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INFLUENCE OF STAND COMPOSITION ON SOIL ORGANIC CARBON  
STABILIZATION AND BIOCHEMISTRY IN ASPEN AND CONIFER  
FORESTS OF UTAH

by

Mercedes Román Dobarco

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

of

DOCTOR OF PHILOSOPHY

in

Ecology

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Logan, Utah

2014

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## ABSTRACT

Influence of Stand Composition on Soil Organic Carbon Stabilization and Biochemistry  
in Aspen and Conifer Forests of Utah

by

Mercedes Román Dobarco, Doctor of Philosophy

Utah State University, 2014

Major Professor: Dr. Helga Van Miegroet  
Department: Wildland Resources

Quaking aspen (*Populus tremuloides* Michx.) is an iconic species in western United States that offers multiple ecosystem services, including carbon sequestration. A shift in forest cover towards coniferous species due to natural succession, land management practices, or climate change may modify soil organic carbon (SOC) dynamics and CO<sub>2</sub> emissions. The objectives of this study were to: (i) assess the effects of overstory composition on SOC storage and stability across the aspen-conifer ecotone, (ii) use Fourier transform infrared spectroscopy attenuated total reflectance (FTIR-ATR) to assess whether SOC storage is associated with preferential adsorption of certain organic molecules to the mineral surfaces, and (iii) develop models using near-infrared reflectance spectroscopy (NIRS) to predict aspen- and conifer-derived SOC concentration. Mineral soils (0 – 15 cm) were sampled in pure and mixed aspen and conifer stands in Utah and subjected to physical fractionation to characterize SOC stability (i.e., SOC protected against microbial decomposition), long term laboratory

incubations (i.e., SOC decomposability), and hot water extractions (i.e., SOC solubility). Vegetation cover had no effect on SOC storage ( $47.0 \pm 16.5 \text{ Mg C ha}^{-1}$ ), SOC decomposability (cumulative released  $\text{CO}_2\text{-C}$  of  $93.2 \pm 65.4 \text{ g C g}^{-1} \text{ C}$ ), SOC solubility ( $9.8 \pm 7.2 \text{ mg C g}^{-1} \text{ C}$ ). Mineral-associated SOC (MoM) content was higher under aspen ( $31.2 \pm 15.1 \text{ Mg C ha}^{-1}$ ) than under mixed ( $25.7 \pm 8.8 \text{ Mg C ha}^{-1}$ ) and conifer cover ( $22.8 \pm 9.0 \text{ Mg C ha}^{-1}$ ), indicating that aspen favors long-term SOC storage. FTIR-ATR spectral analysis indicated that higher MoM content under aspen is not due to higher concentration of recalcitrant compounds (e.g., aliphatic and aromatic C), but rather to stabilization of simple molecules (e.g., polysaccharides) of plant or microbial origin. NIRS models performed well during calibration-validation stage (ratio of standard deviation of reference values to standard error of prediction (RPD)  $\geq 2$ ). However, model performance decreased during independent validation (RPD = 1.2 – 1.6), probably due to the influence of soil texture, mineralogy, understory vegetation, and land history on SOC spectra. Further improvement of NIRS models could provide insight on SOC dynamics under potential conifer encroachment in semiarid montane forests.

## PUBLIC ABSTRACT

Influence of Stand Composition on Soil Organic Carbon Stabilization and Biochemistry  
in Aspen and Conifer Forests of Utah

Mercedes Román Dobarco, Doctor of Philosophy

Social concern about climate change and the elevated level of atmospheric CO<sub>2</sub> demands understanding carbon (C) storage and dynamics in forest soils, especially since soils are the largest C reservoir in terrestrial ecosystems, storing two thirds of total C. Quaking aspen (*Populus tremuloides* Michx.) is an iconic species in western United States that offers multiple ecosystem services, such as habitat and forage for wildlife and cattle, biodiversity, water yield, and C storage. A decline in quaking aspen cover has been documented during the last decades, possibly due to fire suppression and ungulate browsing. A shift from aspen- to conifer-dominated forests may modify the amount and properties of soil organic carbon (SOC) in montane forests in Utah, affecting the C balance at ecosystem or even regional level. This study tested the influence of overstory composition on SOC storage, stability (i.e., protection against microbial decomposition), and chemical composition along the transition between aspen and conifer forests in northern and southern Utah. This study indicates that increasing presence of aspen in the overstory is associated with greater SOC stability (i.e., longer residence time), but that site conditions also play an important role. Understanding the factors that control SOC dynamics can facilitate management recommendations towards increasing long-term C sequestration at those sites where vegetation exerts the strongest influence on SOC storage.

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Mercedes Román Dobarco

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## CHAPTER 1

### INTRODUCTION

Concern about climate change and the elevated concentration of CO<sub>2</sub> in the atmosphere has increased the interest in quantifying carbon sequestration by forest ecosystems and understanding the processes controlling the stability of soil organic carbon (SOC). Changes in vegetation cover (Jobbagy and Jackson, 2000) or forest management practices (Jandl et al., 2007) can modify the balance between inputs and outputs of carbon within the soil. Over time, this balance determines the total amount of SOC stored. Because soils contain two thirds of the total carbon in terrestrial ecosystems (Dixon et al., 1994), losses or gains of SOC can result in great variations in carbon balance at a regional scale (Schlesinger and Andrews, 2000). However, the response of SOC stocks to climate, land management, or vegetation shifts depends on the persistence of the SOC, which in turn is influenced by SOC intrinsic properties and interaction between SOC and the soil matrix (Six et al., 2002; Wagai et al., 2008; Schmidt et al., 2011). It is necessary to assess the differences in SOC properties among different vegetation species, including transitional stages, to improve our understanding of how changes in vegetation cover may alter the storage of SOC. This research will use forest systems of quaking aspen (*Populus tremuloides*) and mixed coniferous species (*Abies lasiocarpa*, *Abies concolor*, *Picea engelmannii*) to investigate the influence of species composition on SOC storage and stabilization mechanisms.

## **SOC stabilization**

The term SOC comprises all the organic materials found in the soil, derived from various biological sources (plant, faunal or microbial biomass) and in different states of decomposition (Baldock, 2002). Plant debris accumulates on the soil surface (aboveground litter) or is incorporated at variable depth in the soil (belowground litter), and undergoes physical and chemical changes due to the activity of soil fauna and microorganisms. Soil microbes perform a key role in SOC dynamics; microbes use SOC as source of energy and for biomass growth, and consequently release CO<sub>2</sub> as result of heterotrophic respiration (mineralization) and transform organic compounds into microbial biomass. Hence, the organic compounds used as metabolic substrate can be transformed, mineralized, or altered (i.e., enzymatic activity partly degrades a biomolecule, modifying its structure and composition, but the resulting product is not necessarily incorporated into the microbial biomass pool) (Baldock, 2002). Microbes generate new organic compounds (e.g., extra cellular polysaccharides, enzymes, cellular wall materials, etc.) that eventually are incorporated into the extra cellular space and interact with mineral particles and other organic compounds (Fig. 1.1). Multiple theories explain the recombination of soil biomolecules, either by biotic polymerization (enzyme mediated) or abiotic processes (condensation reactions involving the formation of covalent bonds or self-aggregation of small molecules into supramolecular structures), into what is commonly known as humic substances (Essington, 2003). However, the traditional concept of humic substances as relatively large and chemically undecipherable macromolecules is being replaced by the idea of SOC as aggregates of chemically

defined small biomolecules, like polysaccharides, proteins, organic acids, etc. (Schmidt et al., 2011; Schimel and Schaeffer, 2012).

As a result of the input of fresh organic matter and microbial activity, SOC exists in a continuum of compounds characterized by different chemical compositions and complexity, spatial distribution within the soil matrix, and decomposition rates (Trumbore, 1997; Baldock and Skjemstad, 2000; Rovira et al., 2010). SOC can be best conceptualized as an assemblage of various SOC pools, characterized by different turnover rates (Chan et al., 2002; Rovira et al., 2010). SOC pools with longer turnover rates are more stable (i.e., protected against decomposition) and thus contribute more effectively to carbon sequestration in the long term and may be less sensitive to changes in vegetation cover or management practices (Chan et al., 2002). A common methodology to assess the proportion of SOC of different persistence (Sollins et al., 1996; von Lützow et al., 2006) is to divide the soil into fractions based on physical, chemical, or biological fractionation techniques. However, the complexity involved in SOC stabilization makes it very difficult to determine functional SOC pools with homogeneous turnover rates despite the multiple fractionation methods available (von Lützow et al., 2007).

Stabilization of SOC can be defined as the mechanisms that protect SOC against microbial decomposition and hence lengthen the mean residence time of SOC in the soil. The mechanisms of SOC stabilization have been defined as (Six et al., 2002):

- 1) biochemical stabilization
- 2) chemical stabilization
- 3) physical protection

Biochemical stabilization depends on the intrinsic chemistry of organic matter, influenced by the presence and spatial configuration of certain functional groups (chemical recalcitrance) and chemical complexing processes (condensation reactions) that hinder microbial decomposition (Sollins et al., 1996; Six et al., 2002).

Chemical stabilization is due to strong binding of organic compounds to the surface of mineral particles becoming stable organo-mineral associations (Kleber et al., 2007). Different mechanisms responsible for chemical stabilization take place depending on soil forming processes, soil pH, soil exchange complex, soil mineralogy, surface functional groups of mineral particles (e.g., -OH, -O<sup>-</sup>) and functional groups of organic compounds (e.g., -COOH, -NH<sub>2</sub>) (von Lützow et al., 2006).

Physical protection defines the isolation of organic matter inside aggregates, limiting the accessibility of bacteria and fungi to the organic substrates (Sollins et al., 1996; Baldock and Skjemstad, 2000). The spatial distribution of soil particles, and therefore pore size distribution, determines the mobility of microbes and the diffusion of water, enzymes, and oxygen through the intra-aggregate space (von Lützow et al., 2006). Microbes, soil fauna and roots contribute to the cycling of aggregates by modifying the spatial distribution of particles or through binding agents like root exudates or extra-cellular polysaccharides.

When SOC is present as free particulate organic matter (POM) (i.e. less transformed plant, animal or microbial debris, with recognizable cellular structure), its persistence in the soil depends on its chemical recalcitrance and environmental constraints limiting decomposition (e.g. low soil temperature, low oxygen availability). POM is therefore more vulnerable to changes in climatic conditions and land



management practices (Six et al., 2002). In this sense, the factors controlling decomposition in the forest floor are relatively similar to the factors driving decomposition rates of free POM in the mineral soil, with more limitations for oxygen diffusion in the latter. The conditions for decomposition in the forest floor are very different to those in the mineral soil, where accessibility of microbes to organic substrates is more likely limited by the spatial arrangement of soil particles or sorption to mineral surfaces (Schimel and Schaeffer, 2012), rather than by the chemical composition of organic matter *per se*. Although there is evidence indicating that physical and chemical protection mechanisms are more effective in preventing decomposition than biochemical recalcitrance (von Lützow et al., 2006; von Lützow et al., 2007; Rovira et al., 2010; Kleber et al., 2011), the hierarchy of stabilization mechanisms is soil type specific (Spielvogel et al., 2008).

Soil mineralogy determines which organic compounds or biomolecular classes are preferentially adsorbed to mineral particles (Wattel-Koekkoek et al., 2001). Nonetheless, the biochemical composition of the original fresh debris may influence the potential of derived biomolecules (whether they have been altered, processed by microbes, or remain unmodified) to bond to mineral surfaces. The presence of certain functional groups, their hydrophobic or hydrophilic character, and the spatial conformation of organic compounds will condition their affinity with mineral particles (Gu et al., 1995; Kleber et al., 2007); simultaneously, higher input of a biomolecule will promote its stabilization in the mineral soil (Kaiser and Guggenberger, 2003). Hence, organic matter from different species will be stabilized in different proportion by the three stabilization mechanisms. For example, in Mediterranean forests, Scots pine (*Pinus sylvestris* L.) and Pyrenean oak

(*Quercus pyrenaica* Willd.) showed similar values of mineral-associated SOC, but higher amounts of POM under Scots pine (Díaz-Pinés et al., 2011). Laganière et al. (2011) found similar absolute values of SOC in the mineral-associated fraction of aspen and black spruce stands in boreal forests, with higher proportions of SOC in the spruce light fraction (6.13 %) than in the aspen light fraction (3.61 %). Mixed stands had intermediate values of both fractions.

Quaking aspen (*Populus tremuloides*) is a widespread species in North America that offers valuable ecosystem services like habitat and forage for livestock and game (DeByle, 1985), biodiversity (Kuhn et al., 2011), water yield (LaMalfa and Ryle, 2008), and carbon sequestration. Previous research on SOC stocks under aspen and conifer stands in northern Utah found a significantly higher content of total SOC under aspen stands ( $96.2 \pm 26.7 \text{ Mg C ha}^{-1}$ ) than under adjacent conifer stands ( $66.9 \pm 18.6 \text{ Mg C ha}^{-1}$ ) in the first 60 cm of mineral soil (Woldeselassie, 2009). Aspen-derived SOC was more stable than SOC from conifer soils, with higher proportion of mineral-associated SOC ( $55\% \pm 13\%$  in aspen vs.  $41\% \pm 13\%$  in conifers); whereas, in conifer soils the proportion of SOC present in the light fraction was higher ( $52\% \pm 23\%$  in conifers vs.  $39.5\% \pm 11\%$  in aspen) (Woldeselassie et al., 2012). Aspen decline due to land management practices (e.g., ungulate grazing and fire suppression) (Bartos and Campbell, 1998; White et al., 1998; Hessel, 2002) and climate change (Morelli and Carr, 2011; Hanna and Kulakowski, 2012), in combination with natural succession to conifers, could thus lead to an increase in the proportion of SOC in less protected fractions. When conifers establish in aspen stands, the amount and chemical nature of plant debris that enters the soil changes, altering soil chemistry (Binkley and Sollins, 1990; Calder et al., 2011) and carbon

balance (Berger et al., 2010). The proportion of SOC contained in different fractions varies from conifer to broadleaved species (Díaz-Pinés et al., 2011). It also differs between aspen and conifers, with differences further influenced by climate. The relative distribution of SOC among various fractions in aspen and black spruce in boreal forests (Laganière et al., 2011) differed from that found for aspen and conifers forest in the intermountain west (Woldeselassie et al., 2012), where the percentage of SOC in the light fraction is higher for both vegetation types (39.5 -52 % in the Intermountain West vs. 3.61 – 6.13 % in boreal forests).

### **Soil properties under mixed stands**

While we know that there are differences in the amount and distribution among fractions of SOC between aspen and conifers soils, much less is understood about the properties of SOC in mixed aspen-conifer stands from the Intermountain West. Soils under mixed stands, or in transition between two vegetation types (e.g. seral aspen stands with conifers present), may have intermediate properties relative to soil properties under pure vegetation types (e.g., broadleaved species vs. coniferous species). On the other hand, some soil characteristics may reflect the non-additive effects of the overstory species (i.e., the value of a soil property under an ideally mixed overstory is not intermediate to the values found under pure stands of both species) when the influence of one species, through microclimate, biomass input and chemistry, or nutrient cycling, is dominant. Very often, the rate at which plant detritus is decomposed in a mixed stand cannot be predicted from the dynamics of single-species litter, possibly due to changes in litter chemistry and structure affecting microbial activity (Gartner and Cardon, 2004).

The presence of palatable substrate may enhance the decomposition of more recalcitrant organic matter (priming effect) (Kuzyakov et al., 2007), and conversely, the presence of tannins and phenols can reduce the decay of otherwise easily decomposable litter (Berger et al., 2010). There is a vast literature on soil properties under mixed stands in temperate areas. For example, studies of mixed beech-spruce forests in Europe found that some features like soil respiration and soil nutrient content (Berger et al., 2010) had intermediate values to pure beech and pure spruce stands; whereas litter decomposition, carbon release (Berger et al., 2010), forest floor thickness and acidity, flux of nitrate (Rothe et al., 2002), root distribution or water uptake (Glatzel et al., 2000) were not additive with respect to species proportions. Royer-Tardif and others (2010) found that the structure of the microbial community under mixed stands of aspen and jack pine (*Pinus banksiana* Lamb.) from boreal forests of Canada was intermediate to that under aspen and jack pine pure stands. In general, the literature available on soil properties in mixed stands is not extensive for semi-arid areas, and is mostly limited to soil chemistry and organic matter concentration (Bartos and Amacher, 1998; Buck and St. Clair, 2012). Previous studies on SOC pools and SOC stabilization mechanisms under aspen and conifer forests in northern Utah focused on differences between pure stands (Woldeselassie, 2009; Woldeselassie et al., 2012), but the knowledge about SOC under mixed aspen-conifer stands in semi-arid environments is very limited. A first step towards understanding the potential effects of conifer encroachment on SOC pools is to characterize the properties of SOC in mixed stands and compare them with SOC properties in pure aspen and pure conifer soils. Moreover, studying the transition of SOC properties along a gradient with varying proportion of aspen and conifers on the

overstory can help to forecast the fate of SOC under different scenarios of conifer encroachment.

### **The signature of vegetation on SOC**

Characterizing the signature of vegetation in SOC would provide a better understanding of SOC dynamics under mixed stands and the fate of stabilized SOC with vegetation shifts. I define signature as the characteristic chemical composition, or assemblage of organic compounds that can be differentiated among species.

Soil organic matter undergoes successive transformations during decomposition. Chemical composition of litterfall and roots differs among forest species and, in interaction with the microbial community and the soil matrix, determines what biomolecules will be protected in each SOC fraction. Stabilized biomolecules can be residues of plant origin that remain after microbial degradation (e.g. small biomolecules released to the soil solution during the breakdown of litter; root exudates; subunits of larger molecules like lignin derived from partial degradation), or can have microbial origin (cellular components released after cell lysis, metabolic byproducts, etc). Less decomposed materials (e.g. POM) will have a chemical composition more similar to that from the initial debris, while more decomposed organic matter will differ in greater extent from the initial debris. In their review, von Lützow and others (2007) point out that mineral-associated SOC has a high degree of decomposition and is mainly derived from microbial metabolites. Complexed organic compounds (i.e. humic substances) are derived from microbial metabolites and components of microbial cell walls, produced by non-enzymatic polymerization of simple lipids (de Leeuw et al., 2006; Lorenz et al.,

2007), or are the remains from the selective degradation of less recalcitrant compounds (Sollins et al., 1996). Often, microbial community structure and biomass differ among vegetation types (Lucas-Borja et al., 2012) due to differences in environmental conditions (soil temperature, moisture, and pH) and the nature of the organic matter input (Leckie, 2005). Schimel and Schaeffer (2012) suggest that although microbial community composition may not control the rate at which soil organic matter is decomposed, major phylogenetic groups can influence the allocation of resources (e.g. extracellular polysaccharides, extracellular enzymes, cell wall compounds). If microbial communities associated with different overstory species produce an assemblage of organic compounds different enough from each other, this may be interpreted as the indirect fingerprint of overstory vegetation on SOM. Depending on the chemical composition of the microbial metabolites and microbial debris, and how recalcitrant plant debris is, it may be possible to detect differences in SOC chemical composition signature among vegetation types.

The chemical composition of SOC can be characterized with multiple techniques, each providing different information (Poirier et al., 2005). Among these methods, spectroscopic techniques in combination with chemical analyses have been extensively used to determine the chemical composition of litter and soil organic matter at the molecular level (Kögel-Knaber, 2002). Fourier transform infrared spectroscopy (FTIR) has been extensively used to determine specific chemical groups in organic matter, and has been successfully applied to study the stabilization of organic matter by adsorption to mineral surfaces (Lehmann et al., 2007) or to differentiate the chemical composition among SOC organomineral and light fractions (Poirier et al., 2005). This technique has great potential for characterizing SOC derived from different species (e.g. aspen vs.

subalpine fir) based on its chemical composition, and combined with exploratory techniques for multivariate data (e.g., principal component analysis, cluster analysis) can be used to evaluate the similarities of SOC from mixed stands with SOC from pure stands. FTIR applied to SOC fractions can determine whether the content of SOC in different fractions can be explained by the presence of particular functional groups (e.g., alkyl-C, aromatic and carboxyl C) (Lorenz et al., 2007; Kaiser et al., 2012), and if their proportion differs among species.

Near infrared reflectance spectroscopy (NIRS) is a non-destructive, inexpensive, rapid and empirical technique that is commonly used in food and chemical industries and agricultural science to determine the composition of organic compounds. In soil science, it has been applied to estimate SOC concentration, microbial biomass (Côteaux et al., 2003), relative abundance of functional groups (Terhoeven-Urselmans et al., 2006), concentration of C, N, and P in litter at different stages of decomposition (Gillon et al., 1999), SOC pools from the RothC model (Michel and Ludwig, 2010), SOC fractions (Côteaux et al., 2003; Cozzolino and Morón, 2006) and origin of mixed-species forest floor (Gruselle and Bauhus, 2010). Under the assumption that SOC derived from different tree species has a different chemical composition, NIRS can be applied to develop prediction models for the concentration of SOC derived from different species (e.g. aspen SOC vs. conifer SOC), and particularly, to predict the proportion of SOC of different origin under mixed stands. The application of NIRS for the identification of species origin on soil SOC is novel and promising, but it is not exempt of difficulties. Gruselle and Bauhus (2010) found that the ability of NIRS to predict species origin decreased for more decomposed litter. Thus, given the complex factors affecting the

chemical composition of SOC in the mineral soil horizons (soil mineralogy and texture, vegetation age and status, land history, and degree of transformation of the SOC), the applicability of NIRS for mixed species SOC may be limited, but needs to be investigated.

### **Study objectives**

The objectives of this study were:

1. To detect the effects of forest cover [aspen (*Populus tremuloides*) vs. conifers (*Abies lasiocarpa*, *Abies concolor*, *Pseudotsuga menziesii*)], and stand composition on SOC stock, content and distribution of SOC among fractions, and SOC decomposability.
2. To characterize the chemical composition of SOC derived from aspen and conifer species and test differences in SOC chemistry across the aspen-conifer ecotone. A secondary objective was to assess whether the content of mineral-associated SOC is related to higher concentration of recalcitrant compounds and/or preferential stabilization of organic molecules.
3. To develop statistical models using NIRS to predict SOC concentration derived from aspen and coniferous species.

The underlying hypotheses were that SOC storage and the proportion of mineral-associated SOC decreases from aspen to conifer dominated stands, and that SOC decomposability and lability conversely increase with conifer encroachment. I expected that chemical differences in SOC among species would be clearer in the light fraction (i.e., less decomposed and transformed material) than in the mineral-associated fraction



(i.e., more decomposed and processed organic matter). In addition, I expected an increase in the concentration and relative contribution to SOC of recalcitrant organic molecules (i.e., aliphatic C, aromatic C) with the degree of decomposition and aspen dominance in the overstory. SOC beneath mixed stands of aspen and coniferous species will have qualities from both vegetation types, but it is possible that species contribution to carbon dynamics and SOC properties will not be additive, and rather follow a non-linear trend, or exhibit changes at overstory composition thresholds.

## **Methods**

### *Study approach*

Previous research by the forest soil lab at Utah State University has investigated the differences in SOC properties (SOC stock, stability and decomposability) between aspen and coniferous species at study sites located in northern Utah (T.W. Daniel Experimental Forest, and Deseret Land and Livestock) (Woldeselassie, 2009; Olsen and Van Miegroet, 2010; Woldeselassie et al., 2012). To expand the spatial scope of the research I included study sites from southern Utah (Cedar Mountain) and northern Utah (Franklin Basin) for the analyses performed in Chapter 2 and Chapter 3. The sample dataset used for development of NIRS prediction models in Chapter 4 consisted of soil samples from four different study areas in Utah: Cedar Mountain (CM), Franklin Basin (FB), Deseret Land and Livestock (DLL) and T.W. Daniel Experimental Forest (TWDEF) (Fig. 1.2).

This study characterized the effect of vegetation on SOC properties using two different study designs. The first design is based on the influence that a single tree (or a

cluster of trees) can exert on soil properties under its canopy (Rhoades et al., 1997; Berger et al., 2010). Vegetation was treated as a categorical variable (aspen, mixed, and conifer). In the summers of 2010 and 2011 various sites were selected based in the presence of mixed aspen-conifer stands. Three transects were laid at each site, and within each transect, two soil cores were sampled in the mineral soil (0 – 15 cm) beneath aspen, mixed or conifer canopy. The elevation, slope, and aspect was similar along each transect.

The second design was done at the plot scale to include the effect of forest composition and stand structure on SOC properties (Li et al., 2010). Overstory composition was represented as a continuous variable, allowing study of the transition of SOC properties along the continuum from pure aspen to pure coniferous stands. A total of twenty-four plots of 10-m radius were sampled in different sites at CM in the summers of 2011 and 2012; four to five plots per site within a minimum distance of 30 m from each other (Fig. 1.3). Stand characteristics in the 10 m surrounding the plot were similar to those within the plot to avoid edge effects (changes in substrate, light, etc.). Five soil cores (0–15-cm depth) were sampled within each plot and combined into one composite sample. Tree species, status (dead or alive) and diameter at breast height (DBH) (i.e., stem diameter at a 1.30-m height) of all trees >3 cm in diameter were recorded and used to calculate live basal area (LBA) by species ( $\text{m}^2 \text{ha}^{-1}$ ) and live stem density ( $\text{n ha}^{-1}$ ).

### *Study areas*

Cedar Mountain (CM) is a high elevation plateau (1800 m – 3200 m) located within the Kolob Terrace in southwestern Utah. Mean annual precipitation for the area is

812 mm (1981 – 2010) (NRCS, 2013). Cedar Mountain has monsoonal storms in the late summer, but most of the precipitation is in the form of snowfall, occurring from October through April (Evans, 2010). Average monthly temperature ranges from -3.8 °C in December and 15.5 °C in July for the period 1993-2012 (NRCS, 2013). Mountain grasslands consisting of Letterman needlegrass (*Stipa lettermani*) and Kentucky bluegrass (*Poa pratensis*) alternate with woodlands of trembling aspen (*Populus tremuloides*) as the predominant communities (Tshireletso et al., 2010). Subalpine fir (*Abies lasiocarpa*), Douglas-fir (*Pseudotsuga menziesii*) and white fir (*Abies concolor*) appear scarcely in the landscape, mixed with aspen or forming small stands at the edges of the plateau. Patches of Gamble oak (*Quercus gambelii*) occur in lower elevation areas (Evans, 2010). Soil types are commonly Alfisols or Mollisols (McNab and Avers, 1994; Rogers et al., 2010), developed mainly on sedimentary rock and igneous rock (Mittanck, 2012). Most of Cedar Mountain is privately owned, except for the northeastern area, which is part of the Dixie National Forest.

Franklin Basin (FB) is a montane-subalpine area (1770 – 3030 m) located between the Bear River Range and the Wasatch Range in the central Rocky Mountains, distributed between northeastern Utah and southeastern Idaho (Kusbach, 2010). Franklin Basin is approximately 40 km northeast of Logan, Utah. The two closest SNOTEL stations, Franklin Basin (Idaho) and Tony Grove Lake (Utah), indicate a mean annual precipitation of 1161 mm and 1232 mm for the period 1981-2010 (NRCS, 2013). Monthly average temperature ranges between -6.9 °C in December to 16.4 °C in July for the period 1993-2012 (NRCS, 2013). Forest ecosystems are represented by quaking aspen, and mixed conifer stands of Douglas-fir, subalpine fir, and limber pine (*Pinus*

*flexilis*). Non-forested areas are occupied by curl-leaf mountain mahogany (*Cercocarpus ledifolius*) or mountain big sagebrush (*Artemisia tridentata ssp. vaseyana*), (Mittanck, 2012). At higher elevations the soils are composed of residuum derived from limestone, whereas colluvium derived from sandstone predominates at valley bottoms (Mittanck, 2012).

The T. W. Daniel Experimental Forest (TWDEF) is located at an elevation of 2600 m, in northeastern of Utah. Franklin Basin and TWDEF are relatively close. Mean annual precipitation is 950 mm, 80 % as snow (Olsen and Van Miegroet, 2010). Monthly temperature ranges from -10°C in January to 14°C in July (Schimpf et al., 1980; Skujins and Klubek, 1982; Woldeselassie et al., 2012). Forested communities include aspen forest and conifer forest, predominantly Engelmann spruce (*Picea engelmannii*) and subalpine fir. Non-forest communities include open meadows consisting of a mixture of grasses and forbs, and areas dominated by sagebrush (Olsen and Van Miegroet, 2010). Predominant soil orders are Mollisols and Alfisols developed in eolian deposits that overlay on residuum and colluvium from the Wasatch formation (Van Miegroet et al., 2005; Olsen and Van Miegroet, 2010; Woldeselassie et al., 2012).

Deseret Land and Livestock (DLL) is a cattle ranch located in Northeastern Utah. Elevations range between 1889 and 2700 m (Woldeselassie, 2009). Mean annual precipitation is 910 mm for the period 1981 - 2010 (NRCS, 2013), mostly as snowfall. Monthly average temperature ranges between -5 °C in January and 16°C in July for the period 1990-2012 (NRCS, 2013). Grasslands of crested wheatgrass (*Agropyron cristatum*) and western wheatgrass (*Pascopyrum smithii*) dominate at lower elevations (Mangus, 2011). Sagebrush steppe in mid elevation areas, consisting mainly of Wyoming

big sage (*Artemisia tridentata* spp. *wyomingensis*), transitions towards semi-open brush and grasslands with patches of trembling aspen, Douglas-fir and subalpine fir in higher elevation areas (Woldeselassie, 2009; Mangus, 2011). Soils orders are Mollisols, Entisols, Aridisols and Inceptisols (Washington-Allen et al., 2004; Woldeselassie, 2009). Study sites within DLL were located in two small watersheds named Upper Frost and Bear Canyon (DLL Frost and DLL Bear hereafter in the text). A complete description of sites location and soil morphology can be found at Woldeselassie (2009).

### **Objective 1: Effects of forest cover and stand composition on SOC storage and stability**

Several soil and SOC properties were measured in the laboratory on transect and plot samples between 2011 and 2013 and later analyzed as response variables with linear mixed effects models, or with simple correlation coefficients. Bulk soil SOC stocks ( $\text{Mg ha}^{-1}$ ) for the first 15 cm of mineral soil were calculated with TOC concentration, bulk density and fine earth percentage ( $< 2 \text{ mm}$ ) of the middle core sections (5 – 10 cm). Soil texture analysis was performed with the pipette method (Gee and Bauder, 1986).

The storage and distribution of SOC among pools of varying degree of chemical protection was measured with a fractionation method that combined wet sieving and electrostatic attraction (modification of Kaiser et al., 2009). Laboratory incubations were an indirect way of measuring biologically available SOC (i.e. easily decomposable SOC), assuming microbes mineralize accessible (unprotected) SOC first (McLauchlan and Hobbie, 2004). Approximately 15 g of fresh soil were incubated in the laboratory for 10 months, following the protocol of Paul et al. (2001) as modified per Woldeselassie et al. (2012). Unprotected SOC was also estimated with hot-water extractable organic carbon

(HWEOC), a pool considered by some authors as a good indicator of biologically available SOC (Ghani et al., 2003; Bu et al., 2010).

**Objective 2: Characterizing the chemical composition of SOC from mixed aspen-conifer forests with Fourier transform infrared spectroscopy.**

FTIR in the mid-infrared range was used to identify the presence and abundance of functional groups in bulk SOC and SOC fractions. Air-dried bulk soil samples, SOC fractions, and mineral matrix samples were scanned on with FTIR-ATR (Nicolet 6700, Thermo-Fisher, Pittsburgh, PA, USA). Mineral matrix samples were obtained following organic matter removal using a modification of the protocol described by Kaiser et al. (2002). Subtraction of mineral matrix spectra from bulk soil spectra allowed identifying spectral characteristics of organic matter.

FTIR spectra were analyzed initially by visual comparison of spectra peak distribution and height to detect potential differences in SOC chemical composition among vegetation types (aspen, mixed and conifer). Two approaches were used to further analyze the spectral data: 1) test the effect of fixed independent variables (e.g., vegetation cover, contribution of aspen to LBA (%)) on the normalized peak height at selected wavenumbers (dependent variables), and 2) multivariate exploratory data analysis (e.g., principal component analysis) on a wide wavenumber range. By combining both methods it was possible to identify the functional groups responsible of spectral variability and study differences in chemical composition among vegetation types. The comparison of mineral-associated SOC spectra informed of which functional groups are preferentially stabilized in organo-mineral associations.

### **Objective 3: Predicting SOC concentration derived from different species with near infrared reflectance spectroscopy**

Samples collected from transects at CM, FB, TDWEF and DLL were used for modeling the SOC concentration with near infrared reflectance spectroscopy (NIRS). Sample preparation starts by generating artificial mixtures in the laboratory, by mixing varying proportions of aspen soil, conifer soil and a third soil component, to avoid autocorrelation typical of two components mixtures (Gruselle and Bauhus, 2010). Near infrared spectra were acquired over the wave number range from 4000 to 11000  $\text{cm}^{-1}$  on a Tensor 37 spectrometer (Bruker Optics GmbH, Ettlingen, Germany), at the Institute of Silviculture, University of Freiburg (Germany) in the summers of 2011 and 2012.

NIR-spectra were used to generate models with multivariate calibration, using a slightly modified methodology from Gruselle and Bauhus (2011). Prediction models were developed with partial least squares regression using the software OPUS 6.5 (Bruker Optics GmbH, Ettlingen, Germany) at the University of Freiburg (Germany). Model robustness was tested with an independent validation set.

### **Organization of chapters**

The results of this study are organized in three different chapters:

Chapter 2. *Soil organic carbon storage and stability in the aspen-conifer ecotone in montane forests in Utah, USA*. Results on SOC storage, SOC decomposability, and SOC lability along the aspen-conifer natural gradient are presented in this part of the dissertation. The relevance of specific SOC stabilization mechanisms in relation to overstory composition and soil texture is connected to the content and relative distribution of SOC among fractions.

Chapter 3. *Characterizing the chemical composition of SOC from mixed aspen-conifer forests with Fourier transform infrared spectroscopy*. This chapter includes the results for FTIR spectral analysis, and specifically addresses: 1) whether FTIR spectra of SOC and SOC fractions can discriminate among overstory composition, and 2) whether there are differences in the relative contribution of functional groups on mineral-associated SOC among vegetation types and across the gradient aspen-conifer.

Chapter 4. *Predicting the C concentration derived from aspen and conifers on soil organic carbon with near-infrared reflectance spectroscopy (NIRS)*. The methodology for developing NIRS prediction models for aspen- and conifer-derived SOC, results for model prediction performance, and a discussion of factors influencing the characteristics of NIR spectral properties are presented in chapter 4.

Chapter 5. *Summary and conclusions*. The most important findings and relevant conclusions are summarized, and implications for further research are presented in this part of the dissertation.

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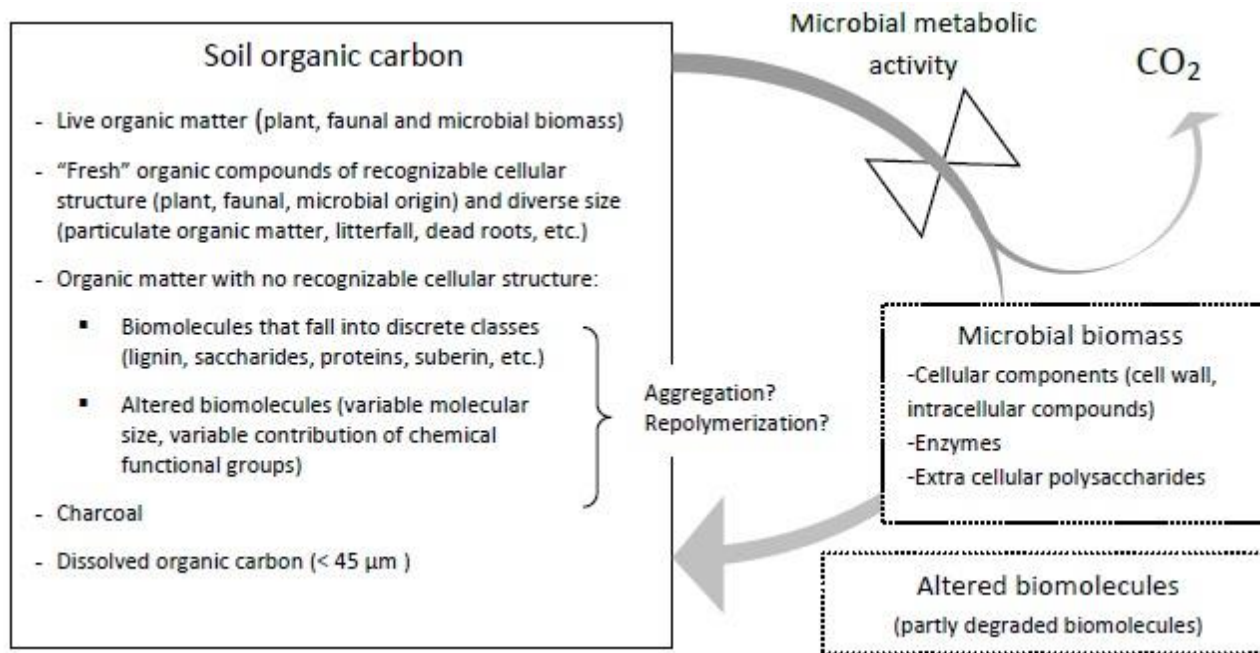


Fig. 1.1: Microbial activity modifies the chemical nature of soil organic matter and contributes to the output of C from the system as CO<sub>2</sub>. Classes included in the SOC pool are modified from Baldock (2002).

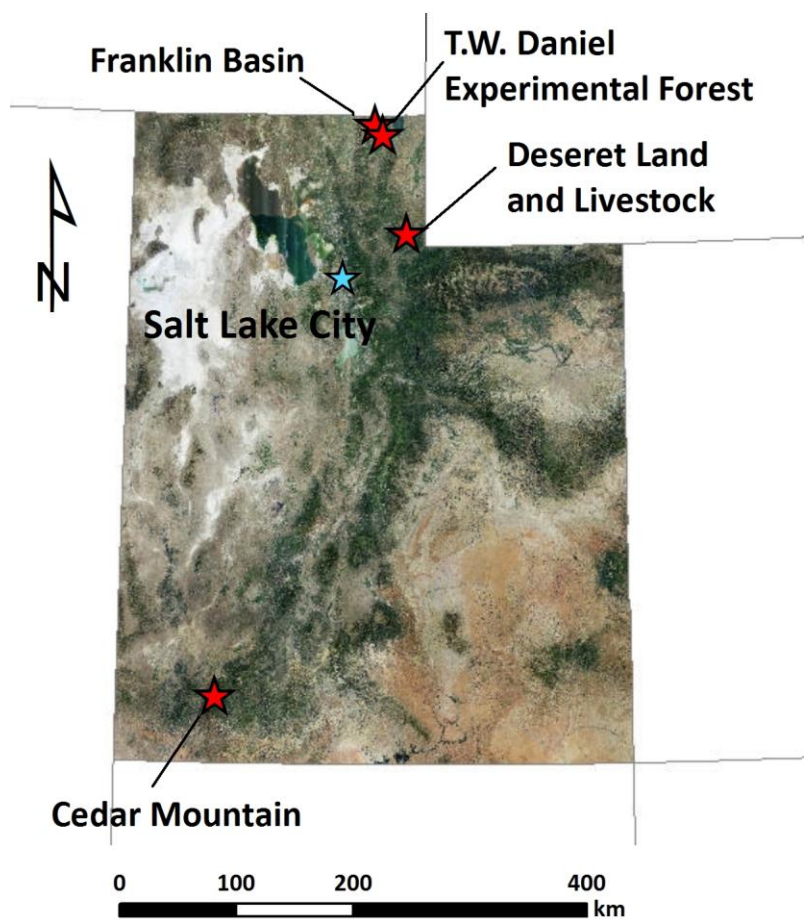


Fig. 1.2: Location of study areas in Utah.

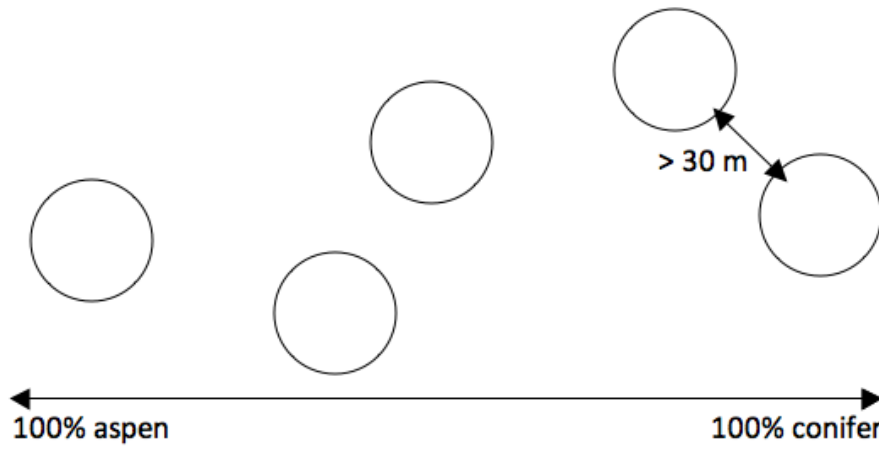


Fig. 1.3: Plots of 10 m radius sampled at Cedar Mountain covered the natural gradient from pure aspen to pure conifer stands. Minimum distance within plots was 30 m.

## CHAPTER 2

SOIL ORGANIC CARBON STORAGE AND STABILITY IN THE ASPEN-  
CONIFER ECOTONE IN MONTANE FORESTS IN UTAH, USA<sup>1</sup>**Abstract**

To assess the potential impact of conifer encroachment on soil organic carbon (SOC) dynamics and storage in montane aspen-conifer forests from the interior western US, we sampled mineral soils (0–15 cm) across the aspen-conifer ecotones in southern and northern Utah and quantified total SOC stocks, stable SOC (i.e., mineral-associated SOC (MoM)), labile SOC (i.e., light fraction (LF), decomposable (CO<sub>2</sub> release during long-term aerobic incubations) and soluble SOC (hot water extractable organic carbon (HWEOC)). Total SOC storage ( $47.0 \pm 16.5 \text{ Mg C ha}^{-1}$ ) and labile SOC as LF ( $14.0 \pm 7.10 \text{ Mg C ha}^{-1}$ ), SOC decomposability (cumulative released CO<sub>2</sub>-C of  $5.6 \pm 3.8 \text{ g C g}^{-1}$  soil) or HWEOC ( $0.6 \pm 0.6 \text{ mg C g}^{-1}$  soil) did not differ substantially with vegetation type, although a slight increase in HWEOC was observed with increasing conifer in the overstory. There were statistically significant differences ( $p = 0.035$ ) in stable MoM storage, which was higher under aspen ( $31.2 \pm 15.1 \text{ Mg C ha}^{-1}$ ) than under conifer ( $22.8 \pm 9.0 \text{ Mg C ha}^{-1}$ ), with intermediate values under mixed ( $25.7 \pm 8.8 \text{ Mg C ha}^{-1}$ ). Texture had the greatest impact on SOC distribution among labile and stable fractions, with increasing stabilization in MoM and decreasing bio-availability of SOC with increasing silt + clay content. Only at lower silt + clay contents (40%–70%) could we discern the

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influence of vegetation on MoM content. This highlights the importance of chemical protection mechanisms for long-term C sequestration.

## **Introduction**

Efforts to optimize C sequestration in forest ecosystems have mainly focused on enhancing stand biomass productivity and density by adapting rotation length, thinning intensity and tree species composition. Less attention is often paid to the effect of forest management and changes in species composition on soil organic carbon (SOC) storage and dynamics. Soils store two thirds of total C in terrestrial ecosystems [1], which is equivalent to 1400–1500 Pg C in the first meter [2,3]. Even small changes in SOC storage or dynamics, whether induced by anthropogenic or natural factors, can alter the ecosystem C balance [4], with significant impact on atmospheric CO<sub>2</sub> levels at the regional scale.

SOC storage at the landscape scale is determined by the interaction of climate, soil properties, vegetation, relief, land use history, disturbance regime and the chemical composition of soil organic matter [5]. The balance between C input, primarily as litter or rhizodeposition, and C output via soil respiration determines whether forest soils are C sources or sinks [6,7]. For particular site conditions (e.g., soil properties, aspect, climate, etc.), forest species composition and stand development determine the amount, allocation (aboveground and belowground) and chemistry of organic matter inputs [6,8–10]. Soil environmental conditions (e.g., temperature, water and O<sub>2</sub> availability, pH), the abundance and type of microbes and the chemical composition of organic matter, in turn, regulate SOC decomposition rates [11]. Biochemical recalcitrance

(i.e., resistance to microbial decomposition due to intrinsic molecular make-up) has greater control over decomposition rates in the litter layer. In the mineral soil, the persistence of SOC is further enhanced by the mineral matrix through additional protection mechanisms, such as the isolation of organic matter inside aggregates (i.e., physical protection) and surface interactions between organic compounds and mineral particles, mainly from the silt and clay fraction (i.e., chemical protection) [12]. The interaction of these protection mechanisms and soil microclimate creates a continuum of SOC pools with different chemical composition and residence time [13,14] that differs among forest species [7,15].

Quaking aspen (*Populus tremuloides* Michx.) is an iconic species of the Intermountain West, USA. Aspen is typically a seral species, eventually replaced by more shade-tolerant species, like Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) at lower elevations or subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) at higher elevations [16]. As a pioneer species, aspen regeneration frequently depends on small or coarse-scale disturbances, like fires or landslides [17]. However, aspen is ecologically versatile and has multiple modes of regeneration and stand development [17,18]. Aspen can form stable, uneven aged stands that regenerate continuously or through gap-phase regeneration [17] and is also found in coexistence with conifers in mixed stands for several decades or centuries [17,18]. Decline in aspen extent in the Intermountain West (so-called sudden aspen decline or SAD) has been attributed to natural succession coupled with fire suppression, ungulate grazing and climate change [19–23].

Aspen and conifer stands from semi-arid montane and subalpine forests differ considerably in soil microclimate [24–27], hydrology [24], litter quality [27,28], soil chemistry [27,29], soil microbial community structure [27] and SOC content and dynamics [25–27]. Woldeselassie et al. [26] found that montane aspen stands in northern Utah had higher SOC stock ( $96.2 \pm 26.7 \text{ Mg C ha}^{-1}$ ) than adjacent conifer stands ( $66.9 \pm 18.6 \text{ Mg C ha}^{-1}$ ) in the top 60 cm of mineral soil. SOC under aspen was also more persistent than SOC from conifer soils and had a higher proportion of mineral-associated SOC ( $55\% \pm 13\%$  in aspen vs.  $41\% \pm 13\%$  in conifers) [25,26]. A shift towards mixed and conifer-dominated stands could thus modify SOC dynamics and potentially reduce long-term SOC storage (i.e., SOC sequestration potential).

Several studies have addressed the properties of SOC under mixed aspen-conifer stands in the boreal climate [15,30], but studies of SOC storage and stability in mixed stands in semi-arid climates are largely missing. In particular, we do not know whether changes in SOC properties occur gradually or abruptly at critical composition thresholds. It is also possible that mixed aspen-conifer stands have distinct SOC dynamics and, thus, represent an alternate state. The objective of this study was to assess the influence of forest composition on SOC storage and SOC stabilization in the mineral soil at the aspen-conifer ecotone in montane forests of Utah. The underlying hypotheses were: (1) SOC storage will decrease from aspen to conifer dominated stands; (2) with increasing conifer encroachment, a greater proportion will be stored as labile SOC; and (3) the proportion and quantity of protected SOC will conversely decrease with conifer encroachment. Understanding the processes controlling SOC storage and stabilization along the aspen-

conifer gradient will provide insight to forecast the fate of SOC with conifer encroachment or climate change-induced vegetation shifts and may also inform management decisions when focusing on C sequestration as an ecosystem service.

## **Materials and Methods**

### *Study Sites*

To test our hypotheses, we chose study sites from southern Utah (Cedar Mountain) and northern Utah (Franklin Basin), expanding the geographical scope of previous studies.

Cedar Mountain (CM) is a high elevation plateau (1800–3200 m) located within the Colorado Plateau region in southwestern Utah [31] (Figure 2.1). Mean annual precipitation is 812 mm [32], most of it as snow from October through April. Monsoonal storms are common in late summer [33]. The average monthly air temperature ranges from  $-3.8$  °C in December to  $15.5$  °C in July [32]. Mountain grasslands consisting of Letterman needlegrass (*Stipa lettermani* Vasey) and Kentucky bluegrass (*Poa pratensis* L.) alternate with woodlands of quaking aspen as the predominant communities [34]. Subalpine fir, Douglas fir and white fir (*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.) appear scarcely in the landscape, mixed with aspen or forming small stands at the edges of the plateau. Patches of Gambel oak (*Quercus gambelii* Nutt.) occur in lower elevation areas [33]. Soil types are commonly Alfisols or Mollisols [31,35] developed mainly on sedimentary rock and igneous rock [36].

Franklin Basin (FB) is a montane-subalpine area (1770–3030 m) located between the Bear River Range and the Wasatch Range in the central Rocky Mountains, distributed



between northeastern Utah and southeastern Idaho [37] (Figure 2.1). The precipitation regime is snow dominated, with a mean annual precipitation of 1197 mm [32]. The monthly average temperature ranges from  $-6.9$  °C in December to  $16.4$  °C in July [32]. Forest ecosystems are represented by quaking aspen and mixed conifer stands of Douglas fir, subalpine fir and limber pine (*Pinus flexilis* E. James). Non-forested areas are occupied by curl leaf mountain mahogany (*Cercocarpus ledifolius* Nutt. ex Torr. & A. Gray) or mountain big sagebrush (*Artemisia tridentata* Nutt. ssp. *vaseyana* (Rydb.) Beetle) [36]. Soils are commonly Alfisols and Mollisols, developed on limestone or quartzite sandstone.

#### *Study Design and Field Sampling*

This study used two sampling designs to characterize the influence of the overstory on SOC properties at different spatial scales. In the first design, the influence of a single tree or a small tree cluster on soil properties was evaluated [38,39]. The second design used plots of a 10-m radius as sampling units to characterize stand composition along the gradient from pure aspen stands to pure conifer stands.

In October of 2011, four sites at CM and two sites at FB were sampled following the first design, hereafter referred to as “transects”, with three transects per site (Table 2.1). Within each transect, two soil cores (5-cm diameter; 0–15-cm depth) were taken in the mineral soil beneath aspen, mixed and conifer cover. The cores were divided by 5-cm intervals at the field and stored separately. Slope, elevation and aspect were similar within each transect. Pure conifer clusters were absent in one site at CM (CM1), while samples from one transect at another site (CM57) were excluded from the inventory, due

to discrepancies in sampling and storage protocol. We did not describe soil pedons in our sites, but the characteristics of the topsoil were in agreement with those described in previous studies [26,37]. We generally observed a thin O horizon (i.e., superficial horizon dominated by organic matter, > 20% by weight of SOC) and a relatively deep A horizon (i.e., mineral topsoil with accumulation of organic matter, < 20% by weight of SOC) under aspen, whereas conifer soils had a thicker O horizon and a shallower and lighter A horizon.

The second design, hereafter named “plots”, was applied in CM the summers of 2011 and 2012. Potential sampling areas were identified a priori with the National Agricultural Imagery Program (NAIP) 1-m orthophoto (2009) and topographic maps using ArcGIS 9.3 (ESRI, Redlands, CA., USA). Sampling areas were selected based on the existence of conifer, mixed and aspen patches of at least a 40-m diameter under similar slope, elevation and aspect conditions. Four to five plots of a 10-m radius were located at five different locations, for a total of 24 plots (Table 2.2). The minimum distance between adjacent plots was 30 m, and the conditions of overstory composition and structure in the surrounding 10-m buffer were homogenous to those within the plot to avoid edge effects. Five soil cores (5-cm diameter; 0–15-cm depth) were randomly sampled within each plot and combined into one composite sample per plot. Two additional cores were collected and the 5–10 cm excised to calculate bulk density. Tree species, status (dead or alive) and diameter at breast height (DBH) (i.e., stem diameter at a 1.30-m height) of all trees >3 cm in diameter were recorded and used to calculate live basal area (LBA) by species ( $\text{m}^2 \text{ha}^{-1}$ ) and live stem density ( $\text{n ha}^{-1}$ ). Overstory

composition was classified by the percentage of live basal area occupied by aspen in three categories: aspen dominated (>75% LBA aspen), mixed (25%–75% LBA aspen) and conifer dominated (<25% LBA aspen).

### *Laboratory Analyses*

Transects (0–5 cm) and plot composite samples (0–15 cm) were sieved (2-mm mesh) and stored at 4 °C to minimize microbial decomposition. Middle core sections (5–10 cm) from transects and plots were oven dried at 105 °C for 24 h, sieved (2-mm mesh), weighed to determine bulk density and the percent of fine earth mass. Fine fraction samples were ground with a mortar and pestle and analyzed for total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) with a Skalar PrimacsSLC Analyzer (Skalar, Inc., Breda, The Netherlands). Average TOC concentration from the 5–10 cm, depth, bulk density and fine earth percentage were used to calculate SOC stocks ( $\text{Mg C ha}^{-1}$ ) for the first 15 cm of mineral soil. As bulk density increases with depth, while SOC concentration decreases, we considered the 5–10 cm section to represent the average properties of the entire 0–15 cm core. Soil texture analysis was performed with the pipette method [40] in transect (0–5 cm) and composite plot (0–15 cm) samples.

A multitude of fractionation methods have been developed with the purpose of dividing SOC into fractions with presumably different turnover rates [41,42]. In this study, we used a simplified size fractionation method: 20 g of air dried soil (0–15-cm depth for plot samples, 0–5-cm depth for transect samples) were processed under the premise that free, large particulate organic matter and SOC associated with mineral particles of different sizes and mineralogy differ in the degree of stabilization and

turnover time. The mineral-associated SOC in the clay and silt fraction (MoM) was separated by wet sieving through a 53- $\mu\text{m}$  sieve, with the  $>53 \mu\text{m}$  fraction further divided into a light fraction (LF) and mineral-associated SOC in the sand fraction (MA  $> 53$ ) using electrostatic attraction, following a modification from Kaiser et al. [43]. The LF is generally composed of free and intra-aggregate particulate organic matter (i.e., relatively fresh organic matter, mainly of plant origin). MoM is considered to be more protected and to have a long residence, whereas SOC in the sand fraction is weakly bonded and has lower residence, but may also be partly composed of relatively recalcitrant charred material [41,44]. All fractions were ground with a mortar and pestle and analyzed for TC content with Skalar PrimacsSLC Analyzer (Skalar, Inc., Breda, The Netherlands), which constituted TOC, given that IC content was negligible in the bulk samples. The C recovery and relative contribution of each fraction to bulk SOC was calculated from the fractions' relative weights, TC concentrations and bulk soil TOC concentration. C recovery was on average  $98.0\% \pm 11.3\%$ , with five samples somewhat outside this range.

SOC decomposability (i.e., biologically available SOC) was determined with long-term (10 months) aerobic laboratory incubations of fresh soil samples (0–5 cm transect, 0–15 cm plot) following the protocol of Paul et al. [45], as modified by Woldeselassie et al. [26]. Cumulative  $\text{CO}_2\text{-C}$  respired was expressed on a dry soil weight basis ( $\text{mg CO}_2\text{-C g soil}^{-1}$ ) and normalized to C content ( $\text{mg CO}_2\text{-C g C}^{-1}$ ) as an indicator of qualitative differences in SOC. Only plot samples collected in 2011 ( $n = 16$ ) were incubated.

Hot water extractable organic carbon (HWEOC), considered by some authors as a good indicator of biologically available SOC [46,47], was determined by mixing field-moist soils with distilled water in falcon tubes (1:10 soil-water (w/v)) and heating the slurry in a hot bath at 85 °C for one hour. The solution was filtered through Whatman GF/F filters (pore size ~0.7 µm) and the extractant analyzed for dissolved organic carbon (DOC) with a Phoenix 8000 Carbon Analyzer (Tekmar-Dohrmann, Mason, OH., USA). Specific ultraviolet absorbance at 254 nm (SUVA) of HWEOC, an estimate of DOC aromaticity which is used as an indicator of chemical recalcitrance [48], was measured with a Genesys 10 spectrophotometer (Thermo Scientific, Madison, WI., USA).

#### *Statistical Analyses*

Relationships between SOC properties were explored with Spearman's rank correlation coefficient using the R package, Hmisc, version 3.10–1.10 (R Foundation for Statistical Computing, Vienna, Austria) [49].

Linear mixed effects (LME) models were applied to test the effect of vegetation and soil texture on the SOC properties in both datasets. In the transect dataset, vegetation class (aspen, mixed and conifer) was treated as a categorical fixed effect and silt + clay content (%) as a continuous fixed effect variable. The site and transect were considered random effect variables, with transect nested within site to account for the dependency among samples from the same transect and site. LME models applied to the plot dataset included overstory composition (aspen percent of LBA) and soil texture (silt + clay (%)) as continuous fixed effect variables and site as a random effect variable.

LME models with vegetation class or overstory composition as explanatory variables were applied to sand (%), silt (%) and clay (%) as response variables to ensure that potential differences in SOC properties across the vegetation gradient were not merely due to the occurrence of aspen and conifers in different soil conditions.

Data were transformed with the logarithm in base 10, the square root, the reciprocal transformation or the reciprocal square root when the assumptions of normality and homogeneity of variance were not met. Linear mixed models were applied with the R package, lmer, Test 2.0–3.0 [50]. Fixed effects were tested with Type III ANOVA, using Satterthwaite approximation for the degrees of freedom of the denominator for the F statistics. Bonferroni pairwise comparisons were used to test differences among estimated means when the main effect of vegetation class was statistically significant ( $p < 0.05$ ). The estimated slope for silt + clay content in transect LME models was reported when the ANOVA test found it statistically significant, to inform on the magnitude and direction of the effect. Estimates for the intercept and slopes for overstory composition and silt + clay content were reported for all the plots LME models.

## **Results and Discussion**

The plots at Cedar Mountain represented a broad gradient from pure aspen stands to conifer stands, with varying degrees of conifer encroachment and stand structure (Table 2.2). Across all plots, mean aspen LBA was  $25.3 \pm 17.0 \text{ m}^2 \text{ ha}^{-1}$ , a similar value to the  $20.7 \pm 13.8 \text{ m}^2 \text{ ha}^{-1}$  reported by Rogers et al. [31] for stable aspen stands in Cedar Mountain. The mean conifer LBA (min–max) was  $24.4 (0\text{--}100.6) \text{ m}^2 \text{ ha}^{-1}$ . The average tree density (min–max) was  $642 (0\text{--}2499)$  live conifer  $\text{ha}^{-1}$  and  $822 (0\text{--}4286)$  live aspen

ha<sup>-1</sup>, higher than the 315 ± 201 aspen stems ha<sup>-1</sup> reported previously for this area [31]. The relative dead basal area was higher for aspen (33.5% ± 32.5%) than conifer (5.5% ± 10.6%) across all sampling sites. Nine plots had aspen dominated overstory (LBA aspen 21–50 m<sup>2</sup> ha<sup>-1</sup>), four of them showing signs of conifer encroachment; six plots had conifer dominated overstory (LBA conifer 17–101 m<sup>2</sup> ha<sup>-1</sup>); nine plots had mixed overstory (total LBA 20–94 m<sup>2</sup> ha<sup>-1</sup>). The distribution by diameter classes suggested that seven mixed plots may continue the succession towards conifer stands.

The SOC concentration in surface soils did not differ by vegetation class in the transect samples (Table 2.3), with an overall mean (± SD) of 48.1 ± 18.1 mg C g soil<sup>-1</sup>. In the plots, SOC concentration ranged between 10.0 and 150 mg C g soil<sup>-1</sup>, with a mean (± SD) of 46.8 ± 27.1 mg C g soil<sup>-1</sup>. The SOC content (Mg C ha<sup>-1</sup>) in the transects was not statistically different among vegetation classes, with an overall mean (± SD) of 47.2 ± 16.8 Mg C ha<sup>-1</sup>, but followed the trend: aspen > mixed > conifer. The SOC content in the plots ranged between 14.4 and 80.9 Mg C ha<sup>-1</sup>, with a mean (± SD) of 46.8 ± 16.2 Mg C ha<sup>-1</sup>. SOC content did not follow any pattern nor did it change abruptly at a critical LBA threshold across the aspen-conifer gradient, but it varied across sampling sites (Figure 2.2 and Table 2.3). Our values for SOC content are comparable to those found by Woldeselassie et al. [26], who similarly did not find significant differences in SOC content in the top 15 cm of mineral soil between aspen (49.5 ± 7.9 Mg C ha<sup>-1</sup>) and conifer stands (54.9 ± 20.3 Mg C ha<sup>-1</sup>) in montane forests from northern Utah.

Although we did not find differences in SOC storage along the vegetation gradient, differences in distribution among labile and stable SOC fractions may be more

relevant for C sequestration. Across all samples, over half of the SOC was stored in the more persistent MoM fraction (mean  $\pm$  SD: 56.1%  $\pm$  12.7% for transects; 60.5%  $\pm$  13.4% for plots), with around one-third stored as LF (mean  $\pm$  SD: 37.0%  $\pm$  11.9% for transects; 30.4%  $\pm$  10.3% for plots). Mineral-associated SOC in the sand fraction was a minor contributor, accounting for <10% of total SOC (mean  $\pm$  SD: 6.9%  $\pm$  3.3% for transects; 9.1%  $\pm$  5.5% for plots) and, therefore, was not further considered in the statistical analyses. MoM content in the transect soils was statistically significantly higher under aspen (31.18 Mg C ha<sup>-1</sup>) than under conifer (22.84 Mg C ha<sup>-1</sup>), with mixed stands having intermediate values (25.67 Mg C ha<sup>-1</sup>) (Table 2.3). This pattern was not visible along the aspen-conifer gradient in the plots (Figure 2.3b and Table 2.4), nor was there an obvious LBA threshold. However, there was a positive correlation between MoM C concentration (mg C g<sup>-1</sup> soil) and aspen contribution to LBA (%) (Figure 2.3a). The relative distribution of SOC among the different fractions (expressed by the percent of SOC) was similar across the vegetation types in plots and transects, probably due to the high variability in LF content in our sites. These results somewhat contradict previous observations from montane semi-arid [26] and boreal aspen-conifer forests [30], where aspen stands had a significantly higher proportion of SOC in the MoM fraction than mixed and conifer stands.

On the other hand, silt + clay (%) had a significant positive effect on MoM content in the plots (Figure 2.3c and Table 2.4) and on the relative proportion of SOC as MoM in both transects (Table 2.3) and plots (Table 2.4), indicative of the formation of organo-mineral associations on silt and clay particles. Individual LME models by



dominant overstory in the plots (e.g., aspen (aspen LBA > 75%), mixed (aspen LBA 25%–75%) and conifer (conifer LBA > 75%)) suggest that at relatively low silt + clay (%), there is a vegetation effect, with aspen soils storing more MoM than soils in conifer and mixed forests. At higher silt + clay (%), the effect of vegetation is negligible or the potential for SOC stabilization is driven by soil texture rather than vegetation (Figure 2.3c). Collectively, these results suggest that SOC stabilization in the mineral-associated fraction is favored by the presence of aspen [26]. This greater accumulation of MoM under aspen may be driven either by a higher concentration of organic matter in the mineral-soil solution interface or a higher affinity between aspen-derived organic compounds and clay minerals or both. Our results indicate that whereas total SOC stocks in the upper mineral soil may not be affected by conifer encroachment, the amount of protected (i.e., more persistent) SOC in soils with low and intermediate silt + clay contents may be below the full potential under conifer compared to similar soils under pure aspen.

The amount and relative contribution of LF was highly variable within and among sites in transects and plots (Figure 2.4) and was not significantly affected by either vegetation cover (LBA) or soil texture (Tables 2.3 and 2.4). The variability of LF among sites likely reflected differences in litter input, root growth and decomposition that were not captured by overstory characteristics in this study. This was somewhat unexpected, as LF is considered responsive to changes in overstory species and land use [7]. In their aspen-conifer comparison, Laganière et al. [30] and Woldeselassie et al. [26] had

previously found a higher proportion of unprotected SOC under conifer stands than under aspen stands.

The two methods used to characterize relatively labile SOC, i.e., decomposability (long-term incubations) and solubility (hot water extractions) were positively, albeit weakly, correlated. Correlation coefficients were  $r = 0.34$  ( $p = 0.024$ ) when data were expressed as concentrations on a soil dry weight basis and  $r = 0.35$  ( $p = 0.019$ ) when expressed per gram of SOC in the soil. This suggests that although the methods are not equivalent, HWEOC can be used as a fast and practical proxy of microbially available SOC when time and/or resources are limited. Our data showed no quantitative or qualitative differences in SOC decomposability with vegetation cover (Table 2.3) or the relative abundance of aspen in the overstory (Figure 2.5a and Table 2.4). Cumulative CO<sub>2</sub> release rates (5 to 7.5 g C g<sup>-1</sup> soil) were in a similar range as those reported by Olsen and Van Miegroet [25] for the 0–10 cm of mineral soil under aspen (3.8 g C g<sup>-1</sup> soil) and conifer stands (5.1 g C g<sup>-1</sup> soil). Although in that study, no significant differences in SOC decomposability were found among vegetation classes in surface soils, vegetation had a significant effect at a 10–20-cm soil depth, with conifer soils containing more decomposable SOC than aspen. Similarly, Woldeselassie et al. [26] found that aspen-derived SOC was qualitatively less decomposable (67.7 mg C g C<sup>-1</sup>) than conifer-derived SOC (130.9 mg C g C<sup>-1</sup>).

HWEOC concentration, expressed on a soil dry weight basis or per gram of SOC, did not differ among vegetation classes in the transect samples (Table 2.3). At the plot level, overstory composition had a significant effect on HWEOC concentration per gram

of soil ( $p = 0.044$ ) or as a fraction of SOC ( $p = 0.006$ ). The small negative slopes (Table 2.4) suggest a slight, but statistically significant, decrease in SOC lability with the presence of aspen in the overstory (Figure 2.5b).

Variability in cumulative CO<sub>2</sub> release per gram of soil was not explained by texture in either transects or plot samples (Tables 2.3 and 2.4). Relative silt + clay content had a significant negative effect ( $p = 0.045$ ) on the decomposability of SOC (CO<sub>2</sub> g<sup>-1</sup>C) in the transects (Table 2.3), indicated by a negative slope for log-transformed CO<sub>2</sub>-C release ( $\beta = -0.016$ ). In the plot data, the effect of silt + clay (%) on decomposable SOC was non-significant when controlling for other factors (Table 2.4). However, the simple correlation between silt + clay (%) and SOC decomposability followed the same negative pattern (Figure 2.5c). The fraction of readily decomposable SOC (mg C g<sup>-1</sup> C) was also negatively correlated with either silt (%) ( $r = -0.47$ ,  $p = 0.001$  transects;  $r = -0.51$ ,  $p = 0.044$  plots) or clay (%) separately ( $r = -0.43$ ,  $p = 0.004$  transects;  $r = -0.79$ ,  $p < 0.001$  plots). The percentage of SOC as MoM was further negatively correlated with decomposable SOC per gram of soil ( $r = -0.30$ ,  $p = 0.047$  transects;  $r = -0.70$ ,  $p = 0.002$  plots) or per gram of C (Figure 2.5d), suggesting that SOC was qualitatively less decomposable as a result of physical-chemical protection.

Silt + clay content (%) had no effect on HWEOC in transects or plot samples (Tables 2.3 and 2.4). However, in transect samples, absolute (mg DOC g<sup>-1</sup> soil) and relative HWEOC concentration (mg DOC g<sup>-1</sup>C) were negatively correlated with silt + clay content ( $r = -0.40$ ,  $p = 0.007$  and  $r = -0.59$ ,  $p < 0.0001$ , respectively) (Figure 2.6) and MoM (% SOC) ( $r = -0.81$ ,  $p < 0.0001$  and  $r = -0.77$ ,  $p < 0.0001$ , respectively).

These relationships further support the hypothesis that organo-mineral associations reduce the biological availability of SOC and, therefore, overall SOC stability.

There were significant negative correlations between SUVA and HWEOC concentration ( $\text{g DOC g}^{-1}\text{soil}$ ) ( $r = -0.44$ ,  $p = 0.020$ ) in the transects and between SUVA and SOC decomposability ( $\text{CO}_2$  release  $\text{g}^{-1}\text{soil}$  or  $\text{CO}_2$   $\text{g}^{-1}\text{C}$ ) (respectively:  $r = -0.69$ ,  $p = 0.005$  and  $r = -0.68$ ,  $p = 0.005$ ) in the plots. In other words, higher concentrations of recalcitrant DOC were associated with low bio-availability, as measured during incubation. We interpret these results as an indication that biochemically labile SOC is preferentially used by microbes and depleted from the SOC pool, leaving more recalcitrant SOC to accumulate [51]. Conversely, when labile compounds are more abundant in the soil, the overall concentration of aromatic SOC decreases through relative dilution.

Finally, the negative relationship between total SOC storage and SOC lability expressed as either HWEOC (Figure 2.7a) and cumulative respired  $\text{CO}_2\text{-C}$  (Figure 2.7b) is consistent with Woldeselassie et al. [26], suggesting that the presence of labile SOC in the soil is not conducive to long-term SOC storage. Rather, our results point to the importance of the mineral matrix and especially clay and silt in SOC stabilization and long-term C sequestration. Organic molecules change their spatial conformation when adsorbed to these mineral surfaces, effectively decreasing SOC decomposition (i.e., C loss) by limiting the access of organic substrates to microbes or microbial enzymes [52]. Surface interactions between SOC and mineral particles thus results in the longer residence time of the MoM fraction and the accumulation of stable SOC. The relationship

between clay content and total SOC storage has been used in regional and global SOC assessments [3,53–55]. In Mediterranean climates, clay content and soil properties contributing to stabilization mechanisms (e.g., polyvalent cations involved in organo-mineral associations) have also been found to favor SOC storage in evergreen oak forests [56].

The effect of forest species on total SOC stocks has been investigated in temperate and boreal forests, but without strong evidence or consistent differential SOC storage patterns between conifers and hardwoods [57,58]. Even fewer studies in the literature have focused on forest species effects on stable SOC pools. Unprotected SOC pools seem more responsive to changes in overstory composition, but there are not always significant effects of species composition on long-term C sequestration. Díaz-Pinés et al. [7] found that Scots pine (*Pinus sylvestris* L.) stored more SOC in unprotected fractions than Pyrenean oak (*Quercus pyrenaica* Willd.), while mineral-associated SOC content was similar across the pine-oak ecotone. Similarly, Laganière et al. [30] did not find differences in mineral-associated SOC between aspen and black spruce (*Picea mariana* (Mill.) Britton et al.), but reported more SOC in less protected fractions under black spruce. The content of recalcitrant SOC did not differ significantly between *Cunninghamia lanceolata* and *Michelia macclurei* in plantations in subtropical China [59]. In contrast, significantly higher mineral-associated SOC was found under *Acacia implexa* than under *Eucalyptus melliodora* [60] in native Australian forests.

While our study clearly supports the role of soil texture in site SOC sequestration potential, we further show that vegetation cover, in this case, the transition from aspen to

conifer forests, leads to divergent SOC stabilization. However, the effect of overstory composition on SOC stabilization may be less pronounced in ecosystems where abiotic factors dominate belowground SOC dynamics, such as the presence/absence of mineral adsorption sites. Soils in our study sites were mostly loams, and within that textural class, differences in silt + clay content were a major factor controlling SOC stabilization. At higher clay + silt contents (i.e., >70% silt + clay; silty clay to silt loam), the sheer abundance of sorption sites may have compensated for the potential differences in organic matter input and chemistry associated with differences in overstory cover, and vegetation management may prove less effective in creating large differences in belowground SOC storage and stabilization. However, our results suggest that below this range (i.e., 40%–70% silt + clay), vegetation management towards preserving aspen in the landscape may lead to more long-term SOC storage.

Collectively, our results support the importance of MoM in long-term SOC storage, which is favored by the dominance of aspen in the overstory. Woldeslassie et al. [26] proposed that faster turnover of aspen litter, combined with rapid hydrological transport during snowmelt creates a pulse of DOC that enhances C adsorption to mineral surfaces. Slower decomposition of conifer needles, associated with O horizon accumulation, would conversely result in lower DOC concentrations and, thus, lower adsorption compared to aspen soils. Furthermore, rapid turnover of aspen litter may contribute to SOC stabilization through strong binding of microbial byproducts and dead microbial biomass to mineral surfaces [61]. Several studies have found that microbially-derived compounds are stabilized in the clay fraction [62,63], becoming part of an SOC

pool with a long mean residence time. Thus, rapid turnover of litter and a lack of O horizon accumulation is indeed compatible with SOC stabilization in mineral soil. Conversely, thick O horizons (as typically observed under conifers) speak to litter recalcitrance, but not necessarily to SOC stability in the mineral soil. We observed some differences in SOC quality with vegetation type, but the few analyses performed in this study are insufficient to draw strong conclusions on the role in SOC stabilization of litter quality differences in aspen vs. conifers, an area that we are currently investigating. While SOC stability depends on the simultaneous action of biochemical recalcitrance and physico-chemical protection, biochemical recalcitrance may play a secondary role in SOC storage [64].

Belowground C allocation via rhizodeposition and fine root turnover may be another important contributor to the greater SOC stabilization under aspen [65]. Aspen develops a widespread shallow lateral root system, from which root suckers originate as a mechanism of asexual regeneration [66]. However, in the Canadian boreal forest, fine root net primary production and the relative contribution to total detritus input were lower for aspen than for jack pine (*Pinus banksiana* Lamb.) or black spruce (*Picea mariana* (Mill.) Britton et al.) [67]. Aspen root volume does not decline significantly in the initial stages of conifer encroachment and can contribute to 25%–50% of total fine root biomass in conifer dominated tree clusters [66,68]. Shepperd and Smith [66] reported changes in large root (>4 mm in diameter) volume and non-structural carbohydrate concentrations with stand age in the central Rocky Mountains of Colorado, and more recently, Hudler [69] showed an increase in soluble C compounds in the roots of aspen with increasing

aspen LBA in Southern Utah. These root-derived non-structural compounds may constitute another pathway of C inputs to the soil. The lack of correlation between aboveground and belowground C allocation patterns may explain why using aspen LBA was not a strong predictor for many SOC properties. Changes in soil microbial community composition and abundance [27], microclimate [25], and hydrology [24] induced by conifer encroachment may further modify the species-specific mechanisms of SOC stabilization in aspen forests.

## **Conclusions**

While differences in SOC storage across the aspen-conifer gradient were not always clear cut, potentially due to the high variability in abiotic factors (e.g., soil parent material, texture or landscape position), our results nevertheless suggest that aspen stores more SOC in association with silt and clay, increasing the pool of longer residence time SOC. In conifer-dominated stands, on the other hand, SOC is more susceptible to losses through microbial decomposition. This suggests that conifer encroachment may lead to an increase in less-protected SOC, which may turn over faster, depending on environmental conditions (e.g., soil temperature, soil moisture), accelerate decomposition of existing SOC (so-called priming effect) and result in a progressive decline in total SOC storage. On the other hand, SOC in the mineral-associated fraction may be less affected by conifer encroachment in sites with high silt and clay content. Management strategies pursuing C sequestration in forest ecosystems should therefore not seek to simply increase SOC content, but rather enlarge SOC pools with a longer residence time,



i.e., stabilized through adsorption to the mineral surfaces, as they are less sensitive to disturbances or changes in environmental conditions [64].

The addition of large amounts of more labile SOC forms, at best, contributes to a temporary increase in SOC storage, as they are likely to turn over within a matter of years. Although the geographic scope of our study does not allow us to make broad generalizations for the entire distribution range of aspen in the western US, we observed 25%–30% more mineral-associated SOC in the top soil under aspen compared to adjacent conifer stands. Especially, for finer textured soils conducive to SOC stabilization, management efforts to increase stable SOC pools in the topsoil of montane and subalpine forests should concentrate on the conservation and regeneration of aspen.

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Table 2.1. Topographic characteristics (mean  $\pm$  SD) and parent material of transects at Cedar Mountain (CM) and Franklin Basin (FB). Parent material not available (n.a.).

<b>Site</b>	<b>Elevation (m)</b>	<b>Slope (degrees)</b>	<b>Aspect</b>	<b>Parent Material</b>
CM 1	2552 $\pm$ 8	14 $\pm$ 9	N	Basalt
CM 2	2756 $\pm$ 12	24 $\pm$ 12	N	Basic and intermediate igneous rock
CM 57	2773 $\pm$ 11	24 $\pm$ 11	W	n.a.
CM 111	2685 $\pm$ 9	46 $\pm$ 2	N	Limestone, sandstone and shale
FB 1	2098 $\pm$ 9	10 $\pm$ 4	NE	Quartzite, sandstone and limestone
FB 2	2196 $\pm$ 18	15 $\pm$ 5	E	Limestone



Table 2.2. Topographic and overstory characteristics of plots at Cedar Mountain. LBA, live basal area.

Site	Plot	Elevation (m)	Slope (degrees)	Aspect	Live Basal Area (m <sup>2</sup> ha <sup>-1</sup> )		Contribution of Aspen to LBA (%)	Live Stem Density (n ha <sup>-1</sup> )	
					Aspen	Conifer		Aspen	Conifer
CM 5	CM 5-1	2764	4	NE	28.9	6.1	82.5	1,055.6	223.9
CM 5	CM 5-2	2766	8	NE	0.0	67.0	0.0	0.0	2,499.4
CM 5	CM 5-3	2746	8	NE	35.5	17.5	66.9	649.2	519.4
CM 5	CM 5-4	2759	9	NE	21.4	0.3	98.4	710.0	41.8
CM 8	CM 8-1	2651	19	NW	43.0	0.0	100.0	1,353.0	0.0
CM 8	CM 8-2	2656	16	N	8.8	50.1	15.0	241.1	551.2
CM 8	CM 8-3	2704	23	NW	56.9	37.3	60.4	563.5	1,390.0
CM 8	CM 8-4	2700	26	NW	27.6	9.7	73.9	827.5	157.6
CM 8	CM 8-5	2685	26	NW	11.3	100.6	10.1	118.2	630.4
CM 15	CM 15-	2636	28	W	57.0	36.6	60.9	641.1	1,518.5
CM 15	CM 15-	2638	22	W	0.0	47.4	0.0	0.0	914.2
CM 15	CM 15-	2688	20	NW	38.6	12.6	75.4	468.7	692.9
CM 15	CM 15-	2654	24	W	28.0	4.1	87.1	634.7	1,025.2
CM 15	CM 15-	2636	26	W	27.0	6.7	80.0	305.9	183.5
CM 17	CM 17-	2725	4	W	1.4	36.7	3.6	32.0	1,439.4
CM 17	CM 17-	2738	4	NW	35.3	7.9	81.8	4,286.2	287.9
CM 17	CM 17-	2725	7	W	21.8	1.2	94.6	1,615.5	64.6
CM 17	CM 17-	2714	3	W	13.1	6.7	66.3	1,340.6	127.7
CM 17	CM 17-	2710	3	W	0.9	16.6	5.3	223.4	861.8
CM 20	CM 20-	2883	4	N	49.9	0.0	100.0	3,262.6	0.0
CM 20	CM 20-	2901	3	N	26.7	15.3	63.5	408.6	367.7
CM 20	CM 20-	2907	4	N	27.5	46.0	37.4	383.8	639.7
CM 20	CM 20-	2903	4	NW	12.4	34.1	26.6	127.9	671.7
CM 20	CM 20-	2895	5	N	33.4	25.6	56.6	481.1	609.4

Table 2.3. Mean values and standard deviation of the soil properties of transect samples by vegetation class, and the p-values from the Type III ANOVA testing the main effects of vegetation and silt + clay content (%). Different letters indicate statistically significant differences among the means at a 5% probability level. TOC, total organic carbon; SOC, soil organic carbon; MoM, mineral-associated SOC in the clay and silt fraction; MA > 53  $\mu\text{m}$ , mineral-associated SOC in the sand fraction; LF, light fraction; Cum. CO<sub>2</sub>-C, cumulative released CO<sub>2</sub>-C in long term incubations; HWEOC, hot water extractable organic carbon; DOC, dissolved organic carbon; SUVA, specific ultra violet absorbance at 254 nm; P, P value.

	<b>Bulk Density (g cm<sup>-3</sup>)</b>	<b>TOC (mg C g<sup>-1</sup>) (0–5 cm)</b>	<b>TOC (mg C g<sup>-1</sup>) (5–10 cm)</b>	<b>SOC (Mg C ha<sup>-1</sup>) (0–15 cm)</b>	<b>MoM (Mg C ha<sup>-1</sup>) (0–15 cm)</b>	<b>MA &gt; 53 <math>\mu\text{m}</math> (Mg C ha<sup>-1</sup>) (0–15 cm)</b>	<b>LF (Mg C ha<sup>-1</sup>) (0–15 cm)</b>	<b>MoM (% SOC)</b>	<b>MA &gt; 53 <math>\mu\text{m}</math> (% SOC)</b>
<b>Aspen</b>	0.85 ± 0.15	67.1 ± 23.4	49.9 ± 22.0	51.8 ± 22.6	31.2 ± 15.1 a	3.2 ± 1.9	17.5 ± 11.5	59.0 ± 14.7	6.8 ± 3.6
<b>Mixed</b>	0.83 ± 0.15	69.7 ± 18.0	49.1 ± 16.2	47.4 ± 10.0	25.7 ± 8.8 ab	3.2 ± 1.6	18.5 ± 5.9	53.5 ± 11.0	7.5 ± 3.7
<b>Conifer</b>	0.82 ± 0.13	78.3 ± 21.8	44.4 ± 18.1	40.9 ± 13.3	22.8 ± 9.0 b	3.0 ± 1.8	15.1 ± 6.9	55.6 ± 12.0	6.7 ± 2.9
<b>P Vegetation</b>	0.842	0.264	0.674	0.274	0.035		0.544	0.200	
<b>P Silt + Clay</b>		0.009	0.102	0.583	0.255		0.718	0.050	
	<b>LF (% SOC)</b>	<b>Cum. CO<sub>2</sub>-C (mg C g soil<sup>-1</sup>)</b>	<b>Cum. CO<sub>2</sub>-C (mg C g C<sup>-1</sup>)</b>	<b>HWEOC (mg DOC g soil<sup>-1</sup>)</b>	<b>HWEOC (mg DOC g C<sup>-1</sup>)</b>	<b>SUVA (abs × 100 mg C<sup>-1</sup>)</b>	<b>Clay (%)</b>	<b>Silt (%)</b>	<b>Sand (%)</b>
<b>Aspen</b>	34.2 ± 13.3	5.1 ± 3.0	86.2 ± 61.8	0.6 ± 0.4	9.4 ± 6.3	2.7 ± 0.4	23.2 ± 7.7	35.9 ± 13.6	40.9 ± 19.3
<b>Mixed</b>	39.9 ± 11.4	7.4 ± 5.8	109.3 ± 87.4	0.6 ± 0.4	8.9 ± 4.6	2.7 ± 0.3	22.6 ± 10.0	40.0 ± 14.3	37.6 ± 20.1
<b>Conifer</b>	36.9 ± 10.5	6.2 ± 2.0	81.5 ± 27.3	0.9 ± 1.1	11.3 ± 10.6	2.8 ± 0.8	25.0 ± 8.7	42.8 ± 13.5	32.2 ± 19.7
<b>P Vegetation</b>	0.160	0.228	0.530	0.398	0.640	0.625	0.162	0.691	0.376
<b>P Silt + Clay</b>	0.190	0.209	0.045	0.364	0.120	0.488			

Table 2.4. Linear mixed effects (LME) model estimates for the intercept, the slope for the contribution of aspen to LBA (%), the slope for silt + clay (%) and the variance explained by the site and residuals for different SOC properties from the plot samples.

<b>Response Variable</b>	<b>Intercept</b>	<b><i>t</i>-Value</b>	<b><i>p</i></b>	<b>Aspen LBA (%)</b>	<b><i>t</i>-Value</b>	<b><i>p</i></b>	<b>Silt + Clay (%)</b>	<b><i>t</i>-Value</b>	<b><i>p</i></b>
<b>SOC (Mg C ha<sup>-1</sup>)</b>	33.06 ± 20.55	1.61	0.143	0.07 ± 0.09	0.79	0.438	0.17 ± 0.31	0.55	0.598
<b>MoM (Mg C ha<sup>-1</sup>)</b>	-4.45 ± 9.36	-0.48	0.639	0.04 ± 0.05	0.71	0.489	0.51 ± 0.14	3.81	0.001
<b>LF (Mg C ha<sup>-1</sup>)<sup>1</sup></b>	-0.28 ± 0.08	-3.25	0.005	4.43 × 10 <sup>-4</sup> ± 3.18 × 10 <sup>-4</sup>	1.39	0.181	-6.18 × 10 <sup>-4</sup> ± 1.25 × 10 <sup>-3</sup>	-0.49	0.628
<b>MoM (%)</b>	28.79 ± 13.23	2.18	0.048	3.59 × 10 <sup>-3</sup> ± 0.05	0.07	0.946	0.52 ± 0.20	2.66	0.019
<b>LF (%)</b>	43.09 ± 10.96	3.93	0.001	-0.01 ± 0.04	-0.31	0.757	-0.20 ± 0.16	-1.23	0.235
<b>Cum. CO<sub>2</sub>-C (mg C g soil<sup>-1</sup>)<sup>2</sup></b>	1.24 ± 0.86	1.44	0.177	3.32 × 10 <sup>-3</sup> ± 2.96 × 10 <sup>-3</sup>	1.12	0.287	-3.96 × 10 <sup>-3</sup> ± 0.01	-0.33	0.744
<b>Cum. CO<sub>2</sub>-C (mg C g C<sup>-1</sup>)<sup>2</sup></b>	4.84 ± 0.78	6.20	<0.0001	1.38 × 10 <sup>-3</sup> ± 2.53 × 10 <sup>-3</sup>	0.55	0.598	-0.01 ± 0.01	-1.08	0.301
<b>HWEOC (mg DOC g soil<sup>-1</sup>)</b>	0.52 ± 0.25	2.14	0.048	-1.98 × 10 <sup>-3</sup> ± 9.14 × 10 <sup>-4</sup>	-2.17	0.044	1.04 × 10 <sup>-3</sup> ± 3.62 × 10 <sup>-3</sup>	0.29	0.778
<b>HWEOC (mg DOC g C<sup>-1</sup>)</b>	16.66 ± 5.09	3.28	0.006	-0.06 ± 0.02	-3.13	0.006	-0.05 ± 0.08	-0.60	0.560
<b>SUVA (abs × 100 mg C<sup>-1</sup>)<sup>2</sup></b>	0.78 ± 0.52	1.50	0.161	2.48 × 10 <sup>-4</sup> ± 2.05 × 10 <sup>-3</sup>	-0.12	0.906	0.03 ± 0.01	4.01	0.002
<b>Clay (%)</b>	22.68 ± 3.52	6.45	<0.001	-0.01 ± 0.03	-0.42	0.681			
<b>Silt (%)</b>	40.57 ± 4.31	9.41	<0.0001	-0.05 ± 0.03	-1.48	0.157			
<b>Sand (%)</b>	36.74 ± 6.25	5.88	0.001	0.06 ± 0.05	1.27	0.220			

<sup>1</sup> Transformed with the reciprocal root ( $-1/x^{0.5}$ ); <sup>2</sup> transformed with the logarithm in base 10.

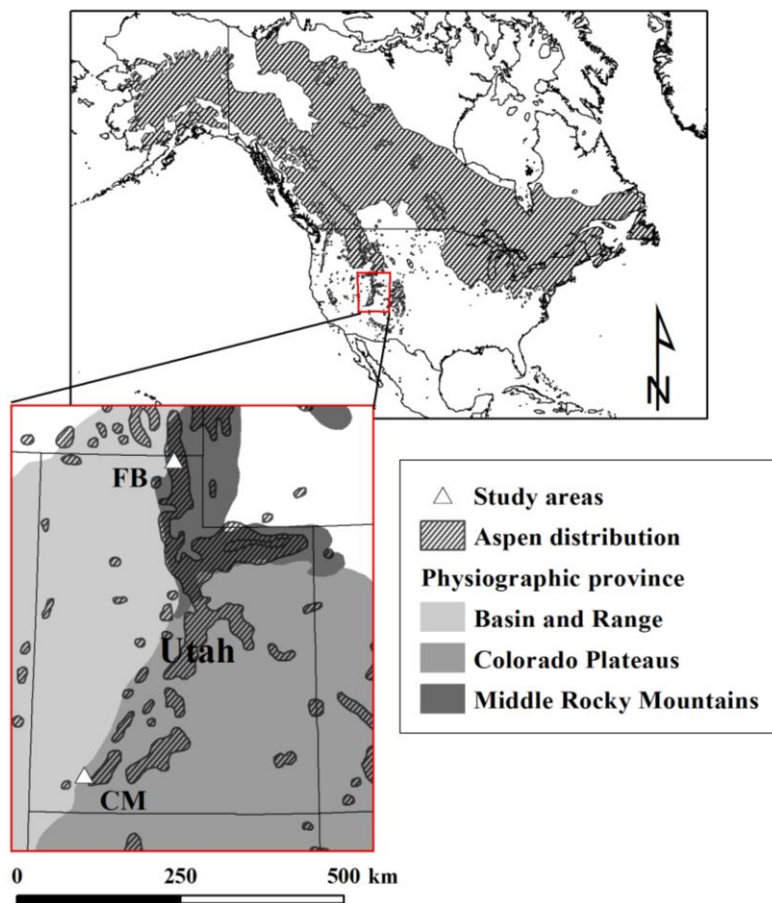


Figure 2.1. Aspen distribution in North America and the location of study areas.

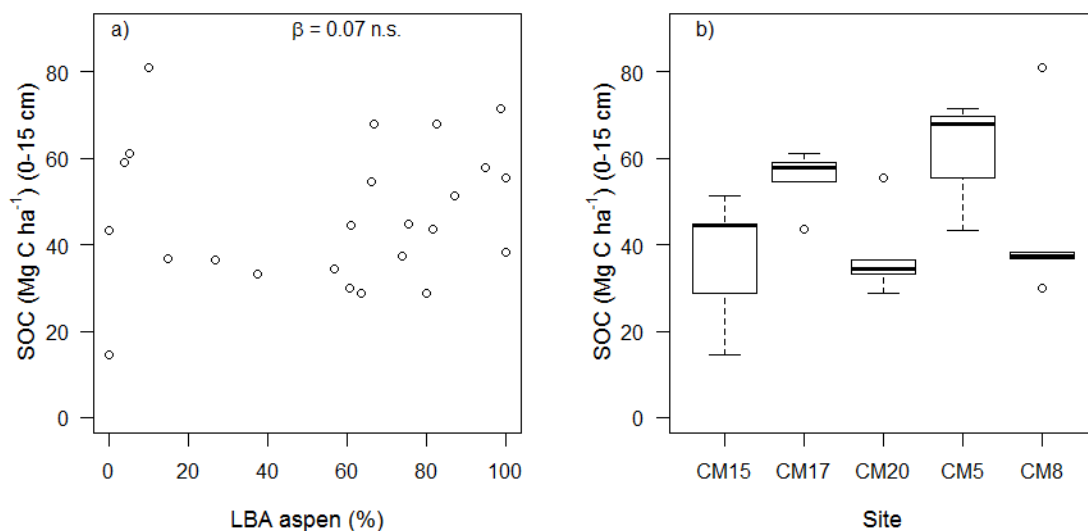


Figure 2.2. SOC content ( $\text{mg C ha}^{-1}$ ) (0–15 cm) for the plots at Cedar Mountain (a) vs. aspen contribution to LBA (%) and (b) by site.  $\beta$  represents the slope for aspen LBA (%). Statistically non-significant, n.s. The boxes represent the 25 and 75 percentiles. The median is represented by the horizontal bold line. The whiskers represent the 10th and 90th percentiles, and the circles correspond to outliers.

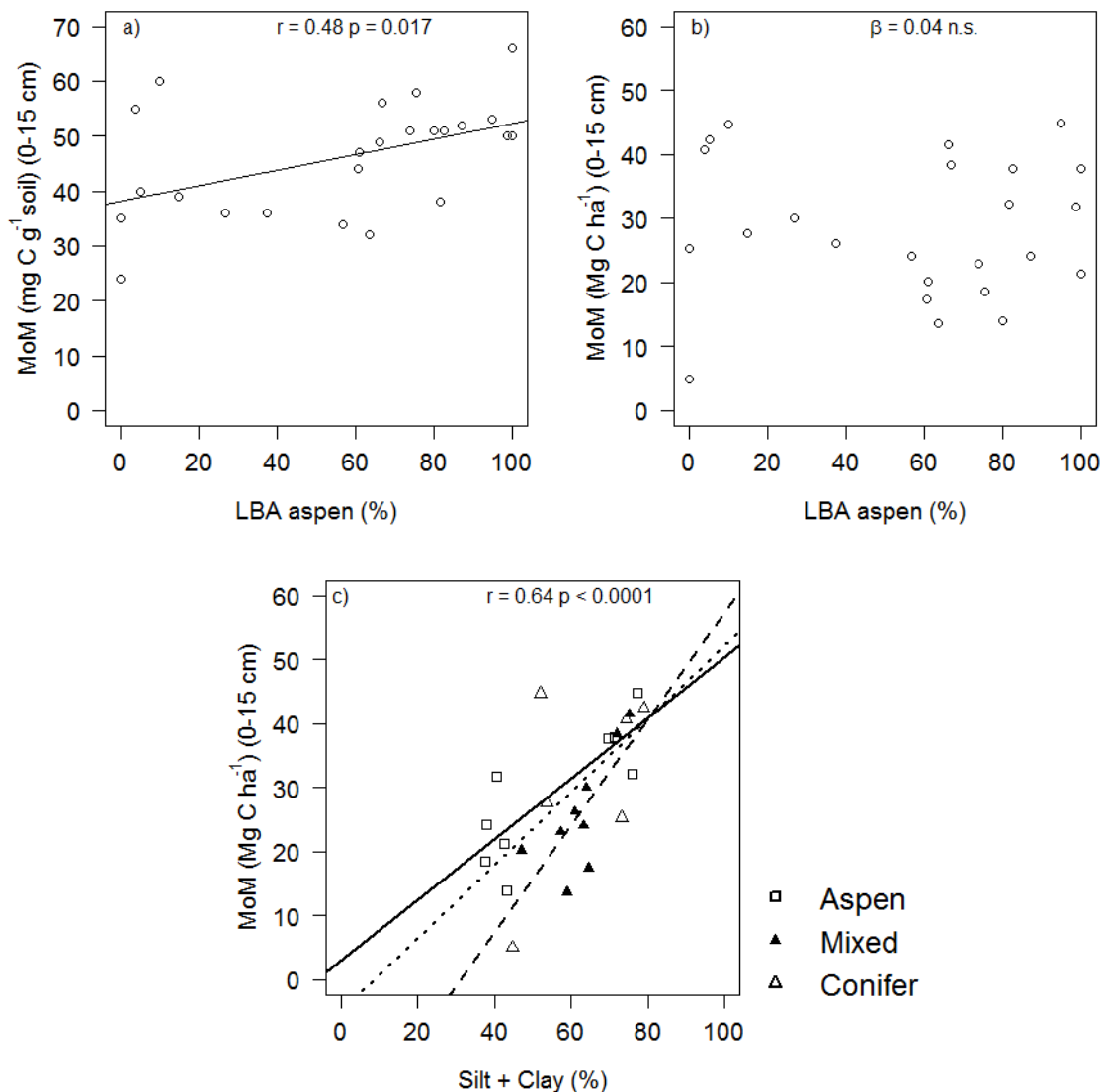


Figure 2.3. (a) MoM C concentration ( $\text{mg C g}^{-1}$  soil) vs. aspen contribution to LBA (%). (b) MoM content ( $\text{Mg C ha}^{-1}$ ) vs. aspen contribution to LBA (%).  $\beta$  represents the slope for aspen LBA (%) (c) MoM content vs. silt + clay content by dominant overstory in Cedar Mountain plots. Regression line — corresponds to aspen dominated overstory; - - - to mixed overstory;  $\cdot \cdot \cdot \cdot$  to conifer dominated overstory.

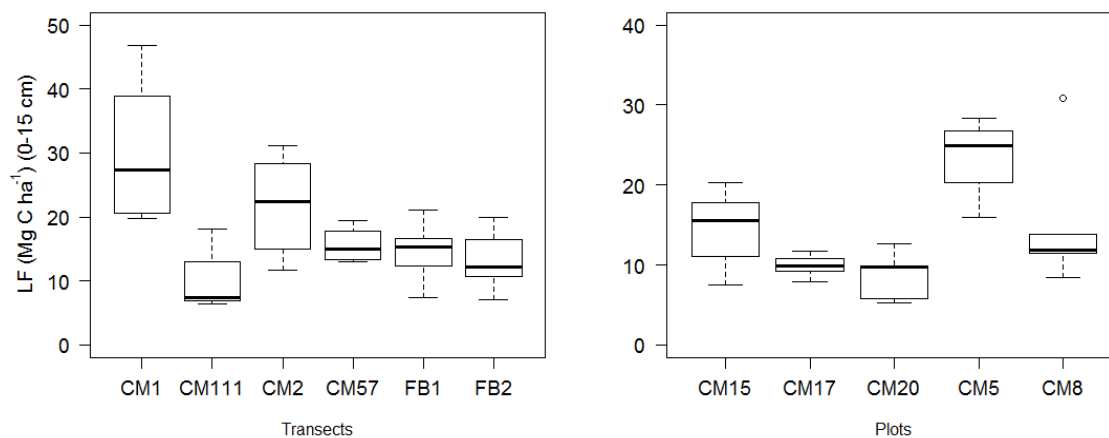


Figure 2.4. (a) Boxplots of LF stocks by sampled sites in Cedar Mountain and Franklin Basin transects; (b) Boxplots of LF stocks by sampled sites in the Cedar Mountain plots. The boxes represent the 25th and 75th percentiles. The median is represented by the horizontal black line. The whiskers represent the 10th and 90th percentiles, and the circles correspond to outliers.

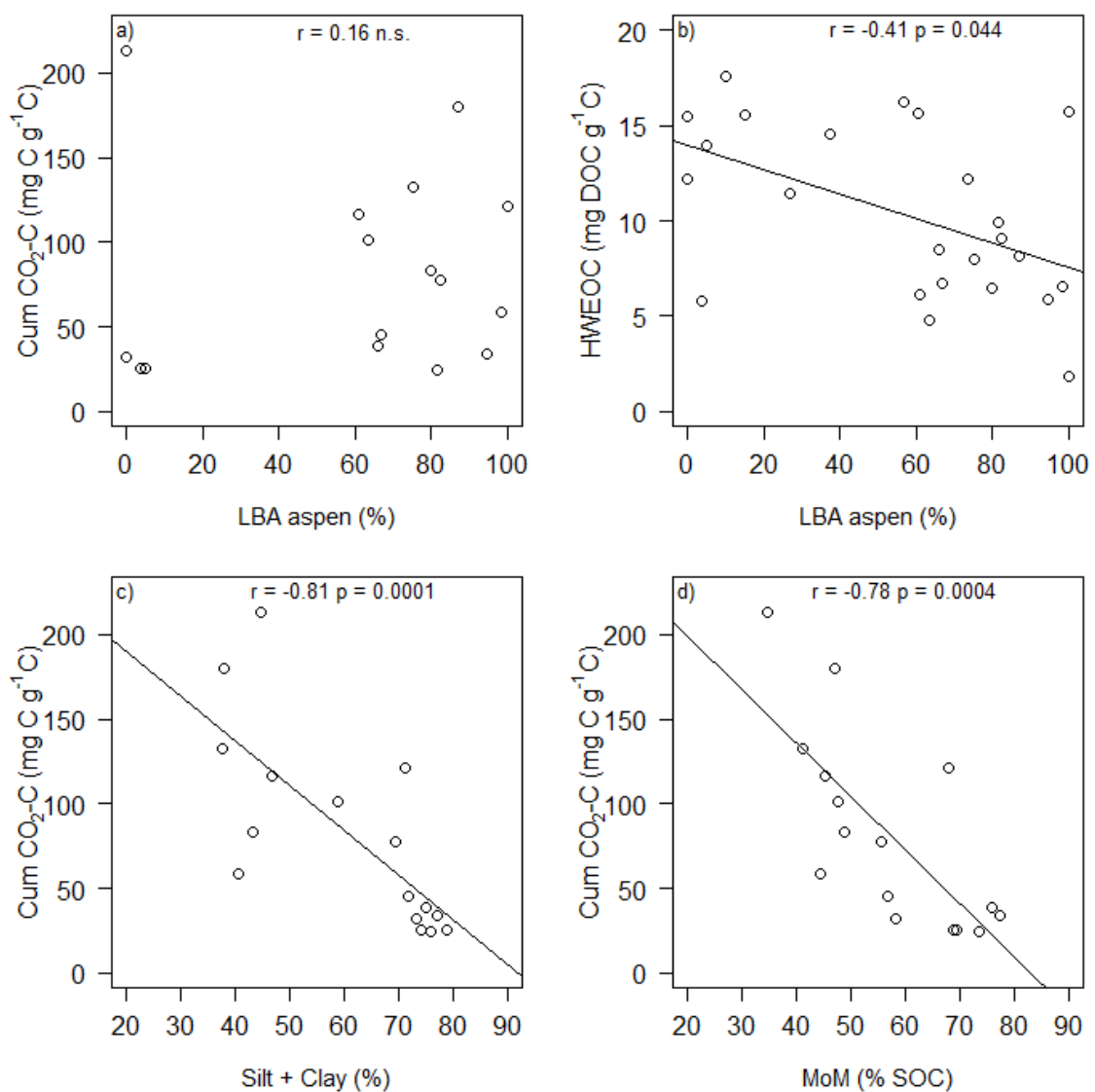


Figure 2.5. (a) SOC decomposability vs. contribution of aspen to LBA; (b) relative HWEOC concentration vs. the contribution of aspen to LBA; (c) SOC decomposability vs. silt + clay (%) or (d) MoM (% SOC).



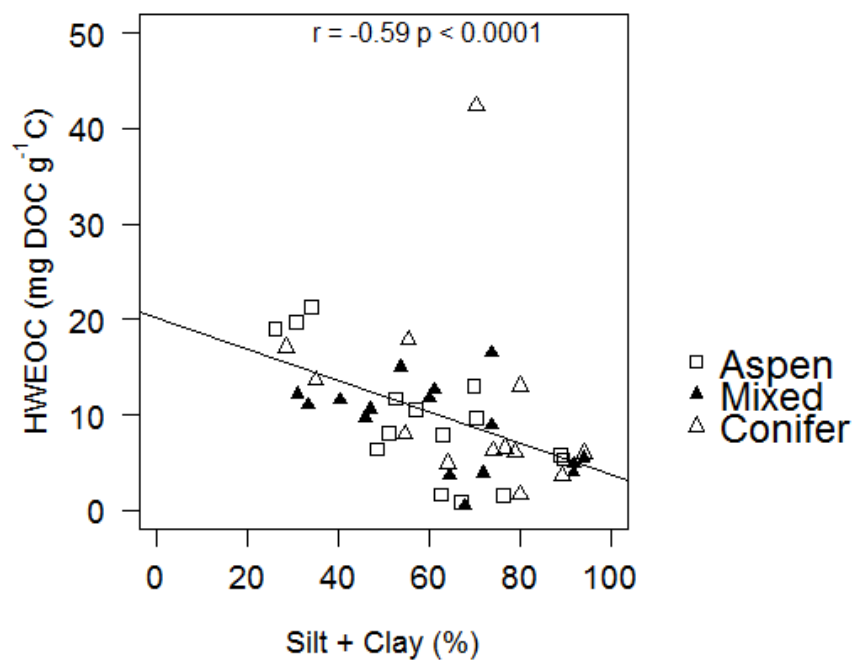


Figure 2.6. The inverse relationship between silt + clay content (%) and HWEOC expressed as the fraction of SOC in transect samples.

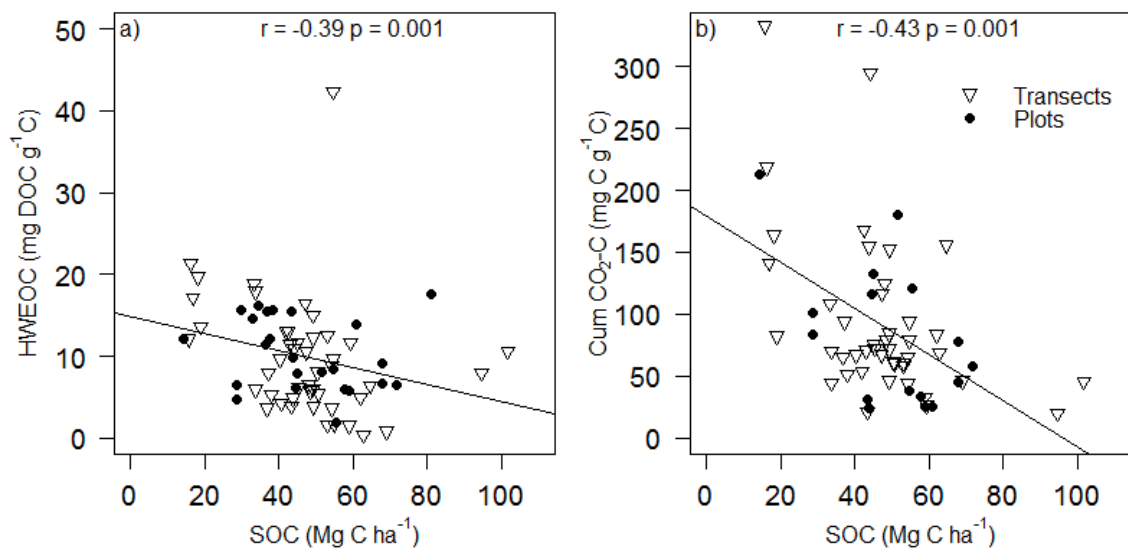


Figure 2.7. (a) SOC storage (Mg C ha<sup>-1</sup>) vs. relatively soluble SOC (b) vs. relative decomposable SOC in transects and plots.

## CHAPTER 3

CHEMICAL COMPOSITION OF SOIL ORGANIC CARBON FROM MIXED  
ASPEN-CONIFER FORESTS CHARACTERIZED WITH FOURIER  
TRANSFORM INFRARED SPECTROSCOPY<sup>2</sup>**Introduction**

The chemical composition and residence time of soil organic carbon (SOC) pools is determined by the simultaneous action of stabilization mechanisms (i.e., processes that lengthen SOC turnover time) and microbial decomposition. Stabilization mechanisms are generally grouped into biochemical recalcitrance, physical protection, and chemical stabilization (Six et al., 2002). Biochemical recalcitrance relies on molecular-level characteristics of organic compounds (e.g., presence of functional groups, spatial conformation) that hinder microbial decomposition (Sollins et al., 1996). Physical protection limits the access of microbes and enzymes to organic matter due to occlusion in aggregates, limited oxygen diffusion or soil moisture (Six et al., 2002; von Luetzow et al., 2006). Chemical stabilization results from the interaction between organic molecules and mineral surfaces or metal oxides and hydroxides. Preferential organo-mineral interactions influenced by soil mineralogy and soil forming processes selectively stabilize certain organic compounds (Wattel-Koekkoek et al., 2001; Rumpel et al., 2004; Spielvogel et al., 2008). The stability of SOC in superficial soil horizons is relevant for C sequestration since they are more sensitive to changes in vegetation cover and land

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<sup>2</sup> Coauthored by Mercedes Román Dobarco, Astrid Jacobson and Helga Van Miegroet

management practices (Guo and Gifford, 2002; Hutchinson et al., 2007; Seddaiu et al., 2013).

Forest species influence the quantity and chemical composition of organic matter input, which in interaction with soil abiotic (e.g., soil climate, hydrology, mineralogy and texture) and biotic factors (e.g., soil microbial community composition and abundance, soil fauna) may lead to specific patterns of SOC stabilization and differences in chemical composition of SOC pools across vegetation gradients. In montane mixed forests of Utah, soils (0-15 cm) under trembling aspen (*Populus tremuloides* Michx.) have higher absolute content (see Chapter 2) or relative content of mineral-associated SOC (i.e., greater proportion of total SOC) (Woldeselassie et al., 2012) than soils under adjacent conifer stands. The C concentration of mineral-associated SOC also increases with predominance of aspen in the overstory (see Chapter 2). Greater SOC stabilization in aspen stands may be explained by the individual or combined effect of 1) higher C input as litterfall, dead roots or root exudates, 2) differences in C allocation (i.e., below- vs. aboveground detritus) and pathways of SOC stabilization, and 3) higher concentration of recalcitrant compounds or precursor molecules of secondary recalcitrant compounds (sensu von Luetzow et al., 2006) and/or 4) higher relative abundance of organic molecules preferentially adsorbed to mineral surfaces. Previous studies on aspen and conifer species in boreal forests found higher ratio of belowground to total detritus production for conifers than aspen, but similar values of fine root production (Ruess et al., 1996; Steele et al., 1997). Aspen and conifer litter in boreal ecosystems also show differences in the relative abundance of organic compounds and decomposition patterns

(Otto and Simpson, 2006; Strukulj et al., 2012). Although there are no data available for semi-arid montane mixed forests, differences in C input and allocation, or in litter chemistry may influence SOC stabilization and storage in these ecosystems.

Direct input of dead fine roots and root exudates, microbial metabolites and cell components, and dissolved organic matter derived from decomposing litter are the main sources of SOC. Aliphatic compounds derived from cutin, suberin, and waxes are believed to be very recalcitrant and to accumulate in the soil through selective preservation (Sollins et al., 1996; Baldock, 2002; Lorenz et al., 2007). Otto and Simpson (2006) found higher concentration of hydrolyzable lipids derived from cutin, suberin, and waxes in aspen leaves than in lodgepole pine needles (*Pinus contorta* Douglas ex Loudon), but the concentrations of cutin and suberin derived compounds were fairly similar in aspen A horizon and pine O horizon, suggesting that preservation of aliphatic C is not generalizable for all forest systems (Strukulj et al., 2012). While some plant-derived compounds may be inherently recalcitrant, other compounds become recalcitrant after microbial resynthesis or abiotic complexation, and are often preserved in fine particle size fractions where the high specific surface and reactivity of clay minerals enhance organo-mineral associations (von Luetzow et al., 2006). Mineral-associated SOC has a relatively low C:N ratio, and is enriched in aliphatic C and organic compounds of microbial origin (Poirier et al., 2005). Microbial carbohydrates are also stabilized in the fine fraction of superficial horizons (Spielvogel et al., 2008). Microbial metabolites and cell debris thus perform an important function for long term SOC stabilization. Since microbes are in intimate contact with mineral particles, microbially derived molecules are

more able to reach adsorption sites than plant derived molecules, and develop strong bonds through carboxylic groups, aliphatic C, and amide groups from lipids and proteins (Baldock, 2002; Chenu and Stotzky 2002; Kleber et al., 2007; Lehmann et al., 2007; Keiluweit et al., 2012).

The more rapid decomposition of aspen litter compared with conifer needles (Stump and Binkley, 1993) may generate greater input of simple molecules of plant origin and microbial compounds involved in organo-mineral associations (e.g., polysaccharides, organic acids, amino sugars, etc.), which combined with water infiltration may enhance SOC stabilization through the adsorption of dissolved organic carbon (Woldeselassie et al., 2012). Stump and Binkley (1993) did not observe differences in root lignin, cellulose contents, and root decomposition between aspen, lodgepole pine (*Pinus contorta* var. *latifolia* Engelm. Ex S. Watson), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in the central Rocky Mountain of Colorado. However, the positive correlation between soluble C compounds in aspen roots and aspen live basal area observed in southern Utah (Abbey Hudler, personal communication, 2014) suggests that root derived non-structural carbohydrates could contribute to SOC stabilization directly or after microbial resynthesis. While these hypotheses need to be tested, these mechanisms may explain greater content of mineral-associated SOC under aspen stands, and may influence its chemical composition.

Fourier transform infrared spectroscopy (FTIR) has been used to characterize the chemical composition of mineral-associated and light fractions of SOC (Poirier et al.,

2005; Lehmann et al., 2007). This technique has been used to discriminate organic matter from forest, pasture or agricultural soils from Brazil (Haberhauer et al., 2000; Tivet et al., 2013), and between temperate forest soils and moorland (Chapman et al., 2001). We used FTIR-ATR (attenuated total reflectance) to address two objectives: (1) characterize the chemical composition of soil organic matter, and of the light and mineral-associated SOC fractions across the aspen-conifer ecotone in montane forests in Utah; and (2) investigate whether higher content of mineral-associated SOC under aspen stands is related to higher concentration of recalcitrant compounds (i.e., aliphatic C) and/or preferential stabilization of certain molecules (i.e., polysaccharides, amino-sugars, etc.).

## **Material and methods**

### *Study areas*

The mixed aspen-conifer forest study areas are located in southern (Cedar Mountain) and northern Utah (Franklin Basin). Cedar Mountain (CM) is a high elevation plateau (1800-3200 m) located within the Colorado Plateau region of southwestern Utah. Mean annual precipitation is 812 mm (NRCS, 2013). Cedar Mountain has monsoonal storms in the late summer, but most of the precipitation is in the form of snowfall, occurring from October through April (Evans, 2010). Average monthly air temperature ranges from -3.8 °C in December to 15.5 °C in July (NRCS, 2013). Forest vegetation is dominated by trembling aspen; whereas subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), and white fir (*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.) are scarce in the landscape, forming mixed or pure stands at the edges of the plateau or northern slopes (Evans, 2010). Aspen stands at Cedar

Mountain are considered stable (i.e., self-replacing, no conifer encroachment) despite lacking multilayer structure (Rogers et al., 2010). Soil types are commonly Alfisols or Mollisols (McNab and Avers, 1994; Rogers et al., 2010), developed predominantly on sedimentary rock (sandstone, siltstone, mudstone, carbonaceous shale) and igneous rock (basalt) (Averitt, 1962; Rowley et al., 2008; Biek et al., 2010). Franklin Basin (FB) is a montane-subalpine area (1770–3030 m) located between the Bear River Range and the Wasatch Range in the central Rocky Mountains, distributed between northeastern Utah and southeastern Idaho (Kusbach, 2010). The precipitation regime is snow dominated, with a mean annual precipitation of 1197 mm (NRCS, 2013). Monthly average air temperature ranges between -6.9 °C in December and 16.4 °C in July (NRCS, 2013). Forest ecosystems are represented by trembling aspen and mixed conifer stands of Douglas fir, subalpine fir, and limber pine (*Pinus flexilis* E. James). During the late 1800's, intense logging and frequent fires favored the establishment of pure aspen stands (Rogers et al., 2011). Moister climate, moderate sheep grazing, and fire suppression during the 20th century created conditions leading to conifer encroachment (Rogers et al., 2011). Aspen communities in this area are predominantly seral, except where topographic conditions favor stable aspen stands (Rogers et al., 2014). Soils are commonly Alfisols and Mollisols, developed on limestone or quartzite sandstone (Kusbach, 2010).

### *Sampling design*

Two sampling designs were used in this study, using overstory vegetation as a categorical variable in the first, and as a continuous variable in the second. For the first design, hereafter referred as “transects”, four sites at CM and two sites at FB were



selected based in the presence of mixed aspen-conifer stands. Three transects were laid at each site, within a minimum distance of 20 m from each other. Within each transect, two soil cores (5 cm of diameter; 0–15 cm depth) were taken in the mineral soil beneath aspen, mixed, and conifer cover in similar conditions of slope, elevation and aspect (Table 3.1). Soil cores were divided in the field into 5 cm intervals.

The second design investigated the changes in SOC chemical composition along the continuum from pure aspen to pure coniferous stands, and hereafter will be named “plots”. A total of 24 plots of 10 m radius were located in five different sites at CM (Table 3.2). Five soil cores (5 cm of diameter; 0–15 cm depth) per plot were combined into a composite sample to represent the influence of stand composition and structure. Species, status (i.e., dead or alive), and diameter at breast height (i.e., stem diameter at 1.30 m height) were recorded for all trees of diameter bigger than 3 cm. Stand measurements were used to calculate live basal area (LBA) by species ( $\text{m}^2 \text{ha}^{-1}$ ) and aspen contribution to LBA (LBA aspen %). Of the 24 plots sampled, nine plots had aspen dominated overstory (i.e., > 75 % LBA aspen), six plots had conifer dominated overstory (i.e., > 75 % LBA conifer), and nine plots had mixed overstory (i.e., 25–75 % LBA aspen) (Table 3.2). Hence, variability in stand structure and species composition along the gradient aspen-conifer was relatively well represented.

### *Laboratory analyses*

Transect middle core sections (5–10 cm) were oven dried at 105°C for 24 hours, weighed, sieved (2-mm mesh), and used to calculate bulk density and SOC storage in the 0–15 cm as presented in Chapter 2 of this dissertation. Transect (0–5 cm) and plot

composite samples (0–15 cm) were sieved (2-mm sieve) and air dried to avoid alterations in SOC composition. Organic-free mineral matrix samples (MM) (0–15 cm plots, 5–10 cm transects) were obtained using sodium hypochlorite (NaOCl) (6%) at room temperature in three consecutive extraction cycles of 12-12-6 hours as described in Román Dobarco et al. (2014) following a modification from Kaiser et al. (2002). A simplified fractionation method was used to separate the mineral-associated SOC and light fraction (LF). Briefly, 20 g of air dry soil (0–5 cm transects, 0–15 cm plots) were mixed with distilled water (1:5 soil-water w/w) and shaken for 16 hours. The soil slurry was sieved through a 53 $\mu$ m sieve with distilled water, separating mineral-associated SOC smaller than 53 $\mu$ m (MoM) from the fraction bigger than 53 $\mu$ m. Electrostatic charged Petri dishes were used to attract light particles from the >53 $\mu$ m fraction following a modification of Kaiser et al. (2009), corresponding to free and occluded LF. Mineral-associated SOC in the sand size fraction (> 53  $\mu$ m) was not included in the spectroscopic analysis because its contribution to SOC was small (Chapter 2). This fraction had a mean C concentration ( $\pm$  SD) of 20.9  $\pm$  17.6 mg g<sup>-1</sup> soil C, compared to the 60.0  $\pm$  18.3 mg g<sup>-1</sup> soil C in the MoM, and 238.2  $\pm$  72.9 mg g<sup>-1</sup> soil C in the LF (Román Dobarco et al., unpublished data). Bulk soil (BS) samples (transects 5-10 cm, plots 0-15 cm), MM, MoM, and LF samples were finely ground with mortar and pestle and scanned with FTIR-ATR (Nicolet 6700, Thermo-Fisher, Pittsburgh, PA, USA). Each spectrum was composed of 500 scans and recorded from 4000 to 650 cm<sup>-1</sup> at 8 cm<sup>-1</sup> intervals. Three replicates per sample were scanned and aggregated following automatic baseline and diamond-ATR corrections.

*Spectral processing and statistical analysis*

The variance between MM and BS spectra from transect samples was calculated with Omnic 7.1 (Thermo Electron Corp., Waltham, MA) to identify the peak with the lowest variability in the region 900-600  $\text{cm}^{-1}$ . Spectra with analogous MM should have similar absorbance in the range 900-600  $\text{cm}^{-1}$  for a given MM concentration, since this region is mainly attributed to mineral particles (Haberhauer et al. 2000). Therefore, the height of the peak around 797  $\text{cm}^{-1}$  was used to normalize the MM spectra for the concentration of mineral matrix before and after NaOCl treatment with the factor:

$$c = \frac{HBS_{797}}{HMM_{797}}$$

Where  $HBS_{797}$  is the corrected peak height (i.e., height measured from a baseline fitted between 740  $\text{cm}^{-1}$  – 715  $\text{cm}^{-1}$ ) at 797  $\text{cm}^{-1}$  from the BS spectra, and  $HMM_{797}$  the corrected peak height at 797  $\text{cm}^{-1}$  from the MM spectra. Spectral subtraction was used to obtain the spectra of organic matter (OM):

$$OM = BS - cMM$$

OM, LF, and MoM spectra were analyzed using Omnic 7.1 (Thermo Electron Corp., Waltham, MA) and the R package ChemoSpec (Hanson, 2013). Prominent peaks located at 1975  $\text{cm}^{-1}$ , 2029  $\text{cm}^{-1}$ , 2097  $\text{cm}^{-1}$  and 2159  $\text{cm}^{-1}$  appeared in most spectra and were assigned to carbon monoxide based on the Omnic 7.1 spectral library. The region 2700–1700  $\text{cm}^{-1}$  was excluded from the analysis because the information attributable to organic matter was masked by carbon monoxide noise. Functional groups were assigned to spectral regions based on FTIR literature on humic substances and metal-humic

complexes (Celi et al., 1997; Elkins and Nelson, 2002; Senessi and Loffredo, 2005), organo-clay complexes (Parker and Frost, 1996), peat, forest floor, forest mineral soil or arable soils (Haberhauer et al., 2000; Chapman et al., 2001; Priha et al., 2001; Solomon et al., 2005; Artz et al., 2006; Schindler et al., 2007; Tatzber et al., 2007; Rusu et al., 2010; Dick et al., 2011; Matejkova and Simon, 2012) and SOC fractions (Augris et al. 1998; Kaiser and Ellerbrock, 2005; Poirier et al., 2005; Lehmann et al., 2007) (Table 3.3).

The chemical complexity of soils leads to overlapping peaks and infrared bands which can potentially correspond to more than one functional group. However, spectral information can be combined with multivariate statistical analysis to cluster soil samples of similar spectral characteristics and identify the spectral regions where differences among samples lie. Principal component analysis (PCA) was performed with ChemoSpec on OM, MoM, and LF spectra to highlight differences in chemical composition among similar substrates (i.e., OM, LF, and MoM). The loading vectors of the first two principal components (PC) were interpreted and used to characterize underlying differences in chemical composition among samples based on their position on the scores plot (Bonnier and Byrne, 2012). The loading coefficients inform on the source of spectral variability as they represent the correlation between the original variables (wavenumbers) and the PC (Lattin et al., 2003; Bonnier and Byrne, 2012). The PCs are defined by the variables with which are most highly correlated (Lattin et al., 2003) and can have a positive (negative) loading coefficient for a given wavenumber, such that spectra with higher absorbance at this wavenumber will have a greater positive (negative) score. The sign and magnitude of the score would be related to the relative contributions of these components. Bonnier and

Byrne (2012) showed that the sign of the loading coefficients for a PC were related to the direction of the gradient in the relative contribution of components (e.g., albumin, collagen, histone) to Raman spectra along the PC. The relation between PC loadings and the position of samples on the scores plot can be used to distinguish samples with marked differences in spectral features (Bonnier and Byrne, 2012), but the interpretation of loadings and their relationship with differences in chemical composition for complex samples is somewhat obscure and uncertain, requiring cautious interpretation.

We tested the effect of overstory vegetation on the normalized height of important spectral peaks (i.e., 2920, 2850, 1648, 1542, 1165, 1087, 1034, 1004  $\text{cm}^{-1}$ ), on the relative peak heights, on the polysaccharides to carboxylate and aromatic C ratio (i.e.,  $\frac{A_{1087}}{\sum A_{1500}}$ ), and polysaccharides to aliphatic C ratio (i.e.,  $\frac{A_{1087}}{A_{2920}}$ ) of MoM spectra. The relative peak height is obtained dividing each peak height by the sum of all peaks (i.e.,  $rA_{1648} = A_{1648} / \sum A_{1030-2920}$ ). While the normalized peak height (i.e. absorbance divided by the organic C concentration of MoM samples) is related to the concentration of functional groups, the relative peak height informs on the relative contribution of these functional groups to the soil sample. The effect of vegetation on these metrics was tested with linear mixed effects (LME) models. In transect spectra, vegetation type was treated as a fixed factor, and site and transect as random effect factors. For plot spectra, aspen contribution to LBA (% aspen LBA) was treated as fixed effect factor, and site as random factor. LME models were applied with the R package lmerTest (Kuznetsova et al., 2014). The p-values of fixed effects are determined with F test based on Sattethwaite's approximation

of degrees of freedom, and the random effects with likelihood ratio test (Kuznetsova et al., 2014).

## Results

### *General spectra characteristics*

Average transect samples OM spectra by vegetation cover do not show marked differences in peak location or shape (Fig. 3.1). Aspen OM spectra have higher absorbance in the band 1000–1030  $\text{cm}^{-1}$  than mixed and conifer, which may be attributed to C-O groups and polysaccharides. Similarly, average transects LF and MoM spectra follow the trend aspen > mixed > conifer in the region 1000–1100  $\text{cm}^{-1}$  (Fig. 3.2), indicating that aspen has greater concentration of polysaccharides and C-O groups across all sample types. OM and MoM spectra show a prominent peak around 1000  $\text{cm}^{-1}$  that can be partly caused by Si-O bonds from mineral particles and is consequently less pronounced in LF spectra. There are different trends across sample types for the absorbance in the aliphatic C region (2850–2950  $\text{cm}^{-1}$ ): while the absorbance decreases from conifer > mixed > aspen for OM spectra (Fig. 3.1), LF follows the opposite trend with aspen > mixed > conifer, and MoM spectra overlap (Fig. 3.2). Compared to MoM spectra, differences in absorbance among vegetation types are more marked in LF, mostly in the region 1000–1160  $\text{cm}^{-1}$  and less pronounced in 2850–2950  $\text{cm}^{-1}$ . The OM and LF spectra further have small peaks in the region 1400–1650  $\text{cm}^{-1}$ , indicative of aromatic C (1510  $\text{cm}^{-1}$ ), amide (1474  $\text{cm}^{-1}$ , 1510  $\text{cm}^{-1}$ , and 1541  $\text{cm}^{-1}$ ), carboxylic groups (1621  $\text{cm}^{-1}$ ), and aliphatic C (1397  $\text{cm}^{-1}$  and 1456  $\text{cm}^{-1}$ ) (Fig. 3.1 and Fig. 3.2). It was not

possible to average the spectra of plot samples by vegetation type since it was characterized as continuous variable.

MoM spectra of transect and plot samples display differences in the region 1000–1200  $\text{cm}^{-1}$  when averaged by site across all vegetation types (Fig. 3.3 and Fig. 3.4). Across transect and plot samples, those CM sites developed over sedimentary rock (i.e., CM1, CM57, CM111, CM8, and CM15) exhibit a double peak at 1000  $\text{cm}^{-1}$  and 1028  $\text{cm}^{-1}$  indicative of Si-O bonds and polysaccharides. CM sites where the parent material is basalt (i.e., CM2, CM5, CM17, and CM20) and FB sites, where soils developed from limestone or quartzite, have a less pronounced peak at 1028  $\text{cm}^{-1}$ . These spectral differences across sites may be caused by the intrinsic signal of the mineral matrix, but possibly also by preferential adsorption of polysaccharides by minerals derived from sandstone or mudstone. Spectra of CM sites on sedimentary rock (i.e., CM1, CM57, CM111, CM8, and CM15) exhibit higher absorbance in the shoulder around 1100  $\text{cm}^{-1}$  than FB sites and CM sites on basalt. This shoulder may indicate complexes between organic molecules and metals (metal-O vibration of bound hydroxylated and or hydrated metal ions with humic substances at 1080–1130  $\text{cm}^{-1}$ ) (Senesi and Loffredo, 2005). Visual examination of spectra within a site or transect showed differences among vegetation classes, but there was not a consistent pattern across sites.

#### *Principal component analysis*

PCA of OM spectra from transect samples did not cluster by vegetation cover (Fig. 3.5). The first PC explains 79% of the variance, and seems to be correlated to higher absorbance in the aliphatic region, causing an overall tilt of spectra baseline indicated by

the loading vector (Fig. 3.6.a). The second PC explains 14% of the variance, and can be interpreted as an indicator of molecules with C-O bonds ( $1000\text{ cm}^{-1}$ ), and to a lesser extent of aliphatic C, carboxyl, aromatic C, and amide ( $1200\text{--}1700\text{ cm}^{-1}$ ) (Fig. 3.6.a). As to site differences: Spectra from CM2 are clearly discriminated from other sites (Fig. 3.5.a), likely caused by lower absorbance on the  $2700\text{--}3100\text{ cm}^{-1}$  region, while spectra from FB1 and FB2 have higher absorbance in the  $2700\text{--}3100\text{ cm}^{-1}$  region. Spectra from CM57 and CM111 are scattered through the scores plot, due to higher intra-group variability on absorbance.

The first two principal components of transect samples explain respectively 72 % and 13 % for LF spectra, and 63 % and 21 % for MoM spectra. Spectra are clustered by site rather than by vegetation cover (Fig. 3.5.b and Fig. 3.5.c). LF spectra from CM57 and CM111 have higher absorbance around  $1080\text{ cm}^{-1}$  and  $1165\text{ cm}^{-1}$  compared to the other sites, suggesting higher contribution of polysaccharides and aliphatic C. These differences are indicated by peaks of positive sign at  $1088\text{ cm}^{-1}$  and  $1165\text{ cm}^{-1}$  in the loading vector for PC2 (Fig. 3.8.a), and is reflected in higher scores for CM57 and CM111 on the second axis (Fig. 3.5.b). The scores plot of the MoM spectra show a cluster composed of CM2, FB1 and FB2 with higher scores on the PC2 axis, and a second cluster of CM1, CM57, and CM111 with lower PC2 scores, and slightly higher PC1 scores (Fig. 3.5.c). The PC2 loading vector can be interpreted as a contrast between polysaccharides (negative peaks at  $1034\text{ cm}^{-1}$  and  $1087\text{ cm}^{-1}$ ) and amide, aromatic C, ester, adsorbed C=O to clay particles ( $1541\text{ cm}^{-1}$ ), ketones, quinones, deprotonated carboxylate groups ( $1651\text{ cm}^{-1}$ ), and aliphatic C ( $2850\text{--}2950\text{ cm}^{-1}$ ), with positive



coefficients (Fig. 3.8.b). Average MoM spectra by site showed that CM1, CM57, and CM111 had a peak at  $1028\text{ cm}^{-1}$  and a more prominent shoulder around  $1100\text{ cm}^{-1}$  than CM2, FB1, and FB2. Similarly to the interpretation of loadings in Bonnier and Byrne (2012), spectra with higher absorbance around  $1034\text{ cm}^{-1}$ ,  $1087\text{ cm}^{-1}$ , where PC2 loading has negative coefficients, may have smaller PC2 scores (Fig. 3.8.b). This is the case with the discrimination in the second axis of CM1, CM57, and CM111 vs. CM2, FB1, and FB2. Likewise, positive PC2 scores for CM2, FB1, and FB2, may indicate lower absorbance at  $1034\text{ cm}^{-1}$ ,  $1087\text{ cm}^{-1}$  and  $1100\text{ cm}^{-1}$ , and relatively higher absorbance in the spectral region  $1400\text{-}2950\text{ cm}^{-1}$  spectra (Fig. 3.5.c and Fig. 3.8.c).

The scores plot for OM spectra from the plot samples does not indicate clear clusters by site (Fig. 3.7.a). The first component explains 93% of variability, and the second 3%. The loading vector of PC1 has positive coefficients for all variables, but with increasing magnitude towards the aliphatic region (Fig. 3.6.b). Thus, spectra with greater absorbance at  $2700\text{--}3100\text{ cm}^{-1}$  (e.g., CM5 and CM17) have greater PC1 scores. LF spectra from plot samples are scattered in the scores plot (Fig.3.7.b), probably caused by greater chemical heterogeneity of partly decomposed LF components and microclimate differences across plots. The first two PC for plot LF spectra explain 51% and 25 % of variability. The loading vector of PC1 for LF characterizes the shape of the main peak at  $1000\text{--}1100\text{ cm}^{-1}$ . The shoulder at  $1092\text{ cm}^{-1}$  has negative coefficients while the peak at  $997\text{ cm}^{-1}$  has positive coefficients. Therefore, spectra with greater concentration of polysaccharides may have smaller PC1 scores (Fig. 3.8.c). PC2 of LF can be interpreted as a contrast between polysaccharides and functional groups with C-O bonds ( $1006\text{ cm}^{-1}$ ,

1029  $\text{cm}^{-1}$ , 1107  $\text{cm}^{-1}$ ) versus phenols (1388  $\text{cm}^{-1}$ ), deprotonated carboxylate group (1388  $\text{cm}^{-1}$ , 1584  $\text{cm}^{-1}$ ), aromatic C and amide (1584  $\text{cm}^{-1}$ ) (Fig. 3.8.c). The first two PC for plot MoM spectra explain 67% and 22% of the variability, with two clusters of spectra along the first axis differentiating CM8 and CM15 from CM20, CM17, and CM5 (Fig. 3.7.c). The PC1 loading vector plot of the MoM spectra indicates Si-O vibrations, C-O groups from ether, ester, acids (1008  $\text{cm}^{-1}$ ), polysaccharides (1031  $\text{cm}^{-1}$  and 1094  $\text{cm}^{-1}$ ) and aliphatic C (1165  $\text{cm}^{-1}$ ) with positive coefficients. CM8 and CM15 had higher absorbance in these peaks (Fig. 3.4), and consequently have higher PC1 scores (Fig. 3.7.c). Positive loading coefficients for PC2 of MoM plot spectra indicate Si-O vibrations and polysaccharides (1005  $\text{cm}^{-1}$  and 1029  $\text{cm}^{-1}$ ), amide, aromatic C, ketone, quinone, carboxylate, ester, adsorbed C=O (1508  $\text{cm}^{-1}$ , 1522  $\text{cm}^{-1}$ , 1541  $\text{cm}^{-1}$ , 1558  $\text{cm}^{-1}$ , 1652  $\text{cm}^{-1}$ ), and a peak at 1636  $\text{cm}^{-1}$  may indicate a bond between Al and unprotonated carboxylate (COO-Al) (Fig. 3.8.d). The position of spectra along the PC2 axis indicates variability of absorbance within sites (Fig. 3.7.c).

#### *Linear mixed models on peak heights*

In transect MoM spectra, vegetation has no effect on normalized absorbances or relative peak heights (Table 3.4 and Table 3.5), indicating similar concentration and relative contribution of main functional groups among vegetation types. Overstory composition has no effect on normalized absorbance and relative peak height for most variables in plot MoM spectra (Table 3.6 and Table 3.7). The statistically significant effect of aspen LBA % (Table 3.6 and Table 3.7) and a decrease in the absorbance and relative peak height at 2850  $\text{cm}^{-1}$  and 2920  $\text{cm}^{-1}$  with aspen dominance (Fig. 3.9 and Fig.

3.10) suggests that the concentration of aliphatic C increases slightly with conifer encroachment, and that SOC changes qualitatively as a result of overstory composition. These results indicate that greater content of MoM under aspen is not due to the accumulation of recalcitrant, aliphatic C.

In transect samples, site has a significant effect on the polysaccharides to carboxylate and aromatic C ratio (i.e.,  $\frac{A_{1087}}{\Sigma A_{1500}}$ ), and polysaccharides to aliphatic C ratio (i.e.,  $\frac{A_{1087}}{A_{2920}}$ ), which are higher for CM1, CM111, and CM57 than for CM2, FB1, and FB2 (Table 3.4). These ratios inform of a less aromatic and aliphatic character of SOC associated to minerals derived from sedimentary rocks at CM. Relative peak height is affected by site for all variables (Table 3.7). Site again has a statistically significant effect for plot samples on absorbance at 1508, 1521, 1541, 1558, 1636, 1652, 2850, and 2920  $\text{cm}^{-1}$ , following the general trend  $\text{CM8} < \text{CM15} < \text{CM5} \approx \text{CM17} < \text{CM20}$  (Table 3.6). These results suggest enrichment in aromatic C, amides, carboxylate, and aliphatic C in soils developed from basalt, possibly due to higher abundance of Fe oxides in these soils able to bond with organic molecules via ligand exchange or cation mediated interactions. Conversely, the proportion of polysaccharides relative to carboxylate and aromatic C (i.e.,  $\frac{A_{1094}}{\Sigma A_{1500}}$ ), and aliphatic C (i.e.,  $\frac{A_{1094}}{A_{2920}}$ ) is higher for CM8 and CM15 (Table 3.6). There is also a site effect on relative peak height for several wavenumbers. CM8 and CM15 have higher relative heights at 1094  $\text{cm}^{-1}$  (polysaccharides, C-O-C bonds), 1165  $\text{cm}^{-1}$  (aliphatic C and carboxylate) than CM5, CM17, and CM20. Relative peak heights for aromatic C, amide, carboxylate, esters, ketones, quinones, and adsorbed C=O (1541,

1558, 1636, and 1652  $\text{cm}^{-1}$ ) are higher for CM5, CM17, and CM20 than at CM8 and CM15 (Table 3.7).

## Discussion

The chemical composition of SOC characterized with FTIR differs in the concentration of polysaccharides, C-O groups, and aliphatic C across the aspen-conifer ecotone. The relative increase in aliphatic C with conifer encroachment and the slightly higher normalized absorbance (absorbance  $\text{g}^{-1}$  C  $\text{g}$  soil) of the polysaccharide band in MoM samples under aspen suggests that greater content of MoM under aspen may not be caused by accumulation of aliphatic C, but by other SOC stabilization mechanisms. Prominent peaks indicative of polysaccharides and C-O from ethers, esters, and acid groups in MoM spectra suggest that chemical stabilization, rather than intrinsic recalcitrance, is the main mechanism of SOC protection in aspen forests. Strukelj et al. (2012) found that the ratio alkyl: O/N-alkyl for aspen litter and balsam fir (*Abies balsamea* (L.) Mill.) decreased with decomposition while the proportion of alkyl C increased for white spruce (*Picea glauca* (Moench) Voss), supporting the notion that selective preservation of aliphatic C varies with species composition. Soil organic matter extractions from A horizons under European beech (*Fagus sylvatica*, L.) and silver fir (*Abies alba* Mill.) had dominant contribution of alkyl and O-alkyl C, the latter slightly more abundant under beech than under fir (Certini et al., 2004). On the other hand, carbohydrate content and origin did not differ significantly between A horizons from Norway spruce (*Picea abies* L. (Karst.)) forest and mixed deciduous forest (Guggenberger et al., 1994). Aliphatic compounds derived from waxes, cutin, and suberin

from conifer needles may be preserved in the soil due to biochemical recalcitrance and organo-mineral interactions. At the same time, higher C concentration and content in the MoM fraction under aspen overstory (see Chapter 2) suggests that relatively simple molecules of plant and microbial origin (e.g., C-O compounds, polysaccharides) contribute significantly to SOC stabilization, or that there is higher input of organic matter under aspen. Although it may seem contradictory that a lower proportion of recalcitrant compounds co-occurs with greater MoM-SOC storage, presumably labile compounds like polysaccharides can have mean residence times of decades (Schmidt et al., 2011). Our findings support the idea that litter biochemistry is not the dominant factor in SOC stabilization, but rather that overall ecosystem interactions determine SOC composition and stabilization (Schmidt et al., 2011).

Initial differences in litter chemistry between aspen and conifers converged into a similar chemical composition of MoM within sites. Similarly, a Finnish study found that PCA performed on FTIR spectra of mineral soil samples from *Pinus sylvestris*, *Picea abies*, and *Betula pendula* did not differentiate overstory species, but discriminated two forest sites (Priha et al., 2001). Discrimination of vegetation cover using FTIR is possible for the litter layer, or when differences in land use or site conditions associated to vegetation are more abrupt (Haberhauer et al., 2000; Chapman et al., 2001; Tivet et al., 2013). Less distinct patterns in mineral soils may arise from clay minerals and oxides in the clay and silt fraction themselves absorbing radiation in the mid-infrared (Reeves, 2012). Despite exclusion of spectral areas typically dominated by mineral matrix characteristics (e.g.,  $< 950 \text{ cm}^{-1}$  and  $> 3600 \text{ cm}^{-1}$ ), the band  $1000\text{-}1100 \text{ cm}^{-1}$  reflects the

composition of the mineral matrix (Si-O bonds from clays and quartz) and of organic matter (C-O bonds from ester, ether, and carboxyl). Therefore, it is not possible to state conclusively that discrimination of MoM from plots is due solely to differences in organic matter. The MoM spectra from plot samples clearly discriminated sites located on basalt (CM5, CM17, and CM20) from those on sedimentary rock (CM8 and CM15). Spectral differences among sites may also indicate preferential adsorption of polysaccharides at CM8 and CM 15, and enrichment of aromatic C, ketones, amides, and aliphatic C at CM5, CM17, and CM20. Basalt may contain more content of Fe oxides and hydroxides promoting strong bonds with carboxylates, aliphatic C, and amides (Chorover et al., 2004; Wagai et al., 2008; Jones and Singh, 2014). The shoulder at  $1100\text{ cm}^{-1}$  may indicate stabilization of SOC via organo-metal complexes (Senesi and Loffredo, 2005) with minerals derived from sandstone and mudstone at CM8 and CM15. Without specific data on mineralogy and Fe and Al (hydro) oxides content it is not possible to propose specific interaction mechanisms between organic compounds and mineral surfaces, but it is probable that very different parent material (e.g., basalt and sedimentary rock) develop different dominant mineralogy. Topographic variables like elevation and slope may have further influenced the composition of SOC through differences in microclimate or soil forming processes (e.g., water runoff and soil erosion). Plots located at CM8 and CM15 had greater slope ( $22.0^\circ \pm 4.4^\circ$  at CM8,  $24^\circ \pm 3.2^\circ$  at CM15) than the other three sites ( $4.9^\circ \pm 4.1^\circ$  averaged across sites). CM8 and CM15 were also located at lower elevation ( $\sim 2650\text{ m}$ ) than CM5 ( $\sim 2759\text{ m}$ ), CM17 ( $\sim 2722\text{ m}$ ), and CM20 ( $\sim 2898\text{ m}$ ). Lower contribution of recalcitrant SOC indicated by higher polysaccharide to aliphatic C, or

polysaccharide to aromatic C ratios at steeper sites may indicate relatively younger soils as result of erosion, and thus less accumulation of aliphatic C through time.

For the transects, slight to significant variations occur in topography, microclimate, and/or parent material among the transects and among sites that may be driving SOC speciation. The interaction between soil forming factors can lead to differences in SOC stabilization mechanisms for soils developed on similar parent material caused by varying degree of mineral weathering (Rasmussen et al., 2006). Higher relative contents of aliphatic C, aromatic C, carboxylate and amide in CM2, FB1, and FB2, where the parent material is basalt (CM2), sedimentary rock (quartzite, sandstone, and limestone) (FB1), or limestone (FB2), result in diverse bonding mechanisms. Jones and Singh (2014) found enrichment of aliphatic C and amides in quartz dominated fractions from topsoils stabilized through H-bonding and to a lesser extent by ligand exchange with siloxane (i.e., Si-O-organic matter). Calcium performs an important role in SOC stabilization in alkaline soils, either through the formation of cation bridges (i.e., mineral-Ca-organic matter), chelation (i.e., Ca-organic matter), or cross-linking (organic matter-Ca-organic matter) with negative functional groups (e.g., hydroxyl or carboxyl) (Oades, 1988; von Lützow et al., 2006; Kunhi Mouvenchery et al., 2012). It is possible that in soils developed from limestone at FB calcium contributes to the formation of micro-aggregates in the nano or microscale included in the MoM fraction (Six et al., 2004).

The interpretation of loadings with contrasting peaks (e.g., PC1 of MoM-SOC from plot samples) can inform intuitively on the relative contribution of organic

compounds. A caveat of PCA loading analysis is that as an exploratory technique, it does not distinguish the intra-group from the inter-group variability. An advantage of this method is the rapid identification of spectral regions with high variance, and of differences in composition among clustered spectra. Provided that the loadings have meaningful information they should be used as support of other statistical analysis.

## **Conclusions**

In montane aspen-mixed forests, the speciation and stabilization of SOC seems more strongly controlled by site conditions rather than litter biochemistry. We found relative increase of aliphatic C with conifer encroachment, and increased proportion of polysaccharides and C-O groups under aspen overstory. These results suggest that species may influence SOC stabilization and speciation through differences in C input, microbial community composition and transformation of plant debris, and microclimate. Greater storage of MoM under aspen may be caused by adsorption of simple molecules resulting from litter breakdown, fine root turnover, or rhizodeposition. At the same time, it is possible that the microbial community is an important driver of SOC stabilization through interactions with mineral particles and input of microbial biomass and byproducts (e.g., polysaccharides). In mixed montane forests, selection of species with higher proportion of aliphatic C (i.e., suberin and cutin) may not be conducive to higher storage of SOC, as recalcitrance seems to play a secondary role relative to chemical stabilization. Future studies on SOC stabilization in semi-arid montane ecosystems should investigate the relationship between mineralogy and organic matter compounds, and evaluate the role of microbial turnover in organo-mineral associations.



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Table 3.1. Topographic characteristics (mean  $\pm$  SD) and geology of transects at Cedar Mountain and Franklin Basin.

Site	Elevation (m)	Slope (degrees)	Aspect	Geological substrate
CM1	2552 $\pm$ 8	14 $\pm$ 9	N	Mudstone with minor sandstone and conglomerate <sup>†</sup>
CM2	2756 $\pm$ 12	24 $\pm$ 12	N	Basalt <sup>‡</sup>
CM57	2773 $\pm$ 11	24 $\pm$ 11	W	Mudstone, sandstone <sup>§</sup>
CM111	2685 $\pm$ 9	46 $\pm$ 2	N	Sandstone, siltstone, mudstone, claystone, carbonaceous shale, coal, and marl <sup>¶</sup>
FB1	2098 $\pm$ 9	10 $\pm$ 4	NE	Unconsolidated conglomerate consisting of locally derived cobbles, boulders, and angular blocks (quartzite, sandstone, and limestone) <sup>#</sup>
FB2	2196 $\pm$ 18	15 $\pm$ 5	E	Dolomitic limestone, and limestone <sup>#</sup>

<sup>†</sup> Averitt, 1962

<sup>‡</sup> Rowley et al., 2008

<sup>§</sup> Biek et al., 2012

<sup>¶</sup> Biek et al., 2010

<sup>#</sup> Dover, 2006



Table 3.2. Topographic and overstory characteristics and geology of plots at Cedar Mountain.

Site	Plot	Elevation (m)	Slope (degrees)	Aspect	Live basal area (m <sup>2</sup> ha <sup>-1</sup> )		Contribution of aspen to LBA (%)	Parent material
					Aspen	Conifer		
CM 5	CM 5-1	2764	4	NE	28.9	6.1	82.5	Basalt †
CM 5	CM 5-2	2766	8	NE	0.0	67.0	0.0	
CM 5	CM 5-3	2746	8	NE	35.5	17.5	66.9	
CM 5	CM 5-4	2759	9	NE	21.4	0.3	98.4	
CM 8	CM 8-1	2651	19	NW	43.0	0.0	100.0	Sandstone, mudstone, siltstone, carbonaceous shale, marl ‡
CM 8	CM 8-2	2656	16	N	8.8	50.1	15.0	
CM 8	CM 8-3	2704	23	NW	56.9	37.3	60.4	
CM 8	CM 8-4	2700	26	NW	27.6	9.7	73.9	
CM 8	CM 8-5	2685	26	NW	11.3	100.6	10.1	
CM 15	CM 15-1	2636	28	W	57.0	36.6	60.9	Sandstone, mudstone §
CM 15	CM 15-2	2638	22	W	0.0	47.4	0.0	
CM 15	CM 15-3	2688	20	NW	38.6	12.6	75.4	
CM 15	CM 15-4	2654	24	W	28.0	4.1	87.1	
CM 15	CM 15-5	2636	26	W	27.0	6.7	80.0	
CM 17	CM 17-1	2725	4	W	1.4	36.7	3.6	Basalt†
CM 17	CM 17-2	2738	4	NW	35.3	7.9	81.8	
CM 17	CM 17-3	2725	7	W	21.8	1.2	94.6	
CM 17	CM 17-4	2714	3	W	13.1	6.7	66.3	
CM 17	CM 17-5	2710	3	W	0.9	16.6	5.3	
CM 20	CM 20-1	2883	4	N	49.9	0.0	100.0	Mudstone, sandstone § Colluvium (olivine basalt) §
CM 20	CM 20-2	2901	3	N	26.7	15.3	63.5	
CM 20	CM 20-3	2907	4	N	27.5	46.0	37.4	Olivine basalt §
CM 20	CM 20-4	2903	4	NW	12.4	34.1	26.6	
CM 20	CM 20-5	2895	5	N	33.4	25.6	56.6	

† Rowley et al., 2008

‡ Biek et al., 2010

§ Biek et al., 2012

Table 3.3. Functional group assignment by broad spectral regions.

<b>Region (cm<sup>-1</sup>)</b>	<b>Functional groups / Organic compounds</b>
1030-1080	Polysaccharides
1000-1100	C-O bond of ethers, esters, phenols, alcoholic groups or acid groups
1150-1170	Aliphatic chains (C-OH stretching)
1200-1260	Protonated carboxylic groups (asymmetric C-O stretch and OH deformation of COOH), phenols and tertiary alcohols (C-OH bending), aryl ethers
1380-1415	Unprotonated or complexed carboxylic groups (symmetric or antisymmetric stretching of COO <sup>-</sup> ), alkyl CH deformations (CH <sub>2</sub> , CH <sub>3</sub> ), phenols (OH deformation and C-O stretching)
1510-1540	Amide (N-H deformation and C=N stretching); (NH stretching vibration)
1600-1640	"Carboxylate" group (stretching asymmetric vibrations of C=O from acids, esters, ketones, quinones; aromatic C=C stretching vibrations)
2830-2860	Asymmetric C-H stretching vibrations from aliphatic methyl and methylene
2920-3000	Symmetric C-H stretching vibrations from aliphatic methyl and methylene
3220-3240	Amide (N-H deformation and C=N stretching); (NH stretching vibration)
3400-3450	Stretching vibration of bonded and non bonded OH groups (water)

Table 3.4. Mean normalized absorbance ( $\pm$  SD) (absorbance  $\text{g}^{-1}$  C g soil) by vegetation for transect MoM-SOC spectra.

Vegetation	A <sub>1004</sub>	A <sub>1034</sub>	A <sub>1087</sub>	A <sub>1165</sub>	A <sub>1508</sub>	A <sub>1521</sub>	A <sub>1541</sub>
Aspen	4.22 $\pm$ 1.61	3.96 $\pm$ 1.50	2.08 $\pm$ 0.74	1.04 $\pm$ 0.38	0.41 $\pm$ 0.18	0.47 $\pm$ 0.19	0.59 $\pm$ 0.23
Mixed	3.96 $\pm$ 1.44	3.74 $\pm$ 1.26	2.03 $\pm$ 0.55	1.01 $\pm$ 0.25	0.41 $\pm$ 0.13	0.47 $\pm$ 0.14	0.58 $\pm$ 0.17
Conifer	3.39 $\pm$ 1.22	3.25 $\pm$ 1.07	1.85 $\pm$ 0.57	0.95 $\pm$ 0.29	0.38 $\pm$ 0.13	0.44 $\pm$ 0.14	0.54 $\pm$ 0.16
<i>p</i> Vegetation	0.403	0.440	0.618	0.754	0.906	0.886	0.860
<b>Site</b>							
CM1	4.74 $\pm$ 2.11	4.37 $\pm$ 1.91	2.24 $\pm$ 0.91	1.05 $\pm$ 0.42	0.38 $\pm$ 0.13	0.44 $\pm$ 0.15	0.56 $\pm$ 0.19
CM2	3.67 $\pm$ 1.95	3.47 $\pm$ 1.85	1.76 $\pm$ 0.88	0.90 $\pm$ 0.45	0.45 $\pm$ 0.21	0.52 $\pm$ 0.23	0.63 $\pm$ 0.27
CM57	3.55 $\pm$ 1.15	3.54 $\pm$ 1.05	2.08 $\pm$ 0.55	1.03 $\pm$ 0.26	0.33 $\pm$ 0.06	0.39 $\pm$ 0.07	0.48 $\pm$ 0.09
CM111	4.59 $\pm$ 1.18	4.25 $\pm$ 1.03	2.09 $\pm$ 0.50	0.93 $\pm$ 0.24	0.34 $\pm$ 0.15	0.40 $\pm$ 0.16	0.49 $\pm$ 0.19
FB1	3.09 $\pm$ 0.82	2.99 $\pm$ 0.76	1.82 $\pm$ 0.43	0.99 $\pm$ 0.25	0.38 $\pm$ 0.12	0.44 $\pm$ 0.13	0.55 $\pm$ 0.15
FB2	4.05 $\pm$ 0.97	3.78 $\pm$ 0.83	2.12 $\pm$ 0.42	1.13 $\pm$ 0.20	0.49 $\pm$ 0.13	0.55 $\pm$ 0.13	0.67 $\pm$ 0.15
<i>p</i> Site	1	1	1	1	1	1	1
Vegetation	A <sub>1558</sub>	A <sub>1636</sub>	A <sub>1648</sub>	A <sub>2850</sub>	A <sub>2920</sub>	Ratio $\frac{A_{1094}}{\sum A_{1500}}$	Ratio $\frac{A_{1094}}{A_{2920}}$
Aspen	0.65 $\pm$ 0.25	1.06 $\pm$ 0.41	1.16 $\pm$ 0.45	1.80 $\pm$ 0.78	2.45 $\pm$ 1.02	1.01 $\pm$ 0.25	0.88 $\pm$ 0.20
Mixed	0.65 $\pm$ 0.18	1.03 $\pm$ 0.27	1.13 $\pm$ 0.30	1.74 $\pm$ 0.46	2.36 $\pm$ 0.60	1.00 $\pm$ 0.21	0.88 $\pm$ 0.18
Conifer	0.60 $\pm$ 0.17	0.96 $\pm$ 0.28	1.05 $\pm$ 0.31	1.63 $\pm$ 0.52	2.23 $\pm$ 0.66	0.95 $\pm$ 0.20	0.84 $\pm$ 0.18
<i>p</i> Vegetation	0.847	0.776	0.825	0.806	0.813	0.886	0.818
<b>Site</b>							
CM1	0.63 $\pm$ 0.21	1.02 $\pm$ 0.35	1.10 $\pm$ 0.38	1.68 $\pm$ 0.62	2.28 $\pm$ 0.82	1.10 $\pm$ 0.19	0.97 $\pm$ 0.09
CM2	0.71 $\pm$ 0.29	1.13 $\pm$ 0.49	1.23 $\pm$ 0.55	1.95 $\pm$ 0.93	2.62 $\pm$ 1.23	0.76 $\pm$ 0.09	0.66 $\pm$ 0.02
CM57	0.54 $\pm$ 0.10	0.85 $\pm$ 0.19	0.93 $\pm$ 0.20	1.41 $\pm$ 0.38	1.93 $\pm$ 0.51	1.19 $\pm$ 0.22	1.08 $\pm$ 0.11
CM111	0.54 $\pm$ 0.19	0.83 $\pm$ 0.21	0.92 $\pm$ 0.25	1.33 $\pm$ 0.31	1.88 $\pm$ 0.41	1.24 $\pm$ 0.18	1.11 $\pm$ 0.15
FB1	0.61 $\pm$ 0.14	1.00 $\pm$ 0.23	1.08 $\pm$ 0.25	1.73 $\pm$ 0.41	2.35 $\pm$ 0.52	0.94 $\pm$ 0.13	0.77 $\pm$ 0.04
FB2	0.74 $\pm$ 0.16	1.21 $\pm$ 0.24	1.32 $\pm$ 0.26	2.09 $\pm$ 0.41	2.80 $\pm$ 0.53	0.88 $\pm$ 0.09	0.76 $\pm$ 0.07
<i>p</i> Site	1	1	1	0.93	1	<b>0.001</b>	<b>&lt; 0.0001</b>

Table 3.5. Relative peak height ( $\pm$  SD) by vegetation class for transect MoM-SOC spectra.

Vegetation	A <sub>1004</sub>	A <sub>1034</sub>	A <sub>1087</sub>	A <sub>1165</sub>	A <sub>1508</sub>	A <sub>1521</sub>
Aspen	0.21 $\pm$ 0.03	0.20 $\pm$ 0.02	0.10 $\pm$ 0.01	0.05 $\pm$ 0.005	0.02 $\pm$ 0.004	0.02 $\pm$ 0.004
Mixed	0.20 $\pm$ 0.02	0.19 $\pm$ 0.02	0.11 $\pm$ 0.01	0.05 $\pm$ 0.01	0.02 $\pm$ 0.004	0.02 $\pm$ 0.004
Conifer	0.19 $\pm$ 0.02	0.19 $\pm$ 0.02	0.11 $\pm$ 0.01	0.06 $\pm$ 0.005	0.02 $\pm$ 0.003	0.03 $\pm$ 0.003
<i>p</i> Vegetation	0.076	0.197	0.267	0.130	0.624	0.553
<b>Site</b>						
CM1	0.23 $\pm$ 0.01	0.21 $\pm$ 0.01	0.11 $\pm$ 0.01	0.05 $\pm$ 0.003	0.02 $\pm$ 0.004	0.02 $\pm$ 0.004
CM2	0.19 $\pm$ 0.01	0.18 $\pm$ 0.01	0.09 $\pm$ 0.002	0.05 $\pm$ 0.002	0.02 $\pm$ 0.003	0.03 $\pm$ 0.003
CM57	0.21 $\pm$ 0.02	0.21 $\pm$ 0.01	0.12 $\pm$ 0.01	0.06 $\pm$ 0.01	0.02 $\pm$ 0.004	0.02 $\pm$ 0.004
CM111	0.25 $\pm$ 0.02	0.23 $\pm$ 0.01	0.11 $\pm$ 0.01	0.05 $\pm$ 0.005	0.02 $\pm$ 0.004	0.02 $\pm$ 0.003
FB1	0.18 $\pm$ 0.01	0.18 $\pm$ 0.01	0.11 $\pm$ 0.004	0.06 $\pm$ 0.003	0.02 $\pm$ 0.003	0.03 $\pm$ 0.003
FB2	0.19 $\pm$ 0.01	0.18 $\pm$ 0.01	0.10 $\pm$ 0.01	0.05 $\pm$ 0.003	0.02 $\pm$ 0.002	0.03 $\pm$ 0.002
<i>p</i> Site	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0.01</b>	<b>0.002</b>
Vegetation	A <sub>1541</sub>	A <sub>1558</sub>	A <sub>1636</sub>	A <sub>1648</sub>	A <sub>2850</sub>	A <sub>2920</sub>
Aspen	0.03 $\pm$ 0.005	0.03 $\pm$ 0.005	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	0.12 $\pm$ 0.02
Mixed	0.03 $\pm$ 0.004	0.03 $\pm$ 0.004	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.09 $\pm$ 0.01	0.12 $\pm$ 0.01
Conifer	0.03 $\pm$ 0.004	0.04 $\pm$ 0.004	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	0.10 $\pm$ 0.01	0.13 $\pm$ 0.02
<i>p</i> Vegetation	0.591	0.408	0.359	0.500	0.773	0.482
<b>Site</b>						
CM1	0.03 $\pm$ 0.005	0.03 $\pm$ 0.005	0.05 $\pm$ 0.004	0.05 $\pm$ 0.005	0.08 $\pm$ 0.004	0.11 $\pm$ 0.01
CM2	0.03 $\pm$ 0.004	0.04 $\pm$ 0.004	0.06 $\pm$ 0.003	0.07 $\pm$ 0.003	0.10 $\pm$ 0.003	0.14 $\pm$ 0.005
CM57	0.03 $\pm$ 0.004	0.03 $\pm$ 0.004	0.05 $\pm$ 0.004	0.06 $\pm$ 0.004	0.08 $\pm$ 0.003	0.11 $\pm$ 0.01
CM111	0.03 $\pm$ 0.004	0.03 $\pm$ 0.003	0.04 $\pm$ 0.003	0.05 $\pm$ 0.003	0.07 $\pm$ 0.01	0.10 $\pm$ 0.01
FB1	0.03 $\pm$ 0.003	0.04 $\pm$ 0.003	0.06 $\pm$ 0.002	0.06 $\pm$ 0.002	0.10 $\pm$ 0.004	0.14 $\pm$ 0.01
FB2	0.03 $\pm$ 0.002	0.04 $\pm$ 0.002	0.06 $\pm$ 0.002	0.06 $\pm$ 0.002	0.10 $\pm$ 0.01	0.13 $\pm$ 0.01
<i>p</i> Site	<b>0.003</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>

Table 3.6. Mean normalized absorbance ( $\pm$  SD) (absorbance  $\text{g}^{-1}$  C g soil) by site for plot MoM-SOC spectra. P values are given for sample set without CM15-2 (outlier)

Site	A <sub>1006</sub>	A <sub>1031</sub>	A <sub>1094</sub>	A <sub>1165</sub>	A <sub>1508</sub>	A <sub>1521</sub>	A <sub>1541</sub>
CM5	5.7 $\pm$ 1.0	5.4 $\pm$ 1.0	2.4 $\pm$ 0.6	1.2 $\pm$ 0.3	0.8 $\pm$ 0.2	0.9 $\pm$ 0.2	1.1 $\pm$ 0.2
CM8	5.5 $\pm$ 0.9	5.4 $\pm$ 0.9	2.9 $\pm$ 0.6	1.5 $\pm$ 0.3	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.2
CM15	6.9 $\pm$ 2.6	6.9 $\pm$ 2.8	3.9 $\pm$ 2.2	1.9 $\pm$ 1.2	0.8 $\pm$ 0.7	0.9 $\pm$ 0.7	1.0 $\pm$ 0.7
CM15 (no outlier)	5.7 $\pm$ 0.6	5.6 $\pm$ 0.6	2.9 $\pm$ 0.3	1.4 $\pm$ 0.2	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.8
CM17	6.1 $\pm$ 2.1	5.6 $\pm$ 1.8	2.4 $\pm$ 0.7	1.2 $\pm$ 0.3	0.7 $\pm$ 0.2	0.8 $\pm$ 0.2	1.0 $\pm$ 0.2
CM20	6.5 $\pm$ 1.4	6.2 $\pm$ 1.3	2.8 $\pm$ 0.4	1.4 $\pm$ 0.2	0.7 $\pm$ 0.2	0.8 $\pm$ 0.2	1.0 $\pm$ 0.2
<i>p</i> aspen LBA (%)	0.884	0.264	0.855	0.622	0.957	0.841	0.831
<i>p</i> Site	1	0.999	0.9	0.7	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>

Site	A <sub>1558</sub>	A <sub>1636</sub>	A <sub>1652</sub>	A <sub>2850</sub>	A <sub>2920</sub>	Ratio $\frac{A_{1094}}{\sum A_{1500}}$	Ratio $\frac{A_{1094}}{A_{2920}}$
CM5	1.1 $\pm$ 0.2	1.6 $\pm$ 0.3	1.8 $\pm$ 0.4	2.8 $\pm$ 0.7	3.7 $\pm$ 0.8	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1
CM8	0.7 $\pm$ 0.1	1.2 $\pm$ 0.2	1.4 $\pm$ 0.3	2.2 $\pm$ 0.4	3.0 $\pm$ 0.6	1.2 $\pm$ 0.1	1.0 $\pm$ 0.1
CM15	1.1 $\pm$ 0.7	1.6 $\pm$ 0.8	1.8 $\pm$ 1.0	2.9 $\pm$ 1.4	3.8 $\pm$ 1.8	1.1 $\pm$ 0.2	1.0 $\pm$ 0.1
CM15 (no outlier)	0.8 $\pm$ 0.1	1.2 $\pm$ 0.1	1.4 $\pm$ 0.1	2.3 $\pm$ 0.2	3.0 $\pm$ 0.2	1.2 $\pm$ 0.2	1.0 $\pm$ 0.1
CM17	1.0 $\pm$ 0.2	1.5 $\pm$ 0.2	1.7 $\pm$ 0.2	2.5 $\pm$ 0.7	3.4 $\pm$ 0.7	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3
CM20	1.1 $\pm$ 0.2	1.9 $\pm$ 0.3	2.1 $\pm$ 0.4	3.6 $\pm$ 0.7	4.6 $\pm$ 0.9	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1
<i>p</i> aspen LBA (%)	0.472	0.100	0.174	<b>0.025</b>	<b>0.023</b>	0.864	0.167
<i>p</i> Site	<b>0.01</b>	<b>0.005</b>	<b>0.009</b>	<b>0.01</b>	<b>0.01</b>	<b>0.001</b>	<b>0.002</b>

Table 3.7. Relative peak height ( $\pm$  SD) by site for plots MoM-SOC spectra. P values in parenthesis are given for the analysis without a possible outlier.

Site	A <sub>1006</sub>	A <sub>1031</sub>	A <sub>1094</sub>	A <sub>1165</sub>	A <sub>1508</sub>	A <sub>1521</sub>
CM5	0.202 $\pm$ 0.004	0.189 $\pm$ 0.008	0.085 $\pm$ 0.012	0.040 $\pm$ 0.007	0.030 $\pm$ 0.001	0.032 $\pm$ 0.001
CM8	0.216 $\pm$ 0.018	0.212 $\pm$ 0.012	0.115 $\pm$ 0.005	0.058 $\pm$ 0.005	0.018 $\pm$ 0.002	0.021 $\pm$ 0.002
CM15	0.212 $\pm$ 0.017	0.210 $\pm$ 0.012	0.115 $\pm$ 0.006	0.056 $\pm$ 0.006	0.021 $\pm$ 0.006	0.024 $\pm$ 0.005
CM17	0.215 $\pm$ 0.038	0.198 $\pm$ 0.032	0.085 $\pm$ 0.012	0.041 $\pm$ 0.006	0.027 $\pm$ 0.008	0.030 $\pm$ 0.008
CM20	0.198 $\pm$ 0.010	0.188 $\pm$ 0.008	0.085 $\pm$ 0.008	0.043 $\pm$ 0.006	0.023 $\pm$ 0.002	0.026 $\pm$ 0.002
<i>p</i> aspen LBA (%)	0.099	0.188	0.726	0.680	0.995	0.998
<i>p</i> Site	0.999	0.200	< <b>0.0001</b>	<b>0.001</b>	0.080	0.060

Site	A <sub>1541</sub>	A <sub>1558</sub>	A <sub>1636</sub>	A <sub>1652</sub>	A <sub>2850</sub>	A <sub>2920</sub>
CM5	0.038 $\pm$ 0.002	0.039 $\pm$ 0.002	0.056 $\pm$ 0.004	0.064 $\pm$ 0.003	0.098 $\pm$ 0.008	0.128 $\pm$ 0.008
CM8	0.025 $\pm$ 0.002	0.028 $\pm$ 0.001	0.048 $\pm$ 0.002	0.054 $\pm$ 0.003	0.087 $\pm$ 0.007	0.119 $\pm$ 0.009
CM15	0.029 $\pm$ 0.005	0.031 $\pm$ 0.004	0.047 $\pm$ 0.001	0.053 $\pm$ 0.002	0.086 $\pm$ 0.002	0.114 $\pm$ 0.003
CM17	0.036 $\pm$ 0.009	0.037 $\pm$ 0.008	0.055 $\pm$ 0.009	0.063 $\pm$ 0.009	0.092 $\pm$ 0.025	0.123 $\pm$ 0.025
CM20	0.031 $\pm$ 0.002	0.034 $\pm$ 0.002	0.058 $\pm$ 0.002	0.065 $\pm$ 0.002	0.108 $\pm$ 0.006	0.141 $\pm$ 0.007
<i>p</i> aspen LBA (%)	0.871	0.751	0.179	0.240	0.067 ( <b>0.024</b> )	<b>0.036 (0.011)</b>
<i>p</i> Site	<b>0.030</b>	<b>0.020</b>	<b>0.004</b>	<b>0.005</b>	0.090 (< <b>0.0001</b> )	<b>0.04 (0.0001)</b>

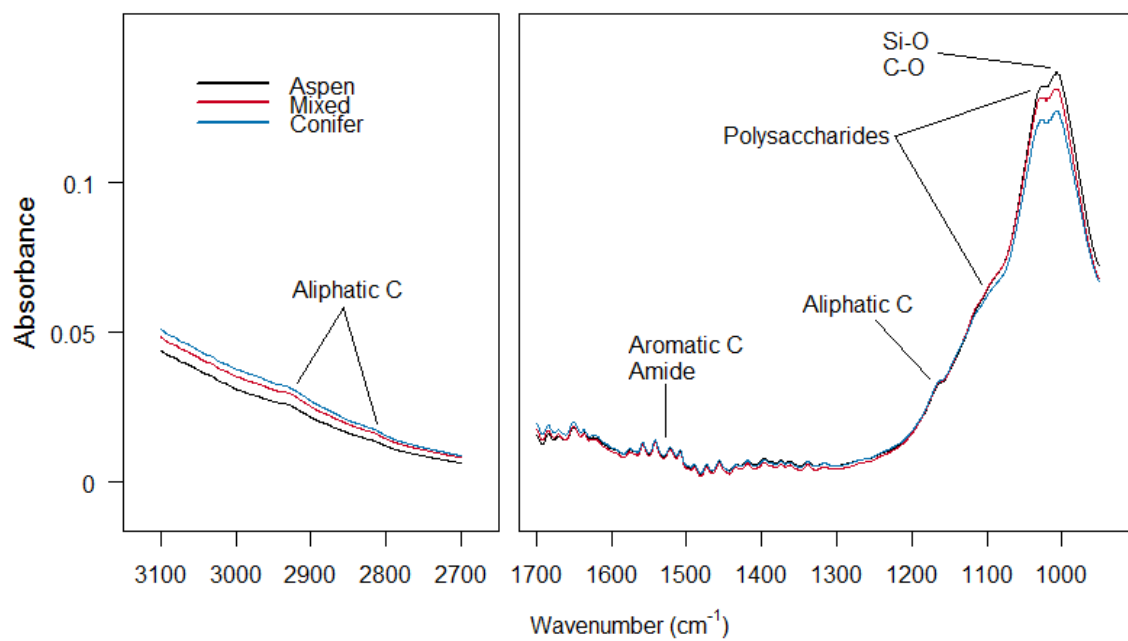


Fig. 3.1. Average organic matter spectra by vegetation class from transect samples (5-10 cm depth).

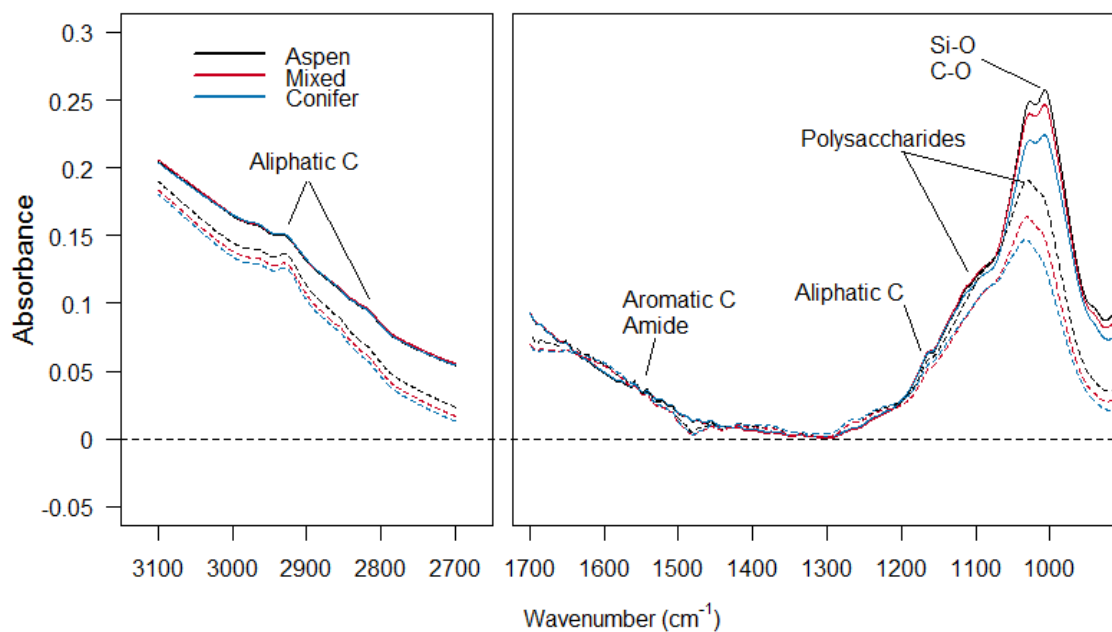


Fig. 3.2. Average spectra of the mineral-associated SOC (—), and light fraction SOC (- - -) from transect samples.



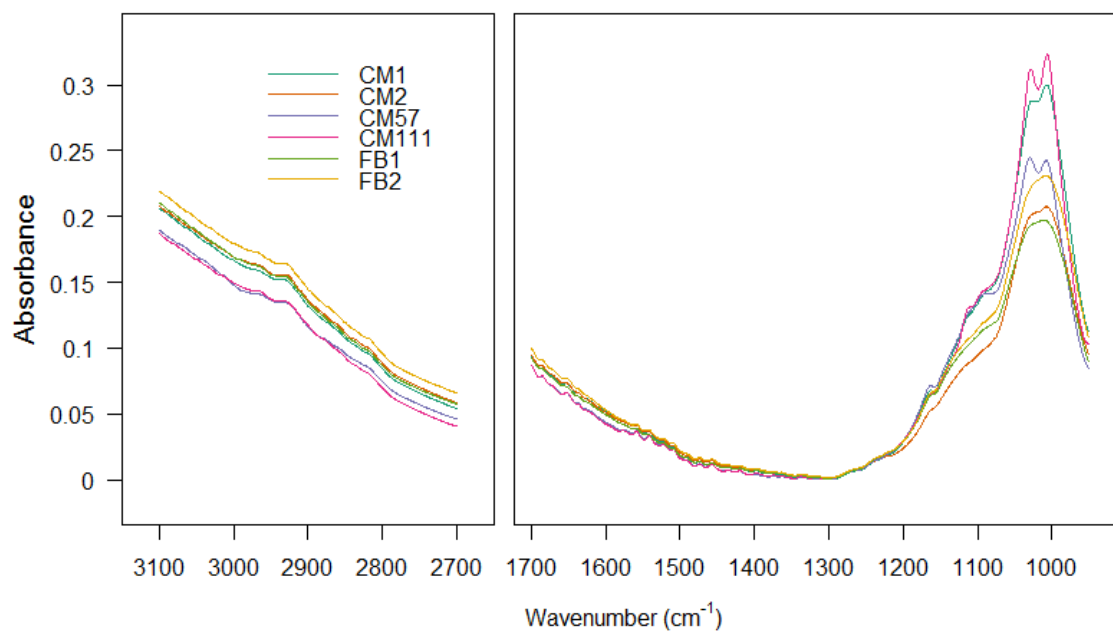


Fig. 3.3. Average spectra by site of mineral-associated SOC from transects.

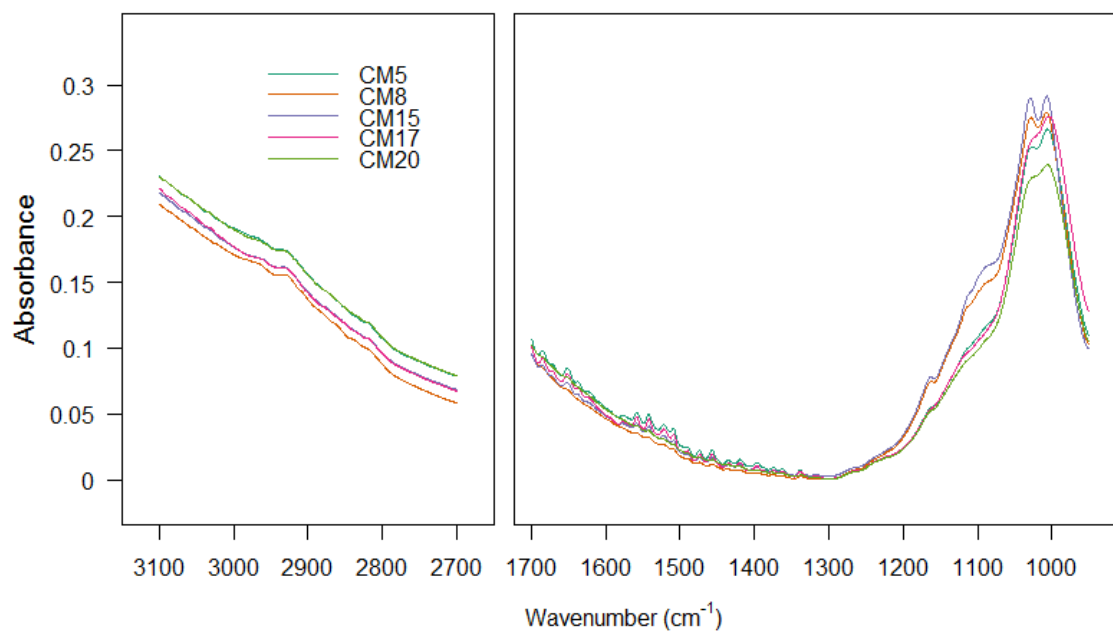


Fig. 3.4. Average spectra by site of mineral-associated SOC from plots.

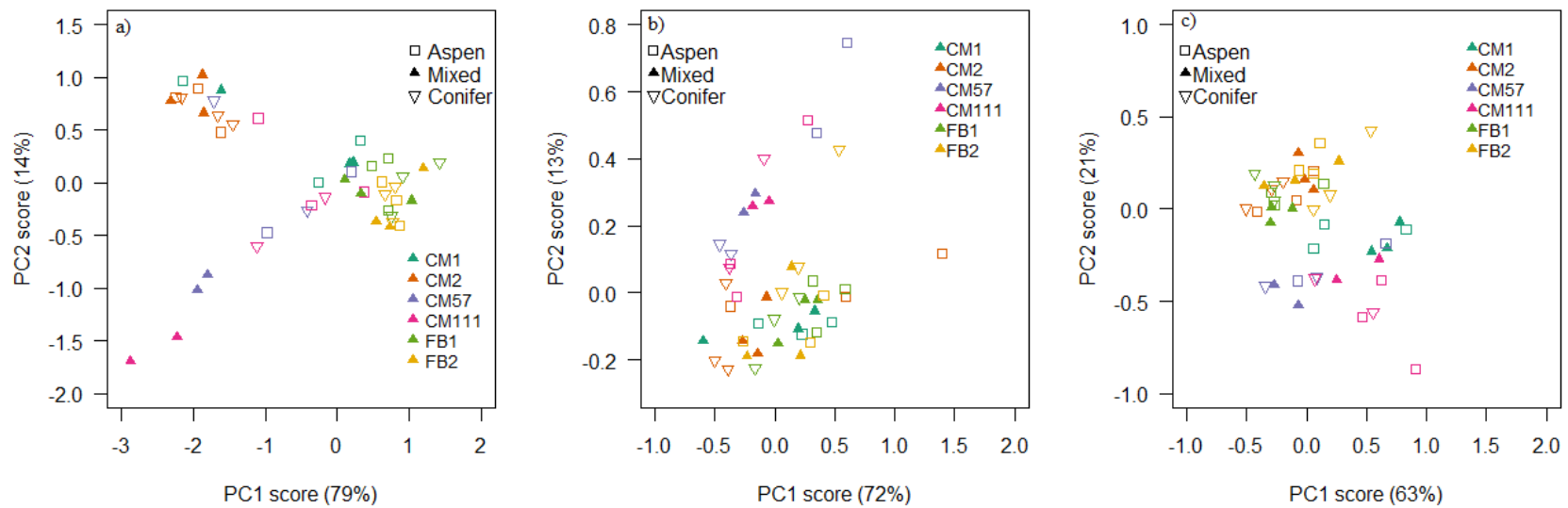


Fig. 3.5. Scores plot of a) organic matter spectra (5–10 cm), b) light fraction SOC (0–5 cm), and c) mineral-associated SOC (0–5 cm) for transect samples.

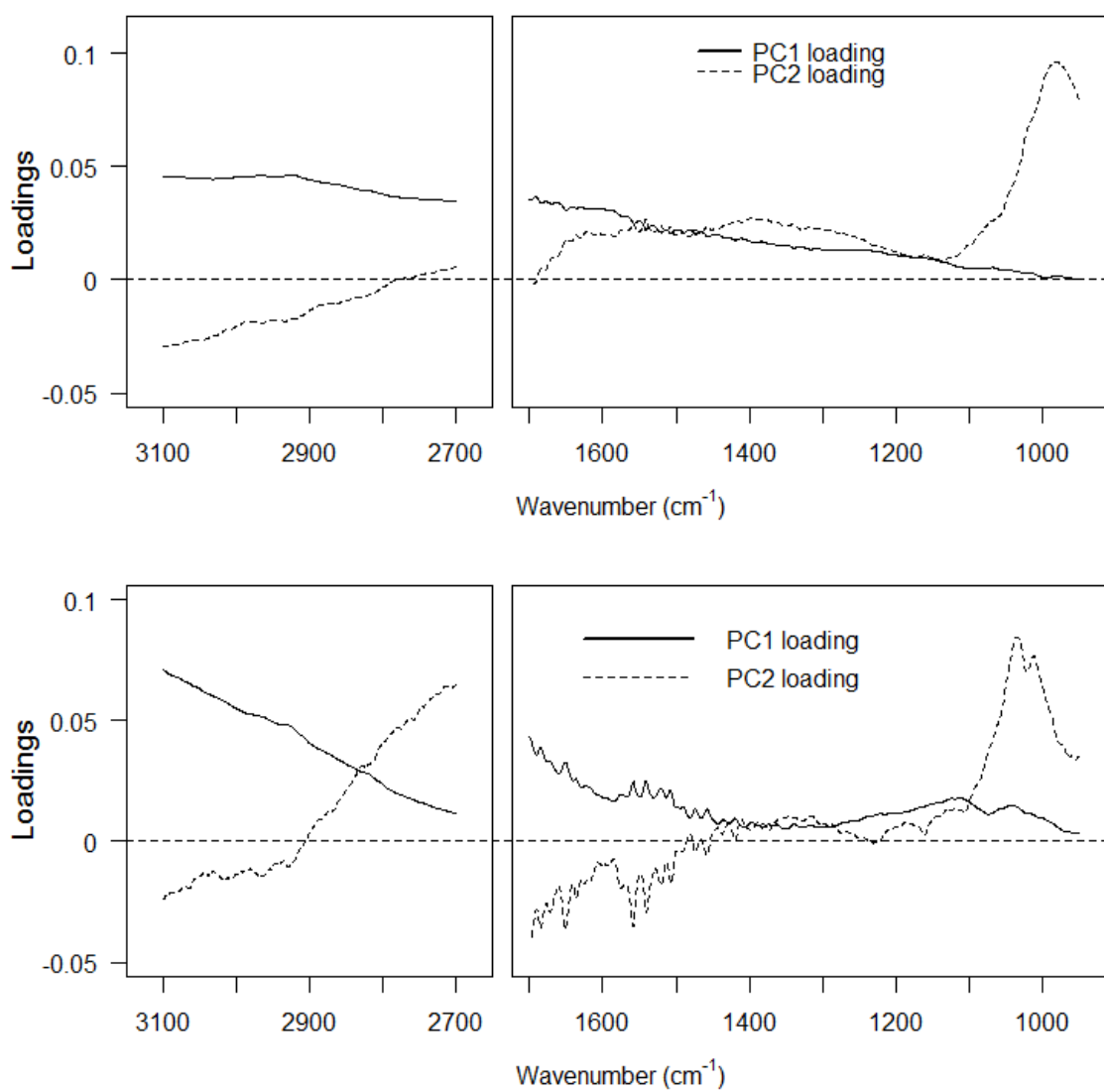


Fig. 3.6. Loading vectors for PC1 and PC2 of organic matter spectra from a) transect samples (5–10 cm), and b) plot samples (0–15 cm).

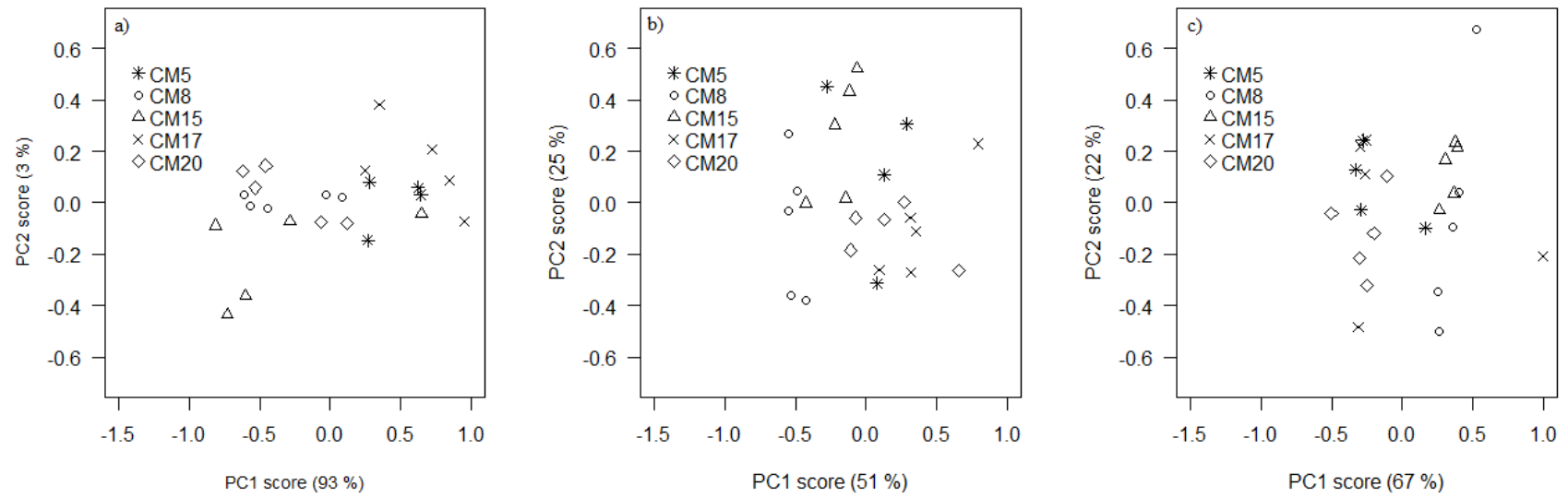


Fig. 3.7. Scores plot of a) organic matter, b) light fraction SOC and c) mineral-associated SOC for plot samples.

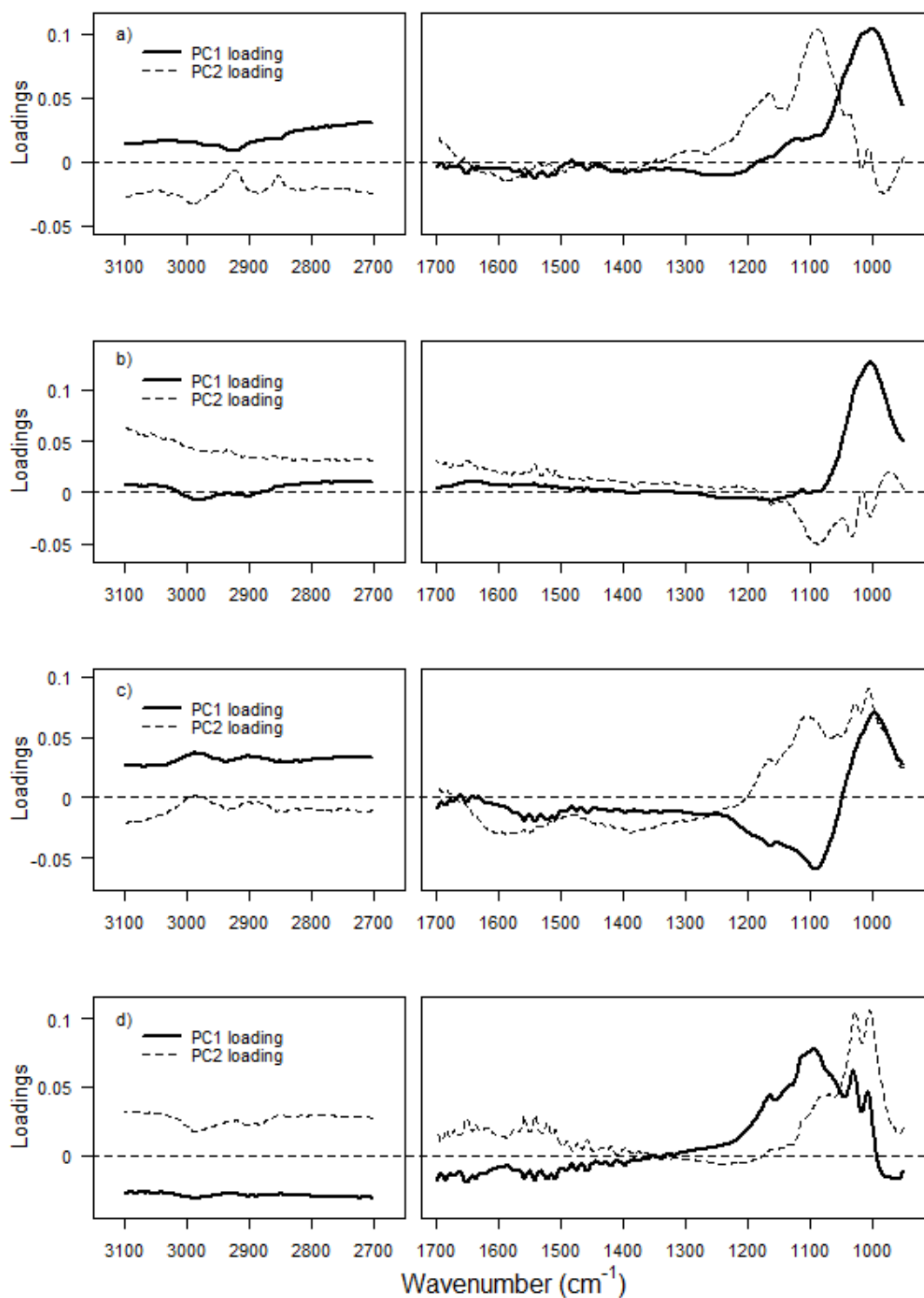


Fig. 3.8. Loading vectors for a) transects light fraction SOC, b) transects mineral-associated SOC, c) plots light fraction SOC, and d) plots mineral-associated SOC.

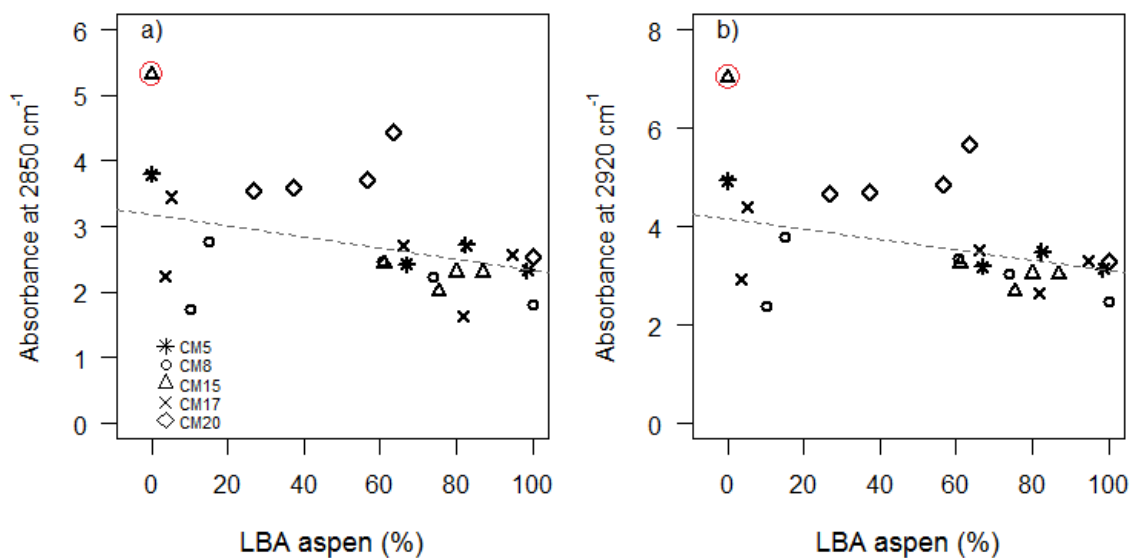


Fig. 3.9. Relationship between contribution of aspen to LBA and a) normalized absorbance at 2850 cm<sup>-1</sup> (absorbance g<sup>-1</sup> C g soil) (aliphatic C) of MoM-SOC spectra; b) normalized absorbance at 2920 cm<sup>-1</sup> (absorbance g<sup>-1</sup> C g soil) (aliphatic C) of MoM-SOC spectra. Grey line indicates the regression line without the outlier (red circle).

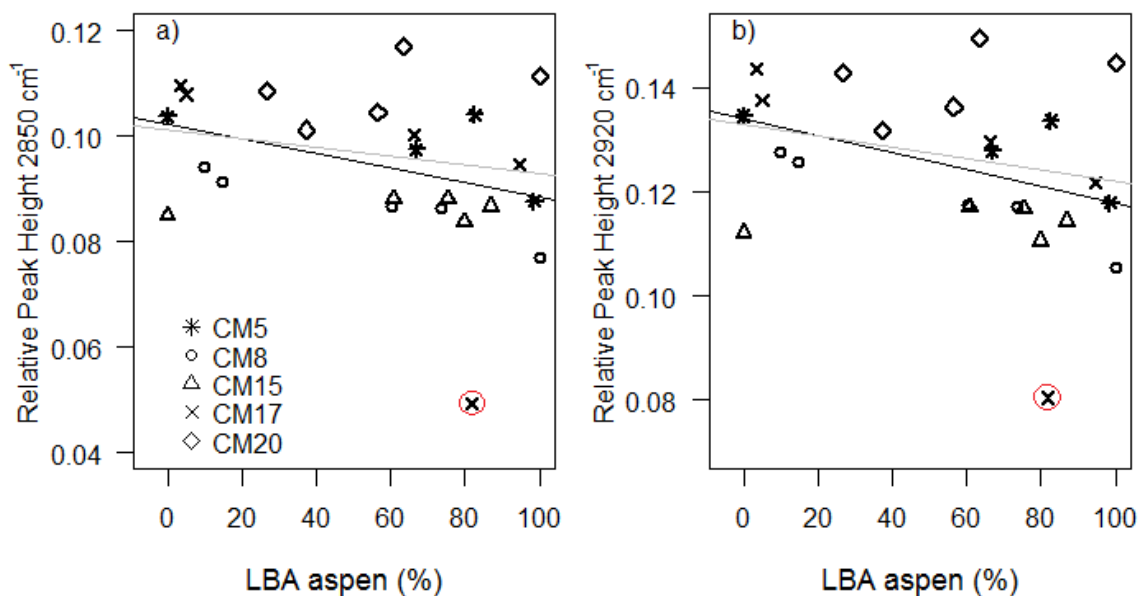


Fig. 3.10. Relationship between contribution of aspen to LBA and a) relative peak height at 2850 cm<sup>-1</sup> [aliphatic methyl (-CH<sub>3</sub>) and methylene (-CH<sub>2</sub>-)] of MoM-SOC spectra; b) relative peak height at 2920 cm<sup>-1</sup> [aliphatic methyl (-CH<sub>3</sub>) and methylene (-CH<sub>2</sub>-)] of MoM-SOC spectra. Black line indicates the regression line for the whole population. Grey line indicates the regression line without the outlier (red circle).



## CHAPTER 4

PREDICTING TREE SPECIES ORIGIN OF SOIL ORGANIC CARBON WITH NEAR-  
INFRARED SPECTROSCOPY<sup>3</sup>**Abstract**

Near-infrared reflectance spectroscopy (NIRS) and partial least squares regression (PLSR) were used to develop prediction models for identifying the species of origin of soil organic carbon (SOC) in semi-arid montane forests of quaking aspen (*Populus tremuloides*) and mixed conifers in Utah. Artificial mixtures of mineral soils (0-15 cm) sampled under pure aspen and pure conifer cover (n = 415) at 4 locations were divided into calibration-validation set (n = 265) for model development and an independent validation set (n = 150) to test model robustness. Models in the 10,000- 4,000 cm<sup>-1</sup> spectral region were developed separately with original soil spectra (OS) and organic matter spectra (OM) using the full and truncated (10 to 90<sup>th</sup> percentile) sample sets. The OS models performed better than OM models, and the best OS models showed good prediction ability at the validation step, with R<sup>2</sup> = 76%, RPD = 2.1 for aspen-SOC, and R<sup>2</sup> = 74%, RPD = 2.0 for conifer-SOC. Model performance decreased at independent validation (R<sup>2</sup> = 33 - 49% and RPD = 1.2-1.6), probably due to unaccounted for site-specific factors and variability in SOC chemical composition within and among aspen and conifer soils. Current models are still somewhat limited for accurately predicting contributions of aspen vs. conifer in independent samples. More detailed site information, such as texture, mineralogy, geology, and land use history is needed to improve models

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<sup>3</sup> Coauthored by Mercedes Román Dobarco, Marie-Cécile Gruselle, Helga Van Miegroet and Jürgen Bauhus. North American Forest Soils Conference Proceedings, *Soil Science Society of America Journal* doi:10.2136/sssaj2013.08.0331nafsc. Reprinted by Permission, ASA, CSSA, SSSA

such that they can be used to provide insight into SOC properties changes along a continuum of aspen to conifer forests in the western US.

**Abbreviations:** CM – Cedar Mountain; CO<sub>2</sub> – carbon dioxide; C-V – Calibration-validation; DLL –Deseret Land and Livestock; FB – Franklin Basin; FD – First derivative; IC –inorganic carbon; IV – Independent validation; MM – Mineral matrix spectra; MTT – Maximum mean monthly temperature; mMT – Minimum mean monthly temperature; NIRS - Near-infrared reflectance spectroscopy; NSP – No spectral preprocessing; OS – Original spectra; OM – Organic matter spectra; PCA – Principal components analysis; PLSR – Partial least square regression; RMSEP – Root mean square error of prediction; RPD – Ratio of standard deviation of reference value to standard error of prediction; SD – Standard deviation; SOC – Soil organic carbon; SLS – Straight line subtraction; TC – Total carbon; TOC – Total organic carbon; TWDEF – T.W. Daniel Experimental Forest; VN – Vector normalization

## **Introduction**

Quaking aspen (*Populus tremuloides* Michx.) is a major species in montane ecosystems of the semi-arid region of western North America (N.A.), occurring predominantly as a pioneer species that is replaced by conifers in later stages of succession (Mueggler, 1985). Fire suppression and ungulate browsing is believed to have caused a loss in aspen cover during the last century (Bartos and Campbell, 1998). Although changes in aspen cover may be within the range of historical fluctuation (Kulakowski et al., 2004), a shift towards coniferous species may modify soil physical, chemical, and biological properties (Ayres et al., 2009), including soil organic carbon (SOC) dynamics and CO<sub>2</sub> emissions into the atmosphere.

Previous studies in montane forests of western N.A. have found that aspen stands store more SOC in the first 60 cm of the mineral soil than adjacent conifer stands (Woldeselassie et al., 2012). The results further indicate that SOC in aspen soils has slower microbial turnover than conifer soils (i.e., more stable SOC), and that aspen have higher proportion of SOC associated with mineral surfaces (Woldeselassie et al., 2012), which increases residence time of SOC (von Luetzow et al., 2007). Differences in input, litter chemical composition, and environmental soil conditions following conifer encroachment can also affect SOC dynamics (Olsen and Van Miegroet, 2010; Woldeselassie et al., 2012). SOC storage and its properties under mixed stands may not necessarily be predicted through linear interpolation between SOC contents and properties of pure aspen and conifer stands. Being able to distinguish the vegetation type legacy on SOC (i.e., the contribution of aspen- and conifer-derived portions in SOC) would greatly contribute to our understanding of changes in SOC storage and dynamics as aspen transitions to mixed aspen-conifer forests. This challenge may be addressed using near-infrared reflectance spectroscopy (NIRS). To our knowledge, no study has used NIRS to distinguish the relative contribution of tree species belonging to different forest types (broadleaves versus conifers) to SOC in the mineral soil.

NIRS is an empirical, non-destructive, inexpensive, and rapid technique that is commonly used in food and chemical industry and agricultural science to determine simultaneously the concentration of various organic and inorganic components. Therefore, NIRS may be an appropriate technique for analyzing chemically heterogeneous SOC. In soil science, NIRS has been applied to predict organic carbon (C) and nitrogen (N) concentrations in agricultural soils (Dalal and Henry, 1986; Morra et al.,

1991), relative abundance of functional groups (Terhoeven-Urselmans et al., 2006), concentration of C, N, and phosphorus in litter at different stages of decomposition (Gillon et al., 1999), or SOC fractions (Coûteaux et al., 2003; Cozzolino and Morón, 2006). NIRS is based on the absorption of infrared radiation (800- 2500 nm) by C-H, N-H, O-H bonds (Foley et al., 1998) as found in organic and inorganic constituents of plant and soil materials. Thus, the NIR spectrum of a material can be interpreted as the overall chemical composition of the soil organic matter (Palmborg and Nordgren, 1996; Coûteaux et al., 2003). When NIRS is combined with chemometrics, it is possible to develop prediction models for NIR-active constituents of known concentrations. As opposed to the characterization of individual compounds by wet chemical analyses, NIRS permits the determination of the chemical composition of heterogeneous samples and does not produce chemical wastes (Cozzolino and Morón, 2006). Gruselle and Bauhus (2010) used NIRS successfully to predict the species of origin of the forest floor in mixtures of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.). Few studies have used NIRS to distinguish the vegetation origin of organic matter in mineral soils. Mineral soil is defined in this study as soil material distinct from O horizon and litter and containing less than 20 % (by weight) of SOC (Soil Survey Staff, 2010), and will hereafter be referred to as soils. Coûteaux et al. (2003) were able to predict  $^{13}\text{C}$  and  $^{15}\text{N}$  derived from labeled wheat straw with NIRS models, three years after the straw was added to coniferous forests soils. Michel and Ludwig (2010) used NIRS models to predict C derived from C3 and C4 plants in pools from the RothC model. Ertlen et al. (2010) used NIRS to discriminate soils originated under grassland or forests. These studies indicate that NIRS can be used to differentiate the origin of SOC by land use type and

plants differing in metabolic pathways. Moreover, they invite the hypothesis that NIR spectra can reflect the types of vegetation and their relative contribution to SOC concentration in the mineral soil where components of plant (aboveground and belowground) litter have been recycled into microbial biomass, and /or result from advanced decomposition.

Our goal was to investigate whether NIRS and chemometrics can be used to predict the concentration of SOC in the soil derived from aspen and coniferous species using soils sampled directly under aspen and conifer canopies at different locations in Utah. We further wanted to test whether the legacy of vegetation on SOC could be adequately predicted in the presence of the mineral matrix of the soil or whether it was necessary to remove the influence of the mineral matrix on NIR spectra prior to NIRS model development.

## **Materials and methods**

### *Study areas and land use history*

Four study areas located in northern Utah [Franklin Basin (FB), T.W. Daniel Experimental (TWDEF), and Deseret Land and Livestock (DLL)] and in southern Utah [Cedar Mountain (CM)] were sampled between 2007 and 2011 to capture the broad range of physical settings encompassed by aspen (Fig. 4.1). FB, TWDEF, and DLL are located in the physiographic province of the Middle Rocky Mountains and CM is located on the Kolob Terrace, within the Colorado Plateau (Fig. 4.1) (Fenneman and Johnson, 1946). These are montane or subalpine ecosystems, with elevation ranging from 1,770 to 3,200 m (Table 4.1). The climate is characterized by cold winters and hot, dry summers. Annual

precipitation across the sites ranges between 812 and 1,197 mm (Table 4.1), decreasing from North to South. Precipitation occurs mainly as snow. Average temperatures of the hottest month are fairly similar across all study areas, ranging between 14.0 and 16.4 °C (Table 4.1). Average temperatures of the coldest month decrease towards the North (Table 4.1), ranging from -3.8°C at CM to -10.0°C at TWDEF. The geology differs somewhat across study areas: soils in CM developed mainly on sedimentary (shale, sandstone, or limestone) and igneous rock (basalt, basic or intermediate igneous rock) (USDA, 2014); at TWDEF and DLL, the parent material is derived from Wasatch conglomerate (Woldeselassie et al., 2012); and at FB, soils developed on sedimentary rock (limestone or quartzite sandstone) (Kusbach, 2010). In our study areas, aspen was present as large pure stands or in patches embedded in mountain meadows, shrublands, or conifer forests. In mixtures, aspen was associated with a variety of coniferous species (Table 4.1). The understory vegetation under aspen stands commonly consists of diverse grasses, forbs (*Delphinium occidentale* (S. Watson) S. Watson or *Achillea millefolium* L.), legumes (*Lupinus* spp.), and shrubs (*Symphoricarpos oreophilus* A. Gray, *Ribes* spp), and are denser than under conifer stands, which often have bare soils or sparse grasses, forbs, and shrubs.

Complete soil profile descriptions for the plots at DLL and TWDEF can be found in Woldeselassie et al. (2012). Soil profile descriptions were not available for the sites in CM and FB. However, Kusbach (2010) described several other soil pedons under aspen and conifer stands at FB, and as reported in Woldeselassie et al. (2012), soils under aspen generally have a thick and pronounced A horizon (~30-50 cm) and are classified as

Mollisols. Conifer soils have a shallower and lighter A horizon (~5-30 cm), and are commonly classified as Alfisols, but also as Entisols or Inceptisols (Table 4.1).

Documentation on land use for the western US is anecdotal before 1900s, and for most of the 20<sup>th</sup> century there is a paucity on land use cartography for our study areas; thus, information on historical vegetation had to be derived from the literature. Rogers et al. (2011) characterized the transition in aspen communities in the last 150 years in the Bear River Range, where FB and TWDEF are located, as being dominated by mixed and conifer stands in the early 1800s, with subsequent expansion of pure aspen stands during the end of the 19<sup>th</sup> century due to a shift in climatic conditions coupled with disturbances associated to the European settlement (e.g., timber extraction, sheep grazing, high intensity fires). During the 20<sup>th</sup> century, fire suppression, cattle and sheep grazing, and a moist climate contributed to the natural succession towards mixed and conifer stands (Rogers et al., 2011). Similarly, grazing and intensive logging of accessible conifer stands were the main land uses in DLL in the late 1800s and early 1900s. Aspen communities at CM are presumably stable stands that self-regenerate continuously or through gap-phase regeneration (Kurzel et al., 2007), while conifer stands are found in the edges of the plateau and northern slopes. Intensive sheep grazing since the European settlement may have profoundly modified the structure of aspen stands (Rogers et al., 2010) and caused a shift in understory composition from forb- to graminoid-dominated (Bowns and Bagley, 1986).

### *Sampling design*

We used two sampling designs: the first sampling campaign (2007) was done at the plot level; subsequently (2009-2011) points were sampled along transects to capture the influence of a single tree on soil properties under its canopy. In July 2007, a total of six paired plots (20 m x 20 m) (designated as “plots” in Table 4.2) were located at TWDEF and in two small watersheds named Upper Frost and Bear Canyon at DLL (DLL Frost and DLL Bear hereafter), under either conifer- or aspen-dominated overstory. Each pair of plots had similar conditions of elevation and slope, and plots were located between 10 and 100 m from each other (Woldeselassie et al., 2012). After removing the litter layer (when present), five soil cores (5 cm of diameter) were taken to a depth of 15 cm in each plot, and combined into one composite sample per plot. Soil sampling was done by depth rather than by horizon, but consisted entirely of A horizon under aspen and of A horizon with some portion of the underlying B, AB or E horizon under some conifers (Olsen and Van Miegroet, 2010; Woldeselassie et al., 2012). A second sampling design (named “transects” in Table 4.2) was applied in the fall of 2009 at three sites (DLL Bear, DLL Frost, and TWDEF) close to the 2007 plots. Three transects were laid out at each location, and within each transect, two soil cores (0 – 15 cm) were sampled beneath conifer or aspen canopy after removing of the litter layer, and composited on site. The elevation, slope, and aspect were similar along each transect. In addition, four sites at CM (CM1, CM2, CM57, and CM111) and two sites at FB (FB1 and FB2) were sampled using transects method in the fall of 2010 and 2011, respectively. In CM and FB, soil cores were taken to a depth of 15 cm and the middle section (5 - 10 cm) was used in subsequent analyses. As SOC characteristics change with depth (bulk density increases, SOC



concentration and particulate organic matter content decrease), we considered the middle section to represent average properties of the entire 0-15 cm core (Román Dobarco and Van Miegroet, unpublished data). In the text, we refer to soils sampled under pure aspen or pure conifer canopy from paired plots or transects as end members.

#### *Sample preparation and spectra measurements*

Soils were oven dried at 105°C, sieved through a 2-mm mesh, finely ground with mortar and pestle and analyzed for total carbon (TC), inorganic carbon (IC), and total organic carbon (TOC) concentrations with a Skalar Primacs<sup>SLC</sup> Analyzer (Skalar, Inc., Breda, The Netherlands). While oven drying may have induced some alterations (e.g., oxidation and loss of volatile organic carbon) in organic matter configuration, earlier lab comparisons between oven-dried and air-dried soils did not indicate change in TC content (Román Dobarco and Van Miegroet, unpublished data). Texture of the original soil samples was determined using the pipette method.

Thirteen pairs of end members were used to generate 362 artificial mixtures in the laboratory (Table 4.2) by mixing known amounts of aspen and conifer soils in a 0 to 100% gradient. A third soil component (TOC = 48.7 mg g<sup>-1</sup> soil), hereafter called external soil, from a garden from Neustadt, Germany, was added (0 to 85% by weight) to avoid autocorrelation typical of simple two-component mixtures (as per Gruselle and Bauhus, 2010). Mixtures were created exclusively within paired plots or transect to control for parent material and soil texture, although the texture differed somewhat between aspen and conifer soils in some pairs (Table 4.2). Pure aspen (n = 29) and pure conifer (n = 24) soil samples were included in the dataset, for a total of 415 samples

(Table 4.2, Fig. 4.2). The spectra of end members and artificial mixtures will be called original spectra (OS) hereafter. Relative SOC concentration ( $\text{mg C g}^{-1}$  soil) of each vegetation type (aspen or conifer) in the artificial mixtures was calculated as follows:

$$SOC_{veg} = \frac{Weight_{veg} \times TOC_{veg}}{\sum_{i=1}^3 Weight_{vegi}}$$

Where  $SOC_{veg}$  is the relative SOC concentration of the vegetation type (aspen or conifer) for which the NIRS models are developed,  $Weight_{veg}$  is the weight of soil (g) of a given vegetation type,  $TOC$  the C concentration ( $\text{mg g}^{-1}$  soil) of the vegetation type in the source sample, and  $i = 1-3$ , the three soils (aspen, conifer, external soil) used in the mixtures.

Organic-free mineral matrix samples were obtained from an aliquot of the original soil samples using a modification of the sodium hypochlorite (NaOCl) extraction protocol described by Kaiser et al. (2002). Briefly, NaOCl (6%) was added to soil in a ratio 50:1 (v/w) and the soil slurry was shaken at room temperature for a total 30 hours, replacing the sodium hypochlorite two times (after 12 and 24 h). The remaining mineral material was rinsed at least four times with deionized water (ratio 44:1 v/w) and centrifuged at 18,000 rpm. Samples were dried at  $40^{\circ}\text{C}$  and ground with mortar and pestle ( $< 1$  mm) prior to spectral analysis. This extraction method effectively removes organic matter with minimal effects on mineral structure as discussed by Siregar et al. (2005). The spectra of the remaining organic-free mineral material will be called mineral matrix spectra (MM) hereafter.

NIR measurements and multivariate statistics were performed at the Institute of Silviculture of the University of Freiburg (Freiburg, Germany). Spectra from artificial

mixtures, end members, and mineral matrix were obtained with a Tensor 37 spectrometer (Bruker Optics GmbH, Ettlingen, Germany). Samples were dried in the oven overnight at 40° C to eliminate any interference of water with the NIRS spectra. Absorbance was measured in 8 cm<sup>-1</sup> interval over the range 12,000 - 3,500 cm<sup>-1</sup> (833 - 2,857 nm). The spectral region 10,000 - 4,000 cm<sup>-1</sup> was actually used by the chemometric software OPUS (see details below) for calibration-validation because the regions outside of this range have limited utility in the calibration due to spectral noise as per Locher et al. (2005). Five to eight spectra per sample (32 scans per spectrum) were obtained and an average spectrum for each sample was calculated with the software OPUS 6.5 (Bruker Optics GmbH, Ettlingen, Germany), which is specific to the Tensor 37 spectrometer. Samples were shaken and well mixed between spectra acquisitions to ensure a mean spectrum representative of the sample variability, as per Gruselle and Bauhus (2010).

The spectrum of the soil organic matter (OM) was obtained by subtracting the spectrum of the mineral matrix (MM) from that of its corresponding original sample (OS) using the OPUS software. For each pair of end members, an average mineral matrix spectrum was calculated using OPUS, assuming small variability in the mineral matrix of the soil among end members pairs within a given site or transect, and then subtracted from their site-specific artificial mixtures. Spectral subtraction to obtain OM spectra improved NIRS prediction models for total N and N mineralization of soil samples (Russell, 2003), and has also been performed previously in Fourier transform infrared spectroscopy (Ellerbrock and Kaiser, 2005).

*NIRS models development*

Prediction models for aspen-SOC and conifer-SOC in artificial mixtures were developed with partial least squares regression (PLSR), the most widely used method in chemometrics for multivariate calibration (Dunn et al., 2002; Locher et al., 2005; Cozzolino and Morón, 2006; Peltre et al, 2011). The methodology used to develop aspen-SOC and conifer-SOC prediction models is shown in Figure 4.2, and involves a calibration-validation (C-V) step, followed by an independent validation (IV) step. The division of the whole spectral data set ( $n = 415$ ) into a C-V and IV set was done *a priori* based on a principal component analysis (PCA) on raw spectra, which allowed examining qualitative differences among sites (as in Cozzolino et al., 2009). This approach was taken to ensure: (1) that the environmental variability of all sample locations throughout UT was represented in the C-V set as well as in the IV set, and (2) that the largest possible spectral variability was represented in the C-V set. Sites assigned to the C-V set were CM1, CM2, CM111, FB1, DLL Frost, and TWDEF ( $n = 265$ ), while the IV set consisted of CM57, FB2, and DLL Bear ( $n = 150$ ) (Table 4.2). Screening of the C-V set showed that the concentrations of aspen-SOC and conifer-SOC were skewed with more observations at low aspen or conifer SOC concentrations (aspen-SOC skewness = 1.8, kurtosis = 3.4; conifer-SOC skewness = 1.5, kurtosis = 2.0). Because few observations at higher concentration in the range will have high leverage during calibration, we considered two different datasets: the initial dataset ( $n = 415$ ), and a spectral subset (i.e., truncated dataset) with reference values between the 10<sup>th</sup> and 90<sup>th</sup> percentiles for each component (aspen-SOC and conifer-SOC;  $n = 291$ ), with the corresponding C-V and IV sets containing 179 and 112 spectra, respectively. The truncated spectral dataset reduced

skewness and was used to develop models for concentration ranges of 2.18 – 36.82 mg C g<sup>-1</sup> soil for aspen and 1.34- 41.40 mg C g<sup>-1</sup> soil for conifer. Kendall rank correlation coefficient between aspen-SOC and conifer-SOC was  $r = -0.18$  for the entire dataset and  $r = -0.02$  for the truncated dataset used in C-V, supporting the assumption that both components were independent from one another in both datasets.

Different mathematical treatments, embedded in the OPUS software, were systematically applied in the C-V step for both the entire and the truncated datasets. These were applied to normalize the C-V spectra before model calibration. The mathematical treatments were: no spectral preprocessing, straight line subtraction (SLS), vector normalization (VN), first derivative (FD) with 13 smoothing points, FD + SLS, and FD + VN. The SLS treatment causes a tilt in the recorded spectrum (Tripathi and Mishra, 2009) while VN entails mean centering and variance scaling and removes the multiplicative interferences of scatter and particle size, and FD removes background and increases spectral resolution (Cen and He, 2007). The full-range spectra within the C-V data set were divided at a 50/50 ratio into spectra for calibration ( $n = 132$ ) vs. validation ( $n = 133$ ) using the PCA technique with the program QUANT embedded in OPUS 6.5. In the truncated dataset, 70% of the spectra were used for calibration ( $n = 125$ ) and 30% for validation ( $n = 54$ ).

Models for aspen-SOC and conifer-SOC were calibrated with OM spectra and OS spectra separately using the optimization routine in the program QUANT for OPUS 6.5, which provided models developed in the spectral regions presented in Table 4.3 and Table 4.4.

Criteria of good performance of the models at the validation stage were: highest coefficient of determination ( $R^2$ ), lowest root mean square error of prediction (RMSEP), highest ratio of standard deviation of reference values to standard error of prediction (RPD), and low rank. The RPD classification proposed by Chang et al. (2001) is often used to assess the prediction ability of NIRS models for soil analysis: good models have  $RPD > 2$ , models with  $1.4 < RPD < 2$  could be improved with other calibration techniques, and models with  $RPD < 1.4$  are non-reliable (Cozzolino and Morón, 2006). Between 5 and 10 best models per mathematical treatment and component were selected after validation. These models were then applied to the IV set (i.e., samples not included in model development), which was the final step in evaluating model performance and our ability to predict species-derived SOC.

## Results and discussion

At the C-V stage, the best aspen-SOC model developed with OS spectra for the initial dataset had  $R^2 = 62\%$ ,  $RMSEP = 9.4 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{VAL} = 1.6$ , and the best conifer-SOC model had  $R^2 = 54\%$ ,  $RMSEP = 10.8 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{VAL} = 1.5$  (Table 4.3). Models developed with OM spectra for the initial dataset performed worse than the OS models for both components (Table 4.3).

Models developed for the truncated dataset with OS spectra at the C-V stage yielded the best results, with  $R^2 = 76\%$ ,  $RMSEP = 4.6 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{VAL} = 2.1$  for the best aspen-SOC model, and  $R^2 = 74\%$ ,  $RMSEP = 5.1 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{VAL} = 2.0$  for the best conifer-SOC model (Table 4.3). These models can thus be considered good for soil analysis, with an RPD above or near 2 (Chang et al., 2001; Cozzolino and

Morón, 2006), and can be used to predict concentration of both components in unknown soil samples from mixed aspen-conifer stands with similar history and physical characteristics. Contrary to our expectation, the best models for both components developed for the truncated dataset with OM spectra (i.e., mineral matrix subtracted) did not improve prediction ability ( $R^2 = 76\%$ ,  $RMSEP = 4.1 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{VAL} = 2.0$  for aspen-SOC and with  $R^2 = 70\%$ ,  $RMSEP = 5.4 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{VAL} = 1.8$  for conifer-SOC, Table 4.3). They performed similarly to models developed for the truncated dataset with OS spectra at the C-V stage.

The best models developed with OS spectra for the initial dataset at the IV phase had  $R^2 = 49\%$ ,  $RMSEP = 10.1 \text{ mg C g}^{-1} \text{ soil}$ , and  $RPD_{IV} = 1.6$  for aspen-SOC, and  $R^2 = 33\%$ ,  $RMSEP = 8.5 \text{ mg C g}^{-1} \text{ soil}$ , and  $RPD_{IV} = 1.2$  for conifer-SOC (Table 4.4). Performance of OS models at IV was noticeably less than at C-V (Table 4.3 and Table 4.4), with  $R^2 = 49$  vs.  $R^2 = 62\%$  for aspen-SOC and  $R^2 = 33$  vs.  $R^2 = 54\%$  for conifer-SOC. A  $RPD_{IV}$  of 1.6 indicated that the best aspen-SOC model requires further improvement. The  $RPD_{IV}$  of the best conifer-SOC model was worse than at the C-V ( $RPD_{IV} = 1.2$  vs.  $RPD_{VAL} = 1.5$ ) (Table 4.3 and Table 4.4) and should be considered as non-reliable for soil analysis. The models developed with OM spectra for the initial dataset at the IV stage were all classified as unreliable for soil analysis, especially for conifer-SOC ( $R^2 = 3\%$ ,  $RMSEP = 10.1 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{IV} = 1.0$ ) (Table 4.4). Furthermore, we observed higher deviation of predicted vs. measured values from the ideal 1:1 line in the OM models for both aspen and conifer components (Fig. 4.3a vs. 3c and Fig. 4.4a vs. 4.4c, respectively), indicating less accuracy of predictions of OM models compared to the OS models. Moreover, predictions of these four models tended

to underestimate the aspen-SOC and conifer-SOC in the higher range of concentrations.

This may be due to the smaller sample size in the higher concentration range.

For the best aspen-SOC and conifer-SOC models based on the truncated dataset, OS and OM models showed a lack of prediction ability at the IV stage as the RPD for these models were all  $\leq 1.6$  (Table 4.4). The best OS model for aspen-SOC on truncated dataset at the IV stage had a  $R^2 = 27\%$ ,  $RMSEP = 6.7 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{IV} = 1.2$  and the best OS model for conifer-SOC had a  $R^2 = 31\%$ ,  $RMSEP = 7.2 \text{ mg C g}^{-1} \text{ soil}$  and  $RPD_{IV} = 1.3$  (Table 4.4). The best OM models developed on truncated dataset at the IV stage had significantly lower  $R^2$  ( $R^2 = 2\%$  for aspen-SOC and  $R^2 = 9\%$  for conifer-SOC) than OS models, but similar RPD and RMSEP (Table 4.4). Furthermore, the best OM models underestimated the concentrations of aspen-SOC and conifer-SOC at the higher end of the concentrations ranges and overestimated the aspen-SOC and conifer-SOC at the lower end of the range (Fig. 4.3d and Fig. 4.4d).

Of all the models developed in this study, the models developed with the truncated dataset and OS spectra offered the best results at the C-V stage. This may be due to a more homogeneous distribution of samples across the concentration range for which the calibrations were developed. A compact dataset improved the fitting of the models in comparison to model calibration for the initial dataset, which was affected by observations at the extremes of the concentration range ( $< 2.18 \text{ mg C g}^{-1} \text{ soil}$  for aspen and  $< 1.34 \text{ mg C g}^{-1} \text{ soil}$  for conifer;  $> 36.82 \text{ mg C g}^{-1} \text{ soil}$  for aspen and  $> 41.40 \text{ mg C g}^{-1} \text{ soil}$  for conifer). From a practical standpoint, our results indicate that the time-consuming organic matter removal from the soil samples prior to spectra acquisition is not necessary because it does not improve the prediction ability of our models.



For both components and spectra types, model performance decreased between the C-V and the IV stages, suggesting that factors other than SOC concentration and species of origin interfered with our analysis. Gruselle and Bauhus (2010) developed models to predict the contribution beech and spruce in the forest floor, using material in varying stages of decomposition and from different sites across the Black Forest (Germany). They were able to achieve a high degree of accuracy for both species at the IV stage ( $R^2$  of 91% for beech and 90% for spruce). Compared to prediction models for the forest floor, our OS models for the soil at the IV stage showed  $R^2$  between 33 – 49 %. We considered that differences in the composition of detritus inputs and microbial communities, as well as variability in biotic and abiotic characteristics within and among our aspen and conifer ecosystems, could have contributed to this lower model performance.

Sources of organic matter in the mineral soil consist of litterfall, dead roots, and rhizodeposits from trees and understory vegetation and their decomposition products. They all potentially influence soil NIR spectra through differences in organic matter chemistry, amount, and allocation within the soil profile. Lower  $R^2$  for SOC models compared to forest floor models most likely reflects the greater complexity emerging from interaction between soils and organic matter, as well as the presence of the mineral matrix with its own spectral signal (Viscarra Rossel and Webster, 2011). Also, we had greater success with aspen-SOC models than with conifer-SOC models. Nevertheless, differentiation of aspen SOC vs. conifer SOC was possible due to initial differences in amount and composition of litter, root, and understory inputs. Studies conducted in boreal forests have found that aspen and conifer species retain distinct chemical characteristics

in foliar litter after 6 year of decomposition (Strukelj et al., 2012), and exhibited differences in fine root net primary productivity, root decomposition rates, and their relative contribution to total detritus input (Finer et al., 1997; Steele et al., 1997). Furthermore, the sensitivity of NIRS may explain lower performance of conifer models than aspen models as the conifer soil samples were derived from stands representing multiple conifers species. Indeed, since NIRS is able to discriminate pine needles from different species (Espinoza et al., 2012), it is possible that having multiple conifer species increased SOC chemical (and spectral) heterogeneity within the conifer component, thereby confounding the calibration of the conifer-SOC model across all UT sites.

We observed a relative clustering of spectra by site , as well as high spread among the spectra of pure aspen soils in the scores plot (data not shown), which can be attributed to two properties of aspen. First, aspen is a species of high ecological plasticity, and in the interior western US alone, thirty-five plant community types have been described for pure aspen (Mueggler, 1988). Understory biomass and diversity is significantly higher under aspen stands than under adjacent conifer stands, possibly due to more favorable conditions of soil moisture, nutrients, and light in aspen stands (Mueggler, 1988; Stam et al., 2008). Hence, aspen soil NIR spectra are expected to be affected by understory to a greater extent than conifer spectra. Second, it is plausible that aspen genetic diversity also contributed to greater spectral variability. Sexual reproduction in aspen is frequent in western US landscapes (Mock et al., 2008; Long and Mock, 2012). Even within a single stand, clone diversity can be high (Hipkins and Kitzen, 2004; Mock et al., 2008; De Woody et al., 2009). Aspen genotype influences root growth (Fischer et al., 2006), foliar and litter chemistry, soil C and N concentrations, microbial enzymatic activity (Madritch

et al., 2009), and microbial community structure (Madritch et al., 2011), all of which may be reflected on soil spectral properties.

Differences in microbial community structure, composition, and activity between aspen and conifer stands, and among conifer species may also have contributed to the differentiation of SOC between aspen and conifer soils with NIRS. While we have no direct measurements on microbial community compositions for our sampling sites, soil fauna and microbial community structure have been shown to differ among aspen and conifer forests in boreal (Laganière et al., 2009; Royer-Tardif et al., 2010) and temperate semi-arid environments (Ayres et al., 2009). Thus, if microbial communities associated with different species produce distinct assemblages of organic compounds, their legacy on SOC chemistry could be identifiable through NIRS.

Our initial assumption of an additive relationship among OS, OM, and mineral matrix (MM) spectra (i.e.,  $OS = OM + MM$ ) was not supported. Clustering of spectra by site (data not shown) further suggests that, apart from biotic factors, site-specific soil characteristics exerted some influence on NIR spectral properties as well. Indeed, NIR spectra can reflect the influence of soil type (Bartholomeus et al., 2008; Viscarra Rossel and Webster, 2011), soil texture (Van Waes et al., 2005), mineralogy (Vendrame et al., 2012), and soil development (Knadel et al., 2013). This presence of a latent site imprint on our spectra, including those controlled for MM, may also suggest selective or differential preservation of certain organic compounds, causing an indirect influence of the mineral matrix on SOC composition (e.g., Kaiser and Guggenberger, 2000). This is consistent with Schmidt et al. (2011), who proposed that SOC persistence is an ecosystem property that emerges from the interaction between biological and

physicochemical features of a given site. Woldeselassie et al. (2012) found that aspen soils had a greater fraction of mineral-associated SOC than conifer soils, and suggested leaching and adsorption of litter decomposition products to the mineral matrix as the main pathway. Our soils consisted mostly of loams, but there were slight differences in texture among sites, ranging from sandy loams to silty clay loams (Table 4.2). Uneven representation of textural classes in the C-V and IV sets (Table 4.2) may thus have contributed to the lower performance of the models at the IV stage. Although we do not have mineralogy data for our study areas, differences in this aspect may have further contributed to the lower accuracy we achieved in our SOC models compared to forest floor models.

## **Conclusions**

The ecology of aspen and conifer forests in the interior western US is closely linked to disturbance regime, which has been intensely modified through land use changes since the European settlement. Thus, spectral properties of aspen and conifer soils do not solely reflect the influence of current overstory and understory diversity, soil microbial community, soil texture and mineralogy; but also carry with them the legacy of past land use. The complex interaction between site environmental conditions, forest dynamics, and historical land use, all contribute to NIR spectral heterogeneity of soil samples, requiring a sufficiently populated spectral library to develop robust models that could be applied across montane forests in western US.

The good model performance ( $R^2 \sim 70\%$ ) of SOC models at calibration-validation indicates that the contribution of vegetation to SOC can be predicted using the artificial

mixtures method. However, in order to develop more powerful models at the independent validation stage (i.e., models with  $RPD > 2$ ) further work with NIRS models applied to aspen and conifer forests should consider: (1) application of other chemometrics methods, besides PLSR to OS spectra, (2) a more systematic testing of SOC spectra across a geographically broad aspen-conifer soils database, and (3) stratification of the spectra datasets based on prior land use history/soil physical characteristics. Acquiring detailed information on historical vegetation cover for stratification purposes is specially challenging in regions with relatively recent land use records, such as Utah. These ecosystems may experience further change of vegetation cover over the next decades due to land management and climate change that may alter SOC dynamics. NIRS may thus prove to be a useful tool in large scale SOC accounting or the prediction of future SOC stock trajectories in these montane forests.

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Table 4.1. General characteristics of the study areas.

Study area†	Latitude	Longitude	Elevation range m	Mean annual precipitation mm	MMT‡ -----°C-----	mMT§	Common soil order	Coniferous species	Sources
FB	41° 56' N	111° 34' W	1770-3030	1197	16.4	-6.9	Mollisols and Alfisols	Subalpine fir ( <i>Abies lasiocarpa</i> (Hook.) Nutt.) Douglas fir ( <i>Pseudotsuga menziesii</i> (Mirbel) Franco) Limber pine ( <i>Pinus flexilis</i> (Willd.) Rostk. & Schmidt)	Kusbach, 2010 NRCS, 2013
TWDEF	41° 51' N	111° 30' W	2600	950	14	-10	Mollisols and Alfisols	Engelmann spruce ( <i>Picea engelmannii</i> Parry ex Engelm.) Subalpine fir ( <i>Abies lasiocarpa</i> (Hook.) Nutt.)	Olsen and Van Miegroet, 2010 Woldeselassie et al., 2012
DLL	41°8' N	111°14' W	1889-2700	910	16	-5	Mollisols, Entisols, Aridisols and Inceptisols	Subalpine fir ( <i>Abies lasiocarpa</i> (Hook.) Nutt.) Douglas fir ( <i>Pseudotsuga menziesii</i> (Mirbel) Franco)	Woldeselassie, 2009 NRCS, 2013
CM	37°31' N	113°8' W	1800-3200	812	15.5	-3.8	Mollisols and Alfisols	Subalpine fir ( <i>Abies lasiocarpa</i> (Hook.) Nutt.) Douglas fir ( <i>Pseudotsuga menziesii</i> (Mirbel) Franco) White fir ( <i>Abies concolor</i> (Cord. & Glend.) Lindl. ex Hildebr.)	McNab and Avers, 1994 Evans, 2010 Rogers et al., 2010 NRCS, 2013

† Study area: FB, Franklin Basin; TWDEF, T.W. Daniel Experimental Forest; DLL, Deseret Land and Livestock; CM, Cedar Mountain.

‡ MTT, Maximum mean monthly temperature.

§ mMT, Minimum mean monthly temperature.

Table 4.2. Aspen and conifer SOC concentrations and texture of end member (e.m.) soils (i.e., aspen and conifer soils) and range of concentrations in the artificial mixtures (a.m.).

Study area†	Site‡	Transect / Plot	Number of a.m.	Aspen e.m.	Conifer e.m.	Aspen soil	Conifer soil	Aspen-SOC	Conifer-SOC
				SOC	SOC§	texture	texture	range a.m.	range a.m.
				-----mg C g <sup>-1</sup> soil-----					
						-----mg C g <sup>-1</sup> soil-----			
CM	CM1	T1	0	64.8	-	Loam	-		
CM	CM1	T2	0	81.1	-	Loam	-		
CM	CM1	T3	0	74.2	-	Loam	-		
CM	CM2	T1	0	39.2	72.4	Silt loam	Silt loam		
CM	CM2	T2	20	41.7	49.6	Loam	Silty clay loam	4.2 - 36.6	2.8 - 42.2
CM	CM2	T3	30	66.2	84.7	n.a.¶	Clay loam	4.1 - 53.4	3.4 - 66.4
CM	CM57 (IV)	T2	22	53.8	35.9	Sandy clay loam	Sandy clay loam	2.4 - 46.3	1.4 - 31.7
CM	CM57 (IV)	T3	20	30.2	24.3	Loam	Loam	2.2 - 24.1	0.9 - 19.4
CM	CM111	T1	20	24.1	17.3	Sandy loam	Sandy loam	1.8 - 21.2	0.7 - 14.9
CM	CM111	T2	0	14.0	-	Sandy loam	-		
CM	CM111	T3	20	10.9	14.1	Sandy loam	Sandy loam	1.1 - 8.7	0.6 - 11.1
FB	FB1	T1	30	62.2	55.9	Loam	Clay loam	2.8 - 49.1	2.5 - 41.9
FB	FB1	T2	30	48.6	61.7	Silt loam	Loam	2.0 - 39.0	1.6 - 57.8
FB	FB1	T3	0	43.1	49.5	Loam	Silt loam		
FB	FB2 (IV)	T1	25	70.6	37.8	n.a.	Silty clay loam	3.2 - 57.7	1.1 - 31.3
FB	FB2 (IV)	T2	0	54.6	43.4	Silty clay loam	Clay loam		
FB	FB2 (IV)	T3	25	50.6	49.2	Silty clay loam	Silty clay loam	6.4 - 42.2	1.8 - 39.2
DLL	DLL Frost	Plot	40	24.2	37.3	Loam	Silt loam	1.2 - 20.6	1.5 - 33.5
DLL	DLL Frost	T1	0	20.2	19.0	Sandy loam	Loam		
DLL	DLL Frost	T2	0	38.1	27.5	Sandy loam	Loam		
DLL	DLL Frost	T3	0	35.4	36.3	Loam	Loam		
DLL	DLL Bear (IV)	Plot	40	35.4	28.8	Loam	Loam	1.7 - 30.3	1.1 - 25.9
DLL	DLL Bear (IV)	T1	0	43.3	66.1	Loam	Loam		
DLL	DLL Bear (IV)	T2	0	27.5	25.7	Sandy loam	Loam		
DLL	DLL Bear (IV)	T3	0	69.2	28.7	Loam	Sandy loam		
TWDEF	TWDEF	Plot	40	24.3	26.6	Sandy clay loam.	Sandy loam	1.3 - 20.7	1.0 - 24.0
TWDEF	TWDEF	T1	0	43.9	44.3	Loam	Loam		
TWDEF	TWDEF	T2	0	46.8	-	Loam	Loam		
TWDEF	TWDEF	T3	0	33.7	42.8	Clay loam	Clay loam		
Total dataset			362	10.9 - 81.1	14.1 - 84.7			1.1 - 57.7	0.6 - 66.4

† Study area: CM, Cedar Mountain; FB, Franklin Basin; DLL, Deseret Land and Livestock; TWDEF, T.W. Daniel Experimental Forest.

‡ (IV), sites included in the independent validation set. All other sites were used for calibration-validation.

§ - no conifer sample available, due to absence of pure conifer stands at CM1, and processing error of the samples at CM11 and TWDEF.

¶ n.a., texture data not available due to insufficient sample.

Table 4.3. Aspen-SOC and conifer-SOC models developed in the calibration-validation phase with original spectra (OS) and organic matter spectra (OM).

Type of spectra	Dataset	Component	Concentration range -----mg C g <sup>-1</sup> soil-----	SD <sub>v</sub> †	Mathematical treatment‡	Range cm <sup>-1</sup>	Rank	R <sup>2</sup> <sub>VAL</sub> §	RMSEP <sub>VAL</sub> ¶	RPD <sub>VAL</sub> #
								%	mg C g <sup>-1</sup> soil	
OS	Initial	Aspen-SOC	0 - 81.1	14.71	FD + SLS	7347.7-6676.5 4829-3992	10	62	9.4	1.6
OS	Truncated	Aspen-SOC	2.18 – 36.82	8.40	FD + VN	5440-4246.6	8	76	4.6	2.1
OS	Initial	Conifer-SOC	0-84.7	16.14	FD + VN	4601.5-3999.8	9	54	10.8	1.5
OS	Truncated	Conifer-SOC	1.34- 41.40	9.85	SLS	6101.8-4597.6	9	74	5.1	2.0
OM	Initial	Aspen-SOC	0 - 81.1	14.71	NSP	8751.6-7498.1 6101.8-4597.6	8	55	9.9	1.5
OM	Truncated	Aspen-SOC	2.18 – 36.82	8.40	FD + VN	7502-6800 5450-4246.6	8	76	4.1	2.0
OM	Initial	Conifer-SOC	0-84.7	16.14	VN	5349.7-4597.6	9	43	13.1	1.3
OM	Truncated	Conifer-SOC	1.34- 41.40	9.85	SLS	5450-4597.6	8	70	5.4	1.8

† SD<sub>VAL</sub>, standard deviation of validation set.

‡ Mathematical treatments: NSP, no spectral preprocessing; SLS, straight line subtraction; FD, first derivative; VN, vector normalization.

§ R<sup>2</sup><sub>VAL</sub>, coefficient of determination at validation.

¶ RMSEP<sub>VAL</sub>, root mean square error of prediction at validation.

# RPD<sub>VAL</sub>, ratio of SD<sub>VAL</sub> to SEP<sub>VAL</sub>.





Table 4.4. Statistics of model performance at the independent validation stage for aspen-SOC and conifer-SOC models developed in the calibration-validation stage with original spectra (OS) and organic matter spectra (OM).

Type of spectra	Dataset	Component	Concentration range	Mathematical treatment†	Range	Rank	SD <sub>IV</sub> ‡	R <sup>2</sup> <sub>IV</sub> §	RMSEP <sub>IV</sub> ¶	SEP <sub>IV</sub> #	RPD <sub>IV</sub> ††
			mg C g <sup>-1</sup> soil		cm <sup>-1</sup>		mg C g <sup>-1</sup> soil	%	-----mg C g <sup>-1</sup> soil-----		
OS	Initial	Aspen-SOC	0 - 81.1	SLS	6761.4 - 6244.6 5446.2 - 4007.5	10	14.2	49	10.1	8.7	1.6
OS	Truncated	Aspen-SOC	2.18 - 36.82	VN	6101.8 - 4597.6	9	7.9	27	6.7	6.6	1.2
OS	Initial	Conifer-SOC	0-84.7	FD	5222.4 - 4987.2 4516.6 - 4285.2 4134.8 - 4007.5	7	10.3	33	8.5	8.4	1.2
OS	Truncated	Conifer-SOC	1.34- 41.40	SLS	5349.7 - 4597.6	7	8.7	31	7.2	6.5	1.3
OM	Initial	Aspen-SOC	0 - 81.1	SLS	6850.1 - 3999.8	8	14.2	44	10.5	10.4	1.4
OM	Truncated	Aspen-SOC	2.18 - 36.82	FD + SLS	10001.3 - 7498.1 6101.8 - 5446.2 4601.5 -4246.6	4	7.9	2	7.7	7.3	1.1
OM	Initial	Conifer-SOC	0-84.7	FD + SLS	7085.4 - 6846.3 5403.7 - 4397	7	10.3	3	10.1	10.1	1.0
OM	Truncated	Conifer-SOC	1.34- 41.40	FD + SLS	7725.7 - 5446.2 4601.5 - 4246.6	5	8.7	9	8.3	6.9	1.3

† Mathematical treatments: NSP, no spectral preprocessing; SLS, straight line subtraction; FD, first derivative; VN, vector normalization.

‡SD<sub>IV</sub>, SD of independent validation set.

§ R<sup>2</sup><sub>IV</sub>, coefficient of determination at independent validation.

¶ RMSEP<sub>IV</sub>, root mean square error of prediction at independent validation.

# SEP<sub>IV</sub>, standard error of prediction at independent validation.

††RPD<sub>IV</sub>, ratio of SD<sub>IV</sub> to SEP<sub>IV</sub>.

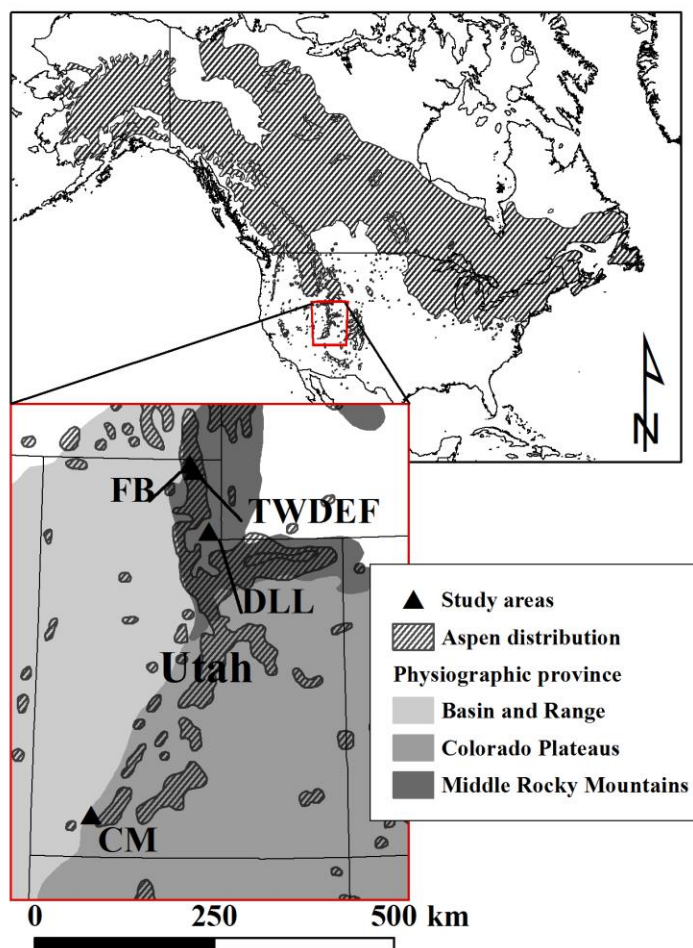
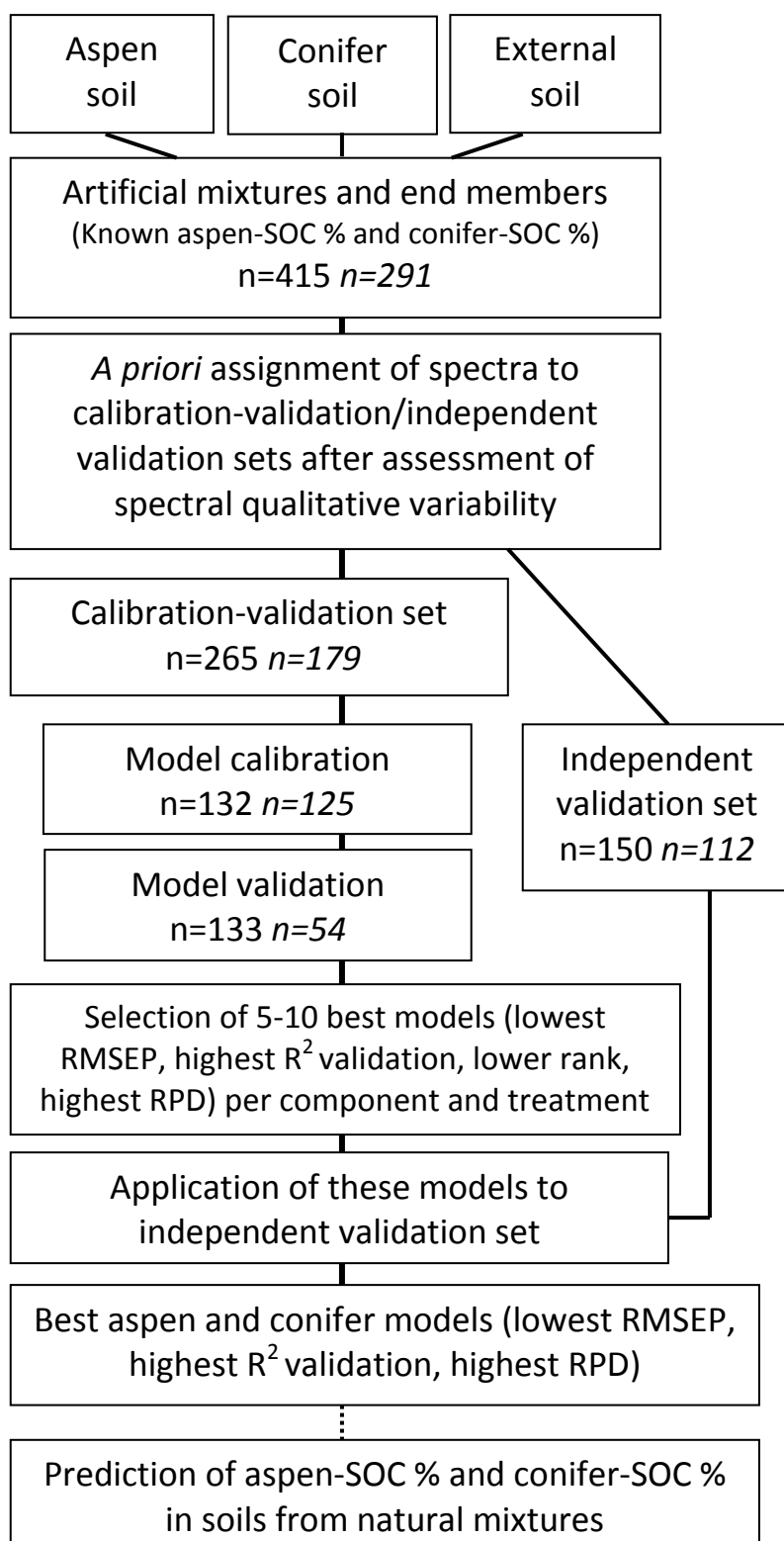


Fig. 4.1. Location of study areas in Utah relative to the physiographic provinces defined by Fenneman and Johnson (1946) and aspen habitat distribution by Little (1971). Study areas: FB, Franklin Basin; TWDEF, T.W. Daniel Experimental Forest; DLL, Deseret Land and Livestock; CM, Cedar Mountain.

Fig. 4.2. Methodology followed for the development and validation of NIRS prediction models (modified from Gruselle and Bauhus, 2010). In italics, sample size of the truncated dataset (i.e., spectra with reference values between the 10<sup>th</sup> and 90<sup>th</sup> percentile). RMSEP, root mean square error of prediction at validation; RPD, ratio of standard deviation of reference values to standard error of prediction. The dashed line indicates the ultimate goal of NIRS models development.



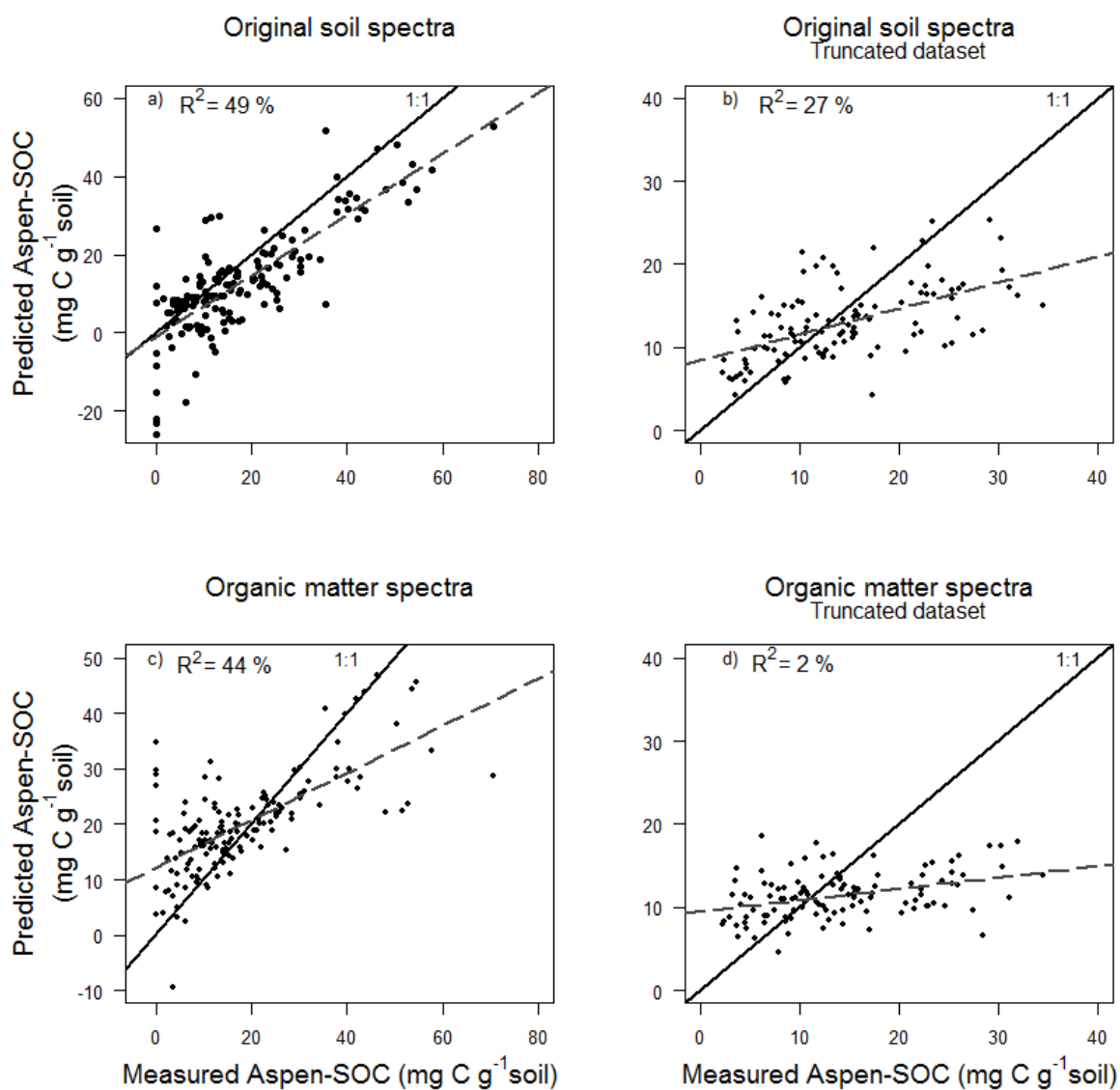


Fig. 4.3. Predicted vs. measured values of aspen-SOC (mg C g<sup>-1</sup> soil) for the independent validation set. Truncated dataset, comprised of the 10<sup>th</sup> to the 90<sup>th</sup> percentile of original reference values. Dashed line indicated the regression line; solid line is the 1:1 line.

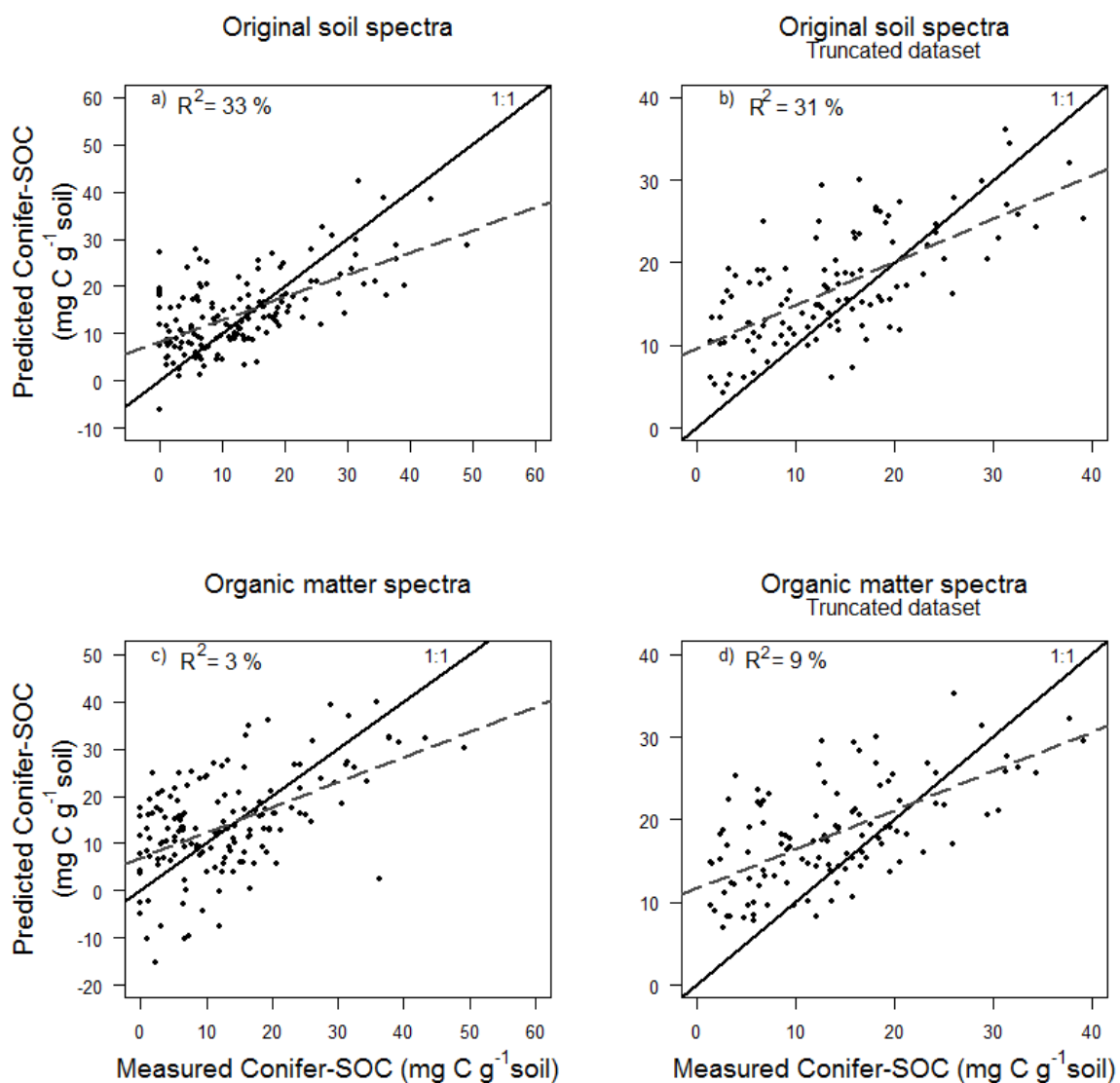


Fig. 4.4. Predicted vs. measured values of conifer-SOC (mg C g<sup>-1</sup> soil) for the independent validation set. Truncated dataset, comprised off the 10<sup>th</sup> to the 90<sup>th</sup> percentile of original reference values. Dashed line indicated the regression line; solid line is the 1:1 line.

## CHAPTER 5

## SUMMARY AND CONCLUSIONS

Changes in the relative distribution of aspen and coniferous species in montane forests as result of land management and climate change may impact storage, stability, and chemical composition of SOC in topsoils. Overstory species determine the amount, nature, and allocation patterns of C input, which in interaction with soil microclimate, soil microbial community composition and activity, soil texture, and mineralogy, regulate C balance and specific patterns in SOC stabilization. We investigated quantitative and qualitative differences in SOC across the aspen-conifer ecotone in Utah to assess potential effects of conifer encroachment on C storage.

In the first section we assessed the effects of forest cover [aspen (*Populus tremuloides*) vs. conifers (*Abies lasiocarpa*, *Abies concolor*, *Pseudotsuga menziesii*)] and stand composition on SOC storage, content and distribution of SOC among fractions, and SOC decomposability. Understanding how biotic (e.g., overstory composition) and abiotic factors (e.g., soil texture) control SOC storage and stability can help to forecast the fate of SOC under future scenarios of conifer encroachment for different site conditions. To expand the spatial scope of previous studies we selected sampling locations in southern Utah (Cedar Mountain) and northern Utah (Franklin Basin). Overstory composition was characterized as either a categorical variable (aspen, mixed, conifer) or as a continuous variable (contribution of aspen to live basal area) with two different study designs. We measured SOC storage, mineral-associated SOC in the silt and clay size fraction (MoM) as estimate of stable SOC, and SOC lability with light fraction (LF) (i.e., free and occluded particulate organic matter), hot water extractable

organic carbon (soluble SOC), and long term laboratory incubations (SOC decomposability). The results indicate that in these ecosystems overstory composition has an effect on SOC stability, expressed as an increase in MoM content with dominance of aspen on the overstory. However, the vegetation effect is somewhat obscured by the effect of soil texture for silt + clay content above 70 %. At relatively high silt and clay contents the sheer abundance of absorption sites and the protective capacity of the mineral matrix may overwhelm potential differences in organic matter input and chemistry associated with different forest cover. Silt and clay content strongly influenced the distribution of SOC among fractions, and explained variability in SOC decomposability and solubility better than vegetation cover. Management efforts pursuing long-term C sequestration should preserve aspen in sites with silt + clay contents between 40-70 %, where overstory species composition influences the storage of stable SOC.

In a second section we characterized the chemical composition of SOC, MoM, and LF with Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) to address two objectives: (1) to assess differences in SOC chemistry across the aspen-conifer ecotone, and (2) to investigate whether higher content of mineral-associated SOC under aspen stands is related to higher concentration of recalcitrant compounds (i.e., aliphatic C) or preferential stabilization of certain molecules (i.e., polysaccharides, amino-sugars, etc.). Spectra were analyzed with qualitative and semi-quantitative methods, allowing to identify the functional groups present across substrate types (i.e., OM, MoM, LF) by vegetation cover, and to estimate differences in concentration and relative contribution of main functional groups to SOC. FTIR spectra of SOC, MoM, and



LF were dominated by peaks indicative of polysaccharides and C-O groups from ether, ester, carboxylates, and Si-O bonds from quartz and clay minerals, followed by aliphatic C from methyl and methylene groups. Weak signals for aromatic C, carboxylate, and amides were more detectable in the light than in the mineral-associated fraction. Across all sample types, the absorbance in the polysaccharide and C-O band followed the trend aspen > mixed > conifer, suggesting that aspen litter is richer in O-alkyl C, and that it is preserved in the mineral-associated fraction through chemical stabilization of plant and microbial derived compounds. There was an increase in the relative contribution of aliphatic C to MoM with conifer encroachment, indicating that recalcitrance is not solely responsible of greater storage of MoM in aspen stands, and that accumulation of aliphatic C may not drive storage of C in as MoM. FTIR spectra were clustered by sites of similar parent material rather than by vegetation cover, suggesting that site conditions exert as a filter for SOC speciation. Despite initial differences in litter chemistry, SOC chemical composition within and among sites is more likely shaped by differences in microclimate, topography, soil chemistry, and especially mineralogy.

Uncertainty about the future of aspen creates the need for a better understanding of SOC dynamics in aspen-conifer forests, and especially being able to elucidate the fate of stable SOC after conifer encroachment. In a third section we developed prediction models for aspen-derived SOC concentration and conifer-derived SOC concentration using near infrared reflectance spectroscopy (NIRS). To that end, we generated a sample set at the laboratory by mixing soils sampled under aspen and conifers from different Utah locations, and a third independent soil component (garden soil). The sample set was divided *a priori* into a calibration-validation set and an independent validation set based

on qualitative differences among spectra grouped by site. Partial least regression was used to calibrate the models. Model performance was good at the validation stage ( $R^2 \sim 70\%$  and ratio of standard deviation to standard error of prediction (RPD)  $> 2$ ) but was less satisfactory at independent validation ( $R^2 \sim 70\%$ , RPD  $< 2$ ). Similarly to the FTIR results, we observed clustering of spectra by site. NIR spectra reflects indirectly the influence of vegetation, but also of land use history, soil texture and mineralogy, soil microbial community, and soil forming processes. Our results indicate that it is possible to develop prediction models for species SOC concentration. However, given the high NIR spectral variability among sites and within species, the aspen-conifer database should cover a broad range of ecological settings in order to develop robust models, or consider stratification of the spectra datasets based on prior land use history/soil physical characteristics. NIRS prediction models could inform on the fate of SOC following changes in land cover as a substitute of other techniques of more difficult application in forest soils (e.g., isotope studies or radiocarbon dating).

The results from this research project highlight how the interaction of abiotic (e.g., soil texture and parent material) and biotic factors (e.g., overstory species composition) control SOC stabilization and chemistry, and influences our ability to detect clear vegetation imprints. The presence of the mineral matrix enables mechanisms of physical and chemical protection of SOC. More C is stored as MoM, becoming more stable, as the availability of mineral surfaces increase. The chemistry of MoM reflects major differences in parent material (e.g., basalt vs. sedimentary rock) and overstory vegetation. While SOC stability depends on the simultaneous action of biochemical recalcitrance and physico-chemical protection, biochemical recalcitrance seems to play a secondary role in

SOC storage in mixed aspen-conifer forests. The mechanisms driving greater storage under aspen may be linked to the fast turnover of aspen litter, either through adsorption of soluble, simple molecules of plant origin or via microbial resynthesis. At the same time, it is possible that rhizodeposition and turnover of fine roots contributes to SOC stabilization as source of non-structural carbohydrates. Other possible factors resulting in higher C sequestration under aspen may be higher differences in C allocation and input, (e.g., belowground vs. aboveground), or differences in microbial community composition and microclimate. Future work should investigate the pathways of C input and SOC stabilization, determine the sources of SOC (e.g., belowground vs. aboveground), and assess the role of the microbial community in organo-mineral interactions.

APPENDICES

## APPENDIX A

Topographic characteristics (mean  $\pm$  SD) and parent material of transects at Cedar Mountain (CM) and Franklin Basin (FB).

Site	UTM X	UTM Y	Elevation (m)	Slope (degrees)	Aspect	Parent material
CM1	314626	4155367	2552 $\pm$ 8	14 $\pm$ 9	N	Mudstone with minor sandstone and conglomerate
CM2	320849	4165698	2756 $\pm$ 12	24 $\pm$ 12	N	Basalt
CM57	332666	4159578	2773 $\pm$ 11	24 $\pm$ 11	W	Mudstone, sandstone
CM111	319937	4149936	2685 $\pm$ 9	46 $\pm$ 2	N	Sandstone, siltstone, mudstone, claystone, carbonaceous shale, coal, and marl
FB1	452200	4643568	2098 $\pm$ 9	10 $\pm$ 4	NE	Unconsolidated conglomerate consisting of locally derived cobbles, boulders, and angular blocks (quartzite, sandstone, and limestone)
FB2	450966	4645352	2196 $\pm$ 18	15 $\pm$ 5	E	Dolomitic limestone, and limestone

## APPENDIX B

Number of plots, mean topographic characteristics, parent material, and range of overstory live basal area and stem density for aspen and conifer species in sites at Cedar Mountain (CM).

<b>Site</b>	<b>UTM X</b>	<b>UTM Y</b>	<b>Elevation (m)</b>	<b>Slope (degrees)</b>	<b>Aspect</b>	<b>Parent material</b>
CM5	320180	4165310	2759 ± 9	7 ± 2	NE	Basalt
CM8	320176	4150109	2679 ± 25	22 ± 4	NW	Sandstone, mudstone, siltstone, carbonaceous shale, marl
CM15	331655	4161737	2650 ± 22	24 ± 3	W	Sandstone, mudstone
CM17	314931	4157543	2722 ± 11	4 ± 2	W	Basalt
CM20	330450	4159867	2898 ± 9	4 ± 1	N	Olivine basalt

APPENDIX C

Coauthor Permission Letters

20 June, 2014

Mercedes Román Dobarco has my permission to include the following paper, which has been published, of which I was co-author, in her doctoral dissertation.

**Román Dobarco, M.**, and H. Van Miegroet. 2014. Soil organic carbon storage and stability in the aspen-conifer ecotone in montane forests in Utah, USA. *Forests*, 5(4): 666-688.

A handwritten signature in black ink, appearing to read 'H. Van Miegroet', with a stylized flourish at the end.

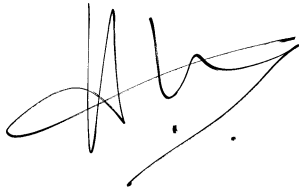
Helga Van Miegroet  
Professor, Wildland Soils and Biogeochemistry  
Department of Wildland Resources



20 June, 2014

Mercedes Román Dobarco has my permission to include the following paper, which is published, of which I was co-author, in her doctoral dissertation.

**Román Dobarco, M.,** M-C. Gruselle, H. Van Miegroet, and J. Bauhus. Predicting tree species origin of soil organic carbon with near-infrared reflectance spectroscopy. North American Forest Soils Conference Proceedings, Soil Science Society of America Journal (in press) doi:10.2136/sssaj2013.08.0331nafsc.

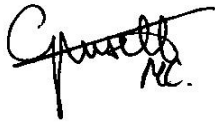
A handwritten signature in black ink, appearing to be 'H. Van Miegroet', with a stylized flourish at the end.

Helga Van Miegroet  
Professor, Wildland Soils and Biogeochemistry  
Department of Wildland Resources

20 June, 2014

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A handwritten signature in black ink, appearing to read 'C. Gruselle' with a stylized flourish and the initials 'M.C.' below it.

Marie-Cécile Gruselle



Albert-Ludwigs-Universität Freiburg . Professur für Waldbau . D-79085 Freiburg

Chair of  
Silviculture

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Freiburg, June 22, 2014

To whom it may concern

#### Permission to reproduce article

Dear Sir/Madam,

Mercedes Román Dobarco has my permission to include the following paper, which is in press and which I co-authored, in her doctoral dissertation.

Román Dobarco, M., M-C. Gruselle, H. Van Miegroet, and J. Bauhus. North American Forest Soils Conference Proceedings, Soil Science Society of America Journal (in press)  
doi:10.2136/sssaj2013.08.0331nafsc.

Sincerely

Digital unterschrieben von Jürgen Bauhus  
DN: cn=Jürgen Bauhus, o=University of  
Freiburg, ou=Chair of Silviculture,  
email=juergen.bauhus@waldbau.uni-  
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Prof. Dr. Jürgen Bauhus

## CURRICULUM VITAE

Mercedes Román Dobarco

(June 2014)

**EDUCATION****Ph.D. Ecology** 2011 – 2014

Utah State University, Logan, Utah, USA

Dissertation title: “Influence of stand composition on soil organic carbon stabilization and biochemistry in aspen and conifer forests of Utah”

Advisor: Dr. Helga Van Miegroet

**M.S. Forestry Engineering, Magna Cum Laude** 2002 – 2010

Universidad Politécnica de Madrid, Madrid, Spain

Thesis title: “Environmental study of the Rocina stream. Improvement measures and economic valuation of the current water use”

Advisor: Dr. Marta González del Tánago

**RESEARCH EXPERIENCE**

Graduate Research Assistant 2011 – Present

Department of Wildland Resources, Utah State University, Logan, Utah,

USA

Advisor: Dr. Helga Van Miegroet

- Analyzed the influence of overstory vegetation on soil organic carbon storage and stabilization using several fractionation techniques

- Characterized the chemical composition of soil organic matter with Fourier transform infrared spectroscopy. Collaboration with Professor Astrid R. Jacobson, Department of Plants, Soils and Climate, Utah State University
- Developed multivariate prediction models for species derived soil organic carbon concentration with near-infrared reflectance spectroscopy. Collaboration with Professor Jürgen Bauhus and Dr. Marie-Cécile Gruselle, Department of Silviculture, University of Freiburg

Undergraduate Research Assistant

2009 – 2010

UPM Hydrobiology Research Group, Madrid, Spain

Advisors: Dr. Marta González del Tánago and Dr. Diego García de Jalón

- Assessed the landscape evolution of the Guadalete River (Cádiz, Spain)
- Compiled and analyzed fluvial restoration case studies for [FORECASTER](#) project
- Reviewed methods for economic valuation of water resources. Applied the residual imputation approach for assessing the economic value of irrigation water for strawberry cultivation in Huelva (Spain)

Undergraduate Research Assistant

2008 – 2009

Collaboration Scholarship with the Center for Energy, Technological and Environmental Research (CIEMAT), Madrid, Spain

Advisor: Dr. Rosa María Inclán Cuartas

- Measured greenhouse gases flow through the soil in Mediterranean forests using static chamber technique

- Measured soil respiration with LI-COR, soil moisture content with time domain reflectometry in Mediterranean dehesas

## COMPLEMENTARY EDUCATION

Getting started as a successful proposal writer and academician, Utah State University, October, 2013.

Restoring the West Conference 2011, Sustaining forests, woodlands, and communities through biomass use, Logan, UT (October 2011)

IWRM Forecaster workshop. Stream physical restoration: Syntheses and methods for basin management, Lyon, France (June 2010)

Sustainable Management of Wetlands (summer course) (July 2009)  
Universidad Internacional de Andalucía

Public Use Planning in Protected Natural Areas (course) (September 2008)  
EUROPARC Spain-La Casa Encendida - Obra Social Caja Madrid

Erasmus exchange student (September 2007 – June 2008)  
Faculty of Forestry and Life Sciences, Czech University of Life Sciences, Prague (Czech Republic)

## PEER-REVIEW PUBLICATIONS

Román Dobarco, M., Jacobson, A., and H. Van Miegroet. Chemical Composition of Soil Organic Carbon from Mixed Aspen-Conifer Forests Characterized with Fourier Transform Infrared Spectroscopy. (in preparation)

Román Dobarco, M., Gruselle, M-C., Van Miegroet, H., and J. Bauhus Predicting Tree Species Origin of Soil Organic Carbon with Near-Infrared Reflectance Spectroscopy. Proceedings of the North American Forest Soils Conference. *Soil Science Society of America Journal* (in press)

Román Dobarco, M., and H. Van Miegroet. 2014. Soil Organic Carbon Storage and Stability in the Aspen-Conifer Ecotone in Montane Forests in Utah, USA. *Forests*, 5(4): 667-689 doi:10.3390/f5040666

Gonzalez del Tánago, M., D. García de Jalón, and M. Román Dobarco. 2012. River Restoration in Spain: Theoretical and Practical Approach in the Context of the European Water Framework Directive. *Environmental Management*, 50 (1): 123 - 139 doi 10.1007/s00267-012-9862-1

**CONFERENCES AND PRESENTATIONS**

BIOGEOMON, Bayreuth (July 2014)

Soil organic carbon dynamics on aspen and conifer soils in Utah. Poster presented.

Román Dobarco, M., and H. Van Miegroet

12th North American Forest Soils Conference, Whitefish, MT (June 2013)

The use of spectroscopic techniques to determine overstory species influence on SOC properties and origin. Poster presented.

Román Dobarco, M., Van Miegroet, H., Gruselle, M-C., Jacobson, A., and J. Bauhus

Remedia Workshop 2013, Zaragoza, Spain (April 2013)

Pasture, tillage and canopy effects on carbon dioxide fluxes in a Spanish dehesa.

Abstract presentation.

Uribe, C., Hernando, L., Román, M., Clavero, M.A., Roig, S., Van Miegroet, H., and R. Inclán

BIOGEOMON, Northport, ME (July 2012)

Changes in storage, stability and spectroscopic properties of soil organic carbon along montane aspen-conifer ecotones in Utah, USA. Poster presented.

Van Miegroet, H., Gruselle, M-C., Bauhus, J., Jacobson, A., and M. Román Dobarco

Intermountain Graduate Research Symposium, Logan, UT (April 2012)

Influence of overstory vegetation on soil organic carbon properties in Cedar Mountain (Utah). Poster presented.

Román Dobarco, M., Van Miegroet, H., and M-C. Gruselle

50<sup>a</sup> Reunion científica de la SEEP, Pastos, paisajes culturales entre tradición y nuevos paradigmas del siglo XXI, Toledo, Spain (May 2011)

Flujos de CO<sub>2</sub> del suelo en una dehesa del centro peninsular. Poster presented.

Uribe, C., Hernando, L., Román, M., Roig, S., and R. Inclán