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Effect of Foliage and Root Carbon Quantity, Quality, and Fluxes on Soil Organic Carbon Stabilization in Montane Aspen and Conifer Stands in Utah

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EFFECT OF FOLIAGE AND ROOT CARBON QUANTITY, QUALITY, AND FLUXES ON SOIL ORGANIC CARBON STABILIZATION IN MONTANE ASPEN AND CONIFER STANDS IN UTAH

by

Antra Boča

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ABSTRACT

Effect of Foliage and Root Carbon Quantity, Quality, and Fluxes on Soil Organic Carbon Stabilization in Montane Aspen and Conifer Stands in Utah

by

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Utah State University, 2017

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Department: Wildland Resources

Forest soils store as much carbon (C) as the vegetation that grows on them, and the carbon in soil is more stable than the C in biomass. Quaking aspen (*Populus tremuloides* Michx.) is the most widespread tree species in North America, and aspen forests in the Western US have been found to store more soil organic carbon (SOC) in the mineral soil than nearby conifers. Fire exclusion and grazing often promote the succession of aspen to conifer dominated forests due to their effect on aspen regeneration. So far the factors driving the differential SOC accumulation, and the effects of the vegetation shift on SOC pools, are not well understood.

In this dissertation I aimed to evaluate how various forest vegetation characteristics – tree type, detritus fluxes, detritus chemistry – affect SOC pools and stability from a global to a molecular level using two contrasting forest types – aspen and conifer. A meta-analysis showed that, while conifer forests worldwide had higher C pools in the forest floor, this

difference did not translate into the mineral soil, suggesting that the mechanisms that control SOC storage differ between both soil compartments. Above- and belowground detritus input fluxes were similar between aspen and conifer forests, and did not explain the higher SOC pools under aspen. A sorption study revealed that the more labile aspen foliage dissolved organic carbon (DOC) was more effectively retained in soil than aspen root, and conifer substrate DOC. Furthermore, soils that contained aspen SOC retained new DOC better than soils with conifer SOC, irrespective of the source of the DOC. Finally, foliage and root specific compounds that were identified for aspen and subalpine fir provide a base for future studies aiming to identify the source of SOC under both overstory types.

Overall, the results of the dissertation suggest that substrate chemistry more than detritus fluxes drive the differences between SOC pools under aspen and conifer forests in Utah. This finding indicates that the link between C input amounts and SOC pools is not as direct as currently assumed in most SOC models. Furthermore, a tree species effect on SOC as distinct as aspen vs conifer is not common between all hardwood and conifer comparisons worldwide, thus suggesting that the effect of vegetation can be overridden by other factors.

(204 pages)

PUBLIC ABSTRACT

Effect of Foliage and Root Carbon Quantity, Quality, and Fluxes on Soil Organic Carbon Stabilization in Montane Aspen and Conifer Stands in Utah

Antra Boča

Soil organic carbon (SOC) positively affects many soil properties (e.g., fertility and water holding capacity), and the amount of carbon (C) in soil exceeds the amount in the atmosphere by about three times. Forest soils store as much C as is found in trees. Tree species differ in their effect on SOC pools. Quaking aspen forests in the Western US often store more stable SOC in the mineral soil than nearby conifers. During the last decades a decline in aspen cover, often followed by conifer encroachment, has been documented. A shift from aspen to conifer overstories may negatively affect the amount and properties of SOC. In this dissertation, I aimed to evaluate the mechanisms that drive the higher SOC pools under aspen compared to conifers. I found that the amount of detritus produced by both forest types could not explain the observed differences. Aspen foliage dissolved organic carbon (DOC) was, however, retained in soil more than conifer DOC, and soils with aspen SOC retained new C more in general. This suggests that it is the chemistry of aspen detritus rather than the amount that drives the higher SOC pools. Root- and foliage-specific biomarkers, identified in this dissertation, could help us elaborate on the source of stable SOC in future studies. The observed SOC differences between aspen and conifers do not represent a general trend between hardwoods and conifers worldwide, suggesting that the factors affecting SOC differ from place to place.

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Antra Boča

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER 1

INTRODUCTION

Soils constitute the largest terrestrial pool of organic carbon (C), which is approximately twice the size of the atmospheric C pool, and three times the size of the biotic pool (Batjes 1996; Lal 2004). Changes in processes that allow for such high C storage can make soils C sources or C sinks for atmospheric C. Thus consideration of soil C is important for goals such as achieving "a balance between anthropogenic emissions by sources and removals by sinks of greenhouse gases" described in Article 4 of the 2012 Paris Agreement. While sounding simple, C pools and fluxes in soils, and the mechanisms that affect them, are not well understood. In fact, there is currently no consensus on the size of soil organic carbon (SOC) stocks, their spatial distribution, and the C emissions from soil (Scharlemann et al. 2014). One of the major soil forming factors, and the main source of organic C in soil is vegetation. Among major vegetation types, forests have made up half of the terrestrial C sink globally over the last 20 years, with forest soils storing similar amounts of C as tree biomass (Pan et al. 2011). Tree species are known to affect SOC stocks and stability (as reviewed by Vesterdal et al., 2013), but the conditions under which these effects occur, and the mechanisms behind them are often still unclear. Considering the size of forest SOC pools, understanding tree species effects on SOC storage is as crucial as understanding C sequestration in their biomass. In this dissertation I investigate several forest overstory characteristics, and their effect on SOC pools and stability by using two adjacent, yet contrasting, forest overstory types.

Apart from the goal to estimate existing SOC pools on a large scale, the question about vegetation effects on SOC has received much attention also because modelling and retrospective approaches predict shifts in spatial distributions of tree species as a result of global change (Kutzbach et al. 1988; Boucher-Lalonde et al. 2012). For example, conifers in temperate and boreal regions are expected to extend the tree line to higher latitudes and altitudes, and may be partly replaced by hardwoods in their current core areas (Overpeck et al. 1991; Cramer et al. 2001; Lenoir et al. 2008). In North America, fire suppression and grazing in areas dominated by the pioneer hardwood species *Populus tremuloides* (Quaking aspen) – the most widely distributed tree on the continent (Little 1971) – have resulted in the expansion of conifers and a decline in aspen forests (Rogers 2002; Kulakowski et al. 2004; Di Orio et al. 2005). Predictions suggest that some areas will become even less favorable for aspen in the future (Worrall et al. 2013) leading to more drastic vegetation shifts. Therefore, there is a need to better understand how forest vegetation – from tree species level to larger functional groups, can be used to estimate existing and future SOC pools and fluxes.

Conifer and deciduous broadleaved tree effects on SOC have been of research and practical interest for decades (e.g., Ovington, 1956; Alban et al., 1978; Gurmesa et al., 2013). Disparities in such traits as leaf structure, photosynthetic capacity, hydraulic network and tissue composition (Chabot and Hicks 1982; Bond 1989; Aerts 1995; Cornelissen et al. 1997; Castro-Díez et al. 2000), suggest differences in forest ecosystem functioning. Therefore, conifers and hardwoods (or broadleaves) have the potential to be important groupings for predicting soil properties (as reviewed by Augusto et al., 2014).

Using vote counting (summing the numbers of statistically significant positive and negative studies), Vesterdal et al. (2013) summarized findings from published studies, and found a strong positive effect of conifer species on forest floor C stocks, while forest vegetation effects on mineral SOC were not as straight forward. One reason that might have prevented the detection of an effect in this study could have been the method used. Vote counting does not provide any information about the magnitude of the effect of interest. A more robust statistical quantification might be more effective.

Forest overstory affects SOC via many pathways (e.g., microclimate, microbial associations, substrate chemistry, etc.), but ecosystem C models assume an especially strong relationship between the amount and type of plant litter inputs and soil C accumulation. Vegetation is the primary source of SOC through above and belowground litter inputs. Aboveground forest litter consists mainly of leaves or coniferous needles (Jensen 1974; Millar 1974). The below-ground source of C is primarily fine root turnover (Rasse et al. 2005) with root exudates inhibiting or accelerating SOC decomposition (Cheng and Kuzyakov 2005). While long-term litter manipulation studies like the Detritus Input Removal and Transfer (DIRT) experiment have found above- and belowground detritus exclusion to reduce C stocks (from 9-18% in 20 years), the doubling of aboveground litter inputs did not affect SOC pools (Lajtha et al. 2014). This indicates that the response of SOC stocks to litter input is neither linear nor immediate, and raises questions about the strength of the relationship between litter input and SOC accumulation.

Decomposition models currently used in all ecosystem C models (more precisely, Earth System Models) are built on the assumption that carbon substrates have intrinsic chemical decomposition rates (Todd-Brown et al. 2013), which depend on chemical properties like C to N or N to lignin ratios of plant substrates. While this has been proven to work well for the forest floor, with, for example, higher C to N ratios indicating higher recalcitrance and leading to longer mean residence times, model calculations based on these assumptions yield erroneous estimates for the mineral soil (Todd-Brown et al. 2013; Wieder et al. 2014). In fact, today there is growing evidence that higher substrate quality enhances C stabilization in mineral soil (Cotrufo et al. 2013; Castellano et al. 2015; Cyle et al. 2016), meaning lower C to nutrient and N to lignin ratios might lead to more stable SOC.

While belowground detritus decomposes in-situ, and, therefore, root C has the advantage of directly interacting with soil particles and soil solution, aboveground C (litter and forest floor) needs to be incorporated into soil. One of the most important pathways for the litter layer to be incorporated into mineral soil is by leaching as dissolved organic carbon (DOC) (Kalbitz and Kaiser 2008). In fact, both, root and foliage C, are redistributed within the soil profile as DOC (Uselman et al. 2007). In its dissolved form, organic carbon can easily interact with mineral surfaces forming one of the most stable SOC fractions in soil – organo-mineral complexes (see Fig. 1-1 for a simplified visual representation of forest soil C cycling). The association with mineral soil particles (sorption, desorption) is the ultimate controller of organic C stabilization in soil over decadal to millennial time-scales (Schmidt et al. 2011; Keil and Mayer 2014). These

interactions vary with the concentration and chemistry of the organic molecules, and soil mineral characteristics (Lilienfein et al. 2004; Kögel-Knabner et al. 2008; Yeasmin et al. 2014). Thus vegetation properties affecting the solubility of detritus and its chemistry can drive SOC pools and their stability.

To evaluate vegetation effects on SOC all other soil forming factors – parent material, climate, topography, and time – need to be kept constant. Aspen and conifer forests in Utah fulfill this requirement. Here the forests are dominated by aspen and various conifer species, often growing in close proximity to each other as a mosaic in the landscape. Van Miegroet et al. (2005) and Woldeselassie et al. (2012) have reported significantly higher and more stable SOC pools under aspen compared to adjacent conifer forests in northern Utah. The proximity of stands in these studies suggests that the difference in SOC pools is a result of either litter input quantity or chemistry, or the interactions of their DOC with soil mineral (silt and clay) surfaces. The large differences in mineral SOC stocks, the contrasting vegetation characteristics, and the close proximity make these forests ideal for investigating how forest vegetation affects SOC pools. Measuring above- and belowground litter input fluxes, and evaluating the interactions between foliage and root DOC with mineral surfaces could be the first step in understanding the drivers of higher SOC pools under aspen vs. conifer.

To further advance our knowledge on the effects of above- and belowground sources on SOC pools and stability, there is promising evidence that a more precise determination of the C source in soil is possible by using foliage- and root-specific biomarkers. Cutin and suberin are two major foliage and root lipid macromolecules that

can be extracted from SOC with alkaline hydrolysis, identified with gas chromatography-mass spectroscopy, and used to determine the source of SOC (Kogel-Knabner et al. 1989; Nierop 1998; Otto and Simpson 2006; Mendez-Millan et al. 2011). For example, Spielvogel et al. (2014) found a strong correlation between suberin and live fine root biomass in soil, and Crow et al. (2009) reported that, based on their foliage and root biomarker signatures, the contribution of above- vs. belowground detritus to SOC differed for a conifer and a hardwood forest. While being potentially very informative, these biomarkers are species-specific (Angst et al. 2016). Therefore, before these biomarkers can be used to determine the importance of above- and belowground detritus for the formation of SOC, they first need to be the identified for the vegetation that is the primary contributor of organic carbon at a site.

In this dissertation I aim to evaluate how various forest vegetation characteristics – functional group, litter fluxes, litter chemistry – affect SOC pools and stability from a global to a molecular level. The specific objectives of the dissertation are to: (i) quantify global observed patterns in SOC pool differences between hardwoods and conifers by using a meta-analysis; (ii) compare aboveground and belowground litter C fluxes under adjacent aspen and conifer stands, and evaluate their importance in explaining SOC pool differences; (iii) compare the sorption and desorption of aspen and conifer leachates on mineral soil; and (iv) identify species-specific foliage and root biomarkers (cutin and suberin) in order to evaluate above- and belowground plant source contributions to the formation of SOC under aspen and conifer overstories. These four objectives constitute individual chapters of this dissertation.

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Figures

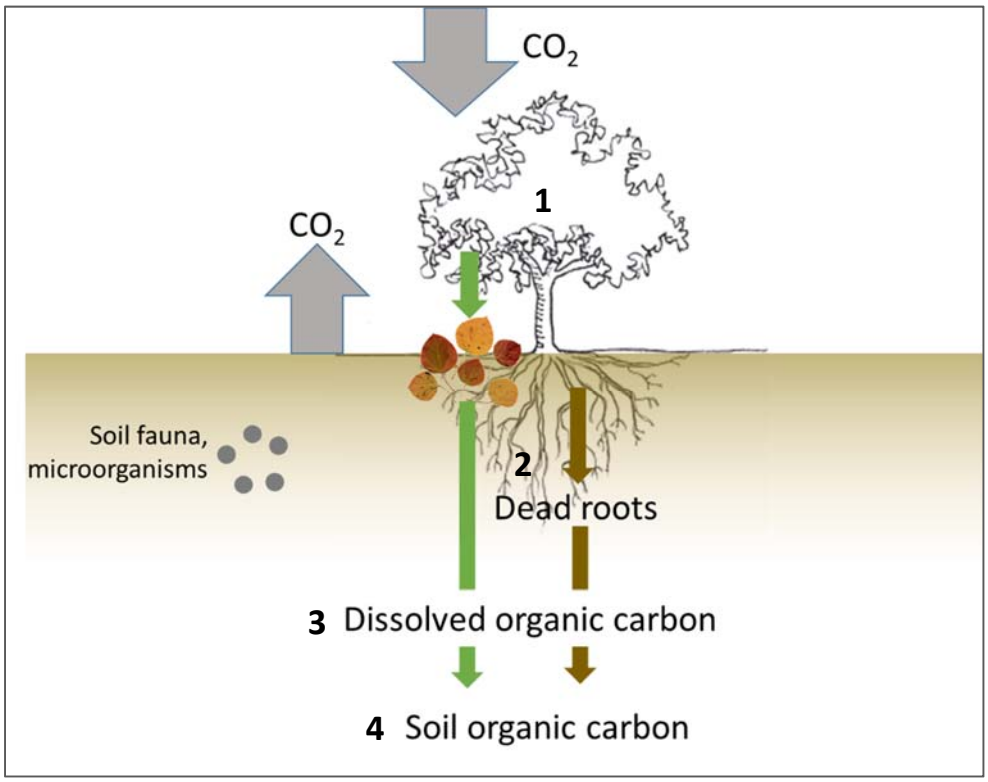

Fig. 1-1. Simplified representation of forest carbon cycling in Utah forests. CO_2 is taken up by trees, which are the major contributors of plant C in forest soils through aboveground litterfall and belowground root turnover. Both sources (green arrow for litter and brown arrow for dead roots) of detritus are re-distributed in soil with snowmelt water as dissolved organic carbon (DOC). In its dissolved form C can sorb to mineral surfaces, and create stable soil organic carbon (SOC) through the formation of organo-mineral complexes. In contrast to litterfall, roots turn over in-situ, meaning their particulate organic matter is already distributed through soil. Due to the lack of large soil fauna, particulate organic matter from litter is not distributed within soil very deep. Microorganisms alter the particulate organic matters that enters the soil, and respire CO_2 during this process, returning C back into the atmosphere. This dissertation focuses on (1) the effect of overstory type on SOC pool size; (2) above- and belowground detritus input flux size; (3) the retention of DOC in soil from above- and belowground detritus, and (4) identification of SOC sources by tracing foliage and root C.

CHAPTER 2

FOREST OVERSTORY EFFECT ON SOIL ORGANIC CARBON STORAGE – A META-ANALYSIS[1]

Abstract

A meta-analysis using 77 studies from 28 countries was performed to assess the effect of hardwood vs. conifer overstory on soil organic carbon (SOC) storage in forest floor (FF), mineral soil and whole soil (FF+mineral soil). Overall FF stocks were 38% higher under conifers, mineral SOC stocks were similar and whole soil SOC was 14% higher under conifers. An analysis with six of the seven most reported tree genera reaffirmed higher FF and whole soil C stocks under conifer stands. Analysis with all seven of the genera showed more pronounced variability in mineral SOC results compared to the overall results. *Eucalyptus* was the only hardwood that stored significantly (17%) more SOC in the mineral soil than adjacent conifers. *Picea* was the only conifer that stored significantly (7%) more SOC in the mineral soil than adjacent hardwoods. Differences in FF SOC stocks had a limited predictive power in explaining the variability of mineral SOC stock differences, suggesting that they are not very closely linked with regards to SOC storage. Only when comparing FF SOC stocks among genera, did precipitation, age difference, soil texture, and previous land use moderate SOC storage differences between conifers and hardwoods. In other cases, neither climate nor

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soil variables could explain differences between SOC stocks. Our findings suggest that using plant-trait driven vegetation categories may be a more descriptive way of detecting vegetation effects on soil SOC.

Introduction

Globally, forest soils play an important role in the terrestrial greenhouse gas balance as they store many times more C than tree biomass (EC/UN-ECE, 2003). Forest soil organic carbon (SOC) stocks are influenced by biotic and abiotic factors, such as climate and soil properties that often interact and regulate C inputs to and losses from the soil. Tree species connect to forest soils in two important ways: distribution and growth of various species depends on climate and soil properties, and soil properties may be strongly influenced by tree species occupying a site.

In the past the main interest in tree species effects on soils has focused on soil fertility parameters and possible environmental issues, for example, following atmospheric deposition and heavy metal accumulation (Vesterdal et al., 2008). From the numerous studies that have investigated the effects of tree species on soil properties across a range of climates (e.g., Binkley and Valentine, 1991; Finzi et al., 1998; Binkley and Menyailo, 2005; Vesterdal et al., 2008; Hansson et al., 2011), including comprehensive reviews (Binkley and Giardina, 1998; Augusto et al., 2002; Vesterdal et al., 2013); only few have explicitly focused on SOC storage effects (Vesterdal et al., 2002, 2013). In many instances, findings were equivocal. With an ongoing debate about climate change and C sequestration, the potential of forests to store C has become of increasing interest in science, policy, and management (Jandl et al., 2007; Vesterdal et

al., 2013). This has led to more efforts in quantifying vegetation effects on soil C storage, since soils constitute the largest terrestrial reservoirs (Schlesinger, 1977), and small changes in SOC pools may influence atmospheric CO_2 levels.

Forest management, including changes in tree species, has been proposed as a measure for mitigating atmospheric CO_2 in national greenhouse gas budgets (Vesterdal et al., 2008). Many European countries currently experience a change in forest policy towards use of native tree species adapted to local climate with natural regeneration (Larsen and Nielsen, 2007). Historically, in areas with high population density, forests have been highly shaped by human influence. For example, the need to counteract wood shortages in some European countries caused forest management to focus on regenerating highly productive forests, often associated with the expansion of coniferous forests beyond the limits of their natural ranges (Spiecker, 2003). Forest use for wood fuel and timber, and forest clearing for agriculture as well as the alteration of disturbance regimes has also caused shifts in forest composition in the U.S. over the last 300 years (McKinley et al., 2011). Current predictions suggest that in many parts of Europe and North America, hardwood species may expand their potential distribution ranges into areas currently dominated by conifers (Thuiller et al., 2006; Mckenney et al., 2007; Price et al., 2013). The opposite pattern can also be observed in areas dominated by pioneer hardwood species like aspen where disturbance suppression has resulted in the expansion of conifers (Rehfeldt et al., 2009; Rogers et al., 2010). Understanding the ecological consequences of these vegetation shifts on the global C balance requires accurate knowledge of forest type effects on SOC storage and stabilization mechanisms.

The differentiation between hardwoods (or broadleaves) and conifers is one of the most basic and most commonly used categorization in forestry. It implies broad differences in plant-traits between both groups and has been the source of extensive and often heated debate among foresters on the impact of tree species on soil properties. Conifers, for example, are generally thought to produce more acidic soils and cation depletions (Dambrine et al., 1998; Berger et al., 2006). However, conclusive evidence of systematic vegetation effects on soils are often lacking (Binkley and Giardina, 1998; Binkley and Fisher, 2012) especially as it pertains to soil C pools (Vesterdal et al., 2013).

The most consistent findings of overstory effects on SOC stocks relate to the forest floor (FF). Many studies have found that the forest floor under conifer stands accumulates more C than under hardwood stands (Vesterdal et al., 2008) for the most part due to the differences in persistence of foliage litter (Binkley and Giardina, 1998). Conifer needles have higher concentrations of lignin, and higher C to nutrient ratios, resulting in slower decomposition of needles compared to hardwood litter (Augusto et al., 2002; Vesterdal et al., 2002; Hansson et al., 2011), which leads to higher C accumulation rates in the forest floor of conifer stands compared to hardwood stands.

Published data on SOC stocks in mineral soil have not yet yielded such consistent results. For example, Ovington (1956) found no significant differences between 20 year old conifer and hardwood SOC stocks in SE England; Oostra et al. (2006) found higher SOC stocks under hardwoods than under spruce in S Sweden. In dry montane forests in Utah, Woldeselassie et al. (2012) found that aspen store more mineral SOC than adjacent conifer stands. However, in the more mesic conditions in Canada, aspen store less SOC

overall than adjacent conifer stands, but when comparing different depths, aspen store more C in the deeper horizons (Laganière et al., 2013). This raises several questions: (i) does more C in the forest floor imply greater SOC storage in the mineral soil; (ii) does more rapid turnover of hardwood foliage lead to lower SOC stocks in the mineral soil; and (iii) is the effect consistent geographically?

The meta-analyses and reviews by Guo and Gifford (2002), Paul et al. (2002) and Laganière et al. (2010) concluded that afforestation with coniferous species resulted in lower SOC stocks than the afforestation with hardwood species. However, these reviews compared stands under varying climatic and soil conditions, and therefore, may not reflect solely the effect of forest overstory types on soil properties like SOC. Furthermore, most reviews acknowledged the difficulty in generalizing or quantifying broad patterns about tree species effect on SOC stocks. This raises the question whether differences over broad groups of tree species such as hardwood vs. conifer are detectable or whether more specific taxonomic levels, e.g., genus, would give clearer results?

The aim of this study was to investigate whether overstory type (conifer vs. hardwood or broad taxonomic groups such as tree genera) affects SOC stocks in clear and consistent ways. Specifically, we address the following study questions: (i) do hardwood stands consistently store more or less SOC than conifer stands under similar climatic and soil conditions; (ii) are differences in SOC storage patterns between different forest covers consistent throughout the soil profile, i.e., similar in forest floor and mineral soil; (iii) are there tree genera that stand out in terms of higher or lower SOC storage relative

to their comparison group; and (iv) are differences in SOC storage between hardwood and conifer stands or among taxonomic groups influenced by abiotic site conditions (e.g., climate, soil properties)?

Methods

Literature Search

Peer-reviewed and "gray" literature was searched mostly via on-line databases ISI Web of Science and Google Scholar. Among others, the keywords used were "tree species, forest, soil organic carbon, pool, stock" as well as names of specific countries like "South Africa, Russia, New Zealand, Brazil, etc." We also searched for references in papers that addressed SOC in forest soils. The analysis contains data from six unpublished studies, and two studies (one in Japan, one in Brazil) that were obtained after personal communication with researchers from these countries.

The search was done using English keywords; therefore, the hits included only studies that had keywords and abstracts in English. This introduces a language bias and is a major reason for missing data. However, searching with keywords from different languages and national databases were beyond the practical limits of this study. Our search resulted in more than 10,000 hits from which we extracted 77 studies that matched the following eligibility criteria: (i) study reported soil C stocks (or data from which stocks can be estimated) for forest or woodland stands; (ii) the comparison stands were dominated (~80%) by hardwoods or conifers in terms of species composition, stem density and/or canopy cover; (iii) the comparison stands were adjacent and therefore shared similar climatic and soil/parent material conditions; (iv) stand age ≥ 15 years; and (v) SOC data were

reported for at least 5 cm of mineral soil. The studies originated from 28 countries and reported SOC stocks for adjacent hardwood and conifer stands at 93 sites (Appendix A). Acceptable comparisons were paired plot designs, single-tree studies (soils under multiple individual tree canopies), and chronosequences that compared adjacent hardwood vs. conifer stands. For our analysis, we used ancillary information provided in the studies to select only those comparison pairs where abiotic factors (climate, elevation, aspect, soils) were as similar as possible.

We used soil C pool size as the response variable for this analysis. When only C concentrations and bulk densities were reported we calculated the SOC stocks from these values. If data were reported in a graph, we used Plot Digitizer 2.6.2. (http://plotdigitizer.sourceforge.net/) to extract the relevant information. To explain potential patterns in SOC stock differences between hardwoods and conifers, we also extracted metadata (predictor variables) from each publication (Table 2-1) for a moderator analysis.

Comparisons of SOC pools were done at the level of the whole soil (FF + mineral soil), FF, mineral soil, surface mineral soil (< 30 cm) and deep mineral soil (> 30cm). However, most studies (54 out of 77) reported C pools for < 30 cm. In the genus-level analysis we analyzed differences between individual hardwood and conifer genera for the whole soil, FF, and mineral soil (without separation in surface and deep). The decision to analyze the total mineral soil without separation by depth was made so that a sufficient number of response ratios (effect size that measures the magnitude of difference between SOC stocks under hardwoods and conifers) were obtained for the individual genera.

Several studies reported C stock data for the whole depth of 0 to 50 or even 100 cm, excluding them from the surface mineral soil analysis.

The studies we selected encompassed 31 hardwood genera including a group that contained stands with more than one genus (classified in the data set as "Hardwood") and 17 conifer genera including a group that contained more than one genus (classified in the data set as "Conifer"). The genera that were reported the most were *Betula, Eucalyptus* (mineral soil only)*, Fagus, Quercus, Larix, Picea,* and *Pinus* (number of effect sizes (k) > 25). We compared these individual genera to the corresponding comparison group (e.g., *Betula* vs. conifers or *Larix* vs. hardwoods). This analysis could not be performed with other genera due to a low number of effect sizes.

Statistical Analyses of Response Ratios

Meta-analysis encompasses statistical methods used to summarize research findings across disparate studies (Gurevitch and Hedges, 1999), by using relative effect sizes, i.e., standardized, directional measures of the mean change (Harrison, 2011). This is typically done between a "control" and a "treatment". The groups compared in this study do not constitute true experimental control or treatments; however, vegetation is the only variable that is different between the comparable sites. Since the overarching goal was to find patterns in SOC storage differences among vegetation groups, we selected conifers as our control or norm against which to evaluate relative change in SOC storage by hardwoods.

We measured the magnitude of difference in the SOC stocks between hardwoods and conifers across studies using the ln-transformed response ratio (R) as the effect size:

$$\ln R = \ln(X_{hardwood}/X_{conifer})$$

where, $X_{hardwood}$ represents the mean SOC stock value of hardwood stands and $X_{conifer}$ represents the mean SOC value of conifer stands for a given site. After back transformation [$e^{\ln(R)}$] , R can be conceptualized as the proportional or percentage change in SOC stocks relative to its control value (as per Nave et al., 2013). Meaning, if the value after back transformation is 1, then that corresponds to 0% change. If the value is below 1, then that corresponds to more SOC under conifers, and can be depicted as % change compared to 0% change calculated as ($e^{\ln(R)} - 1$)*100.

When analyzing data at the genus level, R was based on the mean SOC stock value of a specific hardwood genus over the mean SOC stock value of different conifer genera for a site or the SOC stock value of different hardwood genera over the mean SOC stock value of a specific conifer genus for a site. Consider, for example, a study reporting SOC pools *for Betula, Acer, Populus, Pinus* and *Picea* on one site. In the general hardwood-conifer meta-analysis, $X_{hardwood}$ was the mean SOC pool value for *Betula, Acer* and *Populus* over the analyzed depths (whole soil, FF, mineral soil, surface mineral soil, deep mineral soil), and $X_{conifer}$ the corresponding mean SOC pool value for *Pinus* and *Picea*. Consequently, in this case, the number of response ratios (k) is 1 (i.e., 1 comparison for the mean SOC pool under hardwoods vs. mean SOC pool under conifers) per analyzed depth. Some studies reported data for two separate sites with adjacent conifer and hardwood stands. For example, Olsson et al. (2012) reported data for one site in southwest Sweden and one site in northern Finland. For this study, k is two – one for Sweden and one for Finland. When genus effect was evaluated, k depended on the

number of genera compared. In the above example, k would be 6 as three hardwood genera (*Betula, Acer, Populus*) were compared against two conifer genera (*Pinus, Picea*). In reporting the results by hardwood genus, response variables against all conifers were averaged; if reported as conifer genus, responses of all hardwoods against this conifer genus were averaged.

A parametric, weighted meta-analysis should always be the first choice when error terms and sample size data are reported (Gurevitch and Hedges, 1999). Unfortunately, many of the identified publications did not report these data, mostly lacking information on variance. In order to include as many studies as possible, we performed an un-weighted meta-analysis, where all studies in a dataset were assigned an equal variance. Distributional statistics were generated by bootstrapping using the package "boot" in the software R (Canty and Ripley, 2013). Bootstrapping allows estimating distributional statistics by iteratively permuting and resampling the dataset. Since it makes no parametric assumptions and generates distributional statistics from available data, bootstrapping typically produces wider, more conservative confidence intervals (Adams et al., 1997). The difference between SOC pools was considered significant when the 95% confidence intervals (CI) did not overlap with 0% change (i.e., no change) in SOC pools.

Our data synthesis generated 93 response ratios for mineral soil in the general analysis, 248 response ratios for mineral soil in the genus-level comparison, 44 response ratios for forest floor in the general analysis and 195 response ratios for forest floor in the genus-level comparison.

Significance of predictor variables

Much as one can partition variance in an analysis of variance (ANOVA), one can also partition the total heterogeneity (Qt) in the distribution of observations into within-class (Qw) and between-class (Qb) homogeneity (Gurevitch and Hedges, 2001). To define factors that drive the difference between SOC pools under hardwoods and conifers, Qb is a measure of the variation in mean effect size between classes (i.e., between classes of the predictor variables, such as previous land use, parent material etc.), which is distributed as a χ^2-statistic with degrees of freedom equal to the number of classes minus 1 (Gurevitch and Hedges, 2001). A categorical factor that defines groups of R with large Qb is a better predictor of variation than a categorical factor with low Qb, and accordingly has a lower P value. In this study, we used Qb and P statistics to check for best predictors of variation.

Categorical (e.g. soil texture, previous land use) and continuous (e.g., mean annual temperature (MAT), mean annual precipitation (MAP), % clay) predictors were used in the analysis to explain SOC stock differences between hardwoods vs. conifers at the general or genus level (Table 2-1). As the description of parent material and mineralogy across studies was often vague, we had to use broad descriptors for this category (e.g., sedimentary, glacial, andic, etc.; Table 2-1). Likewise, we attempted to use soil taxonomic units to the extent possible, which resulted in using only US taxonomy soil orders, and ended up excluding many studies from the soil taxonomy analysis that used different classification systems, due to the difficulty in reconciling different soil classification systems.

In the general analysis (i.e., hardwood vs. conifer comparisons), continuous variables that differed among stands from one site (e.g., soil pH, stem density, etc.) were averaged for each site. Other variables like MAT, MAP, climate class, parent material, and soil texture had to be similar *a priori* for a site to be included in this analysis and could be used unmodified. Previous land use was often only coarsely or incompletely described. Only sites where all hardwood stands shared the same previous land use and all conifer stands shared the same previous land use were included in the general moderator analysis (no averaging possible). For the specific genus-level analysis on SOC stock differences between individual hardwood or individual conifer genera, all variables from Table 2-1 were considered without modification.

Continuously varying factors were tested as predictors of variation using continuous meta-analyses, which is similar to the variance-partitioning process of Qb analysis, in that the heterogeneity among k observations is partitioned into a fraction explained by a linear model (Qm) and that which constitutes the residual error variance (Qe). As such, continuous meta-analysis is the same as the ANOVA F-test for significance of linear regression models (Hedges and Olkin, 1985 from Nave et al., 2013). In all tests we accepted results with $P < 0.05$ as statistically significant. The meta-analyses statistics for the moderator analysis were performed using the R package "metafor" (Viechtbauer, 2010).

Results and Discussion

Patterns of SOC stock differences

SOC stocks in the FF were significantly higher (38%) under conifer than hardwood stands (Fig. 2-1). This statistically significant difference in the FF affected the whole soil C results with conifers having overall higher SOC stocks (14%) compared to hardwood stands. SOC stocks in the mineral soil (0 to 30 cm, 30 to 100 cm and 0 to 100 cm) showed no significant difference between hardwoods and conifers.

None of the potential moderator variables selected (Table 2-1) proved significant in explaining the variability of the effect sizes among hardwood-conifer comparisons across studies in the general analysis of FF, mineral soil and whole soil (FF + mineral soil) (data not shown). In other words, the difference between hardwood and conifer FF or mineral soil SOC stocks could not be explained by any other (constrained and unconstrained) sources of variation.

When each of the most commonly reported genera was compared to its comparison group, FF SOC stocks were consistently lower under the hardwood genera than conifers, with differences ranging from 28% to up to 140% lower (Fig. 2-2b). The same pattern was observed, albeit less pronounced, in the mineral soil (8 to 20 % lower) and whole soil (17 to 32 % lower) (Fig. 2-2a and 2-2c). For the conifer genera, SOC stocks were higher in the forest floor (up to two times) and whole soil (up to 30%); but, except for *Picea*, no significant difference in the mineral soil was found compared to the hardwood comparison group (Fig. 2-2c).

Betula stored significantly less SOC than adjacent conifers at all soil levels (Fig. 2-2), indicated by the lack of overlap between the 95% CI and zero, with differences more pronounced in the forest floor (76% lower) than in the mineral soil (14% lower). Studies reporting SOC stocks for *Betula* stands were mostly located in the temperate, boreal and arctic zones, with *Larix, Picea* or *Pinus* as the main comparison groups. While across all studies, *Betula* stands on average contained less SOC in the whole soil, forest floor, and mineral soil than conifer stands in these climatic zones; this was not always the case, and the opposite pattern was found at some plots in individual sites (Alriksson and Eriksson, 1998; Hansson et al., 2011; Mueller et al., 2012).

A similar pattern was observed for *Fagus* dominated stands, where SOC stocks were on average 26 % lower in the FF and 19 % lower in the mineral soil compared to adjacent conifer stands (Fig. 2-2). The SOC stock comparisons were predominantly reported in the temperate zone and against stands dominated by *Abies, Larix, Picea, Pinus* and *Pseudotsuga*. Once again, the overall effect across all experimental units was not always reflected at individual sites with several studies reporting the opposite pattern (Ladegaard-Pedersen et al., 2005; Zhiyanski et al., 2008; Mueller et al., 2012).

Quercus-dominated stands showed the largest differences in FF SOC stocks (two to three times smaller C pools than in conifer FF) and smallest differences in mineral SOC stocks (8% less) compared to adjacent conifer stands, with all effects statistically significant (Fig. 2-2). Among the four hardwood genera analyzed, *Eucalyptus* stood out as the only hardwood genus with significantly higher SOC stocks (17% more) in the mineral soil than adjacent conifer stands (Fig. 2-2c). The majority of values (k = 21 out of

26) for *Eucalyptus* soils were derived from the temperate zone and these stands were mostly compared to soils under *Pinus*. Exclusion of this genus from the general hardwood-conifer analysis (k = 83) or from genus-level comparison with *Pinus* (k = 123) did not alter the overall conclusion, i.e., the SOC stocks under hardwoods were lower than SOC stocks under conifers. This is most likely due to the comparatively small number of response ratios for *Eucalyptus,* i.e., 10 in the general analysis and 21 in the *Pinus*-based analysis.

FF SOC stocks under *Larix* were almost twice as large as under the hardwood comparison group. In the mineral soil, this difference was reduced to only 8%, and no longer statistically significant (Fig. 2-2). *Larix* stands were mostly compared to stands dominated by *Betula, Fagus,* and *Quercus,* as well as to seven other genera stands and were located mostly in temperate climates; some values were reported in the boreal and arctic zones.

FF SOC stocks under *Pinus* were about 46% higher than under hardwoods. Mineral SOC stocks, on the other hand, showed no significant difference relative to the hardwood comparison groups (Fig. 2-2). Interestingly, when mineral soils under *Pinus* were compared specifically to *Quercus,* we found significantly more SOC (~12 %) under *Pinus*.

Only *Picea* stands stored significantly more mineral SOC (7%) than adjacent hardwood stands with the CI remaining below zero. In the FF, *Picea* stored more than twice the amount of C compared to the hardwood comparison group (Fig. 2-2). When

Quercus stands were compared to *Picea* stands, however, no statistically significant difference in SOC stocks in the mineral soil was observed.

To our knowledge this is the first broad scale analysis of forest overstory composition effects on SOC pools that uses a quantitative approach. Our analysis numerically reaffirmed earlier findings in the literature of higher FF C accumulation under conifer stands (e.g., Binkley and Giardina, 1998; Vesterdal et al., 2013). Even though we found that whole soil (FF + mineral soil) carbon stocks under conifer stands were often higher than under hardwood stands, this was not always the case. Several studies (e.g., Finzi et al., 1998; Oostra et al., 2006; Vesterdal et al., 2008), have shown that differences in FF C stocks can be countered by an opposite accumulation pattern of C in the mineral soil, resulting in total SOC stocks that are not significantly different among overstory types.

Relationship between predictor variables and FF C stock differences

As was the case with the general hardwood-conifer comparison, none of the predictor variables used in the genus-level analysis tested significant (data not shown) for SOC stocks in the mineral soil. In the FF genus-level analysis, age difference (hardwood age – conifer age), elevation, MAT, MAP, previous land use, and soil texture initially emerged as significant. When hardwood stands were older than adjacent conifer stands, the difference between SOC stocks in the FF was smaller and in some cases hardwood stands stored more SOC in the FF. While statistically significant, this positive effect of age difference was mostly driven by 49 response ratios (i.e., 25% of the dataset) where the age among comparison stands was indeed different (Fig. 2-3a). However, the

variability in effect size was very large when there were no differences in age among the comparison stands, which encompassed the majority of the data set. Therefore, the ecological relevance of age as a predictor of difference in SOC stocks among compared groups is questionable.

In our FF data-set, elevation, MAT, MAP were highly correlated, and when colinearity was accounted for, MAP was the only significant variable in the model. The results showed that differences between conifer and hardwood FF C stocks are bigger at lower precipitation (Fig. 2-3b). This relationship, however, was based on two-thirds of the FF response ratios data-set in temperate and boreal climatic zones. Keeping in mind that MAP is positively related to MAT in this analysis, these results indicate that there are fewer differences between hardwood and conifer FF SOC stocks on warmer moister sites than on colder drier sites. Fissore et al. (2008) found that the difference in mineral SOC stocks between hardwoods and conifers decreased with increasing temperature. They suggested that forests with higher MAT experience higher decomposition rates. Liu et al., (2004) found litterfall increased more in hardwood than conifers with increasing temperature and precipitation. They suggested that conifers are better adapted to low-temperature climates, therefore have a higher productivity than hardwoods, resulting in higher litterfall. They did not find productivity differences in production in temperate regions and hypothesized that higher litterfall in hardwood forests was due to differences in biomass allocation patterns.

In the FF analysis among genera, previous land use was reduced to only two levels (cropland and forest) due to the limited number of response ratios in the other

categories. Nevertheless, the results showed that the differences in FF C stocks among genera were more pronounced when stands had been converted from agricultural land than when stands had been under forest cover previously (either the same or different) (data not shown, p-value <0.001). Most of the stands (38 out of 44) were 20 to 40 years old and all were on loamy or clayey soils. Conversion of agricultural land to forest offers more homogenous initial soil conditions among the comparison groups as no FF is present, and FF C stocks more clearly reflect differences in litter chemistry and decomposition rates among the planted species. Our results suggest that, when managing forests for increasing SOC storage, species choice may be a more critical decision during afforestation, than in the case of forest conversion. However, this applies only to FF, which is a more labile C pool compared to mineral SOC. We found no effect of previous land use on mineral SOC stock differences.

Finally, soils emerged as a modifier in terms of texture, such that differences between conifer and hardwood FF C stocks were smaller on sandy soils compared to loamy and clayey soils.

It is difficult to distinguish between the effect of previous land use and soil texture on FF C stocks as all sandy soils for the FF analysis had been previously under forest cover. However, Vesterdal and Raulund-Rasmussen (1998) reported increasing FF C contents with decreasing mineral soil nutrient status in Danish stands of oak and Norway spruce and attributed this mainly to differences in decomposition rates.

Contrast between FF and mineral soil SOC stock differences

Our meta-analyses indicated pronounced differences in FF SOC storage between hardwood and conifer stands but these were highly variable in the genus analysis. Mineral SOC stock differences, on the other hand, were far less pronounced (non-significant in the general analysis) and considerably less variable, suggesting that SOC in the mineral soil is more robust and less sensitive to changes of aboveground vegetation cover. FF has traditionally been considered the main source of organic C to the mineral soil (Schmidt et al., 2011) and recent ^{13}C studies have provided evidence for this aboveground litter contribution (Rubino et al., 2010). However, mineral SOC has been also shown to correlate more with fine root growth and turnover and less with foliage input (Russel et al., 2004). Unfortunately, root data are seldom reported, and this gap in our dataset did not allow us to analyze the effect of fine root mass and turnover on mineral SOC stocks. Furthermore, as Schmidt et al. (2011) have pointed out, C dynamics in the FF and mineral soils are subject to quite different controls. Environmental conditions and biochemical recalcitrance, i.e., litter origin, primarily control microbial decomposition rates in the litter layer. On the other hand, the presence of a mineral matrix further regulates the persistence of SOC in the mineral soil through physical and chemical protection mechanisms (Six et al., 2002), and biochemical characteristics (associated with vegetation composition) are thought to play a secondary role (Rovira et al., 2010). When testing FF as a predictor variable, FF explained only 6% of the variability in mineral SOC stocks in the general analysis and less than 1% in the genus-level analysis. This lack of predictive power, together with the somewhat divergent

accumulation patterns of FF vs. mineral SOC stocks under hardwood and conifer stands suggests that both ecosystem compartments are not that closely linked with regard to SOC storage.

Relationship between predictor variables and mineral soil C stock differences

Our analysis failed to show a relationship between abiotic site conditions (climate, soil texture, previous land use, etc.) and SOC stock differences in the mineral soil and the general hardwood vs. conifer analysis. This does not imply that these factors are not important as several studies have shown the effect of climate and soil texture on SOC stocks (Jobbágy and Jackson, 2000; Six et al., 2002; Fissore et al., 2008). We think that the lack of any relationship arose from the coarseness of the data available. For example, data on exact proportions of clay and silt by depth were scarce, and we had to rely on broad texture descriptors or use values that were averaged across the entire site. In addition, the depth increments measured varied among all studies (0 to 5; 10; 15; 20 cm), as did the final depths for which SOC data were reported. This might result in different effect sizes than if all studies had reported data to the same depth. A study by Baritz et al. (2010), comparing C stocks in forest soils in Europe, also showed that the effect of climate and soil texture could not be detected over a broader geographic area. Finally, variables like previous land use, parent material, or soil order were probably too general to enable detection of their influence on the reported SOC stocks.

Potential limitations of this study

Overall, our analysis shows that it is difficult to detect the influence of biotic and abiotic factors on mineral SOC stocks over a wide geographical range. Potential reasons for this are that the number of studies used in this analysis is not sufficiently large to draw clear conclusions and/or that the information provided in the studies are reported at too coarse of a scale. A more extensive analysis, using databases like the International Soil Carbon Network (ISCN) would be a great source of data for answering these kinds of questions, provided they contain specific (genus-level) vegetation descriptions. Such information is seldom available in large databases.

Furthermore, the search method introduced a language bias in this analysis and therefore limits the number of studies conducted outside of Europe and North-America. Also, the un-weighted analysis, as performed in this study, is very conservative and of low sensitivity; thus, one has to be careful in interpreting the results. Increases in analysis power of 50–100% can easily be obtained in weighted analysis compared to un-weighted tests of the significance of the mean (Gurevitch and Hedges, 1999). However, using the weighted approach would have excluded one third of all studies due to lack of information on variance. We made the decision to give higher priority to the inclusion of more studies, as it would provide more information on the variability in SOC stocks over a broader geographical scale. This was of higher interest than more precisely quantifying variability within individual sites.

Most studies reported sample sizes, which allowed an approximation of the sampling variance (see e.g., Hedges and Olkin, 1985). However, the definition of

replicates turned out to be more problematic than expected. Evaluating true replication for all studies and, hence weighting according to sample size, was not possible due to limited information.

Conclusions

Our whole soil analysis showed that conifer stands generally store more SOC than hardwood stands, mostly driven by higher FF C accumulation under conifers. However, at the level of the mineral soil, no differences in SOC storage between conifer and hardwood stands were found, irrespective of whether the focus was on surficial or deeper soil layers. This shows that a broad generalization of hardwood vs. conifer overstory effect on SOC storage in the mineral soil is not possible based on the information available and method used. One has to be careful in interpreting the "whole soil" data as SOC pool estimates in many studies did not extend beyond 30 cm, with some going only to 5 cm depth.

The individual genus-level analysis revealed more pronounced differences in mineral SOC stocks between hardwood and conifer stands not observed in the general analysis. It also highlighted genus differences in FF C accumulation. This implies that broad categories such as hardwoods and conifers may not be appropriate groupings for understanding vegetation composition effects on soil properties such as C storage. Vegetation affects soil properties by its morphology and dominant plant traits (De Deyn et al., 2008). Therefore, it would probably be more useful to divide vegetation using plant-trait driven categories. Using genus was a first attempt in that direction. Further analyses may reveal better surrogates for plant traits than the genus level used in this

study. By understanding the mechanisms and drivers for SOC sequestration under different species, genera, or families, we could make better predictions of different ecosystem services and implement these findings into forest policy and management practices.

This study utilized the limited number of basic variables that were available and known from observational and experimental studies to influence SOC storage. Additional parameters, such as above- and belowground detritus input, type of clay minerals, etc. might be worth considering in future analyses, provided that such information is available. The number of studies reporting aboveground litterfall, for example, was insufficient for this variable to be included in this analysis. Carbon fluxes were not explicitly part of this investigation and large knowledge gaps remain concerning the sources of litter, decomposition, mixing, leaching, or stabilization of organic matter through aggregation and sorption in soils. A more consistent approach towards sampling and analysis across studies, as well as availability of more detailed data would allow to improve this type of analysis. Data from common garden experiments where all factors, except vegetation are similar, give us most insights into C pathways in forest ecosystems.

We did not detect a relationship between FF and mineral SOC stocks, suggesting that different factors control C fluxes between these two ecosystem compartments. In addition, our results suggest that mineral SOC stocks might be more influenced by belowground litter input than FF.

Finally, as did Guo and Gifford, (2002), we conclude that as the quantity of available data is not large and the methodologies used are diverse, the conclusions drawn must be regarded as working hypotheses from which to design future targeted investigations that expand the database.

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Tables and Figures

Table 2-1. Predictor variables tested using meta-analysis

Factor	Levels
Hardwood genus	*Acer, Alnus, Betula, Brachystegia, Carpinus, Carya, Castanea, Castanopsis Eucalyptus, Fagus, Fraxinus, Gleditsia, Hyeronima, Laurus, Liquidambar, Liriodendron, Michelia, Mytilaria, Nothofagus, Ormosia, Pentaclethra, Populus, Quercus, Schima, Sclerolobium, Tilia, Ulmus, Virola, Vochysia*, "Hardwood"
Conifer genus	*Abies, Araucaria, Cedrus, Chamaecyparis, Cunninghamia, Cupressus, Fokienia, Juniperus, Larix, Picea, Pinus, Podocarpus, Pseudotsuga, Thuja, Tsuga*, "Conifer"
Soil texture	Loamy; sandy; clayey
Soil fine texture	sandy; fine loamy; coarse loamy; fine clayey; very fine clayey
Clay (%)	Continuous
Silt (%)	Continuous
Soil depth	(l) forest floor; (u) surface soil; (d) deep soil
Previous land use	forest, grassland, cropland (as pairs)
Stand establishment	Natural; plantation, afforested
Age difference	continuous (range: 0-58 to 163 years)
Elevation	continuous (range: 10 – 2700 m a.s.l.)

Table 2-1 *continued*

Koeppen-Geiger climate class	Af; Am; Aw; BSk; Cfa; Cfb; Cfc; Csa; Csb; Cwa; Cwb; Dfa; Dfb; Dfc; Dwa; Dwb; ET
Mean annual temperature	continuous (range: -3.4 - 25.8ºC)
Mean annual precipitation	continuous (range: 29 - 3960 mm)
Parent material	Glacial;igneous; sedimentary; metamorphic; lacustrine; eolian;andic (volcanic ashes and tuffs)
pH difference	Continuous (range: -1.2 1.54)
Stem density difference	Continuous (range: -75 1409)
DBH difference	Continuous (range: -20.62 20.6)
Basal area difference	Continuous (range: -52.5 6.6)
US soil taxonomy	Alfisol ; Oxisol ; Ultisol ; Inceptisol; Spodosol

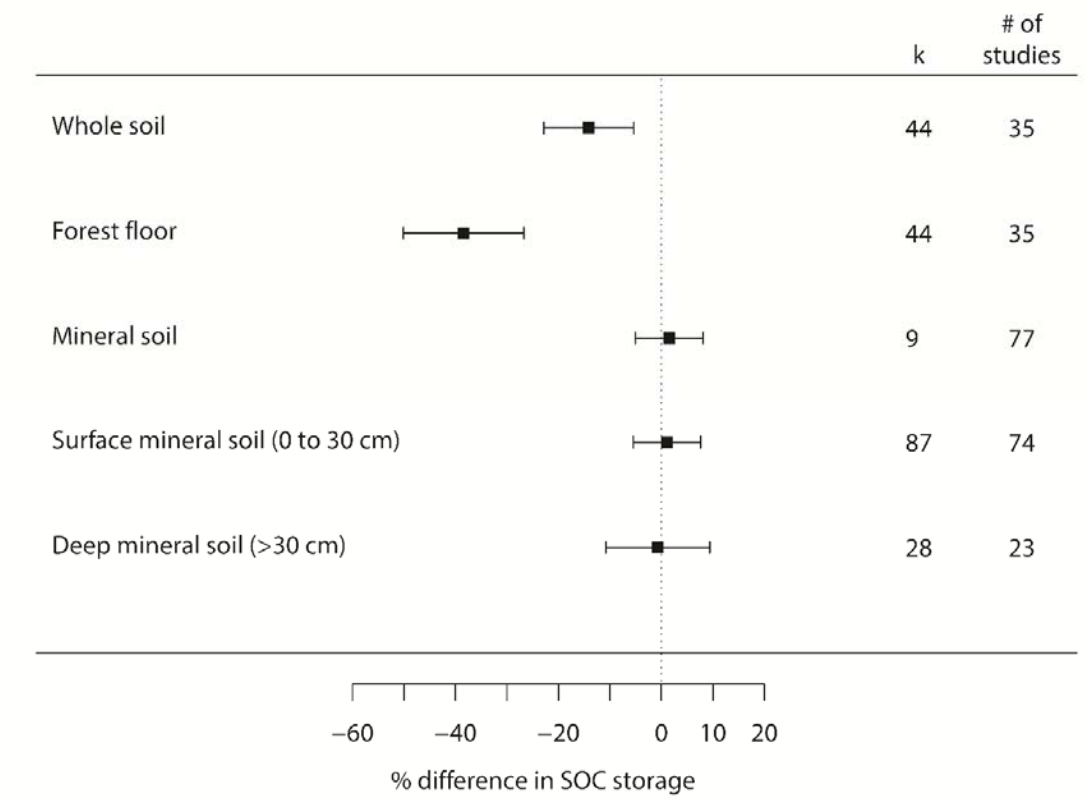
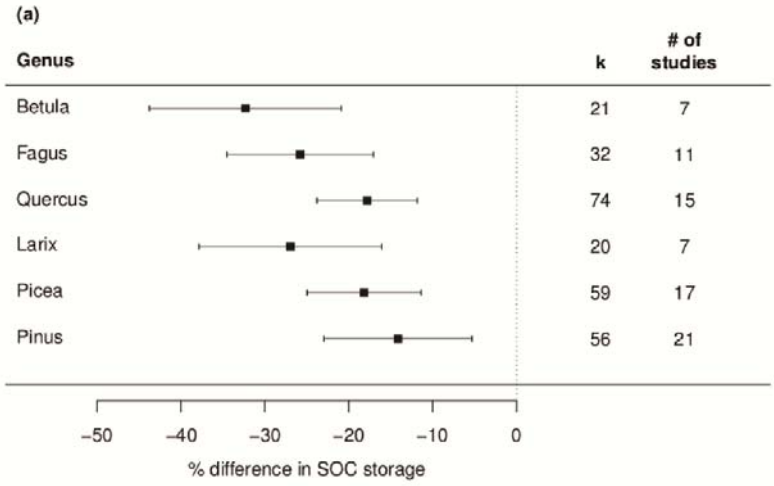
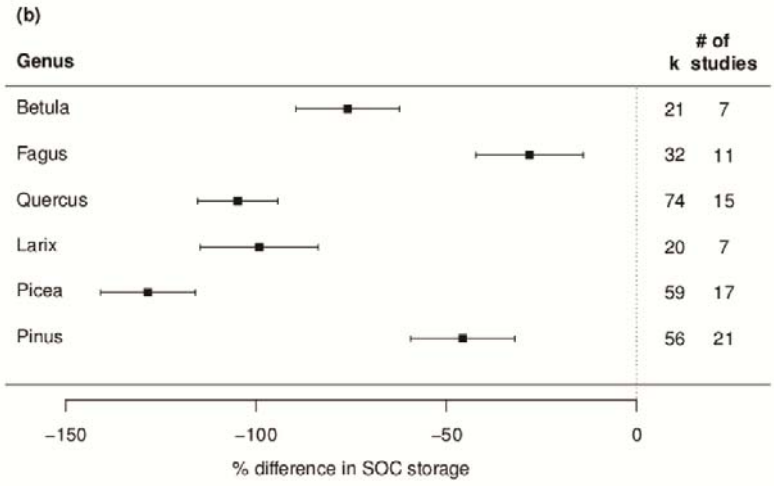


Fig. 2-1. Soil Organic C (SOC) stock differences between conifer and hardwood stands. Negative values indicate more C stored under conifer stands and positive values indicates more C stored under hardwood stands (k = number of response ratios).


(a)
Genus
k
# of studies
Betula 21 7
Fagus 32 11
Quercus 74 15
Larix 20 7
Picea 59 17
Pinus 56 21
−50 −40 −30 −20 −10 0
% difference in SOC storage


(b)
Genus
k
# of studies
Betula 21 7
Fagus 32 11
Quercus 74 15
Larix 20 7
Picea 59 17
Pinus 56 21
−150 −100 −50 0
% difference in SOC storage

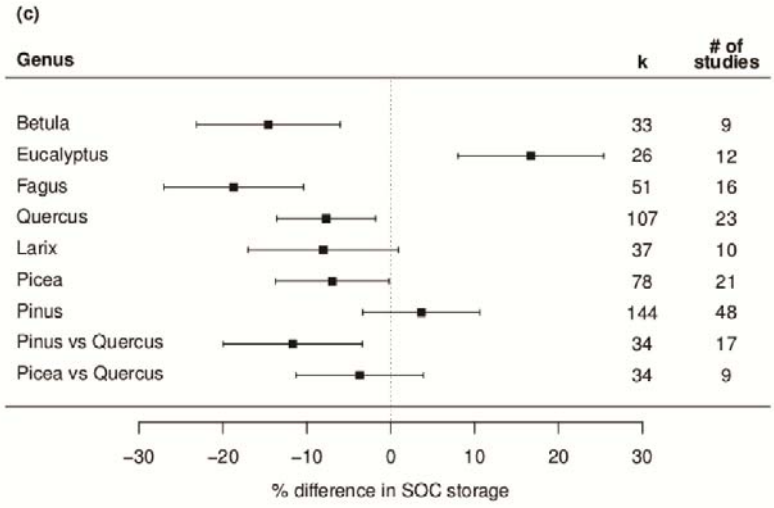

(c)
Genus
k
# of studies
Betula 33 9
Eucalyptus 26 12
Fagus 51 16
Quercus 107 23
Larix 37 10
Picea 78 21
Pinus 144 48
Pinus vs Quercus 34 17
Picea vs Quercus 34 9
−30 −20 −10 0 10 20 30
% difference in SOC storage

Fig. 2-2. Soil organic C (SOC) stock differences in (a) whole soil (FF+ mineral soil), (b) forest floor, and (c) mineral soil under stands of specific tree genera compared to the comparison group. Negative values indicate more SOC under conifer stands; positive values indicate more SOC under hardwood stands. In (c), the comparison between two genera is given for *Pinus* vs. *Quercus* and *Picea* vs. *Quercus* stands as these were the only paired genera with a sufficient number of response ratios (k).

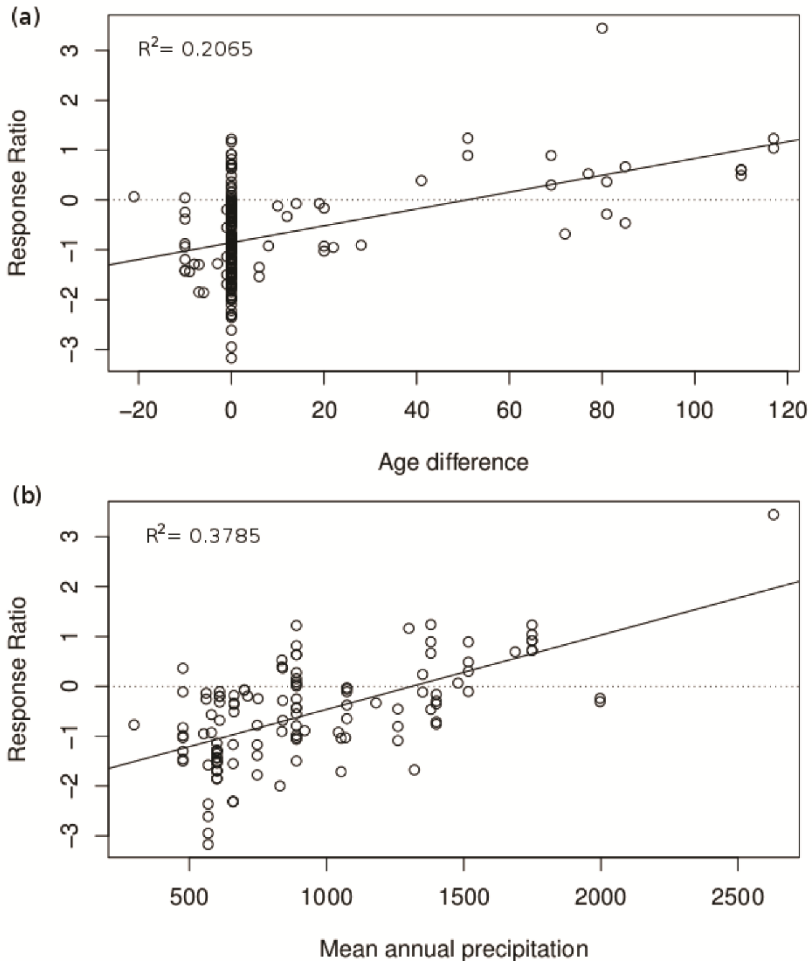


Fig. 2-3. Relationship between hardwood and conifer genera forest floor C response ratios and (a) age difference (calculated as hardwood stand age – conifer stand age; number of response ratios [k] = 192, with about 40 values being non-zero); and (b) mean annual precipitation (k = 123, with most comparisons being located in the temperate and boreal zones).

CHAPTER 3

CAN CARBON FLUXES EXPLAIN DIFFERENCES IN SOIL ORGANIC CARBON STORAGE UNDER ASPEN AND CONIFER FOREST OVERSTORIES?[2]

Abstract

Climate- and management-induced changes in tree species distributions are raising questions regarding tree species-specific effects on soil organic carbon (SOC) storage and stability. Quaking aspen (*Populus tremuloides* Michx.) is the most widespread tree species in North America, but fire exclusion often promotes the succession to conifer dominated forests. Aspen in the Western US have been found to store more SOC in the mineral soil than nearby conifers, but we do not yet fully understand the source of this differential SOC accumulation. We measured total SOC storage (0–50 cm), characterized stable and labile SOC pools, and quantified above- and belowground litter inputs and dissolved organic carbon (DOC) fluxes during snowmelt in plots located in N and S Utah, to elucidate the role of foliage vs. root detritus in SOC storage and stabilization in both ecosystems. While leaf litterfall was twice as high under aspen as under conifers, input of litter-derived DOC with snowmelt water was consistently higher under conifers. Fine root (<2 mm) biomass, estimated root detritus input, and root-derived DOC fluxes were also higher under conifers. A strong positive relationship between root and light fraction C content suggests that root detritus mostly

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fueled the labile fraction of SOC. Overall, neither differences in above- and belowground detritus C inputs nor in detritus-derived DOC fluxes could explain the higher and more stable SOC pools under aspen. We hypothesize that root–microbe–soil interactions in the rhizosphere are more likely to drive these SOC pool differences.

1. Introduction

With an increasing emphasis in forestry practices on ecosystem services other than wood, including climate change mitigation, there is a need to better understand tree species effects on soil organic carbon (SOC) sequestration. As forest soils store as much, if not more, carbon than aboveground biomass [1], information about tree species effects on SOC storage is as crucial as understanding C sequestration in biomass. This becomes especially important given climate change and management-induced changes on the distribution of tree species [2].

Vegetation is the primary source of SOC through above- and belowground litter inputs. In forests, aboveground litterfall consists mainly of leaves or coniferous needles [3,4] while belowground carbon (C) primarily originates from fine root turnover associated with trees [5,6]. Tree species-specific effects on SOC stocks have been documented in temperate and boreal forests (as reviewed by Vesterdal et al. [7]) showing clear species effects on the forest floor, but only limited support for species-specific effects on mineral SOC. In the Intermountain West, quaking aspen (*Populus tremuloides* Michx.), the most widespread hardwood species on the North American continent, grow on soils significantly higher in mineral SOC stocks compared to neighboring conifer stands, despite higher forest floor SOC pools in the latter's systems [8]. This pattern

occurs across different conifer species—subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), Douglas fir (*Pseudotsuga menziesii* Mirb.), and Engelmann spruce (*Picea engelmanii* Parry ex Engelm.). The spatial proximity of aspen and conifer stands further suggests that this difference is mainly due to the effect of vegetation rather than climate or soil properties. However, mechanisms behind this vegetation impact are not yet fully understood. In light of aspen decline observed in many areas of the western US [9–11], often accompanied by conifer encroachment, elucidating the mechanisms and pathways of SOC storage and stabilization is crucial for future carbon balance predictions and modeling efforts.

To understand how the shift in vegetation from aspen to conifer stands will affect SOC stocks, we first must identify and quantify the C input and output processes that control these SOC stock differences in aspen and conifer stands. The objective of this study is, therefore, to quantify and compare the role of foliage and root detritus in SOC storage and stabilization under aspen and conifer forest soils typical of the Intermountain West, USA. We specifically aim to assess (i) whether SOC storage and stability patterns under both overstories are consistent across a wider geographical range; (ii) how SOC properties and stocks differ with depth; and (iii) what the relative role of foliage and root detritus input is in terms of SOC stabilization under both overstories.

To address these questions, we determined belowground SOC distribution and fluxes under aspen and conifer stands at multiple sites in northern and southern Utah. As previous studies had shown aspen–conifer SOC differences at three locations in northern Utah [8,12], we added four sites at Cedar Mountain (CM) in southern Utah to test

whether these initial patterns were consistent across a wider geographical range. We assessed the quantity and quality of SOC and measured fine root mass at all sites sampled. For logistical reasons, we were able to measure major C fluxes only in northern Utah, which constituted our intensively studied core study site, with CM as complementary sites.

2. Materials and Methods

2.1. Site Description

The sampling for this study was conducted at the T.W. Daniels Experimental Forest (TWDEF) located approximately 30 km northeast of Logan in northern Utah, and at CM in SW-Utah (Figure 3-1, Table 3-1).

TWDEF is a Utah State University research forest located on U.S. Forest Service land at 2600 m elevation. Climate data from the past eight years at the Daniel SNOTEL site [13] indicate an average low temperature around −7.1° C in December, and an average high temperature of 15.8° C in July. Mean annual precipitation is 1031 mm with about 70% accumulating as snow. Snowmelt typically occurs from mid-April or early May to mid-or late-June. Monthly rainfall is low between May and October, with lowest monthly precipitation (<50 mm) typically occurring in July. Forested communities include aspen and conifer stands, predominantly subalpine fir and Engelmann spruce stands. These secondary forests have been dated to be around 100 to 200 years old [14]. The aspen and conifer stands are in close proximity to each other (Figure 3-1), and characterized by similar elevation, aspect, climate, geomorphology, and geology. The

soils in the study area are carbonate-free and generally well drained, formed in eolian deposits overlying residuum and colluvium from the Wasatch formation (tertiary: middle and lower Eocene) dominated by roughly stratified, poorly sorted conglomerate a few hundred meters thick [15]. Soils have been classified as Mollisols under aspen stands and as Alfisols under conifer stands [16]. Summer grazing by cattle and sheep has occurred since the late 1800s [17], but was greatly reduced coincident with fire suppression since 1910 [14]. The research sites are located in a fenced area to exclude cattle. The area was fenced off in 2005 to protect the equipment from livestock damage. The site is well instrumented and studied, and our study capitalized on additional data on snow cover, water dynamics, soil respiration, soil temperature and moisture from prior and ongoing studies at the site.

Cedar Mountain is located southeast of Cedar City on a high-elevation plateau (1800–3200 m) that falls within the greater Colorado Plateau region. It encompasses approximately 275 km^2 of the Kolob Terrace formation of the Markagunt Plateau. Precipitation averages 823 mm annually, and monthly temperature means range from −3.8 °C in December to 15.3 °C in July [18]. Snowfall delivered primarily by Pacific-origin westerlies comprises most of the precipitation, occurring during the months of October through April. Additionally, the study area receives monsoonal rainfall during the summer months (mid-July through September) [19]. Soil types vary generally from Mollisols to Alfisols [20]. Major forest vegetation types in the study site consist of a mosaic of aspen, aspen–conifer mixtures, and conifer forests. The CM conifer plots in this study were dominated by Douglas fir, white fir (*Abies concolor* (Gord.) Lind. ex

Hild.), and subalpine fir. Higher elevation sites across the Markagunt were historically dominated by Engelmann spruce [21], but now include large areas of aspen-dominated forest. The study sites ranged from 2680 to 2986 m in elevation. Past research suggests that Cedar Mountain has been subjected to long-term grazing, primarily from domestic sheep, which has altered herbaceous understory communities [22]. The sampling plots (aspen and conifer pairs) at CM were a subset of plots sampled in a previous study [12]. It was not possible to install instruments or measure SOC fluxes at CM due to access limitations and land-use issues (e.g., unplowed roads and actively grazed private property).

2.2. Field Sampling

Soil and vegetation samples were collected in six adjacent aspen- and conifer-dominated stands at TWDEF and four plot pairs (eight plots in total) at CM in late summer and early fall of 2013 and 2014. In 10-meter circular plots, status (dead or alive) and diameter at breast height (DBH) (i.e., stem diameter at 1.30 m in height) of all trees >4 cm diameter were recorded, from which we calculated live basal area (LBA) by species ($m^2 \cdot ha^{-1}$). Stands were designated as either conifer- or aspen-based on a threshold of >75% LBA of the overstory. In addition, we calculated live stem density ($n \cdot ha^{-1}$). At TWDEF, understory was cut in one subplot (1 × 1 m) per plot, dried at 50 °C, weighed, ground, and analyzed for total C with a Skalar PrimacsSLC Analyzer (Skalar, Inc., Breda, The Netherlands) to estimate understory aboveground C input.

Soils were sampled within the same 10-meter circular plots by excavating three pits per plot to a depth of 50 cm and removing subsamples at 10 cm increments. Soils

were put in plastic bags and stored in coolers until transported to the laboratory where they were stored at 5 °C until further analysis. In addition, three soil cores per plot were taken using a split corer from 0–15, 15–30, and 30–45 cm in depths, and the middle 5 cm part of the core was excised to calculate bulk density (BD). Forest floor C content in the aspen and conifer plots was determined by excavating three O horizon samples per plot within 15 × 15 cm-frames. The samples were stored in plastic bags during transport, dried at 50 °C in the laboratory, ground, and analyzed for total C as described above.

At all sampling sites we collected six root cores in each plot up to 50 or 60 cm depth in late summer and early fall of 2013 and 2014. At CM and one TWDEF plot, cores were taken with a 5 cm diameter split corer in 15 cm increments. At the other TWDEF plots, 15 root cores were taken with a hydraulic soil corer (Giddings Machine Company, Windsor, CO, USA) up to 50 cm depth. In addition, root–soil cores were collected when 30 rhizotron tubes were installed during summer 2013 and 2014. The hydraulic soil cores were split into 10 cm increments in the lab; the other samples were processed by depth increments collected and adjusted to 10 cm increments for further analysis.

2.3. Laboratory Analyses

Soil samples were sieved (2-mm mesh) and divided in two. One part of the sample was air-dried and the other one stored at 5 °C. Soil BD samples were dried at 105 °C, sieved (2 mm), and the coarse and fine fractions weighed. For three 35–40 cm BD samples that were missing, BD values were estimated using a correction factor based on

values of the other plots (BD at 20-25 cm multiplied by 1.16 for aspen and 1.07 for conifer plots).

Air-dried soils were used to extract three SOC pool fractions with different turnover times using a simplified size fractionation method described by Roman Dobarco and Van Miegroet [12]. In brief, 30 g of air-dried soil was shaken with glass beads for 18 h to break up aggregates. The mineral-associated organic matter in the clay and silt fraction (MoM) was separated by wet sieving through a 53-μm sieve, with the >53 μm fraction further divided into a light fraction (LF) and mineral-associated SOC in the >53 μm sand fraction (MA). The LF was separated using electrostatic attraction, following a modification of the method by Kaiser et al. [23]. All fractions and bulk soil were ground to <250 μm and analyzed for total organic carbon (TOC) and inorganic C (IC) with Skalar PrimacsSLC Analyzer (Skalar, Inc., Breda, The Netherlands). SOC pool sizes in bulk soil and fractions were calculated by multiplying C concentrations with fine soil mass, which, in turn, was calculated from bulk density ($g \cdot cm^{-3}$) and percentage of coarse (>2 mm) content.

In order to determine relative stability, we used two indices of bioavailability: (1) hot water extractable organic carbon (HWEOC) [24,25], and (2) cumulative CO2 evolution per gram SOC during a 10-month soil incubation as a proxy for decomposability. HWEOC was determined by mixing field-moist soils with ultrapure water in 50-mL centrifuge tubes (1:10 soil–water (w/w)), and heating the slurry in a hot water bath at 85 °C for one hour. The solution was filtered through Sterlitech GF/F filters (pore size 0.4 μm) and the supernatant analyzed for dissolved organic carbon (DOC) with

a Phoenix 8000 Carbon Analyzer (Tekmar-Dohrmann, Mason, OH, USA). To measure decomposability field-moist soils from the top 20 cm of TWDEF aspen and conifer stands, adjusted to a gravimetric moisture content of 30%, were incubated at 25 °C for 10 months. Three soil lab replicates of one composite sample per overstory type (composited from three plots) were added to 1 L glass jars with a lid designed to connect to a gas analyzer through a system of tubes and valves. CO2 evolution was measured at weekly intervals with an automated soil gas flux system (LI-8100, LI-COR, Inc., Lincoln, NE, USA) that was connected to incubation jars during the time of measurement. After the measurement, the jars were opened to bring the gas concentrations back to ambient levels.

The root–soil cores were washed using a hydropneumatic elutriator system [26] to remove soil. The material was dried at 50 °C, weighed, and recognizable roots of <2 mm were separated from the organic material. This size was chosen based on suggestions in literature that roots of less than 2-mm diameter are contributing the most to root C turnover in soils [27]. The weight of the fine roots was recorded, and a subset was ground for TOC analysis as described above, and for N analysis with a Europa 20/20 SL isotope ratio mass spectrometer (Sercon, Cheshire, UK).

Soil texture was determined by particle size analysis with the hydrometer method at Utah State University's Analytical Lab. pH was measured by mixing 10 mL soil with 10 mL ultrapure water using the ATI Orion 950 Ross FASTQC Titrator. Soils from the top and bottom 10 cm sampled from each pit were extracted with sodium pyrophosphate (NaPP), acid ammonium oxalate (AAO), and citrate-dithionite (CD) to estimate

organically-bound, amorphous and crystalline Fe and Al. The extracts were analyzed with an Atomic Absorption Spectrometer (Varian AA240 flame atomization). Organically bound Fe and Al were calculated by subtracting NaPP values from AAO values.

2.4. Carbon Fluxes

2.4.1. Aboveground C Input

Five litter traps with an area of 794 cm2 were installed one meter above the soil surface in each plot at TWDEF for fine litter-fall sampling in the snow-free season (June till October of 2014 and 2015). At the end of October (2014 and 2015), ground litter traps were installed to capture litterfall during snow cover presence. The litter from these litter traps was collected after the snow had melted in early June. All litter was dried at 50 °C, the dry weight recorded, and ground to 250-µm diameter before analysis of TOC and total nitrogen. Branches were excluded for C flux calculations.

2.4.2. Soil Solution Fluxes

Silicon carbide suction cup (SIC 20, Decagon Devices, Inc, Pullman, WA, USA) soil pore water samplers (SPW) were installed at 5 and 45 cm depth in three aspen and three conifer plots at TWDEF. Water was sampled by applying negative pressure of 50 kPa to 1 L glass sampling bottles wrapped in duct tape and stored in Styrofoam coolers to reduce light penetration. In 2014, samples were collected twice a week during the snowmelt period (April–June) until no water could be collected (~July 8) to capture seasonal variability. As no fluctuations of DOC concentrations were detected in 2014,

sampling frequency was reduced to once a week during the snowmelt period of 2015, and early weeks of snowmelt in 2016. On sampling days, water was transferred to amber vials, transported to the laboratory where samples were filtered through a 1-μm glassfiber filter, and DOC was measured with Phoenix 8000 Carbon Analyzer (Tekmar-Dohrmann, Mason, OH, USA). Absorbance at 254 nm was measured with a Genesys 10 UV-Vis spectrophotometer (Thermo Scientific, Madison, WI, USA) to calculate Specific Ultraviolet Absorbance (SUVA = abs at 254 $nm \cdot cm^{-1} \times 100$/ DOC $mg \cdot L^{-1}$; units = $L \cdot mg\text{-}1\ C \cdot m^{-1}$) as a proxy for DOC aromaticity [28], hydrophobicity [29], and microbial stability [30].

As the area of collection for SPW samplers is not known, we calculated DOC fluxes in the soil based on snow water equivalent (SWE) data recorded annually in an open meadow at the Daniel SNOTEL site (NRCS—TWDEF, accessed Oct, 2016). In 2016, we independently collected SWE data from aspen and conifer plots at TWDEF by digging two pits per plot, and collecting two snow cores per pit. This enabled us to calculate SWE under aspen and conifers in 2014 and 2015 from the open meadow SNOTEL site data for those years. We used the three-year-average SWE values—595 mm for aspen and 446 mm for conifers—for calculating the DOC input via throughfall, by multiplying the DOC concentration measured in snow with the water volume.

In the soil DOC flux calculations, water flux at 5 cm soil depth was assumed to be equal to SWE. The water volume at 45 cm depth was adjusted based on the ratio between average water volumes collected at 5 and 45 cm depths during the three sampling years—0.75 for aspen and 0.57 for conifers. Average annual DOC flux was calculated using

weighted averages of DOC concentrations and SWE-based water volumes. Dissolved total nitrogen, NO3, and NH4 were measured in samples from three sampling times in 2015 and from two sampling times in 2016. Samples were analyzed with AQ2 Discrete Analyzer (Seal Analytical, Mequon, WI, USA) at USU's Water Research Laboratory.

2.4.3. Belowground C Input

Root detritus C input was estimated indirectly from soil respiration and aboveground litterfall as described by Raich and Nadelhoffer [31]. We used previously published soil summer respiration data at TWDEF [32] to calculate annual soil respiration. Non-summer respiration rates were estimated based on summer rates and average soil temperatures using the equation by Zak et al. [33]:

$$k_1 = k_2\, e^{(t1-t2)/10\, \ln Q10} \quad (1)$$

where k_1 is the calculated mean winter respiration rate, k_2 the average measured summer respiration rate, t_1 the average winter soil temperature, t_2 the average summer soil temperature, and $Q_{10} = 2$. Soil temperature had been measured at 30-min intervals at the sites in three aspen and three conifer plots, all but one conifer corresponding to our measurement plots. The data were collected with temperature-soil moisture sensors (Acclima TDT, Meridian, ID, USA) as part of an ongoing study at TWDEF (S. Jones, unpublished data). In our calculations, the year was split into three periods; Summer: 1 June–30 September; Winter: 1 November–30 April for aspen, and 1 November–31 May for conifers based on snowpack presence; with a transition in October and May for aspen and October for conifers, based on soil temperatures transitioning between subnivean winter soil temperatures and high summer soil temperatures. For each period, the average

daily respiration rate was multiplied by the number of days, and the annual CO2 emission from the soil (Rs) was calculated as the sum of these seasonal values.

We used annual soil respiration data and aboveground litterfall data to calculate root turnover based on the relationship described by Raich and Nadelhoffer [31], and the assumption that heterotrophic and autotrophic (root) respiration each accounted for 50% of total respiration [34,35]:

$$P_b = R_h - P_a = R_s - R_r - P_a = 0.5 \times (R_s - P_a) \quad (2)$$

where P_b = belowground detritus production, R_h = heterotrophic respiration, P_a = aboveground detritus production, R_s = soil respiration, and R_r = root respiration.

In addition, we installed 30 minirhizotron tubes at TWDEF (15 in aspen, and 15 in conifer stands up to 40 cm depth) in summer 2013 and 2014. The tubes were installed at a 45° angle up to 40 cm vertical depth. Images were collected every 1.3 cm down the minirhizotron tube once a month from June till October, 2015, with a minirhizotron camera (Bartz Technology Corporation, Carpinteria, CA, USA). The length, diameter, and status (dead or alive based on appearance) of each root was recorded using the software Rootfly (Version 2.0.2, Clemson University). In images collected in June, roots were marked dead if the color of a root was black. Later roots were marked dead if the color changed with time to dark brown or black, or the root disappeared. The length of fine roots was summed for each 10-cm soil depth for each minirhizotron, and the average fine root length was calculated for each plot. We calculated root length on an area basis by dividing observed root lengths by the product of minirhizotron frame area and depth-of-field of 2 mm, which then was multiplied by the depth of the soil profile sampled [36].

Minirhizotron data were converted from length (m·m−2) to total root dry matter ($g \cdot m^{-2}$) using conversion factors: 51.0 $m \cdot g^{-1}$ for aspen, and 15.0 $m \cdot g^{-1}$ for conifers [37], and root detritus input was calculated from the ratio of dead root mass at the end of the growing season to total root mass.

As part of a separate laboratory experiment, we ground aspen and conifer roots, saturated the biomass with ultrapure water, exposed them to freeze-thaw cycles and leached them to obtain source-specific dissolved organic carbon (DOC) (unpublished data). We used the respective DOC concentrations and root masses to estimate root-derived DOC input in the field.

2.5. Statistical Analysis

Data analysis was conducted using the software R [39]. Statistical comparisons for total SOC stocks (O-horizon plus mineral soil), mineral SOC stocks, C stocks in SOC fractions, average HWEOC values, and root C pools were done for the whole soil profile sampled (sum of all depths). Differences between both overstory types for these dependent variables were compared using a paired t-test. Sites were the unit of replication ($n = 5$) with four sites at CM, and the average of three plots constituting one site at TWDEF. This was done due to the close proximity of all plots at TWDEF, and the concern about pseudoreplication (Figure 3-1). No data transformations were performed. Due to the small sample size, we computed a post-hoc power analysis using the package pwr [40] ($\alpha = 0.05$, $\pi = 0.8$) to evaluate whether a p-value > $\alpha = 0.05$ was due to inefficient sample size. DOC fluxes were analyzed with repeated measures ANOVA with overstory type and depth used as the independent variables, and variation by year as the

error term. Relationships among root and SOC variables were assessed using linear mixed effects (LME) models with the package lme4 [41], with depth being considered as the random variable. To estimate model fit, we calculated marginal and conditional R2 [42] with the package piecewiseSEM [43]. Average values are reported as mean ± standard deviation, unless stated otherwise. Outcomes of statistical analyses are reported by stating the p-value, and t-statistic from the paired t-test, Cohen's d effects size (ES), 95% confidence interval (CI), and suggested sample size (SN) from the power analysis (if $p > \alpha$). Cohen's d was evaluated based on the categories defined by Cohen [44] with 0.2 being small, 0.5 medium, and 0.8 being large. In other words, an effect size of 0.8 can also be interpreted as 47% non-overlap between two distributions. All figures were plotted with the package ggplot2 [45]. All maps were created with ArcGIS 10.2 (ESRI, Redlands, CA, USA).

3. Results

3.1. SOC Distribution under Aspen and Conifer Forest Stands

Total SOC stocks (O-horizon + mineral soil up to 50 cm) under aspen were slightly higher than SOC stocks under conifers: 93.7 ± 16.11 $Mg \cdot ha^{-1}$ under aspen vs. 82.9 ± 27.9 $Mg \cdot ha^{-1}$ under conifers ($p = 0.51$, $t = 0.72$; ES = 0.32, CI = (1.15, 1.79), SN > 78). Mineral SOC stocks were consistently higher under aspen (Figure 3-2) at each site, and were on average 91.55 ± 16.3 $Mg \cdot ha^{-1}$ under aspen vs. 61.25 ± 22.4 $Mg \cdot ha^{-1}$ under conifer stands ($p = 0.08$, $t = 2.31$; ES = 1.03, CI = (0.52, 2.58), SN > 9). (The difference

between plots sampled at CM and TWDEF ranged from 7.4 to 81. 5 $Mg \cdot ha^{-1}$, and was on average 30.3 $Mg \cdot ha^{-1}$.

At all sites, SOC consisted mainly of the more stable MoM fraction (68%–87%) (Figure 3-3). At TWDEF, aspen had a slightly higher SOC proportion in the MoM fraction (72% of mineral SOC) compared to conifers (68%), while conifers had more C in the LF fraction (23%) compared to aspen (11%). At CM, vegetation differences in SOC distribution among the different fractions were less pronounced with the LF fraction, constituting 16% of SOC pools under aspen and 19% under conifers. At TWDEF, MoM stocks (0–50 cm) were 50.9 ± 12.9 Mg $C \cdot ha^{-1}$ under aspen vs. 30.6 ± 5.3 Mg $C \cdot ha^{-1}$ under conifers; with corresponding values at CM of 78.8 ± 16.2 Mg $C \cdot ha^{-1}$ under aspen and 56.6 ± 19.7 Mg $C \cdot ha^{-1}$ under conifers ($p = 0.15$, $t = 1.8$; ES = 0.78, CI = (0.73, 2.29), SN > 15). At TWDEF, slightly higher LF C pools were found under conifer stands (11.0 ± 1.7 Mg $C \cdot ha^{-1}$) than aspen (9.3 ± 1.7 Mg $C \cdot ha^{-1}$), but at CM the opposite pattern was observed with aspen having higher LF C pools (17.2 ± 3.2 Mg $C \cdot ha^{-1}$) than conifers (14.7 ± 7.9 Mg $C \cdot ha^{-1}$), mostly in the topsoil ($p = 0.53$, $t = 0.69$, SE = 0.31, CI = (1.16, 1.78), SN > 83). The MA fraction constituted less than 10% of SOC stocks under both overstories, and ranged from 2 to 5 Mg $C \cdot ha^{-1}$ at the northern and southern sites ($p = 1$, $t = 0.005$, SE = 0.002, CI = (1.46, 1.46), SN > 10,000).

During the 10-month long lab incubation, aspen soils showed lower CO_2 evolution (146.2 $mg \cdot g^{-1}$ soil C or 8.5% of total SOC), than conifer soils (231.4 $mg \cdot g^{-1}$ soil C or 18% of total SOC), indicating lower decomposability of aspen SOC. Results from hot water extractions showed a similar pattern of lability with conifer soils

containing more water soluble (labile) SOC (21.6 ± 8.4 $mg \cdot g^{-1}$ soil C at TWDEF and 13.6 ± 4.6 $mg \cdot g^{-1}$ soil C at CM) than aspen soils (16.1 ± 8.2 $mg \cdot g^{-1}$ soil C at TWDEF and 11.2 ± 2.3 $mg \cdot g^{-1}$ soil C at CM) ($p = 0.03$, $t = -3.29$, $SE = 1.47$, $CI = (0.17, 3.11)$). The water-extractable C, however, constituted only about 1.6% of total SOC in aspen soils and 2.1% of total SOC in conifer soil at TWDEF, and respectively 1.2% and 1.4% at CM. Deeper soils from TWDEF conifer plots, and two conifer plots at CM contained higher labile C amounts in the 40–50 cm depth than in the topsoil. This was not observed for aspen soils where there was no difference in the depth distribution of HWEOC.

Based on the estimated age of forest stands at TWDEF, around 100 years [14], we calculated a net average annual SOC accumulation difference of 225 kg $C \cdot ha^{-1} \cdot year^{-1}$ between aspen and conifer mineral soil. The age of the stands at CM could be assumed to be around 100–150 years based on measurements by Mueggler [46]. Assuming an average stand age of 100 years, the estimated difference in net average annual SOC accumulation between aspen and conifers at CM ranged from 74 to 190 kg $C \cdot ha^{-1} \cdot year^{-1}$. At one site (CM20), the difference was even bigger, 815 kg $C \cdot ha^{-1} \cdot year^{-1}$, possibly due to differences in soil mineralogy, as at CM20 the soil at the aspen stand contained twice as much extractable Fe as the soil at the conifer stand (1400–1700 vs. 400–700 mg $Fe \cdot g^{-1}$ soil). Assuming a stand age of 150 years, the range of net average annual SOC accumulation difference between overstory types was 50–126 kg $C \cdot ha^{-1} \cdot year^{-1}$ for three of the four sampled sites (excluding CM20).

3.2. Relative Role of Foliage Inputs to SOC Storage

Aboveground litterfall in TWDEF aspen stands was 851 ± 207 kg C·ha^{-1} in 2014–2015 and 596 ± 143 kg C·ha^{-1} in 2015–2016, compared to respectively 520 ± 102 kg C·ha^{-1} and 430 ± 62 kg C·ha^{-1} under conifers. Aboveground C input via litterfall was on average 250 kg C·ha^{-1} higher under aspen, and this difference increased to 429 kg C·ha^{-1} when understory aboveground C was added (197 ± 18 kg C·ha^{-1} under aspen vs. 17 ± 7 kg C·ha^{-1} under conifers). The majority of aspen litterfall decomposed within 2 to 3 years based on the O-horizon stock values by Woldeselassie et al. [8] (1.7 ± 0.38 Mg C·ha^{-1}) and this study (2.7 ± 0.87 Mg C·ha^{-1}), respectively. The higher C content in the conifer O-horizon (22.8 Mg C·ha^{-1}) as well as the average aboveground litterfall of 492 kg·ha^{-1} (including understory) indicated a mean residence time (MRT) of 46 years for the conifer O-horizon C pool.

As litterfall needs to be incorporated into soil to become part of mineral SOC, the next step is to assess how, and to what extent, the differences in litter input and turnover are expressed in DOC fluxes into the soil. The majority of the annual precipitation at TWDEF is in the form of snow, therefore, the majority of the soil water flow occurs during snowmelt. The DOC in the snowpack constituted 2%–10% of the DOC fluxes during snowmelt at 5 cm depth under aspen (3.3 kg C·ha^{-1}), and 3%–7% under conifers (7.6 kg C·ha^{-1}). Soil solution DOC concentrations at 5 cm depth under aspen (average range 7.3–23.8 mg·L^{-1} from 2014–2016) were mostly lower than DOC concentrations under conifers (average range 28.4–45.5 mg·L^{-1}), and generally decreased at 45 cm depth for both overstories (average range 8.1–10.1 mg·L^{-1} for aspen, and 25–37.7 mg·L^{-1} for

conifers). Litter-derived DOC fluxes transported into 5 cm soil depth with snowmelt water ranged from 50 to 145 kg·ha^{-1} under aspen, representing only 7% to 20% of annual litterfall C. The litter-derived DOC fluxes under conifers ranged from 130 to 177 kg C·ha^{-1}, constituting 27%–37% of conifer litterfall C (Table 3-2).

As water percolated through the soil during snowmelt, DOC flux declined (Table 3-2), and on average 44.7 kg C·ha^{-1} of DOC was retained (or decomposed) between 5 and 45 cm in aspen soils, compared to 77.1 kg C·ha^{-1} in conifer soils, about 42% higher. The variability in net DOC retention was much higher under aspen (7.1 to 98.8 kg C·ha^{-1}), than under conifers (72.9 to 95.5 kg C·ha^{-1}).

Despite the higher aboveground litterfall, the smaller DOC input fluxes and lower net DOC retention in aspen soils make it unlikely that aboveground litter is the main factor causing the differences in SOC pools between aspen and conifer stands. This, in turn, suggests that differences in root detritus production might be a more important factor.

3.3. Relative Role of Root Inputs to SOC Storage

Fine root (<2-mm diameter) C stocks were higher in conifer soils (4060 ± 960 kg C·ha^{-1} at TWDEF and 5370 ± 610 kg C·ha^{-1} at CM) compared to aspen soils (1940 ± 420 kg C·ha^{-1} at TWDEF and 3520 ± 540 kg C·ha^{-1} at CM; $p = 0.005$, $t = -5.65$, SE = 2.52, CI = (0.57, 4.47)). Root biomass was the highest at the top 10 cm under both overstories at all sites, and decreased with soil depth (Figure 3-4). We found a strong relationship between root mass and LF ($p < 0.001$), with root distribution explaining 26% (marginal R^2, conditional $R^2 = 0.42$) of the variability of the light fraction distribution in

10–50 cm depths. The top 10 cm were excluded from the analysis as this depth experiences direct litterfall inputs that add to the LF fraction of SOC, and, therefore, does not have a strong relationship with root mass.

Based on the average ecosystem-specific annual soil respiration rates (3025 kg $C \cdot ha^{-1} \cdot year^{-1}$ under aspen and 2379 kg $C \cdot ha^{-1} \cdot year^{-1}$ under conifers) and aboveground litterfall values (723.5 ± 175 kg $C \cdot ha^{-1} \cdot year^{-1}$ under aspen and 475 ± 82 kg $C \cdot ha^{-1} \cdot year^{-1}$ under conifers), we calculated annual belowground detritus (root) input as 572 kg $C \cdot ha^{-1} \cdot year^{-1}$ for aspen and 744 kg $C \cdot ha^{-1} \cdot year^{-1}$ for conifers at TWDEF. Compared with the fine root mass data from root cores, this represented 29% of total fine root biomass for aspen and 18% for conifers, suggesting a three- to four-year MRT of aspen fine roots, and a five- to six-year MRT of conifer fine roots.

Minirhizotron image analysis revealed seven times more roots under aspen than conifers (696 under aspen, and 109 under conifers from 15 minirhizotron tubes), and total calculated root mass under aspen was 1592 kg $C \cdot ha^{-1} \cdot year^{-1}$ for aspen and 494 kg $C \cdot ha^{-1} \cdot year^{-1}$ for conifers. At the end of the growing season, 32% of live aspen roots had died vs. 36% under conifers, which corresponded to about 573 kg $C \cdot ha^{-1} \cdot year^{-1}$ in aspen root detritus input, while there was only 158 kg $C \cdot ha^{-1} \cdot year^{-1}$ in conifer root detritus. When root mortality rates from minirhizotron observations were applied to root mass values from root cores, annual root detritus input for aspen was 620 kg $C \cdot ha^{-1} \cdot year^{-1}$ vs. 1462 kg $C \cdot ha^{-1} \cdot year^{-1}$ for conifers at TWDEF, and 1120 kg $C \cdot ha^{-1} \cdot year^{-1}$ for aspen vs. 1933 kg $C \cdot ha^{-1} \cdot year^{-1}$ for conifers at CM.

Our previous estimates of net DOC retention between 5 and 45 cm (45 kg C·ha^{-1} of DOC in aspen and 77 kg C·ha^{-1} in conifer soils) did not consider DOC leaching from roots. Based on the laboratory leaching experiment (unpublished data), we calculated the potential amount of root DOC contributions by combining the DOC concentrations from leachates with the root mass from root cores. We estimated that aspen root detritus could have contributed as much as 39 kg C·ha^{-1}, and conifer roots as much as 77 kg C·ha^{-1} to the DOC flux in the soil. Adding this root-derived DOC flux would increase net DOC retention/decomposition under aspen to 84 kg C·ha^{-1} and 154 kg C·ha^{-1} in the conifer soil.

DOC concentration and chemistry (e.g., degree of hydrophobicity, C/N ratio) are important factors affecting C sorption to mineral surfaces [47,48]. The snowpack DOC in our study had a low aromaticity (SUVA was on average 2.2 L·mg C^{-1}·m^{-1} under aspen, and 1.5 L·mg C^{-1}·m^{-1} under conifers). The SUVA values generally increased as water infiltrated from the forest floor into the mineral soil (SUVA = 3.1 ± 0.89 L·mg C^{-1}·m^{-1} under aspen, and 3.2 ± 0.19 L·mg C^{-1}·m^{-1} under conifers at 5 cm depth), and then decreased with depth (to 2.8 L·mg C^{-1}·m^{-1} at 45 cm under aspen, and to 2.6 L·mg C^{-1}·m^{-1} under conifers), as did C/N ratios (from a range of 22–48 at 5 cm to 18–37 at 45 cm under aspen, and from 44–61 at 5 cm to 22–55 at 45 cm under conifers). Overall, and based on the measured characteristics, DOC quality did not differ much between aspen and conifer. An additional factor affecting sorption–desorption processes in soil is pH [47]. The pH of the solutions sampled was similar under both overstories, and ranged

from 6.4 to 6.9 for conifers and 6.9 to 7.1 for aspen, and was similar during all three sampling years.

At TWDEF, conifer stands were characterized by larger DOC input fluxes from both aboveground and belowground sources, larger DOC leaching losses below 45 cm and overall greater DOC retention/degradation compared to aspen. This pattern (greater retention under conifers), however, is opposite to the actual SOC and MoM accumulation pattern observed, and is thus unable to explain higher SOC storage in aspen soils.

4. Discussion

4.1. SOC Pools, and Biotic and Abiotic Controls on SOC and MoM

By expanding the geographical range of aspen and conifer comparisons in Utah through the addition of the CM sites, we saw big differences between SOC pools at TWDEF and CM. The high values observed in CM aspen (from 81 to 112 Mg $C \cdot ha^{-1}$) are not unique as Woldeselassie et al. [8] reported similar values at Bear and Frost canyons in northern Utah. Woldeselassie [49] further found that even under the same aspen cover, SOC pools could differ highly at fine spatial scales, mostly driven by abiotic factors such as microclimate and soil moisture.

The SOC stocks found in the mineral soils at TWDEF are comparable to values found in other areas in North America [50–53]. However, aspen do not always have higher SOC stocks than conifers [54]. Laganiére et al. [53] found higher mineral SOC pools under aspen in Ontario, but not in Quebec. In none of these reported sites were the differences statistically significant, but the authors argued that this might be due to a

small sample size. Two studies in Minnesota found smaller or similar SOC stocks under aspen compared to adjacent conifers [50,51]. In comparing SOC stocks for the top 50 cm under black spruce, aspen, and jack pine at two sites in Canada, Gower et al. [52] reported black spruce SOC > aspen SOC > jack pine SOC. The results from this Canadian study must be considered with some caution as the soils in that comparison differed in water drainage.

The majority of SOC at TWDEF and CM was associated with the silt and clay fraction, i.e., consisted of MoM, with conifer soils having a slightly higher proportion of C in the LF fraction, and a slightly lower proportion of C in the silt and clay fraction compared to aspen soils. A vegetation difference in SOC distribution, favoring more stable MoM under aspen, has been shown in other studies in Utah [8,12], and Canada [55]. A higher association of C with silt and clay under aspen could partially help to explain why SOC in aspen soils was less decomposable during the 10-month incubation and less soluble (as indicated by lower hot water extractable DOC), both suggesting higher stability. Higher soil decomposability under conifers has been reported before by Olsen and Van Miegroet [32], Woldeselassie et al. [8], and Giardina et al. [56]. Also, Laganiére et al. [55] found a higher proportion of SOC distributed as LF in conifer soils, and higher CO_2 evolution from these soils during incubation [53]. Overall, higher stability of aspen SOC seems to be a consistent finding in literature, as reviewed by Laganiére et al. [54]. The strong correlation between root and LF C suggests that the major source of LF in the deeper depths is root detritus.

Modest sample size potentially played a role in limiting statistical power when mineral SOC, and MoM SOC pools were compared under aspen and conifer stands. The calculated effect size for mineral SOC was 1.03, and for MoM SOC pools it was 0.78. According to the criteria defined by Cohen [44], both qualify as high. A post hoc power analysis revealed that on the basis of the mean, the effect size observed for the mineral SOC pool would require an n of approximately 9 to obtain statistical power at the recommended 0.80 level. For MoM SOC, the approximate n was 15. In fact, when we combined previously published SOC stock data for adjacent aspen and conifer forest stands in Utah [8] with our data, which increased the sample size to 11 pairs, we found that aspen SOC in the mineral soil was consistently higher than conifer SOC ($p = 0.0013$, $t = 4.44$; SE = 1.34, CI = (0.36, 2.32)). Therefore, we are confident that the observed values in our study, at least in the mineral SOC pool, were not due to chance.

In the study by Woldeselassie et al. [8], there is no information on the mineralogy of the soils at Bear and Frost canyons or other abiotic factors that could explain the reported high SOC values. In our study, soils at CM and at TWDEF differed in terms of Fe and Al oxide amounts. The highest SOC pools corresponded with the highest C concentrations and extractable Fe oxide contents (Figure 3-5), illustrating the potential role of mineralogy on SOC storage. The CM stands also have higher root biomass, and, therefore, potentially higher root C inputs contributing to belowground SOC storage. However, the observed positive correlation between root C and LF C suggests that root detritus potentially fuels the less stable LF C pool rather than the more stable MoM pool.

4.2. Aboveground C Input

Forest floor is widely known to be more directly affected by tree species, with conifers having overall higher forest floor C stocks than broadleaved trees [57,58]. In our study, forest floor C stock differences were big enough to partially offset the higher SOC stocks in aspen mineral soils, making the total SOC stocks similar between overstories. However, forest floor is more sensitive to disturbances [59,60], and in a fire prone region such as Utah, the O-horizon does not constitute a long-term C pool. The larger mineral SOC pools under aspen are comparatively less susceptible to fire disturbance, and thus are more likely to contribute to long-term belowground C sequestration.

The aspen litterfall measured in this study was similar to what has been reported by Bartos and Debyle [61] in northern Utah—1397 kg·ha^{-1} of leaves, which corresponds to about 630 kg C·ha^{-1}. The results are also similar to what has been found in Canada by Gower et al. [62]—1672 kg organic matter·ha^{-1} (752 kg C) in their northern study site and 2170 kg organic matter·ha^{-1} (977 kg C) in the southern study site. Conifer litterfall reported by Gower et al. [62] was smaller to what we found in our study—860 kg organic matter·ha^{-1} under pine (387 kg C) and 785 kg organic matter·ha^{-1} (353 kg C) under spruce in the southern site, as well as 619 kg organic matter·ha^{-1} under pine (279 kg C) and 684 kg organic matter·ha^{-1} under spruce (309 kg C) in the northern site. The differences in litterfall are probably due to differences in growing conditions between the boreal forests of Canada, and the semi-arid mountain forests of Utah.

The potential pathways for aboveground C incorporation into mineral soil are by leaching of DOC and/or by biological and physical mixing. While soil fauna has not been

specifically analyzed at TWDEF, past soil pedon analyses conducted at TWDEF [8,16] did not find any signs of megafauna activity. Furthermore, if faunal mixing was prominent, we would expect a more even distribution of the LF with soil depth. We also did not observe any earthworm activity in the sites, nor are we aware of a study from the Intermountain West that has documented such activity. Therefore, we assume that soil fauna plays a minor role in plant detritus incorporation into deeper mineral soil at the studied sites, and most of the aboveground C is incorporated into mineral soil with snowmelt water.

Woldeselassie et al. [8] hypothesized that higher litterfall, and faster turnover of aspen foliage, coupled with freeze-thaw cycles, and slow decomposition under the snowpack could potentially lead to higher DOC fluxes into the soil profile occurring under aspen. Our results did not support this hypothesis. Even though lab experiments indicated that aspen foliage does release ten times more DOC after freezing and thawing than do conifer needles (Boča, Chapter 4), the DOC concentrations and fluxes measured in the field were always smaller under aspen than under conifers. It is possible that some leaching occurs during fall and early winter when daytime temperatures rise above freezing, and small volumes of snowmelt transport high concentration DOC into soil. However, in a two-year study with monthly sampling intervals, Fröberg et al. [63] similarly found consistently higher DOC values under conifers than birch in Sweden without any high concentration peaks under birch. The overall DOC input from litterfall was found to be comparatively small—9% of aspen litterfall, and 30% of conifer litterfall. The contribution of fresh litterfall to mineral SOC has been shown to be

minimal also in an upland oak forest at Oak Ridge National Laboratory using ^{14}C [64]. Despite the higher litterfall values in aspen, the lower DOC input fluxes from the forest floor, and the absence of clear signs of bioturbation make it unlikely that aboveground C is the main source of total and stable SOC in the mineral soil.

4.3. Belowground C Input

Root biomass data for different tree species are known to vary by geographical location due to abiotic growing conditions [36,65], which are the likely drivers of root biomass differences between CM and TWDEF. Our finding that conifers had higher fine root biomass than aspen is partially supported by other studies. For example, Steele et al. [37] found higher fine root biomass under aspen than black spruce at the southern study site, but lower biomass in the northern study site. Hansson et al. [66] found Norway spruce to have three times higher fine root biomass than adjacent pine and birch stands in Sweden. In a review, Vogt et al. [58] found that deciduous forests had lower fine root biomass than conifers and suggested that the capacity of evergreen forests to photosynthesize year round combined with longer foliage retention, may increase their potential to maintain a higher root mass. Our estimated annual root turnover of about 20%–36% (MRT 3–5 years) coincides with estimates by Hansson et al. [66]. Similar to Steele et al. [37] we found no big differences in fine root turnover rates between tree species.

It is interesting that root cores, minirhizotrons, and calculations yielded similar root detritus C input estimates for aspen stands (~600 kg C·ha^{-1}·year^{-1}) at TWDEF, while the various estimates were more variable (200–1500 kg C ha^{-1}·year^{-1}) for conifers.

One reason for the divergent rhizotron-derived estimates is that the tubes under conifers experienced high fungal growth that obscured the detection of roots. In the calculations of root detritus input from soil respiration and aboveground litter input [31], it is uncertain whether the 50:50 partitioning of autotrophic vs. heterotrophic respiration [34,35] is equally valid in both forest types, especially considering that heterotrophic respiration in laboratory incubations was higher for conifer soils. Indeed, differences in C allocation patterns between conifers and hardwoods have been reported in other studies [67]. Also, differences in the type of mycorrhizal associations between conifers and aspen [68] may have resulted in different belowground C allocation patterns [69] that were not captured in our calculations.

The strong positive relationship between root and LF C, and the lack of a significant relationship between MoM and root C, suggests that root detritus most likely fuels the LF fraction of SOC, which is considered less stable. On the other hand, rhizodeposition fuels microbial processes [70]. As studies suggest that microbial-derived compounds dominate MoM [71,72], detritus quality would be expected to influence the processing speed, with higher quality substrates resulting in more SOC being incorporated into MoM [73]. While DOC concentrations derived from root detritus did not differ with vegetation type in our laboratory experiment, the roots themselves showed differences in C/N ratio, with aspen root C/N around 40 vs. 90 for conifer roots, potentially pointing at differential microbial C processing and stabilization as per Cotrufo et al. [73].

The DOC in solution is more likely to add directly to the MoM fraction of SOC. Our estimated root detritus contribution to soil solution DOC showed potentially higher DOC C input from conifer than aspen roots. Vegetation differences regarding DOC inputs derived from aboveground and belowground detritus sources also followed an opposite pattern to what we observed in terms of SOC pools and stabilization. The observed depth differences in SUVA are consistent with our conceptual understanding of how DOC chemistry changes from precipitation to top- and subsoil [74]. Even though we found higher DOC/DON ratios in conifer than in aspen soil solutions, the difference in DOC aromaticity (SUVA) between both overstories was generally minimal, similar to what was found by Fröberg et al. [63] as well as in a global DOC meta-analysis by Michalzik et al. [75]. The decrease of SUVA and C/N values with depth is indicative of potential sorption or decomposition of aromatic compounds [74] or of roots adding less aromatic compounds to the solution. SUVA values recorded during the leaching experiment showed similar values for foliage and root leachates. Hansson et al. [76] also found similar SUVA values from Norway spruce needle and root leachates, but their values were higher than in our study, often increasing with time of decomposition. In our experiment, the substrate was leached once, potentially explaining the lower SUVA values. Collectively, this suggests that root DOC additions should not lower the SUVA of DOC in the percolating solution. We conclude that the observed differences in soil water chemistry between aspen and conifer were too small to cause major differences in sorption and stabilization of that DOC under both overstories. The higher DOC fluxes associated with higher calculated net DOC retention under conifers might initially

suggest concentration driven DOC sorption. However, the DOC flux and retention patterns run contrary and fail to explain the actual SOC and MoM storage, which is higher under aspen.

The above- and belowground plant C pools and detritus input fluxes, as well as the DOC fluxes measured in our study, prove inadequate in explaining the differences in SOC storage and stabilization between aspen and conifer soils. The larger stable SOC and MoM stocks are thus not simply the result of higher above- and belowground litter input or turnover (Figure 3-6). As suggested by Rasse et al. [77], roots probably play a greater role in SOC stabilization than C derived from aboveground sources, but this is not necessarily mediated through detritus dynamics, which seem to feed more into the LF. Rather, the rhizosphere, i.e., living roots and associated microbial populations, may be key in creating the observed differences. Unfortunately, this study did not quantify microbial biomass, diversity and activity in the field.

Tree species differ in their C allocation to roots, and how this C is partitioned between root respiration and fine root biomass. While reviews have suggested that, on average, half of soil respiration is autotrophic from recent photosynthate [78], the reported relative proportion of fixed C that is allocated belowground ranges from 10% to 90% [34]. Differences in C allocation between deciduous and evergreen trees, and trees with ectomycorrhizal (like most conifers) and arbuscular mycorrhizal (aspen have also arbuscular mycorrhizae) associations have been reported in literature [67,79,80]. Therefore, we hypothesize that the differences between SOC pools under aspen and conifer overstories are due to differences in belowground C allocation and microbial

composition and activity in the rhizosphere. While we did not investigate the rhizosphere, studies have shown that quantitatively the C inputs into soil by fine root turnover and exudation can be in the same range [81].

Furthermore, it has been shown that species with thicker roots (such as conifer roots in our study) forage more by mycorrhizal fungi, whereas thin-root species (such as aspen roots in our study) forage more by root proliferation [83]. Higher root proliferation can translate into higher surface area, and more microbial MoM. In fact, Román Dobarco et al. [83] showed that the MoM under aspen largely consists of relatively simple molecules, which could originate from root exudates and microbial decomposition.

5. Conclusions

In this study, we quantified above- and belowground soil C pools and fluxes (Figure 3-6) to test some of the commonplace explanations for differential SOC accumulation patterns between ecosystems. To our knowledge, this is the first attempt to explain differences in SOC storage and stabilization in aspen and conifer systems in North America. Our results clearly demonstrate that aspen store significantly more mineral SOC than conifer stands in Utah, with most of the C associated with the silt and clay fraction, considered the more stable form of SOC. Aboveground C input fluxes are an unlikely factor in creating these differences. Indeed, while aspen have higher aboveground litterfall, only a small fraction of the aboveground litterfall appears to be transported into mineral soil. Nor did we find evidence that root detritus input is the driver of SOC differences between both overstory types. This leaves the logical conclusion that the observed differences in SOC storage and stabilization are more likely

related to plant–microbe–soil interactions that take place in the rhizosphere. Our analysis identifies major gaps in our understanding of SOC dynamics, including the quantification of rhizosphere processes in belowground C sequestration. It also points to new directions for future inquiry, for example, the use of novel techniques, such as foliage- and root-specific biomarker (cutin and suberin) concentrations in bulk soil and MoM to further elucidate the relative role of above- and belowground C sources of SOC stabilization.

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Table 3-1. Site location and stand characteristics.

	Aspen							Conifer						
Site	**UTM Coordinates**	**Elev. (m)**	**Slope (%)**	**Aspect**	**LBA ($m^2 \cdot ha^{-1}$)**	**Stems (ha^{-1})**	**Soil Texture**	**UTM Coordinates**	**Elev. (m)**	**Slope (%)**	**Aspect**	**LBA ($m^2 \cdot ha^{-1}$)**	**stems (ha^{-1})**	**Soil Texture**
CM8	X: 320149 Y: 4150010	2703	23	NW	54.4	639	Loam	X: 320206 Y: 4150075	2699	23	NW	65.7	526	Loam, clay loam
CM16	X: 3316696 Y: 4161467	2680	11	N	19.7	529	Sandy loam	X: 331651 Y: 4161417	2702	8	N	45.9	1298	Loam
CM17	X: 315048 Y: 4157533	2724	4	NW	19.8	2396	Loam	X: 315004 Y: 4157475	2714	9	N	34.6	1403	Loam
CM20	X: 330427 Y: 4159551	2896	11	W	34.7	1057	Sandy loam	X: 330542 Y: 4159749	2892	15	N	45.6	1569	Sandy loam
TWDEF *	X: 0457840 Y: 4634963	2634–2649	1–11	SSE–SE	48.7	1949	Loam, clay loam	X: 0457952 Y: 4634897	2636–2659	1–9	SSE–SE	56.4	3138	Loam, clay loam

* The parameters for TWDEF are ranges of three replicates. LBA, live basal area ($m^2 \cdot ha^{-1}$); UTM - Universal Transverse Mercator

Table 3-2. Dissolved organic carbon (DOC) (kg·ha^{-1}) transport during snowmelt period ± standard deviation (n = 3 plots per overstory type at TWDEF).

Year	DOC kg·ha^{-1}			
	Aspen 5 cm	**Aspen 45 cm**	**Conifer 5 cm**	**Conifer 45 cm**
2014	56.26 ± 2.35	49.20 ± 4.56	177.61 ± 152.82	82.11 ± 97.43
2015	52.81 ± 10.19	24.67 ± 5.91	137.96 ± 33.14	65.11 ± 11.91
2016	145.44 ± 49.23	46.66 ± 9.13	130.49 ± 27.35	67.47 ± 38.39

(Effect of overstory type p = 0.01, $F_{1,28}$ = 7.63; effect of depth p = 0.006, $F_{1,28}$ = 9.02; effect of interaction p = 0.98, $F_{1,28}$ = 0.001; repeated measures ANOVA).

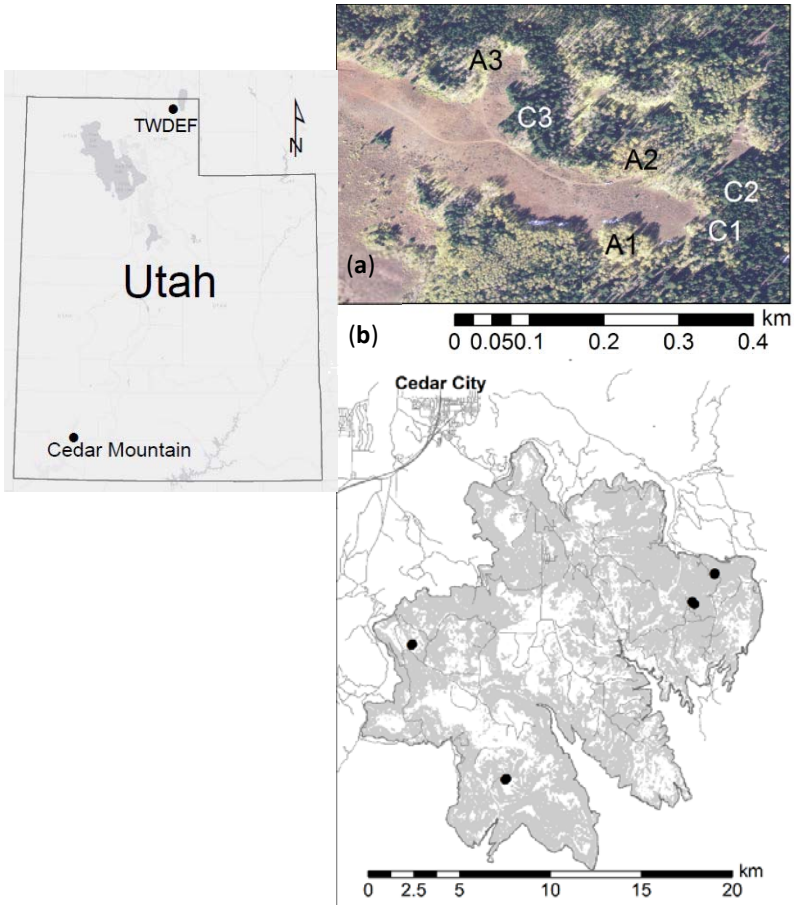

Figure 3-1. Location of sampling sites: (**a**) T.W. Daniels Experimental Forest (TWDEF) site with six intensive measurement plots; and (**b**) pairs of extensively measured plots at four Cedar Mountain (CM) sites.

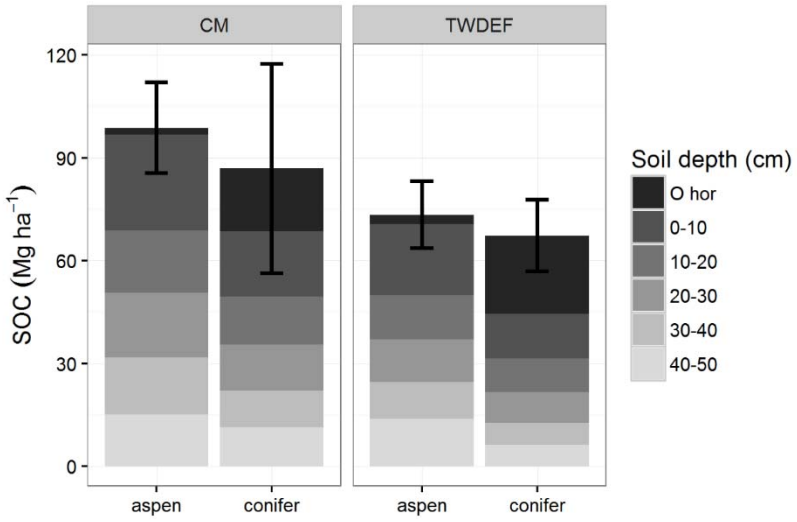

Figure 3-2. Soil organic carbon (SOC) stocks (Mg C·ha^{-1}) for aspen and conifer at CM and TWDEF. Values are averages of four paired sites at CM, and three plot pairs at TWDEF. Error bars are standard deviations for the total SOC stocks (O-horizon −50 cm) across the sites and plots (p = 0.51, ES = 0.32 for total SOC stocks, and p = 0.08, ES = 1.03 for mineral SOC stocks).

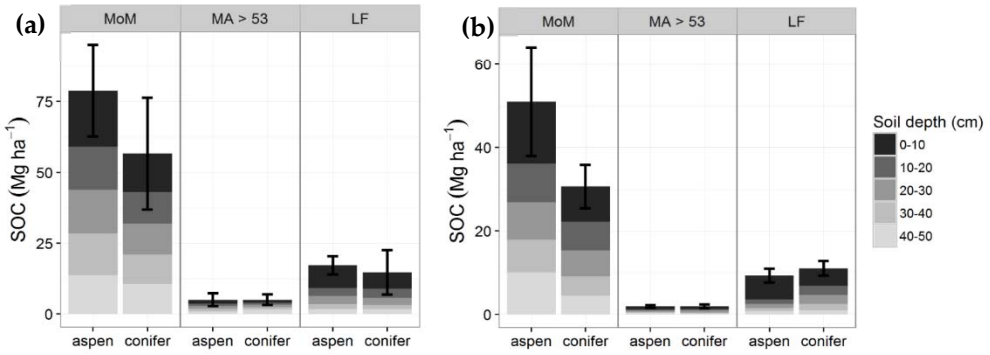

Figure 3-3. Pool sizes of the three major SOC fractions - mineral associated organic matter (MoM) in the silt and clay fraction, MA > 53—mineral associated SOC in the sand fraction, LF—light fraction (Mg·ha^{-1}) at TWDEF (average of three plots) (**a**) and CM (average of four sites) (**b**). Error bars are standard deviations for the whole profile (MoM: $p = 0.15$, ES = 0.78; MA: $p = 1$, SE = 0.002; LF: $p = 0.53$, $t = 0.69$, SE = 0.31).

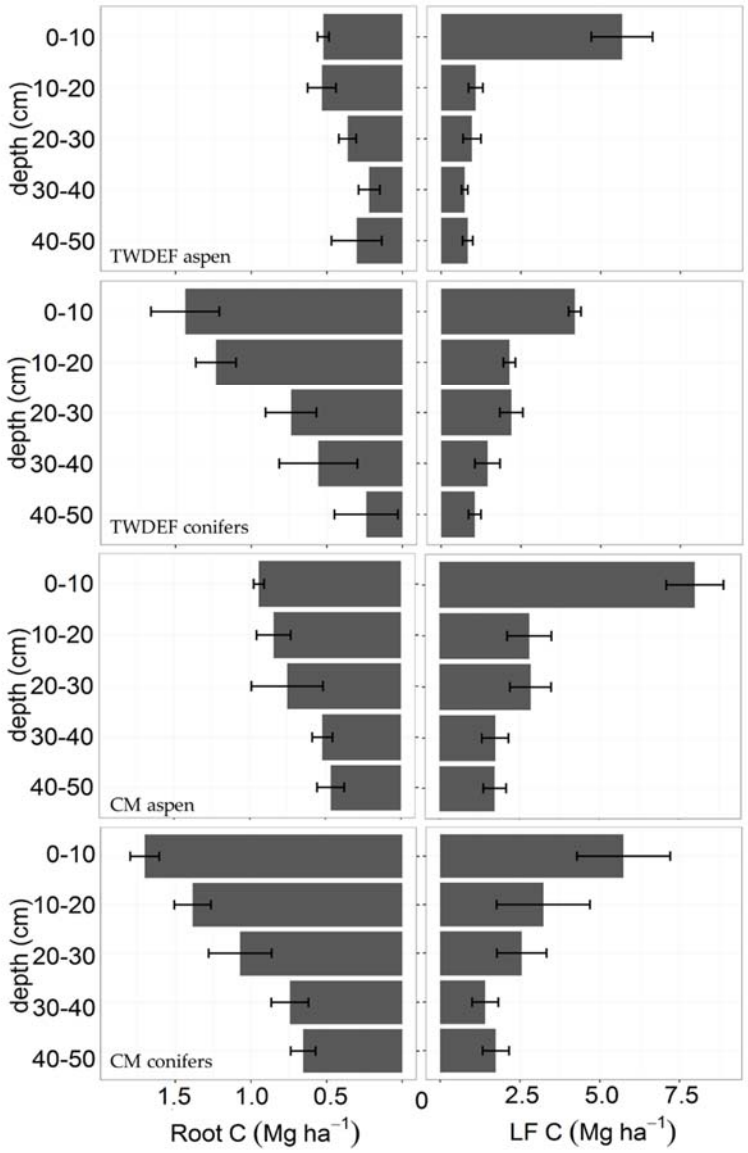

Figure 3-4. Root biomass and LF—light fraction C content (Mg·ha^{-1}) by depth and vegetation type at TWDEF and CM. Error bars are standard errors of three plots at TWDEF and four sites at CM. For the whole soil profile, total root biomass was higher under conifers than aspen (p = 0.005, SE = 2.52), while light fraction pools were similar (p = 0.53, SE = 0.31).

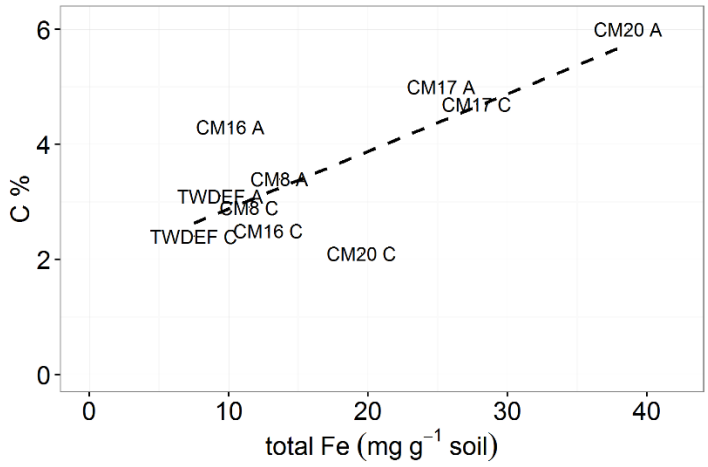

Figure 3-5. Relationship between total extractable Fe (mg·g^{-1} soil) and C concentrations in 0–10 cm depth soils from CM and TWDEF. The labels in the graph correspond to the plot labels in Table 3-1. "A" indicates aspen plots, and "C" indicates conifer plots.

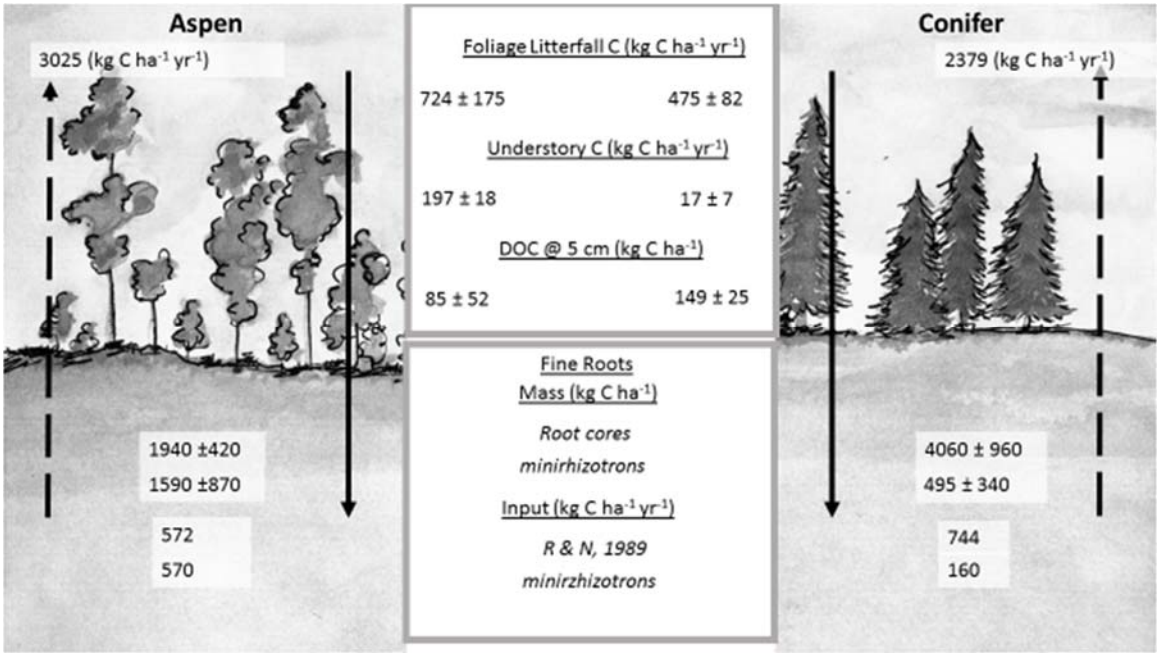

Figure 3-6. C fluxes into and out of soil at TWDEF. All values are averages of three plots ± SD. Foliage litterfall C is an average value for two consecutive years (2014–2016). Understory C was measured in 2015. DOC—dissolved organic carbon flux during snowmelt period averaged for three consecutive years. Fine root mass was measured from root cores and from minirhizotron data. Fine root input (R and N, 1989) was calculated using the relationships reported by Raich and Nadelhoffer [31] assuming 50% heterotrophic respiration, and using minirhizotron data evaluation. Arrows going upward indicate C loss through soil respiration with actual C loss values given on top. Soil respiration data used in the figure were originally reported by Olsen and Van Miegroet [32]. (Illustration by Mercedes Román Dobarco)

CHAPTER 4

ASPEN SOIL ORGANIC CARBON INCREASES RETENTION OF DISSOLVED ORGANIC CARBON IN SOIL.

Abstract

Background and aims

Dissolved organic carbon (DOC) is a major source of C for the formation of stable organo-mineral complexes in soil. In the Intermountain West, aspen soils have higher and more stable soil organic carbon (SOC) pools, even though conifer soils have higher DOC fluxes. This suggests that, instead of concentration, the observed SOC differences could be caused by DOC quality. The goal of this study was to quantify the retention and release of aspen and conifer detritus leachate DOC in various soils.

Methods

Using a batch sorption experiment approach, we compared leachates from four plant sources – aspen leaves, aspen roots, conifer needles, and conifer roots – on soils sampled from aspen and conifer forests.

Results

Retention of aspen foliage DOC was higher than aspen root DOC, as indicated by all four sorption parameters – k and n (describing the sorption curve shape), null point concentration (NPC; net sorption = net desorption), and endpoint (EP, sorption at the highest DOC concentration added). Leachates from conifer needles and roots showed

very similar retention behavior, and root leachate retention from both sources was more similar than foliage leachate retention. Soils sampled from aspen forests showed higher affinity for new DOC than conifer soils (higher sorption rate (n), lower NPC, and higher EP), irrespective of the source.

Conclusions

The results indicate that aspen foliage DOC seems to be an important contributor to the formation of the mollic epipedon often found under aspen forests in Utah. Furthermore, aspen overstories seem to increase the effective C saturation capacity of soils compared to conifers.

Introduction

Dissolved organic matter (DOM) is an important driver of biogeochemical cycling of elements and of soil formation. It is the main pathway for the redistribution of nutrients, pollutants, metals, and through its mobility, it contributes to soil organic carbon (SOC) accumulation in deeper soils (Kaiser and Kalbitz 2012). In its dissolved form C can easily interact with mineral surfaces (Qualls 2000; Guggenberger and Kaiser 2003; Kalbitz et al. 2005). This suggests that dissolved organic carbon (DOC) is a potential source of silt- and clay- bound C (Kalbitz and Kaiser 2008; Schmidt et al. 2011), considered to be one of the most stable fractions of SOC (Keil and Mayer 2014).

In a recent literature review, Laganière et al. (2017) reported that in North America, SOC under quaking aspen (*Populus tremuloides* Michx.) is consistently more stable than under adjacent conifer stands. In the Intermountain West, aspen's southern

distribution range, SOC pools in bulk soil and in the organo-mineral fraction under aspen are considerably higher than under adjacent conifer stands (Woldeselassie et al. 2012; Boča and Van Miegroet 2017). In this region, aspen and conifers are characterized by differences in detritus input quantity, quality, and DOC fluxes, which all are potential drivers of the observed SOC pool differences. Spring snowmelt water fluxes are the major pathway for C redistribution in soil in these areas, due to lack of soil faunal activity. In a recent study Boča and Van Miegroet (2017) reported higher DOC fluxes under conifers than under aspen during snowmelt. Studies have shown that sorption of DOC to mineral particles is concentration and composition dependent (Kaiser and Kalbitz 2012). Higher DOC fluxes, attendant with lower mineral-associated SOC pools under conifers compared to aspen, point at potential differences in sorption characteristics of the detritus leachates from both sources.

The litter layer has traditionally received most attention in literature as a source of DOC in forest soils (as reviewed by Kalbitz and Kaiser 2008). The estimated flux of DOC from the forest floor to the mineral subsoil is about 115–500 kg C ha^{-1} $year^{-1}$ in forest ecosystems, representing up to 35% of the annual litterfall (Kalbitz et al. 2003). Retention in mineral subsoils has been shown to range from 40 to 370 kg DOC ha^{-1} yr^{-1} (Currie et al. 1996; Guggenberger and Kaiser 2003). Cotrufo et al. (2015) reported that DOM produced during the early stages of litter decomposition (labile non-structural compounds) formed new SOC with high efficiency. On the other hand, Kaiser and Guggenberger (2000) have suggested that hydrophobic and more aromatic compounds are preferentially sorbed to mineral surfaces compared to the more labile polysaccharide

derived hydrophilic DOC. Information about DOC composition under broadleaved and conifer trees is inconsistent, with some studies indicating that conifer DOC is more aromatic and broadleaved DOC more labile (Kiikkilä et al. 2011), while others report no differences in regard to aromaticity (Fröberg et al. 2011). This might suggest that the chemistry of DOC is species and location dependent.

Roots are considerably less examined as a source of DOC. Data on root DOC in soils are so scarce that it has prohibited researchers to calculate any estimates of root DOC contribution to SOC (Kalbitz and Kaiser 2008). Based on a soil column experiment, Uselman et al. (2007) suggested that root DOC could contribute to the accumulation of SOC, especially in deeper depths. Uselman et al. (2012) further reported that fine root DOC was less labile than foliage DOC, and that DOC thus became more recalcitrant with increasing root input. Overall, the lack of root DOC data, and their sorption/desorption behavior in soils, hampers our understanding of how DOC fluxes and their variability under varying species compositions affect SOC accumulation. This is especially important in forests with minimal faunal mixing, where DOC fluxes potentially represent a major pathway of C incorporation into deeper soils.

Apart from DOC concentration and composition, DOC sorption has been shown to be highly affected by soil characteristics such as Fe and Al oxide concentrations (Kaiser et al. 1996; Lilienfein et al. 2004; Schneider et al. 2010; Heckman et al. 2011; Kramer et al. 2012). Indeed, Boča and Van Miegroet (2017) found a significant correlation between Fe oxyhydroxide and bulk SOC concentrations in soil when comparing aspen and conifer sites in northern and southern Utah. This might suggest

higher sorption capacity of soils with higher Fe and Al concentrations. Furthermore, Six et al. (2002) have suggested that sorption of C to mineral surfaces is not infinite, but can reach saturation. Therefore, native SOC levels can also alter the sorption of new DOC inputs to mineral surfaces.

The objective of this study was to investigate the retention and release (sorption and desorption) characteristics of foliage and root DOC of two contrasting tree species – quaking aspen and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), in forest soils with contrasting soil properties. Published findings on DOC sorption characteristics do not provide a good explanation as to why conifer stands in Utah have lower and less stable SOC pools even though they have higher DOC fluxes. Aspen and conifer forests are ideal study systems to answer questions regarding the effects of substrate quality and quantity on DOC sorption characteristics. We used a batch sorption approach to investigate: i) whether there are differences in sorption based on type and origin of plant substrate – aspen foliage and roots, conifer foliage and roots; ii) whether native soil C affects the sorption of new C based on initial SOC concentration and type of native SOC present – aspen or conifer in top and subsoil; iii) whether and to what extent biogeochemical soil characteristics such as Fe and Al oxyhydroxide content affect sorption behavior; and iv) how stable the sorbed DOC is as determined by its desorption?

Based on previous findings regarding broadleaf and conifer foliage DOC, we expect that the foliage of aspen and subalpine fir will yield different quantities and quality of DOC, which could result in differences in sorption and desorption behavior. Published studies on root DOC characteristics (Uselman et al. 2007; Hansson et al. 2010;

Uselman et al. 2012) have mainly focused on conifer species, and our study is the first to explicitly investigate root DOC sorption characteristics of contrasting tree types. Finally, by contrasting DOC sorption in low and high Fe and Al oxyhydroxide concentration soils, and deep vs shallow soils we can further elucidate the role of biotic vs abiotic factors in sorption behavior.

Methods

Soil substrates

Soils for the experiment were collected from adjacent aspen and conifer forest stands at T. W. Daniels Experimental Forest (TWDEF) in northern Utah, and at Cedar Mountain (CM, specifically plot CM17) in southern Utah. CM has much higher Fe and Al oxyhydroxide concentrations, and higher SOC concentrations than TWDEF (Table 4-1; Boča and Van Miegroet 2017). A detailed description of the sampling sites and the sampling procedure is given in Boča and Van Miegroet (2017). The soils were collected from the top 10 cm and 40-50 cm of the soil profile to capture differences in native SOC within a given site.

Soil texture was determined by particle size analysis with the hydrometer method at Utah State University's Analytical Lab. pH was measured by mixing 10 ml soil with 10 ml water on the ATI Orion 950 Ross FASTQC Titrator. Soils were extracted in triplicate with sodium pyrophosphate (NaPP), acid ammonium oxalate (AAO), and citrate-dithionite (CD) to estimate organically-bound, amorphous and crystalline Fe and Al. The extracts were analyzed with an Atomic Absorption Spectrometer (Varian AA240

flame atomization, Australia). Amorphous Fe and Al were calculated by subtracting NaPP values from AAO values. Clay mineralogy was determined with an X-Ray diffraction spectrometer (Panalytical X'Pert Pro with monochromatic Cu K-alpha radiation). The soil was ground to <250 µm and analyzed for total organic carbon (TOC) and inorganic C (IC) with Skalar Primacs^{SLC} Analyzer (Skalar, Inc., Breda, The Netherlands).

Leachate preparation and analyses

The plant material used in the experiment was collected at TWDEF and CM at the end of the growing season in 2015, and consisted of senesced aspen leaves, conifer needles, and fine roots (<2 mm diameter) obtained from soil cores in both forest types at both sampling sites. The material was ground with a Wiley mill (20 mesh; Thomas Scientific, New Jersey, USA), analyzed for C with Skalar Primacs^{SLC} Analyzer (Skalar, Inc., Breda, The Netherlands), and for total nitrogen with PDZ Europa ANCA GSL IRMS elemental analyzer (Sercon Ltd., Cheshire, UK).

To obtain DOC stock solutions, 20 grams of ground foliage and root material were saturated with ultrapure water, and subjected to two freeze-thaw cycles for a week to maximize the amount of DOC leached. The thawing was done at 5^{o} C to reduce microbial decomposition of the material, and mimic fall field and snowmelt conditions. After thawing the material a second time, the substrates were leached with 2 L of a 0.08 millimolar KCl solution, which corresponded to an electrical conductivity (EC) of around 10 µS cm^{-1}, similar to the EC detected in snow sampled from the TWDEF site (Boča,

unpublished data). The leaching was done once through a glass fiber filter (Sterlitech 0.45um) by applying vacuum.

Experimental setup

The stock solution of each leachate was analyzed for DOC immediately after the leaching, so that four working concentrations of around 10, 20, 40, and 80 mg L^{-1}, and the first batch of samples could be prepared on the same day as the stock solution. The working solutions were adjusted with KCl to have a constant EC of around 150 µS cm^{-1} (1 millimolar KCl), similar to the highest values detected in soil pore water at TWDEF (Boča, unpublished data), and analyzed for DOC with a Phoenix 8000 Carbon Analyzer (Tekmar-Dohrmann, Ohio, USA). The pH of leachates was measured from stock solutions, which had DOC concentrations of around 150 mg L^{-1}, except for AL, which had to be diluted prior pH measurements due to DOC concentrations in the stock solution close to 1000 mg L^{-1}.

The experiment had four leachate treatments – aspen leaves (AL), aspen roots (AR), conifer needles (CN), and conifer roots (CR), and eight soil types – TWDEF aspen (TA), TWDEF conifer (TC), CM17 aspen (CMA), CM17 conifer (CMC), from 0-10 and 40-50 cm soil depths. These two depths were chosen to represent differences in native SOC concentrations. We assumed that the upper soil is closer to C saturated, while the soil at greater depth is not, mostly due to limitations in C re-distribution. Differences in C concentration between both depths ranged from 1.81 to 2.15% (Table 4-1).

The study was a full factorial experiment (32 combinations of leachate and soil), such that every soil was mixed with every concentration of every leachate (1:10 soil to

solution w/v ratio), and a zero-DOC KCl solution with an EC of 150 µS cm^{-1} was included to measure desorption of native SOC (Fig. 4-1). The experiment was done in triplicate for concentrations 0, 10 and 80 mg L^{-1}, and in duplicate for concentrations 20 and 40 mg L^{-1}. The mixing was done in glass jars with septa caps to allow for measurements of CO_2 evolution after shaking due to heterotrophic activity. The jars were shaken in the dark on an orbital shaker for 24 hours (100 rpm) at room temperature. Due to the sample size the shaking had to be split in two days. The first round of samples (equilibration) were prepared on the same day as the leachates themselves, and the second round was prepared on the next day. After shaking, CO_2 within the jar was measured by inserting needle extensions through the septa and analyzing the gas with a LICOR-8100 gas analyzer (LI-COR, Inc., Nebraska, USA). After accounting for the volume of ambient air in the tubing, the CO_2 in the headspace and dissolved in water was converted to C by using the ideal gas law to account for DOC losses via mineralization. Afterwards, all samples were filtered through a 0.4 µm glass fiber filter (Sterlitech), and analyzed for DOC as described above.

Leachate (pre-sorption) and post-sorption solution quality was assessed with fluorescence spectrometry using an Aqualog fluorometer (Horiba Jobin Yvon, Japan). Fluorescence excitation wavelengths ranged from 248 to 800 nm, at an increment of 6 nm, while the emission spectrum was obtained at an increment of 8 nm with medium CCD Gain. Fluorescence spectra were Raman normalized, corrected for the inner-filter effect, and blank-subtracted using filter blanks. Each sample was diluted to not exceed 0.3 cm^{-1} absorbance at 254 nm.

UV-vis and fluorescence data were used to calculate spectroscopic indices that represent variation in the chemical character of DOC (Gabor et al. 2014a). We calculated the humification index (HIX) and specific ultraviolet absorbance at 254 nm (SUVA = abs @ 254 nm cm^{-1} x 100/ DOC mg L^{-1}; units = L mg C^{-1} m^{-1}). A higher value of the humification index (HIX) corresponds to lower hydrogen to carbon (H:C) ratios and indicates a greater degree of humification. SUVA was calculated by normalizing absorbance at 254 nm to the DOC concentration and is reported in units of L mg^{-1} m^{-1}. SUVA has been used as a proxy for DOC aromaticity (Weishaar et al. 2003), hydrophobicity (Dilling and Kaiser 2002), and microbial stability (Kalbitz et al. 2003).

Desorption

At the end of the adsorption experiment, soils in glass jars were incubated for 7 days at 5° C. Post-incubation they were extracted once with 40 mL of 1 millimolar KCl solution to determine desorption of the sorbed material. The desorption solutions underwent the same preparation procedure and measurements as the sorption solutions described above. For data analysis, DOC values were corrected for the amount of DOC in solution that could not be decanted from the sample (approximately 5 mL) at the end of the adsorption experiment.

Sorption-desorption data analyses

We analyzed DOC retention patterns (adjusted for mineralization) by fitting initial mass (IM; Nodvin et al., 1986), Langmuir and Freundlich curves to the measured retention isotherms based on the modifications suggested by Lilienfein et al. (2004) and

Vandenbruwane et al. (2007) for sorption isotherms. The release of native organic C as DOC (from extraction with zero-DOC solution) needs to be subtracted from the original Langmuir and Freundlich equations. For example, in the case of Freundlich the parameter "a" was added, representing a non-zero intercept:

$$q_e = k \times C_i^{1/n} - a \qquad [1]$$

In equation [1] q_e is the mass of DOC (mg) released/retained per mass of soil (kg), C_i is the DOC concentration added (initial DOC; Kothawala et al. 2008), and "a" is the y intercept, which describes the DOC released at a zero-DOC concentration. Parameter k affects the slope of the Freundlich curve, especially at low concentrations. Parameter n describes the shape of the curve with 1 indicating a linear shape. Parameter k alone does not reflect the entire slope because it can be offset by n. For example, a high k with a high n means the slope of the curve is high at low concentrations, but levels off quickly. On the other hand, if k is high, and n is close to 1, the curve will have a high slope throughout all concentrations. We used nonlinear regression to estimate the parameters k and n using the function *nls* in the package *Stats* in R (R Development Core Team 2015).

Initial mass isotherms resulted in the worst fits based on root mean square error (RMSE), Akaike information criterion (AIC), and the correlation coefficient (R^2). Langmuir and Freundlich isotherms had the best fits (Appendix B). More than half of the data were better explained with the Freundlich equation, and the rest with the Langmuir equation, but the difference was very small. As the fit of different curves with DOC sorption data has been evaluated in other publications (Vandenbruwane et al. 2007;

Kothawala et al. 2008) and was not the objective of this study, we report statistical results for the Freundlich isotherm parameters only. The parameters of all three fitted curves are provided in Appendix B. We used the Freundlich curve to also determine the null point concentration (NPC; DOC concentration added at which net sorption equals net desorption).

We tested differences between leachate type and soil properties in regards to NPC, endpoint (EP; C sorbed at the highest concentration of DOC added), parameters k and n with a factorial analysis of variance (ANOVA) testing for main effects and two-way interactions with $\alpha = 0.05$. We also performed post-hoc Tukey HSD tests to determine differences between individual leachate types. The soil properties considered were soil forest type, which represented different native SOC (aspen vs. conifer); site, which represented different Fe and Al oxyhydroxide levels; and depth, which represented differences in how far removed the soils are from their effective C saturation levels (topsoils closer to C saturation, and subsoils further away). The Fe and Al oxyhydroxide differences between both sites (CM and TWDEF), however, correlated also with differences in SOC concentration (Boča and Van Miegroet 2017). We further tested the relationship between initial SOC% and the four retention response variables with a multivariate regression. Multivariate ANOVA (MANOVA) was also used to test for differences between the independent variables in regard to fluorescence indices. We performed a multivariate linear regression to test whether any of the applied treatments (leachate or concentration) changed the sorption characteristics of the soil. Data were log

transformed where necessary to ensure equal variances. All statistical analyses were performed with the software R (R Development Core Team 2015). The values depicting results are reported as mean ± standard deviation, unless noted otherwise.

Results

Soils

At each site, soils under both overstory types had similar characteristics (texture, Fe and Al concentration, clay type), except for C concentration and pH, which were always lower under conifers (Table 4-1). The higher C concentration in surficial aspen soils is the defining characteristic of a mollic epipedon under aspen, and is the reason why aspen soils at these sites have often been classified as Mollisols, while soils under conifers have been mostly classified as Alfisols (Van Miegroet et al. 2005; Woldeselassie et al. 2012). Deeper soils always had much lower C concentrations. All soils were loams with some 40-50 cm soils being clay loams. The clay concentration was lowest in the CMC 40-50 cm soils at 18%, but varied from 21 to 29% in the other soils. The main soil difference between sites was in the concentration of non-crystalline and crystalline Fe and Al, which was always considerably higher at CM (Table 4-1).

Leachates

Aspen leaves (AL) yielded the highest DOC concentration among leachates, while the other three substrates released ten times less DOC per gram of material (Table 4-2). Leachates from foliage had higher total N (mg g^{-1} substrate) values than root leachates, even though root biomass had higher N concentrations. The substrates had

similar aromaticity (as indicated by HIX and SUVA), except for AL where the low HIX suggests higher H:C ratios, and a more aliphatic nature of the solution compared to the other leachates.

DOC retention

The retention/release of DOC can be evaluated visually by comparing the shape of the sorption curves. Visually the curves of conifer needle (CN) and conifer root (CR) DOC sorption are very similar, irrespective of soil substrate (Fig. 4-2 and 4-3). Aspen leaf (AL) and aspen root (AR) DOC curves differ from each other for a given soil substrate (Fig. 4-2 and 4-3). Numerically this difference was shown by the curve parameters k and n, which, based on post-hoc Tukeys HSD test, significantly differed between AL and AR, but not between CN and CR (Table 4-3). When sorption curves between both tree species were compared, the post-hoc Tukey's HSD test suggested that conifer and aspen foliage curve shapes did not differ significantly (parameters k and n were not significantly different), while root curve shapes were more distinct (significant difference in parameter k; Table 4-3).

As seen in Table 4-3, among plant substrates AL had the lowest NPC (net retention = net release) values, and the highest EP (C retained at highest DOC concentration) values, indicating higher retention. Both of these values differed significantly from AR. In the post-hoc comparison across all soil substrates, AL did not differ significantly from CN and CR in regard to NPC even though the mean value of NPC of AL was half that of CR. Fig. 4-2 and 4-3 show that for aspen soils the NPC of AL and conifer substrates was very close, while differences in average NPC were most

prominent in conifer soils. A statistically significant interaction between leachate type and forest type for parameter k ($p = 0.04$, $F_{3,13} = 3.9$) was due to the fact that the average slope for the sorption region of AL and CN was greater on conifer soils than aspen soils (60.2 and 47.6 on conifer soils and 45.4 and 36 on aspen soils, respectively), while it was the opposite for AR and CR (20.9 and 27.4 on aspen soils, and 11.5 and 25.4 on conifer soils, respectively). Overall, k was more similar for aspen soils, irrespective of the leachate type, indicating that soil properties might be a larger driver of sorption/retention. On conifer soils parameter k varied more between substrates.

In the statistical tests, the average EP of AL across soils was highest and differed significantly from AR and CN (both are negative), but was not statistically different from the EP of CR. As with parameters k and n, CN and CR did not differ significantly in regard to NPC and EP values.

The sorption isotherms depicted in fig 4-2 and 4-3 have been adjusted for the amount of DOC mineralized and released as CO_2. On average more DOC was lost through mineralization in the root leachate treatments than foliage treatments -14% of added C mineralized for AL treatment vs 21% for AR, 12% for CN vs 19% for CR. This might be one explanation for the lower NPC values for root treatments. The proportion of C mineralized did not differ between aspen and conifer soils, suggesting that the mineralization rate was mostly affected by the leachate type.

NPC and curve shape (parameter n) differed significantly between depths (0-10 vs. 40-50 cm; Table 4-3). Lower n values ($p < 0.01$, $F_{1,13} = 13.85$) for topsoils compared to 40-50 cm depth soils, associated with similar k values indicate steeper curves for

topsoils, and higher retention rates. Interestingly steeper curves did not result in lower NPC values for topsoil. In fact, on average topsoils had significantly higher NPC values than soils from 40-50 cm depth ($p = 0.03$, $F_{1,13} = 5.95$). Overall, the results suggest that in deeper soils in this study, sorption commenced at lower DOC concentrations than in the more surficial soils, but actual retention rates (DOC sorbed as a fraction of DOC present in solution) were higher in the latter. A significant interaction between depth and forest type was found for EP ($p < 0.01$, $F_{1,13} = 12.1$). Fig. 4-2 and 4-3 show that the EP for aspen soils was higher in topsoils than in deeper soils (126.5 and 80, respectively), while for conifer soils the topsoil had lower EP values and the deeper depth had higher EP values (-99.7 and 4.3, respectively). No statistically significant relationship was found between native SOC% and any of the different sorption parameters.

Differences between sites were harder to detect visually, and the ANOVA results revealed that the only significant difference between sites was for parameter k ($p < 0.01$, $F_{1,13} = 19.9$), which was larger for CM (Table 4-3). This indicates that the average slope of the curves was greater for CM, i.e., there was a greater difference between the y-axis intercept and EP. While the other response variables were not significantly different, lower mean NPC values and higher EP values for CM followed our expectations that the CM soils exhibit larger ability to sorb DOC, irrespective of plant origin (Table 4-3; higher Fe and Al oxyhydroxide concentration).

One of the most interesting findings, however, was the consistent difference in sorption capacity between aspen and conifer soils, irrespective of plant origin of DOC. As illustrated in fig. 4-2 and 4-3, aspen soils reach NPC at lower DOC concentrations,

i.e., they start sorbing at lower DOC concentrations, and have overall higher EP values than conifer soils, suggesting greater sorption affinity. The ANOVA results corroborated this observation ($p < 0.01$, $F_{1,13} = 22.96$ for NPC; $p < 0.01$, $F_{1,13} = 48.7$ for EP; $p = 0.02$, $F_{1,13} = 6.8$ for n; Table 4-3). The lower n values for aspen soils indicated steeper retention curves than conifer soils, i.e. greater sorption rates. Finally, aspen SOC was also less water soluble than conifer SOC, as indicated by lower desorption (higher y-axis intercept; Fig. 4-2 and 4-3) despite higher SOC levels already present in aspen soil (Table 4-1). This suggests that aspen SOC forms more stable organo-mineral interactions or organic precipitates than conifer SOC. Due to the higher water solubility of conifer SOC, a higher DOC concentration was needed to reach equilibrium between the solid and solution phases, which lead to higher NPC values for conifer soils. Overall, the lower NPC, higher sorption rate (parameter n) and higher EP, collectively might indicate that soils with native aspen SOC have greater affinity for new DOC (greater retention capacity).

Post-sorption DOC quality

HIX values of the post-sorption solutions could potentially provide additional information on the direction of solid phase-solution interactions. HIX values at the lowest initial DOC concentrations (10, 20 mg L^{-1}) were similar to the DOC released from soil when no DOC was added (0 mg L^{-1}), and were all around 7. These values were also distinctly different from the pre- and post-sorption leachate baseline (Fig. 4-4). Thus, the HIX profile at the lower initial DOC concentrations confirmed a DOC signature derived from SOC desorption. At the initial DOC of around 40 mg L^{-1} HIX decreased to 3 for AL, 2.8 for AR, 3 for CN, and 4.4 for CR. Overall, with higher initial DOC

concentrations HIX became closer to the composition of the original source leachate (solid horizontal line in Fig. 4-4), indicating less influence of desorption of the soil SOC on the post-sorption solution. We found no statistically significant difference between HIX values from solutions of aspen and conifer soils ($p = 0.2$, $F_{1,30} = 1.6$; MANOVA). The NPC values between both soil types, however, differed significantly (Fig. 4-4). This indicates that HIX probably only reflects rough thresholds of signature change, but is not sensitive enough to be used as an indicator for sorption-desorption processes.

SUVA values did not exhibit as distinct of a pattern as HIX. They stayed relatively constant for all concentrations of AR (2.3 ± 0.13), and decreased slightly for CN and CR (2.3 to 1.8 from zero-DOC to 80 mg L^{-1}). For AL, SUVA values initially increased from 2.3 to 2.9 at concentrations 0, 10, and 20 mg L^{-1}, potentially indicating desorption of aromatic material. At higher concentrations (40 and 80 mg L^{-1}) SUVA decreased to 2.4 and 1.8, respectively. Similar to HIX, SUVA did not seem sensitive enough to detect composition changes from sorption-desorption in soil.

DOC desorption

The single-step desorption at the end of the sorption experiment resulted in the same desorption pattern that was observed at the zero-DOC treatment during the sorption experiment ($p < 0.01$, $r^2 = 0.9$; multivariate linear regression), i.e., DOC release patterns of incubated post-treatment soils were affected by the same soil properties as untreated soils (initial SOC concentration, and at similar SOC concentrations conifer soils released more C than aspen soils). The concentrations desorbed ranged from 0 to 7.5 mg L^{-1} for AL, 1 to 9.3 mg L^{-1} for AR, 0.4 to 8.9 mg L^{-1} for CN, and 0.8 to 10.5 mg L^{-1} for CR.

Among the treatments applied the AL treatment had the lowest desorption (2.2 ± 2.5 for AL, 3.6 ± 2.6 for AR, 3.4 ± 2.7 for CN, and 4.1 ± 3 for CR; $p = 0.04$, $F_{3,112} = 2.84$).

After 7 days of incubation, the water-soluble SOC desorption solutions had increased HIX values – AL 18.01 ± 2.6, AR 12.3 ± 1.8, CN 10.8 ± 2.1, CR 12.5 ± 3.8 - compared to sorption solutions shown in Fig. 4-4. This indicates a decrease in H:C ratio and a change in composition towards more aromatic compounds, compared to the more aliphatic nature of the DOC immediately after sorption. Similarly to HIX, SUVA values also increased from an average of 2.2 ± 0.18 to 4.1 ± 0.37 for all leachate treatments, further substantiating a shift to a more aromatic composition. This change could potentially indicate microbial processing of more aliphatic compounds in the soil, and/or desorption of more aromatic compounds in the equilibrium solution.

Discussion

Composition of plant and soil leachates

The amount of DOC leached (mg g^{-1} substrate) from plant tissues in our study was higher than values reported by Kalbitz et al. (2003) for forest floor material. In comparison to values reported by Uselman et al. (2012) DOC yields in our study were similar for aspen leaves, but lower for roots. While we found differences in regard to DOC concentration released from aspen foliage and fir needles, Uselman et al. (2012) reported similar values for the broadleaved species *Quercus kelloggii* and three conifer species. This could be indicative of differences between aspen and oak foliage chemistry or differences in leachate preparation (Uselman et al. 2009).

SUVA of plant leachates in this study was in the range of values found by Kalbitz et al. (2003) for a group they characterized as being highly biodegradable, but slightly higher than values reported by Uselman et al. (2012). HIX values of AR, CN, and CR were similar to HIX_{syn} for high and medium biodegradability, while HIX of AL was much lower than anything that was reported by Kalbitz et al. (2003). Overall, the differences in HIX values we measured suggest a higher aliphatic character of AL DOC compared to the other leachates.

SUVA and HIX values for all equilibrium solutions treated with the zero-DOC solution (pure SOC desorption) were similar to what has been reported by Gabor et al. (2014b). The increase of these indicators in post-incubation soil leachates was similarly observed by Kalbitz et al. (2005). The higher HIX values for post-incubation soil leachates that had been receiving AL DOC suggest a higher degree of humification potentially resulting from decomposition or microbial assimilation of the more aliphatic AL C that was added. It further suggests that the retention/release dynamics observed in this study are not simple chemical solution-solid phase sorption interactions, but may also reflect the influence of microbial processing.

Sorption characteristics of leachates and soils

The greater "sorbability" of AL, especially compared to AR leachates leads us to speculate that, under aspen, foliage DOC might contribute to stabilized SOC more than root DOC. Yet, AL-derived DOC differed statistically only from CN in regard to EP (amount C sorbed at the highest DOC concentration added). It, therefore, remains difficult to ascertain to what extent the observed differences in foliage leachate sorption

dynamics observed in this lab study are responsible for differences in overall SOC pools between aspen and conifer forests in Utah.

The sorption parameters of root leachates of both tree species were on average much more similar than the sorption parameters of foliage leachates (Table 4-3), and less likely to explain the observed differences in SOC pools. The similarities between conifer needle and root leachate sorption in our study, have also been reported by Hansson et al. (2010). The relatively greater similarity between aspen and conifer root leachate sorption behavior compared to foliage leachate sorption appears supportive of findings by Hobbie et al. (2010) who, after comparing 11 different tree species, reported that the chemistry of roots was more similar among different species than the chemistry of foliage. Based on this observation, Uselman et al. (2012) suggested that this could potentially result in similar DOC fluxes and quality from roots of different tree species, which our study partially supports.

The retention of AL DOC in soil was higher in both aspen and conifer soils with a much steeper slope for topsoils. As the proportion of C mineralized for AL was similar to the proportion of C mineralized for CN, and was lower than for root leachates, as indicated by the CO_2 evolution measurements, the greater AL DOC removal from the solution was unlikely due to microbial breakdown *per se*. The higher aliphatic character of AL (as indicated by HIX), however, might suggest a positive role of microbial assimilation in the retention of AL DOC compared to the other substrates. This might be especially true in topsoils where the isotherms did not show any leveling-off of the curve. While strong relationships between SOC and microbial C have been reported (Bradford

et al. 2013), and would suggest that the more C rich topsoil exhibited higher microbial assimilation, interestingly this would only be true for AL, as the curves for the other leachates did not follow the same trend. Furthermore, conifer soils did not exhibit the same pattern either. It is also possible that greater microbial activity, especially in aspen topsoils facilitated changes in aspen SOC that rendered it more prone to retention of new C.

The greater sorption of aspen foliage DOC found in this study, and higher stability of aspen SOC reported in previous studies (Van Miegroet et al. 2005; Woldeselassie et al 2012; Román Dobarco and Van Miegroet 2014; Boča and Van Miegroet 2017) is consistent with the Microbial Efficiency – Matrix Stabilization framework proposed by Cotrufo et al. (2013). It states that due to the higher microbial use efficiency of labile substrates, more microbial degradation products are formed, which in turn form more organo-mineral complexes. Indeed, Román Dobarco (2014) found that most of the mineral stabilized SOC in aspen and conifer soils is of microbial origin.

The biggest surprise of this study were the significant and consistent differences in sorption parameters between aspen and conifer soils, irrespective of the plant origin of the DOC. We are not aware of a study that has shown that SOC can create a positive feedback loop in regard to retention of newly added C.

Conifer soils are known to have more water soluble SOC (Van Miegroet et al. 2005; Román Dobarco and Van Miegroet 2014; Boča and Van Miegroet 2017), which originates either from particulate organic matter in soil or from desorption from mineral

surfaces. Due to the higher water solubility of SOC, higher DOC concentrations are required for the solid and solution phase to reach equilibrium, indicated in this experiment by the higher NPC values for conifer soils. The lower water solubility of aspen SOC reported in earlier studies, indicates that the more decomposable aspen litter yields DOC that forms more stable organo-mineral complexes or organic precipitates. It is unlikely that the lower DOC release from soil during the sorption experiments reflects particulate organic matter from aspen foliage being less water soluble, as in our study the DOC produced per gram of substrate from AL was more than ten times higher than from the other substrates (Table 4-2).

The higher sorption rate (as expressed by n at similar k), and higher EP of aspen soils indicate that aspen SOC is not only more stable, but more receptive to new C. If the greater removal of DOC by aspen soils was due to higher microbial assimilation, it means that microorganisms found in aspen soils are capable of utilizing any type of DOC (aspen or conifer foliage or roots) for growth more efficiently than microorganisms in conifer soils, as the retention of all leachates was enhanced in aspen soils. Overall, we did not observe visible microbial strands in the solutions or on the filters, and DOC analysis of filtered and unfiltered control samples (leachate solution with no soil) did not reveal differences in DOC concentration after 24 h shaking. Therefore, the formation of microbial strands was an unlikely mechanisms for DOC removal. The microorganisms involved in assimilation were probably mostly concentrated in soil biofilms (Burmølle et al. 2011).

Microbial activity might have affected the retention of DOC in another way. Kalbitz et al. (2005) and Mikutta et al. (2007) have shown that even mineral-bound organic matter undergoes transformations. These depend mostly on the binding between minerals and organic matter. Therefore, it is possible that, once sorbed, aspen SOC undergoes different transformations than conifer SOC, rendering it more receptive to new C.

By linking the C saturation concept with the MEMS framework Castellano et al. (2015) suggested that a more labile substrate compared to a more recalcitrant substrate could have a larger effect on SOC increases only at higher C deficit levels of the soil. Meaning, the closer a soil is to C saturation the lower the effect of C added, even if it is highly labile. In our study, aspen soils had higher SOC concentrations (lower C deficit), yet despite these higher SOC levels, continued to retain significantly more new C from both aspen and conifer sources (as expressed by NPC, EP and n). This might suggest that the soils have not reached their effective C saturation (max C concentration at current conditions). Even so, as conifer soils contain less C, they should have a higher C deficit, which should result in higher retention rates of all leachates, but especially AL (the most labile leachate) on conifer soils. Our results did not support this hypothesis. In fact, as mentioned earlier, the soils closer to C saturation retained more C from all leachates compared to the other soils. We hypothesize that in Utah's aspen and conifer soils, the effective C saturation capacity is affected by the quality of the substrate, and the microbial transformations it undergoes to form SOC.

Overall, the higher stability of aspen SOC and its higher retention rate of new C are consistent with the formation of a thicker mollic epipedon under aspen soils

compared to conifer soils (Van Miegroet et al. 2005; Woldeselassie et al. 2012), and provides evidence that the observed differences in SOC pools under aspen and conifer forests in Utah are due to the effect of the overlying vegetation. Interestingly, desorption from soils treated with AL leachates did result in significantly lower desorption concentrations compared to the other treatments. This provides additional evidence that DOC originating from aspen foliage is stabilized in soil, and provides an important contribution for the formation of the mollic epipedon observed in these soils.

Sorption differences between top- and subsoils (lower C saturation in subsoils) have been reported before, and partially followed our expectations. Lilienfein et al. (2004) and Vandenbruwane et al. (2007) also found that sorption curves from deeper soils had lower NPC values, meaning sorption commenced at lower DOC concentrations. While we did not find statistically significant differences in EP between depths, soils from the deeper depth did have higher EP values on average, indicating more C sorbed at the highest DOC concentration added. Considering that we did not find any correlation between SOC% and NPC, the depth effect on NPC could be interpreted as higher availability of mineral sorption sites, as suggested by the slightly higher clay amounts at greater depth in most soils (Table 4-1). In the case of CM conifer soils, however, the clay amount was lower at the deeper depth, yet the same sorption pattern was observed, questioning the importance of the small differences between clay contents.

As to the role of soil physiochemical characteristics on sorption isotherms, we found that soils with higher Fe and Al concentrations (i.e., CM soils) indeed showed on average a higher gain of C, between y-intercept and EP (parameter k). While not

statistically significant, the NPC was lower for CM and the EP higher, which is in agreement with our expectations about higher sorption with higher metal concentrations. These findings are consistent with Vandenbruwane et al. (2007) who reported a strong positive correlation between DOC adsorption capacity and oxalate extractable Fe and Al. In their study adsorption capacity was a parameter from the Langmuir isotherm, which, under a similar n, would roughly relate to parameter k in our study. The potential reason as to why no other sorption parameter seemed to be affected by site was probably the SOC concentration (which was also higher in the CM soils). Indeed, Kaiser and Zech (1998) and Kaiser and Guggenberger (2000) showed that sorption of organic matter on oxide/hydroxide surfaces "masks" the mineral surfaces; therefore, it is likely that the oxide/hydroxide effect was overwritten by the SOC effect.

Sorption experiment result significance for field observations

A strong correlation between NPC and field DOC concentrations has been reported by Lilienfein et al. (2004) and Vandenbruwane et al. (2007), allowing to translate laboratory results into field conditions. The rationale behind this relationship is that field DOC reflects equilibrium conditions with the soil and solution, i.e., it basically reflects the NPC (net sorption = net desorption). The experimental conditions of this study differed greatly from field conditions in regard to temperature, soil to solution ratio, contact time, soil moisture content (we used air-dried soils), which, in turn affected the sorption and desorption processes (Kaiser et al. 2001). Therefore, a one to one relationship between NPC and field DOC should not be expected. Furthermore, in contrast to Lilienfein et al. (2004) and Vandenbruwane et al. (2007) we did correct for C

mineralization during the experiment, which lowered the NPC values. Nevertheless field DOC and NPC results can still be used for comparative purposes of, aspen vs. conifer soils. DOC concentrations at TWDEF, sampled during snowmelt over a three year period (2014 – 2016), were higher in conifer soils (average range 28.4–45.5 $mg \cdot L^{-1}$) than aspen soils (average range 7.3–23.8 $mg \cdot L^{-1}$; Boča and Van Miegroet 2017). The NPC differences between aspen (25.8 and 19.9 $mg \cdot L^{-1}$ for AL and 50.2 and 57.7 $mg \cdot L^{-1}$ for AR in top- and subsoil) and conifer soils (102.4 and 52.1 $mg \cdot L^{-1}$ for NC and 503.7 and 83.6 $mg \cdot L^{-1}$ for CR in top- and subsoil) indicate that for sorption to commence, conifer soils require higher DOC concentrations than aspen soils. This means that the higher field DOC concentrations under conifers, which have been reported in a previous study (Boča and Van Miegroet 2017), do not necessarily result in higher sorption of DOC under field conditions. This is in agreement with Michalzik et al. (2001) who found no significant relationship between DOC concentrations and SOC pools in temperate forests. The overall lower NPC values for foliage leachates compared to root leachates at both depths further indicates that the effect of foliage on deep mineral-bound SOC might be stronger than root effect, and greater than suggested by DOC input fluxes (Boča and Van Miegroet 2017).

Conclusions

In the last decade a dominant view has developed that environmental and biological controls operating within the mineral soil matrix dominate SOM stabilization rather than the quality (i.e., molecular structure or elemental composition) of litter. This study provides compelling evidence that litter quality matters in SOC stabilization via

sorption and microbial transformation processes, albeit in more complex ways than simple recalcitrance to microbial decomposition.

Collectively our findings suggest that the more labile DOC originating from aspen, once incorporated into soil, facilitates retention of new C, i.e., it provides a positive feedback loop to SOC storage and stabilization. This, in turn, indicates that litter quality in these forests affects the effective C saturation capacity of the soil, with aspen soils having a higher effective C saturation capacity than conifer soils.

Based on our findings, the presence and maintenance of aspen forests in the landscape is favorable to the belowground C storage function of ecosystems. Encroachment by conifers into aspen stands, will not necessarily lead to a quick loss of C from soils, as the aspen SOC present in the soil is also receptive to C from conifer leachates. This would suggest that for aspen soils to lose their C sequestration function with conifer encroachment, most of the aspen SOC has to be replaced.

While the differences between aspen and conifer SOC pools observed in Utah are not consistent throughout the whole distribution range of aspen forests, aspen SOC, however, does seem to be more stable than conifer SOC throughout the distribution range (Laganière et al. 2017). It remains unclear whether the differences in DOC sorption dynamics described in this study fully account for the SOC stability differences measured in the field. More targeted studies, using tools to reliably identify the origin of the stable SOC under aspen will be required to more conclusively establish a direct link between litter input quality and SOC stability.

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Table 4-1. Selected soil properties from TWDEF and CM study sites.

	Texture	pH (H_2O)	Fe (mg g^{-1})			Al (mg g^{-1})			Clay minerals	C %
			Org	*Non-cryst*	*Cryst*	*Org*	*Non-cryst*	*Cryst*		
TWDEF A 0-10	Loam (23% clay)	6.1	0.79 ± 0.08	2.21 ± 0.6	6.39 ± 0.97	1.83 ± 0.24	0.55 ± 0.05	1.39 ± 0.05	Illite, Kaolinite, Muscovite, Vermiculite	3.11
TWDEF A 40-50	Clay loam (28% clay)	6.1	0.68 ± 0.06	2.22 ± 0.68	6.96 ± 1.24	0.91 ± 0.03	0.99 ± 0.31	1.35 ± 0.07		1.02
TWDEF C 0-10	Loam (24% clay)	5.5	0.87 ± 0.4	1.22 ± 0.49	5.43 ± 0.43	1.33 ± 0.46	0.64 ± 0.03	1.40 ± 0.16	Illite, Dickite, Kaolinite, Vermiculite	2.42
TWDEF C 40-50	Clay loam (29% clay)	5.4	0.81 ± 0.29	1.27 ± 0.39	4.99 ± 0.85	1.09 ± 0.13	0.71 ± 0.12	1.19 ± 0.04		0.61
CM17 A 0-10	Loam (21% clay)	5.4	1.09 ± 0.28	9.18 ± 0.43	15 ± 0.6	1.22 ± 0.17	2.95 ± 0.42	2.37 ± 0.31	Illite, Kaolinite, Vermiculite, Mica *	5.02
CM17 A 40-50	Loam (25% clay)	6.4	2.82 ± 0.25	8.25 ± 0.34	16.77 ± 1.07	2.15 ± 0.05	3.04 ± 0.12	2.57 ± 0.10		3.13
CM17 C 0-10	Loam (23% clay)	5.3	1.53 ± 0.08	10.02 ± 1.53	16.23 ± 1.81	2.09 ± 0.05	2.79 ± 0.29	2.93 ± 0.07	Illite, Kaolinite, Vermiculite *	4.72
CM17 C 40-50	Loam (18% clay)	5.9	3.4 ± 0.05	7.54 ± 0.9	16.58 ± 1.61	2.67 ± 0.04	2.54 ± 0.07	2.70 ± 0.12		2.57

* Due to the high concentration of non-crystalline Fe and Al oxides, the clay mineralogy could not be fully described with XRD in CM17 soils

Table 4-2. Selected properties of pre-sorption leachates derived from foliage and root biomass.

	Biomass			Leachates				
	C%	N%	C/N	mg DOC g^{-1} substrate	mg total N g^{-1} substrate	pH	HIX	SUVA
AL	43	0.58	74	136	0.94	5.6	0.06	0.9
AR	38	0.95	40	10.9	0.53	6.7	0.37	0.8
CN	43	0.45	96	10.5	0.75	6.5	0.73	0.8
CR	40	0.50	80	11	0.24	6.2	0.58	1. 1

Table 4-3. Average values of calculated parameters for each level of each factor. (Bolding indicates statistically significant differences at alpha = 0.05; letters indicate differences between levels of a factor.)

Factors	Levels	K	n	NPC (mg L^{-1} initial DOC)	EP (mg C kg^{-1} soil)
Site					
	TWDEF	**24.78 ± 17.8**	2.09 ± 0.75	116.87 ± 128.56	16.02 ± 121.21
	CM	**43.82 ± 20.22**	2.11 ± 0.62	74.27 ± 49.06	39.56 ± 123.03
Depth					
	0-10	35.01 ± 22.26	**1.84 ± 0.56**	**118.04 ± 115.71**	13.44 ± 157.46
	40-50	33.58 ± 20.56	**2.36 ± 0.7**	**73.1 ± 73.73**	42.15 ± 69.94
Forest type					
	Aspen	32.41 ± 16.88	**1.88 ± 0.45**	**51.11 ± 27.46**	**103.24 ± 104.81**
	Conifer	36.19 ± 25.03	**2.31 ± 0.8**	**140.04 ± 122.19**	**-47.66 ± 83.63**
Leachate					
	AL	**52.8 ± 17.42^{a}**	**2.61 ± 0.99^{a}**	**57.59 ± 51.24^{a}**	**114.26 ± 144.51^{a}**
	AR	**16.17 ± 8.42^{c}**	**1.67 ± 0.33^{c}**	**125.96 ± 91.5^{b}**	**-28.36 ± 94.33^{b}**
	CN	**41.8 ± 23.33^{ab}**	**2.27 ± 0.47^{ab}**	**83.43 ± 56.37^{ab}**	**-4.8 ± 91.51^{b}**
	CR	**26.43 ± 12.19^{b}**	**1.85 ± 0.33^{bc}**	**115.31 ± 158.6^{ab}**	**30.07 ± 114.13^{ab}**

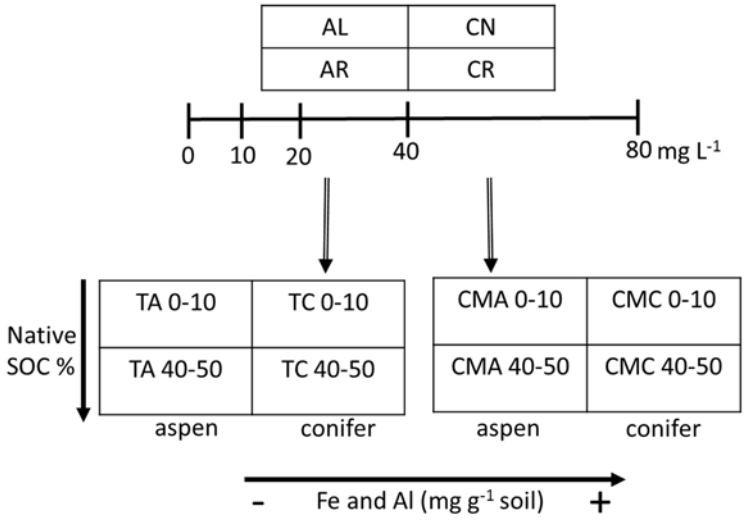

Fig. 4-1 Experimental design of the sorption experiment. Leachates from four plant substrates – aspen leaves (AL), aspen roots (AR), conifer needles (CN), and conifer roots (CR) were added to aspen and conifer soils at five concentrations (0, 10, 20, 40, 80 mg L^{-1}). The two depths (0-10 and 40-50 cm) represented differences in native SOC concentration, and the T (for TWDEF) and CM sites represented differences in sesquioxide concentration. All measurements were done in triplicate for 0, 10, and 80 mg L^{-1} treatments, and in duplicate for 20 and 40 mg L^{-1} treatments

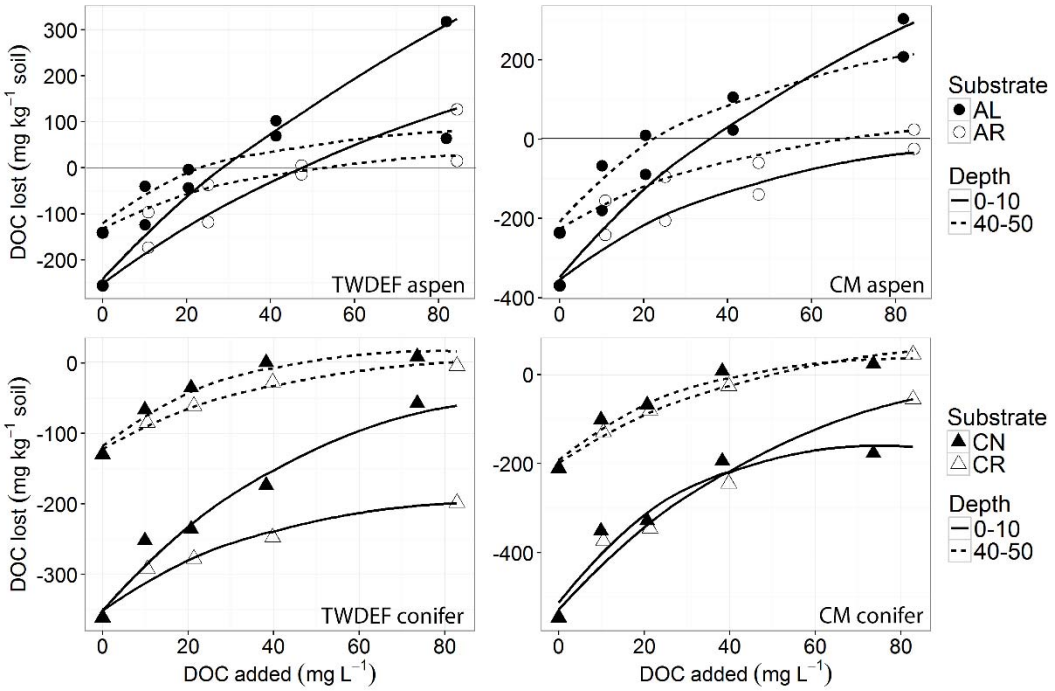

Fig. 4-2 Freundlich isotherms representing release/retention of dissolved organic carbon (DOC) from aspen leaves (AL) and aspen roots (AR) on aspen soils (upper two graphs), and of conifer needles (CN) and conifer roots (CR) on conifer soils (lower two graphs) from TWDEF and CM (sites are representative of differences in Fe and Al oxyhydroxide concentration)

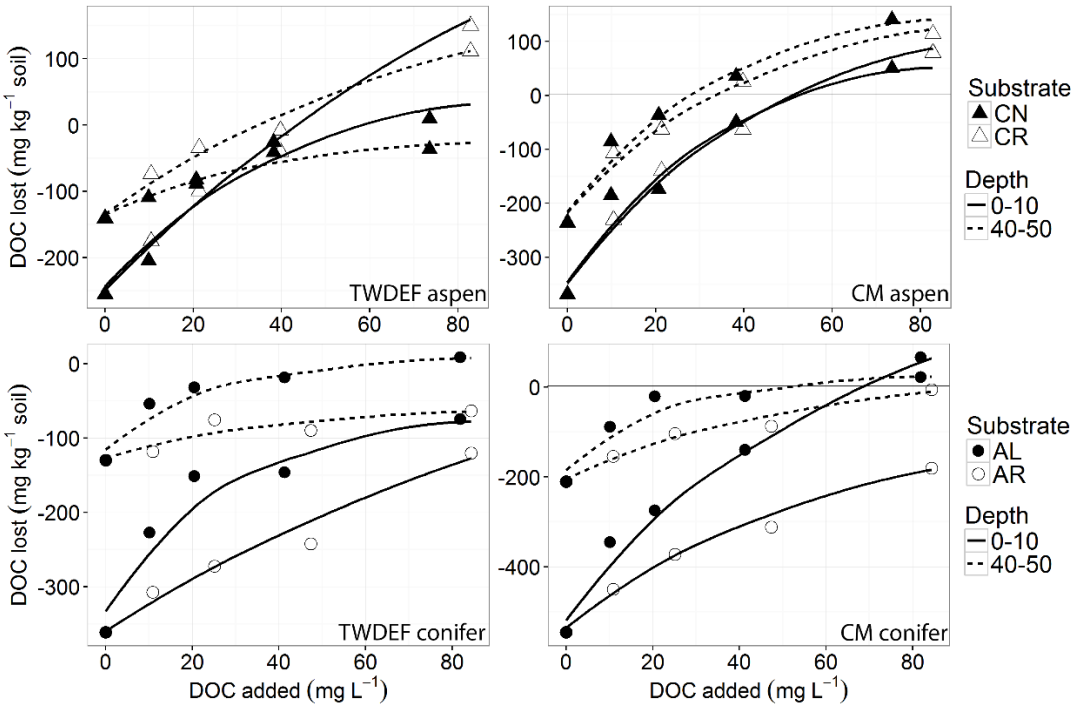

Fig. 4-3 Freundlich isotherms representing release/retention of dissolved organic carbon (DOC) from conifer needles (CN) and conifer roots (CR) on aspen soils (upper two graphs), and of aspen leaves (AL) and aspen roots (AR) on conifer soils (lower two graphs) from TWDEF and CM (sites are representative of differences in Fe and Al oxyhydroxide concentration)

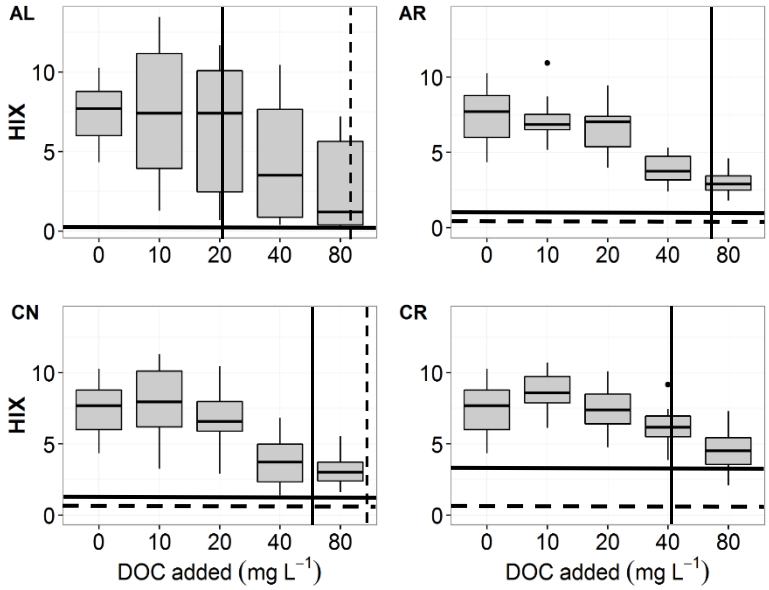

Fig. 4-4 HIX values of post-sorption solutions for all four leachates – AL, AR, CN, CR. The dashed horizontal lines indicate HIX values of fresh, pre-sorption leachates (AL = 0.06, AR = 0.37, CN = 0.73, CR = 0.58). The solid horizontal lines indicate HIX values for pure leachates after 24 hours of shaking (AL = 0.06, AR = 1.17, CN = 1.34, CR = 2.99). The solid vertical lines indicate the average NPC for aspen soils (AL = 24.6, AR = 70.4, CN = 65.2, CR = 44.2 mg L^{-1}), and the dashed horizontal lines indicate average NPC for conifer soils (AL = 90.5, AR = 181.5, CN = 101.7, CR = 186.4 mg L^{-1})

CHAPTER 5

CUTIN AND SUBERIN BIOMARKERS SPECIFIC FOR ASPEN AND CONIFER FOLIAGE AND ROOTS.

Introduction

Models of ecosystem carbon (C) balance generally assume a strong relationship between net primary productivity (NPP), litter inputs, and soil C accumulation (Gottschalk et al., 2012). While long-term litter manipulation studies like the Detritus Input Removal and Transfer (DIRT) experiment have found above- and belowground detritus exclusion to reduce C stocks (from 9-18% in 20 years; Lajtha et al., 2014), the doubling of aboveground litter inputs either did not have any effect or accelerated soil organic matter decomposition, and reduced soil organic carbon stocks under a hardwood forest (Lajtha et al., 2014; Pisani et al., 2016). When comparing a hardwood and a coniferous forest, Crow et al. (2009) reported the major source of topsoil SOC to be foliage for the hardwood forest, and roots for the coniferous forest. This suggests that the relationships between SOC stocks and litter inputs are not only non-linear, but also differ based on forest type. Therefore, to understand how vegetation, and its changes, affect SOC we need to identify the sources that contribute most to SOC.

Quaking aspen is the most widespread tree in North America (Little, 1971). In western North America, fire exclusion has promoted the encroachment of conifers into aspen (*Populus tremuloides* Michx.) forests (Rogers, 2002; Kulakowski et al., 2004; Di Orio et al., 2005). Worrall et al. (2013) suggested that changes in climate will further change aspen distribution ranges. In Utah, aspen stands have been shown to contain more

SOC than adjacent coniferous forests (Woldeselassie et al., 2012; Boča and Van Miegroet, 2017). A recent review of studies on the North American continent also found that SOC in the mineral soil under aspen is consistently more stable than under conifer stands (Laganière et al., 2017). As in all of these studies the comparisons were between adjacent aspen and conifer forests that had similar climate, topography, parent material, and time of establishment, the differences must logically be driven by vegetation. This, in turn, raises the question, which plant inputs – foliage or roots – drive these differences. Such information is vital for understanding how climate or management induced vegetation shifts (aspen to conifer) will affect the large SOC pools under aspen. A recent analysis of above- and belowground detritus C fluxes under aspen and conifer stands in Utah did not find a clear relationship between litter input quantity and SOC pool differences under both overstory types (Boča and Van Miegroet, 2017). In a sorption study reported in this dissertation, we found that sorption in soil of aspen leaf leachate differed from that of root and conifer needle leachates. It was higher at low DOC concentrations, and was almost linear for the topsoil. Furthermore, soils containing SOC that had originated from aspen detritus inputs showed higher sorption capacity than soils that contained conifer SOC, indicating that vegetation inputs change the sorption characteristics, and affect the stabilization of new C in these soils.

Recent studies have proposed aliphatic lipids derived from plant waxes and biopolymers, such as suberin and cutin, as biomarkers for above- and belowground C in soils (Kögel-Knabner, 2002; Otto and Simpson, 2006; Clemente et al., 2011; Spielvogel et al., 2014). Cutin and suberin are biomacromolecules common in most vascular plants.

Cutin is a major component of leaf cuticles (Holloway, 1982), while suberin occurs in the periderm of roots and barks (Kolattukudy and Espelie, 1989). Besides cutin reflecting fresh foliage detritus inputs (Otto and Simpson, 2006; Feng and Simpson, 2007), and suberin being highly correlated with live fine root distribution in soil (Spielvogel et al., 2014) they are also considered comparatively stable. Within the last decade studies have shown that aliphatic compounds originating from cutin and suberin are preserved in soil (Feng and Simpson, 2007; Clemente et al., 2011) through accumulation in finer particle fractions (Clemente et al., 2011). Compositionally, cutin and suberin are similar with only few distinct (exlusive) monomers and polymers, but the concentrations of many of these differ greatly between cutin and suberin derivatives. Hence, the two ways to compare foliage and root contribution to SOC is to compare absolute concentrations of exclusive monomers with soil depth, or to calculate compound specific ratios for plant tissues, e.g., x,16-di$OHC_{16}/\Sigma C_{16}$ or Σcutin/Σsuberin, and compare their changes with soil depth (Kogel-Knabner et al., 1989; Otto and Simpson, 2006; Crow et al., 2009). When suberin and cutin monomer ratios or exclusive compounds are compared they are assumed to have similar degradation rates.

Cutin and suberin have been successfully used to distinguish between above- and belowground SOC sources. Most studies have compared different land uses (Otto and Simpson, 2006; Clemente et al., 2011), the effect of different agricultural crops (Mendez-Millan et al., 2010) or have focused on describing species-specific biomarker differences (Otto and Simpson, 2006; Mueller et al., 2012). Pisani et al. (2016) demonstrated that cutin and suberin biomarkers well reflected treatment induced detritus input changes in a

20-year detritus input and removal treatment experiment (DIRT). Crow et al. (2009) were one of the first that used cutin and suberin to explain how above- and belowground C pathways in a hardwood forest in Pennsylvania vs. a conifer forest in Oregon affected SOC pool characteristics. The forest sites, however, differed in regard to many soil forming factors, and thus the link between vegetation and soil in this study was not straight forward. In our study, however, the close proximity between aspens and conifers in Utah offers an ideal experimental setting (similar to a common garden) to study the effect of forest overstory on C cycling and sources of stabilized SOC. Comparing the relative abundance and distribution of cutin and suberin in soils under similar site conditions, but contrasting forest vegetation and SOC pools, can provide valuable information on how differences in detritus input affect SOC. Specifically, it can provide us with more insight into the connections between C input quantity and quality and SOC storage, which are crucial for predicting potential future changes in SOC stocks under vegetation shifts.

In order to be able to determine the main C sources that contribute to the SOC pool in a given ecosystem, we must first identify from an array of foliage and root derived compounds, those that are most source-specific. The objective of this study is to identify cutin and suberin constituents that can serve as foliage- and root-specific biomarkers, and assess their presence in SOC of aspen and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) soils. This study constitutes a first step in a series of sequential biomarker studies, and will be followed by an analysis of biomarker degradation in soil. Both will form the basis for a third follow-up study aimed at identifying the source of

SOC in aspen and conifer soils by linking biomarker data to foliage and root detritus input data that have been described in a previous study by Boča and Van Miegroet (2017). The research described in this chapter is therefore only the beginning, and identifies compounds of interest based on their concentration in plant material and presence in soil.

Methods

Sample collection

Freshly senesced aspen foliage and subalpine fir needles were collected with littertraps during two consecutive years (2014 and 2015) as part of a study measuring C fluxes at the T. W. Daniels Experiment forest (TWDEF) in northern Utah (Boča and Van Miegroet, 2017). Roots were sampled with root cores up to 50 cm depth in late summer and early fall of 2013 and 2014. Fifteen root cores were taken with a hydraulic soil corer (Giddings Machine Company) up to 50 cm depth where possible, and with a 5 cm diameter split corer in 15 cm increments where the machine could not get in. The hydraulic soil cores were split into 10 cm increments in the lab; the other samples were processed by depth increments collected and adjusted to 10 cm increments for further analysis. Soils were sampled from the top 20 cm under aspen and conifer stands at TWDEF, sieved through a 2 mm-mesh size sieve, and air-dried.

The plant material was ground with a Wiley mill (Thomas Scientific, New Jersey, USA) to pass through a 20 mesh screen. Soil samples were ground with a mortar and pestle to pass a 250 μm sieve. All samples were analyzed for total organic carbon, and

total nitrogen with PDZ Europa ANCA GSL IRMS elemental analyzer (Sercon Ltd., Cheshire, UK).

Sample analyses

Cutin and suberin biomarkers were extracted from ground aspen leaves, aspen roots, conifer needles, conifer roots, and soil from both overstories. One gram of ground plant biomass, and 10 grams of soil was first extracted with an accelerated solvent extractor (ASE) using methylene di-chloride (DCM) and methanol to remove solvent extractable "free" lipids, following the method by Wiesenberg et al., (2004). The extracts were dried, stored in a freezer, and were not used in this study. These "free" lipids are not considered to be part of cutin and suberin, but they do contain molecular markers indicative of the source vegetation, and are often used in paleoecology to distinguish between plant functional types or even species of past vegetation covers (Otto et al., 2005; Zech et al., 2010). Once dried, they can be stored in a freezer until further analysis.

In the next step, 100 mg of each ASE extracted plant biomass sample, or 1 gram of soil, was processed further with alkaline hydrolysis (1N KOH in methanol). The samples dissolved in methanolic KOH (100 mg or 1 g in 10 mL) were heated for 4 hours at 80^{o}C, after which they were filtered through a Sterlitech glass fiber filter (1µm pore size). The extract was mixed with 100 mL ultrapure water, acidified to pH 2, and extracted with liquid-liquid separation using 3 times 20 mL DCM. The DCM extracts were dried under nitrogen using an automated evaporation system (TurboVap® LV, Biotage, Sweden). Dried extracts re-dissolved in 0.5 ml of pyridine were sylilated with

BSTFA (N,O-bis (trimethylsilyl)-trifluoroacetamide) containing 1% of trimethylchlorosilane (TMCS) at 70º C for 1 h.

Silylated saponification products were separated with a gas chromatograph (GC) HP6890 equipped with a Restek™ Rtx™-5MS Capillary column (30 m, 0.25 mm internal diameter, 0.25 μm film thickness), using a He constant flow of 1.5 ml min^{-1}. A 1 μl aliquot was injected in splitless mode, at a temperature of 300ºC. The GC oven temperature was programmed at 100ºC for 2 min, then from 100 to 150ºC at 10ºC min^{-1}, from 150 to 200ºC at 5ºC min^{-1}, and finally at a rate of 2ºC min^{-1} from 200 to 300ºC and then from 300 to 325 ºC at 5ºC min^{-1} (followed by post run at 325ºC for 2 min) . The mass spectrometer (MS; Agilent HP5973) was operated in the Electron Impact (EI) mode (70 eV, Emission 30.9, EI Energy 69.9, EM Volts 1388, scan range m/z 50–650, and 7min solvent delay). The chromatograms were analyzed using the software OpenChrom (Wenig and Odermatt, 2010) by comparing the fragmentation pattern of each peak with a mass spectra library (NIST), published mass spectra in literature, by calculating the target ions using a homologous series approach, and, where possible, with authentic standards. Compounds that could not be identified, but were found to be source-specific were named according to their retention time and target ion, and their fragmentation patterns are shown in Appendices 1 and 2. All compounds were quantified based on an external calibration curve with ω-hydroxyhexadecanoic acid. A known amount of nonadecanoic acid was added to each sample before liquid-liquid extraction to evaluate the recovery of compounds. Cutin- and suberin-specific monomers were designated as tissue specific biomarkers based on the following criteria: (i) their

contribution to a certain plant tissue was at least tenfold higher compared to their contribution to other plant organs, and (ii) they were present not only in plant tissue, but also in soil, and (iii) they constituted at least 0.3% of the total source-specific compounds found in soil (as modfied from Mueller et al., 2012; Spielvogel et al., 2014).

Results and Discussion

Aspen biomarkers

We found a total of 19 compounds that were source-specific for aspen foliage or roots and were present in aspen soil (Table 5-1); 11 compounds were root-specific, and 8 were leaf-specific. This is similar to what was reported by Otto and Simpson (2006), who found 7 foliage-specific, and 11 root-specific compounds for aspen. In a study comparing two deciduous and two coniferous species in Europe, Spielvogel et al. (2014) reported only 3 and 4 leaf-specific compounds, and 8 and 6 root-specific compounds for European beech and pedunculated oak, respectively. The extracted compounds constituted about 10-15% of the total C in plant tissue and soil, which is similar to what has been reported by Otto and Simpson (2006). Similar to other studies (Otto and Simpson, 2006; Mueller et al., 2012; Spielvogel et al., 2014; Angst et al., 2016), we found that only few lipids were exclusive for leaves or roots, meaning they were not found at all in the comparison plant tissue. Most compounds were found in both tissues.

In contrast to most studies, we decided to also report unidentified compounds. The major reason why unidentified compounds have been excluded from prior studies was the inability to clearly distinguish whether these compounds were truly plant specific

or of microbial origin. Such a priori exclusion is not necessary in our study as we will be able to determine later, through a biomarker degradation study, whether a compound is more likely of plant or microbial origin. For example, if the concentration of a compound will increase during the incubation-degradation it is more likely to be of microbial or mixed microbial and plant origin.

In a study comparing cutin and suberin in grasslands, aspen and pine forests, Otto and Simpson (2006) reported cutin for aspen to be the sum of mid-chain hydroxy C_{14}, C_{15}, C_{17} acids and C_{16} mono- and dihydroxy acids and diacids. Mid-chain substituted hydroxyalkanoic acids (8or10,16-dihydroxy-C_{16} acid , 9,ω-dihydroxy-C_{16} acid, and7or8-hydroxy-C_{16} diacid often referred to as x,16-dihydroxy or hydroxyl with x indicating the position of the substitution) have been reported as cutin biomarkers also in other studies (Mueller et al., 2012; Spielvogel et al., 2014; Angst et al., 2016; Pisani et al., 2016) with x,16-dihidroxy hexadecanoic acid often reported to be of the highest concentration in foliage extracts. Consistent with these prior studies, we found 8or10,16-dihydroxy hexadecanoic acid to have the highest concentration of all compounds in foliage extracts, and confirmed its designation as a cutin (foliage) biomarker. We also found 7or8,16-hexadecanoic diacid to be a molecular marker of aspen foliage. In contrast to the study by Otto and Simpson (2006), we did not find 16-hydroxy hexadecanoic acid to be foliage specific, as it was present in roots at higher concentrations than in foliage (165 for foliage vs 332 $\mu g\ g^{-1}C$ for roots). This discrepancy was probably due to the fact that Otto and Simpson (2006) did not directly measure suberin in aspen roots, but rather used previously published data from other studies suggesting that all ω-hydroxyalkanoic acids

were mostly root derived. We also did not find any mid-chain hydroxy C_{14}, C_{15}, and C_{17} acids in our aspen samples. It is possible, however, that one of our unidentified compounds is one of these acids. The mid-chain hydroxy C_{15} acid was also found by Spielvogel et al. (2014) to be a cutin biomarker.

We did identify C_{14}, C_{26} and C_{28} fatty acids and 1-octadecanol as foliage-specific. Otto and Simpson (2006), however, mentioned that alkanoic acids and alkanols are derived of vascular plant or microbial origin, and, therefore, cannot be used as pure plant biomarkers. We included them in our list of potential biomarkers because the continuation of this study includes the characterization of the degradation patterns of each of the identified biomarkers. We are confident that we will be able to better distinguish between plant vs microbial origin of these compounds at the completion of the degradation study.

The x-hydroxy alkanoic acids (mid-chain substituted) with chain lengths of C_{20}, C_{22}, C_{24}, and C_{26}, and the α,ω alkanedioic acids with chain lengths of $C_{18:1}$, C_{20}, C_{22}, C_{24} released from aspen roots corresponded well with previously suggested suberin-specific monomers (Otto and Simpson, 2006; Mueller et al., 2012; Spielvogel et al., 2014; Angst et al., 2016). Similar to these studies, we also found 1,18-hydroxy octadecenoic acid (ω-OH-C18:1) to be root-specific. Mueller et al. (2012) and Spielvogel et al., (2014), however, reported discrepancies in the concentration of this compound in plant tissues and in soil between angiosperm and gymnosperm overstories. For example, Mueller et al. (2012) found similar concentration of ω-OH-C18:1 acid in plant tissues of angiosperms and conifers, while the concentration in soil beneath angiosperms was approximately

twofold of that in soil beneath conifers. Spielvogel et al. (2014), by contrast, found different concentrations in plant tissues, but similar concentrations in soil; one reason why this compound was not considered a biomarker in their studies. We see a similar discrepancy in the results of our study. While aspen foliage and conifer needles have similar concentrations of ω-OH-C18:1 (1257 μg g^{-1}C for AL vs 1250 μg g^{-1}C for CN ; Table 5-1 and 5-2), aspen roots have approximately a three times higher concentration than conifer roots (73,089 μg g^{-1}C vs 27,586 μg g^{-1}C; Table 5-1 and 5-2). Nevertheless, the concentration in soil is slightly higher under conifers (1143 μg g^{-1}C) than under aspen (928 μg g^{-1}C). We, however, decided to keep this compound on our list, and evaluate its change with decomposition.

We found two benzyls that fulfilled the criteria to be considered root-specific – p-hydroxybenzoic acid (Pd) and m-hydroxybenzoic acid (mBd). According to Goñi et al. (2000) these benzyls likely originate from the degradation of proteins or tannins, which can have multiple origins, and are, therefore, non-source specific biomarkers. As mentioned earlier we decided to list all compounds in Table 5-1 that fit the criteria described in the Methods to evaluate their changes during degradation. Contrary to Otto and Simpson (2006) we found ferulic acid (Fd) to be root-specific for aspen. While ferulic acid (Fd) has been reported as an ester-bound moiety in the ligno-cellulose complex of grasses (Lam et al., 2001), the Fd detected in the present study likely does not originate from lignin, because the applied base hydrolysis cleaves esters, but not the ether bonds of the lignin macromolecule. Fd is also known to be a phenolic constituent of suberin (Kolattukudy and Espelie, 1989; Bernards, 2002), which is more likely its origin

in this study. Otto and Simpson (2006) did not report Fd as a root biomarker because they found it in foliage, and because they did not extract roots, they could not compare the differences in concentrations. We have included it on our list as a potential root biomarker.

The compounds with the highest concentration in aspen soil were the two cutin monomers 8or10,ω-diOH C16 (5381 μg g^{-1}C) and 7or8-OH C16DA (1400 μg g^{-1}C), and the two suberin monomers ω-OH-C22 (1236 μg g^{-1}C) and ω-OH-C18:1 (928 μg g^{-1}C). This is similar to what was reported by Otto and Simpson (2006). The majority of compounds identified as aspen foliage and aspen root specific decreased by 80 to 90% in soil from the concentrations observed in plant tissues. Four compounds - ω-OH-C18:1, 8or10,ω-diOH C16, C18:1 DA, p60.0_451 – decreased by 95 to 98%, and p42.0_317 decreased by 99%. This suggests potential differences in degradation rates for some compounds, which would affect their use in comparing cutin and suberin ratios. The long-chain hydroxy fatty acid ω-OH-C26 was the only compound that showed a higher concentration in soil (105 μg g^{-1}C) than in plant tissue (64 μg g^{-1}C in roots; Table 5-1). This could indicate a preferential accumulation of this compound or an additional source. The biomarker degradation study that will follow this study will be able to explain this increase.

The last unidentified aspen root peak – p60.0_451 has a very distinct signal in aspen roots and soil (Appendices 1, root and soil chromatograms), but is absent from foliage. It is also present in conifer roots, but at lower concentrations (Table 5-2; Appendix D). Based on external standards available to us the closest compound that

eluded at a similar time was oleanolic acid (at 62.5 min), which is a triterpenoid. A literature search of possible other triterpenoids yielded no successful results. We ran the root extract on a high resolution GC-MS at Oregon State University's Mass Spectrometry Center, and found that the precise molecular weight (MW) of the compound was 451.2481. It is difficult to say how the compound changed during base hydrolysis and silylation. The MW of oleanolic acid, for example, increased by approximately 144, which is a little less than two trimethylsilyl groups (MW 73.1891). So far we have not been successful in identifying it.

Conifer biomarkers

We found a total of 24 compounds that were source-specific for subalpine fir foliage and roots (Table 5-2); 5 compounds were foliage specific, and 19 were root specific. As for aspen, most compounds were not exclusive to one tissue type, but were found in both tissues.

We do not know of a study where dodecanol (lauryl alcohol) has been reported as a foliage or root-specific biomarker. In this study it was completely absent from aspen tissue and soil, as well as from conifer roots. Vascular plants normally contribute predominantly long-chain (C_{16}–C_{32}) alkanols to the soil (Otto and Simpson, 2006). It is likely that the dodecanol was of microbial or fungal origin from microorganisms that were present on the conifer needles analyzed.

14-hydroxytetradecanoic acid has been reported by Spielvogel et al. (2014) as a cutin monomer in Norway spruce needles. Otto and Simpson (2006) also found it in relatively high concentrations in pine needles. The compound 9,16-dihydroxy

hexadecanoic acid from conifer needles eluted at the same retention time (RT) as 8or10,16-dihydroxy hexadecanoic acid from aspen foliage. Overall, mid-chain substituted 16-dihydroxy hexadecanoic acids were the compounds with the highest concentration for both foliage types (131,583 μg $g^{-1}C$ in aspen leaves and 193,438 μg $g^{-1}C$ in conifer needles). As mentioned earlier, this is in agreement with many other studies that have reported x,16-dihydroxy hexadecanoic acid as a cutin biomarker in gymnosperm and angiosperm foliage (Otto and Simpson, 2006; Mueller et al., 2012; Spielvogel et al., 2014; Angst et al., 2016). In addition, similar to findings by Goñi and Hedges (1990), Matzke and Riederer (1990) and Mueller et al. (2012) we found that leaves of aspen contained no 9,16-diOH C_{16} acid isomer and substantial quantities of 10,16-diOH C_{16} acid, while the opposite was true for subalpine fir.

9,10,18-trihydroxyoctadecanoic acid (triOH-C_{18}) has been reported by Otto and Simpson (2006) as a constituent of pine needles. However, they argued that it is only partially an original monomer, with the other part being derived from the hydrolysis of 9,10-epoxy-18-hydroxy octadecanoic acid. TriOH-C_{18} was found in aspen and conifer tissues, but it was not source-specific for aspen. In the past some studies have suggested that it is foliage-specific, but Mueller et al. (2012) and studies cited in their paper refuted this suggestion for multiple tree species. They showed that for some species this compound was mostly foliage associated, while for others the concentration was similar between leaves and roots, and for some it was produced overwhelmingly in roots. Finally, while we found a compound that we could not identify – p33.1_415, it did not seem to be

any of the mid-chain substituted hydroxy and dihydroxy C_{14}, C_{15}, C_{16} and C_{18} acids that have been identified as foliage-specific in other studies.

The compound with the highest concentration in roots was 17-hydroxyheptadecanoic acid (54,112 μg g^{-1}C; Table 5-2; Fig. 5-1). This compound has never been reported as a root biomarker, but we have detected it not only in subalpine fir, but also in Engelmann spruce roots (data not shown). The fragmentation pattern in Fig. 5-1 clearly suggests a ω-hydroxy fatty acid (a difference of 16 between the target ion and the closest ion on the left). Furthermore, the fact that the calculated target ion for this compound is 415, which matches the fragmentation pattern, and that this compound lies between ω-OH-C_{16} and ω-OH-C_{18} makes us confident that it is 17-hydroxyheptadecanoic acid.

We are not aware of a study where $C_{12:1}$DA, C_{21}FA, C_{23}-ol, w-OH-C_{19}, w-OH-$C_{20:1}$, w-OH-C_{21}, $C_{20:1}$DA, and w-OH-C_{23} (Table 5-2) have ever been reported as a foliage or root biomarkers. We included the fragmentation patterns of each of them in Appendix D.

We found 7 compounds that we classified as source-specific for both aspen and conifer roots – w-OH-$C_{18:1}$, $C_{18:1}$DA, w-OH-C_{20}, w-OH-C_{22}, C_{22}DA, C_{24}DA, and p60.0_451. These compounds (except the unidentified one) have been found to be root-specific in many other studies (Otto and Simpson, 2006; Mueller et al., 2012; Spielvogel et al., 2014; Angst et al., 2016; Pisani et al., 2016). Indeed, when comparing 11 tree species Mueller et al. (2012) found that across all species, α,ω-diacids and ω-OH acids with chain length ≥20 were typically much more abundant in roots than leaves.

Furthermore, their results showed n-alcohols and n-acids with chain length ≥ 27 were primarily or exclusively present in leaves. We, however, did not find many compounds that had that long of a chain length, and no compound that was found to be foliage specific for aspen and fir. The study by Mueller et al. (2012) also showed that roots of different species are more similar in regard to biomarkers than foliage.

The compounds with the highest concentration in conifer soil were the root-specific compounds ω-OH-C_{17}, ω-OH-$C_{18:1}$, $C_{18:1}$DA, p60.0_451 (3815, 1143, 1222, 1133 µg g^{-1}C, respectively), and the foliage-specific compound 9,16-diOH C16 (2422 µg g^{-1}C). The three compounds ω-OH-$C_{18:1}$, $C_{18:1}$DA, and x,16-diOH C_{16} have been reported to have high concentrations in conifer soil also by Spielvogel et al. (2014) and Otto and Simpson (2006) with x,16-diOH C_{16} to be the compound with the highest concentration in conifer soil. Similar to aspen soils, the majority of compounds identified as conifer foliage- and conifer root-specific decreased by 80 to 90% in soil when compared to the concentrations observed in plant tissues. One foliage-specific compound ω-OH-C14, and four root-specific compounds ω-OH-C18:1, ω-OH-C19, C18:1DA, ω-OH-C20:1 decreased by 93 to 98%, while C12:1DA and p60.0_451 decreased by only 70 and 50% respectively. 9,16-diOH C16 decreased by almost 99%, but still was one of the most abundant compounds in soil. No compound showed an increase in concentration.

There are only few published biomarker degradation studies. The study by Angst et al. (2016) is the only one that described suberin decomposition. They reported that the percentage of all acids remaining at the end of an 84 day incubation was approximately 33% and 19% for beech leaves and roots, and 43% and 23% for spruce needles and roots.

This is much higher than the values observed in soil, and does not provide a good estimate on the long-term stability of these compounds. Opsahl and Benner (1995) found that the most abundant cutin monomer x,16-OH C16 decreased to about 20% of the initial concentration after 4 years in mangrove leaves, and to about 1% in cypress needles, which corresponded also to the overall cutin loss (20% in mangrove leaves, and 1% in cypress needles). Both studies suggest that the degradation of cutin and suberin monomers is species specific. Therefore, after identifying cutin and suberin compounds for aspen and subalpine fir, our next step will be to characterize their degradation patterns to better understand the relationship between cutin and suberin stability originating from aspen and conifer forests.

Conclusions

In this study we identified a considerable number of aspen and subalpine fir foliage- and root-specific compounds. As they were found also in soil they can be used as molecular markers in determining the source of SOC in aspen and conifer forests. Many of the cutin and suberin compounds identified in this study corresponded well with findings from other studies. Specifically, mid-chain hydroxy acids have often been identified as foliage specific, and ω-hydroxy fatty acids and diacids have been often reported to be suberin specific.

We did also find compounds that were not reported in published studies, or were excluded as source indicators for reasons discussed in the text. Considering markedly lower concentrations of all compounds (expressed on a per C unit basis) in soil compared to plant tissues, it is logical to conclude that all of these compounds degrade. This may be

problematic for using cutin to suberin ratios in soil to relate to specific plant tissue origin, especially if degradation rates are unequal for cutin and suberin derivatives. Therefore, the next step of this study is to evaluate the degradation patterns in mineral soil of the compounds identified here.

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Tables and Figures

Table 5-1. Aspen foliage-specific (AL) and root-specific (AR) biomarker concentrations in plant tissue and soil (AS) identified from base hydrolysis extractions as trimethylsilyl ethers. Grey shading indicates root specific compounds, and no-shading indicates foliage specific compounds.

			(μg g^{-1}C)		
Compound name	**Abbreviation**	**RT (min)**	**AL**	**AR**	**AS**
p-hydroxybenzoic acid	Pd	9.2	112	5340	576
m-hydroxybenzoic acid	mBd	11.4	n.d.	144	50
Unidentified	p14.6_284	14.6	n.d.	509	102
Tetradecanoic acid	C14FA	15.3	1171	63	239
Unidentified	p19.6_331	19.6	630	n.d.	73
Ferulic acid	Fd	20.1	96	1213	232
1-octadecanol	C18-ol	21.5	2006	163	213
18-hydroxy octadecenoic acid	ω-OH-C18:1	33.0	1257	73,089	928
8or10, 16-dihydroxy hexadecanoic acid	8or10,ω-diOH C16	33.5	131,583	623	5381
1,18-octadecenoic diacid	C18:1 DA	35.7	479	10,761	471
7or8,16-hexadecanoic diacid	7or8 OH C16DA	36.3	4680	134	1400
20-hydroxy eicosanoic acid	ω-OH-C20	40.1	116	3158	522
Unidentified	p42.0_317	42.0	22,339	1339	61
1,20 eicosanoic diacid	C20 DA	42.9	38	1290	159
22-hydroxy dodecosanoic acid	ω-OH-C22	46.3	520	6722	1236
Hexacosanoic acid	p47.7_454 C26FA	47.7	539	43	119
1,22-dodecosanoic diacid	C22DA	48.9	91	1601	208
Octacosanoic acid	p53.6_482 C28FA	53.6	1364	44	116
1,24-tetracosanoic diacid	C24DA	54.7	n.d.	156	125
26-hydroxy hexacosanoic acid	ω-OH-C26	57.8	n.d.	64	105
Unidentified	p60.0_451	60.4	n.d.	19,566	598

Table 5-2. Conifer foliage—specific (CN) and root-specific (CR) biomarker concentrations in plant tissue and soil (CS) identified from base hydrolysis extractions as trimethylsilyl ethers. Grey shading indicates root specific compounds, no shading indicates foliage-specific compounds.

			(μg g^{-1}C)		
Compound name	**Abbreviation**	**RT (min)**	**CN**	**CR**	**CS**
Dodecanol	C12-ol	10.2	853	n.d.	76
Dodecenoic diacid	C12:1DA	21.5	n.d.	298	91
14-hydroxytetradecanoic acid	w-OH-C14	22.8	13,435	1386	210
Unidentified	p25.9_353	25.8	n.d.	280	52
17-hydroxyheptadecanoic acid	ω-OH-C17	28.9	1510	54,112	3815
Heneicosanoic acid	C21FA	31.3	n.d.	848	64
18-hydroxyoctadecanoic acid	w-OH-C18	31.8	n.d.	814	78
18-hydroxy octadecenoic acid	ω-OH-C18:1	32.7	1250	27,586	1143
Unidentified	p33.1_415	33.1	3151	n.d.	231
9,16 dihydroxy hexadecanoic acid	9,16-diOH C16	33.5	193,438	16,127	2422
1-tricosanol	C23-ol	34.7	92	6235	471
19-hydroxynonadecanoic acid	ω-OH-C19	35.1	82	883	55
18-octadecenoic diacid	C18:1DA	35.4	1508	19,938	1222
20-hydroxy eicosenoic acid	ω-OH-C20:1	38.9	n.d.	883	52
20-hydroxy eicosanoic acid	ω-OH-C20	39.9	n.d.	8212	819
21-hydroxy heneicosanoic acid	ω-OH-C21	41.2	n.d.	2150	160
Eicosenoic diacid	C20:1DA	41.6	n.d.	492	49
9,10,18-trihydroxyoctadecanoic acid	triOH-C18	43.0	7243	n.d.	813
Unidentified	p43.5_149	43.3	n.d.	7753	570
22-hydroxy dodecosanoic acid	ω-OH-C22	46.0	498	8667	955
23-hydroxy tricosanoic acid	ω-OH-C23	47.1	146	4515	347
1,22-dodecosanoic diacid	C22DA	48.6	219	3918	429
1,24-tetracosanoic diacid	C24DA	54.7	n.d.	491	50
Unidentified	p60.0_451	59.8	81	2146	1133

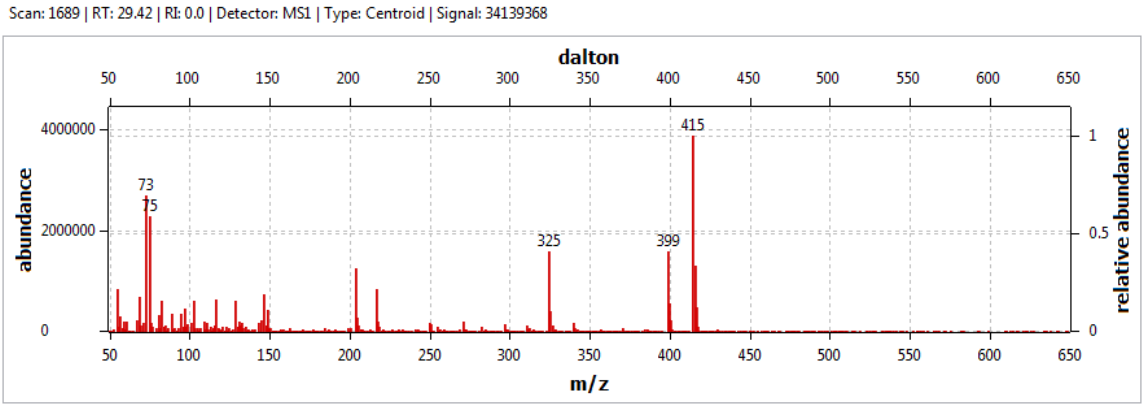


Fig. 5-1. Fragmentation pattern of 17-hydroxyheptadecanoic acid in conifer roots.

CHAPTER 6

SUMMARY AND CONCLUSIONS

As shown throughout this dissertation, soil organic carbon (SOC) under aspen in Utah has been shown to be significantly higher than under conifers. The similar climate, topography, parent material, and time of establishment of these forests suggest that the differences are driven by vegetation. In this dissertation I aimed to examine the effect of above- and belowground detritus inputs of these two contrasting forest types – aspen vs. conifer – on their respective SOC pools.

In the first chapter of this dissertation I compared SOC stocks under adjacent hardwood and conifer forests worldwide to determine how vegetation type affects forest floor and mineral SOC. While conifer forests stored significantly more SOC in the forest floor, this vegetation effect did not translate into mineral soil, as mineral SOC stocks were similar between both overstory types. A genus level analysis revealed some genera that showed an overall positive effect on SOC pools (*Eucalyptus* and *Picea*, as well as *Pinus* when compared to *Quercus*) in comparison to their conifer or hardwood neighbors. Interestingly, even in cases when an overall effect of vegetation was not found for a specific overstory type, there were always exceptions to the general trend where a strong effect was reported. This indicates that forest vegetation effects on SOC should be investigated on a local and regional scale if SOC storage is a management goal. Meaning, the same genus or species can have a different effect on SOC pools under different environmental conditions. This chapter allowed to put aspen and conifer forests in the Intermountain West, USA into a broader perspective. Such large differences, as found

under aspen and conifer in Utah, have been observed in other places, but an overall forest type effect was not found. In fact, cases from Canada and Minnesota showed that even the effect of aspen vs. conifer, that has been observed in Utah, is not present on a continental scale.

In the second chapter I compared aboveground and belowground detritus C fluxes between aspen and conifer stands in Utah. With my work I expanded the spatial scope of previous studies, which were all located in northern Utah, by measuring SOC pools for up to 50 cm depth at four sites at Cedar Mountain in southern Utah. I confirmed previous findings that aspen have higher mineral SOC pools, and most of this C is associated with the silt and clay fraction, which makes it also more stable. While aspen had higher aboveground litterfall, the amount of C transported into the mineral soil with snowmelt water was lower than under conifer stands. Fine root biomass C was twice as high under conifers as under aspen when calculated from root core samples. Minirhizotron data, however, revealed the opposite pattern. The results did not provide a clear indication of whether above- or belowground detritus input was driving the differences between SOC pools under aspen and conifer forest stands. This suggests that detritus C input fluxes in the sites studied do not necessarily have a direct relationship with the size of the SOC pools, and that SOC sequestration under aspen and conifer forests in Utah is driven more by the chemistry of the organic matter in either its water soluble form or as particulate organic matter

In the third chapter I compared the retention (sorption and microbial assimilation) of aspen and conifer foliage and root leachates on aspen and conifer soils, using a batch

sorption study approach. I found that aspen leaves (AL) differed significantly from aspen root sorption, with all four sorption parameters – k and n (describing the sorption curve shape), null point concentration (NPC; net sorption = net desorption), and endpoint (EP, sorption at the highest DOC concentration added) –indicating a higher sorption of AL. Leachates from conifer needles and roots showed very similar sorption behavior, and root leachate sorption from both sources was more similar than foliage leachate sorption. Sorption commenced at lower DOC concentrations for deeper soils with lower SOC concentrations, and Al and Fe concentrations, as expressed by site differences, affected the shape of the sorption curves (parameter k). Soil forest type – aspen vs. conifer – was the soil factor with the strongest effect on leachate retention. Soils sampled from aspen stands showed lower initial desorption and higher sorption than soils from conifer stands for all of the DOC solutions applied. This finding suggests that aspen SOC has a positive effect on the retention of new C.

To further evaluate how detritus inputs (quantity and quality) are linked to SOC stabilization, a more accurate characterization of the direct contributions of foliage- and root-derived compounds to the mineral associated SOC is required. In recent years foliage- and root-specific biomarkers (cutin and suberin) have been applied in various soils to determine the source of SOC. As the first step in identifying the plant source of SOC under aspen and conifer stands in Utah, in the fourth chapter of this dissertation, I identified foliage- and root-specific biomarkers for aspen and subalpine fir. In total I found 19 cutin and suberin constituents that were source-specific for aspen foliage and roots, and 24 for conifer foliage and roots. For aspen 11 compounds were root specific,

and 8 were leaf specific. For conifers 5 were needle-specific, and 19 were root-specific. Several mid-chain hydroxy acids identified in this study matched well with foliage-specific biomarkers identified in other studies. Similarly, I also identified several ω-hydroxy fatty acids and diacids that have been reported as suberin-specific in other studies. I also found several compounds to be source-specific that have not been reported in other studies, e.g., odd numbered ω-hydroxy fatty acids in conifer roots or mid-chain alcohols and benzyls in aspen and conifer foliage. Most foliage and root-specific compounds were found in both tree species examined, but were not always found to be root or foliage specific for both of them, e.g., 9,10,18-trihydroxyoctadecanoic acid was found to be needle-specific, but not aspen foliage-specific. Chapter 4 is only the first from several studies that will aim to investigate the source of SOC under aspen and conifer stands. The next step will be to evaluate biomarker degradation from a 10-month soil incubation, which will provide insights about the origin of the identified and unidentified compounds.

While there is no overall forest overstory effect on SOC when comparing hardwoods and conifers, exceptions to this general pattern are common in the world's forests. Aspen and conifer forests in the Intermountain West present one of these exceptions with aspen having higher and more stable mineral SOC pools than conifers. Most SOC models assume that equilibrium C stocks are linearly proportional to C inputs, and, given a similar climate, outputs are determined by the quality of the litter. Following this assumption the higher aspen aboveground litterfall would be countered by the higher conifer belowground C flux, rendering the NPP of aspen and conifer forests in Utah

similar. Given the lower quality of conifer foliage, the decomposition would be lower, resulting in higher predicted SOC pools. The field observations, however, show the opposite pattern. The explanation for this observation seems to lie in the quality of the substrate dominating the C fluxes. Aspen C, especially the very labile aspen foliage DOC, seems to increase the effective C saturation of soils compared to conifer C. The results reported in this dissertation show the importance of vegetation type and litter quality for SOC pools and their stability. The more labile substrate resulting in the higher and more stable SOC pools supports the Microbial Efficiency – Matrix Stabilization framework proposed by Cotrufo et al. (2013). The more labile substrate, after being incorporated into soil, positively affects retention of new C, and suggests that not only abiotic soil properties drive the effective C saturation of soil, but also the quality of the inputs.

While some questions were answered during this dissertation many new ones were formed. The next steps should include separating the effects of sorption and microbial assimilation on DOC retention in aspen and conifer soils, and the evaluation of microbial assimilation vs. mineralization rates for foliage and root substrates. More work needs to be done in identifying the importance of various detritus flux incorporation and stabilization pathways into soil, from DOC to particulate organic matter.

APPENDICES

APPENDIX A – PUBLICATIONS USED AS DATA SOURCES IN META-ANALYSIS

Table A-1. Data sources used in the meta-analysis of SOC storage differences between hardwood and conifer stands

Reference	Location	Dominant hardwood genera	Dominant conifer genera
Alban et al., 1978	Minnesota USA	*Populus*	*Picea, Pinus*
Alriksson and Eriksson, 1998	NE Sweden	*Betula*	*Larix, Picea, Pinus*
Andreux et al., 2002	Central France	*Fagus*	*Pseudotsuga*
Armas-Herrera et al., 2012	Canary Islands Spain	*Laurus*	*Pinus*
Ashagrie et al., 2005	Central Ethiopia	*Eucalyptus*	*Podocarpus*
Berger et al., 2010	NE Austria	*Fagus*	*Picea*
Bini et al., 2013	S Brazil	Mixed hardwoods	*Araucaria, Pinus*
Borken et al., 2002	Central Germany	*Fagus*	*Picea, Pinus*
Charro et al., 2010	W Spain	*Quercus*	*Pinus*
Chen et al., 2005	SE China	*Castanopsis,* mixed hardwoods, *Ormosia*	*Cunninghamia, Fokienia*
Chen et al., 2012	NE China	Mixed hardwoods	*Cunninghamia*
Cole et al., 1995	Washington USA	*Alnus*	*Pseudotsuga*
Compton and Boone, 2000	Massachusets USA	Mixed hardwoods	Mixed conifers
Compton et al., 1998	Massachusets USA	*Populus, Quercus*	*Pinus*
Cook, 2012	S Brazil	*Eucalyptus*	*Pinus*
Díaz-Pinés et al., 2011	Central Spain	*Quercus*	*Pinus*

Table A-1 continued

Dijkstra and Fitzhugh, 2003	Connecticut USA	*Acer, Fagus, Fraxinus, Quercus*	*Tsuga*
Gartzia-Bengoetxea et al., 2009	NE Spain	*Fagus, Quercus*	*Pinus*
Goh and Heng, 1987	Central New Zealand	*Nothofagus*	*Pinus*
Gomes da Silva et al., 2009	Central Brazil	*Eucalyptus, Sclerolobium*	*Pinus*
Gurmesa et al., 2013	Denmark	*Fagus, Quercus*	*Larix, Picea*
Hansson et al., 2011	SW Sweden	*Betula*	*Picea, Pinus*
Huygens et al., 2005	Central Chile	*Nothofagus*	*Pinus*
Ichikawa et al., 2004	Central Japan	Mixed hardwoods	*Cryptomeria*
Jiang et al., 2010	S China	*Liquidambar*, mixed hardwoods, *Schima*	*Pinus*
Kasel and Bennett, 2007	SE Australia	*Eucalyptus*	*Pinus*
King and Campbell, 1994	Central Zimbabwe	*Brachystegia, Eucalyptus*	*Pinus*
Kulakova, 2012	SE Russia	*Quercus*	*Pinus*
Ladegaard-Pedersen et al., 2005	Denmark	*Fagus,Quercus*	*Abies, Larix, Picea, Pinus, Pseudotsuga*
Laganiere et al., 2013	Ontario & Quebec Canada	*Populus*	*Picea, Pinus*
Lakshmanan, 1962	Ohio USA	*Acer, Carya, Liriodendron*, mixed hardwoods, *Quercus*	*Pinus*

Table A-1 continued

Lee et al., 2009	N South Korea	*Quercus*	*Pinus*
Lemenih et al., 2004	Central Ethiopia	*Eucalyptus*	*Cupressus*, mixed conifers
Lemma et al., 2006	SW Ethiopia	*Eucalyptus*, mixed hardwoods	Cupressus, Pinus
Li et al., 2005	NE Puerto Rico	Mixed hardwoods	*Pinus*
Liang et al., 2007	Michigan USA	*Acer, Tilia*	*Tsuga*
Luan et al., 2010	S China	Mixed hardwoods	*Cunninghamia*
Matos et al., 2010	NE Germany	*Quercus*	*Pinus*
Michalzik & Gruselle, unpublished data, 2013	Central Germany	*Fagus*	*Pinus*
Morris et al., 2007	Michigan USA	Mixed hardwoods	*Pinus*
Mueller et al., 2012	Central Poland	*Acer, Betula, Carpinus, Fagus, Quercus, Tilia*	*Abies, Larix, Picea, Pinus, Pseudotsuga*
Nihlgard, 1971	S Sweden	*Fagus*	*Picea*
Noh et al., 2012	Central South Korea	*Quercus*	*Abies*
Olsen and Van Miegroet, 2010	Utah USA	*Populus*	Mixed conifers
Olsson et al., 2012	N Finland	*Betula*	*Picea, Pinus*
Oostra et al., 2006	S Sweden	*Carpinus, Fagus, Fraxinus, Quercus, Ulmus*	*Picea*
Ovington, 1956	S United Kingdom	*Alnus, Betula, Castanea, Fagus, Nothofagus, Quercus*	*Abies, Chamaecyparis, Larix, Picea, Pinus, Pseudotsuga, Thuja, Tsuga*

Table A-1 continued

Paul et al., 2003	*Ontario Canada; Ohio USA*	*Acer, Mixed hardwoods*	*Pinus*
Priha and Smolander, 1999	S Finland	*Betula*	*Picea, Pinus*
Richards et al., 2007	E Australia	Mixed hardwoods	*Araucaria*
Riestra et al., 2012	Central Argentina	*Eucalyptus, Gleditsia*	*Pinus*
Ritter, 2007	E Iceland	*Betula*	*Larix*
Roman-Dobarco, unpublished data, 2013	Utah USA	*Populus*	Mixed conifers
Russell et al., 2007	E Costa Rica	*Hyeronima, Pentaclethra, Virola, Vochysia*	*Pinus*
SanClements et al., 2010	Maine USA	Mixed hardwoods	Mixed conifers
Schulp et al., 2008	Central Netherlands	*Fagus, Quercus*	*Larix, Pinus, Pseudotsuga*
Scott and Messina, 2010	Texas USA	*Quercus*	*Pinus*
Sevgi et al., 2011	NW Turkey	*Quercus*	*Abies, Cedrus, Picea, Pinus*
Shugalei, 2005	Central Russia	*Betula, Populus*	*Larix, Picea, Pinus*
Shukla et al., 2006	New Mexico USA	*Quercus*	*Juniperus, Pinus*
Sigurðardóttir, 2000	E Iceland	*Betula*	*Larix, Pinus*
Son and Gower, 1992	Wisconsin USA	*Quercus*	*Larix, Picea, Pinus*
Stolpe et al., 2010	Central Chile	*Nothofagus*	*Pinus*

Table A-1 continued

Turner and Kelly, 1977	E Australia	*Eucalyptus*	*Pinus*
Turner et al., 1985	SE Australia	*Eucalyptus*	*Pinus*
Turner and Lambert, 1988	E Australia	*Eucalyptus*	*Pinus*
Turner and Lambert, 2000	E Australia	*Eucalyptus*	*Pinus*
Ulrich et al., 1971	Central Germany	*Fagus*	*Picea*
Vesterdal et al., 2002	E Denmark	Mixed hardwoods, *Quercus*	*Picea*
Vesterdal et al., 2008	Denmark	*Acer, Fagus, Fraxinus, Quercus, Tilia*	*Picea*
Wang and Wang, 2007	SE China	Mixed hardwoods	*Cunninghamia*
Wang et al., 2007	SE China	*Michelia*	*Cunninghamia*
Wang et al., 2010	S China	*Castanopsis, Michelia, Mytilaria*	*Pinus*
Woldeselassie et al., 2012	Utah USA	*Populus*	Mixed conifers
Yang et al., 2005	SE China	Mixed hardwoods	*Cunninghamia*
Curiel Yuste et al., 2005	NE Belgium	*Quercus*	*Pinus*
Zhiyanski et al., 2008	Central Bulgaria	*Fagus*	*Picea*

Table B-1. Initial Mass, Langmuire and Freundlich fitted isotherm model parameters for each soil and leachate type

	Initial mass					Langmuir					Freundlich			
	AL	AR	CN	CR		AL	AR	CN	CR		AL	AR	CN	CR
TA 0-10														
Intercept	-207.1	-234.5	-214.7	-228.8	**Intercept**		-255.7			**Intercept**		-255.7		
Slope	6.71	4.41	3.57	4.68	**Q**	1285	1266	418.33	1142	**k**	25.48	11.15	27.48	13.65
					b	0.0097	0.0051	0.027	0.0065	**n**	1.41	1.25	1.84	1.31
NPC	30.86	53.18	60.13	48.89	**NPC**	25.61	49.63	58.24	44.39	**NPC**	25.83	50.19	60.6	46.46
R^2	0.966	0.986	0.76	0.976	**R^2**	0.998	0.997	0.96	0.992	**R^2**	0.9998	0.999	0.93	0.996
RMSE	31.35	13.54	43.01	18.44	**RMSE**	8.8	7.78	20.51	12.24	**RMSE**	2.55	4.8	27.72	8.96
AIC	54.64	46.24	57.8	49.34	**AIC**	41.93	40.7	50.4	45.24	**AIC**	29.56	35.87	53.41	42.12
40-50														
Intercept	-77.6	-111	-121.1	-115.7	**Intercept**		-140.7			**Intercept**		-140.7		
Slope	2.18	1.8	1.38	2.8	**Qo**	256.56	226.79	156.73	538.1	**k**	53.9	20.21	13.54	12.32
					b	0.065	0.031	0.032	0.01	**n**	3.12	2.09	2.03	1.48
NPC	35.57	61.67	87.77	41.31	**NPC**	18.67	52.67	0.967	35.39	**NPC**	19.93	57.67	115.75	36.74
R^2	0.57	0.75	0.74	0.95	**R^2**	0.975	0.98	273.34	0.961	**R^2**	0.95	0.95	0.94	0.99
RMSE	43.92	25.84	17.49	16.02	**RMSE**	12.19	8.52	7.22	13.12	**RMSE**	17.29	13.54	10.02	9.21
AIC	58.01	52.71	48.8	47.93	**AIC**	45.2	41.61	39.96	45.93	**AIC**	48.69	46.25	43.23	42.39

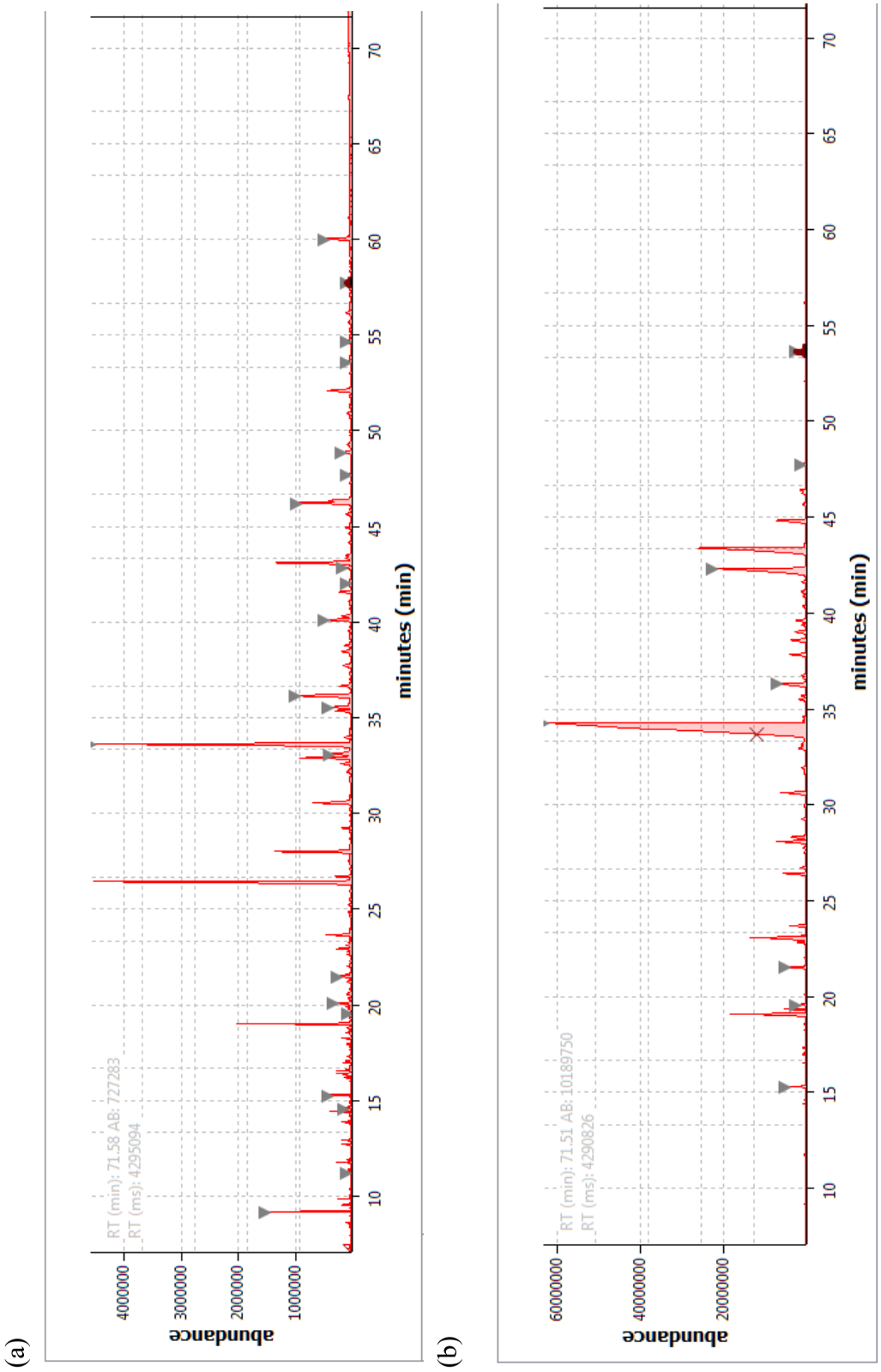
Table B-1 continued

TC 0-10														
Intercept	-280	-349.8	-322.6	-328.5	**Intercept**		-361.3			**Intercept**		-361.3		
Slope	2.86	2.66	3.75	1.71	**Q**	319.52	1736	543	213.48	**k**	71.61	5.21	22.22	22.19
					b	0.074	0.0019	0.016	0.034	**n**	3.19	1.17	1.66	2.23
NPC	97.9	131.49	86.25	192.09	**NPC**	N.D.	138.33	124.29	N.D.	**NPC**	171.69	142.57	102.44	503.66
R^2	0.63	0.968	0.924	0.82	**R^2**	0.977	0.969	0.964	0.978	**R^2**	0.98	0.975	0.984	0.996
RMSE	51.32	12.57	23.91	19.53	**RMSE**	14.9	14.16	18.87	7.97	**RMSE**	14.55	12.76	12.63	3.3
AIC	59.57	45.51	51.93	49.91	**AIC**	47.2	46.7	49.57	40.94	**AIC**	46.97	45.66	45.55	32.12
40-50														
		-		-										
Intercept	-86.71	119.27	-92.02	104.17	**Intercept**		-129.87			**Intercept**		-129.87		
Slope	1.35	0.71	1.68	1.38	**Qo**	150.09	88.3	175.39	173.2	**k**	41.17	7.03	32.13	17.21
					b	0.093	0.03	0.061	0.033	**n**	3.65	1.99	2.83	2.19
NPC	64.23	167.99	54.77	75.49	**NPC**	69.06	N.D.	46.77	90.83	**NPC**	66.23	331.47	52.08	83.6
R^2	0.59	0.63	0.65	0.79	**R^2**	0.99	0.835	0.993	0.998	**R^2**	0.998	0.82	0.974	0.986
RMSE	25.87	13.22	25.8	17.61	**RMSE**	4.61	10.21	4.29	2.24	**RMSE**	2.26	10.71	8.11	5.23
AIC	52.72	46	52.69	48.88	**AIC**	35.48	43.42	34.76	28.24	**AIC**	28.33	43.91	41.12	36.73
CMA 0-10														
	-	-	-	-										
Intercept	294.66	318.65	290.74	297.53	**Intercept**		-369.05			**Intercept**		-369.05		
Slope	7.55	3.66	5.1	4.94	**Qo**	1281	529.3	583.6	671	**k**	38.87	29.9	54.51	42.81
					b	0.013	0.02	0.032	0.023	**n**	1.56	1.84	2.11	1.88
NPC	39.03	87.06	57.01	60.23	**NPC**	31.13	115.15	53.75	53.14	**NPC**	33.49	101.91	56.57	57.39
R^2	0.947	0.904	0.83	0.876	**R^2**	0.981	0.966	0.965	0.997	**R^2**	0.994	0.989	0.981	0.998
RMSE	44.75	30.51	50.4	46.08	**RMSE**	30.98	20.87	26.46	8.9	**RMSE**	17.83	12.05	19.5	7.06
AIC	58.2	54.37	59.39	58.49	**AIC**	54.52	50.57	52.95	42.05	**AIC**	49	45.08	49.89	39.73

Table B-1 continued

CMA 40-50														
Intercept	-144.4	-198.7	-166.4	-171.4	**Intercept**		-235.7			**Intercept**		-235.7		
Slope	4.83	2.82	4.56	3.82	**Qo**	585	396.4	515	496.2	**k**	63.29	22.24	48.35	40.87
					b	0.036	0.021	0.033	0.028	**n**	2.25	1.81	2.1	2.05
NPC	29.98	70.45	36.49	44.87	**NPC**	18.75	69.87	25.58	32.32	**NPC**	19.27	71.74	27.85	36.32
R^2	0.81	0.9	0.85	0.84	**R^2**	0.998	0.986	0.987	0.994	**R^2**	0.998	0.994	0.999	0.995
RMSE	57.72	24.05	42.2	41.11	**RMSE**	7.1	10.56	14.42	9.49	**RMSE**	6.84	6.59	3.98	8.03
AIC	60.75	51.99	57.61	57.35	**AIC**	39.79	43.76	46.88	42.69	**AIC**	39.41	39.05	34	41.02
CMC 0-10														
	-	-	-	-										
Intercept	457.55	508.02	444.81	479.02	**Intercept**		-545.93			**Intercept**		-545.93		
Slope	6.81	4.06	4.41	5.36	**Qo**	965.3	701.5	458.09	831.3	**k**	51.16	20.31	87.36	36.3
					b	0.02	0.012	0.062	0.016	**n**	1.78	1.54	2.9	1.71
NPC	67.19	125.13	100.86	89.37	**NPC**	65.09	292.44	N.D.	119.6	**NPC**	67.64	158.97	203.18	103.05
R^2	0.91	0.948	0.649	0.918	**R^2**	0.987	0.989	0.967	0.972	**R^2**	0.999	0.996	0.964	0.99
RMSE	53.03	24.54	68.26	40.08	**RMSE**	22.94	13.16	24.09	26.86	**RMSE**	7.8	7.65	25.2	16.4
AIC	59.9	52.19	62.42	57.1	**AIC**	51.52	45.96	52.01	53.1	**AIC**	40.73	40.54	52.46	48.16
40-50														
	-	-	-	-										
Intercept	132.88	186.75	151.07	168.03	**Intercept**		-210.78			**Intercept**		-210.78		
Slope	2.25	2.22	2.86	2.84	**Qo**	254.74	352.4	301.07	378.9	**k**	76.92	13.28	48.8	26.08
					b	0.1	0.015	0.054	0.025	**n**	3.95	1.64	2.66	1.92
NPC	59.06	84.12	52.82	59.17	**NPC**	47.95	99.22	43.23	50.15	**NPC**	53.61	93.12	49	55.26
R^2	0.515	0.917	0.689	0.861	**R^2**	0.981	0.968	0.984	0.999	**R^2**	0.972	0.981	0.974	0.997
RMSE	49.23	17.13	40.74	28.18	**RMSE**	11.27	12.21	10.53	2.04	**RMSE**	13.63	9.49	13. 72	5.15
AIC	59.15	48.6	57.26	53.58	**AIC**	44.41	45.21	43.73	27.32	**AIC**	46.13	42.69	46.38	36.59

APPENDIX C – ASPEN CHROMATOGRAMS AND FRAGMENTATION PATTERNS



(c)

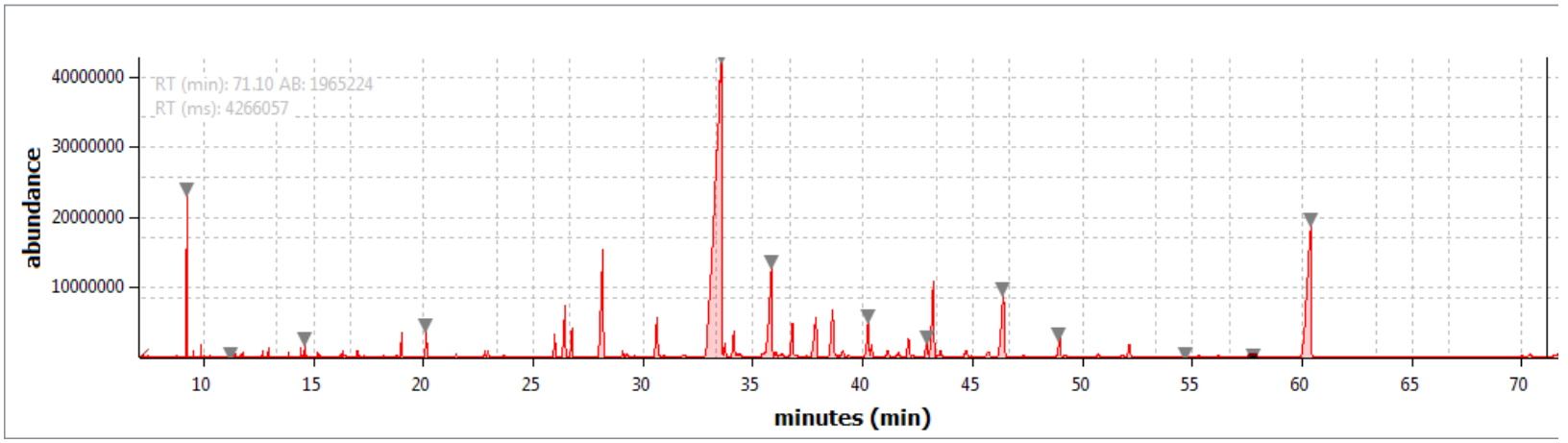
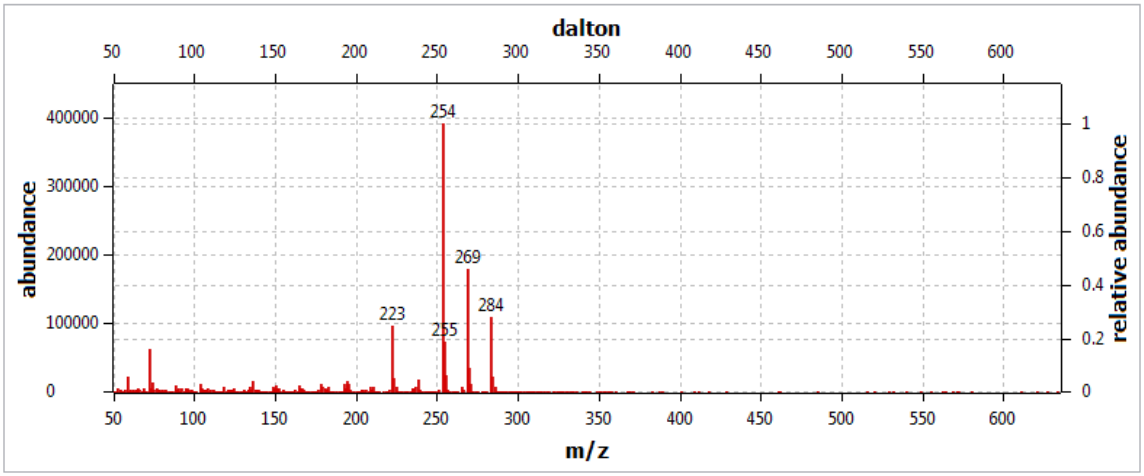
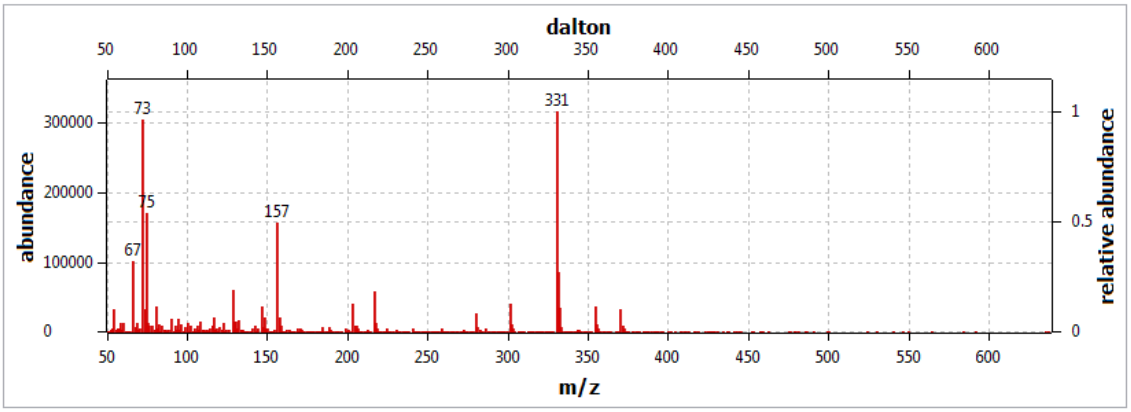


Figure C-1. Full chromatograms of an aspen soil (a), aspen foliage (b) and aspen root (c) extract. The peaks labelled with the grey triangles correspond to the peaks listed in Table 5-1.

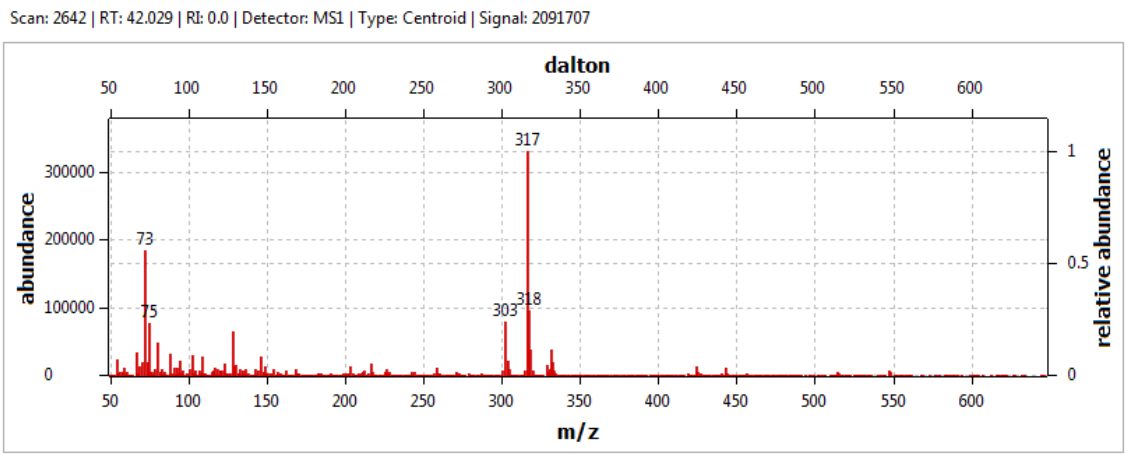
(a) p14.6_284 – aspen roots



(b) p14.6_284 – aspen roots: p19.6_331 – aspen foliage (355 and 371 are the final ions)



(c) p42.0_317 – aspen leaves (332 is the final ion)



(d) p60.0_451 found in aspen roots

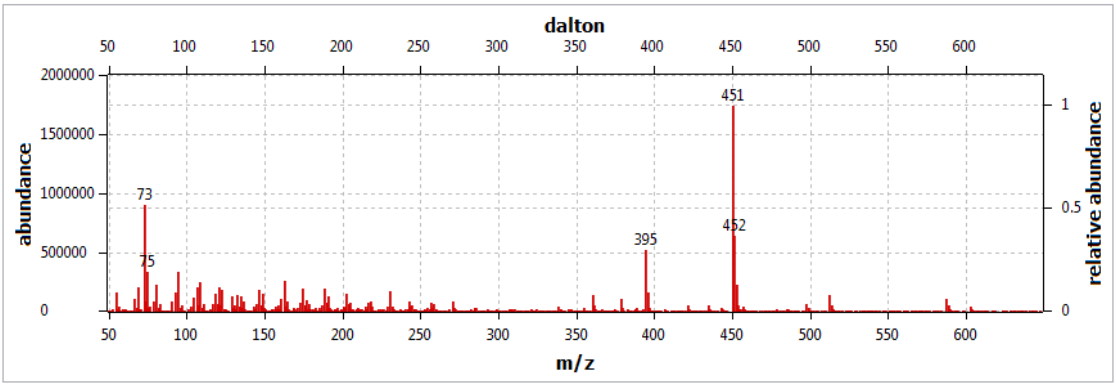
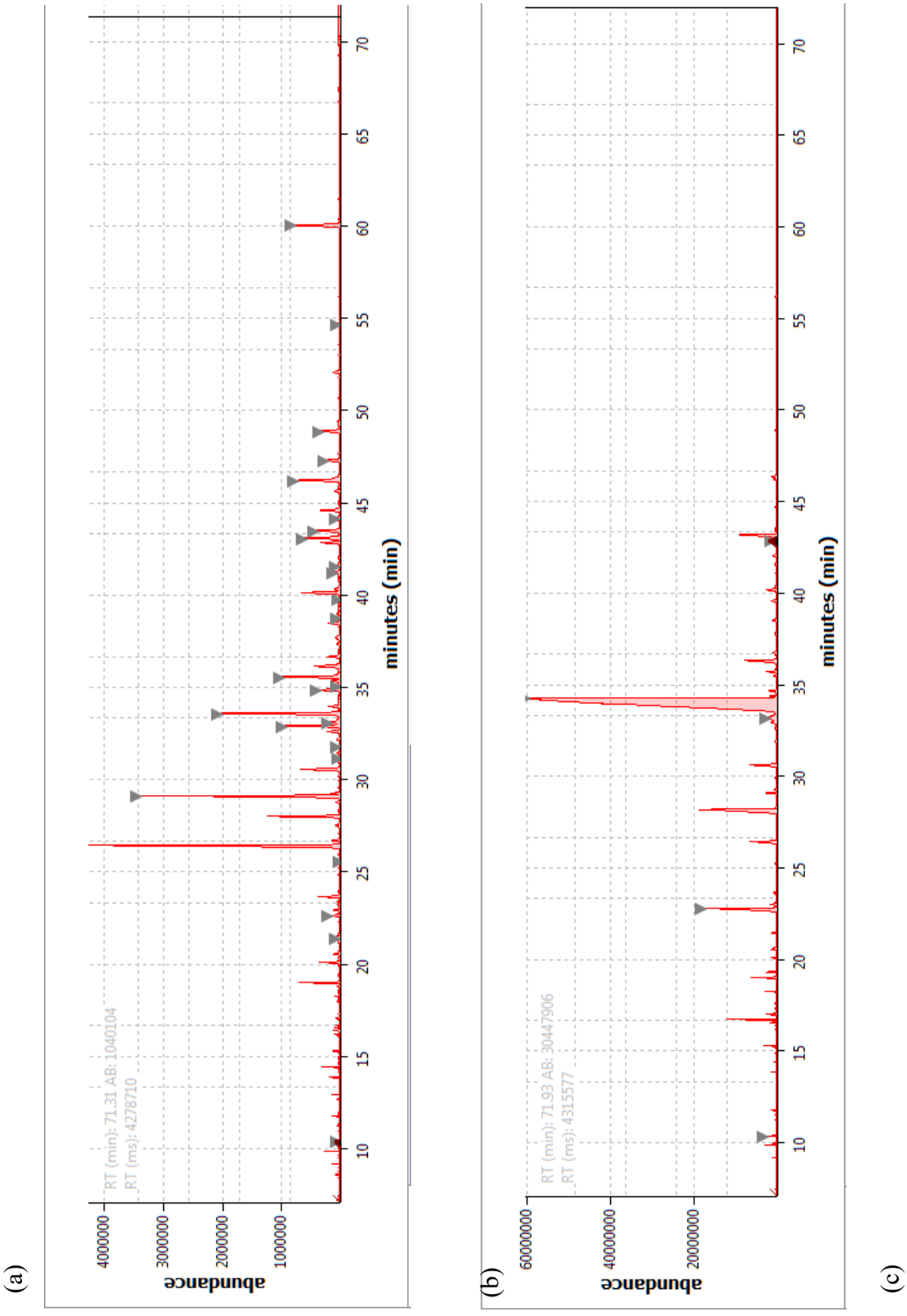


Figure C-2. Fragmentation patterns of unidentified or rarely reported compounds extracted from aspen material – a) p14.6_284; b) p14.6_284; c) p42.0_317; d) p60.0_451.

APPENDIX D – CONIFER CHROMATOGRAMS AND FRAGMENTATION PATTERNS



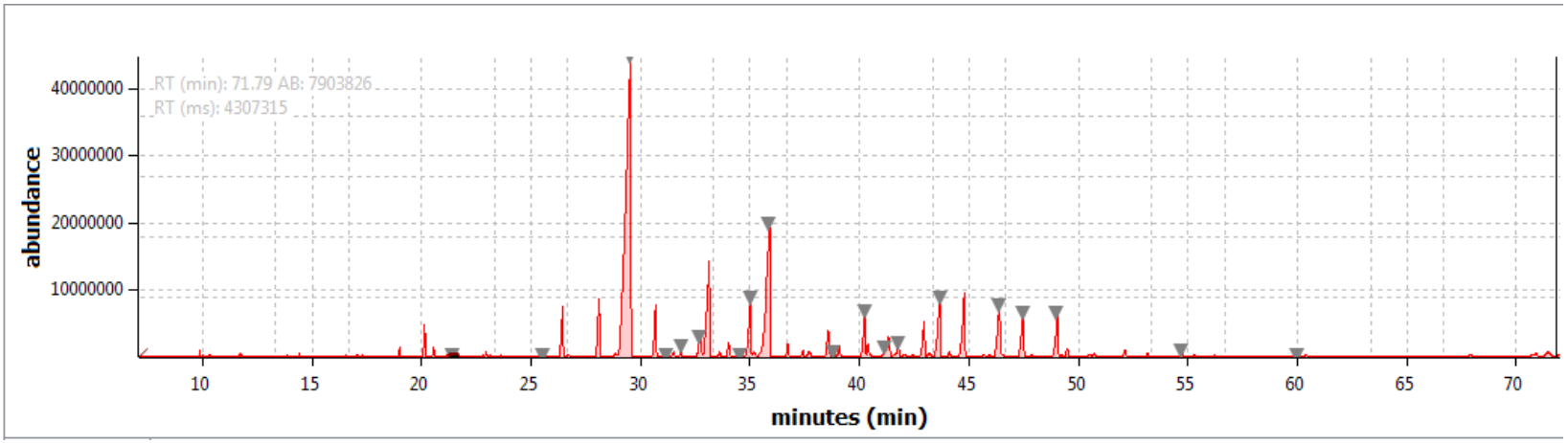
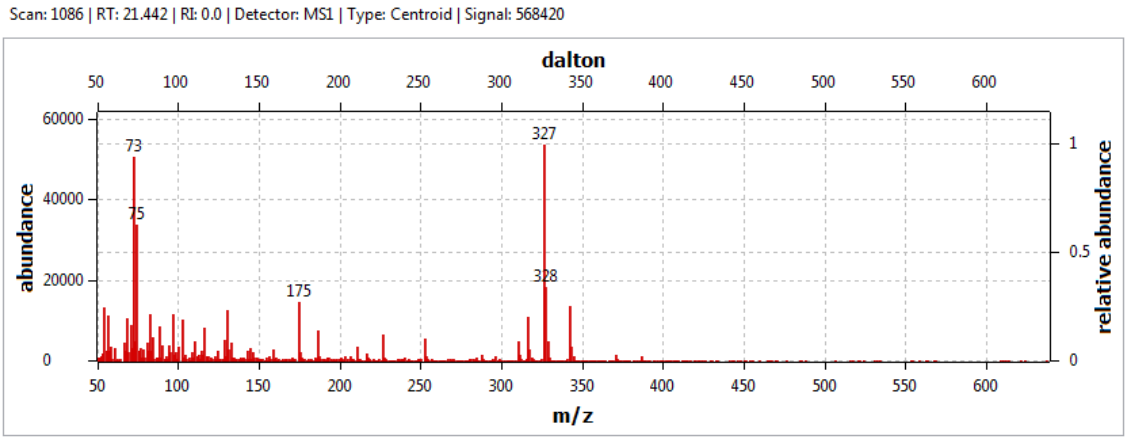
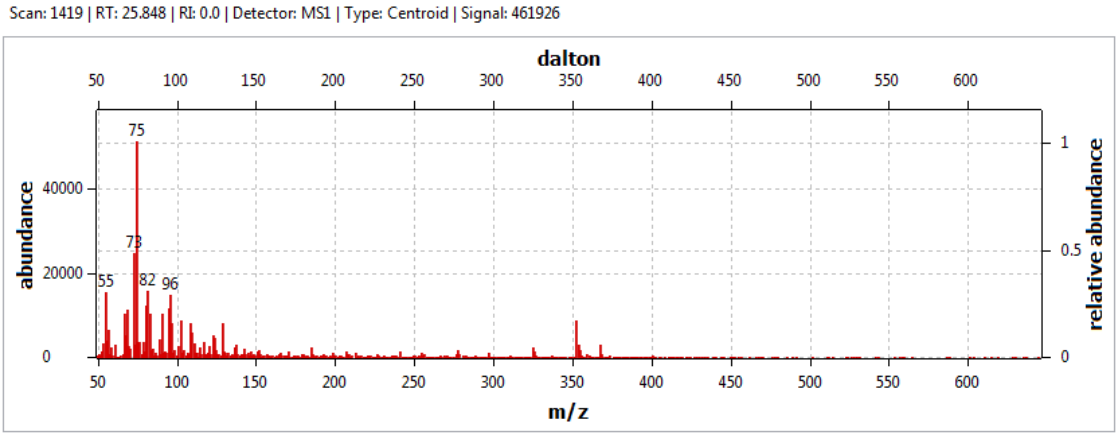


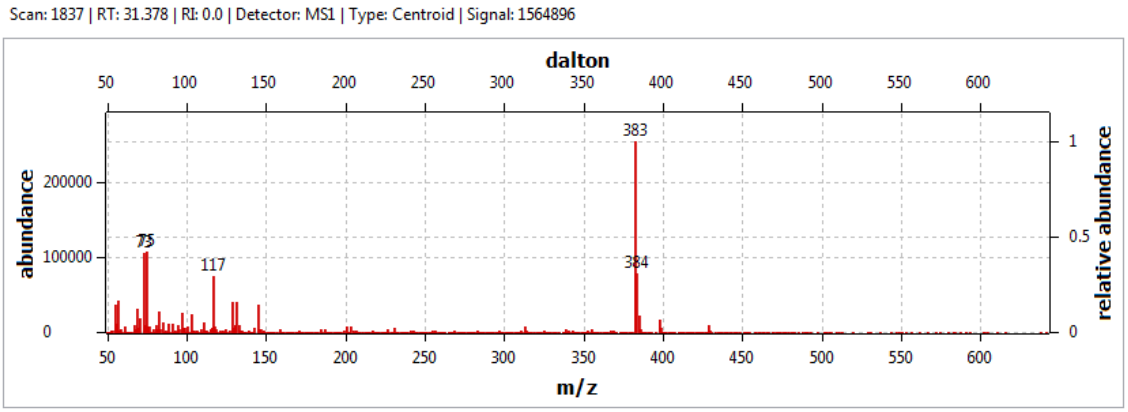
Figure D-1. Full chromatogram of a conifer soil (a), foliage (b) and root (c) extract. The peaks labelled with the grey triangles correspond to the peaks listed in Table 5-2.



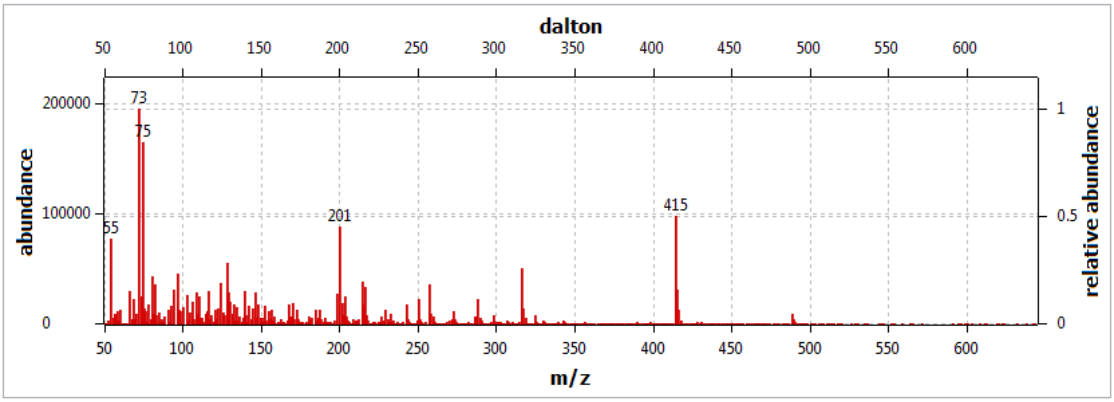
(a) C12:1DA – fir roots (ions 317, 327, 343)



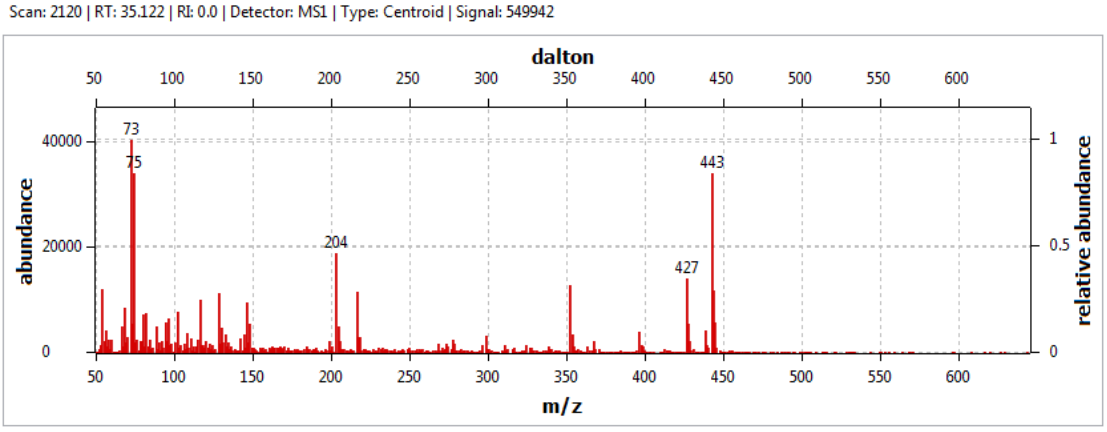
(b) p25.9_353 – fir roots (ions 325, 353, 368)



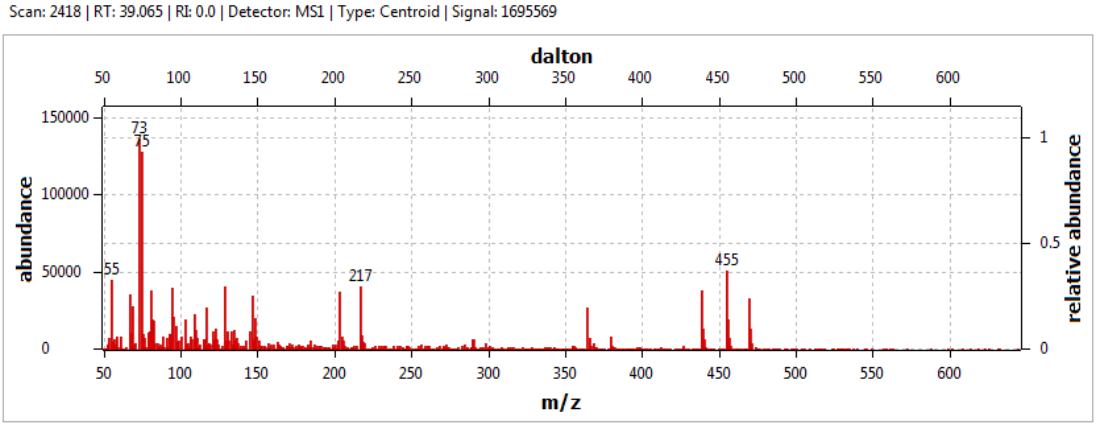
(c) C21FA – fir roots



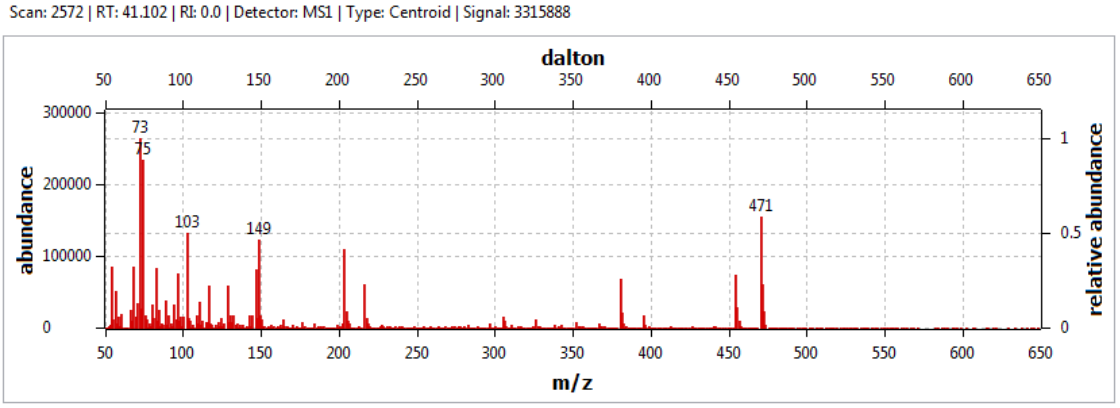
(d) p33.2_415 - needles (ions 317, 415, 489)



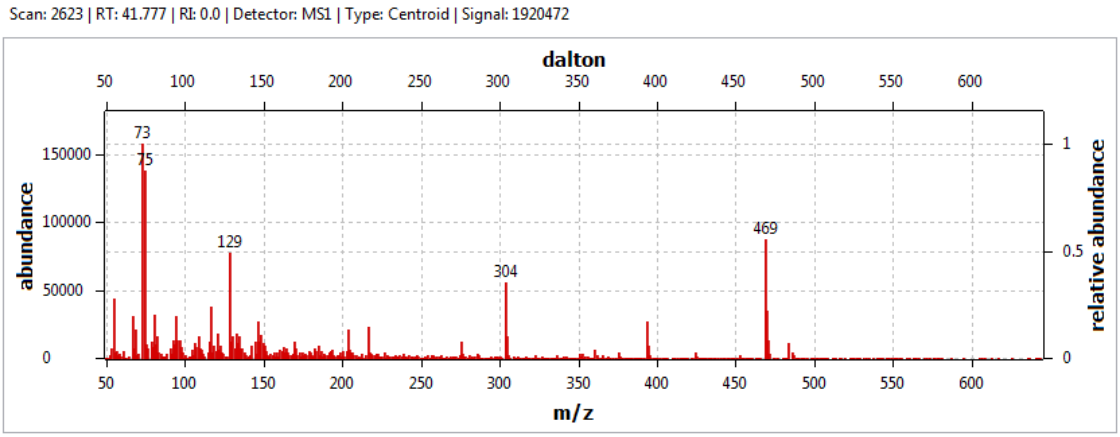
(e) ω-OH-C19 – fir roots (ions 353, 427, 443)



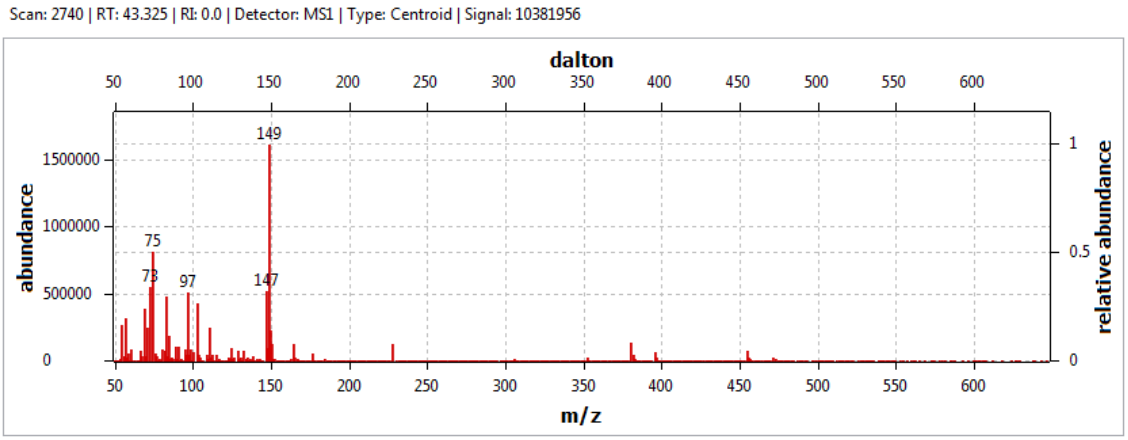
(f) ω-OH-C20:1 – fir roots (ions 365,439,455,470)



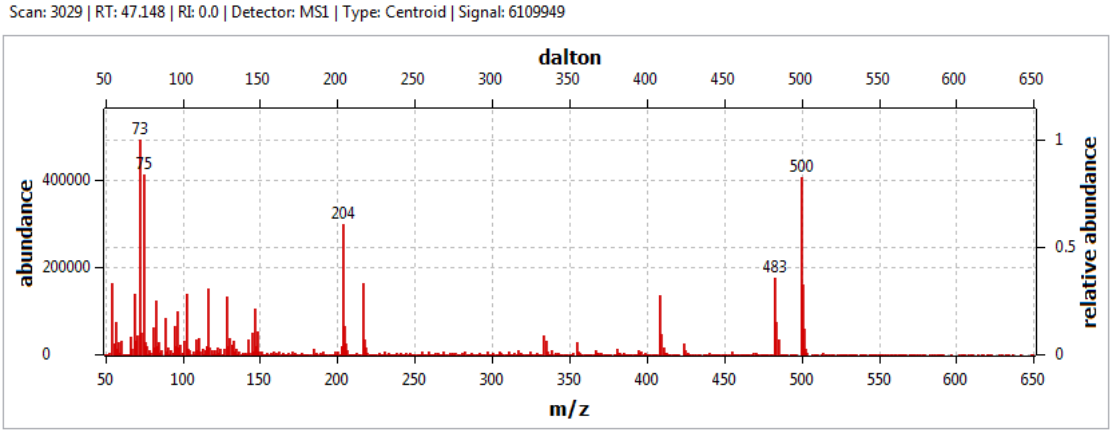
(g) ω-OH-C21 – fir roots (ions 381, 455, 471)



(h) C20:1 DA – fir roots



(i) p43.5_149 – fir roots (ions 381, 396, 455, 472)



(j) ω-OH-C23 – fir roots

Figure D-2. Fragmentation patterns of unidentified or rarely reported compounds extracted from subalpine fir material – a) C12:1DA; b) p25.9_353; c) C21FA; d) p33.2_415; e) ω-OH-C19; f) ω-OH-C20:1; g) ω-OH-C21; h) C20:1 DA; i) p43.5_149; j) ω-OH-C23.

APPENDIX E

Permission-to-reprint and permission-to-use letters

Dear Ms. Lynch,

I am in the process of preparing my dissertation in the Department of Wildland Resources at Utah State University. I hope to complete my degree program at the end of this summer.

The article "Forest overstory effect on soil organic carbon storage: a meta-analysis", of which I am first author, and which appeared in the Soil Science Society of America Journal (Aug 18, 2014; Issue S1; pages 35-47), reports an essential part of my dissertation research. I would like permission to reprint it as a chapter in my dissertation, which may require some revision. Please not that USU sends every dissertation to ProQuest to be made available for reproduction.

I will include acknowledgement to the article on the first page of the chapter, as shown below. Copyright and permission information will be included in a special appendix. Please let me know if you would like a different acknowledgement.

An approval of this request via email would suffice.

Kind regards,

Antra Boča

Acknowledgement:

This chapter was published in Soil Science Society of America Journal on August 18, 2014, and should be cited as

Boča A., Van Miegroet H., M.-C. Gruselle. 2014. Forest Overstory Effect on Soil Organic Carbon Storage: A Meta-Analysis. Soil Sci. Soc. Am. J. 78(S1): S35-S47

Danielle Lynch Jul 7 (11 days ago)

Hello Antra,

Congrats on finishing up your dissertation! You are welcome to republish your article. We do ask that you fill out a form on Copyright.com. Simply go to http://www.copyright.com/search.do?operation=detail&item=122807347&detailType=advancedDetail, select Thesis/Dissertation under *Republish or Display Content*, then make sure you select "Author of requested content" under "Describe who will republish the content". This just helps us keep track of who is republishing our material and where, and you of course will not be charged. Have a great day!

Danielle

From: Antra Boča [mailto:antraboca@gmail.com]
Sent: Thursday, July 6, 2017 10:23 PM
To: Danielle Lynch <dlynch@sciencesocieties.org>
Subject: permission to reprint article from SSSAJ

Date: July 7, 2017

Antra Boča has my permission to include the following paper, which is published, of which I was co-author, in her doctoral dissertation.
Boča, A., Van Miegroet, H., Gruselle, M.-C., 2014. Forest Overstory Effect on Soil Organic Carbon Storage: A Meta-analysis. Soil Sci. Soc. Am. J. 78, S35–S47. doi:10.2136/sssaj2013.08.0332nafsc

Signature

Name: Marie-Cécile Gruselle

CURRICULUM VITAE

Antra Boča

Education

08/2012 – 06/2017	Doctoral Candidate, PhD program in Ecology, Quinney College of Natural Resources (QCNR), Utah State University (USU) Dissertation title: "Effect of foliage and root carbon quantity, quality and fluxes on soil organic carbon stabilization in montane aspen and conifer stands in Utah". Major adviser: Prof. Dr. Helga Van Miegroet
10/2009 – 11/2011	M.Sc. Forest Ecology and Management, Faculty of Forest and Environmental Sciences, University of Freiburg, Germany M.Sc. thesis "Determination of Phosphorus in forest soils by the Hedley method and near-infrared spectroscopy (NIRS)". Major adviser: Prof. Dr. Jürgen Bauhus
01/2007 – 05/2007	Exchange semester, Erasmus Program, Environmental Engineering, Technical University of Denmark
09/2004 – 06/2008	B.Sc. Environmental Sciences, Faculty of Geography and Earth Sciences, University of Latvia B.Sc. thesis "Sewage sludge management in Bauska district (Latvia) and Frederikssund municipality (Denmark) – commonalities and differences" Major adviser: Prof. Gunta Spriņģe

Publications

Boča A., Van Miegroet H. Can carbon fluxes explain differences in soil organic carbon storage under aspen and conifer forest overstories? Forests 8, 118; doi:10.3390/f8040118

Laganière J., **Boča A.**, Van Miegroet H., Paré D. Consistent effect of aspen on soil carbon across its North American range. Forests, 8, 113; doi:10.3390/f8040113

Niederberger J., Todt B., **Boča A.**, Nitschke R., Kohler M., Kühn P., Bauhus J. (2015) Use of near-infrared spectroscopy to assess phosphorus fractions of different plant availability in forest soils. Biogeosciences 12: 3415-3428

Boča A., Van Miegroet H., Gruselle MC (2014) Forest overstory effect on soil organic carbon storage – a meta-analysis. Soil Sci. Soc. Am. J. 78: S35-S47.

Submitted
Boča A., Jacobson A., Van Miegroet H. Aspen soil organic carbon increases retention of dissolved organic carbon in soil. (*June, 2017, Plant and Soil*)

Presentations

Oral presentations

Boča A. (2017) Impact of forest tree species on soil organic carbon. International Union of Forest Research Organizations Anniversary Congress. Freiburg, Germany. September 19-22. (*Invited talk; session "What is the potential for CO_2 mitigation of forest soils?")*

Boča A., Van Miegroet H. (2016) Differences between aspen and conifer soil organic carbon pools explained by carbon fluxes. Soil Science Society of America Annual Meeting. Phoenix, AZ, USA. November 6-9. (*Rapid Talk)*

Boča A. (2016) Vegetation change effects on soil organic carbon properties in forests of the Intermountain West. Restoring the West conference. Logan, UT, USA. October 18-19. (*Invited talk*)

Boča A., Ignite (2016) "Of earth they were made, and into earth they return"., USU Research Week, April 15 (*selected as one of nine speakers*). Published on YouTube May 23, 2016 (https://www.youtube.com/watch?v=z5zuJ_CMLIo)

Boča A. (2015) The effect of foliage and roots on soil organic carbon quantity, quality and fluxes in montane aspen and conifer stands in Utah. Department of Wildland Resources Graduate Project symposium, April 17.

Boča A. (2015) The soils under Utah's forests: aspen vs conifers. Society of American Foresters Intermountain Chapter conference. Logan, Utah, USA. April 3. (*Invited talk)*

Boča A. (2013) Contribution of foliage vs root carbon to the stabilized SOC pool in semiarid forest soils in Utah. USU, Department of Wildland Resources Pre-project symposium, April 19.

Poster presentations

Boča A., Van Miegroet H. (2016) Soil organic carbon pools and fluxes in montane aspen and conifer stands in Utah. USU Student Research Symposium. April 14. (*Best Graduate Poster Award in Life Sciences*)

Boča A., Hatten J., Van Miegroet H. (2015) Relationship of tree above-and belowground C inputs and cutin and suberin presence in soil. 5th International Symposium on Soil Organic Matter. Göttingen, Germany. September 20-24.

Boča A., Hatten J., Van Miegroet H. (2014) Root contribution to SOC pools in Utah forest soils: Root-specific compound analysis. International Union of Forest Research Organizations World Congress. Salt Lake City, Utah, USA. October 6-11.

Boča A., H. Van Miegroet, MC Gruselle (2013) Linking overstory, soil and climate to explain SOC storage in forest soils – a meta-analysis. North American Forest Soils Conference. Whitefish, Montana, USA. June 16-20.

Funding

Fellowships and scholarships

09/2012 – 04/2016	Presidential Doctoral Research Fellowship for PhD studies at USU
09/2016 – 05/2017	Seely-Hinckley Scholarship, USU
09/2016 – 05/2017	School of Graduate Studies Dissertation Fellowship, USU
02/2016	Jeb Stuart Scholarship, USU QCNR
10/2009 – 09/2011	DAAD (German Academic Exchange Service) scholarship for MS studies at the University of Freiburg
01/2007 – 05/2007	Erasmus scholarship for exchange studies at the Technical University of Denmark

Grants and Awards

07/2016 – 07/2017	McIntire-Stennis research grant (*co-PI – biomarker analysis in soil pore water, foliage and root sorption experiment*)
06/2016 – 06/2017	Ecology Center Graduate Research Award, USU (*biomarker methods comparison study at the University of Bern*)
04/2016	Student Association Graduate Enhancement Award, USU
02/2016 – 06/2017	Dissertation Enhancement Award, USU (*study of plant biomarker stability in mineral soil*)
05/2014 – 12/2015	Utah Agricultural Experiment Station grant entitled " Contribution of foliage and roots to stabilized soil organic carbon pools in Utah forests determined by plant biomarker analysis" (*; co-PI –quantification of carbon pools and fluxes within aspen and conifer systems and biomarker distribution in soil organic carbon fractions.*)
06/2014 – 06/2015	Ecology Center Graduate Research Award, USU (*exploratory biomarker research: cutin- and suberin extraction from soil*)

Travel Awards

11/2016	Robert J. Luxmoore Student Travel Award, Soil Science Society of America meeting in Phoenix, AZ
2016	Ecology Center, Office of Research and Graduate studies, Department of Wildland Resources, USU

2015	Ecology Center, Center for Women and Gender, Office of Research and Graduate studies, Department of Wildland Resources, Quinney College of Natural Resources, USU
2014	Ecology Center, USU
2013	Ecology Center, Office of Research and Graduate Studies, USU
06/2013	North American Forest Soils Conference in Whitefish, MT Student Travel Award

Teaching and mentoring Experience

08/2016 – 12/2016	Teaching Assistant, Monitoring and Assessment in Natural Resource and Environmental Management (WILD 4750), USU
2015 – present	Mentoring of undergraduate research project; mentee – Brian Rozick, USU (Project: *Can SOC be used as an indicator for soil nutrient status and management at Cedar Mountain, UT?*)
April, 2016	Mentoring of a high-school intern from Netherlands for a two week internship
Fall 2014,2015,2016	Invited guest lecture on soil formation, Physical Geography (GEOG 1000 BPS), USU
Spring 2015 & 2017	Invited Lecturer: Module on soil organic matter, Wildland Soils (WILD 5350/6350), USU
Summer 2015	Mentoring of two international (Brazil and Germany) interns each for a 8 week internship
Spring 2013	Mentoring of undergraduate Capstone Project; mentee - Nicole Shepard, USU
11/2010 - 12/2010	Teaching Assistant, Natural Hazards and Risk Management, University of Freiburg

Work and short-term research experience

06/2016 – 08/2016	Visiting researcher at Institute of Geography, University of Bern, Switzerland (*plant biomarker extraction method comparison with Prof. Dr. Sandra Spielvogel*)
09/2015 & 06/2016	Visiting researcher at College of Forestry, Oregon State University (*plant biomarker stability and distribution in soil with Prof. Jeff Hatten*)
03/2011-11/2011	Research Assistant in the project "Development of methods to characterize plant-available P in large scale forest soil inventories", Institute of Silviculture, University of Freiburg.
05/2009-09/2009	Acting Head, Division for Nature Protection, Department of Environment, Riga City Council.
10/2008-05/2009	Specialist in Forest Issues, Division for Nature Protection, Department of Environment, Riga City Council

10/2007-10/2008	Analyst, Department of Spectral Analysis, Environmental Analysis Laboratory, State Agency for Environment, Geology and Meteorology in Latvia.

Leadership positions

2015 – 2016	USU QCNR Graduate Student Council chair
2014 – 2016	USU QCNR Graduate Student Council medical liaison
2014 – 2015	USU Ecology Center Seminar Series committee Co-chair
2013 – 2014	USU Ecology Center Seminar Series committee member
2013	Organizing committee member for the Restoring the West Conference (Oct 17-18, 2013); USU campus

Outreach Activities

04/15/2016	**Boča A.,** Ignite (2016) "Of earth they were made, and into earth they return", USU Research week (*selected as one of nine speakers*). Published on YouTube May 23, 2016 (https://www.youtube.com/watch?v=z5zuJ_CMLIo)
03/2016	USU Utah 4-H career workshop for 7th graders (*presented research techniques used in the Wildland Soils lab*)
10/29/2014	Speaker at Cedar Ridge Middle School Career Fair, Hyde Park, UT (*presentation: What does a soil scientist do?*)
03/09/2013	Judge at Cache County Science and Engineering Fair

Research Skills and Experience

Field work: soil, soil bulk density, and root sampling by cores and pits; soil pore water sampling, minirhizotron installation and data collection, litterfall sampling, forest stand measurements, vegetation sampling techniques, soil classification based on U.S. Soil Taxonomy and FAO World Reference Base.

Laboratory work:

- Biogeochemical techniques: quantification of soil C, N with TOC/TN analyzer, and of phosphorus via colorimetric detection with UV-Vis, aquatic C and N concentrations with DOC/DN analyzer, elemental analysis with atomic absorbance spectrometry and interactively coupled plasma mass spectrometry; liquid sample analysis with fluorescence spectrometry; solid sample analysis with near-infrared spectroscopy.
- Molecular techniques: plant biomarker extraction from plant tissues and soil; gas-chromatography mass-spectroscopy
- Mineralogical techniques: clay mineral preparation for XRD analysis; Fe and Al extraction from soils

Data analysis: R – meta-analysis; multiple and multivariate regression, categorical data analysis, mixed effects models, multivariate statistical methods like random forests, classification trees, PCA, MANOVA; SQLite for data management.
Language skills: Latvian (native), English, German (proficient), Russian (colloquial)