VEGETATION EFFECTS ON SOIL ORGANIC MATTER IN FORESTED ECOSYSTEMS

EDITED BY: Sandra Spielvogel, Jérôme Laganière, Laurent Augusto and
Jeff Allen Hatten

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VEGETATION EFFECTS ON SOIL ORGANIC MATTER IN FORESTED ECOSYSTEMS

Topic Editors:
Sandra Spielvogel, University of Kiel, Germany
Jérôme Laganière, Canadian Forest Service, Canada
Laurent Augusto, INRA Centre Bordeaux-Aquitaine, France
Jeff Allen Hatten, Oregon State University, United States

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EDITORIAL
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Editorial: Vegetation Effects on Soil Organic Matter in Forested Ecosystems

Jérôme Laganière[1]*, *Laurent Augusto*[2], *Jeff Allen Hatten*[3] *and Sandra Spielvogel*[4]

[1] Laurentian Forestry Centre, Natural Resources Canada, Canadian Forest Service, Quebec, QC, Canada, [2] INRA Centre Bordeaux-Aquitaine, Bordeaux, France, [3] Department of Forest Engineering, Resources and Management, College of Forestry, Peavy Forest Science Center, Oregon State University, Corvallis, OR, United States, [4] Department of Soil Science, Institute for Plant Nutrition and Soil Science, Christian-Albrechts-University of Kiel, Kiel, Germany

Keywords: soil organic matter, forest vegetation, biogeochemical cycles, carbon stabilization, soil biota, soil respiration

Editorial on the Research Topic

Vegetation Effects on Soil Organic Matter in Forested Ecosystems

Soil organic matter (SOM) plays a crucial role in forest ecosystems including nutrient provision, carbon sequestration, water regulation, soil structuring, and biodiversity promotion, thus placing SOM as an indicator of soil health. Vegetation is the main source of SOM in soils through above- and belowground inputs of organic matter. Vegetation also substantially influences various soil properties such as temperature, moisture, pH, composition and activity of the soil biota (Binkley and Giardina, 1998; Mueller et al., 2015; Schelfhout et al., 2017). Hence, a shift in vegetation composition through natural succession, forest management and/or global changes may alter SOM dynamics and related ecosystem services. Although the importance of plants in driving SOM formation and carbon cycling is now well-established, predicting SOM from vegetation composition in a variety of ecosystems is still challenging due to poor understanding of mechanisms and of potential interactions with biophysical attributes modulating vegetation-SOM relationships (Augusto et al., 2015; Mayer et al., 2020).

This Research Topic brought together research conducted in various ecosystems and climates, from the boreal forest of Canada to the Mediterranean region and wet tropical forest of Costa Rica. The contributions received were also very broad in term of spatial scale (from stand to landscape), SOM roles examined (e.g., carbon storage and stability, tree nutrition, soil respiration) and approaches used (from laboratory incubations to long-term field monitoring and meta-analysis).

In a 40 years old common garden experiment with five forest types in Argentina, Morazzo et al. observed significant differences in soil chemical and physical attributes, including SOM content, as well as in the morphology of soil profiles, in such as way that two forest types generated a change in the taxonomic order of the soil over time. In Italian mountain forests, Panico et al. found that together with site exposure and altitude, vegetation cover is a good predictor of soil characteristics including soil organic carbon. Tree species also have major effects on soils through their roots. In the tropical forest of Costa Rica, Russell et al. showed that litter inputs of fine roots and their biochemistry, especially the abundance of syringyl phenols, explain differences in organic carbon stocks among species. At a larger scale, Jandl et al. used data from the Austrian Forest Soil Survey to estimate current and projected soil carbon stocks at the country's scale under different forest managements and climate change scenarios. The concept letter by Desie et al. shows how threshold behavior in soil pH mediates tree species effects on carbon cycling and highlights that this parameter should be considered when managing forest composition to promote carbon sequestration.

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Frank Hagedorn,
Swiss Federal Institute for Forest,
Snow and Landscape Research
(WSL), Switzerland

****Correspondence:***
Jérôme Laganière
jerome.laganiere@canada.ca

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The contrasting influence of broadleaves on soils relative to conifers has been highlighted in past research (Prescott et al., 2000; Vesterdal et al., 2008; Augusto et al., 2015). Specifically, research on trembling aspen has shown that the abundance of this broadleaf species in North American forests can positively affect stand productivity, biodiversity, nutrient cycling and water retention (Laganière et al., 2017). We further learn from Boca et al. that soils developed under aspen retain more dissolved organic carbon than soils developed under conifers, which has implications for carbon stability via the formation of organo-mineral associations. The direct impact of a vegetation shift on substrate utilization efficiency and incorporation into microbial biomass is investigated in the Lloret and Quideau paper using ^{13}C labeled substrates. Their lab experiment indicates that a shift from spruce to aspen could be accompanied by litter C losses through microbial respiration but that incorporation into microbial biomass and eventually into the more persistent soil carbon pool may not be affected. In Nickels and Prescott, the stability of soil organic matter produced by aspen-dominated, conifer or grass vegetation in post-mining reclaimed ecosystems is investigated. The authors found that the type of vegetation established has important implications for SOC persistence in these systems.

Managing forests in mixed-species stands could also be a way of enhancing productivity and carbon sequestration as well as promoting resilient soil functions (Binkley et al., 2003; Hooper et al., 2005; Laganière et al., 2015). A meta-analysis by Rehschuh et al. showed that diversification of European beech forests, and especially beech–conifer admixtures, may benefit soil carbon and nitrogen storage and serve as an adaptation measure for drought-sensitive beech forests under climate change. Another study looking at species mixtures, the Bélanger et al. study, illustrates how the presence of balsam fir and American beech in sugar maple dominated–forests affect soil respiration in opposite directions, through their different ecophysiological activity, light interception and litter quality.

Collectively, results from these studies highlight that vegetation influences SOM accumulation, persistence and release through various mechanisms. Continuous research effort is needed to gain more insight into the role of vegetation on SOM cycling in the ecosystems of the world. Such knowledge is crucial to increase our ability to manage vegetation composition to maintain and enhance SOM, and to promote more resilient ecosystems services in a changing world.

AUTHOR CONTRIBUTIONS

JL prepared the draft. LA, JH, and SS revised the draft. All authors approved the final submission.

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ORIGINAL RESEARCH
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Aspen Soils Retain More Dissolved Organic Carbon Than Conifer Soils in a Sorption Experiment

Antra Boča[1,2], Astrid R. Jacobson[3] and Helga Van Miegroet[2]*

[1] *Faculty of Environment and Civil Engineering, Latvia University of Life Sciences and Technologies, Jelgava, Latvia,* [2] *Department of Wildland Resources, Utah State University, Logan, UT, United States,* [3] *Department of Plants, Soils and Climate, Utah State University, Logan, UT, United States*

The effect tree species have on soil organic carbon (SOC) has been hotly debated but, so far, few clear patterns have emerged. One example of a differing tree species effect on SOC are aspen forests in North America, which have been found to have more stable SOC than adjacent conifer forest stands. An important source for the formation of stable organo-mineral complexes in soil is dissolved organic carbon (DOC). DOC concentrations in mineral soil are often higher under the thick O-horizons of conifer forests than under aspen forests, but this does not correspond to more stable mineral SOC. This suggests that, instead of DOC concentration, DOC quality could be driving the observed differences in SOC. Therefore, we quantified the retention of contrasting forest detritus DOC in soils. Using a batch sorption experiment approach, we compared the retention of detritus leachates from four sources – aspen leaves (AL), aspen roots (AR), conifer (subalpine fir) needles (CN), and conifer (subalpine fir) roots (CR) – on soils sampled under aspen and conifer (subalpine fir and Douglas fir) overstories. The calculated sorption isotherms showed a higher retention of AL DOC than AR DOC, as indicated by all four sorption parameters – k and n (curve-fitting parameters), null point concentration (NPC; net sorption = net desorption), and endpoint (EP, retention at the highest initial DOC concentration). Leachates from CN and CR showed very similar retention behavior, and between the two species the retention of root leachates was more similar than the retention of foliage leachates. Soils sampled from aspen forests showed higher affinity for new DOC than conifer soils [higher sorption rate (n), lower NPC, and higher EP] regardless of the DOC source. The findings suggest that the higher DOC sorption on aspen soils might be a major driver for more stable SOC under aspen stands in North America.

Keywords: aspen, dissolved organic carbon, foliage, roots, sorption, tree species

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Jérôme Laganičre,
Canadian Forest Service, Canada

Reviewed by:
Sylvie Quideau,
University of Alberta, Canada
Lars Vesterdal,
University of Copenhagen, Denmark

***Correspondence:**
Antra Boča
antraboca@gmail.com

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INTRODUCTION

As forest soils store as much carbon as aboveground biomass (Pan et al., 2011), information on tree species' effects on soil organic carbon (SOC) storage is of interest to ecologists, ecosystem modelers, and forest managers. Most synthesis studies on this topic have not found globally consistent patterns (Vesterdal et al., 2013; Boča et al., 2014; Lin et al., 2017), but some species or functional groups stand out in terms of SOC storage and stabilization.

In a 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. This is an important finding considering that quaking aspen is the most widely distributed tree species in North America (Little, 1971), and its current decline (Rogers, 2002; Di Orio et al., 2005) is often accompanied by conifer encroachment (Potter, 1998). While no consistent difference in SOC pools was detected by Laganière et al. (2017), in several areas in the state of Utah, United States, higher SOC stability was also associated with higher total SOC pools compared to nearby conifer stands (Woldeselassie et al., 2012; Boča and Van Miegroet, 2017). An analysis of C fluxes in these ecosystems (higher litterfall under aspen, higher DOC concentrations under conifers, higher fine root biomass under conifers, similar root turnover under both overstories), however, could not explain the differences in SOC pools under both overstory types (Boča and Van Miegroet, 2017). This leaves two mechanisms as potential drivers: (i) difference in dissolved organic matter (DOM) quality and sorption, and/or (ii) difference in root exudation. In this region, as in most of the Intermountain Western US, the majority (>75%) of the precipitation falls as snow. Spring snowmelt creates the largest soil water fluxes that affect the whole soil profile (fall rains mostly affect only topsoil) (LaMalfa and Ryle, 2008). This makes spring snowmelt water fluxes a likely pathway for C redistribution in soil, as soil macrofauna have not been observed in these areas (Ayres et al., 2009; Boča and Van Miegroet, 2017). We are not aware of a study that has compared root exudation under aspen and conifer trees. Higher specific root length of aspen compared to conifers (e.g., Steele et al., 1997; Bauhus and Messier, 1999) could, however, suggest more dynamic belowground processes under aspen. In this study, we examined the first mechanism: DOM quality and sorption.

In its dissolved form, organic matter can be transported through the soil profile and sorbed to mineral surfaces or incorporated into microbial biomass attached to these surfaces, thus participating in the formation of stable mineral-bound organic matter (Qualls, 2000; Kalbitz et al., 2005; Kalbitz and Kaiser, 2008). Factors affecting sorption are: (i) Fe and Al oxyhydroxide concentrations in soil (e.g., Moore et al., 1992; Lilienfein et al., 2004; Heckman et al., 2011); (ii) native SOC concentration, which affects the potential of soils to retain more C (Hassink, 1997; Six et al., 2002; Stewart et al., 2007); and (iii) the quantity and quality of dissolved organic carbon (DOC). Higher concentrations of DOC are known to result in higher total retention of C in laboratory sorption experiments, but DOC fluxes in the field do not correlate with SOC pools (as reviewed by Michalzik et al., 2001). Field measurements in Utah revealed higher DOC concentrations in soils under conifers (Boča and Van Miegroet, 2017). Considering that the examined plots were less than 50 m apart, and had similar soil characteristics (Van Miegroet et al., 2005; Olsen and Van Miegroet, 2010; Boča and Van Miegroet, 2017), the higher DOC under conifers should have resulted in higher C concentrations and higher mineral-associated SOC. Yet the opposite pattern was observed (Román Dobarco and Van Miegroet, 2014; Boča and Van Miegroet, 2017). This again suggests that other factors, such as DOC quality, are potentially more important drivers for sorption in this case.

The litter of quaking aspen is considered more labile than conifer litter due to differences in nutrient and lignin concentrations (Moore et al., 2006). In a litter decomposition study, Prescott et al. (2000) also suggested leaching losses as a major reason for faster aspen vs. conifer litter degradation. In the first year of decomposition, the mass loss of 35% from aspen litter (Prescott et al., 2000) was similar to the 32% leachable content from aspen litter observed by Taylor et al. (1989). DOM quality has been proposed as a major factor affecting organic matter sorption in soil with hydrophobic and more aromatic compounds being preferentially sorbed to mineral surfaces compared to more labile polysaccharide-derived hydrophilic DOM (Kaiser and Guggenberger, 2000; Kalbitz et al., 2005). Recently, Cotrufo et al. (2015) showed that SOC can be formed with high efficiency through microbial processing of DOM produced during the early stages of litter decomposition (labile non-structural compounds).

Root detritus is considerably less examined as a source of DOM, prohibiting researchers from calculating 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, and later reported that fine root DOM was less labile than foliage DOM (Uselman et al., 2012). Hansson et al. (2010) reported no differences in aromaticity and sorption rates between root and needle DOM, but did find lower DOC production rates from roots. Both studies examined root and foliage DOM from coniferous species. We are not aware of any published data on root DOM quality from contrasting tree species. Finér et al. (1997) reported faster aspen root decomposition compared to adjacent conifers, but, as no data on root quality were recorded, it is unclear whether faster decomposition reflects differences in root DOM quality. Overall, the sorption of root DOM is a knowledge gap that needs to be filled.

The objective of this study was to investigate the retention and release (sorption and desorption) by forest soils of foliage- and root-derived DOC from two contrasting tree species – quaking aspen and subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.] – in the montane regions of Utah in the western US. The proximity of the aspen and conifer forest stands in Utah's mountains, and their contrasting litter quality, make them ideal study systems to answer questions regarding the effects of substrate quality on DOC sorption. We used a batch sorption approach to quantify DOC retention based on: (i) the source and quality of leachate derived from aspen and fir, and (ii) soil properties at different sites and soil depths. We hypothesized that: (i) aspen foliage DOC will be more labile in its chemical composition than root DOC and fir needle DOC, and thus will be more effectively retained in the soil; (ii) native DOC will sorb better on native soil; (iii) topsoils will experience lower sorption due to higher initial SOC concentrations than subsoils; and (iv) soils with higher Fe and Al oxyhydroxide concentrations will experience higher sorption.

MATERIALS AND METHODS

Soil Sampling and Analysis

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. A detailed description of the sampling sites and the sampling procedure is provided in Boča and Van Miegroet (2017). In brief, TWDEF is located at 2600 m elevation with an average annual temperature of 3.1°C and mean annual precipitation of 1031 mm, with about 70% accumulating as snow (NRCS[1]; USU Doc Daniel SNOTEL station). Cedar Mountain is a high−elevation plateau (1800–3200 m) with an average annual precipitation of 823 mm and a mean annual temperature of 4.7°C [NRCS (see footnote 1); Kolob and Webster flat SNOTEL stations). Forests at both sites most likely originated from natural regeneration about a century ago (Wadleigh and Jenkins, 1996; DeRose and Long, 2007). Soils at both sites have been classified as Mollisols and Alfisols under aspen stands and as Alfisols under conifer stands, according to USDA soil taxonomy (McNab and Avers, 1994; Van Miegroet et al., 2005; Olsen and Van Miegroet, 2010).

The CM soils had two to three times higher total Fe and Al oxyhydroxide and SOC concentrations than those at TWDEF (**Table 1**; Boča and Van Miegroet, 2017). As oxyhydroxides affect sorption behavior (e.g., Heckman et al., 2011; Kramer et al., 2012), we considered site as a factor in further analyses. The soils were collected from the top 10 cm (topsoil) and 40–50 cm (subsoil) of the soil profile to capture differences in native SOC concentration within a given overstory type. The lower sampling depths correspond to the ABt and BAt horizons under aspen and Bt horizons under conifers.

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 Fe and Al that were organically bound, or present in short range ordered (non-crystalline) and crystalline hydrous mineral phases (Sparks et al., 1996). The extracts were analyzed with an Atomic Absorption Spectrometer (Varian AA240 flame atomization, Australia). Concentrations of non-crystalline Fe and Al oxyhydroxides were calculated by subtracting NaPP values from AAO values, and concentration of crystalline Fe oxides was calculated by subtracting AAO from CD. 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 and inorganic C with Skalar Primacs^{SLC} Analyzer (Skalar, Inc., Breda, Netherlands).

Leachate Preparation and Analyses

The plant material used in the experiment was collected at TWDEF and CM at the end of the 2015 growing season, and consisted of senesced aspen leaves, subalpine fir needles, and fine roots (<2 mm diameter) obtained from soil cores in both forest types at both sampling sites. The needles used were older, and collected from the Oi layer of the O-horizon. They were mixed with freshly senesced needles based on calculations of annual litterfall additions to the O-horizon. This was done to ensure that we are comparing similar material (i.e., Oi layer) for aspen and fir. We used a mix of older and fresh fir needles because, in contrast to aspen stands, most of the DOC under conifers originates from an O-horizon, which is mostly dominated by older foliage material in various stages of decomposition (Fröberg et al., 2003). The material was ground with a Wiley mill (20 mesh; Thomas Scientific, NJ, United States), analyzed for C with Skalar Primacs^{SLC} Analyzer (Skalar, Inc., Breda, Netherlands), and for total nitrogen with PDZ Europa ANCA GSL IRMS elemental analyzer (Sercon Ltd., Cheshire, United Kingdom).

DOC stock solutions were obtained following a method developed prior to the experiment. In brief, 20 g of ground foliage or root material were saturated with ultrapure water and subjected to two freeze-thaw cycles for a week to facilitate the release of DOC from substrates. Freezing and thawing are common processes in the field sites during fall when air temperatures drop below 0°C during night, and rise above freezing during the day[2] (NRCS SNOTEL – Kolob station). The thawing temperature was set at 5°C to reduce microbial decomposition of the material. 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 during spring 2014 and 2015 (Boča, unpublished data). The leachates were created by vacuum-filtering a litter-water slurry through a glass fiber filter (Sterlitech 0.4 μ m).

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} could be prepared on the same day as the stock solution. The DOC concentrations used were within the range of DOC concentrations observed in soil pore water at TWDEF (Boča and Van Miegroet, 2017). 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, and analyzed for DOC with the wet oxidation persulfate UV method using a Phoenix 8000 Carbon Analyzer (Tekmar-Dohrmann, OH, United States). The pH of leachates was measured in stock solutions, which had DOC concentrations of around 150 mg L^{-1}. The only exception was the stock solution derived from aspen leaves, which had DOC concentrations close to 1000 mg L^{-1}, and, hence, had to be diluted prior to pH measurements.

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[1] Natural Resources Conservation Service [NRCS]. Snow Telemetry (SNOTEL) Precipitation and Air Temperature Data for Webster Flat, Kolog, Tony Grove Lake (Utah) (1981–2010). Available online at: http://www.wcc.nrcs.usda.gov/snow/ (accessed November 25, 2014).

[2] http://twdef.usu.edu/TWDEF/

TABLE 1 | Selected soil properties from TWDEF and CM study sites.

Site/horizon	Texture	pH (H_2O)	Fe (mg g^{-1})			Al (mg g^{-1})		Clay minerals	C%
			Org	*Non-cryst*	*Cryst*	*Org*	*Non-cryst*		
TWDEF A 0–10	Loam (23% clay)	6.1	0.79 ± 0.08	2.21 ± 0.6	3.39 ± 0.46	1.83 ± 0.24	0.55 ± 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	4.07 ± 0.88	0.91 ± 0.03	0.99 ± 0.31		1.02
TWDEF C 0–10	Loam (24% clay)	5.5	0.87 ± 0.4	1.22 ± 0.49	2.88 ± 0.42	1.33 ± 0.46	0.64 ± 0.03	Illite, Dickite, Kaolinite, Vermiculite	2.42
TWDEF C 40–50	Clay loam (29% clay)	5.4	0.81 ± 0.29	1.27 ± 0.39	3.29 ± 0.5	1.09 ± 0.13	0.71 ± 0.12		0.61
CM A 0–10	Loam (21% clay)	5.4	1.09 ± 0.28	9.18 ± 0.43	4.74 ± 0.6	1.22 ± 0.17	2.95 ± 0.42	Illite, Kaolinite, Vermiculite, Mica[a]	5.02
CM A 40–50	Loam (25% clay)	6.4	2.82 ± 0.25	8.25 ± 0.34	5.7 ± 1.07	2.15 ± 0.05	3.04 ± 0.12		3.13
CM C 0–10	Loam (23% clay)	5.3	1.53 ± 0.08	10.02 ± 1.53	4.68 ± 1.81	2.09 ± 0.05	2.79 ± 0.29	Illite, Kaolinite, Vermiculite[a]	4.72
CM C 40–50	Loam (18% clay)	5.9	3.4 ± 0.05	7.54 ± 0.9	5.64 ± 1.61	2.67 ± 0.04	2.54 ± 0.07		2.57

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

Experimental Setup

The experimental setup is depicted in **Figure 1**. In brief, the experiment had four leachate treatments – aspen leaves (AL), aspen roots (AR), conifer (subalpine fir) needles (CN), and conifer (subalpine fir) 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. The conifer stands, from which soils were sampled, were dominated by subalpine fir at TWDEF, and by Douglas fir (*Pseudotsuga menziesii* Mirb.) at CM.

In this experiment, the two depths represent differences in initial C concentration under the same overstory, which is thought to affect soil C saturation/deficiency. Considering that the forests investigated had not been managed for timber production for at least a century, we assumed that SOC levels were at steady-state. Following the same assumptions as studies that developed the C saturation capacity concept (Hassink, 1997; Six et al., 2002; Stewart et al., 2007), we assumed that the upper soil was closer to SOC saturation and thus had lower C retention capacity while the soil at greater depth had a higher C deficiency.

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 pure KCl solution (DI water with no DOC) with an EC of 150 μS cm^{-1} was included to measure the desorption of native SOC (**Figure 1**). The experiment was done in triplicate for concentrations of 0, 10, and 80 mg DOC L^{-1}, and in duplicate for concentrations of 20 and 40 mg DOC L^{-1}. The mixing of soil and solution was done in glass jars with septa caps to allow for measurements of CO_2 evolution from heterotrophic activity after shaking. The jars were shaken in the dark on an orbital shaker for 24 h (100 rpm) at room temperature. Due to the sample size, the shaking (equilibration) had to be split in 2 days. The first round of samples 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 jars was measured by inserting needle extensions through the septa and analyzing the gas with a LICOR-8100 gas analyzer (LI-COR, Inc., NE, United States). Afterward, all samples were filtered through a

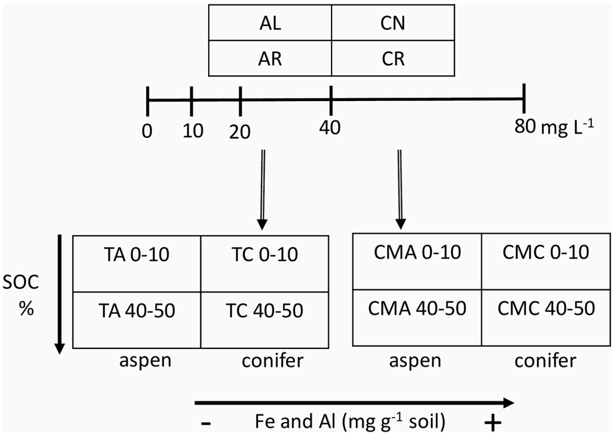

FIGURE 1 | Experimental design of the sorption experiment. Leachates from four plant substrates – aspen leaves (AL), aspen roots (AR), fir needles (CN), and fir roots (CR) - were added to aspen (A) and conifer (C) soils at four concentrations plus blank (0, 10, 20, 40, and 80 mg L^{-1}). The two depths (0–10 and 40–50 cm) represented differences in native SOC concentration, and the T (for TWDEF) and CM sites represented differences in oxyhydroxide 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.

0.4 μm glass fiber filter (Sterlitech) and analyzed for DOC as described in subsection "Leachate Preparation and Analyses."

Fluorescence Analysis

Leachate (pre-sorption) and post-sorption solution quality was assessed with fluorescence and absorbance spectroscopy 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 range was 249 – 828 nm at an increment of approximately 4.5 nm. As the Aqualog measures fluorescence and absorbance simultaneously, so absorbance was measured at the same wavelengths as excitation. Each sample was diluted to not exceed 0.3 cm^{-1} absorbance at 254 nm (Miller et al., 2010) to minimize inner-filter effects; this corresponded to approximately 7–10 mg DOC L^{-1}. The samples were measured at their natural pH as we were interested in the characterization of the natural DOM (see Cuss et al., 2014, for more information on pH effects on plant leachate fluorescence).

The fluorescence spectra were Raman normalized, corrected for the inner-filter effect, and blank-subtracted before calculating several spectroscopic indices and building a parallel factor analysis (PARAFAC) model (Murphy et al., 2013). All corrections and calculations were performed using the MATLAB (version R2017a) software. We calculated the humification index (HIX – at ex 254 nm, area of peak under em 435–480 nm divided by peak area under em 300–345 nm), the fluorescence index (FI – em 470 nm/em 520 nm at ex 370 nm) (Gabor et al., 2014a), and, by using UV-Vis data, specific ultraviolet absorbance at 254 nm (SUVA = abs @ 254 nm cm^{-1} × 100/DOC mg L^{-1}; units = L mg C^{-1} m^{-1}) as these have been utilized in other studies to characterize soil derived DOM (Gabor et al., 2014b; Strid et al., 2016). A higher value of the humification index (HIX) corresponds to lower hydrogen to carbon (H:C) ratios and indicates a greater degree of humification (Gabor et al., 2014a). The FI is used as an indicator of precursor material, with lower values indicative of DOM that is plant-dominated in origin, and higher values indicative of DOM that is predominately from microbial (originally algal) sources, with a difference in value of 0.1 considered to be significant (McKnight et al., 2001). 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).

To better characterize the quality of the DOM solutions, we analyzed the fluorescing compounds by building a PARAllel FACtor analysis (PARAFAC) model following the guidelines described by Murphy et al. (2013). PARAFAC uses all of the data contained in excitation-emission matrices (EEMs) to identify and quantify independent underlying spectral features, termed "components." A component represents a single fluorophore (compound that absorbs or re-emits light) or a group of highly related fluorophores (Fellman et al., 2010; Murphy et al., 2013; Aiken, 2014). A PARAFAC model was built using the drEEM toolbox (version 4.0). As input for the model we used all sorption and desorption (see subsection "Desorption") data, as well as data from pure leachates. After validation, all components were compared with information from a global model database using the OPENFluor plugin (Murphy et al., 2014) in OPENChrom® (Wenig and Odermatt, 2010; version Dalton) to estimate the chemical identity of each component. More detailed information regarding fluorescence measurements and model building is provided in **Supplementary Material 1**.

Desorption

At the end of the adsorption experiment, soils were dried in glass jars (same jars as used for the sorption process) for 7 days at 5°C. Drying, a natural process in the examined soils, can potentially affect OM retention in soil (Borken and Matzner, 2009). We chose the drying temperature to reflect the natural soil temperature observed in the field (Scott Jones, unpublished data; raw data available at *twdef.usu.edu*). After drying, soils 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 in subsection "Experimental Setup" and "Fluorescence Analysis."

Data Analyses

Before fitting a sorption equation, we adjusted the data for CO_2-C lost through mineralization. We calculated the CO_2 concentration in the headspace using the ideal gas law, and by taking into account the pH of the solution we calculated the CO_2 in the liquid phase. First, to correct for the "Birch effect" (Jarvis et al., 2007), and for the mineralization of native SOC, the CO_2 measured for each sample was corrected by the CO_2 measured for the same soil when only the pure KCl solution was added. The corrected values were then used to calculate sorption/desorption using the following equation:

$$iDOC - sDOC - CO_2\text{-}C = rDOC \tag{1}$$

where *iDOC* is the initial DOC concentration added, *sDOC* is the DOC concentration measured in the solution after 24 h of shaking, and CO_2-*C* is the amount of C lost via mineralization. The resulting *rDOC* is the DOC retained in soil or released from soil in the cases where no sorption occurred, which is why we further refer to DOC retention/release in the text. Finally, *rDOC* was converted to mg C retained/released per kg of soil.

To describe the sorption behavior of leachate DOC on soil, we fitted a non-linear function, loosely based on the Freundlich isotherm, using initial DOC concentrations and *rDOC* values. Following suggestions by Lilienfein et al. (2004) and Vandenbruwane et al. (2007) we fit the non-linear curves by subtracting the native organic C released as DOC (from extraction with the KCl solution) from the original equation, meaning, the parameter "*a*" was added, representing a non-zero intercept:

$$rDOC = k \times C_i^{1/n} - a \tag{2}$$

In equation [2], *rDOC* is the mass of DOC (mg) released/retained per mass of soil (kg), C_i is the DOC concentration added [initial DOC; as per Kothawala et al. (2008) who used it for the Langmuir equation], and "*a*" is the y intercept, which describes the native DOC released with the pure KCl solution (Vandenbruwane et al., 2007). Parameters *k* and *n* are curve-fitting parameters that together describe the shape of the curve. We used non-linear regression to estimate the parameters *k* and *n* using the function *nls* in the R package *Stats* (R Development Core Team, 2015, version 3.1.2).

We tested differences between leachate type and soil properties in regard to null point concentration (NPC; initial DOC concentration at which net sorption equals net desorption), endpoint (EP; C sorbed at the highest concentration of DOC added), and parameters *k* and *n* with a factorial analysis of variance (ANOVA) testing for main effects and two-way interactions with $\alpha = 0.05$. When significant, we performed *post hoc* Tukey HSD tests to determine differences between individual leachate types. The soil properties considered were: soil type, which represented differences in soils affected by aspen and conifer overstories; site, which was representative of differences in Fe and Al oxyhydroxide levels as well as site SOC concentrations (Boča and Van Miegroet, 2017); and depth, which represented differences relative to effective C saturation levels (topsoils closer to C saturation, and subsoils further away). Data were transformed where necessary to ensure equal variances and normal distribution of the residuals. We further tested the relationship between initial SOC concentration and the four retention response variables with a multivariate regression.

To compare PARAFAC results, we calculated the proportion that each PARAFAC component explained from total fluorescence. This approach allowed us to compare shifts in component dominance (solution quality) without having to consider the non-linear effect of concentration on fluorescence intensities. A high heterogeneity of variances in residuals among several factors (mostly leachate type and soil type) prohibited the use of an ANOVA, and, therefore, differences in components were analyzed for main effects only using the non-parametric Kruskal–Wallis test. If leachate type was found to have significant differences, we used the Wilcoxon signed rank test for pairwise comparisons with a Holm adjustment for the *p*-value to compare between the leachates. All statistical analyses were performed with the software R version 3.1.2 (R Development Core Team, 2015). The values depicting results are reported as mean ± standard deviation, unless noted otherwise.

RESULTS

Soil Characterization

As seen in **Table 1**, at each site, soils under both overstory types were fairly similar. All soils were loams with some soils at 40–50 cm depth being clay loams. The clay concentration was lowest in the CMC 40–50 cm soils at 18%, and varied from 21 to 29% in the other soils. The clays at TWDEF were dominated by a mixture of 1:1 and 2:1 clays (kaolinite, illite, vermiculite with smaller peaks of dickite and muscovite). Similar XRD spectral peaks were also detected for CM soils, but due to the high oxyhydroxide concentration, which interfered with the XRD measurements, the clay mineralogy could not be fully described. At CM CD extractable "free" (sum of all three fractions) Fe and AAO extractable Al (sum of organically bound and non-crystalline fractions) oxyhydroxide concentrations were very similar among overstories and averaged around 16 mg g^{-1} for Fe and around 4.9 mg g^{-1} for Al oxyhydroxides. At TWDEF, conifer soils had lower non-crystalline and crystalline Fe oxide concentrations (around 2.2 mg g^{-1} non-crystalline Fe oxides for aspen vs. 1.2 mg g^{-1} for conifers, and around 3.7 mg g^{-1} crystalline Fe for aspen vs. 3.1 mg g^{-1} for conifers), while the Al concentrations were similar (**Table 1**). The largest measured differences between aspen and conifer soils were in terms of C concentration and pH, which were always lower under conifers, with differences ranging from 0.3 – 0.6 percentage points for C, and 0.1 – 0.7 units for pH (**Table 1**). The main soil difference between sites was in the concentrations of non-crystalline and crystalline Fe and Al oxyhydroxides, which were three to four times higher at CM. The higher oxyhydroxide concentration also corresponded to higher C concentrations at CM compared to TWDEF with differences ranging from 1.9 to 2.3 percentage points. At both sites, C concentrations in the topsoils were approximately 2 percentage points higher than in the corresponding subsoils (**Table 1**).

Leachate Characterization

Aspen leaves (AL) yielded the highest DOC concentration among leachates (136 mg DOC g^{-1} substrate), while the other three substrates released ten times less DOC per gram of material (**Table 2**). Leachates from foliage had approximately two to three times higher total *N* values than corresponding root leachates, even though root biomass itself had higher (0.95% vs. 0.58% for AR and AL) or similar (0.5% vs. 0.45% for CR and CN) N concentrations. The leachates had similar SUVA values – 0.8 to 1.1. AL had the lowest HIX value (0.06) suggesting higher H:C ratios and a more aliphatic nature of the solution compared to the other leachates, and the highest FI index (2.01). Foliar leachates had higher FI than those derived from roots (**Table 2**).

We created a 4-component PARAFAC model, which validated via split-half analysis, and after normalization of the input data, accounted for 98.7% of the observed variation in DOC fluorescence. From the four components of the PARAFAC model the first two (C1 and C2) were identified as humic-like (similar to C3 and C2 in Stedmon et al., 2007) while C3 and C4 were protein-like (C4 similar to peak in panel X8 in Murphy et al., 2011; C3 similar to C4 in Gueguen et al., 2014). Intensities reported in **Table 2** for each component (C1 – C4) indicate that the fluorescence signal of AL was almost entirely explained by C4 (86.5%), while the remaining components explained only 13.5%. By contrast, C4 had very small intensities in the AR and CN leachates and was entirely missing from the CR leachate. For AR and CN, the protein-like C3 explained most of the fluorescence (67 and 54%, respectively), followed by the humic-like components C1 and C2. For CR, C1 and C3 explained similar proportions of fluorescence (40 and 37%), and C2 explained the rest.

After 24 h of shaking, the raw intensities of the leachate solutions decreased (data not shown) due to a decrease in DOC concentration, but the relative contribution of each component did not change with mineralization. For the AL leachate, HIX did not change but it increased for the other three leachates to an end-value of 1.17 for AR, 1.34 for CN, and 2.99 for CR. Conversely, the FI decreased significantly for AL (1.87), but remained fairly constant for the other three leachates (end-value

TABLE 2 | Selected properties of plant biomass and pre-sorption leachates derived from foliage and root biomass (C1–C4 are four components of the PARAFAC model and values are percentages of total fluorescence).

	Biomass			Leachates									
	C%	N%	C/N	mg DOC g^{-1} substrate	mg total N g^{-1} substrate	pH	HIX	SUVA L mg C^{-1} m^{-1}	FI	C1	C2	C3	C4
AL	43	0.58	74	136	0.94	5.6	0.06	0.9	2.01	5.9	0.2	7.4	86.5
AR	38	0.95	40	10.9	0.53	6.7	0.37	0.8	1.41	20.2	11.9	66.6	1.3
CN	43	0.45	96	10.5	0.75	6.5	0.73	0.8	1.84	28.8	14.1	54.3	2.8
CR	40	0.50	80	11	0.24	6.2	0.58	1.1	1.51	40.3	22.9	36.9	0

1.47 for AR, 1.9 for CN, and 1.5 for CR). SUVA almost doubled for all leachates with end-values of 1.2 for AL, 1.8 for AR, 1.8 for CN, and 2.3 for CR.

DOC Retention/Release

The sorption isotherms depicted in **Figures 2**, **3** were adjusted for the amount of DOC mineralized and released as CO_2 (exact values are reported in the **Supplementary Materials 2**, **3**). On average, more DOC was lost through mineralization in the root leachate treatments than foliage treatments −13% of added C mineralized for AL treatment vs. 18% for AR, 12% for CN vs. 18% for CR. Overall, similar proportions of DOC were mineralized in aspen and conifer soils (16 and 15%, respectively).

The sorption behavior of leachate types was significantly different based on all four sorption metrics analyzed – the curve parameters (n and k) and NPC and EP (**Table 3**). *Post hoc* Tukey's HSD test indicated statistically significant differences between AL and AR in regard to all four parameters analyzed, but no significant differences were detected between CN and CR (**Table 4**). **Figures 2**, **3** show the similarities between CN and CR on almost all soils, while the sorption isotherms diverge much more strongly between AL and AR.

Based on the sorption isotherm parameters k and n, AL had the steepest sorption isotherms, suggesting the highest retention. This was followed by CN > CR > AR (**Table 4**), with the latter two being significantly different from AL. Overall, AL had the lowest NPC (DOC concentration where net retention = net release) and the highest EP (C retained at highest DOC concentration added) values. For NPC, the only significant difference was between AL and AR, while for EP, AL differed significantly from AR and CN (**Table 4**).

One of the most interesting findings of this study was the consistently higher DOC retention in aspen soils compared to conifer soils, irrespective of the source of DOC (**Table 4**). For a given leachate type, aspen soils reached NPC at lower DOC concentrations, i.e., they started to retain C at lower DOC concentrations and had overall higher EP values. Conifer soils often did not reach NPC with the DOC concentrations used in this study, especially for topsoils. The ANOVA on curve parameters corroborated this observation for NPC, EP, and n (**Tables 3**, **4**). The lower n values for aspen soils indicated steeper retention curves than for conifer soils, i.e., greater sorption.

Null point concentration and curve shape (parameter n) differed significantly between top- and subsoil (**Table 3**). The lower n values for topsoils were associated with similar k values (**Table 4**), indicating steeper curves for topsoils, i.e., higher retention rates. However, the steeper curves did not result in lower NPC, as topsoils on average had significantly higher NPC values than subsoils (**Tables 3**, **4**).

The ANOVA results showed that the only significant difference between sites was for parameter k (**Table 3**). It was larger for CM than TWDEF (**Table 4**), again indicating higher DOC retention rates in CM.

We found statistically significant interactions between leachate type and soil type for parameter k, and between depth and soil type for EP (**Table 3**). The interaction between leachate type and soil type for parameter k was due to the fact that root-derived DOC had higher k in aspen soils (20.9 for AR and 27.4 for CR on aspen soils, and 11.5 for AR and 25.4 for CR on conifer soils), and foliage DOC had higher k in conifer soils (60.2 for AL and 47.6 for CN on conifer soils and 45.4 for AL and 36 for CN on aspen soils). The interaction between depth and soil type for EP indicates that maximum retention was higher in aspen topsoils than in aspen subsoils (126.5 mg C kg^{-1} soil and 80 mg C kg^{-1} soil, respectively), while in conifer soils the depth pattern was the opposite. No statistically significant relationships were found between native concentration of SOC and any of the different sorption parameters.

Post-sorption DOC Quality

We calculated fluorescence indices and PARAFAC components to evaluate the effect of leachate quality on DOM sorption patterns. FI values of the sorption solutions did not change in relation to initial DOC concentration and overall ranged from 1.39 to 1.6 for all soils irrespective of the leachate treatment. HIX values (high values mean greater degree of humification and a low H:C ratio) at the lowest initial DOC concentrations (10, 20 mg DOC L^{-1}) reflected a soil signature (expressed as HIX at 0 mg DOC L^{-1} in **Figure 4**) of around 7. Values at these concentrations were also distinctly different from the pre- and post-sorption leachate baseline (<1 for AL, AR and CN, and <3 for CR; **Figure 4**). At the initial DOC concentration of around 40 mg L^{-1} the average HIX decreased to 3 for AL, 2.8 for AR, 3 for CN, and 4.4 for CR. We found no statistically significant differences between HIX values from solutions of aspen and conifer soils.

SUVA values stayed relatively constant for all concentrations of AR (2.3 ± 0.13), and decreased slightly for CN and CR (from 2.3 in the KCl treatment to 1.8 at 80 mg DOC L^{-1}). For AL, SUVA values initially increased from 2.3 to 2.9 at concentrations

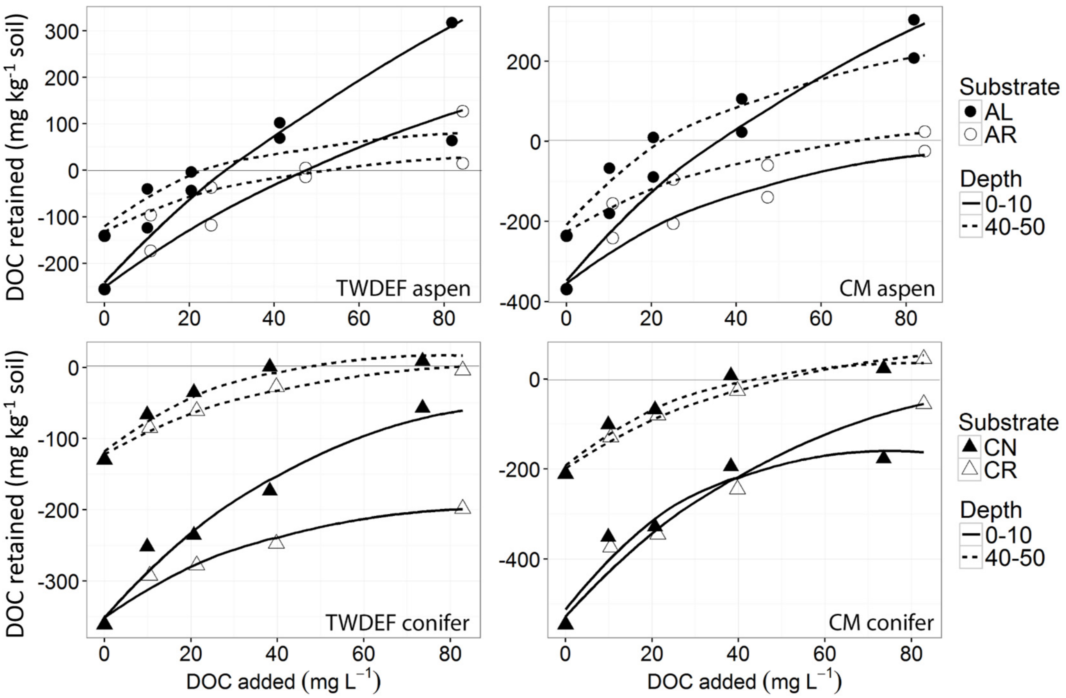

FIGURE 2 | Non-linear isotherms representing release/retention of dissolved organic carbon (DOC) from aspen leaves (AL) and aspen roots (AR) on aspen soils **(upper two graphs)**, and of fir needles (CN) and fir roots (CR) on conifer soils **(lower two graphs)** from TWDEF and CM sites. The *y*-axis indicates DOC retention in the area above zero, and DOC release in the area below the zero-line. The SE of laboratory replicates was mostly $< 5\%$.

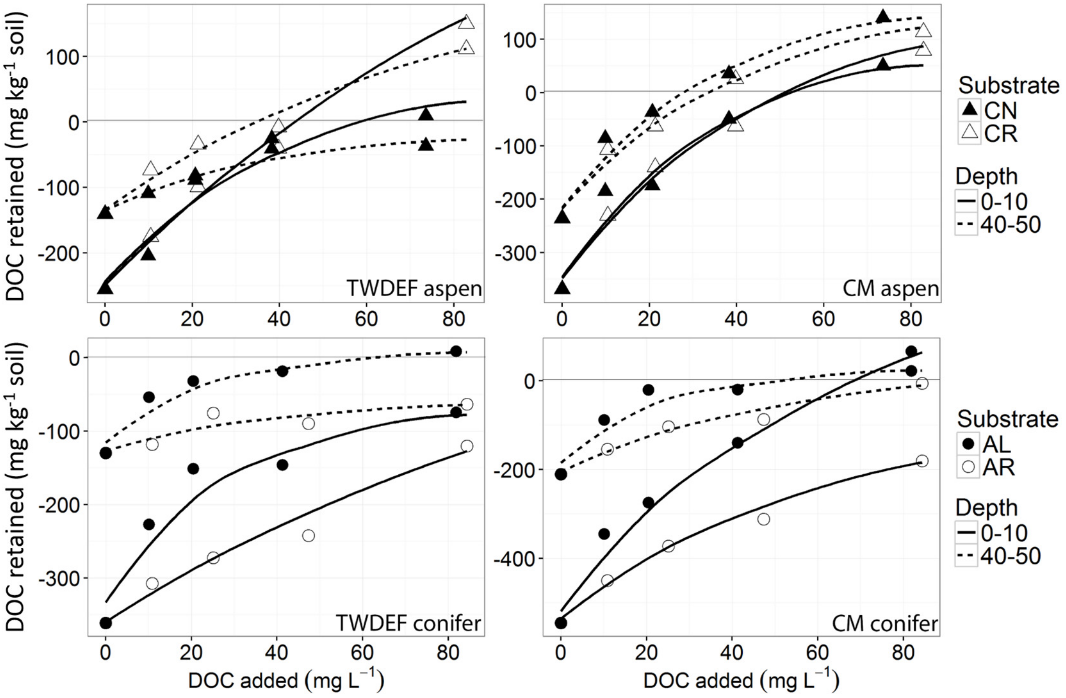

FIGURE 3 | Non-linear isotherms representing release/retention of dissolved organic carbon (DOC) from fir needles (CN) and fir roots (CR) on aspen soils **(upper two graphs)**, and of aspen leaves (AL) and aspen roots (AR) on conifer soils **(lower two graphs)** from TWDEF and CM sites. The *y*-axis indicates DOC retention in the area above zero, and DOC release in the area below the zero-line. The SE of laboratory replicates was mostly $< 5\%$.

TABLE 3 | ANOVA summary table for calculated sorption parameters (Bolding indicates statistically significant differences at α = 0.05).

		k		n		NPC		EP	
	df	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Leachate	3,13	**21.9**	**<0.01**	**7.32**	**<0.01**	**3.77**	**0.04**	**8.34**	**<0.01**
Soil type	1,13	0.01	0.94	**6.8**	**0.02**	**23**	**<0.01**	**48.7**	**<0.01**
Depth	1,13	0.07	0.79	**13.9**	**<0.01**	**5.95**	**0.03**	1.76	0.21
Site	1,13	**33.9**	**<0.01**	0.26	0.62	1.7	0.22	1.19	0.3
Leach × Soil	3,13	**3.86**	**0.04**	2.43	0.11	0.92	0.46	1.57	0.24
Leach × Depth	3,13	1.05	0.4	2.15	0.14	0.84	0.49	2.81	0.08
Leach × Site	3,13	1.84	0.19	1.24	0.34	0.19	0.9	1.05	0.4
Soil × Depth	1,13	0.75	0.4	0.01	0.92	1.52	0.24	**12.1**	**<0.01**
Soil × Site	1,13	0.28	0.61	1.23	0.29	1.46	0.25	0.09	0.77
Depth × Site	1,13	0.48	0.5	1.64	0.22	0.76	0.4	2.63	0.13

k and n are curve fitting parameters. NPC, null point concentration; EP, endpoint.

TABLE 4 | Average values ± standard error of the mean of calculated parameters for each level of each main effect (Bolding indicates statistically significant differences atα = 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	**24.8 ± 4.5**	2.09 ± 0.19	116.8 ± 32.1	160 ± 30.3
	CM	**43.8 ± 5.1**	2.11 ± 0.15	74.3 ± 12.3	39.6 ± 30.8
Depth					
	0–10	35.0 ± 5.6	**1.84 ± 0.14**	**118.0 ± 28.9**	13.44 ± 39.4
	40–50	33.6 ± 5.1	**2.36 ± 0.18**	**73.1 ± 18.4**	42.15 ± 17.5
Soil type					
	Aspen	32.4 ± 4.2	**1.88 ± 0.11**	**51.1 ± 6.9**	**103.2 ± 26.2**
	Conifer	36.2 ± 6.3	**2.31 ± 0.20**	**140.0 ± 30.6**	**−47.7 ± 20.9**
Leachate					
	AL	**52.8 ± 6.2^{a}**	**2.61 ± 0.35^{a}**	**57.6 ± 18.1^{a}**	**114.3 ± 51.1^{a}**
	AR	**16.2 ± 3.0^{c}**	**1.67 ± 0.12^{c}**	**126.0 ± 32.4^{b}**	**−28.4 ± 33.4^{b}**
	CN	**41.8 ± 8.3^{ab}**	**2.27 ± 0.17^{ab}**	**83.4 ± 19.9^{ab}**	**−4.8 ± 32.4^{b}**
	CR	**26.4 ± 4.3^{b}**	**1.85 ± 0.12^{bc}**	**115.3 ± 56.1^{ab}**	**30.07 ± 40.4^{ab}**

k and n are curve fitting parameters. NPC, null point concentration; EP, endpoint.

0, 10, and 20 mg L^{-1}, and decreased to 2.4 and 1.8 at higher concentrations (40 and 80 mg L^{-1}, respectively).

The non-parametric comparison of fluorescence components for all main effects (**Table 5**) showed that the humic C1 peak and protein-like C3 and C4 peaks differed significantly by leachate type. C1 proportion was highest for CR and C3 was highest for CN, while both constituted the smallest proportion of total fluorescence in samples treated with AL (**Table 6**). Most samples treated with AL were dominated by C4, which was much less abundant or completely absent in samples treated with the other leachates (**Table 6**). Overall C4 was absent in 40% of all samples, mostly from the CR treatment (missing in 80%). The proportion of C4 was highest in samples treated with the highest AL DOC concentrations and was missing in a few topsoil samples treated with the lowest AL DOC concentrations. On average, AL treated subsoils had 13 percentage-point higher C4 proportions than topsoils. Overall, AL treated samples showed a high variability between individual component proportions due to different responses of top- and subsoils (**Supplementary Figure 11**).

The proportions of all components differed significantly by depth (**Tables 5**, **6**). Topsoils had higher proportions of C1 and C2 than subsoils. In contrast the proportion of C3 was higher for subsoils than topsoils. C2 also differed significantly between sites, with CM soils having higher proportions compared to TWDEF soils (**Table 6**).

The proportions of the first two components showed a similar trend with initial DOC, as did HIX (**Supplementary Figures 3**, **4**). This means that for C1 and C2 the proportions decreased with increasing initial DOC concentration by 12 and 10 percentage-points, respectively. In contrast, for the protein-like C3 and C4 the proportions increased by, on average, 17 (for AR, CN, CR) and 30 (for AL) percentage-points, respectively.

DOC Desorption

In the final step we evaluated whether and to what extent there was a difference in the strength with which the sorbed SOC was held in the soils. As seen by the *y*-axis intercepts in **Figure 2**, aspen SOC was generally less water soluble than conifer SOC,

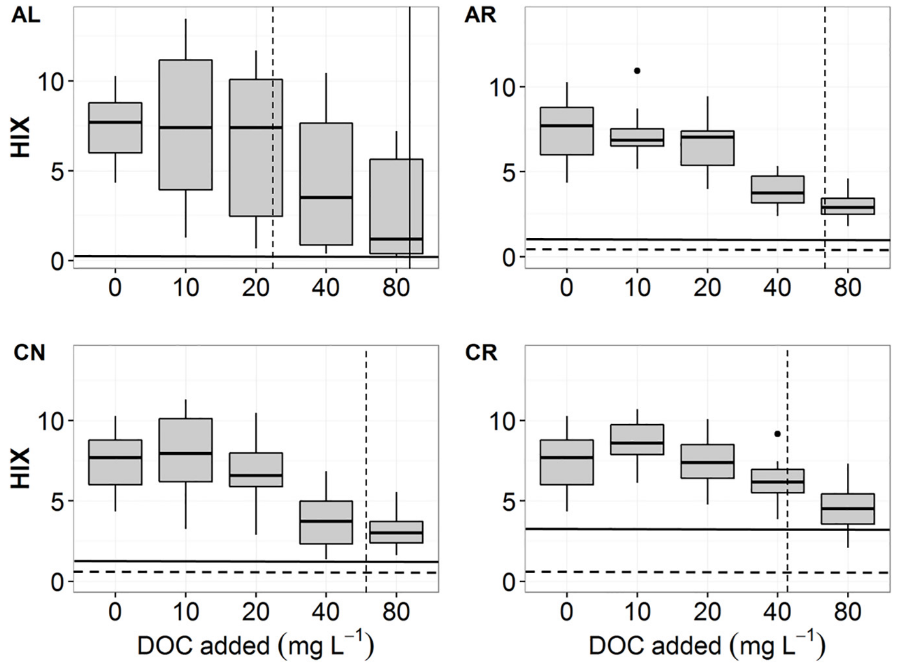

FIGURE 4 | HIX values of post-sorption solutions for all four leachates – AL, AR, CN, CR. The dashed horizontal lines indicate HIX values of fresh, pre-sorption leachates (AL = 0.06, AR = 0.37, CN = 0.73, CR = 0.58). The solid horizontal lines indicate HIX values for pure leachates after 24 h of shaking (AL = 0.06, AR = 1.17, CN = 1.34, CR = 2.99). The dashed vertical lines indicate the average NPC for aspen soils (AL = 24.6, AR = 70.4, CN = 65.2, CR = 44.2 mg L^{-1}). Average NPC for conifer soils is indicated with a solid vertical line for AL (AL = 90.5 mg L^{-1}), but could not be visualized for the other leachates due to being out of range (AR = 181.5, CN = 101.7, CR = 186.4 mg L^{-1}).

TABLE 5 | Kruskal–Wallis test result summary for fluorescence components in post-sorption DOM samples (Bolding indicates statistically significant differences at α = 0.05).

		C1		C2		C3		C4	
	df	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Leachate	3	**9.33**	**0.025**	2.58	0.46	**19.51**	**<0.01**	**15.59**	**<0.01**
Soil type	1	0.07	0.79	1.84	0.17	0.02	0.88	0.24	0.62
Depth	1	**12.82**	**<0.01**	**11**	**<0.01**	**3.84**	**0.05**	**8.9**	**<0.01**
Site	1	1.74	0.19	**10.51**	**<0.01**	2.27	0.13	2.89	0.09

despite higher intrinsic SOC levels in aspen soils than conifer soils (**Table 1**). On average, the desorption of this intrinsic SOC with the KCl solution yielded 9.2 ± 3.1 mg DOC g^{-1} C for aspen soils vs. 14 ± 5.6 mg DOC g^{-1} C for conifer soils.

In the single-step desorption following the sorption experiment (after 7 days of drying), aspen and conifer soils released similar DOC concentrations (3 ± 1.8 mg DOC L^{-1} from aspen soils and 3.6 ± 3.5 mg L^{-1} from conifer soils). However, as sorption on conifer soils was much weaker than on aspen soils, absolute retention was still almost twice as high for aspen soils as it was for conifer soils (10 mg DOC L^{-1} vs. 6.6 mg DOC L^{-1}). Among the leachate treatments, soils that had been treated with AL showed the lowest desorption with 2.2 ± 2 mg L^{-1}, while the other treatments released higher DOC concentrations with 3.6 ± 2.6 mg L^{-1} for AR, 3.4 ± 2.7 mg L^{-1} for CN, and 4.1 ± 3 mg L^{-1} for CR ($p = 0.04$, $F_{3,112} = 2.84$).

The soil solutions after the desorption process had changed qualitatively, and 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 any of the sorption solutions shown in **Figure 4**. Similarly to HIX, SUVA values also increased from an average of 2.2 ± 0.18 to 4.1 ± 0.37 for all leachate treatments, substantiating a shift to a more aromatic composition. The FI values of the solution did not change from the ones found after the sorption process (on average 1.5).

In contrast to the sorption samples the fluorescence indices in the desorption samples were overall similar among all soils and treatments, suggesting a similar quality of the SOM in solution. The proportion of the first two fluorescence components (C1 and C2) was higher in desorption samples (49 ± 2%; 43 ± 5%, respectively) than sorption samples at various initial concentrations of DOC (44 ± 8%; 30 ± 9%, respectively). In

TABLE 6 | Average values ± standard error of calculated fluorescence parameter proportions for each level of each main effect (Bolding indicates statistically significant differences at α = 0.05; letters indicate differences between levels of a factor).

Factors	Levels	C1 %	C2	C3	C4
Site					
	TWDEF	42 ± 1.5	**26 ± 1.3**	19 ± 1.4	14 ± 3.1
	CM	46 ± 0.9	**35 ± 1.1**	14 ± 1	5.4 ± 1.8
Depth					
	0–10	**48 ± 0.7**	**35 ± 0.9**	**14 ± 0.9**	**3 ± 1.3**
	40–50	**39 ± 1.4**	**26 ± 1.4**	**19 ± 1.5**	**16 ± 3.2**
Soil type					
	Aspen	45 ± 1	33 ± 1.2	16 ± 1.2	6.8 ± 2.1
	Conifer	43 ± 1.4	28 ± 1.4	17 ± 1.3	12 ± 2.9
Leachate					
	AL	**37 ± 2.7**^{ab}	24 ± 2.9	**8 ± 0.8**^{a}	**31 ± 5.8**^{a}
	AR	**44 ± 1**^{b}	32 ± 1.1	**18 ± 1.4**^{b}	**5 ± 0.8**^{b}
	CN	**44 ± 1.2**^{b}	31 ± 1.4	**23 ± 2.1**^{b}	**2 ± 0.4**^{b}
	CR	**49 ± 0.6**^{a}	35 ± 0.9	**16 ± 1.4**^{b}	**0.3 ± 0.1**^{c}

contrast, there was a decline in C3 from the sorption samples (16 ± 7%) to desorption solutions (6 ± 4%). The average proportion of C4 in the desorption samples ranged from 1.2 to 2.6%, with almost half of the samples completely lacking C4 (even the soils treated with AL), and the other half showing extremely small intensities ($<5\%$ of total fluorescence). None of the components in the desorption samples were significantly different as a function of leachate, soil type, and site. The proportion of C1 was significantly higher for topsoil than subsoil (50 ± 1.5% vs. 48 ± 2%; $\chi^2 = 11.25$, $p < 0.01$).

DISCUSSION

Understanding how tree species affect SOC pools is crucial for building better C models and reaching various ecosystem-service goals. This is true especially now, when the distribution of tree species is changing at local and global scales due to climate change and forest management practices (McKenney et al., 2007; IPCC, 2019 - 2.2.4). SOC under aspen forests has been shown to be more stable compared to adjacent conifer forests in various studies in North America (as reviewed by Laganière et al., 2017). The results of our study indicate that this stability might be due to enhanced sorption of DOC from aspen foliage. Furthermore, aspen DOM seems to help create mineral soil conditions that are more favorable to the sorption of incoming DOC, irrespective of the source.

Most sorption parameters in this study indicated that DOC derived from aboveground litter (foliage and needles) showed a higher retention than DOC of root leachates. The magnitude of this was, however, different for the two species. While the retention of AL differed significantly from the retention of AR, CN (subalpine fir needles) had only a slightly higher sorption than CR (subalpine fir roots). This, along with similar results for conifers reported by Hansson et al. (2010), suggests that the relative contribution of foliage and root DOC to mineral-associated organic matter can differ based on the tree species that dominate a forest stand. For aspen soils, the DOC contribution to mineral-associated SOC is most likely dominated by foliage leachates, while in conifer soils the contribution is represented by an equal mix of both.

The soils sampled from aspen and conifer stands in this study had similar soil mineral properties, and the soil horizons sampled (ABt and BAt in aspen and Bt in conifer soils at TWDEF) differed mostly in regard to the amount and type (aspen vs. conifer) of organic matter. Thus, the higher sorption and lower desorption in aspen soils, irrespective of the leachate type added, indicates that this effect is likely caused by SOM properties. The effect occurred even though aspen soils had higher SOC concentrations and was also more pronounced in the topsoils (steeper sorption slopes and higher maximum retention), which are more C-rich than subsoils.

Detritus and Soil DOM Quality

To test the hypothesis that DOM quality drives the sorption of DOM in mineral soil, we calculated various spectroscopic indices – SUVA, HIX, FI, and fluorophores from a PARAFAC model – and measured DOC and TN concentrations, and CO_2 release during the shaking process in the batch sorption experiment. The results did not always point in the same direction. In general, SUVA and HIX values indicated an aliphatic and labile nature of all plant leachates. FI suggested a more microbially derived nature of the foliage than root leachates. The leachate from aspen leaves stood out from the others in terms of HIX, FI, and the fluorescence components. They suggested that AL was of a more aliphatic nature than the other leachates. The DOC/TN ratio was, however, much higher for AL (145), and the mineralization of AL (not mixed with soil) was similar to the mineralization of CN leachates (similar proportion of DOC mineralized).

The high proportion of the protein-like C4 fluorescence component in AL clearly distinguished this leachate type from the others. The high C:N ratio (145) of the AL solution makes it unlikely, however, that this component was dominated by protein-like compounds. Other compounds, like tannins and lignin phenols, have been found to fluoresce with similar spectral signatures to proteins and amino acids (Aiken, 2014). Even though aspen foliage leachate has been found to contain much higher amounts of phenolic compounds compared to conifer needles (Startsev et al., 2008), the low HIX and high FI values indicate a highly aliphatic nature of AL leachates, which in turn would rule out high aromatic compound concentrations. Dominance of protein-like components in leachates of senesced litter together with low HIX and high FI has also been reported by Beggs and Summers (2011). Similar to our study, Beggs and Summers (2011) found that a similar protein-like peak to C4 was lost due to biodegradation from leachate formed from dead needles that had not been shed by the tree. Meanwhile, needles collected from the O-horizon in their study did not loose this component with biodegradation, suggesting different chemistry of the fluorophores representing this component. Overall, while a few studies have examined the fluorescence spectra of plant leachates with PARAFAC models (Beggs and Summers, 2011; Cuss et al., 2014; Wheeler et al., 2017), any interpretation of the protein-like peaks is still based on aquatic research findings in fresh or marine waters. Thus, there is a need to evaluate the assumption of amino acid contribution to the protein-like peaks with more detailed chemical composition data from terrestrial DOM.

Fluorescence indices for the description of leachate-soil exchange processes were only of limited value. The HIX values at small initial-DOC concentrations were similar to the soil-HIX signature (KCl solution treatment), which indicated that the soil-leachate mixture was mostly dominated by the desorbed SOM (**Figure 3** and **Supplementary Figures 3–6**). The high proportion of C1 and C2 components in these solutions, which suggest a highly processed DOM nature, also confirmed this. At higher initial-DOC concentrations, the plant signature (solid horizontal line in **Figure 4**) became more dominant, and the solutions had high proportions of the protein-peaks C3 for AR, CN, CR, and C4 for AL. The soil-leachate mixture, however, never acquired a purely plant-like signature, suggesting that even at high DOC concentrations, where sorption should be favored, desorption of native SOM occurred.

Leachate and Soil Interactions

In a field study conducted at TWDEF (Boča and Van Miegroet, 2017), we measured higher DOC concentrations and losses between 5 and 45 cm depths under conifers. The SOC pools under conifer stands were, however, much smaller than under aspen stands. This experiment elucidated why field DOC levels might not be good indicators for DOC sorption and SOC stocks in the aspen-conifer forests in Utah. The lower NPC of AL in aspen soils (25.8 and 19.9 mg$\cdot$L^{-1} in top- and subsoil, respectively) suggests that high concentrations are not necessary for the retention of AL DOC to occur in these soils. Conversely, the high NPC of subalpine fir leachates on conifer soils (102.4 and 52.1 mg$\cdot$L^{-1} for CN and 503.7 and 83.6 mg$\cdot$L^{-1} for CR in top- and subsoil) suggests that the conifer field DOC concentrations (28.4–45.5 mg$\cdot$L^{-1} under conifers and 7.3–23.8 mg$\cdot$L^{-1} under aspen) might not be sufficient for DOC retention to commence in these soils. The lower sorption of subalpine fir DOC to conifer soils might suggest a lower stability of conifer SOC due to fewer mineral-organic associations. This is in agreement with results from long-term incubation experiments that have shown conifer soils having higher heterotrophic respiration than aspen soils (Giardina et al., 2001; Woldeselassie et al., 2012; Laganiére et al., 2013).

Our study does not allow us to elucidate the mechanisms behind higher DOC sorption in aspen soils. Based on other published literature we can hypothesize that the higher sorption was either due to an unmeasured property of the mineral soil or due to the properties of SOM. In regard to unmeasured properties we can only speculate that there could be differences in the very fine clays between soils that might have developed due to differing mineral weathering trajectories caused by the overlaying vegetation (Taylor et al., 2009). In regards to organic matter, one explanation could be that aspen, with their more nutrient rich foliage, facilitate a more rapid formation of mineral-associated organic matter via microbial pathways (Craig et al., 2018; Lavallee et al., 2018). Considering that aspen soils were more receptive to DOC irrespective of the source, the microbial biomass attached to minerals in patches already containing old C (Vogel et al., 2014) could be more active and/or efficient in aspen than in conifer soils. The similar proportion of mineralized DOC on both soils, found in this study, does not necessarily indicate similar carbon use efficiency, especially for substrates of contrasting quality (Manzoni et al., 2012), and, therefore, cannot exclude a difference in microbial activity in both soils. Indeed, as mentioned above, long-term laboratory incubations have shown that more SOC is mineralized in conifer soils than aspen soils (Giardina et al., 2001; Woldeselassie et al., 2012; Laganiére et al., 2013), suggesting a difference in microbial functioning. Furthermore, community-level physiological profiling data showed that microbial biomass increases with increasing aspen basal area (Román Dobarco et al., 2020). Finally, a second organic pathway could be organic matter precipitation by forming supramolecular organic-organic associations (Sutton and Sposito, 2005). Further studies with more detailed methods are needed to test these hypotheses.

Differences in pH have been commonly observed under angiosperm and gymnosperm overstories (Augusto et al., 2014), and soils in our study were no exception. Most laboratory findings assume a reduction of the sorption capacity with increasing pH (as reviewed by Michalzik et al., 2001). In our study, pH was slightly higher in aspen soils, which should lead to lower sorption. This, however, was not the case, suggesting that either the difference in pH was not big enough to cause measurable differences in sorption or the effect of DOM chemistry was stronger. In terms of potential impacts from differences in Fe oxihydroxide concentrations between the two overstory soils at TWDEF (**Table 1**), any effect of this parameter should remain smaller than its significance in causing differences between sampling locations. Large Fe and Al oxyhydroxide differences were observed between CM and TWDEF but sorption differed only minimally between these sites.

While in this experiment we used needles and roots only from subalpine fir, the results could still be representative of other conifers. The higher SOC pools under aspen compared to various conifer species in Utah (Woldeselassie et al., 2012; Román Dobarco and Van Miegroet, 2014; Boča and Van Miegroet, 2017), and higher SOC stability under aspen compared to various conifers in North America (Laganière et al., 2017), certainly point in that direction. Concomitantly, the absence of a consistently higher aspen effect on SOC pools in North America might indicate that other factors, like climate, might also control the species' effect. This could happen by climate causing variations in plant functional traits (Reich et al., 2003) or by differently affecting the strength of mechanisms for SOC accumulation.

Limitations

The initial DOC concentration in the aspen foliage leachate was ten times higher than the other leachates, indicating that freshly senesced aspen leaves contained more water-soluble compounds. These concentrations obtained under laboratory conditions were much higher than those observed in the field (Boča and Van Miegroet, 2017). While freezing of plant material can increase concentrations of dissolved organic C, N, phenolic compounds, and proteins (Kiikkilä et al., 2012), such conditions have also been observed in the natural environment, where the material was collected (Boča and Van Miegroet, 2017). We believe that grinding the biomass resulted in elevated concentrations of DOM in the leachates, but this was necessary to perform the experiment. While not completely representative of field conditions, the chosen approach for leachate generation still allows for a relative comparison of sorption between leachate types.

The NPC values from the laboratory experiment are unlikely to represent NPC values in the field due to the experimental setup (sieved soils, shaking process, and filtration). These values, however, allowed us to do a relative comparison between the sorption behavior of both soils, and they clearly showed fundamental differences between them.

CONCLUSION

In this study, we compared DOC sorption in soils of two contrasting forest overstory types – quaking aspen and conifers (subalpine fir and Douglas fir). We found that aspen soils retained more DOC than conifer soils, irrespective of the leachate type. Furthermore, aspen soils retained aspen foliage DOC especially well. While higher DOC concentrations overall increased sorption, this depended on the quality of the leachate source and the native SOC already present in soil. The findings suggest that while aspen forests have lower DOC concentrations in soil pore water measured in the field than conifers, sorption in aspen forest soils can commence at these lower concentrations. Furthermore, the study provides a foundation on which to build further investigations for understanding the exact mechanisms that allow for more efficient incorporation of labile DOM into SOC.

The results also indicate that 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, however, will not necessarily lead to immediate or quick soil C losses, as aspen SOC currently present in the soil is also receptive to sorption of DOC from conifer leachates. This dynamic could change in the long term if aspen SOC is replaced by conifer SOC.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AJ and HV conceived the experiment. AB, AJ, and HV designed the experiment. AB executed the experiment, conceptualized, and wrote the manuscript as lead author. All the authors contributed to the interpretation of the findings and the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2020.594473/full#supplementary-material

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Impact of European Beech Forest Diversification on Soil Organic Carbon and Total Nitrogen Stocks–A Meta-Analysis

Stephanie Rehschuh*[1], *Mathieu Jonard*[2], *Martin Wiesmeier*[3], *Heinz Rennenberg*[4] and *Michael Dannenmann*[1]

[1] Institute of Meteorology and Climate Research–Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Garmisch-Partenkirchen, Germany, [2] Earth and Life Institute, Université catholique de Louvain, Louvain-la-Neuve, Belgium, [3] Chair of Soil Sciences, TUM School of Life Sciences, Technische Universität München, Freising-Weihenstephan, Germany, [4] Chair of Tree Physiology, Institute of Forest Sciences, University of Freiburg, Freiburg, Germany

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Jérôme Laganière,
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University of Göttingen, Germany

****Correspondence:***
Michael Dannenmann
michael.dannenmann@kit.edu

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Drought-sensitive European beech forests are increasingly challenged by climate change. Admixing other, preferably more deep-rooting, tree species has been proposed to increase the resilience of beech forests to drought. This diversification of beech forests might also affect soil organic carbon (SOC) and total nitrogen (TN) stocks that are relevant for a wide range of soil functions and ecosystem services, such as water and nutrient retention, filter functions and erosion control. Since information of these effects is scattered, our aim was to synthesize results from studies that compared SOC/TN stocks of beech monocultures with those of beech stands mixed with other tree species as well as monocultures of other tree species. We conducted a meta-analysis including 38 studies with 203, 220, and 160 observations for forest floor (i.e., the organic surface layer), mineral soil (0.5 m depth) and the total soil profile, respectively. Monoculture conifer stands had higher SOC stocks compared to monoculture beech in general, especially in the forest floor (up to 200% in larch forests). In contrast, other broadleaved tree species (oak, ash, lime, maple, hornbeam) showed lower SOC stocks in the forest floor compared to beech, with little impact on total SOC stocks. Comparing mixed beech-conifer stands (average mixing ratio with regard to number of trees 50:50) with beech monocultures revealed significantly higher total SOC stocks of around 9% and a smaller increase in TN stocks of around 4%. This equaled a SOC accrual of 0.1 Mg ha^{-1} yr^{-1}. In contrast, mixed beech-broadleaved stands did not show significant differences in total SOC stocks. Conifer admixture effects on beech forest SOC were of additive nature. Admixing other tree species to beech monoculture stands was most effective to increase SOC stocks on low carbon soils with a sandy texture and nitrogen limitation (i.e., a high C/N ratio and low nitrogen deposition). We conclude that, with targeted admixture measures of coniferous species, an increase in SOC stocks in beech forests can be achieved as part of the necessary adaptation of beech forests to climate change.

Keywords: tree species, mixed forests, forest management, carbon storage, review

INTRODUCTION

European beech (*Fagus sylvatica*)–the dominant species of the potential natural forest vegetation and the main broad-leaved forest tree species in Central Europe (Ellenberg, 1988; Bolte et al., 2007) is increasingly affected by climate change due to longer, more intense and more frequent summer droughts. This has already led to physiological constrains (Rennenberg et al., 2006) as well as nutrient limitations including impaired ecosystem nitrogen cycling (Geβler et al., 2004; Jonard et al., 2015; Dannenmann et al., 2016). Several studies showed that the admixture of different tree species (e.g., oak, pine, and silver fir) to European beech can reduce drought susceptibility of beech (Pretzsch et al., 2013; Metz et al., 2016; Magh et al., 2018). These admixtures to beech might not only alter soil water dynamics and availability, but also change soil organic carbon (SOC) and nutrient cycling and accumulation in soils, compared to monoculture stands. A targeted selection of tree species has a large potential for SOC accumulation (Mayer et al., 2020) with up to 2–5 fold organic carbon (C) stock increase in the forest floor and up to two fold in mineral soil (Vesterdal et al., 2013). Organic C in soils is relevant for a wide range of soil functions and ecosystem services such as water and nutrient retention, erosion control and climate regulation (Lal, 2006; Lal et al., 2018). In this context, forests are increasingly discussed as option to mitigate greenhouse gas emissions (Hulvey et al., 2013). According to the United Nations framework convention on climate change of 1992 and the Kyoto protocol of 1997, C sequestration in terrestrial sinks by silvicultural management, including a change in tree species, has been accepted to offset carbon dioxide emissions for national carbon budgets.

However, information on admixing tree species to increase forest stand resilience in the context of climate change, and the potential consequences for SOC and total nitrogen (TN) stocks are scarce, but urgently required for forest management and policy. Effects of tree species on SOC and TN stocks have been studied mostly using common garden experiments or retrospective analyses (comparison of paired stands or single trees). Earlier research mostly addressed the differences in SOC stocks of monoculture forest stands (Ovington, 1956; Vesterdal and Raulund-Rasmussen, 1998; Gurmesa et al., 2013). In most cases coniferous and broadleaved stands were compared, with conifers revealing generally higher organic C stocks in the forest floor with rather inconsistent effects in the mineral soil (Vesterdal et al., 2013). Later, also interest in mixed forests increased (Borken and Beese, 2005; Cremer et al., 2016; Dawud et al., 2017). The most recent reviews on the effects of tree species on SOC stocks in temperate forests were conducted in the last decade (Vesterdal et al., 2013; Boča et al., 2014), however, comprehensive summaries on SOC stocks in mixed forest stands do not exist. So far, only the contribution of tree mixtures and tree species diversity to aboveground carbon sequestration have been reviewed (Hulvey et al., 2013).

Due to the high relevance of European beech in Europe, our central aim was to synthesize results from studies that compared SOC and TN stocks of beech monocultures with those of mixed beech stands as well as monocultures of other tree species. Our objectives were to: (1) detect effects of admixing other tree species to beech stands on SOC and TN stocks, (2) compare these stocks under beech monocultures with those of other monocultures, (3) identify soil and climatic factors that indicate the potential for SOC/TN stock increase due to admixture, and (4) identify knowledge gaps and future research needs. We hypothesized that (1) admixture effects on SOC stocks cannot be linearly derived from stocks in the corresponding monoculture stands due to non-additive and synergistic species mixture effects, and (2) that admixture of coniferous trees to beech stands results in larger increases of SOC and TN stocks than admixture of other broadleaved species. Finally, we expected that (3) the admixing effects vary depending on both soil and climate conditions.

MATERIALS AND METHODS

Data Compilation

A literature search was performed up to 13.12.2019 using different databases (see **Supplement 1** for flow diagram of protocol). Besides others, key words used and differently combined were *tree species, beech, tree diversity, soil organic carbon, carbon, stocks, storage* as well as different countries and different tree species. The articles had to match the following criteria to be included in the meta-analysis: (1) The experiments used paired stands/designs (i.e., common garden experiments or retrospective analyses with adjacent stands on the same parent material, similar history and stand age) of European beech and at least one other tree species/tree species mixture with beech; (2) organic C stocks in the forest floor and/or mineral soil were reported or could be calculated; and (3) sampling designs included replicated measurements ($n \geq 3$). In case, TN stocks were reported or could be calculated, we also collected information on this parameter. Data were extracted from tables or graphs by digitalization using GetData Graph Digitizer (version 2.26, Russian Federation). If a study included multiple observations at different sites, each site was regarded as independent study and included in the analysis. We recorded the data only once, if different studies were using the same data/ study site. Original SOC/TN stock data were converted to standard units (Mg ha^{-1}). In case, only SOC/TN concentrations were reported, stocks were calculated using bulk density (BD) and sampling depth. If BDs were not determined (2 cases), they were estimated using the pedotransfer function from Post and Kwon (2000) (Equation 1) based on the organic matter (OM) content.

$$BD = \frac{100}{\frac{OM\ (\%)}{0.244} + \frac{100 - OM\ (\%)}{1.64}} \text{ with } OM\ (\%) = \frac{C\ (\%)}{0.58} \quad (1)$$

Sampling depth for SOC stocks of the various studies ranged from 2 to 100 cm (**Table 1**). The mean sampling depth was 35 cm and for the comparison of SOC/TN stock data from various studies with different soil depths, a depth of 50 cm was selected. Although this does not reflect the full picture of SOC stocks, differences in SOC stocks due to species composition generally occur in the upper mineral soil (Jandl et al., 2014; Jonard et al.,

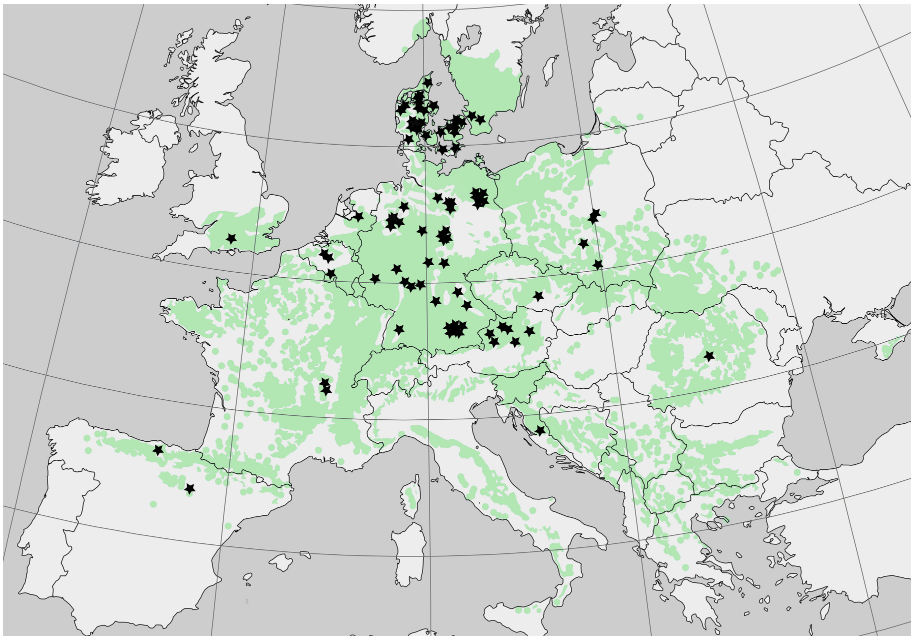

FIGURE 1 | Distribution of forest sites included in the meta-analysis ($n = 102$) illustrated by stars. The green color indicates the potential natural distribution area of European beech. Source: http://www.euforgen.org/species/fagus-sylvatica/.

2017). Original SOC/TN stock data presenting stocks for a depth <50 cm (21 studies, see **Table 1**) were extrapolated to 50 cm using the depth distribution functions from Jobbágy and Jackson (2000, 2001) (Equations 2, 3), where Y is the cumulative proportion of the SOC/TN stock from the soil surface to the depth d (cm). The relative rate of SOC stock decrease with depth (β) was calculated separately for deciduous stands (0.9656), coniferous (0.9673) and mixed beech-coniferous stands (0.9665). The SOC/TN stock in the upper 50 cm (X50) was calculated using β, the original SOC/TN stock (Mg ha^{-1}) (Xd0) and the original soil depth available in individual studies (cm) (d0) (Equation 3).

$$Y\,(\%) = 1 - \beta^{d} \tag{2}$$

$$X_{50} = \frac{1 - \beta^{50}}{1 - \beta^{d0}} * X_{d0} \tag{3}$$

A test based on available measured profile data revealed that this extrapolation to 50 cm had neither effects on SOC and TN stocks nor on assessment of tree species effects (**Supplement 2**).

Studies were also included when only organic C stocks for the forest floor or the mineral soil were reported. From reports presenting both, forest floor plus mineral soil, total SOC stocks were calculated. The dataset was split into three subsets: forest floor only, mineral soil only (50 cm), and total soil profile to 50 cm. Thus, we obtained a meta-data set of 203, 220, and 160 observations for forest floor, mineral soil and total soil profile from 38 studies, respectively (list of data sources see **Table 1**). European beech and comparisons with other species, were investigated from its northern to it's southern distribution limit. However, studies in Mediterranean and boreal climate region were very limited (two studies in the Mediterranean) (**Table 1**). Data were collected from 13 countries, with dominating research in Germany and Denmark (**Figure 1**).

We compiled information of SOC/TN stocks for 12 different tree species and seven types of mixtures with beech, each compared with monocultures of European beech (**Table 1**). Information of little studied species (e.g., *Larix decidua*) were merged with information on species of the same genus (e.g., *Larix kaempferi*) under their genus name (larch), if species effects showed the same trend. Species included are as follows (English names are used in text and figures): Norway and Sitka spruce (*Picea abies* & *P. sitchensis*), Douglas fir (*Pseudotsuga menziesii*), silver and Grand fir (*Abies alba & A. grandis*), larch (*Larix decidua & L. kaempferi*), pine (*Pinus sylvestris; P. contorta; P. nigra*), ash (*Fraxinus excelsior*), sycamore maple (*Acer pseudoplatanus*), lime (*Tilia platyphyllos & T. cordata*), oak (*Quercus robur & Q. petraea*), and hornbeam (*Carpinus betulus*). Main research from larch was on Japanese larch (*Larix kaempferi*), from oak on pedunculate oak (*Quercus robur*) and from spruce on Norway spruce (*Picea abies*). The age of the forest stands ranged between 27 and 160 years. If not stated otherwise, "broadleaved" refers to "broadleaved other than beech." From the 14 studies investigating mixed stands, eight indicated the mixing composition: The percentage of beech in the mixtures ranged between 40 and 60%, with an average of 49%. Since the information on the mixture composition was limited, the dataset did not allow us to derive conclusions for different mixing ratios.

For each study we compiled meta-data as predictor variables, i.e., information regarding location, climate, stand, and soil properties (**Supplement 1**). For unification, we transferred the various soil taxonomies from national soil classifications used by the individual studies to the international classification of the

World Reference Base for Soil Resources (IUSS Working Group WRB, 2015). Soil texture was summarized in four categories from sandy to loamy clay/clayey loam. Furthermore, the percentage of the sand content was extracted. If only the soil texture class was given, the percentage of sand was estimated using the soil texture triangle. We selected the months May to October as an approach to represent growing season climate and to calculate the drought index for each of this month (Equation 4). The drought index was later averaged for the whole 6 months period.

$$\text{Drought index (month)} = \frac{monthly\ sum\ of\ precipitation}{monthly\ mean\ temperature + 10} \quad (4)$$

Information on nitrogen deposition was collected from external sources (e.g.,[1] for Belgium or[2] for Germany).

Data Calculation, Statistical Analysis and Presentation

Since standard deviations were mostly not presented in the desired soil depth and a sum of standard deviations from each single soil layer would have created even a higher bias, an unweighted meta-analysis was used as has been common in previous SOC stock studies (Guo and Gifford, 2002; Li et al., 2012; Boča et al., 2014). For all publications included in the meta-analysis, the response ratio was calculated as the absolute annual difference of SOC stocks between the various tree species and mixtures in comparison to European beech monoculture (in the following shortened by using the phrase "tree-species-induced differences in SOC stocks") (Equation 5). In addition, the percentage differences in SOC stock were calculated.

$$\text{absolute difference in SOC stock } \left(Mg\ ha^{-1}\ yr^{-1}\right) = \frac{SOC\ stock\ of\ respective\ species\ \left(Mg\ ha^{-1}\right) - \ SOC\ stock\ of\ beech\ \left(Mg\ ha^{-1}\right)}{stand\ age\ (years)} \quad (5)$$

As the number of observations was relatively scarce for some tree species, we combined results to different groups (conifers, broadleaved, mixed beech-conifers, and mixed beech-broadleaved). The difference between the SOC stock of the control (beech monoculture) and the treatment (various other tree species and mixtures) was considered significant at $p < 0.05$ when the 95% confidence interval did not include 0% difference (i.e., no change) in SOC stocks.

Multivariate models were developed to explore the impact of potential soil and environmental controls on tree-species-induced organic C stock differences in the forest floor and the mineral soil. To correct the percentage differences in SOC stocks for heteroscedasticity, values were log transformed. For the selection of the most relevant variables to be included in the model, we used multiple stepwise regressions with Akaike information criterion (AIC) for best model selection. The following potential predictor variables were considered for the extent of tree-species-induced SOC stock differences: stand type (categorical), forest floor/mineral soil organic C stock of the reference beech monoculture stand, forest floor/mineral soil C/N ratio, forest floor organic C stock of the respective stand, sand content in the mineral soil, nitrogen deposition, stand age of the paired stands, mean annual temperature (MAT) and mean annual precipitation (MAP) (all numeric).

Statistical analyses and data presentation were performed using R 3.5.3 (R Core Team, 2017). To test for normal distribution and homogeneity of variances the Shapiro-Wilk test and the Levene test were used at $p < 0.05$. The one-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test (for normally distributed data) and the Kruskal–Wallis test followed by the Bonferroni *post hoc* test (for non-normally distributed data) was applied to compare mean values among various groups with $p < 0.05$ set as threshold for significant differences.

To test for interactive effects of species mixtures, we analyzed experimental designs that included a beech monoculture, a mixed beech stand and a monoculture of the admixed species. The Friedman test, a non-parametric paired test, followed by the Wilcoxon-Nemenyi-McDonald-Thompson *post hoc* test was used to test for significant differences between the SOC stocks of the mixed stand, beech monoculture and the monoculture of the admixed species. To test for the type of mixing effect (additive vs. non-additive) we compared the expected SOC stocks for the mixture (calculated based on the proportions for the corresponding monocultures) with the observed SOC stocks for the mixtures. From the regression patterns mixing effects can be derived (**Figure 2**). After assessing data distribution and homogeneity of variance, the paired t-test allowed to test whether the expected SOC stock was significantly different from the observed SOC stock.

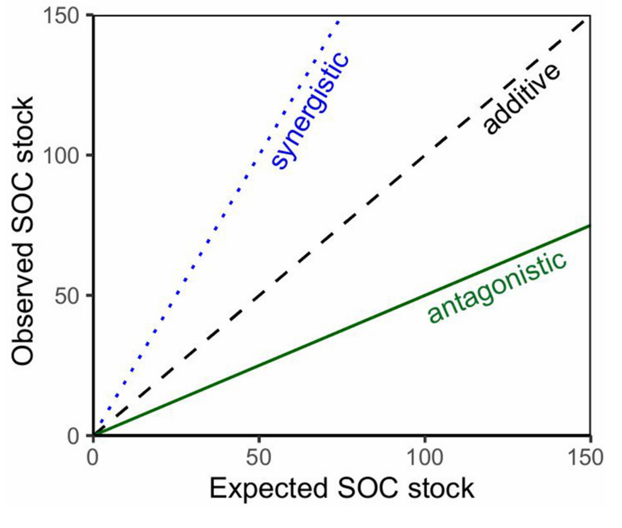

FIGURE 2 | Mixing effects derived from the relation of expected SOC stocks of mixed forest stands (based on calculated stocks using the corresponding monocultures) and observed SOC stocks for the mixed stands.

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[1] https://www.milieurapport.be/milieuthemas/vermesting-verzuring/vermesting/stikstofdepositie

[2] https://gis.uba.de/website/depo1/

TABLE 1 | References included in the SOC stock database for meta-analysis.

Authors(s) (year)	Location	Tree species	Sampled layers (sampling depth of MS in cm)	No. of study site(s)
Andivia et al. (2016)	Czech Republic	Beech, Spruce, Mixed Beech-Spruce	FF, MS (10)	1
Andersen et al. (2004)	Denmark	Beech, Spruce, Oak, Grand fir	MS (90)	4
Bagherzadeh et al. (2008)	Germany	Beech, Spruce, Mixed Beech-Spruce	FF	1
Berger et al. (2010)	Austria	Beech, Spruce, Mixed Beech-Spruce	FF, MS (10)	6
Berger et al. (2015)	Austria	Beech, Spruce, Mixed Beech-Spruce	MS (10)	2
Błońska et al. (2018)	Germany, Poland	Beech, Pine, Mixed Beech-Pine	FF, MS (25)	3
Borken and Beese (2005)	Germany	Beech, Spruce, Mixed Beech-Spruce	FF	1
Borken et al. (2002)	Germany	Beech, Spruce, Pine	MS (10)	2
Butterbach-Bahl et al. (2002)	Germany	Beech, Spruce	FF, MS (20)	1
Christiansen et al. (2012)	Denmark	Beech, Ash, Oak	FF, MS (100)	3
Cremer et al. (2016)	Germany	Beech, Spruce, Mixed Beech-Spruce, Douglas fir, Mixed Beech-Douglas fir	FF, MS (60)	3
Dawud et al. (2017)	Denmark	Beech, Douglas fir, Mixed Beech-Douglas fir	FF, MS (20)	2
Elberling and Ladegaard-Pedersen (2005)	Denmark	Beech, Spruce	FF, MS (100)	1
Gartzia-Bengoetxea et al. (2009)	Spain	Beech, Oak	MS (5)	1
Girona-García et al. (2018)	Spain	Beech, Pine	FF, MS (10)	1
Gurmesa et al. (2013)	Denmark	Beech, Spruce, Oak, Larch	FF, MS (30)	8
Unpublished C and N content determined by a CHN analyzer; soil sampling see Jonard et al. (2007)	Belgium	Beech, Oak, Beech-Oak	FF, MS (2)	1
Ladegaard-Pedersen et al. (2005)	Denmark	Beech, Spruce, Douglas fir, Silver fir, Grad fir, Oak, Pine, Sitka spruce, Larch	FF (one site), MS ((both sites:) 50)	2
Langenbruch et al. (2012)	Germany	Beech, Ash, Lime, Mixed Beech-Ash, Mixed Beech-Lime, Mixed Lime-Ash	FF, MS (20)	2
Leuschner et al. (2013)	Germany	Beech, Pine	FF, MS (60)	5
Lorenz and Thiele-Bruhn (2019)	Germany	Beech, Douglas fir, Pine	FF, MS (30)	1
Mareschal et al. (2010)	France	Beech, Douglas fir, Spruce, Pine	MS (15)	1
Mueller et al. (2012)	Poland	Beech, Silver fir, Spruce, Douglas fir, Oak, Pine, Lime, Larch, Maple	MS (20)	1
Neirynck et al. (2000)	Belgium	Beech, Oak (1 site), Lime (1 site), Maple (1 site)	MS (22)	2
Nihlgård (1971)	Sweden	Beech, Spruce	FF, MS (65)	1
Nitsch et al. (2018)	Germany	Beech, Oak, Mixed Beech-Pine	MS (55)	10
Oostra et al. (2006)	Sweden	Beech, Spruce, Oak, Ash, Hornbeam	FF, MS (20)	1
Ovington (1956)	Great Britain	Beech, Spruce, Douglas fir, Grand fir, Oak, Larch	FF, MS (60)	1
Prietzel and Bachmann (2012)	Germany	Beech, Douglas fir	FF, MS (50)	9
Rehschuh et al. (2019)	Germany	Beech, Beech-Silver fir	FF, MS (90)	1
Rehschuh et al. (unpublished); Methods and results for SOC/TN stock analysis see **Supplement 3**	Croatia	Beech, Silver fir, Beech-Silver fir	FF, MS (45)	1
Schmidt et al. (2015)	Germany	Beech, Oak, Lime, Hornbeam	MS (10)	1
Schulp et al. (2008)	The Netherlands	Beech, Douglas fir, Pine, Larch	FF, MS (20)	1
Trum et al. (2011)	France	Beech, Spruce, Douglas fir, Oak	FF	1
Vesterdal et al. (2008)	Denmark	Beech, Spruce, Ash, Lime, Maple	FF, MS (30)	6
Vesterdal and Raulund-Rasmussen (1998)	Denmark	Beech, Spruce, Douglas fir, Grand fir, Sitka spruce, Oak, Pine	FF	7
Zederer et al. (2017)	Germany	Beech, Spruce	FF (3 sites), MS (all sites) (5)	4
Zhiyanski et al. (2008)	Bulgaria	Beech, Spruce	MS (50)	1

Soil layers: FF-forest floor, MS-mineral soil.

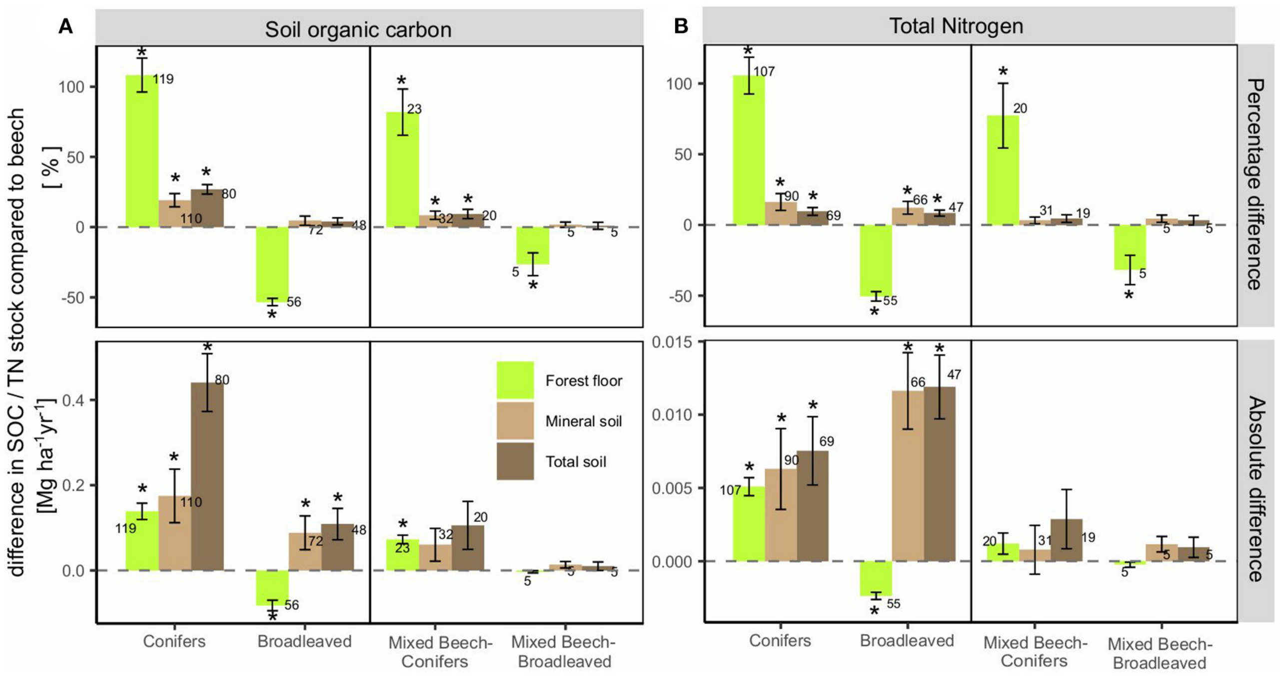

FIGURE 3 | Average percentage and absolute annual differences of **(A)** SOC and **(B)** TN stocks summarized for monocultures (conifers, broadleaved) and mixtures with beech (mixed beech-conifers/mixed beech- broadleaved) compared to beech monocultures. Average mixing ratio: 50%/50%. Numbers next to the bars are the number of observations. Error bars show the standard error. Asterisks indicated significant differences compared to beech monocultures. Note that in some cases the difference for the total soil is not the sum of those for the forest floor and mineral soil, since not all studies included observations for both layers.

RESULTS

We found generally more observations comparing beech monoculture with other monocultures than comparing beech monoculture with mixed-beech stands (**Supplement 4**). Especially for spruce and oak, mixtures with beech were rarely studied: While there were ∼40 observations comparing spruce and oak monocultures with beech monocultures, only 10–13 and one observations compared beech vs. spruce-beech and oak-beech mixed stands, respectively. Generally, tree-species induced effect sizes (expressed as % SOC stock difference) were largest in the forest floor (**Supplement 5**). Because of the small size of the forest floor C pool compared to the mineral soil C pool, annual changes in SOC accrual for the entire soil profile (Mg $\mathrm{ha^{-1}}$ $\mathrm{yr^{-1}}$ of C) were however mostly dominated by effects in the mineral soil (**Figure 3**).

Tree Type Effects on SOC Stocks

Conifer monocultures showed significantly higher SOC accumulation in all soil layers when compared with beech monocultures (**Figure 3A**). Broadleaved monoculture stands other than beech had substantially less organic C in the forest floor compared to beech, but a larger SOC accrual in the mineral soil and total soil profile (50 cm) (0.09 Mg C $\mathrm{ha^{-1}}$ $\mathrm{yr^{-1}}$, $p < 0.05$). Comparing mixed stands to beech monocultures generally revealed similar effects as indicated from comparing monocultures (**Figure 3A**). Mixed beech-conifer forests had on average 9% more SOC in the total soil profile, which translated into an increased SOC accrual of 0.1 Mg C $\mathrm{ha^{-1}}$ $\mathrm{yr^{-1}}$ (**Figure 3A**). In contrast, mixtures of beech with other broadleaved tree species on average showed similar SOC stocks as compared to beech monocultures.

Effects of Single Tree Species on SOC Stocks

Among the coniferous tree species, monocultures of larch, spruce, Douglas fir as well as silver/Grand fir showed the largest increases in total SOC stocks compared to beech monoculture, while only pine monocultures had similar SOC stocks as beech monoculture (**Figure 4**, **Supplement 5A**). Coniferous forest floor organic C stocks were generally considerably higher when compared to beech with up to 200% or 0.32 Mg $\mathrm{ha^{-1}}$ $\mathrm{yr^{-1}}$ in larch forests, that was also the case for mineral organic C stocks that revealed up to 45% or 0.30 Mg $\mathrm{ha^{-1}}$ $\mathrm{yr^{-1}}$ higher stocks (silver & Grand fir stands) (**Figure 4**). Monoculture stands of oak, ash, lime, and maple stored significantly less organic C in the forest floor than beech monocultures (41–72%), but more in the mineral soil (up to 28%), which overall slightly higher SOC stocks in broadleaved monocultures other than beech (**Figure 4**, **Supplement 5A**).

European beech stands mixed with the conifers spruce, Douglas fir, pine and silver/Grand fir showed considerably higher organic C stocks in the forest floor (2–101%, 60% on average, **Figure 5**, **Supplement 5B**) compared to beech

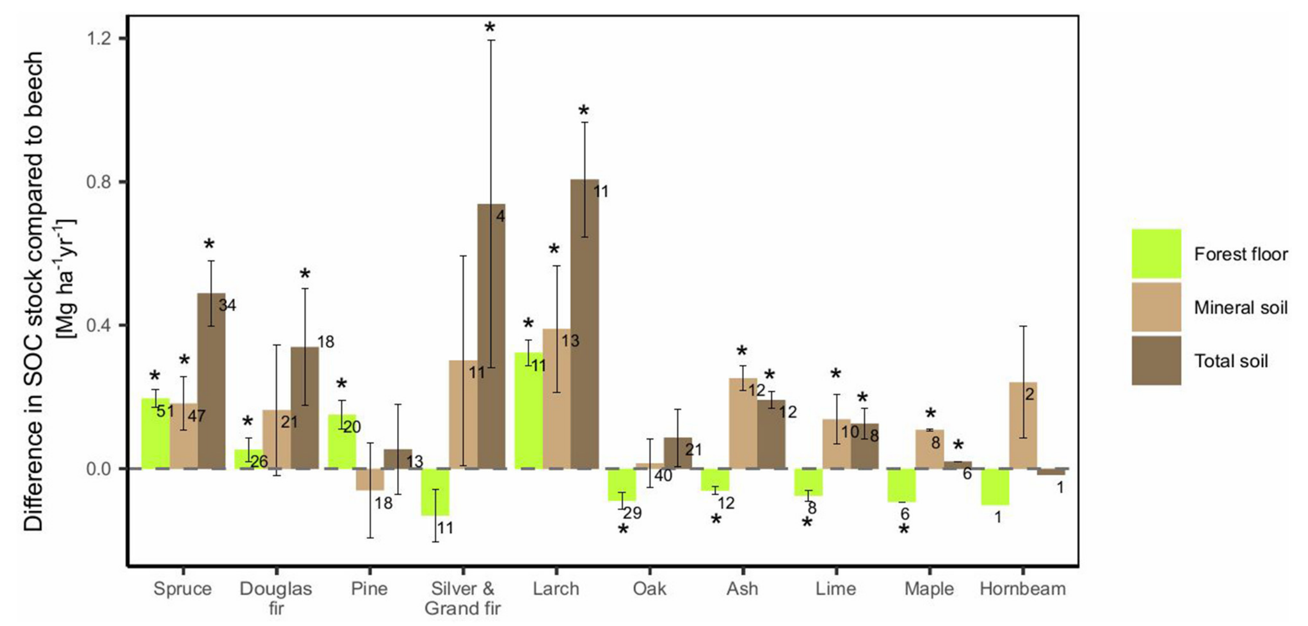

FIGURE 4 | Average absolute annual differences of SOC stocks of monoculture stands compared to beech monoculture. Numbers next to the bars are the number of observations. Error bars show the standard error. Asterisks indicated significant differences compared to beech monoculture. Note that in some cases the difference for the total soil is not the sum of those for the forest floor and mineral soil, since not all studies included observations for both layers.

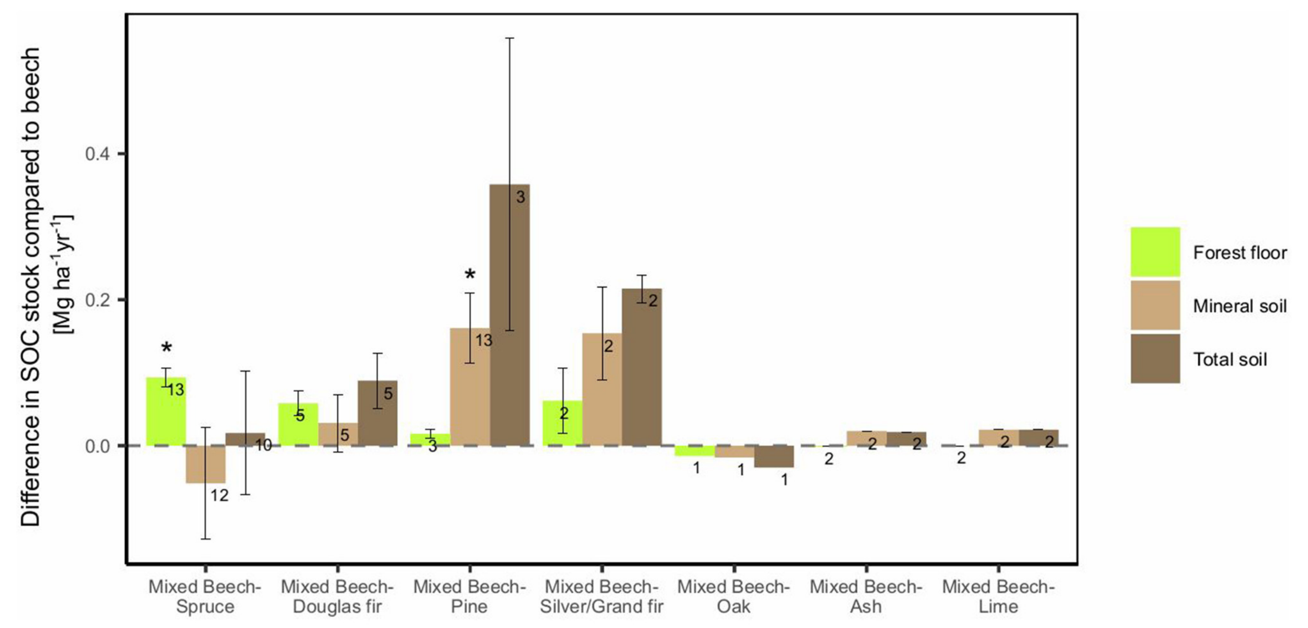

FIGURE 5 | Average absolute annual differences of SOC stocks of mixed forest stands compared to beech monocultures. Numbers next to the bars are the number of observations. Error bars show the standard error. Asterisks indicated significant differences compared to beech monocultures. Note that in some cases the difference for the total soil is not the sum of those for the forest floor and mineral soil, since not all studies included observations for both layers.

monoculture. Up to doubled organic C stocks were found in forest floor of mixed beech-spruce and mixed beech-Douglas fir stands compared to beech monocultures (**Supplement 5B**). In mineral soil, these effects were most pronounced for pine. This resulted in considerably higher annual differences in total SOC sequestration, in particular for mixed beech-pine stands (increase of 0.36 Mg ha^{-1} yr^{-1} of C) (**Figure 5**). Mixed beech-broadleaved stands are much less studied with the scarce data

TABLE 2 | Results of the multiple stepwise regression analyses to identify the factors driving the differences in organic C stocks of other tree species/mixtures compared to beech in the forest floor and the mineral soil.

Forest floor				**Mineral soil**			
	Total R^2 and partial R^2 for each variable	***p*-value**	**Coefficient**		**Total R^2 and partial R^2 for each variable**	***p*-value**	**Coefficient**
Overall model	**0.67**	**<0.0001**		**Overall model**	**0.40**	**<0.0001**	
Selected parameters:				Selected parameters:			
Reference forest floor C stock (stock of beech)	0.08	<0.0001	−0.021	Intercept		<0.0001	4.85
Stand category:	0.66			Reference mineral SOC stock (stock of beech)	0.14	<0.0001	$-3.4 * 10^{-3}$
- Conifers		<0.0001	1.124	Sand %	0.10	0.012	$-2.0 * 10^{-3}$
- Broadleaved		0.062	−0.41	Mineral soil C/N ratio of respective stand	0.22	<0.001	0.03
- Mixed beech-conifer		<0.0001	1.146	N deposition	0.05	<0.001	−0.01
- Mixed beech-broadleaved		<0.0001	4.3	Stand age of paired stand	0.06	<0.001	$-2.0 * 10^{-3}$

Percentage differences were log transformed in order to account for heteroscedasticity. The table shows the R^2 and p-values for the overall models as well as the partial R^2 and significances of each variable included in the model.

available indicating marginal SOC changes for mixtures of beech with oak, ash and lime, thereby confirming patterns observed based on comparisons of monocultures (**Figure 3A**).

The studies that allowed a comparison of beech monoculture with both, mixed stands and monoculture of the admixed species, were analyzed separately. For the forest floor organic C stocks, we found a significant, additive mixing effect for both types of admixtures (conifers, broadleaved species), i.e., the organic C stocks of the mixed stand could be deduced from the mixing ratio (**Supplements 6A,D**). For the mineral soil, no species composition effect was observed (**Supplement 6B**) and therefore, no mixing effect. For total SOC stocks, we found no species composition effect and therefore no mixing effect when considering admixtures with broadleaved, but a significant ($p < 0.05$) and additive species mixing effect for the mixtures with conifers (**Supplements 6C,E**).

Total N Stocks and C/N Ratios

Tree-species induced differences of TN stocks showed mostly similar patterns as those observed for SOC stocks when compared to beech monoculture (**Figure 3B**). However, in the mineral and total soil, broadleaved stands showed higher increases in TN stocks compared to beech than conifer stands, which is in contrast to observations of SOC stock differences. The limited number of observations in mixed stands showed TN stock increased in mixed beech-conifer stands by 4% and no change due to admixture of other broadleaved species (**Figure 3B**).

Strong correlations between the differences of SOC and TN stocks of other monocultures and mixed stands compared to beech were detected (forest floor: $R^2 = 0.88$, mineral soil: $R^2 = 0.89$, total soil: $R^2 =$ to 0.69) (**Supplements 7A,B**). For conifers and mixed beech-conifers, the relative TN stock changes were found to be lower than relative SOC stock changes and the C/N ratio increased in all soil layers (**Supplement 7C**). For broadleaved species, C/N ratios for the mineral and total soil tended to decrease compared to beech, while mixed beech-broadleaved forest floor C/N ratios were higher than beech C/N ratios.

Controls of Tree-Species Induced SOC Differences

Forest Floor

The multiple stepwise regression analyses showed that the stand type (broadleaved, conifer, mixed beech-conifer, and mixed beech-broadleaved) explained a large part of the tree-species-induced forest floor organic C stock differences. Further, the forest floor organic C stock of the reference beech stand also had predictive power in the multivariate model. In total, 67% of the variance was explained by these factors (**Table 2**). Differences of organic C stocks between other tree species/mixtures and beech were highest at forest floors with low initial forest floor organic C stocks of 0.4–10 Mg ha^{-1} and decreased with increasing initial organic C stock (**Figure 6A**).

Mineral Soil

For the tree-species-induced SOC stock differences in mineral soil, the C/N ratio of the mineral soil of the respective species, SOC stock of mineral soil of the reference beech stand, percentage of sand content in soil, the stand age of the paired stands and nitrogen deposition explained 40% of the variance (**Table 2**). The mineral soil C/N ratio was with the highest partial R^2 the most important predictor of tree-species-induced mineral SOC stock differences. The multivariate model revealed an exponential relation with increasing tree-species-induced SOC stock differences at increasing mineral C/N ratio (**Figure 6B**). Furthermore, largest differences in SOC stocks due to tree species appeared at stands with a low nitrogen deposition (8–20 kg $\text{ha}^{-1}\text{yr}^{-1}$) and at younger forest stands, aged 40–50 years (**Figures 6E,F**).

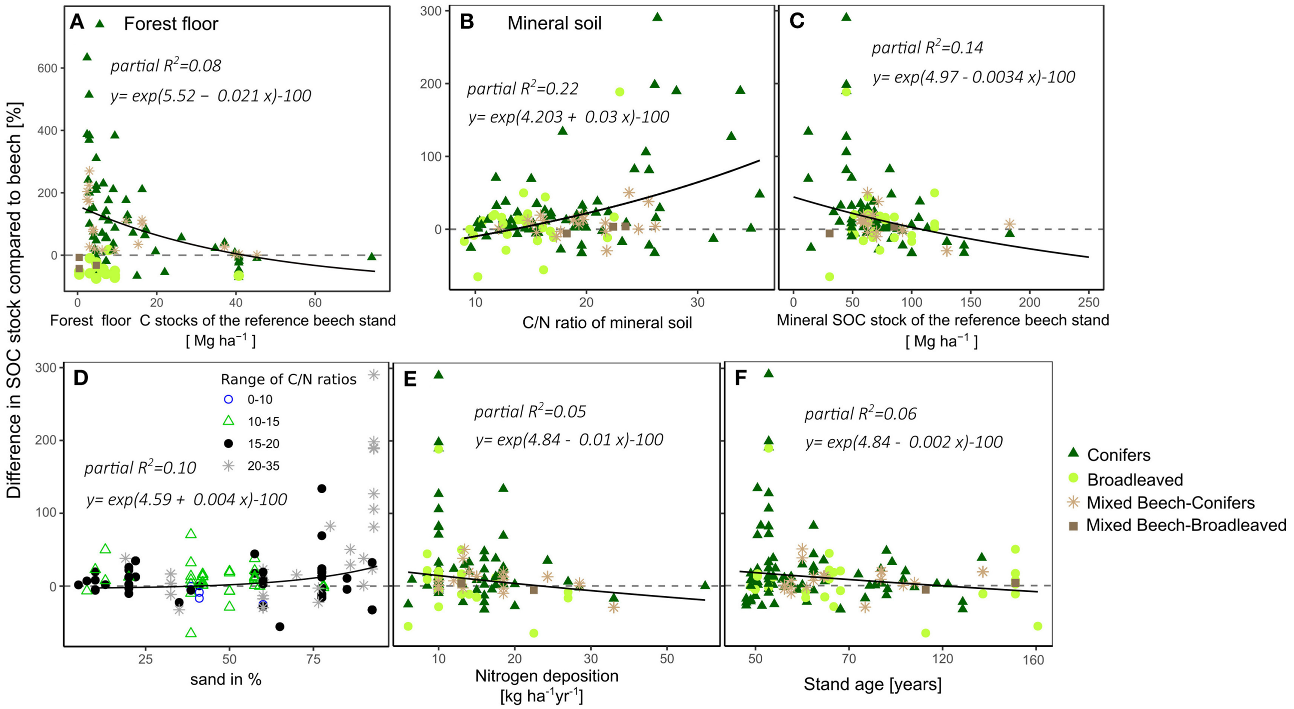

FIGURE 6 | Effects of statistically significant drivers on tree-species-induced differences in forest floor C stocks **(A)** and mineral SOC stocks **(B–F)**. For forest floor C stock differences, this was the forest floor organic C stock of the reference beech stand **(A)**. For the mineral soil differences, important drivers were **(B)** the C/N ratio of mineral soil, **(C)** the reference mineral SOC stock of the beech stand, **(D)** the soil sand content, **(E)** nitrogen deposition, and **(F)** the stand age. The solid lines represent the multivariate model predictions with average values assumed for all of the other variables in the model. The equations were obtained from the full multivariate model using the function: $y = exp(4.85 - 3.4 * 10^{-3}\ a - 2.0 * 10^{-3}\ b + 0.03\ c - 0.01\ d - 2 * 10^{-3}\ e) - 100$, with the constants a- Mineral SOC stock of the reference beech stand [Mg ha^{-1}] = 78, b- sand in % = 60, c- C/N ratio of the mineral soil = 16.8, d- Nitrogen deposition [kg ha^{-1}yr^{-1}] = 13.2, e- stand age (years) = 65. The effect of the sand percentage is partly confounded with the other effects. Therefore, the effect of sand content is illustrated using C/N ratios.

Regarding the influence of SOC stocks of beech reference stands, largest tree-species-induced differences in C sequestration were found for low SOC stocks of 12–50 Mg ha^{-1}, with exponentially decreasing differences at increasing beech SOC stock (**Figure 6C**). Finally, the percentage of sand was an important predictor (partial $R^2 = 0.1$). On sandy soils up to three-fold higher SOC stocks were observed (mean: 57%) for other species or beech mixtures compared to beech monocultures. With a decrease in soil sand content, tree-species-induced differences in SOC stock decreased (**Figure 6D**). When subdividing the meta-dataset in 4 texture categories from high (sandy) to low (clayey loam/loamy clay) grain sized soils, the largest tree-species-induced SOC stock increase was observed for sandy soils (**Supplement 8A**). Consequently, also soil type affected tree-species induced SOC changes with Podzols and Regosols showing significantly higher C sequestration rates compared to the other soil types (Acrisol, Cambisol, Chernozem, Histosol, Leptosol, and Luvisol) (**Supplement 8C**). In this context, legacy effects on SOC stocks were found from the previous land use. Afforested heath and mining lands showed significantly higher C sequestration rate differences of other tree species compared to beech than previous agriculture or forest lands (**Supplement 8B**).

In contrast to soil parameters, climate parameters, including MAT, MAP, precipitation during growing season and the drought index showed no predictive power for tree-species-induced SOC stock differences (see **Table 2** for important parameters).

DISCUSSION

Additive Admixing Effects on SOC and TN Stocks

Tree species admixing effects on SOC stocks could differ from those deduced from comparison of monoculture stands due to non-linear interactions e.g., in litter decomposition. Borken and Beese (2005) investigated two kinds of mixtures of beech with spruce and found similar effects on forest floor organic C stocks, no matter if 30 or 70% spruce were admixed to beech. From this single study, it may be assumed that the mixing ratio does not have large impact on forest floor organic C stocks, possibly due to synergistic or antagonistic interactions. In the present meta-analysis however, additive species mixing effects were observed for the forest floor organic C stocks of mixed stands (**Supplements 6A,D**, **Figure 5**). Based on these observations, our hypothesis (1) on synergistic mixture effects cannot be confirmed for the forest floor organic C stocks.

Considering the mineral soil, for mixtures with beech (1) intermediate values between the monoculture stands (Jonard et al., 2007; Cremer et al., 2016), (2) largest SOC stocks in mixed stands (coniferous-beech stands) (Andivia et al., 2016; Błońska et al., 2018; Rehschuh et al., 2019, unpublished), and (3) no detectable differences between beech monoculture and mixed stands (Langenbruch et al., 2012; Berger et al., 2015; Dawud et al., 2017) were observed in individual experimental studies. Synergistic effects of mixed stands on mineral SOC could be due to a more complex structure of the stand and greater density of crowns and rooting systems due to the exploiting of deeper soil layers by beech fine roots when mixed e.g., with spruce (Bolte and Villanueva, 2006; Cremer et al., 2016) with increased belowground biomass and an increased root turnover (Andivia et al., 2016; Błońska et al., 2018). No effects of the admixtures of other tree species to beech were attributed to the prevention of any larger niche differentiation and complementarity because of relatively similar tree species traits (Dawud et al., 2017). The largely additive effects observed in this synthesis point to an overall relatively low importance of such non-linear mixing effects. Consequently, our hypothesis (1) can also not be confirmed for the mineral and total soil, since the SOC stocks of the mixtures can be additively derived from the corresponding monoculture stocks.

Tree-Species Induced SOC and TN Stock Differences

Forest Floor

Our study revealed higher forest floor organic C stocks in mixed beech-conifer stands but lower forest floor OC stocks in beech stands mixed with other broadleaved tree species (**Figure 4**). That conifers reveal higher organic C stocks in forest floors compared to broadleaved stands such as beech (Prescott and Vesterdal, 2013) is related not only to the more recalcitrant litter (Scheu et al., 2003) with higher lignin and lower nutrient concentrations (especially calcium) (Raich and Tufekcioglu, 2000; Hobbie et al., 2006; Ahmed et al., 2016), but also to the lower faunal and microbial activity due to lower pH-values (Hobbie et al., 2006).

For broadleaved stands, also previous studies reported species-induced differences in forest floor organic C stocks. Vesterdal et al. (2013) showed that relatively low organic C stocks were present under ash, lime and maple, whereas beech and oak were species with organic C stocks intermediate between conifers and other broadleaved species. Beech litter was found to have highest lignin contents and highest C/N ratios with ∼53 out of 5 deciduous tree species studied (amongst lime, maple, ash) (Jacob et al., 2010). In the present study, oak forest floors showed with 28 out of 29 observations faster litter decomposition and, hence, lower organic C stocks than beech.

Generally, tree-species induced differences in forest floor organic C stocks can be either due to different litter production or different litter decomposition. Although trees produce similar (Andivia et al., 2016) or even higher litter amounts in broadleaved stands (Butterbach-Bahl et al., 2002), organic C stocks are higher under stands with conifers intermixed, pointing to the importance of processes related to litter decomposition (Augusto et al., 2015). Slower decomposition and, therefore, slower biogeochemical cycles are observed, when litter with lower nutrient concentrations (usually from conifers) are admixed to higher quality litter and *vice versa* (Chapman et al., 1988). Jonard et al. (2006) found a positive relationship between the forest floor litter mass and the proportion of the most recalcitrant litter. In a litter bag experiment with differently mixed litter of 3–5 broadleaved tree species, Jacob et al. (2010) showed that decomposition rates decreased with increasing proportion of beech, pointing to poorest litter quality of beech leaves among the broadleaved species studied. These results further show that decomposition rather depends on the tree species identity than on tree species diversity (Jacob et al., 2010; Dawud et al., 2016). However, Joly et al. (2017) reported that tree species diversity indirectly affects decomposition due to modified micro-environmental conditions. Apparently, litter fall dynamics and distribution also play an important role for litter decomposition (Rothe and Binkley, 2001; Dawud et al., 2017). In sum, we provide evidence that effects of admixing other tree species to beech forests rather exerts additive effects on forest floor organic C stocks, that can be deduced based on the litter quality of admixed tree species and the mixing ratio. Consequently, conifers are most suitable to increase forest floor organic C stocks of beech stands.

Mineral and Total Soil

The mineral soil showed smaller relative (expressed in % change) but larger absolute (expressed in Mg ha^{-1} yr^{-1}) tree-species induced changes compared to the forest floor (**Figures 4**, **5**, **Supplement 5**). This is due to larger residence time, resistance to disturbance, and overall pool sizes of mineral soil compared to forest floor organic C stocks (Hedde et al., 2008; Vesterdal et al., 2013). On average, we detected a plus in SOC sequestration in the mineral soil of 0.2 and 0.1 Mg ha^{-1} yr^{-1} for conifers and other broadleaved species, respectively (**Figure 3A**) compared to beech. These results are generally in line with the findings of Boča et al. (2014), who found conifers compared to beech dominated stands to have on average 26 and 19% more organic C in the forest floor and mineral soil, respectively. Also, Vesterdal et al. (2013) summarized that in temperate climate more mineral SOC is stored under ash, maple, lime, and ulm than under beech. Besides leaf litter inputs, root debris, rhizodeposition (Kuzyakov and Domanski, 2000; Jandl et al., 2007; Trum et al., 2011; Schleuß et al., 2014) and microbial-derived compounds (Angst et al., 2018) are important C inputs into mineral SOC pools, while C output is controlled by decomposition and leaching processes. Usually it is assumed that largest differences due to tree species occur in the top mineral soil because of the great influence of litter incorporation. However, in ancient forests of >230 years also significant differences were found in the subsoil (Nitsch et al., 2018). Generally, the soil organic matter (SOM) of subsoil horizons might be more dynamic than previously thought, possibly driven by root C input (Tefs and Gleixner, 2012). Land use legacy can affect SOC stocks (Li et al., 2012) and thus is a potential confounding factor in meta-analyses comparing different forest stands. Here we minimized such bias by the selection of adjacent paired stands only, and by refusing studies which reported such differences in previous land use.

Because only few studies included in the present meta-analysis revealed SOC stocks of the lower subsoil (see **Table 1**), it was not possible to analyze SOC stocks for deeper soil layers >0.5 m. However, the role of the subsoil in tree-species induced SOC stock differences is assumed to be rather limited (Jandl et al., 2014). Nonetheless, root chemistry is essential. Angst et al. (2019) found that SOC stabilization under different tree species mainly varies due to differences in tissue chemical composition, especially of roots. Tree species with roots rich in nitrogen, but low in calcium contents and low in recalcitrant compounds such as lignin are thought to increase SOM stability (e.g., European larch, Scots pine, and Douglas fir) (Angst et al., 2019). This is because easily decomposable SOM promotes microbial induced stabilization of SOC via organo-mineral compounds (Cotrufo et al., 2013). Therefore, for the selection of tree species to be admixed to beech, in addition to quantifying SOC stocks, also the root litter quality should be considered.

It has been frequently discussed, if there is a difference in the vertical allocation of SOC stocks between conifers and broadleaved species rather than a difference in total SOC stocks. It is suggested that less C in the mineral soil offsets higher C stocks in the forest floor and *vice versa* (Vesterdal et al., 2008, 2013; Wiesmeier et al., 2012; Prescott and Vesterdal, 2013). In the present study we show that this is possibly evident for broadleaved species other than beech as well as mixed beech-broadleaved stands, showing a slightly negative relation of forest floor organic C stocks and mineral SOC stocks (**Supplement 9**). However, for coniferous, beech monoculture and mixed-beech-coniferous stands this assumption was not confirmed. Apparently, increases in forest floor organic C are not generally offset by lower mineral SOC storage, but accompanied by larger SOC stocks in mineral soil as well. Around 80% of the conifer stands observed showed higher C stocks in the mineral soil than beech stands. Also in the meta-analysis of Boča et al. (2014), forest floor and mineral organic C patterns were not closely linked when testing forest floor organic C as predictor for mineral SOC stocks. From these contrasting findings, it appears that different species groups have to be distinguished for assumptions on vertical SOC distribution and total stocks.

The present meta-analysis shows that coniferous and other broadleaved stands can sequester significantly more C in the total soil profile than beech stands. Mixed beech-broadleaved stands only showed small differences in total SOC compared to beech. However, mixtures of beech with conifers showed considerably higher total annual SOC accumulation, on average 0.1 Mg ha^{-1} yr^{-1} with pine, Douglas fir and silver fir as potential tree species to be intermixed in existing beech forests. Thus, hypothesis (2) of this study was confirmed. For the adaption of beech forests to climate change, admixtures of coniferous tree species could enhance soil C sequestration.

While the admixture of conifers has positive effects on C stocks, nonetheless, tradeoffs should also be mentioned here, such as a decrease in soil pH (Augusto et al., 2015) and a decrease in earthworm biomass and diversity (Ammer et al., 2006). Further, there might be undesired impacts on the soil biological net methane sink, which decreases slightly due to conifer admixture to beech (Menyailo and Hungate, 2003; Rehschuh et al., 2019).

Close Coupling of Tree-Species Induced SOC and TN Stock Changes

Differences in SOC and TN stocks compared to beech monocultures were generally closely coupled (**Supplements 7A,B**). However, there are differences between the groups of tree species types. The relatively higher TN gain in other broadleaved than conifer stands (**Figure 3**), i.e., a lower C/N ratio of the mineral soil compared to conifers, could be explained by lower C/N ratios in the fresh leaf litter of broadleaved species. Vice versa, Conifers showed significantly higher C/N ratios compared to beech in all soil layers (**Supplement 7C**), which in the long term could result in progressive nitrogen limitation (Li et al., 2012). From this observation it appears that admixture of conifers to beech would result in higher C/N ratios. Whether this leads to changes in nitrogen availability however remains uncertain, e.g., due to counterbalancing effects of atmospheric N deposition.

Which Beech Sites Are Most Promising to Increase SOC Stocks Due to Admixture of Other Trees?

We assessed a range of predictors of tree-species induced differences in SOC stocks in order to identify indicators for sites, where admixture of other tree species to beech is most promising. Clearly, the admixed or compared tree species were decisive for forest floor organic C gain compared to beech monocultures, thereby reflecting the importance of tree species-associated recalcitrance of litter on C accumulation in forest floors. However, also a range of soil, site and beech stand properties regulated the potential SOC gains due to other tree species. High carbon gain at low forest floor and mineral soil organic C stock of the reference beech stand (**Figures 6A,C**) might reflect enhanced productivity of admixed tree species and a relatively fast change of forest floor organic C due to production of more recalcitrant litter.

Also with increasing mineral soil C/N ratio (**Figure 6B**) as well as at stands with lower nitrogen deposition (**Figure 6E**), larger tree-species induced increases in SOC stocks were found. This might be due to the fact, that SOM decomposition is reduced due to nitrogen limitation and this effect is stronger for species with more recalcitrant woody and leaf debris. Further, largest differences in mineral SOC sequestration rates between various other tree species and beech were found in soils with a sandy texture such as Podzols and Regosols, and sites with the historical land cover/use of heath and mining, that typically go along with low SOC stocks (**Figures 6C,D** and **Supplement 8**). Dawud et al. (2017) investigated beech, Douglas fir and mixtures on sandy and clayey soils and found greater impacts of tree species admixture in sandy soils. Also in another study on C sequestration in forest soils, sandy soils showed higher enrichments in C compared to clayey and calcareous soils (Grüneberg et al., 2014). In general, larger SOC stocks and a better stabilization due to associations of C in organo-mineral complexes and aggregation are found in clayey and loamy soils rather than in sandy soils (Jandl et al., 2007; Schleuß et al., 2014; Angst et al., 2018). SOC stocks in the mineral-associated organic matter fraction are mainly controlled by pedogenic properties (Grüneberg et al., 2013). Hence, we assume that clayey and loamy soils buffer tree species effects better than sandy soils because the soil properties might more strongly control SOC stocks than the chemical recalcitrance of litter inputs. In contrast, in sandy soils with relatively low stabilization of organic C in aggregates and organo-mineral associations, the input of chemically recalcitrant litter from coniferous trees as well as a higher productivity will result in higher relative differences of SOC (see also Grüneberg et al., 2014). In sum, on sandy and low carbon soils, such as Podzols, Regosols, previous heath and mining lands, as well as on soils with nitrogen limitation, admixtures of other tree species to beech are most effective in terms of C sequestration, thereby confirming hypothesis (3) part one.

With hypothesis (3) part two, we expected an effect of climate parameters such as MAT, MAP, precipitation during growing season and the drought index on the magnitude of tree-species induced differences, which could not be confirmed (**Table 2**). Climate is assumed to have a higher effect on mineral SOC stocks than tree species (Vesterdal et al., 2013), i.e., with drier climate, lower microbial activity is expected and thus an enrichment in SOC stocks. A legible effect of climate in our study might be explained by the fact, that most of the studies included were conducted in temperate regions with similar MAP or MAT and only two in the subtropics. Apparently, for the sampled dataset with its relatively narrow climate variability, the effect of tree species on SOC stocks is not substantially influenced by climate parameters.

CONCLUSION

Based on a meta-analysis we show that the admixture of conifers to beech forests can increase carbon accumulation in soils by on average 9% which corresponds to 0.1 Mg ha^{-1} yr^{-1}. The highest potential to increase SOC of beech forests is found for soils with sandy texture, low organic matter content, and nitrogen limitation (low N deposition and high C/N ratios). As this meta-analysis highlighted a lack of studies on the most climate-change-sensitive beech stands at the Southern distribution limit, future studies should fill this gap. Furthermore, an improved understanding of C and N stabilization mechanisms in soil, and quantification of both above- and belowground carbon sequestration, are desirable for developing further improved guidelines for forest compositions with high ecosystem carbon sequestration in a changing climate.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SR, MD, and HR devised the idea of the study. SR extracted the data and conducted the analysis of the results with contribution of MD and MJ. SR wrote the original article with review and editing from all other authors. All authors discussed the results.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.606669/full#supplementary-material

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Linkage Between Site Features and Soil Characteristics Within a Mediterranean Volcanic Area

***Speranza Claudia Panico*[1], *Valeria Memoli*[1], *Lucia Santorufo*[1]*, *Francesco Esposito*[1], *Anna De Marco*[2], *Rossella Barile*[3] and *Giulia Maisto*[1]**

[1] Department of Biology, University of Naples Federico II, Naples, Italy, [2] Department of Pharmacy, University of Naples Federico II, Naples, Italy, [3] Vesuvius National Park, Via Palazzo del Principe c/o Castello Mediceo, Ottaviano, Italy

Altitude, exposure, and plant cover may have a significant impact on the soil system, affecting its abiotic characteristics and, in turn, soil microbial composition and activity. In the Mediterranean area, the relationships among environmental features and soil characteristics are still scarcely investigated. The present study aimed to evaluate the effects of altitude, slope exposure, and plant cover on soil abiotic characteristics and the responses of the soil microbial community. Surface soil was sampled at 32 field points of the Vesuvius Mountain (Southern Italy) at two slope exposures (North and South), two altitudes (600 and 900 m a.s.l), and under two different plant covers (pines and shrubs), and it was analyzed for soil abiotic and biotic characteristics. The results showed that soil characteristics mainly differed according to site altitude, but some characteristics also changed according to site exposure and plant cover. The soil organic carbon (C_{org}) showed significant high values at low altitude, south exposure, and under pines and played a role in influencing the soil microbial community. In soil covered by pines, the greatest soil C_{org} amount matched with the highest values of C/N ratio and fungal biomass. Finally, high C_{org} and water availability significantly enhanced the microbial activities.

Keywords: plant cover, altitude, exposure, volcanic soils, microbial community, microbial activity

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Jeff Allen Hatten,
Oregon State University, United States

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Jörg Luster,
Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Switzerland
Lukas Kohl,
University of Helsinki, Finland

****Correspondence:***
Lucia Santorufo
lucia.santorufo@unina.it

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INTRODUCTION

Soil is defined as a complex mixture of eroded rock, mineral nutrients, organic matter, water, air, and billions of living organisms, whose combination is difficult to predict and depends on multiple environmental features (Miller, 2007). Among these features, altitude and slope exposure influence the local climate, which, in turn, affects soil characteristics (Griffiths et al., 2009).

Particularly in the Mediterranean area, moderately wet and cold winters are coupled with dry and hot summers, but the intensity of the drier periods, increasing from high to low latitudes, can vary widely and directly influence different soil characteristics (Tsui et al., 2004; Sardans and Peñuelas, 2013).

The temperature and moisture generated from the elevation and the exposition may affect soil nutrient availability, erodibility, moisture content, infiltration capacity, leaching and deposition processes, cation exchange capacity, soil organic matter dynamics, and stabilization and pH (Lemenih and Itanna, 2004; Griffiths et al., 2009; Saeed et al., 2014).

Furthermore, different altitude and slope exposure have an impact on plant community distribution as vegetation types and thus strictly interact with soil processes or function, modifying its chemical and physical characteristics (Griffiths et al., 2009; Thakur et al., 2015). In addition, vegetation density and type, affecting the litter quantity and quality, soil pH, and chemical composition (Menyailo et al., 2002; Mendes et al., 2014; Cline and Zak, 2015), directly or indirectly play important roles on soil microbial composition and activity (Rajala et al., 2012; Bardelli et al., 2018). For example, McCulley and Burke (2004) observed, in soils covered by grass, that altitude directly impacted microbial biomass amount, whereas, in boreal forest soils, topographic gradient changed thick forest floors, pH, and C/N ratio and the microbial community structures (Högberg et al., 2007; Seibert et al., 2007). So, differences in soil abiotic characteristics due to altitude, exposure (Swallow et al., 2009; Bach et al., 2010), and plant cover (Panico et al., 2020) have a significant impact on ecosystem dynamics and may lead to differences in soil microbial communities (Tajika et al., 2020).

Particularly interesting are the soils formed along the slopes of a volcano for their capacity to store the highest amount of organic C among all mineral soil types (Lilienfein et al., 2003; Egli et al., 2008; De Marco et al., 2013a). In fact, these soils are generally characterized by high litter input due to primary productivity (Dahlgren et al., 2004) but have also a high stabilization of soil organic matter by non-crystalline inorganic soil components (Torn et al., 1997). On the other hand, volcanic soils are characterized by the natural pedo-geochemical background with high heavy metal concentrations inherited from the parent rock material (Adamo and Zampella, 2007).

Although the impacts of the environmental features (i.e., altitude, exposure, and plant cover) on soil formation and evolution are known, the specific relationships between these and soil abiotic and biotic characteristics have been poorly studied (Liu et al., 2003; Brockett et al., 2012; Wang et al., 2016). In fact, the few studies performed in the Mediterranean area (Rutigliano et al., 2009; Iovieno et al., 2010; Lucas-Borja et al., 2012) are not interested at soil–vegetation relationships along an altitudinal gradient and among different slope exposures. Anyway, the low water and nutrient availability in soils of Mediterranean area affect plant cover and soil biological activity and diversity which, in turn, are related to soil hydrological and erosion behavior throughout the slopes (Ruiz-Sinoga et al., 2010, 2011). Moreover, the assessment of altitude and slope exposure incidence and their prevailing role as driving features for changes in soil characteristics is becoming fundamental especially in Mediterranean ecosystems due to their fragility and exposure to climate change conditions (Sardans and Peñuelas, 2013).

Therefore, the aims of this research were (i) to assess the effects of altitude, slope exposure, and plant cover (the main site features) on soil abiotic and biotic characteristics within a Mediterranean volcanic area and (ii) to analyze the responses of soil microbial community to soil abiotic characteristics at different altitude, slope exposure, and plant cover. The hypotheses behind the research were that sites of distinct altitude/exposure/vegetation directly affect the soil abiotic characteristics and indirectly the soil microbial community and activities. To achieve the aims, soils were sampled at the Vesuvius National Park (Southern Italy), a volcanic mountain covered by typical Mediterranean plants. The study was performed along two slope exposures (North and South) and two altitudes (600 and 900 m a.s.l.) characterized by two representatives (Vacchiano et al., 2012) plant covers (pines and shrubs). The soil samplings were carried out during spring, i.e., a growing season with non-restrictive temperatures and water availability, in three sampling campaigns (2015, 2016, and 2017). The Vesuvius National Park is a volcanic area located not so far from densely populated urban areas and characterized by intensive human activities. The investigated soils are particularly rich in Cr, Cu, Ni, and Pb because of both their pedogenetic origin and air deposition deriving by the surroundings with high human density and activity (De Nicola et al., 2003; Maisto et al., 2006; Memoli et al., 2018a). Besides, in previous researches performed in the same investigated area, many nutrients showed low ready availability (namely, acid-soluble, reducible, and oxidizable fractions) and did not exert meaningful effects on soil microbial biomass and activity (Memoli et al., 2018b). By contrast, ecotoxicological effects on soil biota were due to trace elements, particularly Cr, Cu, Ni, and Pb (Maisto et al., 2011; Memoli et al., 2018b).

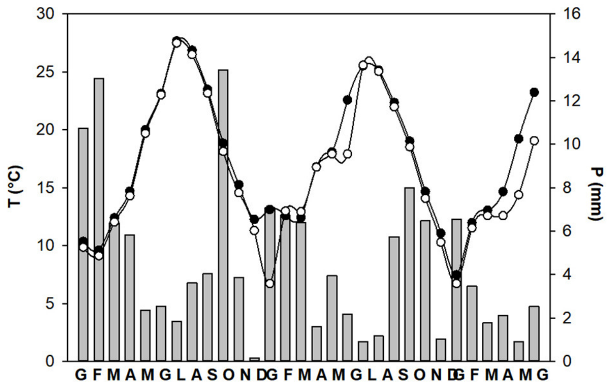

FIGURE 1 | Monthly mean of temperature (T, expressed as °C) and precipitation (P, expressed as mm) values of north (filled symbols and bars) and south (empty symbols and bars) slopes related to the study area from January 2015 to June 2017.

MATERIALS AND METHODS

Study Area and Experimental Design

The study was performed on the Vesuvius Mountain (Naples, Southern Italy), characterized by Mediterranean climatic conditions with dry summers and rainy autumns and winters; mean annual temperature is 9.8°C, and annual precipitation is 940 mm according to long-term averages (years 1961 to 1990) from the closest meteorological station at Osservatorio Vesuviano (605 m a.s.l.; 40°49′N; 14°24′E). The monthly mean of temperature (°C) and precipitation (mm) of the north and south slopes of the study area in the sampling period (2015–2017) is reported in **Figure 1**.

The slopes of Mount Vesuvius are a complex mosaic of areas afforested mainly by *Pinus pinea* and sites characterized by shrubs (such as *Myrtus communis* L., *Laurus nobilis* L., *Viburnum tinus* L., *Cistus* sp., *Ginesta* sp.) of the Mediterranean maquis (De Nicola et al., 2003; De Marco et al., 2013b). The soils are Lepti-Vitric Andosols according to the FAO soil classification (Gennaro and Terribile, 1999; IUSS Working Group WRB, 2014); in the studied areas, soils derived by volcanic deposits of the recent cycles (1804–1906). These deposits were Leucititic Tephrite-Phonolites similar in chemical composition (Santacroce, 1987; Santacroce and Sbrana, 2003).

The soil samplings were carried out during three sampling campaigns conducted during spring in 2015, 2016, and 2017. Surface soils (0–10 cm) were collected at 32 sites: 16 along the south-exposed (S) and 16 north-exposed (N) slopes. Along each slope, 8 sites were chosen at low altitudes (L-600 m a.s.l.) and 8 at high altitudes (H-900 m a.s.l.), and for each altitude 4 soils were sampling under pines (P) and 4 soils under shrubs (S). At each site, 12 subsamples of soils were collected and mixed together in order to obtain a homogeneous sample to perform the analyses.

Soil Physico-Chemical Analyses

In the laboratory, the soil samples were sieved (2 mm) and divided in portions to measure, in triplicates: pH, water holding capacity (WHC), bulk density (BD), water content (WC), organic C (C_{org}) and N concentrations, Cr, Cu, Ni, and Pb total concentrations, and available fractions.

Soil pH was measured in water suspension (1:2.5 = v:v = soil:water) by the electrometric method (Colombo and Miano, 2015). WC was determined by drying fresh soil at 105°C until reaching a constant weight, and the water holding capacity (WHC) was determined by the gravimetric method according to Aceves et al. (1994). Bulk density (BD) was assayed on undisturbed soil cores of known volume after drying for 48 h at 105°C. Soil C_{org}, in samples previously treated with HCl (10%), and N concentrations were evaluated by a CN elemental analyzer (Thermo Finnigan).

Total concentrations of Cr, Cu, Ni, and Pb were measured and oven-dried (105°C until constant weight) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) after acidic digestion (HF 50% and HNO3 65% at 1:2=v: v) in a microwave oven (Milestone mls 1200—Microwave Laboratory Systems). The available metal fractions were extracted with diethylenetriamine pentaacetic acid, $CaCl_2$, and triethanolamine at pH 7.3 ± 0.05 (Lindsay and Norvell, 1978). Metal concentrations were measured by atomic absorption spectrometry, via graphite furnace (SpectrAA 220 FS; Varian, Sidney, Australia).

Soil Biological Analyses

The biological analyses, performed within a week after sampling, in triplicates, on fresh samples stored at 4°C, were microbial and fungal biomasses and soil basal respiration (BR). The microbial biomass (MB) was evaluated as microbial carbon, according to Anderson and Domsch (1978), by the method of substrate-induced respiration (SIR). SIR was determined using glucose 1% as the substrate and the evolved CO_2 in 72 h incubation at 25°C in the dark (Anderson and Domsch, 1978). The evolved CO_2 was adsorbed in NaOH and measured by two-phase titration with HCl (Froment, 1972). The fungal biomass (FB) was assayed by membrane filter technique (Sundman and Sivelä, 1978), after staining with Aniline Blue, determining hypha length by the intersection method (Olson, 1950) with an optical microscope (Optika, B-252).

BR was determined by measuring the CO_2 evolved in the 10-day incubation at 25°C in the dark (Anderson and Domsch, 1993) and expressed as mg CO_2 g^{-1} d.w.

The soil metabolic quotient, qCO_2, was calculated as ratio between the C-CO_2 obtained by basal respiration and C_{mic} (Cheng et al., 1996), and the coefficient of endogenous mineralization, CEM, was calculated as ratio between the CO_2 obtained by basal respiration and C_{org} (Rutigliano et al., 2002).

Statistical Analyses

To test the normality of the data distribution, the Shapiro–Wilk test was performed.

In order to highlight the direct influences of altitude, slope exposure, plant cover, and sampling time on soil abiotic (pH, WC, O.M., C/N, BD, WHC, total and available metal contents) and biotic (MB, FB, BR, qCO_2, CEM) characteristics, linear mixed effect models (LME) were performed. For each soil characteristic, the influence of altitude, slope exposure, and plant cover, considered as fixed effects, and of sampling time, considered as random effect, was calculated using restricted maximum likelihood (REML), the better estimation of variance components for the present dataset. The significant impacts and interactions among altitude, slope exposure, plant cover, and sampling time on soil characteristics were calculated with the comparison of models, using the likelihood ratio test with the Anova function (for $\alpha = 0.05$).

In order to assess the significant impacts of soil abiotic characteristics on soil biotic ones, multiple linear regressions were carried out. The responses of soil biotic properties (MB, FB, BR, qCO_2, CEM), defined as dependent variables, to soil abiotic properties (pH, WC, C_{org}, C/N, BD, WHC, total and available metal contents), defined as independent variables, were tested. Before the linear mixed effect models and the multiple linear regressions' performance, the linearity of the data, the independence, the homogeneity, and the normality of residuals were tested. Only the normality was not always satisfied for some variables, and in that case a logarithmic transformation was done.

The R 3.6.2 programming environment (R Core Team 2016) was used to perform the statistical analyses, considered significant at least for $P < 0.05$, the linear mixed effect models (lme4 package), and the multiple linear regressions.

RESULTS

Characteristics of Soils at Different Altitudes, Exposures, and Plant Cover

The results showed that soil characteristics were mainly dependent on altitude, partially dependent on slope exposure and plant cover, and to a lesser to sampling time (**Tables 1–3**). In particular, soil pH was high at high altitude (**Table 1**); WC at low altitude (**Table 1**); WHC at low altitude, along

TABLE 1 | Mean values (±s.e.) of pH, water content (WC, expressed as % d.w.), water holding capacity (WHC, expressed as % d.w.), and bulk density (BD, expressed as mg cm^{-3}).

Slope	**North**				**South**			
Altitude	**Low**		**High**		**Low**		**High**	
Plant cover	**Pine**	**Shrubs**	**Pine**	**Shrubs**	**Pine**	**Shrubs**	**Pine**	**Shrubs**
pH	6.91 (±0.17)	7.16 (±0.06)	6.57 (±0.23)	7.05 (±0.18)	6.48 (±0.21)	6.70 (±0.16)	6.69 (±0.22)	7.01 (±0.19)
WC	25.9 (±5.85)	22.1 (±4.61)	26.6 (±5.36)	11.9 (±1.94)	62.1 (±7.88)	55.8 (±6.28)	17.8 (±4.93)	13.8 (±5.20)
WHC	92.9 (±9.35)	55.7 (±8.65)	59.9 (±7.45)	23.9 (±4.01)	122 (±10.1)	108 (±9.73)	34.9 (±2.89)	37.1 (±3.66)
BD	1.23 (±0.01)	1.50 (±0.01)	1.49 (±0.05)	2.03 (±0.11)	1.20 (±0.15)	1.27 (±0.14)	1.61 (±0.03)	1.56 (±0.09)
C_{org}	4.20 (±0.87)	3.36 (±0.85)	8.82 (±0.72)	3.61 (±0.36)	13.8 (±1.76)	8.56 (±1.72)	3.82 (±0.59)	4.28 (±0.70)
N	0.56 (±0.08)	0.40 (±0.09)	0.62 (±0.07)	0.49 (±0.08)	0.54 (±0.05)	0.45 (±0.10)	0.33 (±0.11)	0.27 (±0.04)
C/N	21.8 (±4.20)	13.4 (±3.24)	21.7 (±2.53)	11.3 (±1.64)	32.9 (±4.08)	23.0 (±3.66)	16.4 (±1.19)	13.4 (±2.74)
Total Cr	14.4 (±1.30)	9.19 (±1.26)	22.4 (±2.38)	30.5 (±3.05)	21.7 (±1.78)	18.9 (±2.07)	23.1 (±2.11)	21.4 (±1.74)
Total Cu	138 (±4.36)	228 (±6.50)	126 (±3.67)	97.6 (±3.48)	83.2 (±2.84)	74.9 (±3.01)	92.9 (±4.02)	82.9 (±1.89)
Total Ni	10.7 (±0.09)	9.32 (±1.08)	18.8 (±1.54)	15.7 (±1.55)	11.5 (±1.06)	12.0 (±1.11)	13.9 (±1.21)	13.0 (±1.74)
Total Pb	123 (±0.08)	114 (±0.08)	97.4 (±2.64)	60.7 (±3.05)	107 (±0.08)	92.8 (±0.08)	42.7 (±2.11)	44.8 (±2.74)
Available Cr	0.01 (±0.001)	0.002 (±0.001)	0.001 (±0.001)	0.001 (±0.001)	0.006 (±0.001)	0.03 (±0.001)	0.001 (±0.001)	0.001 (±0.001)
Available Cu	10.2 (±1.67)	10.2 (±1.29)	4.31 (±1.54)	3.77 (±2.03)	4.51 (±1.08)	5.80 (±1.83)	2.57 (±1.28)	2.51 (±1.61)
Available Ni	0.12 (±0.04)	0.16 (±0.05)	0.02 (±0.01)	0.06 (±0.01)	0.22 (±0.09)	0.39 (±0.11)	0.03 (±0.01)	0.06 (±0.01)
Available Pb	18.2 (±2.03)	5.81 (±0.35)	1.93 (±0.45)	3.95 (±0.35)	10.6 (±1.13)	31.9 (±2.10)	2.29 (±0.11)	5.39 (±0.86)

Organic carbon (C_{org}, expressed as % d.w.), nitrogen concentration (N, expressed as % d.w.), C/N ratio, total concentration, and available fraction of Cu, Cr, Ni, and Pb (expressed as $\mu g\ g^{-1}$ d.w.) measured in soils collected at low and high altitudes, North and South exposure, and under shrubs and pine cover.

south exposure and under pine cover (**Table 1**); and BD at high altitude, along north exposure and under pine cover (**Table 1**).

Soil C_{org} content was high at low altitude along south exposure and under pine cover (**Figure 1**); finally, the C/N ratio was high at low altitude and showed wide variability among the soils along south and north exposure and under different plant covers (**Figure 1**).

Also, soil total heavy metal (Cr, Cu, Ni, and Pb) concentrations highlighted wide variability among all investigated soils (**Table 1**). The high Cu concentrations were observed in soils collected along south exposure (**Table 1**). Concerning the heavy metal available fractions, all of them showed high concentrations at low altitude (**Table 1**); additionally, Cu showed high concentrations in soil collected along south exposure (**Table 1**).

Among the soil biotic characteristics, MB was high at low altitude (**Table 2**), FB at low altitude and under pine cover (**Table 2**), and BR at low altitude and along south exposure (**Table 2**).

The variability of FB and MB was comparable according to different altitudes, slope exposures, and plant covers (**Table 2**); instead, BR, qCO_2, and CEM mainly showed wide variability particularly in soils collected at low altitude, north exposure, and under pines as compared to those collected, respectively, at high altitude, south exposure, and under shrubs (**Table 2**).

Relationships Among Environmental Features and Soil Characteristics

The linear mixed-effect model showed that soil characteristics were mainly dependent on altitude, partially dependent on slope exposure and plant cover (fixed effects) and to a lesser extent on sampling time (random effects) (**Table 3**). In particular, altitude significantly influenced the great part of the investigated soil characteristics, with the exception of N, C/N, total Cu

TABLE 2 | Mean values (±s.e.) of microbial biomass (MB, expressed as mg C g^{-1} d.w.), fungal biomass (FB, expressed as mg g^{-1} d.w.), basal respiration (BR, expressed as mg CO_2 g^{-1} d.w.), metabolic quotient (qCO2, expressed as mg C-CO_2 mg^{-1} C_{mic}), and coefficient of endogenous mineralization (CEM, expressed as mg C-CO_2 g^{-1} C_{org}) measured in soils collected at low and high altitudes, North and South exposure, and under shrubs and pine cover.

Slope	North				South			
Altitude	**Low**		**High**		**Low**		**High**	
Plant cover	**Pine**	**Shrubs**	**Pine**	**Shrubs**	**Pine**	**Shrubs**	**Pine**	**Shrubs**
MB	1.59 (±0.12)	1.40 (±0.06)	1.71 (±0.13)	1.29 (±0.18)	2.10 (±0.21)	1.55 (±0.16)	1.09 (±0.22)	1.31 (±0.19)
FB	0.34 (±0.01)	0.32 (±0.02)	0.47 (±0.02)	0.36 (±0.01)	0.51 (±0.03)	0.40 (±0.02)	0.32 (±0.04)	0.27 (±0.01)
BR	0.16 (±0.01)	0.13 (±0.02)	0.32 (±0.01)	0.21 (±0.01)	0.46 (±0.02)	0.33 (±0.01)	0.16 (±0.01)	0.17 (±0.01)
qCO2	0.06 (±0.01)	0.07 (±0.01)	0.07 (±0.01)	0.04 (±0.01)	0.15 (±0.02)	0.12 (±0.01)	0.09 (±0.02)	0.06 (±0.01)
CEM	2.40 (±0.17)	3.50 (±0.15)	2.47 (±0.12)	1.84 (±0.16)	1.61 (±0.18)	1.26 (±0.89)	2.27 (±0.59)	1.54 (±0.34)

concentration, available Cr and Ni concentrations, FB, qCO_2, and CEM (**Table 3**); slope exposure significantly influenced the WC, C_{org}, total Cu and Pb concentrations, available Cu concentration, and BR (**Table 3**); and plant cover significantly influenced the WC, C_{org}, total Cr, Cu, and Ni concentrations, and BR (**Table 3**). A great part of the WC, WHC, and total Cr, Cu, Ni, and Pb concentration variabilities were also due to the sampling time (**Table 3**). In addition, the interactions among the altitude, slope exposure, and plant cover significantly influenced all the soil characteristics (**Table 3**), whereas the interaction between altitude and slope significantly influenced WC, C_{org}, C/N, and available Cu fraction (**Table 3**). The interaction between altitude and plant cover significantly influenced WC, C_{org}, total Cu concentration, and BR (**Table 3**), whereas the interaction between slope and plant cover significantly influenced pH, WC, WHC, BD, C_{org}, total Cr and Pb concentration, available Cu, Ni, and Pb fractions, MB, and BR (**Table 3**). Overall, the influence of altitude, slope exposure, and plant cover on soil abiotic characteristics was higher than that on the biotic ones (**Table 3**).

The multiple linear regressions highlighted that C_{mic} was positively correlated with soil N and C_{org} content and negatively with C/N ratio (**Table 4**); FB was negatively correlated with soil Cr available fraction and positively with C/N ratio and Ni available fraction (**Table 4**); BR was positively correlated with soil WHC (**Table 4**); and qCO_2 was positively correlated with soil C/N ratio and negatively to Ni total content (**Table 4**).

DISCUSSION

Relationships Between Site Features and Soil Abiotic and Biotic Characteristics

In the studied area, altitude more than slope exposure and plant cover seems the main discriminating feature in defining directly the characteristics of the investigated soils. In fact, as shown by the linear mixed effect analyses, a significant influence on soil abiotic properties was highlighted according to the different altitudes. In fact, altitude seemed to affect the soil structure and porosity as shown by the highest values of WHC and the lowest values of BD measured in soil at low altitude. The lower BD in soil indicates a higher degree of soil organic matter, good granulation, aeration, and higher infiltration (Dar and Somaiah, 2015; Saeed et al., 2019). The high C_{org}, observed at low altitude and south exposure, could be due to the greater plant canopy which will be responsible for the falling of a high amount of leaves on soil floor (Hutchins et al., 1976; Kao and Chang, 2001) as well as to the inputs of litter deriving by phenomena of leaching along the slope (Mukai et al., 2016). The C_{org} observed in soil at low altitude could enhance the soil stabilization (Ruiz-Sinoga et al., 2012) and be the main responsible for the high soil water content and C/N ratio (Tipping et al., 2016). By contrast, the high pH detected at high altitude could be also due to the low values of C_{org} and humic acids, known to lead to a decrease of soil pH (Finzi et al., 1998).

The observed variability of total and available element concentrations suggested the direct impact of altitude and slope exposure on their distribution. This finding confirms previous studies hypothesizing that the content of elements in the Vesuvius area was influenced by lithogenic factors and microclimatic conditions due to site features (Memoli et al., 2018a, 2019a). However, the high Ni and Cr availability found at low altitude and south exposure could be due also to the high C_{org} content and the low pH, as the capability of organic compounds to retain soil elements (Vega et al., 2004; Nunes et al., 2014) and the role of low soil pH to enhance element availability are known (Acosta et al., 2010). In addition, the high element availability at low altitude could be also due to the accumulation of soil components, produced at high altitude, which were transported along the slope through leaching phenomena (Acosta et al., 2010). Nevertheless, it cannot be overlooked that sites at low altitude, being nearer suburban areas, could receive by air depositions pollutants rich in potential

TABLE 3 | Summary of mixed-effect model analyses (F-value: *F*) among altitude (Alt), slope exposure (Slp), and plant cover (Veg), as fixed effects, and sampling time, as random effects, on abiotic (pH, water content—WC, water holding capacity—WHC, bulk density—BD, organic carbon content—C_{org}, nitrogen concentration—N, C/N ratio, total concentration and available fraction of Cu, Cr, Ni, and Pb) and biotic (microbial biomass—MB, fungal biomass—FB, basal respiration—BR, metabolic quotient—qCO_2, coefficient of endogenous mineralization—CEM) characteristics of soils collected at the Vesuvius National Park. Asterisks indicate significant impacts of fixed effects and their interactions on soil characteristics (Anova test—model comparison).

Soil characteristics		**Fixed effects**			**Random effects**	**Residual effects**	**Interactions among fixed effects**			
		Altitude	**Slope**	**Plant cover**	**Sampling time**					
								Alt	Slp	Alt × Slp × Veg
pH	*F*	6.30*	3.13	2.13	0.03	0.14	Slp	n.s.		***
							Veg	n.s.	*	
WC	*F*	29.1***	8.17***	12.7***	166***	185	Slp	*		***
							Veg	*	***	
WHC	*F*	20.9***	2.07	3.65	165*	1,190	Slp	n.s.		***
							Veg	n.s.	***	
BD	*F*	25.0***	4.24	0.09	0.01	0.05	Slp	n.s.		**
							Veg	n.s.	**	
C_{org}	*F*	60.8***	52.4***	48.1***	0.22	10.8	Slp	***		***
							Veg	***	***	
N	*F*	0.002	0.46	3.13	<0.001	0.12	Slp	n.s.		***
							Veg	n.s.	n.s.	
C/N	*F*	1.16	4.08	7.29	<0.001	<0.001	Slp	*		***
							Veg	n.s.	n.s.	
Total Cr	*F*	22.5***	1.49	9.99***	46.3***	24.8	Slp	n.s.		***
							Veg	n.s.	*	
Total Cu	*F*	2.85	12.3***	4.70*	1,610**	2,673	Slp	n.s.		
							Veg	*	n.s.	***
Total Ni	*F*	10.9***	0.27	4.41*	76.2***	16.6	Slp	n.s.		***
							Veg	n.s.	n.s.	
Total Pb	*F*	13.7***	4.24*	0.44	903***	1,338	Slp	n.s.		***
							Veg	n.s.	*	
Available Cr	*F*	3.96	1.73	1.77	<0.001	<0.001	Slp	n.s.		*
							Veg	n.s.	n.s.	
Available Cu	*F*	8.82**	7.34**	2.62	2.31	46.6	Slp	*		***
							Veg	n.s.	**	
Available Ni	*F*	17.7	3.88	3.50	<0.001	0.01	Slp	n.s.		***
							Veg	n.s.	***	
Available Pb	*F*	13.2***	0.76	0.82	2.58	47.9	Slp	n.s.		***
							Veg	n.s.	***	
MB	*F*	14.5***	0.96	3.39	0.01	0.26	Slp	n.s.		***
							Veg	n.s.	***	
FB	*F*	4.24	0.14	0.55	<0.001	0.00	Slp	n.s.		***
							Veg	n.s.	n.s.	
BR	*F*	13.5***	12.7***	6.25*	0.01	0.02	Slp	n.s.		***
							Veg	*	*	
qCO_2	*F*	2.42	5.38	2.86	<0.001	<0.001	Slp	n.s.		***
							Veg	n.s.	n.s.	
CEM	*F*	0.06	0.44	1.25	0.53	2.33	Slp	n.s.		***
							Veg	n.s.	n.s.	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

toxic elements as Pb and Cu (Memoli et al., 2019b), which form weakly binds to soil particles and can become readily available (Massas et al., 2009). The high bulk density observed at high altitudes disagrees with other authors (Saeed et al., 2014, 2019), and it could be due to the low amount of soil C_{org} (Athira et al., 2019).

TABLE 4 | Significant results of multiple linear regression analyses of soil biotic (microbial biomass: MB, fungal biomass: FB, basal respiration: BR, metabolic quotient: qCO_2) characteristics in relationship with soil abiotic (water holding capacity: WHC, organic carbon: C_{org}, nitrogen concentration: N, C/N ratio, total and available concentration of Cu, Cr, Ni, and Pb) characteristics.

Soil biotic characteristics	Multiple regression model
MB	$0.697587\,N + 0.063019\,C_{org} - 8.017878\,C/N + 2.818380$ $R^2 = 0.6699$ p-value: $3.614*10^{-5}$
FB	$0.098770\,C/N - 0.842864\,Cr_{(Aivai)} + 0.113271\,Ni_{(Aivai)} + 0.017856$ $R^2 = 0.2286$ p-value: 0.01625
BR	$0.002461WHC - 0.053642$ $R^2 = 0.2529$ p-value: 0.005552
qCO_2	$0.8066798\,C/N - 0.0034506\,Ni_{(Tot)} + 0.0964434$ $R^2 = 0.5623$ p-value: $4.767*10^{-5}$

Besides altitude and slope exposure, plant covers (namely, shrubs and pines) also play a role in influencing some soil characteristics, such as C_{org}, WHC, and C/N. Particularly, the soil under pine was richer in C_{org} than soil under shrubs, as observed in soil at low altitude and at south exposed sites as compared to those at high altitude and north exposure. In addition, pine cover was responsible, as compared to shrub cover, for greater litter accumulation and higher soil water retention. Under pines, the greatest soil C_{org} corresponded to the highest values of C/N ratio, which indicated an increase of organic matter recalcitrance. In fact, the trend of C_{org} in soils with different altitudes, exposure, and plant cover was similar to that of the C/N ratio, although this parameter showed significant differences only for soils at different altitudes.

The altitude, slope exposure, and plant cover partially explain the variability of some soil abiotic characteristics, as they were also influenced by the sampling time. In particular, the content of water in soil varied with the sampling time as the precipitation rate could influence the quantity of water in soil. Metal concentrations also can vary with time, as they are influenced by anthropic activities, such as tourism (Memoli et al., 2019b), and by climatic factors, such as wind intensity and direction.

Relationships Between Soil Abiotic and Biotic Characteristics

The direct influence of altitude and slope exposure on soil abiotic characteristics indirectly affect the composition and activity of soil microorganisms. In fact, the high soil WC and C_{org} in the soil at low altitude favored the abundance and activity of microorganisms (both bacteria and fungi), conversely to what occurred at high altitude. The close dependence between the soil microbial biomass and the soil C_{org} was also confirmed by the multiple linear regressions that showed the positive correlations between MB and C_{org} and N contents. The findings agreed with those reported by numerous studies that highlighted the fundamental role of organic compounds and N as resources for microorganisms (Aneja et al., 2004; McMahon et al., 2005; Williams et al., 2006). In addition, soil microorganisms are involved in C and N cycles (Zeraatpishe and Khormali, 2012; Aislabie and Deslippe, 2013; Wang et al., 2013) and contribute to organic matter stabilization (Six et al., 2000). Moreover, the results also highlighted that soil water availability and capacity to retain water significantly affected microbial respiration (Wang et al., 2013).

Anyway, although in soil collected at low altitude the microbial biomass was abundant and C_{org} was available, an inadequate mineralization occurred (i.e., CEM values were similar in the soils both at low and high altitudes). This could be probably due to both the dominance of organic compounds difficult to degrade, as suggested by the high C/N (Yüksek et al., 2013), and the presence of high Ni and Cr available concentrations (Chu, 2018). The increase of the organic matter complexity and recalcitrance (high values of C/N ratio) as well as the availability of potential toxic elements could create stress conditions for the microbial community, measured by high qCO_2 values (Mataix-Solera et al., 2002; Panico et al., 2020; Zhao et al., 2020). Although this parameter did not significantly differ in soil at different altitudes, slope exposures, and plant cover, because of the high variability of the collected data, a positive correlation was found between qCO_2 and C/N ratio. In addition, the results of multiple linear regressions highlighted that the variations of the soil organic matter quality significantly affected the microbial and fungal biomass. So, the quality of organic matter negatively affected the microbial biomass (Li et al., 2012) and positively the fungal one. The selective role of organic matter quality was particularly evident in soils under pine where its quality affected the composition of microorganisms (Bardgett and van der Putten, 2014; Panico et al., 2020). This was confirmed by the statistically higher FB in soils under pines than under shrubs. Pine litter, in fact, constituted by complex compounds and waxes, favored fungi rather than bacteria (Virzo De Santo et al., 2002), as the former are particularly able to feed on recalcitrant substrates (De Marco et al., 2013b, 2016).

Additionally, as microorganisms are known to be sensitive to the variations of temperature (Kirschbaum, 2006), the higher microbial biomass observed at low altitude could be also due to the occurrence of favorable microclimatic conditions (Memoli et al., 2019a). The results of RDA performed using the soil abiotic and biotic characteristics highlighted similar trends in soil at low altitude and at south exposure. The similar amount of C_{org} observed between soils at low altitude (15.5% d.w.) and south exposure (15.1% d.w.) could be due to the better microclimatic conditions. In fact, at these sites, the expected warmer conditions together with higher water soil availability (Tamai, 2010; He et al., 2016) could enhance plant productivity and, in turn, litter fall. Soil organic matter and water availability affected the soil microbial community, as it was always significantly correlated with microbial biomass (Hackl et al., 2005) and microbial activity (Wang et al., 2013). In addition, water availability seemed to drive soil community composition (Stefan et al., 2014) as fungi and bacteria differently responded to soil moisture according to other studies (De Vries et al., 2006; Bapiri et al., 2010). These findings could suggest that bacteria and fungi occupy different niches, avoiding competition in using resources (Panico et al., 2020).

The soil fungal biomass was also negatively affected by high Cr availability, showing more sensitivity to this metal as compared to the other ones (Marzaioli et al., 2010). Anyway, the heavy metal effects on the soil microbial community can often be minimized or masked by fluctuations in soil characteristics, mainly C_{org}, which may contribute to counterbalance the negative effects of heavy metal on the soil microbial community (D'Ascoli et al., 2006).

CONCLUSIONS

In the investigated Mediterranean volcanic area, altitude appeared the main factor in influencing soil characteristics. In fact, numerous soil characteristics significantly differed between soils at low and high altitudes. However, also site exposure and plant cover affected some abiotic characteristics.

Organic matter quantity and quality were the main soil abiotic characteristics affected by site altitudes, exposures, and plant covers. These organic matter characteristics associated with high water availability enhanced the fungal rather than the bacterial component of the soil microbial community. However, the content of metals, impacted by site features, played a role in influencing negatively the activity of microorganisms.

An overall evaluation highlighted that, in the studied Mediterranean volcanic area, the altitude and slope exposure have a crucial role in affecting directly soil abiotic characteristics and indirectly the biotic ones.

Finally, the resultant soil–plant cover and soil–environmental feature interrelationships could be more complex than either of the two considered separately and other studies are necessary to expand knowledge especially in Mediterranean ecosystems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

GM: conceptualization. SP, LS, and AD: writing. SP and LS: statistical analyses. SP, FE, LS, AD, VM, and GM: writing revision. GM, AD, and RB: validation. All authors contributed to the article and approved the submitted version.

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Tree Species of Wet Tropical Forests Differ in Their Tissue Biochemistry and Effects on Soil Carbon Dynamics

Ann E. Russell[1]*, *Rachel F. Marek*[2] *and Daniel C. Olk*[3]

[1] *Department of Natural Resource Ecology and Management, Iowa State University, Ames, IA, United States,* [2] *IIHR-Hydroscience & Engineering, Department of Civil and Environmental Engineering, University of Iowa, Iowa City, IA, United States,* [3] *USDA-ARS, National Laboratory for Agriculture and the Environment, Ames, IA, United States*

Given the hypothesized effects on soil organic matter (SOM) of polyphenols in plant tissues, differences among tree species in their biochemical composition could influence the turnover and accrual of SOM in various ways. The extent to which the biochemical composition of leaf and fine-root tissues differ among tropical tree species, and the effects on soil dynamics, are largely undocumented, however. We used cupric oxide analyses of plant tissues and soil in long-term, replicated, mono-dominant 15-year-old plantations at La Selva Biological Station, Costa Rica, to test for differences among six tree species. We related these results to companion studies in this experimental site to evaluate relationships between interspecific differences in tissue biochemistry and SOM dynamics. Newly senesced leaves and fine roots of the six species differed in their concentrations of three lignin-derived families of phenols, the cinnamyls, syringyls and vanillyls ($p < 0.0001$ for all tests). Cinnamyl and syringyl phenols in soil differed significantly among species ($p = 0.0408$, 0.0071, respectively), whereas vanillyl phenols did not ($p = 0.83$). The degree of decomposition of syringyl and vanillyl phenols in soil also differed ($p = 0.0015$, 0.0027, respectively), as evidenced by the ratio of carboxylic acid to aldehyde compounds, based on the concept that carboxylic acids are a common by-product of oxidative decomposition of lignin by microorganisms. In our study in a single site, i.e., the same soil type, climate, and growth form of vegetation, total phenols in soil ranged from 5 to 21 mg g^{-1} organic carbon (OC) across the 20 plots, and the endpoints were both broad-leaved evergreen species; even the means across species, 7–12 mg g^{-1} OC, covered half the range of values reported in another study across a broad latitudinal range of sites. This study's tree species differed in traits that influenced at least four factors that explained their differential effects on soil organic carbon (SOC) pools: (1) Fine-root detrital inputs; (2) Fine-root syringyl concentrations; (3) Soil pH; and (4) Macroaggregate structure. This trait-based approach provides a process-based understanding of how trees species influence SOC dynamics, and the consequences for ecosystem properties, under land-use change that involves shifts in species composition.

Keywords: fine root growth, phenolic compounds, soil carbon (C) storage, tropical tree species, soil organic carbon pools

Edited by:
Laurent Augusto,
INRA Centre Bordeaux-Aquitaine, France

Reviewed by:
Xiao-Tao Lu,
Institute of Applied Ecology, Chinese Academy of Sciences (CAS), China
Antra Boca,
Latvia University of Agriculture, Latvia

***Correspondence:**
Ann E. Russell
arussell@iastate.edu

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INTRODUCTION

Humid tropical forests account for ∼19.6 million km^2 of the Earth's area (Asner et al., 2009) and contain some of the Earth's largest soil organic carbon (SOC) stocks (Field et al., 1998; Jobbágy and Jackson, 2000). Development of these SOC stocks has been, and continues to be a function of climate, organisms, parent material, topography, time, and human factors (Jenny, 1941). Of all these factors, we focus on organisms, specifically tree species, because trees are the dominant growth form in tropical forests, which have been undergoing unprecedented rates of land-use change over the last few decades. These changes include fragmentation, degradation, regeneration of secondary forests following deforestation, and logging of remaining forests (Lewis et al., 2015). These changes generally involve shifts in tree species composition, yet it is not clear how these shifts influence SOC stocks.

Tree species can differ in a variety of traits that could influence the quantity and turnover of organic matter (OM) inputs to soil, that would in turn influence SOC stocks differentially as a result of shifts in species composition. These traits include the rate and chemistry of detrital inputs from litterfall and roots, the decomposition of these plant tissues in soil, and the fueling of microbial and fungal populations that influence SOC dynamics and stocks.

Evaluating plant traits that influence tissue decomposition, and tracing the flow of plant-based carbon (C) into soil and its stabilization there, has been a topic of much debate because so many factors can potentially contribute to the persistence of OM inputs to soil. In their review of multiple studies Schmidt et al. (2011) concluded that C residence time was only secondarily influenced by the molecular structure of detrital inputs; rather, the persistence of OM in soil was the result of complex interactions between the biota (including microbes and fungi) and abiotic factors such as climate, mineralogy, soil acidity, and water availability. In a litter-manipulation experiment, Ma et al. (2019) found that differences in temperature sensitivity of the active SOC pool were related to lignin content and the microbial community, whereas for the slow pool temperature sensitivity was related to environmental factors such as pH and soil C/N ratio.

To evaluate the relative impacts of the quantity and biochemistry of plant detrital inputs on SOC dynamics and stocks, we conducted studies in a field experiment in lowland Costa Rica that contains monocultures of six tropical tree species in four blocks, with species selected such that they differed in their root and litter attributes (Fisher, 1995). Established in 1988, this is one of the oldest replicated experiments containing broad-leaved evergreen tree species in the humid tropics. Because all tree species were planted in plots on the same soil type under similar environmental conditions and land-use history, this study focuses on the effects of the tree species on accrual of SOC after 15 years. Previous studies in this site allow for a rich database of traits to evaluate in relation to SOC stocks, including fine-root growth, litterfall, microbial studies, and various soil studies. For the study reported herein, we measured lignin-derived phenols (i.e., biomarkers that could be measured in plant tissues and soil), and evaluated SOC pools and their turnover times using a two-pool model. Given that lignin is a major constituent of forest litter, biomarkers such as cinnamyl, syringyl, and vanillyl phenols have been used to identify broad categories of vegetation in forest litter, e.g., angiosperms vs gymnosperms (Kögel, 1986), or pasture vs forest (Heim et al., 2010). The oxidative degradation of lignin with cupric oxide (CuO) has also been used to characterize the pattern of lignin decomposition (Kögel, 1986; Feng and Simpson, 2011). The acid-to-aldehyde (Ad/Al) ratio of syringyl and vanillyl units has been found to increase with the degree of lignin degradation (Kögel, 1986; Hedges et al., 1988; Opsahl and Benner, 1995; Otto and Simpson, 2006).

To compare and integrate findings about plant tissue biochemistry with SOC dynamics, long-term laboratory incubation-based methods can be used to estimate active and slow turnover pools of SOC and their residence times (Paul et al., 2001). This method has been used to compare SOC dynamics and C sequestration under different soil types and land uses and management (e.g., Six et al., 2002; McLauchlan et al., 2006), although to our knowledge laboratory incubations have not yet been coupled with biochemical analyses of tropical forest SOC.

We addressed four broad hypotheses in relation to differences in tropical tree species traits and their influence on SOC dynamics and stocks:

1. Species differ in the biochemistry of their newly senesced leaves and fine roots, and the differences persist in SOC. While differences between angiosperms and gymnosperms or grasses and trees are well-documented (Kögel, 1986; Heim et al., 2010), we are not aware of any other studies comparing broad-leaved evergreen tropical trees.
2. Species differ in the decomposability of their fine roots and soil, as indicated by the Ad/Al of their syringyl and vanillyl concentrations in soil. While this was true for studies comparing different growth forms and forest types (Guggenberger et al., 1995; Heim et al., 2010), it has not been tested among tropical tree species.
3. The size and turnover of active and passive SOC pools differ among tree species. In a previous study in this site, C mineralization rates differed among the species, as determined from 30-day laboratory incubations (Russell et al., 2007). However, we have not previously evaluated sizes of the active, slow and passive SOC pools and their turnover.
4. Differences among species in both the quantity and biochemistry of detrital inputs influence the dynamics and stocks of SOC. Results from previous studies in this site indicate that the tree species differ in the quantity and decay of fine-root inputs to soil (Valverde-Barrantes et al., 2007, 2009; Raich et al., 2009). However, we have not previously addressed the relative effects of the quantity and biochemistry of detrital inputs and their relationship with the sizes and turnover of SOC pools.

MATERIALS AND METHODS

Study Site and Field Studies

Study Site

This study was conducted in a lowland Tropical Wet Forest, *sensu* Holdridge life zones (Hartshorn and Hammel, 1994), within experimental plots established in 1988 at La Selva Biological Station, Costa Rica. Mean annual precipitation (MAP) is 3,960 mm, with no month receiving < 100 mm; mean annual temperature (MAT) is 25.8°C (Sanford et al., 1994). The soil has most recently been classified as an Oxisol, a Mixed Haplic Haploperox (Kleber et al., 2007). The site is hilly, with elevations ranging from 51 to 84 m above mean sea level (Raich et al., 2007). Mean soil temperature (5 cm depth) was similar to air temperature (10 cm above forest floor), 24.4 and 24.1°C, respectively, based on hourly measurements over 24-h periods on >100 days from 2004 to 2010 in these plots (Raich and Valverde-Barrantes, 2017). Of these >2,600 soil temperature observations, 50% fell within a range of −1.3 to +2.0°C of air temperature.

The field experiment was established on a 12-ha site that had been mature wet tropical forest until deforestation in 1955. The site was then converted to cattle pasture dominated by the perennial grasses *Panicum maximum* L. and *Melinis minutiflora* Pal. and grazed until abandonment in 1987. In 1988 a randomized complete block experiment was established. The design contained twelve 0.25-ha (50 m × 50 m) plots in each of four blocks; each plot was divided into four quadrants to facilitate stratified random sampling within plots. Each of the 11 plots was planted with a single tree species, with a tree spacing of 3 × 3 m; the 12th plot was left unplanted as a control (Fisher, 1995). For details about site management, and early tree growth and soil properties, see González and Fisher (1994); Fisher (1995); and Haggar et al. (1997). To provide a reference for soil property measurements, we established four 50 m × 50 m plots in the adjacent mature forest, and four 14 m × 8 m plots in the adjacent abandoned pasture.

By 2003, only six of the 11 tree species planted had survived with complete canopy coverage, the six species in this study. We refer to these species by two or four-letter codes (in parentheses), based on the first letters of their genus and species: *Hieronyma alchorneoides* Allemao (Hial, Ha); *Pentaclethra macroloba* (Willd.) Ktze, (Pema, Pm); *Pinus patula* ssp. *tecunumanii* (Eguiluz & J.P. Perry) Styles (Pipa, Pp); *Virola koschnyi* Warb., (Viko, Vk); *Vochysia ferruginea* Mart. (Vofe, Vf); and *Vochysia guatemalensis* Donn. Sm (Vogu, Vg). All species are native to this region, except *Pinus*; *Pentaclethra* is the only nodulated legume. All of the broad-leaved species had arbuscular mycorrhizal associations (Kivlin and Hawkes, 2016b). When this study occurred, the trees had the following averages: stem densities, 200–500 stems ha^{-1}; basal area, 15–40 $\text{m}^2\ \text{ha}^{-1}$; tree diameters 22–37 cm; and heights, 19–33 m (Raich et al., 2007; Valverde-Barrantes et al., 2007).

Plant and Soil Sampling

Newly senesced, fallen leaves were collected from the ground in March 2004. The status of "newly senesced" was determined based on leaf color and integrity, as described by Raich et al. (2007). At least 20 leaves (fascicles in *Pinus*) were collected for each species in each plot and combined within a plot to yield $n = 1$ sample per plot. Fine roots (0–2 mm diameter) were sampled in June-July 2004 by carefully excavating and tracing roots to six individual, randomly selected trees within each plot. The six samples per plot were bulked to produce a single sample in each of the 24 plots. Root samples were washed before drying. All plant tissue samples were dried at 65°C, ground in a Wiley mill, and then pulverized to a fine powder using a LABCONCO Spex 8000 Mixer/Mill.

Fine root growth over this same period had been measured in a previously reported study (Valverde-Barrantes et al., 2007, 2009). To summarize, fine-root growth (0–15 cm depth) was measured using the ingrowth core method, using 20 cores per tree species. Fine-root growth was assumed to equal fine-root production because fine-root biomass did not vary significantly 2-year timeframe of that study.

Soil was sampled in the 0–15 cm layer, following the protocol of Russell et al. (2007) in June-July 2003. Within each quadrant of each plot, 20 punch-tube samples, 3.2-cm in diameter, were collected at randomly selected points and then bulked into a single sub-sample, for a total of four sub-samples per plot. For this study, the four sub-samples per plot were combined into a single sample per plot, for a total of 24 soil samples. These soil samples had been air-dried and roots were removed as the soil was passed through a 2-mm sieve. A well-mixed sub-sample was finely ground with mortar and pestle for subsequent analyses. Soil sub-samples were dried at 105°C to determine the air-dry to dry-weight conversion factor.

By 2008, it had become apparent that *V. ferruginea* was succumbing to a fungal disease. All of the tissue concentrations analyses we report here are for live tissues, and thus would not have been affected by the disease. However, we could not rule out the possibility that this disease could have caused premature fine-root die-off earlier than 2008, and could have thus created an artifact in the fine-root growth and soil data. Thus, we did not include *V. ferruginea* in any analyses involving soil or fine-root growth. This study was not designed to compare differences in land use, but for reference purposes, we used the same protocols to sample soil in the adjacent mature forest and abandoned pasture.

Laboratory Studies

Lignin-derived Phenols

Cupric Oxide extractable, lignin-derived phenols were measured by adapting the procedure of Goñi and Hedges (1992). Samples of 100 mg soil or 6 mg roots were oxidized with alkaline CuO and 2 M NaOH for 3 h in a stainless steel minibomb. Bombs were loaded into a specially designed carousel in a refitted Hewlett-Packard 5890A gas chromatograph (GC). The heating rate was 4.2°C/min for 30 min from a start temp of 27°C, and a final temp of 150°C was held for 150 min. Following heating, ethyl vanillin in a concentration of 240 ng μl^{-1} (25 μl for soils, 100 μl for roots) was added to each sample as the internal standard. The minibombs were closed again, shaken by hand, then centrifuged for 5 min at 3,500 rpm, and their supernatant transferred to test tubes. Three ml of 6 M HCl was added to each bomb to neutralize the NaOH and each test tube was vortexed. About 2.5 g NaCl was added to each test tube, then capped and mixed by hand. About 3 ml ether was added, then again capped and mixed by hand. Test tubes were shaken for 2 min to accumulate the phenols in the ether, then centrifuged for 2 min at 2,000 rpm. The ether layer containing the phenols was then carefully pipetted from the surface of each solution into a new test tube. Na_2SO_4 (~2.5 g) was added to each tube to remove residual H_2O from the ether solution. Tubes were shaken, and if necessary stored under refrigeration for overnight.

For derivitization and chromatography, samples were first filtered through quartz wool then concentrated by rotary evaporation to about 1 ml volume, then transferred to 4-ml vials, and dried to nearly air-dryness under a stream of Ar gas. The precipitate was then dissolved in 150 μl pyridine/50 μl methyl-3,4-dimethoxybenzoate (soils) or 300 μl pyridine/100 μl methyl-3,4-dimethoxybenzoate (roots). The methyl-3,4-dimethoxybenzoate served as a recovery standard a 50 μl aliquot of each solution was added to a new 4-ml vial and mixed with 50 μl of the derivitizing agent bis(trimethylsilyl)trifluoroacetamide and then placed in a heating block at 70°C for 30 min, after which the samples were loaded into the GC. Gas chromatography was carried out with a Hewlett-Packard 6890 GC equipped with a derivatized, non-packed injection liner, a HP-5 (5% crosslinked phenylmethyl siloxane) capillary column (30-m length, 0.32-mm column id., 0.25-μm film thickness) and a flame ionization detector. The following conditions were employed for phenol separation: injector temperature, 300°C; temperature ramp, 100°C ramped to 220°C at 4°C/min, 220°C ramped to 270°C at 25°C/min and held for 10.1 min; detector temperature 300°C. Results are reported in the conventional units of SOC enrichment, that is mg phenol g OC^{-1}.

Soil C Pool Sizes and Turnover Rates

To determine sizes of active and slow pools and their respective turnover rates, we used long-term incubations of soil at 23°C, similar to field ambient temperature, coupled with a chemical approach, described by Paul et al. (2001). We incubated 16 field replicates of soil for each species, using sieved (2 mm), air-dried subsamples of 3 g, from each of the 4 quadrants × 4 blocks, as described in Russell et al. (2007). To summarize, the subsample was mixed with acid-washed sand (1:1 w/w) and adjusted to 60% water-filled pore space, using deionized water that contained 2 mL of inoculant (5 g field-moist soil, combined from all plots, homogenized with 50 mL distilled water). We measured CO_2-C release rates periodically over 400 day (before CO_2 concentrations reached 4%); the flask headspace was flushed through an infrared gas analyzer (LI-820, LI-COR Biosciences, Inc., Lincoln, NE, United States). The air supply was piped through a CO_2-free water bath to keep the incubation-tube atmosphere hydrated. The mean lab temperature was very close to ambient field temperature, so we did not perform a temperature conversion of the data.

We determined the resistant fraction by refluxing 1-g soil subsamples in 6 M HCl at 115°C for 16 h on a controlled-temperature digestion block as described by Paul et al. (2001). The refluxed samples were then washed three times with deionized water, dried at 55°C and finely ground in a mortar and pestle. Resistant soil C was measured by dry combustion, using a Thermo-Finnigan EA Flash (Series 1112, EA Elantech, Lakewood, NJ, United States).

Data Calculations and Analyses

Soil Pool Parameter Calculations

We used curve analyses of CO_2 evolved for each sample incubated over 400 days (n = 96) to determine the size and turnover rates of the active (C_a) and slow (C_s) SOC pools as in Paul et al. (2001). We did not have carbon dating for these soils, so we used a two-pool constrained model in the regression analysis:

$$\text{Model } C_{(t)} = C_a * k_a^{(-ka*days)} + (SOC - C_r - C_a) * \ k_s^{(-ks\,*\,days)}$$

where $C_{(t)}$ is the total C pool at time t, C_a and k_a are the size and decay rate of the active pool. C_s is the size of the slow pool (SOC – C_r – C_a), where C_r is the size of the resistant pool as determined by acid hydrolysis and k_s is the turnover of the slow pool. The mean residence times (MRT_a and MRT_s) were calculated as 1/k for each of the two soil pools. We used non-linear regression to calculate the parameters C_a, k_a, and k_s, using PROC NLIN in the Statistical Analysis System (SAS Institute Inc [SASI], 1999). A Marquardt fit was used with initial parameter settings of: k_a = 0 to 0.3 by 0.05; k_s = 0.001 to 0.005 by 0.001; and C_a = 100 to 2,000 by 100.

Analysis of Variance

The plot was the experimental unit; for variables with measurements at the quadrant level, the mean value for the plot was used in analyses. Soil data from *V. ferruginea* and the reference plots (mature forest and abandoned pasture) were not included in the analyses. To evaluate whether the tree species had effects on more than one response variable among soil phenols and SOC (pools and total), we first conducted a multivariate analysis of variance. Interactions among the response variables were significant, so we then tested response variables individually, using a generalized linear mixed effects model with both fixed and random effects in SAS (Littell et al., 1996). We tested for homogeneity of variances and normality of distributions. Variables that did not meet these assumptions included root and soil cinnamyl, and soil syringyl and vanillyl phenols, which met the assumptions when natural log transformed. Thus, analyses were conducted on the transformed data; we present the un-transformed values, however. Differences were considered to be significant at $\alpha \leq 0.05$. Pairwise comparisons for significant overall *F*-tests were performed using *p*-values adjusted by Tukey's Honestly Significant Difference method.

Multiple Regression Models

We used a multiple regression model to evaluate the relative effects of the quantity, biochemistry and turnover of OM on one response variable, total SOC stocks. We used partial residual analysis to quantify the effect of a single explanatory variable on the response variable, total SOC stocks, *after* the effects of other possible explanatory variables in the model had been accounted for (Neter et al., 1996). With only n = 20 plots for this analysis, we were limited to only six explanatory variables in the model. There is a rich data set for this field experiment, so to select the six explanatory variables from the set of many possibilities, we used Pearson correlation analyses to evaluate the strength and significance of pair-wise comparisons of variables (**Supplementary Table 1**). Variables from other microbial and fungal studies in these plots were not included in the set of possible variables because previous studies had indicated a lack of difference among the tree species in microbial and fungal parameters (Kivlin and Hawkes, 2016a,b); also, microbial and fungal variables were not correlated with total SOC stocks (Russell et al., 2018). For plant tissue biochemistry, we evaluated fine roots but not leaves, given that fine roots had been more correlated with SOC stocks than were leaves in another study at La Selva (Russell et al., 2004). Based on the results of the correlational analyses, the six explanatory (=predictor) variables were included in a multiple regression model They were: (a) fine-root syringyls (mg 100 mg OC^{-1}); (b) fine-root growth (Mg C ha^{-1} $year^{-1}$); (c) acid/aldehyde of soil syringyls (mg 100 mg OC^{-1}); (d) MRTs (year); (e) active SOC pool (mg kg^{-1}); and (f) soil syringyls (mg 100 mg OC^{-1}); all variables relate to the 0–15 cm soil layer.

RESULTS

Phenols in Plant Tissues and Soils

Across the tree species in this study, mean total cinnamyl phenol concentrations (mg g OC^{-1}) ranged from 1.5–3.4 in leaves, 0.8–3.7 in fine roots, and 0.9–1.7 in soil (**Table 1**). Mean total syringyl phenols (mg g OC^{-1}) ranged from 0.3–6.7 in leaves, 1.8–13.4 in fine-roots, and 1.5–5.6 in soil. Mean total vanillyl phenols (mg g OC^{-1}) ranged from 3.8–16.0 in leaves, 8.3–17.5 in fine roots, and 3.6–5.5 in soil. Thus, for cinnamyl and separately for vanillyl phenols, concentrations in leaves and fine roots were of similar magnitudes. In contrast, the average concentration across all plots of syringyl phenols in fine roots was double that of leaves, 7.8 and 3.8 mg g OC^{-1}, respectively. Leaf and fine-root syringyl concentrations were significantly correlated (r = 0.62, p = 0.0098), as were vanillyl phenols (r = 0.79, p = 0.0003), but cinnamyl phenol concentrations were not significantly correlated (r = 0.18, p = 0.4936; **Supplementary Table 1**). The ratio of the phenolic compounds in fine roots-to-soil was calculated for each species. Across species, cinnamyl root concentrations ranged from 0.9 to 1.7 times that of soil, whereas the ratio was higher for syringyl phenols, 1.2 to 3.3 across species and for vanillyl phenols, 2.2 to 3.3.

Regarding comparisons among species, all three phenolic classes differed significantly among the tree species for both leaf and fine-root tissues (**Tables 1**, **2**). For soil, species did not differ significantly in vanillyl phenol concentrations; for cinnamyl and syringyl phenols, the differences among species were significant, but not as strongly as for plant tissues. The sum of cinnamyl, syringyl, and vanillyl phenols (CSV) ranged from 5 to 21 mg g^{-1} OC across the 20 plots (published dataset), with a mean of 7–12 mg g^{-1} OC across species (**Table 1**). In comparison, the CSV sums for soil in the mature forest and abandoned pasture reference samples were 11 and 8 mg g^{-1} OC, respectively, with *Vochysia* most closely resembling mature forest values.

The Ad/Al of both syringyl and vanillyl phenols in soil differed significantly among species (**Tables 1**, **2**), with that of *Pinus* higher than *Hieronyma*, *Pentaclethra* and *Vochysia* for syringyl phenols and *Hieronyma*, *Pentacletha* and *Pinus* greater than that of *Virola* and *Vochysia* for vanillyl phenols. In the mature forest, the Ad/Al for syringyl phenols in soil was lower than that of the abandoned pasture, with *Pinus* and *Virola* more closely resembling that of the abandoned pasture, and the other three species closer to the mature forest values. There was not a significant relationship of the Ad/Al of syringyl phenols in soil with either root or soil syringyl concentrations (**Figure 1** and **Supplementary Table 1**). Acid/aldehyde ratios of roots and soil were not significantly correlated, for either syringyl or vanillyl phenols; nor was the soil Ad/Al correlated with total SOC for either biochemical family (**Figures 1**, **2** and **Supplementary Table 1**).

SOC Pools and Turnover and Fine-Root Growth

The mean size of the C_a (active pool) ranged from 880 to 1,281 mg kg^{-1} across species; the C_s (slow pool) was 25 to 32 times larger, ranging from 27,000 to 32,000 mg kg^{-1} (**Table 3**). Both the active and slow pools were significantly larger in *Vochysia* and *Hieronyma*. The C_a pool was smallest in *Virola* and the C_s pool smallest in *Pinus* and *Pentaclethra*. The tree species differed significantly in the turnover of the slow pool, reported here as the mean residence time (MRT_s): it was longest in *Virola* and shortest in *Hieronyma* (**Table 3**). In contrast, the MRT_a was similar across species. Fine-root growth differed significantly among the species, ranging from 1.2 to 3.9 Mg C ha^{-1} $year^{-1}$ in *Pinus* and *Hieronyma*, respectively (**Table 3**; Valverde-Barrantes et al., 2007, 2009).

Correlation Analysis Results

Across species, fine-root growth was significantly correlated with the C_a, MRT_s, and total SOC (**Figures 1**, **2** and **Supplementary Table 1**). In contrast, root syringyl phenols were not significantly correlated with these measures of SOC turnover, or with the Ad/Al of soil syringyl phenols or total SOC stocks. Within the total SOC, the relationship between the pool size and its turnover differed with the pool: C_a and MRT_a were significantly correlated, but C_s and MRT_s were not (**Supplementary Table 1**).

Partial Regression Results

Based on the results of the correlation analyses, six explanatory variables were included in the multiple regression model to analyze their effects on total SOC stocks: (a) Fine-root growth (FRG); (b) Fine-root syringyls (RtSyr); (c) Acid/Aldehyde ratio of Soil Syringyls (AdAlSyr); (d) mean residence time of the slow pool (MRT_s); (e) size of the active SOC pool (C_a); and (f) Soil Syringyls (SoSyr). Of those six variables, fine-root growth, fine-root syringyls and the size of the active SOC pool were significant after the other variables have been taken into account (**Table 4**). Comparisons of various multiple regression models indicated that for the simplest models (only one variable), the size of the active pool and fine-root growth explained the most variability

TABLE 1 | Phenolic compounds and their Acid/aldehyde (Ad/Al) ratios in six tropical tree species in 15-year-old plantations.

Substrate	Species	Family of phenolic compound			Total phenols	Ad/Al of phenolic compound	
		Cinnamyl (C) mg g^{-1} OC	Syringyl (S) mg g^{-1} OC	Vanillyl (V) mg g^{-1} OC	CSV mg g^{-1} OC	Syringyl	Vanillyl
Leaves	Hial	1.55 ± 0.05 **d**	5.65 ± 0.25 **b**	6.55 ± 0.25 **b**	13.78 ± 0.55 **c**	0.160 ± 0.001 **b**	0.155 ± 0.005 **bc**
	Pema	3.07 ± 0.19 **ab**	6.73 ± 0.26 **a**	6.37 ± 0.27 **b**	16.18 ± 0.32 **b**	0.183 ± 0.003 **b**	0.170 ± 0.006 **b**
	Pipa	3.40 ± 0.08 **a**	0.30 ± 0.01 **d**	15.95 ± 0.38 **a**	19.62 ± 0.44 **a**	0.670 ± 0.040 **a**	0.170 ± 0.001 **b**
	Viko	1.50 ± 0.06 **d**	3.27 ± 0.03 **c**	4.80 ± 0.10 **c**	9.60 ± 0.14 **e**	0.143 ± 0.003 **b**	0.203 ± 0.003 **a**
	Vofe	2.73 ± 0.15 **bc**	3.53 ± 0.06 **c**	4.93 ± 0.10 **c**	11.18 ± 0.27 **d**	0.160 ± 0.001 **b**	0.158 ± 0.003 **bc**
	Vogu	2.40 ± 0.15 **c**	5.27 ± 0.22 **b**	3.80 ± 0.06 **c**	11.51 ± 0.35 **d**	0.123 ± 0.003 **b**	0.140 ± 0.006 **c**
Fine roots	Hial	1.20 ± 0.15 **b**	7.00 ± 0.62 **b**	10.90 ± 0.76 **bc**	19.10 ± 1.40 **b**	0.213 ± 0.027 a	0.207 ± 0.028 a
	Pema	0.80 ± 0.06 **c**	8.63 ± 0.77 **b**	11.60 ± 0.70 **bc**	21.03 ± 0.86 **ab**	0.210 ± 0.012 a	0.208 ± 0.015 a
	Pipa	1.08 ± 0.08 **bc**	1.80 ± 0.75 **c**	17.48 ± 1.12 **a**	20.35 ± 0.63 **ab**	0.415 ± 0.175 a	0.238 ± 0.011 a
	Viko	1.70 ± 0.16 **b**	8.33 ± 0.73 **b**	12.88 ± 0.71 **b**	22.90 ± 1.47 **ab**	0.230 ± 0.022 a	0.233 ± 0.019 a
	Vofe	3.67 ± 0.58 **a**	13.40 ± 1.15 **a**	8.33 ± 0.61 **c**	25.40 ± 0.99 **a**	0.177 ± 0.003 a	0.213 ± 0.003 a
	Vogu	2.63 ± 0.55 **ab**	9.40 ± 0.70 **ab**	11.47 ± 0.32 **bc**	23.50 ± 0.98 **ab**	0.307 ± 0.082 a	0.187 ± 0.027 a
Soil	Hial	0.90 ± 0.06 **ab**	2.78 ± 0.14 **ab**	3.65 ± 0.25 a	7.28 ± 0.35 a	0.493 ± 0.017 **b**	0.678 ± 0.025 **a**
	Pema	0.90 ± 0.07 **ab**	2.75 ± 0.21 **ab**	3.55 ± 0.10 a	7.16 ± 0.19 a	0.475 ± 0.006 **b**	0.670 ± 0.026 **a**
	Pipa	0.85 ± 0.10 **b**	1.45 ± 0.06 **b**	5.50 ± 0.36 a	7.85 ± 0.46 a	0.775 ± 0.069 **a**	0.718 ± 0.048 **a**
	Viko	0.98 ± 0.11 **ab**	2.55 ± 0.28 **ab**	4.75 ± 0.19 a	8.25 ± 0.48 a	0.613 ± 0.069 **ab**	0.523 ± 0.031 **b**
	Vogu	1.68 ± 0.42 **a**	5.63 ± 1.76 **a**	5.13 ± 1.61 a	12.40 ± 3.59 a	0.480 ± 0.017 **b**	0.550 ± 0.024 **b**
	Forest	0.97	4.19	6.09	11.26	0.444	0.522
	Pasture	1.42	2.40	4.01	7.83	0.664	0.594

Notes. Means ± SE of n = 20 plots for leaves and roots, n = 16 for soil. Means within each comparison followed by the same letter do not differ significantly among species (Tukey's HSD, α = 0.05). Bold letters denote significant differences. Tree species are identified by four letters, the first two letters of the species and genus listed in the text. "Forest" refers to mature forest. "Pasture" refers to abandoned pasture. All root and soil data are for the 0–15 cm depth interval.

TABLE 2 | Summary table of ANOVA results for families of phenolic compounds and their Acid/Aldehyde (Ad/Al) ratios in six tropical tree species.

Substrate	df	Family of phenolic compound						Total phenols		Ad/Al of phenolic compound			
		Cinnamyl		Syringyl		Vanillyl		CSV		Syringyl		Vanillyl	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Leaves	3, 5	40.94	**<0.0001**	258.60	**<0.0001**	437.47	**<0.0001**	146.32	**<0.0001**	94.11	**<0.0001**	25.92	**<0.0001**
Roots	3, 5	22.08	**<0.0001**	18.60	**<0.0001**	18.13	**<0.0001**	3.96	0.0236	0.94	0.4884	0.84	0.5448
Soil	3, 4	3.27	**0.0408**	5.34	**0.0071**	1.42	0.83	1.84	0.1869	8.76	**0.0015**	7.64	**0.0027**

Bold letters denote significant differences.

(**Table 5**). However, in 2-variable models, the addition of root syringyls explained substantially more of the variability, without over-fitting the model. The addition of Ad/Al soil syringyls in a 3-variable model had a similar effect. Beyond that, addition of the other variables had diminishing explanatory effects.

DISCUSSION

Effects of Tropical Tree Species on Phenolic Biochemistry

Lignin signatures based on CuO analysis have been used to distinguish between vegetation types (Hetherington and Anderson, 1998), effects of land-use change (Guggenberger et al., 1994), and invasions of a new growth form, as in shrub encroachment in grasslands in Mongolia (Zhou et al., 2018). Most studies of plant phenolic contents in relation to SOC dynamics take place in the temperate zone or higher latitudes. Moreover, studies of plant tissues usually concern aboveground plant components, despite that root-derived C dominates SOC stocks in comparison with aboveground detrital inputs, as reviewed in Schmidt et al. (2011).

Comparison of a cropping rotation, grassland, mixed deciduous forest and spruce forest in a pre-Alpine region, found that the sum of lignin-derived soil phenols, CSV, ranged from 11 to 23 g/kg (Guggenberger et al., 1994). Across 18 sites that encompassed a broad range of latitudes and soil types from Saskatchewan (Canada) to Texas (United States), in which MAT ranged from 0.9 to 23.4°C and MAP from 300 to 1,308 mm, CSV phenols in soil ranged from 9 to 26 mg g^{-1} OC (Amelung et al., 1999). In our study in a single site, i.e., the same soil type, climate, and growth form of vegetation, CSV phenols in soil ranged from 5 to 21 mg g^{-1} OC across the 20 plots; even the means across species,

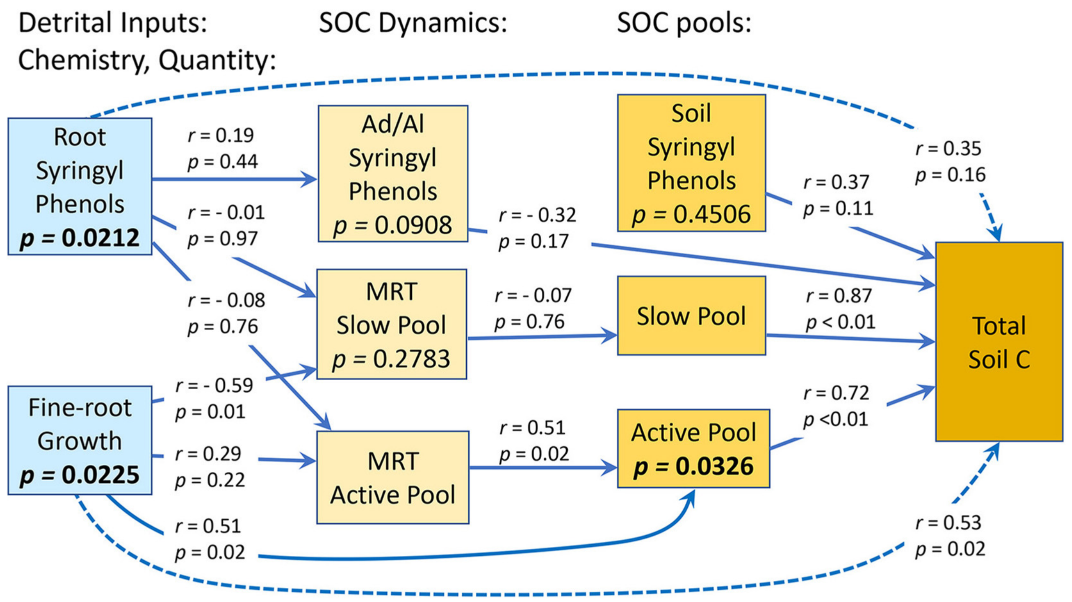

FIGURE 1 | Relationships between quantity and chemistry of detrital inputs to soil, and SOC dynamics and pools in an experiment containing five tropical tree species. Results of correlations are shown on arrows connecting the pair of variables in the analysis. *P*-values in boxes are from partial residual analyses. *N* = 20 plots.

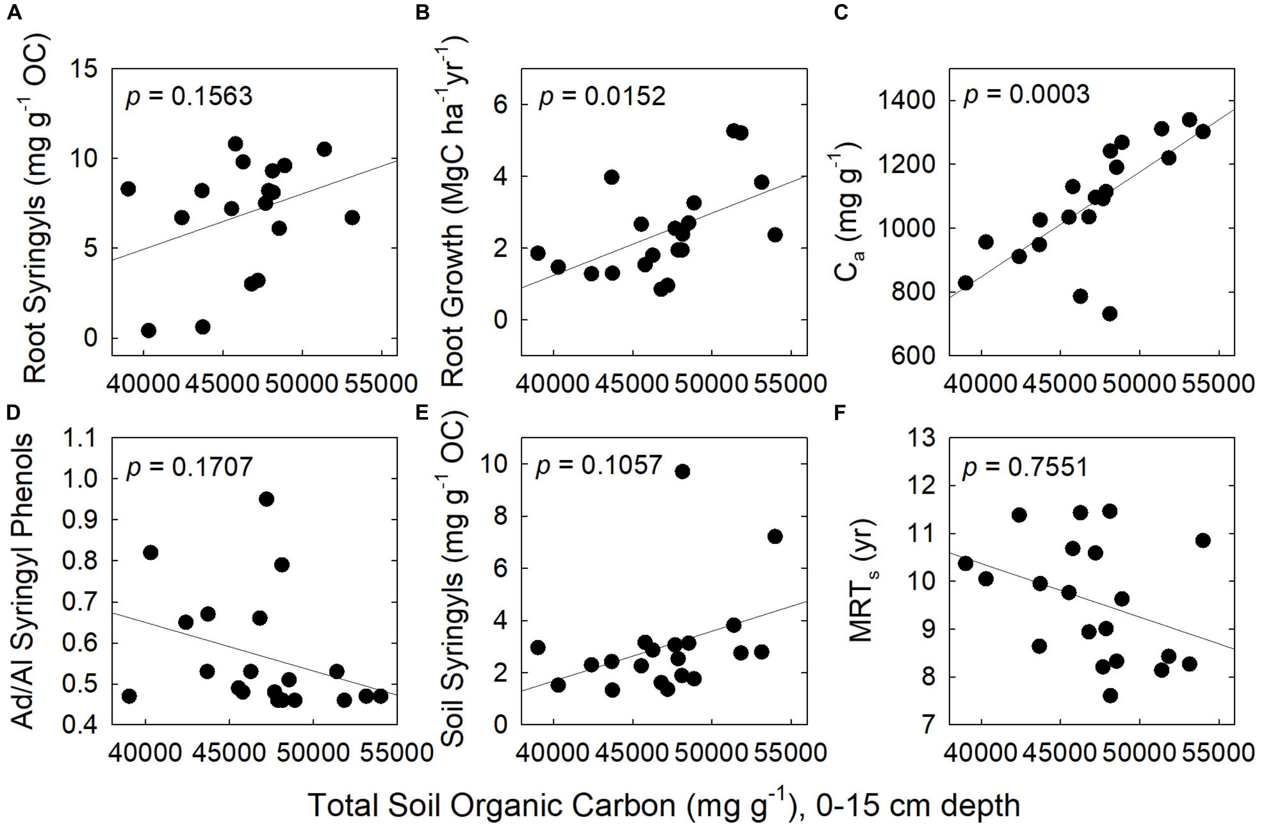

FIGURE 2 | Correlations between total SOC and six explanatory variables. They are: **(A)** Root syringyl phenols; **(B)** Fine-root growth; **(C)** Size of the active soil pool, C_a; **(D)** Acid/Aldehyde of Syringyl phenols in soil; **(E)** Soil syringyl phenols; and **(F)** Mean Residence Time of the slow pool, MRT_S. *N* = 20 plots.

TABLE 3 | Soil organic carbon (SOC) pools and their turnover and fine-root growth (FRG) in five tropical tree species.

Variable	Species					Reference		Statistics	
	Hieronyma	***Pentaclethra***	***Pinus***	***Virola***	***Vochysia***	**Forest**	**Pasture**	***F***	***p***
C_a pool	1175 ± 82 **ab**	1027 ± 69 **bc**	1028 ± 29 **bc**	880 ± 80 **c**	1281 ± 16 **a**	1454	1012	9.53	**0.0010**
C_s pool	26908 ± 755 **ab**	28392 ± 1026 **b**	27903 ± 856 **b**	28553 ± 654 **ab**	32051 ± 990 **a**	31274	32893	3.65	**0.0286**
C_r pool	18512 ± 1289 a	15130 ± 1257 a	15569 ± 965 a	16676 ± 923 a	17271 ± 498 a	17580	12675	2.55	0.0938
Total SOC	49295 ± 2113 a	44549 ± 1920 a	44500 ± 1605 a	46109 ± 1301 a	50603 ± 1326 a	50308	46580	2.76	0.0668
MRT_a	7.86 ± 0.42 a	7.63 ± 0.452 a	8.01 ± 0.32 a	7.34 ± 0.85 a	8.43 ± 0.21 a	11.28	5.24	1.74	0.2053
MRT_s	8.42 ± 0.08 **b**	9.96 ± 0.37 **ab**	9.88 ± 0.34 **ab**	10.62 ± 0.80 **a**	9.06 ± 0.74 **ab**	7.40	8.16	5.21	**0.0115**
k_a	0.128 ± 0.007 a	0.133 ± 0.009 a	0.125 ± 0.005 a	0.143 ± 0.017 a	0.119 ± 0.003 a	0.089	0.191	=MRT_a	=MRT_a
$k_s \times 10^{-4}$	3.25 ± 0.03 **b**	2.76 ± 0.11 **ab**	2.78 ± 0.10 **ab**	2.63 ± 0.23 **a**	3.08 ± 0.24 **ab**	3.70 E^{-4}	3.36 E^{-4}	=MRT_s	=MRT_s
FRG	3.92 ± 0.51 **a**	2.00 ± 0.24 **bc**	1.15 ± 0.14 **c**	1.90 ± 0.26 **bc**	3.32 ± 0.68 **ab**	n.d.	n.d.	11.22	**0.0005**

Data are from long-term incubations of soil, 0–15 cm depth. SOC pools: C_a = active; C_s = slow; and C_r = resistant. Units are: mg g^{-1} for all soil C pools; day^{-1} for k_a and k_s (decay rates); days for MRT_a; years for MRT_s; and Mg C ha^{-1} $year^{-1}$ for FRG. Different letters within a row denote significant differences among species (α = 0.05) by Tukey's HSD. There were n = 20 plots in the analyses, with df for blocks = 3 and species = 4. Data for reference vegetation not included in analyses. Data for total SOC were published previously (Russell et al., 2007). Fine-root growth data were reported previously in Valverde-Barrantes et al. (2007, 2009). No data = n.d. Bold letters denote significant differences.

7–12 mg g^{-1} OC (**Table 1**), covered half the range found in the broad latitudinal study by Amelung et al. (1999). The broad range found in our study was not driven by different growth forms either: values for the only gymnosperm species in the experiment fell in the middle of the range, with the endpoints of the range both represented by broad-leaved evergreen tropical tree species.

In a study of 18 sites across a range of biomes, from taiga and tundra to temperate forest and tropical forest, Vancampenhout et al. (2009) concluded that tropical soils were low in lignin. However, two of three forests in that study were dry forests. Low soil lignin content could be explained by lower detrital inputs in the drier forests, given the results of another study in which net primary productivity (NPP) in tropical forests declined with MAP for sites with MAP less than ∼2,500 mm (Schuur, 2003). It is also possible that lignin degraded faster in the drier forests, given the finding that lignin was subject to accelerated degradation via photodegradation in a semi-arid environment because lignin effectively absorbed light over a wide range of wavelengths (Austin and Ballaré, 2010). Thus, the lower lignin contents in tropical dry forests could be the result of an abiotic factor that increases lignin degradation.

TABLE 4 | Partial regression results for the effects of six explanatory factors on SOC stocks.

Variable	*p*	Type II SS
Fine-root growth	**0.0225**	40.31
Fine-root syringyl phenols	**0.0212**	41.26
Ad/Al Soil Syringyl phenols	0.0908	19.89
MRT_s	0.2783	7.58
C_a	**0.0326**	34.30
Soil Syringyl phenols	0.4503	3.58

Notes. The six explanatory variables included in this model were: (a) Fine-root growth (FRG); (b) Fine-root syringyls (RtSyr); (c) Acid/aldehyde ratio of Soil Syringyls (AdAlSyr); (d) mean residence time of the slow pool (MRT_s); (e) size of the active SOC pool (C_a); and (f) Soil Syringyls (SoSyr). These analyses are based n = 20 plots. The p values and Type II Sums of Squares) refer to significance of the reduction in the sum of squared error when adding a parameter to the model that already has the other five parameters in it. Bold letters denote significant differences.

SOC Pools and Their Turnover

In our study, the Ad/Al ratio of both syringyl and vanillyl phenols in soil differed significantly among species. For syringyl phenols, this ratio decreased significantly as fine-root growth increased ($r = -0.51$, $p = 0.0210$). This suggests that in a species such as *Vochysia*, which had a low Ad/Al ratio, the soil pool of syringyl phenols was being replenished relatively more with inputs of fine-root detritus. *Hieronyma,* however, also had relatively high fine-root growth, but a relatively higher Ad/Al ratio, suggesting that its fine-root detritus decomposed relatively faster. Thus, while the Ad/Al ratio provides a measure of the state of decomposition of

TABLE 5 | Multiple regression results for the effects of six explanatory factors on SOC stocks.

Number in model	R^2	AIC	SBC	Variables in model
1	0.4531	36.89	38.67	C_a
1	0.2719	42.04	43.82	FRG
2	0.5424	35.68	38.35	RtSyr, C_a
2	0.4950	37.46	40.13	FRG, C_a
3	0.6144	34.60	38.16	RtSyr, C_a, AdAlSyr
3	0.5543	37.21	40.77	RtSyr, C_a, SoSyr
4	0.6377	35.48	39.93	RtSyr, C_a, AdAlSyr, MRT_s
4	0.6192	36.38	40.82	RtSyr, FRG, C_a, AdAlSyr
5	0.6552	36.59	41.93	RtSyr, C_a, AdAlSyr, SoSyr, MRT_s
5	0.6378	37.48	42.82	RtSyr, FRG, C_a, AdAlSyr, MRT_s

Notes. The six explanatory variables included in this model were: (a) Fine-root syringyl phenols (RtSyr); (b) Fine-root growth (FRG); (c) Acid/aldehyde ratio of Soil Syringyl phenols (AdAlSyr); (d) mean residence time of the slow pool (MRT_S); (e) size of the active SOC pool (C_a); and (f) Soil Syringyl phenols (SoSyr). Analyses are based n = 20 plots. The Aikake Information Criterion (AIC) and Schwartz Baysian Criterion (SBC) provide criteria to compare and select models among a finite set of models, based on their goodness of fit and simplicity. All data are for the 0–15 cm depth.

OM within the soil, when comparing different treatments (e.g., land use, species, and history), the interpretation of these data requires information about the quantity of detrital inputs to the soil pool in each of the treatments.

The size of the active pool (0-15 cm), averaged across all species in our site, 1,078 mg kg^{-1} (**Table 3**) was nearly double that reported for an agricultural soil (0-20 cm) at Kellogg Biological Station in Michigan, 570 mg kg^{-1} (Paul et al., 2001). The slow, resistant and total SOC pools in our site were > 5-, 3-, and 4-fold greater, respectively, than those reported for the Michigan site. The MRT in that site for the active and slow SOC pools were 20 days and 12 years under laboratory temperatures, respectively, compared with 8 days and 10 years for MRT_a and MRT_s, respectively, averaged across species in this study (**Table 3**). When the Michigan data were converted to field temperatures, however, the MRT_a was 60 days and MRT_s was 37 years. For the tropical soils in this study, the laboratory and field incubation temperatures were nearly the same; thus these tropical soils were more dynamic and had larger SOC pools.

Tree Species Effects on Accrual and Persistence of SOC

Given that lignin is generally considered to be recalcitrant, owing to its complex chemical structure that protects litter from microbial attack (Swift et al., 1979), variation in lignin content among species is expected to translate into species-specific variation in the decomposability of their tissues. Indeed, in multiple studies of decomposition of aboveground plant tissues, higher lignin content has been highly correlated with slower decomposition of detritus (Melillo et al., 1982; Berg et al., 2000; Cornwell et al., 2008). Lignins were also expected to degrade slowly in soil, but the evidence regarding lignin's recalcitrance within soil has been varied. A review by Vancampenhout et al. (2009) indicated a lack of persistence of lignins in soil. In a comparison of lignin biomarkers in a pasture and rainforest soil in subtropical Australia, Heim et al. (2010) found that only small portions of rainforest-derived lignin persisted 100 years after conversion to pasture, indicating that lignin biomarkers didn't provide a proxy for stable SOC. In tropical soils, higher temperature is thought to increase decomposition of lignin, and thus explain their low lignin contents. As indicated above, however, the soils in our study site had lignin contents on par with temperate-zone sites. Mineral-organic associations can stabilize a large fraction of SOC (Mikutta et al., 2009), however, and this could provide a mechanism for stabilizing lignin-derived phenols in our study site's Oxisol. In addition, given the high rates of NPP (Russell et al., 2010), detrital inputs were replenishing soil lignin stocks in this site. This study was not designed to distinguish between these two mechanisms of lignin retention and accrual in soil, however.

Many studies have demonstrated that leguminous trees increase SOC stocks, as reviewed by Mayer et al. (2020). Given our results that the only nodulated legume species in the study, *Pentaclethra*, had low SOC stocks (**Table 3**), we suggest that the generalization about tree species with N-fixing root associates may well hold for sites that are relatively limited in available N, but not necessarily in tropical sites where N is relatively less limiting for microbial processes (Camenzind et al., 2018). Our data also suggest that lower inputs from roots was an important factor that explained differences among tree species in the accrual of SOC stocks (**Figure 1**). In biomarker studies in temperate zone forests, roots have also been shown to be more important contributors to SOC than aboveground detritus (Crow et al., 2009; Spielvogel et al., 2014). Root activities in soil may afford them physical protection through mycorrhiza and interactions with metal ion (Rasse et al., 2005). By allocating C to microbes, roots may also prime microbial processes, and thus speed up decomposition of older SOC and thus, availability of nutrients for plant uptake (Fontaine et al., 2007; Kuzyakov, 2010), thereby stimulating plant growth and the detrital inputs to soil.

Tree species identity, i.e., species-specific effects, have been shown to influence SOC stocks across a range of experiments (Vesterdal and Raulund-Rasmussen, 1998; Hansson et al., 2011; Osei et al., 2021). Nevertheless, in their evaluation of a large set of factors that could provide indicators of SOC storage, Wiesmeier et al. (2019) concluded that there was no consensus among previous studies regarding the effects of tree species on SOC storage. Some studies distinguish between broad taxonomic categories of tree species, and then find differences in SOC storage between the categories, e.g., gymnosperms and broad-leaved species, without differences within a category (Mayer et al., 2020). In this study, however, we found differences among the four broadleaved evergreen tropical species in their pools of SOC and turnover times.

Kleber et al. (2011), in their comparisons of three different soil types, found that the turnover rate of SOC was not determined solely by the thermodynamic stability of the detrital inputs, such that chemical "recalcitrance" of SOC was not a good predictor of its turnover. Schmidt et al. (2011) emphasized that although compound chemistry is a driver of decomposition, its relative importance must be considered within the context of other environmental factors. Lehmann and Kleber (2015) found that prediction of SOC turnover required an understanding of the interactions between the substrate, microbes, and abiotic drivers; as such, OM quality was a poor predictor of OM persistence. In our study, there was not a significant correlation between total SOC stocks and the Ad/Al ratio of soil, for either syringyl or vanillyl phenols (**Supplementary Table 1**). These ratios can represent the state of oxidation of these phenolic compounds, and thus indicate the intensity of microbially driven oxidation or the length of time the phenols have been in soil, rather than intrinsic phenol stability. That interpretation is consistent with the results from these experimental plots that microbial composition did not differ among tree species (Kivlin and Hawkes, 2016a,b).

In contrast with phenol Ad/Al ratios in soil, fine-root syringyl concentrations did explain differences in total SOC stocks across species in our study, after other variables were taken into account in the partial residual analysis (**Table 4** and **Figures 1**, **2**). This effect of biochemistry on SOC stocks was likely promoted in part by high NPP, and accentuated by the short-term recalcitrance of syringyl phenols; these two factors together would cause syringyl input rates to exceed their degradation rates. Fine-root growth was significantly correlated with fine-root syringyl phenol content ($r = 0.71$, $p = 0.03$, and **Supplementary Table 1**), indicating the importance of input rates. However, of the three families of root phenols in this study, only the syringyl phenols, the "woodiest" of the three, were also identified as a significant factor; this indicates a role of their specific chemical nature or location within roots, in addition to quantity of inputs alone. In contrast, the more degradable cinnamic phenols had much lower accumulations in soil (**Table 1**), further suggesting that biochemistry played a role in soil organic matter dynamics.

Given the experimental design of our study, however, abiotic factors, e.g., MAT, MAP, parent material, time of soil development, relief, and previous land-use history were similar across plots. Certainly, individual species can influence microclimate and microbial communities that could influence SOC dynamics. Given that the tree species in this experiment had similar canopy coverage and height, microclimate differences were not expected to differ significantly. Other results from this study site indicated that soil bacteria richness, composition and soil fungal abundance, richness, and composition did not differ among the tree species (Kivlin and Hawkes, 2016a,b). Based on this and other studies in this field experiment, we suggest that at least four other factors explained the differences among the tree species in SOC pools and dynamics. (1) Differences among the species in fine-root productivity (Valverde-Barrantes et al., 2007) and decay rate (Raich et al., 2009) resulted in differential inputs of detritus to the soil pool. (2) Differences in fine-root syringyl phenols explained some of the variation in total SOC pools across species (**Tables 1**, **4**). (3) The species differed in their effects on soil pH, which in turn influenced the availability of cations that are limiting in this Oxisol, and hence the quantity and chemistry of detrital inputs to soil (Russell et al., 2017). As a result of *Vochysia*'s trait of accumulating aluminum, this species de-acidified the soil. We hypothesized that increasing the soil pH above the point of zero charge in this soil with variable-charge clays dispersed soil organo-mineral colloids, thereby liberating cations available for plant uptake. In contrast, as a result of its N-fixing association, *Pentaclethra* reduced soil pH below the point of zero charge, which was hypothesized to reduce cation availability for uptake. (4) The species also differed in their effects on macroaggregate structure (Russell et al., 2018), which would have differentially influenced the protection of OM in soil.

Given the rates and extent of land-use change in humid tropical forests over the last several decades (Asner et al., 2009) that have generally resulted in loss of biodiversity, the need is ever more urgent to understand the effects of tree species on soil C. Our results from these studies indicate that, as Schmidt et al. (2011) suggested, the explanation for differences among the tree species in their effects on SOC stocks and dynamics is complicated and involves a mix of factors. Nevertheless, developing a framework for evaluating the effects of species advances our conceptual framework of carbon cycling (Russell et al., 2010), and offers managers an important tool, selection of tree species, for forest regeneration projects or establishment of plantation. Our study illustrates that a trait-based approach can provide a process-based understanding of how trees can influence SOC dynamics at a finer-tuned taxonomic level than the Division, e.g., Angiosperms vs. Gymnosperms, and the consequences for ecosystem properties such as SOC stocks.

CONCLUSION

We compared tropical tree species in terms of the quantity of detrital inputs and tissue biochemistry and evaluated the effects of these differences on SOC pools and turnover. The study was conducted in a 15-year-old field experiment containing six tree species, including one gymnosperm and one nodulated legume, grown in monocultures in four blocks. Even within the category of broad-leaved evergreen tree species, the species differed in their concentrations of phenols in tissues and soil. In this single site with the same soil type, MAT and MAP, the means of total phenols across the study species encompassed half the range of values found in another study that covered sites across a broad latitudinal range (Canada to Texas). We used partial residual analysis to quantify the effect of a single explanatory variable on SOC stocks; results indicated that differences among species in syringyl phenols in fine roots, and in the quantity of detrital inputs from fine roots explained differences among species in SOC stocks. Previous studies in this site indicated that the tree species also influenced SOC accumulation differentially as a result of species-specific effects on soil pH and macroaggregate structure. Together, these results highlight the complexity of tree species effects on soil, and the need for a trait-based approach in the selection of tree species when ameliorating degraded sites after land-use change.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. The datasets for this study are available: doi: 10.5061/dryad.fbg79cntt.

AUTHOR CONTRIBUTIONS

AR and DO conceptualized the study and acquired funding. RM collected the data under supervision of AR and DO. AR analyzed the data, designed and ran the model, and prepared the figures and tables. All authors contributed to interpretation of the findings. AR and RM wrote the original draft, and all authors participated in reviewing and editing the manuscript.

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SUPPLEMENTARY MATERIAL

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frontiers in Forests and Global Change

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Balsam Fir and American Beech Influence Soil Respiration Rates in Opposite Directions in a Sugar Maple Forest Near Its Northern Range Limit

Nicolas Bélanger[1,2]*, Alexandre Collin[1,2], Rim Khlifa[1,2] and Simon Lebel-Desrosiers[1,2]

[1] Centre d'étude de la forêt, Université du Québec à Montréal, Québec, QC, Canada, [2] Département Science et Technologie, Téluq, Université du Québec, Québec, QC, Canada

Conifers and deciduous trees greatly differ in regard to their phylogenetics and physiology as well as their influence on soil microclimate and chemical properties. Soil respiration (R_S) in forests can therefore differ depending on tree species composition, and assessments of the variation in R_S in various forest types will lead to a more thorough understanding of the carbon cycle and more robust long-term simulations of soil carbon. We measured R_S in 2019 and 2020 in stands of various species composition in a sugar maple forest near the northern range limit of temperate deciduous forests in Quebec, Canada. Seasonal variations in soil temperature had the largest influence on R_S, but conditions created by the stands also exerted a significant effect. Relative to the typical sugar maple-yellow birch forest (hardwoods), R_S in stands with >20% of basal area from balsam fir (mixedwoods) was increased by 21%. Whilst, when American beech contributed >20% of litterfall mass (hardwood-beech stands), R_S was decreased by 11 and 36% relative to hardwoods and mixedwoods, respectively. As a whole, R_S was significantly higher in mixedwoods than in other forest types, and R_S was significantly higher in hardwoods than in hardwood-beech stands. Sugar maple and American beech at the study site are near their northern range limit, whereas balsam fir is near its southern limit. R_S in mixedwoods was therefore higher than in hardwoods and hardwood-beech stands due to high root activity in the presence of fir, despite colder and drier soils. We estimated that root respiration in mixedwoods was more than threefold that in hardwoods and hardwood-beech stands. The lower R_S in hardwood-beech stands compared to hardwoods points to the lower soil temperature as well as the poor quality of beech litter (low decomposability) as indicated by a generally lower heterotrophic respiration. Other than soil temperature, regression models identified mixedwoods, soil water potential and Mg^{2+} activity in the soil solution as important predictor variables of R_S with about 90% of its variation explained. Our study shows the benefits of combining forest-specific properties to climatic data for more robust predictions of R_S.

Keywords: cool temperate forests, tree species composition, CO_2 efflux, soil respiration, root respiration, litter quality, soil water

Edited by:
Jérôme Laganière,
Canadian Forest Service, Canada

Reviewed by:
Antra Boca,
Latvia University of Agriculture, Latvia
Richard Drew Bowden,
Allegheny College, United States

****Correspondence:***
Nicolas Bélanger
nicolas.belanger@teluq.ca

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INTRODUCTION

Soil respiration (R_s) is the second largest CO_2 flux in forests after plant respiration and in turn, it has a high potential to modify atmospheric CO_2 levels (Schlesinger and Andrews, 2000; Raich et al., 2002). It is constituted of heterotrophic (R_h) and autotrophic (R_a) respiration. The R_h component comes from the respiration of the soil faunal and microbial communities that physically breakdown and biochemically decompose dead organic matter, whereas the R_a component comes from root and rhizosphere respiration. Soil temperature is the main abiotic control of R_s in forests (Davidson and Janssens, 2006; Subke and Bahn, 2010). The temperature sensitivity of R_s is often expressed as Q_{10} − it is the proportional change in respiration with a 10°C increase in soil temperature (Curiel Yuste et al., 2004). Soil moisture also interacts with soil temperature to influence R_s in forests (Cisneros-Dozal et al., 2006; Moyano et al., 2012). Soil temperature and moisture may vary seasonally and across years, thereby modifying the temperature sensitivity of R_s in forest ecosystems (Davidson et al., 1998). Other factors also influence the seasonal patterns of R_s in forests such as variation in root activity during the growing season and fresh litter additions in the fall (Boone et al., 1998; Lavigne et al., 2004; Prévost-Bourré et al., 2010).

Changes in forest structure and species composition that lead to a modification in soil conditions are thus expected to influence R_s. Soil respiration tends to increase with stand age due to root growth and respiration (Subke et al., 2006), but this trend can be absent in forests where crown closure reduces soil temperature (e.g., Gough et al., 2005). Forest species composition may also influence R_s by affecting: (1) soil chemical properties (e.g., pH, N, and P availability), (2) root biomass, respiration and phenology, (3) litter inputs, quality and decomposability, and (4) microbial communities and activity (Raich and Tufekcioglu, 2000). Side-by-side comparisons of R_s between deciduous and coniferous stands generally suggest higher fluxes under deciduous trees (Tewary et al., 1982; Weber, 1985, 1990; Raich and Tufekcioglu, 2000; Curiel Yuste et al., 2004; Fahey et al., 2005), but lower R_s under deciduous (Lee et al., 2010) or no difference in R_s between forest types (Reiners, 1968; Raich and Potter, 1995; Davidson et al., 1998; Borken et al., 2002; Fernández-Alonso et al., 2018) have also been reported.

The generally lower quality of conifer litter and R_h relative to deciduous tree species was a common explanation for the lower R_s measured in coniferous stands, whereas differences in phenology and physiology between tree species explained no change or higher R_s in coniferous stands.

Worldwide, temperate deciduous forests were estimated to hold 7–8% of global terrestrial C stocks (Pregitzer and Euskirchen, 2004). Cool temperate deciduous forests in eastern Canada offer high C sequestration potential because they are among the most productive ecosystems in the country due to their relatively long growing season and sufficient precipitation falling during the growing season (Kurz and Apps, 1999; Stinson et al., 2011). Also, there is little fire disturbance or forest harvesting (mostly uneven age management) that could release large amounts of C back to the atmosphere (Saucier et al., 2009a). Fahey et al. (2005) provided R_s estimates for various deciduous and coniferous stands in the cool temperate Hubbard Brook Experimental Forest, New Hampshire. However, there is no study that investigated the variations in R_s near the northern limit of temperate deciduous forests in northeastern North America. These forests are associated with common tree species changes due to the presence of deciduous species to the south and coniferous (boreal) species to the north. Furthermore, northeastern North American deciduous forests are characterized by replacement patterns between sugar maple (*Acer saccharum*) and American beech (*Fagus grandifolia*). This replacement pattern is also observed at their northern limit (Collin et al., 2017). Light, water and nutrient availability as well as reproduction rates govern the replacement patterns between the two species. These factors can be modified due to natural disturbances such as ice storms, disease and herbivory (Arii and Lechowicz, 2002; Bohn and Nyland, 2003; Nyland et al., 2006), forest management practices (Nolet et al., 2008; Bannon et al., 2015) and atmospheric acid deposition (Bailey et al., 2004; Long et al., 2009). Water limitations for tree growth are suspected to increase in frequency and intensity under climate change in eastern North America (Gustafson and Sturtevant, 2013), whereas sugar maple is likely more sensitive than American beech to water stress (Nolet and Kneeshaw, 2018). Overall, the recent increase in the dominance of beech in the understory of maple stands suggests that beech stands will occupy forest land in the future (Hane, 2003; Gravel et al., 2010). Similar to conifer litter, the high recalcitrance of American beech litter (Melillo et al., 1982; Côté and Fyles, 1994) has the potential of reducing R_s by slowing down litter turnover and thus decreasing R_h (Bowden et al., 1993; Cisneros-Dozal et al., 2007).

Climate change is causing physiological constraints that have the potential of impacting the survival, growth and distribution of plant species globally (Rosenzweig et al., 2008; Chen et al., 2011), including northeastern North American tree species (Beckage et al., 2008; Charney et al., 2016; Sittaro et al., 2017; Iverson et al., 2019; Boivert-Marsh and de Blois, 2021). In addition to understanding how tree growth and thus forest C uptake will be affected by climate change, analyses of how forest species compositions affect R_s are needed for more robust predictions of temporal and spatial changes in ecosystem C pools and atmospheric CO_2 (Raich and Tufekcioglu, 2000; Buchholz et al., 2014). As sugar maple, American beech and conifers differ in regard to their physiology, biogeochemistry (e.g., litter quality/decomposition) and phylogenetics (angiosperm vs. gymnosperm), R_s is expected to vary depending on the proportion of these tree species within a forest. The objective of this study was to assess R_s across a range of plots that captured variations in the abundance of conifers and American beech within a forest dominated by sugar maple near the northern range limit of temperate deciduous forests in eastern North America. Mostly due to their lower litter qualities compared to maple litter, it was hypothesized that an increase in the abundance of conifers and beech would decrease R_s in this maple forest.

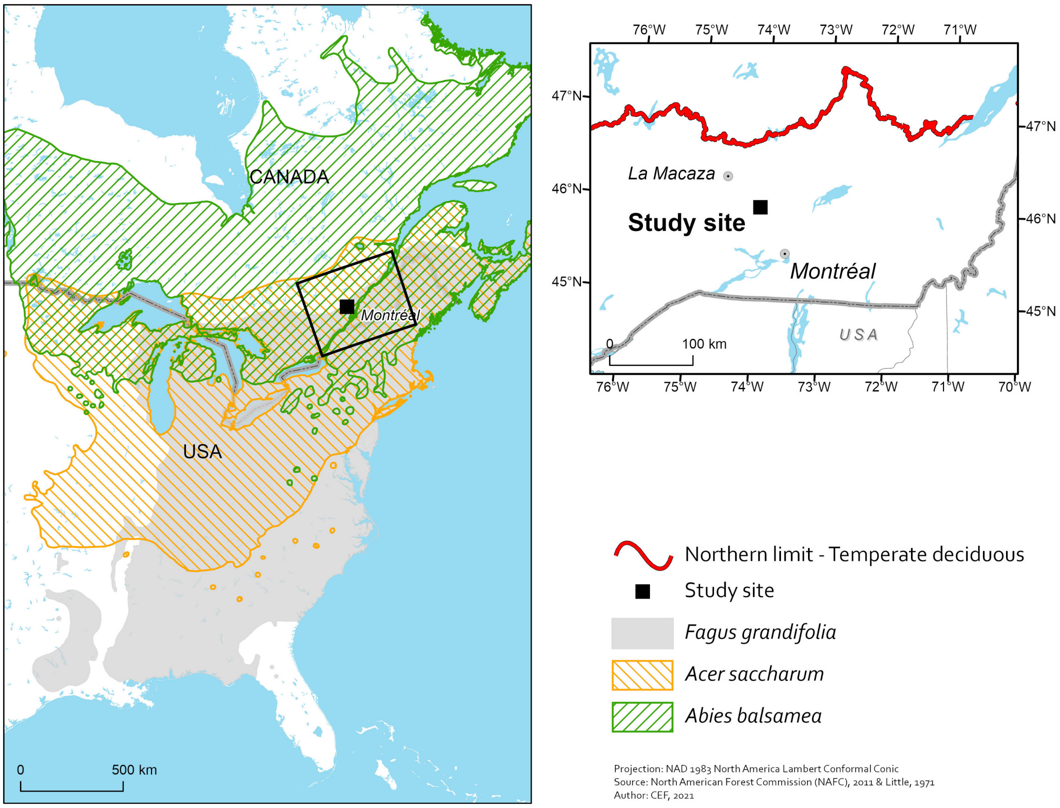
MATERIALS AND METHODS

Study Site

The study was conducted at the *Station de Biologie des Laurentides* (SBL) of *Université de Montréal* in St. Hippolyte, Quebec (**Figure 1**). The SBL is found within the northern limit of the maple-yellow birch (*Betula alleghaniensis*) bioclimatic domain of the lower Laurentians. The maple-yellow birch domain is the northernmost deciduous forest domain in Quebec (Saucier et al., 2009b). A mosaic of tree species is found at the site. It is composed mostly of sugar maple, red maple (*Acer rubrum*), American beech, yellow birch (*Betula alleghaniensis*), white birch (*Betula payrifera*), largetooth aspen (*Populus grandidentata*), eastern white cedar (*Thuja occidentalis*), white pine (*Pinus strobus*), and red spruce (*Picea rubens*) (Savage, 2001). Because SBL is only 35 km south of La Macaza, i.e., the doorway to temperate mixedwoods at that specific longitude in Quebec (**Figure 1**), stands dominated with tree species that are typically boreal [e.g., balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*)] are not uncommon at the site. Also, stands exhibiting sugar maple regeneration failures and expansion of beech are frequent at SBL (Collin et al., 2017). The mean annual temperature at SBL simulated with the BioSIM model (Régnière and Bolstad, 1994) between 2003 and 2013 was 4.9°C, mean degree-days were 2845, mean days without frost were 153 and mean precipitation was 1,270 mm, with about 30% falling as snow. Soils were developed from glacial till made mostly of anorthosite and felsic rocks of the Precambrian Shield (Bélanger et al., 2012). They are classified as Orthic Humo-Ferric and Ferro-Humic Podzols with a sandy loam texture and the forest floor is characterized by a moder humus form (Soil Classification Working Group, 1998).

Experimental Design and Stand/Plot Characterization

In 2018, eight stands with four 3 × 3 m plots within each stand (total of 32 plots) were selected in three zones distributed within a 18 ha area. Stands were selected to capture as much variation as possible in regard to tree species composition. Stands were a minimum of 0.5 ha. The four plots within each stand were delineated in the center of the stand (within a maximum of 15 m between the most distant plots) under a very similar tree species composition. A series of ecological variables were collected at the stand level between 2017 and 2020, allowing for a detailed classification of tree species composition. These included (1) basal area by species based on trees with a stem diameter at breast height above 9 cm (**Table 1**) and (2) litterfall mass by species



Projection: NAD 1983 North America Lambert Conformal Conic
Source: North American Forest Commission (NAFC), 2011 & Little, 1971
Author: CEF, 2021

FIGURE 1 | Location of *Station de biologie des Laurentides* (Study site) in southern Quebec, Canada, along with the distributions of sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*) and balsam fir (*Abies balsamea*) (left-hand map) as well as the northern limit of temperate deciduous forests (right-hand map).

TABLE 1 | Total basal area and tree species contributions to total basal area in each stand in 2018.

Zone	Stand	Forest type	Basal area m^2 ha^{-1}	Contribution (%)							
				AS	*AB*	*BA*	*BP*	*FG*	*AR*	*PG*	*AP*
1	1	Mixedwood	35.5	11.3	29.6	0.00	32.4	0.00	21.1	2.80	2.80
1	2	Hardwood	35.5	35.2	9.90	0.00	32.4	0.00	19.7	2.80	0.00
2	3	Hard.-beech	29.0	87.9	0.00	6.90	0.00	5.20	0.00	0.00	0.00
2	4	Mixedwood	35.5	45.1	29.6	2.80	2.80	2.80	11.3	0.00	5.60
3	5	Hard.-beech	22.5	51.1	8.90	17.8	0.00	20.0	0.00	0.00	2.20
3	6	Hardwood	31.0	54.8	6.50	11.3	14.5	12.9	0.00	0.00	0.00
3	7	Mixedwood	40.5	58.0	25.9	4.90	8.60	0.00	2.50	0.00	0.00
3	8	Hard.-beech	34.0	76.5	0.00	5.90	2.90	14.7	0.00	0.00	0.00

N.B. Hard.-beech is hardwood-beech, AS is Acer saccharum (sugar maple), AB is Abies balsamea (balsam fir), Betula alleghaniensis (yellow birch), BP is Betula papyrifera (white birch), FG is Fagus grandifolia (American beech), AR is Acer rubrum (red maple), PG is Populus grandidentata (large-tooth aspen), and AP is Acer pensylvanicum (striped maple). The sum of AS, AB, BP, BA, FG, AR, PG, and AP equals 100%.

TABLE 2 | Mean tree species contributions to total litterfall mass in each stand in 2018.

Zone	Stand	Forest type	LAI (m^2 m^{-2})	Contribution (%)							
				Leaves	Needles	*AS*	*Betula*	*FG*	*AR*	*PG*	*AP*
1	1	Mixedwood	3.20	54.1	45.9	1.14	21.3	0.09	31.3	0.00	0.25
1	2	Hardwood	2.58	98.2	1.83	59.0	8.26	0.00	20.5	10.4	0.00
2	3	Hard.-beech	2.66	99.9	0.08	59.5	10.6	21.4	5.22	1.22	2.00
2	4	Mixedwood	2.72	72.7	27.3	44.4	16.0	9.87	0.00	0.01	2.37
3	5	Hard.-beech	3.01	98.4	1.64	31.4	35.0	29.0	2.36	0.30	0.32
3	6	Hardwood	3.03	97.9	2.08	37.1	28.2	14.9	11.3	3.33	3.07
3	7	Mixedwood	2.93	90.8	9.23	36.4	3.02	1.39	48.2	1.39	0.39
3	8	Hard.-beech	2.62	100	0.00	36.4	2.48	31.5	29.6	0.00	0.00

N.B. Hard.-beech is hardwood-beech, LAI is leaf area index, Leaves is all leaves, Needles is mostly Abies balsamea (balsam fir), AS is Acer saccharum (sugar maple), Betula is Betula alleghaniensis and Betula papyrifera (yellow and white birch, respectively), FG is Fagus grandifolia (American beech), AR is Acer rubrum (red maple), PG is Populus grandidentata (large-tooth aspen), and AP is Acer pensylvanicum (striped maple). The sum of Leaves and Needles or the sum of Needles and AS, Betula spp., FG, AR, PG, and AP equals 100%.

(**Table 2**). At the center of each stand, litterfall was collected in a plastic bin (0.9 cm × 0.7 cm × 0.5 cm) that was perforated at the bottom and filled with silica sand (depth of about 5 cm) to drain the bin and thus prevent the trapped litter to immerse in water between samplings. Litter was collected each fall and dried in an oven at 65°C for 48 h before being weighted by species. Leaf area index was also measured in the center of each plot on a sunny morning of mid-August 2020 when the canopy was fully developed using a CI-110 Plant Canopy Imager (CID Bio-Science, Camas, WA).

Stands were classified as mixedwoods when basal area of balsam fir contributed to more than 20% of total basal area (i.e., stands 1, 4, and 7, **Table 1**). However, basal area by species may not be fully representative of deciduous litterfall type in the plots. For example, the high American beech sapling density in some stands was not captured in the basal area measurements, despite that beech litterfall is high (i.e., stands 3 and 8, **Table 2**). Because R_h is a significant component of R_s in temperate deciduous forests [25–35% of R_s according to Bowden et al. (1993) and Cisneros-Dozal et al. (2007)], we used litterfall mass by species to separate between hardwoods and hardwood-beech stands. In this respect, hardwood-beech stands were identified as having at least 20% of their total litterfall mass from beech (stands 3, 5, and 8), whereas hardwoods had less than 20% (stands 2 and 6, **Table 2**). In the case of stand 2, there was no presence of beech.

Soil temperature and water availability at a 10 cm depth were measured every 15 min in each plot from two temperature sensors (Spectrum Technologies, United States) and two water potential sensors (Irrometer 200SS-5, Watermark, United States), all connected to the same WatchDog 1650 Micro Station data logger (Spectrum Technologies). Plant Root Simulator (PRS) probes (Western Ag Innovations, Canada) were used to assess ionic activity (i.e., NO_3^-, NH_4^+, $H_2PO_4^-$, Ca^{2+}, Mg^{2+}, K^+, Al^{3+}, Fe^{3+}, and Mn^{2+}) in the soil. Four pairs of cation and anion probes were carefully inserted vertically into the forest floor at random locations within each plot. They were installed in early June of 2019 and 2020 and collected 6 weeks later. The PRS probes represent a dynamic measurement of ions flowing through the soil over time compared to conventional soil extraction methods that provide a measurement of soil nutrient availability at a particular point in time. The probes are now frequently used in forest ecology research (Hangs et al., 2004; Bilodeau-Gauthier et al., 2013). Once extracted from the soil, the probes were cleaned with deionized H_2O and stored in the fridge in zipseal bags until analysis. Probes were eluted for 1 h with 0.5 M HCl. NH_4-N and NO_3-N were determined colorimetrically by continuous flow analysis (Autoanalyser III, Bran and Luebbe, United States), whereas other ions were determined by inductively coupled plasma atomic emission spectroscopy (Optima 3000-DV, PerkinElmer, United States).

Gas Sampling, Laboratory Analysis, and Flux Calculations

Four circular bases were installed in each plot in July 2018. The bases were set out in a square shape pattern with a distance of 1.5 m between them. They were fabricated from high-density PVC 15 cm inside diameter pipes (i.d., surface area of 177 cm^2) with smooth 6 mm thick side walls. The bases were cut to a length of 12 cm and were inserted at a soil depth of 6 cm. The flux chambers were fabricated from the female connection end of the pipes (i.d. of 15.5 cm, surface area of 189 cm^2) with a double seal locked-in gasket, thus securing a tight seal with the base. The chamber was closed with a 4 mm thick Teflon sheet and the exterior was wrapped with reflective insolation to avoid overheating. The combination of the base and the length of the chamber results in a headspace volume of 4.56 L. The risk of pressurization of the flux chamber was eliminated by (1) venting with a Tygon tube (4 mm i.d.) that passed through the top of the chamber and (2) avoiding to sample under windy conditions. Gas sampling was done from an injection site (Bung Interlink IV, Baxter, United States). Debris were removed only when they obstructed the placement of the chamber on the base.

In 2019, gas sampling started in June and finished just before soil freezing in November 2019. It was done in all the plots within each stand and at five different dates ($n = 160$). Despite the large number of plots (4 plots per stand, 8 stands, and 32 plots in total), sampling was done within a day (∼6 h). In 2020, we opted for a higher sampling frequency but did not sample all stands or all four plots in each stand each time. We sampled two plots in all stands on June 9, June 22, July 9, July 22, August 26, and September 23, all plots in stands 1, 2, 3, and 4 (i.e., block 1) on June 15, June 30, July 21, and August 11, and one plot per stand on October 22. A rotation of plots was done for a better assessment of spatial variability within each stand. In total, 160 gas samples were collected at 11 different dates in 2020, but 4 samples were discarded due to sampling errors ($n = 156$).

Gas sampling was done as soon as the flux chamber was deposited on the base (t_0). To do so, 5 ml of gas sample was withdrawn from the chamber using a 50 ml polypropylene syringe equipped with a 25-gauge 5/8-inch needle. The gas samples collected from each chamber (4×) were injected in the same pre-evacuated (ca. 0.005 atm.) 12 ml Exertainer® vial (LabCo, United Kingdom). The same procedure was repeated at 4 (t_4), 8 (t_8), 16 (t_{16}), and 24 (t_{24}) min. The gas samples were stored under a positive pressure of approximately 1.7-atm, i.e., respectively 20 ml of headspace gas into a 12 ml vial, to minimize any gaseous exchange with atmospheric air. The sampling scheme (i.e., pooling gas samples) followed the recommendation of Arias-Navarro et al. (2013) as a means to better capture spatial variability of soil gas efflux within the plots.

To provide an estimate of the contribution of the autotrophic (R_a) and heterotrophic (R_h) components to R_s, we used a root exclusion approach similar to Kelting et al. (1998). In mid-May 2020, we created four 1 × 1 m root-free plots by severing all the roots to a depth of 40 cm. This depth was considered to capture most of the roots because Lajeunesse (1990) measured that 70% of root mass at SBL is found within the first 25 cm of soil, i.e., the foret floor and upper podzolic B horizon. We had one plot in stands 1, 2, 3, and 4, being, respectively, mixedwood, hardwood, hardwood-beech and mixedwood. All four plots were delineated in proximity of the other sampling plots. There was barely any vegetation in those plots and any new growth was removed by clipping. We used the same sampling procedure as described above. No barrier was used to further limit root development because CO_2 efflux measurements were planned to be performed in 2020 only. Sampling of these plots started on June 15, exactly 1 month after trenching, and continued on June 30, July 21, August 11. This sampling was done at the same time as the sampling scheme described above. For each forest type and sampling date, root respiration (R_{root}) was calculated as the difference between R_s (i.e., mean of the standard 3 × 3 m plots) and soil respiration in the root-free plots (1 × 1 m plot), whereas R_h was assumed as the net flux from the trenched plots. The percent contributions of R_{root} and R_h to R_s were also calculated. Soils can become wetter in the trenched plots due to the absence of water uptake by roots and this can affect soil respiration patterns when they exhibit extremes in moisture levels (Hanson et al., 2000). In addition, soil respiration from the trenched plots could include an added flux of CO_2 because of a possible increase in the decomposition of dead roots [rhizosphere (or rhizomicrobial) respiration], especially a few months after trenching (thus the use of R_{root} and not R_a). This means that R_{root} could be an underestimation of root respiration, whereas R_h could be overestimated. Bowden et al. (1993) provided a strong argument that the contribution of decomposition of dead roots in trenched plots is small, at least in the short term. However, because we do not have estimates of root decomposition in the trenched plots, we need to interpret the data with care. The estimates were therefore used as a means to exhibit the main contrasts in R_{root} and R_h patterns among the three forest types studied.

Carbon dioxide and CH_4 analysis of gas samples was carried out within 48 h of completion of each sampling campaign. Cavity-ring down (CRD) spectroscopy (G2201-*i* Isotopic Analyzer, Picarro, United States) and ultra-zero air as the carrier gas were used for analysis. Before analysis, water vapor from gas samples was selectively removed using a monotube Nafion^{TM} gas dryer (MD-050-12-2, PermaPure, United States).

Raw CO_2 fluxes were estimated by fitting t_0, t_4 (exp. 2 only), t_8, t_{16}, and t_{24} data to a linear model using the HMR package in R (Pedersen et al., 2010). We used a linear model for all flux samples for two main reasons: (1) it was the best fit for over 95% of samples, and (2) it provides more consistent flux results for short enclosure times and in cases of a concave flux curve, i.e., the most common non-linear pattern for the remaining 5% of samples (Kutzbach et al., 2007; Görres et al., 2014; Kandel et al., 2016). Selecting a single linear model thus avoided calculating differences in CO_2 fluxes due to differences in models and not CO_2 fluxes *per se*. Air temperature and pressure were used to adjust raw gas fluxes (Rochette and Bertrand, 2007). The well drained soils resulted in small CH_4 sinks and therefore, CH_4 fluxes were not investigated any further.

Data Analysis

To confirm the influence of soil temperature on R_s, we first fitted single exponential models of R_s with soil temperature using the equation $R_s = a \times e^{(b\ \times soiltemp)}$, where *a* and *b* are parameters to be estimated, *e* is the base of the natural logarithm (2.71828) and *soil temp* is measured soil temperature in °C. Models were fitted for each forest type and for all forest types combined (general model) for both years combined. We calculated R^2 from the linear regression between measured vs. predicted R_s. The Q_{10} value for each model was obtained with the equation $Q_{10} = e^{(b\ \times\ 10)}$. The residuals of the general model were then modeled using a forward stepwise regression with the following independent variables: soil water potential, total basal area, LAI, and NO_3^-, NH_4^+, $H_2PO_4^-$, Ca^{2+}, Mg^{2+}, K^+, Al^{3+}, Fe^{3+}, and Mn^{2+} activity in the soil solution as measured by PRS probes. Forest types were also tested after they were converted into dummy variables, i.e., mixedwoods (0) vs. other forest types (1), hardwood-beech stands (0) vs. other forest types (1), and hardwoods (0) vs. other forest types (1). Only the first three selected variables were kept in the model. This procedure may leave out some variables that are not necessarily unimportant. However, our purpose was to find a small set of logical predictor variables that did an adequate job of prediction while being relevant to biological theory (Sokal and Rohlf, 2012). All predictor variables individually improved the fit between the observed and predicted values at $P < 0.01$. The marginal sum of squares (type III) was also used to measure the predictive information contained in the predictor variables individually while considering the other predictor variables in the model. The variance inflation factor (VIF) was used to detect any co-linearity between the independent variables in the model. No VIF exceeded a value of 2.8. Transformation of the raw data was not necessary to assure equal variance and normality in the distribution of the residuals. Predicted R_s was plotted against measured R_s and the R^2 of the linear regression was calculated as an indication of the capacity of this two stage modeling approach to explain the variation in R_s.

To assess the effect of forest types on R_s, we used two linear mixed effect models with forest type as the fixed factor in both models. In the first model, sampling date was used as the random factor, whereas soil temperature was used as the random factor in the second model. We proceeded this way to consider that soil temperature differences may be produced by the forest types and their canopies and, in turn, this could lead to differences in R_s. A square root transformation was used in both models to normalize the residuals, whereas predicted values were computed for subsequent display. To assess the effects of forest types on soil temperature and water potential, we first computed daily averages from May 15 to October 15 in 2019, and from May 15 to August 31 in 2020, i.e., the data that we could secure while avoiding logistical problems associated with snow cover in both years. Technical problems with dataloggers reduced the continuity of the data in 2020 and thus, we did not use time-series data for September and October for further analysis. With these data, we used linear mixed effect models with forest type as the fixed factor and sampling date as the random factor. A logarithmic transformation was used to warrant normality of the residuals. Finally, one-way ANOVA was used to detect differences in R_{root} and R_h between forest types as well as their respective contributions (in %) to R_s. Block identity was not tested in any of the linear mixed effect models and ANOVAs because it was not possible to obtain all forest types within each zone and thus, zones should not be considered as a blocking structure *per se*. Significant differences between forest types detected with these tests were further depicted with *post hoc* multiple comparisons using the Tukey's honest significant difference (HSD) test. Linear mixed models and ANOVAs were, respectively, conducted with the R statistical software (version 4.0.4) and the "Analysis" module in SigmaPlot 12.0.

RESULTS

Soil respiration rates varied from as low as 21 mg CO_2 m^{-2} h^{-1} in hardwood-beech stands on November 8, 2019, to as high as 726 mg CO_2 m^{-2} h^{-1} in mixedwoods in July 9, 2020 (**Figure 2**). The large variation in R_s was associated to seasonality, increasing from June to July–August and decreasing again to the lowest values just before snow in late October of 2020 or early November of 2019 (**Supplementary Figure 1**). The seasonal effect was more apparent in 2020 because of a higher sampling frequency. Relatively strong exponential relationships were found between soil temperature and R_s for each forest type and all forest types combined (general model) (**Figure 2**). Model parameter estimates, Q_{10} and R^2 are shown in **Table 3**. The modeled curve for mixedwoods was consistently above all other models. Conversely, the modeled curve for hardwood-beech stands was below all other models, although the regression lines between hardwoods and hardwood-beech stands crossed each other at a soil temperature of 15.5°C. Soil temperature alone explained 59% of the variance in R_s rates (see general models in **Table 3**). The predictive ability was higher for hardwood-beech stands and mixedwoods (64–65%) and lower for hardwoods (55%). Computed Q_{10} values were 2.26 for hardwoods, 2.44 for mixedwoods, 2.58 for all forest types, and 2.97 for hardwood-beech stands (**Table 3**).

The forward stepwise regression model of the residuals of the general exponential model (i.e., soil temperature vs. R_s) explained an additional 19% of the variation in R_s (**Table 4**). The dummy variable representing mixedwoods (0) vs. other forest types (1) was selected as the first predictor variable in all models and explained between 11% of the variation in R_s. Soil water potential was selected as the second predictor variable (5.3%), whereas Mg^{2+} activity in the soil solution was selected as the last predictor variable (3.0%). In the end, the linear relationship between measured and predicted R_s over the two study years suggest that the combination of both models (i.e., exponential model followed by multiple linear model) captured 89% of the total variation in R_s. A graphical illustration between measured and predicted values shows that this approach underestimated lower R_s values and overestimated higher R_s values (**Figure 3**).

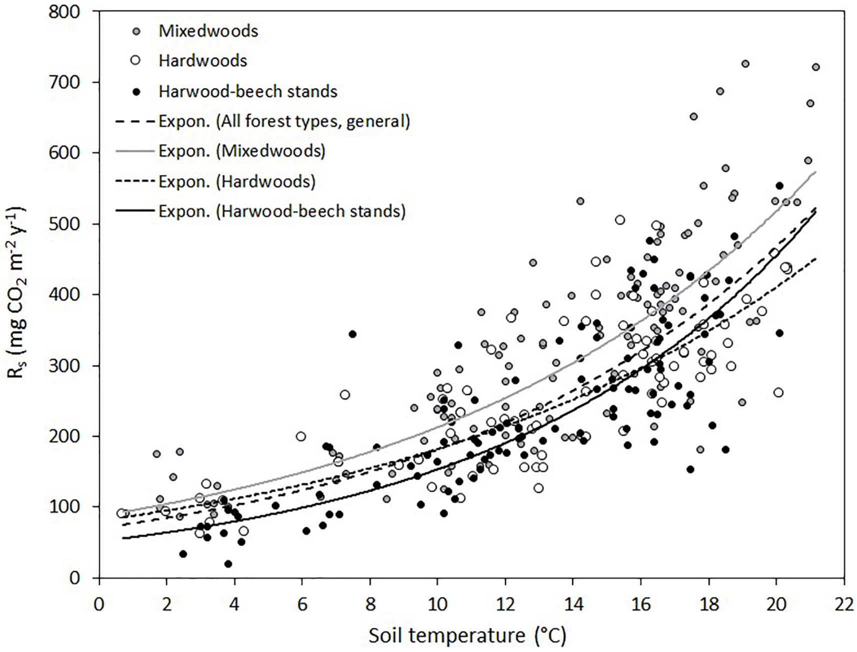


FIGURE 2 | Exponential relationships between soil temperature and soil respiration rates (R_S) for each forest type individually and for all forest types combined (general model). Parameter estimates and Q_{10} of equation $R_s = a \times e^{(b \times soiltemperature)}$ are presented in **Table 3**.

Over the two study years, R_s in mixedwoods was 21 and 36% higher than hardwoods and hardwood-beech stands, respectively (**Figure 4**). The difference in R_s between hardwood and hardwood-beech stands was 11%. Mixed model analysis with sampling date as a random variable suggest a significant difference in R_s between all forest types, whereas mixed model analysis using soil temperature as a random variable suggest a significantly higher R_s in mixedwoods than in hardwoods and hardwood-beech stands, but not between hardwoods and hardwood-beech stands (**Table 3**).

Soil temperature exhibited a seasonal variation similar to R_s, increasing from June to July–August and decreasing again to < 10°C in October and < 4°C in November (**Supplementary Figure 2**). Soils reached higher temperatures in 2020 than in 2019. In 2019, soil water potential reached a maximum in early August (**Supplementary Figure 2**). In 2020, a series of early heatwaves and drought events explain the unusually high soil temperature and very high water potential recorded in June. Overall, soil water potential in 2020 reached higher values than in 2019. Linear mixed effect models detected significantly lower soil temperature in mixedwoods, followed by hardwood-beech stands, and then hardwoods, whereas soil water potential was significantly lower in hardwood-beech stands, followed by hardwoods and then mixedwoods (**Supplementary Figure 2**).

Root-free plots were used to estimate R_{root} and R_h in the three forest types and their contributions (%) to R_s. Root respiration in mixedwoods was approximately threefold higher than in hardwoods and hardwood-beech stands, whereas R_{root} in hardwood-beech stands was 22% higher than in hardwoods (**Table 5**). The R_h component in mixedwoods and hardwood-beech stands generated a smaller CO_2 flux than hardwoods by about 26 and 11%, respectively. Due to the low degrees of freedom of the ANOVAs, however, the only significant difference was found between R_{root} of mixedwoods and R_{root} of hardwood and hardwood-beech stands. Root respiration contributed about 20% of R_s in hardwoods, 25% in hardwood-beech stands, and 50% in mixedwoods (**Table 5**). The remaining CO_2 flux thus originated from the heterotrophic component. The contributions of R_{root} and R_h to R_s were, respectively, significantly lower and higher in hardwoods and hardwood-beech stands than in mixedwoods.

TABLE 3 | Parameter estimates and Q_{10} values of exponential models (**Figure 3**) between soil temperature respiration rates for each forest type individually and for all forest types combined (general model).

Forest type	a	b	Q_{10}	R^2	n
All forest types, general	70.1	0.0949	2.58	0.59	315
Hardwood-beech stands	51.6	0.1090	2.97	0.65	111
Hardwoods	80.8	0.0813	2.26	0.55	79
Mixedwoods	87.4	0.0890	2.44	0.64	125

R^2 were computed from the linear regression between measured vs. predicted soil respiration rates.

DISCUSSION

The seasonal variation in soil temperature in this sugar maple forest near the northern distribution limit of temperature deciduous forests was conducive to relatively strong exponential

TABLE 4 | Multiple linear regression of the residuals of the general exponential relationship between soil respiration and soil temperature.

	R^2	Adj. R^2	*P*	SEE	Intercept	Mixedwoods	Soil water potential	Magnesium activity
	0.19	0.19	<0.001	79.7	9.77	−58.4	−0.49	0.23
Delta R^2						0.112	0.053	0.030
Partial P						<0.001	<0.001	<0.001
SSmarg						243,992	167,548	72,955

Adj. R^2 is adjusted R^2 and P is the P-value, SEE is the standard error of estimate, and the last three columns are the predictor variables (i.e., Mixedwoods is a dummy variable [i.e., mixedwood (0) vs. other forest types (1)], soil water potential, and magnesium activity in the soil solution. Full lines report performance and parameters of the models, whereas delta R^2, partial P and SSmarg [i.e., marginal sum of squares (type III)] provide an assessment of the predicting role of each variable to the model.

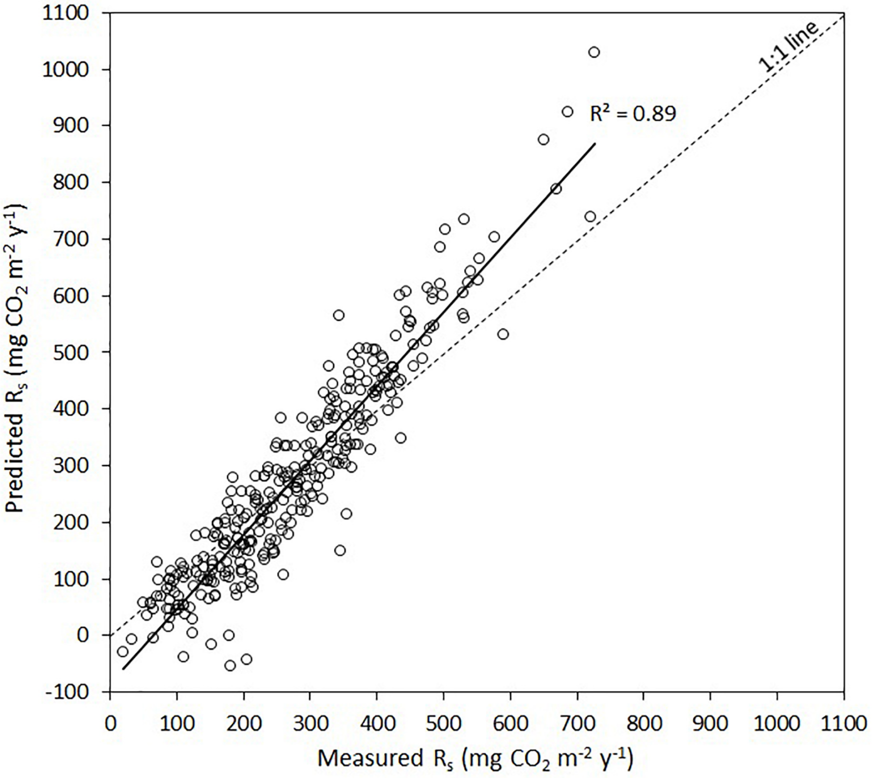


FIGURE 3 | Measured soil respiration rates (R_S) vs. predicted R_S after the two stage modeling approach, i.e., exponential of the raw R_S data and soil temperature (**Figure 2** and **Table 3**) followed by a multiple linear regression of the residuals (**Table 4**). The solid line is the linear regression between measured vs. predicted R_S, whereas the dashed line is the 1:1 line.

relationships between soil temperature and R_s for all forest types. This result was expected because, at all spatial scales, soil temperature is the main abiotic control of R_s and its associated processes (Kutsch et al., 2009) and the key variable for predicting R_s in the context of climate change (Subke and Bahn, 2010). However, further statistical analyses of our data indicated that, over the 2 years of measurements at SBL, tree species composition has had a substantial influence on R_s as well. First, forest stands where balsam fir contributed at least 20% of basal area (i.e., mixedwoods) significantly increased R_s, whereas forests where American beech contributed at least 20% of litterfall (i.e., hardwood-beech stands) significantly decreased R_s. These results contradict our initial hypothesis that conifers would lower R_s in this typically maple dominated forest, but they validate our hypothesis that beech would lower R_s.

Influence of Mixedwoods on R_S

Most studies that investigated the influence of forest type on R_s were conducted at spatially broad experimental scales and thus emphasized the direct effect of temperature and forest productivity across landscapes instead of forest type *per se*. Because R_s is largely dependent on the translocation of photosynthates produced aboveground to the roots (R_a component, Högberg et al., 2001), the expected trend is for R_s to increase from colder to warmer forests. As such, meta-analyses show an increase in R_s from boreal to temperate to tropical forests (Raich and Schlesinger, 1992; Subke et al., 2006). However, within

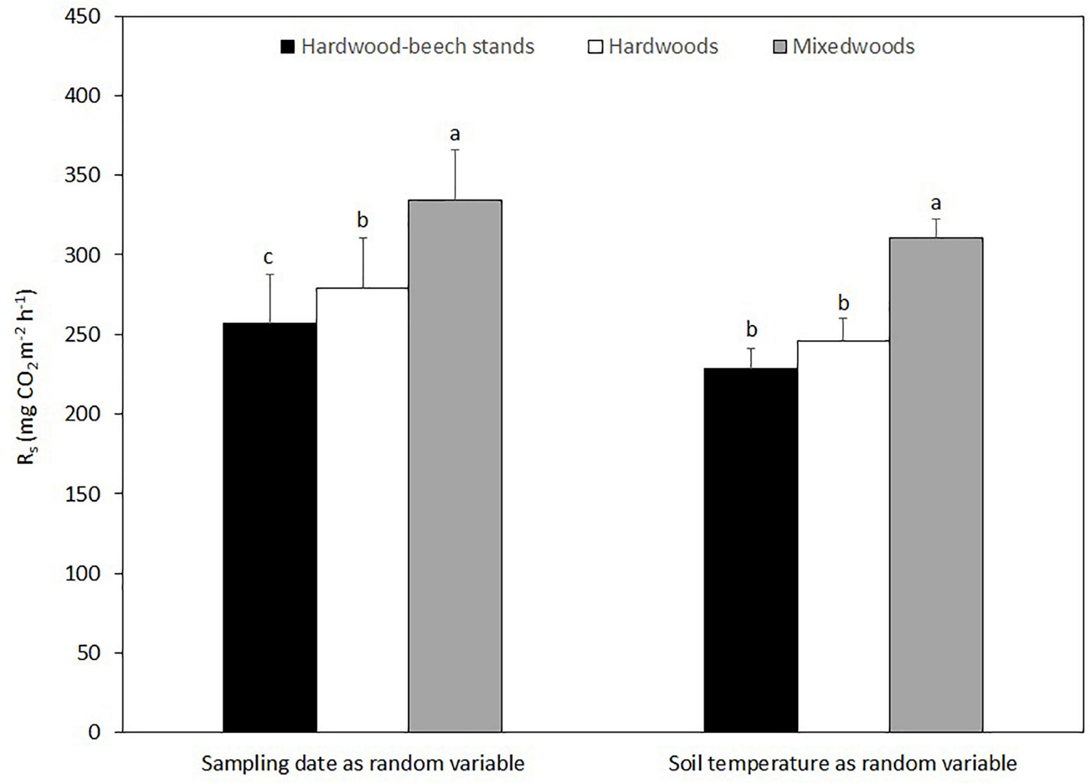


FIGURE 4 | Mean soil respiration rates (R_S) predicted from the linear mixed effect models for each forest type using sampling date and soil temperature as the random variables. Predicted values were back-transformed as a square root transformation was used in both models to assure normality of the residuals. Standard errors are shown. Different letters indicate a significant difference at $P < 0.01$ between forest types using the Tukey's honest significant difference test.

TABLE 5 | Root (R_{root}) and heterotrophic (R_h) respiration fluxes from the root exclusion plots in summer 2020.

Forest type	**Respiration flux (mg CO_2 m^{-2} y^{-1})**		**Contribution (%) to R_s**		
	R_h	**R_{root}**	**R_h**	**Rroot**	**S.E.**
Hardwood-beech stands	296 (± 35.7)	103 (± 27.6)b	74.3a	25.7b	5.56
Hardwoods	331 (± 1.76)	84.5 (± 30.3)b	80.1a	19.9b	5.75
Mixedwoods	262 (± 24.2)	282 (± 25.7)a	48.2b	51.8a	2.26

Contributions of both components to total soil respiration were also calculated. Estimates are means and standard errors (in parentheses or under S.E.) of the four sampling dates (June 15, June 30, July 21, and August 11). N.B. The autotrophic component does not account for rhizosphere respiration and thus, it is described as root respiration (R_{root}). This means that autotrophic respiration is underestimated and R_h is overestimated. Different letters indicate a significant difference at P < 0.01 between forest types using one-way ANOVA.

a constrained geographical location, an increasing abundance in conifers can also modify the soil system to conditions (e.g., lower temperatures, pH and nutrient availability) that are less suitable for microbial degradation of soil organic matter and leaf litter (Binkley and Giardina, 1998; Binkley and Fisher, 2012; Prescott and Grayston, 2013; Joly et al., 2017). Such conditions would be expected to lower R_h, i.e., a significant component of R_s (25–35%) in temperate deciduous forests (Bowden et al., 1993; Cisneros-Dozal et al., 2007). Therefore, in a side-by-side study of deciduous and coniferous stands with similar photosynthetic rates, it would seem reasonable to find higher R_s under deciduous stands because of the greater organic matter turnover (Raich and Tufekcioglu, 2000; Curiel Yuste et al., 2004; Fahey et al., 2005). At SBL, Collin et al. (2017, 2018) observed more acidic and nutrient-poor soils under mixedwoods and conifer dominated forests than hardwoods and hardwood-beech stands. Furthermore, in a study of four sites in the sugar maple-basswood (*Tilia Americana*) and sugar maple-yellow birch domains of Quebec, including SBL, Bélanger et al. (2019) found that decomposition rates of sugar maple leaf litter were slower under pure coniferous stands than under hardwood and hardwood-beech stands, and that decomposition rates under mixedwoods were intermediate. We thus hypothesized that the slower leaf litter decomposition in mixedwoods at SBL would lead to lower R_s than hardwoods, but consistently higher R_s under mixedwoods compared to the other forest types contradicts this hypothesis.

In a similar cool temperate climate in Korea, Lee et al. (2010) measured lower R_s under deciduous forests than coniferous forests. They explained that there were greater photosynthetic constraints on leaves of deciduous trees. In turn, this led to limited photosynthate transport and lower root activity and R_a. We do not have reliable dendrochronological data to infer differences in productivity among tree species and forest types at SBL. However, biomass data provided by Maliondo et al. (1990) suggest that balsam fir and white spruce stands produce equivalent or more biomass annually than sugar and red maple stands in northern temperate deciduous forests in eastern Canada. In this respect, mixedwoods at SBL, with balsam fir as the main coniferous species, are expected to perform very well. Conversely, growing conditions are sub-optimal for maple spp. and beech at SBL as they develop near their northern range limit and are supported by acidic and N-poor soils (Collin et al., 2017, 2018). We thus argue that R_s in mixedwood plots were balanced by a greater contribution of R_a relative to hardwoods and hardwood-beech stands. This is well supported by our partitioning analysis of R_{root} and R_h. Despite colder and drier soils under mixedwoods, R_{root} was substantially and significantly higher (about threefold) in mixedwoods than in the other forest types studied. We also estimated that R_{root} in mixedwoods was about 50% of R_s compared to only 20 and 25% in hardwoods and hardwood-beech stands, respectively. Raich and Schlesinger (1992) suggested that correlation between ecosystem productivity and R_s was largely due to a regional/continental effect of climate, whereas Valentini et al. (2000) argued that ecosystem productivity can overshadow the influence of climatic variables on R_s at large spatial scales. Similarly, Reichstein et al. (2003) were able to partition the contributions of climate and vegetation types on R_s, but this was over an array of sites in Europe and America that encompassed a large gradient in soil microclimate and vegetation productivity. In our study, a dummy variable that characterized the mixedwood plots (0) (vs. hardwood-beech and hardwood plots 1) was the first predictor variable in a multiple linear regression of the residuals produced from the exponential model between R_s and soil temperature. We were thus able to separate the large temperature effect on R_s from the vegetation effect reflecting differences in productivity between mixedwoods and deciduous forests at a very small spatial scale.

Raich and Potter (1995) and Fernández-Alonso et al. (2018) reported no difference in R_s between deciduous and coniferous forests. In a Mediterranean ecotone forest of central Spain, Fernández-Alonso et al. (2018) partitioned the contribution of R_a and R_h to R_s and found that deciduous stands had a lower R_h and a greater R_a than coniferous forests, but R_s rates were similar between the two forests. This is an opposite scenario to the one at SBL where fir, not maple spp. or beech, produced higher R_s due to higher R_a. As discussed above, this finding is reasonable given that growing conditions at SBL are more suitable for fir than sugar maple and beech. It thus appear that patterns in R_s between deciduous and coniferous forests under similar growth conditions cannot be easily generalized and that further side-by-side studies are needed to fully elucidate the factors, notably soils and climate, that govern differences in R_s between trees with diverging phylogenetics and physiology.

Influence of Hardwood-Beech Stands on R_s

Based on data extracted from Gosz et al. (1973); Melillo et al. (1982), Côté and Fyles (1994), and Moore et al. (1999), rates of American beech leaf litter decomposition are estimated to fall toward the lower end of the species present at the study sites: birch spp. ≥ red maple, aspen spp. ≥ sugar maple, spruce spp. > white pine > balsam fir, American beech > eastern white cedar. Our results are consistent with this sequence because the R_h component is generally lower when leaf litter at SBL is enriched with beech and fir. However, due to the small sample and variability in the data, differences were not significant. In this respect, the poor quality and low decomposability of beech litter can be associated only in part to the significantly lower R_s under hardwood-beech stands compared to hardwoods where there is no significant addition of beech litter.

Collin et al. (2017, 2018) measured low canopy openness and light transmission under hardwood-beech stands compared to hardwoods and mixedwoods at SBL. For example, canopy openness in July was as low as 10.7% in hardwood-beech stands compared to 23.8 and 30.6% in hardwoods and mixedwoods, respectively. This resulted in light transmission of < 5 mol m^{-2} d^{-1} at the soil surface under hardwood-beech stands and > 10 mol m^{-2} d^{-1} under the other forest types. Castin-Buchet and Andre (1998) and Kunhamu et al. (2009) suggested that low light transmission can supress microfaunal and microbial activity in the leaf litter by negatively affecting immediate air temperature and relative humidity. At SBL, this is corroborated by significantly lower soil temperature and higher soil water potential in hardwood-beech stands than in other forest types. Differences in soil water potential also appeared to increase during drier periods. In this respect, the lower light transmission in these stands during the full leaf period likely limited overheating of the understory vegetation and soils and in turn, reduced water losses from evapotranspiration. In addition, two results at SBL suggest that differences in R_s were due to the lower soil temperature and/or a different response to changes in soil temperature (i.e., greater sensitivity) under hardwood-beech stands compared to other forest types: (1) mixed model analysis with soil temperature as a random variable detected no difference in R_s between hardwoods and hardwood-beech stands, and (2) the Q_{10} value produced with data from hardwood-beech stands (2.97) was quite lower than Q_{10} values produced for hardwoods (2.26) and mixedwoods (2.44). We conclude that low light transmission at the soil surface and low surface soil temperature during the full leaf period were acting in combination with the recalcitrant beech leaf litter in hardwood-beech stands to suppress R_h and R_s relative to hardwoods without beech or with only small proportions of the species in the canopy.

Annual soil respiration rates under four stands dominated by American beech at Hubbard Brook, New Hampshire, were estimated at 629 g C m^{-2} y^{-1} compared to 728 g C m^{-2} y^{-1} for three stands dominated by either sugar maple or white birch (Fahey et al., 2005). Although there are some differences in the approach to classify the stands between studies, forests at SBL and Hubbard Brook are quite similar in composition and grow on acidic soils. The main difference is that beech is at approximately 250 km south of its distribution at Hubbard Brook, whereas it is very near its northern limit at SBL. Thus, there are likely similar mechanisms associated with the presence of beech that are decreasing R_s rates relative to the other forest types at these sites.

Influence of Soil Water and Magnesium on R_s

Soil water potential was also an important effect on R_s at SBL as it was the second predictor variable in the multiple regression model. The inclusion of soil water potential in the model is consistent with previous findings. Soil temperature, relative to soil moisture, generally exerts a more consistent control on R_s during the growing season in temperate and boreal forests (Maier and Kress, 2000; Gough et al., 2005; Kumpu et al., 2018). However, soil moisture can become an important control of R_s during dry periods, namely by affecting the decomposition of leaf litter and thus R_h (Epron et al., 1999; Fang and Moncrieff, 2001; Cisneros-Dozal et al., 2007; Vogel et al., 2013; Santonja et al., 2015). In 2020, there were two heatwaves recorded before June 21 and the second one affected southern Quebec and SBL for more than 7 days. This is the first time that two heatwaves were recorded to hit Quebec before the summer solstice. An exceptional drought accompanied these heatwaves, with 70% less rain than normal in June. A shorter heatwave hit Quebec in mid-July and then conditions cooled off and precipitation was normal thereafter. This series of events led to unusually high soil temperature and water potential in June, only about 1 month after complete snow melt at the site, and likely explain the inclusion of an indicator of soil water availability in the multiple regression model.

Soil nutrient availability effects on R_s also appeared important at SBL as Mg^{2+} activity in the soil solution was selected as the third predictor variable in the multiple regression model. More specifically, Mg^{2+} activity in the soil solution may reflect the benefits of an improved soil acid-base status on decomposer biota and activity (Blagodatskaya and Anderson, 1998; Bååth and Anderson, 2003; Han et al., 2008) under hardwoods. Indeed, 4 years of PRS data (2017–2020) in the plots at SBL suggest significantly higher Ca^{2+} and Mg^{2+} activities under hardwoods than under hardwood-beech stands and mixedwoods (Bélanger, unpublished data). Interestingly, soil solution Ca^{2+} activity, another indicator of alkalinity, was selected as the fourth predictor variable in the model (results not shown). The higher activity of these base cations in the soil solution in hardwoods are likely associated with increased biocycling, especially by the abundant *Betula* spp. in these plots (**Table 1**, Bélanger et al., 2004).

CONCLUSION

We assessed R_s within a sugar maple forest near the northern limit of deciduous temperate forests and where the abundance of conifers and American beech varied. Seasonal variations in soil temperature exerted the largest influence on R_s in these stands, but the results also illustrate how admixtures of balsam fir and beech are affecting R_s in opposite directions. The admixture of fir consistently yielded the highest R_s, whereas the admixture of beech yielded the lowest R_s. The larger R_s in the presence of fir is associated to increased photosynthate transport and root activity, whereas the lower R_s in the presence of beech is due to the creation of a soil microclimate favored by the dense canopy as well as the poor litter quality (low decomposability). Other factors explaining the variation in R_s include low soil moisture during subsequent heatwaves and an improved soil acid-base status due to biocycling by birch trees. Sugar maple was used in this study as the “control” species, but understanding the effects of a change in tree species composition on R_s is relevant to all biogeographic contexts. Such studies will help to better predict future temporal and spatial changes in the biogeochemistry of forest ecosystems, including C pools and atmospheric CO_2 concentrations, and their changing trajectories in terms of species composition under climate change (Lafleur et al., 2010; Ettinger and HilleRisLambers, 2013).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

NB conceived and designed the experiment and drafted the manuscript as the lead author. AC, RK, and SL-D acquired the data and revised and edited the manuscript. NB and AC analyzed the data. All authors contributed to the interpretation of the findings.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.664584/full#supplementary-material

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Pedogenic Threshold in Acidity Explains Context-Dependent Tree Species Effects on Soil Carbon

Ellen Desie[1]*, *Bart Muys*[1], *Boris Jansen*[2], *Lars Vesterdal*[3] *and Karen Vancampenhout*[1]

[1] Department of Earth and Environmental Sciences, KU Leuven, Leuven, Belgium, [2] Department of Ecosystem and Landscape Dynamics, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, Netherlands, [3] Department of Geosciences and Natural Resource Management, University of Copenhagen, Frederiksberg, Denmark

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***Correspondence:**
Ellen Desie
ellen.desie@kuleuven.be

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Despite the general agreement that maximizing carbon storage and its persistence in forest soils are top priorities in the context of climate change mitigation, our knowledge on how to steer soil organic carbon (SOC) through forest management remains limited. For some soils, tree species selection based on litter quality has been shown a powerful measure to boost SOC stocks and stability, whereas on other locations similar efforts result in insignificant or even opposite effects. A better understanding of which mechanisms underpin such context-dependency is needed in order to focus and prioritize management efforts for carbon sequestration. Here we discuss the key role of acid buffering mechanisms in belowground ecosystem functioning and how threshold behavior in soil pH mediates tree species effects on carbon cycling. For most forests around the world, the threshold between the exchange buffer and the aluminum buffer around a pH-H_2O of 4.5 is of particular relevance. When a shift between these buffer domains occurs, it triggers changes in multiple compartments in the soil, ultimately altering the way carbon is incorporated and transformed. Moreover, the impact of such a shift can be amplified by feedback loops between tree species, soil biota and cation exchange capacity (CEC). Hence, taking into account non-linearities related to acidity will allow more accurate predictions on the size and direction of the effect of litter quality changes on the way soil organic carbon is stored in forest soils. Consequently, this will allow developing more efficient, context-explicit management strategies to optimize SOC stocks and their stability.

Keywords: SOC, tree species effect, litter quality, soil acidity, soil process domain, acid buffering, CEC, context-dependency

INTRODUCTION

The potential of forests, and particularly the soils beneath them, in combating climate change is increasingly recognized in science (Bastin et al., 2019), policy (European Commission [EC], 2019), and management (Mayer et al., 2020). Boosting soil organic matter (SOC) in forest soils not only mitigates the continued increase of atmospheric CO_2 concentrations, but also provides associated benefits, including increased soil fertility, productivity, water holding capacity and therefore better adaptation in the face of climate change (Tiessen et al., 1994; Minasny et al., 2017). Tree species selection has been identified as a promising avenue to steer carbon cycling by the forester (Prescott and Vesterdal, 2013). A considerable body of literature has addressed the link between overstory tree species and functioning of the forest soil, in terms of productivity, nutrient availability, biological activity and soil carbon cycling (Finzi et al., 1998; Augusto et al., 2002; Reich et al., 2005; Mueller et al., 2015; Schelfhout et al., 2017). However, the reported effects of litter quality on belowground functioning often vary in magnitude or even in direction (Ehrenfeld et al., 2005). Accordingly many papers conclude that litter effects are site- or context-dependent (Eviner and Hawkes, 2008; Kooijman and Martinez-Hernandez, 2009). To reach the full potential that is predicted in terms of boosting soil carbon stocks via forest management, it is therefore crucial to understand what drives this context-dependency (Jandl et al., 2007; Jackson et al., 2017; Solly et al., 2020). Or, as Prescott and Vesterdal, 2013 emphasize, the question is no longer "what is the effect?", but "under which conditions is this effect happening"?

Context-dependency was anchored in soil carbon research by Schmidt et al. (2011), who argued that carbon persistence in soils is an ecosystem property. Lehmann et al. (2020) moreover highlighted the role of substrate complexity in soil carbon cycles, but the nexus between litter quality, the edaphic context and soil carbon decomposition and stabilization to this date remains poorly explored. Previous studies have already indicated the role of soil pH (Vedy, 1973; Beck et al., 1969; Gurmesa et al., 2013; Solly et al., 2020), texture (Vesterdal and Raulund-Rasmussen, 1998; Angst et al., 2018b; Desie et al., 2020c), cation exchange capacity (CEC) (Rasmussen et al., 2018; Desie et al., 2019), soil type (Kögel-Knabner and Amelung, 2021), and parent material (Heckman et al., 2009; Angst et al., 2018a) on various aspects of soil organic matter dynamics, but integration of these variables into an overall conceptual model remains elusive. Moreover, a complex system approach to ecosystem science (sensu Messier et al., 2013) is needed to recognize tipping points or non-linearity in a system's behavior. Indeed, non-linearity has been identified as a key feature of ecosystems (Scheffer et al., 2001) and accordingly it is important in the success (or failure) of forest ecosystems as measures for global climate change mitigation.

In this concept letter, we explore the importance of a well-described pedogenic threshold in soil acidity at a pH-H_2O of ca. 4.5 and a base saturation (BS) of 30% for soil carbon = processes (**BOX1**). This threshold is known as the limit between the exchange buffer range ("acidic" or mesotrophic domain) and the aluminum buffer range ("acid" or oligotrophic domain) (Ulrich, 1991). Literature agrees that a shift from the exchange to the aluminum domain can result in multiple shifts in belowground functioning (Ulrich, 1991; Ponge, 2013). In this letter, we discuss how a change in tree species composition can trigger such a shift in forest floor and soil functioning and argue how that must ultimately also affect carbon dynamics in the soil, particularly the quantity, composition and stability of forest soil carbon can be affected. A change in overstory species composition has multiple effects on belowground ecosystem functioning, including a potential change in (leaf/root) litter input quantity and quality (Guo et al., 2005; Mueller et al., 2015), rooting patterns (Spielvogel et al., 2014; Cremer et al., 2016), microclimate (Joly et al., 2017), associated mycorrhiza (Heděnec et al., 2020), and etc. Here, we focus on the impact of leaf litter quality which has been identified as a strong driver of soil pH (van Breemen et al., 1997). Other indirect tree species effects were not in the scope of this concept letter, although remain key for understanding plant-soil interactions. Secondly, soil buffer mechanisms and ecological feedbacks can mitigate or amplify shifts in belowground functioning and thereby explain context dependent litter effects. This would also imply that there is a "window of opportunity" to steer carbon sequestration outcomes via the selection of tree species based on litter quality. We will discuss (i) how leaf litter-induced acidification can cause rapid changes in forest soils; (ii) how these changes in belowground functioning affect processes relevant to carbon cycling; and (iii) how context-dependency leads to a "window of opportunity" for steering carbon cycling through selection of tree species.

WHEN TREE SPECIES ACIDIFY THE SOIL, THAT CASCADES THROUGH BELOWGROUND FUNCTIONING

A change in overstory species composition that results in a decrease in litter quality, i.e., either characterized by a decrease in base cation content or an increase in C/N ratio, lignin, and cutin content (Cornwell et al., 2008), can lead to a litter-induced shift between the exchange and aluminum soil process domain and trigger sudden changes in soil geochemistry thereby altering the composition, diversity and functioning of biotic communities (**Figure 1**).

The composition of meso- and macrofaunal communities is one of the first biotic compartments to react. The activity of burrowing macro-fauna such as endogeic and anecic earthworm species is reduced below a pH of ca. 4.5 (De Wandeler et al., 2016; Schelfhout et al., 2017) with the exception of acid tolerant ants and termites (Taylor et al., 2019). Smaller mesofauna such as enchytraeids, collembola and mites become more dominant (Briones, 2014; Korboulewsky et al., 2016), leading to reduced soil bioturbation and a vertical decoupling of organic matter and nutrient cycles in the soil (Muys et al., 1992; Ponge et al., 2010). This provokes a clear spatial disconnection between microbial communities in the litter layer and underlying mineral soil layers: most of the microbial catabolic potential is concentrated in the litter layer when the soil further acidifies (Desie et al., 2019). Not only the location of dominance, but also the species composition of decomposers shifts below a pH of ca. 4.5: fungi become dominant over bacteria (Blagodatskaya and Anderson, 1998; Phillips et al., 2013; Heděnec et al., 2020) and the diversity of bacterial communities decreases (Fierer and Jackson, 2006). This also leads to more pronounced substrate-decomposer interactions (Palozzi and Lindo, 2018), e.g., there is an increased cooperation between plants and nutrient mining symbionts, which are often species specific (Eastwood et al., 2011). Corresponding with aboveground

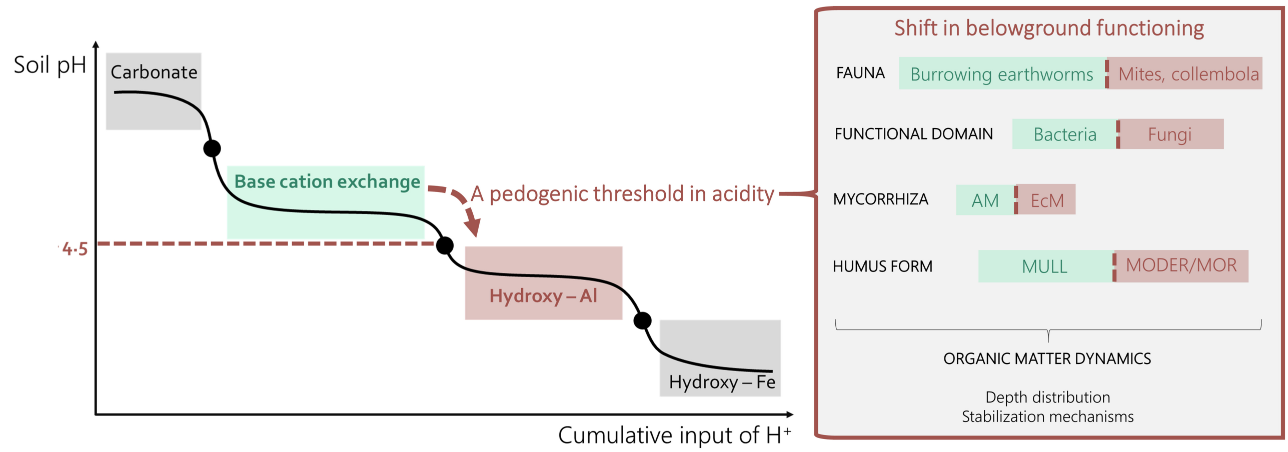

FIGURE 1 | Left: Soil pH-H_2O is buffered by several subsequent mechanisms. These soil process domains are interchanged by pedogenic thresholds (**BOX1**). **Right:** A shift from the exchange domain to the aluminum domain cascades through belowground functioning and ultimately affects SOC dynamics.

changes in species composition, the mycorrhizal associations may change: Ectomycorrhiza (ECM) are typically more related to tree species with low quality litter and more acidic conditions whereas arbuscular mycorrhiza (AM) depend on saprotrophic microbes for nutrient release and are therefore associated with base cation-rich, fast decaying litter and higher soil pH (that allow bacterial communities to thrive) (Phillips et al., 2013; Hedĕnec et al., 2020; Peng et al., 2020). Overall, when the soil shifts to the Al buffer domain, ecological strategies focus more on mitigating nutrient limitations (Wardle et al., 2004; Ribbons et al., 2018) mirrored by a more "organic based nutrient economy" instead of a mineral nutrient economy (Lin et al., 2017). Moreover, the aboveground shift in tree species can also correspond with changes in canopy and rooting characteristics that further impact the belowground ecosystem and amplify or mitigate other litter effects (Spielvogel et al., 2014; Joly et al., 2017).

. . . AND ULTIMATELY AFFECTS SOC DYNAMICS

Both the input of low quality litter and the changed environmental conditions (low pH, high Al, low base cations availability, in addition to microclimatic conditions) change the community of decomposers leading to a slower decomposition pathway and the accumulation of organic matter on the soil surface (Blagodatskaya and Anderson, 1998). Therefore, we argue that this abiotic shift in soil process domain and the following chain in biotic reactions eventually translates in a change in soil carbon dynamics, i.e., a change in the vertical distribution of SOC, its chemical composition and its stability.

The most visible and widely studied result of all above changes in faunal and microbial life in the soil is a **change in the forest floor** or litter layer. Most studies agree that forest floors become thicker when litter quality decreases (Toutain, 1981). Moreover the litter layer also changes functionally with a shift in soil process domain: when pH drops below 5, moder and mor humus forms become dominant and mull humus forms no longer occur (Ponge et al., 2010), indicating that humus form would already be a good indicator of carbon cycling (Andreetta et al., 2011).

The accumulation on top of the soil profile and the reduced activity of bioturbators leads to a **different vertical distribution** of carbon within in the soil profile as a consequence of a shift in soil process domain (Vesterdal et al., 2013). In accordance with the accumulation in the forest floor, many studies report an increase in topsoil total carbon stocks with a change to lower litter quality (Vesterdal et al., 2008; Boča et al., 2014; Augusto et al., 2015). For deeper soil layers, the results are less clear, yet some studies report a decrease in subsoil carbon stocks, although more subtle, as bioturbation and organic matter incorporation are inhibited (Oostra et al., 2006; Mareschal et al., 2010; Frouz et al., 2013). Subsoil carbon stocks are probably relatively more influenced by other tree species effects (e.g., rooting patterns and root litter and exudates) and soil texture (vertical migration of DOC) (Rumpel and Kögel-Knabner, 2011).

Moreover, the change in decomposer community causes a **change in the chemical composition** as lignin is favorably decomposed by a dominantly fungal community (Vancampenhout et al., 2009; Brock et al., 2019). Concurrently, interactions between different types of organic molecules in the soil may also change. Typically, the addition of high-quality litter leads to increased decomposition of low-quality litter in non-acid soils (positive priming), while negative apparent priming is observed in acid systems (the most advantageous food source is processed first) (Heitkötter et al., 2017; Zhou et al., 2021).

Finally, also the **stability of stored carbon is altered** when the pH drops below ca. 4.5. First of all, the vertical distribution of carbon will affect its persistence as carbon in the forest floor and topsoil layers is more exposed to environmental disturbances, such as increased microbial decomposition because of higher soil temperatures in canopy gaps or direct oxidation by fire (von Lützow et al., 2006; Jandl et al., 2007). Secondly, we argue that with this change in biochemical conditions and the players present in the process, also the stabilization

BOX 1 | Pedogenic thresholds in soil acidity.

Acidity has long been recognized as a key factor of belowground forest ecosystem functioning (Ulrich, 1991), linked to differences in the biotic compartment for a range of soils (Ponge, 2003). Moreover, it is one of the best described edaphic properties prone to tipping points in soil geochemistry (Ulrich, 1987) and thereby a good example of non-linearity in belowground ecosystem functioning. Most of the time, a temporary increase in H^+ ion production or exogenic input has little or no effect, as these protons are neutralized by a range of soil buffer mechanisms. These include dissolution of carbonates between pH-H_2O 8.6 and 6.2, weathering of silicates between pH-H_2O 6.2 and 5.0, mainly exchange of base cations between pH-H_2O 5.0 and 4.5 and Al-hydroxides below pH-H_2O ca. 4.5 (Ulrich, 1991). When a certain buffer is exhausted, pH drops suddenly in response to a small additional increase in acidity. These steep intervals in soil pH response curve are termed pedogenic thresholds (Chadwick and Chorover, 2001). As most forests around the world are characterized by low pH values (**Supplementary Figure 1**), many of them might be very close to the threshold value of 4.5 (Slessarev et al., 2016). Hence, understanding the effect of overstory species around that threshold is of particular relevance for forest carbon cycles around the globe.

mechanisms within the mineral soil are altered. Microbial activity is inhibited by low pH values and thus there is reduced microbial entombing for SOC stabilization (Cotrufo et al., 2013; Liang et al., 2017; Angst et al., 2021). The absence of burrowing macro- and mesofauna moreover leads to reduced aggregate stabilization and reduced organo-mineral interactions (Briones, 2014), resulting in less carbon stored in mineral associated fractions and more in large particulate organic matter (POM) (Laganière et al., 2011; Angst et al., 2018b; Desie et al., 2019; Giannetta et al., 2019). Chemical recalcitrance and metal-humus complexation become the main SOC stabilization mechanisms at play (Heckman et al., 2009; Clarholm and Skyllberg, 2013). Corresponding with this, Hobbie et al. (2007) reported greater impact of acidic hydrolyzing cations (Al and Fe) on the exchange complex for carbon stabilization in mineral soil layers via cation bridging and flocculation and indirectly by inhibiting microbial activity (due to the low pH and high Al). Hence, organic matter decomposition becomes nutrient driven rather than energy driven (Grime et al., 1988; Camenzind et al., 2018; Wiesmeier et al., 2019). Altogether, these studies illustrate that with a change in soil process domain (when the pH drops below ca. 4.5) the carbon cycle is altered. Although carbon sequestration by accumulation in the forest floor may increase, the mechanisms controlling SOC stabilization shift as well and the stability of the carbon stored in the soil decreases.

Still, the impact of a pedogenic threshold in terms of **total carbon stocks** is less clear: studies reporting differences in total carbon over the entire soil profile are inconsistent (Mayer et al., 2020). This can partly be explained by the longer timespan needed to alter total carbon stocks: decomposer communities react fast to changing conditions thereby more quickly resulting in changes in the forest floor and stabilization mechanisms, whereas in terms of total carbon stocks, legacies can mask the effect of changed conditions for a relative long time. That could also explain why the impact of litter quality on mineral soil carbon stocks is more pronounced in recently reclaimed mining sites, where legacy effects are small (Frouz et al., 2013). Also, carbon saturation and/or differences in root litter carbon input (that are relatively more pronounced in the mineral soil) may explain the absence of clear trends in total mineral soil carbon stocks per soil process domain. Finally, only few studies follow and integrate the continuum from forest floors to deep mineral soil layers (Thomas et al., 2021).

Incorporating the impact of acid buffering explicitly into soil carbon research could provide more insight in the mechanisms controlling organic matter cycles, including the effect on total mineral carbon stocks, and how to steer it by management. Along that line, Schmidt et al. (2011) emphasized that the way carbon is processed (and its persistence) does not merely depend on intrinsic properties of the input (e.g., the litter quality), but is to a great extent determined by the surrounding environment, i.e., it can be considered an ecosystem property. This corroborates findings of Duchaufour (1990) and Raulund-Rasmussen and Vejre (1995) that already stressed the important influence of the environment and edaphic factors on carbon cycling. In forest ecosystems in particular, the pervasive role of acidity is long recognized (Beck et al., 1969; Ulrich, 1991) and broadly applicable (**Figure 1**; **Supplementary Figure 1**; Slessarev et al., 2016). However, based on existing literature, it is hard to evaluate effects of tree species on soil carbon relative to the soil process domain they create, as few studies provide data on texture, soil pH and base saturation per tree species (or per reported effect). Also, the myriad of methods and setups to evaluate tree species effects makes meta-analysis challenging. Future studies of tree species effects on SOC should clearly describe and parameterize the context (including the dominating soil process domain) and evaluate tree species effects relative to this context. In that regard, reporting the soil type as a resulting structure of the processes at play might once again prove valuable (De Vos et al., 2015; Kögel-Knabner and Amelung, 2021). Nevertheless, it remains a challenge to find set-ups, e.g., multisite common garden experiments, that allow to evaluate tree species and context independently (Vesterdal et al., 2013).

A WINDOW OF OPPORTUNITY FOR MANAGEMENT

If and when a change in litter quality can induce the above described shift in soil process domain and the corresponding cascade in belowground functioning, depends on intrinsic soil properties and is simultaneously mitigated by mechanisms of mineral and biological nature (Ulrich, 1987; Ponge, 2013). Combined, these mechanisms underpin the context-dependency of litter effects on carbon cycling.

First, we discuss how the mechanism of mineral nature causes context-dependency and leads to a window of opportunity for management. As indicated by Slessarev et al. (2016), all climatic zones that are suitable for forest growth, typically characterized by a precipitation surplus that induces leaching of nutrients, move naturally toward the threshold between the exchange and the aluminum domain (**Figure 1** and **Supplementary Figure 1**). In this range, the shape of soil response curves to increased acidity essentially depend on two factors: (i) the total amount of negative charge on the exchange complex (i.e., CEC), and (ii) the relative proportion of basic cations (Ca^{2+}, Mg^{2+}, K^{+}, or Na^{+}) on that exchange complex that have already been replaced by H^{+} or Al^{3+} (i.e., the base saturation or, inverted, the exchangeable acidity) (Ulrich, 1991; Blume et al., 2016).

In soils with a very low CEC, e.g., extremely sandy soils low in organic matter or carbonates, there is very little exchange buffer capacity and pH will not exhibit strong threshold behavior because of the variable charge, which is dependent on the acid strength of specific organic matter functional groups (Schwertmann et al., 1987). The response curve therefore approaches an inverse logarithmic relation (**Figure 2A**). In these forest soils the potential to alter carbon processing via tree species selection is rather limited. Note that in this concept we assume CEC to remain constant over the entire soil profile. In practice, however, there are situations where CEC and BS vary with depth, which results in more complex results; e.g., in acidified topsoil with low CEC (**Figure 2A**), tree species can still induce a shift if base cations can be taken up from deeper unaffected layers. This added complexity when integrating depth was not included in our concept, however, it remains key in the understanding of belowground functioning and carbon cycles (Rumpel and Kögel-Knabner, 2011).

If the CEC buffer is larger, the replacement of basic cations by H^{+} or Al^{3+} will initially keep the pH almost constant upon addition of low quality litter (via tree species selection). When most of the base cations have been replaced (typically when the base saturation drops below 30%), the curve shows a clear threshold behavior, i.e., when the ecosystem shifts from the exchange domain to the aluminum domain (**Figure 2B**). In these zones the choice for a certain tree species can have high consequences in terms of acidity and carbon cycling.

Finally, if the CEC is very high (this limit was set at 24meq/100 g clay for agricultural soils (Driessen et al., 2001)), the response curve can fold back upon itself (sensu Scheffer et al., 2001; **Figure 2C**). This is when Al^{3+} saturation will induce increasing hysteresis as a consequence of the stronger sorption of Al on the CEC (Bolt and Bruggenwert, 1978). In this last case, the impact of changing litter quality – solely – is insufficient to shift the soil process domain and affect carbon processes.

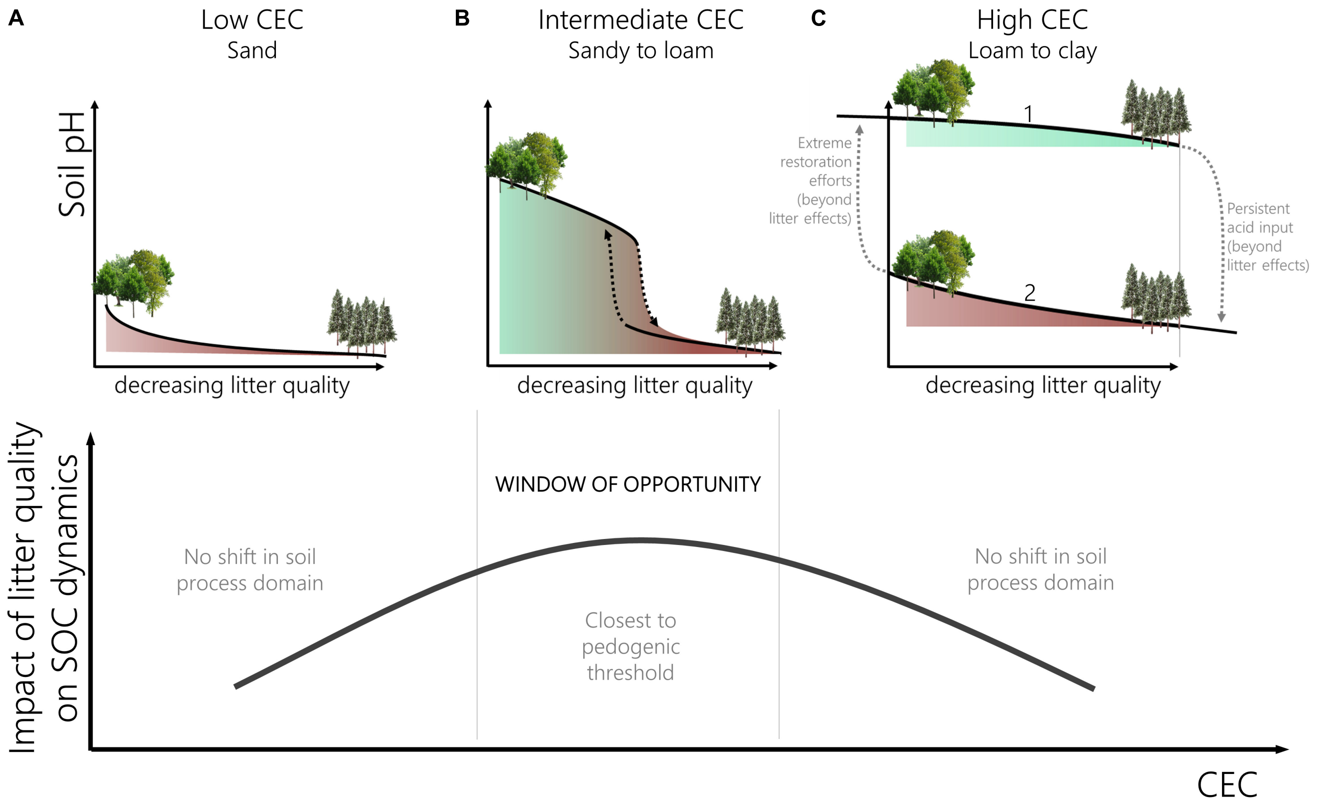

FIGURE 2 | Top: the response curve of soil pH to a change in litter quality (e.g., the input of acidity) differs depending on the context [here cation exchange capacity (CEC)]. The response can be reverse-exponential **(A)**, show threshold behavior **(B)** or indicate two alternative stable states **(C)**. A distinction can be made for the latter: soils with high CEC and high base saturation (BS) (C1) remain in the base exchange domain upon a decrease in litter quality whereas soils with high CEC and low BS (C2) remain in the *aluminum* domain even with rich litter input. Note that the CEC is assumed constant over the entire profile whereas in practice variability in CEC with depth can occur, which would lead to increased complexity. Bottom: the closer to a pedogenic threshold the higher the impact of tree species litter quality on SOC dynamics indicating a window of opportunity where management efforts to optimize carbon sequestration are most efficient.

A high CEC is therefore a mitigating property as long as the exchange buffer capacity has not been exhausted, but will become an amplifying factor once the system has tipped to the acid domain (Verstraeten et al., 2018; Desie et al., 2019). Translated to practice, **Figure 2C** illustrates that in some cases (soils with high CEC) it is crucial to avoid a shift in soil process domain and management efforts should focus on avoiding degradation because once acidified the potential to affect functioning (and carbon cycling) by litter quality alone is low. Simultaneously, management measures that aim to restore acidified soils or drastically alter the way carbon is processed should focus on soils with intermediate CEC's, as there the impact is largest (see window of opportunity below).

The potential of exchange capacity to drive context-dependent litter effects has been evidenced in previous research, often proxied by clay context (Angst et al., 2018b) or geology (Heckman et al., 2009). For example, Vesterdal and Raulund-Rasmussen (1998) already suggest larger impact of tree species effects on sandy soils, and also, van Oijen et al. (2005); Ribbons et al. (2018), Verstraeten et al. (2018), report more pronounced tree species effects in intermediate sites. Contrary, in sites that are extremely poor the impact of admixing rich litter (and thus tree species litter quality) remains limited (Desie et al., 2020c). This explains another dimension of context-dependent litter effects: the closer a system is to a pedogenic threshold, the more likely the shift will occur, the greater impact external drivers will have and multiple changes are triggered by only a small change in environmental forcing, e.g., a change in litter quality. In intermediately buffered soils, litter quality, hence, has most impact on the ecosystem state, whereas in soils with high contents of free carbonates the impact of litter quality is limited. This can also be seen in the presence of humus forms (Andreetta et al., 2016): in extremely poor sandy soils even under high quality litter tree species only moder and mor humus forms are found (Gurmesa et al., 2013), whereas on very Ca rich soils, we can even find mulls under oak (Andreetta et al., 2011). In between, there is a zone where tree species ultimately determine the dominant humus form, and the full range can be found: mull, moder, mor (Desie et al., 2020b). In these zones, the impact of litter quality on soil carbon sequestration would also be largest. We therefore argue that there is a **window of opportunity** where the impact of tree species selection is highest: depending on the edaphic setting, a shift in litter quality as represented by a shift in tree species can be a useful tool for the manager to restore a certain state (Desie et al., 2020c) but may lead to a very undesired situation if a shift to a lower state is induced (Desie et al., 2019). This knowledge can help harmonize management efforts and allows to develop more efficient, context-explicit management strategies to optimize SOC stocks and their stability.

Secondly, there are mechanisms of biological nature that can counteract a shift in soil process domain (Ulrich, 1987; Ponge, 2013). Ponge (2013) argues that humus forms can be seen as ecosystem strategies (e.g., mull the "dissipative pathway" versus mor the "conservative pathway") acting as basins of attraction to which ecosystems evolve and where plant-soil interactions (including the presence and functionality of the present biotic communities) maintain and even reinforce the state. An example of such a mechanism is the positive feedback loop between burrowing earthworms, humus form and soil pH that maintains the system in its present state: by promoting litter turnover rate, thereby increasing topsoil pH and creating suitable living conditions for themselves, burrowing earthworms promote their own abundance and activity (Desie et al., 2020a). However, as this feedback loop can reinforce litter effects, it can also amplify a negative (or positive) spiral when aboveground litter input changes and the mitigating mechanism again becomes the amplifying mechanism. The impact of such feedbacks is most important at intermediate pH values as this is where small changes induced by a certain species can differentially affect other species and trigger faster changes (**Figure 2**; Ehrenfeld et al., 2005). For example, the above discussed feedback loop is only active below a pH of 5, underpinning another driver of context-dependent litter effects.

These complex response curves imply that a change in litter quality may or may not have an immediate and strong effect on soil acidity and SOC dynamics: depending on the CEC, the remaining base saturation and the present biotic communities, the increase in acidifying litter may take shorter or longer to push a system over the threshold and alter the way carbon is processed. In the case of an alternative stable states curve (high CEC with high hysteresis, **Figure 2C**), legacy effects come into play: e.g., reducing the acid load and boosting the base cation input by planting rich litter species will not suffice to remove the large quantities of Al sorbed to the CEC (Desie et al., 2020c) and a shift in belowground carbon processing is not made. Acidity response curves may therefore be an interesting lens to build a conceptual framework for better understanding context-dependency in litter effects on belowground organic matter cycling in forest soils.

CONCLUSION

In this concept letter, we highlight how threshold behavior in soil acid buffering can drive context-dependency of litter effects on belowground ecosystem functioning to shape organic matter dynamics. Acidity is a key driver of forest ecosystem functioning, with clear non-linear relations between buffer domains. A shift in soil process domain in between the exchange and aluminum buffer domain (around a pH of 4.5), has pervasive impact on multiple compartments of the belowground ecosystem (e.g., abiotic conditions and soil biota communities) and will also change carbon dynamics. Particularly the quantity of carbon stored in the litter layer, the vertical distribution of SOC and its stability respond to a shift in soil buffer domain. The impact on total carbon stocks and SOC persistence will require more research. We suggest that studies of vegetation effects on organic matter dynamics should identify and explicitly discuss the context of the reported effects and parameterize it, if possible, based on key soil properties (e.g., soil texture, CEC, base saturation, exchangeable aluminum in addition to soil pH). Moreover, using acidity as the "lense" to study vegetation effects, i.e., the relative evaluation of soil process domain vs. tree species effects, would provide much needed insight into soil the CEC buffer is lar carbon dynamics. Finally, as a plus, the non-linearity associated with acidity also implies that we can target management efforts to where they will be most effective, i.e., in the window of opportunity where tree species selection has most impact on belowground functioning. This knowledge can help harmonize management efforts to optimize SOC stocks and their stability.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

ED, BM, and KV designed the concept. All authors contributed critically to the final manuscript

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.679813/full#supplementary-material

Supplementary Figure 1 | Left: world forest cover (FAO, 2010). **Right:** World soil pH map (IGBP-DIS Soils Data Task).

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Soil Organic Carbon Stocks in Mixed-Deciduous and Coniferous Forests in Austria

Robert Jandl[1]*, Thomas Ledermann[2], Georg Kindermann[2] and Peter Weiss[3]

[1] Austrian Forest Research Center BFW, Institute of Forest Ecology, Vienna, Austria, [2] Austrian Forest Research Center BFW, Institute of Silviculture, Vienna, Austria, [3] Environmental Agency Austria, Vienna, Austria

Question: We compared the soil organic carbon stock of the forests of an entire country. The objective of our research was establishing the differences between coniferous or deciduous forests with respect to soil carbon stocks. The question is relevant because coniferous forests are increasingly damaged by abiotic and biotic disturbances that are related to climate change. Deciduous forests are considered to be less vulnerable. Their soils are expected to be more persistent and reliable sinks for carbon dioxide.

Methods: Soil data are available from the Austrian Forest Soil Survey. Soils have been sampled on sites of the Austrian Forest Inventory. The data were stratified according to geology (calcareous vs. silicatic bedrock), orientation of the slopes, and forest type (coniferous vs. mixed-deciduous forest). These data were used to establish ground truth of soil organic carbon stocks. Further, we had simulation results of a coupled forest growth/soil carbon model. The scenarios built on the results of the Forest Inventory 2007/09 and reflect a business-as-usual forest management vs. a climate-change adaptation scenario where forest managers replace coniferous with deciduous forests if site conditions permit it. The simulations were performed with the forest growth simulator CÂLDIS and the soil carbon model Yasso07.

Results: Based on the Austrian Forest Soil Survey carbon stocks of coniferous forests were consistently higher than in mixed-deciduous forests. This result applies both for the organic litter layer and the mineral soil to a depth of 50 cm. The depth gradients of carbon were similar in both forest types. The simulation under a strong warming scenario showed an increase in the carbon stocks of soils when conifers are replaced by deciduous tree species. In the 150-year simulation the majority of forest sites will become suitable for deciduous forests. The build-up of a large soil organic carbon stock is driven by the stronger harvesting pressure on the remaining coniferous forests. Deciduous forests were in lesser demand and developed under a light forest intervention regime. However, toward the end of the century, when the temperature level is far above present levels, the soil organic carbon stocks declined.

Keywords: Austrian forest soil survey, coniferous forest, mixed-deciduous forest, soil organic carbon stock, climate change mitigation, climate change adaptation

Edited by:
Jeff Allen Hatten,
Oregon State University, United States

Reviewed by:
Nicole Wellbrock,
Thuenen Institute of Forest Ecology,
Germany
Jörg Luster,
Swiss Federal Institute for Forest,
Snow and Landscape Research
(WSL), Switzerland

***Correspondence:**
Robert Jandl
robert.jandl@bfw.gv.at

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1. INTRODUCTION

Organic carbon storage in forest soils is a potential contributor to climate change mitigation. Temperate cool forest soils store on average 120 t C/ha and are an important carbon sink (Post et al., 1982; Pan et al., 2011). Changing preferences for tree species as part of the adaptation of forest management to climate change can affect the size of the soil organic carbon stocks. Historically, many deciduous forests in Central Europe have been replaced with Norway spruce (*Picea abies*) stands in order to optimize productivity. The so-called "secondary Norway spruce stands" presently proof to be particularly vulnerable to biotic and abiotic disturbances and options of eventually replacing them with less vulnerable deciduous forests are investigated (Seidl et al., 2017; Hlásny et al., 2019; Jandl, 2020; Lindner et al., 2020; Mayer et al., 2020). Moreover, climate change is leading to shifts in the habitat of forest types and deciduous tree species are expected to move into higher elevation where forests are presently dominated by Norway spruce (Hanewinkel et al., 2012; Bircher et al., 2015; Lexer et al., 2015).

Soil organic carbon stocks are determined by several site factors and also by the amount of above- and belowground litterfall (forest productivity), rooting depth (allocation of organic matter), and decomposition rate of organic material (chemical quality). The overall effect of these three factors is complex (Liski et al., 2005; Andivia et al., 2016). Field observations give evidence for different soil organic carbon stocks of coniferous vs. deciduous forests. Under pine (*Pinus* sp.) and Norway spruce forests an organic layer of litter material builds up because needles are more recalcitrant toward decomposition than leaves. Under deciduous forests a shallower organic layer with a higher turnover rate forms (Achilles et al., 2020). Yet, deciduous trees tend to develop deeper rooting systems, thereby supplying the subsoil with organic matter (deB Richter jr. and Markewitz, 2001; Berger et al., 2002, 2006; Schmid and Kazda, 2002; Rehschuh et al., 2021). The effect of storing organic carbon in the litter layer of coniferous forests could be partially compensated by the deeper distribution of organic carbon in soils of deciduous forests. Evidence for the dominant effect of root-derived carbon for the formation of stable soil C has been provided (Rasse et al., 2005). The relevance of subsoil organic carbon has been often stressed and it has been pointed out that considerable quantities of organic carbon are encountered at depths that are often not captured by soil surveys that are confined to the organic litter layer and the uppermost part of the mineral soil (Harrison et al., 2011; deB. Richter and Billings, 2015).

Much research has been conducted on the organic carbon stocks in European forest soils. Differences between soil types have been identified and the relative importance of tree species was high in the litter layer, and of lesser relevance for the organic carbon stock in the mineral soil (De Vos et al., 2015). A detailed analysis of German forest soils showed significantly higher carbon stocks in the litter layer of spruce forests, as compared to beech (*Fagus sylvatica*). In the mineral soil the difference was statistically not significant (Grüneberg et al., 2014, 2019). The findings are supported by the comprehensive analysis of Swiss soil data that have been presented for the litter layer and the mineral soil to a depth of 1.2 m (Gosheva et al., 2017). Large-scale studies of tree species effects on soil carbon stocks in Central Europe are scarce. Comparisons on a national scale are hampered by the fact that forest management strategies are overriding the effect of natural site factors. Whether a site is presently stocked with coniferous or deciduous forests only partially reflects site conditions and eventually limiting ecological factors, but rather reflects the choice of forest managers favoring a particular forest type.

Some information on the impact of tree species on soil carbon stocks is available from common garden experiments. A comparison conducted in Southern Sweden showed significantly more carbon under Norway spruce than under beech in the organic layer and the upper 20 cm of the mineral soil (Oostra et al., 2006). Six common gardens in Denmark supported the findings for the litter layer. In the upper 30 cm of the mineral soil no differences were found (Vesterdal et al., 2008, 2013).

Stand conversion experiments provide further evidence for smaller organic carbon stocks in the organic layer under deciduous forests as compared to coniferous forests. Converting pine forests to beech reduces the thickness of the organic layer and the signature of Norway spruce on the chemical quality of the organic layer is evident (Fischer et al., 2002; Prietzel and Bachmann, 2012). Establishing coniferous forests on abandoned agricultural land leads to a buildup of the organic layer (Hager, 1988; Strohschneider, 1991; Markewitz et al., 2002; Smal et al., 2019).

There is a clear indication that soils of deciduous forests have consistently lower soil organic carbon stocks than soils of coniferous forests (Osei et al., 2021). Deciduous forests are favored over coniferous forests mostly for the sake of stand stability and resilience toward expected climate change effects. Changing the tree species composition has ecological and economic consequences for the forestry sector. The price to be paid for presumingly more stable forests is high. The technology of the forest product chain needs to be adapted and the timber production of deciduous forests and mixed-species forests is substantially lower than the productivity of Norway spruce forests. Based on existing knowledge it is not clear yet, whether forest managers are better off when accepting a higher risk of climate-change related damages of coniferous forests or when they are opting for less productive yet more stable deciduous forests (Braun et al., 2016; Paul et al., 2019; Knoke et al., 2020).

In our analysis we investigate the evidence for differences in soil organic carbon stocks under coniferous vs. mixed-deciduous forests of Austria. We investigate whether the differences that are reported from case studies are evident in our large-scale dataset from a national forest soil survey that presumingly covers a wider range of site conditions than silvicultural experiments. Secondly, we evaluate three scenarios of a forest simulation experiment. Other than a case study or a common garden experiment, the simulation reflects the response of soil organic carbon to a combined effect of climate change, forest management, and the response of the market to an altered supply of timber. In our simulation we changed the tree species composition according to

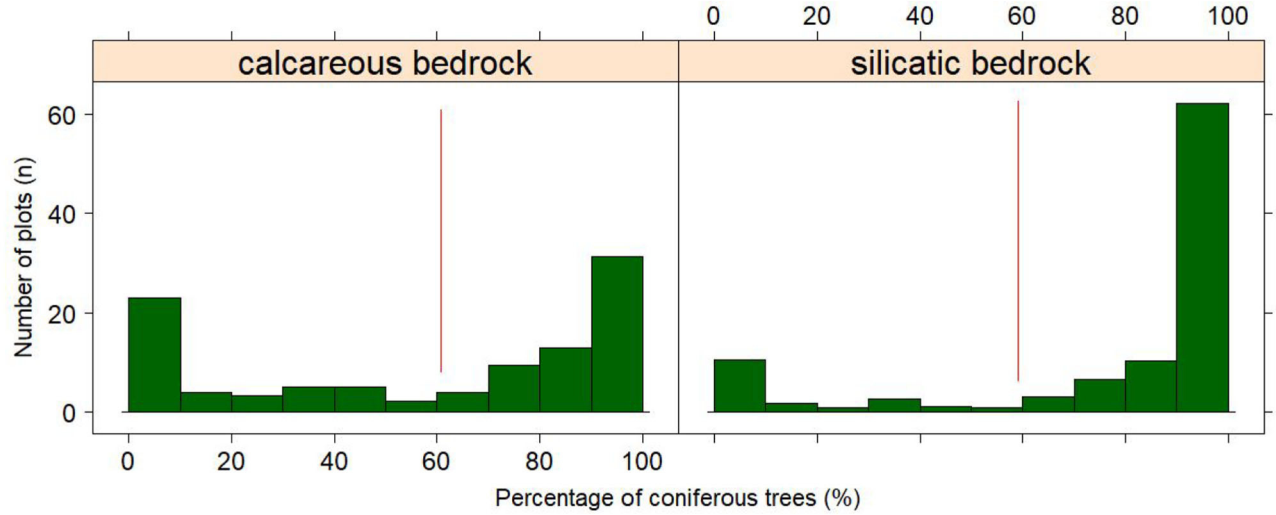

FIGURE 1 | Distribution of tree species combinations in Austria on calcareous vs. silicatic bedrock. The vertical red reference lines indicate the pragmatically defined threshold between mixed-deciduous and coniferous forests.

arising opportunities after final harvests. In order to easier detect the consequences of changes in tree species a simulation period of 150 years was chosen.

2. METHODS

2.1. Characteristics of Austrian Forests

According to the Austrian Forest Inventory 2016/18 the country has a forest cover of 4.02 *Mio* ha or almost 50% of the surface, an average standing stock of stemwood of 351 m^3, and an average productivity of 8.9 m^3 ha^{-1}. Presently, coniferous tree species comprise 61.4% of the forest area [Norway spruce 49.2%, pine 5%, European larch (*Larix decidua*) 4.4%, Silver fir (*Abies alba*) 2.5%, and others], and deciduous trees 24.5% [European beech 10.2%, oak (*Quercus* sp.) 2.1%, and others]. The remaining 14.4% of the surface are legally forest land that is currently not forested. Typically, these are open meadows, forest roads, or timber manipulation areas (BFW, 2021). The economically most relevant trees species is Norway spruce. It is managed with high efficiency and the forest value chain is adapted to a continuous supply of spruce timber. The forest-based sector of the economy contributes 1.9% to the gross domestic product. This value is twice the European average (Schwarzbauer, 2018). Yet, the figure is declining due to a continuous shift toward the tertiary sector of the economy (Braun et al., 2020).

2.2. Austrian Forest Soil Survey

The Austrian Forest Soil Survey is placed on a regular grid with 8 km side length and comprises 526 plots. The soil sampling plots are a subset of the assessment grid of the Austrian Forest Inventory. Therefore, the available soil information is supported with detailed information on forest stands such as tree species composition, stand age, and standing stock. We also used the available site information on geological bedrock, altitude, and growth district. The advantage of the sampling design is the representativity for the entire country, the disadvantage is that rare soil or forest types are not necessarily well-captured.

Soils have been sampled and chemically analyzed according to ICP Forest standards (ICP, 2020). The soil organic carbon stock in the organic surface layer and the mineral soil to a depth of 50 cm was calculated based on the measured carbon concentration, the estimated bulk density from a nationally derived function, and the fine earth fraction (Foldal et al., 2021). The rock content has been visually estimated in the field. Some plots were removed from the dataset due to incomplete records. The available sample size for our analysis was finally 511 plots. Ancillary variables to soil carbon concentration and soil organic carbon stocks were altitude, geology (calcareous and silicatic bedrock, respectively), slope exposition, and forest type. Slope exposition was stratified into “East/West” (161 sites) for slopes facing to E, W, SE, SW, “South/even” (163 sites) for south-facing slopes and sites on even terrain, and “North” (190 sites) for N-, NW-, NE-facing slopes. With hindsight to the dominance of Norway spruce forests we defined the forest type as “coniferous” when conifers contributed more than 60% to the standing stock of stemwood, and otherwise “mixed-deciduous” (**Figure 1**). This split gave 136 mixed-deciduous forests and 375 coniferous forests. A further stratification was considered impractical due to small populations in some strata.

2.3. Simulation Experiment

In a simulation experiment we investigated the development of Austrian forests. We used two scenarios of climate change in order to capture a range of possible futures. In the period 1980–2010 the mean air temperature in Austria was 6.3 °C and the annual precipitation was 1,084 mm. The regionally downscaled climate change indicated a warming trend between 2 °C and 3.5 °C, depending whether a path of RCP 4.5 or RCP 8.5 will be followed (Jacob et al., 2014; Chimani et al., 2016). The RCP

4.5 scenario is an ambitious pathway that assumes the successful implementation of climate change mitigation measures, even though the Paris target of 1.5 °C is exceeded (Rogelj et al., 2018). RCP 8.5 is described as pessimistic scenario reflecting little success on curbing the annual emissions of greenhouse gases (Riahi et al., 2011). Both scenarios show only a slight change of the annual precipitation; a seasonal change in precipitation patterns is possible.

The climate data were used to run the forest growth model CÂLDIS. The model is based on the growth simulator PrognAUS that has been initially developed from data of the Austrian forest inventory period 1981/85 and was refined with new modules based on later inventory periods until 2007/09 (Monserud and Sterba, 1996). The single-tree model yields information on stem volume, stem diameter and height for every tree at annual time steps. In order to derive the input of organic matter to the soil we used the output of CÂLDIS. The simulation period started at year 2010 and was based on data of the Austrian Forest Inventory 2007/09 that represents the most recent complete dataset and reflects the current forest management (Ledermann et al., 2017). Forest practitioners have several options to cope with climate change. In the RCP 4.5 scenario we assumed that no adaptation is required. The RCP 8.5 scenario was used on one hand without changes of forest management and on the other hand with the gradual change of the tree species composition in order to replace increasingly vulnerable coniferous with deciduous forests. The scenarios are summarized below.

- RCP 4.5 Business-as-usual: The forests are experiencing a moderate warming. The 2 °C target of the Paris Agreement is not fully reached. Forest management follows the presently encountered path.
- RCP 8.5 Business-as-usual: The forests are experiencing an unprecedented warming trend. The mean annual temperatures will rise until the end of the century by ∼3.5 °C as compared to 2010 and will continue rising thereafter. No modification of the forest management plan is envisioned.
- RCP 8.5 Change of tree species from conifer-dominated to deciduous-dominated forests: The forests are experiencing an unprecedented warming trend. The forestry sector responds by changing the tree species composition.

The forest management scenario was based on the demand of the timber market that is reflected in the data of the Austrian Forest Inventory 2007/09. The future demand for timber was defined by an assumed economic development until the year 2150 that has been described previously (Braun et al., 2016). The required timber resources were supplied according to the demand of a business-as-usual scenario for the entire simulation period. We avoided assumptions on technological developments in timber processing in order to keep the simulation outputs overall sufficiently constrained.

In the model the option of changing of tree species was explored whenever a forest stand was harvested. The replacement of conifers with deciduous tree species followed a defined rule based on the expected mean annual temperature 50 years after the reforestation as provided by the respective climate scenario (**Table 1**). We opted for a simulation period beyond 2100 in order to ensure that most managed forests were harvested at least once.

TABLE 1 | Replacement of coniferous tree species based on the expected mean annual temperature in 50 years after reforestation.

Mean annual temperature 50 years after reforestation	Future tree species composition
< 6°	Coniferous tree species
6–7°	Coniferous trees and maple (*Acer pseudoplatanus*)
7–8°	Coniferous trees and maple and beech
8–11°	Maple and beech and oak
11–12°	Maple and oak
>12°	Oak

In order to keep the number of influencing factors low, a constant forest area was assumed. This condition does not fully reflect reality, because the forest area is annually increasing by 3,000 ha, mostly due to abandonment of high elevation pastures and marginal agricultural land (Gschwantner, 2019).

The soil organic carbon stock was simulated with Yasso07 (Liski et al., 2009). The crucial parameter for Yasso07 is the influx of organic carbon from trees into the soil. No measured flux data were available. Therefore, the influx was estimated in a two-step procedure. Firstly, the standing stock of the above- and belowground biomass, i.e., stem mass, branch mass, mass of needles or leaves, and the mass of fine ($\varnothing < 2$ mm) and coarse roots ($\varnothing > 2$ mm) was calculated with a set of biomass equations. Stem volume was converted to stem mass with tree-species specific wood densities. The tree-species-specific equations for the mass of branches, needles and leaves, and total roots have been derived from Austrian experiments and are completely referenced in a previous paper (Jandl et al., 2018). The biomass equations use stem diameter, stem height, and canopy height of individual trees as input parameters. The mass of fine roots was pragmatically chosen to be 2% of the total root biomass. Secondly, the annual flux of organic carbon from the respective biomass compartments to the soil was estimated by assigning each biomass compartment a certain turnover rate. The assumptions on the longevity of different tree compartments were based on observations from intensive monitoring plots and from literature information, as also previously described (Jandl et al., 2018). The forest inventory data do not provide information on the biomass of herbaceous plants and bushes. Their eventual contribution to the flux of organic carbon from plants to the soil is therefore not reflected in our simulations. Later, the individual biomass compartments are assigned chemical properties and sizes that are affecting the decomposition rate of soil organic matter (Didion et al., 2016; Hernández et al., 2017).

The next step was the stratification of trees into the classes "Standing stock," "Mortality," and "Harvest." Each stratum was treated differently with respect to the release of organic material from the biomass into the soil. The class "Standing Stock" releases organic matter in the form of aboveground and belowground litterfall. Deciduous trees and larch are shedding all photosynthetic active tissue each year and have therefore a turnover of 1 year. For pine and Norway spruce we had observations on the longevity of needles, ranging from 2 years of pine (*Pinus silvestris*) to 5 years for Norway spruce. Therefore, the annual input from needles and leaves to the soil was quantified. The annual flux of branch biomass to the soil is difficult to estimate. It widely depends on local conditions (exposure to storms) and the number and severity of storm events. Missing site-specific information we based our estimate on a pragmatically chosen fixed value (Jandl et al., 2018). The turnover of coarse roots was the same as the turnover of branch biomass. The turnover of fine roots is a relevant, yet most elusive flux of carbon (Brunner et al., 2013). Fine roots are short-lived, highly decomposable and supply a significant amount of organic matter to the soil (Trumbore and Gaudinski, 2003). Based on many controversial discussions with root experts we defined the turnover time of fine roots to be 1 year. The class "Harvest" assumed the common strategy in Austria: Trees are cut and de-limbed. Branches, needles and leaves, and roots remain on site. A small portion of the stem, the canopy, is also remaining on site. The merchantable stem is exported. All remaining compartments enter the soil and are decomposing according to the quality- and size-depending rules of Yasso07. The class "Mortality" followed a similar logic as "Harvest." The difference is that stems (logs) remained on site. Salvaging of previously dying trees was reported in the data of the National Forest Inventory. Therefore, it could be defined at which point in time the dead trees were either removed from the site or entered the soil as slowly decaying logs.

Yasso07 does not distinguish between carbon in the litter layer and carbon in the mineral soil, neither does it distinguish between soil horizons. The output of Yasso07 gives some resemblance to the terminology of soil science. Yet, the match between strata in Yasso07 and soils is not fully defined and is left to the imagination of the model user. The unconstrained soil depth of the Yasso07 model informs, how much carbon is expected to reside in the entire soil in order to meet steady-state conditions. The model does not define at which soil position exactly the carbon is sitting.

2.4. Data Analysis

The data of the Austrian Soil Survey were statistically analyzed. An Analysis of Covariance was used to identify differences in soil carbon stocks between coniferous and mixed-deciduous forests at different geological substrates (two categories) and elevation (continuous variable), and exposition of slopes (four categories). Further, the difference in different strata was analyzed with a *t*-test. Soil data were analyzed with the AQP package (Beaudette et al., 2013). All data analyses were done with R version 4.0.3 (codename: "Bunny-Wunnies Freak Out"; R Core Team, 2017).

3. RESULTS

3.1. Austrian Forest Soil Survey

Owed to the geological structure of Austria two thirds of the forests are on silicatic bedrock. Overall, coniferous forests are dominating (73% of the investigated sites) over mixed-deciduous forests (27%). On silicatic bedrock, 82% of the forests are coniferous, and only 18% are mixed-deciduous. On calcareous bedrock the ratio is rather even with 42% deciduous and 58% coniferous forests. The soil organic carbon stocks of the Austrian Forest Soil Survey are shown in **Table 2**. The mean total organic carbon stock is 104 t C/ha with 17% residing in the organic surface layer and 83% in the upper 50 cm of the mineral soil. Mineral soils derived from calcareous rocks have significantly higher organic carbon stocks than silicatic soils. The difference in organic carbon stocks in the litter layer is statistically not significant due to the high variability within the dataset. The stratification by bedrock material shows consistently higher soil organic carbon stocks in the litter layer of coniferous forests whereas the stocks in the mineral soils of both forest types are more even. The highest soil organic carbon stocks are found in coniferous forests on calcareous bedrock.

Figure 2 and the difference between the median and the mean value in **Table 2** show left-skewed distributions of the soil organic carbon stocks for both forest types. The highest frequency of soil organic carbon stocks in mixed-deciduous forests is around 75 t C/ha and somewhat lower in coniferous forests. Low soil organic carbon stocks are mainly found on shallow soils, which are commonly found independently of the bedrock material. Very high soil organic carbon stocks are reported for peatlands, which are rare in Austria, yet more commonly encountered on silicatic bedrock because the population of silicate sites is larger and therefore has a higher chance to include rare soil types.

The elevational gradient of the total soil organic carbon stock is shown in **Figure 3**. On calcareous bedrock there is a gradual elevational increase of soil organic carbon in mixed-deciduous forests, and no increasing gradient in coniferous forests. On silicatic bedrock a strong elevational trend is found for both forest types. Yet, there is a wide scatter around the regression lines. The results of the analysis of covariance are presented in **Table 3**. The organic carbon pool in the forest floor material is always higher in coniferous as compared to mixed-deciduous forests, as shown by the numerical difference in the "intercept." There are statistically significant slopes ("Coefficient for Altitude") for all strata.

The effect of slope exposition is shown in **Figure 4**. Obviously, slope exposition is among the key stratification parameters. Yet, in our dataset we found some evidence for the influence of exposition on the soil organic carbon stocks. Yet, other dominant factors such as bedrock, forest type, and elevational zone are over-shadowing its effect (**Table 2**).

The depth gradients of organic carbon in the mineral soil is shown in **Figure 5**. Coniferous forests have higher carbon stocks in the upper part of the mineral soil. Deeper in the soil mixed-deciduous forests hold slightly more organic carbon. Yet, the difference is statistically not significant.

3.2. Modeling

The replacement of coniferous with deciduous tree species in order to adapt forests to a warmer world on a national scale is a long process. A replacement can be implemented either after the final harvest or after major disturbances of the stand structure. In **Figure 6**, we show the gradual increase in deciduous trees in time slices until the year 2150 when we apply the rules

TABLE 2 | Soil organic carbon stocks (t C/ha) in Austrian forest soils stratified according to geological bedrock (silicatic vs. calcareous material), forest type (coniferous vs. mixed-deciduous), and slope exposition.

		Litter layer (t C/ha)		**Mineral soil 0–50 cm (t C/ha)**	
Stratum	***n***	**Median**	**Mean ± s_e**	**Median**	**Mean ± s_e**
All sites	511	13.5	16.9 ± 0.6	72.3	87.1 ± 2.4
Bedrock					
Silicatic	332	14.8	17.6 ± 0.7	62.3	76.5 ± 2.6
Calcareous	179	10.9	15.4 ± 1.2	104.4	112.0 ± 4.4
Significance			n.s.		***
Forest type					
Mixed-deciduous	136	8.0	12.9 ± 1.4	66.0	83.7 ± 5.0
coniferous	375	15.5	18.2 ± 0.7	73.7	88.2 ± 2.7
Significance			n.s.		***
Exposition					
East/West	159	12.9	16.8 ± 1.1	72.6	83.8 ± 3.8
North	189	14.3	17.4 ± 1.1	77.6	97.1 ± 4.3
South/even	163	14.4	16.3 ± 1.0	63.0	79.5 ± 3.9
Significance			n.s.		n.s.
Forest types on silicatic bedrock					
Mixed-deciduous	60	6.3	11.3 ± 1.8	51.4	67.1 ± 6.4
Coniferous	272	16.2	18.9 ± 0.8	64.5	78.4 ± 2.9
Significance			***		n.s.
Forest types on calcareous bedrock					
Mixed-deciduous	76	8.4	14.4 ± 2.1	78.5	100.4 ± 7.0
Coniferous	103	12.5	16.3 ± 1.4	114.8	121.3 ± 5.5
Significance			***		*

The median, the mean value, and its standard (s_e) and the significance of differences between groups are shown (n.s., not significant; $p > 5\%$; "": significant; $p < 5\%$; "***": highly significant; $p < 0.1\%$).*

provided in **Table 1**. The present dominance of coniferous forests includes sites where conifers are dominating for natural reasons and where Norway spruce stands have been established for economic reasons. Until the year 2050 the sites in low elevation are already dominated by deciduous forests. By the end of the century coniferous forests merely prevail in the Inner Alps at high elevation sites. A further 50 years later the map is similar to the situation in 2100. This reflects the remaining sites in Inner Alpine areas and high elevation where site conditions are not suitable for deciduous forests in the foreseeable future.

The simulated total soil organic carbon stock is constantly rising under the scenario of moderate warming (RCP 4.5, forest management following business-as-usual). In the next 70 years the soil organic carbon stock increases by almost 20%. Stronger warming (RCP 8.5) leads to increasing soil organic carbon stocks during several decades. However, after a peak toward the end of the century the soil organic carbon stocks decline and eventually drop below the presently encountered levels (**Figure 7**).

4. DISCUSSION

Forest sciences have a rich body of information on the effect of tree species on soil properties. Differences on the soil organic carbon stocks are driven by the species-specific growth rates and the allocation of photosynthates to above- or belowground tissue. Common garden experiments and case studies are taking place under site conditions that support an experiment but are not necessarily representative for regions (Binkley, 2020). Moreover, in a common setting in Central Europe the distribution of tree species is not primarily driven by the expected formation of a stock of soil organic carbon but rather by management decisions of forest practitioners. Niche models are a powerful tool for the prediction of future tree species compositions that are based on site conditions and to some extent on the competitive advantages of different tree species (Zimmermann et al., 2009; Chauvier et al., 2021). Yet, forest management decisions are not reflected.

The data of the Austrian Forest Soil Survey do not allow a deep stratification of the data set to the identification of the effects

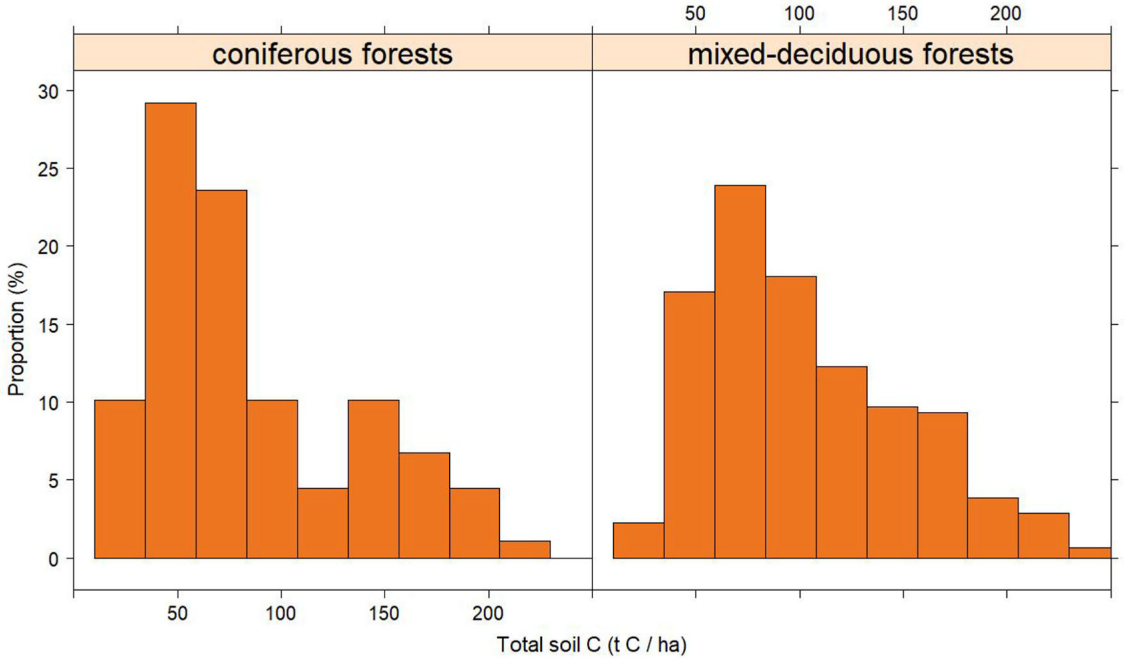

FIGURE 2 | The total soil organic carbon stock (t C/ha) of coniferous and mixed-deciduous forests in Austria based on data of the Austrian Forest Soil Survey.

of single tree species except for Norway spruce because strata sizes are forbiddingly low. Our analysis is therefore hampered by pooling all deciduous tree species into one group, even though differences in the decomposition rate among deciduous trees are known (Gustafson, 1943). Further, deciduous forests in our study are not strictly composed of deciduous trees, but hold a fair share of coniferous tree species. Hence, we are referring to "mixed-deciduous forests." Strictly deciduous forests are only found in some regions in the east of the country. In other regions, the dogma of "as much coniferous tree species as possible, as much deciduous tree species as required" has been widely followed. The dogma is based on the demand of the Austrian timber market, where timber from conifers, in particular Norway spruce, is in higher esteem than timber from deciduous trees. Lumping all deciduous trees into one group does not have a decisive impact on our topic. The influence of external effects on leaf decomposition rates is larger than the difference between the litter of different deciduous tree species (Cornelissen et al., 2007).

Ground truth based on the Austrian Forest Soil Survey reinforces existing knowledge that coniferous forests have higher soil organic carbon stocks than mixed-deciduous forests. This applies both for the organic surface layer and the upper 50 cm of the mineral soil and independent of the geological bedrock material (**Table 2**). The depth gradient of carbon in the mineral soil shows higher organic carbon stocks in the upper soil profile of coniferous forests, because there the rooting density is higher and some carbon leaches out of the organic surface layer. Our data do not confirm the theory, that deciduous tree species lead to higher carbon stocks in deeper parts of the mineral soil due to their tendency to root deeper (**Figure 5**). Most soils in Austrian forests are rather shallow. Deeply weathered soils in low-elevation areas are predominantly under agricultural use where slope inclinations allow for it. The majority of soil organic carbon resides in the organic litter layer and the upper 30 cm of the mineral soil. The expectations of deeper distribution of organic carbon due to the decomposition of organic matter due to deep-rooting deciduous trees as compared to the shallow rooting Norway spruce is not supported by the analysis of national data due to insufficient information on subsoil horizons. The relevant information may be more efficiently derived from case studies where the experimental setup is specifically addressing deep soil horizons.

However, the encountered differences between forest types ("coniferous forests" vs. "mixed-deciduous forests") are not as clear-cut as the results from common-garden experiments and case studies would suggest. We emphasize that our observation of differences in total soil organic carbon stocks are partially an effect of tree species, partially owed to site conditions, and mostly driven by forest management decisions. Firstly, the distribution of total soil organic carbon stocks for different forest types is rather similar. The difference is caused by a higher frequency of coniferous forests on sites with above-average soil carbon stocks such as peatlands (**Figure 2**). Secondly, even more important, is the geographical location of different forest types. Deciduous forests are mostly confined to low-elevation sites because many deciduous tree species cannot tolerate site conditions in higher elevation. Coniferous forests are encountered in low elevation where they are often replacing naturally dominating deciduous tree species, and in high elevation, where they are dominating for ecological reasons. In addition, the Inner Alps,

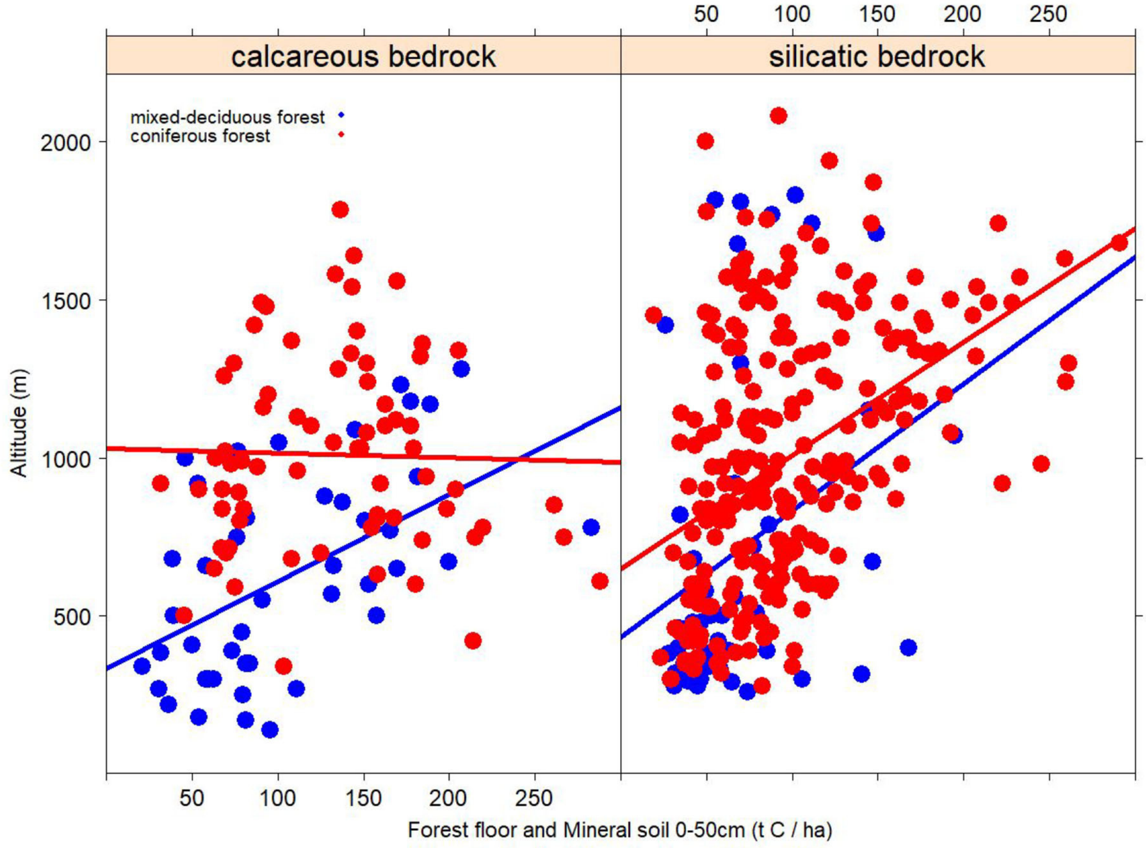

FIGURE 3 | The total soil organic carbon stock differentiated between sites on calcareous bedrock and silicatic bedrock. The elevational gradient is indicated by a linear trend line. Different forest types (coniferous vs. mixed-deciduous forests) are shown in different colors.

that are characterized by a continental cool climate, are naturally dominated by coniferous tree species. At low-elevation sites generally a quicker turnover of soil organic carbon is expected. At high elevation under cooler conditions the productivity of forests and the soil microbial activity is low. Our data corroborate that site conditions in high elevation are supporting the accumulation of soil organic carbon. Finally, the spatial distribution of tree species is mostly reflecting the decisions of the forest manager at work in the particular region who is choosing his tree species under consideration of economic effects of his forest-ecological system understanding.

Modeling results allow analyzing the long-term consequences of climate warming under realistic conditions where the ecological dynamics of forest ecosystems are embedded in management decisions of the timber producing sector. The difference to case studies and common-garden experiments is that on top of tree species characteristics forest management decisions are reflected in the results. The gradual increase in soil organic carbon stocks in a moderate warming scenario (RCP 4.5) reflects that currently the productivity of many mountain forests is constrained by the brevity of the growing season. Increasing temperature as the current growth-limiting factor will increase forest productivity in mountain forests (Lexer et al., 2015; Pretzsch et al., 2020). Under conditions of increasing biomass production an increase in the total soil carbon stock is expected (**Figure 7**). Even under increased warming rates (RCP 8.5; business-as-usual) this effect prevails for several decades. However, eventually climate conditions become problematic for Norway spruce and the growth rate declines. As a consequence of decreasing litter input to the soil and higher microbial activity the soil organic carbon stock declines.

The "deciduous tree species scenario" was embedded in the extreme climate change scenario RCP 8.5. With hindsight to the strong warming trend of the RCP 8.5 and envisioned accumulating disturbances of forests we assumed that all forest managers are changing their management strategy. The strategy was replacing Norway spruce with oak, beech, or maple, depending on the extent of climate change (**Table 1**). We pragmatically omitted the option of admitting non-native tree species because we lack the required species-specific model parameters. Thereby, we adhere to the claim of nature conservation of not-introducing additional tree species, even though we entertain this future option based on a more solid knowledge basis that is currently emerging. However, we followed the scheme of assisted migration because we simply introduced the deciduous tree species instead of

TABLE 3 | Linear regression functions for the estimation of the soil carbon stock in different strata, as obtained by a covariance analysis with elevation as continuous and bedrock type as categorical variable.

Stratum	Forest type	Intercept	Coefficient for altitude	R^2
All bedrocks				
Forest floor	Mixed-deciduous	1.0	0.02	
Forest floor	Coniferous	12.2	0.01	
		n.s.	***	0.11
Mineral soil	Mixed-deciduous	48.6	0.05	
Mineral soil	Coniferous	50.5	0.04	
		***	***	0.12
Total soil	Mixed-deciduous	59.86	0.05	
Total soil	Coniferous	59.09	0.04	
		***	***	0.12
Calcareous bedrock				
Forest floor	Mixed-deciduous	−6.12	0.03	
Forest floor	Coniferous	6.02	0.01	
		n.s.	***	0.22
Mineral soil	Mixed-deciduous	53.47	0.07	
Mineral soil	Coniferous	131.40	−0.01	
		***	***	0.13
Total soil	Mixed-deciduous	33.85	0.12	
Total soil	Coniferous	137.97	−0.01	
		***	n.s.	0.19
Silicatic bedrock				
Forest floor	Mixed-deciduous	5.57	0.01	
Forest floor	Coniferous	13.65	0.01	
		n.s.	*	0.05
Mineral soil	Mixed-deciduous	35.67	0.04	
Mineral soil	Coniferous	32.20	0.03	
		***	***	0.18
Total soil	Mixed-deciduous	54.78	0.02	
Total soil	Coniferous	43.46	0.05	
		***	n.s.	0.22

*n.s.; not significant; *significant $p < 5\%$; ***highly significant $p < 0.01\%$.*

enabling their slow natural expansion into a new habitat (Chakraborty et al., 2019).

In **Figure 7**, it is shown that in "RCP 8.5—deciduous tree species" soil organic carbon stocks are increasing as compared to "RCP 8.5—business-as-usual" in the second half of the century. This trend rather reflects several processes that cannot be disentangled with the available data. Firstly, the most productive forest sites are currently populated by coniferous forests. Deciduous tree species may be able to establish comparably high soil organic carbon stocks. An unintended effect is the lack of demand for timber from deciduous forests. A known challenge of the conversion of conifer to deciduous forests is the lack of demand for timber from deciduous trees, because the wood-technology options are still limited and hardwood timber still cannot replace timber of Norway spruce. Therefore, deciduous forests are under-used until the remaining conifer forests can supply the demand for timber and can develop with less disturbance. However, we cannot rule out that the simulation is overestimating the increase of soil organic carbon stocks under deciduous trees. It is possible that our biomass functions for the foliage of deciduous trees are delivering values on the high end of the natural spectrum. Hence, a high estimate of leaf biomass translates into high annual influx of organic material *via* aboveground litterfall. A second potential source for bias is the

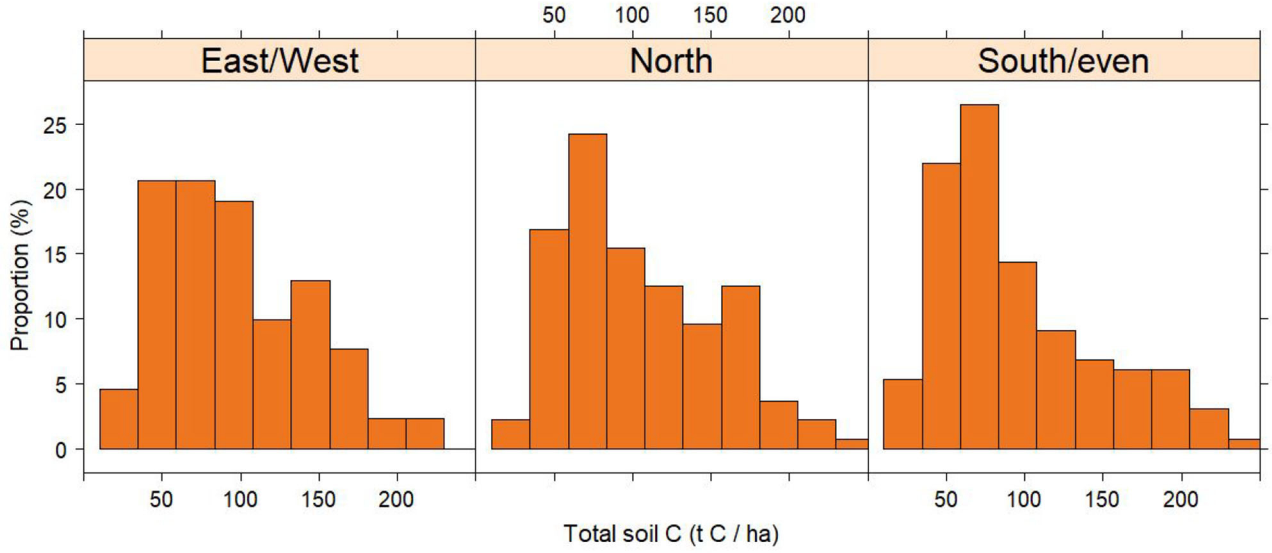

FIGURE 4 | The total soil organic carbon stock (t C/ha) of coniferous and mixed-deciduous forests in Austria based on data of the Austrian Forest Soil Survey. The data are stratified according to slope orientation.

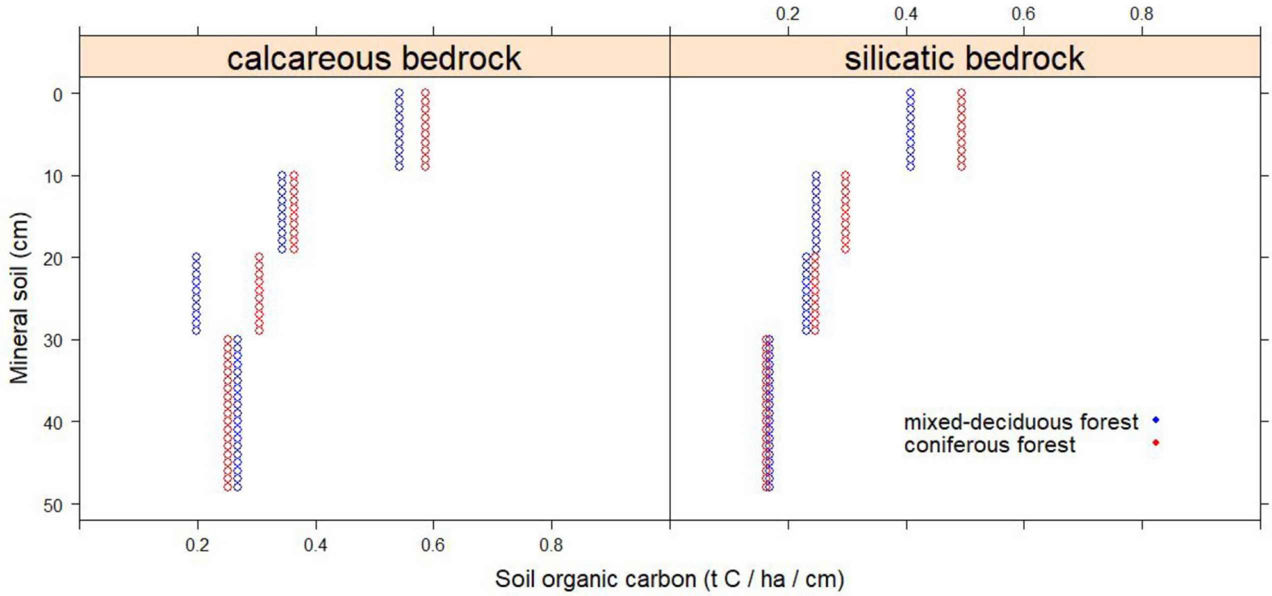

FIGURE 5 | Depth gradients of soil organic carbon in the mineral soil of coniferous and mixed-deciduous forest stands differentiated between sites on calcareous vs. silicatic bedrock.

uncertainty of the belowground litterfall *via* root turnover that is pertinent in soil carbon models (Brunner et al., 2013). Thirdly, the results are quantitatively emphasized because the scenario holds that all forest managers adopt the same adaptation strategy in order to cope with climate change.

Irrespective of tree species selection the forest soil organic carbon stock declines in a strong warming scenario due to the establishment of a new equilibrium level of lower productivity of forests and increasing decomposition rates of soil organic matter. This finding reflects that carbon sequestration in the biomass and the soils of forest ecosystems is only a temporary solution. Concepts such as increasing the forest area on a global scale can buy additional time but in the long term are not capable to resolve the challenge of climate change (Bastin et al., 2020).

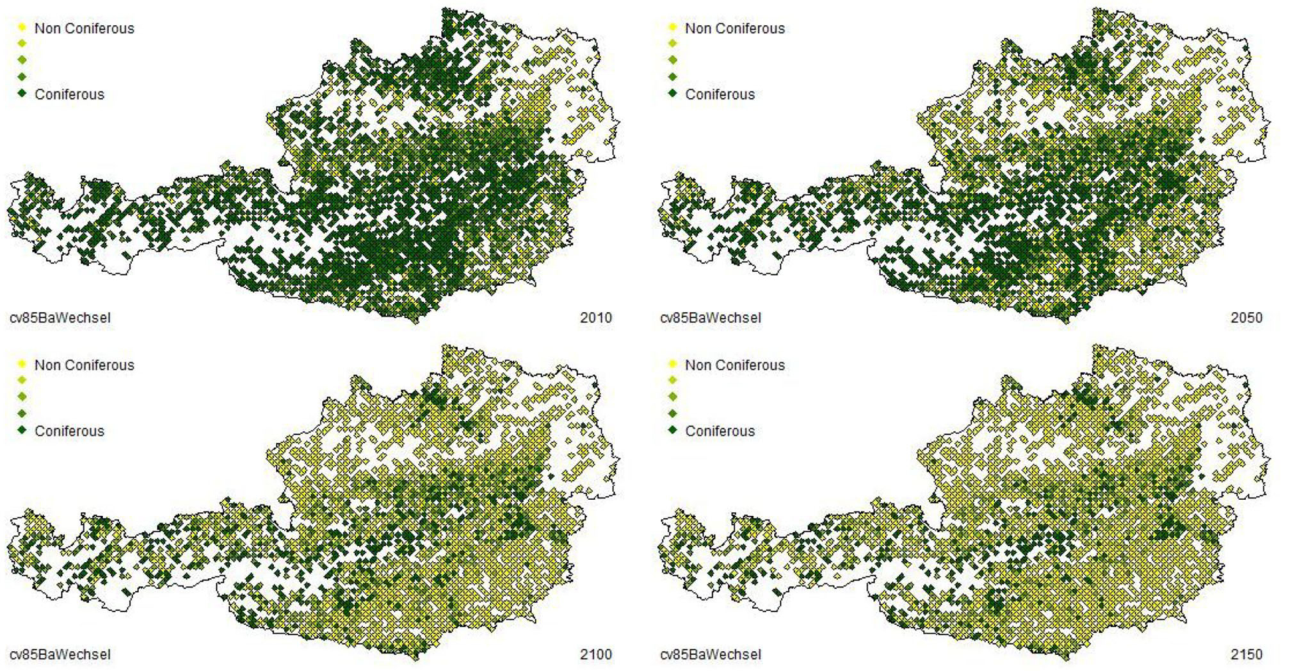

FIGURE 6 | Forest adaptation to climate change *via* replacement of coniferous with deciduous tree species, depending on expected climatic conditions. The used climate scenario (RCP 8.5) reflects a strong warming trend. The initial conditions are presented for the year 2010 **(upper panel, left)**, the distribution of tree species in 2050 **(upper panel, right)**, 2100 **(lower panel, left)**, and 2150 **(lower panel, right)** is shown. The color gradient describes the proportion of deciduous forests from 0 (yellow), 20, 40, 60, 80, 100% (increasing darkness of greens).

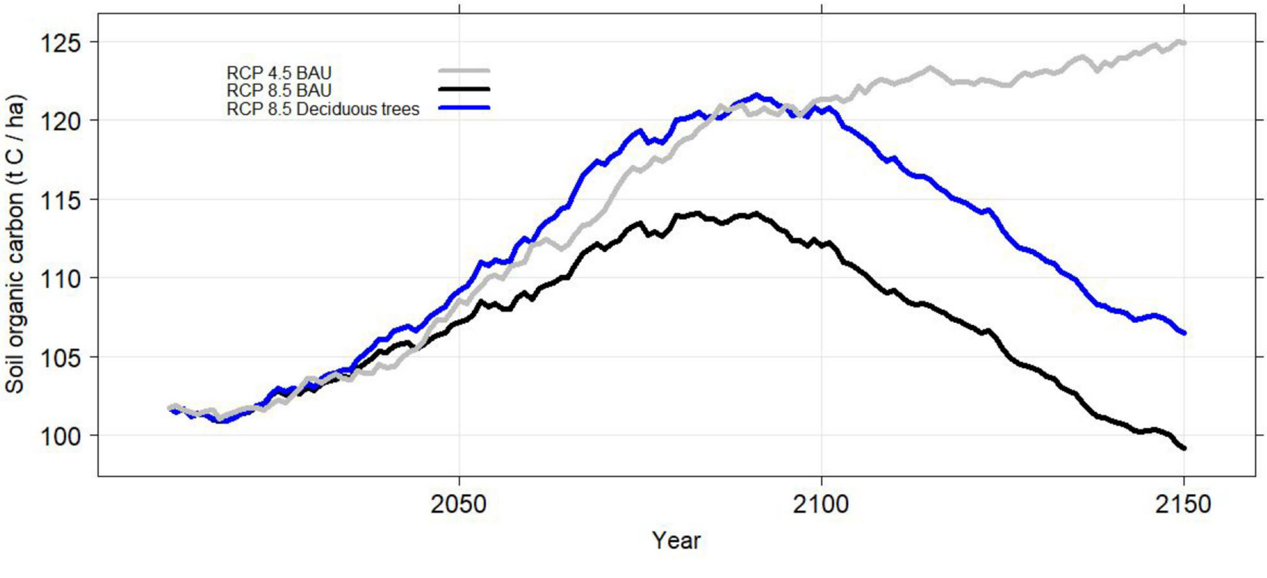

FIGURE 7 | Temporal trend of the soil organic carbon stock of Austrian soils in a simulation experiment. The scenarios are a moderate temperature increase (RCP 4.5 Business-as-usual forest management), and a strong warming trend (RCP 8.5 Business-as-usual forest management, and RCP 8.5 replacement of coniferous forests with deciduous forests). The y-axis ranges from 100 to 125 t C/ha.

Figure 6 shows the current dominance of coniferous forests in Austria. Only in the Eastern and South-Eastern part of the country as well as along major valleys deciduous trees are currently more abundant than coniferous tree species. In a strong climate warming scenario within only 50 years, i.e., half a lifetime of trees, deciduous trees are gradually replacing conifers, because sites at medium altitudes in mountains are becoming increasingly suitable for their growth. This scenario is indicative, but more a reflection of a gedanken experiment than a real future situation. The scenario assumes that all forest owners follow the mindset of abandoning coniferous tree species when prognosticated climate conditions advise against them. In a real world situation we rather expect that a group of forest managers follows the ecological advise, whereas others adhere to traditional beliefs of forestry, and will continue favoring coniferous tree species at sites that are no longer suitable for them. Such a position is perfectly understandable, given that no convincing model has been presented showing that timber from deciduous trees can replace the Norway-spruce dominated economic model. In reality, the climate-change induced shift from coniferous forests to mixed-deciduous and deciduous forests will be a slower process. In the simulation experiment, after the year 2100 the tree species composition is not changing further. There are only some few forests sites in high elevation that are not suitable for deciduous trees. Except for that, forests will be dominated by deciduous tree species. Such a scenario has many implications, such as the aesthetics of the entire landscape, the provision of protective services by forests in mountain communities, and the regulatory effects of forests to air and water quality. The implications are not fully discussed here. Yet, the role of forests for society will need to be re-evaluated.

We emphasize that we are showing an extreme scenario that shows a potential for the future distribution of forests dominated by deciduous trees. It can be overruled by alternative forest management decisions. Main reasons are the driven by the demand side of the timber market. Due to the technological properties of timber from Norway spruce, forest managers will seek options to grow Norway spruce and may accept an increasing production risk. Current wood technology does not have the means to replace coniferous wood with timber from deciduous trees. In addition, the implemented rule for switching from coniferous to deciduous tree species may be instrumental, yet overly simple. In high-elevation forests the decision to implement a change of tree species may be more complex. An example is the exposure of trees to eventual extreme events such as early and late frost that are not reflected in the described rule. We therefore insist, that our scenarios depict a possible/potential future. Regional or local forest management decisions will rely on more refined assumptions on the appropriate choice of tree species.

DATA AVAILABILITY STATEMENT

The datasets can be requested from the corresponding author. Data access is provided upon reasonable requests and in compliance with the data policy of the Austrian Forest Research Center.

AUTHOR CONTRIBUTIONS

RJ wrote the manuscript. He participated in the discussions and provided input with respect to soil carbon modeling. He further analyzed and interpreted the data of the Austrian Forest Soil Survey. TL simulated forest growth and provided input to the writing. GK simulated the Yasso07 runs and gave critical input to the manuscript. He programmed the visualization of the effect of tree species change. PW coordinated the project that provided the forest and forest soil simulations. He also established the first benchmark for organic carbon stocks in Austrian forests and forest soils. All authors contributed to the article and approved the submitted version.

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Afforestation With Different Tree Species Causes a Divergent Evolution of Soil Profiles and Properties

German Morazzo[1], Diego Rene Riestra[2], Emmanuel Leizica[2,3], Lucila Álvarez[2] and Elke Noellemeyer[2]*

[1] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Santa Rosa, Argentina, [2] Facultad de Agronomía, Universidad Nacional de La Pampa, Santa Rosa, Argentina, [3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Edited by:
Jeff Allen Hatten,
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United States

Reviewed by:
Antra Boca,
Latvia University of Agriculture, Latvia
Per Gundersen,
University of Copenhagen, Denmark

***Correspondence:**
Elke Noellemeyer
noellemeyer@agro.unlpam.edu.ar;
enoellemeyer@gmail.com

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Land-use change and specifically a change in the type of vegetation cover affects soil morphology, chemistry, biology, and nutrient regimes. Numerous studies have documented that in land-use conversions from agricultural land to forest, or from plantations to restored natural savanna most soil attributes and functions undergo changes. The purpose of the present study was to evaluate the changes brought about by afforestation of degraded croplands and to understand the impact of forest vegetation on soil evolution in a semiarid region where soils originally co-evolved with a savanna biotope. We used long-term experiments (>40 years) of five tree species: *Pinus halepensis* (PH), *Pinus halepensis* inoculated with ecto-mycorrhiza at planting (PM), *Pinus pinea* (PP), *Eucalyptus* spp. (E), *and Gleditsia triacanthos* (G) and compared these to an agricultural soil (A) at the same site near Santa Rosa, La Pampa in the semiarid center of Argentina. Soil profiles were described, and samples taken for chemical and physical analyses of soil properties [organic matter (OM), pH, cation exchange capacity (CEC) and exchangeable cations, particle size distribution (texture), aggregate stability (MWD), bulk density (BD), porosity (TP), and water holding capacity (WHC)]. We found a strong effect of tree species on soil profile morphology, even taxonomy, and on all studied variables. PM and G had highest OM, CEC, neutral pH, higher TP, WHC, while PH, PP, and E had acid pH, lower base saturation, OM, TP, and WHC. The effect of tree species on the soil profile was noticeable a depth of about 40 cm, comprising the A and AC, but not the C horizons. The results showed that to obtain reasonable results of OM sequestration under forest systems, tree species should be chosen to include legumes to improve C/N stoichiometry for C fixation, or inoculation with mycorrhiza to promote microbial transformation of forest litter.

Keywords: semiarid central Argentina, organic matter, base saturation, Mollisols, hydrophobicity

INTRODUCTION

Land-use change and specifically a change in the type of vegetation cover affects soil morphology, chemistry, biology, and nutrient regimes. Numerous studies have documented that in land use conversions from agricultural to forest systems (Lemenih, 2004), or from plantations to restored natural savanna vegetation (Johnson-Maynard et al., 2002) most soil attributes and functions undergo changes. These changes are driven by environmental factors (climate, parent material) and by the forest species and management. Different forest species resulted in a wide span of accrued soil organic carbon levels (Crow et al., 2008; Gurmesa et al., 2013; Prescott and Grayston, 2013; Tang and Li, 2013; Vesterdal et al., 2013). The effect of species also was documented for changes in soil physical and chemical properties such as pH, CEC, and nutrient status (i.e., N and P stocks) (De Vries et al., 2003). A novel concept regarding soil development or soil evolution is defining soils as extended composite phenotypes that co-evolve with the biota associated to each soil in a process of natural selection that provides benefits to those organisms that adapt or "engineer" special habitats or niches within a given soil environment (Phillips, 2009). This concept, based on the notion of soils as "biomantles" or "excited skin" of our planet (Phillips, 2009), is much more dynamic than the traditional view of unidirectional soil development, dependent on the combination of soil forming factors present in a determined location. If soils were indeed extended composite phenotypes, a strong biological change such as caused by the substitution of the dominant vegetation species, should be reflected by significant qualitative changes in soils (in addition to quantitative changes in soil properties) as for instance an effect on soil morphology. Some studies already pointed out that Corsican pine (*Pinus nigra*) caused incipient podzolization in soils of Tuscany, while at the same time this process was not observed under silver fir (Certini et al., 1998).

In many areas of the world afforestation was promoted to restore degraded agricultural lands (Masera et al., 2003; Mendham et al., 2003; Farley et al., 2004; Niu and Duiker, 2006; Laik et al., 2009; Wei et al., 2013). However, in many semiarid regions of the world original landcover were grasslands or savannas, under which very fertile soils, typically Mollisols, evolved. The Haplustolls, Calciustolls, and Paleustolls of the central Argentinean Dry Pampas developed under savanna vegetation, which consists of a dense grass layer of C3 short bunchgrasses, forbs, and legume trees and shrubs. These soils were converted to croplands by immigrants during the first half of the past century, and like the history of the North American farmlands, prolonged droughts triggered severe soil loss through wind erosion during the 1930s and 1950s. Afforestation was promoted to recover the degraded soils and the most ubiquitous species were Aleppo pine, Stone pine, Black locust, and Eucalypt. A previous study on afforested soil in this region (Riestra et al., 2012) already reported on the effect of these species on some soil chemical and physical properties, concluding that legumes, such as Black locust, or the inoculation with mycorrhiza in Stone pine stands were favorable for carbon sequestration and soil structure.

The present study proposed to deepen our understanding of the changes brought about by afforestation of degraded croplands and to understand the impact of forest vegetation on soil evolution and profile characteristics in a semiarid region where soils originally co-evolved with a savanna biotope. We therefore hypothesized that soils under different forest species would show divergence in their chemical, physical, and biological properties, even in the relatively short time span after this land cover conversion took place. We also wondered whether the 50-year time span was long enough to bring about morphological changes in the soil profiles, and to which profile depth the effect of forest species would reach.

MATERIALS AND METHODS

Site Description

The study was carried out at the provincial forest nursery at Santa Rosa, La Pampa (**Figure 1**; Coordinates: 36°34′17″S; 64°16′56″W), on a sandy loam Entic Haplustoll (Usda and Nrcs, 2010). The soils are formed on quaternary aeolian loess deposits forming extensive undulating plains (Zárate and Tripaldi, 2012) in a semiarid climate with a mean annual temperature of 15.5°C and mean annual rainfall of 750 mm (Casagrande et al., 2006). The original vegetation is a dry forest or savanna composed of mainly legume trees of the Prosopis species (e.g., *Prosopis Caldenia, P. flexuosa*) and a dense grass cover (e.g., *Stipa tenuis, Poa ligularis, Piptochaetium napostaensis*).

The treatments corresponded to four tree species: Aleppo pine, *Pinus haleppensis* (PH) and *Pinus haleppensis* inoculated with ecto-mycorrhiza at planting (PM); Stone pine, *Pinus pinea* (PP); Eucalypt, *Eucalyptus* spp. (E); and Black locust, *Gleditsia triacanthos* (G). The inoculated Aleppo pine treatment was included in the forest nursery trials since it was thought that this might improve seedling vigor, seedlings were planted in soil inoculated with mycorrhiza mycelia that were originally brought from Croatia (Poduje, personal communication). It is to be noted that in pine stands that were not grown on inoculated substrate no evidence of fungi can be found. While there most likely exist native ectomycorrhiza in the soils, apparently these do not colonize conifers, perhaps since the native vegetation does not include any coniferous species.

In addition, a control plot which had been cultivated and planted to annual crops for the same time (Ag) was sampled, resulting in six treatments. The size of each forested plot was approximately 3 ha, and the tree distance were 3 by 3 m for PH and PM, 3.5 by 3.5 m for PP, 3 by 3.5 m for E, and 4 by 4 m for G (see **Supplementary Material** for a map of the forest plots). All stands were more than forty years old at sampling, and had only been pruned, but never cut or thinned. No weed control had been practiced, and the lighter stands had a sparse grass cover.

Soil Sampling and Analytical Procedures

At each treatment site surface samples at two depth layers (0–6 and 6–12 cm) were collected with steel cylinders with a fixed volume of 1,060 cm^3. For each treatment, 8 replicate soil samples were taken at random, taking care that in the

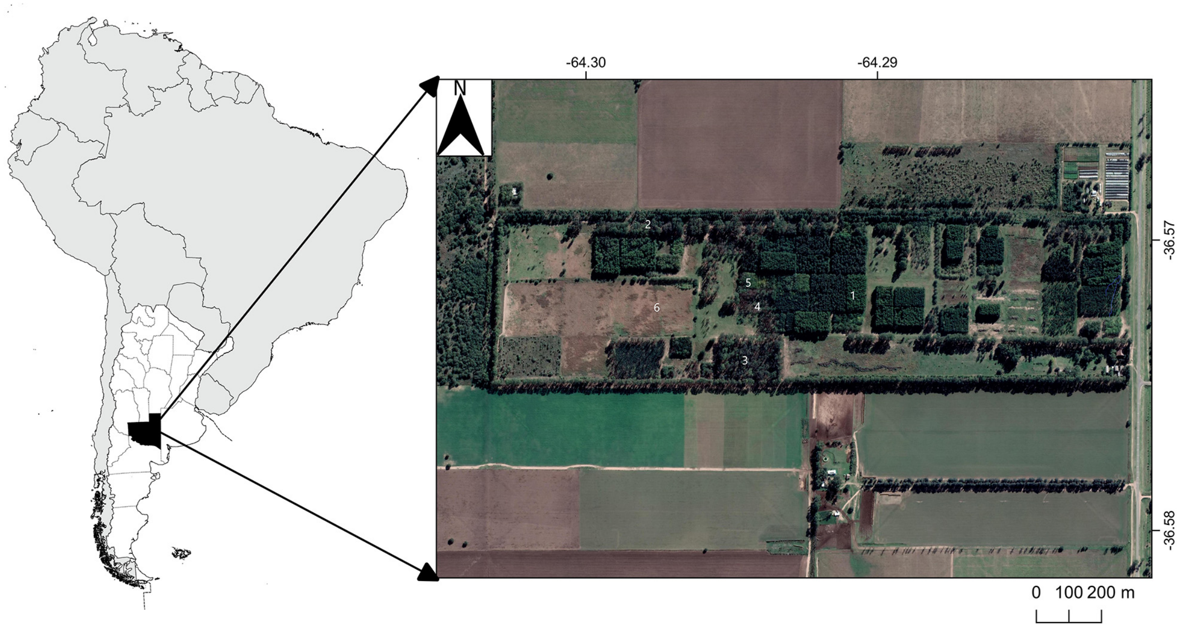

FIGURE 1 | Map of location of the sampling area and aerial view of the afforestation plots near Santa Rosa, La Pampa Argentina. The numbers indicate the treatments: (1) Aleppo pine (PH), (2) Aleppo pine with ectomycorrhiza (PM), (3) Eucalypt (E), (4) Stone pine (PP), (5) Black locust (G), (6) Agriculture (Ag).

forest treatments sampling points were equidistant between tree trunks. These samples were used to represent the A-horizon's chemical properties, considering that changes in land-use are most like to be detected in the uppermost soil layers, and that under soils under native and implanted forest show a strong stratification of SOC and related properties (Noellemeyer et al., 2008, 2006; Gili et al., 2010). In addition, a soil pit was dug in each stand and the soil profile was described according to the soil survey manual (National Soil Survey Center, 2012; Soil Survey Staff, 2014). Soil samples were taken from the center of each horizon for physical-chemical analysis. The sampling was carried out in spring/summer 2008 and refers to the same treatments described by Riestra et al. (2012).

Soil samples were oven-dried at 105°C to constant weight, and the dry weight of each soil core was used to calculated bulk density (BD, Mg m^{-3}) as follows:

$$\mathrm{BD} = \mathrm{M/V}$$

Where M is the dry weight of the soil contained in the cylinder, and V is the volume of the cylinder. Soils in this region do not contain stones or gravel, wherefore these are not considered in the determination of BD.

Part of the soil sample was sieved to 2 mm, and roots or any other plant residue particles were excluded from the samples for analytical determinations. Total organic carbon (OC, g kg^{-1}) was determined by wet oxidation with potassium dichromate in sulfuric acid and colorimetric valuation (Skjemstad et al., 2003) and total nitrogen was determined by the semimicro Kjeldahl procedure. Both were made on 0–6 and 6–12 cm samples separately, whereas pooled samples from both depth layers (0–12 cm) were used for all other determinations. Particle size fractions were determined by the Robinson Pipette method and soil texture was obtained with the textural triangle (Gee and Bauder, 1986). Particle density (PD, Mg m^{-3}) analysis was carried out using the standard pycnometer technique (Blake and Hartge, 1986), and total porosity (TP,%) was calculated as:

$$\mathrm{TP} = \mathrm{PD/BD} * 100$$

Field capacity moisture content (FCM,%), and the moisture retention at different tensions were determined using a sandbox and pressure plates (Reynolds, 2007a).

The remaining sample that was not sieved through 2 mm was placed in a battery of sieves with mean diameters of 8, 4, 3, 2, and 1 mm. The soil aggregates that were retained by the largest sieve corresponded to the > 8 mm aggregate class, while the ones that passed the 1 mm sieve were < 1 mm class; both fractions were weighed and then discarded. The remaining aggregate size fractions were: 4–8, 3–4, and 2–3, which were utilized for determining the structural stability of the soil (De Boodt et al., 1967). The structural stability index (SSI) was calculated as the inverse of the mean weight diameter loss of the different aggregate size fractions after wet sieving.

Additional 6 cylinders were extracted (244.1 cm^{-3}) in the A-horizons in order to determine the saturated hydraulic conductivity (K) in the laboratory, using a constant load permeameter in undisturbed samples (Reynolds, 2007a). Infiltration rate was determined using the double ring infiltrometer (Reynolds, 2007b), with a 21.5 cm inner diameter and 30 cm outer diameter cylinder inserted 10 cm into the soil. Four measurements were carried out at each site, on a 20 m transect, with infiltrometers equidistant at 5 m and at equal distances to tree trunks.

Statistical Analysis

Data obtained from soil samples of the horizons described in the soil profiles were not replicated and therefore no statistical analysis was performed on them while the samples taken by the steel cylinder had 8 replicates each and were analyzed by one-way analysis of variance and Tukey test ($p < 0.05$) to determine significant differences between means. A principal component analysis (PCA) was carried to with the data of pH, CEC, C/N, BD, SSI, and IR from replicated samples to establish the relationships among variables and between these variables and the different treatments. All statistical analyses were carried out with InfoStat/P software (Di Rienzo et al., 2017).

RESULTS

Soil Morphology

Table 1 shows the morphological and chemical characteristics of soil profiles under different forest treatments compared with an agricultural soil as control. Profiles were similar with regards to their total depth (106–140 cm), except for G, which was 200 cm deep, and they also had a similar horizon sequence of A, AC, C, C_k. All forest soils, except G, had organic horizons above the mineral A-horizon, varying in thickness between 2 and 5 cm, while E also had an O_e –horizon. PH and G developed stronger A-horizons (A_1, A_2), which reached a depth of 30 and 50 cm, respectively, while all other profiles had A-horizons that did not exceed 24 cm depth.

The color of soil horizons varied between 10 YR 3/2 moist, and 10 YR 3/3 dry in surface soil, and 10 YR 3/4–5/3 moist, and 10 YR 4/4–5/3 dry in C-horizons (data not shown). Aggregate structure followed a gradient from sub-angular blocks in A-horizons to angular blocks in deeper ones. The exception was G where a granular structure was found in the A_1 horizon. This treatment also showed most abundant roots in this horizon while these were very scarce in all other profiles.

Soil Chemical Properties

Base saturation (BS) and carbon contents (C) of all A-horizons, except E and PP, were well above the established limits for Mollisols (> 50% BS and ≥ 1% organic matter) (**Table 1**). The highest value for BS in A-horizons was found in G (97%), followed by Ag (85%) and PH (70%), while the lowest values corresponded to PP (49%) and E (49%), just below the threshold to be classified as a mollic epipedon. These data matched with the pH values, which were highest in Ag and G (both 7.0), whilst all other forest A-horizons had more acidic pH, with lowest values in PP (4.6) and E (4.9). The acidification of these profiles reached a depth of about 30–40 cm, below which all profiles had similar pH values of between 7 and 8. Cation exchange capacity values also were higher in all A-horizons of forest soils, ranging from 17 to 20 $\text{Cmol}_c\ \text{kg}^{-1}$, compared to the Ag treatment (16 $\text{Cmol}_c\ \text{kg}^{-1}$). Highest exchangeable Ca concentrations were found in the A-horizon of G (13.8 $\text{Cmol}_c\ \text{kg}^{-1}$), and lowest ones corresponded to PP and E (4.2 and 3.1 $\text{Cmol}_c\ \text{kg}^{-1}$, respectively).

The A-horizons also varied widely in their C contents in the upper 6 cm, ranging from 7.6 g kg^{-1} in PP to 31.3 g kg^{-1} in G (**Table 1**), and all forest treatments except PP, had higher C contents than Ag (9.1 g kg^{-1}).

The differences between forest and agriculture soils, as well as among forest profiles were noticeable down to the AC-horizons, below this depth (C horizons) all profiles showed similar values in BS, C, pH, CEC, and exchangeable Ca. The high Ca values of C-horizons, and correspondingly high BS (>100%) were due to free Ca-carbonate that was not eliminated before exchangeable cation extraction. The presence of free Ca-carbonate in the soil mass varied among profiles from a depth of around 50 cm in PH, PP and G to about 100 cm in Ag and E.

The comparison of OC and N contents in the replicated samples (**Table 2**) revealed that PM and G had significantly higher carbon (34.0 and 32.7 g kg −1, respectively) and nitrogen (4.4 and 4.8 g kg^{-1}, respectively) contents in the uppermost layer than the other forest treatments (PH 24.3 and 2.8, E 17.9, and 1.6, and PP 13.5, and 1.2 g kg^{-1} of C and N, respectively) and the agriculture plot (9.3–0.8 g kg^{-1} for C and N, respectively) (**Table 2**). The highest C/N ratios were found in E, PP, and Ag (11.2, 11.3, and 11.3, respectively), and the lowest one in G with 6.7, and PM and PH were intermediate with values of 7.1 and 8.7, respectively. Although PM and PH soils had different OC and N contents, their C/N ratio was similar. However, in the 6–12 cm depth layer there were no significant differences among treatments for OC, with values ranging from 10.5 to 11.9 g kg^{-1}, but N contents were significantly higher in PM and G (0.98 and 1.02 g kg^{-1}, respectively), resulting also in lower C/N ratios for these treatments. In all forest soils a strong stratification of OC and N was observed, for instance topsoil OC in PM and G was 3 and 2.7 times higher than OC in the 6–12 cm layer, respectively. This was not the case for Ag (0.9 times higher), and of all forest treatments PP soil was least stratified (1.2 times higher value in 0–6 than in 6–12 cm).

Soil Physical Properties

Soil particle size distribution varied very little for the A horizons of the treatments (**Table 3**) and all soils had a sandy loam texture throughout their profiles (data not shown). The physical conditions of the A-horizons differed considerably among the treatments (**Table 4**). The highest bulk density was found, as expected, in the Ag soil, and the lowest values were for the E and G treatments. For the two Aleppo pine treatments, the one with mycorrhiza showed lower BD, than the non-inoculated one, which had a similar value as PP. Particle density was also different among the treatments, although more similarities were found than for BD. Thus, E had the lowest value, followed by PM (1.96 and 2.11 Mg m^{-3}, respectively) while the highest PD was found in G and Ag. Total porosity was highest in the G treatment (59.1%), and the difference to the lowest values in the Ag, PH and PP soils was more than 20% less porosity for the latter. The moisture contents at field capacity were highest in G and PM, whereas PP had an even lower value than the PM and Ag soils (11.9, 13.2, and 13.8%, respectively).

Similarly, the SSI was best in PM and G soils (1.26 and 1.30, respectively), PH, E, and PP were intermediate (1.16, 1.13, and 0.96, respectively), while the Ag soil had a much inferior value

TABLE 1 | Properties of the soil profiles under different forest species (total organic carbon-TOC; cation exchange capacity-CEC).

Treatment	Horizon	Depth	TOC	pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CEC	Base saturation
		cm		Cmol$_c$ kg^{-1}					(%)	
Pinus halepensis	O_i	0–4	–	–	–	–	–	–	–	–
PH	A1	4–18	18.1	5.5	6.7	1.9	0.6			
								2.2	17.2	67
	A2	18–30	8.1	5.8	6.7	2.8	0.6	2.7	15.2	84
	AC_b	30–55	4.6	7.1	7.6	2.8	0.6	2.1	16.4	80
	C_{Kb}	55–107	–	7.9	25.5	0.9	0.7	1.3	16.8	168
Pinus halepensis with mycorrhiza PM	O_i	0–4	–	–	–	–	–	–	–	–
	A	4–21	29.7	6.3	8.0	2.3	0.	2.5	19.2	70
	AC_d	21–40	9.5	6.8	7.1	3.2	0.6	2.8	18.4	75
	C	40–73	–	7.1	9.8	2.7	0.6	2.5	17.6	89
	C_k	73–116	–	8.0	27.2	0.8	0.7	2.3	16.8	185
Eucalyptus spp. E	O_i	0–3	–	–	–	–	–	–	–	–
	O_e	3–5	–	–	–	–	–	–	–	–
	A	5–20	11.8	4.9	3.1	2.3	0.8	2.3	17.2	49
	AC	21–40	8.6	5.5	4.9	2.9	0.8	2.4	16.0	69
	C	40–97	–	7.7	8.5	2.3	0.6	2.0	16.8	80
	C_k	97–106	–	–	–	–	–	–	–	–
Pinus pinea PP	O_i	0–2	–	–	–	–	–	–	–	–
	A	2–24	7.6	4.6	4.2	2.0	0.7	2.6	19.2	49
	AC	24–47	4.7	5.0	5.4	2.3	0.5	2.6	19.2	56
	C_k	47–139	–	7.9	25.9	1.3	0.7	2.7	19.6	156
Gleditsia triacanthos G	A_1	0–18	31.3	7.0	13.8	1.7	1.4	2.6	20.0	97
	A_2	18–50	9.3	6.4	7.1	1.9	1.2	2.6	19.6	65
	AC_k	50–71	3.9	7.8	24.1	0.9	0.4	2.7	19.2	146
	C_k	71–200	–	8.2	25.9	2.1	0.5	2.9	19.2	164
Agriculture Ag	Ap	0–27	9.1	7.0	8.0	2.3	0.6	2.7	16.0	85
	AC	27–47	4.5	6.8	5.8	2.8	0.6	3.0	14.8	83
	C	47–104	–	7.9	8.9	3.6	0.6	1.6	16.0	92
	C_K	104–127	–	8.1	24.6	1.3	0.6	1.6	16.0	176

Samples were taken from the center of the horizons.

(0.47). In short, Ag and PP soils had the worst physical condition, while the best soil structure was found in G and PM.

The hydraulic properties (**Figure 2**) reflected the differences in soil structure and porosity. The best hydraulic conductivity was found in PM and G soils (17.5 and 15.4 cm h^{-1}, respectively), and the lowest conductivity corresponded to E and Ag soils (2.7 and 5.8 cm h^{-1}, respectively). The difference in magnitudes was important since PM had 6.5 times and G 5.7 times the hydraulic conductivity of the soil under eucalypt forest. Similarly, the infiltration rate was lowest in E and PP (111- and 78-mm h^{-1}, respectively), which represented on average 2.7 times less than the best treatments (PH, PM, and G).

The principal component analysis (**Figure 3**) explained 90.7% of the variability of the chosen variables (pH, CEC, C/N, SSI, BD, and IR). The first PC was defined positively by C/N and BD, and negatively by CEC, SSI, and IR, while the second PC was primarily defined by pH and IR (**Table 5**). Among the forest treatments, G and PM were associated with high IR, CEC, and SSI, while on the opposite side PP an E were associated to high C/N and BD. An intermediate position was shown by PH. On the second PC, Ag and to a lesser degree G were different from the other treatments and more related with higher pH while E and PP were in the opposed segment of the bi-plot, indicating lowest pH values and IR. The correlation matrix among the original variables (**Table 6**) showed that SSI and BD were strongly and negatively related (R^2 = –0.93) as was to be expected, like the negative relation between BD and IR (R^2 = –0.74). However, these indicators of soil structure were also related to chemical properties such as CEC (BD vs. CEC: R^2 = –0.72) and C/N (IR vs. C/N: R^2 = –0.94; and C/N vs. BD: R^2 = 0.91). This indicated that soil physical, biological, and chemical indicators were interrelated and connected.

DISCUSSION

The fact that soil texture showed no meaningful variation among soil profiles indicated that parent material and its particle size distribution was sufficiently similar as to be able to attribute differences in chemical and physical properties to the effect of land use and forest species. The data obtained showed that the effects of the five forest treatments on the original Haplustoll soil generated significant differences in the morphology of their soil profiles and their chemical and physical attributes.

The most important divergences were seen under E and PP forestations, where acidification of the A and AC horizons resulted in a change in the taxonomic classification, assigning these soils to the Alfisol soil order, due to the fact that with less than 50% base saturation a soil does not classify as a Mollisol (Soil Survey Staff, 2014). The calcium loss and acidification brought about by coniferous forest species has been shown in other climatic and soil conditions, and several mechanisms including an increase in organic acids, acid deposition, and absorption of nutrients have been proposed as the reasons for this (Mareschal et al., 2010). It has also been shown that poor leaf litter quality contributes to the absence of a burrowing earthworm community, which retards leaf litter decomposition and, consequently, leads to forest-floor build-up and soil acidification (Schrijver et al., 2012). Certini et al. (1998) attributed the acidity of stemflow under pine to cause the leaching of cations and organic matter. Both these latter processes most likely occurred in the Eucalypt and Stone pine plots of our experiment. Similar trends of enhanced acidification under Eucalypt plantations have been reported in Ethiopian abandoned farmland soils, where the authors concluded that soil properties deteriorated under Eucalypt (Lemenih, 2004). While this deterioration was strongest in the E and PP soils, PH also had an acid pH and lower base saturation than the G and Ag soils. A general and stronger trend of leaching of base cations and subsequent acidification in forested lands compared to grasslands was the result of a long-term assessment in Canadian soils (Cho et al., 2019). Our results showed that soil acidification will occur even in calcareous soils that were originally covered by grassland steppes or savannas. The scarcity of results on afforestation effects on steppe soils makes is difficult to estimate whether this trend can be generalized. However, Jobbágy and Jackson (2003) also found that afforested grassland soils of the humid Pampas suffered acidification and calcium loss to a depth of between 35 and 60 cm. In our case the only legume among the forest species and the mycorrhiza-inoculated pine were able to retain base cations and conserve a neutral to slightly alkaline pH, similar to the values of the natural grassland Mollisols of this region (INTA et al., 1980). This might be related to differences in other chemical and biological properties the G and PM soils, compared to the E, PP and PH treatments. The former had significantly higher carbon and nitrogen contents in the topsoil compared to the latter, and lower C/N ratios indicating that the litter quality and nitrogen availability were improved in G and PM, while E, PP, and PH had lower quality litter and lower N inputs. Högberg (2007) reported that most boreal and temperate

TABLE 2 | Total organic carbon (OC), total nitrogen (N) contents and carbon to nitrogen ratio (C/N) in the 0–6 cm and 6–12 cm depth layers of A-horizons under the five forest treatments (PH, *Pinus halepensis*; PM, *Pinus halepensis* with mycorrhiza, E, *Eucalyptus* spp.; PP, *Pinus pinea*; G, *Gleditsia triacanthos*) and the agriculture plot (Ag).

Treatment Depth (cm)	OC		N		C/N	
	0–6	6–12	0–6	6–12	0–6	6–12
	g kg^{-1}					
PH	24.3b	10.5a	2.76b	0.78b	8.7b	13.5a
PM	34.0a	11.2a	4.37a	0.98a	7.9bc	11.4b
E	17.9c	10.3a	1.60c	0.72b	11.2a	14.3a
PP	13.5d	10.5a	1.20c	0.76b	11.3a	13.8a
G	32.7a	11.9a	4.84a	1.02a	6.7c	11.7b
Ag	9.3e	10.3a	0.84d	0.88ab	11.3a	11.7b

Different letters within a column indicate significant differences among means ($p < 0.05$).

TABLE 3 | Particle size distribution and texture class in the uppermost 0–12 cm of A-horizons under the five forest treatments (PH, *Pinus halepensis*; PM, *Pinus halepensis* with mycorrhiza, E, *Eucalyptus* spp.; PP, *Pinus pinea*; G, *Gleditsia triacanthos*) and the agriculture plot (Ag).

Treatment	Sand	Silt	Clay	Texture class
	(g kg^{-1})			
PH	620a	226a	145a	Sandy loam
PM	640a	223a	123a	Sandy loam
PP	645a	218a	136a	Sandy loam
E	638a	221a	117a	Sandy loam
G	653a	214a	121a	Sandy loam
Ag	659a	209a	131a	Sandy loam

Different letters within a column indicate significant differences among means ($p < 0.05$).

TABLE 4 | Soil physical properties of the upper 6 cm of A-horizons of forest (PH, *Pinus halepensis*; PM, *Pinus halepensis* with mycorrhiza, E, *Eucalyptus* spp.; PP, *Pinus pinea*; G, *Gleditsia triacanthos*) and agricultural (Ag) soil profiles at the Santa Rosa site.

Treatment	Bulk density	Particle density	Total porosity	Field capacity moisture content	Structural stability index
	Mg m^{-3}		%	% w/w	
PH	1.12b ± 0.10	2.14ab ± 0.14	47.9c ± 3.51	12.2bc ± 2.04	1.16b ± 0.33
PM	1.05c ± 0.12	2.11b ± 0.06	50.4b ± 5.53	14.2a ± 1.05	1.26a ± 0.45
E	0.97d ± 0.09	1.96c ± 0.11	50.8b ± 4.22	14.2a ± 1.52	1.13b ± 0.55
PP	1.13b ± 0.04	2.20ab ± 0.07	48.9bc ± 1.49	11.9bc ± 0.73	0.96c ± 0.18
G	0.96d ± 0.10	2.35a ± 0.11	59.1a ± 3.22	13.9a ± 2.40	1.30a ± 0.53
Ag	1.21a ± 0.05	2.24a ± 0.09	45.5c ± 3.17	11.6c ± 0.93	0.47d ± 0.12

SD values are shown as ±. Different letters within a column indicate significant differences among means ($p < 0.05$).

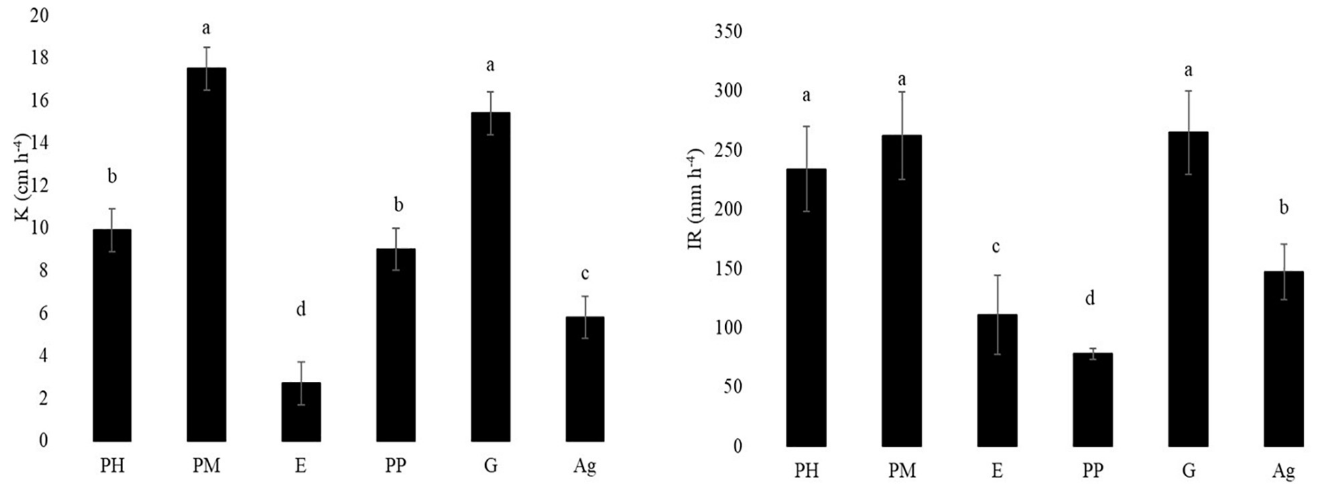

FIGURE 2 | Hydraulic conductivity (K, cm h^{-1}) and infiltration rate (IR, mm h^{-1}) in the five forest treatments (PH- Pinus halepensis, PM- Pinus halepensis with mycorrhiza, E-*Eucalyptus* spp., PP-Pinus pinea, G-Gleditsia triacanthos) and the agriculture plot. Different letters indicate significant differences among means ($p > 0.05$), bars indicate SD.

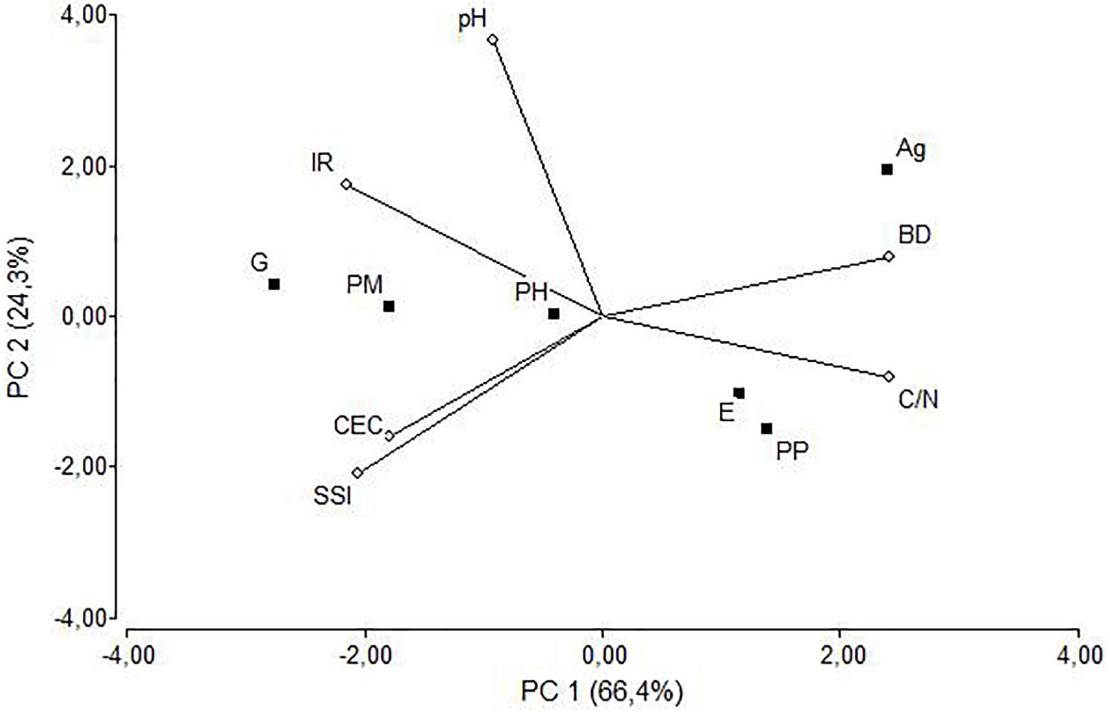

FIGURE 3 | Bi-plot of the principal component analysis for the five forest treatments and the agricultural soil. Variables are pH, cation exchange capacity (CEC), infiltration rate (IR), structural instability index (SSI), bulk density (BD), and carbon to nitrogen ratio (C/N).

forests are nitrogen limited and respond with higher carbon sequestration to anthropogenic nitrogen inputs. The N-limitation in E, PP and PH might therefore be one of the reasons for their lower carbon contents, due to the restriction this imposes on microbial activity. This has been shown for agricultural soils in this region, where crop sequences without legumes accumulated litter on the soil surface, but had lower topsoil carbon contents (Frasier et al., 2016), similarly Almagro et al. (2021) found that higher N availability in residues promoted C and N stabilization in soil aggregates. In the PM soil, the enhanced decomposing activity due to the presence of ectomycorrhiza facilitated the nutrient cycling and carbon stabilization in the soil, as has been reported for different types of forests (Wallander et al., 2011; Clemmensen et al., 2013; Li et al., 2015). Lin et al. (2017) found

TABLE 5 | Correlation between the original variables and the principal components.

	PC 1	PC 2
IR	−0.86	0.42
CEC	−0.72	−0.39
pH	−0.37	0.89
C/N	0.98	−0.19
SSI	−0.83	−0.51
BD	0.97	0.19

Variables are pH, cation exchange capacity (CEC), infiltration rate (IR), structural instability index (SSI), bulk density (BD), and carbon to nitrogen ratio (C/N).

TABLE 6 | Correlation between variables used in the principal component analysis.

	IR	CEC	pH	C/N	SSI	BD
IR	1					
CEC	0.34	1				
pH	0.63	0.04	1			
C/N	−0.94	−0.6	−0.52	1		
SSI	0.54	0.67	−0.18	−0.71	1	
BD	−0.76	−0.72	−0.19	0.91	−0.93	1

Variables are pH, cation exchange capacity (CEC), infiltration rate (IR), structural instability index (SSI), bulk density (BD), and carbon to nitrogen ratio (C/N).

that trees which live in symbiosis with ectomycorrhiza usually have greater forest-floor C stocks, but also might accrue more C in the mineral horizons as occurred here in the case of PM compared to its uninoculated counterpart, PH. The authors attribute this to the fact that ectomycorrhiza on one hand have a lower decomposition rate than other soil microorganisms, and on the other hand they can take up organic N compounds from soil organic matter, thus enabling the transformations of low-quality forest litter. This also seems to explain the slightly lower C/N ration in the PM soil compared to PH.

The C/N ratios observed in these forest soils were considerably lower than those reported from a study on European forest soils under different tree species, where black locust forest floor had a ratio of less than 20, while evergreen trees had higher ones (>30). In the mineral topsoil these ratios were lower, for instance an average ratio of 17.6 was found for Aleppo pine (Cools et al., 2014). But these authors also found a strong effect of environmental conditions such as mean annual temperature and precipitation, indicating that warmer and drier climates generally had lower soil C/N ratios than cold and humid regions. Soil type also influenced C/N, with lowest values in Chernozem and Kastanozem A horizons. The soils of the present study would be classified as Kastanozem (IUSS et al., 2015) and under natural savanna vegetation have C/N ratios between 6 and 8 (Noellemeyer et al., 2006).

The amount of carbon stored under Eucalypt and Stone pine forest was lower than under Black locust and ecto-mycorrhiza-inoculated Pine plantations, while the Aleppo pine soil had an intermediate value of OC and showed an intermediate position with regards to other soil properties analyzed by the PCA. The strong correlations between biological (C/N), chemical (CEC, pH), and physical properties (SSI, IR) showed that all three dimensions of soil fertility are interrelated and that the effect of the vegetation to sequester more carbon also results in improved soil structure and chemical properties. The strong feedback between soil physical and biological processes in these soils was already shown by Fernández et al. (2019) in a comparison of natural vegetation and agricultural sites.

Soil physical properties under the different forest species also showed significant divergence. Lower organic C stocks and pH, and consequently less Ca^{2+} in the soil solution for colloidal stabilization, affected soil structure and pore system (Rawlins et al., 2016; Helliwell et al., 2017). Therefore E, PP, and PH had consistently poorer values for soil physical quality indicators (i.e., higher BD, lower porosity, lower SSI) than G and PM. This reflects the strong interaction between soil pores and soil carbon processes (Kravchenko and Guber, 2017). The deficient hydrological integrity of the soils under E, PP, and PH is clear from their extremely low hydraulic conductivity and infiltration rates, compared to PM and G, indicating high runoff even in rainfall events with low intensity. The low infiltration and hydraulic conductivity are strongly conditioned by the poor soil structure, including pore size distribution and morphology (Fernández et al., 2021). This is probably aggravated by the hydrophobicity of the forest floor under these species. Ellerbrock et al. (2005) found that for soil organic carbon contents <10 g kg^{-1} wettability increased with OC content, while it decreased for OC contents >10 g kg^{-1}, but they also attributed wettability to organic matter composition, presumably the higher lipid contents of the litter floor under eucalypt and conifers would cause higher water repellency (Mataix-Solera and Doerr, 2004; Bodí et al., 2013). However, the hydrophobicity also depends strongly on the soil moisture content, and stronger water repellency was observed at lower moisture contents (Greiffenhagen et al., 2006), which would be aggravated by the fact that evergreen trees were shown to have a drier soil regime since the throughfall rate of rainwater is lower than in deciduous trees (Augusto et al., 2015).

Thus, in terms of chemical, biological, and physical fertility afforestation with Eucalypt and uninoculated conifers did not result in an improvement. On the contrary, these species deteriorated soil quality, similar to the results of Liu et al. (2021), who concluded that the use of native shrubs and grasses was more effective for restoring soil fertility on degraded croplands than afforestation.

CONCLUSION

Our results confirmed that Eucalypt and Pine plantations without ectomycorrhiza have a deleterious effect on soil biological, chemical, physical conditions, and on the soil profile morphology, resulting in a different taxonomic order for these soils, due to their low base saturation. On the other hand, the soils under Black locust and Aleppo pine with mycorrhiza showed significantly better physical and hydraulic conditions, which is to be expected given their higher organic matter and nitrogen contents, and lower C/N ratio, indicating that microbial activity was not limited by substrate quality nor by habitat restrictions. The inoculation of pine with ectomycorrhiza and the higher nitrogen availability under the legume species resulted in a positive change of soil biological and physical conditions, enhancing the importance of biological activity for essential soil functions to be preserved. However, considering the scarceness of information of the effects of plantations on steppe and grassland soils, further studies are needed to confirm the proposed processes and effects caused by different forest species on dryland Mollisols.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

GM and DR carried out field work and lab analysis. EL and LÁ helped with data analysis and manuscript preparation. EN coordinated the study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.685827/full#supplementary-material

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Microbial Substrate Utilization and Vegetation Shifts in Boreal Forest Floors of Western Canada

*Emily Lloret[1,2] and Sylvie Quideau[1]**

[1] Renewable Resources Department, University of Alberta, Edmonton, AB, Canada, [2] Univ. Lille, IMT Lille Douai, Univ. Artois, Yncrea Hauts-de-France, ULR 4515 – LGCgE, Laboratoire de Génie Civil et Géo-Environnement, Lille, France

Boreal forest soils are highly susceptible to global warming, and in the next few decades, are expected to face large increases in temperature and transformative vegetation shifts. The entire boreal biome will migrate northward, and within the main boreal forest of Western Canada, deciduous trees will replace conifers. The main objective of our research was to assess how these vegetation shifts will affect functioning of soil microbial communities and ultimately the overall persistence of boreal soil carbon. In this study, aspen and spruce forest floors from the boreal mixedwood forest of Alberta were incubated in the laboratory for 67 days without (control) and with the addition of three distinct ^{13}C labeled substrates (glucose, aspen leaves, and aspen roots). Our first objective was to compare aspen and spruce substrate utilization efficiency (SUE) in the case of a labile C source (^{13}C-glucose). For our second objective, addition of aspen litter to spruce forest floor mimicked future vegetation shifts, and we tested how this would alter substrate use efficiency in the spruce forest floor compared to the aspen. Tracking of carbon utilization by microbial communities was accomplished using ^{13}C-PLFA analysis, and ^{13}C-CO$_2$ measurements allowed quantification of the relative contribution of each added substrate to microbial respiration. Following glucose addition, the aspen community showed a greater ^{13}C-PLFA enrichment than the spruce throughout the 67-day incubation. The spruce community respired a greater amount of ^{13}C glucose, and it also had a much lower glucose utilization efficiency compared to the aspen. Following addition of aspen litter, in particular aspen leaves, the aspen community originally showed greater total ^{13}C-PLFA enrichment, although gram positive phospholipid fatty acids (PLFAs) were significantly more enriched in the spruce community. While the spruce community respired a greater amount of the added ^{13}C-leaves, both forest floor types showed comparable substrate utilization efficiencies by Day 67. These results indicate that a shift from spruce to aspen may lead to a greater loss of the aspen litter through microbial respiration, but that incorporation into microbial biomass and eventually into the more persistent soil carbon pool may not be affected.

Keywords: boreal, forest floor, vegetation shifts, labeled substrate, phospholipid fatty acid analysis, SIP–PLFA, microbial communities

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Laurent Augusto,
INRA Centre Bordeaux-Aquitaine,
France

Reviewed by:
Susan Ziegler,
Memorial University of Newfoundland,
Canada
Antra Boca,
Latvia University of Agriculture, Latvia

***Correspondence:**
Sylvie Quideau
sylvie.quideau@ualberta.ca

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INTRODUCTION

The boreal forest is the largest terrestrial ecosystem and the largest terrestrial sink of carbon on Earth (Watson et al., 2000). Indeed, boreal forests account for over half of the forest carbon stocks globally (DeLuca and Boisvenue, 2012). The boreal forest forms a "green belt" between latitude 45° and 70° N, and in Canada, it stretches over 307 million ha from the Yukon to Newfoundland and Labrador. Due to the extreme climatic conditions occurring in this region, the boreal forest is characterized by a low diversity of tree species, including *Abies*, *Larix*, *Picea*, *Pinus*, and *Populus* (Brandt et al., 2013). In the boreal mixedwood forests of western Canada, *Picea glauca* (white spruce) and *Populus tremuloides* (trembling aspen) are the most common species (Lieffers et al., 1996). The boreal is particularly sensitive and vulnerable to global warming, and in the next few decades, is expected to face transformative changes; it is likely that the region will see a 2°C temperature increase by 2050 compared to 2000 (Price et al., 2013). Ongoing increases in atmospheric CO_2 and warming have already been linked to vegetation shifts where early-successional deciduous broadleaves have been replacing late-successional conifers since the 1950s (Searle and Chen, 2017). Predictions for precipitation are not as straightforward, although frequency and intensity of extreme events, including drought, are expected to increase. Boreal tree species exhibit a wide range of responses to the changing climate, and broadleaved trees are less sensitive to moisture deficit than conifers (Hogg et al., 2017; D'Orangeville et al., 2018). In the mixedwood boreal forest of Western Canada, this will mean an accelerated shift from white spruce to trembling aspen stands.

The composition and activity of forest floor microbial communities are the result of complex interactions between biotic and abiotic factors including climate, tree species, quality and quantity of organic matter input, soil fauna, nutrient availability, and land disturbance (e.g., Myers et al., 2001; Grayston and Prescott, 2005; Quideau et al., 2013; Cleveland et al., 2014). Differences in microbial communities associated with individual plant species are hypothesized to result from variations in the quantity and quality of plant carbon inputs (Grayston and Prescott, 2005), and coniferous forest floors (i.e., surficial soil organic horizons containing litter at various stages of decomposition) typically have lower microbial biomass and activity than those under deciduous cover (Bauhus et al., 1998). Similarly, in the mixedwood boreal forest of Western Canada, forest floors from white spruce and aspen stands have distinct microbial communities and distinct properties, including greater microbial biomass and higher pH and nutrient availability in aspen forest floors (Hannam et al., 2006; Swallow and Quideau, 2013). In a systematic review comparing aspen and coniferous stands across North America, Laganière et al. (2017) concluded that forest floor carbon stocks were larger in conifer dominated stands in the majority of studies. In the mixedwood of central Alberta, Kishchuk et al. (2014) reported average soil carbon stocks of 25 and 42 Mg ha^{-1}, for aspen and spruce forest floors, respectively. On the other hand, soil carbon under aspen appears to be more stable than under spruce (Laganière et al., 2017), and this has recently been linked to preferential retention of aspen foliage leachates compared to spruce needle leachates (Boča et al., 2020). What remains unclear, however, is how current and future vegetation shifts may be affecting carbon storage and persistence in boreal soils.

The home-field advantage (HFA) concept, which stipulates that litter decomposes faster in an area dominated by plants from which it is derived (i.e., at home) rather than under an alternative plant cover, has been proposed as a widespread phenomenon occurring in most forest ecosystems (Ayres et al., 2009). According to the HFA theory, a rapid shift in vegetation could mean a slow decomposition of the new aspen litter by the microbial community already present in the native spruce forest floor. On the other hand, when labile organic substrates are added to soil, microbial activity is commonly stimulated and a short-term change in the turnover of native organic matter [termed "priming effect" (PE)] may occur in which both added substrate and indigenous soil organic matter (SOM) are degraded (Waldrop and Firestone, 2004; Kuzyakov, 2010). A positive PE following vegetation shifts could potentially increase decomposition as fresh, more labile aspen litter is added to native spruce forest floor. Furthermore, an increasingly accepted paradigm highlights the importance of microbial anabolism, and substrate utilization efficiency (SUE), in retaining plant-derived carbon and eventually increasing the persistence of carbon in soils (Cotrufo et al., 2013; Waring et al., 2020; Neupane et al., 2021). Several interacting factors, including the composition of soil microbial communities and substrate stoichiometry, can alter SUE (Rinnan and Baath, 2009; Keiblinger et al., 2010; Kohl et al., 2021). Global carbon stocks in boreal forests are estimated to reach 1095 Pg of carbon (Bradshaw and Warkentin, 2015). As about a quarter of these stocks are stored in boreal forest floors themselves (Kurz et al., 2013), it is important to assess how aspen replacing spruce may alter the carbon balance of boreal forest floors.

Stable isotope probing (SIP) of phospholipid fatty acids (PLFAs) found in microbial membranes allows tracking of ^{13}C-labeled substrates as they become incorporated into soil microbial communities (Waldrop and Firestone, 2004; Watzinger, 2015; Feland and Quideau, 2020). In this study, aspen and spruce forest floors from the mixedwood boreal forest of Alberta were incubated following addition of ^{13}C-enriched glucose, aspen leaves and aspen roots that had been previously ^{13}C-labeled, and results were compared to control forest floors (no substrate added). Our first objective was to assess if differences between aspen and spruce forest floors would foster differences in soil microbial metabolism, as indicated by estimating SUE following addition of ^{13}C-glucose. Secondly, addition of ^{13}C-labeled aspen litter to spruce forest floor mimicked the effects of anticipated future vegetation shifts in the boreal mixedwood forest of Western Canada. Hence, the second objective of our study was to test whether the addition of fresh aspen litter to spruce forest floor would alter microbial utilization processes, and in turn, to ascertain implications in terms of overall carbon persistence in these soils. Respired $^{13}C\text{-}CO_2$ was measured to quantify the percent of CO_2 coming from each added substrate, and carbon assimilation by different members of the forest floor microbial community was tracked using PLFA-SIP.

MATERIALS AND METHODS

Soil Collection and Aspen Seedling Labeling

Soil samples (0–10 cm) were collected from two mature aspen and white spruce stands (>70-year-old) located in the boreal forest of Northern Alberta, Canada (**Table 1**). The region experiences a mean annual temperature of 0.7°C and a mean annual precipitation of 45 cm. These two stands were selected within 2 km of each other to minimize climatic variation and were established on similar fine-textured Luvisols (IUSS Working Group WRB, 2015). Both sites were selected from previous studies investigating plant and soil microbial communities in the area (Norris et al., 2013, 2016; Quideau et al., 2013). The two soil types were collected in preparation of the laboratory incubation by sampling ten locations within each stand and then composited to obtain homogenous samples. Forest floors averaged 19.5 cm for the spruce and 7 cm for the aspen stand (**Table 1**); while samples were contained within the forest floor layers (i.e., soil organic layers with >17% C) for the spruce stand, the aspen samples also included some underlying mineral soil. Soil samples were kept cool and transported to the laboratory at which time they were sieved to 4 mm (Kalra and Maynard, 1991), and stored in the dark until the start of the incubation experiment.

Enriched aspen leaves and roots were obtained by growing seedlings in a greenhouse as described by Norris et al. (2012). After five weeks of growth, seedlings were labeled once a week for three consecutive weeks with 99.9% $^{13}C\text{-}CO_2$ (Cambridge Isotope Laboratories, Inc. Andover, MA, United States). The enriched $^{13}CO_2$ gas was applied to each seedling for 30 min each week, and seedlings were harvested one week after the last pulse. Leaves and roots were separated, dried, ground and stored until the start of the incubation experiment. The abundance of ^{13}C in the labeled plants was measured to average 228 ± 18‰ for the aspen leaves and 128 ± 35‰ for the aspen roots.

Laboratory Incubation

Prior to the start of the laboratory incubation, deionized water was added to soils to reach a moisture content equivalent to field capacity (−12 kPa), as estimated by the pressure plate method (Swallow and Quideau, 2013). Resulting moisture contents were 98 ± 3%, and 210 ± 13% for the aspen and spruce forest floors, respectively (**Table 1**). Sixty experimental units were constructed for each forest floor type (aspen and white spruce) and were left to equilibrate for two weeks before the labeled substrates were added. Moisture content was maintained during the preconditioning period as well as during the incubation itself by regular weighing and application of any lost moisture. Each experiment unit consisted of 2 g of equivalent dried soil in a small tube resting at the bottom of a 1 L airtight glass jar. Lids were fitted with Luer Lock valves to allow for gas sampling.

The incubation experiment began with the addition of the labelled substrates, which were either dissolved (glucose), or suspended as fine powder (leaves and roots) in deionized water. After addition to the soils, each tube was shaken to promote homogenous incorporation of the substrate. For each soil type, 15 experimental units were used as control, where no substrate was added. For the other 45 experiment units, 15 were used for incubations with a ^{13}C labeled synthetic substrate (Sigma Aldrich, 99% D-glucose-1-^{13}C), 15 for incubations with ^{13}C-labeled aspen leaves, and 15 with ^{13}C-labeled aspen roots. The quantity of substrate to add was calculated to correspond to the same amount of carbon for each substrate, which was chosen as 0.4 mg C g^{-1} of dry soil to optimize respiration fluxes as shown by Larionova et al. (2007). Soil samples were incubated in the dark at room temperature (20 °C) for 67 days. For the entire duration of the experiment, every 2–3 days, the jars were opened and ventilated for 30 min to maintain aerobic conditions. Before opening the jars, gas samples were taken to estimate accumulated CO_2 from five randomly chosen units for the two soil types and each incubation type (control, glucose, aspen leaves, and aspen roots). In addition, the same flasks were sampled nine times for isotopic composition analysis of CO_2 ($\delta^{13}CO_2$), namely after 0.2, 2, 7, 14, 18, 25, 46, 60, and 67 days of incubation. Lastly, five experimental units for each incubation type and each soil were destructively sampled after 0.2, 14, and 67 days of incubation. These were immediately frozen at −80°C to be later freeze-dried prior to PLFA analysis.

Soil Respiration Measurements

For quantitative CO_2 and $\delta^{13}CO_2$ measurements, two aliquots were taken from the experimental unit headspaces. One aliquot was transferred to an evacuated borosilicate Labco Exetainer (Labco Limited, High Wycombe, United Kingdom), and accumulated (respired) CO_2 was analyzed on a HP5890 Series II gas chromatograph and a HP3396 Series II Integrator

TABLE 1 | Key properties of the aspen and spruce forest floor materials used during the 67-day laboratory incubation.

		Aspen forest floor	Spruce forest floor
Location		N56 57.5, W111 38.9	N56 56.6, W111 44.3
Forest floor thickness	(cm)	7.0	19.5
Moisture content	(%wt)	98 ± 3	210 ± 13
Soil organic carbon	(mgC g^{-1})	207	455
Soil organic nitrogen	(mgN g^{-1})	9.8	18.9
$\delta^{13}C$*	‰	–27.8	–27.5
Total PLFAs	(nmol g^{-1})	4,800 ± 600	3,900 ± 700

*From Norris et al. (2013, 2016).

(Hewlett-Packard, Santa Clara, CA, United States), using helium as the carrier gas. The amount of respired CO_2 in each incubation unit headspace was corrected for the background CO_2 concentration present in the room, and converted from ppmv to gC using the ideal gas law.

The second aliquot was transferred to a helium flushed positive pressure Labco Exetainer, and $\delta^{13}CO_2$ of respired CO_2 was measured using a CTC Combi PAL autosampler (CTC Analytics AG, Zwingen, CH) leading to a Porapak Q column on a Finnigan Gas Bench II (Thermo Electron Corporation, Waltham, MA, United States) attached to an Isotope Ratio Mass Spectrometer (IR-MS). The $\delta^{13}C$ values of CO_2 were reported against the Vienna Pee Dee Belemnite standard (isotopic ratio: $R_{VPDB} = 0.0111802$) and were corrected for atmospheric contribution on each sampling date according to:

$$\delta^{13}\mathrm{C_r} \times [\mathrm{CO_2}]_\mathrm{r} = \delta^{13}\mathrm{C_i} \times [\mathrm{CO_2}]_\mathrm{i} - \delta^{13}\mathrm{C_a} \times [\mathrm{CO_2}]_\mathrm{a}$$

where the subscript "r," "I," and "a" refers to the CO_2 "respired," the total CO_2 measured in the "incubation unit" and the contribution from the "atmosphere," respectively. Atmospheric CO_2 concentrations and $\delta^{13}C$ values were measured in replicate prior to sampling of the experimental units; over the 67-day incubation period, concentration averaged 505 ± 106 ppmv, and $\delta^{13}CO_2$ was $-9.9 \pm 1.2‰$.

Phospholipid Fatty Acid Analysis

Phospholipid Fatty Acid analysis was conducted on 0.9 g of freeze-dried soil using a Bligh and Dyer extractant containing a 0.15M citrate buffer at the methanol:chloroform:buffer 1:1:0.9 ratio according to Quideau et al. (2016). Prior to extraction, PC(19:0/19:0) nonadecanoate surrogate standard (Avanti Polar Lipids Inc., Alabaster, AL, United States) was added to each sample to assess recovery. Extracted lipids were separated on solid phase extraction (SPE) silica columns (Agilent Technologies, Santa Clara, CA, United States), and re-dissolved in 1:1 chloroform:methanol prior to mild alkaline methanolysis and subsequent extraction with hexane to synthesize fatty acid methyl esters (FAMEs), as specified in White et al. (1997). An instrument standard of 10:0Me (methyl decanoate, Aldrich, St. Louis, MO, United States) was added prior to identification and quantification on a HP5890A Series II GC equipped with a HP7673 Injector, and an FID detector (Hewlett-Packard, Santa Clara, CA, United States). Peaks were identified using the Sherlock Microbial Identification System Version 4.5 software (MIDI, Inc., Newark, NJ, United States). PLFAs were described using the standard X:YωZ nomenclature, and their abundance in each sample was expressed as nmol PLFA g^{-1} of soil.

The $\delta^{13}C$ values (‰) of individual PLFAs were measured using a 6890N Agilent GC (Agilent Technologies, Santa Clara, CA, United States) equipped with an HP-Ultra 2 column (50 m length, 0.2 mm i.d. and 0.33 μm film by J&W Scientific Columns from Agilent Technologies), and linked to a Thermo Finnigan GC Combustion III (Thermo Finnigan, Bremen, Germany) and the IRMS. Retention times for individual PLFAs were calibrated using FAME mixes GLC-30 and C4-C24 Unsaturated (Sigma Aldrich). In addition, retention times were compared to those from the GC-FID to ensure their correct assignment. Peaks with low intensity (<200 mV) were discarded due to low signal to noise ratios. Measured values were expressed in the δ-notation (‰) and corrected for the additional methanol group added during the last step of the PLFA extraction. Values were calibrated against three 20:0 isotope standards (USGS70, USGS71, and USGS72).

Microbial biomarkers were equated to PLFAs containing between 14 and 20 carbons, and included: saturated straight chained PLFAs (e.g., 15:0); saturated PLFAs with branching on the second (e.g., i15:0) or third (e.g., a15:0) carbon from the aliphatic end; saturated PLFAs with mid-chain (10-methyl) branching (e.g., 16:010Me); monounsaturated PLFAs (e.g., 16:1ω5); polyunsaturated PLFAs (18:3ω6); and cycloPLFAs (e.g., 17:0cyclo). Biomarkers were assigned to microbial groups according to the literature (e.g., Zelles, 1999; Myers et al., 2001; Frostegård et al., 2010; Quideau et al., 2016), where 10Me PLFAs are typically associated with Actinobacteria, branched saturated PLFAs to gram positive bacteria, monounsaturated PLFAs to gram negative bacteria, and polyunsaturated PLFAs to fungi. The number of selected biomarkers was 35 when using results from the MIDI software (mol%), and $\delta^{13}C$ values (‰) were obtained for 22 of these.

Data Analysis

Data collected from the daily CO_2 measurements from each individual flask were summed to estimate cumulative CO_2 fluxes over the entire incubation period, and average values and standard deviations were calculated for the two forest floor types with and without substrate addition ($n = 5$). Statistical differences among respiration rates from different substrates and forest floor types (mgC-CO_2/gCsoil) were tested for each sampling date using a one-way ANOVA followed by a Tukey's HSD post-hoc test. A Bonferroni adjustment was used for multiple comparisons to maintain the family-wise Type I error rate at 0.05.

The percentage of respired CO_2-C coming from the added substrate ($\%C_{sub}$) was calculated with a mixing model as in Waldrop and Firestone (2004):

$$\%\mathrm{C_{sub}} = \left(\frac{\delta_\mathrm{C} - \delta_T}{\delta_\mathrm{C} - \delta_\mathrm{S}}\right) \times 100$$

where δ_C is the $\delta^{13}C$ value of the respired CO_2 from the control (no added substrate) soils, δ_T is the $\delta^{13}C$ respired CO_2 in the treated soils, and δ_S is the $\delta^{13}C$ of the labeled substrate. The cumulative substrate-derived CO_2 was also calculated in mgC-CO_2/g soil by multiplying the amount of respired CO_2 by the percentage of respired CO_2-C coming from added substrate (%Csub) for each date. A Student's t-test was conducted to detect statistical differences between aspen and spruce for each added substrate.

The difference in SOM mineralization following substrate addition, named "PE," was calculated by comparing the amount of unlabelled CO_2 production with and without substrate addition according to the equation from Blagodatsky et al. (2010):

$$\mathrm{PE} = \mathrm{CO_2}^{\mathrm{SOM}} - \mathrm{CO_2}^{\mathrm{control}}$$

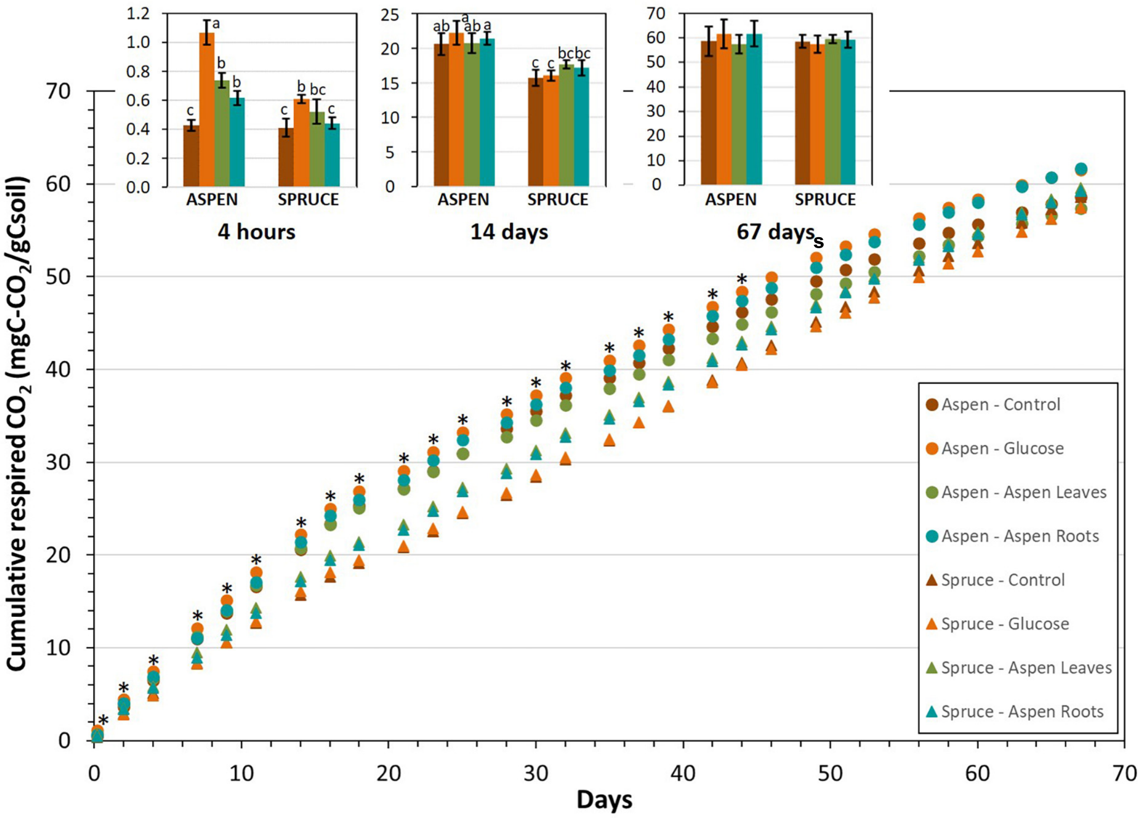


FIGURE 1 | Average cumulative respired C-CO$_2$ from the aspen (circles) and spruce (triangles) soil incubation units for all substrates (glucose, aspen leaves and aspen roots) during the 67-day laboratory incubation. Asterisks indicate dates where statistical differences among samples were detected using a one-way ANOVA. In addition, the inset bar graph shows average cumulative respired CO$_2$ from the aspen and spruce soil incubation units at three dates (4 h, 14 days, and 67 days), with error bars showing standard deviations over five replicates and different letters indicating significant differences (p < 0.05).

where CO$_2$ control is evolved CO$_2$ from the control soil, and CO_2^{SOM} is the respired CO$_2$ coming from SOM mineralisation in the amended soil according to:

$$CO_2{}^{SOM} = (100 - \%C_{sub}) \times [CO_2]_r$$

where $\%C_{sub}$ is the percentage of respired CO$_2$-C coming from the added substrate, and $[CO_2]_r$ is the total respired CO$_2$. Data were further examined by calculating arithmetic means and standard deviations.

Microbial community structure was examined by non-metric multidimensional scaling (NMDS) ordination on the obtained PLFA values (mol %, Hellinger transformed) using the PC-ORD software version 5 (MjM Software Design, Gleneden Beach, OR, United States). Statistical differences between whole PLFA profiles were assessed using a Sorenson (Bray-Curtis) distance measurement followed by multi-response permutation procedure (MRPP; Mielke and Berry, 2001) in PC-ORD. Outputs from the MRPP test include overall significance (p); degree of separation between groups (T), where a greater value indicates a greater separation; and an indicator of within-group homogeneity (A), where homogeneity increases as values become closer to 1. Lastly, the relation between specific PLFAs and variation in the ordination space was displayed using correlation vectors when $r^2 > 0.5$.

For individual PLFAs, the difference in ^{13}C (ε_{T-C} in ‰) between the substrate-amended samples and the control samples was calculated using the following equation:

$$\varepsilon_{T-C} = \left(\frac{1,000 + \delta^{13}C_{PLFA(treated)}}{1,000 + \delta^{13}C_{PLFA(control)}} - 1 \right) \times 1,000$$

where $\delta^{13}C_{PLFA(treated)}$ is the isotopic composition of PLFA in the amended soil; and $\delta^{13}C_{PLFA(control)}$ is the isotopic composition of PLFA in the control (no substrate) soils.

The percent of labeled substrate-C (%) incorporated into each PLFA was estimated according to the following equation (Ziegler and Billings, 2011):

$$\%\text{Substrate incorporated in PLFA} = \frac{\left(\delta^{13}C_{PLFA(treated)} - \delta^{13}C_{PLFA(control)}\right)}{\left(\delta^{13}C_{Substrate} - \delta^{13}C_{PLFA(control)}\right)} \times 100$$

where $\delta^{13}C_{PLFA(treated)}$ is the isotopic composition of PLFA in the amended soil; $\delta^{13}C_{PLFA(control)}$ is the isotopic composition of PLFA in the control (no substrate) soil; and $\delta^{13}C_{substrate}$ is

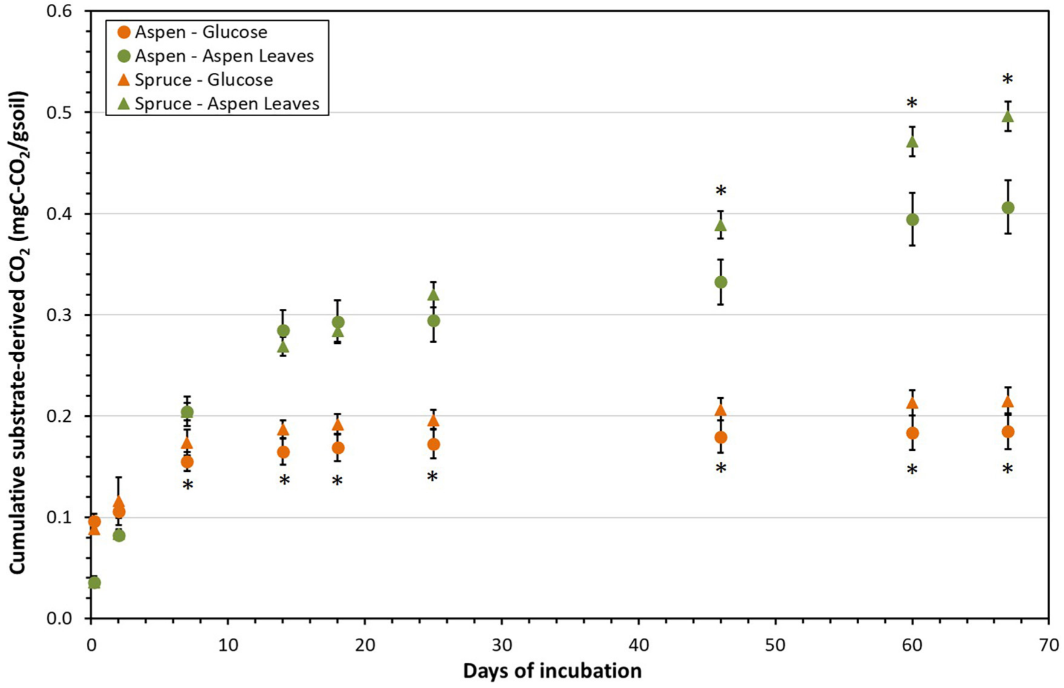


FIGURE 2 | Cumulative substrate-derived CO_2 fluxes (mgC-CO_2/gsoil) from the aspen (circles) and spruce (triangles) soil incubation units following addition of glucose and aspen leaves during the 67-day laboratory incubation. Asterisks indicate dates where significant differences were detected between aspen and spruce ($p < 0.05$).

TABLE 2 | Priming effect (PE%), corresponding to % increase or decrease in soil C mineralized after 4 h and 2 days of incubation following addition of glucose, aspen leaves and aspen roots.

	Aspen forest floor			Spruce forest floor		
Days	**Glucose**	**Leaves**	**Roots**	**Glucose**	**Leaves**	**Roots**
0.2	43.2 ± 15.7	33.6 ± 19.3	16.5 ± 11.7	2.2 ± 2.9	−3.6 ± 9.6	−11.3 ± 5.3
2	8.2 ± 8.7	−1.4 ± 6.8	−2.8 ± 5.2	−11.5 ± 17.0	9.6 ± 7.7	9.9 ± 9.3

Errors are standard deviations over five experimental replicates.

the isotopic composition of the labeled substrate. Data were also expressed in µgC/g soil to calculate the PLFA-based SUE as follows (%):

$$\text{PLFA}-\text{based SUE} =$$

$$100\times\frac{\text{Substrate incorporated in PLFA}}{(\text{substrate incorporated in PLFA}+\text{substrate derived}CO_2)}$$

Average SUE values were computed both for total PLFA biomass, and for given groups of bacterial PLFAs (10Me, branched saturated, monounsaturated).

RESULTS

Respiration Fluxes

Daily respiration rates decreased over time during the 67 days of the laboratory incubation (**Figure 1** and **Supplementary Tables 1**, **2**). The decrease occurred most rapidly during the first 7 days of incubation and then became progressively less. For instance, daily respiration rates from the aspen control incubation units decreased by 30% between Day 1 and Day 7 of the incubation period, but only by 9% between Day 7 and Day 16. Respiration fluxes from the aspen forest floors continued to decrease after Day 15 and reached a plateau by the end of the incubation (**Figure 1**). On Day 67, daily respiration rates from the aspen control units were more than five times lower than rates at the beginning of the incubation (**Supplementary Table 1**). Similar trends were apparent for the amended aspen incubation units (**Figure 1** and **Supplementary Table 1**). While substrate addition boosted daily respiration rates in the first 2 days by 100–200% compared to the control (**Supplementary Table 1**), and resulted in significantly higher cumulative fluxes (**Figure 1**), all respiration rates from the aspen incubation units reached comparable low values by the end of the 2-month incubation.

During the first two weeks of incubation, cumulative respiration fluxes for the aspen incubation units increased faster than for the spruce, indicating that the aspen forest floor samples

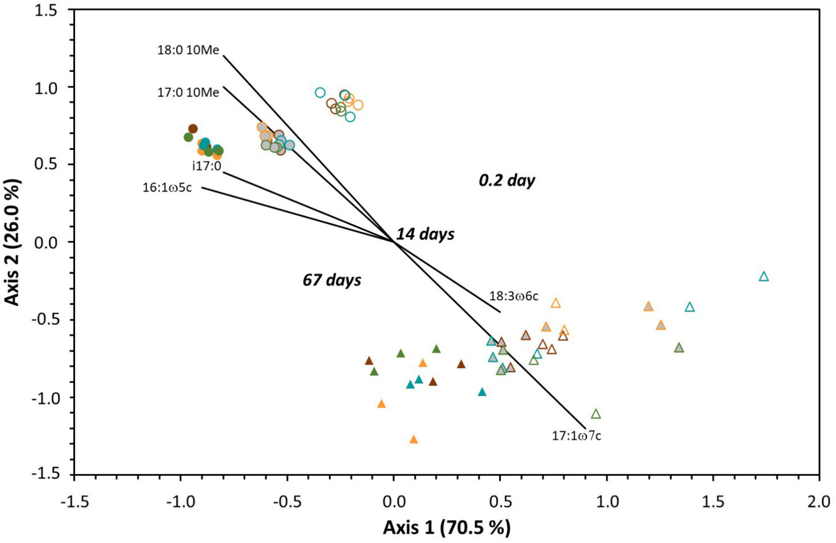


FIGURE 3 | Non-metric multidimensional scaling ordination of PLFA profiles extracted from the aspen (circles) and spruce (triangles) soil incubation units after different incubation times, including 4 h (empty symbols), 14 days (grayed symbols), and 67 days (filled symbols). Substrates are indicated with different colors, including brown for control, orange for glucose, green for aspen leaves, and blue for aspen roots.

TABLE 3 | Multi-response permutation procedure (MRPP) results for PLFAs extracted from the aspen and spruce soil incubation units during the 67-day laboratory incubation.

	T	*A*	*p*-Value
Forest floor (aspen or spruce)	−44.50	0.45	$<10^{-5}$
Incubation time (4 h, 14 days, 67 days)	−7.85	0.11	$<10^{-4}$
Treatment (control, glucose, leaves or roots)	1.68	−0.03	1.00

respired more during that period than the spruce forest floors (**Figure 1**). However, following this initial period, the daily rates for spruce decreased at a slower rate than for aspen, and showed no evidence to have reached a plateau by the end of the incubation (**Figure 1** and **Supplementary Tables 1**, **2**). Consequently, by Day 67, cumulative respiration rates from the control spruce incubation units (58.6 ± 2.7 mgC-CO_2/gCsoil) had reached values comparable to those from aspen (58.6 ± 6.0 mgC-CO_2/gCsoil). Similarly, cumulative respiration rates of the incubation units amended with the labelled substrates, which ranged from 57.4 ± 3.6 to 61.4 ± 5.3 mgC-CO_2/gCsoil did not significantly differ from the control units (**Figure 1**).

The control incubation units (with no added substrate) for the aspen and spruce forest floors maintained respired δ^{13}C-CO_2 values around −29.1 ± 5.4 and −24.8 ± 2.3‰, respectively, throughout the incubation period (**Supplementary Tables 1**, **2**). For both forest floors, as expected, the highest δ^{13}C-CO_2 values were observed for the incubation units amended with glucose, but these decreased rapidly within the first week of incubation. Similarly, a relatively large portion of the respiration fluxes, between 32% for spruce and 43% for aspen came from the added glucose in the first 4 h following substrate addition, but values decreased rapidly thereafter (**Supplementary Figure 1**). In both forest floor types, the majority of respiration fluxes coming from glucose occurred during the first week of incubation. After 2 days, glucose corresponded to about 10% of the respired CO_2 fluxes for both forest floor types, and continued to decrease for the spruce incubation units to reach negligible values after one week. After 4 h of incubation in the aspen units, the percent of respired CO_2 coming from the added substrates reached 23% for aspen leaves and 19% for aspen roots (**Supplementary Figure 1**). Corresponding values in the spruce incubation units were 15% for aspen leaves and 14% for aspen. Similarly to the glucose substrate, both aspen leaf and aspen root substrates tended to represent a higher percentage of the total respiration fluxes for the aspen forest floors compared to the spruce.

The cumulative substrate-derived CO_2 fluxes (mgC-CO_2/gsoil) were higher for glucose than for aspen leaves during the first 2 days of incubation, but the opposite was true for the remainder of the incubation (**Figure 2**). Respiration fluxes from the added glucose became significantly higher in the spruce incubation units compared to the aspen units on Day 14 of the laboratory incubation, and this remained the case for the rest of the incubation. A slightly different trend emerged following aspen leaf addition, where cumulative substrate-derived CO_2 fluxes tended to be higher from the aspen units during the initial two weeks of the incubation, but then became greater from the spruce units. The spruce incubation systems showed statistically higher aspen leaf-derived CO_2 fluxes after Day 46, and this remained true for the rest of the incubation; i.e., for Days 60 and 67 (**Figure 2**).

A positive PE was observed in most incubation systems 4 h following the addition of substrates, with the exception of the spruce forest floors following addition of aspen leaves and aspen roots (**Table 2**). The enhanced degradation of native organic matter following substrate addition was larger in the aspen incubation units (43, 34, and 17%, for glucose, leaves, and roots substrates, respectively; **Table 2**), but the PE decreased very rapidly, and was not detected past 2 days of incubation.

Phospholipid Fatty Acid Profiles

For both aspen and spruce forest floor incubations, total PLFAs stayed relatively constant during all 67 days of incubation. Total PLFAs were slightly higher in the aspen forest floor incubations, with a value around 4,600 nmol g^{-1} of soil, compared to the spruce forest floor incubations (≈3,700 nmol g^{-1} of soil), but there was no significant difference between the two forest types.

The microbial soil community structure was assessed using an NMDS ordination of the PLFA data (**Figure 3**) and statistical patterns within the data were tested using a MRPP (**Table 3**). Ordination on the PLFA data yielded a two-dimensional solution with a final stress of 6.8 after 62 iterations. Testing for significance of the grouping variables (forest floor, incubation time, and treatment) identified significant differences between the two forest floor types and among the incubation times. The biggest separation was linked to forest floor type ($p < 10^{-5}$) followed by incubation time ($p < 10^{-4}$). As can be seen on **Figure 3**, points corresponding to the aspen forest floors clustered closely together for each incubation time, while the spruce data points were more spread out. However, for both forest floor types, there was a comparable shift in the composition of the microbial communities with time (**Figure 3**). Separation between forest floor types was also very clear, and remained for the entirety of the two months of incubation. On the other hand, there was no

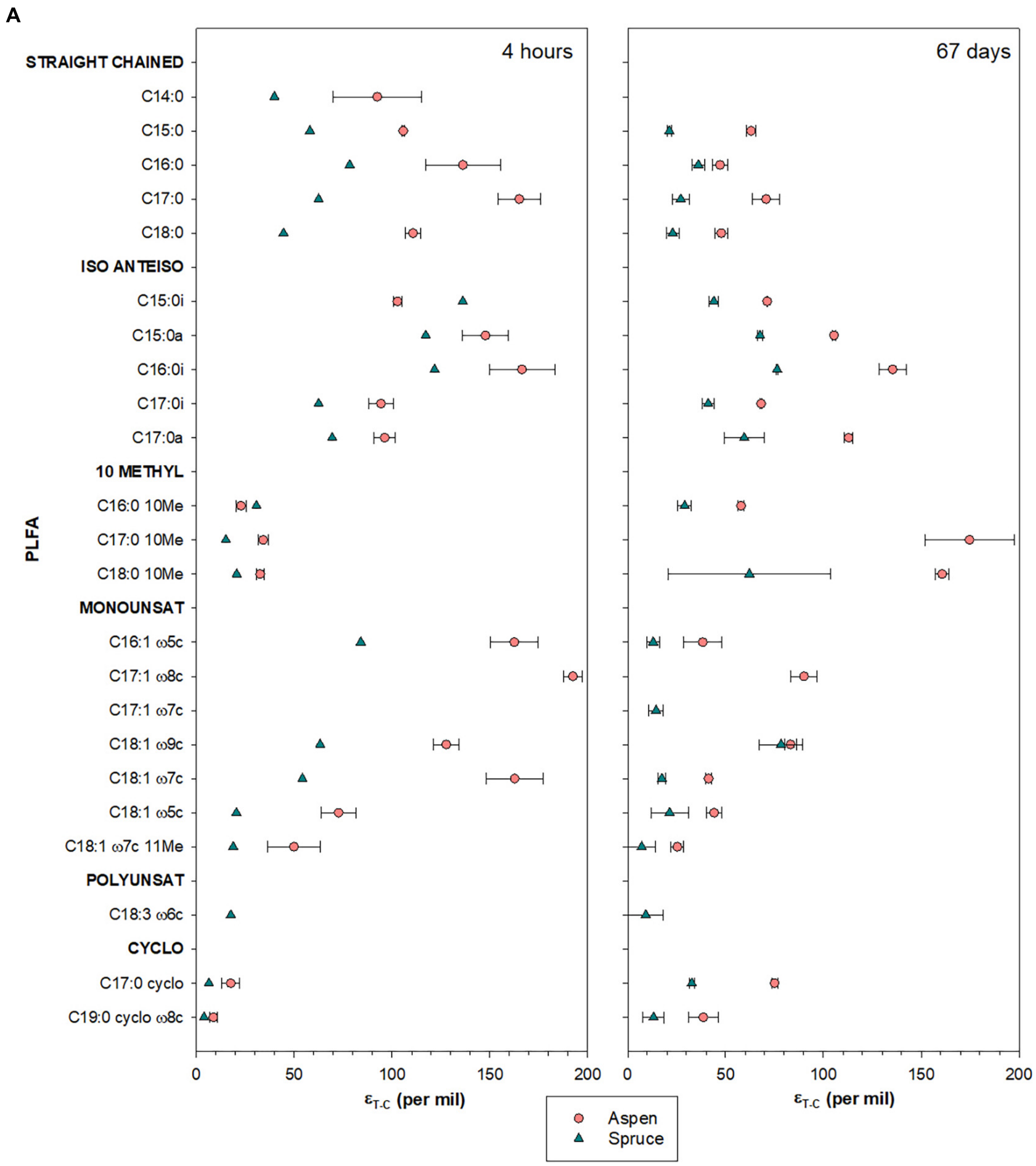
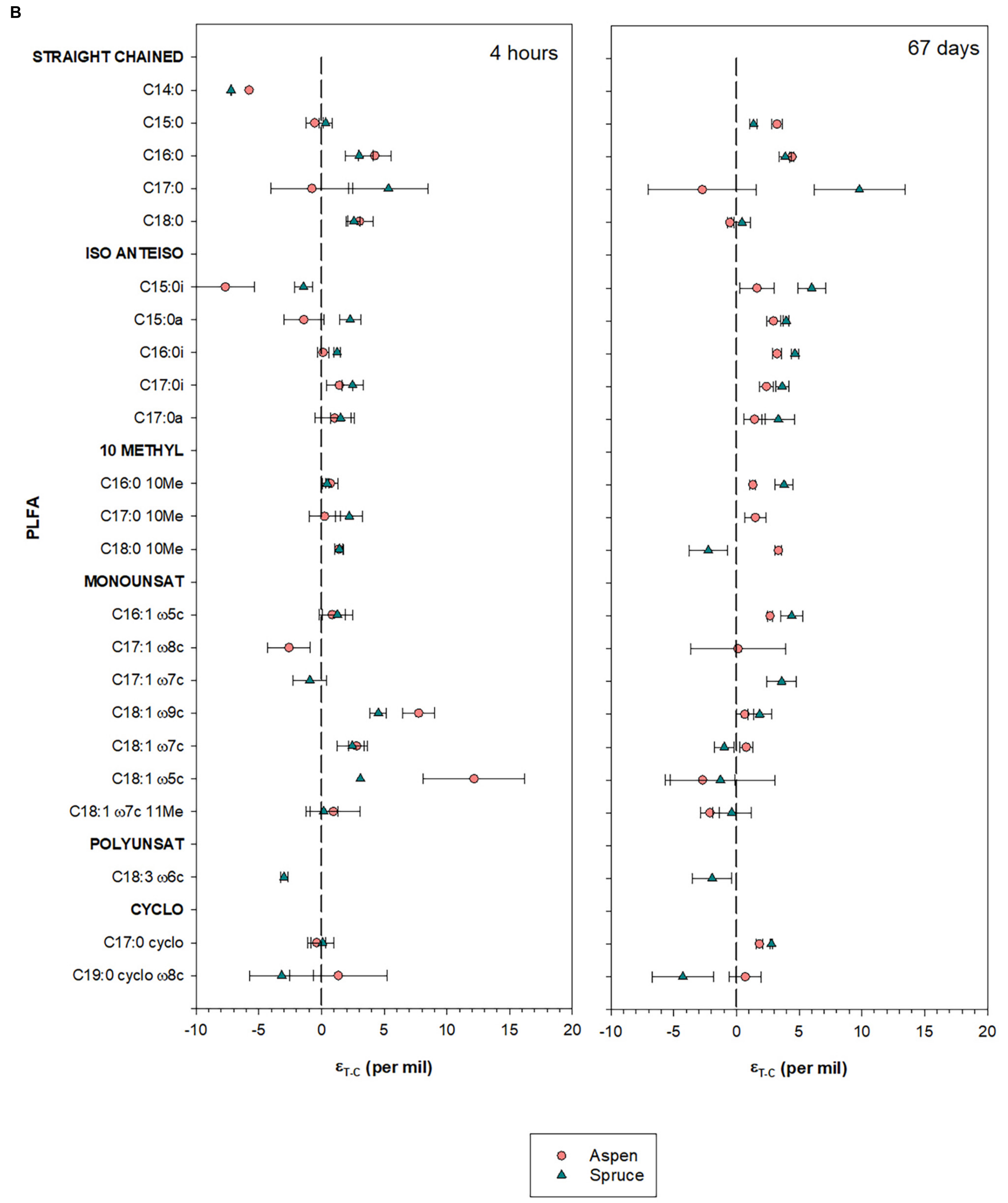


FIGURE 4 | Continued



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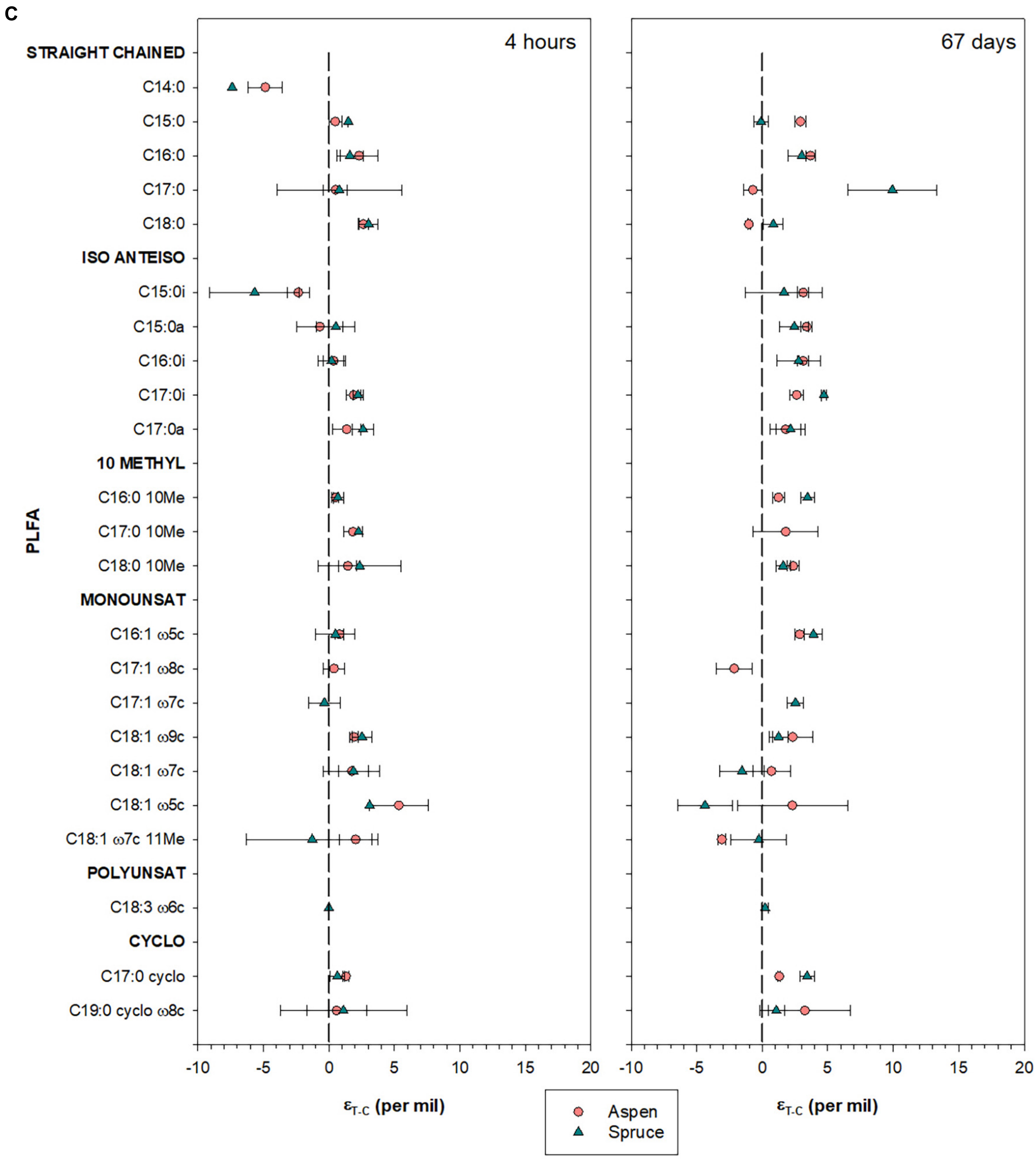

FIGURE 4 | Differences in isotopic composition of selected PLFA biomarkers (ε_{T-C}) between substrate-amended and control incubation units after 67 days of incubation. Results are presented for both aspen and spruce soil incubation units, and added substrates included: glucose **(A)**, aspen leaves **(B)**, and aspen roots **(C)**. Average values are presented with standard deviations over five experimental replicates. However, for some PLFAs, only one reliable measurement could be obtained with GC-C-IRMS analysis, and results are presented as a single point with no standard deviation.

significant difference linked to the substrates added for either aspen or spruce (**Table 3**).

For the control forest floor incubations, $\delta^{13}C_{PLFA}$ values ranged from −35.1 to −24.1‰ within the aspen incubation units, and from −34.1 to −22.9‰ within the spruce forest floor incubations (**Supplementary Tables 3**, **4**). As expected, $\delta^{13}C_{PLFA}$ values for the forest floors amended with glucose presented higher ^{13}C enrichment values than the forest floors amended with other substrates (**Figure 4**). These enrichment values were clearly higher for aspen forest floor incubations than for spruce forest floor incubations for nearly all PLFAs, and were generally higher after 4 h than after 67 days of incubation (**Figure 4A**). However, different PLFA groups responded differently. Monounsaturated and straight chained saturated PLFAs became highly enriched after 4 h, while 10-methyl

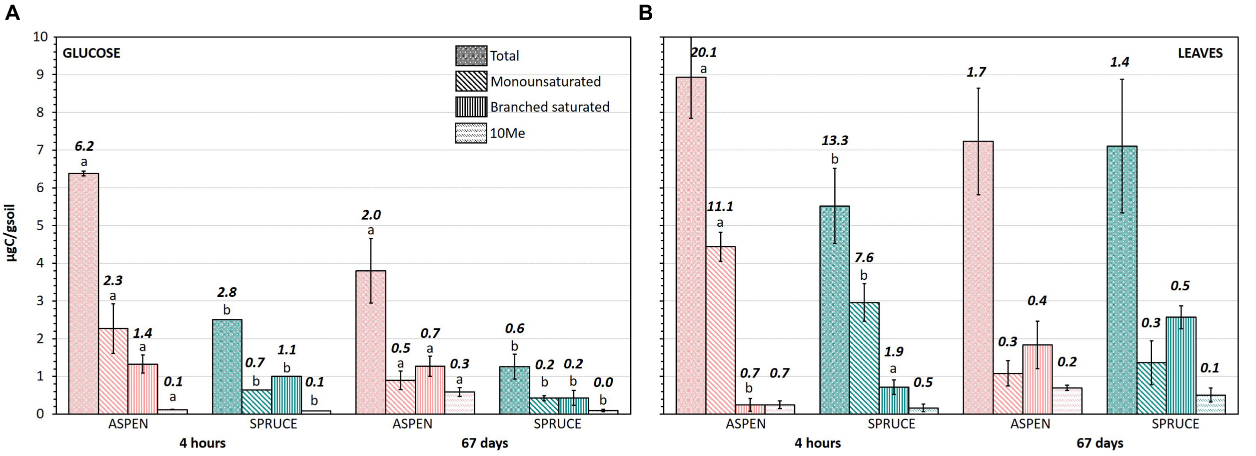

FIGURE 5 | Incorporation of **(A)** glucose and **(B)** aspen leaves into 10Me, branched saturated, monounsaturated, and total PLFAs for both aspen and spruce soil incubation units. Error bars show standard deviations over five replicates and different letters indicate significant differences between the aspen and spruce soil incubation units for each date (4 h and 67 days) and each PLFA grouping ($p < 0.05$). The value indicated on top of each bar corresponds to the PLFA based-SUE value (%).

PLFAs proportionally became more enriched after 67 days (**Figure 4A**).

Enrichment following addition of ^{13}C-labeled aspen leaves and aspen roots was much smaller than was the case following glucose addition, and in several cases PLFA δ^{13}C values were similar to those of the control incubation units, especially after only 4 h of incubation (**Figures 4B,C**). There was no clear difference between aspen and spruce forest floors following addition of the ^{13}C-labeled aspen roots (**Figure 4C**). On the other hand, after 67 days of incubation, a slight enrichment was observed for most PLFAs in the case of the forest floors amended with aspen leaves (**Figure 4B**). This was particularly true of the saturated PLFAs with terminal branching, which tended to become more enriched in spruce compared to aspen forest floors (**Figure 4B**). This was the opposite trend to what was observed following glucose, where all microbial PLFAs were more enriched in the case of the aspen forest floor (**Figure 4A**).

Incorporation of substrate-C into microbial PLFAs (µgC/gsoil) showed both temporal shifts and differences linked to forest floor type (aspen and spruce), substrate added (glucose and aspen leaves), and the group of PLFAs considered (**Figure 5**). All PLFA groupings (10Me, branched saturated, monounsaturated, total) showed significantly higher incorporation of glucose-^{13}C for the aspen than the spruce forest floors. As the spruce forest floors respired more of the glucose (**Figure 2**), the resulting PLFA-based SUE values were clearly higher for aspen than for spruce, in particular for the monounsaturated PLFAs preferentially associated with gram negative bacteria (**Figure 5**). Right after addition of the aspen leaves, substrate incorporation was also statistically higher for the aspen than the spruce forest floors in terms of total PLFAs, but this did not hold true for all PLFA groupings; terminally branched PLFAs (characteristic of gram positive bacteria) showed a statistically higher enrichment in the case of the spruce forest floors. After 67 days, the aspen and spruce forest floors reached comparable SUE values (**Figure 5**).

DISCUSSION

Microbial Substrate Utilization Following Glucose Addition

Glucose addition did not significantly alter PLFA profiles in either aspen and spruce forest floors (**Figure 1**), and PEs, when detected, were short-lived (**Table 2**). Similarly, no change in PLFA profiles was detected in another study with a one-time addition of either starch or xylose to an oak woodland soil, at a level of substrate addition (400 µg C/g of soil) comparable to our study (Waldrop and Firestone, 2004), or in incubations where small quantities of carbon substrates (glucose, glutamate, oxalate or phenol) were added to a soil from a Douglas-fir/ hemlock dominated site (Brant et al., 2006). Microbial communities in both aspen and spruce forest floors changed with time during the 67-day incubation (**Figure 3** and **Table 3**), but this temporal shift was similar for the control and the labeled materials (**Figure 3**). In both forest floors, concentrations of saturated PLFAs, which tend to be more abundant in gram positive bacteria, generally decreased during the 67 days of incubation while monounsaturated PLFAs more typical of gram negative bacteria increased (**Supplementary Tables 3**, **4**). The ratios corresponding to monounsaturated/saturated PLFA$_S$ increased from 1.09 (4 h) to 1.12 (14-days) to 1.24 (67 days), corresponding to a 14% increase, in the aspen incubation units; and from 0.99 to 1.04 and finally 1.07 (i.e., a smaller 8% increase) in the spruce incubation units. In addition, there were larger changes in specific PLFAs for the aspen than the spruce community (**Figure 3**). For instance, a shift in the iso/anteiso ratio, which has been linked to changes in bacterial membrane fluidity (Watzinger, 2015) could be detected for aspen but not for spruce; specifically, the (i15:0+i17:0)/(a15:0+a17:0) ratio increased from 1.2 (4 h) to 1.3 (14 days) to 1.4 (67 days) for aspen, while there was not any clear change for spruce.

Individual PLFAs diverged in their temporal response to ^{13}C-glucose addition, where non-specific, straight chained PLFAs (e.g., 16:0, 17:0) as well as PLFAs with terminal branching associated with gram positive bacteria (e.g., a15:0a, i16:0i), and in particular monounsaturated gram negative PLFAs (e.g., 18:1ω7) readily picked up the label after 4 h, while 10Me PLFAs took longer to become enriched (**Figure 4**). This trend is similar to results from incubations with ^{13}C-labeled glucose conducted over short time periods (0.75–48 h) where a rapid enrichment of PLFAs associated with gram positive bacteria (e.g., a15:0), and the gram negative 18:1ω7 was detected (Ziegler et al., 2005; Swallow and Quideau, 2020). The 10Me18:0 became enriched following depletion of the original glucose, which was evidence of the importance of Actinobacteria in recycling glucose originally incorporated in other bacterial biomass (Ziegler et al., 2005). Incorporation of the ^{13}C label was increased in Actinobacteria PLFAs 5 days following ^{13}C-glucose addition while δ^{13}C values in 16:0 and 18:0 PLFAs were reduced (Dungait et al., 2011). Similarly, in our incubation, 10Me PLFAs maintained strong ^{13}C enrichment even after 67 days of incubation (**Figure 5**).

When comparing the two forest floor types, the aspen community was clearly more efficient than the spruce at utilizing glucose for growth: all PLFAs became more ^{13}C enriched in aspen than in spruce forest floors (**Figure 4**), and incorporation of ^{13}C-glucose was greater in the aspen PLFAs, especially for gram negative at the beginning, and gram positive, including Actinobacteria, at the end of the incubation (**Figure 5**). These results indicate rapid and preferential glucose anabolism by gram negative bacteria in the aspen forest floor. Actinobacteria, better able to utilize more complex C substrates (Ziegler and Billings, 2011), such as those in microbial necromass, played a larger role later in the incubation. Furthermore, the aspen community respired less of the added glucose, and corresponding PLFA-based SUEs were clearly higher for the aspen (**Figure 5**). Together with an enhanced ability to retain labile C through mineral sorption (Boča et al., 2020), a greater microbial utilization efficiency of labile C substrates such as glucose likely plays a role in the greater and more persistent C stocks typically observed in aspen mineral horizons compared to spruce (Laganière et al., 2017; Sewell et al., 2020).

Microbial Response to Aspen Litter Addition

Distinct differences in microbial communities between the two forest floor types (**Figure 3**) have previously been reported in comparable Luvisolic soils from the Mixedwood forest of western Canada (e.g., Hannam et al., 2006; Swallow and Quideau, 2013; Thacker and Quideau, 2021). Similarly to these studies, differences in our study appeared to be linked to specific PLFAs rather than to broader groupings (e.g., gram positive bacteria). In the same way to what we report here where Actinobacteria PLFAs (10Me17:0, 10Me18:0) and the gram positive i17:0 were correlated to aspen (**Figure 3**), 10Me16:0, 10Me18:0 and i16:0 PLFAs have been associated with aspen forest floors (Swallow and Quideau, 2013). The gram negative 17:1ω7, which correlated with spruce in our study (**Figure 3**), has previously been recognized as an indicator PLFA for spruce forest floors (Hannam et al., 2006). Furthermore, differences between the two forest floors remained throughout the two months of incubation, which is in agreement with the work from Hannam et al. (2007), who did not detect any change in microbial community structure when forest floors of aspen and spruce were incubated in the field for 1 year following reciprocal transfer to either spruce or aspen stands. Microbial communities in aspen and spruce forest floors remained distinct 17 years following clearcutting of aspen and spruce stands where aspen was naturally regenerating in both stand types (Thacker and Quideau, 2021). In the case of ecosystems from warmer climates (e.g., Potthast et al., 2010), or more drastic vegetation and/or land management changes (e.g., Quideau et al., 2013), changes in PLFA profiles are typically detected following vegetation shifts. In the boreal forest of Western Canada, however, this does not appear to be the case, and the long-lasting legacy of the original forest floor seems to buffer microbial communities against rapid structural changes following vegetation shifts.

A greater amount of the added aspen leaves was respired by the spruce community (**Figure 2**). Thus, results were opposite of what would have been expected according to the HFA theory (Ayres et al., 2009), which stipulates that the microbial communities in the aspen forest floors would be better adapted to mineralize the aspen litter when compared to the communities present in the spruce forest floors. It is entirely possible that different results would be obtained if a litter decomposition study were to be conducted *in situ*, as divergent conditions including higher moisture and lower temperature may be slowing down decomposition processes under spruce (Sewell et al., 2020). But under laboratory conditions where abiotic limitations were lifted, there was no apparent inhibition of aspen leaf decomposition by the spruce community. Interestingly, greater aspen leaf respiration by the spruce community did not necessarily correspond to greater microbial biomass uptake, except in the case of gram positive bacteria (**Figure 5**). From a soil carbon balance point of view, this meant a faster loss of aspen leaf carbon from the spruce forest floors, but a comparable carbon retention in microbial biomass, as suggested by the comparable SUE in both forest floors by the end of the incubation period. Further, while our study did not detect any differences between aspen and spruce in terms of overall bacterial or fungal biomass, it is possible that species-specific differences exist, such as those observed in fungal species between spruce and beech forests

(Asplund et al., 2019), which could have impacts on soil carbon persistence. Differences in soil carbon storage have been linked to distinct mycorrhizal fungal communities in boreal forest soils (Clemmensen et al., 2015). In all cases, observations from our study indicate that a transition from spruce to aspen will initially increase the loss of aspen leaf litter through microbial respiration, although incorporation into microbial biomass and eventually more persistent soil carbon may not be affected. Follow-up work is needed to test if these results hold true over a longer time period.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

EL and SQ conceived and designed the methodology. EL performed the laboratory incubation, statistical analyses, and wrote the first draft of the manuscript. Both authors discussed the results and contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.700751/full#supplementary-material

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Soil Carbon Stabilization Under Coniferous, Deciduous and Grass Vegetation in Post-mining Reclaimed Ecosystems

Meghan C. L. Nickels*† and Cindy E. Prescott†

Department of Forest and Conservation Sciences, University of British Columbia, Vancouver, BC, Canada

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Sandra Spielvogel,
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Antra Boca,
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Georg August University of Göttingen,
Germany

***Correspondence:**
Meghan C. L. Nickels
laidlaw.meghan@gmail.com

†*These authors have contributed equally to this work*

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Vegetation plays an important role in determining soil organic carbon (SOC) stocks, and influences the mechanisms through which SOC is stabilized within the soil. The type of vegetation selected for use in reclamation may therefore influence the accumulation rate and residence time of SOC in these ecosystems. Earlier studies at reclaimed sites in the Alberta Oil Sands demonstrated that reclaimed ecosystems planted with deciduous trees accumulated the most soil organic matter in the top 10 cm of reclamation material, followed by grass sites, while coniferous sites accumulated the least SOM. The objective of this study was to assess differences in SOC stabilization in the upper 10 cm of soil among revegetated deciduous, coniferous and grass ecosystems 20–40 years following reclamation. We compared soil C in unprotected, physically protected, and chemically protected forms among the three reclamation treatments using density flotation to isolate free particulate (unprotected) SOC from the soil sample, and size fractionation to separate the remaining sample into heavy particulate (physically protected) SOC and mineral-associated (chemically protected) SOC. In addition to this analysis, we used NaOCl oxidation to distinguish chemically resistant and chemically oxidizable C stocks. Chemically resistant C was consistent across all vegetation treatments at approximately 25% of total soil C, while the remaining 75% was chemically oxidizable. Total SOC stocks were also not significantly different among vegetation treatments. Deciduous sites had 57.8 Mg ha^{-1} SOC, grass sites had 52.7 Mg ha^{-1} SOC, and coniferous sites had 43.7 Mg ha^{-1} SOC. Two-thirds of total SOC at grass sites was in protected forms, compared to half of total SOC at coniferous sites and one-third of total SOC at deciduous sites (33.6, 22.6, and 15.6 Mg ha^{-1}, respectively). Grass sites had significantly more physically protected SOC than deciduous sites while deciduous sites had more unprotected SOC than grass sites. Our findings indicate that the type of vegetation selected for reclaimed areas has important implications for soil carbon in persistent versus unprotected pools.

Keywords: soil organic carbon, reclamation, aggregates, Populus, MAOM, Alberta oil sands, hPOM, fPOM

INTRODUCTION

Most organic carbon (C) in terrestrial ecosystems, approximately 1,400 petagrams, is stored in soil (Scharlemann et al., 2014), and restoration of disturbed ecosystems presents an opportunity to sequester atmospheric CO_2 into persistent forms of soil C (Lal et al., 2018). Stocks of SOC reflect the balance between inputs of C (e.g., dead plant material, root and microbial exudates, and soil organism necromass) and losses of C (e.g., microbial respiration, leaching, and erosion). The SOC pool includes diverse compounds that have different residence times and respond differently to environmental factors such as climate or land use. Forms of SOC that have short residence times and respond quickly to changes in climate or land use are referred to as labile or fast-cycling SOC, while forms that have longer residence times and respond more slowly are typically referred to as stable SOC (Soucémarianadin et al., 2018). These forms are not inherently stable; rather they are in a state of "dynamic stability" due to biological, environmental, and physicochemical constraints on decomposition (Dynarski et al., 2020), and so can be better described as persistent or slow-cycling forms.

Slow-cycling SOC pools are created through three main pathways (Lützow et al., 2006). First, some organic matter inputs have complex chemical structures that make them resistant to decomposition and result in long residence times (chemically resistant C). Second, organic matter can become chemically protected by binding to mineral surfaces, making it inaccessible to decomposers and resulting in long residence times (mineral-associated organic matter or MAOM-C). Third, organic particles can become physically protected through incorporation into soil aggregates where they are partially protected from microbial enzymes (heavy particulate organic matter or hPOM-C). Within this physically protected pool, SOC is stored in different size classes of aggregates that offer varying degrees of protection. The strength of the binding agents that hold aggregates together increases as the aggregate size decreases, resulting in microaggregates being associated with older, more persistent SOC while macroaggregates are associated with younger, faster-cycling SOC (Jastrow et al., 1996; Six et al., 2004). Further, the roots and hyphae that bind macroaggregates together are thought to act as nuclei for microaggregate formation within macroaggregates, particularly in soils where organic matter (OM) is an important binding agent (Oades, 1984; Six et al., 2004). Uncomplexed SOC that is not physically or chemically protected from microbes is referred to as the free light fraction, or free particulate organic matter (fPOM-C) and is a fast-cycling C pool. Fast-cycling soil C can promote the production of persistent SOC by stimulating production of microbial biomass, residues and decay products (Kleber et al., 2011; Dungait et al., 2012; Cotrufo et al., 2013).

Vegetation type influences the rate of input, the chemical structure of C compounds entering the soil, and the relative magnitude of the various SOC stabilization pathways. Grasses typically have abundant fine and very fine roots, which are more likely to interact with aggregates and clay minerals than medium or coarse roots, facilitating the generation of chemically and physically protected SOC (Prescott et al., 2019). Grassland establishment on degraded soils increases soil C stocks as much or more than afforestation (Poeplau et al., 2011; Wei et al., 2012), and conversion of grasslands to forests often cause SOC stocks to decline (Guo and Gifford, 2002; Poeplau et al., 2011). Grass ecosystems had greater stocks of chemically protected SOC (Eclesia et al., 2012) and physically protected SOC than nearby forests (Guidi et al., 2014). In forest ecosystems, deciduous trees typically have deeper and more extensive root systems than conifers, which contribute more organic matter to the soil (Finér et al., 2007; Laganière et al., 2010). As a result, broadleaved species tend to have greater C stocks in the mineral soil, whereas coniferous species accumulate more C in the forest floor (Peng et al., 2020). Deciduous tree species are often associated with greater macrofaunal activity than coniferous forests (Józefowska et al., 2016), which mix surface organic material with mineral soil (Frouz et al., 2006). Soil macrofauna also add mucus, which acts as a cementing agent, holding organic and mineral particles together and creating biogenic structures like earthworm casts that contribute to aggregation (Bottinelli et al., 2015). By stimulating microbial growth, earthworm mucus also increases microbial necromass and production of extracellular polymeric substances which promote the creation of micro- and macroaggregates (Angst et al., 2019a).

Reclaimed ecosystems can sequester substantial amounts of soil C if they are managed to promote soil C stabilization (Akala and Lal, 2001). The type of vegetation planted on reclaimed sites (e.g., grasses, broadleaved trees or coniferous trees) can influence rates of soil C sequestration and stabilization. Degraded soils restored to grassland or pasture often accumulate soil C faster than those restored to forest (Akala and Lal, 2001; Wei et al., 2012; Frouz et al., 2013). Reclaimed sites planted with deciduous trees accumulate SOC more rapidly and deeper in the soil profile than sites with coniferous trees (Frouz et al., 2009, 2013; Laganière et al., 2010; Vindušková and Frouz, 2013). At 93 restoration sites summarized by Frouz et al. (2013), mean rates of SOC accumulation were significantly lower in coniferous forests than in grasslands or deciduous forests.

Reclaimed ecosystems provide an opportunity to isolate the effects of vegetation on SOC stabilization pathways while minimizing the influence of confounding factors such as differences in parent material, soil properties and site characteristics that typically determine the distribution of vegetation communities and SOC pools on the landscape (Angst et al., 2018; Wiesmeier et al., 2019). In reclaimed ecosystems in the oil sands region of Alberta, Canada, Anderson et al. (2019) reported the greatest accumulations of soil organic matter (SOM) at reclaimed sites planted with deciduous trees, followed by sites seeded with grasses, while reclaimed sites planted with coniferous trees had the least SOM. This difference in reclaimed vegetation treatments was attributed to greater macrofaunal activity and higher root biomass at deciduous and grass treatments, compared to coniferous treatments. All reclaimed vegetation treatments had substantially more organic matter in the mineral soil than natural sites in the area (Anderson et al., 2019). We sampled the same reclaimed ecosystems dominated by deciduous trees, coniferous trees and grass to determine the proportion of SOC in each vegetation treatment in fast and slow-cycling pools – specifically in uncomplexed, physically protected, chemically protected, and chemically resistant SOC pools. We hypothesized that reclaimed grass sites would have the most physically and chemically protected SOC, and that aggregation would be greater in soils at grass and deciduous sites than at sites with coniferous vegetation.

MATERIALS AND METHODS

Study Sites

The study sites are located in the Athabasca Oil Sand region in the Boreal Mixedwoods region of Alberta, Canada. The area has long, cold winters and short, warm summers. Mean monthly air temperatures range from 17.1°C in July to −17.4°C in January (Environment Canada, 2020). Mean annual precipitation is 418.6 mm and two-thirds of this precipitation falls as rain during the growing season (Environment Canada, 2020). This is an undulating landscape of uplands dominated by aspen (*Populus tremuloides* Michx.) and white spruce (*Picea glauca* [Moench] Voss.), with jack pine (*Pinus banksiana* Lamb.) in well drained areas, and lowlands dominated by black spruce (*Picea mariana* [Mill.] BSP) and larch (*Larix laricina* [Du Roi] K. Koch). Upland soils are primarily Eutric Brunisols and Gray Luvisols that have developed on glaciofluvial, glaciolacustrine and glacial till deposits (Soil Classification Working Group, 1998). Organic soils are common in lowlands and consist of decomposed plant remains, primarily sphagnum (Sphagnum spp.), feather mosses (various species) and Labrador tea (*Rhododendron*). Because of the abundance of organic soils in the Oil Sands disturbance footprint, the salvaged coversoils used in reclamation are typically composed of peat or a mixture of peat and mineral upland soils. Wildfire is the dominant natural disturbance regime in the Boreal mixedwood region (Bergeron et al., 2014).

Fifteen sites were selected in reclaimed portions of oil sands mining leases north of Fort McMurray, AB (56°39′12′′ N, 111°13′24′′ W). Five sites had been planted with coniferous tree species (mostly white spruce), five with deciduous tree species (mostly trembling aspen) and five had been seeded with grasses (primarily Festuca spp. and *Bromus inermis* [Leyss.]). Forested sites had at least 80% tree cover and grass sites had less than 10% tree cover and less than 20% shrub cover (Anderson et al., 2019). Vegetation composition of the study sites is detailed in Anderson et al. (2019). Briefly, understory cover at deciduous sites consisted of grasses (20%), shrubs (15%), forbs (6%), and mosses (4%), while coniferous site understory was primarily mosses (46%), followed by forbs (11%), shrubs (5%) and very few grasses (1%). Other than planting prescription, the 15 study sites received similar reclamation treatments and had been reclaimed approximately 30 years earlier. All were classified as mesic upland sites and had a peat-mineral mix cover-soil over overburden or secondary materials. Peat-mineral mixes are defined as mineral soils with less than 17% organic carbon, and the peat-to-mineral ratio targeted for reclamation covers ranges from 50:50 to 70:30 (Alberta Environment Water, 2012). Initial SOC content of cover material was not available for the study sites. Cover-soil depths ranged from 10 to 100 cm, average depths and ranges within each vegetation treatment were similar (34 cm on average), and differences in cover-soil depths were not a confounding factor. All sites had a soil texture of either Sandy loam or Loamy sand. Only sites that were reclaimed at least 20 years prior were selected for sampling. At the time of sampling, deciduous and coniferous sites ranged from 21 to 29 years since reclamation, while grass sites ranged from 28 to 41 years.

Soil Sampling

Study sites were sampled in 2013. At each site, one 10-m^2 plot was subdivided into 10 1-m^2 subplots and three subplots were randomly selected for sampling. At each subplot, a 30-cm deep soil pit was dug and described, including a brief description of organic and mineral horizons. Organic surface horizons were removed and soil structure samples were carefully extracted from the top 10 cm of each subplot using a long-tipped trowel to avoid soil compaction and minimize the crushing of soil aggregates. A subsample of soil homogenized from the three soil pits was put on ice and stored at −18°C until laboratory analyses were performed. All other bulk soil samples were stored at 4°C until they could be further processed. The top 10 cm of mineral soil was selected as vegetation species influences on soils tend to appear sooner in the upper layers. Additionally, Anderson et al. (2019), found differences in SOM contents among these vegetation treatments only in the top 10 cm.

Bulk Density, Root Biomass and Extractable Microbial Biomass Carbon

Bulk density was determined by cutting a 10-cm^3 block of soil from each soil pit and carefully extracting all soil from the hole. Field moist samples were then weighed and air-dried at 105°C to determine gravimetric water content. Bulk density was averaged by site and corrected for gravel and coarse fragments using the equation described by Page-Dumroese et al. (1999) to calculate fine bulk density. Root biomass was measured by hand-sorting roots into four size classes: very fine (<1 mm), fine (1–2 mm), medium (2–5 mm) and coarse (>5 mm). Root samples were then washed, dried in a forced-air oven at 60°C, and then weighed. Bulk density results were used to express root biomass in megagrams per hectare. Extractable microbial biomass carbon (eMBC) was measured in fresh soil samples the same day they were sampled using the chloroform-fumigation direct-extraction method from Tate et al. (1988). Extracts were frozen, stored at −20°C, and sent to the Analytical Service Laboratory of the University of Alberta where total organic carbon was measured using a TOC-TN autoanalyzer (Shimadzu, Kyoto, Japan). Bulk density results were used to express extractable eMBC in megagrams per hectare (Mg ha^{-1}). Microbial biomass C was not calculated because a calibration factor (kEC) from natural soils would not be reliable for these peat-rich materials.

Aggregate Size Separation

Field-moist soil blocks were gently broken apart by hand and passed through an 8-mm sieve before air-drying. Subsamples of air-dried soil (50 g) were wet-sieved using the method described in Elliot (1986) and adapted by Six et al. (2000) to separate bulk soil into four aggregate size classes: large macroaggregates

(2,000 – 8,000 μm; large), macroaggregates (250 – 2,000 μm; macro), microaggregates (53 – 250 μm; micro) and silt and clay particles (<53 μm; S&C). Briefly, samples were slaked in distilled water for 5 min to disrupt unstable aggregates through the buildup and release of air and pressure, leaving only water-stable aggregates (Laganière et al., 2010). Samples were then sieved through a series of sieves (2,000, 250, and 53 μm), and material that remained on each sieve was collected in pre-weighed aluminum drying pans. Any floating organic material that was left in the 2,000-μm sample was discarded because it is considered too large to be part of the SOC fraction (Six et al., 2002). Samples were dried in a forced-air oven at 60°C and weighed. Individual fraction weights are expressed as a percentage of dry soil weight.

Size-Density Fractionation

Density and size fractionation methods described by Laganière et al. (2011) and adapted from Gregorich et al. (2006) were used to separate soil into uncomplexed (fPOM), physically protected (hPOM), and chemically protected (MAOM) C pools. Briefly, 25-g soil samples, previous sieved through an 8-mm sieve and air-dried, were gently shaken in a 1.7 g cm^{-3} solution of sodium iodide (NaI) for 20 min and allowed to settle for 48 h. The suspended fPOM was then recovered from the surface of each sample using vacuum-filtration equipment. This recovered light fraction was rinsed several times with a 0.01-M calcium dichloride ($CaCl_2$) solution and distilled water, then oven-dried at 60°C and weighed. Size separation was used to separate the remaining soil into heavy and mineral-associated fractions. The sample was rinsed as described above and centrifuged for 15 min at 2,000 rpm several times. Aggregates were then dispersed in the sample using glass beads and a reciprocal shaker, and the dispersed sample was wet-sieved through a 53-μm sieve. The sample remaining on the sieve (hPOM) and the sample that passed through the sieve (MAOM) were oven-dried at 60°C and weighed. Organic matter content was measured in each size-density fraction using the loss-on-ignition method (Rabenhorst, 1988). The LOI method used in this study was tested using calibrated controls (Masse et al., 2017), and was selected to facilitate comparison between previous work on the same study sites that used the same method. Organic C content was determined using the coefficient 1.724 (Rabenhorst, 1988). Bulk density results were used to express soil C pools in megagrams per hectare (Mg ha^{-1}).

Separating Chemically Oxidizable and Chemically Resistant Carbon Pools Using Oxidation

Soil C was separated into chemically oxidizable and chemically resistant pools using sodium hypochlorite (NaOCl) oxidation (described by Zimmermann et al., 2007). This analysis was used to divide total soil C into an old, more recalcitrant C pool and a younger, more labile C pool. A 25-g sample of air-dried, sieved (2 mm) soil was added to 50 mL of 6% (wt/wt) NaOCl. Samples were oxidized for 16 h at

TABLE 1 | Site and soil properties measured in the three vegetation treatments (mean ± standard error).

Treatment	**Forest floor depth**	**Very fine roots (<1 mm)**	**Fine roots (1–2 mm)**	**Medium roots (2–5 mm)**	**Coarse roots (>5 mm)**	**Total roots**	**Fine bulk density**	**eMBC**	**S&C (<53 μm)**	**Micro (53–250 μm)**	**Macro (250–2,000 μm)**	**Large (>2,000 μm)**	**pH**
Units	***cm***	***Mg ha^{-1}***	***Mg ha^{-1}***	***Mg ha^{-1}***	***Mg ha^{-1}***	***Mg ha^{-1}***	***g cm^{-3}***	***Mg C ha^{-1}***	***% of soil dry weight***	***% of soil dry weight***	***% of soil dry weight***	***% of soil dry weight***	
Coniferous	2.5 ± 0.4*b*	2 ± 0.3	0.8 ± 0.2	1.2 ± 0.2	2.7 ± 1.4	6.7 ± 1.4	0.89 ± 0.09	1.1 ± 0.1	3.2 ± 0.7*b*	12.5 ± 2.4**b**	29.1 ± 2.9	41.7 ± 4.6	6.5 ± 0.09**b**
Deciduous	4.4 ± 0.7*a*	6.3 ± 2.7	1 ± 0.1	0.9 ± 0.2	2.6 ± 1.2	10.7 ± 3.6*a*	0.66 ± 0.10	0.9 ± 0.1	2.5 ± 0.6**b**	15 ± 2.5*b*	38.1 ± 6.2	27.4 ± 7.1	6.4 ± 0.15**b**
Grassland	3.5 ± 0.6	2.5 ± 0.7	0.7 ± 0.3	0.6 ± 0.3	0.5 ± 0.3	4.3 ± 0.7*b*	0.91 ± 0.06	0.8 ± 0.2	4.7 ± 1.1**a***a*	21.3 ± 5.3**a***a*	38.6 ± 5.2	24 ± 6.9	7.1 ± 0.06**a**

Significant differences ($p \leq 0.05$) among treatments are noted using bold letters, and differences in which $p \leq 0.1$ are noted using italicized letters. eMBC, extractable microbial biomass carbon; S&C, silt-and-clay-sized fraction; Micro, microaggregates; Macro, macroaggregates; Large, large macroaggregates.

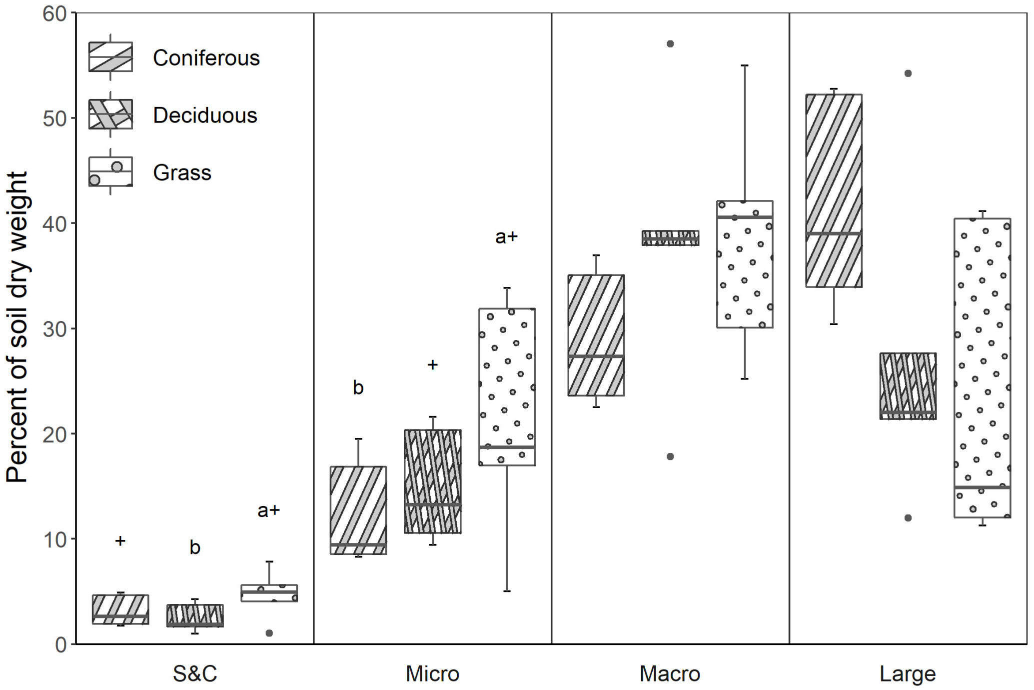

FIGURE 1 | Distribution of aggregate size classes (g aggregate g^{-1} dry soil) among the three vegetation treatments, as a percentage of the total soil dry weight (S&C = silt-and-clay-sized fraction; Micro = microaggregates; Macro = macroaggregates; Large = large macroaggregates). Significant differences ($p \leq 0.05$) among treatments are noted using a letter above the boxplot, and differences in which $p \leq 0.10$ are symbolized using +.

25°C, then washed with distilled water, dried at 60°C and weighed. The difference between the initial sample weight and the NaOCl-treated sample weight represents the chemically oxidizable organic material. Total C and total N were measured in NaOCl-treated samples and untreated samples by dry combustion using an elemental analyzer (Elementar Vario EL Cube, Hanau, Germany). Changes in mass and C contents before and after oxidation were used to calculate chemically resistant C and chemically oxidizable C, which are expressed as megagrams per hectare. Total N content was used to determine C:N ratios of chemically resistant and chemically oxidizable C pools.

Soil texture, cation exchange capacity, pH and soil inorganic carbon measured at the study sites were reported by Masse et al. (2017). Soil inorganic carbon was found to be negligible at all sites.

Statistical Analyses

Data from the three subplots at each site were averaged to determine mean soil C stocks, aggregate size fractions, root biomass and bulk density. Composite soil samples were used to determine microbial biomass, CEC, exchangeable cations, conductivity and pH. One-way analysis of variance (ANOVA) was used to determine significant differences among vegetation treatments ($n = 5$). Data that did not meet assumptions of normality were log-transformed to ensure that assumptions were met. Tukey HSD tests were used to compare differences among treatments. A significance threshold of $\alpha = 0.05$ was used for all statistical analyses, although differences up to $\alpha = 0.1$ are noted in recognition of the considerable variability associated with the use of actual reclamation treatments in the field. Canonical redundancy analyses (RDA) were used to identify variables that significantly explained variation among vegetation treatments in soil C fractions and aggregate size-classes. The following variables were hypothesized to explain variation in soil C fractions: microaggregates, small macroaggregates, C:N ratios, depth of the organic surface horizon, pH, CEC, biomass of very fine roots, eMBC, understory cover, total cover, and the clay-and-silt fraction. Additionally, very fine roots, total SOC, C:N ratios, pH, CEC, understory cover, eMBC, total cover, Ca, Mg and the clay-and-silt fraction were hypothesized to explain variation in aggregate size-classes. Cover data was not available for two of the sites (one deciduous and one grass), therefore only 13 sites were included in RDA analysis. Significant variables were selected using a forward-selection algorithm: vegan's ordistep function

TABLE 2 | Total soil organic C stocks among the three reclaimed vegetation treatments, as well as organic C stored within different size-density fractions in the soil, in Mg C ha^{-1}.

Treatment	MAOM	hPOM	fPOM	Total SOC stock
Coniferous	12.7 ± 1.9	9.9 ± 0.9*b*	21.1 ± 2.2*b*	43.7 ± 2.5
Deciduous	7.3 ± 2.6*b*	8.3 ± 2**b**	42.2 ± 9.5**a***a*	57.8 ± 6.7
Grassland	16.9 ± 3.8*a*	16.6 ± 2.5**a***a*	18.8 ± 4.8**b**	52.7 ± 3.7

Significant differences ($p \leq 0.05$) among treatments are noted using bold letters, and differences in which $p \leq 0.1$ are noted using italicized letters. MAOM, mineral-associated organic matter; hPOM, heavy particulate organic matter; fPOM, free particulate organic matter.

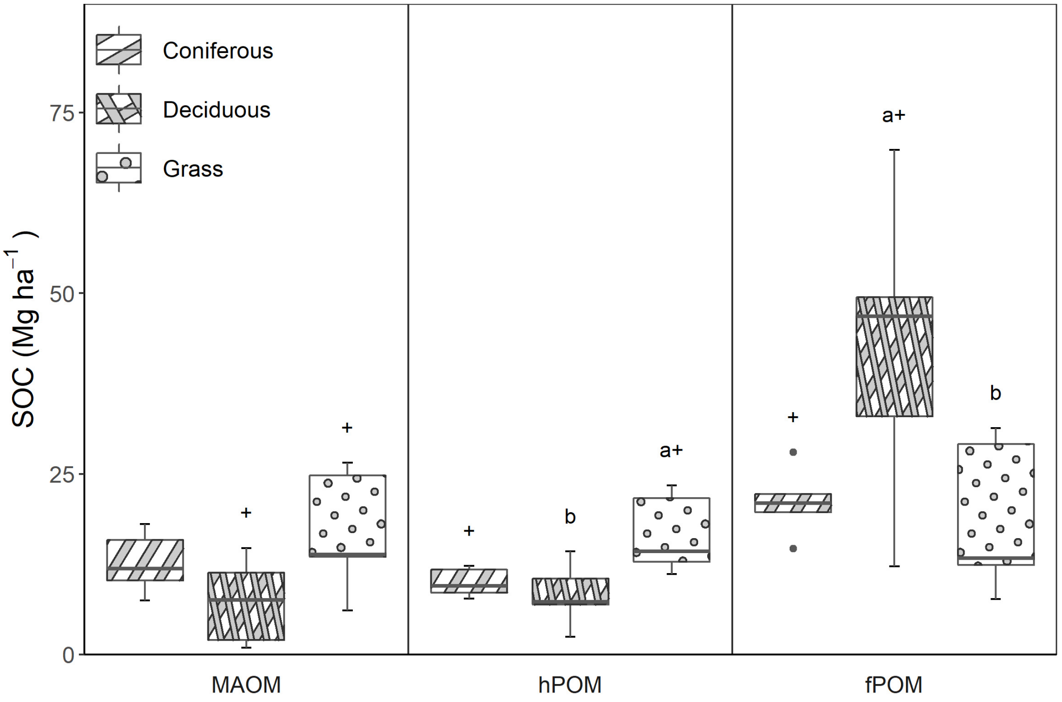

FIGURE 2 | Stocks of soil organic C in MAOM (mineral-associated organic matter), hPOM (heavy particulate organic matter) and fPOM (free particulate organic matter) pools among the three reclaimed vegetation treatments. Significant differences ($p \leq 0.05$) among treatments are noted using a letter above the boxplot, and differences in which $p \leq 0.10$ are symbolized using +.

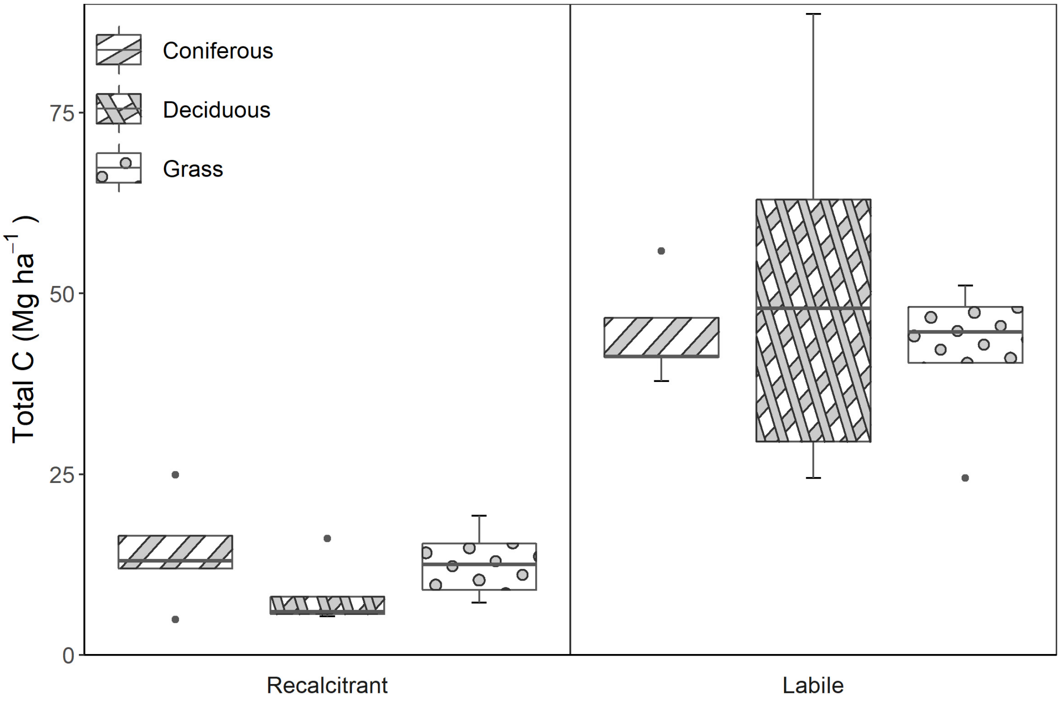

FIGURE 3 | Total C in chemically resistant and chemically oxidizable pools in the three reclaimed vegetation treatments. There were no significant differences among treatments.

(Oksanen et al., 2020). The RDA model, axis, and explanatory variables were tested using a permutation test (Bordcard et al., 2011). R software (ver.4.0.3) was used for all analyses (R Core Team, 2020).

RESULTS

The surface organic layer was deepest at deciduous sites and shallowest at coniferous sites (**Table 1**, $p = 0.08$). Total root biomass was greater at deciduous sites than at grass sites ($p = 0.08$), primarily due to the abundance of very fine roots at deciduous sites. More than half of total root biomass at deciduous and grass sites was in the form of very fine roots, while coarse roots made up the biggest portion of root biomass at coniferous sites (40%). Soil bulk density and eMBC were similar among treatments (0.82 ± 0.05 g cm^{-3} and 0.92 ± 0.08 Mg C ha^{-1}, respectively). Grass sites had a mean soil pH of 7.0, while coniferous and deciduous sites were significantly more acidic (6.5 and 6.4, respectively).

Large macroaggregates were the most abundant size class of aggregates in soils at coniferous sites whereas macroaggregates were most abundant at deciduous and grassland sites (**Figure 1**). Differences in aggregate size-classes among treatments were only significant for small size-fractions. Microaggregates represented a greater portion of the soil dry weight at grass sites than at coniferous sites ($p = 0.04$) or deciduous sites ($p = 0.08$). The silt-and-clay-sized fraction was also greater at grassland sites than at deciduous ($p = 0.01$) and coniferous ($p = 0.07$) sites.

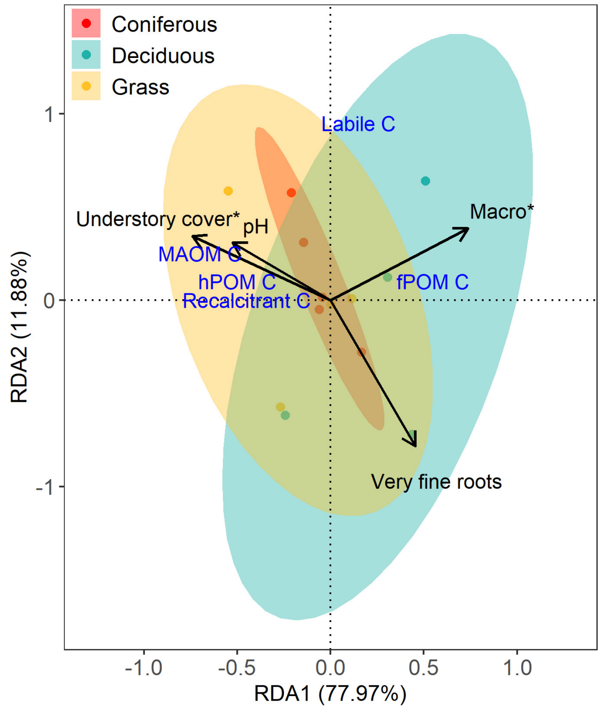

FIGURE 4 | Canonical redundancy analysis (scaling 2) demonstrating the relationships among vegetation and soil properties, and soil C pools in the studied soils. Blue text represents response variables and black text and arrows represent the environmental variables that explained variance in the response variables among study sites. Significant explanatory variables ($p \leq 0.05$) are indicated by a star.

Stocks of total SOC were greatest at deciduous sites, followed by grass sites, and then coniferous sites (**Table 2**). No significant differences in total SOC were found among vegetation treatments, however, SOC was distributed differently among size-density fractions in the different vegetation treatments (**Figure 2**). At deciduous sites, two-thirds of total SOC was in unprotected forms (fPOM-C), compared to half of total SOC at coniferous sites and one-third of total SOC at grass. Grass sites had a larger hPOM-C pool than coniferous ($p = 0.07$) or deciduous ($p = 0.02$) sites. Stocks of MAOM-C were greater at grass sites than deciduous sites ($p = 0.09$), while coniferous and grass sites were similar. Total stocks of protected SOC were 33.6 Mg ha^{-1} at grass sites, 22.6 Mg ha^{-1} at coniferous sites, and 15.6 Mg ha^{-1} at deciduous sites.

Across all vegetation treatments, at least 75% of all soil C was chemically oxidizable, while the remaining 25% was chemically resistant (**Figure 3**). Treatment means for chemically oxidizable C ranged from 41.7 Mg ha^{-1} at grass sites to 50.7 Mg ha^{-1} at deciduous sites, while the chemically resistant pool ranged from 8.22 ha^{-1} at deciduous sites to 14.23 ha^{-1} at coniferous sites. No significant differences were observed among the three vegetation treatments.

Canonical redundancy analysis indicated that 59% of the variation in the distribution of C fractions across the 13 study sites used in this analysis could be explained by environmental variables (**Figure 4**; $F = 6.62$, $p = 0.001$), specifically understory cover ($F = 7.77$, $p \leq 0.01$) and macroaggregates ($F = 9.79$, $p \leq 0.01$). Very fine roots and pH contributed to the explained variation, but were not significant explanatory variables. RDA1 was the only significant axis ($F = 16.72$, $p \leq 0.01$). Macroaggregates explained 30% of the variation and were positively correlated with the uncomplexed C fraction (fPOM) and deciduous sites. Understory cover explained 27% of the variation in C fractions and was positively correlated with protected pools of C (hPOM-C and MAOM-C) and grass sites. The three vegetation treatments overlapped, indicating similarity in C pools across vegetation treatments, although grass and deciduous treatments diverged slightly. Canonical redundancy analysis was also used to explore whether environmental variables could explain the distribution of aggregate size classes, but the model was not significant.

DISCUSSION

Consistent with our hypothesis that reclaimed sites with grass would have the most physically protected SOC, grass sites had more physically protected C (hPOM-C) than coniferous or deciduous treatments. These results suggest that soil carbon at grass sites may be more resistant to losses through disturbances such as wildfire than soil carbon at afforested sites. Microaggregates were also greatest in the grass treatment, while macroaggregates and large macroaggregates did not differ among treatments. These results suggest that the greater C in protected pools at grass sites can be attributed to SOC protected within microaggregates. Guidi et al. (2014) also found that the greater SOC in grasslands compared to adjacent afforested areas was primarily due to SOC stored in small aggregates. Accumulation of SOC under grasses is commonly attributed to greater fine roots compared to forest soils, and therefore greater association between root biomass and mineral surfaces (Poeplau et al., 2011; Wei et al., 2012). However, fine and very fine root biomass were similar between vegetation treatments in this study. Very fine roots were also not a significant variable in explaining the variation in SOC pools, suggesting that differences in root biomass among vegetation treatments were not a strong driver of aggregation or SOC stabilization in the top 10 cm. The greater stocks of C in small aggregates at grass sites may be a consequence of the dominance of arbuscular mycorrhizae (AM) in the ecosystems. AM-associated plants, including most grass and herb species, release proteins such as glomalin, which act as binding agents, increasing aggregate stability, and contributing to greater pools of physically and chemically protected SOC (Rillig, 2004; Bedini et al., 2009; Dignac et al., 2017). Boreal forest tree species, both coniferous and deciduous, are dominated by ectomycorrhizal (ECM) fungi (Policelli et al., 2020).

We also hypothesized that reclaimed sites with grass would have the most chemically protected SOC (MAOM-C). Although MAOM-C was not significantly different among vegetation treatments, average values were larger at grass sites than deciduous sites. Other studies have reported no measurable change in MAOM-C after 40–50 years, and suggested that ecosystem development on a decadal timeframe is too short to detect changes in this slow-cycling C pool (Lajtha et al., 2014; Angst et al., 2019b).

Contrary to our hypothesis, soils at deciduous sites did not have greater aggregation; instead they had twice as much uncomplexed C (fPOM-C) as coniferous or grass treatments. Our expectation of greater soil aggregation, and therefore greater physically protected C, at sites with deciduous trees was based on common characteristics of deciduous/broadleaved tree species such as rapidly decomposing leaf litter (rich in nutrients, calcium, and soluble compounds) which promotes burrowing earthworms, and AM fungal associates (Reich et al., 2005; Phillips et al., 2013). However, aspen leaf litter does not decompose much faster than spruce needle litter (Prescott et al., 2000, 2004; Jerabkova et al., 2006), and in the boreal mixedwood forests of western Canada, trembling aspen roots are dominated by ECM fungi (Visser et al., 1998). Burrowing earthworms are not abundant, rather the soil macrofauna are detritivores that comminute litter and transform it into fecal pellets with minimal mixing into mineral soil. Intact macrofaunal fecal material was abundant in the forest floor and the upper 10 cm of mineral soil at deciduous sites (Anderson et al., 2019), and would have contributed to the abundance of fPOM at these sites. The greater amount of fPOM-C at deciduous sites can also be attributed to greater litter input in deciduous stands in boreal mixedwood forests (Jerabkova et al., 2006) and the 9-year retention time of needles of spruce (Anderson et al., 2019) which forestalls the development of forest floors in spruce stands.

Understory cover explained 27% of the variation in C pools and was positively correlated with protected pools of C (hPOM-C and MAOM-C) and grass sites. At grass sites, understory cover was often equivalent to total cover and consisted of grasses and to a lesser extent, herbaceous species. Understory vegetation at deciduous sites was dominated by grasses and shrubs whereas mosses dominated on coniferous sites (Anderson et al., 2019). This finding indicates an important role of understory vegetation, particularly grasses, in generating stocks of protected soil C (MAOM-C and hPOM-C) during early phases of restoration in these ecosystems. Deciduous sites had greater grass covers than coniferous sites, but this did not translate into greater protected pools of C at deciduous sites. This can be attributed to grass cover being on average only 20% (2–60% range) at deciduous sites compared with 75% grass cover (44–96% range) at grass sites. We propose that the grass cover at deciduous sites was not great enough to significantly increase protected C pools, and as canopy closure is reached at these sites, grasses will likely be replaced by the natural regeneration of herbaceous species and shrubs that may not have the same effect of generating stocks of protected soil C. As protected forms of SOC are more resistant to loss through disturbances such as wildfire than are unprotected forms of C, inclusion of native grasslands in reclaimed landscapes could assist in long-term soil development and C sequestration.

Although there were some indications of different SOC stabilization pathways among the vegetation treatments, there was also significant overlap in their distributions in the multivariate analyses. This could be attributable to peat from the original peat-mineral mix in reclaimed soils masking smaller changes in the quantity and chemical composition of inputs from the three vegetation treatments. Nuclear magnetic resonance (NMR) spectra indicated that SOM in reclaimed soils under different vegetation treatments was chemically homogeneous, while natural soils in the Oil Sands region were more variable and their chemical compounds were more closely tied to the different vegetation types (Turcotte et al., 2009). These results suggest that the reclaimed ecosystems have not developed to the point where SOC clearly reflect the dominant stabilization processes associated with the type of vegetation planted on the site, as would be expected in natural ecosystems (Crow et al., 2009; Dawud et al., 2016; Angst et al., 2019b).

CONCLUSION

We compared soil organic C pools at reclaimed sites revegetated with deciduous trees, coniferous trees or grass in the boreal forest. Although total SOC stocks did not differ, the relative stabilization of SOC differed among vegetation treatments. At reclaimed grass sites, most SOC was physically and/or chemically protected, whereas most SOC at reclaimed deciduous sites was in unprotected forms (coniferous sites were intermediate). Our findings indicate that the type of vegetation selected for reclaimed areas has important implications for soil carbon in persistent versus unprotected pools.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2021.689594/full#supplementary-material

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