

PROJECT NARRATIVE

1. INTRODUCTION

1.1 Overview – Long-Term Goals and Project Objectives

Soils comprise the largest pool of terrestrial organic carbon (C). Forests cover about 30% of the earth's terrestrial surface and forest soils account for a similar proportion of the global terrestrial pool of soil organic carbon (SOC) (Scharlemann et al., 2014).

Disturbances such as harvesting and forest fires can remove substantial portions of a forest's aboveground C stores; however, in most cases these extreme disturbances have little impact on SOC pools (Nave et al., 2010, 2011; James and Harrison, 2016). Very few studies have been conducted to examine SOC pools and their ability to resist change or determine how resilient to change these pools are (i.e. recover C at rates similar to those lost). A new paradigm to explain SOC dynamics is emerging (Schmidt et al., 2011; Lehmann and Kleber, 2015). We propose to use this emerging paradigm to examine this apparent resilience a global forestry study.

The long-term goal of this research group is to understand the mechanisms by which SOC is stabilized and destabilized in forested systems. The overall objective of this proposal is to elucidate the mechanisms that impart resilience to forest SOC after extreme disturbances. Our central hypothesis is that organo-mineral complexes are resistant to disturbance-induced degradation and that any SOC vulnerable to disturbance is readily replaced by residual roots. Additionally, previous work has shown that in many forest systems there is little incorporation of aboveground C to soils and therefore aboveground disturbances may have little influence on the stability of SOC stocks since there is typically a large portion of belowground residuals (McFarlane et al., 2013). By meeting this objective, we will be able to understand which soils are most vulnerable to losses and degradation and develop management strategies that maintain or enhance forest SOC in all managed settings.

Two specific Objectives and working hypotheses have been developed to guide this research:

Objective #1: Determine the mechanisms of resistance in SOC severe disturbances across a wide range of soils and forest types.

Forest soil can resist SOC loss due to severe disturbances as a result of stabilization mechanisms that have been elucidated recently. The new paradigm of SOC stabilization purports that stabilized SOC is that which is associated with a mineral surface or entrained within soil aggregates, while older paradigms suggest that the molecular structure of SOC regulates its resistance to degradation. We will examine the dynamics of SOC stabilization across archives of soils collected from a widely applied biomass removal study. This study encompasses a broad spectrum of soils, forests, and climates and will allow us to determine

if the mechanisms of stabilization and resistance to change are different by soil or forest type. We will use density fractionation, mineralogical characterization, and biomarkers to characterize the SOC. Using these methods, we will be able to pinpoint the resistance of mineral and aggregate stabilized pools as well as the organic constituents' (e.g. charcoal/biochar, lignin) resistance to biomass loss. We hypothesize that those forests that initially have a higher proportion of mineral and aggregate stabilized SOC will resist changes associated with loss of aboveground biomass but that the molecular structure of SOC will have no effect on resistance to loss.

Objective #2: Determine the mechanisms of resilience in SOC to severe disturbances across a wide range of soils and forest types.

Forest soils can be resilient against C losses by recovering C within stabilized pools within a short period of time. The mechanisms of resilience could be a result of: 1) increased inputs of dead biomass created as a result of the disturbance; and/or 2) a higher rate of stabilization of the C that is available. By examining the nature of SOC and studying the assemblages of biomarkers, ¹⁴C abundances, and ratios of stable isotopes in stabilized and unstabilized pools of SOC across a range of soils from a broadly applied biomass experiment, we will determine the source of new C in recently disturbed soils. Using these methods, we will determine the quantity and source (belowground or aboveground and fresh versus degraded) of C contributing to the resilience of the SOC.

1.2 Review of Relevant Literature

Soil organic carbon is a very dynamic component of the soil; each year, the amount of SOC processed by microorganisms within the soil is roughly equal to the amount of inputs from plant detritus. SOC quantity and quality are linked to important soil functions including nutrient mineralization, aggregate stability, trafficability, and water retention and hydrologic processes. In turn, these soil functions are correlated with a wide range of ecosystem properties. For example, high SOC is associated with high plant productivity (Oldfield et al., 2017), with subsequent implications for wildlife habitat, distribution and abundance. Consequently, ecosystem services can be degraded when SOC is altered or lost from forest or range sites. Soils comprise the largest pool of terrestrial organic C globally, with an estimated 2,270-2,770 Pg of C in the top 2-3 m of soil (Jackson et al., 2017). Because the SOC pool is large compared to other carbon pools (especially the atmosphere), a small change in SOC can cause a large change in the atmospheric CO₂ levels. Conversely, sequestering a few percent to this large C pool translates into a substantial increase that is globally relevant (e.g. Nave et al., 2018).

Mechanisms of mineral soil organic carbon stability. Our understanding of SOC distribution and vulnerability has been limited by the traditional SOC conceptual model. A

new conceptual framework of SOC stabilization and destabilization is currently being developed that improves our ability to predict SOC behavior. Historically it has been thought that the ability of an organism to effectively decompose organic material was directly related to the material's molecular composition (e.g., lignin content) and concentration of nutrients (e.g. nitrogen). These concepts are still very useful when describing decomposition dynamics of organic soils or organic soil horizons. However, unstabilized SOC in O horizons and belowground particulate organic matter represents about 1–12% of forest soil carbon (Sollins et al., 2006; Binkley and Fisher, 2013). Mineral associated carbon is associated with mineral surfaces and represents more than 90% of all soil carbon and has the longest residence time (i.e., oldest ^{14}C age) (Fisher Richard and Binkley Den, 2000; Gaudinski et al., 2000; Jobbágy et al., 2000). *Therefore, traditional concepts describing the dynamics of SOC are useful for only a fraction of the soil carbon and they break down when describing the majority of SOC of mineral associated SOC.*

The previous paradigm suggested that organic matter entering the soil had three possible fates: 1) mineralized, 2) incorporated into microbial biomass, or 3) stabilized as humic substances (Schulten H.-R., 1998). Humic substances were described as refractory, dark colored, heterogeneous, organic compounds of high molecular weight which could be separated into fractions based on their solubility in acidic or alkaline solutions (Sutton and Sposito, 2005). Advances in analytical technology have revealed that SOC is largely comprised of identifiable biopolymers and the perceived existence of humic substances was an artifact of the procedures used to extract the material (Kelleher et al., 2006; Marschner et al., 2008; Kleber and Johnson, 2010; Lehmann and Kleber, 2015).

The classical paradigm also has a difficult time predicting the long-term behavior of soil carbon pools. Compounds thought to be chemically recalcitrant and resistant to decomposition (e.g., lignin) sometimes turned over rapidly, while compounds thought to be labile (e.g. sugars) were demonstrated to persist for decades (Grandy et al., 2007; Kleber and Johnson, 2010; Schmidt et al., 2011). These inconsistencies support the assertion that the classical paradigm is not an appropriate framework for determining the vulnerability of soil carbon to change. As a result of such shortcomings, the conceptual model that soil scientists use to describe SOC and stabilization is undergoing a paradigm shift towards one that is able to better predict SOC dynamics by exploring the complex interactions between microorganisms and minerals in the soil.

This new framework for understanding SOC stability postulates that SOC exists across a continuum of microbial accessibility ranging from free, unprotected particulate materials and dissolved organic matter to organics that are stabilized against biodegradation through association with mineral surfaces and/or occlusion within soil aggregates (Figure 2 in Lehmann and Kleber, 2015). Under this model, interactions of the microbial community and soil minerals, rather than characteristics inherent to the SOC itself, are the primary regulators of the pathways of organic matter stabilization and

biodegradation. *These factors may more accurately predict the behavior of SOC pools and are also more easily assessed, leading to new possibilities for development of management strategies to increase the stability and/or abundance of SOC.*

Sorption to mineral surfaces and occlusion within aggregates are the basic mechanisms of SOC stabilization under the emerging paradigm. Whether organic matter is occluded or adsorbed to the surface of mineral soil particles depends partly on SOC quality. In the past SOC quality has been simplified to the C:N or lignin:N. However, development of the concept of substrate use efficiency, i.e. the proportion of substrates assimilated versus mineralized or respired, has found that the ability of an organism to effectively decompose and transform the structural components of organic matter into stabilized SOC is related to SOC quality and the composition of the microbial community itself (Cotrufo et al., 2013).

Stabilization of carbon entering the soil system also depends on the carbon content of the soil horizon it enters. Carbon entering a carbon-rich or carbon-saturated surface horizon may accumulate at a constant rate but have a very short residence time. On the other hand, carbon entering a carbon-poor soil horizon has more potential to be stabilized in a mineral-associated pool because of the larger proportion of reactive mineral surface area. Because surface soil horizons in forest soils are near saturation, decreases in inputs may result in a loss of carbon, while an increase in inputs is not likely to result in any change to mineral stabilized carbon pools. Mineral soil below the A-horizon has much lower carbon concentrations and therefore may have the capacity to stabilize newly added carbon. *We propose to examine mechanisms of resilience in SOC in surface and sub-surface mineral soils to determine if carbon saturation concepts are validated across forest type and depth.*

SOC sources. Post-disturbance dynamics of SOC are dependent on the source of organic matter. Litterfall, root turnover and rhizodeposition are the major inputs of carbon to soil carbon pools and are closely related to net primary productivity (Schlesinger 1997). Many studies have concluded that most SOC within the mineral soil is derived from belowground sources such as roots (Rasse et al., 2005; Mendez-Millan et al., 2010; Schmidt et al., 2011; Jackson et al., 2017). Jackson et al. (2017) found that roots account for over 80% of mineral soil carbon. The mechanisms for the conversion of roots to SOC include their chemical composition and their proximity to mineral particles that can stabilize the organic matter through aggregate formation and mineral-organic matter interactions. The aforementioned studies have all focused on agricultural soils, which do not have O-horizons with abundant aboveground litter. The few studies that have analyzed forest soils have found a much higher dependence on aboveground sources. Biomarkers are one way to elucidate the sources of SOC in soils. Using substituted fatty acids in a hardwood forest, Crow et al., found that in the top 5cm of the mineral soil in a younger forests had a 50/50 split in the surface while older forests had about 75% from aboveground sources. This finding suggests that older forests have a higher fraction of SOC sourced from the O-

horizon. Possibly as a result of a higher efficiency of producing DOC from older and deeper O-horizons. In another study, Crow et al. found that the top 5cm of mineral soil a deciduous forest appeared to derive about 80% of the surface mineral soil carbon from roots, while a coniferous forest appeared to be dominated by aboveground sources. Forest development (i.e. age) and species composition appear to control whether the source of carbon is from above or belowground sources, but there are very few studies describing these trends. *By characterizing sources of carbon across a wide range of forests with and without aboveground materials we will elucidate the source of SOC and the source of SOC's resilience in response to severe aboveground disturbance.*

Effects of disturbances on SOC. Clear-cut harvesting and high severity forest fire alter soil carbon cycling by cutting off the supply of root and litter inputs (killing trees, removing/consuming biomass). Furthermore, the action of clearing aboveground biomass, disturbs the soil surface and changes temperature and moisture regimes, which tends to increase heterotrophic respiration rates (Figure 1). A few large studies, meta- analyses, and review articles conclude that the net-effect of harvest is a reduction in SOC, with forest and soil type determining the magnitude of C loss (Johnson and Curtis, 2001; Jandl et al., 2007; Nave et al., 2010). Nave et al. (2010) reported an 8% average reduction in SOC stocks after

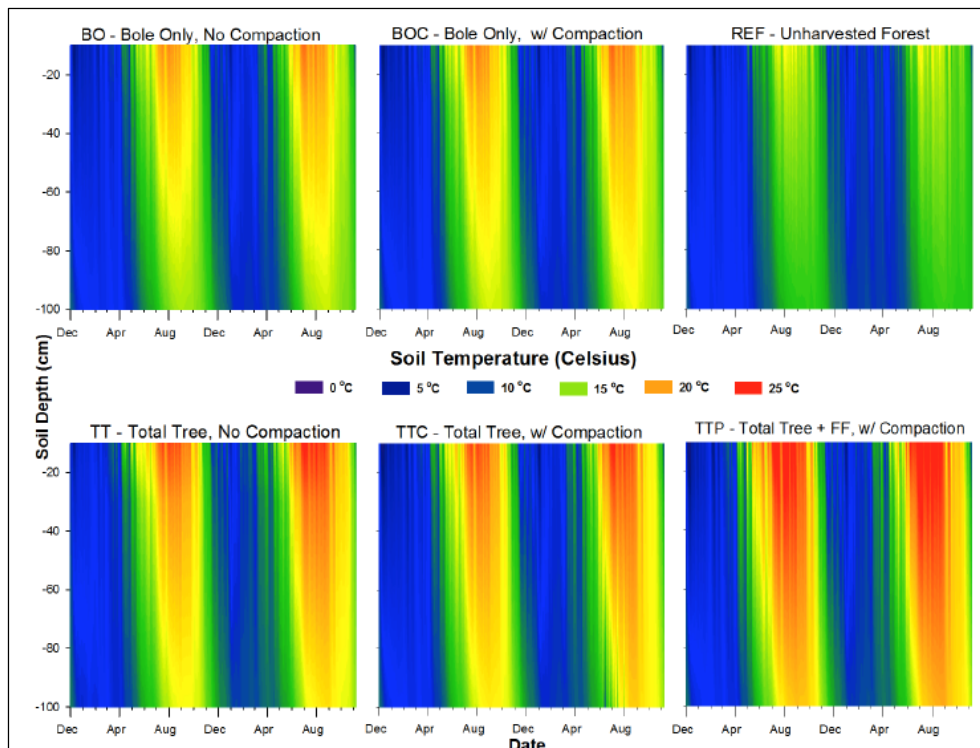
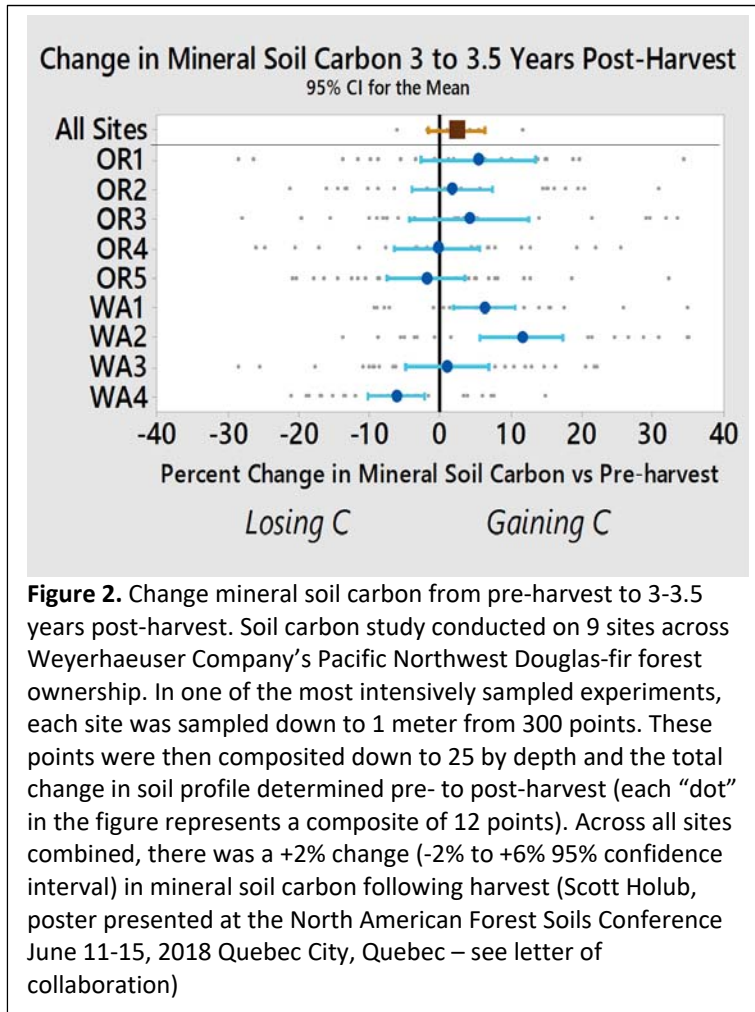


Figure 1. Two-year soil temperature patterns following intensive organic matter and compaction manipulations at the NARA LTSP site. Soil probes were installed at 10, 20, 30, and 100cm mineral depth recorded hourly but represented on daily time steps. A linear average is used to interpolate between all probes (from Gallo, 2016) .

harvesting over all forest and soil types studied. Most of this impact was a result of losses to the O-horizon. Holub (2018) showed no net effect of forest harvesting on SOC in managed Douglas-fir stands 3.5 years after harvesting (Figure 2 from Gallo, 2016). Reductions in SOC as a result of forest harvesting has been shown to come as a



result of soil disturbance during harvesting and site preparation (Achat et al., 2015; James and Harrison, 2016). With a few exceptions, across many soils, mineral associated SOC is not impacted by harvesting. The exceptions include Inceptisols, deep soil horizons from Spodosols (e.g. Bh and Bs horizons), and surface horizons from Ultisols. Stabilization of SOC in Inceptisols may be limited by the amount of active iron and aluminum minerals available for SOC stabilization. While the surface soil horizons of Ultisols and the illuvial soil horizons of Spodosols may be especially reliant upon aboveground sources of carbon. *We will examine both the factors of stabilization (e.g. active iron and aluminum minerals) as well as the source (e.g. above versus belowground) of SOC in order to determine the mechanisms of resistance and resilience of SOC in response to disturbance.*

Fire, whether prescribed or wild, has important effects on SOC that are tied to fire intensity and duration of heating (Neary et al., 1999). Fire that mineralizes surface organic matter will reduce total carbon pools (SOC plus forest floor) and organic matter inputs to the soil. At least partial consumption of the O horizon is common in wildfires, especially in shrubby or forested sites with high fuel loading near the surface. Depending on soil bulk density and parent material, soil heating is usually strongly attenuated with depth in the mineral soil profile, and depths as shallow as 2.5 cm may be well-buffered from SOC combustion during surface fires (DeBano et al., 1998). The impact of fire (particularly moderate to high-severity) on soils is very difficult to assess due to the lack of available controls or pre-fire sampling, and very few studies have actually managed to examine pre- and post-severe-fire effects on soil across many soil types to examine the factors influencing post-fire SOC resilience. *Here we will utilize several sites where organic matter has been mechanically removed and appropriate controls and pre-treatments sampling and*

data collection have been conducted and archived as a way to examine the secondary effects of fire (changing the post-fire soil temperature and moisture environment) on SOC.

Resistance, resilience, and SOC. Vulnerability of soil carbon (mineral soil and O-horizons) refers to the susceptibility of soil carbon to change in the face of a perturbation. This could include either increases or decreases, but usually the concern is with loss of soil carbon. Vulnerability of soil carbon can be described in terms of resilience and resistance. Soil carbon could resist losses as the result of a perturbation, or it could be resilient and recover soil carbon lost due to the perturbation. A system that is not affected by disturbance is thought to be resistant to change. However, a system that loses soil carbon as a result of a disturbance, and regains lost soil carbon post-disturbance, is resilient but not resistant.

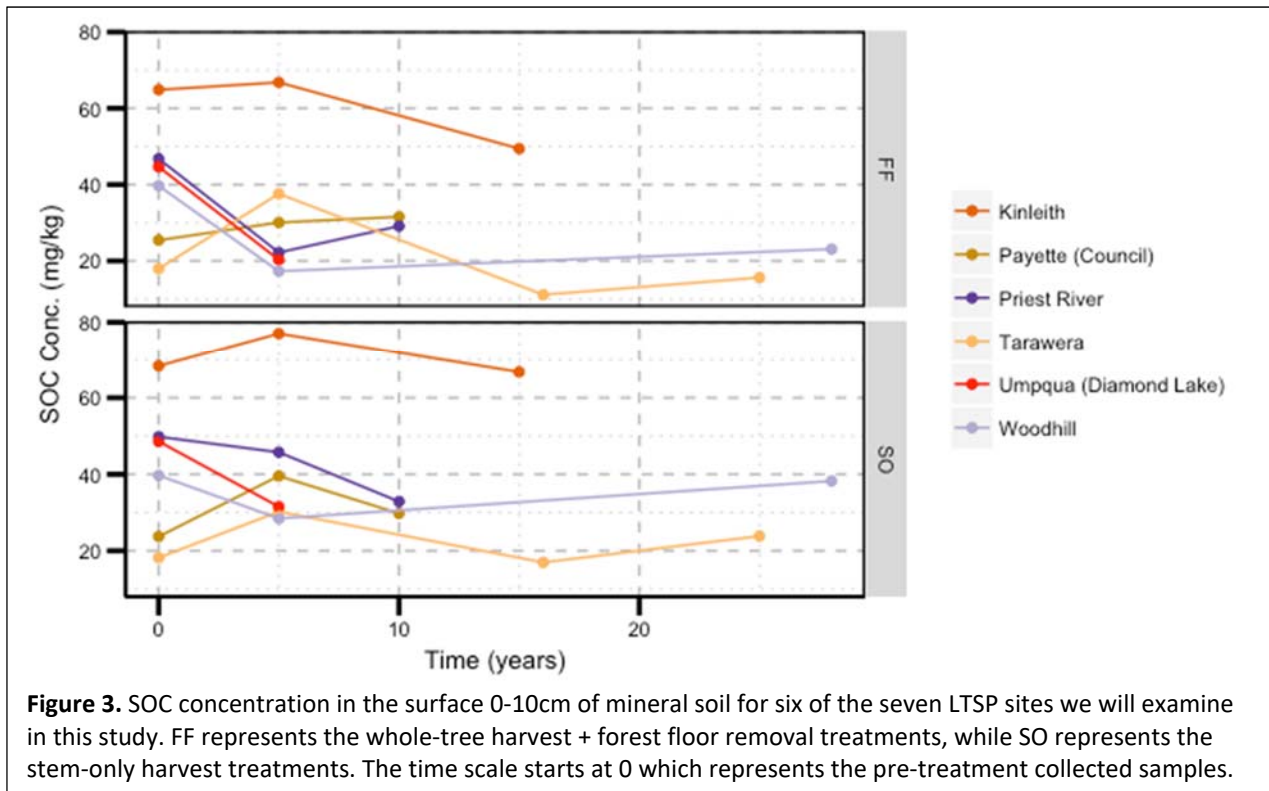
With this new SOC paradigm comes a fresh approach to study and predict the response of SOC to disturbance or management. The stability of any pool is dependent on the magnitude of, and controls on, its fluxes. The inputs are the quantity and quality of C fixed by the primary producers and altered by abiotic processes (i.e. fire), while the outputs are regulated by microbial accessibility, and microbial activity. Anything that changes the 1) quantity of organic matter inputs, 2) quality of organic matter inputs, 3) microbial accessibility, or 4) microbial activity will affect the magnitude and stability of the soil carbon pool.

- 1) Quantity of organic matter inputs - shifts in productivity or by the removal of biomass by fire or harvesting.
- 2) Quality of organic matter inputs - change in species, allocation of production (esp. below vs. aboveground production); large inputs of above or belowground residuals as a result of disturbance.
- 3) Microbial accessibility - destruction of aggregates; destabilization of redox-active minerals; etc.
- 4) Microbial activity - Change in soil temperature and moisture.

Our proposed research will examine these important controls on SOC stabilization and destabilization as a result of a common experimental manipulation across a suite of soils and forests that will determine the site- and soil-specific controls on SOC dynamics in response to disturbance.

1.3 Preliminary Data

We propose to utilize sites from a long-term soil productivity study that examined the effects of extreme aboveground organic matter manipulations at many sites across the world. We have selected seven sites with frequent sampling and available archives of



samples and data (Figure 3). These sites also display very different responses to stem-only (SO) harvesting and complete removal of all aboveground biomass (whole-tree harvesting + forest floor removal; FF). Payette and Tarawera show little loss over the first decade of treatment of FF or SO and would represent those sites that may be resistant and resilient after severe disturbance. The gains in SOC in Payette and Tarawera after both SO and FF treatments suggest that belowground residuals are contributing to the resilience of these soils. The increase in SOC concentration of Kinleith after SO, but not FF suggest that aboveground residuals were important at this site for contributing to its resilience. On the other hand, Priest River, Umpqua, and Woodhill appear to lose SOC after both SO and FF treatments suggesting that these sites are neither resistant nor resilient. Priest River and Woodhill appear to maintain more SOC after SO treatment so maybe slightly resilient due to aboveground residuals. *We propose to examine the mineralogy, trends in source, and SOC mean age across time across these sites to elucidate the mechanisms that create resistance and resilience to SOC loss in the face of fairly extreme biomass removal.*

We have performed some preliminary work at our NARA site in Western Oregon (Figure 4). Total carbon increases for both treatments immediately post-treatment suggesting an influx of belowground residuals to the SOC pool (FF treatments had no aboveground residuals). After 2 years we assessed cutin and suberin biomarkers representing shoot and root contributions, respectively, and found that there is evidence of a large input of suberin biomarkers indicative of root contributions in both treatments. The work we propose here will determine if resilience as a result of belowground residuals is a

common effect across a range of sites exposed to similar organic matter removal treatments.

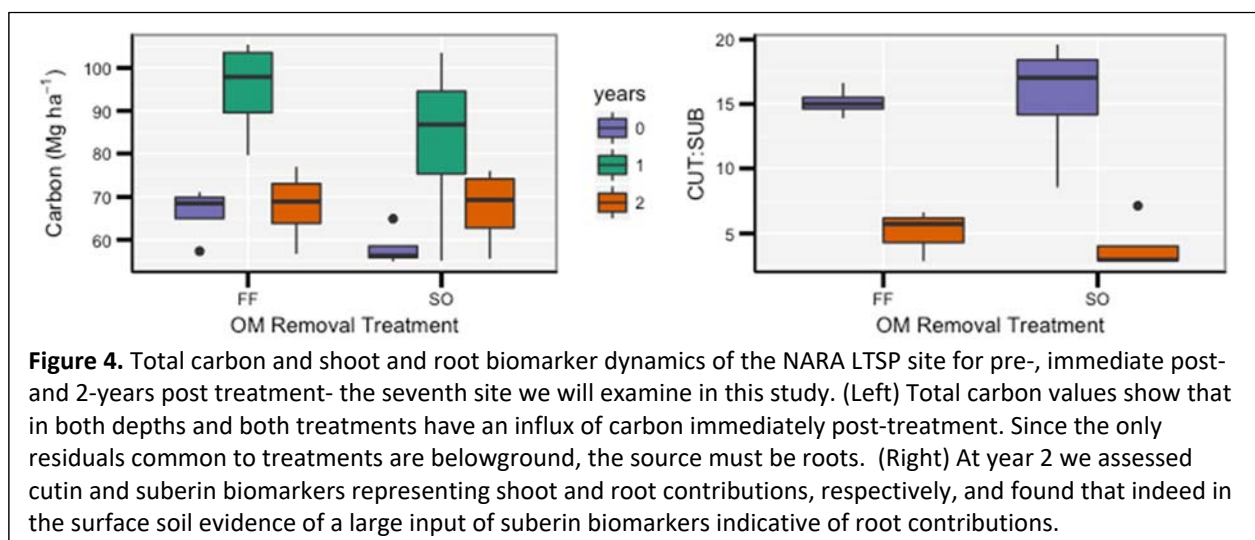


Figure 4. Total carbon and shoot and root biomarker dynamics of the NARA LTSP site for pre-, immediate post- and 2-years post treatment- the seventh site we will examine in this study. (Left) Total carbon values show that in both depths and both treatments have an influx of carbon immediately post-treatment. Since the only residuals common to treatments are belowground, the source must be roots. (Right) At year 2 we assessed cutin and suberin biomarkers representing shoot and root contributions, respectively, and found that indeed in the surface soil evidence of a large input of suberin biomarkers indicative of root contributions.

2. RATIONALE & SIGNIFICANCE

2.1 Rational

The Bioenergy, Natural Resources, and Environment Program’s function is to support work that is to “improve, and maintain healthy agro-ecosystems and the natural resources that are essential to the sustained long-term production of agricultural goods and services.” The program area’s description further explores this concept, stating that: “Sustainable management of agroecosystems requires the maintenance of the supporting natural resources and ecosystem services (ecosystem services are the benefits people obtain from ecosystems that fall into four categories of supporting, provisioning, regulating and cultural, including genetic resources, water quality, air quality, pollinator habitat, carbon sequestration, nutrient cycling and recreation).” The project we have described here will improve the management of soil carbon. By understanding the mechanisms that impart resilience to forest SOC stocks after extreme disturbances we will be able to understand which soils are most vulnerable to losses and degradation and develop management strategies that maintain or enhance forest SOC resistance and/or resilience in all managed settings.

The Sustainable Agroecosystems: Functions, Processes and Management program goals are to support projects that represent “foundational research to advance scientific understanding of processes and interactions”. Projects in this program area “should lead to substantial improvements in soil health” by “using *innovative approaches, tools and technologies* to enhance the understanding of the physical and biogeochemical processes affecting the fluxes, fate and transport, transformation, and storage of the critical

components of agroecosystems, as well as chemicals and agents that threaten them”. By developing a stronger understanding of forest SOC resilience to perturbation we will better be able to inform managers of the soils, sites, and situations that 1) require special care to maintain SOC or reduce SOC losses, and 2) are particularly resilient to the activity they are proposing (e.g. prescribed fire, timber harvest), and then 3) begin to develop strategies that enhance SOC storage in soils.

2.2 Significance

Soils of the US store about 73.4 Pg OC in the top 1 m (Sundquist et al., 2009). Agricultural soils contain only about 37% of the SOC in the US, so most of the SOC is in non-intensively managed lands such as forests (34%) and rangelands (29%). The ability to manage forest SOC is very limited in forest soils as a result of our lack of knowledge regarding the mechanisms on how disturbances or management impact SOC. Granted there are many papers on harvesting and fire impacts on soil carbon. James and Harrison (2016) used 112 publications to examine 945 responses (experimental units normalized by a control) in a meta-analysis to examine *harvesting impacts*. Nave et al. (2011) accessed 57 publications to acquire 468 responses in their meta-analysis of *fire effects* on SOC. Both studies found that indeed, harvesting and fire impact mineral associated SOC, but *despite all these studies examining harvesting and fire’s impact on SOC very few if any have explored the mechanisms behind the response or apparent lack of response* (for example Figure 4 would show a lack of response if we did not measure immediately after harvesting and did not measure biomarkers). Both of the aforementioned meta-analyses were left with significant questions about why some sites and soils were impacted and others were left unchanged by these significant perturbations to the ecosystem carbon cycle. We propose here to utilize a common experimental design installed across a range of soils and climates to determine the mechanisms responsible for creating resilience or resulting in vulnerability in SOC’s response to disturbance. We will use the latest knowledge in the emerging SOC paradigm and most recently developed techniques in examining SOC sources and dynamics to determine the resilience and vulnerability of SOC to disturbance.

3. APPROACH

Fires and forest harvesting intrinsically remove organic matter; these exports impact soil carbon stores of forests. Post-fire and -harvest impacts will manifest as changes to organic matter inputs and modification of the post-disturbance soil environment (i.e. soil moisture and temperature). Therefore, organic matter manipulation experiments are needed to elucidate. Many studies focused on the effects of forest harvesting have been conducted utilizing treatments ranging from bole-only removal to whole-tree to whole-tree

plus forest floor removal to examine the impacts of organic matter removal on biomass harvesting sustainability. These studies have been named Long-Term Soil Productivity experiments and have been installed all over the world. We will utilize archived samples from several Long-Term Soil Productivity (LTSP) Experiments located across the globe in a wide variety of soil, climate, and forest types in order to examine the trends across important SOC stabilization and destabilization factors.

Table 1. Sites available with pre- and post-treatment soil samples. *this study

Site	Country	MAT (°C)	MAP (mm/yr)	Planted Species	Sampled Years (0=pre-treatment)	Soil Order (USDA)	Soil Texture
Payette (Council)	USA	3.9	551	<i>Tsuga Heterophylla</i> and other species	0, 5, 10, 26*	Alfisol	Clay Loam
Priest River	USA	7	817	<i>Abies grandis</i>	0, 5, 10, 26*	Alfisol	Loam
Umpqua (Diamond Lake)	USA	10.4	1216	<i>Pseudotsuga menziesii</i>	0, 5, 26*	Ultisol	Sandy Loam
NARA	USA	11.4	1600	<i>Pseudotsuga menziesii</i>	0, 1, 2, 6*	Inceptisol and Ultisol	Loam to Silty Clay Loam
Kinleith	New Zealand	13.2	1420	<i>Pinus radiata</i>	0, 5, 15	Andisol	Loam
Tarawera	New Zealand	14	1820	<i>Pinus radiata</i>	0, 5, 16, 25	Entisol	Sandy Loam
Woodhill	New Zealand	14.3	1330	<i>Pinus radiata</i>	0, 5, 28	Entisol	Sand

Site Descriptions: The seven sites to be examined in this study are described in Table 1. These sites were selected due to their range in soils and response to these perturbations (Figure 3 and 4), and also for the presence of data and soil sample archives. All sites have pre-treatment soils present in their respective archives and available for this study (see letters of collaboration). All studies also have data and soils samples collected at year 5 of the study (we will collect year 6 soil samples at NARA as part of this study). All sites also have samples collected, or will as a result of this study, samples and data from year 10 or older.

The New Zealand sites are all second rotation radiata pine (*Pinus radiata*) plantation forest. The US sites are either Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*)



Figure 5. Location of 7 sites we propose to study in this proposal. (Upper left) 3 sites in the Pacific Northwest of the United States. (Lower Right) 4 sites in New Zealand. These sites span a $>10^\circ$ range in MAT, $>1\text{m}$ in MAP, and 5 soil orders. Furthermore, Figure 3 shows that these sites also span a range in SOC response to disturbance.

or mixed conifer with western hemlock (*Tsuga heterophylla*). Soils span a range of ages from the youngest Entisols to the older Alfisols and Ultisols. These soils also span a range of parent materials. Tarawera, Umpqua, and Kinleith have igneous parent materials including basalt tephra, pumice, and pumaceous tephra, respectively. These volcanic parent materials resulted in the development of andic characteristics at Kinleith which is classified as an Andisol. Both Payette and Preist River are both influenced by volcanic ash in their surface, but are underlain by basalt and glacial till, respectively. The soils at Woodhill are forming in eolian sand, while the soils at NARA are forming in colluvium and residuum derived from sandstone and tuffaceous rock.

Treatment descriptions: In almost all cases stands were clear-cut harvested and at least two common harvest residue treatments were installed at each site were: **whole-tree harvest plus forest floor removed (FF)** and **stem-only harvest with harvest residues (SO)**. At most sites there were 3-4 replicates (3-4 blocks) made up of 400 sq. m and larger plots. Each site was planted with the commercial tree appropriate for the area (Table 1).

Hypotheses testing: Forest soils can resist losses in SOC or be resilient against C losses by recovering C within stabilized pools within a short period of time. Soils that are resistant to losses will show little loss of total carbon (clearly), and little evidence that SOC

was replaced with younger carbon from a different source. On the other hand, soils that are resilient to SOC losses will show little loss of total carbon, but some of the carbon will be replaced by whatever source that is creating the resilience (above or belowground residuals of post-disturbance primary production). By examining the nature of SOC and studying the assemblages of biomarkers, ^{14}C abundances, and the ratios of stable isotopes in stabilized and unstabilized pools of SOC across a range of soils from a broadly applied biomass experiment we will determine the source of new C in recently disturbed soils. Using these methods, we will determine the quantity and source (belowground or aboveground and fresh versus degraded) of C contributing to the resilience of the SOC.

By utilizing the LTSP treatments we will be able to further pinpoint mechanisms involved in the resistance or resilience of SOC to change. The FF treatment will allow us to examine the effect of complete aboveground biomass removal on mineral SOC resistance and resilience and would mimic the effects of fire and other extreme disturbances without the effects of ash and soil heating. By comparing the SO to the FF we will be able to compare the effect of aboveground residual biomass (O-horizon and harvest residuals) on contributing to the resilience of SOC to disturbance.

Table 2. Hypothetical responses of SOC to LTSP treatments (Stem-only (SO) and whole-tree + forest floor removal (FF))

Mechanism	Expected shift in source	Expected shift in $\Delta^{14}\text{C}$ age
Resist Δ in SOC	No change Negative relationship between SOC loss and aggregate and mineral associated SOC, clay content + active iron and aluminum	SO & FF: SOC age = pre-treatment + time since disturbance
Resilience from aboveground residuals	SO: \uparrow shoot biomarkers immediate to 5 years post-treatment FF: No change	SO: SOC age < pre-treatment immediate to 5 years post-treatment FF: SOC age = pre-treatment + time since disturbance
Resilience from aboveground production	SO & FF: \uparrow shoot biomarkers a decade post-treatment	SOC age < pre-treatment a decade post-treatment
Resilience from belowground residuals	SO & FF: \uparrow root biomarkers immediate to a few years post-treatment	SO & FF: SOC age < pre-treatment immediate to 5 years post-treatment
Resilience from belowground production	SO & FF: \uparrow root biomarkers a decade post-treatment	SO & FF: SOC age < pre-treatment a decade post-treatment

The suite of variables we will collect on SOC age and source will also allow us to examine whether multiple mechanisms are at play (for example clay + active iron and aluminum may modulate losses in SOC). Density fractions will allow us to examine the mechanisms further by examining specific mechanisms of stabilization within the SOC pool.

3.1 Objective #1: – Determine the mechanisms of resistance in SOC severe disturbances across a wide range of soils and forest types.

3.1.1 Introduction

The new paradigm of SOC stabilization purports that stabilized SOC is that which is associated with a mineral surface or entrained within soil aggregates. Furthermore, our understanding of the role of mineralogy continues to evolve, with recent work highlighting iron- and aluminum-oxyhydroxides as having a disproportionate amount of control on SOC stabilization in forested systems (Rasmussen et al., 2018). We hypothesize that those forests that initially have a higher proportion of mineral and aggregate stabilized SOC and/or a high proportion of iron- and aluminum-oxyhydroxides will resist changes as a result of loss of aboveground biomass and that the form of SOC will have no effect on resistance to loss.

3.1.2 Approach

We will analyze **two mineral soil depths** (0-15 and 15-30cm typically) and **O-horizons** across at-least **three timepoints** (0, 5, and >10 years typically) from **two treatments** (FF & SO) **replicated 3-4 times/site** on the **seven sites** listed in Table 1. We will determine the stable isotopic composition and C & N content on all soil samples (~290 samples). We will analyze the iron- and aluminum-oxyhydroxides and particle size distribution from each of the pre-treatment mineral soil samples (~85 samples). We will composite across replicates and perform density fractionations on 84 mineral soil samples resulting in 3 fractions – unstabilized light fraction (LF), and the stabilized heavy (HF) and aggregate occluded fractions (oLF) (~250 density fractions). We will use ANOVA and multivariate regression techniques to determine the factors most important for controlling SOC dynamics in response to disturbance.

3.1.3 Expected Outcomes

We hypothesize that soils that are resistant to change will have greater portions of their soil carbon associated with the heavy or occluded light fractions. This framework is basically not too different from what would be predicted from the theory of carbon saturation (i.e. a larger portion of SOC is destabilized with increasing concentrations) since soils with lower carbon will have a larger portion of SOC associated with the stabilized mineral fraction as HF or oLF. Our framework (unlike the C-saturation theory) will allow to examine the specific mechanisms by stabilization mechanism (mineral sorption or occlusion). We also predict that soils with high quantities of iron- and aluminum-oxyhydroxides and clay will resist changes in SOC due to FF or SO treatments.

3.2 Objective #2: Determine the mechanisms of resilience in SOC to severe disturbances across a wide range of soils and forest types.

3.2.1 Introduction

The source of SOC in mineral soils has been found to be dominated by roots in agricultural soils, but maybe more dominated by aboveground sources in forests (Rasse et al., 2005; Crow et al., 2009b; a; Mendez-Millan et al., 2010; Jackson et al., 2017). Little work has been done in forests or at depth. We will characterize the source of SOC across seven forests and further explore the dynamics of SOC as a result of disturbance to elucidate the source of resilience.

3.2.2 Approach

Similar to section 3.1.1 We will analyze **two mineral soil depths** (0-15 and 15-30cm typically) and **O-horizons** across at-least **three timepoints** (0, 5, and >10 years typically) from **two treatments** (FF & SO) **replicated 3-4 times/site** on the **seven sites** listed in Table 1. We will composite across replicates and perform density fractionations on 84 mineral soil samples resulting in 3 fractions – unstabilized LF, and the stabilized HF and oLF (~250 density fractions). All composited whole soils and density fractions will be analyzed for free lipids, CuO oxidation products, black carbon, and ¹⁴C-derived mean age.

Since we are examining treatments with and without aboveground residues we will be able to isolate the impact those ecosystem components have on the resilience of SOC to change. We will examine free-lipid and CuO oxidation products to determine the dominant source of SOC across treatments, depth, and time since treatment. Because black carbon may resist decomposition due to its chemical structure (and not association with mineral surfaces) we will assess the BPCA content of all whole soils and density fractions. Finally, by assessing the ¹⁴C age of SOC we can determine if fresh or old carbon is replacing native carbon as a result of these disturbances.

3.2.3 Expected Outcomes

We expect that at pre-treatment sites with SOC dominated by aboveground sources will be resilient to SO treatments but not FF. Sites that are dominated by belowground sources at pre-treatment will be resilient to both SO and FF. By measuring soils collected 10+ years from treatment we will be able to determine if subsequent production of biomass has resulted in any resilience to SOC by replacing SOC lost as a result of the harvest due to faster growth of crop-trees.

3.4 Specific methods and procedures

Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), carbon and nitrogen contents, and C:N - All of the sites have C:N contents on the <2mm size fraction from previous studies. Using the elemental composition from the stable isotopic analysis we will perform an internal calibration of this data to correct bias from inter-lab variation. The stable isotopic compositions of C and N of soils will be determined on pre-acidified samples using high temperature combustion coupled with isotope ratio mass spectrometry (Goni et al., 2005; Hatten et al., 2012). These

analyses will be conducted at the Oregon State University College of Earth, Ocean, and Atmosphere Science Stable Isotope Lab.

Sequential biomarker analysis free lipids and CuO Oxidation - Dried soils and sediments will be analyzed using a sequential procedure for free lipids and CuO oxidation products (Goñi and Montgomery, 2000; Hatten et al., 2012; Goñi et al., 2013; Pisani et al., 2014, 2015, 2016; Hatten and Goñi, 2016) and the extracts analyzed by GC/MS to obtain the yields of a variety of products derived from different biochemical precursors and biological sources. We will quantify several classes of reaction products, including n-alkanes, long-chain alcohols, and free-fatty acids that are markers of microbial and vascular plant inputs and can be used to distinguish between above and belowground sources of SOC (Pisani et al., 2014, 2015, 2016). CuO oxidation releases biomarkers such as vanillyl phenols, syringyl phenols and cinnamyl phenols that are characteristically derived from different lignin sources (Hedges and Mann, 1979). We will also quantify several cutin- and suberin-derived C16 to C18 hydroxy fatty acids that make up the chemical backbone of cuticles and Casparian strip in roots and other hydrophobic structures in plant leaves and roots (Goni and Hedges, 1990; Crow et al., 2009b; a; Goñi et al., 2013). In addition to these vascular plant biomarkers, we will quantify the yields of various benzoic acids, which are derived from a variety of sources, including tannins, degradation products of phenolic compounds, and thermally altered carbon. Finally, we will quantify products derived from microbial sources including specific amino acids (Goñi and Hedges, 1995).

Black carbon analysis - We will analyze benzene poly-carboxylic acids (BPCA) after nitric acid digestion using the improved methods of Wiedemeier (2013). BPCA analysis will allow us to examine the controls that a recalcitrant component (i.e. black carbon or char) may have on SOC dynamics.

Radiocarbon - Soil samples will be graphitized in preparation for ^{14}C abundance measurement at the USDA Carbon, Water & Soils Research Lab in Houghton, Michigan. Samples will be dried, weighed into quartz tubes and sealed under vacuum. Samples will be combusted at 900 °C for 6 h with cupric oxide (CuO) and silver (Ag) in sealed quartz test tubes to form CO₂ gas. The CO₂ will be then reduced to graphite through heating at 570 °C in the presence of hydrogen (H₂) gas and an iron (Fe) catalyst (Vogel et al., 1987). Graphite targets will be analyzed for radiocarbon abundance and corrected for mass-dependent fractionation using measured $\delta^{13}\text{C}$ values according to (Stuvier and Polach, 1977).

Density fractionation - Composited bulk soils will be density fractionated (Swanston et al., 2005) to separate bulk SOC into three operationally defined pools: the free light fraction (LF), the occluded or intra-aggregate fractions (oLF), and the mineral-associated or dense fraction (HF). Previous work has indicated that density fractionation is an effective means of separating SOC into distinct pools of differing molecular composition and MRT (Crow et al., 2007; Wagai et al., 2009a; b; Schrumpf et al., 2013). Flotation in a dense fluid (Na

polytungstate) utilizes differences in density between organics and minerals to separate organics unassociated with mineral surfaces (LF) from organics intimately associated with mineral surfaces (HF). The incorporation of aggregate disruption by sonication or crushing into density separation methods allows for the isolation of a third fraction of SOC, the occluded fraction (oLF). The oLF, as isolated in the laboratory, is assumed to be sourced from within soil microaggregates which are dispersed during the density separation procedure. LF organics are generally composed of particulate organics which have undergone varying degrees of microbial processing along with pyrogenic matter, while HF organics are generally highly degraded materials and are enriched in microbial biomass and metabolites. Conceptually, LF organics represent the unstabilized pool of SOC, while HF and oLF organics represent pools of stabilized SOC protected from biodegradation by bonding to mineral surfaces and physical isolation of substrate C from microbial communities, respectively. The relative distribution of bulk SOC among these three fractions will be used as a proxy to represent the proportion of SOC in each soil that is not stable (LF), stabilized through organo-mineral complexation (HF), and stabilized through occlusion within aggregates (oLF).

Selective dissolution - Fe- and Al-humus complexes, Fe and Al and Si short-range order hydroxides, and crystalline Fe oxides will be measured through selective dissolution with pyrophosphate, ammonium oxalate, and citrate dithionite solutions (Dahlgren, 1994).

Particle size analysis - Particle size distribution will be determined by pipette method following standard methods described in Soil Survey Laboratory Methods Manual (Soil Survey Staff, 2009)

3.6 Timetable

Year ->	2019		2020				2021				2022	
Quarter	Su	F	W	Sp	Su	F	W	Sp	Su	F	W	Sp
Sample and field data acquisition												
Acquire samples from collaborators archive	■	■	■	■								
Travel to sites to collect endmember samples, and addition data and soil samples	■	■	■	■	■	■						
Laboratory Analysis												
Stable Isotope and C&N analysis					■	■	■	■				
Particle Size and Selective Dissolution analysis					■	■	■	■				
Density Fractionation							■	■	■	■		
Biomarker and BPCA analysis							■	■	■	■		
¹⁴ C analysis							■	■	■	■		
Project Dissemination												
Attend PI and national meetings			■				■				■	
Project meetings (Corvallis, OR)					■				■			■
Prepare dissertation								■	■	■	■	
Prepare manuscript											■	■