hardwood sites analyzed for a total of 48 plots. Similar to what was seen when analyzing the full 18 sites (see Figure 2.4 on page 56) there was a strong relationship between earthworm presence and forest floor depth within these 8 hardwood sites. When analyzed linearly by plot, the relationship is significant (r^2= 0.35, P<0.001, Figure 2.5). When analyzed categorically (Table 2.5), plots with no earthworms ("none") have substantially deeper forest floors (Oe/Oa) than those with 4-6 species (2.8±0.4 cm (none), 0.1±0.1 cm (4-6) P<0.001), The variation for this metric was high (Figure 2.6). The range of forest floor measurements, even within a single site without earthworms such as WAT, was broad (Table B.1).

The C analysis of these mineral soils demonstrated an increase in C with an increase in the number of species noted, related to the reduction in the forest floor. When analyzed linearly by plot, the relationship of species richness with total soil C is stronger than that noted with the forest floor (r^2= 0.41, P<0.001, Figure 2.5). When analyzed categorically by site, WAT, where no earthworms are seen, is significantly different than HIN, which has an average species richness of 5 species noted per plot (P<0.001), however their is only a slight insignificant trend noted between all other sites. When analyzed categorically by plot (Figure 2.6) the trend is more substantial. Plots without earthworms ("none") had almost half the C in the mineral soil as plots with many earthworm species noted ("4-6") (28.4±3.3 gC/$kg_{bulksoil}$ (none), 54.4±5.3 gC/$kg_{bulksoil}$(4-6) P<0.001), however the middle plots, with 1-3 species noted, were not significantly different from either of the other categories (40.4±3.4 gC/$kg_{bulksoil}$ (1-3)).

2.4.3 Microaggregates and the Physically Protected C Pool

Four different microaggregate (53-250 µm) pools were analyzed; fmA (free, non-associated microaggregates), mAlg (microaggregates occluded within large macroaggreages, lgMA, >2000 µm), mAsm (microaggregates occluded within small macroaggregates, smMA, 250-2000 µm), and the total mA (the sum of fmA, mAlg, and mAsm pools).

2.4.3.1 Shifts in Physical Distribution of Microaggregates

Plots with many earthworm species (4-6) had almost triple the proportion of soil composed of lgMA (0.15±0.02 $kg_{fraction}/kg_{bulksoil}$ (none), 0.39±0.04 $kg_{fraction}/kg_{bulksoil}$ (4-6), P<0.001). The fmA fraction was 0.09±0.02 $kg_{fraction}/kg_{bulksoil}$ less in plots with no earthworms (0.11±0.01 $kg_{fraction}/kg_{bulksoil}$ (none), 0.02±0.01 $kg_{fraction}/kg_{bulksoil}$ (4-6), P<0.001), while the mAlg fraction was 0.06±0.02 $kg_{fraction}/kg_{bulksoil}$ greater (0.03±0.01 $kg_{fraction}/kg_{bulksoil}$ (none), 0.09±0.01 $kg_{fraction}/kg_{bulksoil}$ (4-6), P<0.001). This slight disparity, along with a small but insignificant decrease in the mAsm fraction, resulted in a 20% decrease in the total mA in the plots under heavy earthworm influence (0.24±0.01 $kg_{fraction}/kg_{bulksoil}$ (none), 0.19±0.01 $kg_{fraction}/kg_{bulksoil}$ (4-6), P<0.001). The proportion of macroaggregates (lgMA and smMA) composed of microaggregates (mAlg and mAsm, respectively) was also investigated. Though there was a decrease in the microaggregate proportion of both macroaggregate pools with higher earthworm influence, these differences were not significant for either lgMA or smMA (Table 2.5)

2.4.3.2 Microaggregate Associated C pool

Plots under heavy earthworm influence had significantly more C associated with the total microaggregate pool (15.3±1.6 $gC_{microaggregation}/kg_{bulksoil}$ (none), 29.4±2.4 $gC_{microaggregation}/kg_{bulksoil}$(4-6), P<0.001). This increase corresponds with the increase in total soil C (28.4±3.3 $gC/kg_{bulksoil}$ (none, n=19), 54.4±5.3 $gC/kg_{bulksoil}$(4-6, n=13) P<0.001), and no difference is noted in the proportion of total soil C composed of microaggregate associated C (54% for both "none" and "4-6", see Figure 2.7).

An increase to the pool of microaggregate associated C could occur by either a change in the total proportion of soil composed of microaggregates (see above) or through an alteration of the C content within the microaggregates. For the fmA fraction, there was a noted decrease in the physical pool of 80%, corresponding with the 54% decrease in the C associated with this pool (4.8±0.4 $gC_{fmA}/kg_{bulksoil}$ (none), 2.2±0.03 $gC_{fmA}/kg_{bulksoil}$ (4-6), P<0.001), leaving a gap of approximately 30% which is likely due to C enrichment. The mAsm pool had a slight, though insignificant decrease in its proportion of soil mass, yet there was an average of 4.9±2.3 $gC_{mAsm}/kg_{bulksoil}$ more found in plots under heavy earthworm influence (6.9±0.8 $gC_{mAsm}/kg_{bulksoil}$(none), 11.8±1.5 $gC_{mAsm}/kg_{bulksoil}$(4-6), P<0.001), likely due entirely to a C enrichment of this fraction. The heavily earthworm influenced plots had significantly more C associated with the mAlg fraction than those plots with no earthworms, a difference of 425% (3.6±0.8 $gC_{mAlg}/kg_{bulksoil}$(none), 15.3±1.9 $gC_{mAlg}/kg_{bulksoil}$, P<0.001) This increase is greater than the 300% increase noted in the physical proportion of mAlg (see above). Had there been no enrichment of C, this 300% increase in physical mAlg structures could account for approximately 10.6 $gC_{mAlg}/kg_{bulksoil}$, leaving approximately 4.7 $gC_{mAlg}/kg_{bulksoil}$, or 31% of the total mAlg associated C pool attributed solely to the C enrichment of the fraction. The sum of the hypothe-

sized C enrichment in mAsm and mAlg fractions is approximately 9.6 gC/kg$_{bulksoil}$, accounting for almost 70% of the total C difference between plots without earthworms and those under heavy earthworm influence.

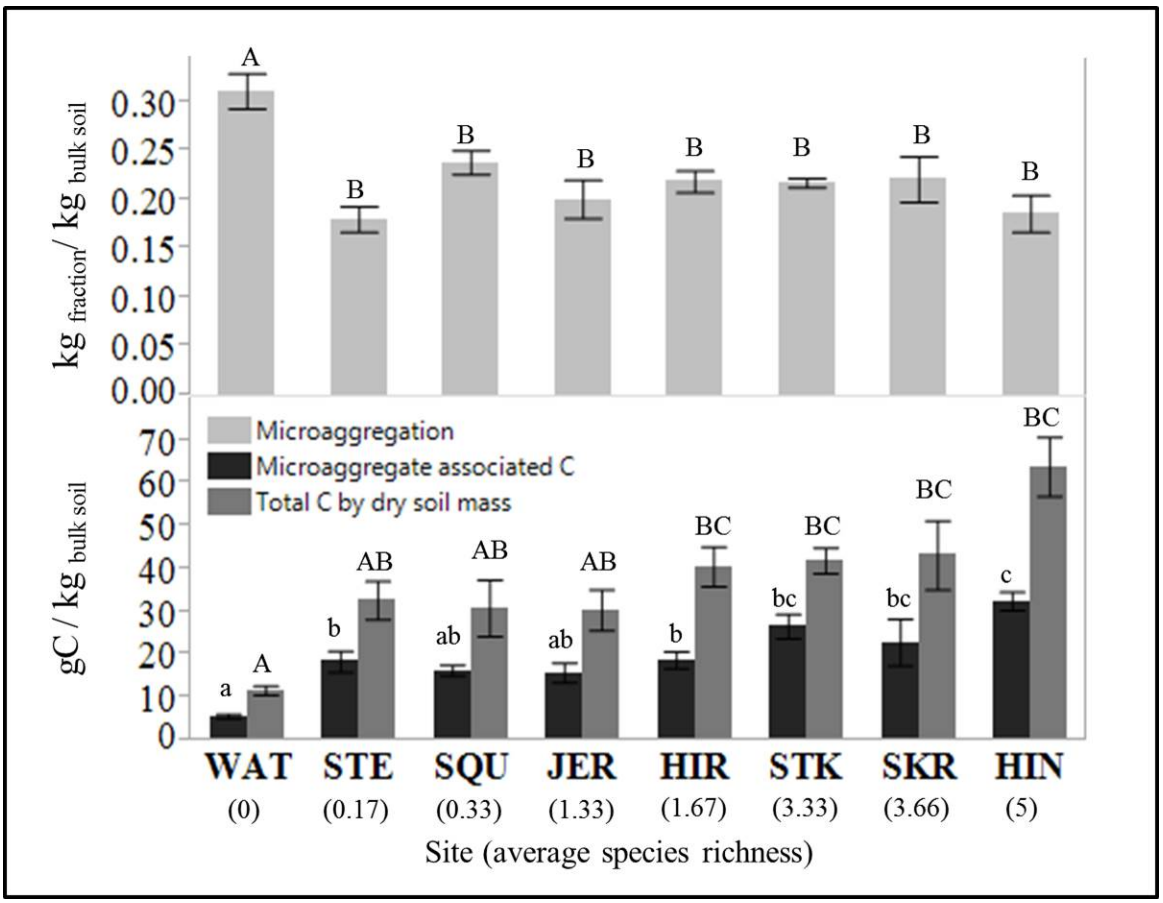

Figure 2.7: Comparison of microaggregate proportion by dry mass, microaggregate associated C (gC/kg$_{bulksoil}$ - assumed protected fraction) and the soil's total C (gC/kg$_{bulksoil}$) for 8 hardwood sites with varying earthworm species richness. All data are averages of the 6 plots surveyed per site with the error bars representing SE. Average species richness is represented by the number of species seen at each plot, averaged for the entire site. Different letters represent statistically ($P<0.05$) significant differences between sites for the three metrics represented (Tukey-Kramer HSD).

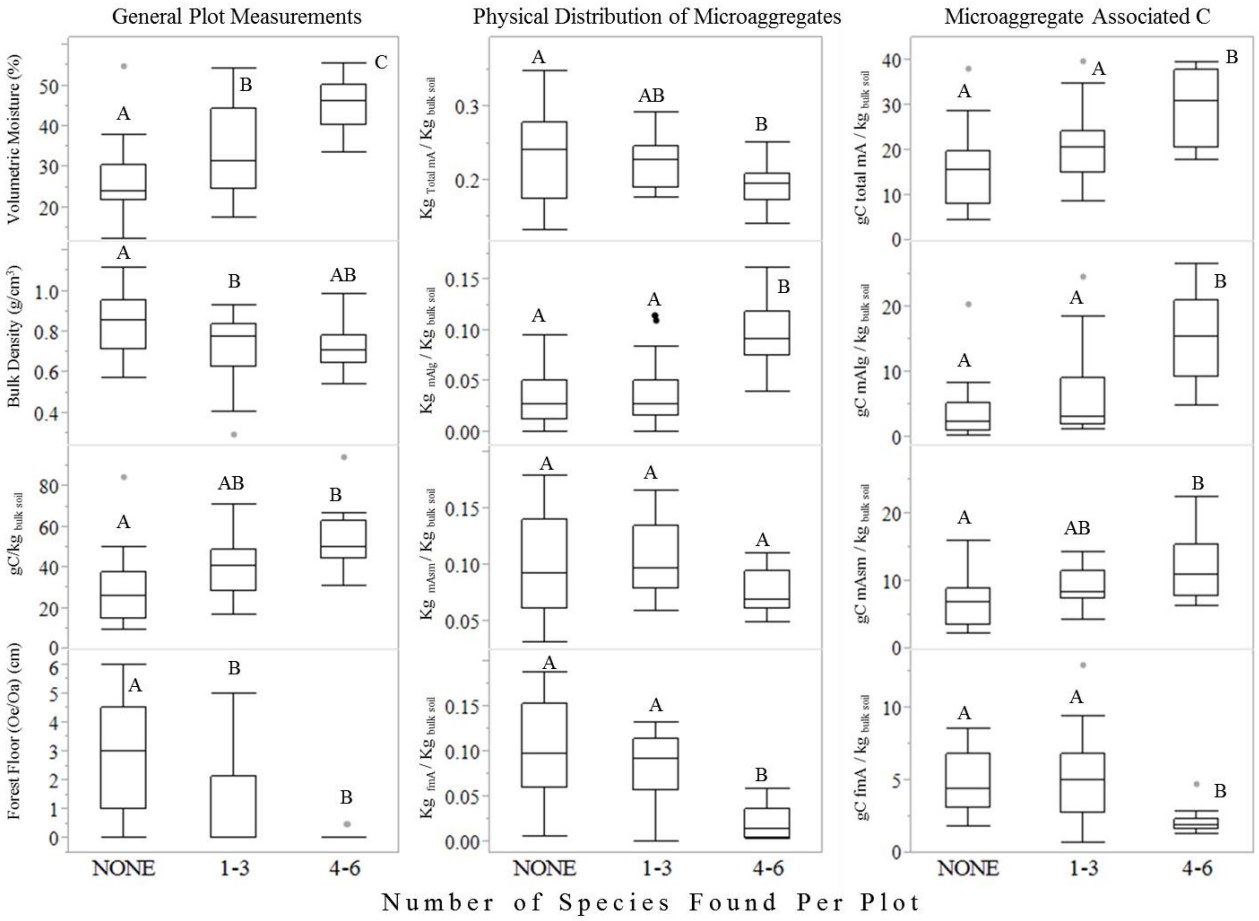

Figure 2.6: Various measurements for species richness categories: None (n=19), 1-3 (n=18), 4-6 (n=11). fmA: free microaggregates, mAlg: microaggregates occluded within the large macroaggregates (lgMA > 2000 µm), mAsm: microaggregates occluded within small macroaggregates (smMA 250-2000 µm), Total mA: the sum of fmA, mAlg, and mAsm. (*) indicate statistical significance ($P<0.05$), box plot represents mean and minimum and maximum values in each category.

General Plot Measurements	Worm Category			Physical Distribution of Microaggregates	Worm Category		
	NONE (n=19)	1-3 (n=18)	4-6 (n=11)		NONE (n=19)	1-3 (n=18)	4-6 (n=11)
Volumetric Moisture (%)	26.2 ± 1.8	33.33 ± 2.64	45.0 ± 2.0	fmA ($kg_{fraction}/kg_{bulksoil}$)	0.105 ± 0.011	0.080 ± 0.010	0.021 ± 0.006
Bulk Density (g/cm^3)	0.84 ± 0.03	0.71 ± 0.04	0.73 ± 0.04	mAlg ($kg_{fraction}/kg_{bulksoil}$)	0.032 ± 0.005	0.041 ± 0.009	0.091 ± 0.011
$gC/kg_{bulk\ soil}$	28.4 ± 3.3	40.4 ± 3.4	54.4 ± 5.3	mAsm ($kg_{fraction}/kg_{bulksoil}$)	0.098 ± 0.009	0.103 ± 0.007	0.075 ±0.006
Forest Floor (Oa/Oe) depth (cm)	2.8 ± 0.4	1.2 ± 0.4	0.1 ± 0.1	fmA + mAlg + mAsm ($kg_{fraction}/kg_{bulksoil}$)	0.236 ± 0.013	0.223 ± 0.008	0.187 ± 0.012
Microaggregate Associated C	Worm Category			Microaggregates within Macroaggregates	Worm Category		
	NONE (n=19)	1-3 (n=18)	4-6 (n=11)		NONE (n=19)	1-3 (n=18)	4-6 (n=11)
fmA ($gC / kg_{bulk\ soil}$)	4.8 ± 0.4	5.1 ± 0.7	2.2 ± 0.3	lgMA ($kg_{fraction}/kg_{bulksoil}$)	0.138 ± 0.019	0.183 ± 0.035	0.395 ± 0.039
mAlg ($gC / kg_{bulk\ soil}$)	3.6 ± 0.8	6.6 ± 1.6	15.3 ± 1.9	mAlg/lgMA ($kg_{fraction}/kg_{fraction}$)	0.257 ±0.011	0.246 ± 0.011	0.228 ± 0.013
mAsm ($gC / kg_{bulk\ soil}$)	6.9 ± 0.8	9.3 ± 0.7	11.8 ± 1.5	smMA ($kg_{fraction}/kg_{bulksoil}$)	0.332 ± 0.019	0.359 ± 0.014	0.333 ± 0.024
fmA + mAlg + mAsm ($gC / kg_{bulk\ soil}$)	15.3 ± 1.6	20.9 ± 1.9	29.4 ± 2.4	mAsm/smMA ($kg_{fraction}/kg_{fraction}$)	0.167 ± 0.015	0.163 ± 0.020	0.085 ± 0.011

Table 2.5: General plot measurements and microaggregate properties (±SE) for different species richness catagories. fmA: free microaggregatio, mAlg: microaggregation occluded within the large macroaggregation (lgMA > 2000 µm), mAsm: microaggregatiom occluded within small macroaggregation (smMA 250-2000 µm), Total mA: the sum of fmA, mAlg, and mAsm.

2.5 Discussion

2.5.1 Current Worm Distribution in Vermont

Earthworm communities were found in the majority of sites noted in the 2008 and 2009 site establishment. However, there were two sites, WAT and SMB in which worms were noted during site establishment (see Table 2.1) but were not found during the surveys done in 2012 (WAT) and 2013 (SMB) (Table 2.4). There are several possible explanations for this. Due to earthworm presence not being the primary focus of the work done during plot establishment, it is possible that these species were misidentified, being instead some other soil organism. In unpublished data used in Juillerat (2011) it is noted that only one specimen was found at each of these sites. These earthworms were described as being "found at [the] bottom of Oa [horizon], about 2 cm long" for WAT, and "found in [litter] bag the next day, partially decomposed" for SMB. The descriptions of these two specimens indicate that they would likely have been epigeic species. If present in few numbers, this group of small species, known to hide within leaf litter, may have been missed during the earthworm survey described above.

Conversely, earthworms found at STE, SQU, and PCB, were not noted in 2008/2009. As stated earlier, because earthworm presence/absence was not the primary goal of site establishment, it can not be said for certain whether these species were present in 2008/2009, or if they represent newly established communities. Site STE contained 2 presumed juvenile epigeic species, both in the same sampling location, noted to be a very short distance from a recently establish harvesting skid trail. Site PCB contained just 1 juvenile endogeic species, found in a depressed, C enriched wet area, likely to be a seasonal river bed. Site SQU contained specimens of endogeic species *A. tuberculata,* as well as a few large endogeic juveniles. Earthworms were found at

2 physically separated locations in SQU, one site having a very high moisture and C content (see Table B.1 below), with the other site located down slope from the previous.

Hale et al. (2005) described a dynamic of earthworm invasion in the hardwood forests of Minnesota where epigeic and epi-endogeic species invaded first, integrating the forest floor into the mineral horizons. This action directly facilitating the establishment of endogeic species, which live and feed on the newly C enriched mineral soils. If the three sites above (STE, SQU, PCB) do, in fact, represent newly establish populations, only one (STE) would appear to be following that invasion trend, with PCB, and SQU having first established endogeic species. Of these two sites, it may be speculated that SQU will maintain the endogeic populations found during the survey, due to the high numbers noted, as well as the presence of both adults and juveniles. The same may not be said about PCB. With only one specimen noted it remains unclear if this worm presence is an anomaly, or indicative of a well established population. The patchiness of earthworm populations (Curry, 2004; Lee, 1985) makes surveys such as the one we conducted limited in conclusions we can draw about the presence of current populations.

For the sites that had earthworms noted during both initial site establishment (2008/2009) and the above survey (2012/2013) (EML, HIN, HIR, JER, MBR, SKR, STK), community composition and densities were diverse and varied (Table 2.4). Most of these sites, which have been under earthworm influence for a minimum of 3 years, contained all functional groups, except EML and JER which did not have anecic species (identified by presence of middens) noted. The time, vector, and order of earthworm invasion is not known for these sites, and it will take follow-up surveys to draw any conclusions on the dynamics of these populations.

2.5.2 Impact of Earthworm Diversity on the Forest Floor

Hale et al. (2005) suggested that earthworm invasions may have a succession pattern, species composition changing with time since invasion. It is possible that the presence of one ecological group created an environment allowing the invasion of another group, creating an invasion "front". Her research showed that recovery of forest floor is more difficult in the later stages of invasion, when many species are working together. Not all earthworm species impact forest soils equally, for instance the epi-endogeic species *Lumbricus rubellus* has been shown to increase the rate of forest floor removal to a greater extent than species of other ecological groups (Fahey et al., 2013; Hale et al., 2005), however as the diversity of species increases, and various ecological groupings begin acting in concert, the impact of invasion intensifies (Frelich et al., 2006).

Our data support the above notion that as the number of earthworm species increases (what we termed "species richness"), so does the impact on the forest floor (Bohlen and Scheu, 2004; Frelich et al., 2006; Hale et al., 2005; Loss et al., 2013). Though our sites are highly variable there was a significant correlation (r^2= 0.39, P<0.001) with number of species and depth of the forest floor (Figure 2.4). The greatest pull in this correlation comes from either those plots with 3 or more species and no forest floor, or conifer sites. The deepest forest floors were all found in conifer sites, whose litter chemistry is known to slow down decomposition and increase residence time (Currie et al., 2002). While the acidity of coniferous forests is thought to be inhospitable for most earthworm populations (Curry, 2004), earthworms are known to be found in these ecosystems on occasion. However in our study this was not the case. The impact of the translocation and mixing of the forest floor into lower mineral horizons is discussed further below in the aggregate and C analysis of the 9 sites surveyed in 2012

2.5.3 Aggregate and C Analysis

Despite high variation within our data sets, earthworm effects on aggregation and C were still apparent. The lgMA fraction was substantially higher in plots under high earthworm influence (4-6), supporting the many studies that found aged earthworm castings are stable, and form the basis for macroaggregates in earthworm-invaded soils (Bossuyt et al., 2005; Sanchez-de Leon et al., 2014; Shipitalo and Protz, 1989). Increased lgMA resulted in a high proportion of the soil composed of mAlg, however the earthworm effect on total microaggregates was negligible among sites, and statistically negative when individual plots were investigated categorically. Carbon enrichment (see 2.4.3.2 on page 60) supports the notion that earthworms are reworking the soil's microaggregates even in the fractions whose proportions were unaltered.. An example of this is noted in the smMA and mAsm fractions. Though a negligible physical change was noted in the mAsm fraction, there was a significant increase in the C associated with this fraction. The influence of earthworms on this fraction would have been missed had only physical properties of aggregate distribution been investigated.

Many microaggregate and soil C properties had no significant differences, and occasionally not even noticeable trends, between the plots with no earthworms and those which had a species richness of 1-3. The only measurement where the 1-3 richness catagory had an effect was in the forest floor. If there was ever more than 1 species, the plots within this richness category would contain the species *Lumbricus rubellus* (see Appendix Table (B.1)). This species, an epi-endogeic species, is known to impact the forest floor to a greater degree than other worm species (Hale et al., 2005) due feeding on the litter of the surface while living and burrowing in the mineral soil. This action rapidly relocates the forest floor downward into the mineral soil an

action which is supported in our data. The plots with no worms had a much thicker forest floor that either those with 1-3 species, or those with 4-6 species. However, for both physical soil aggregation, and the enrichment of these fractions with C, significant differences only occur in the plots of highest species richness, the presence of *Lumbricus rubellus* apparently not having the same impact on a soil's aggregation as it does on the depletion of the forest floor. The highest richness category (4-6) is predominately associated with the additions of various endogeic species (see Appendix Table B.1). This supports the idea that endogeic species are the ecological grouping most responsible for soil aggregation processes, and hence the incorporation of organic matter into stable microaggregate structures (Lavelle and Spain, 2001).

It is not surprising that microaggregates were not increased under higher earthworm influence. Forest soils are known to contain high levels of microaggregation, low disturbance frequency allowing for microaggregate formation within macroaggregates (Elliott and Coleman, 1988; Oades, 1984; Six et al., 2000; Tisdall and Oades, 1982) Six et al. (2002) found that 20% of the difference in soil organic carbon (SOC) stocks between agricultural and afforested ecosystems could be explained by differences in the microaggregate fraction, suggesting that these structures may be one of the driving mechanisms of long term C storage in forest soils. It is possible the proportion of microaggregates found in most forest soils is at a maximum, this level determined by site specific soil properties. Assuming that the microaggregates present in a soil are destroyed during passage through the earthworm gut (Shipitalo and Protz, 1989), which may not be equally true for all earthworm species in all soil types, it of interest that microaggregate proportions were not found to be substantially lower. Earthworms have been shown to increase microaggregates in agricultural soils, however this is likely due to the fact that frequent tillage does not allow for microaggregate formation naturally (Beare et al., 1994; Fonte, Winsome, and Six, 2009; Plante and

McGill, 2002; Six et al., 2000), making the levels seen prior to earthworm influence lower than the physical capability of the soil.

The C occluded within microaggregates is not protected indefinitely. The binding agents maintaining soil structure are not inert and are therefore subject to decomposition(Frey, 2005). As the structural stability of those aggregates becomes compromised, occluded C may become available for microbial degradation (Baldock, 2002). Theoretically, for every system there exists an ideal rate of aggregate turnover which would allow for organic matter occlusion and protection, while still limiting the re-exposure of previously occluded C (Plante and McGill, 2002). The physical stability of microaggregation means that they will not turnover readily and may require a force such as earthworm ingestion to reform. Due to the high levels of aggregation, and low levels of turnover present in many forests, it may be aggregate turnover, rather than aggregate creation, that will enhance C stabilization in forests showing the potential for C sequestration. Earthworm invasion with its preferential occlusion of C within microaggregation, may potentially be able to accomplish that.

2.6 Conclusions

The history of earthworms at these investigate sites is unknown, and with many of the sites having an agricultural land-use history (see Table 2.1) it is likely that some of these forests developed under the influence of earthworm communities. In this situation the term "invasion" does not apply, being now the steady state of the ecosystem. We conclude that while earthworm community presence had little to no effect on a forest soil microaggregates, through a turnover of microaggregates, earthworms, particularly endogeic species, appeared to have an effect on the pool of physically stabilized C. These findings highlight the fact that forest soils typically contain high

microaggregate levels, and due to various inherent soil properties earthworms may not increase microaggregate proportions to the same extent as noted in agricultural soils. Though our studies did not utilize mass balance to account for C lost due to mineralization, the proportion of C mixed into the mineral soil that was preferentially occluded in microaggregation was significant, and it is likely this pool will demonstrate increased residence time relative to bulk soil C.

CHAPTER 3

INFLUENCE OF *APORRECTODEA TUBERCULATA* ON THE CARBON, NITROGEN, AND AGGREGATION PROPERTIES OF A LOAM FOREST SOIL – A MESOCOSM STUDY

3.1 Abstract

The impact of earthworm presence on the soil carbon (C) dynamics of previously uninhabited northeastern forests is still largely unknown. Currently, earthworm presence is understood to both enhance soil respiration, and create stable microaggregates, processes assumed to have conflicting effects on long-term C storage. To date, studies investigating earthworm created microaggregates, and the occluded C, have rarely been done in undisturbed forest soils. A paired mesocosm study (n=5) was conducted investigating the impact of the endogeic earthworm species *Aporrectodea tuberculata* on the physical proportion of microaggregates, and the associated mineral soil C, of a minimally disturbed forest soil. Pairs analyzed after 4 weeks of incubation, had no significant aggregation effects. At 4 months, paired cores with earthworms (WW) showed a 67% increase in large macroaggregates (>2000 µm, lgMA), as a proportion of total soil dry weight, compared to cores without earthworms (NW) (0.110±0.017 $kg_{fraction}/kg_{bulksoil}$(NW), 0.183± 0.012 $kg_{fraction}/kg_{bulksoil}$(WW), p=0.026), and a 10% decrease in small macroaggregates (250-2000 µm, smMA) (0.439±0.034 $kg_{fraction}/kg_{bulksoil}$ (NW), 0.395±0.010 $kg_{fraction}/kg_{bulksoil}$ (WW), p=0.024). While distribution was seen to shift in various microaggregate pools (free and occluded within macroaggregates), net microaggregates in the soil, as a proportion of total soil dry weight, was unaltered. After 4 months, the mineral soil of WW cores had an average of 60% more C than the NW cores (16.23±0.55

gC/kg$_{bulksoil}$ (NW), 26.01±1.98 gC/kg$_{bulksoil}$ (WW) p=.005) due to the relocation of the forest floor. The C associated with the microaggregate fractions increased an average of 56% (9.14±0.33 gC$_{fraction}$/kg$_{bulksoil}$(NW), 14.57±0.68 gC$_{fraction}$/kg$_{bulksoil}$ (WW), p=0.006). Of this, 95% was found in the microaggregates occluded within the lgMA fraction, which was almost almost 4 times greater in the WW cores (1.71±0.28 gC$_{fraction}$/kg$_{bulksoil}$ (NW), 6.62±0.32 gC$_{fraction}$/kg$_{bulksoil}$ (WW), P<0.001). This investigation found that over 50% of the increase in total mineral soil C (9.79±gC/kg$_{bulksoil}$, WW-NW) was accounted for from C associated within the physically protected microaggregate fractions (5.16±0.23 gC$_{fraction}$/kg$_{bulksoil}$, WW-NW), indicating that, though this species of earthworm did not alter the proportion of microaggregates in these soils, they occluded a substantial proportion of C within those physical fractions. In this particular forest soil, the actions of *Aporrectodea tuberculata* increased the physically protected C pool through microaggregate restructuring and C enrichment, and not through an increase in the soil's proportion of microaggregates.

3.2 Introduction

Concern over the global impact of increased carbon dioxide (CO_2) levels in the atmosphere has encouraged recent research aimed at enhancing our understanding of the carbon (C) cycle. More C resides in soil (1,500 Gt) than in all terrestrial biomass (560 Gt) and the atmosphere (720 Gt) combined (Birdsey, 1992), and an intimate relationship exists between the soil and the atmosphere, soil respiration accounting for roughly 20% of total CO_2 emissions (Rastogi et al., 2002). Approximately 12,000 years ago the last glaciation event covered most of the northeastern United States, eliminating the soils and associated fauna. The forests of this area therefore developed without influence from native earthworms, due in part to the slow northward

expansion of southern species and their inability to adapt to the cold winters of the north (Bohlen and Scheu, 2004). Since the introduction of earthworms from Europe and Asia via ship ballast and imported horticultural products (Gates, 1976) , various species have slowly moved from agricultural settings, where they are welcome, to forests, where their impact is less understood and typically undesirable (Hale et al., 2005). This movement of earthworms into forests is expected to increase in the coming decades, yet it is still unclear how earthworm presence in these ecosystems will impact soil stabilization of C. Forests of the northeast are often actively managed, offering an opportunity to influence their role as a sink or source for CO_2 in the future. Furthering our understanding of the impact earthworms will have in these ecosystems may aid those management decisions.

Earthworms are typically placed into three groups, each inhabiting a specific ecological role and influencing soil aggregation and C turnover differently (Doube and Brown, 1998). Epigeic species live and feed on the litter at the surface, rarely burrowing into the mineral soil. These species have little or no effect on a soil's aggregation, though may play an important role in C turnover. Anecic species feed on fresh litter from the surface, pulling it into the soil surrounding their deep, permanent burrows. This extensive burrowing system contributes to the stabilization of aggregation, as well as the downward transport of fresh litter within there castings. Endogeic species live and burrow in the upper areas of the mineral soil, feeding on mineral-associated organic matter. Endogeic species are considered to be the primary group of earthworms influencing soil aggregation and the stabilization of C (Lavelle and Spain, 2001). These different types of earthworms, working as a community and individually, are referred to as "ecosystem engineers", and are capable of drastically altering the chemical, physical, and microbial soil environment (Lavelle et al., 1997).

Most studies investigating the *in situ* impact of earthworm invasion into native

northern forests have found a reduction of the forest floor (Alban and Berry, 1994; Bohlen et al., 2004; Lyttle et al., 2011) as it becomes integrated by earthworm ingestion and egestion into lower mineral horizons. At any given time, it is hypothesized that the majority of bacteria in the soil are in a metabolically inactive, though alert, state due to low nutrient availability (Coleman, 2001). Earthworm ingestion homogenizes soil, bringing bacterial communities into close contact with their food source, which results in increased soil nutrient cycling (Bohlen and Scheu, 2004; Groffman et al., 2004). Studies have found that microbial communities within the castings of earthworms are greatly altered relative to bulk soil (Brown et al., 2000). Earthworm ingestion disrupts fungal mycorrhizal relationships (Dempsey et al., 2011) and appears to enhance populations of bacteria capable of surviving through the anoxic environment of the earthworm gut (Drake and Horn, 2007).

There is little doubt that, through the above processes, invading earthworms are increasing the mineralization of C in the short term (Lubbers et al., 2013). However earthworms have many secondary effects which profoundly alter soil function, and the impacts of these on long term C storage capacity is still unclear. In a prominent 14-year field study, Alban and Berry (1994) reported that earthworms decreased total soil C by 600 kg per ha per year, however Zhang et al. (2013) noted that this reduction was only seen for the first 2 years, before C levels were maintained at a new equilibrium. Bohlen et al. (2004) found that earthworm invasion decreased soil C storage by 28% in the upper 12 cm of a sugar maple dominated forest in New York, while Wironen and Moore (2006) had findings from a similar forest type in Quebec, Canada suggesting that earthworm presence increased total soil C to a depth of 30 cm. According to a meta-analysis (237 observations from 57 publications) synthesizing the effect of earthworm presence on soil organic carbon (SOC) and greenhouse gas emissions (CO_2 and NO_2), Lubbers et al. (2013) found that earthworm presence increased

CO_2 emissions by 33%. However, as the length of these studies increased, the CO_2 emissions were seen to decrease, indicating that the initially high CO_2 emissions may be followed by a period of C stabilization; a temporal component often not considered in these studies. Additionally, it would be expected that such an increase in CO_2 emissions would lead to a decrease in soil organic carbon (SOC), yet this was not seen to be the case (Lubbers et al., 2013).

There are likely many ways C is stabilized within soils, however the mechanisms of stabilization, as well as methods of measurement, are still being debated. The pool of stabilized C is small in relation to total mineral soil C pool, and is therefore difficult to determine chemically (Zhang et al., 2013). One frequently cited stabilization mechanism is the physical segregation of bacterial communities, and their enzymes, from C occluded within microaggregates (mA, 250-53 µm) (Adu and Oades, 1978; Dungait et al., 2012; Elliott and Coleman, 1988; Sanchez-de Leon et al., 2014; Schmidt et al., 2011; Six et al., 2002; Stewart et al., 2007). Six et al. (2002) found that 20% of the difference in SOC stocks between agricultural and afforested ecosystems could be explained by differences in the microaggregate fraction, suggesting that these structures may be one of the driving mechanisms of long term C storage in forest soils. Presuming that mA-occluded C does, in fact, represent a pool of stabilized C within the soil, the ability to operationally isolate these structures, as outlined in Six et al. (2000), allows for one mechanism of C stabilization to be accurately analyzed.

Shipitalo and Protz (1989) demonstrated that during passage through the gut of anecic species *Lumbricus terrestris,* existing mA structures are destroyed by peristalsis, and organic debris and clay particles become coated in polysaccharides, providing the nuclei for newly formed mA. This process of mA structure formation has been observed in both homogenized (Bossuyt et al., 2005; Sanchez-de Leon et al., 2014) and undisturbed agricultural soils (Pulleman et al., 2005), utilizing various earthworm

species and methods of measurement. It has been proposed through these studies that earthworm enhancement of stable aggregation is a mechanism by which they may stabilize C in the long term, mitigating their effect on increased soil respiration. There is, however, a lack of information on how earthworms will alter the aggregation and C qualities in undisturbed, earthworm-free, forest soils. In a recent study, Fahey et al. (2013) tracked the fate of ^{13}C in plots of a sugar maple forest with varying degrees of earthworm influence, and found that earthworms were directing much of the fresh ^{13}C into water stable aggregation, though no data were available on the quantity and quality of that aggregation prior to earthworm invasion.

In a paired mesocosm study we investigated the impact of one common endogeic earthworm species, *Aporrectodea tuberculata,* on the quantity and quality of microaggregates in undisturbed soil cores from an earthworm-free, northern hardwood forest. We hypothesized that earthworm presence would 1.) increase the proportion of large macroaggregates (>2000 µm, lgMA) as a proportion of total soil dry mass, 2.) enrich the lgMA fraction with microaggregates, increasing occluded microaggregates (mAlg) as a proportion of lgMA fraction dry weight, 3.) increase total microaggregate (occluded and free) as a proportion of total soil dry mass 4.) increase total C within the mineral fraction through the earthworm mediated relocation of the Oa horizon, 5.) increase the pool of microaggregate protected C through increasing both the quantity of microaggregates, and the C contained within the microaggregate fraction.

3.3 Methods

3.3.1 Site Characteristics

The Waterworks Property (WAT) is a 666 acre northern hardwood forest located in the town of Bristol Vermont in the Champlain Valley. Forest composition is primar-

ily *Acer rubrem* (red maple), *Acer saccarum* (sugar maple), and *Fagus grandifolia* (American beech). The cores used in this study were excavated at 73°7'58.68"W 44°9'48.142"N, at an elevation of 237 meters with an average slope of 18 degrees on a long west-facing hill-slope. The soils at this location are a coarse-loamy Marlow non-spodic variant. Previous surveys (see Chapter 2) found a single worm in 2008 with no indication of earthworm influence in a later survey. Approximately 500 m from the retrieval location, at the bottom of the hill-slope, several common species, including *Apporectodea tuberculata*, were observed. It could be assumed that, with time, these soils will be invaded by earthworms. Previous aggregate and C analysis indicated that the Waterworks soils are uniform with little variation in aggregate properties, horizon, and C properties. This lack of earthworm influence, in combination with low soil variability made this site an ideal candidate for the following paired study.

Hinesburg Town Forest (HIN) is a northern hardwood forest which was converted from agriculture approximately 80 years ago. The soils are also Marlow non-spodic variant and have been heavily influenced by the presence of earthworms (approximately 145 worms per m^2). *Apporectodea tuberculata* adults and juveniles were retrieved from Hinesburg Town Forest (73°2'17.603"W 44°19'45.78"N) on July 11th, 2013. To acclimate specimens for the mesocosms, worms were placed in soil from the Waterworks property at 15^{o}C for three weeks prior to incubation within the undisturbed soil cores.

3.3.2 Retrieval of Soil Cores

Segments of 30 cm standard-20 green PVC drain pipe were used for collection on July 22^{nd} 2013. A central location was chosen in the Waterworks property based on homogeneity of vegetation, and slope of the surrounding area. Retrieval of the cores from the field occurred in 6 randomly selected locations (A-F), determined by

a random number chart for distance and direction from the center point (A) (Figure 3.1). At each of the 6 locations, 5 cores were hammered into the ground. Moisture and temperature values were taken in mineral soil by W.E.T. sensor (Delta T Devices, Cambridge, UK) (Table 3.1). The cores were carefully excavated, sealed, and carried back to the lab. If a core was badly damaged during retrieval it was removed from the study. The thick forest floor at location B resulted in inadequate mineral soil mass for aggregate analysis, and so all cores from this location were removed from the study. Four cores were included from each of the remaining 5 locations (20 total).

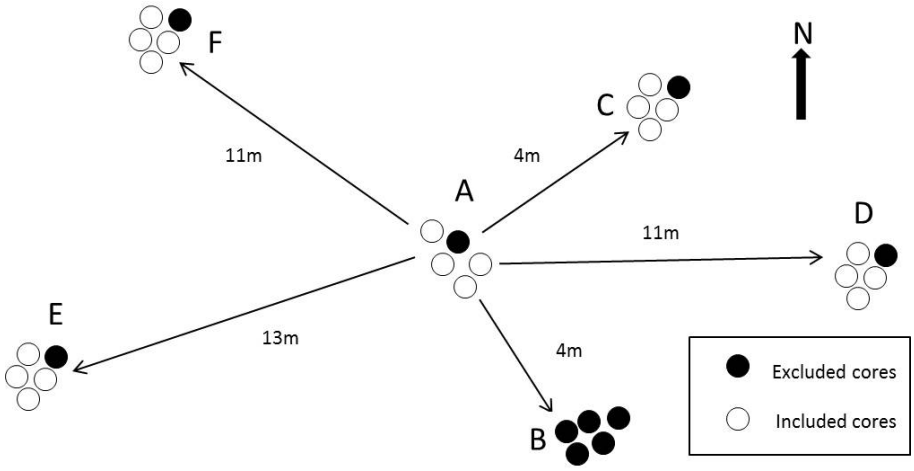

Figure 3.1: Layout of core retrieval area. The center of the plot (Location A) was chosen based on slope and homogeneity of surrounding area. All subsequent locations determined by randomly assigned distance and angle from Location A. Filled circles represent cores excluded from the study.

Table 3.1: Soil conditions of each retrieval location (A-F) on day of core removal July 22^{nd} 2013.

Retrieval location	Moisture (%)	Temp (^{0}C)	Depth of Forest Floor (cm)
A	15.3	17.7	7.5
C	10.9	18.4	5
D	12.4	19.2	9
E	14.7	18.8	6
F	14.8	20.1	6

3.3.3 Experimental Design

Soil cores were brought back to the lab and the soil moisture was slowly increased to approximate field capacity. Based on retrieval location, depth of mineral soil, weight, and appearance, cores were paired together, resulting in 2 pairs for each retrieval location, 1 pair for each opening time. On August 24^{th} 2014, 1 juvenile and 3 adult worms were placed in RO water, blotted with filter paper, weighed, and placed randomly in 1 core from each of the 10 pairs. Density of worms added (approximately $2400/m^2$) was roughly 3.3 times the density found at HIN ($725/m^3$) with a total weight ranging from 4.85 - 6.05g. The experimental cores were placed in 15^oC, surrounded by a series of non-experimental cores to account for possible edge effects, and covered with black plastic to reduce light. Cores were kept at a constant weight, with tap water being added every 3 - 7 days. On September 25^{th} 2013 (4-weeks after inoculation) and January 8^{th} 2014 (4-months after inoculation), one pair of cores was randomly chosen from each retrieval location, deconstructed, and analyzed.

3.3.4 Core Deconstruction

Prior to deconstruction, cores were allowed to dry for 3 days at 4 weeks and 12 days at 4 months. Cores were opened by cutting through PVC with a table saw. Cores were then immediately taped back together, to be fully deconstructed no more than 18 hrs later. Care was taken not to disturb the mineral soil, however loss and PVC contamination was seen in the forest floor, specifically the Oi horizon. During deconstruction, cores were carefully re-opened and soil was moved away from the core edge with a knife. When worms were noticed, they were immediately removed and placed in reverse osmosis (RO) water. Depth of soil horizons were recorded before

the litter layers (Oi, Oe/Oa) were removed, and placed into aluminum pans to dry. The remaining soil was gently passed through an 8 mm sieve, weighed, and laid out to dry for 48 hours after which small samples were removed for moisture analysis. Coarse fragments and roots were separated, washed, and laid out to dry. Worms, if present, were blotted with filter paper and weighed. After 48 hrs, dry weights of the empty core, Oi, Oe/Oa, coarse fragments, and roots were recorded.

3.3.5 Aggregate Analysis

A complete synopsis of the following procedures may be found in Figure 3.2 on page 83.

3.3.5.1 Water Stable Aggregate Fractionation

Representative, 50 g samples were removed from the mineral soil of each core. Wet sieving was done 4 or 3 times (4 Week and 4 Month time points, respectively) for each core according to the methods found in Six et al. (2002), modified from Elliott (1986). This process was done at 8 weeks and 1 week of air drying for the 4 week and 4 month incubations respectively. Briefly, 50 g of air dried soil was submerged in reverse osmosis (RO) water on top of a 2000 µm sieve for 5 minutes to induce slaking. The sieve was moved in and out of the water, in approximate 3 cm circular motions, 50 times over the course of 2 minutes, and the material that remained on the sieve was back washed into a clean container with RO water. Any floating organic matter from the 2000 µm was not considered to be soil, and was therefore decanted and discarded. What remained after decanting was the large macroaggregate fraction (lgMA, >2000 µm). The particles which passed through the 2000 µm sieve were transferred over a 250 µm sieve and the sieving procedure was repeated (50 motions up and down). What was retained on the 250 µm sieve, the small macroaggregate fraction (smMA,

250-2000 µm), was back washed into a clean container with RO water. The particles which passed through the 250 µm sieve were transferred over a 53 µm sieve and the process repeated. What was retained on the 53 µm sieve, the free microaggregate fraction (fmA , 53-250 µm), was back washed into a clean container with RO water, and the silt and clay fraction ($<$ 53 µm), was discarded. The lgMA, smMA, and mA fractions were all back washed through coffee filters (modification, *Home 360 Hannaford Brand #2 cone filters*) which were then placed in 65^{o}C for 18-24 hours. Once dry, the fractions were weighed and carefully brushed away from the coffee filters to be stored in plastic bags until further processing.

3.3.5.2 Microaggregate Isolation

The below method for releasing the occluded microaggregates from the larger aggregate fractions was conducted following the process outlined in Six et al. (2000), for both the lgMA and smMA fractions.

From the above fractionation method duplicates, each macroaggregate fraction was combined into 8g samples. These samples were slaked in RO water on top of a 250 µm sieve for 20 minutes. The submerged 250 µm sieve was then shaken vigorously by hand with 50 stainless steel bearings (4mm diameter) while a continuous flow of RO water passed over the apparatus. This was done in order to wash the smaller material through the sieve quickly, and avoid the further breakup of the microaggregates. After 4 minutes of shaking, the larger aggregates remaining on the sieve were gently prodded with a soft rubber stopper. The prodding, combined with shaking and water flow, continued until all but coarse sand and POM (lgPOM, $>$250 µm or smPOM, 250-2000 µm, dependent on starting fraction) had passed through the sieve. Material which passed through the 250 µm sieve was collected on a 53 µm sieve and wet sieved for 2 minutes (see Water Stable Aggregate Fractionation above), resulting in the

stable materials occluded within the large macroaggregates (mAlg, 250-53 µm) or small macroaggregates (mAsm, 250-53 µm), depending on the starting material. The material which passed through the 53 µm sieve was the silt and clay fraction (< 53 µm), and was discarded. All retained fractions were back washed into clean containers and then through coffee filters before being dried and weighed.

3.3.5.3 Density Fractionation of Light Fraction (LF)

The light fraction (LF) is composed of non-complexed decomposing plant and animal tissues, believed to be more labile, i.e. having a rapid turnover (Evans et al., 2001). The density fractionation procedure assumes that, during the humification process, the more recalcitrant SOM becomes intimately associated with mineral portions of the soil (Barrios et al., 1996). Therefore, any fraction having a density less than the mineral fraction, which is not occluded within a microaggregate, is assumed to be free LF, and more bio-available. In order to get a proper assessment of the amount of protected carbon found within the microaggregates (fmA, mAsm, mAlg), the LF must be removed prior to carbon analysis. The method for this process is outlined in Six et al. (1998) which was modified from Elliott and Cambardella (1991).

The microaggregate fractions were oven dried at 70°C for 18-24 hours. After cooling to room temperature in a desiccator, the samples were weighed and added to a 50-mL graduated conical centrifuge tube already filled with 25 mL of 1.85 g/cm3 (+/- 0.01 g/cm3) sodium polytungstate (SPT). This mixture was then slowly inverted 10 times, bringing the sample into suspension without disruption of the microaggregate structure, the goal being to remove only the LF outside of any microaggregate. The material remaining on the cap and sides of the centrifuge tube was rinsed into the suspension with an additional 10 mL SPT, and after 20 min at equilibrium the samples were centrifuged at 2500 rpm for 60 min. The samples sat at room temperature for 18-

24 hours in order to allow materials to settle completely before the floating material (free LF), as well as most of the SPT, was aspirated onto a 10 µm nylon mesh, rinsed thoroughly with RO water to remove any remaining SPT, and transferred to a small aluminum pan. Samples were dried at 60°C for 18-24 hours, cooled to room temperature in a desiccator, and weighed.

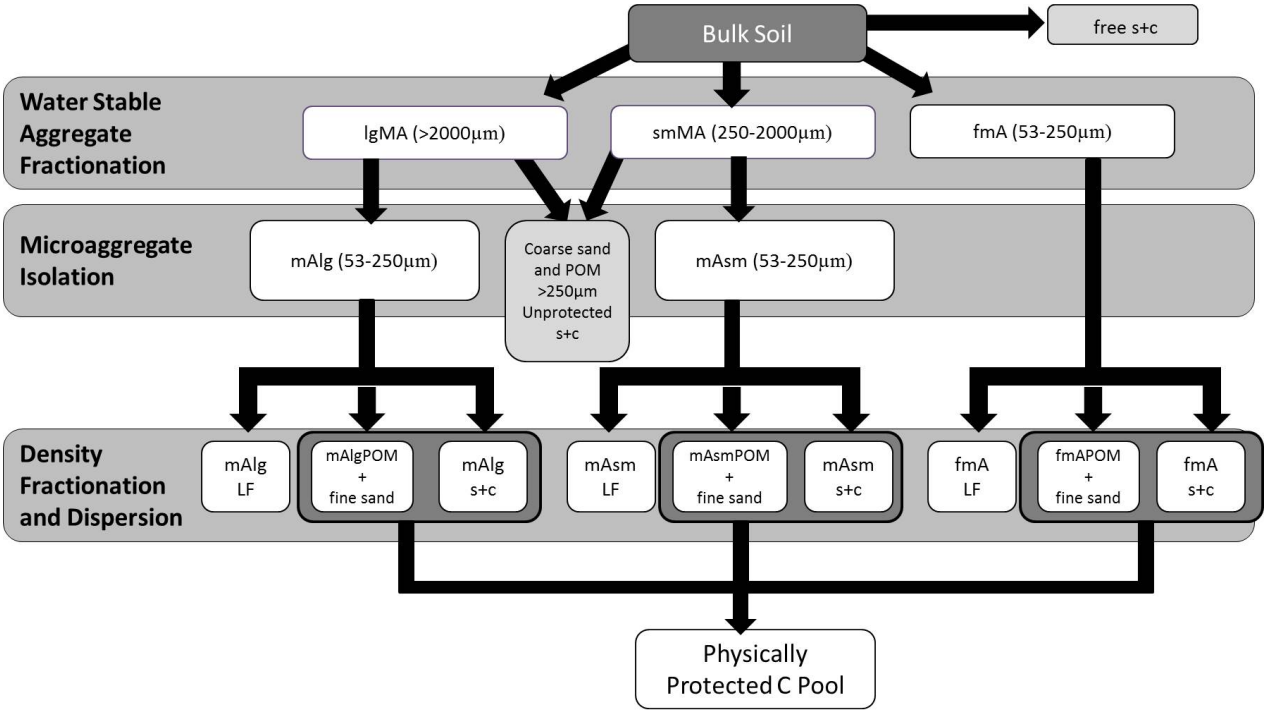

Figure 3.2: Diagram representing processing steps and functional soil fractions obtained from each. s+c: silt and clay fraction <53 µm, LF: organics (<1.85 g/cm^3) between mA fractions, POM: particulate organic matter within mA fractions.

3.3.5.4 Dispersion

The heavy fraction (HF) remaining on the bottom of the conical tube after aspiration was rinsed twice with 50 mL of RO water in order to clean away any remaining SPT. The sample was mixed with 35 mL of 0.5% hexametaphosphate and dispersed by shaking on a reciprocal shaker for 18 hours. The dispersed HF was then passed through a 53 µm sieve, rinsed with RO water, and wet sieved for 2 min. The material

remaining on the sieve was quantified as the intra-microaggregate POM (fmAPOM, mAlgPOM, mAsmPOM), and fine sand. This fraction was transferred to a small aluminum pan and dried 18-24 hrs at 60°C. The material passing through the sieve (fmAs+c, mAlgs+c, mAsms+c) was discarded.

3.3.5.5 Calculations

Due to our assessment of lgMA (>2000 µm), the non-soil fraction (coarse fragments and free POM >2000 µm) was calculated and subtracted from the total soil starting weight for all calculations. Sand has a low likelihood of being incorporated within similar sized aggregates (Six et al., 2000) and therefore weights for all aggregate sizes were corrected for sand content of the same size class. All silt and clay fractions were discarded, values for these fractions were calculated by mass balance. Due to anticipated loss along processing steps, these values are likely an over-estimate of actual values.

3.3.6 Lab and Statistical Analysis

3.3.6.1 Nutrient Analysis

Basic soil nutrient analysis was carried out on all cores following the procedures of the University of Maine Soil Testing Service and the University of Vermont Agricultural and Environmental Testing Laboratory. Soil samples were dried at 45°C, crushed to pass a 2-mm sieve, and extracted with Modified Morgan's solution (0.62 M NH_4OH + 1.25 M CH_3COOH; 4 g, 20 mL, shake 15 minutes). After filtering through Ahlstrom 642 paper, they were analyzed for o-phosphate (molybdate blue procedure) and macro- and micronutrients (inductively coupled plasma spectroscopy, ICP-OES Perkin Elmer Corp, Norwalk, CT, USA). Soil pH was determined in 0.01M

$CaCl_2$ 2:1 v:v; water pH was estimated by adding 0.6 pH units to the salt value. Organic matter was determined by loss on ignition at 375°C (Wolf and Beegle, 2011).

3.3.6.2 Carbon Analysis

Total carbon analysis was conducted at the University of Vermont on a Thermo Scientific Flash EA 1112 NC Analyzer (CE Elantech). The bulk soil and the lgMA fraction were ground by hand to pass through a 250 μm sieve, with coarse rocks and twigs >2000μm removed. All fractions were oven dried to a constant weight at 60^oC prior to analysis. Sub-samples of 20-80 mg from the mineral fractions, and 2-5 mg of the organic fractions were weighed into tin capsules in duplicate. Analyzer calibration and quality control (QC) was conducted using soils obtained from the North American Proficiency Testing program.

Any QC sample with greater than 10% error had samples immediately preceding and following it re-run, along with any samples in which duplication had greater than 10% error. A QC run was included at the end of all sample processing for which 10% of all samples were randomly chosen and re-run.

3.3.6.3 Statistical Analysis

All statistical calculations were performed using JMP 9.0 (SAS Institute Inc.). All analysis was done based on the pairing outlined in the Experimental Design on page 79.

3.4 Results

3.4.1 Earthworm Survival and Core Measurements

All specimens survived and were seen to be active at the end of 4 weeks, though there was a reduction in average fresh weight of approximately 4%. At the end of 4 months, all worms were recovered, with approximately 30% found in diapause, as indicated by specimens being curled into tight balls. All WW cores at 4 months had new juveniles present, accounting for an average fresh weight of 0.48g per core. Even with this added juvenile weight, average fresh weight was seen to decrease by approximately 32%. This reduction in fresh weight, as well as the noted diapause behavior, was possibly due to the length of time cores were allowed to dry prior to deconstruction, which was 12 days at 4 months as compared to 3 days at 4 weeks. Length of drying was increased to ease disturbance during core opening.

3.4.2 Earthworm Effect on Physical Proportion of Aggregates

At the end of 4 weeks of incubation no significant difference in aggregate properties were seen. After 4 months of incubation the main effect of earthworm activity was an increase in the lgMA (>2000 µm) fraction (Fig. 3.3). Cores with earthworms had 67% more lgMA than paired cores without earthworms. Through this action, the smMA (250-2000 µm) fraction was reduced by 10% (Table 3.3).

At 4 months little effect was seen on the proportion of total soil dry mass comprised of the mA fractions (Fig. 3.4). Generally, the proportion of mAlg was numerically higher, due primarily to the significant increase in the lgMA fraction (Fig. 3.3), while the mAsm and fmA proportions decreased, however these trends were not statistically significant, and resulted in no impact on the soil's total mA (Table 3.3).

Plot ID	Experimental Group	Total Mineral Soil Dry Weight (g)	Mineral Soil Percent Moisture (%)	Worm Density (worms/m^3)	Coarse Fragment (>8mm) Weight (g)	Final Horizon Depths (cm)			
						Oi	Oe	Oa	A
A	NW	1172.8	20.2%		85.6	1.5	2.5	3	1
A	WW	1189.2	22.9%	2400	103.4	1.5	2	0	4
C	NW	1117.9	18.6%		356.4	2.5	1	1.5	5
C	WW	1066.8	20.0%	2550	434.4	2.0	1.5	0	7
D	NW	922.8	20.9%		82.8	2.5	1	2	2.5
D	WW	1041.1	22.6%	2940	110.4	2.5	1.5	0	7
E	NW	1369.3	21.3%		83.4	1.5	1	3	4
E	WW	1463.9	23.1%	1820	64.2	2	1.5	0.5	6
F	NW	1238.3	17.1%		142.1	3.5	1	3	2.5
F	WW	1222.9	17.2%	2120	241.6	3	1	0	3.5

Table 3.2: Conditions of core soil properties at the time of deconstruction, after 4-months of earthworm incubation. Soil moisture measured gravimetrically.

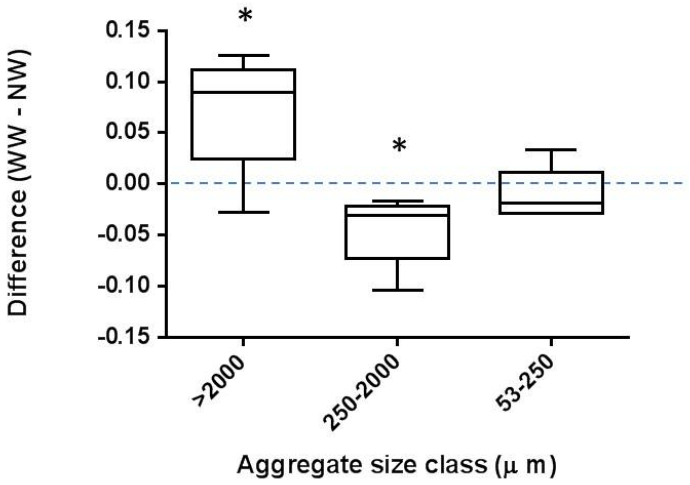

Figure 3.3: Mean difference in aggregate size proportion of soil dry mass in paired cores after 4-months incubation. n=5, (*) represent statistically significant values at $P<0.05$, bars represent minimum and maximum differences from core pairs.

Fraction	Average Proportion of Total Soil Dry Weight		Difference in proportion of total soil dry weight
	NW	WW	WW-NW
lgMA (>2000 µm)	0.110 ± 0.017	0.183 ± 0.012	(+) 0.072 ± 0.026 *
smMA (250-2000 µm)	0.439 ± 0.034	0.395 ± 0.010	(-) 0.044 ± 0.016 *
fmA (53-250 µm)	0.098 ± 0.022	0.087 ± 0.006	(-) 0.010 ± 0.011
mAlg (53-250 µm)	0.051 ± 0.012	0.081 ± 0.007	(+) 0.030 ± 0.016 §
mAsm (53-250 µm)	0.184 ± 0.006	0.165 ± 0.004	(-) 0.020 ± 0.009 *
Total mA (fmA+mAlg+mAsm)	0.332 ± 0.007	0.333 ± 0.007	(+) 0.0004 ± 0.009

Table 3.3: Average difference between paired cores (WW - NW) as a proportion of total soil dry weight with standard error. (*) represents statistical significance at $P<0.05$. (§) Borderline positive earthworm effect in mAlg fraction (p-value = 0.07)

3.4.3 Earthworm Effect on mA associated C

There were no significant earthworm effects on the soil aggregate properties seen after 4 weeks of incubation. While it was apparant that the earthworms were active within the soil, only a small proportion of the total mineral soil volume seemed to have been ingested by the earthworms over the 4 weeks. Any effect the earthworms had within the drilosphere were overshadowed by the bulk soil properties.

At 4 months the A horizon was enhanced through earthworm incorporation of the Oa horizon (Table 3.2). The mineral portion of the WW cores contained, on average, 26.01 gC/kg$_{bulksoil}$ (±1.98 SE) while NW cores contained 16.22 gC/kg$_{bulksoil}$ (±0.55 SE), an earthworm effect on total mineral soil C of 60%. The protected pool of C, defined as the within-mA POM and mA associated silt and clay (s+c), increased by an average of 5.16 gC/kg$_{bulksoil}$ (±0.23 SE), or 55%. Of this increased protected C pool, 95% was due to changes in the mAlgs+c and mAlgPOM fractions, which increased 2.75 gC$_{fraction}$/kg$_{bulksoil}$ (±0.27 SE), and 2.16 gC$_{fraction}$/kg$_{bulksoil}$ (±0.31 SE), respectively. The mA protected pool explains 53% of the difference in total mineral soil C between the cores, while the mA associated LF, an unprotected pool, explains another 33%. The increase in the mA protected C pool was seen despite there being no difference in the soil physical proportion of microaggregates (Fig. 3.4)

3.4.3.1 C of the within-mA associated silt and clay (s+c)

The protected C of the mA associated silt and clay was significantly increased in the mAlg fraction by almost 3 fold (1.42±0.23 gC$_{fraction}$/kg$_{bulksoil}$ (NW), 4.17±0.15 gC$_{fraction}$/kg$_{bulksoil}$ (WW), p=.0003). This increase, along with changes of the distribution in the fmA and mAsm fractions (Fig. 3.5) resulted in an average increase of 3.01 gC$_{fraction}$/kg$_{bulksoil}$ (±0.54 SE) for the total mA associated s+c, an increase of

approximately 40%. This increase in the mA s+c fractions accounted for 60% of the increase in total mA protected C. As a function of total mineral soil C this fraction decreased from 48% (NW) to 41% (WW).

3.4.3.2 Within-mA associated POM (POM)

The protected C of the within-mA associated POM was significantly increased in the mAlg fraction by 9 fold (0.29±0.06 gC/kg$_{bulksoil}$ (NW), 2.46±0.30 gC/kg$_{bulksoil}$ (WW), p=.001). This increase, along with fluctuations in the fmA and mAsm fractions (Fig. 3.5) resulted in an average increase of 2.14 gC$_{fraction}$/kg$_{bulksoil}$ (±0.45 SE) for the total protected mA associated POM, an increase of almost 80%. This increase in mA protected POM accounts for 40% of the increase in total mA protected C, and as a function of total mineral soil C this fraction increased from 10% (NW) to 15% (WW).

3.4.3.3 Between-mA associated light fraction (LF)

The total between-mA associated POM (POM occluded within macroaggregates that is not occluded within microaggregates), quantified by the light fraction (LF) obtained from the mA, mAlg, and mAsm fractions prior to dispersion (Fig. 3.2), was 3 fold greater in the WW cores (0.98±0.09 gC$_{fraction}$/kg$_{bulksoil}$ (NW), 4.21±0.05 gC$_{fraction}$/kg$_{bulksoil}$ (WW) p=.001). This difference was due primarily to the mAlg LF (Fig. 3.6), which increased from 0.16±0.03 gC$_{fraction}$/kg$_{bulksoil}$ to 3.07±0.45 gC$_{fraction}$/kg$_{bulksoil}$, an almost 20-fold increase accounting for 90% of the total LF C difference. As a function of total mineral soil C, the total LF accounted for 16% of the total mineral soil C in the WW cores and 6% in the NW.

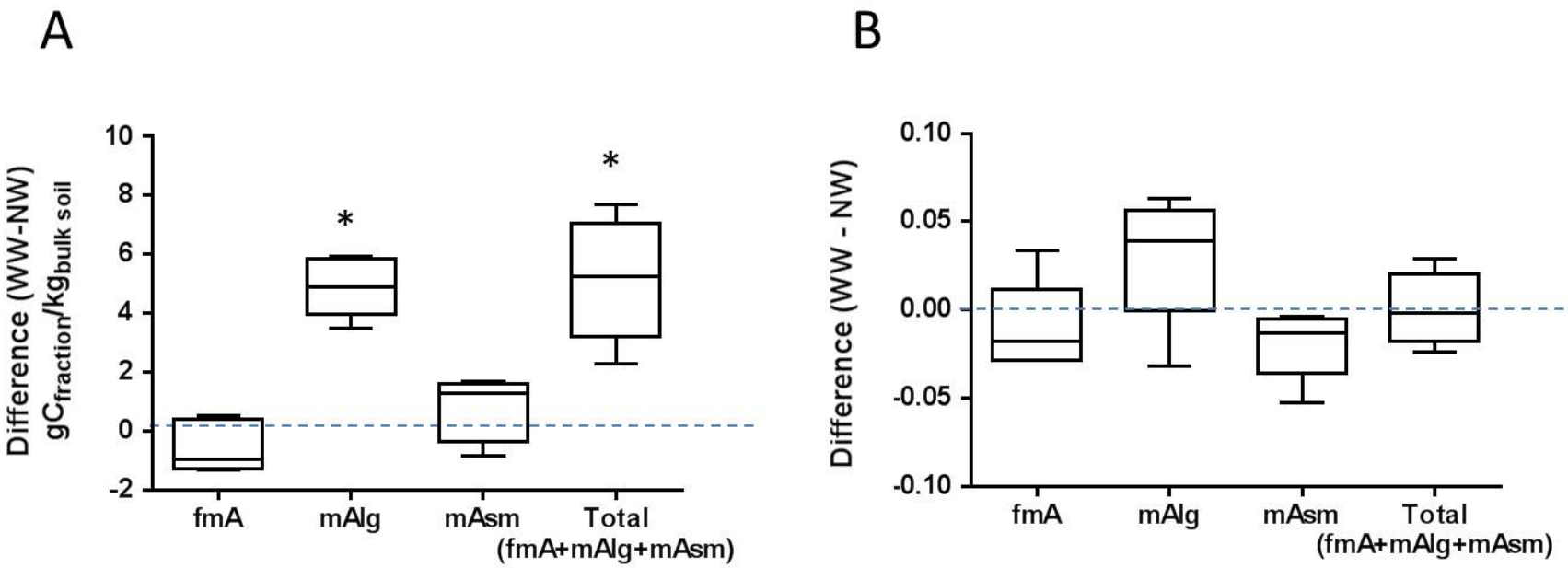

Figure 3.4: Demonstrating the difference in earthworm effect on the quantity and C content of the soil's various microaggregate fractions. Boxes represent the mean difference in values between paired cores, with minimum and maximum differences represented by bars A.) Impact of earthworms on the $gC_{fraction}/kg_{bulksoil}$ of different microaggregate fractions, and total microaggregate fraction. B.) Impact of earthworms on the physical proportion of soil dry mass comprised of different microaggregate fractions, and the total microaggregate fraction. . n=5, (*) represent statistically significant values at $P<0.05$, fmA: microaggregates obtained from the fractionation procedure that are not occluded within macroaggregates, mAlg : microaggregates occluded within the lgMA fraction, mAsm: microaggregates occluded within the smMA fraction

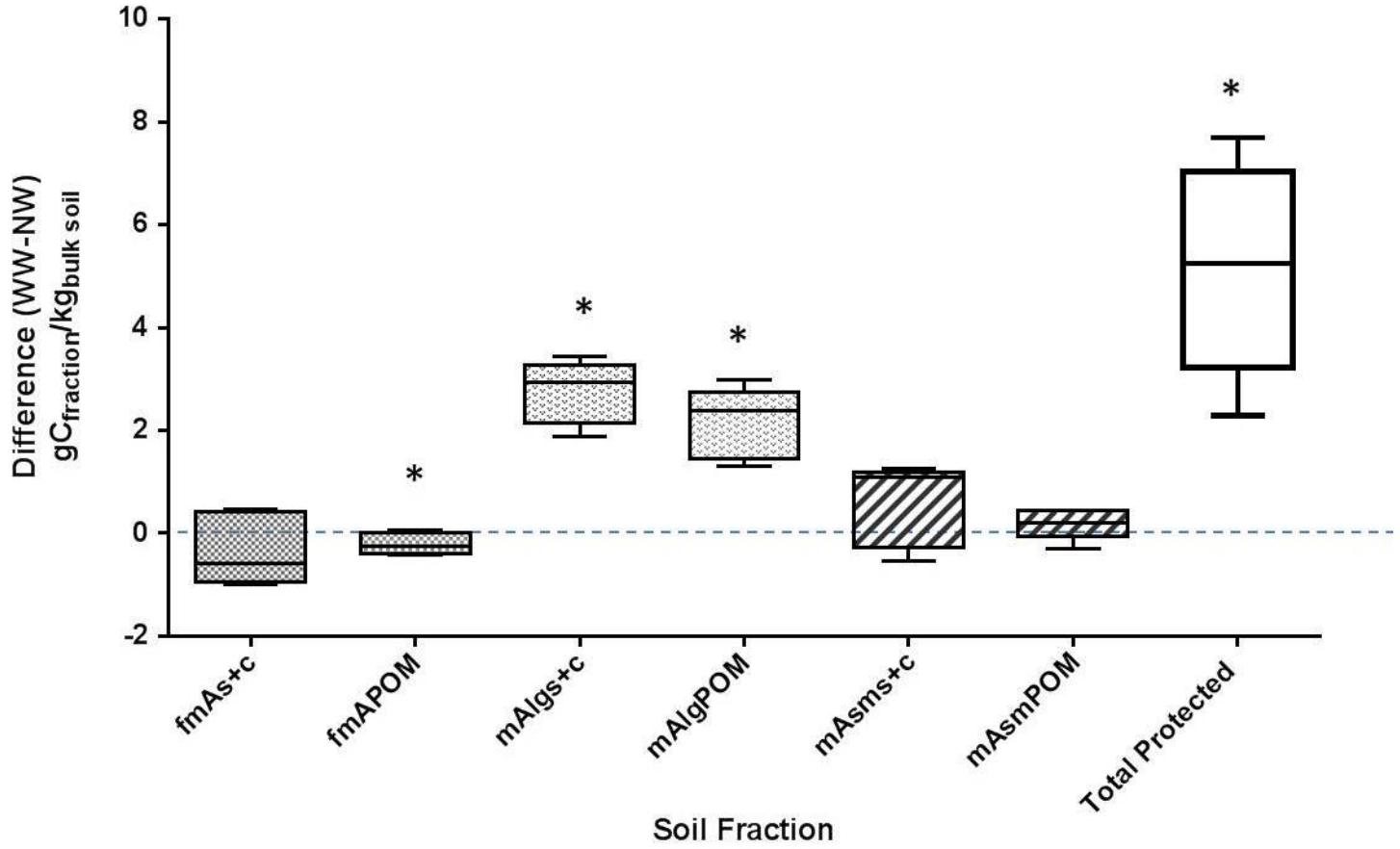

Figure 3.5: Impact of earthworms on all quantified protected C pools. n=5, (*) represent statistically significant values at $P<0.05$. Boxes represent the mean difference in values between paired cores, with minimum and maximum differences represented by bars. fmA: microaggregates obtained from the fractionation procedure which are not occluded within macroaggregates, mAlg : microaggregates occluded within the lgMA fraction, mAsm: microaggregates occluded within the smMA fraction, s+c : protected silt and clay component of microaggregates, POM : protected particulate organic matter occluded within microaggregates, Total Protected: sum of all protected microaggregate fractions.

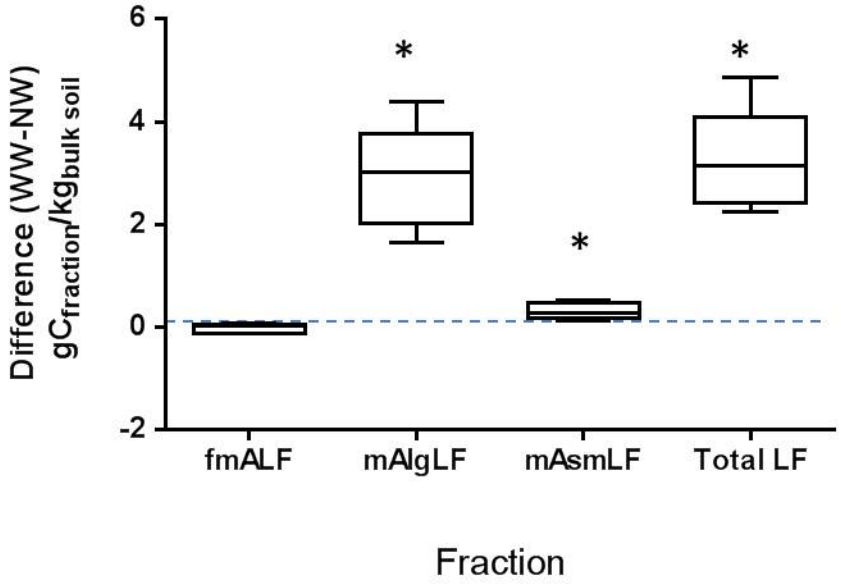

Figure 3.6: Paired core differences in the unprotected C pool, represented by the between-microaggregate POM (LF). n=5, (*) represent statistically significant values at $P<0.05$, boxes represent the mean difference in values between paired cores, with minimum and maximum differences represented by bars.

3.4.4 Nutrients

The mineral soil in WW cores showed an increase in organic matter (OM) of approximately 50%, calcium (Ca) of approximately 75%, and sodium (Na) of approximately 15%.

Measurement		No Worm (NW)	With Worm (WW)	Paired Mean Difference
pH		4.96 ± 1.0	4.90 ± 0.09	(-) 0.06 ± 0.12
OM	%	2.82 ± 0.11	4.16 ± 0.32	(+) 1.34 ± 0.34*
Na	mg/kg	9.80 ± 0.80	11.40 ± 1.12	(+) 1.60 ± 0.68*
Ca	mg/kg	340.40 ± 58.99	593.0 ± 35.95	(+) 252.60 ± 51.41*
K	mg/kg	72.00 ± 6.23	103.00 ± 16.04	(+) 31.00 ± 13.76
Mg	mg/kg	36.60 ± 3.17	66.20 ± 8.75	(+) 29.60 ± 10.84

Table 3.4: Nutrient extraction with Modified Morgan's solution. Differences of paired cores after 4 months of earthworm activity. Means represented with standard error (SE) values. (*) indicates a statistically significant difference of at $P<0.05$.

3.5 Discussion

3.5.1 Aggregation

At 4 months the presence of *Apporectodea tuberculata* significantly increased the proportion of lgMA, and showed influence over the proportion of other fractions. No change was noted after 4 weeks. While many researchers have found that endogeic earthworms enhance larger aggregation after as little as 3 weeks (Bossuyt et al., 2005; Mummey, Rillig, and Six, 2006) these studies almost always utilize an earthworm density much higher than what is found in nature, exaggerating the noted effects (Sanchez-de Leon et al., 2014). In our paired study, the maximum earthworm density used was 2940 worms/m^3(Appendix 3.2), which is three times the highest density seen in an extensive survey recently conducted in the state of Vermont (see Chapter 2). While higher than what is seen in Vermont forest soils, our densities were much lower than what is found in other studies, possibly explaining why we did not see the significant effects expected at 4 weeks. An equal number of worms were placed in

each experimental core (3 adult, 1 juvenile), however, the volume of soil was variable between pairs, and the level of earthworm effect varied with this earthworm density. The pair of cores from sampling plot E contained the lowest earthworm density (1820worms/m^3Appendix on page 87) and also showed a minor decrease in lgMA, an opposite effect as what was seen in all other pairs. This contrary effect, likely due to low earthworm density and soil property variation, was not an outlier and was therefore included in all statistical analysis, however its inverse effect influenced several other fractions. Had the incubation time been longer, or the earthworm density higher in the E pair, we speculate that the mean effects at 4 months would have been much more pronounced.

Contrary to our hypothesis, earthworm presence did not increase the total proportion of microaggregates in the soil, even after 4 months. In this particular soil the impact on the total microaggregate pool was undetectable, though C data suggest that much of the soil's microaggregates within the experimental cores originated from earthworm ingestion. The microaggregate stablized C pool could not have increased in the WW treatment without the breakdown and subsequent reformation of microaggregates with a higher C content. The addition of organic binding agents, in the form of earthworm mucus polysaccharides and microbial exudate, along with peristaltic pressure along the earthworm alimentary canal, has been shown to increase the proportion of stable microaggregates (Bossuyt et al., 2005; McCarthy, Ilavsky, Jastrow, Mayer, Perfect, and Zhuang, 2008; Pulleman et al., 2005; Sanchez-de Leon et al., 2014). It is possible that the microaggregate levels present in these forest soils were high enough that the net effect of earthworm ingestion and reformation of microaggregates was slight.

While the net effect was zero, the microaggregate proportions shifted among fractions. The fmA fraction would have become part of the mAlg or mAsm fractions as it

became occluded within the macroaggregate fractions, however this effect was not significant in the fmA fraction. The proportion of total soil composed of microaggregates occluded within the smMA fraction (mAsm) was significantly decreased ($P<0.05$) due primarily to the significant reduction in the soil's smMA fraction.

We believed that the proportion of total microaggregates would increase primarily through an increase in the proportion of microaggregates occluded within macroaggregates. Earthworms have been shown to facilitate the creation of microaggregates within macroaggregates in the field (Jongmans, Pulleman, and Marinissen, 2001; Pulleman et al., 2005) as well as in the lab (Bossuyt et al., 2005; Mummey et al., 2006) and microaggregates formed within macroaggregates are thought to be the primary mechanism by which microaggregates are increased in soils (Oades, 1984; Six et al., 2000). We saw no effect of earthworms on the proportion of microaggregates within macroaggregates, however, in general the lgMA fraction mass was composed of 6% more microaggregate mass than the smMA fraction, regardless of earthworm presence (0.347±0.015 kg_{mAlg}/kg_{lgMA}, 0.285±0.005 kg_{mAsm}/kg_{smMA}, n=40, p-value <0.001). Perhaps if the study had been allowed to continue for a longer period of time the increase in lgMA alone may have had influence on the proportion of microaggregates in the soil, even if these structures did not themselves have a higher proportion of occluded microaggregates.

3.5.2 Protected C

Analysis was not done for the forest floor horizons or C mineralization and so no balance of total core C was calculated and the amount of C lost to mineralization is unknown. Earthworms are known to relocate C downward into the mineral soil, and the objective of the study was to determine where within the soil's already established structure this species of earthworm would allocate the relocated C. We saw that

53% of C mixed into the mineral soil by earthworms was directed into the stabilized pools within microaggregation, while only 33% was found as unprotected POM. Of this unprotected POM, 90% could be assumed to exhibit minor protection due to its occlusion within the lgMA fraction. As this fraction continues to decompose it will likely become nucleating sites for future microaggregate creation (Baldock, 2002; Six et al., 2002,0). Only 14% of the difference in total mineral soil C between the WW and NW cores was unaccounted for by the pools measured in this study. This remaining pool contains the unprotected POM (250-2000 µm) occluded within the lgMA and smMA fractions, which would have been included in the total mineral soil C measurement, but was removed during the microaggregation isolation procedure (see Figure 3.2 on page 83).

It may be assumed that the majority of relocated C in our experiment originated from the Oa horizon of the forest floor (Table 3.2 on page 87), and that prior to relocation this fraction would have demonstrated a certain level of inherent chemical recalcitrance (Currie et al., 2002). Humic substances, *n*-alkanes (waxes) and lignified tissues, which are found concentrated within the Oa horizon, are placed into the slow or passive pool according to most current C models (Jenkinson and Rayner, 1977; Paustian et al., 1992). In addition to these assumed recalcitrant compounds, a proportion of the Oa horizon humus would be expected to consist of more labile compounds, originating from microbial biomass and microbially modified plant materials (Zou, Ruan, Fu, Yang, and Sha, 2005). While chemical properties will dictate, to some degree, how resistant SOC may be to microbial attack (Bol et al., 1996; Lutzow et al., 2006), the currently held belief is that it is the accessibility of SOC to degradation, rather than its intrinsic chemical quality, which dictates residence time within soils (Dungait et al., 2012; Kleber, 2010; Marschner et al., 2008) This suggests that, with time, most compounds located in this horizon would have come into contact

with a decomposer community able to utilize them.

Zhang et al. (2013) introduced the concept of a "sequestration quotient" in which the simultaneous earthworm effect on mineralization and stabilization is balanced and quantified into a sequestered C pool, the suggestion being that earthworms often increase stabilization to a greater degree than they do mineralization, creating a "C trap". In measuring both C stabilization and C mineralization they found that earthworms lowered the total soil C and increased mineralization after 23 days of incubation, but by the end of the experiment (52 days) soils showed equivalent total soil C, and a lower mineralization rate. While both control and earthworm manipulated soils ended up equivalent in total soil C, the earthworm-worked soil had a much higher proportion of its soil C in protected pools, highlighting that the casts of earthworms are potentially physio-chemically different than that of the bulk soil (Edwards, 2004). With aging, castings may represent a C pool undergoing slower decomposition than the surrounding soil (Lavelle et al., 1997; Martin, 1991).

Zhang et al. (2013) goes on to suggest that the impact earthworms have on the balance of mineralization and stabilization will depend greatly on the SOC content of the starting soil. In C limited systems the majority of organic materials encountered by microbial communities is utilized, and mineralization may be the driving force, while in C rich systems, only the most labile C will be utilized while the remaining may remain stabilized. The mineral soils utilized in our study are C limited (see below), and so while most of the ingested Oa horizon would likely be egested within the casts (Curry and Schmidt, 2007; Edwards and Bohlen, 1996), it is likely that, based on the sequestration quotient explained above, these soils would have shown higher rates of mineralization than stabilization during the duration of our study.

The mineral soil from WAT used in this study inherently contains very low amounts of SOC (approximately 16 $gC/kg_{bulksoil}$), and with only 46 MgC/ha in its

mineral soil is substantially lower than the United States northeastern forest mineral soil average of approximately 90 MgC/ha (Birdsey, 1992). For a related study (see Chapter 2 of this document), WAT was included with 8 other Vermont forest sites which underwent the aggregation analysis outlined above, and it was found that WAT contained almost 60% more microaggregation than the average of the other sites ($0.261 \pm 0.015\ \mathrm{kg}_{microaggregation}/\mathrm{kg}_{bulksoil}$,(WAT), $0.169 \pm 0.004\ \mathrm{kg}_{microaggregation}/\mathrm{kg}_{bulksoil}$ (Remaining sites, n=8), $P<0.001$). In ecosystems where aggregate turnover is slow, incoming organic materials may be degraded before becoming occluded and protected within aggregates (Plante and McGill, 2002; Six et al., 2004). Freeze thaw cycles, wind throw, and bioturbation are the primary modes of soil mixing in temperate hardwood forests, with mineral soil C originating primarily from dissolved organic carbon (DOC), decomposing root tissues, and microbial biomass (Currie et al., 2002). Additionally, the primary tree species found at WAT (Red Maple, Sugar Maple, and American Beech) are known to have a rapid litter turnover time, relative to other prominent tree species (Moore, Trofymow, Taylor, Prescott, Camiré, Duschene, Fyles, Kozak, Kranabetter, Morrison, Siltanen, Smith, Titus, Visser, Wein, and Zoltai, 1999), further limiting the possibility of C occlusion and protection. Limited soil mixing, rapid litter turnover, and a high proportion of physically stable, C deficient microaggregates may be a partial explanation for the low concentration of SOC at the WAT site.

The C occluded within microaggregates is not protected indefinitely. The binding agents maintaining soil structure are not inert and are therefore subject to decomposition (Frey, 2005) As the structural stability of aggregates becomes compromised, occluded C may become available for microbial degradation (Baldock, 2002). Theoretically, for every system there exists an ideal rate of aggregate turnover which would allow for organic matter occlusion and protection, while still limiting the re-

exposure of previously occluded C (Plante and McGill, 2002). Due to the high levels of aggregates, and low levels of turnover present in many forests, it may be aggregate turnover, rather than aggregate creation, that will enhance C stabilization in forests showing the potential for C sequestration. Earthworm invasion with its preferential occlusion of C within castings, may potentially be able to accomplish that. However, it is still unknown how the continuous ingestion of castings, which would occur in highly invaded forests over long periods, will alter the C residence time within microaggregates.

Earthworm communities have many secondary effects in forests which profoundly alter soil function, moving far beyond the scope of this study. Through the removal and relocation of the forest floor, earthworm invasion has been shown to change an understory diverse in herbaceous plants and tree seedlings into one dominated by grasses (*Carex sp.*) (Hale et al., 2006) and other invasive plants (Nuzzo et al., 2009). The forest floor performs many functions within the forest ecosystem, acting as a necessary seed bed for native plants while also regulating the soil's moisture, temperature, and nutrient cycling (Currie et al., 2002). Through bioturbation, earthworms remove the seed bed for native plants, shifting conditions to those which are favorable for plants adapted to germinating in bare mineral soils. This action impacts the future productivity of the forest. The reduction in native tree seedlings, exacerbated by selective browse pressure by deer (Hale et al., 2006), will eventually alter the tree composition shifting primary production, canopy closure, soil temperature, and litter quality. The ecology of the forest soil is dynamic. Various interactions and feedback systems, present in different spatial and temporal settings, results in the full impact of any one earthworm community, in any one forest, on any one metric, being very difficult to predict.

3.5.3 Conclusions

We found that *Aporrectodea tuberculata* significantly increased total mineral soil C, primarily through the relocation of the Oa horizon. The majority of this relocated C was allocated into newly formed microaggregates, and was therefore considered protected with an increased residence time. We found an increase in the proportion of macroaggregates with no change in the proportion of total microaggregates, though C data suggested that much of the microaggregate fraction underwent earthworm ingestion. We suggest that for soils similar to the one studied here, which are C limited and have a high proportion of soil mass composed of stable aggregates, increased aggregate turnover mediated by endogeic earthworms may increase the pool of sequestered C in the long term, though initially a C loss is likely.

Appendices

APPENDIX A

METHODS

SPT recycling

Sodium polytungstate (SPT) is a non-toxic, non-reactive chemical capable of being recycled multiple times for the above procedure. After every step of the process SPT was collected and filtered with coffee filters (*Home 360 Hannaford Brand #2 cone filters*). The resulting volume (approximately 20L) of dilute SPT was then placed in a large polyethylene (Nalgene brand) plastic bin and evaporated at 70°C until the appropriate density was reached (approximately 60 hrs). Proper density was determined by weight in a 5mL volumetric flask. The solution was re-filtered prior to the next usage. To remove any contaminating C that may have accumulated in the solution the liquid was further cleaned by passing through a column containing activated carbon (CITE), quartz wool, and sodium activated resin (CITE). This was done according to the method outlined in Six et al. (1999). This step was very time consuming, and resulted in too much loss, to justify doing after every set of samples, and so was instead done after all samples from one sampling site had been completed (2 procedures between column filtration)

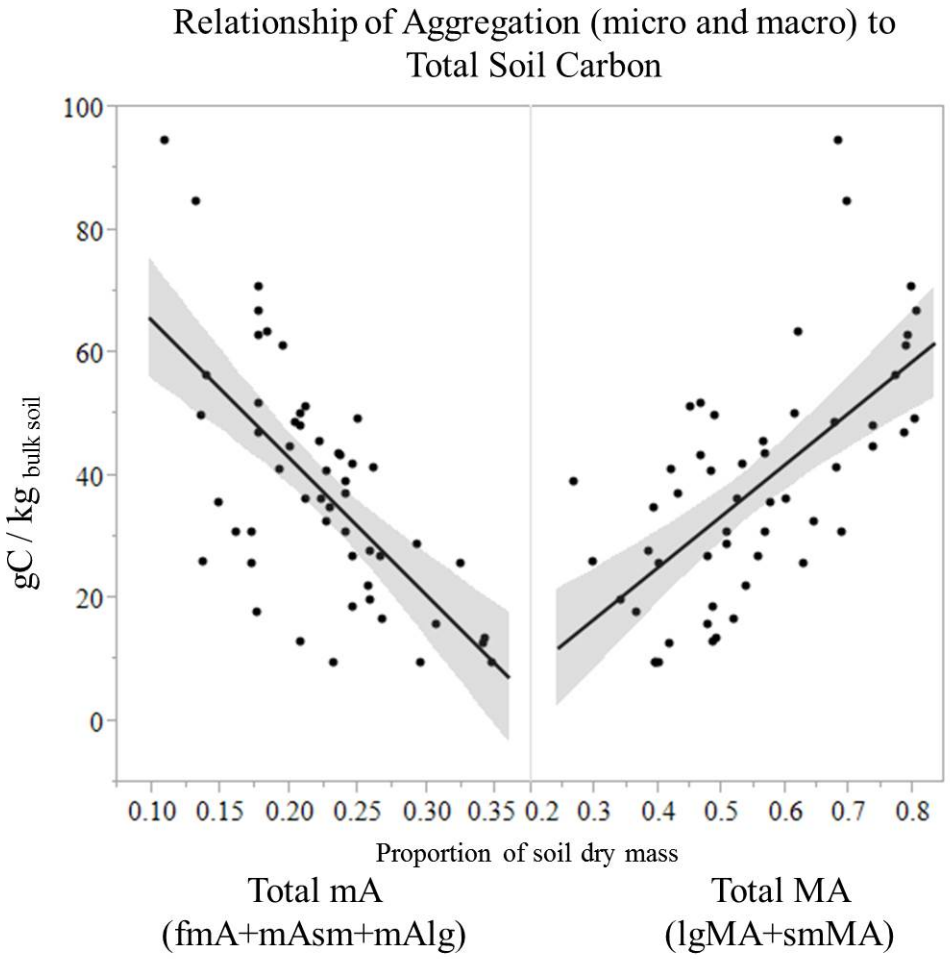

Figure B.1: An interesting trend is seen when looking at Macroaggregates, Microaggregates and Total soil C. These effects are significant even when accounting for differences between plots.

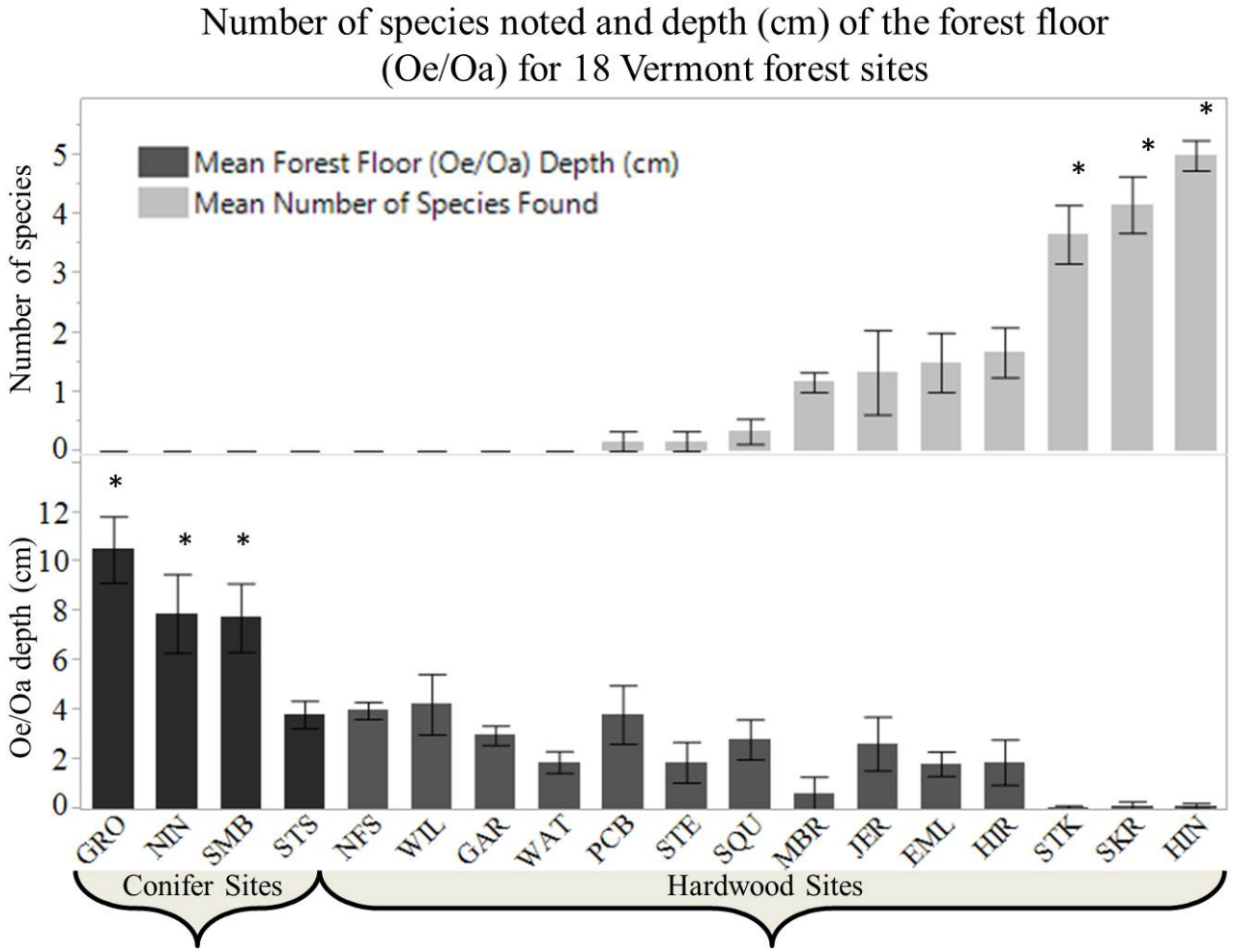

Figure B.2: Sites labeled with (*) represent statistically (P<0.001) different. Forest floor depth and number of species noted represent averages for the site (n=6). Error bars represent SE.

Site (Date Surveyed)	Plot	Adult Species Identified	Volumetric Moisture (%)	Bulk Density (g/cm^3)	Oi Depth (cm)	Oe Depth (cm)	Oa Depth (cm)	$gC/kg_{bulksoil}$	$gC_{fmA}/kg_{bulksoil}$ (protected)	$gC_{mAlg}/kg_{bulksoil}$ (protected)	$gC_{mAsm}/kg_{bulksoil}$ (protected)
WAT 10/11/2012	30^0	----	22.0	0.95	2	3	0	13.46	3.24	0.35	3.30
	90^0	----	15.5	0.95	3.5	1	0	9.52	2.28	0.06	2.30
	150^0	----	14.0	0.89	2	0.5	0	9.45	2.61	0.06	2.37
	210^0	----	12.1	1.00	3.5	0.5	1	12.77	3.00	0.44	2.18
	270^0	----	23.8	0.86	2	3	0	9.59	2.12	0.18	2.27
	330^0	----	23.6	0.95	2	2.5	0	15.67	3.33	0.73	3.65
STE 7/3/2012	30^0	----	31.5	0.95	3.5	2	0	30.93	4.97	5.12	9.09
	90^0	----	23.0	0.96	2.5	2	0	36.25	3.26	5.12	6.18
	150^0	12	46.4	0.91	3	2	0	36.34	3.00	9.32	8.48
	210^0	----	22.5	0.82	2.5	2.5	3	49.75	7.47	7.00	14.17
	270^0	----	26.3	0.96	2.5	0	0	25.80	6.67	3.20	6.79
	330^0	----	28.3	0.67	2.5	0	0	17.66	4.82	1.77	4.46
SQU 9/20/2012	30^0	----	37.9	0.85	1	2	2	21.97	3.85	2.26	6.77
	90^0	----	29.6	0.66	1	1.5	4	25.90	7.15	1.83	7.20
	150^0	----	36.3	0.75	3.5	0.5	0	27.82	8.52	2.28	8.32
	210^0	5	33.1	0.78	0	0.5	3.5	26.74	5.07	3.25	7.48
	270^0	----	22.2	0.59	0.5	1	0	19.88	8.47	1.30	3.80
	330^0	5	54.2	0.29	2	0	2	63.47	3.85	8.82	8.18
JER 6/6/2012	30^0	----	21.3	1.12	2.5	0	1	26.12	6.72	1.08	4.97
	90^0	----	26.3	0.73	3.5	3	3	38.97	7.37	1.86	6.45
	150^0	1	22.1	0.86	6	5	0	26.85	4.11	2.71	7.66
	210^0	5,9,12	52.7	0.93	3	0	0	47.12	0.67	16.37	7.91
	270^0	1,5,6,10	37.4	0.93	2	0	0	30.77	1.92	9.29	7.74
	330^0	----	22.5	1.01	1	3	1	13.03	4.06	2.27	2.34
HIR 5/31/2012	30^0	3,5,9	17.4	0.83	0.5	0	0	43.58	5.44	2.40	12.49
	90^0	2,9	25.0	0.71	4	2	2	43.23	7.52	1.62	10.69
	150^0	5,9	32.3	0.66	0.5	0	0	18.65	2.87	1.19	5.39
	210^0	2	30.2	0.54	0.5	0	0	41.11	9.46	1.27	10.37
	270^0	2,9	27.6	0.62	1.5	0	2.5	51.75	6.23	2.32	11.25
	330^0	----	33.3	0.73	1.5	3	2	45.61	5.88	3.46	13.53
STK 6/19/2012	30^0	2,4,9	34.6	0.81	0.5	0	0	40.69	7.36	6.23	10.28
	90^0	2,4,9	18.1	0.77	1	0	0	34.71	6.60	7.14	7.07
	150^0	2,4,5,6,9	46.1	0.70	2.5	0	0	32.65	2.30	8.75	6.63
	210^0	2,4,6,9	47.6	0.65	0.5	0	0	44.68	1.41	19.40	9.99
	270^0	4,9	46.0	0.40	0.5	0	0	48.26	2.51	18.50	13.70
	330^0	2,4,9	30.5	0.78	2.5	0.5	0	51.21	12.97	6.20	13.27
SKR 6/13/2012	30^0	2,4,8,9,11	33.5	0.76	3	0	0	50.13	4.76	4.86	10.91
	90^0	5,6,9	27.6	0.85	0.5	0	0	28.83	5.01	1.60	7.77
	150^0	2,9,8	22.9	0.81	6	1	0	37.01	5.85	2.95	6.52
	210^0	2,3,9	43.8	0.66	3.5	0	0	70.94	0.84	24.54	14.24
	270^0	2,3,5,8,9	42.2	0.58	2.5	0	0	56.38	1.90	21.13	16.47
	330^0	2,5,9	35.6	0.63	0.5	0	0	16.54	2.20	2.06	4.22
HIN 5/3/2012	30^0	2,4,5,8,9,12	50.3	0.98	0.5	0	0	48.66	2.83	10.05	12.24
	90^0	2,3,4,5,6	55.5	0.74	0.5	0	0	49.25	2.13	20.99	6.22
	150^0	2,3,4,5,6,12	51.9	0.78	0.5	0	0	66.96	1.90	26.52	9.30
	210^0	2,3,5,9,12	49.9	0.54	1	0.5	0	94.72	1.98	14.61	22.42
	270^0	3,5,7,8,9	40.2	0.68	1	0.5	0	62.72	1.33	17.84	13.08
	330^0	2,3,4,5,6	40.7	0.71	0.5	0	0	61.22	1.61	15.28	15.34
STS (conifer site - not included in analysis) 8/31/2012	30^0	----	16.6	0.70	1.5	1.5	1.5	30.69	4.98	5.61	6.95
	90^0	----	21.1	0.64	2.5	2.5	0	41.88	7.02	4.59	8.76
	150^0	----	33.2	0.86	3	3	0	35.66	2.34	8.17	10.97
	210^0	----	29.2	0.82	1	3	2	41.43	4.43	6.40	13.33
	270^0	----	54.9	0.90	1	2	4	84.65	1.78	20.31	15.96
	330^0	----	27.2	0.57	2	1.5	2	25.82	4.14	4.27	7.11

Table B.1: Breakdown of C data from samples collected at each plot with earthworm species found. Species coding is as follows: *Dendrobaena octaedra*-1, *Dendrobaena rubida*-2, *Aporrectodea turgida*-3, *Aporrectodea rosea*-4, *Aporrectodea tuberculate*-5, *Aporrectodea trapazoides*-6, *Octolasion cyaneum*-7, *Octolasion tyrtaeum*-8, *Lumbricus rubellus*-9, *Amynthas agrestis*-10, *Lumbricus terrestris*-11.

APPENDIX B

CHAPTER 2 ADDITIONAL GRAPHS AND TABLES

Site (Date Surveyed)	Plot	Proportion of bulk soil lgMA ($kg_{fraction}/kg_{bulksoil}$)	Proportion of bulk soil smMA ($kg_{fraction}/kg_{bulksoil}$)	Sum Proportion of bulk soil Macroaggregation (smMA+lgMA)	Proportion of bulk soil mAlg ($kg_{fraction}/kg_{bulksoil}$)	Proportion of bulk soil mAsm ($kg_{fraction}/kg_{bulksoil}$)	Proportion of bulk soil fmA ($kg_{fraction}/kg_{bulksoil}$)	Sum Proportion of bulk soil Microaggregation (fmA+mAlg+mAsm)
WAT 10/11/2012	30^0	0.05	0.44	0.49	0.01	0.18	0.15	0.34
	90^0	0.01	0.38	0.40	0.00	0.06	0.18	0.23
	150^0	0.01	0.38	0.39	0.00	0.16	0.19	0.35
	210^0	0.06	0.36	0.42	0.02	0.14	0.18	0.34
	270^0	0.04	0.36	0.40	0.01	0.14	0.15	0.30
	330^0	0.08	0.40	0.48	0.02	0.14	0.15	0.31
STE 7/3/2012	30^0	0.17	0.34	0.51	0.04	0.07	0.06	0.16
	90^0	0.20	0.33	0.52	0.06	0.07	0.09	0.22
	150^0	0.29	0.31	0.60	0.08	0.09	0.04	0.21
	210^0	0.16	0.33	0.49	0.03	0.06	0.05	0.14
	270^0	0.12	0.28	0.40	0.02	0.07	0.09	0.17
	330^0	0.09	0.28	0.36	0.02	0.06	0.10	0.18
SQU 9/20/2012	30^0	0.15	0.39	0.54	0.05	0.13	0.08	0.26
	90^0	0.04	0.30	0.35	0.00	0.07	0.16	0.22
	150^0	0.06	0.32	0.38	0.01	0.11	0.14	0.26
	210^0	0.14	0.34	0.48	0.02	0.11	0.11	0.25
	270^0	0.10	0.24	0.34	0.03	0.07	0.15	0.26
	330^0	0.27	0.35	0.62	0.04	0.08	0.07	0.18
JER 6/6/2012	30^0	0.06	0.24	0.29	0.01	0.03	0.10	0.14
	90^0	0.11	0.16	0.27	0.02	0.03	0.19	0.24
	150^0	0.18	0.38	0.56	0.04	0.10	0.13	0.27
	210^0	0.51	0.28	0.79	0.12	0.06	0.00	0.18
	270^0	0.38	0.31	0.69	0.08	0.06	0.04	0.17
	330^0	0.25	0.24	0.49	0.05	0.05	0.11	0.21
HIR 5/31/2012	30^0	0.10	0.47	0.57	0.03	0.15	0.06	0.24
	90^0	0.06	0.41	0.47	0.01	0.13	0.10	0.24
	150^0	0.07	0.42	0.49	0.02	0.14	0.09	0.25
	210^0	0.04	0.38	0.42	0.00	0.10	0.09	0.19
	270^0	0.06	0.41	0.47	0.01	0.09	0.08	0.18
	330^0	0.12	0.45	0.57	0.03	0.14	0.06	0.22
STK 6/19/2012	30^0	0.15	0.33	0.48	0.04	0.09	0.09	0.23
	90^0	0.13	0.26	0.39	0.02	0.08	0.13	0.23
	150^0	0.36	0.28	0.64	0.11	0.07	0.05	0.23
	210^0	0.48	0.26	0.74	0.12	0.07	0.01	0.20
	270^0	0.40	0.34	0.74	0.11	0.09	0.01	0.21
	330^0	0.12	0.33	0.45	0.02	0.08	0.12	0.21
SKR 6/13/2012	30^0	0.16	0.45	0.61	0.04	0.11	0.06	0.21
	90^0	0.06	0.44	0.51	0.01	0.17	0.11	0.29
	150^0	0.14	0.29	0.43	0.03	0.10	0.10	0.24
	210^0	0.49	0.31	0.80	0.11	0.07	0.00	0.18
	270^0	0.42	0.36	0.77	0.08	0.06	0.00	0.14
	330^0	0.11	0.41	0.52	0.03	0.14	0.10	0.27
HIN 5/3/2012	30^0	0.29	0.38	0.68	0.08	0.10	0.03	0.20
	90^0	0.59	0.21	0.80	0.16	0.07	0.02	0.25
	150^0	0.58	0.23	0.81	0.12	0.05	0.01	0.18
	210^0	0.25	0.43	0.68	0.04	0.07	0.00	0.11
	270^0	0.44	0.35	0.79	0.10	0.07	0.00	0.18
	330^0	0.39	0.40	0.79	0.09	0.09	0.01	0.20
STS (conifer site - not included in analysis) 8/31/2012	30^0	0.26	0.31	0.57	0.08	0.10	0.06	0.24
	90^0	0.19	0.34	0.53	0.06	0.11	0.07	0.25
	150^0	0.25	0.32	0.57	0.04	0.09	0.02	0.15
	210^0	0.23	0.45	0.68	0.05	0.17	0.04	0.26
	270^0	0.40	0.30	0.70	0.06	0.06	0.00	0.13
	330^0	0.26	0.37	0.63	0.09	0.15	0.08	0.32

Table B.2: A breakdown of all physical data by plot. One plot represents the average of two depths (0-10cm and 10-20cm)

APPENDIX C

CHAPTER 3 ADDITIONAL GRAPHS AND TABLES

Fraction		C ($g_{fraction}/kg_{bulksoil}$)			C/N ratio	
		Mean ± SE		Mean difference	Mean ± SE	
		WW	NW	(WW-NW)	WW	NW
Total Soil	*	26.01 ± 1.98	16.22 ± 0.55	(+) 9.79 ± 2.15	16.87 ± 0.75	16.13 ± 0.65
fmA s+c		1.79 ± 0.07	2.11 ± 0.32	(-) 0.32 ± 0.31	14.53 ± 0.63	14.28 ± 0.86
fmA POM	*	0.35 ± 0.05	0.54 ± 0.12	(-) 0.19 ± 0.09	21.93 ± 2.69	22.08 ± 1.91
fmA LF		0.20 ± 0.01	0.22 ± 0.04	(-) 0.013 ± 0.04	26.0 ± 2.12	25.51 ± 2.07
fmA TP		2.14 ± 0.12	2.66 ± 0.42	(-) 0.52 ± 0.39	15.19 ± 0.59	15.27 ± 0.85
mAlg s+c	*	4.17 ± 0.15	1.42 ± 0.23	(+) 2.75 ± 0.27	16.62 ± 0.93	16.54 ± 0.89
mAlg POM	*	2.46 ± 0.30	0.29 ± 0.06	(+) 2.16 ± 0.31	17.61 ± 0.48	17.75 ± 1.39
mAlg LF	*	3.07 ± 0.45	0.16 ± 0.03	(+) 2.92 ± 0.45	21.54 ± 0.42 *	28.72 ± 1.85 *
mAlg TP	*	6.62 ± 0.32	1.71 ± 0.28	(+) 4.91 ± 0.45	16.99 ± 0.70	16.70 ± 0.97
mAsm s+c		4.76 ± 0.37	4.18 ± 0.22	(+) 0.58 ± 0.36	15.28 ± 0.62	14.54 ± 0.65
mAsm POM		1.04 ± 0.09	0.86 ± 0.09	(+) 0.19 ± 0.13	19.19 ± 0.80	17.77 ± 0.93
mAsm LF	*	0.93 ± 0.06	0.61 ± 0.07	(+) 0.32 ± 0.07	26.56 ± 1.29	27.39 ± 1.22
mAsm TP		5.81 ± 0.46	5.04 ± 0.26	(+) 0.77 ± 0.48	15.8 ± 0.63	15.00 ± 0.70
Total s+c	*	10.72 ± 0.42	7.71 ± 0.26	(+) 3.01 ± 0.54	15.65 ± 0.68	14.81 ± 0.69
Total POM	*	3.85 ± 0.32	1.69 ± 0.17	(+) 2.14 ± 0.45	18.25 ± 0.48	18.53 ± 1.02
Total Protected	*	14.57 ± 0.68	9.41 ± 0.33	(+) 5.16 ± 0.23	16.26 ± 0.60	15.35 ± 0.74
Total LF	*	4.21 ± 0.50	0.98 ± 0.09	(+) 3.22 ± 0.45	22.61 ± 0.55 *	27.11 ± 1.21 *

Table C.1: Mean difference of earthworm effect on C ($g_{fraction}/kg_{bulksoil}$) in paired cores (n=5) with standard error. Mean C/N ratio of fractions in cores with earthworms (WW n=5) and without earthworms (NW n=5) with standard error. (*) represents statistically significant differences at $P<0.05$. fmA: microaggregation obtained from the fractionation procedure which is not occluded within macroaggregation, mAlg : microaggregation occluded within the lgMA fraction, mAsm: microaggregation occluded within the smMA fraction, s+c : protected silt and clay component of microaggregation, POM : protected particulate organic matter occluded within microaggregation, LF : unprotected organics found between microaggregation, Total Protected: sum of all protected microaggregate fractions

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