

Soil Carbon and Nitrogen Stocks: Underestimation with Common Sampling Methods, and
Effects of Thinning and Fertilization Treatments in a Coastal Pacific Northwest Forest

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Abstract

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Soil contains substantially more carbon (C) than the atmosphere and vegetation combined. Consequently, the fate of soil organic C (SOC) in response to changes in climate, land use, and management is of great concern. The ability to accurately quantify and compare SOC stocks over time is an important part of understanding carbon-climate feedbacks and constraining climate models, particularly because soils with larger standing SOC stocks are most at risk to release considerable C to the atmosphere. The objectives of this study are to 1) review the global importance of SOC and deep soil biogeochemical processes; 2) compare soil sampling methods and approaches to quantify and compare SOC stocks over time; and 3) examine SOC and nitrogen (N) response to thinning and fertilization treatments in a coastal Pacific Northwest forest. Three soil sampling methods for estimating SOC stocks were compared: clod, core, and

excavation. As it is considered the least biased method, the excavation method was used as the standard by which other methods were compared. Soil was sampled at an intensively managed Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation in northwestern Oregon, USA. Management regimes – no treatment (control), thinning treatments, and fertilization treatments – were randomly assigned to nine 0.2-ha plots established in 1989 in a juvenile stand. Prior to harvest in 2015, soil bulk density and chemical analysis samples were collected in the middle of soil depth layers 0-10, 10-20, 20-50, 50-100, and 100-150 cm. Forest floor samples were also collected. Soil was generally non-rocky (<2% fine to medium gravel content by weight) with a sandy clay texture. The core method significantly (Tukey's HSD, $\alpha = 0.1$) underestimated soil bulk density at all depths below 10 cm. Soil organic carbon concentrations tended to be lowest for the clod method and highest for the excavation method. As all SOC stock calculations rely on the relationship between SOC mass and soil mass, the underestimation of these parameters by clod and core methods, respectively, similarly affects the fixed depth, genetic horizon, and mass based approaches to quantify SOC stocks. The clod and core methods underestimated the SOC stock (Mg ha^{-1}) to a depth of 150 cm by 22% and 36%, respectively. Most of this difference occurred below 20 cm, where the majority of SOC stocks were contained across all soil sampling methods. During a single rotation of ~40 years, thinning treatments significantly reduced SOC (Mg ha^{-1}) and N (kg ha^{-1}) by 25% and 27%, respectively, compared to no treatment. Most of this loss occurred in deeper soil layers (below ~20 cm). Across all management regimes, deeper soil layers comprised the majority of SOC and N stocks. This study shows that 1) commonly used soil sampling methods for measuring soil properties such as SOC should not be assumed to be interchangeable; 2) accurately quantifying and comparing SOC and N stocks requires sampling deep soil; 3) regional and global SOC and N stocks may be largely underestimated due to

shallow sampling and the frequent use of core methods; and 4) forest management can significantly impact both surface and deep SOC and N on decadal timescales.

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An Ode to Soil

Where death turns to life

and all things are made anew

beneath our feet.

CHAPTER 1. A DEEPER LOOK AT SOIL ORGANIC CARBON: A REVIEW

1.1 ABSTRACT

Soil contains substantially more carbon (C) than the atmosphere and vegetation combined. Consequently, the fate of soil organic C (SOC) in response to changes in climate, land use, and management is of great concern. The ability to accurately quantify and compare SOC stocks over time is an important part of understanding carbon-climate feedbacks and constraining climate models, particularly because soils with larger standing SOC stocks are most at risk to release considerable C to the atmosphere. The vulnerability of some ecosystems to rapid SOC decomposition is greater than others and is related to the environmental constraints in place protecting the SOC in the given system. Although often ignored, deep soil comprises the majority of SOC, which is susceptible to decomposition on decadal timescales when the environmental conditions under which it accumulated change. The objectives of this review are to 1) discuss the global importance of SOC stocks; 2) summarize the shifting paradigm of SOC persistence; 3) gather the available information on SOC inputs, dissolved organic C dynamics, and deep soil biogeochemical processes and provide a unified conceptual model of these mechanisms; 4) highlight the dynamic nature of deep soils; and 5) review and emphasize the present need to improve the accuracy of regional and global SOC estimates by sampling deeper soil layers and by including the coarse soil fraction in SOC stock estimates. Ultimately, accurately quantifying SOC stocks and further study of the deep soil environment will provide valuable information about SOC accumulation and mineralization mechanisms, which in turn may help prioritize C sequestration initiatives and provide key insights into carbon-climate feedbacks.

1.2 INTRODUCTION

Soil is estimated to contain about two to three times the amount of carbon (C) stored in the atmosphere and vegetation combined (Ciais et al., 2013; Lehmann and Kleber, 2015). The decomposition of even relatively small amounts of soil organic C (SOC) thus represents a potentially strong positive feedback on global climate change. Soils with larger standing SOC stocks are especially at risk to release considerable C to the atmosphere, as the magnitude of SOC loss due to warming increases with the size of the initial SOC stock (Crowther et al., 2016). Accurately quantifying SOC stocks is an essential part of understanding future responses of soil to global climate change, which in turn will help constrain climate models. However, important knowledge gaps exist regarding the global mass of SOC and its vertical and horizontal distribution (Jandl et al., 2014; Scharlemann et al., 2014). Despite soil below 20 cm containing the majority of the SOC stock, soils are often sampled to 20 cm or less and are rarely sampled below 100 cm (Batjes, 1996; Harrison et al., 2011; James and Harrison, 2016; Jandl et al., 2014; Jobbágy and Jackson, 2000; Mobley et al., 2015; Stockmann et al., 2013). This bias not only contributes to uncertainty in global SOC stock estimates, but also limits our understanding of deep soil processes and SOC protection. Furthermore, many studies exclude the coarse soil (>2 mm) fraction of SOC, which may contain a substantial portion of C content (Corti et al., 2002; Harrison et al., 2011; Zabowski et al., 2011).

1.3 EFFECTS OF CLIMATE CHANGE, LAND USE, AND MANAGEMENT

Global SOC stocks for 0-100 cm in depth are estimated to range from 1220-1576 Pg C (1 Pg = 10^{15} g) (Batjes, 1996; Eswaran et al., 1993; Jobbágy and Jackson, 2000; Köchy et al., 2015; Post et al., 1982; Sombroek et al., 1993; Wieder et al., 2013). In a comparison of 27 studies over the past several decades, Scharlemann et al. (2014) found the median across all estimates for the

global SOC stock in the upper 100 cm of soil was roughly 1500 Pg C. Estimates for global SOC stocks 0-200 cm in depth range from ~2000-2500 Pg C (Batjes, 1996; Jobbágy and Jackson, 2000). The global SOC stock for 200-300 cm in depth was estimated at 351 Pg C (Jobbágy and Jackson, 2000). Notably, far fewer observations exist of the size of SOC stocks for soil depths below 100 cm, causing substantial uncertainty in global SOC estimates (Batjes, 1996; Jandl et al., 2014; Jobbágy and Jackson, 2000).

Organic C is stored in soil wherever primary production rates and organic matter inputs are greater than decomposition rates. This relationship of production versus decomposition is highly dependent on microbial controls, which in turn are highly dependent on climate. In general, maximum decomposition rates occur in warm, humid environments. The northern hemisphere therefore stores more SOC than does the southern hemisphere (Wieder et al., 2013). The soils in the northern permafrost region are estimated to contain ~1700 Pg C (to 300+ cm in depth), nearly 90% of which occurs in perennially frozen soils and deposits (Tarnocai et al., 2009). This global distribution of SOC could prove particularly problematic as the Arctic region is expected to warm more rapidly than the global mean due to climate change (Collins et al., 2013). The thawing of permafrost (i.e., any subsurface materials that remain below 0 °C for two or more consecutive years) due to warming enables the decomposition of ancient SOC stocks. Substantial reductions in the upper 3.5 m permafrost area in the northern hemisphere have been projected by numerous climate models, with reductions ranging between 37% and 81% by 2100 (Collins et al., 2013) and seasonal thaw depths increasing by 30% to more than 50% by 2050 (Anisimov and Reneva, 2006). While SOC in some soils is protected in aggregate interiors or through adsorption to mineral surfaces (Lehmann and Kleber, 2015), permafrost soils are more vulnerable to rapid change because the protection of SOC from microbial decomposition is primarily dependent on

the frozen conditions of the soil (Davidson and Janssens, 2006). Thawed permafrost is also susceptible to erosion and mass movement by mudslides (Brady and Weil, 2008), although soil processes during and after permafrost thaw (such as the development of soil structure) may lead to short- and long-term protection of unfrozen SOC (Schmidt et al., 2011).

Wetlands and peatlands are estimated to contain ~500 Pg C (Bridgham et al., 2006; Gorham, 1991). While peatlands occupy only 3% of the terrestrial global surface (Maltby and Immerzi, 1993), they contain over 80% of this SOC (Bridgham et al., 2006). Similar to permafrost soils, peatlands and wetlands are more vulnerable than mineral soils to rapid change because the protection of SOC in these systems is primarily dependent on the anaerobic conditions under which the soils formed (Davidson and Janssens, 2006). Expected decreases in soil moisture during the summertime in continental areas (Collins et al., 2013; Wetherald and Manabe, 1999) could potentially dry out surface layers of peat, thus making additional SOC available for aerobic decomposition (Davidson and Janssens, 2006). While reduced CH₄ emissions and additional C sequestration in vegetation may offset increases in CO₂ emissions from peatlands under aerobic conditions, potential increases in peat fire frequency and severity due to higher temperatures and peat drying would rapidly release large amounts of C to the atmosphere (Davidson and Janssens, 2006).

Land use and management change have large impacts on SOC dynamics as well. Soils of forest ecosystems are estimated to contain over half the global SOC stock (Batjes, 1996; Jobbágy and Jackson, 2000; Stockmann et al., 2013), and differences between the two large fluxes of soil C inputs and soil respiratory C losses are small (Nave et al., 2010; Schimel, 1995). Therefore, even minor changes in C inputs or losses from forest soils can potentially affect the global C cycle (Lal, 2005; Nave et al., 2010). For example, conversion of forest to agricultural ecosystems

causes large reductions (~40%) in SOC stocks, while potentially large increases (~50%) in SOC stocks can occur if this conversion of ecosystems is reversed (Guo and Gifford, 2002). Forest management practices such as harvesting, thinning, fertilization, tillage, and burning can also substantially impact SOC and forest C budgets (James and Harrison, 2016; Nave et al., 2010). Globally, harvesting reduced SOC stocks by an average ~11%, with recovery times ranging from several decades to more than 75 years depending on soil type (James and Harrison, 2016).

1.4 VERTICAL DISTRIBUTION AND PERSISTENCE OF SOIL ORGANIC CARBON

Soil depth plays an important role in SOC distribution. Although SOC concentrations decrease rather linearly with increasing depth, total SOC stocks are greater in deeper soil layers than at the surface. In fact, due to increased mass in deeper soil layers, well over 50% of global SOC stocks are stored in deeper soil layers (Batjes, 1996; Jobbágy and Jackson, 2000). Soil organic C radiocarbon ages increase with depth, suggesting that SOC is stored longer in deeper soil layers (Fontaine et al., 2007; Paul et al., 1997; Rumpel et al., 2002; Schmidt et al., 2011). Deep SOC is commonly as old as 1,000 or even more than 10,000 years (Schmidt et al., 2011).

Historically, the theory of “humification” – or the formation of complex and stable SOC compounds (generally referred to as “humus”) – was accepted as the process by which SOC stabilization, and thus long-term preservation, took place. However, this theory is based on alkaline extraction of SOC in the laboratory, which is “incomplete, selective and prone to creating artifacts” (Lehmann and Kleber, 2015). In fact, contemporary, *in situ* spectromicroscopic techniques have revealed the presence of much smaller, more chemically simple organic compounds in the soil environment (Schmidt et al., 2011). According to Lehmann and Kleber (2015), no modern analytic techniques have observed “humic substances” in the soil.

The emerging concept of SOC persistence as *an ecosystem property* – rather than simply the result of chemical “recalcitrance” or “stability” – is well-supported in the literature over the past few decades (e.g., Kaiser and Kalbitz, 2012; Lehmann and Kleber, 2015; Schmidt et al., 2011 and references therein). This concept suggests that the physical, chemical, and biological properties of the soil environment interact to protect organic C compounds at different stages along a continuum of organic matter decomposition. Microbial processing increases the solubility and potential for protection of organic C compounds, and protection provided by the surrounding soil environment reduces the probability (and thus rate) of decomposition, creating the capacity of soil to sequester C (Lehmann and Kleber, 2015; Schmidt et al., 2011). Soil organic C protection primarily occurs through abiotic mechanisms involving physical protection and mineral sorption. Physical protection takes place in the fine pores of soil microaggregates in which anoxic conditions prevail or which are inaccessible to or spatially separated from microbial decomposers (Rasse et al., 2005; Six et al., 2002). The clay fraction of soil is predominately responsible for the sorption capacity of a soil, and the iron and aluminum hydrous oxides (secondary minerals containing iron and aluminum, respectively, hydroxides [OH⁻], and weakly bound H₂O) of some clay separates are especially effective sorbents of SOC (Guggenberger and Kaiser, 2003; Kaiser et al., 2002; Kaiser and Kalbitz, 2012; Kaiser and Zech, 2000; Kleber et al., 2007; Lützow et al., 2006). The sorption of organic compounds to minerals is potentially the primary mechanism of SOC protection, particularly in deeper soil layers (Kaiser and Kalbitz, 2012; Lehmann and Kleber, 2015). The fraction of SOC sorbed to minerals has been shown to increase with depth and comprise the majority of C stored in soils (Diochon and Kellman, 2009).

1.5 SOIL ORGANIC CARBON INPUTS

Mechanisms responsible for the phenomenon of SOC residence times increasing with depth are dependent upon multiple complex soil properties and processes that differ on both the biome- and micrometer-scale. Quantifying relative and total organic matter inputs to soil from various sources can provide key insights into the processes of C sequestration and SOC persistence in deeper soil layers (Stockmann et al., 2013). Organic matter inputs to soil include plant litter, roots (including root death/turnover and a wide variety of rhizodeposition processes such as passive and active secretion of exudates and the sloughing of root tissues), and microbial biomass (Jones et al., 2009; Kalbitz et al., 2000; Nguyen, 2003). Relative contributions to SOC from these three sources, and the complex dynamics in their interactions, are largely debated.

1.5.1 Root-derived Carbon

Root-derived C may be a substantial – or dominant – source of deep SOC in some systems. About 40-50% of all C fixed by plants via photosynthesis is allocated belowground (Jones et al., 2009; Lambers, 1987), making the fate of underground C inputs to the soil environment of major importance. Compared to aboveground contributions to mineral SOC, root-derived C contributions are on the order of 1.5-10 times greater, and may make up as much as 75% of SOC stocks (Balesdent and Balabane, 1996; Bird et al., 2008; Bird and Torn, 2006; Kong and Six, 2010; Neff and Asner, 2001; Rasse et al., 2001; Richter et al., 1999). Several mechanisms are potentially responsible for the transfer of root C to the SOC pool, including root-associated microorganisms and mycorrhizae. For example, ¹⁴C bomb-carbon modeling revealed that 50-70% of the SOC in a chronosequence of boreal forested islands was root- or root-associated-microorganism-derived (Clemmensen et al., 2013). In a poplar plantation, mycorrhizal fungal external mycelium were found to contribute ~60% of new SOC over three growing seasons, a

larger contribution than fine root turnover inputs and over twice litter inputs (Godbold et al., 2006). Ectomycorrhizal fungi also appear to obtain all their C from tree roots, thus acting primarily as a transfer of root C to the surrounding soil environment (Wallander et al., 2004).

Although these fresh inputs of root-derived C are susceptible to rapid mineralization, the creation of micrometer-scale pores and aggregates by mycorrhiza and root-hair activities potentially enhance SOC physical protection from microorganisms (Rasse et al., 2005; Stockmann et al., 2013). Root-derived C may also be preferentially retained in soils due to its chemical composition and the fact that it is input belowground, providing it more opportunities for interactions with the mineral soil and sorption to the clay fraction (Mendez-Millan et al., 2010; Rasse et al., 2005; Rumpel et al., 2004). Aboveground contributions to SOC were found to be just 5% of total litter added after 50 years in mixed deciduous forests in the upper 10 cm of mineral soil, and this SOC was subject to rapid turnover (Lajtha et al., 2014). Incubation and *in situ* root growth experiments have shown that the mean residence time of root-derived C in soils is over twice that of shoot-derived C (Rasse et al., 2005). However, the phenomenon of SOC radiocarbon ages increasing with depth is not well explained if roots are considered the primary source of deep SOC (Kaiser and Kalbitz, 2012; Rasse et al., 2005). Although fine-root mean ages also increase with soil depth, the maximum age range is ~11-18 years (Gaudinski et al., 2001), while SOC in deeper soil layers is often thousands of years old (Fontaine et al., 2007; Schmidt et al., 2011). Furthermore, the storage time of C in plants prior to belowground allocation has been found to be <2 years in temperate forest ecosystems (Gaudinski et al., 2001).

1.5.2 Litter-derived Carbon and Dissolved Organic Carbon

One proposed mechanism to resolve “the paradox of soil organic matter being oldest in the youngest part of the soil profile – the deep mineral subsoil” involves the transport of dissolved

organic C (DOC) from the litter layer to deeper soil layers (Kaiser and Kalbitz, 2012). Studies that modeled DOC dynamics in forest ecosystems estimated that DOC-derived SOC represented ~20% to 89% of total SOC stocks (Kalbitz and Kaiser, 2008; Michalzik et al., 2003; Neff and Asner, 2001). Additionally, nearly one-third of deep soil microbial activity may be supported by DOC fluxes to deeper soil layers (Neff and Asner, 2001). Isotopic and spectroscopic evidence suggest that the DOC pool is comprised primarily of smaller, more soluble, highly reactive compounds released from the bulk SOC following microbial processing (Sanderman et al., 2008). Rain events and new inputs of organic matter are important drivers of DOC flux (Neff and Asner, 2001; Sanderman et al., 2008). Waterflow or the saturation of mineral sorption sites in surface soil layers by organic matter inputs preferentially releases less-strongly sorbed DOC from sorption sites (McCarthy, 2005; Sanderman et al., 2008). Dissolved organic C released into the soil solution is susceptible to further microbial processing and transport to deeper soil layers where minerals often have greater surface area (higher clay content and greater abundance of iron and aluminum hydrous oxides) and mineral surfaces are not yet saturated with C (Deb and Shukla, 2011; McCarthy, 2005; Rasse et al., 2005). Soil organic C protection and storage in deeper soil layers occurs through the preferential sorption to mineral surfaces and incorporation into soil aggregates of smaller, microbial-derived, highly oxidized C compounds that have been leached over time from the litter layer (Deb and Shukla, 2011; Kaiser and Kalbitz, 2012; Lehmann and Kleber, 2015; Stockmann et al., 2013). Dissolved organic C in deep soil has been found to approximate the age of SOC at the same depth (Evans et al., 2007; Schiff et al., 1990), supporting the framework of a continuum of organic matter decomposition and protection and release processes as DOC is transported vertically down the soil profile.

1.5.3 Root-derived Carbon and Dissolved Organic Carbon

Microbial processing of root-derived C and the subsequent release of bio-transformed, microbial-derived C into the DOC pool similarly explains the phenomenon of increasing SOC age with depth, while also better accounting for the large amounts of root C inputs to the soil environment compared to litter C inputs. The vertical distribution of both root biomass and the DOC pool suggest that root-derived C may be the dominant DOC source. Proportions of root biomass decline with soil depth, with greater than 50% of root biomass in the upper 30 cm across all terrestrial biomes (Jackson et al., 1996). Trends in DOC flux follow a similar distribution, with a large majority of DOC emanating from the surface soil (0-20 cm in depth) and decreasing with depth in most ecosystems (Neff and Asner, 2001 and references therein). Mean SOC residence times also follow a similar pattern, increasing by ~2,000-10,000 years below 20 cm in depth (Fontaine et al., 2007 and references therein). Unfortunately, the contributions of root-derived C to the DOC pool have not been included in models or reviews of DOC dynamics due to a lack of available data (Kalbitz et al., 2000; Neff and Asner, 2001).

About one-fourth of the C allocated belowground by plants is released into the soil environment by growing roots, a phenomenon called rhizodeposition (Derrien et al., 2005; Jones et al., 2009; Nguyen, 2003). Due to these inputs of organic matter from roots, the portion of soil in the immediate vicinity of roots (known as the rhizosphere) has higher concentrations of SOC and other nutrients and enhanced microbial activity compared to the bulk soil (Grayston et al., 1997; Rasse et al., 2005). Isotopic and biomarker analyses confirm that root-derived C is the primary source of C for soil microorganisms (Kramer et al., 2010; Liang et al., 2002) and that the composition of root C exudates – the primary form of rhizodeposits (Nguyen, 2003) – rapidly approaches that of the bulk SOC (Derrien et al., 2005). This evidence indicates that a substantial

portion of rhizodeposits undergoes microbial processing and bio-transformation into other microbial-derived C compounds (Derrien et al., 2005), which are preferentially stabilized in soils in comparison to the original C inputs (Stockmann et al., 2013). Therefore, the contribution of root-derived C to both the DOC and SOC pools is potentially greater than aboveground C inputs that are often mineralized in the litter layer (Sanderman et al., 2008; Schmidt et al., 2011).

1.6 DEEP (AND DYNAMIC) SOIL ORGANIC CARBON

Losses of old SOC from deeper soil layers represent potentially important long-term feedbacks to climate change. Global surface temperature change by the end of the 21st century is projected to be in the range of +2-4° C, with greater mean warming over land masses than over the ocean and the most warming in the Arctic region (Collins et al., 2013). Although considerable variation can occur in surface and even deeper soil layers, the whole-soil profile approximates the mean annual air temperature (Brady and Weil, 2008). Soils will thus warm at roughly the same rate as surface temperatures over the next century. Several recent studies underscore the importance of the deep SOC response to climate change. For example, in permafrost soils experiencing thaw, old deeper SOC contributions to ecosystem respiration flux increased with soil temperature and ecosystem respiration flux (Hicks Pries et al., 2016). In a temperate forest ecosystem, subjecting the mineral soil profile to 100 cm in depth to 4° C warming increased CO₂ production significantly at all depths and increased total soil respiration by 34-37% (Hicks Pries et al., 2017). Furthermore, soil 30-100 cm in depth contributed to 20-25% of the total soil respiration (Hicks Pries et al., 2017). Despite SOC in deeper soil layers having long residence times, these studies show that SOC traditionally considered as “stabilized” is vulnerable to decomposition under changing environmental constraints (Harrison et al., 2011; Hicks Pries et al., 2017; Schmidt et al., 2011).

Land use change and management have been observed to impact deep SOC stocks on decadal timescales. When deeper soil layers were sampled following the conversion of agricultural to forest ecosystems, deep SOC losses (>15 cm in depth) were found to offset surface SOC gains for at least 40 years after conversion (Mobley et al., 2015; Richter et al., 1999). Deep SOC stocks (60-100+ cm in depth) were also significantly reduced following forest harvest by an average of ~18% globally (James and Harrison, 2016). These reductions in deep SOC following land use change and harvest potentially resulted from a combination of effects and complex interactions in the soil environment that enhanced microbial activity and SOC decomposition (Diochon and Kellman, 2009). Land use change and management can affect soil temperature, moisture, and DOC flux, among other soil properties and processes (Clarke et al., 2015). For example, mean soil temperature and mean daily soil temperature flux at 100 cm in depth have been observed to increase by ~2-4 °C and ~4-6 °C, respectively, in the first two years following harvest compared to an unharvested reference in a temperate forest ecosystem (Gallo, 2016). Soils compacted by harvest treatments experienced greater temperature increases than soils that were not compacted by the same treatments (Gallo, 2016) due to the higher thermal conductivity of compacted soils (Brady and Weil, 2008).

Dissolved organic C pools and fluxes are important drivers of deep soil biogeochemical processes. Increases in DOC flux from surface soil layers can introduce substantial quantities of fresh C compounds into deeper soil layers, potentially enhancing DOC export (Evans et al., 2007; Schiff et al., 1990) and the decomposition of old deeper SOC. The introduction of fresh C compounds can stimulate, or *prime*, microbial activities, alleviating potential energetic barriers to SOC decomposition that may have existed in deeper soil layers where SOC is often thousands of years old (Fontaine et al., 2007). By the end of this century, extreme precipitation events are

expected to become more intense and more frequent over mid-latitude and wet tropical terrestrial ecosystems (Collins et al., 2013). These extreme precipitation events could transport to deeper soil layers sudden and large amounts of DOC comprised primarily of recently-fixed C, especially after prolonged periods without rain events where fresh C compounds accrued in the surface soil (Neff and Asner, 2001; Sanderman et al., 2008). In particular, DOC transported via preferential pathways of interconnected large soil pores can enter deeper soil layers without substantial abiotic and biotic interactions with the soil environment along the way (Deb and Shukla, 2011). Soil organic C concentrations and microbial biomass in preferential flow paths were found to be ~10-70% and 9-92% higher, respectively, than in the surrounding bulk soil (Bundt et al., 2001). Therefore, mineralization rates in these SOC “hot spots” are most likely enhanced (Hagedorn and Bundt, 2002), which could in turn enhance decomposition of the surrounding bulk SOC. Observed reductions in deep SOC due to land use change or management (e.g., Guo and Gifford, 2002; James and Harrison, 2016) may be due in part to increases in DOC flux and the introduction of fresh C compounds to deeper soil layers (Diochon and Kellman, 2009). Groundwater level rise and increased DOC export is also a possible mechanism for the loss of deep SOC following land use change or management such as forest harvest (Evans et al., 2007; Laudon et al., 2009; Schiff et al., 1990).

Rooting depth and morphology play an important part in deep soil biogeochemical processes. Environmental conditions or land use and management practices that modify plant roots – thereby modifying the rhizosphere and biotic and abiotic interactions – affect soil C cycling. While root-derived C is an important SOC source, it may also be a key driver of priming mechanisms in the soil environment. Labile root C inputs can enhance microbial activity and SOC decomposition, a phenomenon referred to as the rhizosphere priming effect (Pausch et al.,

2013; Zhu et al., 2014). Under changing environmental conditions in which deep soil microbial communities are suddenly exposed to additional root C inputs, increased bulk SOC decomposition can occur. For example, elevated atmospheric CO₂ concentrations and increased above- and below-ground plant growth have resulted in losses of old SOC that exceeded the formation of new SOC, potentially due to rooting depth increases and the introduction of root C inputs to deeper soil layers (e.g., Dijkstra and Cheng, 2007; Kuzyakov, 2010 and references therein).

Rhizosphere priming effects may be the primary mechanism of deep SOC loss following reforestation of formerly cultivated land (Mobley et al., 2015; Richter et al., 1999). When relatively shallow-rooted agricultural ecosystems are converted to deep-rooted forests, the introduction of root C inputs to deeper soil layers potentially enhances SOC decomposition in the surrounding bulk soil that was formerly stabilized under the agricultural ecosystem (Dijkstra and Cheng, 2007; Mobley et al., 2015). Projected increases in hot temperature extremes over most land areas during the next century, especially on seasonal timescales (Collins et al., 2013), could similarly induce priming of deep SOC stocks by modifying fine root distribution in the soil. In a study that modeled root allocation in temperate forest ecosystems, increased soil temperatures, reductions in soil moisture, and subsequent increases in soil strength led to fine root colonization of deeper soil layers (Rasse et al., 2001). Forest management practices such as fertilization have also been observed to increase soil rooting depth (Kuzyakov et al., 2000 and references therein). In addition to possible rhizosphere priming effects, the introduction of roots to deeper soil layers increases soil moisture variability and aeration through transpiration, exposing soil microorganisms to potentially more favorable metabolic conditions (Mobley et al., 2015). On the other hand, some studies have shown neutral or negative (i.e., reduced SOC decomposition)

priming effects with fresh C inputs depending on soil properties and nutrition, plant nutrient contents, and relative amounts of fresh C inputs (Blagodatskaya et al., 2007; Carrillo et al., 2017; Graaff et al., 2010; Koranda et al., 2011).

1.7 COARSE SOIL FRACTION CONTRIBUTION TO SOIL ORGANIC CARBON

The coarse soil (>2 mm) fraction has been found to contain as much as 75% of the total SOC stock in rocky forest soils (Harrison et al., 2003). Zabowski et al. (2011) concluded that soils with a coarse fraction greater than 20% (by weight) were likely to have substantial (>10% of total SOC) C content in the coarse fraction. Within the 48 conterminous United States alone, skeletal ($\geq 35\%$ by volume rock content) soils comprise ~33% of the area (Throop et al., 2012). Therefore, the common practice of discarding the coarse fraction of soils could result in substantial underestimates in regional and global SOC stocks if the volume of soil occupied by coarse fragments is considered void (i.e., coarse fragment mass but not volume is subtracted from SOC estimates, as suggested by Throop et al. (2012)) (Corti et al., 2002; Harrison et al., 2011; Zabowski et al., 2011). Although the coarse fraction of SOC has been historically ignored and considered chemically inert (Corti et al., 2002), Koele and Hildebrand (2008) concluded that nutrient exchange mechanisms for both the free and stone-protected fine soil (<2 mm) fraction were essentially the same, and that the coarse fraction contribution to short-term nutritional dynamics was significant. Therefore, it cannot be assumed that coarse fraction SOC is inactive on decadal timescales.

1.8 CONCLUSIONS AND FUTURE DIRECTIONS

Any changes in microbial controls, root biomass and surface area, organic matter, and soil properties (e.g., temperature, moisture, and bulk density) can affect the rates of SOC accumulation and turnover. The magnitude of SOC loss due to warming and priming effects

increases with the size of the initial SOC stock (Crowther et al., 2016; Kuzyakov et al., 2000). Therefore, soils with larger standing SOC stocks are especially at risk to release considerable C to the atmosphere. Although some parts of the world will experience increased primary production as a result of warming and thus increased input of organic C to the soil, net global losses of SOC content are projected due to hastened microbial metabolic processes (Jones et al., 2005). Potential priming effects with greater C input via rhizodeposition could further decrease SOC stocks in the short-term (Dijkstra and Cheng, 2007; Kuzyakov, 2010). However, rhizosphere priming effects are unaccounted for in global models (Dijkstra and Cheng, 2007). In fact, standard models used to predict SOC dynamics do not incorporate direct microbial controls on the vertical and spatial distribution of SOC stocks (Wieder et al., 2013). By modelling microbial processes, Wieder et al. (2013) were able to explain 50% of the variation in global SOC observations in the upper 100 cm of soil compared to traditional models that explained only 28-30% of the variation. These results underscore the importance of including microbial controls on SOC dynamics in global models to help improve the accuracy of projected carbon-climate feedbacks.

Considering that both warming and priming effects are contingent on the size of the original SOC stock, it is imperative that we accurately quantify regional and global SOC stocks. Unfortunately, available data on SOC in the literature is often confined to surface soil layers above 15-30 cm, which contain less than half of the global SOC stock (Batjes, 1996; Diochon and Kellman, 2009; Harrison et al., 2011; James and Harrison, 2016; Jandl et al., 2014; Jobbágy and Jackson, 2000; Lal, 2005; Mobley et al., 2015). Sampling the whole-soil profile – including deeper soil layers to at least 100 cm in depth – is fundamental to understanding soil nutrient dynamics, soil and ecosystem C budget changes over time, and climate change feedbacks

(Hamburg, 2000; Harrison et al., 2011; James et al., 2014; Jandl et al., 2014). The deep SOC stock is not intrinsically “stable” and is vulnerable to (potentially rapid) decomposition when the environmental conditions under which it accumulated change due to global warming, management, or other shifting ecosystem properties (Harrison et al., 2011; Hicks Pries et al., 2017, 2016; James and Harrison, 2016; Schmidt et al., 2011). Biological activity (and associated soil C cycling processes) often extends to at least 100 cm in depth, with some tree roots extending deeper than 60 m (Stone and Kalisz, 1991). Moreover, determining the contribution of the coarse soil (>2 mm) fraction to SOC in rocky soils would substantially improve the accuracy of regional and global SOC stock estimates (Corti et al., 2002; Harrison et al., 2011; Zabowski et al., 2011).

Sampling deeper soil layers and including the coarse fraction in SOC stock estimates would enhance our understanding of soil processes and help reduce uncertainty in climate change projections (Jandl et al., 2014). Due to increasing SOC residence times with depth, losses of deeper SOC result in additions of relatively long-sequestered C to the atmosphere and to a comparably short-term C cycle (Paul et al., 1997; Rumpel et al., 2002; Schmidt et al., 2011). Further study of biogeochemical processes in the deep soil environment will provide valuable information about SOC accumulation and mineralization mechanisms, which in turn may help prioritize C sequestration initiatives and provide key insights into carbon-climate feedbacks. The shifting paradigm of SOC persistence has important implications for future research and ultimately our understanding of the effects of land use change, management, and future climate change on SOC fluxes.

REFERENCES

- Anisimov, O., Reneva, S., 2006. Permafrost and changing climate: The Russian perspective. *Ambio* 35, 169–175. doi:10.1579/0044-7447(2006)35[169:PACCTR]2.0.CO;2
- Balesdent, J., Balabane, M., 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. *Soil Biol. Biochem.* 28, 1261–1263. doi:10.1124/dmd.105.003822.30
- Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47, 151–163.
- Bird, J.A., Kleber, M., Torn, M.S., 2008. 13C and 15N stabilization dynamics in soil organic matter fractions during needle and fine root decomposition. *Org. Geochem.* 39, 465–477. doi:10.1016/j.orggeochem.2007.12.003
- Bird, J.A., Torn, M.S., 2006. Fine roots vs. needles: A comparison of 13C and 15N dynamics in a ponderosa pine forest soil. *Biogeochemistry* 79, 361–382. doi:10.1007/s10533-005-5632-y
- Blagodatskaya, E. V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Appl. Soil Ecol.* 37, 95–105. doi:10.1016/j.apsoil.2007.05.002
- Brady, N.C., Weil, R.R., 2008. *The nature and properties of soils*, 14th ed. Pearson, Upper Saddle River, NJ.
- Bridgman, S.D., Magonigal, J.P., Keller, J.K., Bliss, N.B., Trettin, C., 2006. The Carbon Balance of North American Wetlands. *Wetlands* 26, 889–916. doi:10.1672/0277-5212(2006)26[889:TCBONA]2.0.CO;2
- Bundt, M., Widmer, F., Pesaro, M., Zeyer, J., Blaser, P., 2001. Preferential flow paths: Biological “hot spots” in soils. *Soil Biol. Biochem.* 33, 729–738. doi:10.1016/S0038-0717(00)00218-2
- Carrillo, Y., Bell, C., Koyama, A., Canarini, A., Boot, C.M., Wallenstein, M., Pendall, E., 2017. Plant traits, stoichiometry and microbes as drivers of decomposition in the rhizosphere in a temperate grassland. *J. Ecol.* doi:10.1111/1365-2745.12772
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Quéré, C. Le, Myneni, R.B., Piao, S., Thornton, P., 2013. Carbon and Other Biogeochemical Cycles, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 465–570. doi:10.1017/CBO9781107415324.015
- Clarke, N., Gundersen, P., Jönsson-Belyazid, U., Kjønås, O.J., Persson, T., Sigurdsson, B.D., Stupak, I., Vesterdal, L., 2015. Influence of different tree-harvesting intensities on forest soil carbon stocks in boreal and northern temperate forest ecosystems. *For. Ecol. Manage.* 351, 9–19. doi:10.1016/j.foreco.2015.04.034

- Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. *Science* (80-.). 339, 1615–1618. doi:10.1126/science.1229223
- Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A.J., Wehner, M., 2013. Long-term Climate Change: Projections, Commitments and Irreversibility, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1029–1136. doi:10.1017/CBO9781107415324.024
- Corti, G., Ugolini, F.C., Agnelli, A., Certini, G., Cuniglio, R., Berna, F., Fernández Sanjurjo, M.J., 2002. The soil skeleton, a forgotten pool of carbon and nitrogen in soil. *Eur. J. Soil Sci.* 53, 283–298. doi:10.1046/j.1365-2389.2002.00442.x
- Crowther, T., Todd-Brown, K., Rowe, C., Wieder, W., Carey, J., Machmuller, M., Snoek, L., Fang, S., Zhou, G., Allison, S., Blair, J., Bridgham, S., Burton, A., Carrillo, Y., Reich, P., Clark, J., Classen, A., Dijkstra, F., Elberling, B., Emmett, B., Estiarte, M., Frey, S., Guo, J., Harte, J., Jiang, L., Johnson, B., Kröel-Dulay, G., Larsen, K., Laudon, H., Lavalley, J., Luo, Y., Lupascu, M., Ma, L., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Penuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L., Schmidh, I., Sistla, S., Sokol, N., Templer, P., Treseder, K., Welker, J., Bradford, M., 2016. Quantifying global soil C losses in response to warming. *Nature* 104, 104–108. doi:10.1038/nature20150
- Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165–173. doi:10.1038/nature04514
- Deb, S.K., Shukla, M.K., 2011. A Review of Dissolved Organic Matter Transport Processes Affecting Soil and Environmental Quality. *J. Environ. Anal. Toxicol.* 1, 1–11. doi:10.4172/2161-0525.1000106
- Derrien, D., Marol, C., Balesdent, J., 2005. The dynamics of neutral sugars in the rhizosphere of wheat. An approach by ¹³C pulse-labelling and GC/C/IRMS. *Plant Soil* 267, 243–253. doi:10.1007/s11104-005-5348-8
- Dijkstra, F.A., Cheng, W., 2007. Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecol. Lett.* 10, 1046–1053. doi:10.1111/j.1461-0248.2007.01095.x
- Diochon, A.C., Kellman, L., 2009. Physical fractionation of soil organic matter: Destabilization of deep soil carbon following harvesting of a temperate coniferous forest. *J. Geophys. Res. Biogeosciences* 114, 1–9. doi:10.1029/2008JG000844
- Eswaran, H., Berg, E. Van Den, Reich, P., 1993. Organic carbon in soils of the world. *Soil Sci. Soc. Am. J* 57, 192–194.
- Evans, C.D., Freeman, C., Cork, L.G., Thomas, D.N., Reynolds, B., Billett, M.F., Garnett, M.H., Norris, D., 2007. Evidence against recent climate-induced destabilisation of soil carbon from ¹⁴C analysis of riverine dissolved organic matter. *Geophys. Res. Lett.* 34, 1–5.

doi:10.1029/2007GL029431

- Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450, 277–80. doi:10.1038/nature06275
- Gallo, A.C., 2016. Response of Soil Temperature, Moisture, and Respiration Two Years Following Intensive Organic Matter and Compaction Manipulations in Oregon Cascade Forests. Oregon State University.
- Gaudinski, J.B., Trumbore, S.E., Davidson, E.A., Cook, A.C., Markewitz, D., Richter, D.D., 2001. The Age of Fine-Root Carbon in Three Forests of the Eastern United States Measured by Radiocarbon. *Oecologia* 129, 420–429. doi:10.2307/4223101
- Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., De Angelis, P., Miglietta, F., Peressotti, A., 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant Soil* 281, 15–24. doi:10.1007/s11104-005-3701-6
- Gorham, E., 1991. Northern peatlands: Role in the carbon cycle and probable responses to climatic warming. *Ecol. Appl.* 1, 182–195.
- Graaff, M.-A. De, Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile Soil Carbon Inputs Mediate the Soil Microbial Community Composition and Plant Residue Decomposition Rates. *New Phytol.* 188, 1055–1064. doi:10.1111/j.1469-8137.2010.03427.x
- Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. *Appl. Soil Ecol.* 5, 29–56. doi:10.1016/s0929-1393(96)00126-6
- Guggenberger, G., Kaiser, K., 2003. Dissolved organic matter in soil: Challenging the paradigm of sorptive preservation. *Geoderma* 113, 293–310. doi:10.1016/S0016-7061(02)00366-X
- Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. *Glob. Chang. Biol.* 8, 345–360. doi:DOI 10.1046/j.1354-1013.2002.00486.x
- Hagedorn, F., Bundt, M., 2002. The age of preferential flow paths. *Geoderma* 108, 119–132. doi:Pii s0016-7061(02)00129-5\r10.1016/s0016-7061(02)00129-5
- Hamburg, S.P., 2000. Simple Rules for Measuring Changes in Ecosystem. *Mitigation Adapt. Strateg. Glob. Chang.* 5, 25–37.
- Harrison, R.B., Adams, A.B., Licata, C., Flaming, B., Wagoner, G.L., Carpenter, P., Vance, E.D., 2003. Quantifying deep-soil and coarse-soil fractions: Avoiding sampling bias. *Soil Sci. Soc. Am. J.* 67, 1602–1606.
- Harrison, R.B., Footen, P.W., Strahm, B.D., 2011. Deep soil horizons: Contribution and importance to soil carbon pools and in assessing whole-ecosystem response to management and global change. *For. Sci.* 57, 67–76.
- Hicks Pries, C.E., Castanha, C., Porras, R., Torn, M.S., 2017. The whole-soil carbon flux in response to warming. *Science* (80-.). 1–8. doi:10.1126/science.aal1319
- Hicks Pries, C.E., Schuur, E.A.G., Natali, S.M., Crummer, K.G., 2016. Old soil carbon losses increase with ecosystem respiration in experimentally thawed tundra. *Nat. Clim. Chang.* 6,

214–218. doi:10.1038/nclimate2830

- Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H. a., Sala, O.E., Schulze, E.D., 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108, 389–411. doi:10.1007/BF00333714
- James, J., Devine, W., Harrison, R., Terry, T., 2014. Deep Soil Carbon: Quantification and Modeling in Subsurface Layers. *Soil Sci. Soc. Am. J.* 78, S1–S10. doi:10.2136/sssaj2013.06.0245nafsc
- James, J., Harrison, R., 2016. The Effect of Harvest on Forest Soil Carbon: A Meta-Analysis. *Forests* 7, 308. doi:10.3390/f7120308
- Jandl, R., Rodeghiero, M., Martinez, C., Cotrufo, M.F., Bampa, F., van Wesemael, B., Harrison, R.B., Guerrini, I.A., Richter, D. deB, Rustad, L., Lorenz, K., Chabbi, A., Miglietta, F., 2014. Current status, uncertainty and future needs in soil organic carbon monitoring. *Sci. Total Environ.* 468–469, 376–383. doi:10.1016/j.scitotenv.2013.08.026
- Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* 10, 423–436. doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2
- Jones, C., McConnell, C., Coleman, K., Cox, P., Falloon, P., Jenkinson, D., Powlson, D., 2005. Global climate change and soil carbon stocks; predictions from two contrasting models for the turnover of organic carbon in soil. *Glob. Chang. Biol.* 11, 154–166. doi:10.1111/j.1365-2486.2004.00885.x
- Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere: Carbon trading at the soil-root interface. *Plant Soil* 321, 5–33. doi:10.1007/s11104-009-9925-0
- Kaiser, K., Eusterhues, K., Rumpel, C., Guggenberger, G., Kogel-Knabner, I., 2002. Stabilization of organic matter by soil minerals--investigation of density and particle-size fractions from two acid forest soils. *J. Plant Nutr. Soil Sci.* 165, 451–459.
- Kaiser, K., Kalbitz, K., 2012. Cycling downwards - dissolved organic matter in soils. *Soil Biol. Biochem.* 52, 29–32. doi:10.1016/j.soilbio.2012.04.002
- Kaiser, K., Zech, W., 2000. Dissolved organic matter sorption by mineral constituents of subsoil clay fractions. *J. Plant Nutr. Soil Sci.* 163, 531–535.
- Kalbitz, K., Kaiser, K., 2008. Contribution of dissolved organic matter to carbon storage in forest mineral soils. *J. Plant Nutr. Soil Sci.* 171, 52–60. doi:10.1002/jpln.200700043
- Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Sci.* 165, 277–304.
- Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: Self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry* 85, 9–24. doi:10.1007/s10533-007-9103-5
- Köchy, M., Hiederer, R., Freibauer, a., 2015. Global distribution of soil organic carbon – Part 1: Masses and frequency distributions of SOC stocks for the tropics, permafrost regions, wetlands, and the world. *Soil* 1, 351–365. doi:10.5194/soil-1-351-2015
- Koele, N., Hildebrand, E.E., 2008. The ecological significance of the coarse soil fraction for

- Picea abies* (L.) Karst. seedling nutrition. *Plant Soil* 312, 163–174. doi:10.1007/s11104-008-9654-9
- Kong, A.Y.Y., Six, J., 2010. Tracing Root vs. Residue Carbon into Soils from Conventional and Alternative Cropping Systems. *Soil Sci. Soc. Am. J* 74, 1201–1210. doi:10.2136/sssaj2009.0346
- Koranda, M., Schneckner, J., Kaiser, C., Fuchslueger, L., Kitzler, B., Stange, C.F., Sessitsch, A., Zechmeister-Boltenstern, S., Richter, A., 2011. Microbial processes and community composition in the rhizosphere of European beech - The influence of plant C exudates. *Soil Biol. Biochem.* 43, 551–558. doi:10.1016/j.soilbio.2010.11.022
- Kramer, C., Trumbore, S., Fröberg, M., Cisneros Dozal, L.M., Zhang, D., Xu, X., Santos, G.M., Hanson, P.J., 2010. Recent (<4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. *Soil Biol. Biochem.* 42, 1028–1037. doi:10.1016/j.soilbio.2010.02.021
- Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. *Soil Biol. Biochem.* 42, 1363–1371. doi:10.1016/j.soilbio.2010.04.003
- Kuzyakov, Y., Friedelb, J.K., Stahra, K., 2000. Review of mechanisms and quantification of priming effects (Review). *Soil Biol. Biochem.* 32, 1485–1498. doi:10.1016/S0038-0717(00)00084-5
- Lajtha, K., Townsend, K.L., Kramer, M.G., Swanston, C., Bowden, R.D., Nadelhoffer, K., 2014. Changes to particulate versus mineral-associated soil carbon after 50 years of litter manipulation in forest and prairie experimental ecosystems. *Biogeochemistry* 119, 341–360. doi:10.1007/s10533-014-9970-5
- Lal, R., 2005. Forest soils and carbon sequestration. *For. Ecol. Manage.* 220, 242–258. doi:10.1016/j.foreco.2005.08.015
- Lambers, H., 1987. Growth, respiration, exudation and symbiotic associations: the fate of carbon translocated to the root, in: Gregory, P.J., Lake, J.V., Rose, D.A. (Eds.), *Root Development and Function*. Soc. Exp. Biol. Seminar Ser. 30. Cambridge University Press, Cambridge, pp. 124–145.
- Laudon, H., Hedtjärn, J., Schelker, J., Bishop, K., Sørensen, R., Agren, A., 2009. Response of dissolved organic carbon following forest harvesting in a boreal forest. *Ambio* 38, 381–386. doi:10.1579/0044-7447-38.7.381
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 528, 60–8. doi:10.1038/nature16069
- Liang, B.C., Wang, X.L., Ma, B.L., 2002. Maize Root-Induced Change in Soil Organic Carbon Pools. *Soil Sci. Soc. Am. J* 66, 845–847.
- Lützow, M. V., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions - A review. *Eur. J. Soil Sci.* 57, 426–445. doi:10.1111/j.1365-2389.2006.00809.x
- Maltby, E., Immirzi, P., 1993. Carbon dynamics in peatlands and other wetland soils regional and global perspectives. *Chemosphere* 27, 999–1023. doi:10.1016/0045-6535(93)90065-D

- McCarthy, J.F., 2005. Carbon fluxes in soil: long-term sequestration in deeper soil horizons. *J. Geogr. Sci.* 15, 149–154. doi:10.1360/gso50204
- Mendez-Millan, M., Dignac, M.F., Rumpel, C., Rasse, D.P., Derenne, S., 2010. Molecular dynamics of shoot vs. root biomarkers in an agricultural soil estimated by natural abundance ¹³C labelling. *Soil Biol. Biochem.* 42, 169–177. doi:10.1016/j.soilbio.2009.10.010
- Michalzik, B., Tipping, E., Mulder, J., Gallardo-Lancho, J.F., Matzner, E., Bryant, C.L., Clarke, N., Lofts, S., Vicente-Esteban, M. a. ., 2003. Modelling the production and transport of Dissolved Organic Carbon in forest soils. *Biogeochemistry* 66, 241–264. doi:10.1023/B:BIOG.0000005329.68861.27
- Mobley, M.L., Lajtha, K., Kramer, M.G., Bacon, A.R., Heine, P.R., Richter, D.D., 2015. Surficial gains and subsoil losses of soil carbon and nitrogen during secondary forest development. *Glob. Chang. Biol.* 21, 986–996. doi:10.1111/gcb.12715
- Nave, L.E., Vance, E.D., Swanston, C.W., Curtis, P.S., 2010. Harvest impacts on soil carbon storage in temperate forests. *For. Ecol. Manage.* 259, 857–866. doi:10.1016/j.foreco.2009.12.009
- Neff, J.C., Asner, G.P., 2001. Dissolved organic carbon in terrestrial ecosystems: Synthesis and a model. *Ecosystems* 4, 29–48. doi:10.1007/s100210000058
- Nguyen, C., 2003. Agronomy for sustainable development. *Agronomie* 23, 375–396. doi:10.1051/agro
- Paul, E.A., Follett, R.F., Leavitt, S.W., Halvorson, A., Peterson, G.A., Lyon, D.J., 1997. Radiocarbon Dating for Determination of Soil Organic Matter Pool Sizes and Dynamics. *Soil Sci. Soc. Am. J.* 61, 1058–1067. doi:10.2136/sssaj1997.03615995006100040011x
- Pausch, J., Zhu, B., Kuzyakov, Y., Cheng, W., 2013. Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biol. Biochem.* 57, 91–99. doi:10.1016/j.soilbio.2012.08.029
- Post, W.M., Emanuel, W.R., Zinke, P.J., Stangenberger, A.G., 1982. Soil carbon pools and world life zones. *Nature* 298, 156–159. doi:10.1038/298156a0
- Rasse, D.P., Longdoz, B., Ceulemans, R., 2001. TRAP: a modelling approach to below-ground carbon allocation in temperate forests. *Plant Soil* 229, 281–293. doi:10.1023/A:1004832119820
- Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* 269, 341–356. doi:10.1007/s11104-004-0907-y
- Richter, D.D., Markewitz, D., Trumbore, S.E., Wells, C.G., 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. *Nature* 400, 56–58. doi:10.1038/21867
- Rumpel, C., Eusterhues, K., Kögel-Knabner, I., 2004. Location and chemical composition of stabilized organic carbon in topsoil and subsoil horizons of two acid forest soils. *Soil Biol. Biochem.* 36, 177–190. doi:10.1016/j.soilbio.2003.09.005
- Rumpel, C., Ko, I., Bruhn, F., Kögel-Knabner, I., Bruhn, F., 2002. Vertical distribution, age, and chemical composition of organic carbon in two forest soils of different pedogenesis. *Org. Geochem.* 33, 1131–1142. doi:10.1016/s0146-6380(02)00088-8

- Sanderman, J., Baldock, J.A., Amundson, R., Baldock, J.A., 2008. Dissolved Organic Carbon Chemistry and Dynamics in Contrasting Forest and Grassland Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Source Biogeochem. Biogeochem.* 89, 181–198.
- Scharlemann, J.P., Tanner, E.V., Hiederer, R., Kapos, V., 2014. Global soil carbon: understanding and managing the largest terrestrial carbon pool. *Carbon Manag.* 5, 81–91. doi:10.4155/CMT.13.77
- Schiff, S.L., Aravena, R., Trumbore, S.E., Dillon, P.J., 1990. Dissolved Organic Carbon Cycling in Forested Wetlands: A Carbon Isotope Approach. *Water Resour. Res.* 26, 2949–2957. doi:10.1029/WR026i012p02949
- Schimel, D., 1995. Terrestrial ecosystems and the carbon cycle. *Glob. Chang. Biol.* 1, 77–91. doi:10.1111/j.1365-2486.1995.tb00008.x
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. a., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. a. C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56. doi:10.1038/nature10386
- Six, J., Conant, R.T., Paul, E. a, Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturatin of soils. *Plant Soil* 241, 155–176. doi:10.1023/A:1016125726789
- Sombroek, W.G., Nachtergaele, F.O., Hebel, A., 1993. Amounts, Dynamics and Sequestering of Carbon in Tropical and Subtropical Soils. *Ambio* 22, 417–426.
- Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., Courcelles, V. de R. de, Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric. Ecosyst. Environ.* 164, 80–99. doi:10.1016/j.agee.2012.10.001
- Stone, E.L., Kalisz, P.J., 1991. On the maximum extent of tree roots. *For. Ecol. Manage.* 46, 59–102.
- Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem. Cycles* 23, 1–11. doi:10.1029/2008GB003327
- Throop, H.L., Archer, S.R., Monger, H.C., Waltman, S., 2012. When bulk density methods matter: Implications for estimating soil organic carbon pools in rocky soils. *J. Arid Environ.* 77, 66–71. doi:10.1016/j.jaridenv.2011.08.020
- Wallander, H., Göransson, H., Rosengren, U., 2004. Production, standing biomass and natural abundance of ¹⁵N and ¹³C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia* 139, 89–97. doi:10.1007/s00442-003-1477-z
- Wetherald, R.T., Manabe, S., 1999. Detectability of summer dryness caused by greenhouse warming. *Clim. Change* 43, 495–511. doi:10.1023/A:1005499220385
- Wieder, W.R., Bonan, G.B., Allison, S.D., 2013. Global soil carbon projections are improved by

- modelling microbial processes. *Nat. Clim. Chang.* 3, 909–912. doi:10.1038/nclimate1951
- Zabowski, D., Whitney, N., Gurung, J., Hatten, J., 2011. Total soil carbon in the coarse fraction and at depth. *For. Sci.* 57, 11–18. doi:10.2136/sssaj2004.0612
- Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. *Soil Biol. Biochem.* 76, 183–192. doi:10.1016/j.soilbio.2014.04.033

CHAPTER 2. QUANTIFYING AND COMPARING SOIL CARBON STOCKS: UNDERESTIMATION WITH COMMON SAMPLING METHODS

2.1 ABSTRACT

Soils are an important terrestrial carbon (C) pool, containing more C than the Earth's atmosphere and vegetation combined. Changing climate, land use, and management can impact both surface and deep soil organic carbon (SOC) stocks on decadal timescales, highlighting the importance of accurate measurements and comparisons over time. This study compares three soil sampling methods for estimating SOC stocks: clod, core, and excavation. As it is considered the least biased method, the excavation method was used as the standard by which other methods were compared. Sampling took place at an intensively managed Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation in northwestern Oregon, USA. Soil was generally non-rocky (<2% fine to medium gravel content by weight) with a sandy clay texture. Soil samples were collected in the middle of soil depth layers 0-10, 10-20, 20-50, 50-100, and 100-150 cm. Mean soil bulk density (BD) and SOC concentrations for the excavation method ranged from 0.72 to 1.15 g cm⁻³ and 4.74 to 101.76 g OC/kg soil, respectively. The core method significantly (Tukey's HSD, $\alpha = 0.1$) underestimated BD at all depths below 10 cm. Soil organic carbon concentrations tended to be lowest for the clod method and highest for the excavation method. As all SOC stock calculations rely on the relationship between SOC mass and soil mass, the underestimation of these parameters by clod and core methods, respectively, similarly affects the fixed depth, genetic horizon, and mass based approaches to quantify SOC stocks. The clod and core methods underestimated the SOC stock (Mg ha⁻¹) to a depth of 150 cm by 22% and 36%, respectively. Most of this difference (66% and 70%, respectively) occurred below 20 cm in depth, where the majority of SOC stocks were contained across all soil sampling methods. This study shows that 1) commonly used soil sampling methods for measuring soil properties such as

SOC should not be assumed to be interchangeable; and 2) regional and global SOC stocks may be largely underestimated due to shallow sampling and the frequent use of core methods.

2.2 INTRODUCTION

Soils are an important terrestrial carbon (C) pool, containing about two to three times the amount of C stored in the Earth's atmosphere and vegetation combined (Ciais et al., 2013; Lehmann and Kleber, 2015). Soil organic carbon (SOC) plays a critical role in soil health and quality, i.e., the ability of soil to provide fundamental ecosystem services such as nutrient cycling, water filtration and purification, atmospheric regulation through C sequestration, and a medium for plant growth (Brady and Weil, 2008; Milne et al., 2015). Changing climate, land use, and management can impact both surface and deep SOC stocks on decadal timescales, highlighting the importance of accurate measurements. Quantifying SOC stocks and comparing changes over time would provide key insights into carbon-climate feedbacks, help constrain climate models, and inform decisions regarding land use and best management practices (Jandl et al., 2014; Scharlemann et al., 2014). In addition, the initial size of SOC stocks is an important parameter in forecasting the magnitude of warming and priming effects, as well as predicting responses to management (Crowther et al., 2016; Kuzyakov et al., 2000; Milne et al., 2015). To this end, the ability to sample soil consistently, accurately, and precisely is necessary.

2.2.1 Soil Sampling Bias

The difficulty of quantifying SOC stocks and avoiding soil sampling error and bias may be compounded by varying sampling conditions, the use of different sampling methods, or different researchers collecting the samples. Additional uncertainties in SOC stock comparisons over time occur when changes in land use, management, or ecosystem properties affect the fundamental components used to calculate SOC stocks, thus potentially resulting in over- or under-estimates

of actual SOC changes. In particular, biases in bulk density (BD) measurements or BD changes over time can have greater impacts on SOC stock estimates than any actual changes in SOC concentrations (Hiederer and Köchy, 2011; Köchy et al., 2015; Schrumpf et al., 2011). The BD of soil is frequently used to convert data from a weight to a volume and area basis. It is defined as the ratio of the mass of oven-dry solids to the total or bulk (wet) volume of undisturbed soil, where the volume includes both solids and pores (Soil Survey Staff, 2014). Inconsistencies and potential inaccuracies in BD data represent substantial uncertainties in global SOC stock estimates, as even small changes in BD can lead to large changes in SOC stock calculations and prohibit accurate comparisons over time (Hiederer and Köchy, 2011; Köchy et al., 2015; Wendt and Hauser, 2013). Furthermore, global databases are often missing BD values, or have BD measurements obtained from multiple soil sampling methods (Batjes, 1996; Bridgham et al., 2006; Jobbágy and Jackson, 2000; Tarnocai et al., 2009). While the application of pedotransfer functions for estimating BD has been used in numerous regional and global SOC stock inventories (e.g., Batjes, 1996), recent studies have strongly recommended against the use of pedotransfer functions due to systematic biases, especially concerning forest soils (Schrumpf et al., 2011; Wiesmeier et al., 2012).

2.2.2 Soil Sampling Methods

Differences in soil sampling methods may lead to inaccuracies in the quantification or comparison of SOC stocks over time. Multiple soil sampling tools and methods exist with varying levels of accuracy and ease of use (Jandl et al., 2014). Common methods include clod, core, and excavation. Each of these methods have several variations and can be used to obtain BD, soil mass, and C concentrations. Global databases include soils data obtained primarily

using the clod and core methods (Batjes, 1996; Tarnocai et al., 2009; Throop et al., 2012), making their accuracy and compatibility essential for estimating SOC stocks.

Clod methods obtain intact soil clods carefully separated from the soil *in situ* (Soil Survey Staff, 2014; Tisdall, 1951). Coarse-textured soils are not suitable for these methods, as they do not form clods or do not form clods sturdy enough to remain intact during transport and analysis (Harrison et al., 2003; Howard and Singer, 1981; Soil Survey Staff, 2014). Transport of clods collected in the field is cumbersome compared to core and excavation methods, and laboratory analysis is labor-intensive and destructive (Rossi et al., 2008; Uteau et al., 2013; Van Remortel and Shields, 1993). Clod methods potentially exclude rock content and larger natural pores in between soil aggregates and in between rock fragments and soil aggregates (Harrison et al., 2003; Howard and Singer, 1981; Van Remortel and Shields, 1993). Furthermore, the nature of clod methods biases sampling of soil toward firmer, more compact soil that forms sturdy clods (Van Remortel and Shields, 1993).

Core methods are by far the most commonly used soil sampling methods, as they are generally less labor-intensive compared to other methods (Jandl et al., 2014; Kulmatiski et al., 2003; Throop et al., 2012). Cores vary in length and diameter, are used vertically and horizontally, and can be inserted into the soil by multiple means, including pushing, hammering, or augering. Although core methods are frequently used as the standard soil sampling method (Vogel, 1994; Xu et al., 2015), they have been found to consistently underestimate rock content and BD (Andraski, 1991; Beem-Miller et al., 2016; Harrison et al., 2003; Kulmatiski et al., 2003; Muller and Hamilton, 1992; Tisdall, 1951; Van Remortel and Shields, 1993; Xu et al., 2015). Comparably few recent studies have shown increases in BD due to compaction of soil inside the core (Page-Dumroese et al., 1999). Differences in SOC and other nutrient concentrations

measured by core methods relative to excavation methods have been reported in both negative (Kulmatiski et al., 2003) and positive (Xu et al., 2015) directions. Core methods are incompatible with non-cohesive soils in which the soil does not remain inside the core (Vogel, 1994). Despite limitations and inherent biases, the benefits of reduced time, labor, and site disturbance have resulted in the wide use of core methods (Throop et al., 2012).

Excavation methods are the least used soil sampling methods, as they tend to be the most cumbersome and labor-intensive (Harrison et al., 2003; Jandl et al., 2014). However, these methods are suitable under all soil conditions (Soil Survey Staff, 2014) and are considered the most accurate and least biased of the soil sampling methods (Beem-Miller et al., 2016; Harrison et al., 2003; Muller and Hamilton, 1992; Page-Dumroese et al., 1999; Xu et al., 2015).

Excavation methods are performed numerous ways in the field, such as through total pit or irregular hole excavation. Measurements of excavated pit or hole volume are taken using a frame or replacement with sand, balloons, expanding foam, or water. Comparisons between the frame and sand (Harrison et al., 2003), foam and sand (Muller and Hamilton, 1992), and foam and water (Page-Dumroese et al., 1999) replacement techniques showed no significant differences. Clod and excavation methods (specifically using sand and water replacement techniques) have been reported to yield similar BD values (Howard and Singer, 1981; Tisdall, 1951).

2.2.3 Soil Sampling Depth

Although SOC concentrations tend to decrease with increasing soil depth, SOC stocks are greater in deeper soil layers due to increased mass and total volume of soil. In fact, well over 50% of the global SOC stock is stored below 20 cm (Batjes, 1996; Jobbágy and Jackson, 2000; Tarnocai et al., 2009). Unfortunately, soil sampling is often confined to surface soil layers (above 15-30 cm), biasing SOC stock estimates and providing an incomplete picture of soil C dynamics

(Harrison et al., 2011; Jandl et al., 2014; Stockmann et al., 2013). Because deep SOC represents the largest terrestrial C stock, this bias adds uncertainty to carbon-climate feedbacks and climate models (Jandl et al., 2014). Deep SOC is subject to potentially rapid decomposition under changing environmental conditions such as increasing temperatures and rooting depths, underscoring the importance of sampling deeper soil layers (Harrison et al., 2011; Hicks Pries et al., 2017; Mobley et al., 2015). Furthermore, comparisons of soil sampling methods limited to surface soil layers (e.g., Beem-Miller et al., 2016; Howard and Singer, 1981; Kulmatiski et al., 2003; Xu et al., 2015) are not necessarily representative of the performance of the methods throughout the whole soil profile. Soil properties (e.g., SOC concentrations, BD, and soil texture) and the soil environment (e.g., roots and water content) change with increasing depth, which may alter the performance of soil sampling tools or techniques. Sampling deeper soil layers, and the ability to sample them accurately, is critical to our understanding of SOC fluxes, including persistence, protection, mineralization, and leaching losses.

2.2.4 Soil Organic Carbon Stock Calculations

The conventional methods used to estimate soil nutrient stocks are the fixed depth and genetic horizon methods. Both methods convert SOC concentration to SOC mass per unit area by using the product of SOC concentration, BD, and soil layer thickness, where the soil layer is either a fixed depth or genetic horizon. In general, the fixed depth method requires a smaller sampling size at a given error limit or minimum detectable difference than the genetic horizon method (Grüneberg et al., 2010; Wang et al., 2017). Grüneberg et al. (2010) thus recommend the fixed depth method for measuring and comparing regional SOC stocks over time, but note the benefits of sampling by genetic horizons to study pedogenic controls on SOC stock dynamics. However, Wiesmeier et al. (2012) recommend sampling by genetic horizon for all SOC inventories due to a

biased overestimation of cultivation-induced SOC depletion by the fixed depth method. The fixed depth and genetic horizon methods can result in significantly different SOC stock estimates for the same soil profile, adding ambiguity to global databases and meta-analysis that are comprised of data obtained using both methods (Grüneberg et al., 2010; Wang et al., 2017; Wiesmeier et al., 2012).

Despite their frequent use, numerous concerns have been raised about the ability of the fixed depth and genetic horizon methods to accurately detect SOC stock changes over time. The primary mechanism of bias for both methods results from the confounding effect of BD variability (Ellert and Bettany, 1995; Lee et al., 2009; Mikha et al., 2013; Schrumpf et al., 2011; Wendt and Hauser, 2013; Wuest, 2009). Bulk density changes due to a number of factors, including its negative correlation with SOC concentrations, the swelling and shrinking of some clays in soils, and land use changes or management treatments (Schrumpf et al., 2011). Even small differences in BD can result in misleading conclusions about gains or losses in SOC stocks over time, particularly when SOC concentrations are high (Hiederer and Köchy, 2011; Köchy et al., 2015).

The mass based approach for calculating SOC stocks eliminates the use of BD measurements, attempting to overcome biases associated with changes in BD over time and the imprecision and difficulty of its measurement (Wuest, 2009). This method compares soil mass layers, rather than soil depth layers or genetic horizons (Wendt and Hauser, 2013). In the absence of information on soil erosion or deposition, comparisons of SOC stocks over time are made using reference (i.e., fixed) soil mass layers, thus correcting for the influence of changes in soil mass on nutrient storage estimates (Ellert and Bettany, 1995). Multiple variations of the mass based approach exist and are useful for analyzing management or land use effects on SOC stocks when the

direction of change in BD is known or in the absence of data on the initial conditions (Lee et al., 2009). The mass based approach is increasingly recommended for SOC inventories in order to account and correct for changes in BD with time (e.g., Ellert and Bettany, 1995; Lee et al., 2009; Mikha et al., 2013; Schrumpf et al., 2011; Wendt and Hauser, 2013; Wuest, 2009).

2.2.5 Objectives

The purpose of this study was to determine the accuracy and compatibility of the clod and core soil sampling methods for measuring soil properties used to estimate SOC stocks. These two sampling methods were chosen because they are widely used and generally considered interchangeable (Tarnocai et al., 2009; Throop et al., 2012; Van Remortel and Shields, 1993; Vogel, 1994). Clod (paraffin coating and submerging technique) and hammer-core (54-mm diameter) BD and chemical analysis samples were compared to irregular hole (water-replacement technique) excavation samples. The excavation method was considered the more reliable and least biased method. Samples were taken from an excavated pit at five soil depth layers to 150 cm in depth to determine potential sampling biases among methods in surface and deeper soil, as well as across a range of soil properties. The primary differences between this study and previous studies (e.g., Beem-Miller et al., 2016; Harrison et al., 2003; Howard and Singer, 1981; Kulmatiski et al., 2003; Van Remortel and Shields, 1993; Xu et al., 2015) are the comparison of all three methods (clod, core, and excavation); the depth to which samples were taken; the measurement of BD and SOC concentrations to estimate SOC stocks using both fixed depth and mass based approaches; and the non-rocky soil sampled.

2.3 MATERIALS AND METHODS

2.3.1 Study Site

Soil was sampled at an intensively managed Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation in northwestern Oregon, USA (Figure 2.1, inset). The climate in this region is characterized by cool, wet winters and warm, dry summers. Mean annual temperature at the site from 1981 to 2010 was 9 °C, with an annual maximum and minimum temperature of 14 °C and 4 °C, respectively (PRISM Climate Group, 2017). Mean annual precipitation was ~220 cm over the same period (PRISM Climate Group, 2017). The rotation age of the stand was ~40 years at the time of sampling. The ground was nearly level to gently sloping, with an average slope of 10% and a maximum slope of <30%. Elevation ranged from 620-660 m above sea level. Soil was sampled at nine 0.2-ha plots located across a total area of ~5 ha (Figure 2.1). These plots were established in 1989 as part of a forest management study, and were designed to maximize uniformity within and between plots. The soil most closely resembled the Cumley series (a Palehumult), had a predominately sandy clay texture, and was moderately well drained with a low mean rock fragment content by sample weight (<2% fine to medium gravel). This soil sequence probably developed over hundreds of thousands to millions of years under somewhat stable conditions (Lin, 2011), making it reasonably uniform and an ideal soil for sampling to compare methods under field conditions.

2.3.2 Field Methods

Soil pits were excavated with a shovel to 100 or 150 cm. Major genetic horizons, soil colors, textures, and structures were identified, and roots and stone content were recorded (Table 2.1). Soil samples were collected randomly from within the middle of soil depth layers 0-10, 10-20, 20-50, 50-100, and 100-150 cm. Clod, core, and excavation samples were taken from within the

same pit and from comparable pits within the same 0.2 ha plot. Clod samples were collected in triplicate from each soil layer sampled and were ~200-300 cm³ in volume. Clods were carefully wrapped in plastic wrap, sealed in plastic bags, and stored side by side in padded travel containers. Core samples were collected using a coring cylinder with four parallel inner brass sleeves – two 1-cm sleeves on the ends and two 3-cm sleeves in the middle – and a 5.4-cm inside diameter. The core was hammered horizontally into the soil profile until the end of the core was flush with the soil. After carefully removing the core, the sleeves were removed simultaneously, and the soil trimmed to align with the open edges of the two inner 3-cm sleeves (137.4 cm³). On a few occasions, a cobble protruding from one of the 3-cm rings necessitated the removal of the ring and a smaller soil sample volume (68.7 cm³). Soil collected inside the 3-cm ring(s) was sealed in plastic bags. Excavation samples were collected by carefully excavating an irregular hole (~200-300 cm³) at the desired depth with a sharp soil knife, leveling the hole, lining it with plastic wrap, and recording the volume of water needed to fill the hole. Excavated soil was sealed in plastic bags. Collected soil was returned to the laboratory within 48 hours and stored at 3° C until analysis.

2.3.3 Laboratory Methods

Field-moist clod samples (Tisdall, 1951) were carefully teased apart at natural breaks to form clods between 30 and 60 cm³. After tying thread around the clods, they were quickly dipped in paraffin wax to provide a thin waterproof coat. The volume of the clods was determined by displacement of water in graduated cylinders. Additional volume added by the paraffin wax was considered null. Each clod was subsequently dried in the oven at 105° C for at least 48 hours and weighed. Rock fragment weight and volume was determined by sieving the oven-dried clods to <4.75 mm. Soil removed from the original clods collected in triplicate was composited for

chemical analysis and air-dried to a constant weight along with the core and excavation samples. Because SOC concentrations and BD are not independent variables, soil subsamples used in elemental analysis were taken from BD samples to avoid potential biases (Hamburg, 2000). Air-dried samples were sieved to <4.75 mm (rather than to <2 mm) to avoid discarding a meaningful portion of SOC (Harrison et al., 2003; Holub, 2011). The >4.75 mm fraction was weighed and the volume determined by displacement of water in a graduated cylinder. Representative subsamples of the <4.75 mm fraction were ground with a mortar and pestle and analyzed for total C concentrations (g kg^{-1}) using an automated CHN analyzer (Perkin-Elmer 2400, Norwalk, CT). The precision of the analysis was verified by measuring ~20% of the samples twice, as well as by running quality control samples of known C concentration every 10 samples. The average of samples run twice was used for analysis. Total C concentrations are reported as organic C due to a lack of carbonates measured in the region (Soil Survey Staff, 2016) and strongly acid soils (pH ~5) (Chapter 3.4 of this paper).

Oven-dry weights for the core and excavation samples were obtained by drying subsamples of each sample for at least 48 hours at 105°C and applying conversions to the air-dried weights according to:

$$\text{Oven dry sample weight, g} = (\text{air dry sample weight, g}) \times \left(\frac{\text{subsample oven dry weight, g}}{\text{subsample air dry weight, g}} \right). \quad (2.1)$$

Bulk density was calculated according to:

$$\text{BD} = \left(\frac{\text{oven dry sample weight, g} - \text{rock fragment weight, g (>4.75 mm)}}{\text{soil volume, cm}^3 (\text{solids+pores}) - \text{rock fragment volume, cm}^3 (>4.75 \text{ mm})} \right). \quad (2.2)$$

Soil organic carbon stocks were estimated using the fixed depth/genetic horizon equation:

$$\text{Mg SOC ha}^{-1} = \left(\frac{\text{mg OC}}{\text{g soil}} \right) \left(\frac{\text{g soil}}{\text{cm}^3 \text{ soil}} \right) \left(\frac{\text{cm}}{1} \right) \left(\frac{\text{Mg}}{10^9 \text{ mg}} \right) \left(\frac{10^8 \text{ cm}^2}{\text{ha}} \right), \quad (2.3)$$

where mg OC g soil⁻¹ is SOC concentration, g soil cm soil⁻³ is BD, and cm is soil layer thickness. The mass based approach according to the procedure of Wendt and Hauser (2013) was also used to estimate SOC stocks. For this approach, soil sample mass for each depth layer ($M_{\text{SAMPLE(DL)}}$, g) and method was calculated according to:

$$M_{\text{SAMPLE(DL)}} = \pi \left(\frac{5.4 \text{ cm}}{2} \right)^2 \times \left(\frac{\text{soil layer thickness, cm}}{1} \right) \times \text{BD}, \quad (2.4)$$

where 5.4 cm is the inside diameter of the core. Soil sample masses, SOC concentrations, the inside diameter of the core, and the number of cores per sample were subsequently input into the web-accessible spreadsheet (Wendt, 2012) created by Wendt and Hauser (2013), which fits a cubic spline function to model the relationship between cumulative areal soil mass and cumulative SOC mass. By calculating soil sample mass for each depth layer according to Equation (2.4), the mass of the core samples could be directly compared to the mass of the clod and excavation samples.

Both the fixed depth and mass based approaches used herein to calculate SOC stocks replace the >4.75 mm fraction volume with fine soil (<4.75 mm). While Throop et al. (2012) suggest that this method could overestimate SOC stocks, other studies have found opposite results (Corti et al., 2002; Harrison et al., 2011, 2003; Zabowski et al., 2011). For example, Harrison et al. (2003) found that 75% of the total SOC stock in a rocky forest soil was contained in the coarse soil (>2 mm) fraction. Substantial errors in SOC estimates due to coarse fragment content appear to be limited to rocky soils (Harrison et al., 2003; Throop et al., 2012), specifically soils with a coarse fraction greater than 20% (by weight), which are likely to have considerable (>10% of total SOC) C content in the coarse fraction (Zabowski et al., 2011). Given that the soil sampled herein was generally non-rocky (<2% fine to medium gravel content by weight), replacing the

volume of the >4.75 mm fraction with fine soil is unlikely to cause substantial biases in SOC stock estimates.

An assumption of sampling in the middle of soil depth layers is that the errors associated with not sampling the whole layer tend to have a canceling effect, providing an accurate estimate of the SOC stock in the given layer. Specifically, this method likely overestimates BD values and underestimates SOC concentrations of soil above the sample, while resulting in errors in the opposite direction concerning soil below the sample. The assumption that these errors are canceling applies to the calculation of SOC stocks using both the fixed depth and mass based approaches.

2.3.4 Statistical Methods

Analysis of variance (ANOVA) was used to determine whether soil sampling method affected BD and SOC concentration measurements. When significant differences were detected at $P < 0.1$, Tukey's HSD post-hoc tests were conducted to compare the means. To determine if rock presence was affected by soil sampling method, analysis of deviance was performed. Soil collected from pits that did not form clods at all depths or pits that had clear differences in soil properties and at which all methods were not used equally were eliminated from these tests. Linear regression was used to determine relationships between variables such as BD, SOC concentration, and rock fragment volume. Where necessary, transformations of data were conducted to conform data to the assumption of normality. Data were analyzed using R studio (R Core Team, 2016).

2.4 RESULTS

The clods collected in triplicate from each soil layer and analyzed for BD showed no differences in mean BD values and similar variation (Table 2.2). Averages of the BD values for

clods collected in triplicate were used for all following analysis. Mean soil BD for the clod, core, and excavation methods ranged from 0.79 to 1.12, 0.56 to 0.95, and 0.72 to 1.15 g cm⁻³, respectively, and increased with depth to 100 cm in depth (Table 2.3; Figure 2.2a). Compared to the excavation method, the core method significantly (Tukey's HSD, $\alpha = 0.1$) underestimated BD at all depths below 10 cm. The clod and core methods resulted in significantly different BD values at all soil depth layers sampled. No differences were found between BD values for the clod and excavation methods. The excavation method had high variability compared to both the clod and core methods in the upper 20 cm. Above 50 cm in depth, the clod method had the highest BD values.

Mean SOC concentrations for the clod, core, and excavation methods ranged from 3.2 to 73.0, 3.2 to 78.8, and 4.7 to 101.8 g OC/kg soil, respectively, and decreased with depth (Table 2.4; Figure 2.2b). Soil organic C concentrations tended to be highest for the excavation method. The exception to this trend was in soil depth layer 10-20 cm. In this depth layer, the core method had the highest SOC concentration, which was significantly different from the clod method. The core method had the lowest SOC concentration at 50-100 cm in depth, which was significantly different from the excavation method. Above 50 cm in depth, the clod method had the lowest SOC concentrations.

Soil organic C concentration was a significant ($P < 0.1$) predictor of BD, the two variables being negatively related (Figure 2.3a). When analyzed by depth layer, a significant relationship between these two variables was present only above 50 cm, which corresponded to SOC concentrations between ~15 and 150 g OC/kg soil (Figure 2.3b). No difference was found between methods concerning the probability of rock fragment (>4.75 mm) presence ($P = 0.2438$). The excavation method accounted for 55% of the soil samples collected containing rock

fragments, while the clod and core methods accounted for 27% and 18%, respectively (Figure 2.4a). On a percent weight basis, the clod and excavation methods had the lowest (0.43%) and highest (1.50%) rock content, respectively, and rock content for the core method was closer to the excavation than clod method (1.07%) (Table 2.5; Figure 2.4b). No significant relationships between rock volume and BD or SOC concentration were identified at any soil depth layer for the core method (Figure 2.5). However, due to the relatively small number of samples that contained rock fragments, as well as the typically small volume of rock fragment content, one or two data points tended to exert large influence on the regression analysis. Small sample sizes and limited numbers of samples with rock fragment content inhibited the analysis of relationships between rock volume and other variables for the clod and excavation methods.

Soil organic C stocks calculated using the fixed depth approach were significantly lower for the core method compared to the excavation method at soil depth layers 0-10 and 50-100 cm (-25 and -40 Mg ha⁻¹, respectively) (Table 2.6; Figure 2.6). Differences in SOC stocks between methods at the other depth layers were not significant, often due to low sample size and high variation. Cumulative SOC stocks yielded values of 220.5, 180.3, and 283.7 Mg ha⁻¹ to 150 cm in depth for the clod, core, and excavation methods, respectively (Table 2.7). Compared to the excavation method, the clod and core methods underestimated the SOC stock to a depth of 150 cm by 22% and 36%, respectively. Most of this difference (66% and 70%, respectively) occurred below 20 cm. Across all soil sampling methods, soil below 20 cm in depth comprised over 50% of SOC stocks. Underestimations of the SOC stock by the clod and core methods compared to the excavation method were similar for the mass based approach. Setting the reference mass layers using the excavation method resulted in cumulative SOC stock estimates of 231.3, 190.4, and 294.7 Mg ha⁻¹ to ~150, 182, and 150 cm in depth for the clod, core, and excavation methods,

respectively (Tables 2.8-9). When the reference mass layers for the core method were set to approximate the soil depth layers sampled, the cumulative SOC stock was 181.2 Mg ha⁻¹ to ~150 cm in depth.

Regression Equations (2.5-13) used to predict BD, SOC concentrations, and soil sample mass for each depth layer between the clod, core, and excavation soil sampling methods are listed in Table 2.10 and correspond to Figures 2.7-9. Using the fixed depth equation, adjusting values according to Equation (2.8) and Equations (2.7) and (2.9) for clod and core samples, respectively, resulted in SOC stocks for the clod and core methods that best matched the excavation method. The clod and core methods subsequently underestimated the SOC stock to a depth of 150 cm by 3% and 2%, respectively (Table 2.7). For the mass based approach, adjusting values according to Equations (2.8) and (2.11) for clod samples and Equations (2.9) and (2.12) for core samples resulted in SOC stock underestimations to ~150 cm in depth of 4% and 2%, respectively (Tables 2.8-9). Reference mass layers were set using the excavation method.

2.5 DISCUSSION

While previous studies have found that core methods underestimated BD compared to other soil sampling methods, many of these studies attributed this to the inability of core methods to take accurate soil samples for BD analysis in rocky soils due to coinciding rock fragment content underestimations (Andraski, 1991; Beem-Miller et al., 2016; Harrison et al., 2003; Kulmatiski et al., 2003; Muller and Hamilton, 1992). The soil sampled herein was non-rocky (<2% fine to medium gravel content by weight), and the probability of samples containing rock content (>4.75 mm) was not significantly different between methods. Although a higher percentage of excavation method samples contained rocks, this does not explain the consistent and significant underestimations of BD by the core method compared to the excavation method. Additionally,

core method samples had greater rock content by weight than clod samples, while no significant differences were found between clod and excavation BD measurements.

In a loamy sand soil with <6% rock content by weight, Harrison et al. (2003) found no significant differences between core and excavation (total pit) BD measurements. However, Van Remortel and Shields (1993) found a highly significant difference ($P < 0.001$) between clod and core BD measurements across a wide range of coarse- to fine-textured soil with few rock fragments (<5% fine gravel). Assuming no difference between clod and excavation BD measurements – a conclusion supported by the current study and other studies as well (e.g., Howard and Singer, 1981; Tisdall, 1951) – the underestimation of BD by the core method in non-rocky soils may be limited to or greater in more cohesive, clayey soils. Andraski (1991) concluded that the core method underestimated BD to a greater extent in very loose or cohesive soils, observing a difference of $>0.3 \text{ g cm}^{-3}$ between the core and (balloon) excavation methods in sandy ($>82\%$ sand) and clayey ($>15\%$ clay) soils. The mean underestimation of the core method compared to the clod method observed by Van Remortel and Shields (1993) was somewhat less at 0.09 g cm^{-3} . However, they sampled across a wide range of coarse- and fine-textured soils and found both method and soil texture to be highly significant variables. The mean underestimation by the core method compared to both the clod and excavation methods for the soil sampled herein was $\sim 0.21 \text{ g cm}^{-3}$, somewhat lower than the value reported by Andraski (1991), despite the high clay content ($>35\%$) of the soil sampled.

Soil BD may have been consistently underestimated by the core method due to the act of driving the core into the soil. Repeated hammering likely disturbed and loosened the soil in the core and compacted soil outside the core, particularly in deeper soil layers where soil was more cohesive and had a higher clay content (Andraski, 1991). Additionally, coarse roots may have

played an important role in core method underestimation of BD in the same manner as has been ascribed to rocks in other studies (Andraski, 1991; Beem-Miller et al., 2016; Harrison et al., 2003; Kulmatiski et al., 2003; Muller and Hamilton, 1992), i.e., by catching on the outside edges of the coring device and preventing soil from entering the core. This mechanism could be responsible for low core method BD measurements in the upper 20 cm, where coarse root density was high and soil was less cohesive, allowing roots to be pushed through the soil by the core (Figure 2.10).

Above 50 cm in depth (i.e., at SOC concentrations between ~15 and 150 g OC/kg soil), SOC concentration was a significant predictor of BD, the two variables having a negative relationship that is well supported in the literature (e.g., Hiederer and Köchy, 2011). However, when analyzed across methods, the core method consistently had the lowest BD values above (and below) 50 cm in depth compared to the other two methods, but did not have the highest SOC concentrations at depth layers 0-10 cm and 20-50 cm. This indicates that the low BD values associated with the core method were direct biases and not the result of an interaction with SOC concentration. The clod method, on the other hand, had both the highest BD values and lowest SOC concentrations above 50 cm in depth compared to the other two methods. The most probable explanation for these observed values is that clod methods are biased toward soil that is more cohesive and sturdy, and thus soil with a higher relative clay content than the surrounding bulk soil (Van Remortel and Shields, 1993) (Figure 2.10). This would result in lower SOC concentrations for the clod method, as well as greater BD values within the range that SOC concentration is a significant predictor of BD. Essentially, where SOC concentrations are higher, such as in surface soil layers, replacing lower density organic matter with higher density clay minerals results in increased BD. The clod method had nearly identical BD values to the

excavation method below 50 cm, while still having somewhat lower SOC concentrations. This supports the conclusion that the clod method was biased toward lower SOC concentrations as opposed to higher BD values. Therefore, the results of this study do not suggest that clod methods overestimate BD due to the exclusion of larger natural pores in between soil aggregates and in between rock fragments and soil aggregates. Rather any overestimation in BD appears to be the direct result of a consistent underestimation in SOC concentration, and thus only occurs above the SOC concentration threshold wherein it is a significant predictor of BD.

Less clear is why the core method tended to have lower SOC concentrations than the excavation method, particularly at 50-100 cm in depth where this difference was significant. One possible explanation is that the larger volumes of soil collected by the excavation method were more likely to sample SOC “hot spots” such as preferential flow paths (Bundt et al., 2001; Hagedorn and Bundt, 2002). Additionally, in deeper soil layers where roots are less dense, larger volume samples may include more soil in the immediate vicinity of roots where SOC concentrations are higher than they are in the surrounding bulk soil due to organic matter inputs by roots (Grayston et al., 1997; Rasse et al., 2005). This may in turn help explain why SOC concentration variability (and BD variability, particularly above 50 cm) was generally greater for the excavation than core method.

The fixed depth and mass based approaches yielded similar SOC stock estimates for the non-rocky soil sampled herein. In soils with higher rock content, the difference between the two approaches would depend on how coarse fragment mass and volume are treated in both BD and soil mass calculations (Beem-Miller et al., 2016; Throop et al., 2012). While the mass based approach has been shown to help overcome biases associated with changes in BD over time (e.g., Ellert and Bettany, 1995; Lee et al., 2009; Mikha et al., 2013; Schrupf et al., 2011; Wendt and

Hauser, 2013; Wuest, 2009), the results of this study do not support the conclusion that calculating SOC stocks on a mass basis can overcome the imprecision of soil mass (per unit volume or area) measurements. Even when reference mass layers were set with a more reliable method (i.e., the excavation method), as suggested by Beem-Miller et al. (2016), the extreme underestimation of soil mass by the core method resulted in a compensatory overestimation by the cubic spline model in mean depth sampled and thus a substantial underestimation of the SOC stock – particularly in deeper soil layers – as the modeled relationship assumes a gradual decrease of SOC concentrations with depth. Furthermore, the difference between SOC stock estimates for the clod and excavation methods was due to the underestimation of SOC concentrations by the clod method, which equally affects the fixed depth and mass based approaches.

2.6 CONCLUSIONS

This study shows that clod and core methods consistently underestimate SOC concentrations and BD, respectively, in non-rocky, clayey soils. Soil organic C stock underestimations by these two methods were substantial and were most pronounced in deeper soil layers (below 20 cm), which often comprise the majority of SOC stocks. As all SOC stock calculations rely on the relationship between SOC mass and soil mass, the underestimation of these parameters by clod and core methods similarly affects the fixed depth, genetic horizon, and mass based approaches to quantify SOC stocks. Biases in soil mass per unit volume or area and SOC concentrations may be overcome when calculating SOC stocks by counterbalancing effects. For example, if coarse fragment volume is considered void, an underestimation of coarse fragment content in rocky soils by clod and core methods would increase SOC stock estimates for these methods in comparison to excavation methods (Beem-Miller et al., 2016; Kulmatiski et al., 2003; Throop et

al., 2012; Xu et al., 2015). However, ignoring SOC contained in the coarse soil fraction in rocky soils can also cause underestimations in SOC stock estimates (Corti et al., 2002; Harrison et al., 2011, 2003; Zabowski et al., 2011). The use of clod and core methods interchangeably is questionable and adds uncertainty to SOC databases. Given the substantial amount of SOC in deeper soil layers (which are often not sampled) and the frequent use of core methods, regional and global SOC stocks may be largely underestimated.

REFERENCES

- Andraski, B.J., 1991. Balloon and core sampling for determining bulk density of alluvial desert soil. *Soil Sci. Soc. Am. J* 55, 1188–1190.
- Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47, 151–163.
- Beem-Miller, J.P., Kong, A.Y.Y., Ogle, S., Wolfe, D., 2016. Sampling for Soil Carbon Stock Assessment in Rocky Agricultural Soils. *Soil Sci. Soc. Am. J.* 80, 1411. doi:10.2136/sssaj2015.11.0405
- Brady, N.C., Weil, R.R., 2008. *The nature and properties of soils*, 14th ed. Pearson, Upper Saddle River, NJ.
- Bridgham, S.D., Megonigal, J.P., Keller, J.K., Bliss, N.B., Trettin, C., 2006. The Carbon Balance of North American Wetlands. *Wetlands* 26, 889–916. doi:10.1672/0277-5212(2006)26[889:TCBONA]2.0.CO;2
- Bundt, M., Widmer, F., Pesaro, M., Zeyer, J., Blaser, P., 2001. Preferential flow paths: Biological “hot spots” in soils. *Soil Biol. Biochem.* 33, 729–738. doi:10.1016/S0038-0717(00)00218-2
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Quéré, C. Le, Myneni, R.B., Piao, S., Thornton, P., 2013. Carbon and Other Biogeochemical Cycles, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 465–570. doi:10.1017/CBO9781107415324.015
- Corti, G., Ugolini, F.C., Agnelli, A., Certini, G., Cuniglio, R., Berna, F., Fernández Sanjurjo, M.J., 2002. The soil skeleton, a forgotten pool of carbon and nitrogen in soil. *Eur. J. Soil Sci.* 53, 283–298. doi:10.1046/j.1365-2389.2002.00442.x
- Crowther, T., Todd-Brown, K., Rowe, C., Wieder, W., Carey, J., Machmuller, M., Snoek, L., Fang, S., Zhou, G., Allison, S., Blair, J., Bridgham, S., Burton, A., Carrillo, Y., Reich, P., Clark, J., Classen, A., Dijkstra, F., Elberling, B., Emmett, B., Estiarte, M., Frey, S., Guo, J., Harte, J., Jiang, L., Johnson, B., Kröel-Dulay, G., Larsen, K., Laudon, H., Lavalley, J., Luo, Y., Lupascu, M., Ma, L., Marhan, S., Michelsen, A., Mohan, J., Niu, S., Pendall, E., Penuelas, J., Pfeifer-Meister, L., Poll, C., Reinsch, S., Reynolds, L., Schmidh, I., Sistla, S., Sokol, N., Templer, P., Treseder, K., Welker, J., Bradford, M., 2016. Quantifying global soil C losses in response to warming. *Nature* 104, 104–108. doi:10.1038/nature20150
- Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75, 529–538. doi:10.4141/cjss95-075
- Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. *Appl. Soil Ecol.* 5, 29–56. doi:10.1016/s0929-1393(96)00126-6
- Grüneberg, E., Schöning, I., Kalko, E.K. V, Weisser, W.W., 2010. Regional organic carbon

- stock variability: A comparison between depth increments and soil horizons. *Geoderma* 155, 426–433. doi:10.1016/j.geoderma.2010.01.002
- Hagedorn, F., Bundt, M., 2002. The age of preferential flow paths. *Geoderma* 108, 119–132. doi:Pii s0016-7061(02)00129-5\r10.1016/s0016-7061(02)00129-5
- Hamburg, S.P., 2000. Simple Rules for Measuring Changes in Ecosystem. *Mitigation Adapt. Strateg. Glob. Chang.* 5, 25–37.
- Harrison, R.B., Adams, A.B., Licata, C., Flaming, B., Wagoner, G.L., Carpenter, P., Vance, E.D., 2003. Quantifying deep-soil and coarse-soil fractions: Avoiding sampling bias. *Soil Sci. Soc. Am. J.* 67, 1602–1606.
- Harrison, R.B., Footen, P.W., Strahm, B.D., 2011. Deep soil horizons: Contribution and importance to soil carbon pools and in assessing whole-ecosystem response to management and global change. *For. Sci.* 57, 67–76.
- Hicks Pries, C.E., Castanha, C., Porras, R., Torn, M.S., 2017. The whole-soil carbon flux in response to warming. *Science* (80-.). 1–8. doi:10.1126/science.aal1319
- Hiederer, R., Köchy, M., 2011. Global soil organic carbon estimates and the harmonized world soil database, EUR 25225 EN. Publications Office of the European Union, Italy. doi:10.2788/13267
- Holub, S.M., 2011. Soil Carbon Change in Pacific Northwest Coastal Douglas-fir Forests: Change Detection Following Harvest – Soils Establishment Report. Albany, Oregon.
- Howard, R.F., Singer, M.J., 1981. Measuring Forest Soil Bulk Density Using Irregular Hole, Paraffin Clod, and Air Permeability. *For. Sci.* 27, 316–322.
- Jandl, R., Rodeghiero, M., Martinez, C., Cotrufo, M.F., Bampa, F., van Wesemael, B., Harrison, R.B., Guerrini, I.A., Richter, D. deB, Rustad, L., Lorenz, K., Chabbi, A., Miglietta, F., 2014. Current status, uncertainty and future needs in soil organic carbon monitoring. *Sci. Total Environ.* 468–469, 376–383. doi:10.1016/j.scitotenv.2013.08.026
- Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* 10, 423–436. doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2
- Köchy, M., Hiederer, R., Freibauer, a., 2015. Global distribution of soil organic carbon – Part 1: Masses and frequency distributions of SOC stocks for the tropics, permafrost regions, wetlands, and the world. *Soil* 1, 351–365. doi:10.5194/soil-1-351-2015
- Kulmatiski, A., Vogt, D.J., Siccama, T.G., Beard, K.H., 2003. Detecting Nutrient Pool Changes in Rocky Forest Soils. *Soil Sci. Soc. Am. J.* 67, 1282–1286. doi:10.2136/sssaj2003.1282
- Kuzyakov, Y., Friedelb, J.K., Stahra, K., 2000. Review of mechanisms and quantification of priming effects (Review). *Soil Biol. Biochem.* 32, 1485–1498. doi:10.1016/S0038-0717(00)00084-5
- Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. *Agric. Ecosyst. Environ.* 134, 251–256. doi:10.1016/j.agee.2009.07.006
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 528, 60–8.

doi:10.1038/nature16069

- Lin, H., 2011. Three Principles of Soil Change and Pedogenesis in Time and Space. *Soil Sci. Soc. Am. J.* 75, 2049. doi:10.2136/sssaj2011.0130
- Mikha, M.M., Benjamin, J.G., Halvorson, A.D., Nielsen, D.C., 2013. Soil Carbon Changes Influenced by Soil Management and Calculation Method. *Open J. Soil Sci.* 3, 123–131. doi:10.4236/ojss.2013.32014
- Milne, E., Banwart, S.A., Noellemeyer, E., Abson, D.J., Ballabio, C., Bampa, F., Bationo, A., Batjes, N.H., Bernoux, M., Bhattacharyya, T., Black, H., Buschiazzo, D.E., Cai, Z., Cerri, C.E., Cheng, K., Compagnone, C., Conant, R., Coutinho, H.L.C., de Brogniez, D., Balieiro, F. de C., Duffy, C., Feller, C., Fidalgo, E.C.C., da Silva, C.F., Funk, R., Gaudig, G., Gicheru, P.T., Goldhaber, M., Gottschalk, P., Goulet, F., Goverse, T., Grathwohl, P., Joosten, H., Kamoni, P.T., Kihara, J., Krawczynski, R., La Scala, N., Lemanceau, P., Li, L., Li, Z., Lugato, E., Maron, P.A., Martius, C., Melillo, J., Montanarella, L., Nikolaidis, N., Nziguheba, G., Pan, G., Pascual, U., Paustian, K., Pineiro, G., Powlson, D., Quiroga, A., Richter, D., Sigwalt, A., Six, J., Smith, J., Smith, P., Stocking, M., Tanneberger, F., Termansen, M., van Noordwijk, M., van Wesemael, B., Vargas, R., Victoria, R.L., Waswa, B., Werner, D., Wichmann, S., Wichtmann, W., Zhang, X., Zhao, Y., Zheng, J., Zheng, J., 2015. Soil carbon, multiple benefits. *Environ. Dev.* 13, 33–38. doi:10.1016/j.envdev.2014.11.005
- Mobley, M.L., Lajtha, K., Kramer, M.G., Bacon, A.R., Heine, P.R., Richter, D.D., 2015. Surficial gains and subsoil losses of soil carbon and nitrogen during secondary forest development. *Glob. Chang. Biol.* 21, 986–996. doi:10.1111/gcb.12715
- Muller, R.N., Hamilton, M.E., 1992. A simple, effective method for determining the bulk density of stony soils. *Commun. Soil Sci. Plant Anal.* 23, 313–319.
- Page-Dumroese, D.S., Brown, R.E., Jurgensen, M.F., Mroz, G.D., 1999. Comparison of methods for determining bulk densities of rocky forest soils. *Soil Sci. Soc. Am. J.* 63, 379–383. doi:10.2136/sssaj1999.03615995006300020016x
- PRISM Climate Group, 2017. PRISM Climate Group. URL <http://prism.oregonstate.edu> (accessed 2.17.17).
- R Core Team, 2016. R: A language and environment for statistical computing. Vienna, Austria.
- Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* 269, 341–356. doi:10.1007/s11104-004-0907-y
- Rossi, A.M., Hirmas, D.R., Graham, R.C., Sternberg, P.D., 2008. Bulk Density Determination by Automated Three-Dimensional Laser Scanning. *Soil Sci. Soc. Am. J.* 72, 1591. doi:10.2136/sssaj2008.0072N
- Scharlemann, J.P., Tanner, E.V., Hiederer, R., Kapos, V., 2014. Global soil carbon: understanding and managing the largest terrestrial carbon pool. *Carbon Manag.* 5, 81–91. doi:10.4155/CMT.13.77
- Schrumpf, M., Schulze, E.D., Kaiser, K., Schumacher, J., 2011. How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories? *Biogeosciences* 8, 1193–1212. doi:10.5194/bg-8-1193-2011

- Soil Survey Staff, 2016. Web Soil Survey. Available online. Accessed 05/07/2017.
- Soil Survey Staff, 2014. Soil survey field and laboratory methods manual. Soil survey investigations report No. 51, Version 2.0. Washington, D.C.
- Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., Courcelles, V. de R. de, Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric. Ecosyst. Environ.* 164, 80–99. doi:10.1016/j.agee.2012.10.001
- Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem. Cycles* 23, 1–11. doi:10.1029/2008GB003327
- Throop, H.L., Archer, S.R., Monger, H.C., Waltman, S., 2012. When bulk density methods matter: Implications for estimating soil organic carbon pools in rocky soils. *J. Arid Environ.* 77, 66–71. doi:10.1016/j.jaridenv.2011.08.020
- Tisdall, A.L., 1951. Comparison of methods of determining apparent density of soils. *Aust. J. Agric. Res.* 2, 349–354.
- Uteau, D., Pagenkemper, S.K., Peth, S., Horn, R., 2013. Aggregate and soil clod volume measurement: a method comparison. *Soil Sci. Soc. Am. J.* 77, 60–63. doi:10.2136/sssaj2012.0227n
- Van Remortel, R.D., Shields, D.A., 1993. Comparison of clod and core methods for determination of soil bulk density. *Commun. Soil Sci. Plant Anal.* 24, 2517–2528.
- Vogel, A.W., 1994. Compatibility of soil analytical data: Determinations of cation exchange capacity, organic carbon, soil reaction, bulk density, and volume percent of water at selected pF values by different methods. The Netherlands.
- Wang, H., Wang, W., Chang, S.X., 2017. Sampling Method and Tree-Age Affect Soil Organic C and N Contents in Larch Plantations. *Forests* 8, 1–15. doi:10.3390/f8010028
- Wendt, J.W., 2012. ESM Sample Spreadsheets.Xlsm. URL <https://drive.google.com/file/d/0BzxNFfzLbFxfjSG9RWlpwQ0FXc0k/view?usp=sharing> (accessed 5.16.17).
- Wendt, J.W., Hauser, S., 2013. An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *Eur. J. Soil Sci.* 64, 58–65. doi:10.1111/ejss.12002
- Wiesmeier, M., Spörlein, P., Geuß, U., Hangen, E., Haug, S., Reischl, A., Schilling, B., von Lützow, M., Kögel-Knabner, I., 2012. Soil organic carbon stocks in southeast Germany (Bavaria) as affected by land use, soil type and sampling depth. *Glob. Chang. Biol.* 18, 2233–2245. doi:10.1111/j.1365-2486.2012.02699.x
- Wuest, S.B., 2009. Correction of Bulk Density and Sampling Method Biases Using Soil Mass per Unit Area. *Soil Sci. Soc. Am. J.* 73, 312. doi:10.2136/sssaj2008.0063
- Xu, B., Pan, Y., Johnson, A.H., Plante, A.F., 2015. Method Comparison for Forest Soil Carbon and Nitrogen Estimates in the Delaware River Basin. *Soil Sci. Soc. Am. J.* in press, 227–

237. doi:10.2136/sssaj2015.04.0167

Zabowski, D., Whitney, N., Gurung, J., Hatten, J., 2011. Total soil carbon in the coarse fraction and at depth. *For. Sci.* 57, 11–18. doi:10.2136/sssaj2004.0612

CHAPTER 3. SOIL CARBON AND NITROGEN RESPONSE TO THINNING AND FERTILIZATION TREATMENTS IN A COASTAL PACIFIC NORTHWEST FOREST

3.1 ABSTRACT

Forests provide valuable ecosystem and societal services, including the sequestration of carbon (C) from the atmosphere. The majority of forest ecosystem C stocks are contained in the soil. Management practices can impact the balance of C inputs and losses from soil, affecting the ability of forests to act as a C sink, retain nutrients, and sustainably produce natural resources. This study examines soil organic carbon (SOC) and nitrogen (N) response to thinning and fertilization treatments. Soil was sampled at an intensively managed Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation in northwestern Oregon, USA. Management regimes – no treatment (control), thinning treatments, and fertilization treatments – were randomly assigned to nine 0.2-ha plots established in 1989 in a juvenile stand. Fertilized plots received a total of 1120 kg N ha⁻¹ as urea over 16 years. Thinning treatments were based on relative stand density. Prior to harvest in 2015, soil bulk density and chemical analysis samples were collected in the middle of soil depth layers 0-10, 10-20, 20-50, 50-100, and 100-150 cm. Forest floor samples were also collected. Three pits were sampled per plot and averaged to account for within plot variation. During a single rotation of ~40 years, thinning treatments significantly (Tukey's HSD, $\alpha = 0.1$) reduced SOC (Mg ha⁻¹) and N (kg ha⁻¹) by 25% and 27%, respectively, compared to no treatment. Most of this loss occurred in deeper soil layers (below ~20 cm). Thinning effects on the soil environment (e.g., soil temperature increases) and complex interactions between microorganisms, roots, and nutrients enhancing SOC decomposition and N mineralization potentially explain the observed decrease in SOC and N stocks. Fertilization treatments reduced soil pH compared to both thinning and no treatment to a depth of more than 50 cm, indicating the potential for nitrate leaching. Across all management regimes, deeper soil layers comprised the

majority of SOC and N stocks. This study shows that 1) accurately quantifying and comparing SOC and N stocks requires sampling deep soil; and 2) forest management can significantly impact both surface and deep SOC and N on decadal timescales.

3.2 INTRODUCTION

The world's forests are an important terrestrial carbon (C) sink, sequestering as much as 30% ($\sim 2 \text{ Pg C y}^{-1}$) of annual global anthropogenic CO_2 emissions between 1990 and 2007 (Bellassen and Luysaert, 2014; Pan et al., 2011). In addition to their importance in the global C cycle, forests provide many other valuable ecosystem and societal services. Forest management practices can enhance or reduce the ability of a given forest stand to act as a C sink and provide these services (Lal, 2005; Stockmann et al., 2013). As approximately two-thirds of forests are managed (Bellassen and Luysaert, 2014), understanding how forest management practices affect the global C cycle and the capacity to sustainably produce natural resources is a high priority.

While much research has been conducted regarding the aboveground effects of forest management, comparably little is known about the effects belowground (Clarke et al., 2015). Soil contains the majority of the terrestrial C stock, with $\sim 85\%$, 60% , and 50% of the total C stock in boreal forest, temperate forest, and tropical rainforest ecosystems, respectively (Dixon et al., 1994; Lal, 2005). Therefore, determining the fate of forest soil organic C (SOC) in response to management is an essential part of understanding carbon-climate feedbacks and changes in forest ecosystem C budgets. Gains or losses in SOC affect numerous soil properties essential to maintaining beneficial ecosystem services and productive forest stands, including the water- and nutrient-holding capacity of the soil (Milne et al., 2015).

In a recent meta-analysis of the effects of forest harvest on soil C, James and Harrison (2016) found that harvesting reduced soil C by an average of ~11% globally. Significant losses in both the litter layer (O horizon) and the mineral soil were observed, with particularly large losses in very deep soil (60-100+ cm in depth) compared to more superficial soil. The observation of deep SOC losses on decadal timescales due to management or land use change is not uncommon (e.g., Diochon and Kellman, 2009; Mobley et al., 2015; Richter et al., 1999). Considering that most SOC is contained in deeper soil layers (below 20 cm) (Batjes, 1996; Jobbágy and Jackson, 2000; Tarnocai et al., 2009), and most trees root deeper than 100 cm (Stone and Kalisz, 1991), studying deep soil nutrient dynamics is essential to understanding forest management effects on SOC stocks. Unfortunately, soils are often sampled to 20 cm or less and are rarely sampled below 100 cm (Batjes, 1996; Harrison et al., 2011; James and Harrison, 2016; Jandl et al., 2014; Jobbágy and Jackson, 2000; Mobley et al., 2015; Stockmann et al., 2013).

Globally, thinning treatments resulted in greater losses of mineral soil C than clear-cut harvesting, a difference of about 9% (James and Harrison, 2016). In a long-term reforestation study in the subtropical southeastern USA, Mobley et al. (2015) found that thinning treatments particularly reduced SOC and nitrogen (N) stocks in deeper soil layers where losses exceeded new inputs. Jandl et al. (2007) concluded that, in general, forest thinning increases the stability of a stand at the expense of SOC stocks. However, other studies have found no difference in SOC stocks post-thinning (Clarke et al., 2015 and references therein).

In Pacific Northwest forest ecosystems, N is often the primary limiting nutrient (Blake et al., 1990; Carter and Klinka, 1990; Chappell et al., 1991). The fate of soil N in response to forest management is thus a key concern in this region. In general, N fertilization stimulates biomass production, but the effects on SOC and other soil nutrient dynamics are variable and highly site

dependent due to complex interactions between soil properties, microorganisms, and vegetation (Jandl et al., 2007). Increases in soil N have the potential to enhance aboveground biomass growth and forest productivity, as well as to increase SOC and the retention of other nutrients in the soil through a combination of increased belowground biomass and delayed root decomposition (Li et al., 2015).

The objective of this study was to determine the response of SOC and N to thinning and fertilization treatments, and to understand how any observed response differed vertically in the soil profile to a depth of more than 100 cm. Both the fixed depth and mass based approaches were used to quantify and compare SOC and N stocks.

3.3 MATERIALS AND METHODS

3.3.1 Study Site

Soil was sampled at an intensively managed Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) plantation in northwestern Oregon, USA (Figure 2.1, inset). The plantation was planted in 1977 with 2-year-old Douglas-fir seedlings and was $\geq 90\%$ Douglas-fir throughout the ~40-year rotation. Square plots were established in 1989, the boundaries of which were designed to maximize uniformity within and between plots. Management regimes – no treatment (control), thinning treatments, and fertilization treatments – were randomly assigned to nine 0.2-ha plots spanning a total area of ~5 ha (Figure 2.1). Additionally, the initial trees per hectare were systematically reduced to one-half or one-fourth on randomly assigned plots. The removed trees were left on the forest floor. Fertilized plots received 224 kg N ha^{-1} as urea every four years starting in 1989 for a total of $1120 \text{ kg N ha}^{-1}$ over 16 years. Thinning treatments were based on Curtis' (1982) relative stand density. Thinned trees were either removed or left on the forest floor, depending on the size of the trees at the time of treatment. When trees were removed, slash

from those trees was left on the forest floor (Table 3.1). Landform across all plots was nearly level to gently sloping, with an average slope of 10% and a maximum slope of <30%. The climate is characterized by cool, wet winters and warm, dry summers. Mean annual precipitation from 1981 to 2010 was ~220 cm, and mean annual temperature was 9 °C, with an annual maximum and minimum temperature of 14 °C and 4 °C, respectively (PRISM Climate Group, 2017). Elevation ranged from 620 to 660 m above sea level. Soil was moderately well drained with a low mean rock fragment content by sample weight (<2% fine to medium gravel). The soil sampled was an older, clayey soil (a Palehumult, closely resembling the Cumley series), making it reasonably uniform and an ideal soil for sampling to compare forest management treatments.

3.3.2 Field and Laboratory Methods

Three soil pits per plot were excavated with a shovel to 100 or 150 cm. Major genetic horizons, soil colors, textures, and structures were identified, and roots and stone content were recorded (Table 2.1; Figure 3.1). Soil bulk density and chemical analysis samples were collected in the late summer and early fall of 2015, immediately preceding harvest. Samples were collected randomly from within the middle of soil depth layers 0-10, 10-20, 20-50, 50-100, and 100-150 cm. One forest floor sample was collected from a randomly placed 20 x 30 cm quadrat nearby each soil pit. All soil samples were analyzed separately, and repeated measurements within a plot were subsequently averaged to account for within plot variation. Soil bulk density and chemical analysis samples were collected using a 5.4-cm diameter hammer-core, as well as using clod and excavation (irregular hole, water replacement) methods. Repeated measurements within the same pit and depth layer across methods were analyzed separately and then averaged. A detailed description of soil sampling and laboratory methods is found in Chapter 2.3.2-3. All

soil samples collected in the field were sealed in plastic bags, returned to the laboratory within 48 hours, and stored at 3 °C until analysis.

The volume of field-moist clods (Tisdall, 1951) was determined by the paraffin wax method and displacement of water. Soil subsamples used in elemental analysis were taken from bulk density samples to avoid potential biases, as SOC concentrations and bulk density are not independent variables (Hamburg, 2000). Air-dried samples were sieved to <4.75 mm (rather than to <2 mm) to avoid discarding a meaningful portion of SOC (Harrison et al., 2003; Holub, 2011). The >4.75 mm fraction was weighed and the volume determined by displacement of water in a graduated cylinder. Litter layer samples were weighed, air-dried to a constant weight, reweighed, and ground to less than ~0.5 mm. Representative subsamples of litter layer and <4.75 mm mineral soil fractions were ground with a mortar and pestle and analyzed for total C and N concentrations (g kg^{-1}) using an automated CHN analyzer (Perkin-Elmer 2400, Norwalk, CT). Approximately 20% of the samples were run twice to verify the precision of the analysis, and quality control samples of known C concentration were run every 10 samples. The average of samples run twice was used for analysis. Due to a lack of carbonates measured in the region (Soil Survey Staff, 2016) and strongly acid soils (pH ~5) (Section 3.4.2), total C concentrations are equated to organic C.

Soil pH was measured in a 1:1 (deionized H_2O , mL:soil, g) mix for mineral soil and a 4:1 mix for the litter layer with a digital pH meter (Model PC-700, Oakton Inst., Vernon Hills, IL). Soil mixtures were left to stand undisturbed for at least 30 minutes to allow homogenization before pH was measured. Oven-dry weights for the clod method were determined by drying the clods in the oven at 105 °C for at least 48 hours. Subsamples of each core and excavation sample were

dried for at least 48 hours at 105 °C, and oven-dry weights were determined by applying conversions to the air-dried weights according to:

$$\text{Oven dry sample weight, g} = (\text{air dry sample weight, g}) \times \left(\frac{\text{subsample oven dry weight, g}}{\text{subsample air dry weight, g}} \right). \quad (3.1)$$

Bulk density was calculated according to:

$$\text{Bulk density} = \left(\frac{\text{oven dry sample weight, g} - \text{rock fragment weight, g (>4.75 mm)}}{\text{soil volume, cm}^3 (\text{solids+pores}) - \text{rock fragment volume, cm}^3 (>4.75 \text{ mm})} \right). \quad (3.2)$$

Soil organic C and N stocks were determined using the fixed depth equations:

$$\text{Mg SOC ha}^{-1} = \left(\frac{\text{mg OC}}{\text{g soil}} \right) \left(\frac{\text{g soil}}{\text{cm}^3 \text{ soil}} \right) \left(\frac{\text{cm}}{1} \right) \left(\frac{\text{Mg}}{10^9 \text{ mg}} \right) \left(\frac{10^8 \text{ cm}^2}{\text{ha}} \right), \quad (3.3)$$

and

$$\text{kg N ha}^{-1} = \left(\frac{\text{mg N}}{\text{g soil}} \right) \left(\frac{\text{g soil}}{\text{cm}^3 \text{ soil}} \right) \left(\frac{\text{cm}}{1} \right) \left(\frac{\text{kg}}{10^6 \text{ mg}} \right) \left(\frac{10^8 \text{ cm}^2}{\text{ha}} \right), \quad (3.4)$$

where mg OC g soil⁻¹ is SOC concentration, mg N g soil⁻¹ is N concentration, g soil cm soil⁻³ is bulk density, and cm is soil layer thickness. The mass based approach according to the procedure of Wendt and Hauser (2013) was also used to estimate SOC and N stocks. For this approach, soil sample mass for each depth layer (M_{SAMPLE(DL)}, g) and method was calculated according to:

$$M_{\text{SAMPLE(DL)}} = \pi \left(\frac{5.4 \text{ cm}}{2} \right)^2 \times \left(\frac{\text{soil layer thickness, cm}}{1} \right) \times \text{BD}, \quad (3.5)$$

where 5.4 cm is the inside diameter of the core. Soil sample masses, SOC and N concentrations, the inside diameter of the core, and the number of cores per sample were subsequently input into the web-accessible spreadsheet (Wendt, 2012) created by Wendt and Hauser (2013), which fits a cubic spline function to model the relationship between cumulative areal soil mass and cumulative SOC mass.

The fixed depth and mass based approaches used herein to calculate SOC and N stocks replace the volume of the >4.75 mm fraction with fine soil (<4.75 mm). Multiple studies have found that this method has the potential to overestimate (Throop et al., 2012) or underestimate

(Corti et al., 2002; Harrison et al., 2011, 2003; Whitney and Zabowski, 2004; Zabowski et al., 2011) SOC and N stocks. However, these errors appear to be limited to rocky soils (Harrison et al., 2003; Throop et al., 2012; Whitney and Zabowski, 2004; Zabowski et al., 2011). Because the soil sampled herein was generally non-rocky (<2% fine to medium gravel content by weight), this method is unlikely to cause substantial errors in SOC and N stock estimates. Furthermore, because this study is primarily concerned with determining if there are differences in SOC and N stocks between treatments, it is preferable to remove rock fragment content as a variable by replacing the >4.75 mm fraction with fine soil for SOC and N calculations.

Sampling in the middle of a soil depth layer likely underestimates SOC and N concentrations and overestimates bulk density of soil above the sample, while resulting in errors in the opposite direction concerning soil below the sample (i.e., overestimating SOC and N concentrations and underestimating bulk density). An assumption of this sampling method is that the errors tend to have a canceling effect, giving an accurate estimate of SOC and N stocks in the given layer. This assumption applies to the quantification of SOC and N stocks using both the fixed depth and mass based approaches. As the entire depth of the litter layer was sampled, this assumption does not apply to this layer. Additionally, litter layer SOC and N stocks were only determined according to Equations (3.3) and (3.4).

Total aboveground and root C sequestration were determined using the biomass ratio equation and parameters provided by Jenkins et al. (2003) in Table 6. For a given plot, the quadratic mean diameter (qmd) was used rather than diameter at breast height (dbh). Total aboveground biomass (TAB) for each plot was calculated according to:

$$\text{TAB Mg ha}^{-1} = \frac{\left(\frac{450 \text{ kg}}{\text{m}^3}\right)\left(\frac{\text{stand volume, m}^3}{\text{ha}}\right)\left(\frac{\text{Mg}}{1000 \text{ kg}}\right)}{\text{Exp}\left(-0.3737 - \left(\frac{1.8055}{\text{qmd, cm}}\right)\right)}, \quad (3.6)$$

where 450 kg m^{-3} is the density of Douglas-fir (Miles and Smith, 2009), and the denominator is the biomass ratio equation for softwood stem wood. To represent total aboveground and root C sequestration for thinned plots, the sum of the removed and final aboveground biomass was used for TAB. A ratio of 0.512, which is the average C concentration (g C g^{-1}) for Douglas-fir trees in the Pacific Coast and Rocky Mountain regions (Birdsey, 1992), was used to convert total mass to C mass. Belowground C stocks were determined by summing the SOC stock (calculated using the mass based approach) for a given plot with total root C for the same plot. Total C stocks were determined by summing the above- and below-ground C stocks for a given plot. Merchantable volume was calculated in thousands of board-feet per acre (as 32-ft long logs to a 6-inch top diameter). For thinned plots, removed volume was included in the calculation of merchantable volume only if it was commercially thinned.

3.3.3 Analysis and Statistical Methods

In accordance with the findings detailed in Chapter 2.4 of this paper, core sample bulk density was adjusted for input into Equations (3.3) and (3.4) according to Equation (2.7). Soil organic C concentration for clod and core samples was adjusted for input into Equation (3.3) according to Equations (2.8) and (2.9), respectively. Using raw data from Chapter 2 of this paper, it was determined that soil N concentrations were also underestimated by the clod and core methods compared to the excavation method. Therefore, N concentration for input into Equation (3.4) was adjusted for clod and core samples according to:

$$J(\text{Excavation } N_{\text{conc}}) = (0.9724 \times J(\text{Clod } N_{\text{conc}})) + 0.1304; R^2 = 0.9539, \quad (3.7)$$

and

$$J(\text{Excavation } N_{\text{conc}}) = (0.9036 \times J(\text{Core } N_{\text{conc}})) + 0.1588; R^2 = 0.9025 \quad (3.8)$$

(Figure 3.2). For the mass based approach, data input into the web-accessible spreadsheet (Wendt, 2012) created by Wendt and Hauser (2013) was adjusted for the clod and core methods according to Equations (2.8,11; and 3.7) and (2.9,12; and 3.8), respectively. Reference mass layers were set using the lowest mean soil mass across treatments for each soil depth layer as recommended by Lee et al. (2009) for systems in which the initial conditions (e.g., SOC or bulk density) are not available.

Analysis of variance (ANOVA) was used to determine whether treatment affected SOC and N stocks. When significant differences were detected at $P < 0.1$, Tukey's HSD post-hoc tests were conducted to compare the means. Relationships between numerical variables such as SOC and N concentrations were determined using linear regression. Data were analyzed using R studio (R Core Team, 2016).

3.4 RESULTS

3.4.1 Bulk Density and Soil Organic Carbon Concentrations

In the mineral soil layers, bulk density was consistently highest for the thinning treatment group (hereafter T_{group}) and lowest for the control group (hereafter C_{group}) to a depth of 100 cm (Table 3.2; Figure 3.3a). In the upper 50 cm, this pattern was inversely related to SOC concentrations, with the C_{group} having the highest SOC concentrations and the T_{group} having the lowest (Table 3.3; Figure 3.3b). Soil organic C concentration was a significant ($P < 0.1$) predictor of bulk density in the mineral soil, the two variables following a quadratic relationship (Figure 3.4a). However, a large amount of variation occurred where SOC concentrations were below ~15 g OC/kg soil, which corresponded to depths below 50 cm. When the relationship between SOC concentration and bulk density was analyzed by depths 0-50 cm and 50-150 cm, the former explained slightly more of the variation in bulk density values than the whole mineral

soil (i.e., 0-150 cm in depth), while the latter was not significant (Figure 3.4b,c). Soil organic C concentration and bulk density were not significantly related in the litter layer (Figure 3.4d). Bulk density for the T_{group} was significantly (Tukey's HSD, $\alpha = 0.1$) higher than the C_{group} in the upper 20 cm of mineral soil and significantly higher than the fertilization + thinning treatment group (hereafter FT_{group}) in the 10-20 cm depth layer. The C_{group} had a significantly higher SOC concentration compared to both the T_{group} and the FT_{group} in the upper 20 cm of mineral soil, as well as in the 100-150 cm depth layer compared to the FT_{group}. No significant differences between treatment means were observed for bulk density or SOC concentration in the litter layer.

3.4.2 Soil Nitrogen Concentrations, Carbon to Nitrogen Ratios, and pH

Trends in soil N concentrations between treatments followed a similar pattern as SOC concentrations in the mineral soil, although a few additional means were found to be significantly different (Table 3.4; Figure 3.3c). The C_{group} had a significantly higher soil N concentration compared to the T_{group} at all depths except the 50-100 cm depth layer. Compared to the FT_{group}, the C_{group} had a significantly higher soil N concentration in the upper 20 cm of mineral soil and in the 100-150 cm depth layer. The FT_{group} also had a significantly higher soil N concentration compared to the T_{group} in the 0-10 cm depth layer. Notably, in the litter layer, the T_{group} had a significantly lower soil N concentration compared to the other two treatment groups. No significant differences in the C:N ratio (SOC concentration/N concentration) in the mineral soil were observed. However, the T_{group} had a significantly higher C:N ratio in the litter layer compared to the C_{group} and FT_{group} (39, 32, and 33, respectively) (Table 3.5; Figure 3.3d). The average C:N ratio normalized by depth was 18, 19, and 20 for the C_{group}, T_{group}, and FT_{group}, respectively. Soil organic C concentration was a significant predictor of N concentration in the mineral soil, the two variables being positively related (Figure 3.5a). Soil organic C

concentration explained much of the variation in N concentration in the mineral soil ($R^2 = 0.9886$). These two variables were not significantly related in the litter layer (Figure 3.5b). Soil was strongly acid across all treatments. Normalized by depth, mean soil pH was 5.02, ranging from 5.00 for the FT_{group} to 5.05 for the T_{group}. In the litter layer and upper 100 cm of mineral soil, the FT_{group} had consistently lower pH values than the other two treatment groups (Table 3.6; Figure 3.6). This difference was significant in the 0-10 cm depth layer between the FT_{group} and the T_{group}.

3.4.3 Soil Organic Carbon and Nitrogen Stocks – Fixed Depth

Calculated using the fixed depth approach, SOC and N stocks in the mineral soil were consistently highest for the C_{group} (Tables 3.7-8; Figure 3.7a,b). Compared to the T_{group}, these differences were significant above 50 cm for SOC stocks and at all depths except the 50-100 cm depth layer for soil N stocks. These differences were significant in the 10-20 and 100-150 cm depth layers compared to the FT_{group}. Across all treatment groups, the majority of SOC and N stocks were below 20 cm ($\geq 55\%$ and $>60\%$, respectively) to a depth of 150 cm. The FT_{group} had significantly greater litter layer SOC and N stocks compared to the C_{group}. Litter layer thickness was similar for the FT_{group} and T_{group} and was considerably (though not significantly) thicker for these two treatment groups than for the C_{group} (Table 3.9). Cumulative SOC stocks were significantly less at all depths below the litter layer for the T_{group} compared to the C_{group}, with a difference of 28% to a depth of 150 cm (Table 3.10; Figure 3.7c). The portion of this difference that occurred below 20 cm was 72%. The FT_{group} SOC stock to 150 cm in depth approximated the average of the other two treatment groups and was not significantly different than either. Cumulative N stocks followed essentially the same trends (Table 3.11; Figure 3.7d). The T_{group}

had 29% less soil N than the C_{group} to a depth of 150 cm, with 76% of this difference occurring below 20 cm.

3.4.4 Soil Organic Carbon and Nitrogen Stocks – Mass Based

The mass based estimation of SOC and N stocks yielded similar results as the fixed depth approach (Tables 3.12-15; Figure 3.8). Total differences in SOC and N stocks between the C_{group} and T_{group} were 25% and 27%, respectively, slightly less than for the fixed depth approach. The average depth to the reference mass layers approximated the depths sampled for the C_{group} and were most shallow for the T_{group} (Table 3.16; Figure 3.9). This occurred because the C_{group} and T_{group} generally had the lowest and highest bulk density values, respectively, and reference mass layers were set using the lowest mean soil mass across treatments for each soil depth layer. By correcting for the confounding effect of bulk density, which was significantly higher for the T_{group} than the C_{group} above 20 cm, the mass based approach resulted in greater SOC and N stock differences between these two treatment groups in the surface soil layers compared to the fixed depth approach. However, the majority of the observed difference in SOC and N stocks still occurred in deeper soil layers (below ~20 cm).

3.4.5 Carbon Stocks and Sequestration

Total and belowground C stocks followed the same pattern as SOC stocks, with the C_{group} and T_{group} having the highest and lowest C stocks, respectively. Trends in aboveground and root C sequestration were the opposite, with the T_{group} and C_{group} having the highest and lowest C stocks, respectively. However, none of these differences were significant (Table 3.17; Figure 3.10). Approximately half or more of total C stocks were contained in the soil across treatment groups. Initial trees per hectare was a significant predictor of aboveground, root, and total C stocks, and was positively related to these variables (Figure 3.11a,b,d). Soil organic C stock was also a

significant predictor of total C stock, the two variables being positively related (Figure 3.12).

Soil organic C stock explained more of the variation in total C stock than initial trees per hectare ($R^2 = 0.7143$ versus $R^2 = 0.4503$). There was no relationship between initial trees per hectare and SOC stocks (Figure 3.11c).

3.4.6 Merchantable Volume

Merchantable volume, reported in thousands of board-feet per acre, was significantly greater for the FT_{group} than the T_{group}, a difference of ~11% (Table 3.18). No other variable (e.g., SOC or N stock, initial stems per hectare, and dbh) was significantly related to merchantable volume. Final mean dbh was greatest for the FT_{group} and lowest for the T_{group}, but these differences were not significant (Table 3.18). Initial trees per hectare was a significant predictor of dbh, the two variables being negatively related (Figure 3.13).

3.5 DISCUSSION

It is important to first determine which approach, fixed depth or mass based, provided a more accurate quantification and comparison of SOC and N stocks. While the fixed depth and mass based approaches resulted in similar conclusions overall, the mass based approach appears to have better represented the degree of change along the vertical soil profile by comparing equal soil masses and eliminating bulk density as a factor. Significant differences in bulk density observed between treatment groups were most likely the result of changes in SOC concentrations. This conclusion is supported by the lack of heavy equipment used during thinning treatments (i.e., a lack of soil-compacting operations), as well as the fact that significant differences in bulk density between treatment groups coincided with significant differences in SOC concentrations and occurred in soil layers where SOC concentration was a significant predictor of bulk density. In order to account and correct for changes in bulk density with time

(that have not resulted from soil erosion or deposition), the mass based approach is increasingly recommended for SOC and other soil nutrient inventories (e.g., Ellert and Bettany, 1995; Lee et al., 2009; Mikha et al., 2013; Schrumpf et al., 2011; Wendt and Hauser, 2013; Wuest, 2009). Therefore, SOC and N stocks quantified using the mass based approach and compared among equal soil masses will be considered the more accurate account, as this process removed changes in bulk density as a confounding variable. Further discussion will refer to mass based SOC and N stock estimates.

3.5.1 Soil Organic Carbon and Nitrogen Stocks

Litter layer SOC and N stock differences between the FT_{group} and C_{group} primarily resulted from the greater thickness of the FT_{group} litter layer compared to the C_{group}. Increases in FT_{group} litter layer thickness could have been caused by greater aboveground biomass and potentially increased N availability compared to the C_{group}. These same mechanisms also could have resulted in the increased thickness of the T_{group} litter layer compared to the C_{group}. In the mineral soil, cumulative SOC and N stocks were consistently (and significantly) greater for the C_{group} compared to one or both of the other treatment groups. However, in the absence of information on the initial conditions, it is unclear whether these observed differences were primarily the result of SOC and N losses, gains, or a combination therein.

The T_{group} and FT_{group} contained 77 and 30 Mg ha⁻¹ less SOC, respectively, than the C_{group} to a depth of 150 cm. The difference between T_{group} and C_{group} SOC stocks was significant and would have, theoretically, occurred over a shorter post-treatment timeframe than the difference observed between the FT_{group} and C_{group}. The average length of time between T_{group} thinning treatments and soil sampling was ~11 years. Therefore, assuming equivalent SOC stocks pre-treatment, the rate of post-treatment SOC loss for the T_{group} or gain for the C_{group} was ~700 g C

$\text{m}^{-2} \text{y}^{-1}$ compared to the other treatment group. Given that the average range of SOC accumulation rates in temperate forest soils is ~ 2 to $70 \text{ g C m}^{-2} \text{y}^{-1}$ (Post and Kwon, 2000), it is reasonable to conclude that the difference in SOC stocks between the two treatment groups was primarily due to post-treatment SOC losses. However, potential SOC gains should not be ignored. Following forest harvest, SOC stocks are generally reduced, and recovery can take several decades to 100+ years (James and Harrison, 2016; Jandl et al., 2007). Ultisol soil orders (the soil order sampled herein) in particular appear to experience relatively large and significant SOC stock decreases in the mineral soil post-harvest, with recovery timeframes of well over 75 years (James and Harrison, 2016). Therefore, the C_{group} (and pre-treatment T_{group}) was most likely gaining SOC throughout the length of the rotation. Ascribing an average SOC accumulation rate of $34 \text{ g C m}^{-2} \text{y}^{-1}$ (Post and Kwon, 2000), the T_{group} lost $\sim 666 \text{ g C m}^{-2} \text{y}^{-1}$ post-treatment. Raich and Schlesinger (1992) reported a mean soil respiration rate for temperate coniferous forests of $681 \text{ g C m}^{-2} \text{y}^{-1}$. However, rates ranging between ~ 950 and $1750 \text{ g C m}^{-2} \text{y}^{-1}$ have been measured (Gallo, 2016; Hicks Pries et al., 2017; Tang et al., 2005). The estimated increase in soil respiration rate ($\sim 666 \text{ g C m}^{-2} \text{y}^{-1}$) for the post-treatment T_{group} thus falls within the published range of soil respiration rates for temperate coniferous forests. Furthermore, other mechanisms of SOC and N loss, such as increased export, could help explain observed differences in treatment group SOC and N stocks.

3.5.2 Soil Organic Carbon and Nitrogen Loss

Potential mechanisms that may explain SOC and N losses due to thinning or fertilization treatments include: 1) increased soil temperature and thus enhanced microbial metabolic activity; 2) microbial stimulation, or *priming*, due to increases in rooting depth, dissolved organic carbon

(DOC) flux, or nitrate leaching; and 3) groundwater level rise and subsequent increases in the export of DOC and various forms of N.

3.5.2.1 Soil Temperature

When logging residues were left on-site, forest harvest has been observed to increase mean soil temperature and mean daily soil temperature flux by $\sim 3^{\circ}\text{C}$ and 5°C , respectively, at 10 cm in depth and by $\sim 2^{\circ}\text{C}$ and 3°C , respectively, at 100 cm in depth (Gallo, 2016). Similarly, thinning treatments can result in soil temperature increases due to decreased radiation interception by trees (Chase et al., 2016). Several studies have shown post-thinning increases in soil temperature at 10 cm in depth ranging from 1-6 $^{\circ}\text{C}$ by comparing thinned stands to reference stands (Chase et al., 2016; Cheng et al., 2014; Thibodeau et al., 2000). When different thinning intensities were compared, soil temperature increased with thinning intensity (Chase et al., 2016; Cheng et al., 2014). Thinning treatments have also been observed to increase soil respiration rates. Cheng et al. (2014) measured an increase in soil respiration in the upper 10 cm ranging from $\sim 8\%$ to 20% and increasing with thinning intensity. At the site studied herein, thinning treatments could be considered “intense,” as spacing between remaining trees was relatively large due to low initial stocking. Therefore, potential post-thinning increases in soil temperature and respiration rates may have been on the high end of observed ranges.

Increased bulk density in the surface soil layers of the T_{group} could have also affected soil temperature by increasing heat-transfer rates to deeper soil layers (Brady and Weil, 2008). For example, Gallo (2016) observed greater soil temperature increases post-harvest in soils that received compacting treatments compared to soils that were not subjected to compaction. Hicks Pries et al. (2017) found that mineral soil respiration in a temperate forest ecosystem increased by 34-37% to a depth of 100 cm when subjected to 4°C warming, with soil below 15 cm

contributing to ~50% of the total respiration. The T_{group} followed a similar pattern, with the majority (56%) of SOC losses compared to the C_{group} occurring below ~20 cm.

The microbial demand for N during organic matter decomposition is about a 24:1 C:N ratio (Brady and Weil, 2008). Therefore, increased soil respiration rates increase demand for N, particularly during the decomposition of organic matter with a higher C:N ratio. The significant decrease in T_{group} litter layer soil N concentration compared to the other two treatment groups was likely due to forest floor organic matter additions with high C:N ratios (such as coarse woody debris left as slash on the forest floor) resulting in microbial N scavenging in this layer (Brady and Weil, 2008; Staaf and Berg, 1982). In the surface soil layers – which contain the majority of root biomass (Jackson et al., 1996) – the mean C:N ratio across treatment groups was ~24, suggesting tight N cycling as plants and microorganisms competed for this essential nutrient.

3.5.2.2 Priming Effects

Microbial stimulation, or *priming*, is a mechanism by which potential energetic barriers to SOC decomposition are alleviated by the introduction of fresh C compounds (Fontaine et al., 2007). Similarly, higher rates of nitrification and additional nitrate can result in increased SOC decomposition and DOC production by alleviating microbial nutrient limitations (Carrillo et al., 2017; Kalbitz et al., 2000). The priming phenomenon is particularly relevant in deeper soil layers where SOC is often thousands to tens of thousands of years old (Fontaine et al., 2007; Schmidt et al., 2011). When the environmental conditions under which deep SOC accumulated change, such as through the addition of fresh C compounds, this SOC is vulnerable to decomposition (Fontaine et al., 2007; Harrison et al., 2011; Hicks Pries et al., 2017, 2016; Schmidt et al., 2011). The soil sampled herein may be particularly vulnerable to rapid SOC decomposition when

subjected to changing environmental conditions, as it is an older soil that likely developed and accrued SOC over hundreds of thousands to millions of years under relatively stable conditions (Lin, 2011).

Increases in soil temperature due to thinning treatments may in turn increase fine root colonization of deep soil (Rasse et al., 2001), adding labile root C inputs to deeper soil layers and potentially resulting in rhizosphere (the portion of soil in the immediate vicinity of roots) priming effects (Dijkstra and Cheng, 2007; Zhu et al., 2014). By enhancing SOC decomposition, rhizosphere priming effects may also increase soil N mineralization (after which incorporation of N into microbial biomass, uptake by plants, or export can occur). When inorganic N was available to plants, Dijkstra et al. (2009) found that the priming of SOC by plant root exudates was not related to excess plant available N. However, when inorganic N was depleted, primed SOC and excess plant available N were significantly positively correlated. Apparent tight N cycling at the site studied herein suggests a potentially positive correlation between rhizosphere priming of SOC and N mineralization. Soil roots may also create favorable conditions for microorganisms in deeper soil layers by increasing soil moisture variability and aeration through transpiration (Mobley et al., 2015). Additionally, increased soil rooting depth due to soil temperature rise or fertilization increasing above- and below-ground growth can lead to plants scavenging for N from deeper soil layers than they previously reached (Kuzyakov et al., 2000 and references therein). This could help explain why the loss of soil N from deeper soil layers was proportionally greater than the loss of SOC.

Dissolved organic carbon flux and transport to deeper soil layers is driven largely by rain events and new inputs of organic matter (Neff and Asner, 2001; Sanderman et al., 2008). By removing trees, thinning treatments result in decreased transpiration and rain interception,

possibly increasing DOC flux and the potential for priming effects. Additionally, organic matter left on-site post-thinning (e.g., roots and slash) substantially increases soil C inputs. While forest floor fresh C inputs are often mineralized in the litter layer (Sanderman et al., 2008; Schmidt et al., 2011), C inputs due to the mineralization of root biomass can persist for many years following the harvest of trees (Strahm et al., 2009). Post-thinning increases in channels of decaying roots, particularly coarse roots, could also create preferential flow paths for DOC transport. Along these pathways, DOC has less opportunities for abiotic and biotic interactions, potentially introducing large amounts of fresh DOC to deeper soil layers (Deb and Shukla, 2011). As preferential pathways have been shown to have greater SOC concentrations and microbial biomass than the surrounding bulk soil (Bundt et al., 2001), mineralization rates in these pathways are likely enhanced (Hagedorn and Bundt, 2002), which may lead to increased SOC and N losses from the adjacent bulk soil.

Several studies have observed increased leaching or export of nitrate (and other forms of N) following forest harvest under logging residues (Emmett et al., 1991; Nieminen, 2004; Rosén and Lundmark-Thelin, 1987; Staaf and Olsson, 1994; Strahm et al., 2005). Rosen and Lundmark-Thelin (1987) attributed this phenomenon to a combination of reduced N uptake by roots and increased mineralization of the litter layer. Plots that received both thinning and fertilization treatments at the site studied herein may have been particularly vulnerable to nitrate leaching under slash left on the forest floor post-thinning due to increased N availability. Fertilization treatments tended to decrease soil pH, which indicates that a considerable portion of nitrate resulting from the nitrification of urea was not taken up by plants, causing the net addition of one proton (H^+) to the soil solution per urea compound (Davidson, 1987). Increases in soil acidity due to urea fertilization, high rates of nitrification, and nitrate leaching can negatively affect soil

quality through the priming of SOC and through the co-leaching of other nutrients such as calcium and magnesium (Fox, 2004). Additionally, the high precipitation in the region studied would have generally promoted nitrate leaching.

3.5.2.3 Groundwater Level

The removal of trees via thinning treatments or harvest reduces transpiration, which can result in groundwater level rise and potential increases in the export of DOC and various forms of N (Laudon et al., 2009; Nieminen, 2004). Laudon et al. (2009) observed a >70% increase in DOC export from harvested compared to unharvested sites one year post-harvest. They attributed this increase in DOC export primarily to a raised groundwater level coming into contact with more superficial soil layers. Several of the excavated pits at the site studied herein had redoximorphic features as high as 75 cm in depth, indicating a relatively high water table. A previous study conducted at the same site identified soil features (massive, clay cemented) below 300 cm that could result in a perched water table (James et al., 2015). As Douglas-fir roots commonly extend to at least 300 cm in depth (Stone and Kalisz, 1991), thinning treatments may have sufficiently reduced transpiration to allow a local rise in groundwater level. This rise would have allowed the groundwater to come into contact with more superficial soil layers that have higher DOC and N concentrations, potentially increasing the export of DOC and various forms of N.

3.5.3 Carbon Stocks and Sequestration

Although SOC stocks were significantly greater for the C_{group} than the T_{group}, differences in belowground and total C stocks between these two treatment groups were muted because the T_{group} had greater aboveground and root C stocks. Aboveground and root C sequestration were primarily dependent on initial trees per hectare, and the T_{group} had substantially higher initial trees per hectare, on average, than the C_{group} (952 and 682 trees ha⁻¹, respectively). Interestingly,

there was no relationship between SOC stocks and initial trees per hectare. This is somewhat unexpected because thinning treatments, which did affect SOC stocks, were carried out similarly within about a decade of the reductions in initial trees per hectare. However, several key differences may explain this phenomenon. While thinning treatments occurred after crown closure, thus exposing previously shaded and covered areas, reductions in initial trees per hectare occurred prior to crown closure and would not have drastically changed soil conditions. More substantial understory cover prior to crown closure likely insulated the soil from substantial temperature changes and increased N uptake, decreasing nitrate leaching and helping to retain N on-site following the reductions in initial trees per hectare (Chang and Preston, 2000; Footen et al., 2009). At this early stage in stand development, roots may have been less dense and deeply rooted in the soil, reducing DOC flux and the potential for priming effects compared to thinning treatments implemented years later. Differences in soil microbial communities can also lead to differences in SOC and nutrient dynamics. Smith et al. (2015) found that soil microbial communities differed between younger and older forests in a study examining forests aged 20 years and older. Although initial stems per hectare were reduced at the site studied herein when the stand was aged <15 years, differences in ground and soil conditions – and thus differences in microbial communities – would potentially be greater between juvenile and adult stands than between younger and older adult stands.

3.5.4 Merchantable Volume

Despite greater merchantable volume for the FT_{group} compared to the C_{group}, any net monetary gains were likely minimal due to the additional expenses of thinning and fertilization treatments. The T_{group} resulted in the least financial gain over the length of the rotation (i.e., it had the lowest merchantable volume and incurred thinning expenses) and reduced soil quality and nutrient

stocks for the succeeding rotation. On the other hand, reducing the initial trees per hectare prior to crown closure did not appear to affect SOC and N stocks. This typically low-cost practice could provide benefits such as increased stand stability, health, and dbh, while potentially avoiding negative effects on SOC and N stocks.

3.6 CONCLUSIONS

This long-term forest management study shows that forest management practices such as thinning and fertilization can affect both surface and deep SOC and N stocks on decadal timescales. Thinning treatments reduced SOC and N stocks by 25% and 27%, respectively, with most of this loss occurring below ~20 cm to a depth of ~150 cm. Changing the soil environment by affecting C inputs, roots, and soil properties can enhance microbial activities, increasing SOC decomposition, N mineralization, and SOC and N export. In this study, a combination of these factors and their complex interactions likely resulted in the observed decreases in SOC and N stocks. As the majority of SOC and N stocks are contained in deeper soil layers, accurately assessing SOC and N budgets and comparing changes over time requires sampling soil deeper than 20 cm.

REFERENCES

- Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47, 151–163.
- Bellassen, V., Luysaert, S., 2014. Managing forests in uncertain times. *Nature* 506, 153–5. doi:10.1038/506153a
- Birdsey, R.A., 1992. Carbon storage and accumulation in United States forest ecosystems, Gen. Tech. Rep. WO-59. Radnor, PA. doi:10.5962/bhl.title.94267
- Blake, J.I., Chappell, H.N., Bennett, W.S., Gessel, S.P., Webster, S.R., 1990. Douglas Fir Growth and Foliar Nutrient Responses to Nitrogen and Sulfur Fertilization. *Soil Sci. Soc. Am. J.* 54, 257. doi:10.2136/sssaj1990.03615995005400010041x
- Brady, N.C., Weil, R.R., 2008. *The nature and properties of soils*, 14th ed. Pearson, Upper Saddle River, NJ.
- Bundt, M., Widmer, F., Pesaro, M., Zeyer, J., Blaser, P., 2001. Preferential flow paths: Biological “hot spots” in soils. *Soil Biol. Biochem.* 33, 729–738. doi:10.1016/S0038-0717(00)00218-2
- Carrillo, Y., Bell, C., Koyama, A., Canarini, A., Boot, C.M., Wallenstein, M., Pendall, E., 2017. Plant traits, stoichiometry and microbes as drivers of decomposition in the rhizosphere in a temperate grassland. *J. Ecol.* doi:10.1111/1365-2745.12772
- Carter, R.E., Klinka, K., 1990. Relationships between growing-season soil water-deficit, mineralizable soil nitrogen and site index of coastal Douglas fir. *For. Ecol. Manage.* 30, 301–311. doi:10.1016/0378-1127(90)90144-Z
- Chang, S.X., Preston, C.M., 2000. Understorey competition affects tree growth and fate of fertilizer-applied ¹⁵N in a Coastal British Columbia plantation forest: 6-year results. *Can. J. For. Res.* 30, 1379–1388. doi:10.1139/x00-068
- Chappell, H.N., Cole, D.W., Gessel, S.P., Walker, R.B., 1991. Forest fertilization research and practice in the Pacific Northwest. *Fertilizer Res.* 27, 129–140.
- Chase, C.W., Kimsey, M.J., Shaw, T.M., Coleman, M.D., 2016. The response of light, water, and nutrient availability to pre-commercial thinning in dry inland Douglas-fir forests. *For. Ecol. Manage.* 363, 98–109. doi:10.1016/j.foreco.2015.12.014
- Cheng, X., Han, H., Kang, F., Liu, K., Song, Y., Zhou, B., Li, Y., 2014. Short-term effects of thinning on soil respiration in a pine (*Pinus tabulaeformis*) plantation. *Biol. Fertil. Soils* 50, 357–367. doi:10.1007/s00374-013-0852-0
- Clarke, N., Gundersen, P., Jönsson-Belyazid, U., Kjønås, O.J., Persson, T., Sigurdsson, B.D., Stupak, I., Vesterdal, L., 2015. Influence of different tree-harvesting intensities on forest soil carbon stocks in boreal and northern temperate forest ecosystems. *For. Ecol. Manage.* 351, 9–19. doi:10.1016/j.foreco.2015.04.034
- Corti, G., Ugolini, F.C., Agnelli, A., Certini, G., Cuniglio, R., Berna, F., Fernández Sanjurjo, M.J., 2002. The soil skeleton, a forgotten pool of carbon and nitrogen in soil. *Eur. J. Soil Sci.* 53, 283–298. doi:10.1046/j.1365-2389.2002.00442.x

- Curtis, R.O., 1982. A simple index of stand density for Douglas-fir. *For. Sci.* 28, 92–94.
- Davidson, S., 1987. Combating soil acidity: three approaches. *Rural Res.* 134, 4–10.
- Deb, S.K., Shukla, M.K., 2011. A Review of Dissolved Organic Matter Transport Processes Affecting Soil and Environmental Quality. *J. Environ. Anal. Toxicol.* 1, 1–11. doi:10.4172/2161-0525.1000106
- Dijkstra, F.A., Bader, N.E., Johnson, D.W., Cheng, W., 2009. Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? *Soil Biol. Biochem.* 41, 1080–1087. doi:10.1016/j.soilbio.2009.02.013
- Dijkstra, F.A., Cheng, W., 2007. Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecol. Lett.* 10, 1046–1053. doi:10.1111/j.1461-0248.2007.01095.x
- Diochon, A.C., Kellman, L., 2009. Physical fractionation of soil organic matter: Destabilization of deep soil carbon following harvesting of a temperate coniferous forest. *J. Geophys. Res. Biogeosciences* 114, 1–9. doi:10.1029/2008JG000844
- Dixon, R.K., Brown, S., Houghton, R.A., Solomon, A.M., Trexler, M.C., Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. *Science* (80-.). 263, 185–190.
- Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75, 529–538. doi:10.4141/cjss95-075
- Emmett, B.A., Anderson, J.M., Hornung, M., 1991. The controls on dissolved nitrogen losses following two intensities of harvesting in a Sitka spruce forest (N. Wales). *For. Ecol. Manage.* 41, 65–80. doi:10.1016/0378-1127(91)90119-G
- Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450, 277–80. doi:10.1038/nature06275
- Footen, P.W., Harrison, R.B., Strahm, B.D., 2009. Long-term effects of nitrogen fertilization on the productivity of subsequent stands of Douglas-fir in the Pacific Northwest. *For. Ecol. Manage.* 258, 2194–2198. doi:10.1016/j.foreco.2009.02.033
- Fox, T.R., 2004. Nitrogen mineralization following fertilization of Douglas-fir forests with urea in western Washington. *Soil Sci. Soc. Am. J.* 68, 1720–1728. doi:10.2136/sssaj2004.1720
- Gallo, A.C., 2016. Response of Soil Temperature, Moisture, and Respiration Two Years Following Intensive Organic Matter and Compaction Manipulations in Oregon Cascade Forests. Oregon State University.
- Hagedorn, F., Bundt, M., 2002. The age of preferential flow paths. *Geoderma* 108, 119–132. doi:Pii s0016-7061(02)00129-5\r10.1016/s0016-7061(02)00129-5
- Hamburg, S.P., 2000. Simple Rules for Measuring Changes in Ecosystem. *Mitigation Adapt. Strateg. Glob. Chang.* 5, 25–37.
- Harrison, R.B., Adams, A.B., Licata, C., Flaming, B., Wagoner, G.L., Carpenter, P., Vance, E.D., 2003. Quantifying deep-soil and coarse-soil fractions: Avoiding sampling bias. *Soil Sci. Soc. Am. J.* 67, 1602–1606.
- Harrison, R.B., Footen, P.W., Strahm, B.D., 2011. Deep soil horizons: Contribution and

- importance to soil carbon pools and in assessing whole-ecosystem response to management and global change. *For. Sci.* 57, 67–76.
- Hicks Pries, C.E., Castanha, C., Porras, R., Torn, M.S., 2017. The whole-soil carbon flux in response to warming. *Science* (80-.). 1–8. doi:10.1126/science.aal1319
- Hicks Pries, C.E., Schuur, E.A.G., Natali, S.M., Crummer, K.G., 2016. Old soil carbon losses increase with ecosystem respiration in experimentally thawed tundra. *Nat. Clim. Chang.* 6, 214–218. doi:10.1038/nclimate2830
- Holub, S.M., 2011. Soil Carbon Change in Pacific Northwest Coastal Douglas-fir Forests: Change Detection Following Harvest – Soils Establishment Report. Albany, Oregon.
- Jackson, R.B., Canadell, J., Ehleringer, J.R., Mooney, H. a., Sala, O.E., Schulze, E.D., 1996. A global analysis of root distributions for terrestrial biomes. *Oecologia* 108, 389–411. doi:10.1007/BF00333714
- James, J., Harrison, R., 2016. The Effect of Harvest on Forest Soil Carbon: A Meta-Analysis. *Forests* 7, 308. doi:10.3390/f7120308
- James, J.N., Dietzen, C., Furches, J.C., Harrison, R.B., 2015. Lessons on Buried Horizons and Pedogenesis from Deep Forest Soils. *Soil Horizons* 56, 0. doi:10.2136/sh15-02-0004
- Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., Johnson, D.W., Minkinen, K., Byrne, K.A., 2007. How strongly can forest management influence soil carbon sequestration? *Geoderma* 137, 253–268. doi:10.1016/j.geoderma.2006.09.003
- Jandl, R., Rodeghiero, M., Martinez, C., Cotrufo, M.F., Bampa, F., van Wesemael, B., Harrison, R.B., Guerrini, I.A., Richter, D. deB, Rustad, L., Lorenz, K., Chabbi, A., Miglietta, F., 2014. Current status, uncertainty and future needs in soil organic carbon monitoring. *Sci. Total Environ.* 468–469, 376–383. doi:10.1016/j.scitotenv.2013.08.026
- Jenkins, J.C., Chojnacky, D.C., Heath, L.S., Birdsey, R.A., 2003. National-scale biomass estimators for United States tree species. *For. Sci.* 49, 12–35.
- Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* 10, 423–436. doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2
- Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Sci.* 165, 277–304.
- Kuzyakov, Y., Friedelb, J.K., Stahra, K., 2000. Review of mechanisms and quantification of priming effects (Review). *Soil Biol. Biochem.* 32, 1485–1498. doi:10.1016/S0038-0717(00)00084-5
- Lal, R., 2005. Forest soils and carbon sequestration. *For. Ecol. Manage.* 220, 242–258. doi:10.1016/j.foreco.2005.08.015
- Laudon, H., Hedtjörn, J., Schelker, J., Bishop, K., Sørensen, R., Agren, A., 2009. Response of dissolved organic carbon following forest harvesting in a boreal forest. *Ambio* 38, 381–386. doi:10.1579/0044-7447-38.7.381
- Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. *Agric. Ecosyst. Environ.* 134, 251–256.

doi:10.1016/j.agee.2009.07.006

- Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F., Wu, J., 2015. The effects of simulated nitrogen deposition on plant root traits: A meta-analysis. *Soil Biol. Biochem.* 82, 112–118. doi:10.1016/j.soilbio.2015.01.001
- Lin, H., 2011. Three Principles of Soil Change and Pedogenesis in Time and Space. *Soil Sci. Soc. Am. J.* 75, 2049. doi:10.2136/sssaj2011.0130
- Mikha, M.M., Benjamin, J.G., Halvorson, A.D., Nielsen, D.C., 2013. Soil Carbon Changes Influenced by Soil Management and Calculation Method. *Open J. Soil Sci.* 3, 123–131. doi:10.4236/ojss.2013.32014
- Miles, P.D., Smith, W.B., 2009. Specific Gravity and Other Properties of Wood and Bark for 156 Tree Species Found in North America, Res. Note. NRS-38. Newtown Square, PA.
- Milne, E., Banwart, S.A., Noellemeyer, E., Abson, D.J., Ballabio, C., Bampa, F., Bationo, A., Batjes, N.H., Bernoux, M., Bhattacharyya, T., Black, H., Buschiazzi, D.E., Cai, Z., Cerri, C.E., Cheng, K., Compagnone, C., Conant, R., Coutinho, H.L.C., de Brogniez, D., Balieiro, F. de C., Duffy, C., Feller, C., Fidalgo, E.C.C., da Silva, C.F., Funk, R., Gaudig, G., Gicheru, P.T., Goldhaber, M., Gottschalk, P., Goulet, F., Goverse, T., Grathwohl, P., Joosten, H., Kamoni, P.T., Kihara, J., Krawczynski, R., La Scala, N., Lemanceau, P., Li, L., Li, Z., Lugato, E., Maron, P.A., Martius, C., Melillo, J., Montanarella, L., Nikolaidis, N., Nziguheba, G., Pan, G., Pascual, U., Paustian, K., Pineiro, G., Powlson, D., Quiroga, A., Richter, D., Sigwalt, A., Six, J., Smith, J., Smith, P., Stocking, M., Tanneberger, F., Termansen, M., van Noordwijk, M., van Wesemael, B., Vargas, R., Victoria, R.L., Waswa, B., Werner, D., Wichmann, S., Wichtmann, W., Zhang, X., Zhao, Y., Zheng, J., Zheng, J., 2015. Soil carbon, multiple benefits. *Environ. Dev.* 13, 33–38. doi:10.1016/j.envdev.2014.11.005
- Mobley, M.L., Lajtha, K., Kramer, M.G., Bacon, A.R., Heine, P.R., Richter, D.D., 2015. Surficial gains and subsoil losses of soil carbon and nitrogen during secondary forest development. *Glob. Chang. Biol.* 21, 986–996. doi:10.1111/gcb.12715
- Neff, J.C., Asner, G.P., 2001. Dissolved organic carbon in terrestrial ecosystems: Synthesis and a model. *Ecosystems* 4, 29–48. doi:10.1007/s100210000058
- Nieminen, M., 2004. Export of dissolved organic carbon, nitrogen and phosphorus following clear-cutting of three Norway spruce forests growing on drained peatlands in southern Finland. *Silva Fenn.* 38, 123–132. doi:10.14214/sf.422
- Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A Large and Persistent Carbon Sink in the World's Forests. *Science* (80-.). 333, 988–993.
- Post, W.M., Kwon, K.C.A., 2000. Soil carbon sequestration and land-use change: processes and potential. *Glob. Chang. Biol.* 6, ages.
- PRISM Climate Group, 2017. PRISM Climate Group. URL <http://prism.oregonstate.edu> (accessed 2.17.17).
- R Core Team, 2016. R: A language and environment for statistical computing. Vienna, Austria.

- Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44B, 81–99.
- Rasse, D.P., Longdoz, B., Ceulemans, R., 2001. TRAP: a modelling approach to below-ground carbon allocation in temperate forests. *Plant Soil* 229, 281–293. doi:10.1023/A:1004832119820
- Richter, D.D., Markewitz, D., Trumbore, S.E., Wells, C.G., 1999. Rapid accumulation and turnover of soil carbon in a re-establishing forest. *Nature* 400, 56–58. doi:10.1038/21867
- Rosén, K., Lundmark-Thelin, A., 1987. Increased Nitrogen Leaching under Piles of Slash - a Consequence of Modern Forest Harvesting Techniques. *Scand. J. For. Res.* 2, 21–29.
- Sanderman, J., Baldock, J.A., Amundson, R., Baldock, J.A., 2008. Dissolved Organic Carbon Chemistry and Dynamics in Contrasting Forest and Grassland Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Source Biogeochem. Biogeochem.* 89, 181–198.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. a., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. a. C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56. doi:10.1038/nature10386
- Schrumpf, M., Schulze, E.D., Kaiser, K., Schumacher, J., 2011. How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories? *Biogeosciences* 8, 1193–1212. doi:10.5194/bg-8-1193-2011
- Smith, A.P., Marín-Spiotta, E., Balsler, T., 2015. Successional and seasonal variations in soil and litter microbial community structure and function during tropical postagricultural forest regeneration: A multiyear study. *Glob. Chang. Biol.* 21, 3532–3547. doi:10.1111/gcb.12947
- Soil Survey Staff, 2016. Web Soil Survey. Available online. Accessed 05/07/2017.
- Staaf, H., Berg, B., 1982. Accumulation and release of plant nutrients in decomposing Scots pine needle litter. Long-term decomposition in a Scots pine forest II. *Can. J. Bot.* 60, 1561–1568.
- Staaf, H., Olsson, B. a., 1994. Effects of slash removal and stump harvesting on soil water chemistry in a clearcutting in SW Sweden. *Scand. J. For. Res.* 9, 305–310. doi:10.1080/02827589409382844
- Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., Courcelles, V. de R. de, Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric. Ecosyst. Environ.* 164, 80–99. doi:10.1016/j.agee.2012.10.001
- Stone, E.L., Kalisz, P.J., 1991. On the maximum extent of tree roots. *For. Ecol. Manage.* 46, 59–102.
- Strahm, B.D., Harrison, R.B., Terry, T.A., Flaming, B.L., Licata, C.W., Petersen, K.S., 2005. Soil solution nitrogen concentrations and leaching rates as influenced by organic matter retention on a highly productive Douglas-fir site. *For. Ecol. Manage.* 218, 74–88. doi:10.1016/j.foreco.2005.07.013

- Strahm, B.D., Harrison, R.B., Terry, T.A., Harrington, T.B., Adams, A.B., Footen, P.W., 2009. Changes in dissolved organic matter with depth suggest the potential for postharvest organic matter retention to increase subsurface soil carbon pools. *For. Ecol. Manage.* 258, 2347–2352. doi:10.1016/j.foreco.2009.03.014
- Tang, J., Qi, Y., Xu, M., Misson, L., Goldstein, A.H., 2005. Forest thinning and soil respiration in a ponderosa pine plantation in the Sierra Nevada. *Tree Physiol.* 25, 57–66. doi:10.1093/treephys/25.1.57
- Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G., Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem. Cycles* 23, 1–11. doi:10.1029/2008GB003327
- Thibodeau, L., Raymond, P., Camiré, C., Munson, A.D., 2000. Impact of precommercial thinning in balsam fir stands on soil nitrogen dynamics, microbial biomass, decomposition, and foliar nutrition. *Can. J. For. Res.* 30, 229–238. doi:10.1139/x99-202
- Throop, H.L., Archer, S.R., Monger, H.C., Waltman, S., 2012. When bulk density methods matter: Implications for estimating soil organic carbon pools in rocky soils. *J. Arid Environ.* 77, 66–71. doi:10.1016/j.jaridenv.2011.08.020
- Tisdall, A.L., 1951. Comparison of methods of determining apparent density of soils. *Aust. J. Agric. Res.* 2, 349–354.
- Wendt, J.W., 2012. ESM Sample Spreadsheets.Xlsm. URL <https://drive.google.com/file/d/0BzxNFfzLbFxfjSG9RWlpwQ0FXc0k/view?usp=sharing> (accessed 5.16.17).
- Wendt, J.W., Hauser, S., 2013. An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *Eur. J. Soil Sci.* 64, 58–65. doi:10.1111/ejss.12002
- Whitney, N., Zabowski, D., 2004. Soil total nitrogen in the coarse fraction and at depth. *Soil Sci. Soc. Am. J.* 24. doi:10.2136/sssaj2004.0612
- Wuest, S.B., 2009. Correction of Bulk Density and Sampling Method Biases Using Soil Mass per Unit Area. *Soil Sci. Soc. Am. J.* 73, 312. doi:10.2136/sssaj2008.0063
- Zabowski, D., Whitney, N., Gurung, J., Hatten, J., 2011. Total soil carbon in the coarse fraction and at depth. *For. Sci.* 57, 11–18. doi:10.2136/sssaj2004.0612
- Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. *Soil Biol. Biochem.* 76, 183–192. doi:10.1016/j.soilbio.2014.04.033

Table 2.1. Typical soil profile description. Resembles Cumley series (a Palehumult). Average slope of 10%. Mean elevation ~640 m a.s.l.

Horizons	Depth (cm)	Color (moist)	Texture	Structure	Roots	% Rock
Oi	3.5-0					Gr <2% Cb <5% St <5%
A	0-15	10YR 3/3 dark brown	Sandy clay loam	Medium to coarse granular, weak to moderate	Many fine, medium, and coarse	Gr <2% Cb <5% St <5%
BA	15-30	10YR 3/4 dark yellowish brown	Sandy clay	Medium to coarse subangular blocky, moderate	Many fine and medium; few coarse	Gr <2% Cb <5% St <5%
Bt1	30-80	5YR 4/4 reddish brown	Sandy clay to clay	Coarse to very coarse subangular blocky, moderate to strong	Common fine, few medium; very few coarse	Gr <2% Cb <5% St <5%
Bt2	80-150+	10YR 4/4 dark yellowish brown	Sandy clay	Coarse subangular blocky, moderate	Few fine and medium; very few coarse	Gr <2% Cb <5% St <5%

Table 2.2. Soil bulk density (g cm^{-3}) for clod method comparing the clods sampled in triplicate from each depth layer. Mean \pm one standard error (in parentheses). Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

Soil depth layer (cm)	Clod x		Clod y		Clod z	
	Mean	Sample size	Mean	Sample size	Mean	Sample size
0-10	0.87 ^a (0.03)	10	0.81 ^a (0.04)	10	0.85 ^a (0.05)	10
10-20	0.94 ^a (0.02)	9	0.96 ^a (0.04)	9	0.98 ^a (0.02)	9
20-50	1.03 ^a (0.05)	11	1.02 ^a (0.05)	11	1.05 ^a (0.03)	11
50-100	1.12 ^a (0.03)	11	1.12 ^a (0.03)	11	1.12 ^a (0.03)	11
100-150	0.98 ^a (0.09)	3	0.97 ^a (0.07)	3	1.00 ^a (0.08)	3

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, x-y)	<i>P</i> (Tukey's HSD, x-z)	<i>P</i> (Tukey's HSD, y-z)
0-10	2, 27	0.608	0.552	-	-	-
10-20	2, 24	0.571	0.573	-	-	-
20-50	2, 30	0.046	0.955	-	-	-
50-100	2, 30	0.015	0.985	-	-	-
100-150	2, 6	0.05	0.951	-	-	-

Table 2.3. Soil bulk density (g cm^{-3}) by soil sampling method. Mean \pm one standard error (in parentheses). Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '^'; 0.1 '`'.

Soil depth layer (cm)	Clod		Core		Excavation	
	Mean	Sample size	Mean	Sample size	Mean	Sample size
0-10	0.79 ^a (0.05)	6	0.56 ^b (0.02)	9	0.72 ^{ab} (0.12)	3
10-20	0.98 ^a (0.02)	6	0.68 ^b (0.03)	9	0.92 ^a (0.20)	3
20-50	1.01 ^a (0.04)	10	0.79 ^b (0.02)	10	0.98 ^a (0.05)	5
50-100	1.12 ^a (0.03)	12	0.95 ^b (0.03)	14	1.15 ^a (0.04)	5
100-150	1.07 ^a (0.07)	5	0.90 ^b (0.03)	9	1.07 ^a (0.04)	4

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, cl-co)	<i>P</i> (Tukey's HSD, cl-ex)	<i>P</i> (Tukey's HSD, co-ex)
0-10	2, 15	8.239	0.0039*	0.0035*	0.6362	0.1142
10-20	2, 15	8.607	0.0032*	0.0035*	0.8151	0.0637 [`]
20-50	2, 22	13.29	0.0002**	0.0002**	0.8644	0.0058*
50-100	2, 28	12.64	0.0001**	0.0005**	0.7878	0.0014*
100-150	2, 15	4.256	0.0344 ^{^^}	0.0649 [`]	0.9999	0.0871 [`]

Table 2.4. Soil organic carbon concentration (g OC/kg soil) by soil sampling method. Mean \pm one standard error (in parentheses). Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '``'; 0.1 ''.

Soil depth layer (cm)	Clod		Core		Excavation	
	Mean	Sample size	Mean	Sample size	Mean	Sample size
0-10	73.0 ^a (15.0)	6	78.8 ^a (4.3)	9	101.8 ^a (22.5)	3
10-20	42.4 ^a (2.4)	6	62.7 ^b (5.8)	9	55.9 ^{ab} (7.3)	3
20-50	23.5 ^a (4.8)	10	24.1 ^a (2.7)	10	27.7 ^a (3.7)	5
50-100	7.7 ^{ab} (1.8)	12	4.9 ^b (0.6)	14	11.0 ^a (1.7)	5
100-150	3.2 ^a (0.6)	5	3.2 ^a (0.5)	9	4.7 ^a (1.2)	4

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, cl-co)	<i>P</i> (Tukey's HSD, cl-ex)	<i>P</i> (Tukey's HSD, co-ex)
0-10	2, 15	1.418	0.273	-	-	-
10-20	2, 15	4.127	0.0373``	0.0305``	0.2942	0.8184
20-50	2, 22	0.584	0.566	-	-	-
50-100	2, 28	3.638	0.0394``	0.4832	0.1987	0.0313``
100-150	2, 15	1.079	0.365	-	-	-

Table 2.5. Rock fragment (>4.75 mm) fraction (% weight) by soil sampling method. Mean \pm one standard error (in parentheses). Sample size by soil depth layer was equivalent for each method to account for any potential differences in rock fragment content by depth.

Clod		Core		Excavation	
Mean	Sample size	Mean	Sample size	Mean	Sample size
0.43% (0.28%)	20	1.07% (0.97%)	20	1.50% (0.94%)	20

Table 2.6. Soil organic carbon stock (Mg ha⁻¹) by soil sampling method. Calculated using the fixed depth equation. Mean ± one standard error (in parentheses). Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '''; 0.1 ''.

Soil depth layer (cm)	Clod		Core		Excavation	
	Mean	Sample size	Mean	Sample size	Mean	Sample size
0-10	54.3 ^{ab} (5.9)	6	43.7 ^a (1.5)	9	68.0 ^b (6.7)	3
10-20	41.4 ^a (1.6)	6	42.0 ^a (3.6)	9	49.1 ^a (6.6)	3
20-50	66.7 ^a (10.1)	10	56.7 ^a (6.5)	10	79.7 ^a (7.5)	5
50-100	41.1 ^{ab} (8.4)	12	23.3 ^a (3.0)	14	62.0 ^b (8.3)	5
100-150	17.0 ^a (3.2)	5	14.6 ^a (2.3)	9	24.9 ^a (5.6)	4

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, cl-co)	<i>P</i> (Tukey's HSD, cl-ex)	<i>P</i> (Tukey's HSD, co-ex)
0-10	2, 15	7.734	0.0049*	0.1169	0.1457	0.0046*
10-20	2, 15	0.725	0.501	-	-	-
20-50	2, 22	1.325	0.286	-	-	-
50-100	2, 28	7.002	0.0034*	0.1038	0.1346	0.0031*
100-150	2, 15	2.173	0.148	-	-	-

Table 2.7. Cumulative original and adjusted soil organic carbon stocks (Mg ha^{-1}) by soil sampling method. Calculated using the fixed depth equation. Soil organic carbon concentration for the clod method was adjusted according to Equation (2.8), and bulk density and soil organic carbon concentration for the core method were adjusted according to Equations (2.7) and (2.9) (Table 2.10). Excavation method values were considered the standard and thus were not adjusted.

Soil depth (cm)	Clod		Core		Excavation
	Original	Adjusted	Original	Adjusted	Original
10	54.3	60.8	43.7	61.4	68.0
20	95.7	109.7	85.7	119.8	117.1
50	162.4	192.3	142.4	207.3	196.8
100	203.5	248.4	165.7	248.4	258.7
150	220.5	273.9	180.3	277.1	283.7

Table 2.8. Cumulative original and adjusted soil organic carbon stocks (Mg ha^{-1}) by soil sampling method. Calculated using the mass based approach. Soil organic carbon concentration and soil sample mass for each depth layer for the clod and core methods were adjusted according to Equations (2.8) and (2.11) and Equations (2.9) and (2.12), respectively (Table 2.10). Excavation method values were considered the standard and thus were not adjusted. Reference mass layers were set using the excavation method.

Soil mass (Mg ha^{-1})	Clod		Core		Excavation
	Original	Adjusted	Original	Adjusted	Original
700	52.1	57.7	54.1	54.3	71.6
1650	95.4	106.2	105.2	118.3	125.0
4600	167.5	193.9	151.1	212.7	206.4
10350	213.9	256.5	173.1	259.0	269.3
15700	231.3	282.7	190.4	288.1	294.7

Table 2.9. Original and adjusted depth (cm) to reference soil mass by soil sampling method. Soil organic carbon concentration and soil sample mass for each depth layer for the clod and core methods were adjusted according to Equations (2.8) and (2.11) and Equations (2.9) and (2.12), respectively (Table 2.10). Excavation method values were considered the standard and thus were not adjusted. Reference mass layers were set using the excavation method.

Soil mass (Mg ha ⁻¹)	Clod		Core		Excavation
	Original	Adjusted	Original	Adjusted	Original
700	8.9	9.5	12.2	8.1	9.8
1650	18.8	19.8	25.6	17.7	20.1
4600	48.0	49.1	61.4	48.0	50.1
10350	99.6	100.2	122.0	99.6	100.0
15700	149.8	150.1	182.3	149.5	150.2

Table 2.10. Regression equations used to predict bulk density (BD), soil organic carbon concentration (SOC_{conc}), and soil sample mass for each depth layer ($M_{\text{SAMPLE(DL)}}$) between the clod, core, and excavation soil sampling methods. $M_{\text{SAMPLE(DL)}}$ raw values were obtained according to Equation (2.4). For quadratic Equations (2.7) and (2.13), adjusted R^2 is shown.

Variable	x	y	Equation	R^2	Eq. #
BD	Clod	Excavation	$y = 1.0025x - 0.0145$	0.5427	2.5
BD	Core	Excavation	$y = 0.6446x + 0.4749$	0.2761	2.6
BD	Core	Clod	$y = -1.4693x^2 + 2.9281x - 0.3678$	0.4571	2.7
SOC_{conc}	Clod	Excavation	$\ln(y) = 0.9044(\ln(x)) + 0.5237$	0.9363	2.8
SOC_{conc}	Core	Excavation	$\ln(y) = 0.8255(\ln(x)) + 0.7358$	0.8879	2.9
SOC_{conc}	Clod	Core	$\ln(y) = 0.9355(\ln(x)) + 0.2361$	0.7636	2.10
$M_{\text{SAMPLE(DL)}}$	Clod	Excavation	$y = 1.0212x - 16.315$	0.9604	2.11
$M_{\text{SAMPLE(DL)}}$	Core	Excavation	$y = 1.1326x + 55.681$	0.9418	2.12
$M_{\text{SAMPLE(DL)}}$	Core	Clod	$y = -0.0006x^2 + 1.799x - 57.39$	0.9611	2.13

Table 3.1. Summary of plot treatments. The plantation was planted in 1977 with 2-year-old Douglas-fir seedlings.

Plot	Initial trees ha ⁻¹	Treatment group	Fertilized [†]	Thinning treatment [‡]	Thinning year	Trees left on forest floor [§]
1	1117	Thinning	No	RD55→RD35	2001	Yes
2	297**	Control	No	None	-	-
3	558*	Thinning	No	RD55→RD35	2011	No
4	1181	Thinning	No	RD55→RD35	1999	Yes
5	1196	Control	No	None	-	-
8	554*	Control	No	None	-	-
10	1240	Fert+Thinning	Yes	RD55→RD35; RD55→RD40	1997; 2005	Yes; no
11	558*	Fert+Thinning	Yes	RD55→RD35	2009	No
12	311**	Fert+Thinning	Yes	None	-	-

*initial trees per hectare were reduced to one-half in 1989; removed trees left on forest floor

**initial trees per hectare were reduced to one-fourth in 1989; removed trees left on forest floor

[†]fertilized plots received 224 kg N ha⁻¹ as urea every four years starting in 1989 for a total of 1120 kg N ha⁻¹ over 16 years

[‡]RD = Curtis's (1982) relative density

[§]When trees were removed, slash was left on forest floor

Table 3.2. Soil bulk density (g cm^{-3}) by treatment group. Sample size for all treatment groups and soil depth layers is three. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '^'; 0.1 ''.

Soil depth layer (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	0.05 ^a	0.00	0.06 ^a	0.00	0.06 ^a	0.01
0-10	0.90 ^a	0.03	0.83 ^{ab}	0.01	0.75 ^b	0.03
10-20	1.02 ^a	0.01	0.95 ^b	0.03	0.91 ^b	0.01
20-50	1.06 ^a	0.00	1.01 ^a	0.02	0.99 ^a	0.04
50-100	1.11 ^a	0.01	1.08 ^a	0.03	1.08 ^a	0.03
100-150	1.08 ^a	0.01	1.04 ^a	0.02	1.07 ^a	0.02

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	0.418	0.676	-	-	-
0-10	2, 6	10.44	0.0111 ^{^^}	0.1651	0.0091*	0.1098
10-20	2, 6	9.063	0.0154 ^{^^}	0.0684 [^]	0.0138 ^{^^}	0.4180
20-50	2, 6	1.667	0.266	-	-	-
50-100	2, 6	0.563	0.597	-	-	-
100-150	2, 6	1.551	0.286	-	-	-

Table 3.3. Soil organic carbon concentration (g OC/kg soil) by treatment group. Sample size for all treatment groups and soil depth layers is three. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 ''; 0.1 ''.

Soil depth layer (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	387.4 ^a	7.4	395.0 ^a	9.9	380.6 ^a	6.0
0-10	58.5 ^a	4.0	69.6 ^a	3.0	85.7 ^b	5.1
10-20	41.6 ^a	2.3	47.7 ^a	5.1	63.9 ^b	2.1
20-50	19.5 ^a	2.0	29.7 ^a	3.3	34.2 ^a	6.3
50-100	6.8 ^a	1.2	10.5 ^a	3.5	10.3 ^a	1.3
100-150	5.1 ^{ab}	1.0	3.84 ^a	0.2	6.8 ^b	0.8

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	0.82	0.484	-	-	-
0-10	2, 6	11.22	0.0094*	0.2113	0.0078*	0.0713`
10-20	2, 6	11.1	0.0096*	0.4775	0.0092*	0.0368``
20-50	2, 6	3.11	0.118	-	-	-
50-100	2, 6	0.804	0.491	-	-	-
100-150	2, 6	4.303	0.0693`	0.4873	0.2786	0.0601`

Table 3.4. Soil nitrogen concentration (g N/kg soil) by treatment group. Sample size for all treatment groups and soil depth layers is three. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '``'; 0.1 ''.

Soil depth layer (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	10.26 ^a	0.25	12.14 ^b	0.22	11.93 ^b	0.10
0-10	2.41 ^a	0.13	2.92 ^b	0.07	3.42 ^c	0.15
10-20	1.80 ^a	0.05	2.03 ^a	0.28	2.83 ^b	0.16
20-50	0.93 ^a	0.05	1.27 ^{ab}	0.22	1.57 ^b	0.18
50-100	0.46 ^a	0.08	0.54 ^b	0.15	0.62 ^a	0.06
100-150	0.26 ^a	0.03	0.24 ^a	0.03	0.46 ^b	0.07

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	25.63	0.0012*	0.0015*	0.0028*	0.7550
0-10	2, 6	17.59	0.0031*	0.0543 [`]	0.0025*	0.0594 [`]
10-20	2, 6	7.971	0.0204 ^{``}	0.6880	0.0209 ^{``}	0.0577 [`]
20-50	2, 6	3.7	0.0898 [`]	0.3770	0.0774 [`]	0.4605
50-100	2, 6	0.589	0.584	-	-	-
100-150	2, 6	7.192	0.0255 ^{``}	0.9495	0.0469 ^{``}	0.0324 ^{``}

Table 3.5. Soil organic carbon concentration (g OC/kg soil) to nitrogen concentration (g N/kg soil) ratio (C:N ratio) by treatment group. Sample size for all treatment groups and soil depth layers is three. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '°'; 0.1 '°'.

Soil depth layer (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	39.1 ^a	0.6	33.4 ^b	0.5	32.1 ^b	0.3
0-10	24.6 ^a	0.7	23.9 ^a	0.5	25.2 ^a	0.5
10-20	23.1 ^a	1.3	23.7 ^a	0.8	22.6 ^a	0.5
20-50	19.9 ^a	1.2	23.8 ^a	1.6	21.4 ^a	2.0
50-100	14.8 ^a	0.1	18.0 ^a	0.9	16.3 ^a	1.8
100-150	18.8 ^a	1.8	16.3 ^a	1.2	15.0 ^a	2.0

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	60.4	0.0001**	0.0004**	0.0001**	0.2403
0-10	2, 6	1.287	0.343	-	-	-
10-20	2, 6	0.387	0.695	-	-	-
20-50	2, 6	1.422	0.312	-	-	-
50-100	2, 6	1.773	0.248	-	-	-
100-150	2, 6	1.309	0.337	-	-	-

Table 3.6. Soil pH by treatment group. Sample size for all treatment groups and soil depth layers is three. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey’s HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 ‘****’; 0.001 ‘***’; 0.01 ‘**’; 0.05 ‘’’’; 0.1 ‘’’’.

Soil depth layer (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	5.20 ^a	0.18	4.90 ^a	0.14	5.34 ^a	0.10
0-10	5.05 ^a	0.03	4.88 ^b	0.06	5.02 ^{ab}	0.05
10-20	5.07 ^a	0.03	4.90 ^a	0.07	5.06 ^a	0.06
20-50	5.03 ^a	0.04	4.94 ^a	0.03	5.07 ^a	0.04
50-100	5.02 ^a	0.05	4.99 ^a	0.01	5.01 ^a	0.04
100-150	5.08 ^a	0.07	5.03 ^a	0.03	4.98 ^a	0.05

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey’s HSD, T-FT)	<i>P</i> (Tukey’s HSD, T-C)	<i>P</i> (Tukey’s HSD, FT-C)
Litter	2, 6	2.5	0.162	-	-	-
0-10	2, 6	3.822	0.0851 [`]	0.0887 [`]	0.8666	0.1712
10-20	2, 6	2.743	0.143	-	-	-
20-50	2, 6	2.89	0.132	-	-	-
50-100	2, 6	0.141	0.871	-	-	-
100-150	2, 6	0.973	0.431	-	-	-

Table 3.7. Soil organic carbon stock (Mg ha⁻¹) by treatment group. Calculated using the fixed depth equation. Sample size for all treatment groups and soil depth layers is three. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '^^'; 0.1 '^'.

Soil depth layer (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	7.6 ^{ab}	0.1	9.1 ^a	1.1	6.2 ^b	0.4
0-10	51.4 ^a	1.9	57.6 ^b	2.0	62.1 ^b	0.9
10-20	42.6 ^a	2.8	44.7 ^a	4.0	57.7 ^b	1.7
20-50	60.9 ^a	6.3	89.7 ^{ab}	9.5	96.9 ^b	12.4
50-100	37.8 ^a	6.4	54.6 ^a	16.2	55.5 ^a	7.9
100-150	27.4 ^{ab}	5.2	20.0 ^a	1.1	36.0 ^b	4.4

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.692	0.0593 [`]	0.3149	0.3726	0.0504 [`]
0-10	2, 6	10.32	0.0114 ^{^^}	0.0905 [`]	0.0095*	0.2102
10-20	2, 6	7.457	0.0236 ^{^^}	0.8743	0.0275 ^{^^}	0.0501 [`]
20-50	2, 6	3.848	0.0841 [`]	0.1708	0.0875 [`]	0.8623
50-100	2, 6	0.812	0.487	-	-	-
100-150	2, 6	4.065	0.0766 [`]	0.4386	0.3414	0.0657 [`]

Table 3.8. Soil nitrogen stock (kg ha⁻¹) by treatment group. Calculated using the fixed depth equation. Sample size for all treatment groups and soil depth layers is three. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '``'; 0.1 ''.

Soil depth layer (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	199 ^a	3	264 ^b	28	195 ^a	14
0-10	2117 ^a	64	2414 ^b	41	2530 ^b	41
10-20	1856 ^a	92	1906 ^a	223	2549 ^b	124
20-50	2920 ^a	164	3828 ^{ab}	619	4489 ^b	276
50-100	2562 ^a	422	2847 ^a	686	3367 ^a	421
100-150	1413 ^a	174	1259 ^a	137	2483 ^b	394

ANOVA table						
Soil depth layer (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.549	0.0628 [`]	0.0968 [`]	0.9899	0.0815 [`]
0-10	2, 6	18.34	0.0028 [*]	0.0131 ^{``}	0.0026 [*]	0.2974
10-20	2, 6	6.101	0.0358 ^{``}	0.9719	0.0462 ^{``}	0.0612 [`]
20-50	2, 6	3.831	0.0847 [`]	0.3174	0.0737 [`]	0.5159
50-100	2, 6	0.605	0.576	-	-	-
100-150	2, 6	6.52	0.0313 ^{``}	0.9103	0.0617 [`]	0.0370 ^{``}

Table 3.9. Thickness (cm) of litter layer by treatment group. Sample size for all treatment groups is three. Means accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

Soil layer	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	3.8 ^a	0.1	4.0 ^a	0.3	3.1 ^a	0.6

ANOVA table						
Soil layer	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	1.368	0.324	-	-	-

Table 3.10. Cumulative soil organic carbon stock (Mg ha⁻¹) by treatment group. Calculated using the fixed depth equation. Sample size for all treatment groups and cumulative soil depths is three. Means for each soil depth accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '°'; 0.1 '°°'.

Soil depth (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	7.6 ^{ab}	0.1	9.1 ^a	1.1	6.2 ^b	0.4
10	59.0 ^a	2.0	66.7 ^b	1.2	68.4 ^b	1.1
20	101.6 ^a	3.9	111.4 ^a	3.1	126.1 ^b	2.7
50	162.5 ^a	7.5	201.1 ^{ab}	12.3	223.0 ^b	12.5
100	200.3 ^a	5.3	255.7 ^{ab}	27.2	278.5 ^b	16.7
150	227.7 ^a	7.7	275.7 ^{ab}	26.7	314.5 ^b	13.7

ANOVA table						
Soil depth (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.692	0.0593 [°]	0.3149	0.3726	0.0504 [°]
10	2, 6	11.34	0.0092 [*]	0.0247 ^{°°}	0.0101 ^{°°}	0.7101
20	2, 6	14.12	0.0054 [*]	0.1677	0.0045 [*]	0.0443 ^{°°}
50	2, 6	7.701	0.022 ^{°°}	0.1057	0.0192 ^{°°}	0.3974
100	2, 6	4.626	0.0609 [°]	0.1712	0.0573 [°]	0.6811
150	2, 6	5.898	0.0383 ^{°°}	0.2201	0.0323 ^{°°}	0.3421

Table 3.11. Cumulative soil nitrogen stock (kg ha⁻¹) by treatment group. Calculated using the fixed depth equation. Sample size for all treatment groups and cumulative soil depths is three. Means for each soil depth accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '^^'; 0.1 '^'.

Soil depth (cm)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	199 ^a	3	264 ^b	28	195 ^a	14
10	2315 ^a	65	2678 ^b	35	2725 ^b	52
20	4171 ^a	38	4584 ^a	190	5274 ^b	170
50	7091 ^a	159	8412 ^{ab}	804	9763 ^b	385
100	9653 ^a	354	11259 ^a	1487	13129 ^a	695
150	11066 ^a	316	12519 ^{ab}	1466	15613 ^b	632

ANOVA table						
Soil depth (cm)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.549	0.0628 [`]	0.0968 [`]	0.9899	0.0815 [`]
10	2, 6	18.66	0.0027*	0.0062*	0.0034*	0.8045
20	2, 6	13.96	0.0055*	0.2033	0.0047*	0.0391 ^{^^}
50	2, 6	6.533	0.0312 ^{^^}	0.2516	0.0260 ^{^^}	0.2396
100	2, 6	3.22	0.112	-	-	-
150	2, 6	6.108	0.0357 ^{^^}	0.5521	0.0326 ^{^^}	0.1270

Table 3.12. Soil organic carbon stock (Mg ha⁻¹) by treatment group. Calculated using the mass based approach. Sample size for all treatment groups and soil mass layers is three. Means within each soil mass layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '^^'; 0.1 '^'.

Soil mass layer (Mg ha ⁻¹)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	7.6 ^{ab}	0.1	9.1 ^a	1.1	6.2 ^b	0.4
0-800	47.3 ^a	3.0	56.0 ^a	2.3	66.5 ^b	3.2
800-1700	40.3 ^a	1.2	44.7 ^a	4.0	56.5 ^b	1.5
1700-4600	65.4 ^a	3.3	88.7 ^a	9.9	100.0 ^a	17.0
4600-10100	37.0 ^a	7.5	63.3 ^a	17.5	47.2 ^a	10.4
10100-15400	35.2 ^a	4.1	18.1 ^b	1.0	34.1 ^a	3.5

ANOVA table						
Soil mass layer (Mg ha ⁻¹)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.692	0.0593 [`]	0.3149	0.3726	0.0504 [`]
0-800	2, 6	11.34	0.0092 [*]	0.1571	0.0075 [*]	0.0905 [`]
800-1700	2, 6	10.79	0.0103 ^{^^}	0.4882	0.0099 [*]	0.0388 ^{^^}
1700-4600	2, 6	2.343	0.177	-	-	-
4600-10100	2, 6	1.113	0.388	-	-	-
10100-15400	2, 6	9.346	0.0143 ^{^^}	0.0195 ^{^^}	0.9662	0.0260 ^{^^}

Table 3.13. Soil nitrogen stock (kg ha⁻¹) by treatment group. Calculated using the mass based approach. Sample size for all treatment groups and soil mass layers is three. Means within each soil mass layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '``'; 0.1 ''.

Soil mass layer (Mg ha ⁻¹)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	199 ^a	3	264 ^b	28	195 ^a	14
0-800	1945 ^a	107	2345 ^b	48	2680 ^c	107
800-1700	1724 ^a	34	1903 ^a	227	2532 ^b	143
1700-4600	3037 ^a	14	3790 ^a	656	4568 ^a	451
4600-10100	2494 ^a	442	3179 ^a	768	3101 ^a	562
10100-15400	1829 ^{ab}	200	1240 ^a	87	2379 ^b	336

ANOVA table						
Soil mass layer (Mg ha ⁻¹)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.549	0.0628 [`]	0.0968 [`]	0.9899	0.0815 [`]
0-800	2, 6	16.01	0.0039 [*]	0.0493 ^{``}	0.0032 [*]	0.0931 [`]
800-1700	2, 6	7.389	0.0241 ^{``}	0.7102	0.0246 ^{``}	0.0657 [`]
1700-4600	2, 6	2.77	0.141	-	-	-
4600-10100	2, 6	0.383	0.697	-	-	-
10100-15400	2, 6	6.067	0.0362 ^{``}	0.2475	0.2862	0.0303 ^{``}

Table 3.14. Cumulative soil organic carbon stock (Mg ha⁻¹) by treatment group. Calculated using the mass based approach. Sample size for all treatment groups and cumulative soil masses is three. Means for each soil mass accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '°'; 0.1 '°°'.

Soil mass (Mg ha ⁻¹)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	7.6 ^{ab}	0.1	9.1 ^a	1.1	6.2 ^b	0.4
800	54.9 ^a	3.1	65.1 ^b	1.3	72.7 ^b	3.2
1700	95.2 ^a	3.3	109.8 ^b	3.1	129.2 ^c	4.4
4600	160.6 ^a	5.5	198.5 ^{ab}	13.0	229.2 ^b	17.5
10100	197.6 ^a	8.4	261.8 ^{ab}	29.9	276.3 ^b	17.5
15400	232.8 ^a	6.5	279.9 ^{ab}	30.1	310.4 ^b	14.9

ANOVA table						
Soil mass (Mg ha ⁻¹)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.692	0.0593 [°]	0.3149	0.3726	0.0504 [°]
800	2, 6	11.18	0.0095*	0.0786 [°]	0.0078*	0.1917
1700	2, 6	21.61	0.0018*	0.0684 [°]	0.0015*	0.0225 ^{°°}
4600	2, 6	6.988	0.0271 ^{°°}	0.1781	0.0227 ^{°°}	0.2910
10100	2, 6	4.131	0.0745 [°]	0.1492	0.0791 [°]	0.8748
15400	2, 6	3.922	0.0814 [°]	0.2849	0.0716 [°]	0.5516

Table 3.15. Cumulative soil nitrogen stock (kg ha⁻¹) by treatment group. Calculated using the mass based approach. Sample size for all treatment groups and cumulative soil masses is three. Means for each soil mass accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '***'; 0.001 '**'; 0.01 '*'; 0.05 '^^'; 0.1 '^'.

Soil mass (Mg ha ⁻¹)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	199 ^a	3	264 ^b	28	195 ^a	14
800	2144 ^a	107	2610 ^b	26	2875 ^b	110
1700	3867 ^a	77	4513 ^a	202	5407 ^b	247
4600	6904 ^a	90	8303 ^{ab}	858	9975 ^b	570
10100	9398 ^a	466	11482 ^a	1625	13076 ^a	761
15400	11227 ^a	580	12723 ^{ab}	1631	15455 ^b	678

ANOVA table						
Soil mass (Mg ha ⁻¹)	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Litter	2, 6	4.549	0.0628 [`]	0.0968 [`]	0.9899	0.0815 [`]
800	2, 6	17.01	0.0034*	0.0243 ^{^^}	0.0029*	0.1723
1700	2, 6	16.65	0.0036*	0.1147	0.0029*	0.0360 ^{^^}
4600	2, 6	6.629	0.0302 ^{^^}	0.2952	0.0253 ^{^^}	0.1979
10100	2, 6	2.97	0.127	-	-	-
15400	2, 6	3.986	0.0792 [`]	0.6120	0.0712 [`]	0.2480

Table 3.16. Depth (cm) to reference soil mass by treatment group. Litter layer shows thickness (cm) of layer. Sample size for all treatment groups and cumulative soil masses is three.

Soil mass (Mg ha ⁻¹)	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Litter	3.8	0.1	4.0	0.3	3.1	0.6
800	8.7	0.3	9.4	0.2	10.3	0.3
1700	17.3	0.5	18.8	0.3	19.8	0.3
4600	44.8	1.2	48.0	0.6	50.9	1.9
10100	93.7	2.4	96.4	2.1	101.6	2.0
15400	137.5	3.8	143.1	2.4	146.4	2.6

Table 3.17. Carbon stocks (Mg ha⁻¹) by treatment group. Sample size for all treatment groups and categories is three. Means within each category accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 '****'; 0.001 '**'; 0.01 '*'; 0.05 '``'; 0.1 ''.

Carbon stock	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Roots	45.7 ^a	1.8	42.3 ^a	3.9	40.0 ^a	2.0
Soil	232.8 ^a	6.5	279.9 ^{ab}	30.1	310.4 ^b	14.9
Belowground	278.5 ^a	8.3	322.2 ^a	31.6	350.4 ^a	16.8
Aboveground	213.1 ^a	8.3	198.4 ^a	17.8	187.1 ^a	9.1
Total	491.5 ^a	16.5	520.6 ^a	42.5	537.5 ^a	25.6

ANOVA table						
Carbon stock	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey's HSD, T-FT)	<i>P</i> (Tukey's HSD, T-C)	<i>P</i> (Tukey's HSD, FT-C)
Roots	2, 6	1.077	0.399	-	-	-
Soil	2, 6	3.922	0.0814 [`]	0.2849	0.0716 [`]	0.5516
Belowground	2, 6	2.922	0.13	-	-	-
Aboveground	2, 6	1.081	0.397	-	-	-
Total	2, 6	0.593	0.582	-	-	-

Table 3.18. Merchantable volume (thousands of board-feet per acre as 32-ft long logs to a 6-inch top diameter) and final mean diameter at breast height (dbh, cm) by treatment group. Sample size for all treatment groups and categories is three. Means within each category accompanied by the same letter are not significantly different (Tukey’s HSD, $\alpha = 0.1$). ANOVA table significant codes: <0.0001 ‘***’; 0.001 ‘**’; 0.01 ‘*’; 0.05 ‘.’; 0.1 ‘.’.

	Thinning		Fert+Thinning		Control	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Merchantable volume	41.1 ^a	0.4	45.6 ^b	1.3	43.0 ^{ab}	1.2
Dbh	13.0 ^a	0.9	16.2 ^a	1.4	14.0 ^a	1.6

ANOVA table						
	<i>df</i>	<i>F</i>	<i>P</i>	<i>P</i> (Tukey’s HSD, T-FT)	<i>P</i> (Tukey’s HSD, T-C)	<i>P</i> (Tukey’s HSD, FT-C)
Merchantable volume	2, 6	4.555	0.0626 [^]	0.0539 [^]	0.4553	0.2709
Dbh	2, 6	1.664	0.266	-	-	-

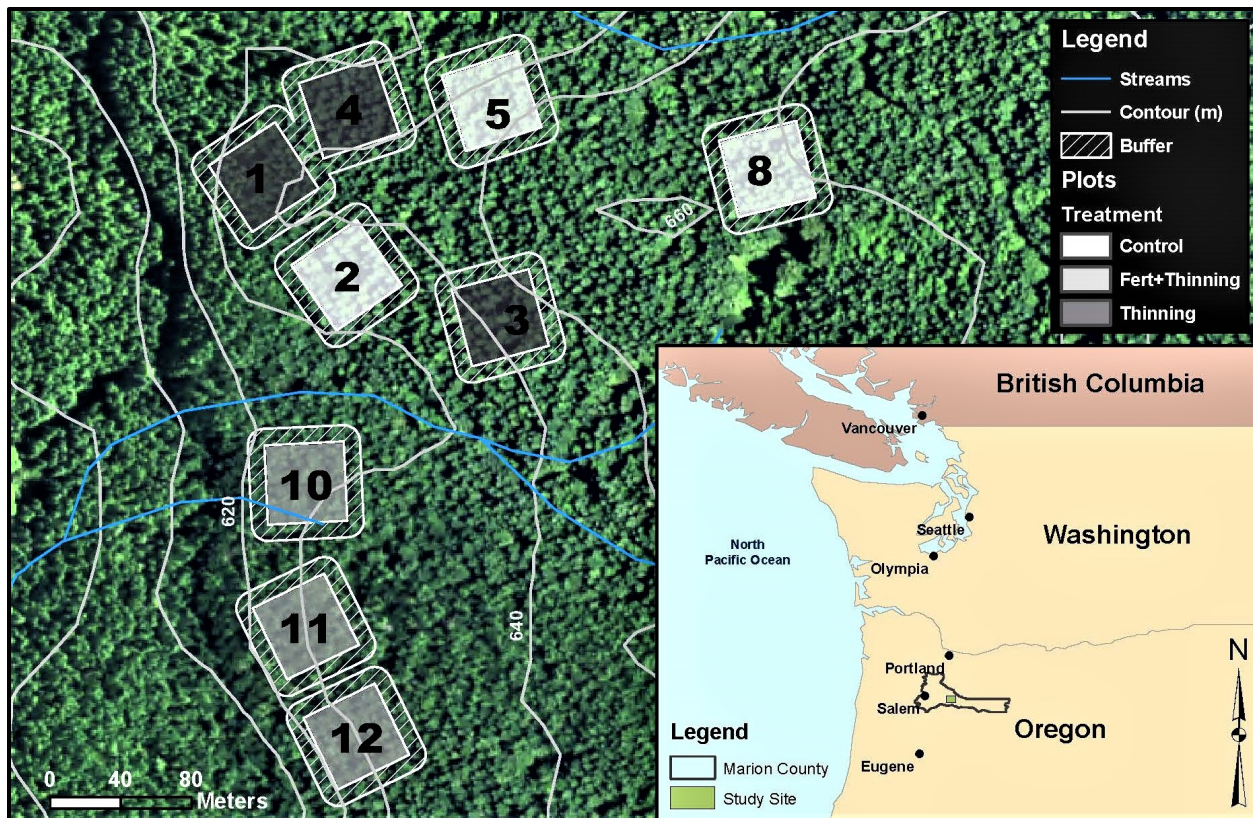


Figure 2.1. Study site plots and location in Marion County, Oregon, USA (inset). The site coordinates are 44.87417, -122.566 (Lat./Long. DD).

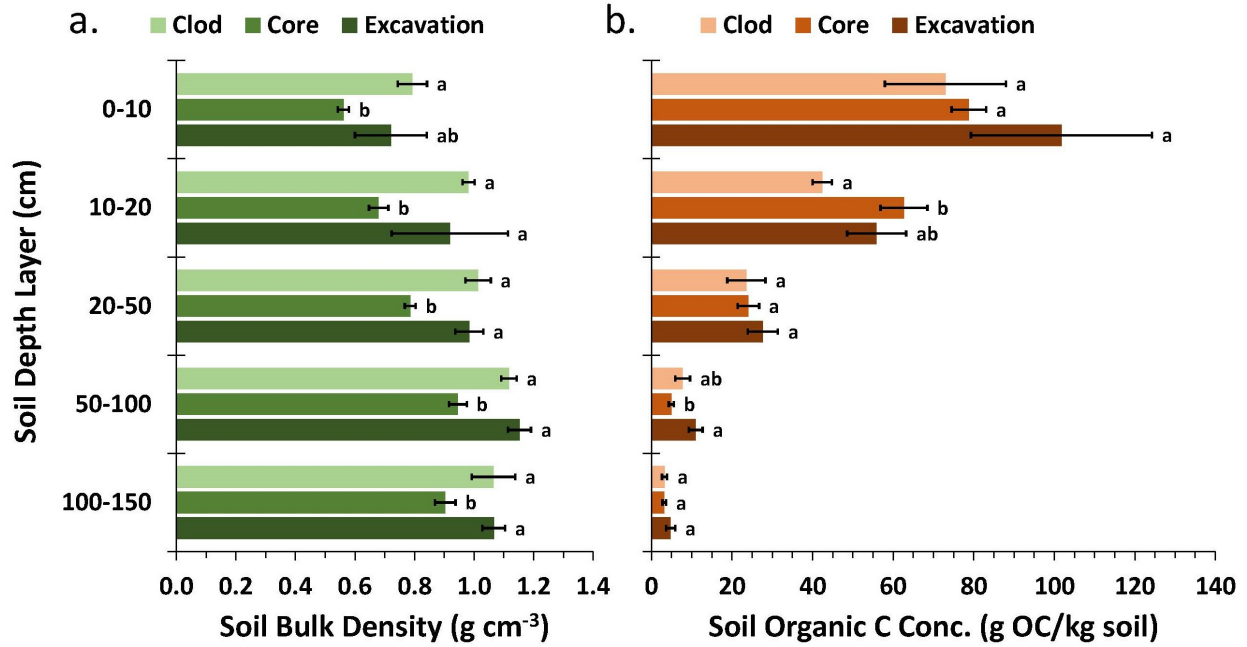


Figure 2.2. Mean soil bulk density (a) and mean soil organic carbon concentration (b) by soil sampling method. Error bars represent \pm one standard error. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

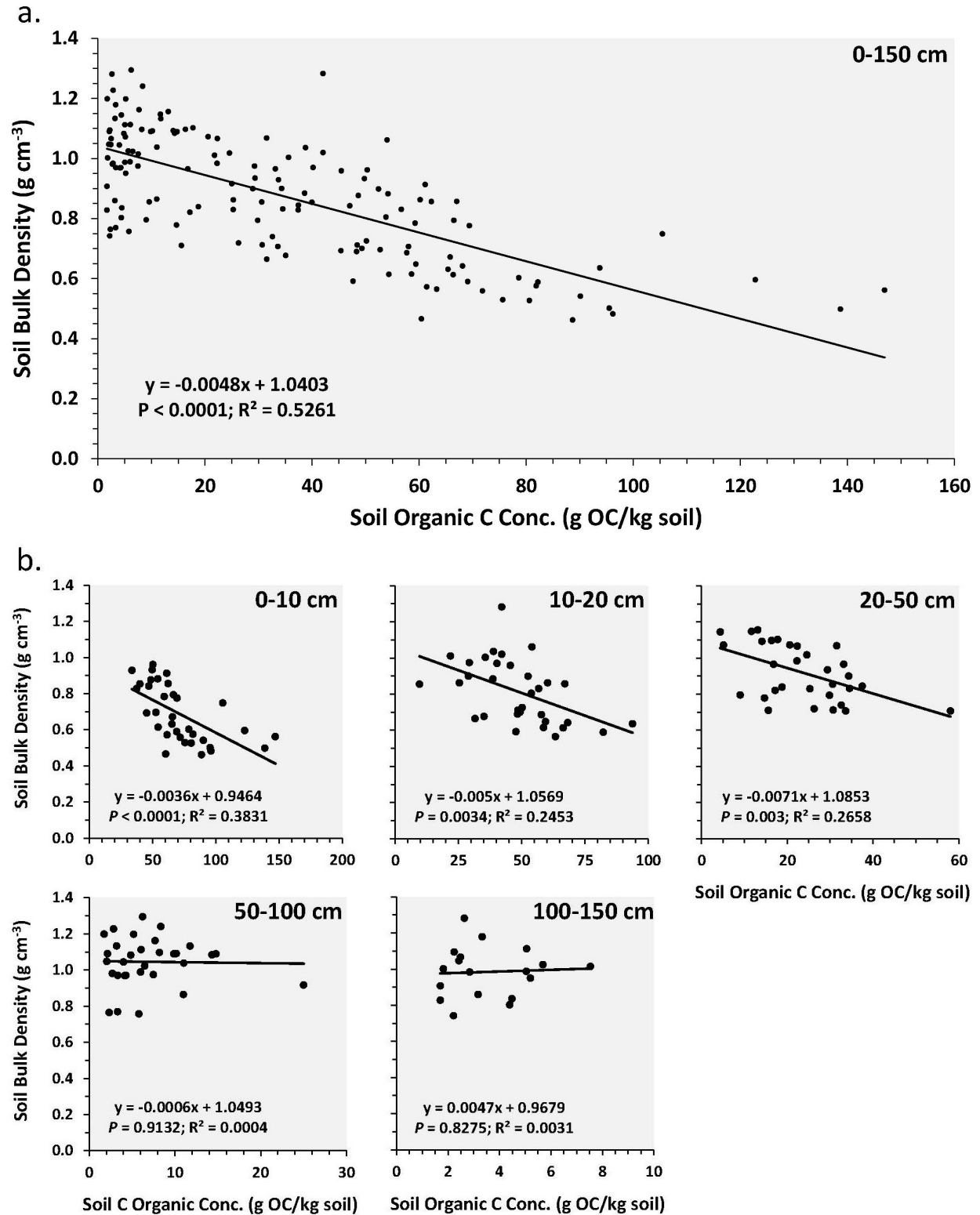
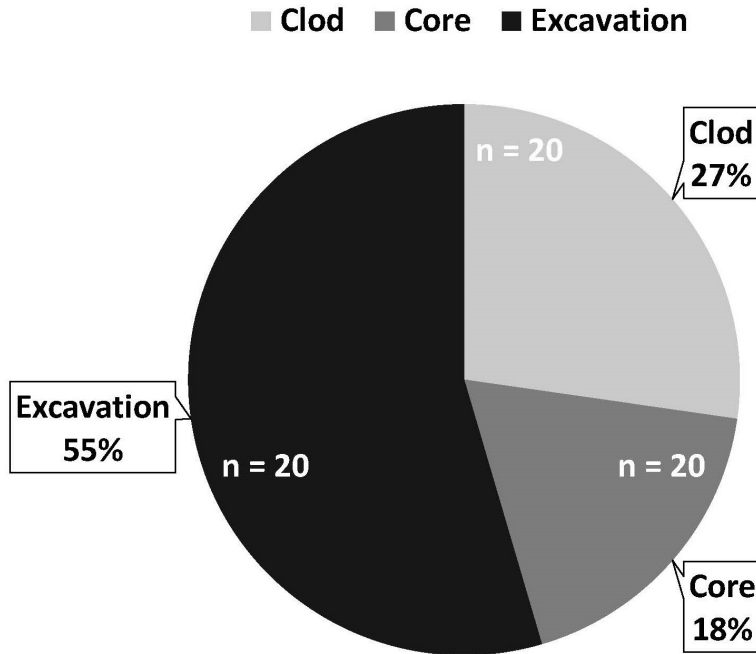


Figure 2.3. Soil bulk density versus organic carbon concentration for the whole mineral soil (a) and analyzed by soil depth layer (b).

a.



b.

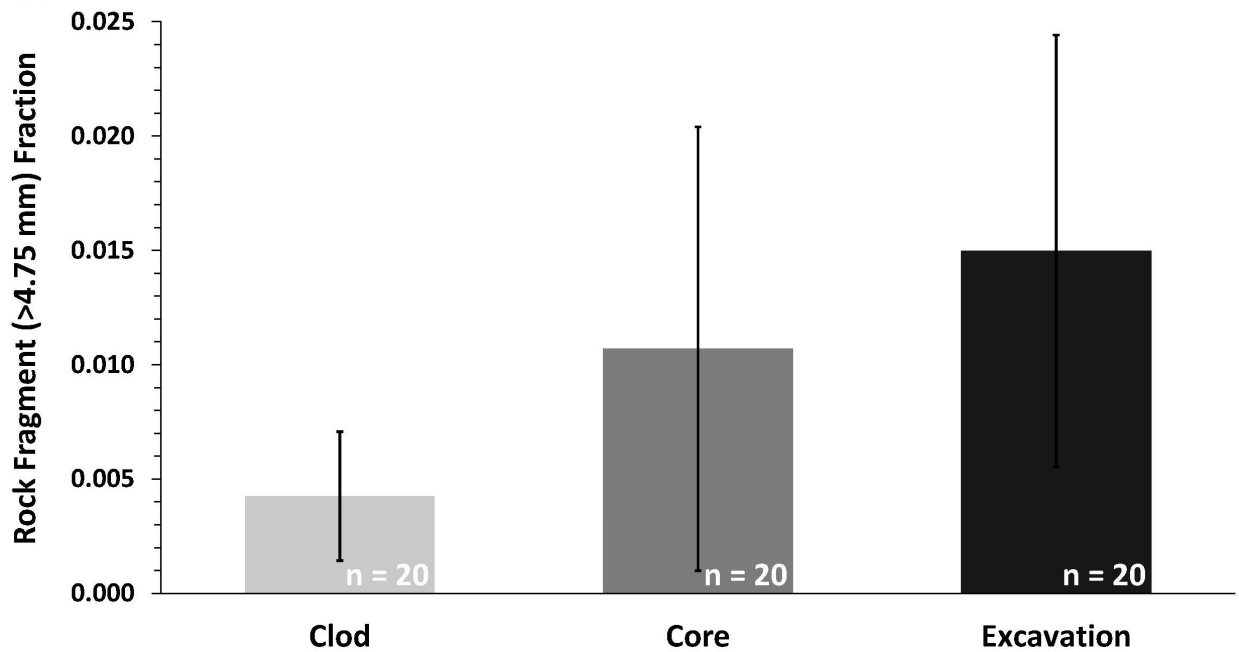


Figure 2.4. Probability of rock fragment (>4.75 mm) presence by soil sampling method (a); analysis of deviance (logistic generalized linear model, binomial), $P = 0.2438$. Rock fragment fraction ((RF weight, g)/(oven dry sample weight, g)) by soil sampling method (b). Sample size by soil depth layer was equivalent for each method to account for any potential differences in rock fragment content by depth.

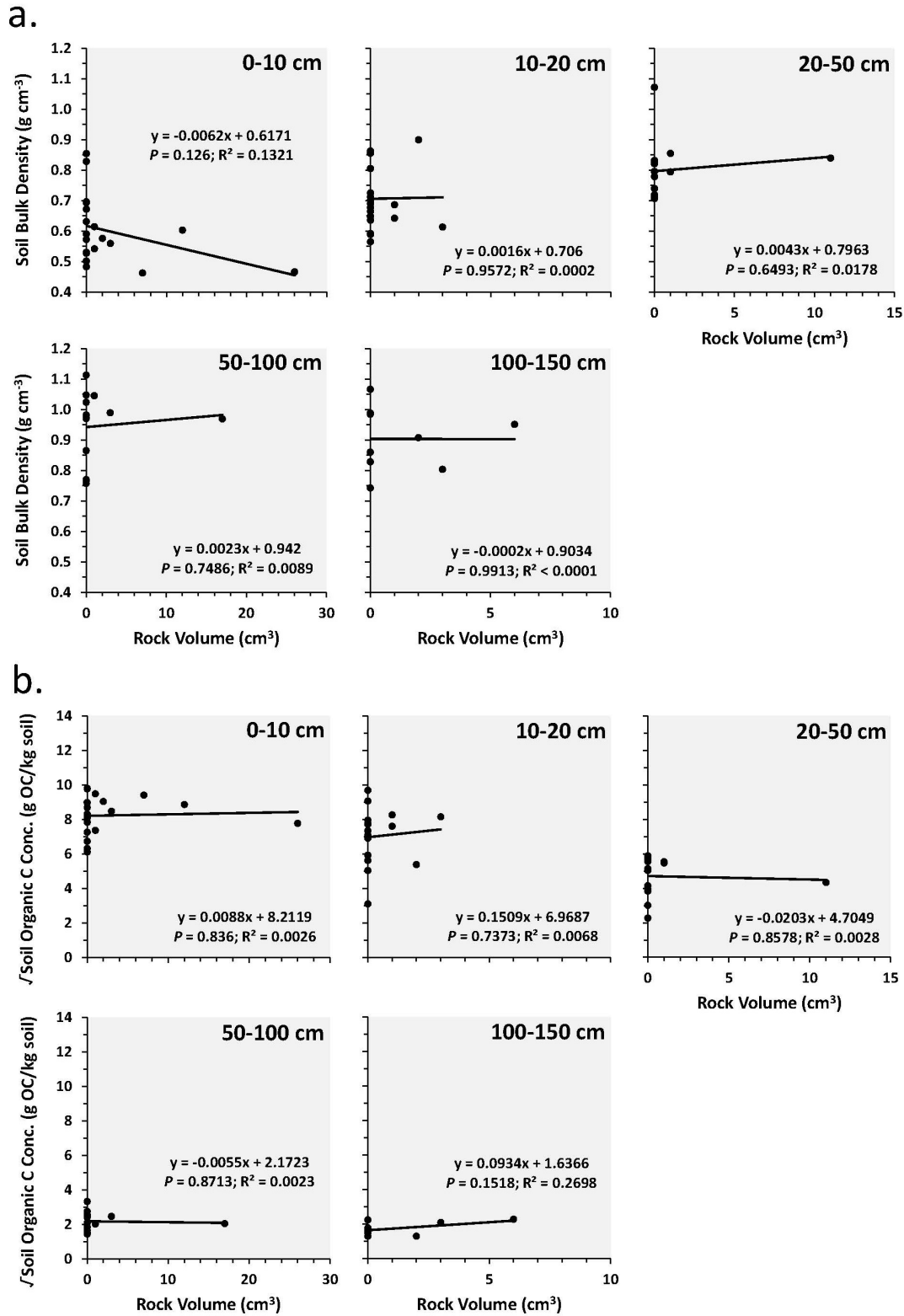


Figure 2.5. Soil bulk density versus rock fragment (>4.75 mm) volume for the core method analyzed by soil depth layer (a). Soil organic carbon concentration versus rock fragment (>4.75 mm) volume for the core method analyzed by soil depth layer (b).

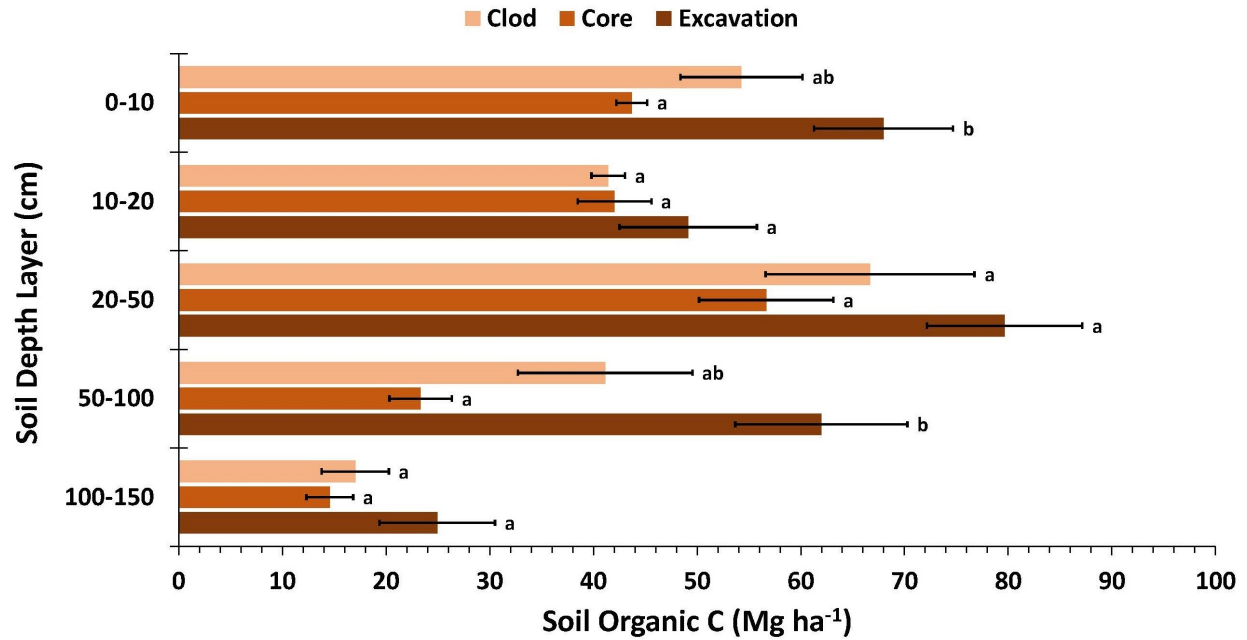


Figure 2.6. Mean soil organic carbon stock by soil sampling method. Calculated using the fixed depth equation. Error bars represent \pm one standard error. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

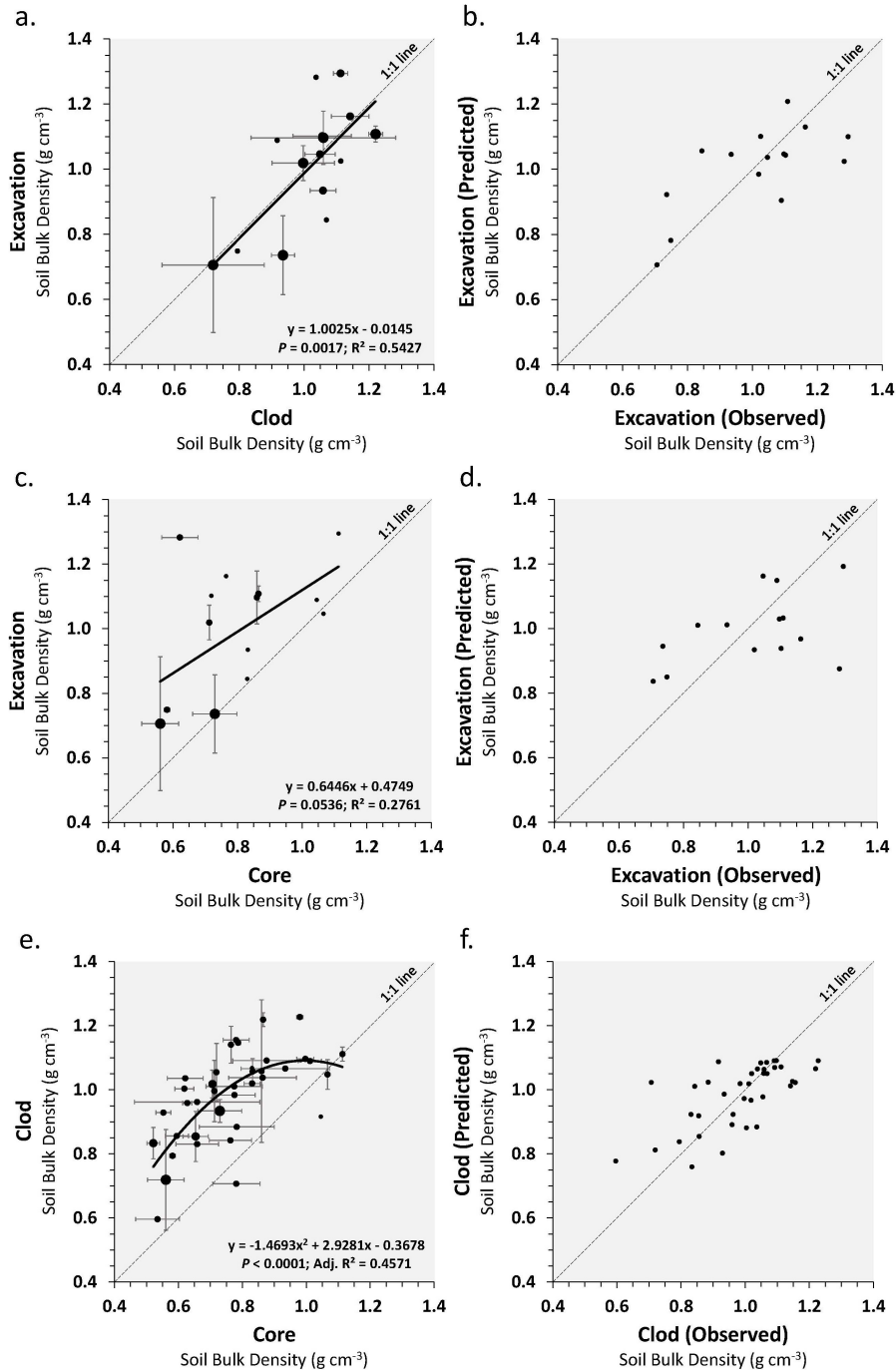


Figure 2.7. Regression equations to predict excavation soil bulk density with clod (a) and core (c) method data. Regression equation to predict clod soil bulk density with core method data (e). Regression models are weighted based on sample size; graduated points represent $n = 2$ to 5. Sample size greater than $n = 2$ denotes that the average of two or more samples from one method was compared to one or more samples from another method collected from the same pit at the same depth or from comparable pits within 0.2-ha plots at the same depth. Error bars represent \pm one standard error where $n > 1$ for the given parameter. Predicted versus observed regression models for the clod-to-excavation (b), core-to-excavation (d), and core-to-clod (f) soil bulk density equations.

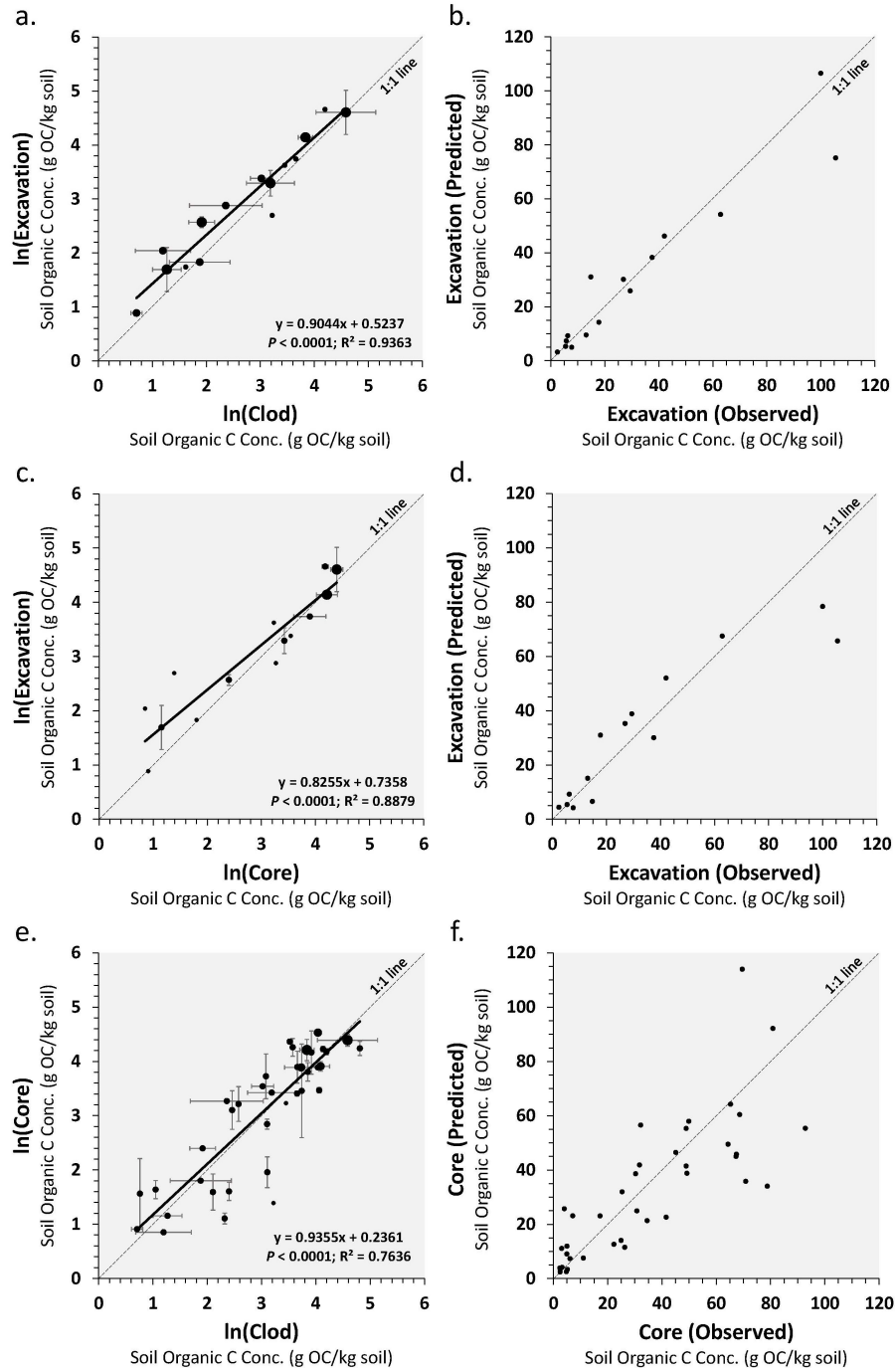


Figure 2.8. Regression equations to predict excavation soil organic carbon concentration with clod (a) and core (c) method data. Regression equation to predict core soil organic carbon concentration with clod method data (e). Regression models are weighted based on sample size; graduated points represent $n = 2$ to 5. Sample size greater than $n = 2$ denotes that the average of two or more samples from one method was compared to one or more samples from another method collected from the same pit at the same depth or from comparable pits within 0.2-ha plots at the same depth. Error bars represent \pm one standard error where $n > 1$ for the given parameter. Predicted versus observed regression models for the clod-to-excavation (b), core-to-excavation (d), and clod-to-core (f) soil organic carbon concentration equations.

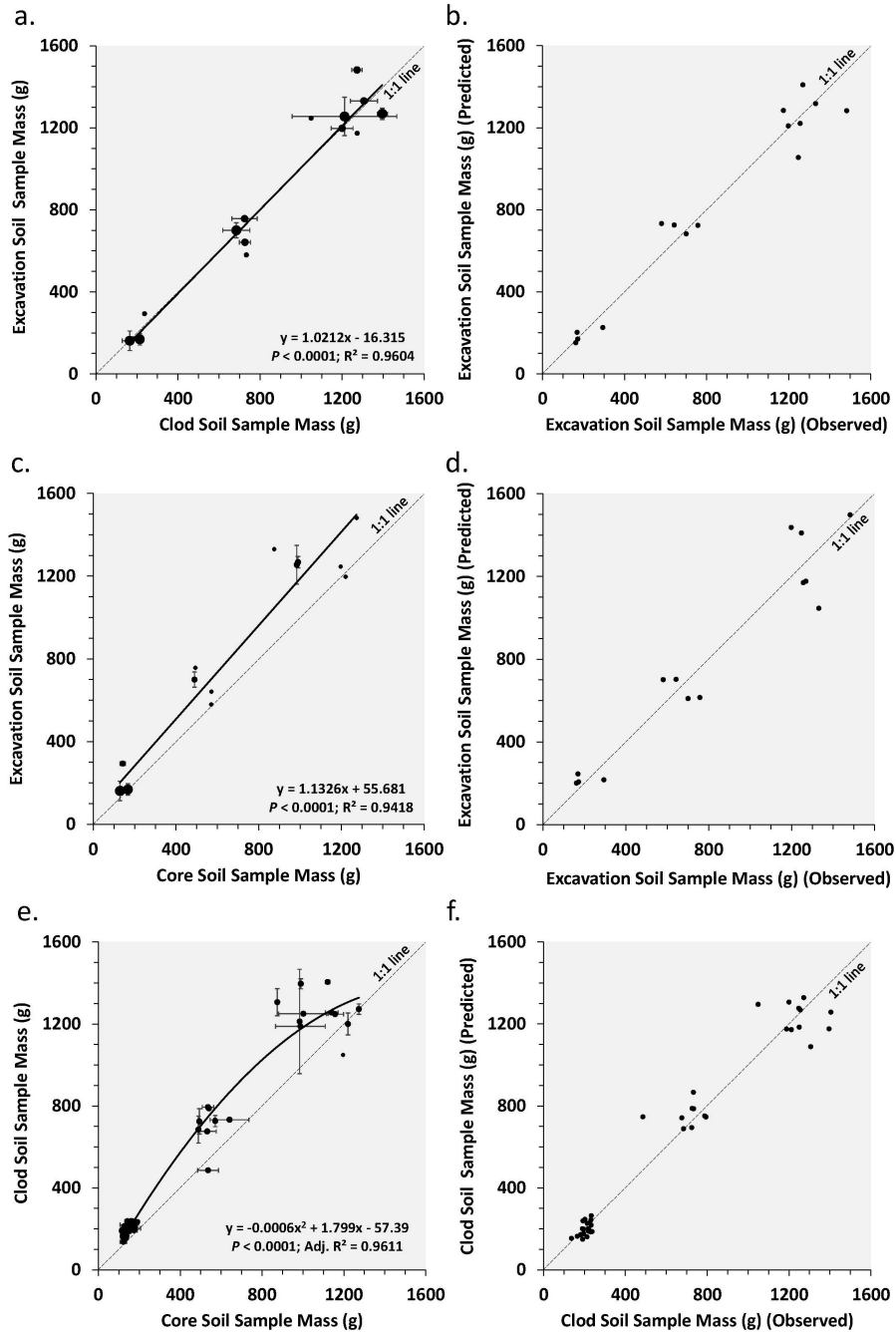


Figure 2.9. Regression equations to predict excavation soil sample mass for each depth layer with clod (a) and core (c) method data. Regression equation to predict clod soil sample mass for each depth layer with core method data (e). Raw values were obtained using Equation (2.4). Regression models are weighted based on sample size; graduated points represent $n = 2$ to 5. Sample size greater than $n = 2$ denotes that the average of two or more samples from one method was compared to one or more samples from another method collected from the same pit at the same depth or from comparable pits within 0.2-ha plots at the same depth. Error bars represent \pm one standard error where $n > 1$ for the given parameter. Predicted versus observed regression models for the clod-to-excavation (b), core-to-excavation (d), and core-to-clod (f) soil sample mass for each depth layer equations.

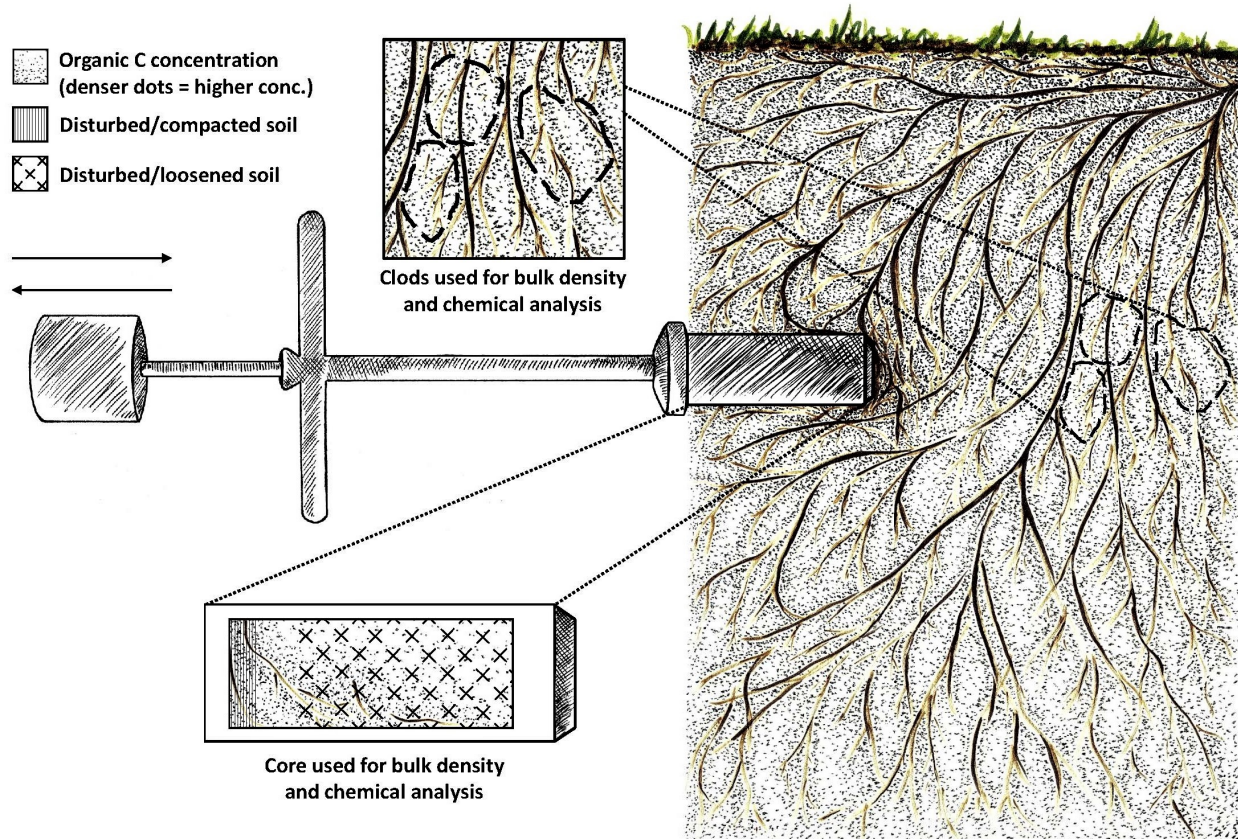


Figure 2.10. Potential biases in soil samples collected by the clod and core methods. Clod samples are shown with a lower organic carbon concentration (and higher clay content) than the surrounding bulk soil due to the selection of more cohesive, sturdy clods. The coring device is shown catching and pushing coarse roots, compacting soil outside the core and preventing soil from entering the core, thus resulting in an underestimation of bulk density.

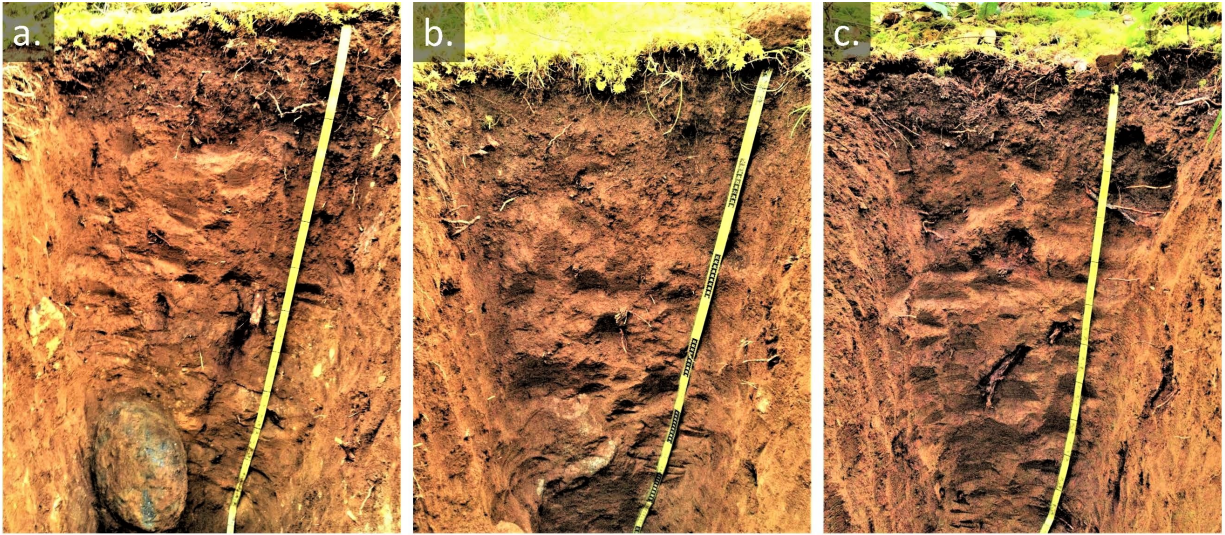


Figure 3.1. Typical soil profiles for the thinning group (a), fertilization + thinning group (b), and control group (c).

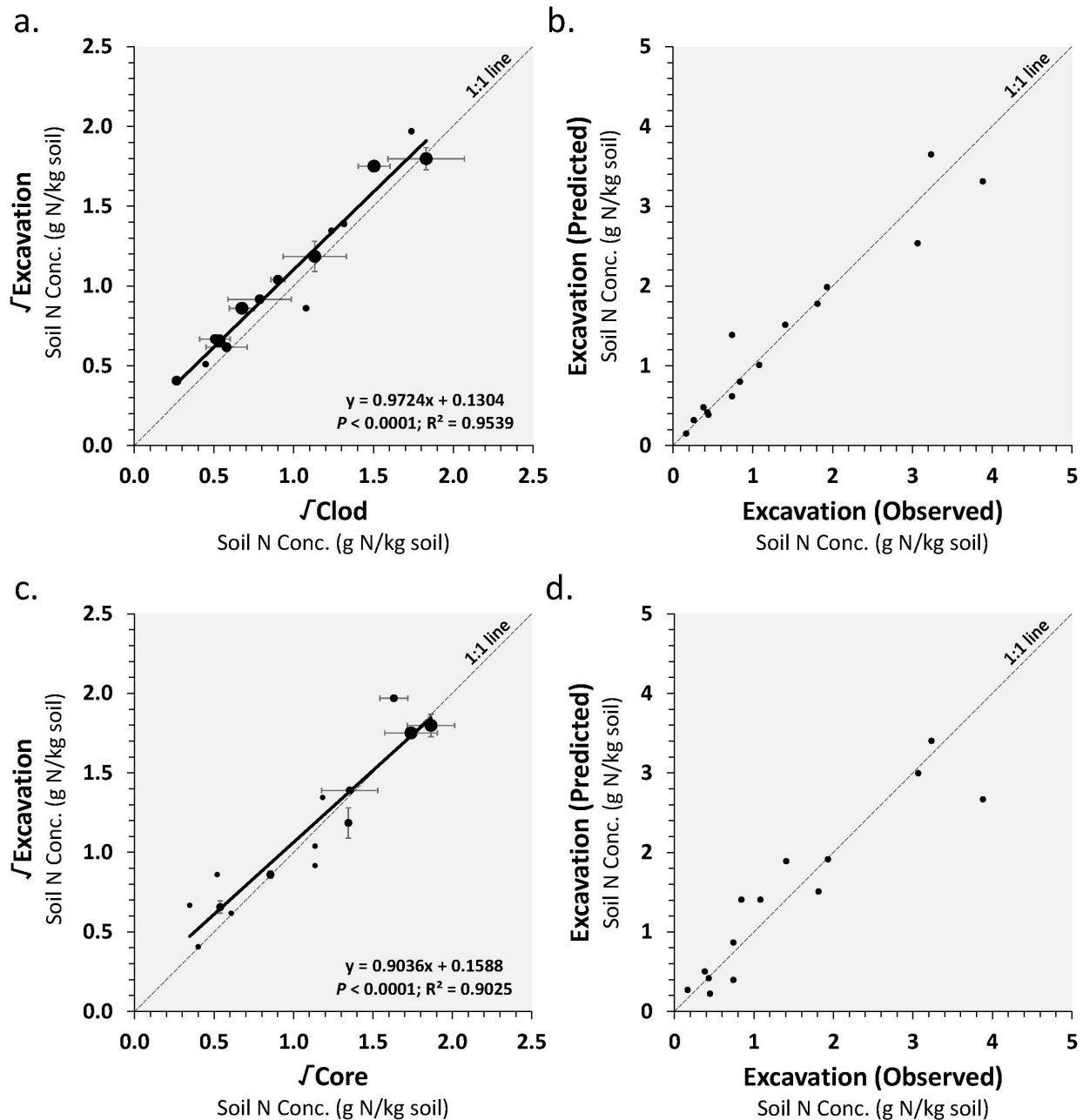


Figure 3.2. Regression equations to predict excavation soil nitrogen concentration with clod (a) and core (c) method data. Regression models are weighted based on sample size; graduated points represent $n = 2$ to 5. Sample size greater than $n = 2$ denotes that the average of two or more samples from one method was compared to one or more samples from another method collected from the same pit at the same depth or from comparable pits within 0.2-ha plots at the same depth. Error bars represent \pm one standard error where $n > 1$ for the given parameter. Predicted versus observed regression models for the clod-to-excavation (b) and core-to-excavation (d) soil nitrogen concentration equations. Raw data from Chapter 2 of this paper.

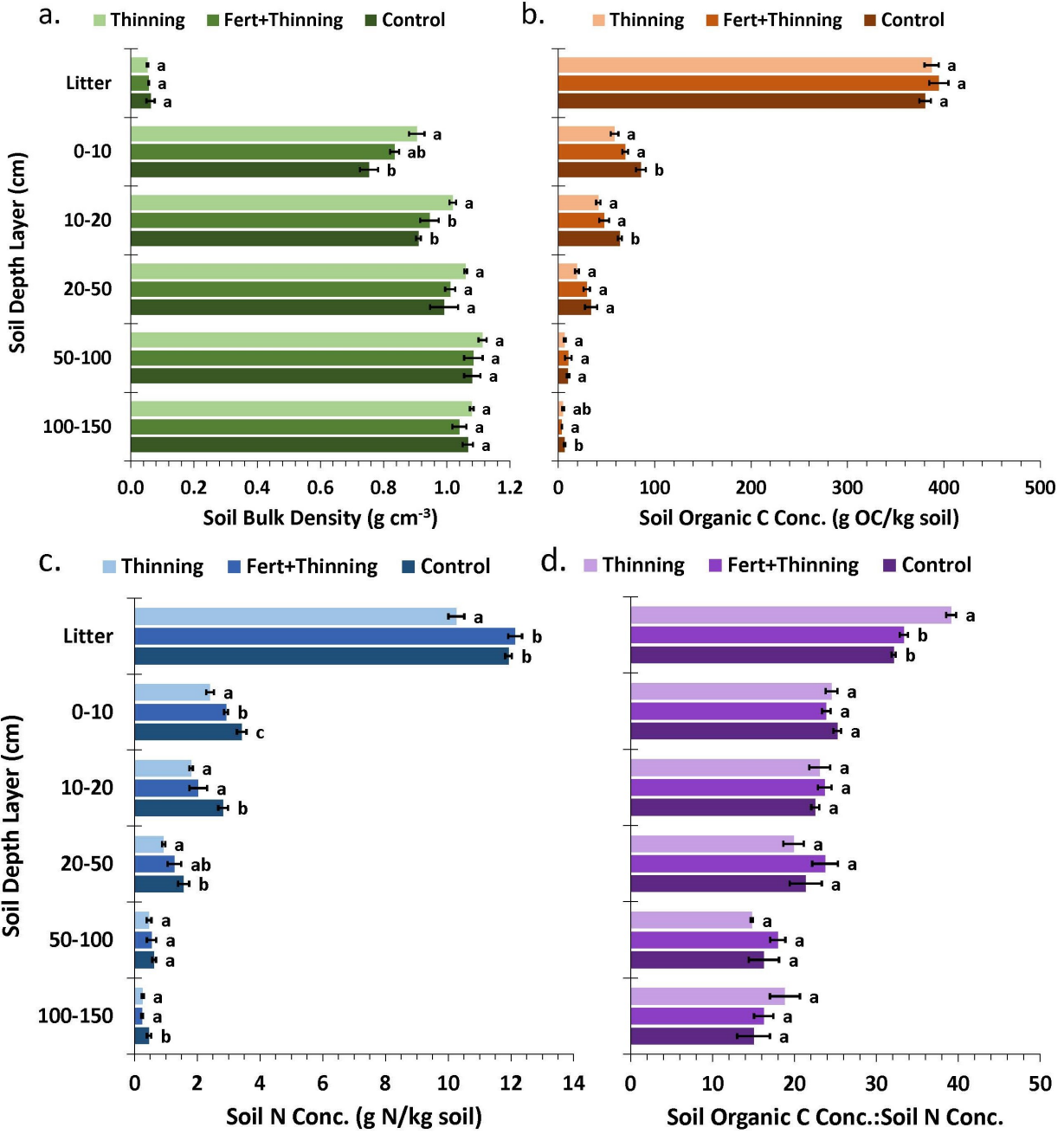


Figure 3.3. Mean soil bulk density (a), mean soil organic carbon concentration (b), and mean soil nitrogen concentration (c), and mean soil organic carbon to nitrogen concentration (d) by treatment group. Sample size for all treatment groups and soil depth layers is three. Error bars represent \pm one standard error. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

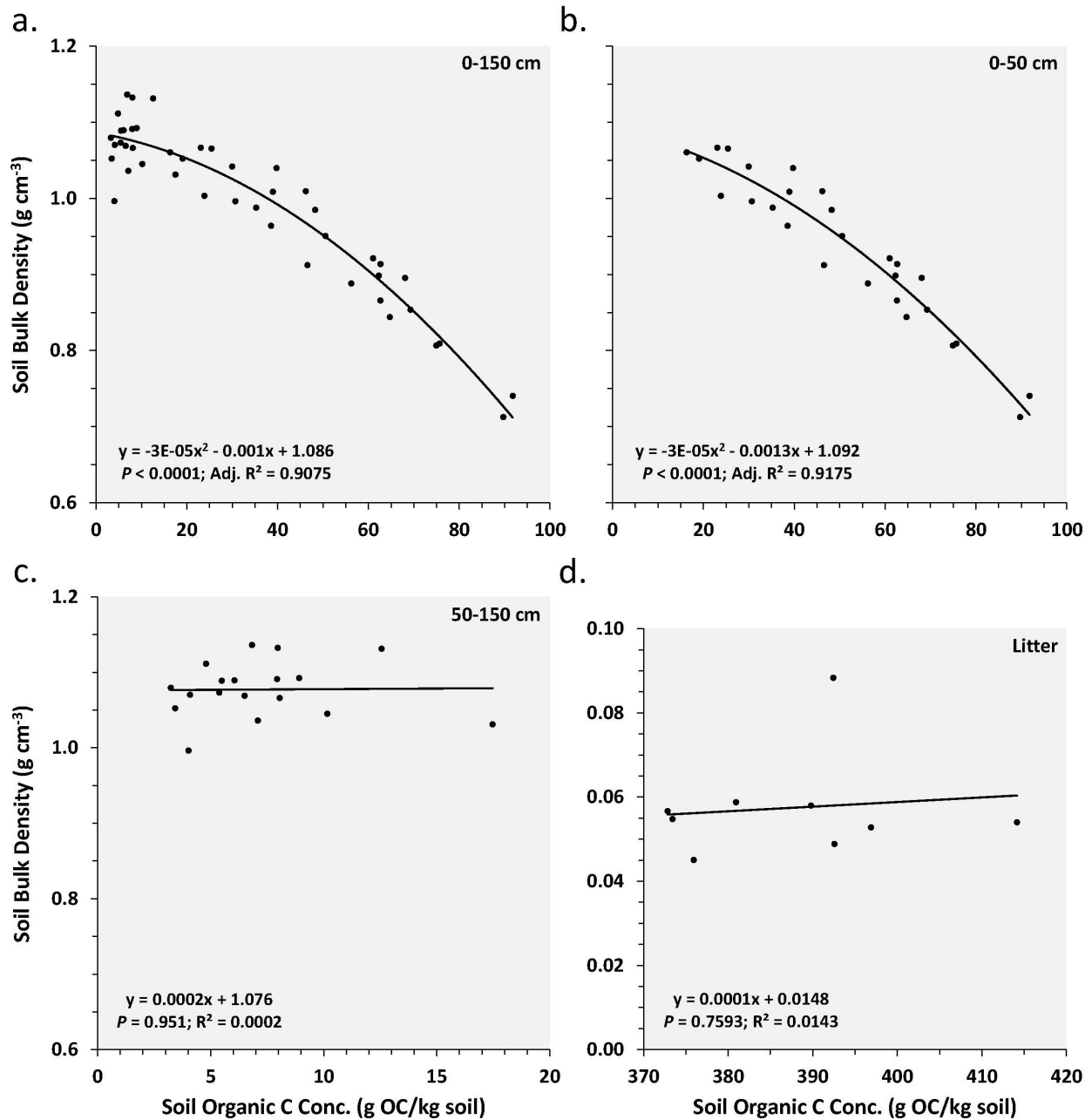


Figure 3.4. Soil bulk density versus organic carbon concentration for the whole mineral soil (a), the 0-50 cm soil depth layers (b), the 50-150 cm soil depth layers (c), and the litter layer (d).

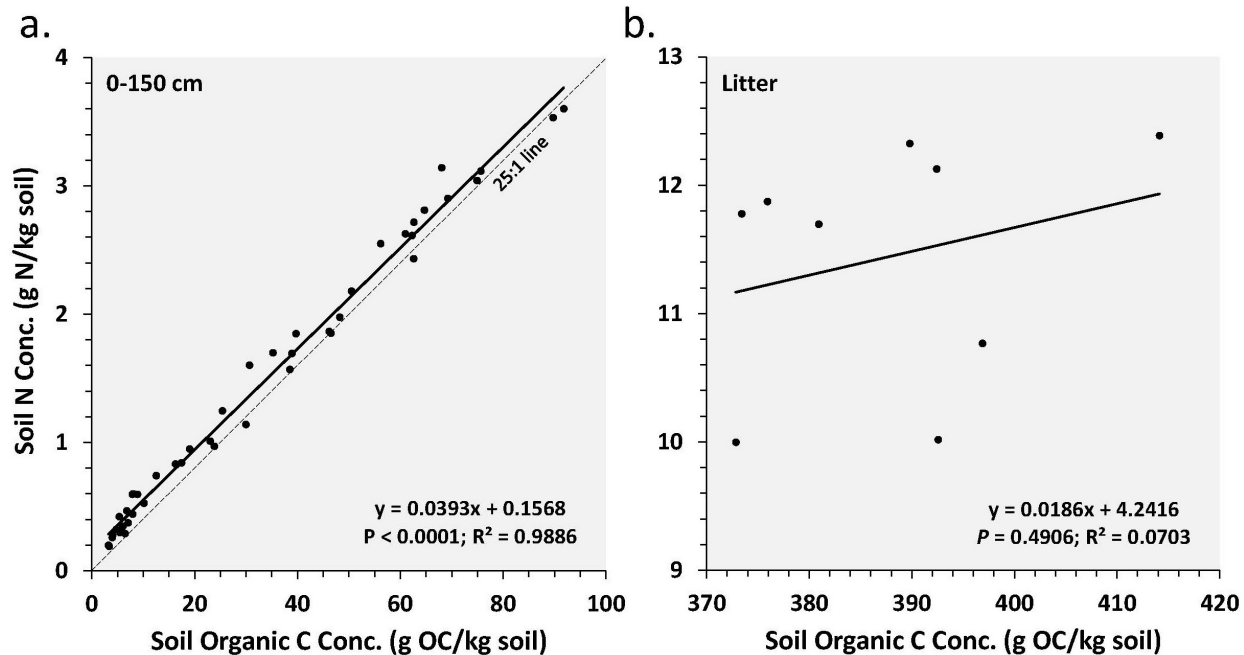


Figure 3.5. Soil nitrogen concentration versus organic carbon concentration for the whole mineral soil (a) and the litter layer (b).

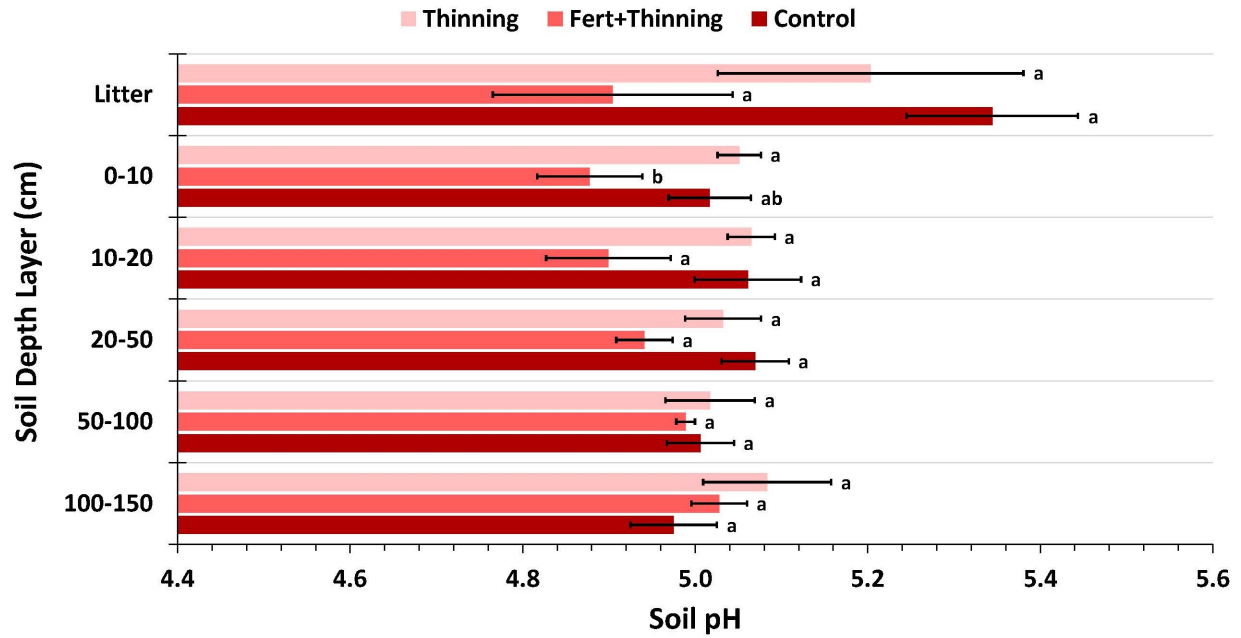


Figure 3.6. Mean soil pH by treatment group. Sample size for all treatment groups and soil depth layers is three. Error bars represent \pm one standard error. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

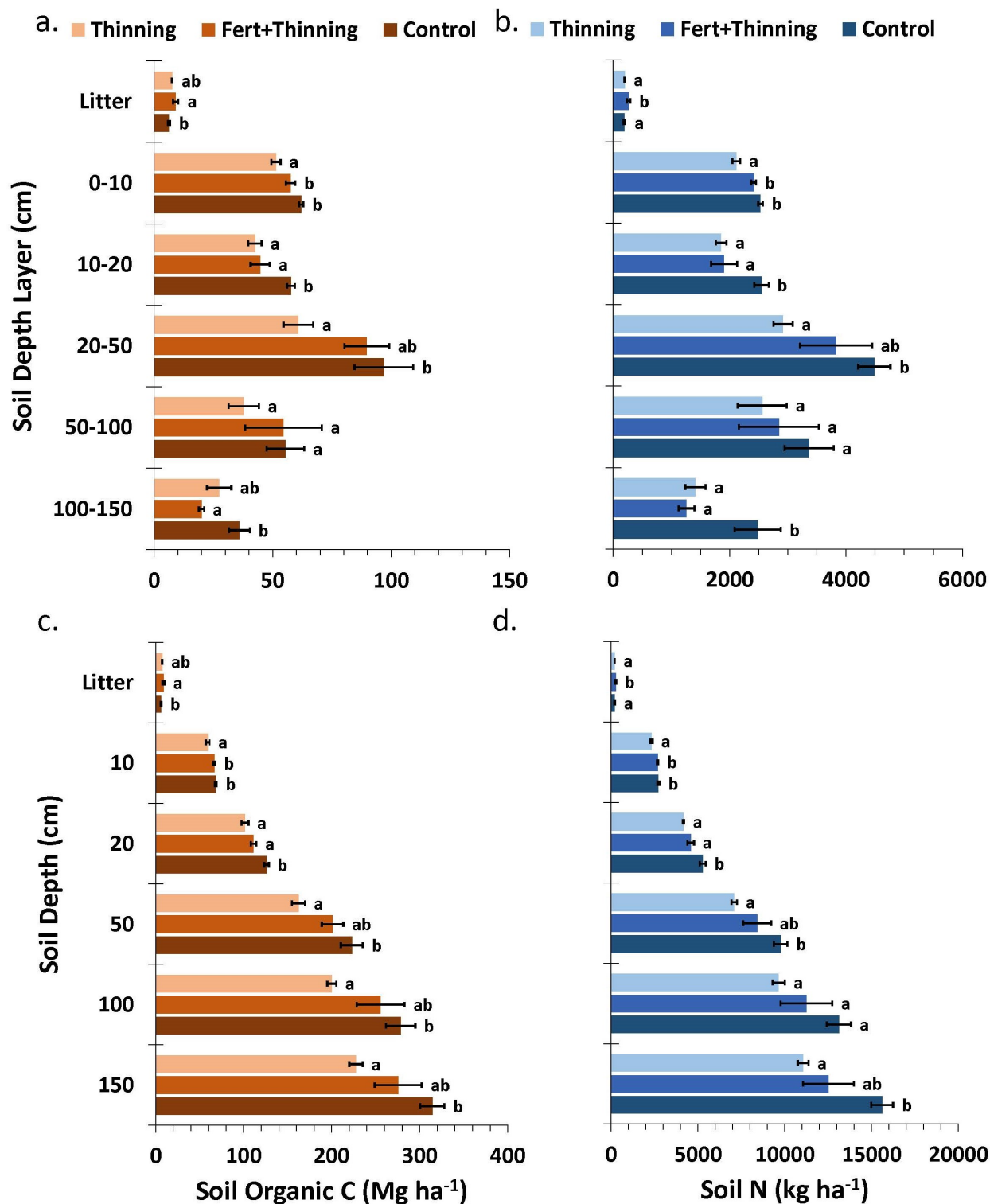


Figure 3.7. Mean soil organic carbon stock (a), mean soil nitrogen stock (b), cumulative mean soil organic carbon stock (c), and cumulative mean soil nitrogen stock (d) by treatment group. Calculated using the fixed depth equation. Sample size for all treatment groups and soil depth layers is three. Error bars represent \pm one standard error. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

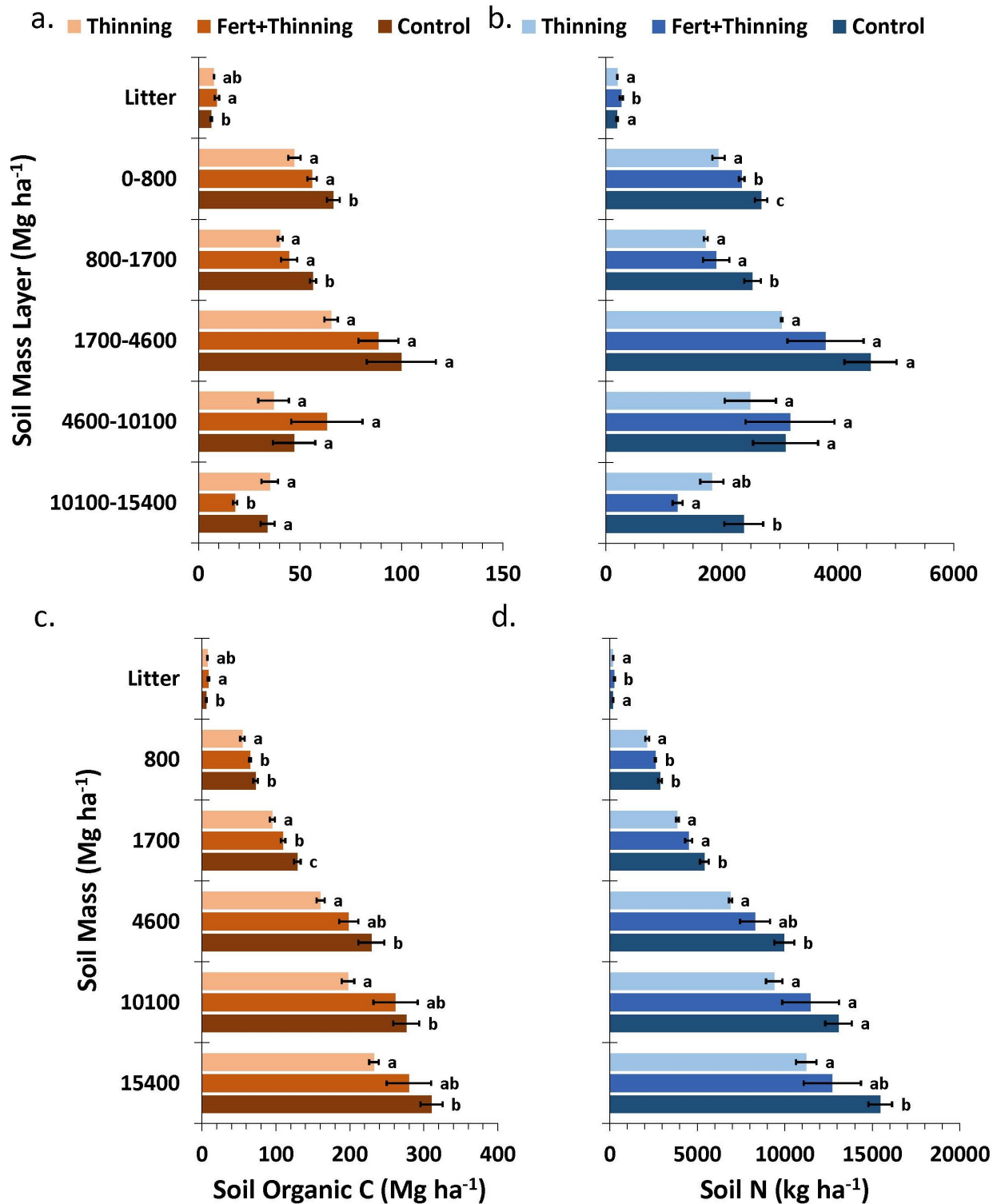


Figure 3.8. Mean soil organic carbon stock (a), mean soil nitrogen stock (b), cumulative mean soil organic carbon stock (c), and cumulative mean soil nitrogen stock (d) by treatment group. Calculated using the mass based approach. Sample size for all treatment groups and soil mass layers is three. Error bars represent \pm one standard error. Means within each soil depth layer accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

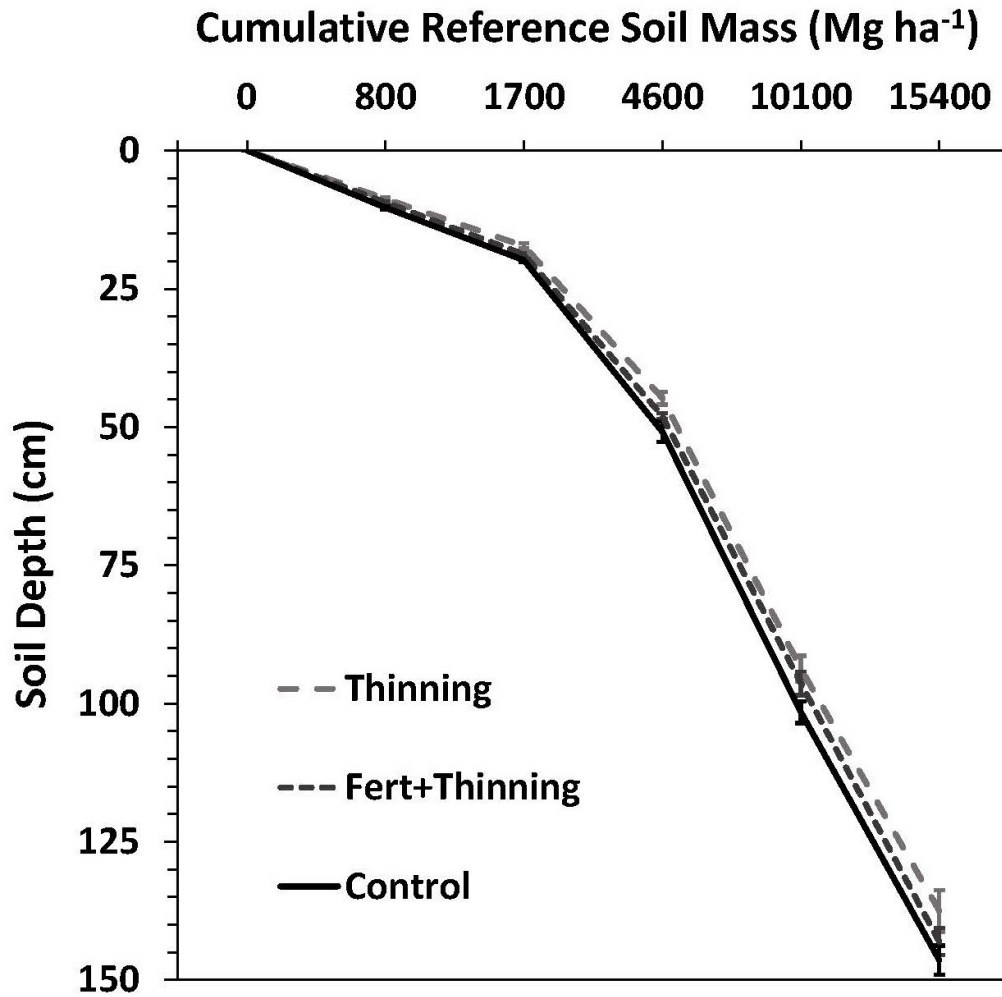


Figure 3.9. Mean depth to reference soil mass by treatment group. Sample size for all treatment groups and cumulative soil masses is three. Error bars represent \pm one standard error. Reference mass layers were set using the lowest mean soil mass across treatments for each soil depth layer.

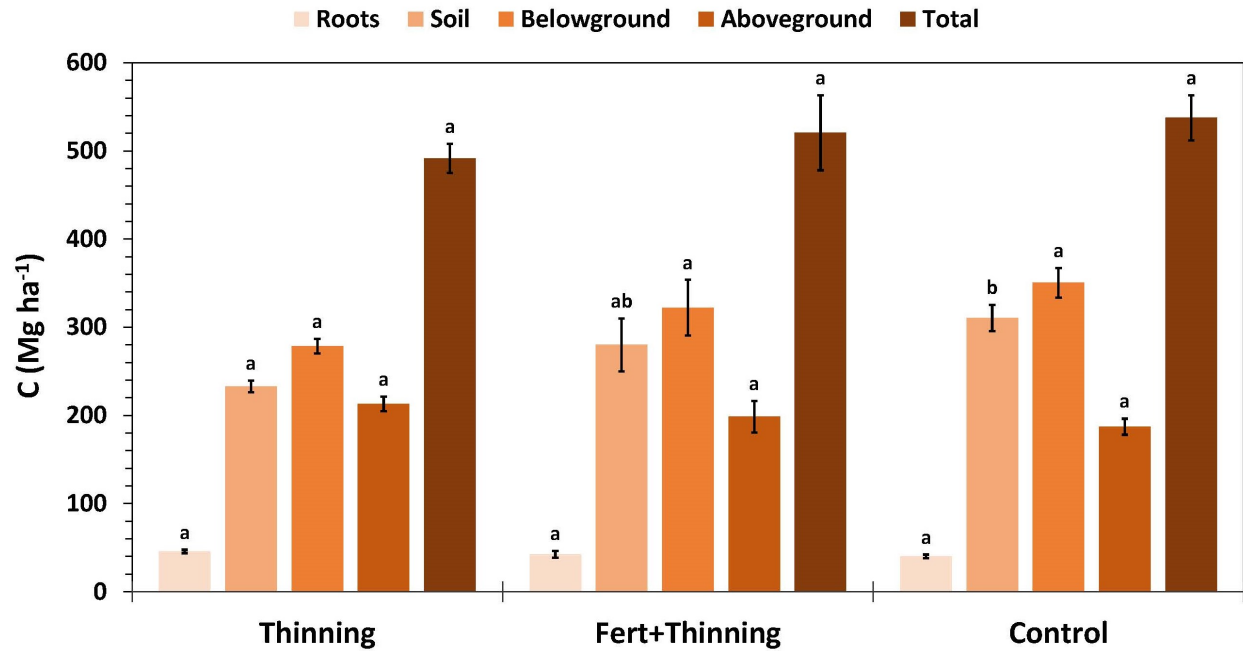


Figure 3.10. Mean carbon stocks by treatment group. Total carbon stocks are the sum of above- and below-ground carbon stocks, where aboveground and root carbon stocks include the biomass of thinned trees, and belowground carbon stocks are the sum of root carbon and soil organic carbon. Soil organic carbon stocks were calculated using the mass based approach. Sample size for all treatment groups and categories is three. Error bars represent \pm one standard error. Means within each category accompanied by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.1$).

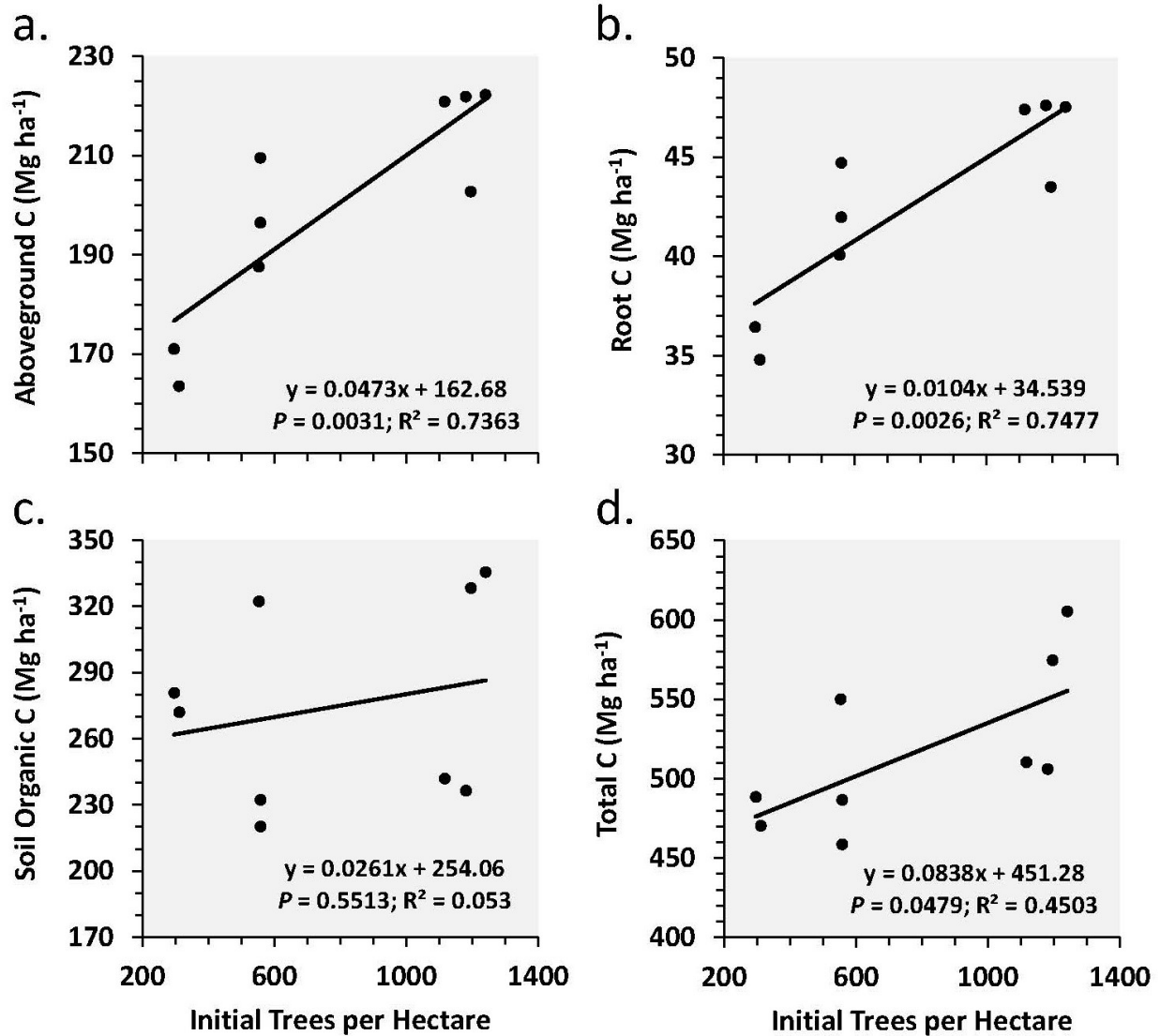


Figure 3.11. Aboveground carbon stock (a), root carbon stock (b), soil organic carbon stock (c), and total carbon stock (d) versus initial trees per hectare for each of the nine treatment plots. Total carbon stocks are the sum of above- and below-ground carbon stocks, where aboveground and root carbon stocks include the biomass of thinned trees, and belowground carbon stocks are the sum of root carbon and soil organic carbon. Soil organic carbon stocks were calculated using the mass based approach.

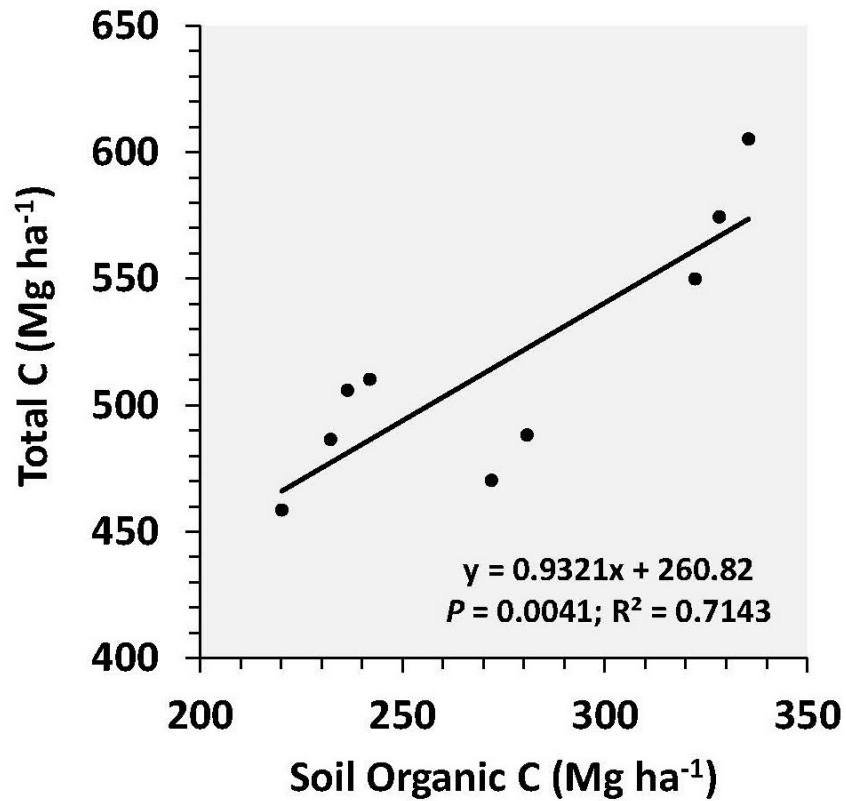


Figure 3.12. Total carbon stock versus soil organic carbon stock for each of the nine treatment plots. Total carbon stocks are the sum of above- and below-ground carbon stocks, where aboveground and root carbon stocks include the biomass of thinned trees, and belowground carbon stocks are the sum of root carbon and soil organic carbon. Soil organic carbon stocks were calculated using the mass based approach.

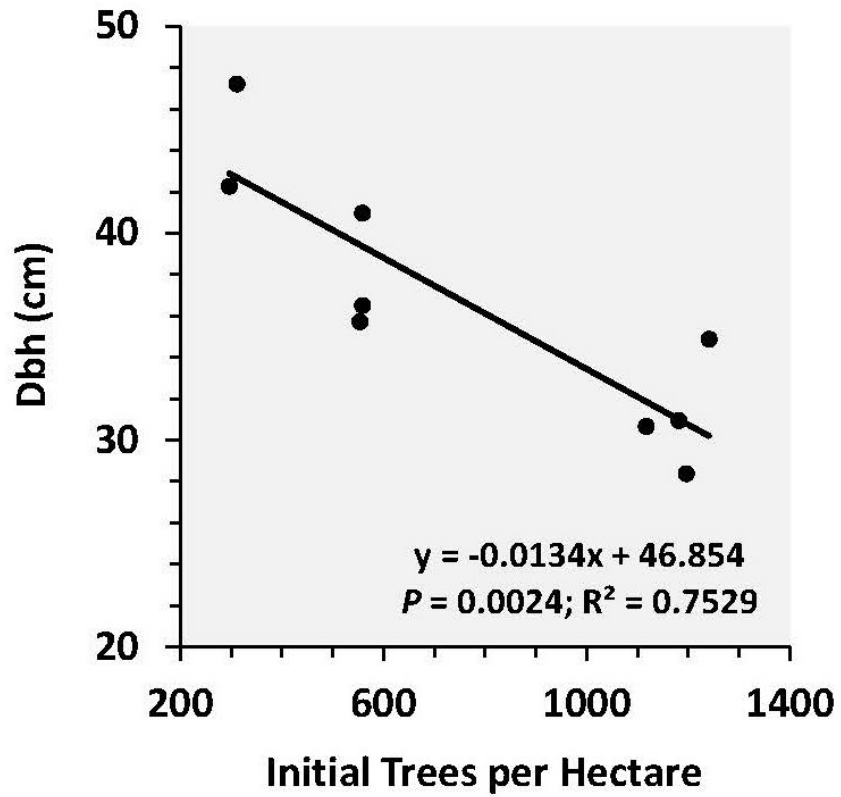


Figure 3.13. Final mean diameter at breast height (dbh) versus initial trees per hectare for each of the nine treatment plots.