Jason James

The Effects of Forest Harvesting and Land-Use Change on Soil Carbon and Nutrient Cycling

Jason James

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Reading Committee:
David Butman, Co-Chair
Rob Harrison, Co-Chair
Drew Gorman-Lewis

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Abstract

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Chairs of the Supervisory Committee:
David Butman & Rob Harrison
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The properties and processes of deep soil horizons remain an important gap in knowledge due to the long history of shallow soil sampling. The majority of soil carbon and nitrogen can be found beneath the A horizon in most soils, particularly those deeper than one meter to bedrock. Such soils are common in many parts of the world, especially the Pacific Northwest where the combination of age (hundreds of thousands of years in many places) and high precipitation lead to rapid development of subsoil pedogenic features. My dissertation seeks to explore deep soils to better understand the relationships between nutrient cycles and the impact of land-use change and forest harvesting on soil carbon. In a series of 36 soil profiles sampled to 3 meters depth across the Pacific Northwest, pedogenesis frequently extended deeper than the upper 2 meters that is arbitrarily defined as the maximum soil depth for soil taxonomy. The combination of

landslides, volcanic activity, and flooding have buried soils in many forests across the region, and these horizons can be important repositories of plant nutrients. In several cases, B horizon development extended deeper than could be excavated with a backhoe (3+ meters). The diversity of parent materials, climate gradients (with both latitude and orography), and soil carbon and nitrogen cycles directly control exchangeable cation cycling across the Pacific Northwest. Soils that experience more precipitation and contain higher levels of carbon and nitrogen hold less exchangeable calcium and magnesium in the whole soil profile, and also have more deeply distributed stocks of exchangeable cations within the profile. Consequently, human disturbances that alter soil carbon can have repercussions for plant nutrition. Millions of acres of forest in the US are actively managed for timber production, but the type and intensity of soil disturbance varies considerably. In a meta-analysis examining the response of soil carbon to forest management from 112 publications, I found that harvesting reduces soil carbon by 11% overall. This loss is predominately driven by O horizon losses (-30%), but there were also losses in surface mineral soil (0-15 cm; -3%). Loss of soil carbon extends deep into the soil with increasing average losses at each depth interval examined; however, very few studies examined soils deeper than 30 cm, leading to extremely wide confidence intervals in deeper soil. Land-use change, even converting one forest type for another, can substantially alter soil carbon cycling, as well. In the Brazilian Cerrado, over half of the natural vegetation has been lost to agriculture, silviculture or urban development, with a substantial portion of the landscape planted with *Eucalyptus* trees. The shift in the aboveground plant community increases aliphatic functional groups in water-soluble organic matter (WSOM), which may lead to reduced microbial biomass in *Eucalyptus* plantations that lack native understory trees. The difference in radiocarbon age between WSOM and bulk soil carbon is smaller under *Eucalyptus* relative to Cerrado, suggesting either mineralization or leaching of aged organic matter under this land use. The consequences of land-use change extend deep into the soil profile, particularly in the Oxisol soils of Brazil which are especially reliant upon soil organic matter for critical ecosystem services like nutrient recycling and water holding capacity.

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Chapter 1. GENERAL INTRODUCTION

Six years ago, on my first day as a graduate student at UW, I climbed down into a deep trench in the middle of a gently sloping forest and looked at a deep soil profile for the first time. Little did I know the importance of the event in shaping my career, both my research interests and future collaborators. The analyses I performed on the samples taken from that pit and twenty other sites across Washington and Oregon became my MS dissertation – and my first two published papers. My fascination with deep soil horizons has shaped all of my work so far and guided me toward a broader understanding of biogeochemistry across ecosystem and disciplinary boundaries.

The second chapter of this dissertation reveals some of the important pedogenic features that can be found in deep regions of the soil. Using an excavator to sample down to 3 meters provided a unique opportunity to document the abundance of biological activity that takes place deep in soil profiles. Subsoil B and C horizons contain a surprising contradiction: they possess the oldest organic matter, often by thousands of years, yet are the youngest part of the soil, newly exposed to the weathering processes that connect geological, hydrological, and biological cycles. A majority of the 36 soils examined over 2 years of sampling contained visible tree roots to the bottom of the profile; indeed, plant roots commonly extend many meters into the soil (Laclau et al., 2013; Stone and Kalisz, 1991). Buried soils were identified in several locations across the coastal Pacific Northwest and can dramatically alter nutrient cycling, water holding capacity, and carbon storage in these soils (Chaopricha and Marin-Spiotta, 2014). For example, root access to buried A horizons below pumice cinders laid down by volcanic eruptions can as much as double the rate of growth in young ponderosa pine trees (Hermann and Petersen, 1968). Despite the difficulty of sampling and studying deeper parts of the soil, it remains one of the principal frontiers in the discipline of soil science.

Deep soil horizons may also be particularly important as repositories for exchangeable cations that leach from surface soil. In chapter three, I explore the drivers of exchangeable cation vertical distribution in these deep soil profiles. The quantity of cations available to plants varies by over an order of magnitude across the Pacific Northwest. While nitrogen is generally considered the most limiting nutrient in the region (Littke et al., 2014), areas with high N fixation rates (Van Miegroet and Cole, 1984) and N-bearing parent materials (Corti et al., 2002) can develop cation (particularly calcium) deficiencies (Perakis et al., 2006). Areas with high precipitation and organic C and N stocks tend to have more deeply distributed divalent cation stocks (Ca^{2+} and Mg^{2+}), likely driven by the availability of NO_3^- and organic ligands that are highly mobile under acidic conditions. This chapter, completed early in my PhD, led me to think much more deeply about the connections between biogeochemical cycles and particularly the role of dissolved organic matter in soil processes.

To further this line of inquiry, I sought to explore the impact of forest management on both solid- and dissolved-phase organic matter. In the fifth chapter, I show the effect on soil organic matter (SOM) of converting native Cerrado forest to *Eucalyptus* plantations in São Paulo State, Brazil. The soils in this region of Brazil are extremely old and highly weathered, forming either Ultisols or Oxisols in most places (Raij et al., 2004). Because kaolinite and other low-charge iron oxides tend to be the predominant clay minerals in soils of this type (Schaefer et al., 2008), forest nutrition relies principally upon SOM. The shift in vegetation due to land use change can result in SOM losses, particularly over multiple harvest rotations and repeated disturbance (Cook et al., 2016). *Eucalyptus* litter, and particularly the water-soluble organic matter that may be leached out of it, appears to be more aliphatic rich than Cerrado forest litter and to support a smaller microbial pool. Changes in radiocarbon with depth in four soil profiles deeper than 1 m suggest that microbial degradation of older SOM could be stimulated by land use change. The processes that control how SOM changes in response to human management are particularly important to understand in the context of both climate change and continuing, world-wide human manipulation of ecosystems.

Forest management has long-term impacts on soil carbon stocks around the globe (Jandl et al., 2007; Nave et al., 2011, 2010). In the fourth chapter, I sought to better quantify the change in mineral soil organic carbon, particularly in deeper parts of the soil profile. Despite this aim, I found that very little literature sampled soils deeper than the top 30 cm of soil. Using a meta-analysis approach, I found that the average carbon loss was larger in deeper layers but had large confidence intervals due to small sample size. Nonetheless, there were still significant losses of mineral soil carbon that persisted decades after disturbance. The impact of harvesting was also not uniform across soil types. Acidic soils seemed to be more prone to carbon losses, particularly Spodosols and Ultisols, while more exchangeable-rich soils (like Alfisols) showed smaller losses. There is growing international recognition of the importance of soil organic carbon (UN FAO, 2017) and an increasing emphasis on managing forests for carbon sequestration (Jandl et al., 2007; Rhemtulla et al., 2009). Because soils are the largest terrestrial carbon pool, the lack of deep soil analysis in the literature is a critical gap in knowledge.

In addition to the chapters included here, I have had the opportunity to collaborate on a number of other projects and to develop skills that round out my scientific background. In collaboration with the US Forest Service and The Nature Conservancy, I served as the lead author on a systematic review of the literature to examine the effects of forest thinning and prescribed fire on total ecosystem carbon (https://doi.org/10.1016/j.foreco.2018.07.029). This study extended my expertise into the areas of fire science and contributed to the US Forest Service endeavor to sustainably manage public forests in western North America in the face of high fuel loads and warming climate.

In conjunction with my fifth chapter examining water soluble organic matter, I analyzed samples with fluorescence spectroscopy, a technique that is increasingly common for characterization of dissolved organic matter in both terrestrial and aquatic systems (e.g. Fellman et al., 2010). I utilized a supervised machine learning technique called partial least squares regression to evaluate several large excitation-emission matrices (EEM) datasets from both my own soil extracts and from a database of riverine DOM samples. I found that EEM characteristics in 15 major rivers in North America sampled over 2 years can predict more expensive and time-consuming chemical measurements such as radiocarbon content and hydrophobic/hydrophilic organic acid content. In addition to this project, I had the chance to learn how to extract purified CO_2 out of dissolved samples (at Yale University). This led me to collaborate on a project with the USGS to examine the radiocarbon age of freshwaters in permafrost seeps, rivers, and lakes in the Yukon Basin, Alaska. Articles documenting the findings of each of these studies are currently in composition.

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Chapter 2. LESSONS ON BURIED HORIZONS AND PEDOGENESIS FROM DEEP FOREST SOILS

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2.1 ABSTRACT

The lower boundary of soil has been a point of contention among soil scientists for decades. Recent evidence suggests that soil is much deeper than is measured by many pedological studies and that arbitrary definitions of maximum soil depth unnecessarily exclude important regions of the soil profile. This paper provides illustrated examples of soil profiles that have important deep soil characteristics or buried horizons. Soil pits were excavated with a backhoe to at least 2.5 m depth at 36 sites throughout the Douglas-fir (*Pseudotsuga menziesii (Mirb.) Franco*) ecoregion of the Pacific Northwest. These soils cover four orders – Andisol, Inceptisol, Alfisol, and Ultisol – and highlighted the hidden diversity of subsoil characteristics throughout the region. The roots of trees and understory species often extended deep into the C horizons of soil. Despite experiencing less pedogenic development than surface horizons, C horizons are important both as the frontier of soil formation and as an important resource for plant growth. In some cases, B horizons are far deeper than the 1 or 2 m depth arbitrarily assumed to represent the whole soil. In other cases, subsoil hides buried profiles that can significantly impact aboveground plant growth. These buried horizons are important repositories of nutrients and carbon that are poorly understood and rarely sampled. Ignoring subsoil precludes incorporating soil burial or deep soil processes into biogeochemical and global carbon cycle models.

2.2 INTRODUCTION

As the primary resource for plant growth and the central medium for nutrient, water, and organic matter recycling, soil plays a key role in determining the productivity of terrestrial ecosystems. In the Pacific Northwest, it has long been observed that the depth of soil directly correlates with forest productivity (Steinbrenner, 1979). Yet, most studies in the ecological literature restrict sampling to within the top meter of soil due to labor, time, and budget constraints (Harrison *et al.*, 2011). For example, in two reviews of over 360 articles examining how land use change affects soil organic matter, median sampling depth was 20 cm, and over 90% of studies restricted themselves to 30 cm sampling depth or less (Post and Kwon, 2000; West and Post, 2002). While soil science has come a long way in explaining the essential functions and services provided by soil, the lack of research into deep soil precludes a complete and mechanistic understanding of how soil affects aboveground ecosystem processes and plant productivity. But in order to address this issue, one must consider one of the most confounding questions in soil science: how deep is soil?

While this question appears on its surface to be simple, the true depth of soil has been a point of great contention among experts in the field. Some assert that *true soil* includes the O, A, E, and B horizons (Plaster, 2014), and that C horizons are actually part of the geological domain because they are "little affected by pedogenic [soil-forming] processes" (Soil Survey Staff 2014). Because the lower boundary of soil is difficult to define for many soils, the Soil Survey Staff (2014) suggests "there is no alternative but to set the lower limit of soil at the arbitrary limit of 200 cm." Such simplification of the depth of soil fails to acknowledge that early soil scientists conceived of soil as "the entire upper weathering layer of the earth's crust," which undoubtedly includes the C horizon (Ramaan, 1928; Glinka, 1931). A more recent investigation conducted at the Calhoun Experimental Forest in South Carolina concluded that the entire profile to 8 m depth bore "the strong imprint of biological activity" in all three phases – gas, liquid, and solid (Richter and Markewitz, 1995). Indeed, the average maximum rooting depth for trees around the globe is 7.0±1.2 m, 5.1±0.8 m for shrubs, and 2.6±0.1 m for herbaceous plants (Canadell *et al.*, 1996). If one conceives of soil as the material at the Earth's surface influenced by the confluence of biota, parent material, climate, topography, and time (Jenny, 1941, 1961), then soil is more voluminous than assumed or measured by much of the soil and ecological literature (Richter and Markewitz, 1995).

Objectives

- To address the lack of sampling and direct observation of deep soil.
- To highlight conditions in which soil burial and deep pedogenesis can occur.
- To illustrate the importance of deep soil to ecosystem processes and cycles so as to spur further research.

2.3 METHODS

We excavated and sampled 22 soils from 2.5 to 3.5 m depth in summer 2012 and 14 more in summer 2014 to address the lack of deep soil information available in the Pacific Northwest. These sites stretch across the coastal Douglas-fir (*Pseudotsuga menziesii (Mirb.) Franco*) ecoregion from the western foothills of the Cascade Mountains to the Pacific Ocean and from southern Oregon to northern Washington, and are all intensively managed for timber production. This paper focuses on a subset of 5 sites that highlight particular subsoil processes and characteristics, such as deep B horizon development or buried soil horizons, that serve as examples of processes at work throughout the Pacific Northwest and beyond (Figure 2.1). Cool, wet winters and warm dry summers characterize the climate in this region. The rain shadow and elevation effects of the Olympic, Coast, and Cascade Ranges largely drive variation in precipitation and temperature between sites.

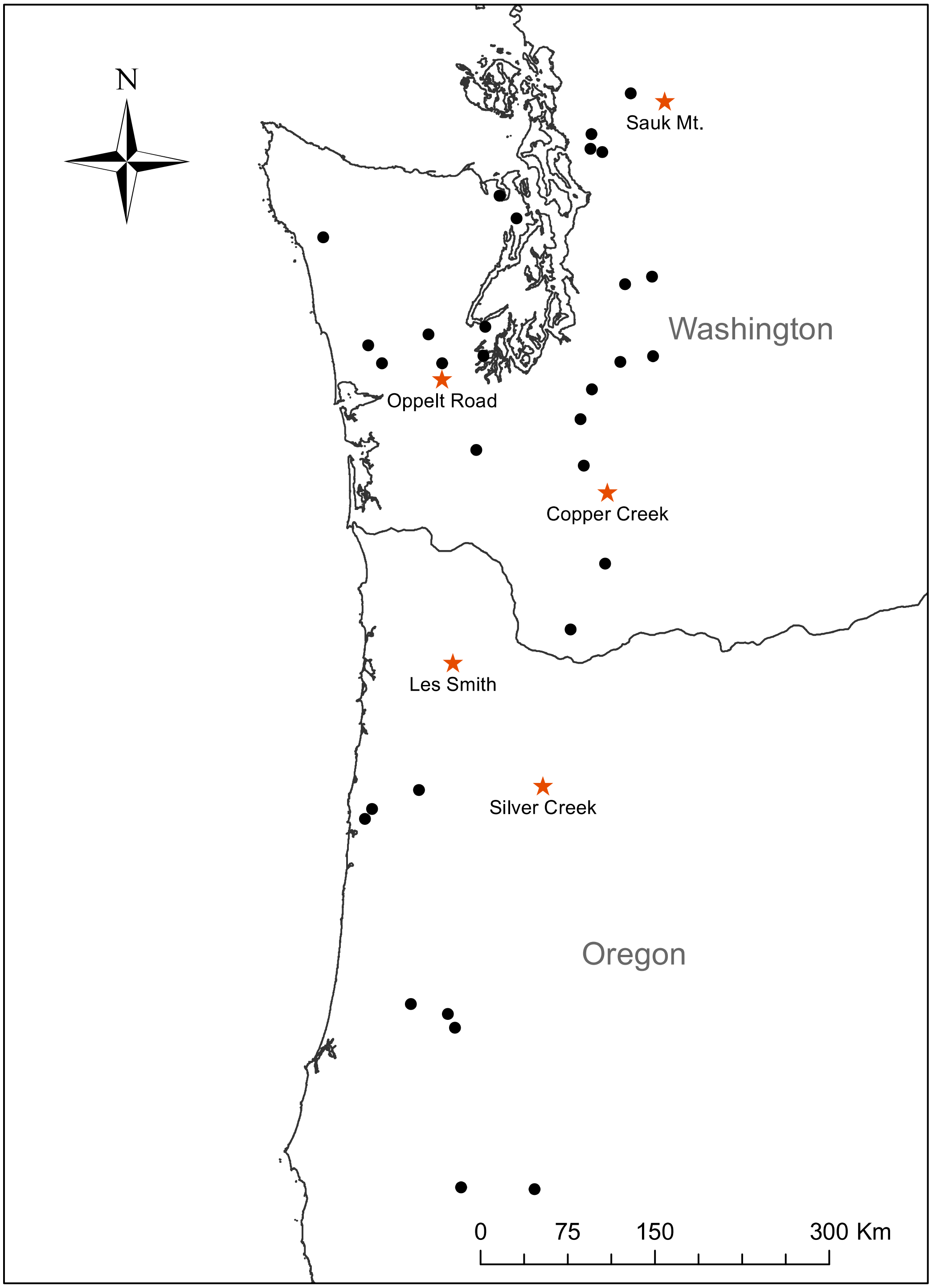

Figure 2.1. Map of 36 deep soil sampling sites in the coastal Pacific Northwest highlighting the 5 sites specifically discussed in this paper.

At each site, a soil pit was excavated with a backhoe, pedon description was recorded, and bulk density samples were taken at the regular depth intervals of 0-10 cm, 10-20 cm, 20-50 cm, 50-100 cm, 100-150 cm, 150-200 cm, 200-250 cm, and 250-300 cm (Figure 2.2). Samples were analyzed for bulk density, pH, Munsell soil color, and texture in the lab. Four soil orders are represented in the dataset - Andisols, Inceptisols, Alfisols, and Ultisols – which reflects the turbulent geological history of the region, including recent glaciation of the Puget Sound basin and active volcanism. This diverse geologic history makes the Pacific Northwest ideal to explore deep soil processes since it provides examples of soils old and young, fine and coarse textured, across steep elevation and precipitation gradients. Collectively, these soils highlight the unseen diversity of subsoil environments and characteristics across the region.

Figure 2.2. Bulk density sampling at Oil City Road, south of Forks, WA. Careful shoring was used to ensure safety while working in the soil pits.

2.4 RESULTS & DISCUSSION

C horizons are actively influenced and exploited by plants, despite less pedogenic development than A or B horizons. This can be illustrated by the soil profile at Oppelt Road (Figure 2.3). The soil is typically developing for southwestern Washington, beyond the southernmost reach of the Cordilleran Ice Sheet during the last ice age. The profile closely resembles the Centralia series (a Palehumult), with an Oi and moderately thick A horizon (0-20 cm) grading into thick argillic horizons (20-133 cm) (Table 2.1). The transition to C horizon below the argillic horizons is gradual, and bulk density remains relatively low despite the transition to coarser texture. Medium to fine tree roots are common down to 193 cm depth, and a few fine roots were observed down to the bottom of the pit at 270 cm. The C horizons, though decidedly less developed than the Bt horizons, are clearly an important source of nutrients and or water which plant roots are actively exploiting. The soil is also highly acidic throughout, indicating significant biogenic weathering, even in deep horizons. The clear imprint of biological activity deep in this soil helps to demonstrate that C horizons are an active frontier for pedogenic development and can impact forest growth.

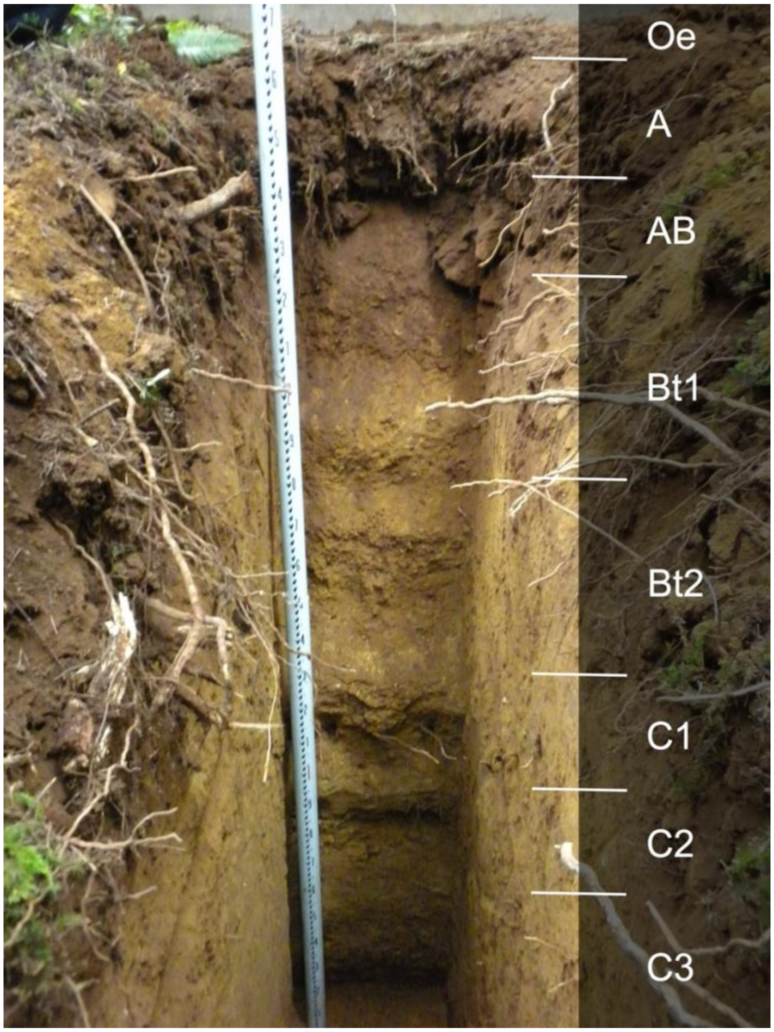

Figure 2.3. Soil profile at Oppelt Road near Elma, WA.

Table 2.1. Oppelt Road pedon description. Resembles Centralia series. Elevation 91 m a.s.l.

Horizons	Depth (cm)	Color (moist)	Texture	Structure	Roots	BD (g cm^{-3})	pH
Oi	1 - 0					0.43	
A	0 - 20	7.5YR 3/3 dark brown	Loam	medium granular	very many coarse, medium, and fine	1.03	4.57
AB	20 - 35	7.5YR 4/6 strong brown	Loam	very large subangular blocky	few large, common medium	1.04	4.57
Bt1	35 - 80	7.5YR 4/6 strong brown	Sandy clay loam	large subangular blocky	few fine	1.13	4.60
Bt2	80 - 133	7.5YR 4/6 strong brown	Sandy clay loam	medium blocky	few fine, common medium and large	1.12	4.86
C1	133 - 171	10YR 4/4 dark yellowish brown	Sandy loam	weak blocky	few large	1.01	4.70
C2	171 - 193	10YR 5/6 yellowish brown	Loamy sand	medium subangular blocky	common medium, many fine	1.01	4.88
C3	193 - 270+	10YR 4/6 dark yellowish brown	Loamy sand	medium subangular blocky	very few fine roots	1.12	4.77

The arbitrary definition of maximum soil depth at 2 m limits our understanding of pedogenic processes, especially weathering and clay formation that can take place beyond this depth. In fact, argillic B horizon development can be quite extensive. Take, for example, the Ultisol at Les Smith (Figure 2.4). Despite excavation to 3 m depth at this site, no C horizon was encountered (Table 2.2). This profile resembles the Olyic series (a Haplohumult), though without evidence of C horizon(s) or paralithic contact. With respect to the latter features, the soil is similar to the geographically associated Tolke series (a Hapludand). Despite deeper Bt horizon development than the Oppelt Road profile, roots appeared much more restricted to surface soil, likely due to lack of macro-pores and high clay content. Despite some root restriction at this site, a live medium-sized root emerged into the pit at 2.75 m, and a few fine roots were evident throughout the Bt2 horizon. Soil pH actually decreases with depth throughout the profile, and weathering of primary minerals extends deep into the soil. Clearly, even excavation of deep soil with a backhoe did not capture the full depth of soil development at this site. In fact, at only 5 of our 35 sites was bedrock reached above 3 m depth. Deep soil can be quite voluminous. For example, seismic testing of the Boistfort series soil at the Fall River Long Term Site Productivity study in central Washington revealed contact with highly weathered basalt at over 5 m depth and contact with hard basalt at greater than 15 m depth (Ward and Terry, 2003).

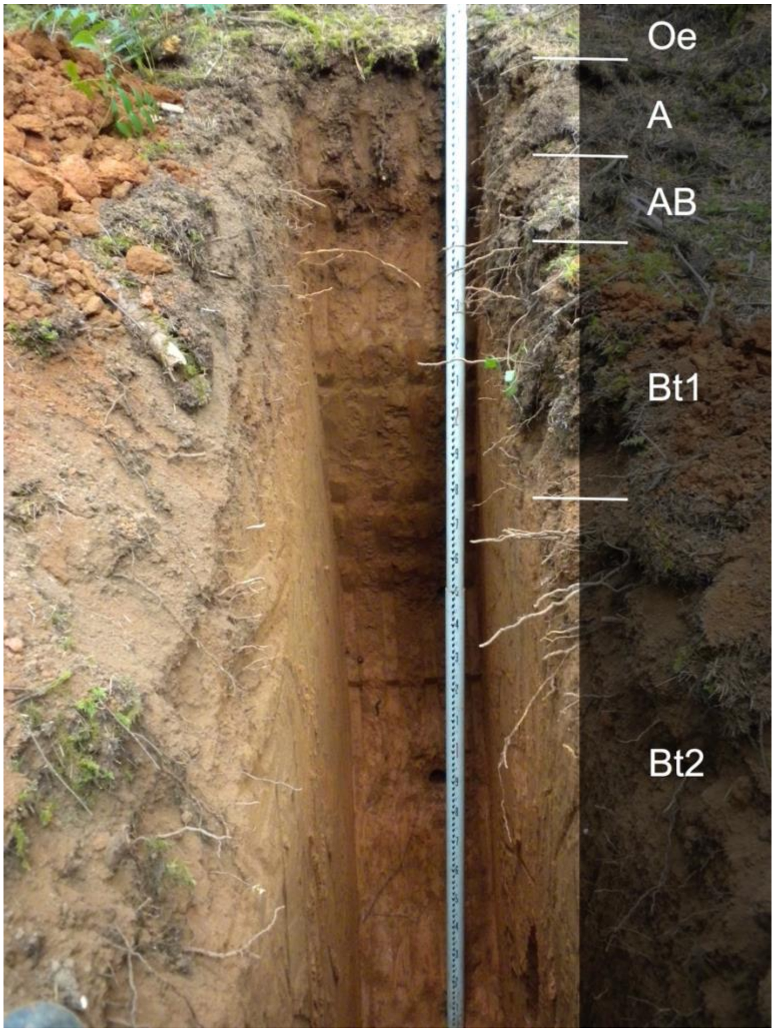

Figure 2.4. Soil profile at Les Smith near Yamhill, OR.

Table 2.2. Les Smith pedon description. Resembles Olyic series. Elevation 451 m a.s.l.

Horizons	Depth (cm)	Color (moist)	Texture	Structure	Roots	BD (g cm^{-3})	pH
Oi	1 - 0					0.12	
A	0 - 21	7.5YR 3/3 dark brown	Silt loam	crumb, hard concretions	very many coarse, medium, and fine	0.83	5.31
AB	21 - 42	7.5YR 4/4 dark brown	Silty clay loam	blocky / granular	many coarse and fine	0.91	5.07
Bt1	42 - 110	7.5YR 4/6 strong brown	Silty clay loam	large subangular blocky	few fine	1.17	4.78
Bt2	110 - 300+	5YR 5/8 yellowish red	Clay loam	very large subangular blocky	very few fine, one medium at 275 cm	1.42	4.72

Deep soil is not restricted merely to well-developed Ultisols in the Pacific Northwest. Soils that have been influenced by recent glaciation and volcanism can have important deep soil characteristics, as shown at Sauk Mountain (Figure 2.5). The soil is developed from coarse glacial outwash and colluvium, with volcanic ash mixed into the A horizon, much like the Barneston series (a Vitrixerand). Disturbance has clearly played an important role in soil development at this site as seen by the two buried soil profiles below the typical Oe, A, Bw, C horizon sequence (

Table 2.3). A strong reddish brown 2Bwb horizon is evident from 90-120 cm, which grades gradually into 2C1 and 2C2 horizons between 120-240 cm. Below this, two dark, relatively clay rich 3Btb horizons are found, evidence of ancient pedogenesis before the overlying material was laid down. Though not clear in the profile photo, digging with a shovel at the bottom of the pit revealed an organic rich 4Bwb horizon below these 3Bt horizons from 305-320+ cm. This site is located along a toe slope of Sauk Mountain just outside North Cascades National Park, and clearly has a history of mass wasting which regularly buries soil. Medium and fine roots extend throughout the soil profile despite high bulk density below 1 m, and the nutrient and water holding capacity of these buried horizons are clearly an important ecosystem resource.

Buried soils are large reservoirs of soil organic matter, yet they are typically not included in regional or global soil inventories (Chaopricha and Marin-Spiotta, 2014; Marin-Spiotta *et al.*, 2014). Soils may be buried through a variety of mechanisms including Aeolian or alluvial deposition, and volcanic ejecta (Chaopricha and Marin-Spiotta, 2014) in addition to soil burial through mass wasting and colluvial processes, as seen at Sauk Mountain. The rate of soil burial can be sensitive to changes in climate. Loess deposits over 6 m deep developed quickly after climate change at the beginning of the Holocene led to decreased vegetative cover and increased airborne dust, which buried organic-matter-rich soil across a large area (Marin-Spiotta *et al.*, 2014). Excluding deep soils from field sampling precludes understanding the extent of soil burial due to geomorphological and climate disturbances, as well as inclusion of buried soil carbon and nutrient stocks in biogeochemical and global carbon cycle models.

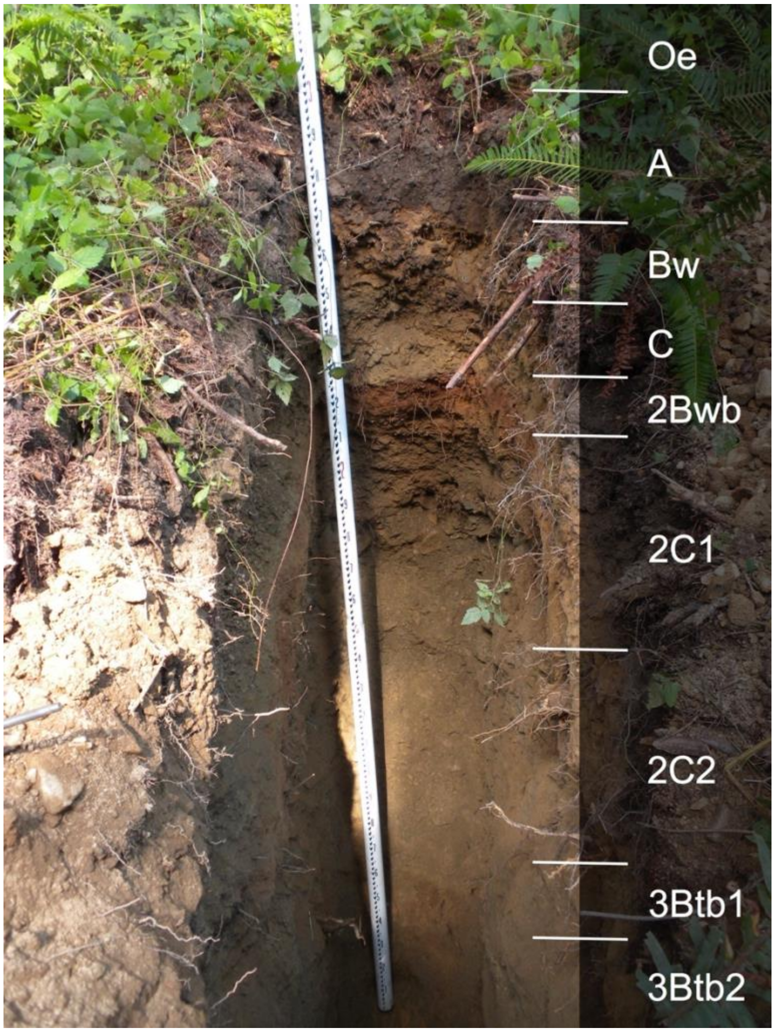

Figure 2.5. Soil profile at Sauk Mountain east of Concrete, WA.

Table 2.3. Sauk Mountain pedon description. Resembles Barneston series in surface. Elevation 442 m a.s.l.

Horizons	Depth (cm)	Color (moist)	Texture	Structure	Roots	BD (g cm^{-3})	pH
Oi	2 - 0					0.14	
A	0 - 25	10YR 3/2 very dark greyish brown	Gravelly sandy clay loam	subangular blocky / fine granular	very many medium & fine	0.98	5.65
Bw	25 - 55	10YR 4/3 dark brown	Gravelly clay loam	weak subangular blocky	many coarse, common medium	1.03	5.65
C	55 - 90	10YR 5/6 yellowish brown	Gravelly sandy loam	single grain	common fine	0.95	5.37
2Bwb	90 - 120	7.5YR 4/6 strong brown	Gravelly sandy loam	moderately strong, medium subangular blocky	many medium and fine	1.19	5.83
2C1	120 - 160	10YR 5/4 yellowish brown	Gravelly sandy loam	very weak small blocky	common medium and fine	1.43	5.83
2C2	160 - 240	2.5Y 5/3 light olive brown	Gravelly loamy sand	weak small blocky / single grain	few medium	1.50	5.97
3Btb1	240 - 275	7.5 YR 4/4 dark brown	Silty clay loam	medium subangular blocky	few coarse, common medium	1.36	5.78
3Btb2	275 - 305	10YR 6/8 brownish yellow	Silty clay loam	large angular blocky	common medium and fine	1.36	5.96
4Bwb	305 - 320+	10YR 5/4 yellowish brown	Sandy clay loam	moderately strong, medium subangular blocky	few medium	1.31	5.96

Plant access to buried soil horizons can be important for fertility and productivity. The soil profile at Copper Creek provides an example of a site where access to buried horizons may be particularly important (Figure 2.6). Located between Mt. St. Helens and Mt. Rainier, this site has experienced repeated volcanic disturbances, most recently ash deposition from the 1980 eruption of Mt. St. Helens. The soil closely resembles the Cispus series (a Humic Vitrixerand). Beneath a relatively thick Oe horizon lies an A horizon from 0-45 cm and Bw horizon from 45-92 cm (

Table 2.4). The C1 horizon is an 8 cm thick cap of volcanic ash that begins to be interlayered with pumice cinders in the C2 horizon. The C3 horizon of pumice lies above a buried A horizon at least 20 cm thick (250-270+ cm). Similar pumice soils occur around Crater Lake in central Oregon, where the eruption of Mount Mazama, approximately 7700 years ago, deposited pyroclastic material over a large swath of Oregon (and ash over much of Washington, Oregon, Idaho, western Montana, and southern British Columbia) (Birdseye and Carson, 1974). The depth of pumice over an older, pre-eruption soil varied from 50 cm to over 3 m, and bridging between individual pumice particles restricted root growth (Geist and Cochran, 1991). By fully excavating sixty young ponderosa pine growing on the soils surrounding Crater Lake, Hermann and Petersen (1969) found sharp increases in tree height and annual increment where root systems penetrated the C horizon pumice and reached the buried soil profile.

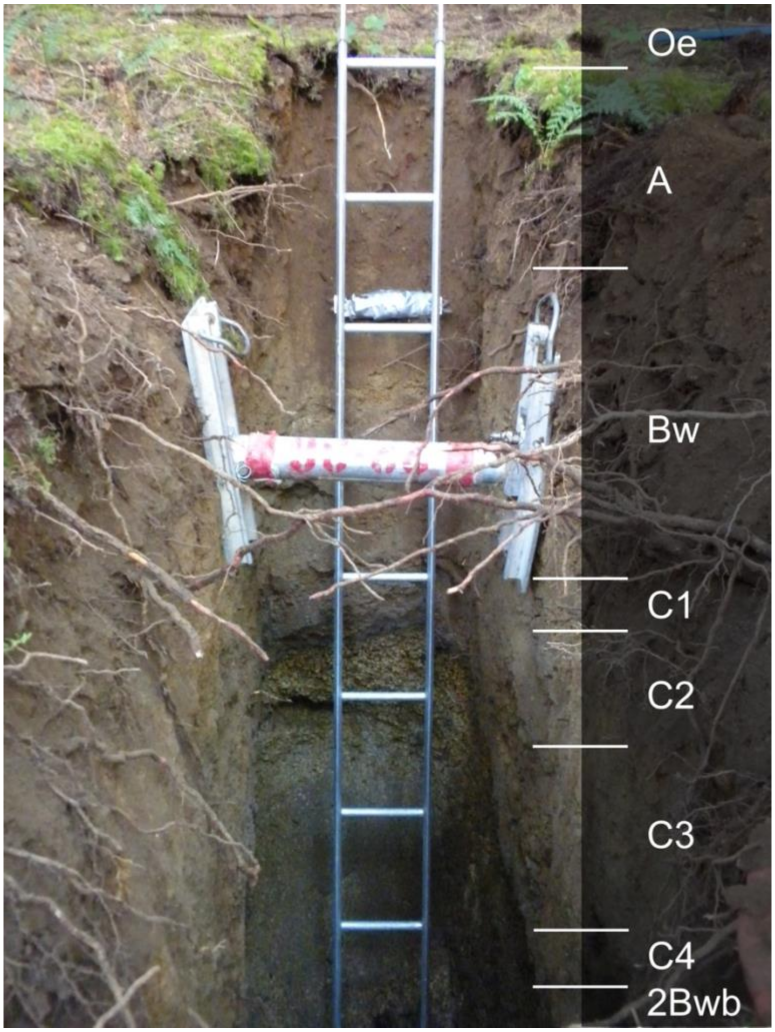

Figure 2.6. Soil profile at Copper Creek south of Morton, WA.

Table 2.4. Copper Creek pedon description. Resembles Cispus series. Elevation 1537 m a.s.l.

Horizons	Depth (cm)	Color (moist)	Texture	Structure	Roots	BD ($g\ cm^{-3}$)	pH
Oe	4 - 0					0.11	
A	0 - 45	10YR 2/2 very dark brown	Sandy loam	weak large granular	many coarse	0.71	4.91
Bw	45 - 92	10YR 4/4 dark yellowish brown	Gravelly loamy sand	weak large subangular blocky	many coarse	0.83	4.95
C1	92 - 100	10YR 6/3 pale brown	Gravelly loamy sand	weak large subangular blocky	very few fine	0.69	5.53
C2	100 - 130	2.5Y 6/6 olive yellow	Gravelly coarse sand	none	few very fine	0.42	5.68
C3	130 - 215	2.5Y 7/6 yellow	Gravelly coarse sand	none	few very fine	0.42	5.76
C4	215 - 230	2.5Y 7/4 pale yellow	Gravelly coarse sand	none	few very fine	0.38	5.90
2Bwb	230 - 250+	10YR 3/6 dark yellowish brown	Loam	med subangular blocky	few medium and fine	0.96	5.74

The processes that lead to soil burial are not always immediately apparent, especially when the disturbance in question occurred thousands of years ago. Take the soil profile at Silver Creek outside of Silverton, OR – which closely resembles the Cumley series (a Palehumult) – as an example (Figure 2.7). Below the moderately decomposed Oi layer and loamy A and AB horizons are two deep Bt horizons from 35 to 240 cm depth (Table 2.5). The Bt2 horizon contains more rock fragments than Bt1, and these rocks are coated with distinct clay films. Rather than grading to C horizon, however, the Bt2 horizon meets a flat, distinct boundary with a yellowish brown, clay-rich 2Btb1 horizon at 240 cm. This transitions gradually into a pale, brownish yellow 2Btb2 horizon, which like the Bt2 horizon above it contains more rock and gravel fragments coated with clay. A few medium and fine roots are found down to the bottom of these 2Btb horizons (300 cm depth). Once again, this 2Btb2 horizon does not grade into C horizon. Instead, it contacts a distinct, massive clay horizon only 2 cm thick (3Btm1, 300-302 cm depth), which itself transitions with an abrupt boundary into a red clay 3Btm2 horizon with paleomorphic features and massive structure from 302-328+ cm (Figure 2.8). Such a feature deep in the soil could perch the water table at this site, providing water for tree growth well into the summer dry down. The development of two distinct Bt horizon sequences above this massive clay feature as well as extensive acidification of the entire profile reveals the age of this soil. At least several million if not tens of millions of years of soil development formed this sequence, coupled with at least two distinct disturbances in pedogenic processes. Further investigation will be necessary to determine the cause of this unusual sequence of horizons.

Table 2.5. Silver Creek profile description. Resembles Cumley series. Elevation 392 m a.s.l.

Horizons	Depth (cm)	Color (moist)	Texture	Structure	Roots	BD (g cm^{-3})	pH
Oi	2.5 - 0					0.20	
A	0 - 15	10YR 3/3 dark brown	Loam	crumb, med to small	very many coarse, medium, and fine	0.79	4.94
AB	15 - 35	10YR 4/4 dark yellowish brown	Silty clay loam	med granular	few coarse, many medium	0.94	4.60
Bt1	35 - 175	5YR 4/4 reddish brown	Silty clay	large to v. large subangular blocky	common fine, few medium, one coarse	0.92	4.29
Bt2	175 - 240	7.5YR 4/4 dark brown	Sandy loam	large subangular blocky, weak	few medium, very few fine	0.96	4.13
2Bt1	240 - 270	10YR 5/8 yellowish brown	Sandy clay loam	med angular blocky	few medium	1.07	4.34
2Bt2	270 - 300	10YR 6/6 brownish yellow	Sandy loam	small subangular blocky	very few fine	1.07	4.20
3Btm1	300 - 302	10YR 5/6 yellowish brown	Clay	massive, clay cemented	none		
3Btm2	302 - 328+	2.5YR 4/6 red	Clay	massive, clay cemented	none	1.13	4.33

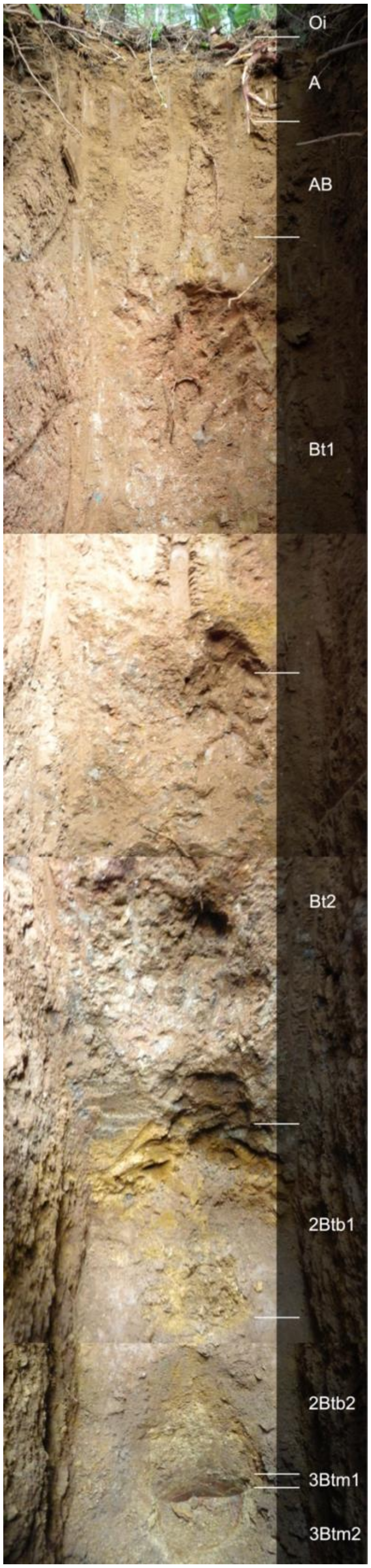

Figure 2.7. Soil profile at Silver Creek outside of Silverton, OR.

Figure 2.8. Close-up of transitions between 2Bt2, 3Btm1, and 3Btm2 horizons at the bottom of the soil profile at Silver Creek. Tick marks on soil knife are 5 mm apart.

The existence of distinct pedogenic features and processes deep in soil is far from trivial for aboveground ecological communities and for ecosystem nutrient budgets. Zabowski et al. (2011) found between 3% and 48% of soil carbon and between 7% and 35% of soil nitrogen (Whitney and Zabowski, 2004) below 1 m depth in soils spanning all twelve soil orders. Across the 36 sites excavated for this study, 24% of soil carbon on average (ranging from 6% to 57%) (James *et al.*, 2014) and 36% of soil nitrogen on average (ranging from 6% to 65%) was found below 1 m (James *et al.*, 2015). Subsoil is an even more important pool for base cations; on average, 66% of exchangeable calcium, 57% of exchangeable potassium, and 76% of exchangeable magnesium are found below 1 m at the 22 sites sampled in 2012 (James, unpublished data, 2014). Ignoring buried horizons and deep soil precludes their inclusion in global biogeochemical and carbon cycle models, and prevents complete understanding of the feedback between soil and climate change or other anthropogenic disturbances.

Little research is available in the literature concerning the specific functions of deep roots of various species despite much documentation of their presence (Canadell *et al.*, 1996; Stone and Kalisz, 1991), but there is growing awareness of the need for further research (Maeght *et al*., 2013). In Brazil, eucalyptus trees preferentially uptake exchangeable cations from roots extending many meters deep into the soil (da Silva *et al.*, 2011). Douglas-fir have been observed to spread roots to depths greater than 3 m in stands as young as 4 years, and up to 10 m deep in old-growth forest (Stone and Kalisz, 1991). Given the considerable extent of tree rooting observed in this study as well as documented in the literature, it seems likely that C horizons and other deep horizons actively exchange with and are in turn altered by plant roots, at once providing crucial resources for ecosystem productivity and further developing the soil.

2.5 CONCLUSION

While setting an arbitrary maximum depth for soil is convenient (Soil Survey Staff, 2014), it provides an excuse to ignore or make assumptions about deep soil horizons. Such generalizations unnecessarily exclude reaches of the soil profile that are both poorly understood and largely under-estimated for their importance to ecosystem water and nutrient cycles, and makes more difficult the process of connecting pedogenic development with ecosystem function. The symbiosis between plants and soil extends many meters in depth, actively transforming the crust of the earth to a living substrate through biogenic weathering (Richter and Markewitz, 1995). There is still an incredible diversity of deep soil processes and characteristics that have yet to be explored. Even in surface soil, attempts to scale point samples to the broader landscape are plagued by spatial variability. Not only are nutrients found in hotspots that vary vertically as well as horizontally in space, they can also form “hot moments” that vary in time (Johnson *et al.*, 2010; Johnson *et al.,* 2011; Woodward *et al.,* 2013). The tradeoff between deeper sampling and frequent spatial sampling presents unique challenges that must be carefully balanced so as to maximize understanding of the whole soil system. Deep soil remains one of the frontiers of soil science, and much further research is necessary to resolve its contribution to terrestrial ecosystem processes and sustaining life on earth.

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Chapter 3. EXCHANGEABLE CATIONS IN DEEP FOREST SOILS: SEPARATING CLIMATE AND CHEMICAL CONTROLS ON SPATIAL AND VERTICAL DISTRIBUTION AND CYCLING

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3.1 ABSTRACT

The vertical distribution of soil exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) results from the integration of multiple processes: weathering of primary minerals, atmospheric input, leaching, and biological cycling. While weathering and atmospheric input affect the location of cation inputs to the soil system, leaching and biological cycling translocate cations in opposing directions within the profile. Little research has been conducted on deep soil relative to surface soil, and thus attempts to verify hypotheses about the drivers of exchangeable cation vertical distributions have not been possible on a broad scale. This study excavated soils down to 2.5 or 3 m at 22 sites across the coastal Pacific Northwest ranging from northern Washington to southern Oregon. Samples were analyzed for soil carbon (C), nitrogen (N), and exchangeable cations. PERMANOVA was used to evaluate the effect of soil chemical (C, N, and pH), environmental (climate, parent material, elevation), physical (texture) and spatial (horizon, depth) gradients on the distribution of exchangeable cation contents and stocks. The majority of exchangeable cation stocks are located in deeper soil horizons. On average, 66% of Ca^{2+}, 76% of Mg^{2+}, 57% of K^+, and 63% of Na^+ stocks were below 1.0 m. Master soil horizon was the most significant predictor of the distribution of cations within the soil profile with substantial separation between A horizon samples and B and C horizon samples. There was a significant interaction between horizon and soil C, with higher soil C concentrations and stocks corresponding with lower Ca^{2+} and Mg^{2+} contents and stocks in horizons of the same type. Between sites, climate and cumulative carbon stocks were the dominant controls over cation distribution, with high C stocks and wet, cool climate leading to low exchangeable Ca^{2+} and Mg^{2+}. These results suggest that leaching with dissolved organic matter could be a driver of exchangeable Ca^{2+} and Mg^{2+} distributions in the Pacific Northwest. On the other hand, K^+ and Na^+ were largely uncorrelated with these environmental gradients. Biological uptake is a more important control over the distribution of exchangeable K^+, while atmospheric deposition drives the relatively uniform distribution of Na^+ both between sites and within profiles.

Keywords: Exchangeable Cations, Deep Soil, Calcium, Potassium, Magnesium, Soil Carbon

3.1 INTRODUCTION

As the interface between biosphere, lithosphere, hydrosphere, and atmosphere, soil is subjected to intense vertical exchange and transformation of materials that results in steep chemical and physical gradients from surface to bedrock (Jobbagy and Jackson, 2001). Stratification of soil into distinct horizons is a direct result of these gradients (Hilgard, 1906; Jenny, 1941), as is the vertical distribution of nutrients, including exchangeable cations such as Ca^{2+}, Mg^{2+}, K^+, and Na^+ (Jobbagy and Jackson, 2001). Four major processes shape the vertical distribution of exchangeable cations in soil: weathering, atmospheric deposition, leaching, and biological cycling (Trudgill, 1988). Release of exchangeable cations from weathering of primary minerals is generally slow (Simonsson et al., 2015), and atmospheric deposition - with the exception of Na^+ - is typically small in the Pacific Northwest (Johnson, 1992). By contrast, rates of biological cycling and leaching can be relatively rapid (Aber and Melillo, 1991; Johnson and Lindberg, 1992).

The vertical transport of cations in soil is affected by plant uptake and leaching in opposite directions. In isolation, plant uptake moves nutrients upwards because a large proportion of nutrients absorbed in roots are transported aboveground, where they are recycled to the soil surface by litterfall, throughfall, and stemflow (Cole and Rapp, 1981; Stark, 1994; Trudgill, 1988). Leaching, on the other hand, moves nutrients downward. The dynamics of this downward cycling for exchangeable cations is complicated by interaction with cation exchange sites (CEC) on soil mineral surfaces that hold nutrients to the soil matrix itself. Consequently, the vertical distribution of nutrients can elucidate the extent to which uptake or leaching control the short-term chemical dynamics of exchangeable cations in soil. The extent to which cations are retained in surface soil and to which uptake controls the vertical distribution of cations can differ by forest type and tree species. For example, Dijkstra and Smits (2002) found greater leaching of Ca^{2+} along with greater pumping of Ca^{2+} from deep soil in sugar maple compared to hemlock forests. In spruce and beech forests, considerably more leaching from surface soil occurs under spruce, and much greater subsurface uptake occurs under beech (Berger et al., 2006).

Soil solution studies suggest that charged chemical species - be they positive or negative - do not move in soil on their own, but instead require a charge balance in solution (Cole et al., 1975; Cole et al., 1967; Johnson and Cole, 1980; Likens et al., 1969). Consequently, the identity and quantity of mobile anions in soil solution controls cation leaching rates. Many anions can satisfy the positive charges of cations in solution, ranging from Cl^- to SO_4^{-2} to NO_3^- to HCO_3^- (Johnson and Cole, 1980). Ugolini and Sletten (1991) found a large deficit in negatively charged chemical species in soil solution when only Cl^-, SO_4^{-2}, and HCO_3^- were measured. Organic acids (along with NO_3^- in some cases) have long been hypothesized to fill the anion deficit in soil solution (Lundstrom, 1993), suggesting a fundamental control of cation dynamics by soil organic matter (SOM). The dynamics governing the behavior of SOM as it relates to exchangeable cations are complex. On the one hand, accumulation of SOM has been well established as a source of CEC sites in the solid phase (Kalisz and Stone, 1980). However, dissolved organic matter (DOM) and, in particular, low molecular weight (LMW) organic acids such as citric and oxalic acid have been identified as important compounds exuded by fungi and plants to induce destabilization of cations from organic complexes for later uptake (Clarholm and Skyllberg, 2013). Across six forest types, Dijkstra et al. (2001) found that between 25% and 43% of negative charge in soil solutions could be attributed to organic acids and implicated these acids as a primary driver of cation leaching from the forest floor into mineral horizons. DOM – especially those hydrophilic compounds rich in carboxylic and phenolic groups - may act as mobile ligands for exchangeable cations that travel with water downward through soil (Johnson and Cole, 1980). Indeed, organic acids have been observed to be major cation leaching agents in acid soils from boreal to tropical/subtropical regions (Johnson et al., 1977; Ugolini et al., 1977). Thus different forms of organic matter are implicated both as a source of exchangeable cation retention and as a means by which nutrients stabilized in SOM are destabilized and subsequently mobilized for either uptake or leaching (Clarholm et al., 2015).

Acidic forest soils are a special subset of soils in which aluminum (Al) can complicate the interpretation of CEC and exchangeable cation extractions (Ross et al., 2008). Soil acidification can mobilize Al^{3+}, which blocks CEC sites from retaining base cations and has been implicated as a mechanism in forest soil Ca depletion (Lawrence et al., 1995). Mycorrhizal fungi take advantage of the affinity for Al^{3+} and LMW organic acids to form strong bridging complexation reactions by exuding LMW organic acids into soils, thus partially neutralizing soil acidity (Clarholm et al., 2015). At or below a pH of 4.5, Ross et al. (2008) argue that little Al^{3+} will be adsorbed to the soil solid phase, and that pH is modeled better if Al^{3+} is treated as a base cation. Consequently, base saturation in this range does not provide a useful metric (Ross et al., 2008).

Nitrogen may also be an important part of cation leaching under certain conditions. Nitrogen saturation is known to occur in forest ecosystems due to either large atmospheric inputs, weathering of N-bearing rocks (Dahlgren, 1994; Holloway and Dahlgren, 2002), or accumulation from N-fixing species (Aber et al., 1998; Aber, 1992; Aber et al., 1989). These phenomena result in increased N mineralization rates, followed by stripping of exchangeable cations from soil through nitrate leaching as the capacity of the ecosystem to take up excess N is overcome (Aber et al., 1989). In the coastal Pacific Northwest, early successional alder trees fix large quantities of nitrogen through a symbiotic relationship with root-nodulating Frankia (Binkley et al., 1994), which can result in long-term acidification of soil and loss of exchangeable cations (Van Miegroet and Cole, 1984; Van Miegroet and Cole, 1985). Perakis et al. (2006; 2013) found that the distribution of N across the Pacific Northwest, which itself reflects forest disturbance and duration of alder colonization, acts as a primary control on exchangeable cation (particularly Ca^{2+}) availability to forest ecosystems. Many such studies focus on the horizontal rather than vertical distribution of nutrients in the soil profile (Perakis and Sinkhorn, 2011), and consequently do not explicitly consider the translocation of cations deeper into the soil profile.

Studies that examine exchangeable cation dynamics in forest soils often focus on the O horizon, or track changes with depth to only 50 or 100 cm. However, Douglas-fir and other dominant or co-dominant tree species in the region extend roots deeply (3+ m) into the soil profile (Stone and Kalisz, 1991). Little is known about the role of deep roots in the nutrition of forests, although maximum rooting depths between 2 and 20 m have been recorded for species from every major biome except for tundra (Canadell et al., 1996). Despite much lower density than surface roots, deep rooting may serve as a safety net by capturing nutrients leaching from surface soil horizons. Indeed, a tracer experiment found functional specialization of deep roots for the uptake of exchangeable cations (K^{+} and Ca^{2+}) from deep regions of the soil (3 m) in highly productive Eucalyptus plantations in Brazil (da Silva et al., 2011). Whether similar rooting specialization exists in Douglas-fir or other forest types has not yet been widely studied.

Nutrient dynamics in deep soil are largely understudied (Harrison et al., 2011; James et al., 2015; Whitney and Zabowski, 2004). Two recent review papers on the impact of land use change on soil organic matter (Post and Kwon, 2000; West and Post, 2002) found that the median depth of soil sampling was 20 cm, with 90% of the 360 referenced studies sampling to 30 cm or less. Consequently, testing the effects of environmental and chemical gradients on the vertical distribution of exchangeable cations deep into soil has not been possible on a broad spatial scale. Such tests often involve simple correlations between variables, which can fail to pick up on more complicated relationships governing changes that occur across the landscape and within the soil profile. Environmental variables are not often distributed normally, and thus techniques that go beyond parametric statistics are needed to evaluate the effects of these variables. The objectives of this paper are:

1. To introduce the use of permutational multivariate analysis of variance (PERMANOVA) and nonmetric multidimensional scaling ordination (NMDS) as tools to analyze complex soil chemical data.
2. To assess the impact of soil C, N and pH on the concentration, stocks and vertical distribution of soil exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in deep forest soils.
3. To assess how other environmental gradients such as climate, horizonation, soil texture, parent material, and soil order affect the contents, stocks, and distribution of soil exchangeable cations.

3.2 METHODS

3.2.1 *Field sampling*

Samples were collected from 22 sites in the coastal Pacific Northwest, ranging from the Pacific Ocean to the Cascade Range and from northern Washington to southern Oregon (Figure 3.1). All sites are intensively managed Douglas-fir stands that have been previously used to study forest response to nitrogen fertilization on individual trees (Littke et al., 2014a; Littke et al., 2014c). One soil pit per site was excavated in unfertilized areas adjacent to fertilized stands. Bulk density samples were taken in the middle of regular depth intervals of 0-10 cm, 10-50 cm, 50-100 cm, 100-150 cm, 150-200 cm, 200-250 cm, and 250-300 cm (this depth only sampled at 6 sites). Major genetic horizon for each sample was assigned based upon field descriptions, which are detailed in James et al. (2015). Briefly, all 0-10 cm samples were taken from A horizons, while the 10-50 cm samples came from A horizons at six sites; the remainder of 10-50 cm samples were from B horizons, as well as eighteen of the twenty-two 50-100 cm samples; and, finally, samples below 100 cm were from C horizons, except at three sites where the B horizon extended to roughly 200 cm.

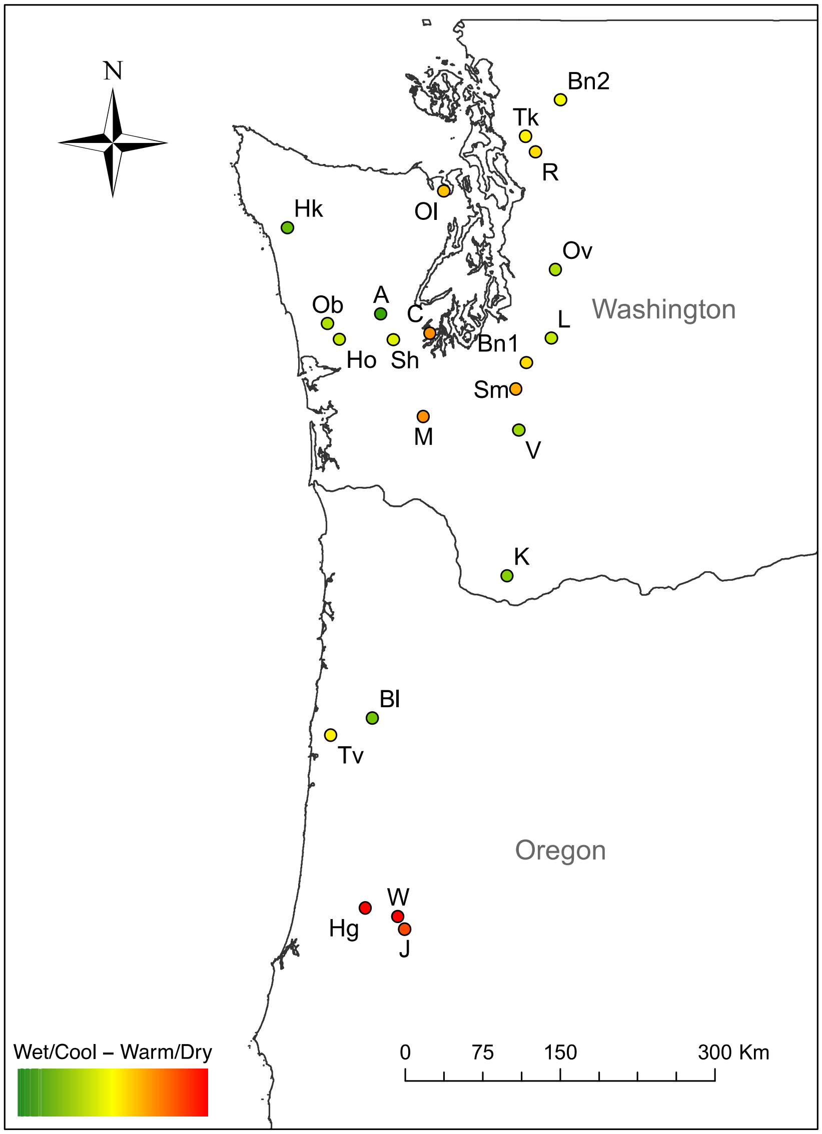

Figure 3.1. Locations of 22 sampling sites in western Washington and Oregon. Symbol colors correspond with site scores on a climate index (Climate PC1, Figure 3.2) running from wet and cool to warm and dry.

3.2.2 *Laboratory Analyses*

Bulk density samples were sieved to 4.75 mm, ground to a fine powder, and analyzed for %C and %N using a PerkinElmer CHN analyzer. Complete explanations of methods and results for soil C and N at these sites can be found in James et al. (2014; 2015). Exchangeable cations

(Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, as well as exchangeable Al^{3+}) were extracted by shaking samples with a 1 M solution of ammonium chloride for 2 hours. Sample solutions were filtered with Whatman 40 filter paper, and analyzed with an inductively coupled plasma mass spectrometer (ICP-MS). Soil stocks for soil C, N, and exchangeable cations were calculated as

$$\mathrm{Stock}_M = [M] * D_b * H \qquad (3.1)$$

where [M] is the concentration of the element M, D_b is the bulk density, and H is the height of the soil layer for which the total stock is being calculated. The effective CEC (CECe) in each sample was calculated as the sum of exchangeable Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Al^{3+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, and Cu^{2+}. Site level variables, including location (latitude and longitude), climate (temperature and precipitation for all four seasons), soil texture (percent sand and clay at 5 and 50 cm), elevation, slope, aspect, and parent material were all collected by Littke et al. (2014b). Soil order was determined by comparing Web Soil Survey mapped soil series (Soil Survey Staff, 2014) with observed profiles and samples from the field (

Table 3.1).

Table 3.1. Soil series, map symbol, subgroup, and texture for 22 forest soils in western Washington and Oregon.

Soil Series	Map Symbol	Subgroup[a]	Texture	Mean pH[b] (range)	Mean CECe[c] (range)
Astoria	A	Andic Humudept	Clay loam	4.9 (4.5 - 5.3)	4.1 (1.2 - 7.4)
Barneston 1	Bn1	Typic Vitrixerand	Gravelly sandy loam	5.4 (5.2 - 5.5)	7.6 (3.7 - 17.2)
Barneston 2	Bn2	Typic Vitrixerand	Gravelly sandy loam	5.3 (5.0 - 5.8)	2.4 (1.6 - 3.3)
Blachly	Bl	Humic Dystrudept	Clay loam	4.8 (4.3 - 5.1)	3.5 (1.7 - 5.0)
Cloquallum	C	Aquandic Dystrudept	Sandy loam	5.4 (4.7 - 6.0)	6.5 (3.5 - 12.7)
Hoko	Hk	Aquic Durudand	Gravelly clay loam	4.5 (3.9 - 4.9)	2.9 (0.9 - 6.0)
Honeygrove	Hg	Typic Palehumult	Gravelly silty clay loam	4.7 (4.5 - 5.0)	11.6 (8.7 - 15.7)
Hoquiam	Ho	Typic Fulvudand	Clay	4.7 (4.4 - 5.0)	11.8 (7.3 - 23.7)
Jory	J	Xeric Palehumult	Silty clay	4.6 (4.4 - 4.8)	4.3 (3.5 - 5.2)
Kinney	K	Andic Humudept	Sandy clay	5.3 (5.0 - 5.9)	10.1 (3.7 - 21.1)
Lemolo	L	Typic Humaquept	Sandy clay loam	5.7 (5.1 - 6.2)	9.2 (7.4 - 10.8)
Melbourne	M	Ultic Palexeralf	Clay	4.6 (4.3 - 4.8)	19.3 (6.5 - 30.3)
O'Brien	Ob	Typic Fulvudand	Sandy clay loam	5.0 (4.3 - 5.2)	2.3 (1.3 - 5.4)
Olete	Ol	Vitrandic Haploxerept	Gravelly loam	6.0 (5.3 - 6.4)	24.5 (8.3 - 50.8)
Ovall	Ov	Andic Humixerept	Gravelly sandy clay loam	4.7 (4.2 - 5.0)	6.9 (2.3 - 12.4)
Ragnar	R	Vitrandic Dystroxerept	Fine loamy sand	5.3 (4.7 - 5.7)	1.5 (0.7 - 3.1)
Scamman	Sm	Aquic Palexeralf	Sandy clay loam	5.2 (4.5 - 5.7)	19.1 (8.3 - 22.9)
Shelton	Sh	Typic Haploxerand	Gravelly loam	5.4 (4.8 - 6.2)	14.9 (5.1 - 25.4)
Tokul	Tk	Aquic Vitrixerand	Gravelly clay loam	5.8 (4.5 - 6.8)	5.4 (3.3 - 6.8)
Tolovana	Tv	Typic Fulvudand	Clay loam	4.2 (4.0 - 4.3)	14.2 (8.8 - 20.3)
Vailton	V	Alic Hapludand	Loam	5.1 (4.7 - 5.3)	3.2 (1.0 - 9.3)
Windygap	W	Xeric Haplohumult	Loam	4.7 (4.5 - 5.1)	15.9 (10.3 - 20.2)

[a] Data from Natural Resources Conservation Service 2012.
[b] pH measured as saturated paste; mean across all depths
[c] effective CEC (eCEC) calculated as the sum of exchangeable Ca^{2+}, Mg^{2+}, K^+, Na^+, Al^{3+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, and Cu^{2+}

3.2.3 *Statistical Analysis*

Principal component analysis (PCA) was used to build a synthetic climate index based upon 8 climate variables – the temperature and precipitation for spring, summer, autumn, and winter seasons. The first and second principal components of the PCA were subsequently used to test the effect of climate on the differences in exchangeable cations between sites. PCA was also used to create a synthetic index for soil texture for each site, which was derived from the percent sand and clay at 5 and 50 cm depths. The first principal component was used to test for the effect of texture on the distribution of exchangeable cations between sites. Texture data was not available for all soil depth samples, and consequently texture was only analyzed for differences between sites, not for changes in cation vertical distribution within the soil profiles.

Permutational multivariate analysis of variance (PERMANOVA) was used to examine the effects of environmental, chemical, physical, and spatial variables on exchangeable base cation concentrations and total stocks both within the soil profile and between sites. To our knowledge, this technique has not been used before in the soil literature outside of microbiology, and a brief explanation should make clear both how this technique works and why it was used here. The PERMANOVA approach is commonly used to statistically assess the effects of experimental or uncontrolled environmental gradients on the overall distribution of ecological communities, which can be made up of hundreds to thousands of biological species (McArdle and Anderson, 2001). Rather than assess the effect of these environmental gradients on one species at a time, PERMANOVA instead analyzes these gradients against a distance matrix that is the product of the differences between sites for all these species at the same time (Anderson, 2001; Anderson and Ter Braak, 2003; McCune and Grace, 2002). The analyses in this paper use Euclidean distance to produce the distance matrix, which is an application of the Pythagorean theorem. To understand how this is done, consider a study in which *n* number of species are measured at *p* number of sites, which produces a *p* x *n* data matrix. Euclidean distance calculates the distance (D_p) between each site using the equation

$$D_p(q,r) = \sqrt{(q_1 - r_1)^2 + (q_2 - r_2)^2 + \cdots + (q_i - r_i)^2 + \cdots + (q_n - r_n)^2} = \sqrt{\sum_{i=1}^{n}(q_i - r_i)^2} \qquad (3.2)$$

where *q* and *r* are separate species measured at each site. This produces a *p* x *p* distance matrix that quantitatively expresses the differences between sites regardless of the number of species measured at each site. PERMANOVA then compares explanatory variables to this distance matrix by permuting the explanatory data many times and comparing the observed test statistic (*Pseudo-F*) to the distribution of *Pseudo-F* values derived from the many possible permutations of the data. Each permutation in the data is made by swapping two (or more) values of the explanatory variables under the null hypothesis that they have no effect on the response variables. In univariate cases where the assumptions of parametric ANOVA are met, PERMANOVA and ANOVA produce identical *F*-statistics and asymptotically similar *p*-values, except that PERMANOVA does not require the assumption that explanatory data is normally distributed (Anderson and Ter Braak, 2003). This paper uses PERMANOVA to examine exchangeable base cation species (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) in an analogous way to biological species.

PERMANOVA assumes that the permuted data is exchangeable under a true null hypothesis, and consequently the precise interpretation of PERMANOVA tests depend upon the method used to control how the data is permuted (Anderson and Ter Braak, 2003). This study employs a nested design in which we are interested in both factors that explain differences in exchangeable cations between sites (i.e. different soils) and factors that explain the vertical distribution of exchangeable cations in repeated measurements within each site (i.e. samples down the soil profile). Consequently, modeling the effects of explanatory variables between sites must be done separately from testing the effects of explanatory variables on the vertical distribution (within-site variability) in soil exchangeable cations. When testing the effects of between-site variables – which include climate PC1 and PC2, soil texture PC1, parent material (sedimentary, igneous, or glacial), total C, total N, mean pH, and soil order (Alfisol, Andisol, Inceptisol, or Ultisol) – PERMANOVA was allowed to freely permute data between each of the 22 sites. The response matrix used for testing between-site explanatory variables was composed of the distances between the site centroids (mean exchangeable base cation values at each site), and thus limited the tests of between-site variables to 21 total degrees of freedom. On the other hand, soil samples in the profiles are repeated spatial measurements and care must be taken to preserve the spatial structure of this sampling scheme. Thus, for testing within-site variables – which include soil C, N, exchangeable Al^{3+}, pH, horizon (A, B, or C), and depth – data were permuted to preserve the spatial ordering (moving either up or down the profile) and were not permuted between sites. Because the primary interest was to test for effects on the vertical distribution of base cations within whole profiles, a site factor was used as the first term in within-site tests to control for the differences between sites. Variables that are statistically significant for between-site tests do not imply control over the vertical distribution of exchangeable cations but only control over differences in mean exchangeable cations across the landscape; likewise, significant within-site explanatory variables are those which differentiate patterns in the vertical distribution of profiles, but not differences between sites. Due to the high variability between sites in both soil base cation content and stocks, soil chemical data was ln-transformed prior to analysis.

The order in which explanatory variables appear in PERMANOVA is important, and the *pseudo-F* statistic and R^2 values for variables change depending on whether they are included first or later in the model (Anderson, 2001). The final PERMANOVA models were built stepwise by adding only the explanatory variable with the highest R^2 at each step. Final p-values for both within- and between-site tests were calculated based upon a *pseudo-F* distribution derived from 10,000 permutations. Variables were only added to the model if they were significant at $\alpha = 0.05$. Analysis of multivariate homogeneity of dispersions (PERMDISP) was used to test for differences due to dispersion for statistically significant grouping factors in the final PERMANOVA models (Anderson et al., 2006; Anderson and Walsh, 2013). Between-site and within-site models for base cation contents ($\text{cmol}_c\ \text{kg}^{-1}$) and total stocks ($\text{kmol}_c\ \text{ha}^{-1}$) were computed separately. Soil content variables were not crossed with soil stock variables either as response or explanatory variables (e.g. %C was used to test models for exchangeable cation contents, but C stocks were used in models of exchangeable cation stocks).

3.2.4 *Data Visualization*

Non-metric multidimensional scaling (NMDS) ordination was used to visualize the significant effects of environmental, chemical, and spatial variables on the distribution of exchangeable base cations. Separate ordinations were computed for exchangeable cation contents and stocks, but only results from the soil stocks ordination are presented here to prevent redundancy. The NMDS ordinations were computed in two dimensions using Euclidean distances between ln-transformed exchangeable cation variables. New random sets of starting coordinates were used for each run of the monoMDS engine until convergence criteria were reached (instability less than $1e^{-5}$). The final ordination converged after 3 runs, with final stress = 0.0305, non-metric $R^2 = 0.999$, and linear $R^2 = 0.997$. Overlaying external environmental variables on the points in NMDS space can visually show their effects on exchangeable cations. When displaying factors that explain differences between sites, the site centroids (average value of exchangeable cations across all depths) are shown, leaving 22 points. However, when displaying the effects of variables that explain the vertical distribution of cations within soil profiles, the full 136-sample dataset is shown. For grouping factors such as soil horizon (where samples are either from A, B, or C horizons in this dataset), there are frequently multiple samples from each horizon in a single soil profile because samples were taken by standard depth intervals.

All models were run in R version 3.1.2 (R Core Team, 2015). Principal components analysis (PCA) for climate and texture data was performed using the `princomp()` function within the base stats package. The `adonis()`, `betadisper()`, and `metaMDS()` functions from the vegan package (version 2.2-1) were used for PERMANOVA, PERMDISP, and NMDS, respectively (Oksanen et al., 2015).

3.3 RESULTS & DISCUSSION

3.3.1 *Synthetic Climate and Texture Variables*

The climate in the coastal Pacific Northwest is characterized by generally warm, dry summers and wet, cool winters. Substantial variation in climate occurs across sites in this study due to wide latitudinal separation between sites, as well as the influence of the Olympic, Coast, and Cascade mountain ranges on precipitation (rain shadow effects) and temperature (elevation effects). Principal Components Analysis (PCA) of climate data reveals that two component axes explain 87% of the variation in climate between sites. The first principal component (PC1) explains 57% of the variance and consists of roughly equivalent loadings for each of the 8 climate variables (TWIN, TSPR, TSUM, TAUT, PWIN, PSPR, PSUM, PAUT) with precipitation negatively weighted and temperature positively weighted (Figure 3.2, Table 3.2). This principal component represents an index of climate running from cold and wet to warm and dry, and follows the trend from high to low leaching rates. In the context of soil taxonomic moisture regimes, climate PC1 could be interpreted as differentiating between more udic conditions (where there is little to no moisture deficit - low PC1 scores) and more xeric conditions (where there is some moisture deficit during the year - high PC1 scores). Differences in temperature between sites were not large enough to span different taxonomic temperature regimes. The second principal component (PC2), which explains an additional 29% of the variance, excludes the summer temperature and precipitation variables and has slightly higher loadings for winter variables (Figure 3.2, Table 3.2). This climate index runs from cool and dry to warm and wet for autumn, winter, and spring seasons, and captures changes in wet season leaching rates. Climate PC2 does not fit patterns recognized as separate soil moisture and temperature regimes. These two indices were subsequently used to examine the impact of climate and leaching on exchangeable cation distribution.

Table 3.2. Loadings for Principal Component Analysis of Climate Variables.

Loadings:		Comp. 1	Comp. 2
Winter Precipitation	PWIN	-0.320	0.440
Spring Precipitation	PSPR	-0.371	0.368
Summer Precipitation	PSUM	-0.369	
Autumn Precipitation	PAUT	-0.395	0.319
Winter Temperature	TWIN	0.221	0.514
Spring Temperature	TSPR	0.396	0.288
Summer Temperature	TSUM	0.390	
Autumn Temperature	TAUT	0.332	0.448

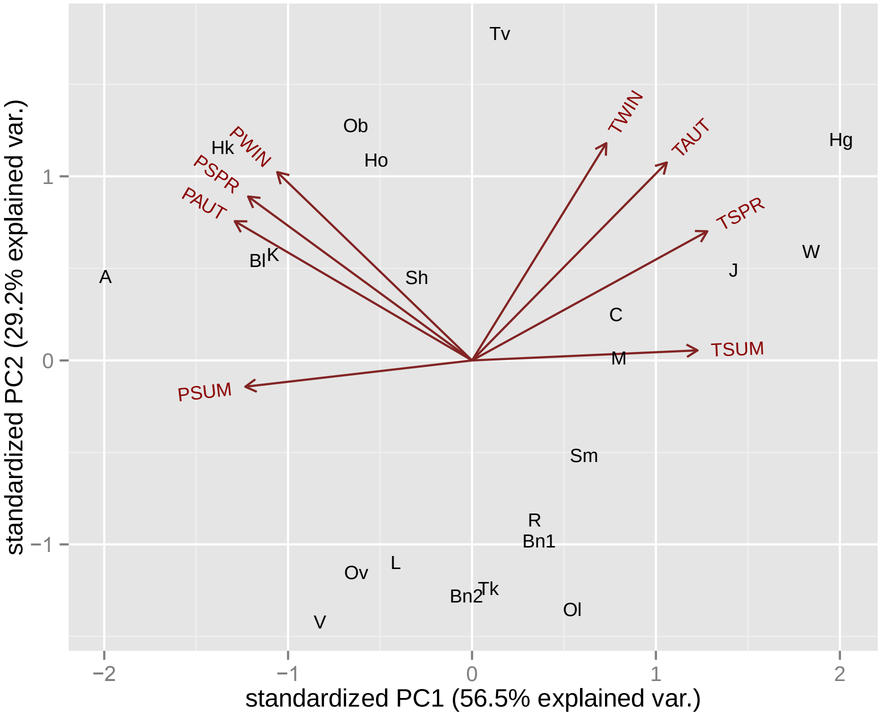

Figure 3.2. Principal components analysis of climate variables. Component 1 (PC1) is composed of roughly equal loadings for all 8 variables (temperature and precipitation for 4 seasons). PC1 runs from generally cool and wet conditions to hot and dry conditions. Component 2 (PC2) is composed of loadings from only autumn, winter, and spring variables. PC2 runs from cool and dry conditions to warm and wet conditions. See Table 3.2 for loadings for each principal component.

The PCA for soil texture yielded a single dominant component that explains 81% of the variance in the 4 texture and depth variables (Clay_5, Clay_50, Sand_5, Sand_50). The loadings for this component are roughly equal for each variable, with negative loadings for clay at 5 and 50 cm depth and positive loadings for sand at 5 and 50 cm depth (Figure 3.3,

Table 3.3). This principal component runs from fine texture to coarse texture and captures all four variables into a single texture index, which was used to test for texture effects on exchangeable cation distribution.

Table 3.3. Loadings for Principal Component Analysis of Soil Texture Variables.

Loadings:		Comp. 1
Clay 5 cm depth	Clay_5	-0.483
Clay 50 cm depth	Clay_50	-0.507
Sand 5 cm depth	Sand_5	0.478
Sand 50 cm depth	Sand_50	0.530

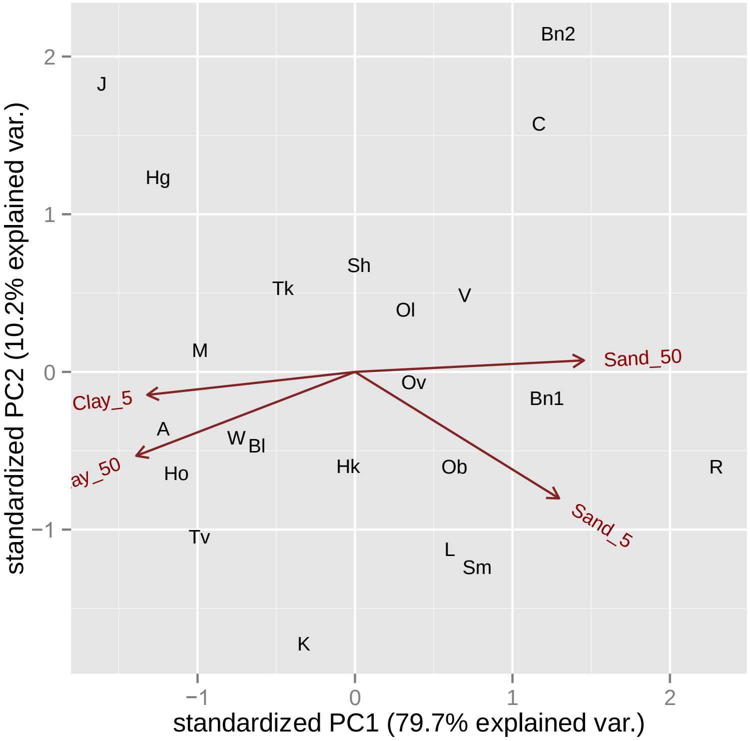

Figure 3.3. Principal components analysis of soil texture variables. Component 1 (PC1) is composed of roughly equal loadings for all 4 texture variables (

Table 3.3) and runs generally from fine texture to coarse texture.

3.3.2 *Exchangeable base cation contents*

Results of PERMANOVA for both between- and within-site tests appear in Table 3.4. The distribution of exchangeable base cation contents between sites is significantly predicted by climate PC1, which accounts for 19% of the between-site variation (*pseudo-F* = 4.59, $p < 0.05$), while climate PC2 was not a significant predictor. Climate PC1 runs generally from cool, wet conditions to hot, dry conditions, and in the context of exchangeable cation cycling can be thought of as an index of leaching. Temperature and precipitation will also affect decomposition rate, leading to variation in the quantity and quality of organic matter at each site. Other variables tested at the between-site level explain relatively large portions of the variation but are not significant or even marginally significant ($p > 0.1$). For example, soil order explains 20% of the variation between sites, but is not significant ($p \approx 0.17$). Neither soil texture PC1 ($R^2 = 0.06$, $p \approx 0.22$) or the average effective CEC ($R^2 = 0.02$, $p \approx 0.75$) were significant. One possible reason for this is the relatively small number of sites (22) and limited degrees of freedom to test for significant effects. These results show that climate (and the associated leaching force associated with different climates) is a primary driver of the differences in exchangeable cation contents between different soils across the landscape.

Table 3.4. PERMANOVA results for distribution of exchangeable cation contents (P-value calculated with 10,000 permutations).

		Df	SS	*Pseudo-F*	R^2	*P* (>F)
Between Site Variation	Climate PC 1	1	23.16	4.59	0.187	0.019
	Residuals	20	101.01		0.813	
	Total	21	124.17			
Within Sit	Site ID	21	655.26	19.10	0.725	< 0.0001

	Horizon	2	42.22	12.92	0.047	< 0.0001
	% Carbon	1	3.04	1.86	0.003	0.2591
	Horizon | % C	2	25.60	7.84	0.028	0.0004
	Residuals	109	178.03		0.197	
	Total	135	904.14			

The site factor – which controls for differences between sites when testing for changes in vertical distribution within the soil profile – is very large, accounting for 73% of the variation (Table 3.4). Nonetheless, soil horizon and an interaction term between horizon and %C are both significant. The soil horizon groupings (A, B, or C horizon) do not differ in their dispersion ($F = 3.02$, $p > 0.05$), and thus the significance of this term can be safely interpreted to be the result of differences between group centroids. In particular, A horizon samples appear distinctly separated from B and C horizon samples with lower concentrations of exchangeable base cations (particularly Ca) in the A horizon compared to B and C horizons. While %C is not a significant predictor of the distribution of soil exchangeable cation contents on its own, within each master horizon the areas with higher %C tend to have fewer exchangeable base cations. This is counter-intuitive given the role of soil organic matter (SOM) as a source of exchange sites in the solid phase. One hypothesis to explain this observed relationship is that the C that reaches the subsoil has been broken down into tiny pieces or even monomers that can be mobile in soil solution. While SOM in the solid phase is considered a source of CEC sites, LMW organic acids in soil solution are mobile anions that can promote leaching loss of exchangeable cations. The role of organic acids as a component of base cation leaching has long been recognized in soil solution studies (Johnson and Cole, 1980; Johnson, 1992; Johnson et al., 1977; Ugolini and Sletten, 1991). These results suggest that pedogenic accumulation and cycling of organic matter may be an important control on the distribution of exchangeable cation contents within acidic forest soils. Detailed soil solution study at these sites would provide additional data to directly observe this phenomenon, but is beyond the purview of this study.

The vertical distribution of C concentration and exchangeable cation contents are shown in Figure 3.4. In sites with high surface and particularly subsurface (50+ cm) soil C concentrations, exchangeable base cation contents remain low throughout the profile (Figure 3.4a). At some sites with moderately high C concentrations - particularly below 100 cm - the concentrations of exchangeable Ca^{2+} and Mg^{2+} begin to increase in the subsurface (Figure 3.4b). Sites with moderately low surface and subsurface soil C tend to have higher surface and subsurface concentrations of Ca^{2+} and Mg^{2+} (Figure 3.4c). Finally, at sites with low soil C concentrations, particularly below 50 cm, exchangeable Ca^{2+} and Mg^{2+} increase dramatically with depth (Figure 3.4d). Very little regular change in exchangeable K^+ or Na^+ can be observed with depth in response to soil C concentration changes. Exchangeable cation contents (especially Ca^{2+} and Mg^{2+}) tend to be lower in A horizons than they are in subsurface horizons, and within each major horizon (A, B, or C) areas with lower %C tend to have higher concentrations of exchangeable Ca^{2+} and Mg^{2+}. This can most dramatically be seen comparing the subsurface (>1 m) samples (corresponding with the B and C horizons) between Figure 3.4a and 3.4d. Between 100 cm and 250 cm depth, soils in Figure 3.4a average 0.477% C by mass, whereas the soils in Figure 3.4d average 0.106% C by mass. Exchangeable Ca^{2+} and Mg^{2+} concentrations, meanwhile, are nearly an order of magnitude less in Figure 3.4a than in 3.4d within the same depth interval.

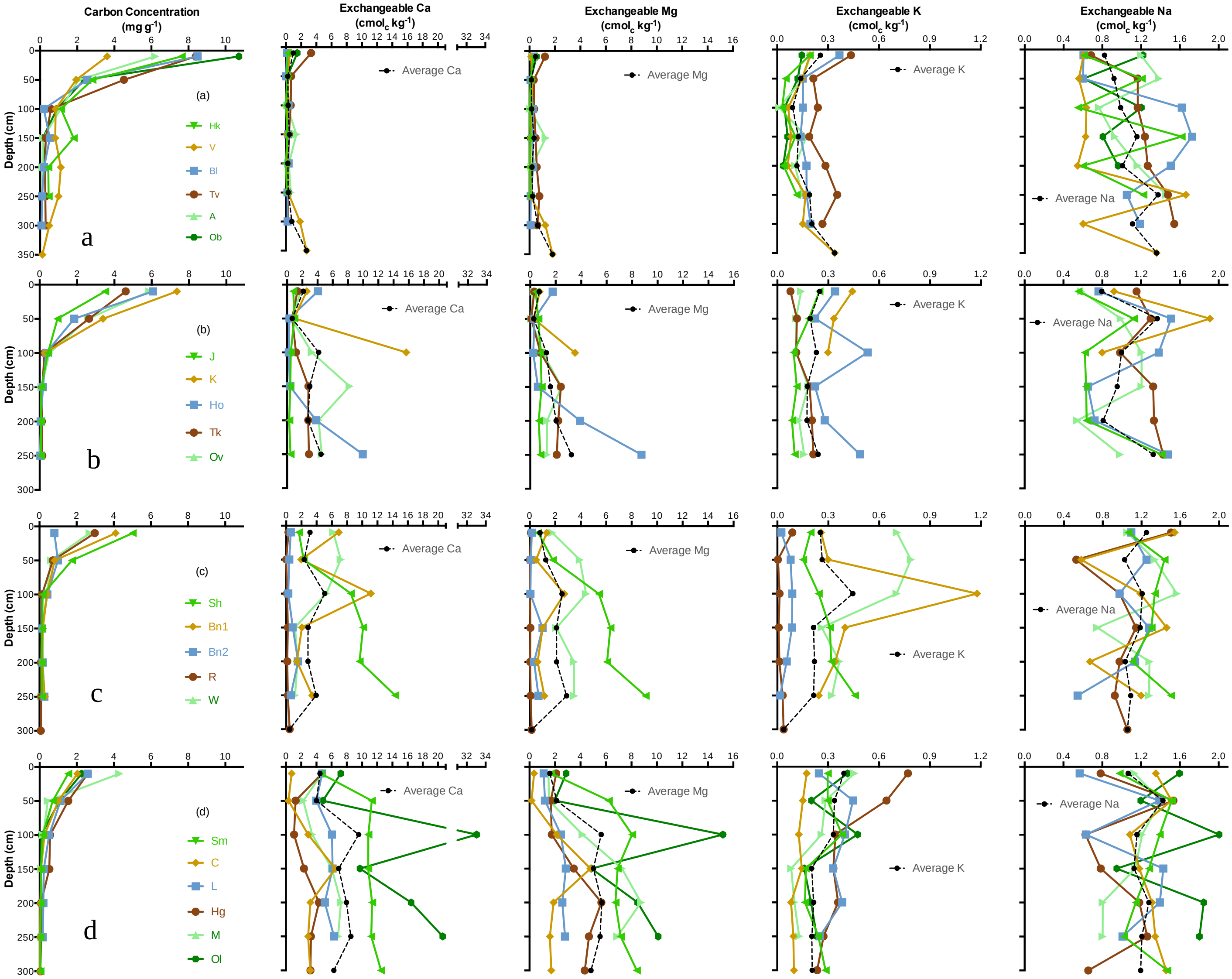

Figure 3.4. The change in exchangeable cation concentrations with depth at 22 soils in the Pacific Northwest split into groups by (a) high soil C concentration, (b) moderately high soil C, (c) moderately low soil C, and (d) low soil C concentration. As soil carbon concentrations decrease, particularly in subsurface horizons, exchangeable Ca^{2+} and Mg^{2+} increase.

The change in the concentration of exchangeable Al with depth across sites is not consistent. It does not increase with depth as soil C decreases, as with Ca^{2+} and Mg^{2+}, nor does it remain consistent with depth, as with K^{+} and Na^{+} (Figure 3.5). At most sites, exchangeable Al remains low throughout the profile. However, substantial concentrations that generally increase with soil depth can be observed at 5 sites, indicating long-term acidification. These sites are some of the oldest in this dataset, representing Ultisols (Windygap, Honeygrove), Alfisols (Melbourne), and Andisols (Tolovana, Hoquiam) developed on old alluvium or sedimentary parent materials with ash influence in the surface. High exchangeable Al^{3+} may indicate greater prevalence of Al and dearth of other cations in parent materials at these sites. The soil pH across the sites in this study ranged from slightly to extremely acidic (

Table 3.1). Consequently, fixation of Ca by soil available P is unlikely to drive Ca cycling in this region.

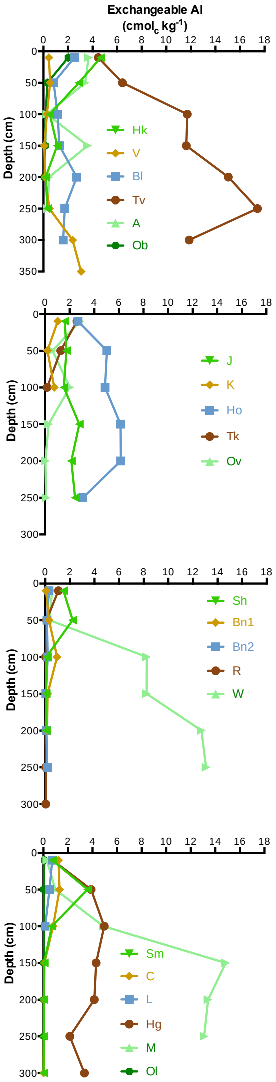

Figure 3.5. The change in exchangeable Al^{3+} with depth, split into groups as in Figure 3.4 for ease of interpretation.

3.3.3 *Exchangeable cation stocks*

Stocks of soil exchangeable Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} are shown in Figure 3.6, as well as the relative contribution of each sampling interval to the total stock at each site. On average there are 762 kmol$_c$ ha^{-1} of Ca (ranging from 21 to 2981 kmol$_c$ ha^{-1}), 496 kmol$_c$ ha^{-1} of Mg (range 11 to

1876 $\mathrm{kmol_c\ ha^{-1}}$), 48 $\mathrm{kmol_c\ ha^{-1}}$ of K (range from 11 to 128 $\mathrm{kmol_c\ ha^{-1}}$), and 261 $\mathrm{kmol_c\ ha^{-1}}$ of Na (range 41 to 484 $\mathrm{kmol_c\ ha^{-1}}$) total across all 22 sites. Subsurface (>1 m depth) stocks represent the majority of the total pool; 66% of exchangeable Ca^{2+}, 76% of exchangeable Mg^{2+}, 57% of exchangeable K^{+}, and 63% of exchangeable Na^{+} are located deeper than 1 m, on average. Douglas-fir can be very deeply rooted, with reports of maximum rooting depth greater than 3 m in many locations (Stone and Kalisz, 1991). Subsoil nutrient stocks are likely a key resource for Douglas-fir growing in this region, and may help explain the increase in forest productivity with increasing effective soil depth (Steinbrenner, 1979).

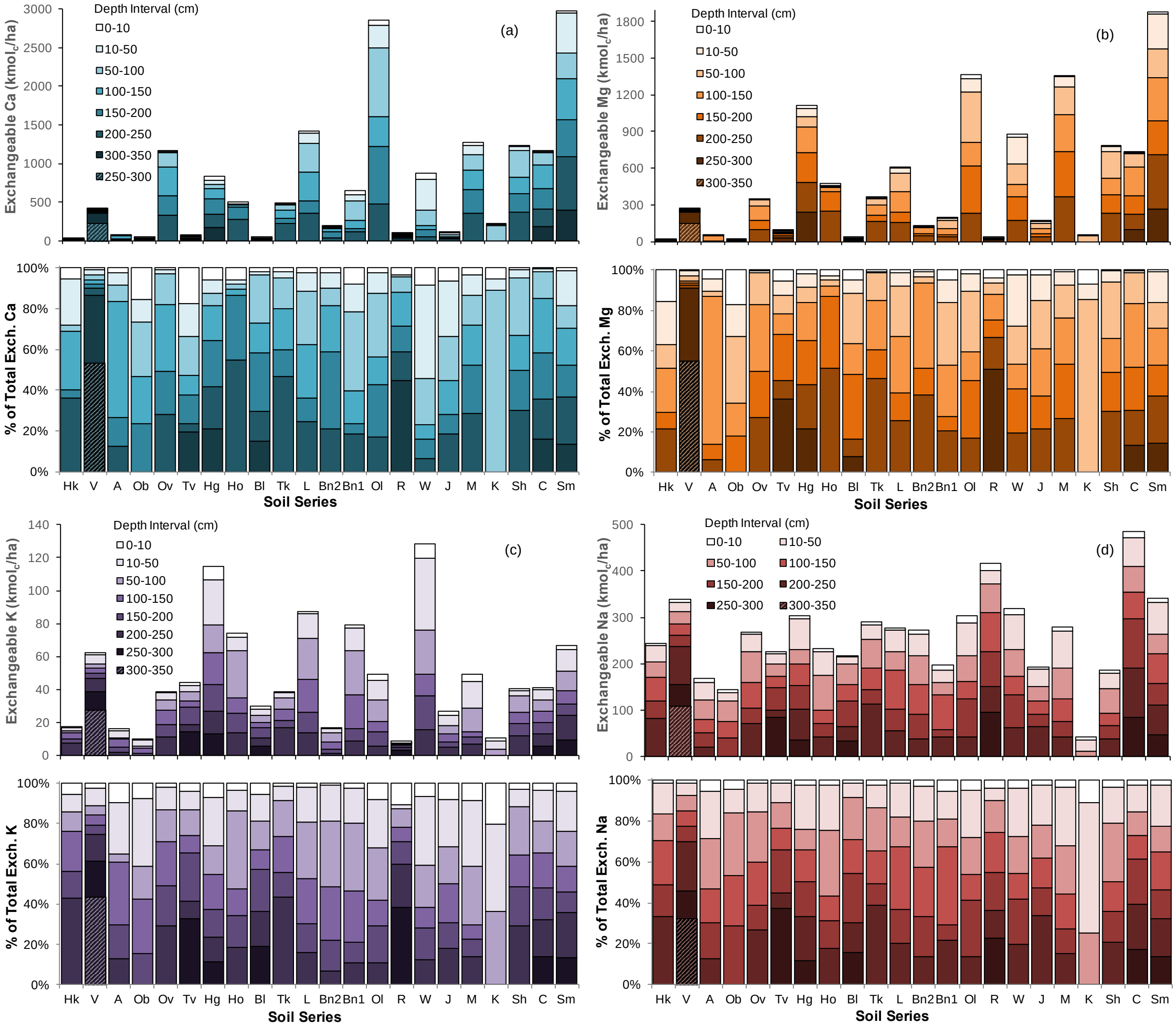

Figure 3.6. Total soil stocks and relative contribution by sampling layer for (a) exchangeable Ca^{2+}, (b) exchangeable Mg^{2+}, (c) exchangeable K^{+}, and (d) exchangeable Na^{+}. The soils are ordered from largest total C stocks (Hk) at left to smallest total C stocks (Sm) at right.

The underlying distribution of base cation quantities in NMDS space is presented in Figure 3.7. All soil chemical data (cations as well as C and N) were ln-transformed prior to analysis, so apparent linear correlations evident in the ordinations are non-linear in nature. Each point in space represents the same rank-order as the distance matrix of exchangeable cations, rather than the actual metric distances. Hulls are drawn around the distribution of points within each major horizon (discussed in detail below). Stocks of Ca^{2+} and Mg^{2+} increase in nearly the same direction, while K^{+} and Na^{+} are more similar to each other. The labeled vectors show that K^{+} and Na^{+} increase orthogonally to Ca^{2+} and Mg^{2+}, indicating that these two sets of cations change independently of each other and, furthermore, that different factors are the dominant controls over their distributions in soil. Environmental and chemical gradients overlain on the NMDS distribution help to visualize the statistical effects seen in PERMANOVA and can aid in interpreting which of the exchangeable cations respond to each gradient.

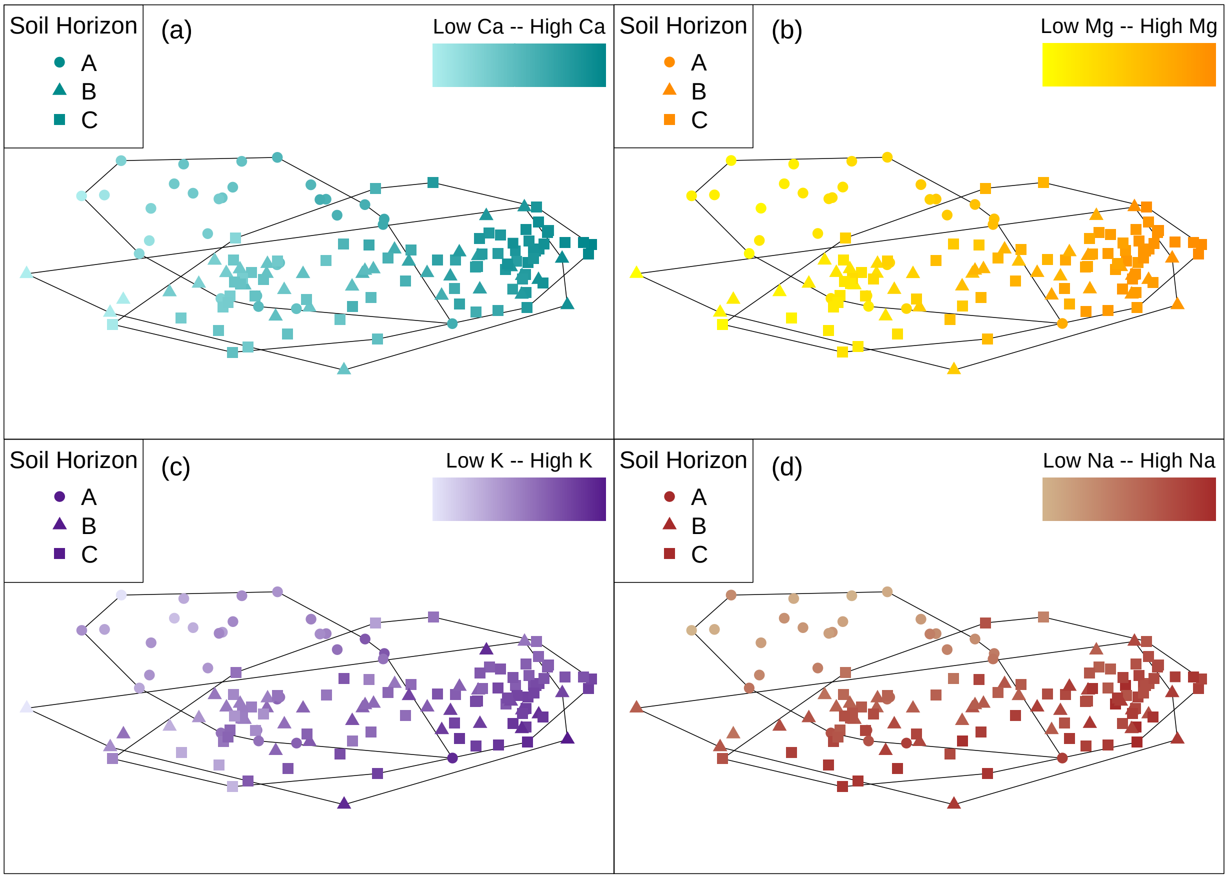

Figure 3.7. Non-metric multidimensional scaling (NMDS) ordination for total soil exchangeable cation data fit in 2 dimensions using a Euclidean distance measure. Data was ln-transformed prior to analysis. NMDS converged on a solution after 3 runs using random starting locations. Final stress = 0.0305. Vectors indicate the direction and magnitude of positive correlation for (a) total exchangeable Ca^{2+}, (b) total exchangeable Mg^{2+}, (c) total exchangeable K^{+}, and (d) total exchangeable Na^{+}. These vectors will be used in the later figures to show the direction of increase for each exchangeable cation. Point symbols represent the horizon from which the sample was taken, and hulls are drawn around the distribution of each horizon in the ordination space.

Both total C and Climate PC1 are significant predictors of the distribution of exchangeable cations between sites (Table 3.5). Cumulative soil C explains 20% of the variation, with an additional 12% explained by Climate PC1. These effects can be seen on the distribution of cations in NMDS space in Figure 3.8. The points in these plots are the site centroids – the point of mean distance between exchangeable cation stocks within each site. Sites with high total soil C tend to occur where exchangeable Ca^{2+} and Mg^{2+} are low (Figure 3.8a). This result is unexpected given the well-recognized positive relationship between SOM and CEC sites (Kalisz and Stone, 1980). One hypothesis to explain this difference between observation and theory is that in sites as acidic as these (

Table 3.1) the pH dependent charges on SOM will be mostly protonated or occupied by Al^{3+} (Gruba and Mulder, 2008), while simultaneously, the sites with high C stocks are likely to release more dissolved organic matter (DOM), which can act as mobile anions that exacerbate leaching loss (Johnson and Cole, 1980; Johnson, 1992). Further reinforcing this hypothesis is our finding that sites with warm, dry climates tend to have larger quantities of Ca^{2+} and Mg^{2+} (Figure 3.8b). The soils that experience less rainfall and higher temperatures have less leaching force exerted on the system, producing less translocation and loss of cations to groundwater. These results suggest that increased leaching accompanied by greater SOM buildup and associated DOM movement could be a fundamental control over the distribution of exchangeable cations across the landscape, particularly for Ca^{2+} and Mg^{2+}. The direction of linear correlation for K^{+} and Na^{+} is nearly perpendicular to the direction of change in both total C and climate PC1, indicating that other processes are dominant controls for these base cation species between sites.

Table 3.5. PERMANOVA results for distribution of exchangeable cation stocks (P-value calculated with 10,000 permutations).

		Df	SS	*Pseudo-F*	R^2	*P* (>F)
Between Site Variation	Total C	1	30.58	5.69	0.203	0.0024
	Climate PC 1	1	18.15	3.38	0.120	0.0342
	Residuals	19	101.96		0.677	
	Total	21	150.69			
Within Site Variation	Site ID	21	658.17	19.55	0.583	< 0.0001
	Horizon	2	237.21	73.98	0.210	< 0.0001
	Nitrogen	1	12.94	8.07	0.011	0.0035
	Carbon	1	9.21	5.74	0.008	0.0115
	Depth	1	3.27	2.04	0.003	0.1775
	Horizon | Carbon	2	23.86	7.44	0.021	0.0006
	Horizon | Depth	2	16.04	5.00	0.014	0.0169
	Residuals	105	168.34		0.149	
	Total	135	1129.03			

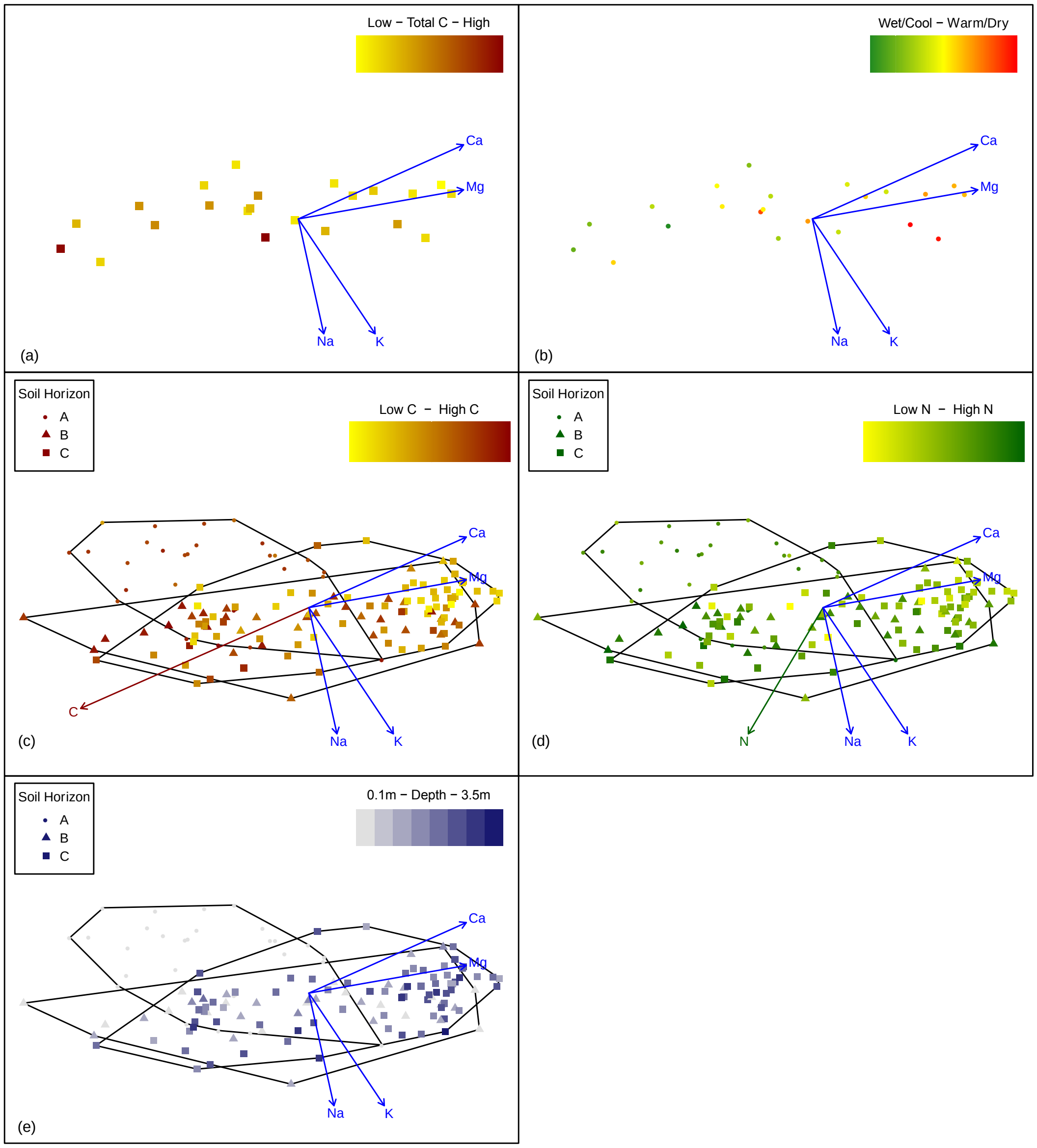

Figure 3.8. Relationships between total soil exchangeable cations and (a) cumulative soil C at each each site, (b) climate PC1 for site centroids, (c) soil C, (d) soil N, and (e) soil depth. These environmental gradients visually show the statistically significant relationships observed in Table 3.5. Soil chemical values (soil C stocks, and soil N stocks) were ln-transformed. For full description of NMDS ordination, refer to Figure 3.7. Vectors indicate positive correlation if they point in the same direction, negative correlation if they point away from each other, and lack of correlation if they are perpendicular. In (a) and (b) plotted points are the centroids of the exchangeable cation stocks for each site. Symbols correspond to the major soil horizon for each sample in (c), (d) and (e).

There are large differences between sites – accounting for 58% of the variation within the soil profiles – which must be controlled for prior to testing the effects of environmental gradients on the vertical distribution of cations. This large site effect is expected given the vast differences between soils, and allows subsequent explanatory variables to be tested for their ability to explain the vertical distribution of exchangeable cations regardless of soil type. Soil horizon along with both soil C and soil N are significant predictors of the distribution of exchangeable cations (Table 3.5). Master soil horizon, on its own, explains 21% of the variation and is statistically a much stronger predictor of cation distribution than is soil depth on its own. The significance of soil horizons could be partially explained by differences in soil volume – the surface stocks (0-50 cm depth) are thinner than subsurface stocks (>50 cm depth), with thicknesses of 10 or 40 cm rather than 50 cm in the subsurface. However, soil horizon is also a significant predictor of cation concentration (Table 3.4), which is independent of soil stock calculations. Furthermore, A horizons are frequently thinner than B and C horizons in the field, as was the case in all sites sampled for this study. Horizon development is driven by pedogenic and biogeochemical mechanisms that have advanced to various depths in different soils, and thus stocks of soil nutrients will naturally vary based upon the volume of each horizon. The significant separation in the distribution of A horizon samples from B and C horizon samples can be seen in Figure 3.8. There was a significant interaction between soil horizon and both soil C ($R^2 = .021$, $p < .05$) and depth ($R^2 = .014$, $p < .05$). Within each horizon, samples with less soil C tend to have more exchangeable base cations, particularly Ca^{2+} and Mg^{2+} (Figure 3.8c). Vectors for K^+ and Na^+ are orthogonal to soil C, suggesting lack of a relationship. Likewise, within each major horizon, deeper samples tend to have more base cations (Figure 3.8e). The interaction between horizon and soil carbon stock reinforces the hypothesis that DOM can be a control over the distribution of exchangeable cations. Not only is soil C a significant control on exchangeable cations between different soils, it is partly responsible for the vertical distribution within each soil. In particular, within each master horizon group (A, B, or C) those samples with higher C tend to contain less exchangeable Ca^{2+} and Mg^{2+} than those with lower C. The interaction of horizon and depth makes intuitive sense given that leaching appears to be a major force acting on the vertical distribution of exchangeable cations, resulting in greater exchangeable cation stocks (particularly Ca^{2+} and Mg^{2+}) in deeper parts of soil horizons.

Soil N has a significant effect on exchangeable cation distribution, second only to horizon (Figure 3.8d). This effect only explains a small portion of the variation, however ($R^2 = 0.011$, $p < .05$). Larger quantities of N occur where exchangeable cations are lower, particularly Ca^{2+} (Figure 3.8d). The finding that exchangeable cations decrease as soil N increases agrees with other studies in the region, most notably Perakis et al. (2006; 2011; 2013). Because N is found in many different forms in soil, this effect could be due to NO_3^- leaching as well as leaching via DOM that has N incorporated in the molecular structure. Soil C and N are highly correlated across all samples in this dataset ($R^2 = 0.71$), though there is surprisingly no significant interaction between these terms in the PERMANOVA model. Though the data presented here cannot tease apart these two mechanisms, most likely both are operating in these soils. Soil N distribution is very surficial compared to exchangeable cations at these sites (31% below 1 m relative to 57-76% below 1 m for exchangeable cations), suggesting that N is more limiting to plant growth and that it is recycled more efficiently within coastal Pacific Northwest ecosystems.

Soil theory states that the dominant role of soil organic matter in cycling of exchangeable cations is as a source of CEC sites in the solid phase that hold these nutrients and keep them from leaching. However, the PERMANOVA results presented here reveal a contrary result: that there is more exchangeable Ca^{2+} and Mg^{2+} in soils with less soil C and in the parts of soil horizons with less C. How might this reconcile with well-established theory? Almost all of these soils are highly acidic (

Table 3.1), which causes the carboxylic and phenolic functional groups that contribute the most to pH-dependent charge to remain protonated or to be occupied by Al^{3+} (Gruba and Mulder, 2008). Consequently, the ability of SOM to retain base cations in these soils is limited. In this context, the role of dissolved organic matter becomes magnified. DOM has been highlighted as an important vector by which C migrates from O and A horizons to subsurface B and C horizons (Kaiser and Kalbitz, 2012). As more complex, surface reactive DOM is retained by sorption to soil minerals, the lighter, more soluble compounds continue into subsoil (Guggenberger and Kaiser, 2003). The DOM likely to reach the deep subsurface horizons include hydrophilic compounds that contain more functional groups and few hydrophobic aliphatic chains, as well as low molecular weight (LMW) organic acids. These compounds could essential serve as mobile CEC sites that gradually leach exchangeable cations deeper into soils as they become mobilized in solution. Scott and Rothstein (2014) hypothesize that DOM in soil is in dynamic exchange, whereby highly sorptive, hydrophobic compounds displace previously sorbed, N-rich hydrophilic compounds on soil surfaces. Several studies have recognized that dissolved organic nitrogen (DON) can leak from terrestrial ecosystems due to its low biodegradability (Hedin et al., 1995; Neff et al., 2003; Perakis and Hedin, 2002), and thus could also serve as a mobile ligand for cations.

The distributions of K^+ and Na^+ differ considerably from Ca^{2+} and Mg^{2+}. The fact that K^+ follows a different trajectory in its vertical distribution – and does not appear to follow soil C, N or horizon as closely as Ca^{2+} and Mg^{2+} – suggests that processes other than leaching are more dominant controls. Jobbagy and Jackson (2001) suggest that at the global scale plant uptake and cycling are more important drivers of K^+ distribution than leaching, despite K^+ being more poorly held to CEC sites than Ca^{2+} and Mg^{2+}. K^+ is found in high concentrations in plant tissues, and there are typically limited supplies available in soil. Consequently, the particular importance of upward translocation through biological cycling of K^+ seems a reasonable hypothesis for the differences in vertical distribution seen here. Based upon the stoichiometry of cation exchange, the release of cations into soil solution is a function of both leaching rate and the total ionic concentration (Johnson and Lindberg, 1992). This relationship is dependent on the charge (and associated hydrated radius) of cations, which implies that K^+ and Na^+ release into solution will be least affected by increases in total anion or cation leaching rate (Johnson, 1992).

The lack of correlation between Na^+ distribution and Ca^{2+} and Mg^{2+} distributions is surprising, since this element is least likely to be cycled upward through biological uptake and most likely to be leached deeper into the profile. There are two conceivable explanations for the uniformity of exchangeable Na^+ stocks both between different soils as well as in its vertical distribution. First, while Na^+ is not recognized as an essential soil nutrient, it has been hypothesized that Na^+ can fill some small roles in plant nutrition usually fulfilled by K^+, including the passive regulation of osmotic pressure across cell walls (Subbarao et al., 2003). This explanation seems unlikely, however, because K^+ concentrations in Douglas-fir foliar tissues are typically an order of magnitude larger than Na^+ (Goodrich and Jacobi, 2012), indicating selective exclusion of Na^+ from uptake. The second, more likely explanation is that increased atmospheric deposition of Na^+ due to proximity to the ocean is driving the more surficial distribution within sites and uniform distribution between sites. Na^+ deposition rates in the coastal Pacific Northwest can be quite high (Cole and Rapp, 1981; Edmonds et al., 1995; Johnson and Lindberg, 1992), resulting in persistent seeping of exchangeable Na^+ through soil. Finding uniform quantities with depth could imply that these soils have reached equilibrium between persistent atmospheric deposition and leaching of Na^+.

This analysis can help guide future research into the mechanisms of these relationships, particularly examining the interplay between cation release from weathering and leaching. Soil solution studies at these sites could help elucidate the chemical speciation of soil C and N that directly results in cation leaching, as well as quantifying other negatively charged species like HCO_3^-, Cl^- and SO_4^{-2}. Across the Pacific Northwest, leaching (possibly via mobile organic acids) is a primary driver of Ca^{2+} and Mg^{2+} differences both between soils across the landscape and within soil profiles, while biological cycling and weathering release from primary and secondary minerals control the distribution of K^+.

3.4 CONCLUSIONS

1. Over 50% of all exchangeable base cations are found below 1 m, on average.
2. Leaching is a primary control over exchangeable Ca^{2+} and Mg^{2+} distribution between sites in the Pacific Northwest, possibly with assistance from mobile organic acids.
3. Soil horizon development substantially affects the distribution of exchangeable base cations within the soil profile, with lower contents and stocks of cations in A horizons relative to B and C horizons. Within horizons, exchangeable Ca^{2+} and Mg^{2+} increase as soil C decreases and depth increases.
4. Exchangeable K^+ is less deeply distributed than Ca^{2+} or Mg^{2+}, indicating plant uptake as a more dominant control.
5. Exchangeable Na^+ is relatively evenly distributed both between soils and within the soil profile in the Pacific Northwest, likely due to persistent atmospheric deposition along with leaching.

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Chapter 4. THE EFFECT OF HARVEST ON FOREST SOIL CARBON: A META-ANALYSIS

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4.1 ABSTRACT

Forest soils represent a substantial portion of the terrestrial carbon (C) pool, and changes to soil C cycling are globally significant not only for C sequestration but also for sustaining forest productivity and ecosystem services. To quantify the effect of harvesting on soil C, we used meta-analysis to examine a database of 945 responses to harvesting collected from 112 publications from around the world. Harvesting reduced soil C, on average, by 11.2% with 95% CI [14.1%, 8.5%]. There was substantial variation between responses in different soil depths, with greatest losses occurring in the O horizon (-30.2%). Much smaller but still significant losses (-3.3%) occurred in top soil C pools (0-15 cm depth). In very deep soil (60-100+ cm), a significant loss of 17.7% of soil C in was observed after harvest. However, only 21 of the 945 total responses examined this depth, indicating a substantial need for more research in this area. The response of soil C to harvesting varies substantially between soil orders, with greater losses in Spodosol and Ultisol orders and less substantial losses in Alfisols and Andisols. Soil C takes several decades to recover following harvest, with Spodosol and Ultisol C recovering only after at least 75 years. The publications in this analysis were highly skewed toward surface sampling, with a maximum sampling depth of 36 cm, on average. Sampling deep soil represents one of the best opportunities to reduce uncertainty in the understanding of the response of soil C to forest harvest.

Keywords: Forest management; harvest; soil carbon; soil order; deep soil; meta-analysis

4.2 INTRODUCTION

Forest ecosystems contain 1240 Pg C [1,2], which represents as much as 80% of aboveground terrestrial C and 70% of all soil organic C [3–5]. The relative proportion of forest C found in soils varies among biomes, ranging from roughly 85% of the terrestrial C pool in boreal forests, to 60% in temperate forests, to 50% in tropical rainforests [1,6]. The net balance of soil C in forests relies upon large rates of detrital inputs (61.4 Pg C yr^{-1}) and respiratory losses (60 Pg C yr^{-1}), which together represent substantial yearly turnover in the soil C pool [7]. By altering the rates of detrital inputs and respiratory outputs in soils, the extent and intensity of forest harvest can have substantial impacts not only on ecosystem function but also on atmospheric chemistry and global climate [6,8,9].

C is one of the principal components of soil organic matter (SOM), a key component of soil that plays an important role in many biological, chemical, and physical properties [10–12]. SOM provides a crucial source of energy and nutrients for soil microbes, buffers soil pH, and helps to stabilize soil structure [12,13]. Along with nitrogen and phosphorus, SOM is considered a critical indicator for soil health and quality.

Thus, soil C is an essential component of forest C accounting, yet many models assume that only surface soil responds to forest management and that soil C returns to equilibrium within 20 years after harvest [14]. Recent national or global assessments of forest C lack any mention of mineral soil C [15–17], implicitly assuming that soil C remains constant after forest harvest. Furthermore, carbon monitoring programs include soil C inconsistently. For example, the American Carbon Registry [18] and the Verified Carbon Standard [19] do not require or specify protocols for soil C measurements. The IPCC inventory standards [20] assume constant mineral soil C in Tier 1, with an option for inclusion of national soil C inventories only if preferred by a particular agency, and the U.S. Forest Service Inventory and Analysis Program [21] specifically limits soil sampling to 20 cm depth. The inclusion of soil in models of ecosystem C following harvest can have significant effects. For example, in a model of the forest C pool change following intensive bioenergy harvest, Zanchi et al. [22] show that the inclusion of soil increases the C payback period by approximately 25 years when substituting forest bioenergy for coal. Thus, the inclusion or exclusion of soil in ecosystem C models and ecological monitoring programs can have a major impact on forest policy when attempting to mitigate climate change through forest management [14].

Ambiguity about the effect of forest harvesting on soil C has persisted in the literature, likely exacerbated by the inherent spatial and temporal variability in soil measurements that can obscure the results of even the most well-designed studies [23]. By gathering the results from many studies that apply similar treatments, meta-analysis can overcome the high levels of spatial and temporal variability to provide cumulative answers that may not have been evident within individual sites [23,24]. Previous meta-analyses on the effect of harvesting on soil C have found either minimal effects on soil C pools [25] or substantial (30%) loss to O horizon pools with little change to mineral soil C [9]. Variation in soil C response has been shown to significantly differ among soil types and different harvesting strategies [9].

Studies of soil C change due to harvest have historically been strongly biased toward surface sampling [26]. Nave et al. [9] reported a mixed response to harvest in deeper soil (20-100 cm depth), ranging from a slight average decrease (-5%) in studies that reported C pools to a large average increase (+20%) in studies that reported only C concentration. Several recent reviews have highlighted the need for greater sampling of deep soil [26–28], especially as the shifting paradigm of SOM research has come to reject the assumption that deep soil C cannot change on timescales relevant to anthropogenic C emissions [29–31]. Resolving the response of deep soil horizons to harvesting is important because these horizons occupy a much greater volume than surface O and A horizons. Even small changes in subsurface C can exacerbate or compensate for changes in surface soil C, and neither the magnitude nor direction of subsoil C change is clear from previous research.

The process of meta-analysis is necessarily cumulative, with each iteration updating previous analyses to further constrain the error in effect size estimates and to extend the scope of analysis. Thus, the objective of our meta-analysis is to update and extend the findings of Nave et al. [9] with respect to five major research questions:

1) What is the overall effect of forest harvesting on soil C pools?
2) How does the effect of forest harvest on soil C change with soil depth?
3) To what extent does the effect of harvesting differ among soil orders?
4) Do site pretreatment strategies or increasing harvesting intensity (i.e. whole tree harvest) moderate or accentuate harvesting impacts on soil C?
5) How long does soil C take to recover from harvest across different soil types?

4.3 MATERIALS & METHODS

Meta-analysis is a cumulative activity which builds upon previous research and meta-analyses on similar research questions. Our meta-analysis builds upon the work of Nave et al. [9] and Johnson and Curtis [25] by updating their results with studies published between 2008 and 2016. The database published by Nave et al. [9] available from < http://www.nrs.fs.fed.us/pubs/34850> was independently recreated from each of 75 references. Metadata for each study was verified, and additional metadata such as the sampling depth of each response ratio was gathered. A total of 8 effect sizes differed in our dataset from the Nave et al. [9] database, all of which were either additional data for mineral soils or a split of one effect size into two based upon sampling depth.

To add to this database with studies published between 2008 and 2016, we searched the peer-reviewed literature for relevant studies using the online database ISI Web of Science with combinations of the terms: forest, timber, harvest, logging, soil C, soil organic matter, and management. No climate criteria was used to screen studies. To be included in the meta-analysis, publications had to report both a control as well as harvested treatments. Both pretreatment soil C and unharvested reference stands were considered acceptable controls, and measurements of reference stands were considered the superior control. For forest chronosequence studies, soil C data from the oldest stand was used as the control. A minimum stand age of 30 years was considered acceptable for control stands, although most studies used controls of considerably greater age. Nave et al. [9] found that studies reporting only soil C concentration data yielded different conclusions about the direction of harvest effects than those studies reporting soil C pool data. Consequently, soil C pool data was used in our meta-analysis when both concentration and pool data were available.

We collected potentially useful predictor variables of soil C response from each publication, including soil order, geographic region, and time since harvest (Table 4.1). Binning of continuous predictor variables (such as precipitation) was carried out in the same intervals as Nave et al. [9] for ease of comparison. Each study was categorized by harvest, residue management, and site preparation strategies. Harvesting technique was categorized as sawlog when only the merchantable bole (stem) was removed from the site or whole tree harvest (WTH) when the tops, limbs, and foliage were removed in addition to the bole. To test the response of soil C at different depths, data from each study was separated into one of five groups: O horizon, top soil (0-15 cm), mid soil (15-30 cm), deep soil (30-60 cm), and very deep soil (60-100+ cm). A sixth group called whole soil was assigned to studies that aggregated mineral soil samples instead of reporting results at separate depths. Several studies aggregated soil data from 0-100 cm, which reduced the number of unique deep and very deep soil observations even though these depths were separately sampled.

Table 4.1. Factors gathered as potential predictor variables in this meta-analysis.

Factor	**Levels**	
Reporting units	Pool (Mg ha^{-1}), concentration (% or mg g^{-1})	
Soil Depth	O horizon	Forest Floor
	Top Soil	0-15 cm
	Mid Soil	15-30 cm
	Deep Soil	30-60 cm
	Very Deep	60-100+ cm
Overstory species	Hardwood, conifer/mixed	
Soil order	Alfisol, Andisol, Entisol, Inceptisol, Mollisol, Spodosol, Oxisol, Ultisol	
Geographic group	NE North America, NW North America, SE North America, SW North America, Europe, Asia, Pacific (Australia, New Zealand)	
Harvest type	Clearcut, thin	
Harvest intensity	Stem only, whole tree	
Residue management	Removed, spread	
Site preparation	Broadcast burn, tillage/scarification	
Soil texture	Fine (mostly silt or clay), coarse (mostly sand), organic	
Time since harvest	Continuous	
Mean Annual Temperature	0-5, 5-7.5, 7.6-10, 10.1-15, 15.1-20, >20 (°C)	
Mean Annual Precipitation	<500, 500-750, 751-1000, 1001-1400, 1401-1800, >1800 (mm)	

Our meta-analysis estimates the magnitude of change in soil C using the ln-transformed response ratio R, which is defined as

$$\ln(R) = \ln\left(\frac{X_T}{X_C}\right) \tag{4.1}$$

where $\overline{X_T}$ is the mean soil C value of treatment (harvested) observations, and $\overline{X_C}$ is the mean soil C value of control observations for a given set of experimental conditions at a specific site and depth. Multiple response ratios were recorded for each publication, with the number of response ratios (k) depending upon the number of experimental conditions imposed and the number of samples taken by depth. For example, a publication that reports the results of two thinning treatments and two clear-cut treatments at three depth increments (forest floor, top soil, and mid soil) versus a control would yield 12 response ratios. R is a unit-less measure of effect size, which allows comparison among studies that report data in different units [32]. By back transforming ln(R), $[(e^{(\ln(R))} - 1) \times 100]$, mean response ratios can be interpreted as the percentage change in soil C relative to the control. Estimates of the standard deviation and sample size for each $\overline{X^T}$ and $\overline{X^C}$ were not available in several publications. Consequently, an accurate estimate of total heterogeneity (Q_T) for the dataset was not possible. Subsequent partitioning of Q_T into within- and among-group heterogeneity (Q_W and Q_A, respectively) for random and mixed effect models (as is customary for meta-analyses) was not possible [24]. Instead, we used nonparametric resampling techniques (bootstrapping) to estimate confidence intervals around mean effect sizes in an unweighted meta-analysis [9]. Adams et al. [33] recommend bootstrapping confidence intervals for ecological meta-analyses, and show that confidence bounds based on this method are more conservative than standard meta-analyses. Bootstrapping was implemented using the bootES package [34] in R [35]. For all statistical tests in our analysis, $\alpha = 0.05$.

Although not exhaustive, the database we compiled from the literature search contained 945 soil C response ratios from 112 publications published between 1979 and 2016. Roughly half the dataset was comprised of response ratios analyzed by Nave et al. [9]. The full dataset is available as Supplementary Material, including maximum sampling depth and the number of response ratios from each paper (Appendix 1).

4.4 RESULTS

4.4.1 *Overall effect & change with depth*

Across all studies, harvesting led to a significant average decrease in soil C of 11.2% relative to control (Figure 4.1). Whether the response to harvest was reported as pools or concentrations had a large impact on the estimated effect of harvest on soil C, with mean response for studies reporting C concentration units (%, mg g, etc.) 16.2% higher (with a 95% CI [20.9%, 11.8%]) than studies reporting C pool units (Mg ha^{-1}, tons ha^{-1}, etc.). Concentration responses are higher than pool responses at all soil depths, except for very deep and whole mineral soil, which did not have enough concentration response ratios to construct separate confidence intervals (Figure 4.1). Consequently, all subsequent analyses focused on the subset of data reporting soil C pools.

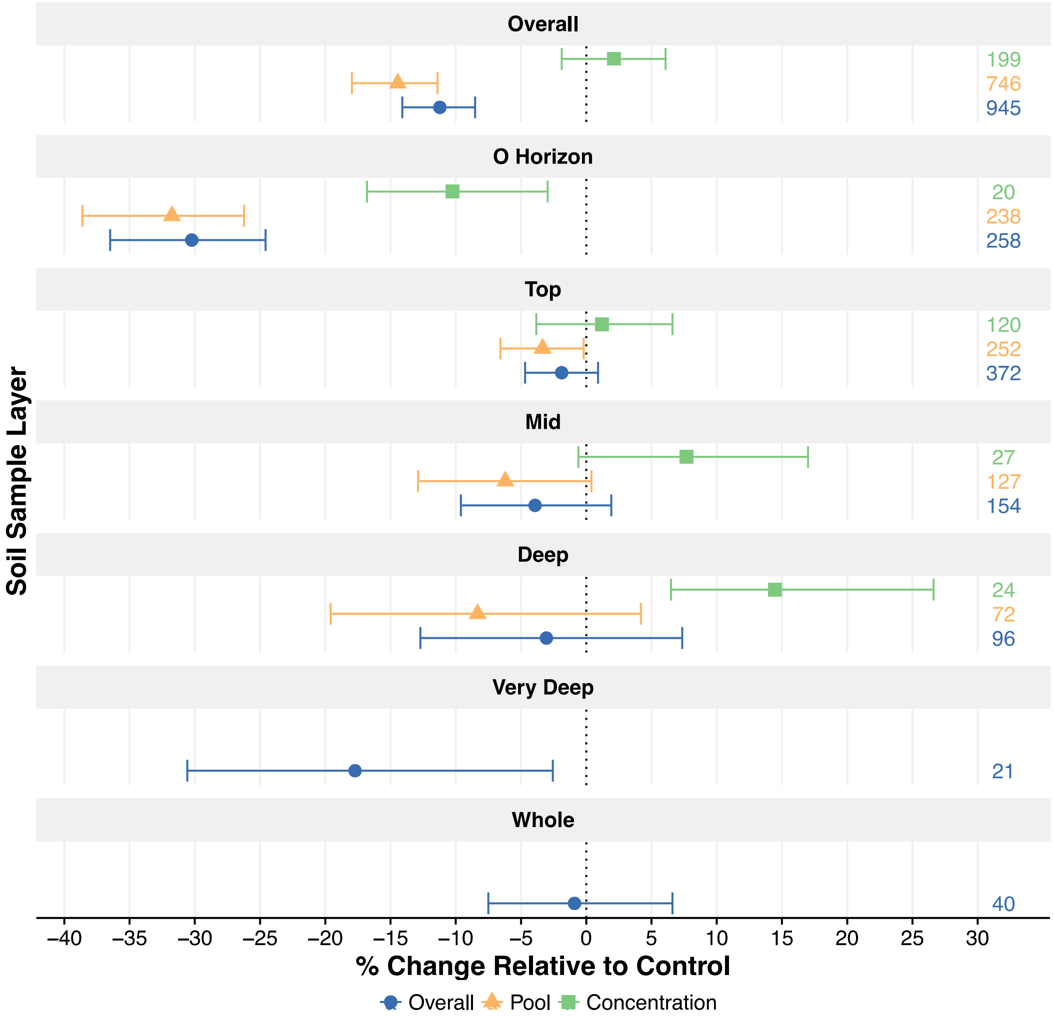

Figure 4.1. Response of soil C to forest harvesting, overall and faceted by soil depth. All points are back-transformed mean effect sizes ± 95% confidence intervals calculated by nonparametric bootstrap. The number of response ratios (*k*) that make up each mean effect is listed on the right. Mean effects with confidence intervals overlapping the dashed line (0%) show no significant change in soil C due to harvesting. Within each facet, mean effect sizes are shown for the overall effect as well as separately for studies reporting C pool units or concentration units.

Several different soil layers show significant losses of C due to harvesting. Overall, O horizons lost 30.2% of their carbon as a result of harvesting. Losses from top soil were much smaller, although the estimated loss when reported in pool units was significant (-3.3%). In mid (15-30 cm) and deep soil (30-60), the average loss of soil C was greater than topsoil, although the smaller number of response ratios for these depths resulted in more poorly constrained estimates. Studies only reporting C concentration observed a 14.5% increase in deep soil (30-60 cm), although the sample size was relatively small. The overall effect in very deep soil (60-100+ cm) was significant, with an average loss of 17.7%. Unfortunately, this region of the profile was not frequently sampled (21 response ratios out of 945 total), and consequently the 95% confidence interval is quite wide.

4.4.2 *Effect of harvesting across soil orders*

The effect of harvesting on soil C differs between soil orders (Figure 4.2). For the Alfisols and Inceptisols, there are significant losses in O horizon C pools (-12.0% and -45.4%, respectively), but no significant loss in the mineral soil. Mollisols lost an average of 17.7%, although neither O horizon nor mineral soil responses were significantly different from 0. In several cases, small samples sizes made separate testing of organic and mineral soil impossible within a single order (Andisols, Entisols, Oxisols). However, in each of these cases the overall effect was significant. Soil C increased by 24.5% on average in Andisols, but decreased by 18.8% in Entisols and 30.9% in Oxisols. The number of response ratios was more concentrated in the Alfisol, Inceptisol, Spodosol, and Ultisol orders, although a large number of publications did not report information on soil classification. The response to harvesting in Spodosols is substantial (-19.0% overall), with significant losses in both the O horizon (-36.4%) and moderately less in the mineral soil (-9.1%). Likewise, Ultisols lost significant soil C in response to harvesting (-24.7% overall), with the most substantial losses occurring in the O horizon (-66.0%) rather than in the mineral soil (-11.9%).

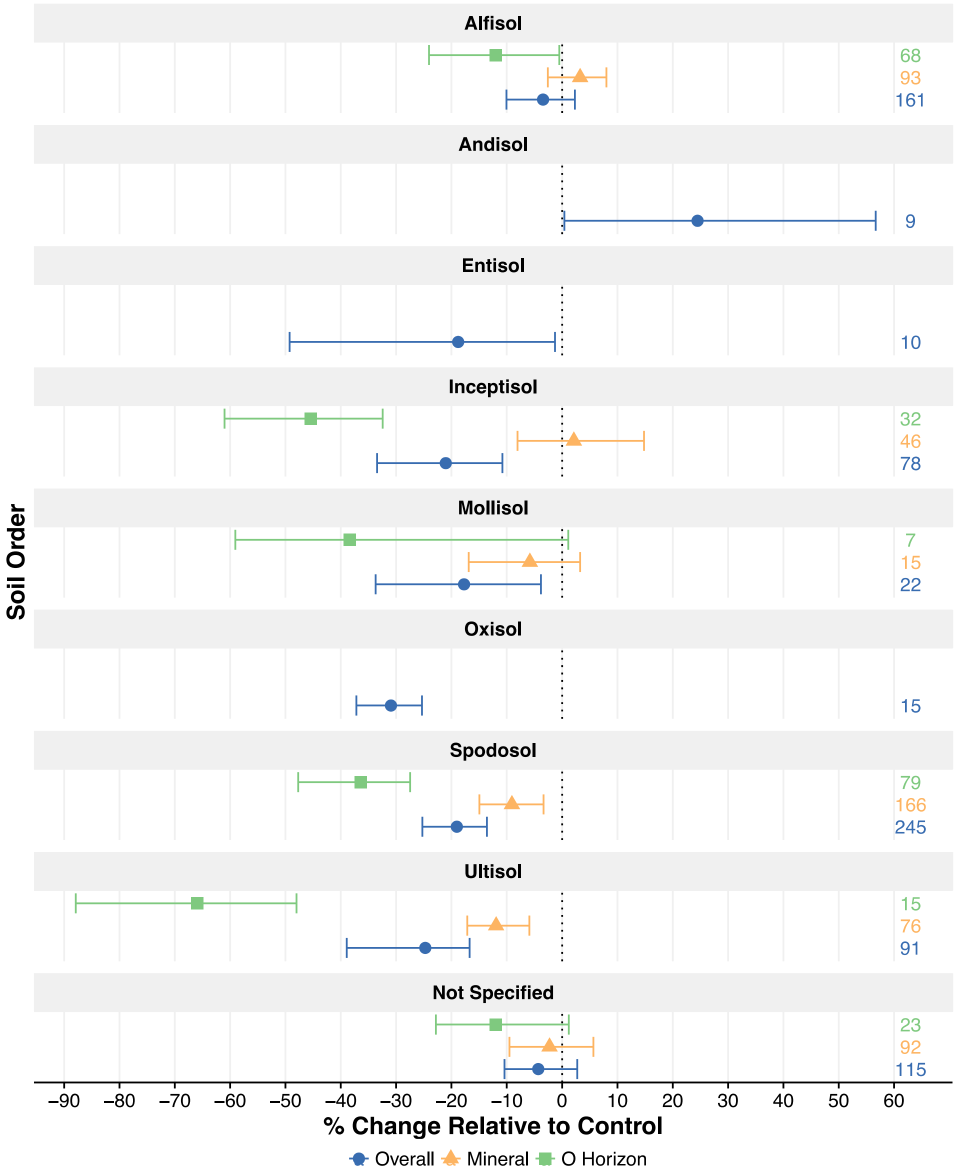

Figure 4.2. Response of soil C to harvesting in different soil orders. Mean effect sizes ± 95% confidence intervals calculated by nonparametric bootstrap are shown for all response ratios in each soil order (Overall) and broken out into mineral soil or O horizon. The number of response ratios (*k*) comprising each mean effect are listed on the right. Effect sizes were calculated only on response ratios reported in pool units (*k* = 746).

4.4.3 *Differences in response to harvest between forest types*

The response of soil C to harvest differs between hardwood and coniferous/mixed forest types (Figure 4.3). The decline in O horizon C pools is significantly greater in conifer/mixed forests (-38.1%) compared to hardwood forests (-25.4%). Differences between forest types were not significant for any mineral soil layer. However, the decline in soil C after harvest was significant for hardwood forests but not conifer/mixed forests in deep soil (30-60 cm) and in studies reporting whole mineral soil C pools. Also in these studies, the difference between hardwood and conifer/mixed forest response to harvest is marginally significant ($p < 0.1$). The number of observations are highly concentrated in O horizon and top soil, consequently limiting the precision of mean effect size estimates in deeper layers. No observations for hardwood forest were made in very deep soil (60-100+ cm).

4.4.4 *Harvest intensity, residue management and site pretreatment*

Differences in forest management strategies can significantly impact the response of soil C to harvesting (Figure 4.4). While there was no significant overall difference observed between thinning and clear-cut harvesting, less C was lost from mineral soils under clear-cut harvesting compared to thinning (+9.3%). Likewise, harvest intensity significantly changed the response of mineral soil C, with soils undergoing whole tree harvesting losing 13.3% less C than bole-only harvesting. Possible mechanisms for these counter-intuitive results are considered in Discussion section 3.5.5.

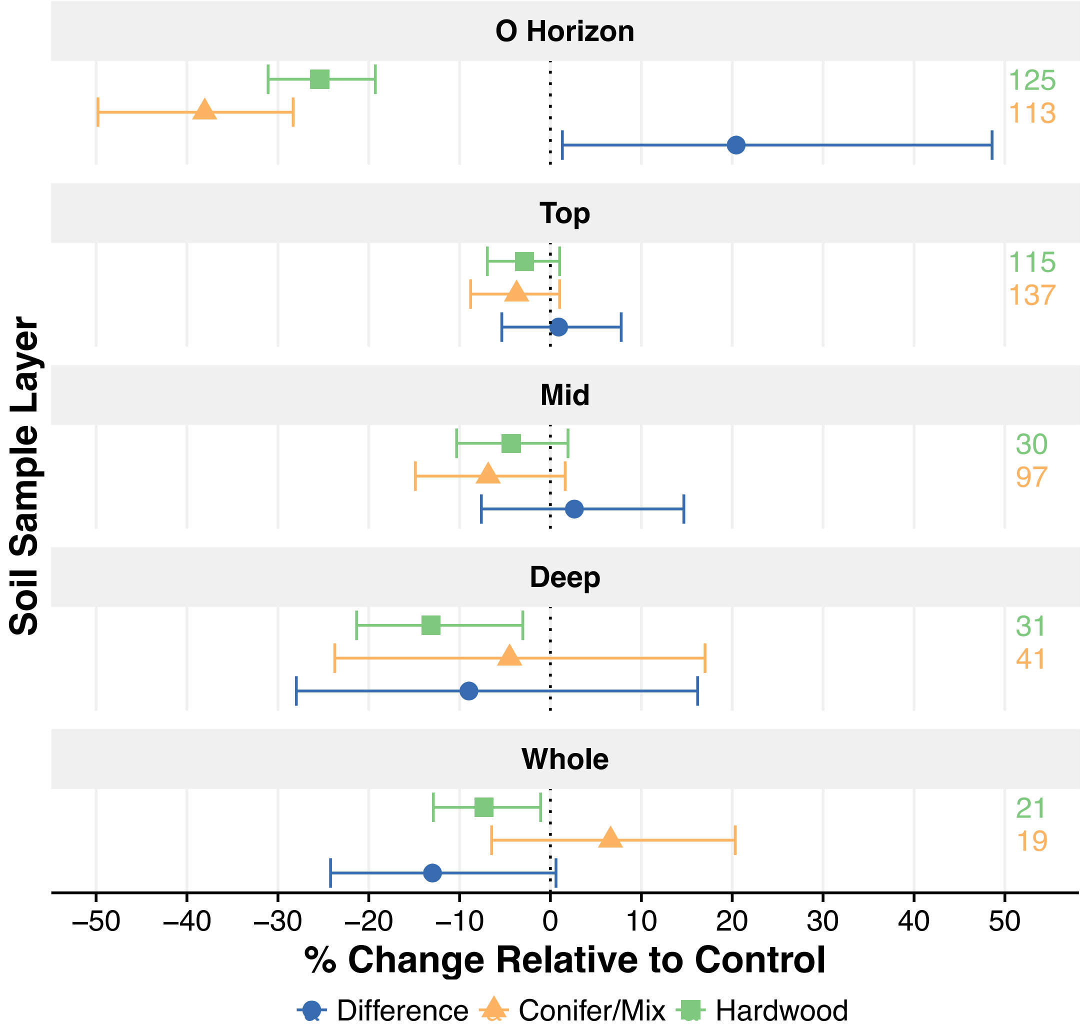

Figure 4.3. Response of soil C harvesting at different depths in soil, broken down by hardwood or conifer/mixed forest types. Mean effect sizes ± 95% confidence intervals calculated by nonparametric bootstrap are shown for hardwood and conifer/mixed forests. Blue circles show the mean difference between these forest types (Hardwood – Conifer/Mix) ± 95% confidence interval for the difference. Differences are calculated on the logarithmic effect size scale, and then back-transformed to % change, and thus do not necessarily add up on the % change scale. The number of response ratios (k) in each forest type at each depth is listed on the right. Data for very deep soil is not shown because there were no observations for this soil layer in hardwood forests.

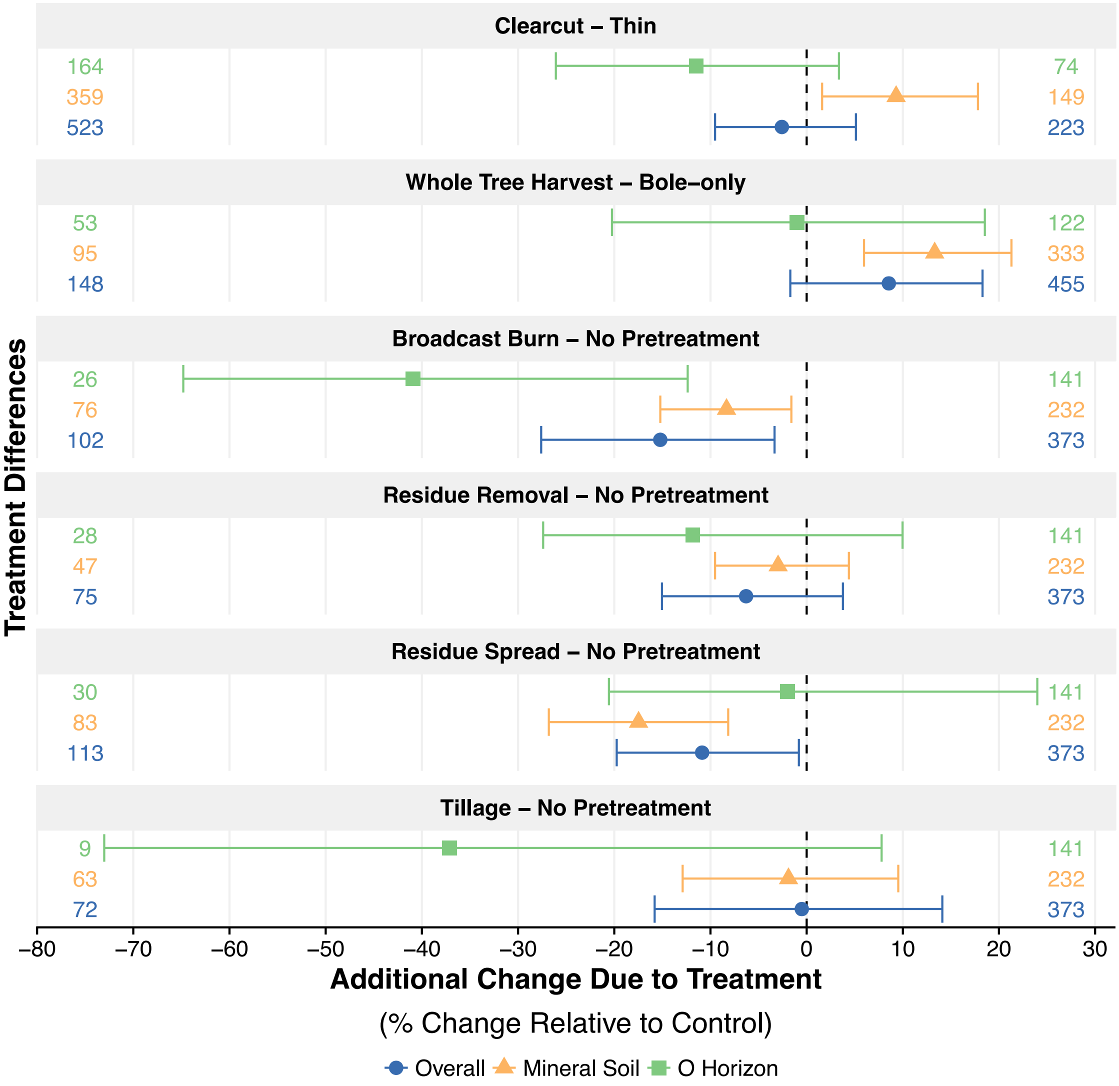

Figure 4.4. Differences in response of soil C to harvesting between treatment strategies. Differences are calculated by subtracting [more intensive treatment] – [less intensive treatment], such that positive differences represent reduced loss of C due to more intensive treatment, and negative differences represent increased losses of C due to more intensive treatment. Point estimates are back-transformed differences between mean effect sizes ± 95% confidence intervals calculated by nonparametric bootstrap. Mean effect differences with confidence intervals overlapping the dashed line (0%) show no significant difference between the harvesting, residual management, or pretreatment strategies. The number of response ratios (*k*) for the intensive treatment in each comparison appear on the left and for the less intensive treatment on the right.

The practice of broadcast burning sites in preparation for planting after a harvest leads to significant additional losses of soil C, with burned soils losing 15.2% more C than soils with no pretreatment. This effect is especially severe in the O horizon (40.9% additional loss than if sites were not burned), and somewhat curtailed in the mineral soil (8.3% additional loss). The wide 95% CI for the estimate of differences in O horizon responses due to burning reflects disparities in burn severity and treatment implementation among different studies.

Spreading of residual materials across harvested sites (by chipping tops and limbs or other methods) resulted in significant additional loss of soil C (-10.9%), with these extra losses occurring mostly in the mineral soil (-17.5%). On the other hand, residue removal resulted in no significant additional losses to soil C.

Tillage is sometimes used to prepare soils for planting after harvest, either to create raised planting beds or to prepare the soil seed bed. This intensive style of site preparation did not result in significant losses in soil C, especially in the mineral soil. However, very large losses were reported in the O horizon (mean effect = -37.1%) with a very wide confidence interval due to a small number of observations. Additional study of the effect of tillage would help to reduce this error.

4.4.5 *Recovery of soil C after harvest*

The recovery time for soil C following harvest differs among soil orders (Figure 4.5). Only 4 soil orders contained enough observations over time to model recovery times: Alfisols, Inceptisols, Spodosols, and Ultisols. We modeled time as a second degree polynomial (Time + Time^2) separately for O horizons and mineral soils for each soil order (

Table 4.2).

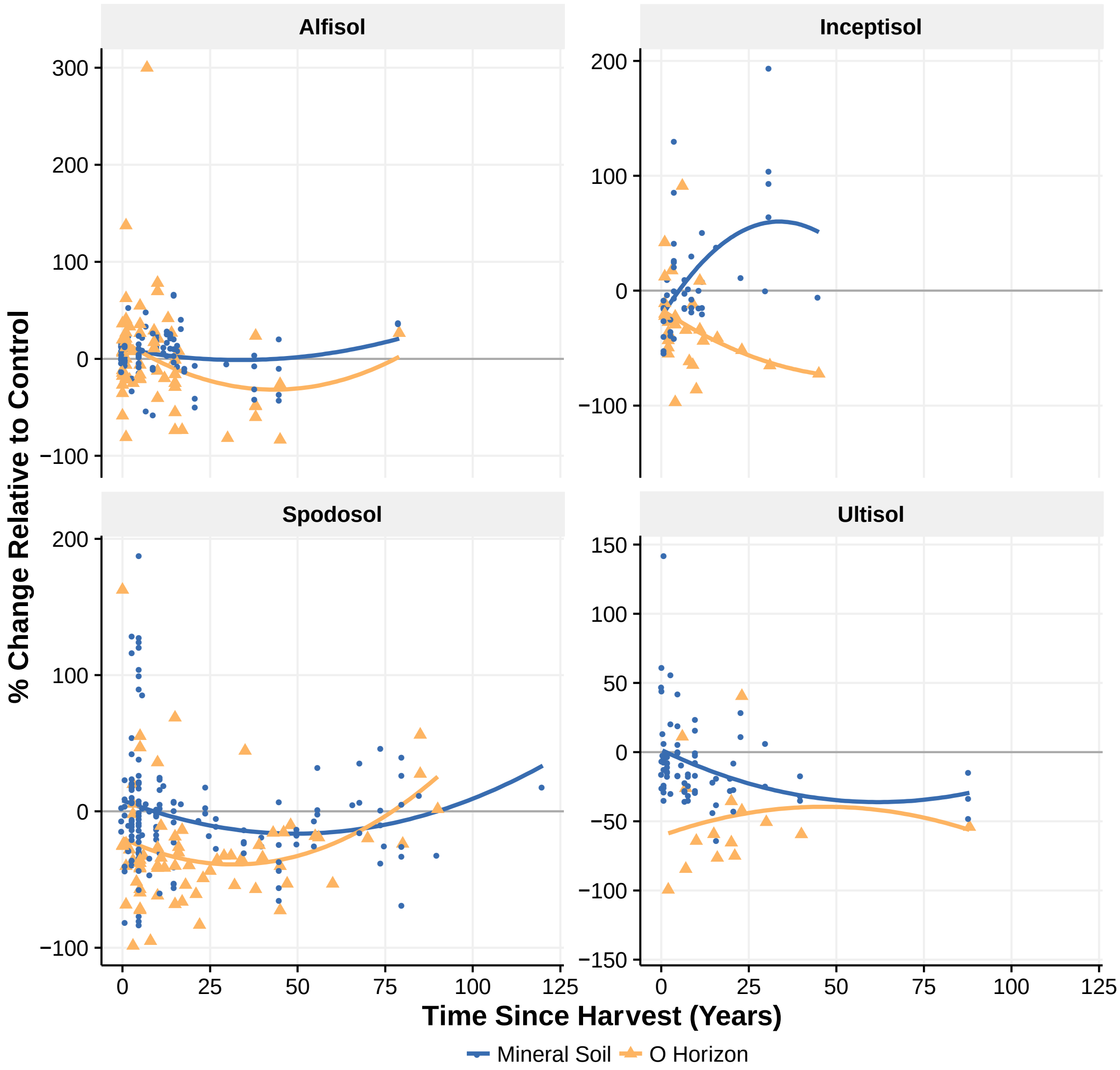

Figure 4.5. Temporal patterns in both O horizon (yellow triangle) and mineral soil (blue circle) C pools for Alfisol, Inceptisol, Spodosol, and Ultisol orders. Other soil orders are not shown due to an inadequate number of response ratios over time. Regression lines show trends with time using a second order polynomial. For the overall model, F = 9.205 on 7 and 532 degrees of freedom, Adj. R^2 = 0.096, and $p < 0.0001$ (

Table 4.2).

Table 4.2. Linear regression coefficients and significance for second degree polynomial model of response of soil C to harvesting over time.

Coefficient	**Estimate**	**SE**	***t*-value**	***p*-value**
Intercept (Alfisol, mineral soil)	12.702	3.587	3.541	0.0004
O horizon	-21.475	3.766	-5.703	<0.0001
Inceptisol	-10.876	5.717	-1.902	0.0577
Spodosol	-14.717	4.320	-3.407	0.0007
Ultisol	-24.776	5.391	-4.596	<0.0001
Time	-67.834	41.56	-1.632	0.10325
$Time^2$	120.412	40.361	2.983	0.0030
Residual SE: 40.24 on 533 DF				
F-Statistic: 10.74 on 6 and 533 df, p <0.0001			$R^2 = 0.108$	Adj. $R^2 = 0.098$

4.5 DISCUSSION

4.5.1 *Overall effect of harvesting on soil C*

Our results reveal that across many publications in the literature there is a significant loss of soil C in response to harvest (-11.2% overall, -14.4% for studies reporting C pools). This estimate is slightly greater than that found by Nave et al. [9], who reported -8% change relative to control. The difference between these estimates derives from additional losses reported in mineral soil, since the effect of harvesting on O horizon C is identical between this study and Nave et al. [9] (-30%). Indeed, while no significant loss of soil C due to harvesting was reported in previous meta-analyses on the subject [9,25], this analysis reveals significant if small losses in various mineral soil layers. Our meta-analysis has roughly double the number of responses than previous meta-analyses on the subject, and consequently has greater statistical power. In particular, this has allowed us to break down the response of mineral soil C to harvest into more depth increments to better characterize how response is moderated or accentuated by depth.

4.5.2 *Depth distribution of soil C response to harvest*

The response of soil C to harvest differs among depths in the soil. O horizons show the most substantial declines (by percentage), although the O horizon is typically a smaller pool of C than mineral soil horizons. Consequently, smaller absolute declines in O horizon C pools can lead to larger response ratios. Forest type significantly alters the response of O horizons to harvesting, with hardwood forests undergoing less drastic losses than conifer and mixed forests (Figure 4.3). This result is in contrast with Nave et al. [9], who found that conifer O horizon soil C declines significantly less than hardwood forest floors. Coniferous forest litter is thought to be more chemically recalcitrant to decomposition because of higher C/N and lignin/N, as well as slower N mineralization rates [36,37]. The trend for more soil C loss from coniferous forest floors could be due to differences in the harvesting techniques utilized for each forest type. On the other hand, less change in soil C in coniferous forests in deeper mineral soils could suggest that some of the additional loss in O horizon C pools is the result of translocation of C into mineral soil rather than mineralization to CO_2. Whatever the case, the mechanism for this difference is not clear and warrants additional study.

In mineral soils, the relative response to harvest is typically less than the O horizon, but this small relative loss might correspond to a larger absolute loss of C in the mineral soil in many forests. The major exception to this pattern are Spodosols, which can contain larger proportions of total soil C in deep, acidic O horizons. Declines in top soil C pools were modest (-3.3%) but still significant (Figure 4.1). Mean effect size estimates become more negative with soil depth, although these estimates are not significant. The overall estimate of change in very deep soil (60-100+ cm) shows substantial and significant loss of C (-17.7%). This estimate, however, only covers a small number of observations (21) from Spodosol, Ultisol, Alfisol, and Inceptisol soil orders and completely excludes hardwood forests. The lack of observations in deeper soil horizons leads to very wide confidence intervals.

On average, the maximum depth of soil sampled by the publications in this meta-analysis was 35.9 cm (Figure 4.6). The average depth of sampling for each response ratio in the database is even more surface-skewed at 21.3 cm. Many of the observations down to 100 cm in the literature only report treatment differences for the whole mineral soil profile (0-100 cm), which eliminates any possibility of understanding the relative response of different horizons or depths. The scarcity of observations in deep soil is incongruous with the increasing loss of soil C with depth relative to control observed in this analysis. More important than the magnitude or significance of the harvest response in very deep soil is the conclusion that much greater attention should be paid to deep soil C pools in both individual forest manipulation experiments and broad-scale C inventory.

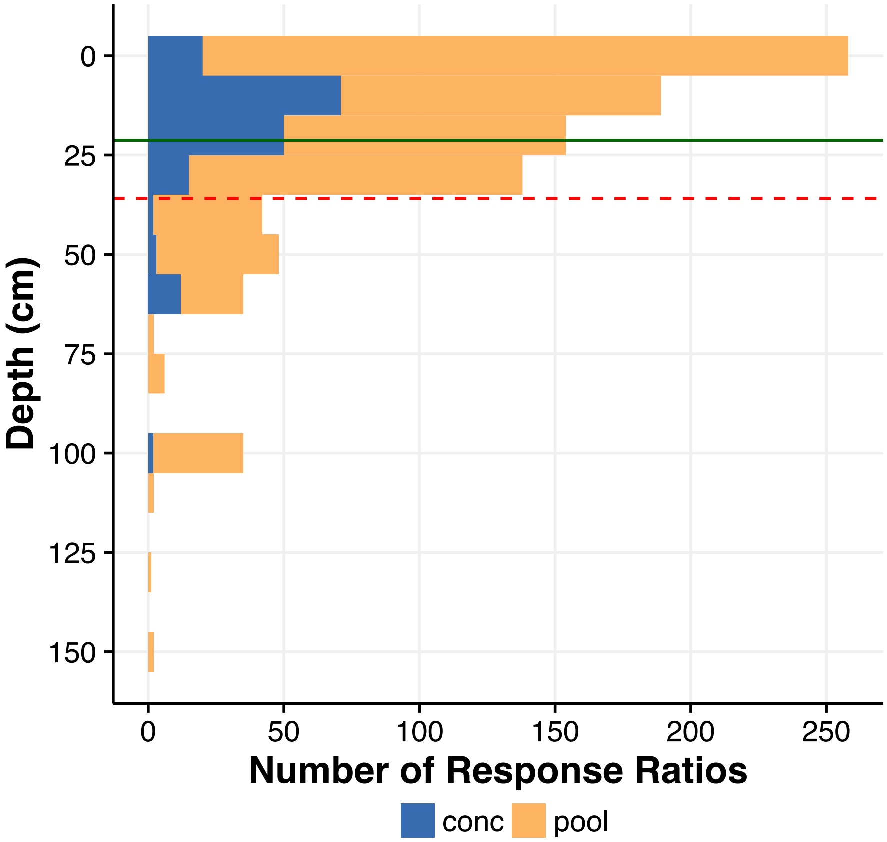

Figure 4.6. Number of response ratios plotted by the maximum depth of sampling for each observation. Response ratios calculated from concentration are in blue, and from pools in orange. The average depth of all response ratios is denoted by the solid green line (21.3 cm, n=945). The average maximum sampling depth for all 112 publications in the meta-analysis is denoted by the dashed red line (35.9 cm).

While soil C in deep soil is much less concentrated than in O and A horizons, subsurface soil represents a much greater volume of soil than surface soil, especially in older/more well developed soil orders like Alfisols, Ultisols, and Oxisols. Some major regions for forestry contain substantial portions of total soil C in deep horizons. For example, 38% of total soil C was below 50 cm and 24.1% below 1m in production forest soils in the Pacific Northwest [38]. The imprint of biological activity extends many meters into soil, even into the C horizon [39]. Globally, the average maximum rooting depth for trees is ~7 m [40], far outreaching even the deepest observations in this database. Harvesting disrupts the continued growth and turnover of roots extended deep into soil by mature trees, which in turn disturbs the steady state of C cycling in deep soil by changing environmental conditions (temperature, moisture) as well as the type and rate of C inputs. Furthermore, the flush of nitrate and dissolved organic matter that frequently follows harvest [41,42] could prime the breakdown of older, subsurface C by providing a spike in nutrient availability and labile energy sources [31,43,44]. Alteration of aboveground ecosystems can cause changes in subsurface soils. For example, Mobley et al. [45] observed that, over a period of several decades following afforestation of agricultural land, modest C gains in surface soil were more than offset by large losses in soil C below 30 cm. Neither the response of deep soil C to harvest nor the mechanisms for that change have been sufficiently resolved in the literature, and future work to address these questions are necessary.

4.5.3 *Differences in soil C response to harvest among soil orders*

Substantial variation in response to harvest was observed among soil orders. Several soil orders had very few response ratios (Andisols, Entisols, Mollisols, and Oxisols), which greatly widens confidence intervals. Nonetheless, significant changes in soil C in response to harvest were observed for all four of these orders. Andisols were the only order to show a significant average increase in soil C in response to harvest. This likely stems from Andisols particular mineralogy, which is often characterized by short-range-order minerals like allophane and imogolite [46–48]. The capacity for these types of minerals to adsorb organic matter makes Andisol soil C especially resistant to loss after harvesting. Alfisols also appear to be resistant to loss of soil C after harvesting, with relatively small loss in O horizons (-12.0%) being the only significant effect. All other soil orders have significant overall losses in soil C, roughly -20% for Entisols, Inceptisols, Mollisols, Spodosols, and Ultisols. The uneven distribution of observations among soil orders (most response ratios in the database are from Alfisols, Inceptisols, Spodosols, and Ultisols) results in substantial differences in the size of confidence intervals among different orders. Unfortunately, many studies did not report soil taxonomic information, and thus 115 response ratios could not be assigned to a soil order. The lack of studies on Andisols is curious given the importance of these soils to forestry in several regions such as the Pacific Northwest, USA and New Zealand. Several studies on Andisol and other under-represented soil orders were excluded from this analysis because of a lack of appropriate controls.

4.5.4 *Recovery of soil C after harvest*

Recovery of soil C after harvesting can take several decades [9]. O horizon pools decline more severely than mineral soil pools, especially in the first several decades (Figure 4.5). In Spodosols, O horizons recovered from harvesting after 60-85 years, while mineral soil recovered over a longer period of 75-100+ years. While the response to harvest was less severe in mineral soils, the longer recovery period implies either lagged response time between forest floor and mineral soils or differences in the decay rate constants leading to longer-term changes in mineral soil C compared to the forest floor. In the case of Alfisols and Inceptisols, soil C in mineral pools increased or stayed the same after harvest, while O horizons declined. However, the observations of harvest effects on Alfisols, Inceptisols, and Ultisols were largely confined to within the first 50 years post-harvest. Consequently, an estimate for the recovery period of soil C pools in these soil orders cannot be assessed with much confidence. Continued observation of existing harvesting experiments in other soil orders must be made to better characterize changes in soil C over time. For Andisols, Entisols, Mollisols, and Oxisols, only a few time points have been documented, and much further study will be necessary to understand recovery of soil C after harvest.

The modeled recovery time has a fairly low adjusted R^2 (0.1) and thus a low predictive capacity. Substantial variation in the response to harvest exists within each soil order, reflecting differences in tree species, harvest intensity, and pretreatment strategies, among other factors. Moreover, soil orders are hardly homogeneous, and differences in the response of soil C among lower levels of classification within each order could be as important as order-level differences. Nonetheless, the substantial and significant differences between orders considered in the model suggest that both the resistance of soil C to change and the recovery period of soil C following harvest (resilience) consistently varies among soil types. Compared with 20-year recovery periods assumed by many models [14], our results indicate that soil C recovery takes place over at least triple that time frame for both O horizons and mineral soil in many cases.

While forests >30 years of age were considered acceptable controls for this analysis, the preponderance of data in this meta-analysis show decreases in soil C relative to control at time = 30 years. Consequently, studies that use mature second growth stands barely over this threshold for experimental controls likely underestimate the response of soil C due to harvesting treatments. Depending upon the site conditions and soil order, control stands of at least 50-75+ years since harvest would be recommended, with older stands being more accurate controls.

4.5.5 *The effect of harvest strategies on soil C*

Differences in harvesting and soil pretreatment strategies significantly impact the loss of soil C after harvest. Curiously, despite the greater relative losses of soil C in O horizons, significant differences between harvest intensities and pretreatment strategies were only found in the mineral soil with the exception of broadcast burning (Figure 4.4). The reduced loss of soil C from mineral soil observed in treatments with greater harvest intensity (+9.3% for clearcut, +13.3% for whole tree harvest) runs counter to the intended effect of these experimental treatments on soil C. One possibility is that increased harvest intensity reduces the quantity of dissolved organic matter and inorganic nutrients leached into the mineral soil, thus reducing the priming [43,49,50] of mineral soil C mineralization through less addition of energy-rich substrates and nutrients. Another possibility is that response of soil C to increased harvest intensity is soil-type specific, and thus an aggregate analysis such as this is subject to bias by unequal sampling of different soil orders. Whatever the case, this dataset cannot identify the specific mechanism(s) driving this difference, and further study is warranted.

Tilling of forest soils prior to planting should intuitively disrupt O horizons to a greater extent than less intensive practices. However, due to the very small number of observations of this practice in the dataset, the large mean treatment effect on soil C was could not be differentiated from 0. By mixing organic material into the surface mineral soil, tilling could increase top soil C in the short term and possibly prime additional breakdown of C over time. In regions where this practice is used, additional research could help to reveal the mechanisms driving change in the soil C of O horizons and mineral following tillage.

Broadcast burning led consistently to additional loss of soil C in both O horizons and mineral soil. The large additional reduction in O horizon C (-40.9%) is expected given that such a treatment is intended to reduce slash on site to facilitate planting. The loss of carbon after harvest extends into deep soil, especially following slash burning (Figure 4.7). Although there are few observations in very deep soil (60-100+ cm), burning appears to especially exacerbate C losses in this layer. This result is despite the direct effects of fire (such as soil heating and nutrient volatilization) being highly attenuated with depth [51,52]. Levels of mineralized nitrogen (NH_4^+ and NO_3^-) and soluble sugars spike within the first year following fire, leading to increased microbial biomass N and N leaching loss [53]. Thus, the flush of nutrients and organic matter into deeper mineral soil following post-harvest broadcast burning has the potential to impact soil C dynamics throughout the soil profile. The number of observations in deep and very deep layers is small, and consequently additional research is necessary to better differentiate between harvesting and fire effects in deep soil horizons.

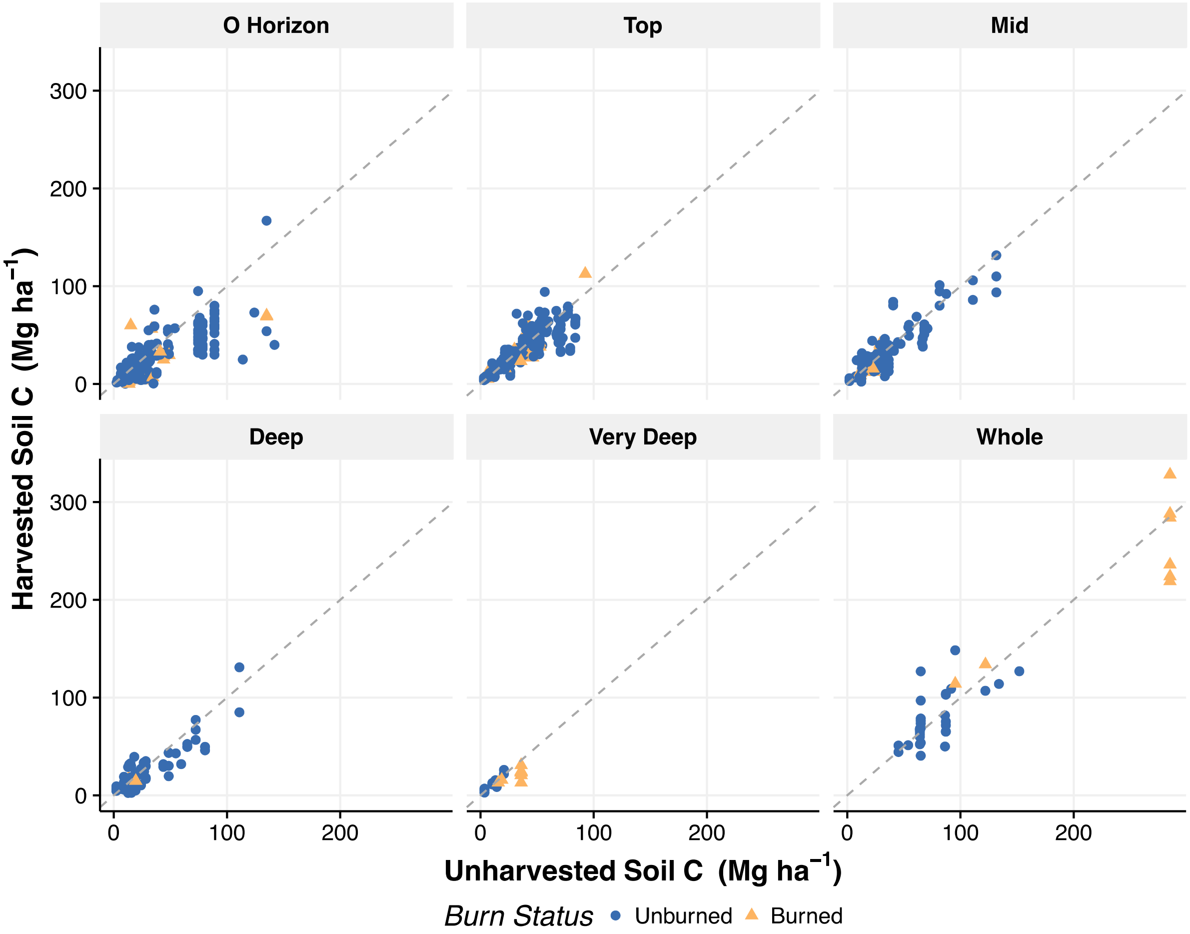

Figure 4.7. Absolute change in soil C due to harvest for each soil depth in this analysis (O horizon, top, mid, deep, very deep, and whole mineral soil). Different points show burned (yellow triangle) and unburned (blue circle) pretreatment strategies. Dashed 1:1 lines in each facet represent no response due to harvest. The total number of responses shown is $k = 746$.

4.6 CONCLUSION

We analyzed 945 responses from 112 publications to examine the effect of harvest on forest soil C around the globe. There is a significant overall reduction in forest soil C following harvest that occurs in both the O horizon and mineral soil. Significant variation in the response to harvesting was observed among different soil depths, among soil orders, between overstory forest types, and between different harvest intensities and pretreatment strategies. Broadcast burning, in particular, appears to exacerbate loss of soil C in both organic and mineral horizons following harvest. The recovery period of soil C following harvest depends upon soil type and takes at least 60 years in many production forests. One of the most important findings of this analysis is a significant loss (-17.7%) of soil C following harvest in very deep soil (60-100+ cm). Deep layers of the soil are greatly under-represented in the literature, and consequently, there is great uncertainty around this estimate. Examination of deep soil horizons in existing manipulative forest studies, in new studies, and in C inventory should be a clear objective for future research.

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Chapter 5. LAND USE CHANGE ALTERS THE RADIOCARBON AGE AND COMPOSITION OF SOIL AND WATER-SOLUBLE ORGANIC MATTER IN THE BRAZILIAN CERRADO

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Jason James, Cole Gross, Pranjal Dwivedi, Tyler Myers, Fernanda Santos, Rodolpho Bernardi, Marianne Fidalgo de Faria, Iraê Amaral Guerrini, Rob Harrison, and David Butman

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5.1 ABSTRACT

The Brazilian Cerrado has undergone extensive land-use change in the past century with large areas converted to agriculture and silviculture. *Eucalyptus* is the dominant planted tree species in Brazil, but the short rotations (6-7 years) and the associated soil disturbances can reduce soil organic matter (SOM), particularly in subsurface soil over long timeframes. SOM is critical to the continued productivity of forests and may be particularly important in Oxisol soils, which underlie much of Brazil. These soils are low in cation exchange sites and rely upon SOM for nutrient and water retention. However, the mechanisms that drive SOM loss under *Eucalyptus* remain unclear. This study examines both bulk soil and water-soluble organic matter chemistry deep into the soil profile (1.3 m) in paired *Eucalyptus* and Cerrado forest stands to identify changes in biodegradability, radiocarbon age, and functional group composition that may explain long-term declines in SOM stocks. We hypothesize that changes in litter quality associated with the conversion of Cerrado forest to *Eucalyptus* plantations increases the content of aliphatic functional groups in water soluble organic matter (WSOM) more quickly than bulk SOM, which drives subsoil accumulation of aliphatic organic moieties through hydrophobic interactions with soil minerals. By utilizing a suite of spectroscopic techniques (^{1}H nuclear magnetic resonance and Fourier transform infrared spectroscopy) alongside isotopic and incubation techniques, this study found substantial shifts toward aliphatic functional group composition in WSOM in both *Eucalyptus* stands and deeper soil horizons. The radiocarbon age of WSOM was younger than bulk SOM at every depth but still substantially diverged from modern in B horizons. This observation is consistent with the theory that WSOM is in dynamic equilibrium with mineral adsorbed SOM, and that old organic matter replaced on mineral surfaces by fresh inputs may be leached or further degraded by microbes. Radiocarbon age of bulk SOM was younger under *Eucalyptus* compared to Cerrado forest and the difference between WSOM and bulk SOM radiocarbon age was much smaller in *Eucalyptus* soils, which together indicate that microbes may be preferentially consuming older SOM after land-use change. The presence of Cerrado understory in *Eucalyptus* may be important to maintaining SOM cycling; WSOM biodegradability and microbial biomass was greatly reduced in the *Eucalyptus* site without any understory. Ecotones with Cerrado understory provide diversity in litter types and rooting strategies, while monoculture *Eucalyptus*, which is commonly found in stand interiors, may reduce the ability for microbes to maintain active nutrient recycling of organic residues.

Keywords: soil organic matter; soil carbon; water soluble organic matter; Cerrado; *Eucalyptus*; radiocarbon

5.2 INTRODUCTION

Long-term storage of soil organic matter (SOM) is one of the irreplaceable ecosystem services that soils provide. In addition to controlling water holding capacity and nutrient recycling, SOM content and composition strongly impacts the chemistry of dissolved organic matter (DOM) in aquatic environments. However, scientific consensus about the mechanisms underlying SOM cycling has been changing rapidly. The humification paradigm that was used for decades to explain and model SOM and DOM dynamics has been increasingly questioned or outright rejected (Lehmann and Kleber, 2015; Marin-Spiotta et al., 2014). Emerging views in SOM research highlight the progressive breakdown of organic substrates into smaller and smaller pieces (Lehmann and Kleber, 2015; Schmidt et al., 2011), coupled with preservation from decomposition through sorption on mineral surfaces (Eusterhues et al., 2003; Kleber et al., 2007, 2004; Mikutta et al., 2006) isolation from microbes by occlusion in (micro)aggregates (Six et al., 2000; Six and Paustian, 2014; Tisdall and Oades, 1982; Verchot et al., 2011), and lack of energy-rich substrates to prime breakdown of more inherently recalcitrant molecules (Chabbi et al., 2009; Fontaine et al., 2007). These processes highlight the importance of DOM as a medium of movement and exchange that explains the vertical distribution of SOM and links terrestrial and aquatic biogeochemistry (Neff and Asner, 2001). The concentration and composition of DOM is a reflection of SOM chemistry due to dynamic equilibrium between physically adsorbed and dissolved phases (Zsolnay, 2003).

To study DOM chemistry, many studies examine water soluble organic matter (WSOM) which is extracted from soil samples in the laboratory and, thus, differs from *in situ* DOM for a number of reasons. First, destruction of the soil pore structure during sampling and laboratory handling can mobilize OM that would otherwise be occluded. Second, the salt extractants used can cause the breakdown of aggregates, lysis of living cells, and desorption from mineral surfaces (Chantigny, 2003). Consequently, WSOM consists of both natural DOM and a pool of potentially but not actually dissolved OM (Hagedorn et al., 2004; Rennert et al., 2007). Furthermore, WSOM is extracted using many different solvents in the literature, most notably 0.5 M K_2SO_4, 2 M KCl, 0.01 M $CaCl_2$, and heated H_2O, which each solubilize different amounts of SOM with variable chemical properties (Gabor et al., 2015; Rennert et al., 2007). Nonetheless, WSOM chemistry has been shown to control critical soil properties and processes, such as the binding of metal ions (Ohno et al., 2008), the mobilization of organic-bound nutrients (Qualls, 2000), and microbial community composition (Gabor et al., 2014).

As DOM/WSOM mobilizes plant-derived compounds from surface soil and leaches them into subsoil, microbes consume and recycle nutrient- and energy-rich compounds (such as amino acids and O-rich polysaccharide chains like cellulose) leaving behind lipids and waxes (cutin) that are poorly soluble and recalcitrant (Pisani et al., 2014). Thus, SOM composition with increasing soil depth reflects more microbially-derived byproducts (Kaiser and Kalbitz, 2012). Roots also provide major inputs of OM into subsoil (Angst et al., 2016) both through the exudation of amino sugars and other compounds to recruit beneficial microbiota and through turnover of root biomass (Poirier et al., 2018). By altering both the quantity and quality of litter and root inputs to soil, shifts in the aboveground plant community due to forest management and land use change can alter rates of SOM/DOM decomposition, with potential implications for soil nutrition and C sequestration (Jandl et al., 2007).

Maintaining or increasing SOM is extremely important to the continued fertility of forests, particularly on extremely weathered tropical soils that contain few cation exchange sites outside of charged SOM molecules (Soares and Alleoni, 2008). SOM is an especially critical property in these soils because of its ability to retain vital nutrients, increase water holding capacity, promote aggregation, and lower runoff and erosion (Schlesinger and Bernhardt, 2012). Oxisols (Brazilian classification: Latosols; FAO classification: Ferralsols) are the most common soil type in the Brazilian Cerrado, underlying 46% of the 2.04 million km^2 land area (Raij et al., 2004). These soils are also particularly sensitive to land use change and disturbance (Batlle-Bayer et al., 2010; Gmach et al., 2018).

The Cerrado biome in Brazil has undergone extensive land use change from its historical condition, with natural vegetation cover reduced by 47% in just the last two decades (1990-2010) (Beuchle et al., 2015). While the Brazilian Government has committed to reducing deforestation in the Cerrado by 40% until 2020 (relative to the rate between 1998-2008; National Plan of Climate Change 2007) and deforestation rates have declined by 43% and 38% in 2016 and 2017, respectively, relative to 2015 (National Institute of Space Research - INPE, 2018), there remain extensive legacy effects on soil processes and ecosystem services due to land use change in this region. A substantial portion of the Cerrado has been converted to silviculture, with *Eucalyptus* representing 71% of total planted tree area in Brazil as of 2012 (FAO, 2015). Periodic disturbances associated with silvicultural activities in *Eucalyptus* plantations reduce SOM over decadal time scales, especially in subsoil (Cook et al., 2016). Moreover, Cook et al. (2016) observed that soils initially high in C saw greater declines over 20 years and 3 harvest cycles, while SOM poor soils showed modest increases. However, the underlying mechanisms and drivers of SOM loss from the conversion of Cerrado vegetation to *Eucalyptus* remain a gap in knowledge, particularly in more dynamic and microbially accessible C pools.

Land-use change and forest management not only impact C cycling in terrestrial systems but can have downstream implications in aquatic environments. Advances in freshwater research have roughly doubled estimates of how much C is leached from terrestrial systems into freshwater streams, rivers, and lakes (0.9 to 1.9 Pg C yr^{-1}) (Cole et al., 2007). Recent studies have shown that highly aged material is an important component of DOM in most rivers, and that this aged material can subsidize modern riverine food webs (Caraco et al., 2010). McCallister and del Giorgio (2012) observed that as the proportion of DOM from terrestrial sources increased, so too did the age of CO_2 respired from temperate freshwater systems. Furthermore, the radiocarbon age of DOM exported in rivers worldwide increases as the proportion of human disturbance (urban and agricultural land) increases in the watershed (Butman et al., 2015), which suggests that humans activities can mobilize SOM in deeper soil horizons.

This study seeks to examine the interface between SOM and WSOM by coupling isotopic, spectroscopic, and incubation techniques to identify the mechanisms underlying soil response to land use change in the Brazilian Cerrado. Here we examine 5 hypotheses about organic matter dynamics, both between forest types and down soil profiles:

1) The composition of WSOM but not SOM will differ between *Eucalyptus* plantations and 'native' Cerrado forest types due to compositional differences in litter affecting fast cycling pools of C more rapidly.
2) SOM & WSOM functional groups will diverge from O horizons increasingly with depth, with a higher proportion of hydrophobic (aliphatic and aromatic) functional groups in subsoil relative to hydrophilic (oxygenated, N-rich, etc.) functional groups.
3) WSOM composition will be correlated to bulk SOM composition due to the exchange of mineral-adsorbed or occluded organic matter with this more actively cycled pool.

4) The biodegradability of WSOM will not change substantially with depth, indicating that factors including mineral sorption, aggregate occlusion, and/or energetic limitations are more important controls on microbial degradation of this organic matter pool than inherent recalcitrance of the material.

5) WSOM will be younger than bulk SOM due to greater exchange with fresh DOM leaching from surface horizons as well as more active microbial cycling; however, WSOM will still get older with depth as some mineral associated or partially occluded SOM is released into solution.

5.3 METHODS

5.3.1 *Field Sites & Soil Sampling*

Field sampling was conducted at two sites in Sao Paulo State, Brazil: the Itatinga Experimental Forest (23°02'02.5"S, 48°37'34.4"W) run by the University of São Paulo in Itatinga, Brazil and the Lageado Farm (22°50'14.0"S, 48°25'18.7"W) on the campus of the São Paulo State University in Botucatu, Brazil. Two soil pits were excavated to a depth of 1.3 m at each site, located in the same topographic position no more than 500 feet apart but in contrasting land-uses. The first land-use type at both sites was a mature (5-to-6-year-old) *Eucalyptus urophylla-grandis* plantation, and the other was an adjacent stand of native vegetation. At Itatinga, the native forest stand was *Cerrado denso* (short-to-moderately tall, closed canopy forest) regrowing after *Eucalyptus* silvicultural management in the 1990s. At Lageado, the native forest stand was undisturbed *Cerradão* forest (medium-tall, closed canopy forest). Understory composition differed considerably between *Eucalyptus* plantations at each site, with virtually no understory growth in the Lageado plantation while a medley of understory shrubs and trees were present in the Itatinga *Eucalyptus* plantation. The soil type at both sites was a very deep (>10 m)

Haplustox (Oxisol in USDA Soil Taxonomy; Ferralsol in the World Reference Base for Soil Resources classification; red-yellow Latosol in the Brazilian Classification System) developed on Cretaceous-age sandstone. Soils were described following USDA Soil Taxonomy.

Bulk density samples were taken by horizon with a punch corer of a known volume (104 cm^3), placed in polyethylene bags, and stored on ice. Forest floor samples were taken from a randomly placed 20x20 cm quadrat adjacent to each soil pit. Samples were immediately returned to the lab, where they were chilled in a refrigerator at 4°C.

5.3.2 *Laboratory Methods*

5.3.2.1 Soil Samples

Soil samples were shipped on ice to the University of Washington following USDA APHIS Plant Protection and Quarantine procedures (Code of Federal Regulations Title 7, CFR Part 352), where they were refrigerated at 4°C prior to extractions for water-soluble organic matter and microbial biomass within two weeks after field sampling. Bulk density was measured by applying a moisture correction to account for water weight in the soil sample. There was no rock content in any samples. Carbon and nitrogen content were measured on a PerkinElmer 2400 CHN Analyzer. Soil pH was measured using Milli-Q water in a 1:2 (*w:v*) ratio.

5.3.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

The quality of SOM was assessed by FTIR following the protocol of Dobarco et al. (2014). In brief, absorbance of infrared light by soil occurs due to stretching of both organic and mineral bonds. To isolate the portion of absorbance that is due to organic matter, we subtracted an organic free mineral matrix spectra from the bulk soil spectra to produce a spectrum where absorbance is attributed to SOM (Margenot et al., 2016). The mineral matrix spectra were obtained through a procedure modified from Kaiser et al. (2002), whereby samples were shaken in 6% sodium hypochlorite solution adjusted to a pH of 9 for 30 total hours, then rinsed 5 times with Milli-Q water. This effectively removed ~85-98% of SOM from soil with minimal alteration of the mineral fraction (Lavkulic and Wiens, 1970; Siregar et al., 2005). FTIR was performed with a PerkinElmer Frontier FTIR spectrometer with an attenuated total reflectance (ATR) attachment.

A conversion factor is needed to scale mineral matrix spectra such that subtraction does not lead to negative peaks in the resulting SOM spectra (Margenot et al., 2016; Parikh et al., 2014). Absorbance in the range of 650-900 cm^{-1} are thought to derive principally from stretching of various mineral bonds. Thus, spectral subtraction was completed with a subtraction factor (c) by the following formula:

$$SOM = BS - cMM \qquad (5.1)$$

$$c = \frac{H_{max}BS_{650-900}}{H_{max}MM_{650-900}} \qquad (5.2)$$

where BS is bulk soil, MM is the mineral matrix, and $H_{max}BS_{650-900}$ and $H_{max}MM_{650-900}$ are the heights of the highest shared absorbance peak for the BS and MM spectra between wavenumbers 650-900 cm^{-1}. Values for c ranged between 0.4 and 0.8.

Prior to spectral subtraction, each of the bulk soil and mineral matrix FTIR spectra were baseline and ATR corrected. After subtraction, the spectra were normalized with the standard normal variate technique (Gautam et al., 2015):

$$sn_i = \frac{s_i - mean_s}{SD_s}; i = 1, 2, 3 \ldots N \qquad (5.3)$$

where sn_i are the normalized points in the spectrum, s_i are the non-normalized points, and N is the number of points in the spectrum.

5.3.2.3 Water-Soluble Organic Matter (WSOM) Extraction

Water-soluble organic matter was extracted by shaking triplicate, 20g soil subsamples with 0.5 M K_2SO_4 (5:1 v:w ratio) in polyethylene bottles for 1 hour. Bottles were then centrifuged at 4000 rpm for 10 minutes before vacuum filtering the supernatant through 0.4 μm Whatman Nuclepore polycarbonate filters into pre-ashed, amber glass bottles. These WSOM extracts were stored at 4°C prior to subsequent analysis. Total organic carbon (TOC) was measured using 680°C combustion catalytic oxidation with a Shimadzu TOC-L on the NPOC setting to remove inorganic carbon.

5.3.2.4 Microbial Biomass

Microbial biomass was measured using the chloroform fumigation direct extraction (CFDE) procedure developed by Vance et al. (1987) and modified by Beck et al. (1997). Briefly, 10 g of field moist soil was fumigated with ethanol-free chloroform in a vacuum chamber for 48 hours. Both the pre- and post-fumigated samples were extracted with 0.5 M K_2SO_4 using the same procedure described above for WSOM. Microbial biomass carbon (MBC) was calculated by the equation

$$\mathrm{MBC} = \mathrm{EC} / \mathrm{kEC} \qquad (5.4)$$

where EC is the difference between C in the pre- and post-fumigated samples, and kEC is a soil specific constant conventionally estimated to be 0.45 (Beck et al., 1997).

5.3.2.5 Biodegradability of Water-Soluble Organic Matter (BWSOM)

Biodegradability of WSOM was assessed following the recommendations of McDowell et al. (2006), specifically by consumption of dissolved organic carbon over 7 days. To counteract the effect of the high concentration of salt in the extracts on microbial growth, 10 ml aliquots of each sample were diluted by half prior to incubation. To these we added 2 ml of a nutrient solution (0.1% NH_4NO_3 and 0.1% K_2HPO_4) and 50µl of inoculum derived from shaking a freshly sampled forest A horizon topsoil (from the University of Washington campus) in Milli-Q water (10:1 v:w) for 10 minutes followed by centrifugation. For each profile, control samples of the inoculum and nutrient solutions were measured immediately for C using the procedure described above for WSOM. We also added nutrients and inoculum to separate vials filled with 20 mg l^{-1} glucose to assess the viability of the inoculum; on average, 87% of the glucose was removed over 7 days. All samples and controls were analyzed in triplicate.

5.3.2.6 Solution state ^1H NMR Spectroscopy

Information on the molecular bonds of hydrogen in solution were obtained by completely freeze-drying 60 ml aliquots of WSOM extracts for each horizon. The solid residues were transferred to a 5 mm NMR tube and re-dissolved in deuterium oxide. ^1H NMR spectra were acquired on a two-channel Agilent Propulse 500 MHz spectrometer, using pre-saturation and PURGE water suppression techniques with 4k scans, 2.6 s acquisition time, and 2.3 s relaxation delay (d1). Processing and integration of spectra followed methods described by Santos et al. (2016), with the integrals grouped into five regions: aliphatic structures ($^{\delta}H$ ~ 0.0-1.9 ppm), functionalized aliphatic ($^{\delta}H$ ~ 1.9-3.1 ppm), oxygenated ($^{\delta}H$ ~ 3.1-4.3 ppm), unsaturated ($^{\delta}H$ ~ 5.3-7.0 ppm), and aromatic functional groups ($^{\delta}H$ ~ 7.0-10 ppm). The relative contribution (%) of each region to the total area in the spectrum was calculated to infer the relative contribution of each group to WSOM composition.

5.3.2.7 Isotopic analysis

WSOM samples were prepared for isotopic analysis following the methods of Raymond and Bauer (2001). A subset of WSOM extracts (alternating mineral soil horizons) at each site were placed in quartz reaction vessels with 0.2 ml of 40% phosphoric acid and attached to a vacuum extraction line. Samples were sparged with ultra-high purity N_2 for 10 minutes to remove inorganic CO_2, bubbled with pure O_2 for 5 min, then sealed and irradiated for 4 hours. This converted all DOC to CO_2 (Williams and Gordon, 1970). Under continuous sparging with N_2, gasses from the reaction vessel were passed through a series of dry ice traps, which removed trace gasses and water vapor. Pure CO_2 was captured at the end of the line in pre-combusted 6-mm Pyrex tubes with 20 mg of pre-baked copper oxide and 5 mg pure silver submerged in liquid nitrogen. The tubes were then sealed with an acetylene torch. Each of the sealed Pyrex tubes were sent to the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at the Woods Hole Oceanographic Institution (WHOI) for isotopic measurement. Mineral soil samples for each horizon were sent to the University of Arizona AMS facility for isotopic analysis due to their ability to receive foreign soil samples (Regulated by 7 CFR 330; USDA APHIS Permit P330-15-00118). For radiocarbon analysis, CO_2 gas samples were converted to graphite targets under 1 atm H_2 at 550°C with an iron catalyst. Results reported from both facilities were corrected for potential fractionation using $\delta^{13}C$ results (Stuiver and Polach, 1977).

5.3.3 *Statistical Analysis*

All statistical analysis was undertaken in R (R Development Core Team, 2017). We calculated modified Pearson correlation coefficients (r) between soil physical and chemical characteristics using a significance test that accounts for spatial autocorrelation between samples in soil profiles (Clifford et al., 1989) with the SpatialPack package in R (Vallejos et al., 2018). The false discovery rate across these multiple comparisons was corrected using the technique of Benjamini and Hochberg (1995) with $\alpha=0.05$.

We used principal components analysis (PCA) to assess the ^1H NMR composition of WSOM and FTIR spectra of bulk SOM. To assess the relationships between compositional data and forest type, soil horizons, and soil physical/chemical attributes (WSOM concentration, soil C and N concentration, bulk density, C:N, radiocarbon age, MBC, BWSOM, and $SUVA_{254}$) we fit vectors on ordinations for continuous variables and used 95% confidence interval ellipses for factors using the envfit function in the vegan package (Oksanen et al., 2017). Significance of individual factors and vectors were assessed by permutation tests restricted within each site and with $\alpha=0.1$.

5.4 RESULTS

5.4.1 *Cerrado vs Eucalyptus*

Differences between Cerrado and *Eucalyptus* forest soil properties are more pronounced at the Lageado site than the Itatinga site. At Lageado, bulk soil C and N concentrations, C:N ratios, and microbial biomass are generally lower in the *Eucalyptus* profile compared to the Cerrado soil (Table 5.1). In contrast, there are only small differences in C and N concentrations at the Itatinga site but there remains a large difference in microbial biomass, particularly in the surface Oi and A horizons. At both sites, WSOM concentration is greater in the mineral soil profile under *Eucalyptus* (Table 5.2). However, the difference in WSOM biodegradability between forest types is much greater at Lageado (18% average loss for *Eucalyptus* vs 64% loss for Cerrado) than at Itatinga (61% average loss for *Eucalyptus* vs 62% loss for Cerrado).

At both sites, the difference in radiocarbon age between bulk soil and WSOM is much greater in the Cerrado soil profile compared to *Eucalyptus* (Figure 5.1). In A horizons, the difference is relatively small but increases substantially in the subsoil. A difference of 0.05 Fm units represents approximately 660 years difference in mean age; at both sites, the difference in radiocarbon age between WSOM and SOM was between 0.05 to 0.10 units greater in the Cerrado compared the *Eucalyptus* forest soils.

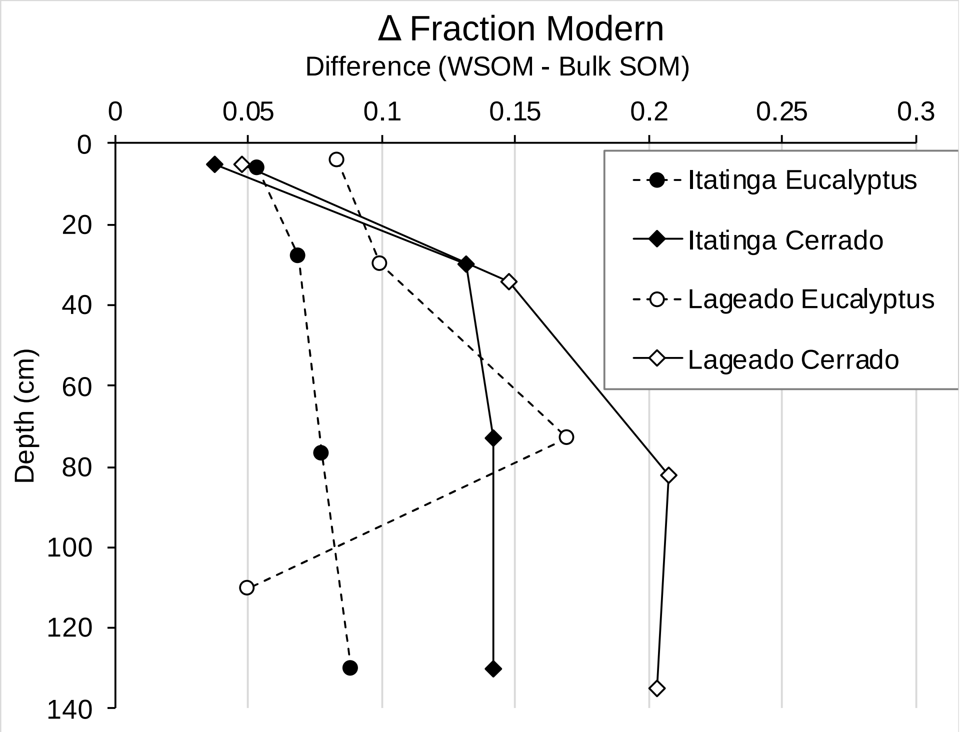

Figure 5.1. Difference between radiocarbon age of WSOM and bulk SOM for four soil profiles in São Paulo State, Brazil. Each 0.05 units difference in Fm corresponds with approximately 660 years difference in estimated radiocarbon age.

Table 5.1. Soil organic matter attributes from 4 soil profiles in paired, contrasting land uses in São Paulo State, Brazil. Microbial biomass carbon (MBC) was measured in triplicate and analytical error is reported as (±1 SD).

Itatinga	Depth (cm)	BD (g cm^{-3})	C Conc. (mg g-soil^{-1})	N Conc. (mg g-soil^{-1})	C:N	C Stock (g m^{-2})	N Stock (g m^{-2})	pH	MBC (µg C g-soil^{-1})	Fraction Modern	^{13}C-SOM (‰)
Cerrado											
Oi	-3	0.03	263.5	18.93	11.9	465	33	5.35	1945.6 (1246.0)		
A	5	1.03	27.5	2.25	10.5	2815	230	4.39	432.2 (10.6)	1.0388	-23.5
AB	14	1.21	21.8	1.67	11.2	2118	162	4.34	297.8 (3.5)		
Bo1	30	0.92	15.1	1.18	11.0	1799	140	4.18	174.1 (4.1)	0.9255	-21.1
Bo2	43	0.98	13.4	0.91	12.6	3156	215	4.39	97.0 (4.6)		
Bo3	73	0.94	10.4	0.72	12.4	3409	237	4.46	59.0 (3.0)	0.7909	-17.4
Bo4	100	0.75	9.1	0.59	13.4	1375	88	4.74	25.2 (3.6)		
Bo5	130	0.77	8.9	0.56	13.7	2712	170	4.83	19.6 (2.7)	0.6407	-16.1
Eucalyptus											
Oi	-3	0.04	390.2	25.47	13.1	421	27	6.7	689.4 (279.1)		
A	6	0.91	29.9	2.46	10.4	3262	269	4.28	324.0 (12.2)	1.0162	-23.8
AB	16	1.19	24.1	1.81	11.4	2281	171	4.17	185.1 (17.5)		
Bo1	28	1.04	15.7	1.30	10.4	2616	215	4.23	147.1 (5.8)	0.922	-20.8
Bo2	50	0.84	13.9	0.99	12.0	3254	232	4.51	60.4 (6.8)		
Bo3	77	0.85	10.6	0.64	14.1	2324	141	4.76	56.1 (11.4)	0.7932	-18.1
Bo4	105	0.75	10.0	0.56	15.3	2241	126	5.13	30.5 (4.1)		
Bo5	130	0.85	10.0	0.53	16.4	2563	134	4.87	29.8 (1.4)	0.6404	-17.5
Lageado											
Cerrado											
Oi	-4	0.02	161.6	10.84	12.8	110	7	5.54	1351.5 (169.6)		
A	5	1.26	17.7	1.43	10.7	2239	180	3.73	429.2 (3.5)	1.0108	-27
AB	17	1.29	11.9	0.98	10.5	2159	176	3.86	215.2 (5.6)		
Bo1	34	0.99	10.0	0.84	10.3	1989	165	4.24	124.8 (6.0)	0.8425	-25.2
Bo2	59	0.93	12.1	0.79	13.3	3286	212	4.36	47.9 (6.3)		
Bo3	82	1.02	12.4	0.64	16.6	2159	111	4.18	76.0 (16.9)	0.6363	-26.2
Bo4	105	0.91	12.9	0.61	18.0	3514	167	4.24	23.7 (2.8)		
Bo5	135	1.17	13.2	0.55	20.6	4617	192	4.25	17.2 (3.9)	0.564	-26.2
Eucalyptus											
Oi	-3	0.02	187.7	6.25	25.8	107	4	5.25	344.0 (1001.3)		
A	4	1.29	14.6	1.39	9.0	1502	143	4.77	80.7 (24.2)	0.9668	-21.3
AB	15	1.70	12.9	1.22	9.1	2854	269	5.15	28.2 (4.1)		
Bo1	30	1.36	12.2	1.31	8.0	2983	319	4.89	87.8 (2.5)	0.9449	-22.6
Bo2	50	1.24	10.2	1.18	7.4	2656	308	4.99	43.4 (4.9)		
Bo3	73	1.17	7.8	0.77	8.7	2396	235	5.45	21.4 (4.3)	0.7282	-23.5
Bo4	93	1.14	7.2	0.68	9.1	1141	107	5.48	18.0 (1.9)		
Bo5	110	0.94	8.8	0.81	9.3	3317	305	5.58	99.4 (20.6)	0.7684	-21.7

Table 5.2. Water-soluble organic matter attributes from 4 soil profiles in paired, contrasting land uses in São Paulo State, Brazil. Samples were extracted in triplicate and are reported as mean (± 1 SD). Isotopic and ^{1}H NMR analyses were performed on composited extracts for each horizon. WSOM = water soluble organic matter; BWSOM = biodegradability of water soluble organic matter; SUVA$_{254}$ = specific UV Absorbance at 254 nm.

						^{1}H NMR Characteristics				
Itatinga	WSOM (μg C g-soil^{-1})	BWSOM (% loss)	SUVA$_{254}$ (L mg-C^{-1} m^{-1})	Fraction Modern	^{13}C-WSOM (‰)	Aromatics (%)	Unsaturated (%)	Oxygenated (%)	Funct. Aliphatics (%)	Aliphatics (%)
Cerrado										
Oi	599.6 (173.1)	88.0 (17.2)	0.56 (0.08)			0.68	0.02	38.18	26.71	34.43
A	34.8 (0.9)	61.9 (1.4)	2.56 (0.43)	1.076	-20.41	2.43	1.30	51.45	12.07	32.75
AB	37.5 (1.0)	56.7 (1.0)	2.15 (0.27)			1.49	1.71	27.72	11.38	57.70
Bo1	33.1 (0.9)	55.5 (0.8)	1.48 (0.03)	1.057	-18.58	6.70	0.87	29.33	13.26	49.84
Bo2	35.1 (0.8)	52.9 (0.3)	1.48 (0.17)			1.29	2.81	38.49	3.87	53.53
Bo3	30.0 (0.2)	52.4 (0.9)	1.13 (0.03)	0.933	-12.68	0.04	0.01	63.55	6.59	29.81
Bo4	22.2 (0.6)	52.8 (0.6)	0.88 (0.12)			1.99	0.70	14.23	14.31	68.77
Bo5	18.5 (0.4)	53.6 (4.4)	0.81 (0.00)	0.782	-26.66	1.47	1.92	39.91	13.92	42.78
Eucalyptus										
Oi	142.8 (49.2)	53.0 (7.5)	2.90 (0.97)			0.31	0.07	22.75	28.11	48.76
A	32.6 (2.9)	51.5 (5.4)	2.55 (0.29)	1.070	-23.74	0.15	0.07	20.70	34.56	44.52
AB	39.6 (1.8)	69.1 (2.2)	1.87 (0.06)			0.55	0.01	46.14	22.96	30.35
Bo1	47.9 (0.8)	59.1 (0.3)	1.33 (0.03)	0.991	-22.89	1.70	0.26	39.17	10.75	48.12
Bo2	39.5 (1.7)	57.5 (1.7)	1.00 (0.24)			13.02	0.13	2.05	0.56	84.25
Bo3	33.3 (3.4)	53.4 (4.7)	0.76 (0.12)	0.871	-14.94	0.01	1.32	3.18	7.34	88.16
Bo4	24.5 (1.0)	52.8 (1.1)	0.75 (0.07)			0.17	0.06	21.99	15.15	62.63
Bo5	18.0 (0.4)	54.9 (2.3)	0.75 (0.09)	0.729	-15.03	6.93	1.33	0.53	6.73	84.48
Lageado										
Cerrado										
Oi	43.4 (6.1)	62.7 (0.7)	2.58 (0.63)			1.34	0.01	32.12	27.43	39.10
A	40.4 (0.8)	69.5 (0.7)	2.62 (0.07)	1.058	-26.66	3.44	2.10	13.50	15.78	65.18
AB	52.3 (1.6)	65.3 (1.1)	1.50 (0.07)			2.83	0.47	16.52	6.31	73.87
Bo1	39.0 (0.7)	65.7 (0.6)	1.03 (0.03)	0.990		13.95	2.00	18.91	3.23	61.91
Bo2	33.0 (1.5)	66.7 (2.6)	1.50 (0.10)	0.907		1.31	0.24	47.94	5.74	44.78
Bo3	31.0 (0.9)	66.0 (2.4)	1.46 (0.01)	0.844	-24.67	3.12	4.23	10.70	5.90	76.05
Bo4	27.2 (0.4)	66.8 (0.9)	1.43 (0.02)			8.91	5.96	20.69	6.75	57.69
Bo5	27.4 (0.7)	66.9 (1.5)	1.40 (0.03)	0.767	-24.69	3.51	0.06	1.74	11.34	83.36
Eucalyptus										
Oi	98.4 (35.5)	55.8 (4.8)	1.43 (0.10)			1.69	1.88	37.18	16.92	42.34
A	42.6 (5.9)	18.8 (8.2)	0.99 (0.06)	1.050	-19.18	0.17	0.28	21.75	32.05	45.75
AB	31.5 (1.0)	13.0 (1.1)	0.73 (0.03)			5.68	0.83	6.89	19.40	67.21
Bo1	35.8 (0.6)	12.9 (0.9)	0.55 (0.04)	1.044	-21.09	1.92	0.69	5.03	26.41	65.96
Bo2	26.9 (1.0)	13.9 (1.9)	0.53 (0.05)	1.015		0.76	1.61	3.55	24.37	69.71
Bo3	20.7 (1.1)	17.8 (3.2)	0.73 (0.28)	0.898	-23.29	2.23	0.53	0.22	15.90	81.11
Bo4	19.1 (0.1)	13.5 (4.5)	0.75 (0.02)			1.52	5.55	1.29	13.82	77.82
Bo5	19.0 (0.4)	19.1 (1.8)	0.92 (0.05)	0.818	-22.92	6.29	22.88	0.78	18.42	51.63

5.4.2 *Soil Physical, Chemical, and Biological Attributes*

Across all profiles, C and N concentrations are highly correlated (Figure 5.2; r = 0.98), are far higher in the O horizon, and decline with depth in the mineral soil in all four profiles (Table 5.1). C and N stocks, on the other hand, are lowest in O horizons, and are close to evenly distributed with depth down to 1.3 m. N stocks are more strongly associated with biological properties of these soils than C stock or C:N, with significant negative correlations with both microbial biomass carbon (MBC) and BWSOM (Figure 5.2). C:N ratios are relatively low in these soils and change little with depth or between forest types. However, they are an important determinant of WSOM biodegradability (BWSOM) across all soil profiles (r = 0.48). Soil pH is highly acidic in all profiles (Table 5.1), and differences in pH are significantly related to BWSOM (Figure 5.2; r = -0.42). BWSOM is positively correlated with microbial biomass carbon (r = 0.42) and $SUVA_{254}$ (r = 0.43) across the soil profiles (Figure 5.2).

Microbial biomass makes up between 0.6% to 2.4% of soil C in surface A and AB horizons but represent a smaller percentage (0.2% to 0.7%) in subsurface Bo horizons. WSOM makes up an even smaller proportion of SOM, ranging from 0.03 – 0.23% in O horizons to 0.12 – 0.44% in mineral horizons.

Radiocarbon age of SOM (Fm-SOM) is moderately related to N stocks (r = 0.31) and weakly related to BWSOM (r = -0.11) (Figure 5.2). The radiocarbon age of WSOM (Fm-WSOM) is not significantly correlated with other soil attributes. ^{13}C-SOM is not significantly related to other soil attributes but is reflected in ^{13}C-WSOM, suggesting little consistent fractionation with the release of a small portion of SOM into solution. On average, ^{13}C-WSOM is enriched by 0.6‰ (SD ±3.6‰) compared to ^{13}C-SOM. There is substantial enrichment in the subsoil of both forest soil profiles at Itatinga (Table 5.1 & 5.2), suggesting that C3 grasses might have occupied this site in the past.

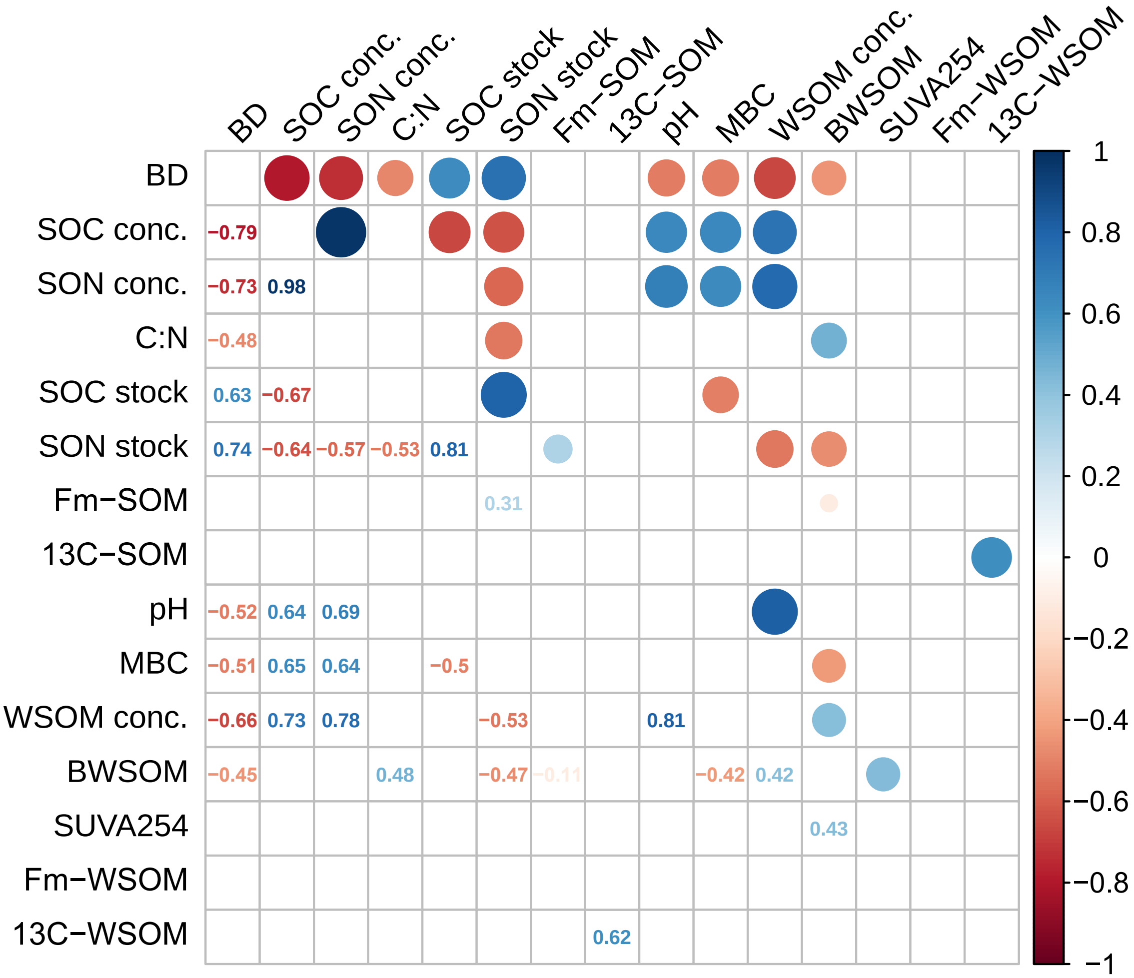

Figure 5.2. Modified Pearson correlation coefficient (r) between soil physical, chemical, and biological properties across four soil profiles in São Paulo State, Brazil. The correlation coefficient is modified to account for spatial autocorrelation between samples taken in the same soil profile. Only statistically significant correlations are reported using the Benjamini and Hochberg (1995) technique to control the false discovery rate for multiple comparisons ($\alpha = 0.05$). Keywords: BD – bulk density; SOC – soil organic carbon; SON – soil organic nitrogen; Fm-SOM – fraction modern for bulk soil organic matter; ^{13}C-SOM - δ^{13}C for bulk soil organic matter; MBC – microbial biomass carbon; WSOM – C concentration of water soluble organic matter; BWSOM – biodegradability (% loss) of water soluble organic matter over 7 days; SUVA$_{254}$ – specific UV absorbance at 254 nm.

5.4.3 ^{1}*H NMR characteristics of WSOM*

In general, the relative proportion of each functional group in WSOM is (in descending order): Aliphatics > Oxygenated > Functionalized Aliphatics >> Aromatics > Unsaturated (Table 5.2). Aliphatic content is negatively correlated to oxygenated functional group content, but nearly orthogonal (and thus un-correlated) with both functionalized aliphatic and aromatic content (Figure 5.3). Aromatics, on the other hand, are negatively correlated with functionalized aliphatics and orthogonal to oxygenated and aliphatic content. Thus, the PCA in Figure 5.3 can be generally broken up into four quadrants corresponding with increasing content of each of these four major functional groups. Unsaturated groups were nearly absent from most samples, and thus do not have significant relationships with other functional groups or external gradients in this dataset.

The dominant functional groups that make up WSOM differ significantly between *Eucalyptus* and Cerrado forest types (Figure 5.3A; $R^2 = 0.11$, $p = 0.025$). Forest type separates primarily along PC2 (23.5% explained variance) with Cerrado forest soil samples corresponding with more oxygenated and/or aromatic groups and *Eucalyptus* soil samples corresponding with more aliphatic and especially functionalized aliphatic groups.

WSOM composition also significantly differs between soil horizons, particularly corresponding with PC1 (Figure 5.3B; $R^2 = 0.21$, $p = 0.006$). O horizon samples have negative PC1 scores, which correspond with higher loadings for oxygenated and functionalized aliphatic groups. The functional composition of WSOM extracts from A and AB horizons transition between organic and subsurface soil horizons and are not significantly different than either (Figure 5.3B, 95% confidence ellipses). Bo horizons are significantly separated from O horizon

WSOM composition with reduced functional aliphatic content and greater aromatic, aliphatic, and in some cases oxygenated functional group content.

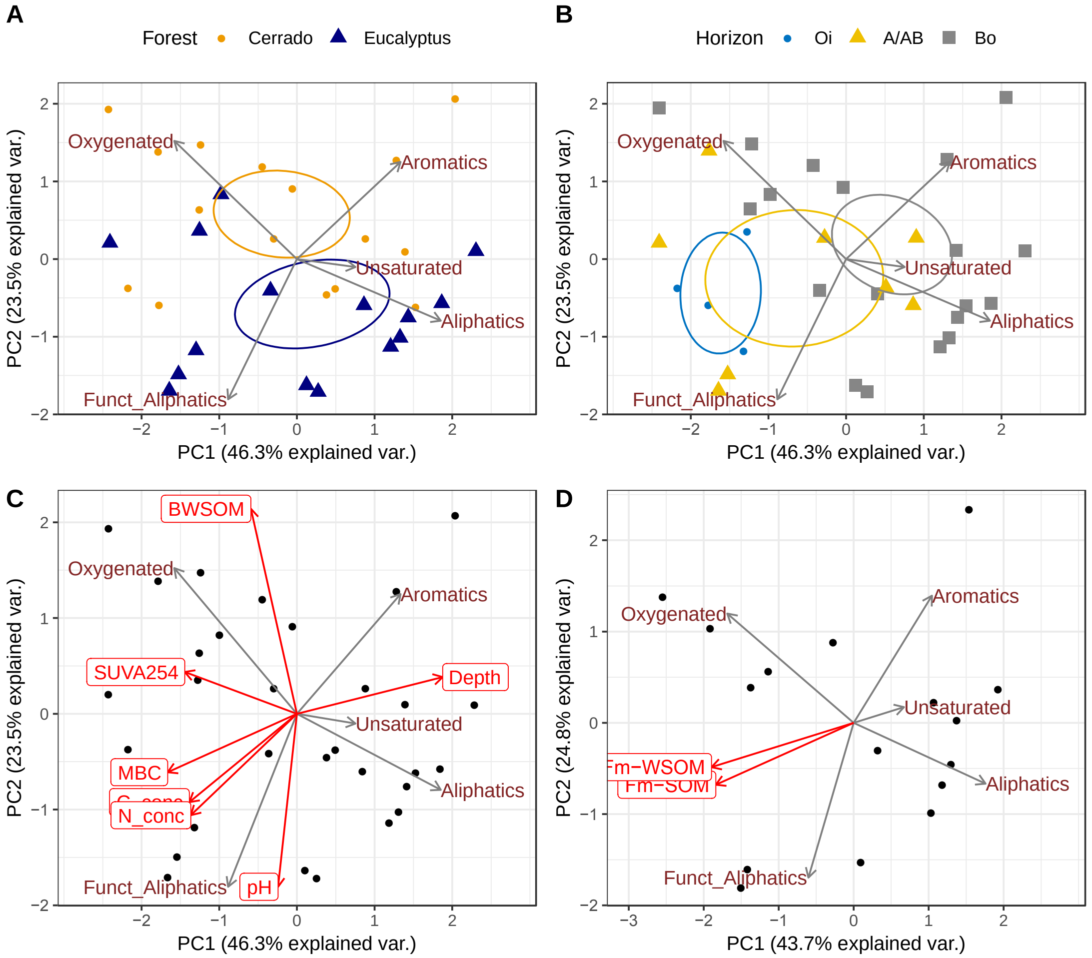

Figure 5.3. PCA biplot showing the influence of (A) forest type, (B) soil horizon, (C) soil physical and chemical characteristics, and (D) bulk soil and DOC radiocarbon age on the functional composition of WSOM assessed by ^{1}H NMR spectroscopy. In all panels, grey vectors show the direction of greatest change in the relative proportion of 5 types of organic compounds. Ellipses in (A) and (B) show 95% confidence intervals around group centroids. Red vectors in (C) and (D) show the direction of greatest change for soil physical and chemical attributes including soil C and N concentration, SUVA$_{254}$, pH, microbial biomass C, biodegradability, and depth. The factors in (A) and (B) and vectors in (C) and (D) are significant predictors of WSOM composition based upon permutation tests restricted within each site at $\alpha = 0.1$. The PCA biplot and significance tests in (D) were computed with only the samples that were analyzed for C isotopes.

A number of soil attributes correlate with ^{1}H NMR composition of WSOM (Figure 5.3C). Depth corresponds largely with PC1 ($R^2 = 0.26$, $p = 0.012$), just like soil horizon. Deeper soil extracts generally have greater aromatic and aliphatic content compared to O horizon and surface soil samples. C concentration ($R^2 = 0.20$, $p = 0.046$) and N concentration ($R^2 = 0.21$, $p = 0.034$) are both negatively related to aromatics and positively related to functionalized aliphatics (Figure 5.3C). Microbial biomass likewise is positively related to functionalized aliphatic content and negatively correlated to aromatics ($R^2 = 0.22$, $p = 0.023$). Despite the previous observation that microbial biomass and biodegradability are correlated, they are related to different portions of WSOM composition. BWSOM is more strongly related to oxygenated groups (such as in polysaccharides) and negatively related to both aliphatics and functionalized aliphatics ($R^2 = 0.35$, $p = 0.001$). pH, on the other hand, strongly separates samples along PC2, but is positively related to aliphatic and functionalized aliphatic content ($R^2 = 0.23$, $p = 0.018$). The final environmental factor significantly related to WSOM composition is $SUVA_{254}$, which is typically interpreted as an index of aromaticity. However, the $SUVA_{254}$ vector is orthogonal to aromatics in WSOM and was instead negatively correlated with aliphatic content ($R^2 = 0.16$, $p = 0.075$).

Vectors showing the relationships between C isotopes and WSOM composition were fit to a second ordination calculated using only the samples where radiocarbon was measured (Figure 5.3A-C with PC1 corresponding with higher aliphatic and aromatic content and lower oxygenated and functionalized aliphatic content, while PC2 corresponds with more aromatics and oxygenated groups and less functionalized aliphatics (Figure 5.3D). Fm-WSOM and Fm-SOM are not strongly correlated with any of the individual functional groups but does strongly follow PC1 and the gradient observed with depth (Fm-WSOM: $R^2 = 0.30$, $p = 0.082$; Fm-SOM: $R^2 = 0.30$, $p = 0.085$).

5.4.4 *FTIR characteristics of SOM*

FTIR spectra for each of the soil profiles show some distinct differences in regions corresponding with known absorbance peaks (Figure 5.4, Table 5.3). The peak in the 1000-1160 cm^{-1} region that corresponds with ester C-O-C stretching, and which is commonly ascribed to polysaccharides in soil (Parikh et al., 2014), is consistently the highest peak in the profiles except in the Cerrado profile at the Lageado site (Figure 5.4C). Polysaccharides appear to be retained down the profile and into the subsoil. On the other hand, absorbance associated with aromatic C=C stretching (1600-1650 cm^{-1}) is more pronounced in the O horizon and surface mineral soils but disappears with depth.

Table 5.3. Characteristic shapes and absorptions for FTIR peak assignments.

	Bond	Shape/ Intensity	Wavenumber Range (cm^{-1})	Source
Polysaccharide	Ester C-O-C stretch	Sharp, strong	1160-1000	Chapman et al. 2001; Kaiser and Ellerbrock 2005
Aromatic	C=C stretch; conjugated carbonyl C=O; quinones	Medium-weak	1650-1600	Dick et al. 2011; Solomon et al. 2005
Aliphatic	C-H stretch	Strong	3000-2800	Dick et al. 2011; Solomon et al. 2005
Amine/Amide	N-H stretch	Sharp, medium	3300-3500	Tatzber et al. 2007
Alcohol/Phenol	O-H stretch	Strong, broad	3200-3600	Dick et al. 2011; Parikh et al. 2014

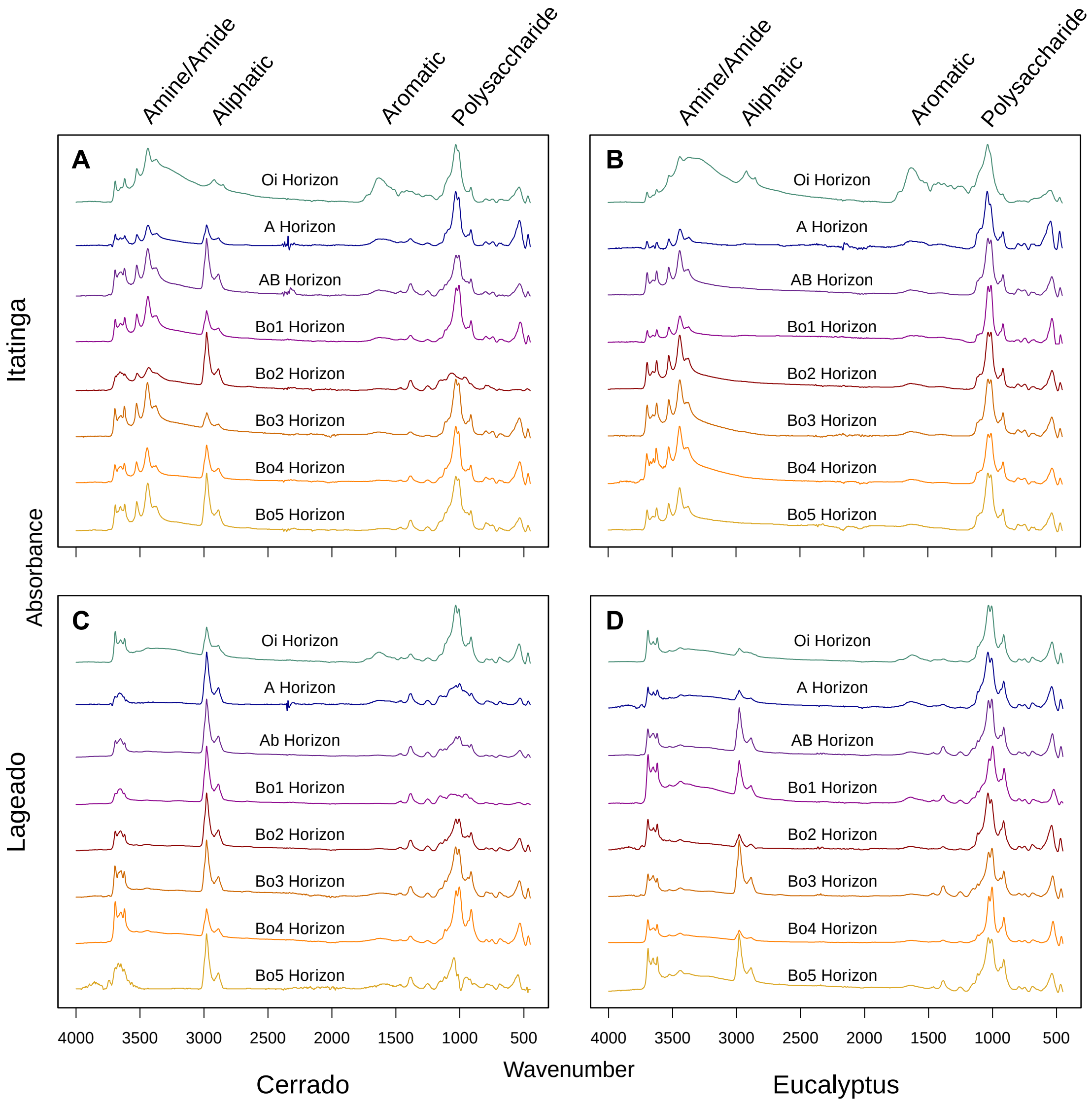

Figure 5.4. Mineral matrix subtracted FTIR-ATR profiles for each of 4 soil profiles from paired Cerrado (A, C) and *Eucalyptus* (B, D) forest soils at two sites (Itatinga: A, B; Lageado: C, D) in São Paulo State, Brazil. Areas in grey highlight approximate regions of absorbance for important compositional peaks representing N containing groups (amines/amides), aliphatic, aromatic, and polysaccharide groups (Table 5.3).

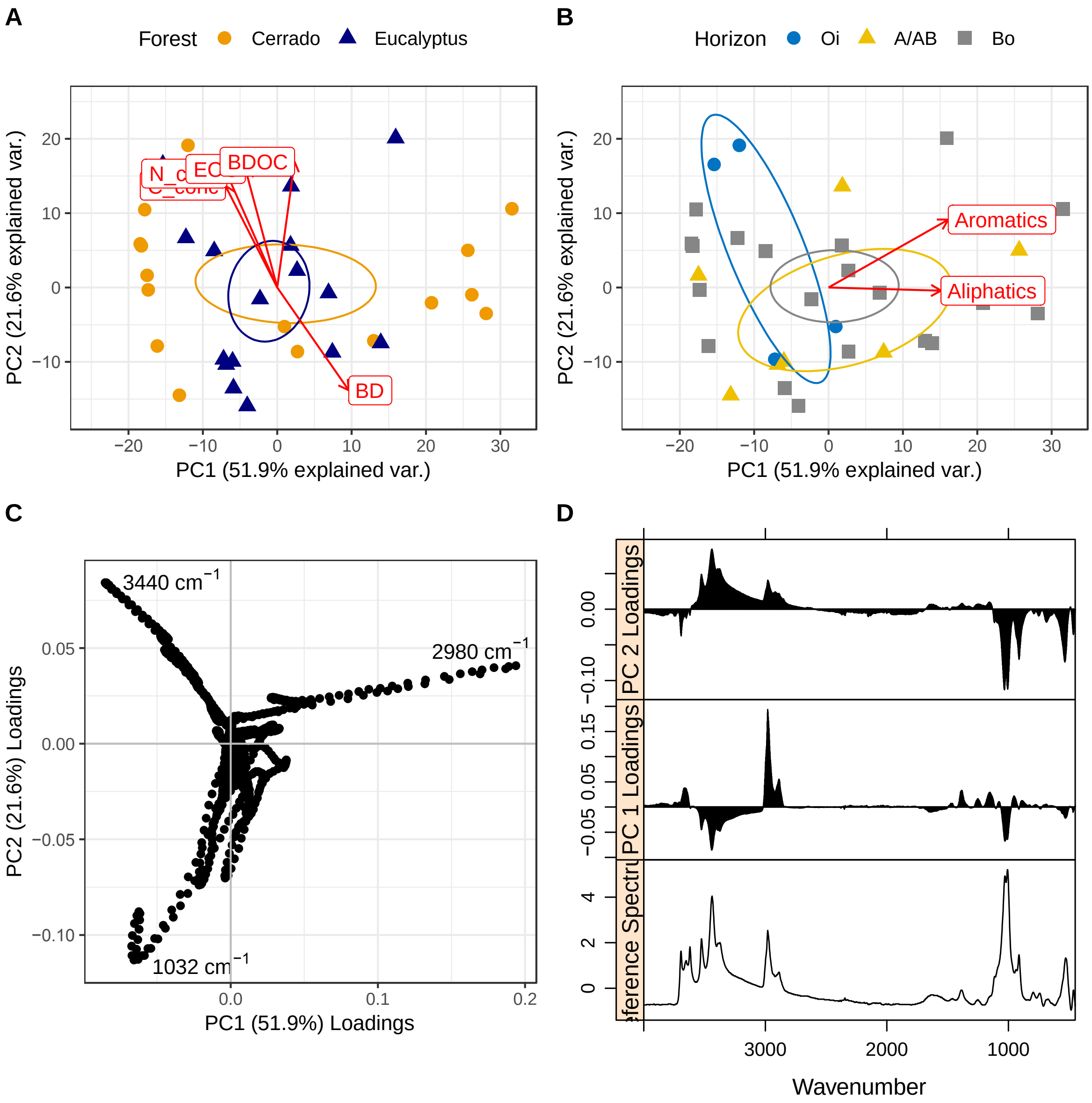

Figure 5.5. PCA ordination showing the relationships between bulk SOM FTIR spectra from four soil profiles in São Paulo State, Brazil and (A) soil physical and chemical characteristics and (B) ^{1}H NMR characteristics of WSOM. The forest type and horizon factors in (A) and (B), respectively, are not statistically significant (based upon permutation tests restricted within each site at $\alpha = 0.1$) but are shown for comparison with Figure 5.3. The vectors in (A) and (B) are significantly correlated with FTIR spectral features based upon permutation tests restricted within each site at $\alpha = 0.1$. The loadings for points along the FTIR spectra are plotted along the ordination axes (C) and spectral axes (D) to aid in interpreting the environmental gradients.

Fewer environmental variables correlate significantly with the PCA ordination of the FTIR profiles compared to the ^{1}H NMR composition of WSOM. Two types of loading plots appear to aid interpretation in terms of both the ordination (Figure 5.5C) and spectral spaces (Figure 5.5D). PC1, which explains just over 50% of the variance in the FTIR data, is strongly related to peaks in the region just below 3000 cm^{-1} that correspond with stretching of aliphatic C-H bonds (Table 5.3). It also has small negative loadings for the aforementioned polysaccharide peak (around 1050 cm^{-1}) and the peaks above 3000 cm^{-1} which include a broad peak between 3200-3600 cm^{-1} (attributed to O-H stretching in alcohol/phenol groups) that is overprinted by the sharp peak around 3400 cm^{-1} (attributed to N-H stretching in amine/amide groups) (Table 5.3). These latter two peaks (polysaccharide and amine/amide groups) strongly separate along ordination PC2, which explains a further 21% of the variance. In the ordination space of Figure 5.5A & 5.5B, points that appear on the righthand side are generally more aliphatic, while samples on the left side have more N- and O- containing groups. Samples toward the top left are relatively enriched in N and OH groups, while those toward the bottom left are enriched in polysaccharides.

Neither forest type (Figure 5.5A) nor soil horizons (Figure 5.5B) separate significantly in the ordination of FTIR profiles, unlike in the ordination of WSOM composition (Figure 5.3A-B) and our second hypothesis. A number of environmental variables did significantly predict changes in FTIR spectra across these profiles. Soil C ($R^2 = 0.17$, $p = 0.032$), N ($R^2 = 0.20$, $p = 0.019$), and WSOM concentrations ($R^2 = 0.19$, $p = 0.014$) are all positively correlated with PC2, particularly corresponding with stronger absorbance associated with OH and NH groups. Bulk density instead is negatively correlated with PC2 ($R^2 = 0.20$, $p = 0.045$). None of these soil attributes strongly relate to the aliphatic content of SOM, which is strongly related to PC1.

The composition of WSOM extracted from these soils was significantly related to aliphatic content (Figure 5.5B). Both aliphatic ($R^2 = 0.19$, $p = 0.07$) and aromatic content ($R = 0.28$, $p = 0.006$) in WSOM are strongly correlated with increased absorbance by aliphatic groups in FTIR spectra for these soils. The small positive relationship between % aromatic content (in WSOM) and PC2 suggests that aromatic moieties (such as phenols) may be released into WSOM from bulk pools as more strongly adsorbing molecules are released from litter and roots.

5.5 DISCUSSION

5.5.1 *Land-Use Change Effects on SOM*

The conversion of Cerrado forest to *Eucalyptus* plantations alters soil carbon cycling by introducing more frequent disturbance, modifying the quantity and quality of litter inputs to SOM pools, and changing rooting strategies and turnover (Batlle-Bayer et al., 2010; Gmach et al., 2018). These changes shift the composition of WSOM: compared to the Cerrado soils, WSOM in *Eucalyptus* plantations contains a significantly higher proportion of aliphatic molecules (Figure 5.3A). While FTIR did separate mineral soil samples based upon aliphatic C-H bonds (PC1 in Figure 5.5A-C), this gradient does not correspond with forest type (Figure 5.5A). Together, these observations are consistent with our first hypothesis: WSOM chemistry reflects the shift in plant composition due to land-use change much more quickly than bulk SOM.

Radiocarbon data also suggest alteration of C turnover in *Eucalyptus* compared to Cerrado soils. The large differences between Fm-WSOM and Fm-SOM in Cerrado subsoils (Figure 5.1) suggest that older compounds are less readily released into solution and remain inaccessible to soil microbes. In contrast, the relatively small differences between Fm-WSOM and Fm-SOM in *Eucalyptus* soils, coupled with relatively young bulk SOM ages at equivalent depths to the Cerrado soils (Table 5.1), suggest that aged SOM has been either mobilized and leached out of the top 1 m+ of soil or converted to CO_2 by soil microbes. Kaiser and Kalbitz (2012) theorize that the steady input of soluble, surface-reactive organic matter near the soil surface forces less strongly binding compounds to move further down and be degraded by microbes, thereby becoming both older in subsoil as well as less strongly binding and prone to future translocation. The Lageado *Eucalyptus* profile shows the greatest evidence for this hypothesis: both microbial biomass and WSOM biodegradability are greatly reduced compared to the neighboring Cerrado soil while C:N ratios are close to 8 throughout the mineral soil profile, which suggests that SOM is highly microbially processed.

One factor that can help explain the differences observed between bulk SOM and WSOM characteristics across these forest types is the degree of Cerrado understory found in the *Eucalyptus* plantations. Increased soil disturbance associated with timber harvesting and decomposition of harvest residues releases a flush of nutrients (Ferreira et al., 2016) which can prime the breakdown of aged SOM in the subsoil (Blagodatskaya and Kuzyakov, 2008; Jenkinson et al., 1985). Leaching loss of this nutrient flush is more likely in the Lageado site which has little-to-no understory. The differences between Cerrado and *Eucalyptus* soils are much smaller at Itatinga at least partially due to the presence of understory Cerrado species beneath the *Eucalyptus* canopy. Ecotones are commonly found on the margins of plantation forests in São Paulo State (Dodonov et al., 2014), at least partly due to small patches of native vegetation occupying steep slopes and riparian zones between stands. In addition to providing connectivity between habitat patches for savanna species, understory vegetation may play an important role in maintaining SOM and preserving nutrient recycling.

5.5.2 *Pedogenic Influence on SOM & WSOM Functional Groups*

The significant separation between Oi and Bo horizon samples in terms of WSOM chemistry (Figure 5.3B), along with the significant shift towards more aromatic and aliphatic groups with increased soil depth (Figure 5.3C) and radiocarbon age (Figure 5.3D), shows that there is substantial microbial transformation of plant-derived compounds deposited on or in the surface soil. This is consistent with our second hypothesis that WSOM composition will diverge increasingly with depth, particularly with the accumulation of hydrophobic compounds in deeper horizons. Over time, the products of microbial processing of WSOM accumulates aromatic and especially aliphatic molecules in the subsoil, altering bulk SOM characteristics (Figure 5.5B). These observations are consistent with our third hypothesis, as well as the conceptual framework of Kaiser and Kalbitz (2012) which states that deeper mineral soil DOM/WSOM is the result of both physico-chemical stripping during transport (due to sorption and co-precipitation) and microbial processing of the organic matter released into solution.

Regardless of forest type, WSOM in these soils is high in aliphatic functional groups (Table 5.2), which are typically hydrophobic and thus not easily mobilized in soil water (Kleber et al., 2007). In extremely old and excessively drained soils like those found in this study (Oxisols), mineralogy is typically dominated by 1:1 silicate clays (such as kaolinite and halloysite) which have relatively little cation exchange capacity and thus little opportunity for ligand exchange or other direct adsorption mechanisms on soil surfaces. Consequently, hydrophobicity may be important in retaining organic molecules in these soils, especially in subsoil Bo horizons. Pisani et al. (2014) reported preferential preservation of aliphatic compounds in tropical soils, with greater acyclic and long-chain aliphatics in sandier horizons. The 0.5 M K_2SO_4 solution we used to extract WSOM likely disrupts hydrophobic interactions, which could help explain both the molecular identity of WSOM observed in this study and the relatively high concentration of C K_2SO_4 removes from soil compared to other extractants (Rennert et al., 2007).

Further supporting the contention that 0.5 M K_2SO_4 disrupts hydrophobic interactions at soil surfaces (or in the zone of hydrophobic interaction as proposed by Kleber et al. (2007)) is the observation that only aromatic and aliphatic functional groups in WSOM were related to the bulk soil FTIR spectra (Figure 5.5B). Aliphatic content in WSOM is closely related to IR adsorption in the region just below 3000 cm^{-1} that is commonly associated with the bending of C-H bonds.

Surprisingly, aromatic content of the WSOM samples was very low (0.04-13% of total; Table 5.1), especially compared to ^1H NMR of NaOH and Na-pyrophosphate extractions (“humic” and “fluvic” acids) in similar Oxisols in southern Brazil (19-28%) (Dick et al., 1999). Rather than being a result of selective extraction with K_2SO_4, these soils appear to be generally aromatic poor outside of the O horizon (Figure 5.4; 1600-1650 cm^{-1} region). $SUVA_{254}$, which is typically understood to be an index of aromaticity, is very low for these WSOM extracts (<1 L mg-C^{-1} m^{-1} for many samples, compared to a typical range of 1-5 L mg-C^{-1} m^{-1} in freshwater DOM samples).

5.5.3 *Limitations on Microbial Growth & Degradation*

Biodegradability of WSOM (BWSOM) is generally highest in O horizons, which produce the freshest and most nutrient rich substrates for consumption, but BWSOM remains consistent with depth in the mineral soil profiles (Table 5.2), which partially confirms our fourth hypothesis. While N is usually considered a limiting nutrient for microbial and plant growth in soils, the positive correlation between BWSOM and C:N ratio (Figure 5.2; r = 0.48) and negative correlation between BWSOM and N stock (Figure 5.2; r = -0.47) suggest that organic matter degradation and cycling in these soils is primarily substrate and/or energy limited rather than nutrient limited. Microbial biomass is also most closely correlated with WSOM ($r = 0.81$), which further supports the hypothesis that microbes are substrate limited in these soils. Moreover, pH is negatively correlated to BWSOM (Figure 5.2; $r = -0.42$) with higher biodegradability of WSOM in more acidic samples. Soil pH is known to be a dominant factor controlling microbial and fungal community structures (Fierer and Jackson, 2006), with fungi dominating in more acidic environments (Rousk et al., 2009). Substrate composition plays a role in the biodegradability of WSOM in these soils as well. BWSOM is correlated with more oxygenated and less aliphatic and functionalized aliphatic groups (Figure 5.3C), and this environmental gradient is orthogonal and unrelated to the amount of microbial biomass or soil C or N concentration.

Cerrado forests appear to produce more labile compounds than *Eucalyptus* forests (both forest type and BWSOM separate along PC2 in Figure 5.3A,C), which could explain the general trend of lower concentrations of WSOM and higher MBC in the Cerrado forest soils (Table 5.1 & 5.2). Moreover, Cerrado WSOM is richer in oxygenated functional groups – which often occur in more energetically rich molecules (such as polysaccharides) – and poorer in aliphatic functional groups. Aliphatic molecules can include long-chain ($\geq C_{20}$) aliphatic lipids from the epicuticular waxes of plants (Jetter et al., 2007) or short chain ($<C_{20}$) aliphatic lipids from soil microbes (Lichtfouse et al., 1995). As the Cerrado soils contain less aliphatic functional groups than the *Eucalyptus* soils, root contributions may be especially important in providing fresh residues in Cerrado ecosystems. Root biomass C (to 2 m depth) represents 66% of total live biomass on average under natural Cerrado vegetation (Batlle-Bayer et al., 2010). While *Eucalyptus* is known to root extremely deeply (Laclau et al., 2013), root density was visibly much higher in each of the Cerrado profiles compared to *Eucalyptus* in this study.

5.5.4 *Radiocarbon Dynamics*

Radiocarbon ages of WSOM samples are closer to modern than the corresponding bulk SOM samples at every depth in all profiles (Figure 5.1; Table 5.1 & 5.2). This is consistent with WSOM representing a substantially more labile and actively cycled pool (Kaiser and Zech, 1997; Kalbitz et al., 2000; Ros et al., 2009). Consistent with our fifth hypothesis, Fm-WSOM still declined with depth. While BWSOM was significantly correlated with Fm-SOC, the relationship is weak (r = -.0.11). Furthermore, BWSOM does not change much with soil depth within each profile, suggesting that older WSOM is not inherently less recalcitrant than more recently fixed C entering the A horizon.

Recent conceptual models for DOM and SOM dynamics theorize that organic molecules adsorbed on mineral surfaces are not permanently affixed but rather are (slowly) exchanged and replaced by mobile, fresh DOM or other microbial byproducts (Kaiser and Kalbitz, 2012; Lehmann and Kleber, 2015). The increase in radiocarbon age of WSOM with depth in this study is consistent with this theory. WSOM may represent an intermediate pool that is particularly active in the interchange of fresh DOM and other fragmented organic detritus for older SOM.

The difference between Fm-SOM and Fm-WSOM is substantially larger in Cerrado forests than *Eucalyptus* forests (Figure 5.1). *Eucalyptus* plantation management increases soil disturbance and can cause increased turnover of SOM and potentially net losses of SOC despite high forest productivity. Cook et al. (2016) estimate a loss of 0.22 Mg C ha^{-1} yr^{-1} in *Eucalyptus* plantations across southern Brazil resampled over three rotations and 20 years. This loss was driven by decreases in subsurface soil C (15-30 cm) rather than surface soil C (0-15 cm). Consequently, smaller differences between Fm-WSOM and Fm-SOM in *Eucalyptus* forest are consistent with the observation that *Eucalyptus* plantations lose subsoil C and suggest that this loss occurs due to reduced C inputs (both litter and root-derived) and/or increased leaching of WSOM relative to Cerrado forests.

5.6 TAKEAWAYS AND NEXT STEPS

This study examined the effect of land-use change in the Brazillian Cerrado on SOM cycling deep into the soil profile (1.3 m). Converting native Cerrado forest to *Eucalyptus* plantations increases both the input and accumulation of aliphatic molecules. WSOM in *Eucalyptus* forest is less labile and does not support as large a microbial community, particularly in the interior of stands that usually have little to no native understory. This is reflected in a loss of older SOM in subsoil horizons and may have implications for nutrient recycling.

The relationships between bulk SOM and WSOM isotopic and functional group composition are consistent with recent conceptual models about the importance of dissolved organic matter leaching in explaining the vertical distribution of SOM characteristics (Guggenberger and Kaiser, 2003; Kaiser and Kalbitz, 2012). The relatively high concentration of aliphatic molecules in WSOM across all four soil profiles also corresponds with observations elsewhere in the literature (e.g. Pisani et al., 2014) and indicates that hydrophobic interactions are an important mechanism for SOM retention in old, weathered soils that underlie much of the Brazilian Cerrado.

The shift in WSOM composition between soil horizons (Figure 5.3B) and with depth (Figure 5.3C) shows that these Oxisol subsoils are enriched in aliphatic compounds. However, the data presented here cannot definitively identify the source of lipids and other aliphatic compounds that dominate SOM composition in these soils. Subsoil WSOM is likely derived primarily from roots (exudates and turnover) or from microbial byproducts (e.g. lipid cell wall fragments), rather than direct DOM leaching from the O horizon. To test this hypothesis, follow up work on these soils will be needed to examine the composition of lipids (such as with biomarkers, e.g. Pisani et al., 2014, and alkaline hydrolysis / gas chromatography – mass spectrometry, e.g. Otto and Simpson, 2006) to identify the relative contribution of roots and shoots to subsoil C storage. Another step that would increase the understanding of how land-use change influences soil C sequestration in the Cerrado is expanding the range of land uses examined, including sugar cane and grazing lands. While this study suggests some mechanisms for soil C loss in *Eucalyptus* plantations relative to Cerrado vegetation, silvicultural management could very well benefit lands degraded by cattle grazing or more intensive uses.

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APPENDIX 1

Publications providing response ratios for the Chapter Four meta-analysis.

Reference	Year	*k*	Max Depth (cm)	Time Since Harvest^a (yrs)	Location
Alban and Perala [54]	1992	7	50	35	MN, USA
Bauhus et al. [55]	2004	6	40	9	Germany
Bisbing et al. [56]	2010	6	100	40	MT, USA
Black and Harden [57]	1995	15	20	23	CA, USA
Boerner et al. [58]	2006	4	10	2	SC, USA
Borchers and Perry [59]	1992	4	15	14	OR, USA
Bravo-Oviedo et al. [60]	2015	8	30	15	Spain
Cade-Menun et al. [61]	2000	12	26	5	BC, Canada
Carter et al. [62]	2002	8	15	2	LA, TX, USA
Chatterjee et al. [63]	2009	19	54	21	WY, USA
Chen et al. [64]	2013	24	100	29	China
Chiti et al. [65]	2016	24	100	24	Ghana, Cameroon, Gabon
Christophel et al. [66]	2013	6	30	15	Germany
Christophel et al. [67]	2015	18	30	33	Germany
Cromack et al. [68]	1999	1	100	10	OR, USA
Dai et al. [69]	2001	3	70	14	NH, USA
DeByle et al. [70]	1980	10	5	3	WY, USA
Deluca and Zouhar [53]	2000	6	8	5	MT, USA
Diochon et al. [71]	2009	28	50	35	NS, Canada
Edmonds and McColl [72]	1989	4	20	3	Australia
Edwards and Ross-Todd [73]	1983	6	45	1	TN, USA
Elliott and Knoepp [74]	2005	3	15	3	NC, USA

Ellis et al. [74]	1982	4	10	2	Tasmania
Ellis and Graley [75]	1983	2	10	1	Tasmania
Esquilin et al. [76]	2008	1	10	14	CO, USA
Falsone et al.[77]	2012	3	130	5	Russia
Fraterrigo et al. [78]	2005	1	15	30	NC, USA
Frazer et al. [79]	1990	4	14	12	CA, USA
Gartzia-Bengoetxea et al. [80]	2009	2	5	10	Spain
Gillon et al. [81]	1999	2	0	1	France
Goh and Phillips [82]	1991	4	60	2	New Zealand
Goodale and Aber [83]	2001	2	10	85	NH, USA
Gough et al. [84]	2007	15	80	41	MI, USA
Grady and Hart [85]	2006	2	15	12	AZ, USA
Grand and Lavkulich [86]	2012	6	80		BC, Canada
Gresham [87]	2002	6	30	10	SC, USA
Griffiths and Swanson [88]	2001	3	10	20	OR, USA
Gundale et al. [89]	2005	4	10	3	MT, USA
Gupta and DeLuca [90]	2012	12	50	5	Wales
Hart et al. [91]	2006	2	15	1	AZ, USA
Hendrickson and Chattarpaul [92]	1989	6	20	3	ON, Canada
Herman et al. [93]	2003	2	9	8	CA, USA
Holscher et al. [94]	2001	2	20	22	Germany
Hwang and Son [95]	2006	2	30	2	Korea
Jang and Page-Dumroese [96]	2015	8	30	38	MT, USA
Johnson [97]	1991	3	20	3	NH, USA
Johnson and Todd [98]	1998	6	45	15	TN, USA
Johnson [99]	1995	12		7	NH, USA
Johnson et al. [100]	1997	14	53	6	NH, USA

Johnson et al. [101]	2014	4	60	1	CA, USA
Jones et al. [102]	2011	12	30	15	New Zealand
Kaye and Hart [103]	1998	2	15	1	AZ, USA
Keenan et al. [104]	1994	1	20	4	BC, Canada
Kelliher et al. [105]	2004	4	50	22	OR, USA
Kishchuk et al. [106]	2014	4	7	6	AB, Canada
Klockow et al. [107]	2013	9	20	1	MN, USA
Klopatek [108]	2002	6	20	30	WA, USA
Knoepp and Swank [109]	1997	4	30	33	NC, USA
Korb et al. [110]	2004	1	10	1	AZ, USA
Kraemer and Hermann [111]	1979	2	10	26	WA, USA
Kurth et al. [112]	2014	72	30	8	MI, MN, USA
Laiho et al. [113]	2003	5	22	5	NC, LA, USA
Latty et al. [114]	2004	2	15	90	NY, USA
Law et al. [115]	2001	3	100	21	OR, USA
Law et al. [116]	2003	9	100	62	OR, USA
Leduc and Rothstein [117]	2007	1	10	5	MI, USA
Maassen and Wirth [118]	2004	2	5		Germany
Mattson and Smith [119]	1993	30	10	11	WV, USA
Mattson and Swank [120]	1989	8	60	5	NC, USA
May and Attiwill [121]	2003	2	10	5	Australia
McLaughlin and Phillips [122]	2006	2	50	17	ME, USA
McKee et al. [123]	2013	8	60	24	AL, USA
McLaughlin [124]	1996	10	50	5	MI, USA
Merino and Edeso [125]	1999	6	15	1	Spain
Moreno-Fernandez et al. [126]	2015	54	50	60	Spain
Mu et al. [127]	2013	18	50	5	China

Murphy et al. [128]	2006	20	60	1	CA, USA
Neher et al. [129]	2003	3	20	2	NC, USA
Norris et al. [130]	2009	15	100	16	SK, Canada
O'Brien et al. [131]	2003	6	50	18	Australia
Powers et al. [132]	2011	20	30	13	MN, WI, USA
Prest et al. [133]	2014	5	50	35	NS, Canada
Prietzel et al. [134]	2004	4	0	1	WA, USA
Puhlick et al. [135]	2016	10	100		ME, USA
Rab [136]	1996	8	10	1	Australia
Riley and Jones [137]	2003	3	10	1	SC, USA
Roaldson et al. [138]	2014	16	20	5	CA, USA
Rothstein and Spaulding [139]	2010	6	30		MI, USA
Sanchez et al. [140]	2007	6	105	2	SC, USA
Sanscrainte et al. [141]	2003	4	70		WA, USA
Saynes et al. [142]	2012	8	5	11	Mexico
Selig et al. [143]	2008	3	30	14	VA, USA
Shelburne et al. [144]	2004	4	10	1	SC, USA
Sheng et al. [145]	2015	5	100	8	China
Skovsgaard et al. [146]	2006	12	30	0	Denmark
Slesak et al. [147]	2012	12	60	5	OR, WA, USA
Small and McCarthy [148]	2005	3	10	7	OH, USA
Stone et al. [149]	1999	1	15	1	AZ, USA
Stone and Elioff [150]	1998	4	30	5	MN, USA
Strong [151]	1997	8	40	18	MN, USA
Strukelj et al. [152]	2015	12	10	5	QC, Canada
Tang et al. [153]	2009	12	60	29	MI, WI, USA
Trettin et al. [154]	2011	6	150	11	MI, USA
Ussiri and Johnson [155]	2007	15	60	8	NH, USA
Vario et al. [156]	2014	6	60	49	NH, USA

Vesterdal et al. [157]	1995	9	0		Denmark
Waldrop et al. [158]	2003	3	0	1	CA, USA
Wu et al. [159]	2010	1	20	10	China
Xiang et al. [160]	2009	8	30	0	China
Yanai et al. [161]	2000	35	0	29	NH, USA
Zabowski et al. [162]	2008	2	20	25	OR, WA, USA
Zhong and Makeshin [163]	2003	2	10	16	Germany
Zummo and Friedland [164]	2011	15	60	3	NH, USA

[a] For chronosequence studies, time since harvest in this table is average across all response ratios for that study.

VITA

Jason James

EDUCATION

Ph.D.	December 2018	University of Washington, Seattle, WA Dissertation: *The Effects of Forest Harvesting and Land-Use Change on Soil Carbon and Nutrient Cycling*
M.S.	June 2014	University of Washington, Seattle, WA Thesis: *Deep Soil: Quantification, Modeling, and Significance of Subsurface Carbon and Nitrogen*
B.A.	May 2012	Oberlin College Majors: Environmental Studies & Creative Writing

PEER REVIEWED PUBLICATIONS

James, J., Bakker, J., Butman, D., Kates, N., Kuhn, C., Littlefield, C., and Miller, C. 2018. The effects of forest restoration on ecosystem carbon in western North America: a systematic review. *Forest Ecology and Management* 429:625-641

Gross, C., **James, J.**, Turnblom, E., and Harrison, R. 2018. Thinning treatments reduce deep soil carbon and nitrogen stocks in a coastal Pacific Northwest forest. *Forests* 9:238

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Menegale, M., Rocha, J., Harrison, R., Gonçalves, J., Almeida, R., Picollo, M., Hubner, A., **James, J.**, and Michelson-Correa, S. Effect of Timber Harvest Intensities and Fertilizer Application on Stocks of Soil C, N, P, and S. 2016. *Forests* 7:319

Faria, M., Guerrini, I., Oliveira, F., Sato, M., Hachich, E., Passos, J., Goulart, L., Silva, T., Gava, J., Furches, J., **James, J.**, and Harrison, R. 2017. Persistence of *Ascaris* spp. ova in tropical soil cultivated with eucalyptus and fertilized with sewage sludge. *Journal of Environmental Quality* doi:10.2134/jeq2016.08.0305

Dietzen, C., Marques, E., **James, J.**, Bernardi, R., Holub, S., Harrison, R. 2017. Response of Deep Soil Carbon Pools to Forest Management in a Highly Productive Andisol. *Soil Science Society of America Journal* doi:10.2136/sssaj2016.09.0305

James, J., Littke, K., Bonassi, T., Harrison, R., 2016. Exchangeable cations in deep forest soils: separating climate and chemical controls on spatial and vertical distribution and cycling. *Geoderma* 279: 109-121

James, J., Dietzen, C., Furches, J., Harrison, R., 2015. Lessons in Buried Horizons and Pedogenesis from Deep Forest Soils. *Soil Horizons* 56:6. Graduate Student Paper Contest Winner

James, J., Knight, E., Gamba, V., Harrison, R., 2015. Deep Soil: Quantification, modeling, and significance of subsurface nitrogen. *Forest Ecology and Management* 336: 194-202

James, J., Devine, W., Terry, T., Harrison, R., 2014. Deep Soil: Accurately Quantifying and Modeling Carbon in Subsurface Layers. *Soil Science Society of America Journal* 78:S1–S10. North American Forest Soils Conference Proceedings.

PUBLICATIONS IN PREPARATION OR REVIEW

Rocha, J., Menegale, M., Rodrigues, M., Gonçalves, J., Pavinato, P., Foltran, E., Harrison, R., and **James, J.** *In review*. Impacts of timber harvest intensity and P fertilizer application on soil P fractions. *Geoderma*

Faria, M., Guerrini, I., Oliveira, F., Sato, M., Hachich, E., Passos, J., **James, J.**, and Harrison, R. *In review*. Survival of thermotolerant coliforms present in municipal biosolids after land application in tropical soil cultivated with Eucalyptus. *Ecotoxicology and Environmental Safety*

James, J., Myers, T., Dwivedi, P., Gross, C., Bernardi, R., Guerrini, I., Harrison, R., and Butman, D. *In preparation*. The Influence of Land-Use Change on the Radiocarbon Age, Lability, and Spectroscopic Characteristics of Soil Carbon and Extractable Organic Matter in Brazilian Oxisols.

Bogard, M., Dornblaser, M., Holtgrieve, G., **James, J.**, Johnston, S., Koch, J., Kuhn, C., Spencer, R., Striegl, R., Wickland, K., and Butman, D. *In preparation*. Exploring Permafrost Soil Carbon Transfer Along Hydrologic Gradients in Interior Alaska.

Norton, S., **James, J.**, Zabowski, D., Littke, K., and Harrison, R. *In preparation*. Carbon content and decay of Douglas-fir stumps in western Washington plantation forests.

NON-PEER-REVIEWED PUBLICATIONS

Holub, S., **James, J.**, 2014. Forest Soils Store Large Amounts of Carbon. In *Western Forester* 59:4 pp. 8-9.

GRANTS & AWARDS

USDA McIntire-Stennis PI: David Butman	Potential soil carbon stocks and turnover across privately-operated forest stands in the PNW: understanding the radiocarbon age, quality, and lability of SOC within Douglas-fir plantations	$193,966	2015-2017
Soil Science Society of America	Robert J. Luxmoore Graduate Student Travel Award	$1,000	2015

University of Washington	David Briggs Endowed Student in Forest Management	$1,000	2014
University of Washington	College of the Environment Student Travel Fund	$500	2014
University of Washington	Stanley Gessel & William Kreuter Fellowship	$25,767	2012-2013
Oberlin College	Robert Haber Gilbert Scholarship	$1,630	2010
Oberlin College	John F. Oberlin Scholarship	$44,000	2008-2012

OTHER WORK EXPERIENCE

Independent Contractor	Arcadis and Ramboll Environ Completed soil descriptions for Arcadis and Ramboll Environ as part of a study to ameliorate lead and arsenic contamination on land owned by the Colville Tribe	October 2017

SCIENTIFIC LEADERSHIP & SERVICE

Moderator	**Soil Science Society of America Annual Meeting:** Forest, Range and Wildland Soils Oral Session III	2016
Lead Workshop Coordinator	**UW ENGAGE Program Board of Directors:** Coordinate & Support C ENV 500: Communicating Science to the Public	2016

ORAL PRESENTATIONS

James, J., Littke, K., and Harrison, R. 2017. *Interaction Between Carbon and Nutrient Cycles in Deep Soils of the Pacific Northwest*. Geological Society of America Annual Meeting, Seattle, WA.

James, J., Gross, C., Dwivedi, P., Harrison, R., and Butman, D. 2017 *Coupling Solid- and Liquid-Phase Soil Organic Matter Analyses to Understand the Consequences of Forest Conversion and Management on Brazilian Oxisols*. Soil Science Society of America Annual Meeting, Tampa, FL.

James, J. and Harrison, R. 2017. *The Effect of Harvest on Forest Soil Carbon*. Stand Management Cooperative Spring Meeting, Vancouver, WA.

James, J. and Harrison, R. 2017. *The Effect of Harvest on Forest Soil Carbon: A Global Meta-analysis*. UN FAO Global Soil Organic Carbon Symposium, Rome, Italy.

James, J. 2016. *How Soil Reveals the Hidden History of the Earth*. Town Hall Seattle, Seattle, WA.

James, J., Dietzen, C., Gross, C. and Harrison, R. 2015. *The Impact of Buried Horizons and Deep Soil Pedogenesis on Soil Carbon Content and Vertical Distribution.* American Geophysical Union Annual Meeting, San Francisco, CA.

James, J., Dietzen, C., Littke K., Gross, C., and Harrison, R. 2015. *Lessons on Buried Soils and Pedogenesis from Deep Forest Soils*. Soil Science Society of America Meeting, Minneapolis, MN.

James, J., Dietzen, C., Littke, K., and Harrison, R. 2015. *Profiles and Chemistry of Deep Forest Soils in the Pacific Northwest*. Northwest Forest Soils Council Meeting, Hood River, OR.

James, J., Littke, K., and Harrison, R. 2014. *The Relationship Between Exchangeable Base Cations, Soil Carbon and Soil Nitrogen in Deep Forest Soils of the Pacific Northwest.* Soil Science Society of America Annual Meeting, Long Beach, CA.

James, J., Devine, W., Terry, T., and Harrison, R. 2014. *The Quantity and Storage Mechanisms of Carbon in Deep Soil Horizons of the Pacific Northwest.* International Union of Forest Research Organizations (IUFRO) World Congress, Salt Lake City, UT.

James, J., Littke, K., and Harrison, R. 2014. *Interactions of Carbon, Nitrogen, and Base Cation Cycles in Deep Forest Soils.* Stand Management Cooperative Spring Meeting, Vancouver, WA.

James, J., Devine, W., Terry, T., and Harrison, R. 2014. *Deep Soil: Sampling, Modeling, and Significance of Carbon in Subsurface Layers*. Northwest Scientific Association Annual Conference, Missoula, MT.

James, J., Littke, K., and Harrison, R. 2013. *Predicting Douglas-fir Response to Fertilizer Using Site-Specific Factors*. University of Washington, Stand Management Cooperative Spring Meeting, Vancouver, WA.

James, J., Littke, K., and Harrison, R. 2013. *Understanding Site-Specific Factors Affecting the Nutrient Demands and Response to Fertilizer by Douglas-fir.* National Science Foundation Center for Advanced Forest Systems Annual Meeting, St. Simons Island, GA.

James, J., Devine, W., Terry, T., and Harrison, R. 2013. *Deep Soils: Accurately Quantifying and Modeling Total Carbon in Subsurface Layers.* Graduate Student Symposium, University of Washington, Seattle, WA.

POSTER PRESENTATIONS

James, J., Gross, C., Dwivedi, P., Bernardi, R., Myers, T., Guerrini, I., Harrison, R., and Butman, D. 2018 *Forest Management and Land-Use Change Alter the Radiocarbon Age, Fluorescence Characteristics, and Lability of* K_2SO_4 *Extractable Organic Matter*. North American Forest Soil Conference, Quebec City, Canada

James, J., Gross, C., Dwivedi, P., Myers, T., Harrison, R., and Butman, D. 2017 *From solid to liquid: Assessing the release of organic matter into soil solution in response to land-use conversion on Brazilian Oxisols*. European Geosciences Union General Assembly, Vienna, Austria.

James, J., and Harrison, R. *The Effect of Harvest on Forest Soil Carbon: A Global Meta-analysis*. 2017. Soil Science Society of America Annual Meeting, Tampa, FL.

James, J., Myers, T., Gross, C., Harrison, R. and Butman, D. 2017. *Assessing the effect of land use conversion to* Eucalyptus *forest on water extractable organic carbon dynamics in Brazilian Oxisols*. Northwest Forest Soil Council Meeting, Hood River, OR.

James, J., Gross, C., Harrison, R., and Butman, D. 2016 *From solid to liquid: assessing the release of carbon from soil into solution in response to forest management*. American Geophysical Union, San Francisco, CA.

James, J., Bonassi, T., Littke, K., and Harrison, R. 2016 *Climate and Chemical Controls on Exchangeable Cations in Acidic Forest Soils*. Soil Science Society of America Journal, Phoenix, AZ. Best Presentation Contest Winner.

James, J., Devine, W. Terry, T., and Harrison, R. 2013. *Deep Soil: Modeling and Significance of Soil Carbon and Nitrogen in Subsurface Layers*. Soil Science Society of America Annual Conference, Tampa, FL.

James, J., Devine, W., Terry, T., and Harrison, R. 2013 *Deep Soil: Modeling and Significance of Subsurface Carbon and Nitrogen*. Northwest Advanced Renewables Alliance (NARA) Annual Meeting, Corvallis, OR.

James, J., Devine, W., Terry, T., and Harrison, R. 2013. *Deep Soils: Accurately Quantifying and Modeling Total Carbon in Subsurface Layers*. North American Forest Soils Conference, Whitefish, MT.

SCIENTIFIC PEER REVIEWS

Applied Soil Ecology – 1 review	2018
Global Change Biology – 1 review	2018
Geoderma – 2 reviews	2017-2018
Forests – 4 reviews	2016-2017
Journal of Geophysical Research - Biogeosciences – 1 review	Fall 2016
Forest Ecology & Management – 1 review	Spring 2015
Soil Science Society of America Journal – 4 reviews	2014-2018

PROFESSIONAL & HONOR SOCIETY MEMBERSHIPS

American Geophysical Union	2015
Northwest Forest Soil Council – Vice President	2014
Society of American Foresters - Washington State Chapter	2013
Northwest Scientific Association	2013
Soil Science Society of America	2013
Xi Sigma Pi - University of Washington Alpha Chapter	2013
Phi Beta Kappa - Oberlin College Zeta of Ohio Chapter	2012