Warming-enhanced preferential microbial mineralization of humified boreal forest soil organic matter: Interpretation of soil profiles along a climate transect using laboratory incubations

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[1] Humified soil organic matter storage in boreal forests is large, and its responses to warming over relatively long timescales is critical for predicting soil feedbacks to climate change. To derive information relevant across decades to centuries from manipulative short-term experiments, we conducted incubations of soils from two forested sites along the Newfoundland-Labrador Boreal Ecosystem Latitude Transect in eastern Canada and assessed linkages between incubation data and these sites' profile characteristics. The sites differ in mean annual temperature by 3.4°C, but vegetation and soil types are similar. Organic soils (Oe + Oa) were incubated for 120 days at 15°C and 20°C, with and without a replaced Oi subhorizon possessing a distinct $\delta^{13}C$ signature. Laboratory warming induced significantly greater mineralization and leaching of humified SOM relative to replaced Oi, congruent with greater warming-induced increases in phenol oxidase activity relative to enzymes associated with labile C acquisition (percent increases of 101% versus 50%, respectively). These data suggest that warming can influence microbial communities and their enzymatic dynamics such that relative losses of humified SOM are disproportionately enhanced. This is consistent with stable isotopic, C:N, and radiocarbon profile differences between the two sites, which suggest a greater degree of microbial processing and greater relative losses of older SOC over the preceding decades at the warmer site, given our knowledge of organic inputs in these soils. This study is a first step toward linking the divergent timescales represented by soil profiles and laboratory manipulations, an important goal for biogeochemists assessing climate change impacts on SOM dynamics.

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1. Introduction

[2] Soil warming typically accelerates soil organic matter (SOM) decay and associated rates of CO_2 flux to the atmosphere [*Rustad et al.*, 2001; *Bergner et al.*, 2004; *Kirschbaum*, 2004; *Bradford et al.*, 2008]. A 4 to 7°C increase in mean annual temperature (MAT) is expected this century in high-latitude ecosystems [*Intergovernmental Panel on Climate Change* (*IPCC*), 2007]. Even a relatively small change in respiratory losses from the large soil organic carbon (SOC) stocks in boreal forest ecosystems (179 Pg C [*Schlesinger*, 1997]) could potentially influence atmospheric CO_2 concentrations. Therefore, quantifying the potential vulnerability of this significant carbon pool to decomposition with warming is important for accurate predictions of SOM feedbacks to climate change. Because SOM is composed of many different compounds exhibiting varying degrees of recalcitrance and turnover times, understanding the temperature dependence of biogeochemical reactions driving transformations of different SOM pools with warming is a particularly challenging task.

[3] Predictions of SOM degradation with warming often invoke the Arrhenius equation [*Kirschbaum*, 1995, 2004], which suggests that decay of humified SOM, possessing a relatively low reactivity and requiring high activation energies for decomposition (E_a), is more sensitive to temperature than more labile compounds. Studies documenting the greater sensitivity of lignin-derived compounds and lower-quality organic materials to warming [*Fierer et al.*, 2005; *Feng et al.*, 2008; *Karhu et al.*, 2010] are consistent with this idea. However, if microbial substrate availability is hindered by dwindling supply or limited soil moisture content and associated diffusion of substrates to enzymatic reaction sites, heterotrophic respiration may not exhibit the temperature sensitivity predicted by enzyme kinetics [*Davidson and Janssens*, 2006].

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Indeed, warming can induce an ephemeral increase in soil respiration, often attributed to depletion of labile SOC [*Melillo et al.*, 2002; *Eliasson et al.*, 2005; *Knorr et al.*, 2005]. The highly varied responses of multiple SOM pools to warming result in continued controversy regarding the relative temperature sensitivity of decomposition of labile versus more humified SOM pools [*Giardina and Ryan*, 2000; *Thornley and Cannell*, 2001; *Niinistö et al.*, 2004; *Fang et al.*, 2005; *Knorr et al.*, 2005; *Bronson et al.*, 2008; *Vanhala et al.*, 2008; *Craine et al.*, 2010]. Further complicating our understanding of these issues, acclimation or adaptation of microbial physiology to soil warming may mitigate the temperature sensitivity of SOM decay on relatively short timescales [*Luo et al.*, 2001; *Bradford et al.*, 2008, 2010], though this concept is controversial [*Hartley et al.*, 2008]. Thus, predicting SOM degradation with temperature variation is highly complex because of a suite of interactions between microbial substrate availability and quality, microbial adaptations in structure and function, and enzyme kinetics [*Davidson and Janssens*, 2006; *von Lutzow and Kogel-Knabner*, 2009; *Karhu et al.*, 2010; *Wetterstedt et al.*, 2010].

[4] An important, additional complication in studies of SOM responses to temperature is the difficulty of linking patterns of decay across temporal and spatial scales. For example, linking laboratory warming experiments to observations of SOM characteristics in intact soil profiles that naturally experience different temperature regimes may help resolve temporal issues. Making such linkages, however, is difficult for two key reasons. First, it is challenging to find soil profiles similar in soil series, disturbance history, current vegetation type and productivity, physicochemical attributes and precipitation regime, but that differ in MAT. Several studies report differences in profile characteristics and SOM turnover across temperature gradients [*Trumbore et al.*, 1996; *Leifeld et al.*, 2009], but the multiple varying factors in addition to MAT usually make it difficult to elucidate the physical or biological processes responsible for observed differences. Comparing such soil profiles would afford us an opportunity to assess the influence of only MAT over relatively long timescales. Further, the timescales over which relevant processes act vary greatly, from seconds to millennia [*Janzen*, 2004]. Short-term responses to warming under laboratory conditions are thus difficult to link to profile characteristics governed by decades or centuries of many interacting processes.

[5] We explore these issues by combining a laboratory approach with field observations in soil profiles of two mesic boreal forests in eastern Canada. The sites are similar in soil type, vegetation, and disturbance history, but differ in MAT by over three degrees. It is likely that in mesic and C-rich soils such as these, substrate availability for microbial transformations likely remains sufficient across much of the growing season to permit enzyme kinetics to play a relatively dominant role in determining patterns of SOM decomposition, compared to diffusion driven substrate limitations. Thus, they are well suited for exploring whether profile characteristics reveal evidence of greater rates of SOM processing in a warmer environment, and whether relatively slow-turnover SOM compounds are more sensitive to temperature than their more labile counterparts. Further, roots are rare in these soils' mineral horizons and instead are concentrated in deep O horizons of these soils (further discussed below). Thus, SOM characteristics in the mineral horizons, where long-term storage is most feasible [*Trumbore*, 2000], are governed primarily by the quantity and composition of compounds transported from the O horizon and the biological and physicochemical processes to which those compounds are subjected [*Dawson et al.*, 1978], and markedly less so by direct vegetation input. As a result, characterizing O horizon responses to temperature may reveal both the microbial mechanisms responsible for such responses, as well as the associated, potential change in composition or quantity of material that becomes available for transport to the mineral horizons.

[6] Invoking our two boreal forest sites, described below in more detail, we ask, can we identify temperature-dependent mechanisms of O horizon decay that, extended across decadal to centennial timescales, are consistent with observations of these same profiles' characteristics in the field? In asking such a question, we necessarily must work across dramatically divergent timescales, a task fraught with difficulties and discussed below. However, attempting to make such linkages is an important first step in expanding the applicability of manipulative studies to observational soil profile data. We address our questions by conducting laboratory incubations of O horizons for which the Oi subhorizon has been replaced with an Oi source possessing a unique δ^{13}C signature, and quantifying CO_2 and dissolved organic C (DOC) released from these pools with warming, as well as quantifying exocellular enzymatic activities. We also define depth distributions of SOM C:N ratios and stable isotopic and radiocarbon signatures from the two sites and assess whether the mechanisms and trends exhibited in laboratory data, if applied across time with all other factors held constant, are qualitatively congruent with observed profile characteristics.

[7] Combining a laboratory incubation approach with field data is not new, nor does it eliminate all of the challenges described above. However, by conducting these explorations at sites comparable in most respects except for MAT, and where inputs to mineral horizons primarily consist of O horizon leachates, we can address several issues critical for predicting soil feedbacks to climate change. First, we can assess microbial substrate choice with warming in O horizons by quantifying the extent to which warming influences decay of labile Oi material versus more humified Oe and Oa subhorizons. This permits a qualitative assessment of the applicability of decay predictions in these soils with warming as derived from enzyme kinetics. Second, we can determine if these dynamics have implications for relatively long-term C storage in these mineral profiles, where SOM is largely derived from O horizon processing compared to direct input from vegetation. Third, we can take a first step toward linking short-term microbial processes to profile characteristics which integrate decades or centuries of inputs, transformations, and outputs, in two soils similar in most respects except MAT.

2. Materials and Methods

2.1. Site Description and Characteristics

[8] The island of Newfoundland and parts of Labrador lie in the boreal forest biome. Soil profiles in the region exhibit relatively high concentrations of SOC, consistent with

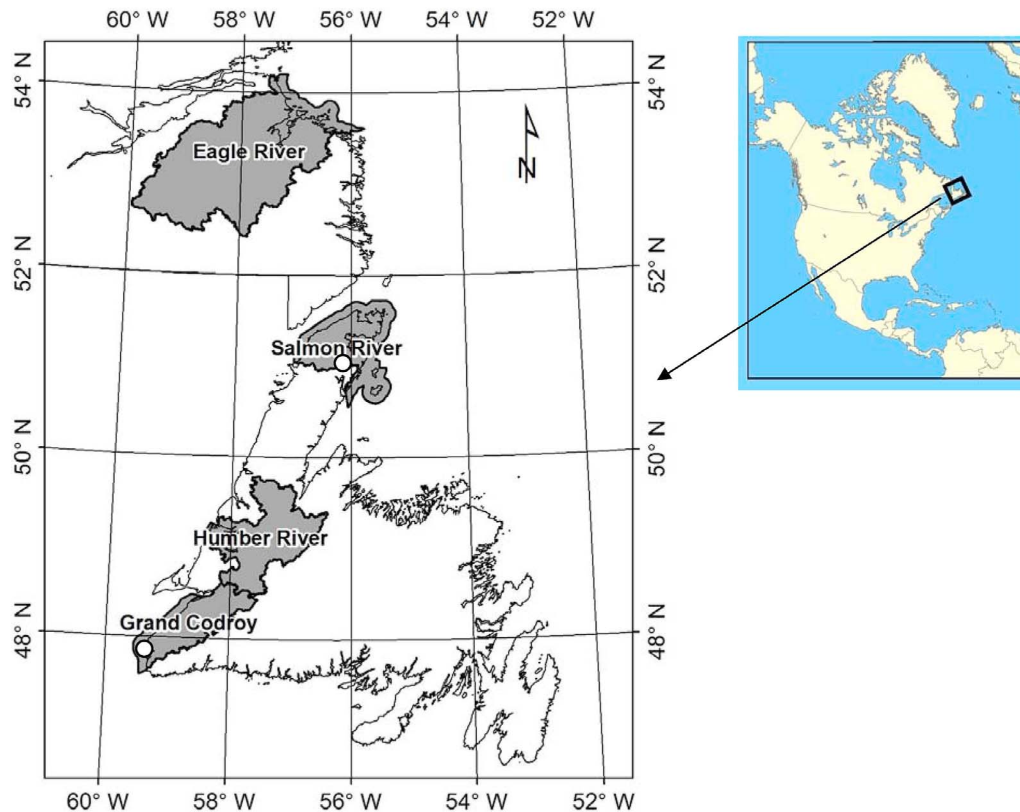

Figure 1. Location of research sites at Salmon River (SR) and Grand Codroy (GC) along the Newfoundland and Labrador Boreal Ecosystem Latitude Transect (NL-BELT) in eastern Canada. Shaded areas represent Canada's National Hydrological Network work units associated with multiple NL-BELT sites.

Schlesinger's [1997] assessment of SOC stocks in boreal systems. Four sites in western Newfoundland and Labrador compose the Newfoundland and Labrador Boreal Ecosystem Latitude Transect (NL-BELT), part of the Canadian Forest Service's National Network of Latitudinal Transects. The sites span a 6°C range in MAT, and all sites have vegetation dominated by balsam fir (*Abies balsamea* (L.) Mill.) ranging in age 60 to 110 y. In the current study, we focus on two of these four sites, Salmon River and Grand Codroy (SR and GC; Figure 1), both of which are similarly aged stands (60–65 years). The more southern, warmer site (GC) has a 3.4°C higher MAT and a 2.4°C higher growing season soil temperature (Environment Canada, Climate normals and averages, http://climate.weatheroffice.gc.ca) and generally lower soil moisture availability than the northern, cooler site (SR, Table 1). The site difference in temperature falls at the lower range of the projected MAT increases predicted for the boreal region this century [*IPCC*, 2007]. Both sites possess Typic Mesisols in the organic horizon and Orthic Humo-Ferric Podzols in the top 10 cm of the moderately well drained mineral soils, equivalent to Histosols and Podzols in the U.S. soil classification system. Mineral soil pH in these forests typically varies between 3.75 and 4.50 [*South*, 1983]. Other site characteristics are presented in Table 1.

2.2. Soil Collections and Laboratory Processing

[9] To assess soil characteristics at both sites and to collect soils for temperature manipulations in the laboratory, we collected fresh litterfall and soil samples from the O horizons (∼20–30 cm at SR, ∼15 cm at GC) and underlying mineral horizon (0–10 cm). At each site, we identified three circular plots (30 m diameter). Within each circular plot, three smaller subplots (5 m radius) were distributed evenly, and three random sampling points were chosen within each of

Table 1. Site Characteristics of Salmon River (SR) and Grand Codroy (GC) Along the Newfoundland and Labrador-Boreal Ecosystem Latitude Transect in Eastern Canada

Site	Coordinate[a]	Meters Above Sea Level[a] (m)	Age (years)	Dominant Vegetation	Soil Type[b]	MAT[a] (°C)	MAP[a] (mm)	GSST[c] (°C)	Degree-Days Above 5°C[a]
SR	51.18°N, 56.01°W	14.0	∼60	*Abies balsamea (L.) Mill.*	Sandy loam Podzols	1.7	1247	13.6	970.1
GC	47.51°N, 59.15°W	13.1	∼65	*Abies balsamea (L.) Mill.*	Sandy loam Podzols	5.1	1534	16.0	1380.4

[a]Canadian climate normals or averages, 1971–2000, http://www.climate.weatheroffice.gc.ca/climate_normals/index_e.html. MAT, mean annual temperature; MAP, mean annual precipitation.
[b]From *South* [1983].
[c]Growing season soil temperature (GSST) averaged over July and August in 2010 based on data from Canadian Forest Service, Atlantic Forestry Centre, at Corner Brook, Newfoundland, Canada.

these subplots. At each sampling point, we collected the entire organic horizon using a predesigned, rectangular frame (700 cm^2). We chose a separate location within each plot for collecting the mineral soil sample (0–10 cm). This sampling scheme generated two sites with three plots at each site, and three replicates representing composited subplot samples within each plot, for a total of 18 organic horizon samples and 6 mineral soil samples. We also collected fresh fir needles by cutting small branches from several dominant trees in each plot. All samples were shipped to the University of Kansas (KU) in coolers and subsequently stored at 4°C until analysis.

[10] We characterized O horizon samples by dividing them into three subhorizons (Oi, Oe, and Oa). Materials in the top layer (~0.5 to ~1 cm in depth) typically varied in color from the underlying soils, and were isolated and homogenized to a sample we identified as the Oi subhorizon, which is primarily composed of fresh and slightly decomposed litter. The lowest O horizon materials were separated into a sample we identified as the Oa subhorizon on the basis of the relatively dark color and high root density in comparison to overlying subhorizons. The remaining material was identified as Oe subhorizon material. In those cases for which Oe and Oa horizons were not clearly differentiated, all material except Oi was regarded as one, Oea subhorizon. The following analyses were conducted on bulk soils at each subhorizon, consisting of multiple C pools and a different ratio of these pools at each subhorizon.

[11] Soil moisture was determined by drying subsamples for 24 h at 105°C. The organic matter content of all soil samples was gravimetrically determined using loss on ignition for 4 h at 550°C. Oven-dried subsamples of O and mineral horizon material (60°C) were ground to a fine powder for analysis of total organic C and N, and $\delta^{15}N$ and $\delta^{13}C$ on a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, California, USA) coupled to a Finnigan MAT253 stable isotope mass spectrometer (Finnigan MAT, Bremen, Germany) at the University of Kansas. Subsamples were similarly prepared for radiocarbon measurements, and sent to the Center for Applied Isotope Studies at the University of Georgia for analysis on an accelerator mass spectrometer (National Electrostatics Corporation, 1.5SDH-1 Pelletron Accelerator Mass Spectrometer, Middleton, Wisconsin). The $\Delta^{14}C$ value was converted to a radiocarbon age (^{14}C yr B.P.) and calibrated to calendar years before present (cal yr B.P., where B.P. = A.D. 1950) using Calib 6.0.1 software [*Stuiver and Reimer*, 1993] and the data set of *Reimer et al.* [2009]. Age calibrations for samples containing greater than 100 percent modern radiocarbon (pMC) were calculated using the CALIBomb program [*Telford et al.*, 2004; *Reimer et al.*, 2009]. The rapid decline of radiocarbon in the atmosphere following the peak of atmospheric nuclear testing allows for high-resolution determinations of age based on excess radiocarbon content. All calibrated ages were converted to a single weighted mean age following the protocol of *Telford et al.* [2004]. We present these data as both weighted mean calibrated ages and $\Delta^{14}C$, to limit problematic inferences about the age of biomass formed during nuclear testing periods. We interpret each age determination as the mean age of all soil carbon pools in a particular bulk soil sample.

2.3. Laboratory Incubation

[12] We composited Oe and Oa subsamples (with no Oi) from replicated subplots on an equal dry weight basis. The incubation was conducted at 15°C and 20°C for 120 days. These temperatures are commonly observed in situ during the warmest weeks of the growing season at the southern site, and represent the high end of the range observed at the northern site. We substituted the well-quantified, natural Oi subhorizons with proportional amounts of loblolly pine (*Pinus taeda L.*) litterfall grown with ^{13}C-depleted CO_2 at the Duke FACE site [*Hendrey et al.*, 1999; *Andrews and Schlesinger*, 2001; *Billings and Ziegler*, 2008]. This permitted us to supply an isotopic tracer to microbial communities via a realistic substrate replacement instead of glucose or other isolated compound, after which preferential substrate utilization is typically observed [*Kuzyakov*, 2010]. The coniferous litter possessed a C concentration of 49.4% and a N concentration of 0.56%, resulting in a C:N of 88.2. Replaced Oi material $\delta^{13}C$ was −34.9‰, and $\delta^{13}C$ of natural Oea subhorizons was −28.5‰. The replaced Oi was ground on a Wiley mill and sterilized using a pressure cooker [*Wright and Jawson*, 2001] to ensure that mineralization was performed by microbial communities native to the incubated Oea horizons, and to promote adequate microbial accessibility of litter and Oea materials on the timescale of these incubations. Though the bulk Oe + Oa material is composed of more labile organic material than the mineral material underlying it, its decades-old age signifies that it represents more humified, microbially processed organic substrates relative to our replaced litter [*Beyer et al.*, 1993]. Thus, our experiment provides a suitable platform for examining warming effects on microbial choice of the two C pools, litter possessing a greater relative abundance of fast-turnover material versus more humified SOC with relatively slower turnover times.

[13] Mesic Oe and Oa subhorizons (equivalent 10.0 g dry weight total) were weighed into PVC cores (5 cm diameter, 7.5 cm tall) and sealed at the bottom with glass fiber paper. The uniquely labeled Oi material (2.5 g) was added to the cores and well mixed with soils on the 1st and 62nd days of the incubation. These treatments are hereafter termed Oi + Oea treatment. The quantity of litter materials (5.0 g) added approximated the mass of replaced Oi material typically associated with the corresponding mass of incubated Oea material. We incubated an equivalent number of Oe + Oa subhorizons without litter additions (hereafter, termed Oea treatments). Water was added to bring the soils from both sites to 75% of their respective soil water holding capacity (4.6 and 6.2 g_{H2O} g_{soil}^{-1} for GC and SR, respectively), a moisture content acknowledged as promoting microbial activity in O horizons [*Linn and Doran*, 1984]. The PVC cores were placed in incubation jars (~1 L) on a layer of glass beads to ensure that the cores did not rest in moisture at the bottom of the jar. A few milliliters of water were added to the jar to keep the atmosphere within the jar relatively humid. The moisture loss in each core was monitored every other day and moisture levels were maintained by adding an equivalent amount of water lost in each PVC core to ensure that diffusion of substrates to enzymatic reaction sites was not limited.

[14] Incubating jars were sealed with airtight lids equipped with a septum for gas sampling, and two 14 mL gas samples were immediately taken from each jar to establish starting conditions in the incubation vessels. One sample was injected into a previously evacuated, airtight vial for analysis of CO_2 concentration. The other sample was injected into a previously evacuated, airtight Exetainer (Labco, Buckinghamshire, UK) for analysis of $\delta^{13}C$ of CO_2. Gas samples were taken and jars were aerated on days 1, 5, 16, 32, 61, 63, 79, 104 and 120. Carbon dioxide concentration data were obtained for every sampling date, and isotopic data were obtained on day 1, 32, 61 and 120. Carbon dioxide concentrations were measured via gas chromatography (thermal conductivity detector, Varian CP3800, Varian Inc., Walnut Creek, California, United States). The $\delta^{13}C$ values of respired CO_2 were measured on a gas headspace sampling system (ThermoFinnigan GasBench II, ThermoFinnigan, Bremen, Germany) coupled to an isotope ratio mass spectrometer (DeltaPlus XP, ThermoFinnigan, Bremen, Germany). We calculated rates of CO_2 production using concentration data, dry weight of the soil subsamples, and the headspace volume of each incubation vessel. The cumulative respiration calculation assumes the respiration rate is constant until the next measurement was made.

[15] We corrected for the influence of lab air on measured $[CO_2]$ and $\delta^{13}C$ of CO_2 in each jar using average values of lab air $[CO_2]$ (550 ppm) and its $\delta^{13}C$ value (−8‰). These numbers represent the average of multiple samplings of lab air during the incubation. On the basis of a mixing model, $\delta^{13}C$ of CO_2 in our incubation jars (Oi + Oea) represents a mixture of CO_2 derived from laboratory air, respired SOM and replaced litter. We first derive $\delta^{13}C$ of mixed CO_2-C from respired SOM and replaced litter (equation (1)) by excluding the influence of laboratory air CO_2-C.

$$\delta^{13}C_{Oi+Oea} = \frac{\delta^{13}C_{Oi+Oea+Air} \times V_{Oi+Oea+Air} - \delta^{13}C_{Air} \times V_{Air}}{V_{Oi+Oea+Air} - V_{Air}} \quad (1)$$

$\delta^{13}C_{Oi+Oea+Air}$ and $\delta^{13}C_{Oi+Oea}$ denote $\delta^{13}C$ of CO_2-C from SOM, litter, and laboratory air, and from SOM and replaced litter, respectively. $V_{Oi+Oea+Air}$ denotes the total concentration of CO_2 respired from SOM, the replaced litter, and ambient laboratory CO_2 introduced into the sample. V_{Air} represents the CO_2 concentration of laboratory air (550 ppm). We then derived the proportion of respired CO_2-C from SOM (Oea) in the total respiration from SOM and replaced litter (equation (2)).

$$P_{Oea} = \frac{\delta^{13}C_{Oi+Oea} - \delta^{13}C_{Oi}}{\delta^{13}C_{Oea} - \delta^{13}C_{Oi}} \quad (2)$$

where P_{Oea} denotes the proportion of respired CO_2-C from indigenous SOM (Oea), and $\delta^{13}C_{Oea}$ and $\delta^{13}C_{Oi}$ denote the $\delta^{13}C$ of SOM and replaced litter, respectively. We assume that the difference between the $\delta^{13}C$ of respired CO_2 and the $\delta^{13}C$ of the substrate from which it is derived is negligible, and that this offset is equivalent for both indigenous SOM and replaced litter. We elected to invoke the most simplistic assumption, in keeping with established protocols [*O'Malley et al.*, 1996; *Phillips et al.*, 2005]. We used estimates of the proportions of material from which CO_2 was derived and total respired CO_2 within each jar to examine the priming effect of Oi replacement; no significant positive priming effects were detected on any sampling date.

2.4. Microbial Biomass and Extracellular Enzyme Activities

[16] Soil subsamples were destructively sampled on day 0, 5 and 120 to estimate microbial biomass. We employed chloroform fumigation-K_2SO_4 extraction [*Brookes et al.*, 1985] and potassium persulfate (0.5M $K_2S_2O_4$) digestion methods to quantify microbial biomass C [*Paul*, 2007]. All K_2SO_4 soil extracts were shaken on a mechanical shaker for 1 h and then filtered through Whatman #40 filter paper. Extractable organic carbon (EOC) in fumigated and unfumigated samples was analyzed colorimetrically on a Lachat autoanalyzer (Madison, Wisconsin, United States), and the difference between fumigated and unfumigated treatments represents microbial biomass C.

[17] At the end of the incubation (day 120), we extracted dissolved organic C (DOC) to assess its concentration and $\delta^{13}C$. Residual soils from each jar were leached with 100 mL of 0.001N $NaHCO_3$ to provide a solution similar in ionic strength to rainfall [*Wickland et al.*, 2007]. The slurry was thoroughly stirred and prefiltered with glass microfiber filters (1.6 μm pore size), and final leachates were filtered through sterile, prerinsed disposable GF/F filters (0.45 μm pore size). Thirty milliliters of the leachate was frozen at −20°C in HDPE bottles for both DOC concentration and $\delta^{13}C$ analysis on an Aurora 1030 TOC Analyzer (OI Analytical; College Station, TX, USA) interfaced with a Delta V plus isotope ratio mass spectrometer (ThermoElectron; Bremen, Germany) via a Conflo III interface (ThermoElectron). The analytical precision for $\delta^{13}C$ analysis was 0.1‰.

[18] On day 120, hydrolytic and oxidative extracellular enzyme assays were performed according to protocols discussed in several studies [*Sinsabaugh et al.*, 2000; *Waldrop et al.*, 2003; *Allison et al.*, 2008]. These measures represent potential enzyme activities indicative of overall enzyme concentrations [*Wallenstein and Weintraub*, 2008] and the potential microbial capacity to process labile and relatively slow-turnover SOM. We used fluorescently labeled substrates to index the enzymes α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), cellobiohydrolase (CBH), β-1,4-xylosidase (BXYL) [*Marx et al.*, 2001]. We also used colorimetric techniques to assess the potential activity of phenol oxidase (PHENOX) and peroxidase (PEROX) [*Saiya-Cork et al.*, 2002]. In this study, we consider labile C acquisition enzymes as the sum of AG, BG, CBH and BXYL.

[19] For these assays, 1.0 g soil sample (fresh weight) was homogenized by mixing with 125 mL of 50 mM sodium acetate buffer (pH 5.3) for 30 s with a hand blender. To quantify extracellular enzyme activities (EEA) for each soil sample, 16 replicate wells containing 200 μL soil slurry and 50 μL of substrate were used. To calculate the quench coefficient, eight wells were used containing 200 μL soil slurry and 50 μL of standard (10 μM 4-methylumbelliferone (MUB)) for hydrolytic enzymes; an additional control (blank) was composed of eight wells pipetted with 200 μL soil slurry. Negative controls consisted of eight wells with 50 μL substrate and 200 μL buffer. Eight wells with 50 μL MUB or 7-Amino 4-methylcoumarin (MC) and 200 μL buffer were used to derive the emission coefficient. We used L-3,4-dihydroxyphenylalanine (DOPA) as a substrate for

phenol oxidase and peroxidase. The plates were incubated at 15°C or 20°C, corresponding to their respective temperature treatments, for approximately 20 h. In each well of all fluorescence plates, 10 μL of 0.5 M NaOH was added to raise the MUB or MC emission coefficients to a detectable level. Fluorescence was assessed using a microtiter plate fluorometer (Molecular Devices, Sunnyvale, California, United States) set to excitation wavelength of 365 nm and emission wavelength of 460 nm. Spectrophotometric activity was quantified with a spectrophotometer (Molecular Devices, Sunnyvale, California, United States). We measured the absorbance at 460 nm for PHENOX and PEROX. Measurements are presented as nmol activity h^{-1} g_{SOM}^{-1}.

2.5. Statistical Analysis

[20] We applied repeated measure ANOVA (PROC MIXED, SAS, Cary, North Carolina, United States) to assess the main effects of temperature, site, and their interaction on soil respiration rate, $\delta^{13}C$ of respired CO_2, proportion of CO_2 respired from Oea, microbial biomass and EEA for multiple enzymes during the incubation for both Oea and Oi + Oea treatments. A separate repeated measure ANOVA was conducted for days 1 to 61 (first litter replacement) and for days 63 to 120 (second litter replacement) in Oi + Oea treatments, respectively. To assess if changes with warming in these same variables varied with site, we applied repeated measure ANOVA on variable differences between two temperatures (values at 20°C minus that at 15°C). Post hoc tests via Tukey-Kramer adjusted p values were also used to assess the effects of temperature, site or their interaction within each date if we observed a significant interaction with date based on repeated measure ANOVA. We also employed a two-way ANOVA test to assess the main effects of temperature, site and their interaction on cumulative respiration (as distinct from respiration rates) and its difference between two temperatures (values at 20°C minus that at 15°C) on each date in Oea and Oi + Oea treatments, respectively. Paired t tests were used to compare $\delta^{15}N$, $\delta^{13}C$ and C:N between sites at each depth, and $\delta^{13}C_{DOC}$ and [DOC] between two temperature treatments. The overall average of each EEA across all dates was tested by two-way ANOVA to examine generalized temperature and site effects.

3. Results

3.1. Soil C and N Pools, Stable Isotopes, and Radiocarbon Age

[21] At both sites, SOC and SON concentrations declined with depth (Table 2). At most depths, both sites exhibited similar SOC and SON concentrations. Though C:N ratios at the warmer site were higher in needles, C:N ratios were significantly lower in the Oi subhorizon, with similar but nonsignificant trends in all other horizons assayed as well. In spite of similar $\delta^{15}N$ signatures of fresh needles at the two sites, mineral soils exhibited significantly higher $\delta^{15}N$ and $\delta^{13}C$ values at GC compared to SR, with similar but nonsignificant trends through the organic horizon (Figure 2). $\Delta^{14}C$ values contrasted between sites in both Oea and mineral soil horizons. At both sites, the radiocarbon age of the SOM increased with depth from ages on the timescale of decades in O horizons to those of several hundred years within the mineral soil horizon. Calibrated radiocarbon ages within the Oi and Oea subhorizons were similar across sites. The weighted mean calibrated radiocarbon age of mineral soil SOC at SR (~600 cal yr B.P.) contrasted substantially with that at GC (~110 cal yr B.P.; Figure 2). Detailed information about radiocarbon ages and the corresponding probabilities are presented in the auxiliary material.[1]

Table 2. Soil Organic Carbon and Nitrogen Concentration and Bulk Mass C:N Ratio in Needle and Soil Samples Collected From Salmon River (SR) and Grand Codroy (GC) in July 2009[a]

Depth	N (%)		C (%)		C:N	
	SR	GC	SR	GC	SR	GC
Needle	1.26	1.11*	50.4	50.2	40.7	45.5*
	(14)	(5.4)	(0.5)	(1.0)	(14)	(5.9)
Oi	1.61	1.85**	49.4	49.2	31	26.8**
	(17)	(16)	(7.7)	(9.5)	(20)	(14)
Oe	1.76	1.74	49	48.4	28.1	28
	(15)	(14)	(6.9)	(3.1)	(20)	(4.4)
Oa	1.6	1.63	47.9	43.1	32	26.6
	(51)	(35)	(10)	(23)	(58)	(5.1)
Min	0.3	0.24	6.59	3.93	21.2	16.7
	(60)	(8.1)	(78)	(18)	(27)	(8.1)

[a]Mean and coefficient of variation (CV, in parentheses) are calculated from nine replicated samples for needle, Oi, Oe, and Oa and three replicated samples for mineral soil. Asterisks indicate significant differences between sites: *, p value < 0.05; **, p value < 0.01.

3.2. Soil CO_2 Efflux and Source

[22] There were significant interaction effects of temperature or site with date on respiration rate for both Oea and Oi + Oea incubations (p values < 0.05). High-temperature treatments exhibited a higher rate of respiration than low-temperature treatments on days 32, 61, 63, 104 and 120 in both Oea and Oi + Oea treatments (Figure 3, insets). The northern SR soils exhibited a higher rate of respiration than southern GC soils on day 1, 5 and 16 in both Oea and Oi + Oea incubations, regardless of incubation temperature. There was a significant interaction of site, temperature and date on respiration in the Oea incubations only, demonstrating a site influence on temperature effects and a temperature influence on site effects; these effects were consistent with the site and warming effects on each date (data not shown). There were significantly positive temperature effects on cumulative respiration on day 5, 16, 79, 104 and 120 in the Oea treatment, and on day 104 and 120 in the Oi + Oea treatments (Figure 3). The north cooler soils (SR) released significantly higher cumulative respiration than the south warmer soils (GC) on all dates in the Oi + Oea treatment and on all but days 104 and 120 in the Oea treatment. A significant interaction of temperature and site was evident on day 16 in the Oea treatment (data not shown). The respiration rate was higher at SR than at GC on each date, and SR produced significantly higher (13%) cumulative respiration on average than GC on day 120, combining the Oi + Oea and Oea treatments.

[23] The $\delta^{13}C$ of respired CO_2-C was significantly greater with warming on days 1, 32, 61 and 120 in the Oea incubations, and on days 32, 61 and 120 in Oi + Oea treatments

[1]Auxiliary materials are available in the HTML. doi:10.1029/2011JG001769.

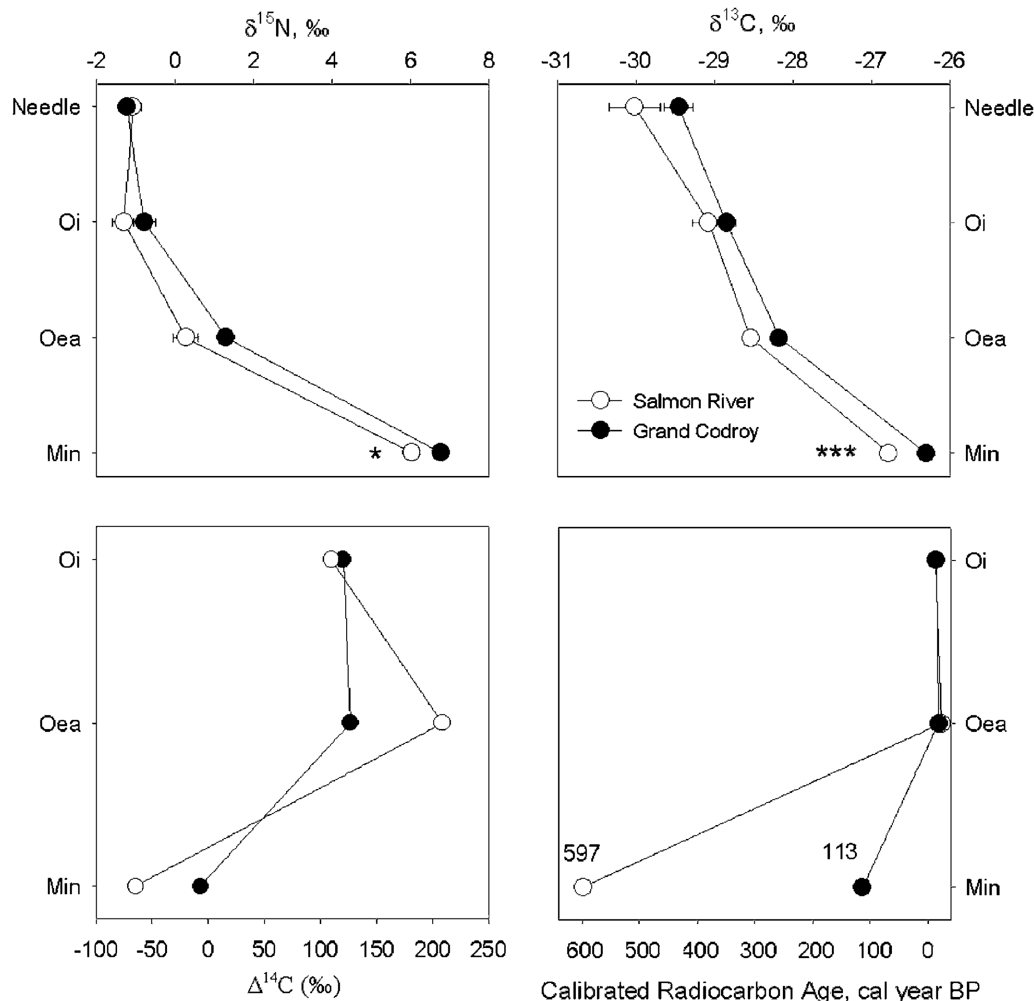

Figure 2. (top) The stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) composition of needle, O horizon, and mineral soil horizons and (bottom) radiocarbon content ($\Delta^{14}C$) and radiocarbon age estimates (cal year B.P.) in O and mineral soil horizons between Salmon River (SR) and Grand Codroy (GC) along the NL-BELT. In Figure 2 (top), the mean and standard error are provided for the needle (n = 9), Oi and Oea (n = 9), and mineral soils (n = 3). In Figure 2 (bottom), weighted mean radiocarbon age estimates in mineral soil horizons are presented. Details regarding radiocarbon calibrations are presented in the auxiliary material. Methods used to calculate the radiocarbon ages and probabilities are presented in section 2. Asterisks indicate the following: *, p value < 0.05; ***, p value < 0.001.

(Table 3). On day 1 in the Oea incubation, there were significant site effects such that the $\delta^{13}C$ of respired CO_2-C was significantly greater in the northern cooler soils (SR) than that in the southern warmer soils (GC) in the Oea treatment (Table 3). The proportion of respired CO_2 derived from Oea in Oi + Oea treatments was significantly greater with warming on days 61 and 120 (Figure 4). There was neither a significant site effect nor an interaction of site with date on these proportions.

3.3. Microbial Biomass and DOC

[24] Microbial biomass C exhibited no significant temperature effects in either Oea or Oi + Oea incubations. We observed a significant site effect on day 0 in the Oea treatment, with 25% more microbial biomass at the northern cooler site (SR) than that at the southern warmer site (GC). Microbial biomass on day 5 was significantly higher than that on day 0 or day 120, but there was no significant difference between days 0 and 120. On day 120, the $\delta^{13}C$ of dissolved organic carbon (DOC) was greater with warming across all treatments (paired t test, $p = 0.03$). There were no significant site or temperature effects on DOC concentration in Oea or Oi + Oea treatments ($p > 0.05$).

3.4. Hydrolytic and Oxidative Enzymes

[25] Extracellular enzymes associated with labile C acquisition significantly increased with warming on day 120 in Oi + Oea materials (Figure 5). Phenol oxidase activities increased significantly with warming in Oi + Oea material on days 5 and 120. Peroxidase activities decreased with warming on day 120, though these changes were not significant. Total oxidase activity (sum of phenol oxidase and peroxidase) showed no significant change with warming in Oi + Oea materials on days 5 and 120. Phenol oxidase

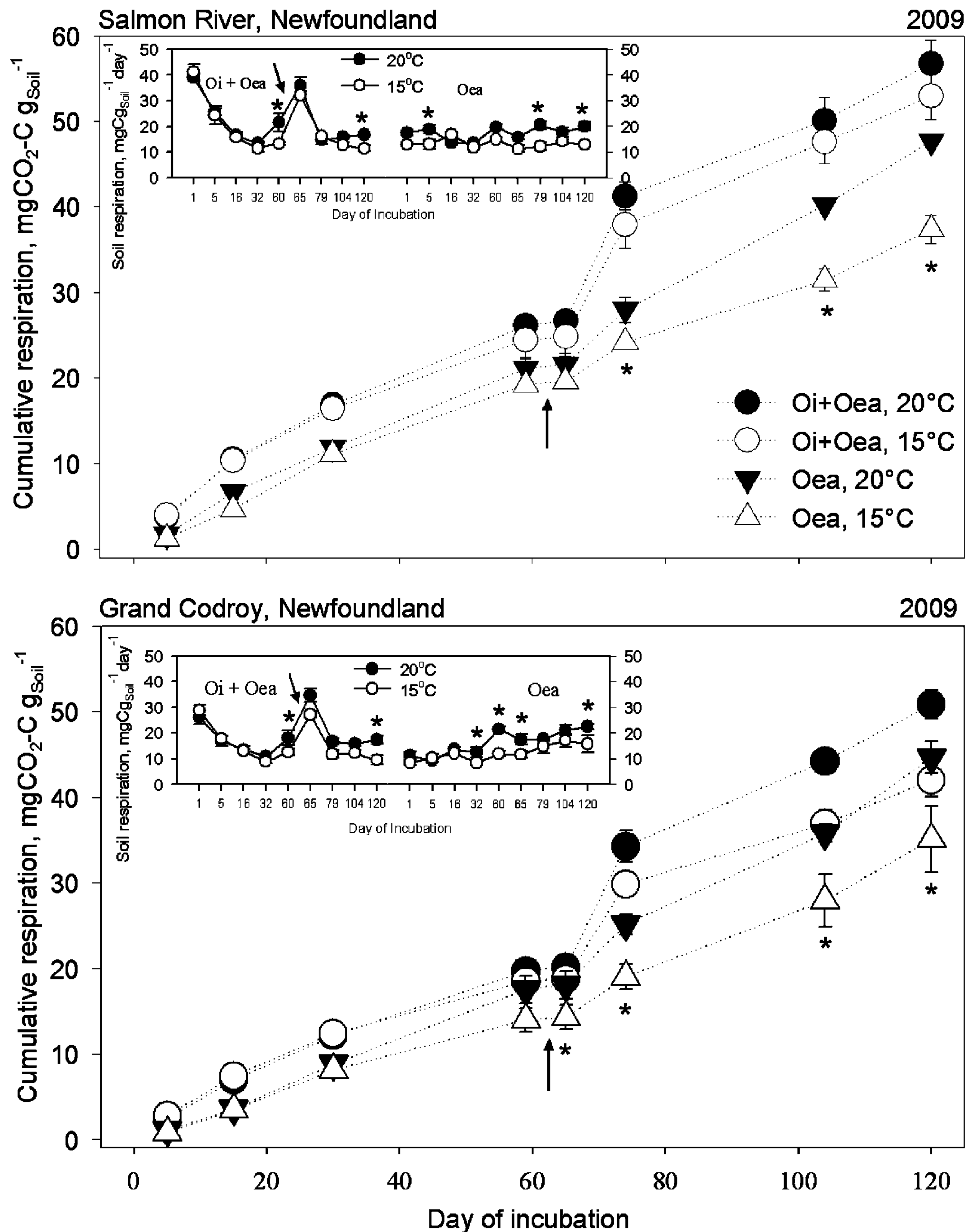

Figure 3. Cumulative soil CO_2 efflux from Salmon River and Grand Codroy soils at two temperatures, with and without replaced Oi subhorizon during a 120 day laboratory incubation. The insets show soil respiration rates applied to estimate cumulative respiration, assuming the rate is applicable until the following measurement. Asterisks denote significant temperature effects at $\alpha < 0.05$. Error bars indicate standard errors of the means (n = 3). The data on day 61 are presented on an earlier date on x axis to distinguish it from day 63. The arrows denote the date of the second litter addition.

Table 3. Stable Carbon Isotope Composition (δ^{13}C) of Respired CO_2-C From Oea or Oi + Oea Treatments on Days 1, 5, 32, 61, and 120 at Two Sites[a]

Site	Litter	Temperature (°C)	Incubation Time				
			Day 1[a]	Day 5	Day 32[b]	Day 61[b]	Day 120[b]
SR[b]	Oi + Oea	15	−33.1 ± 0.4	−33.7 ± 1.1	−32.4 ± 0.9	−31.8 ± 0.3	−31.9 ± 0.4
	Oi + Oea	20	−33.0 ± 0.2	−32.5 ± 0.5	−31.2 ± 0.3	−29.3 ± 0.5	−30.4 ± 0.1
	Oea	15	−32.7 ± 0.5	−34.0 ± 1.9	−31.2 ± 0.3	−30.1 ± 0.1	−29.5 ± 0.1
	Oea	20	−29.6 ± 0.7	−30.1 ± 0.5	−30.1 ± 0.3	−28.5 ± 0.3	−28.2 ± 0.1
GC	Oi + Oea	15	−33.1 ± 0.7	−34.6 ± 0.4	−34.2 ± 0.9	−31.9 ± 0.4	−32.8 ± 0.7
	Oi + Oea	20	−33.0 ± 0.5	−35.2 ± 0.5	−31.9 ± 0.5	−29.4 ± 0.1	−29.8 ± 0.2
	Oea	15	−37.6 ± 1.7	−34.5 ± 1.7	−32.9 ± 1.3	−30.7 ± 0.7	−28.9 ± 0.9
	Oea	20	−31.5 ± 0.7	−35.0 ± 1.3	−29.7 ± 1.0	−27.8 ± 0.0	−27.1 ± 0.1

[a]Significant site effects at $\alpha < 0.05$.
[b]Significant temperature effects on specific date or in specific site at $\alpha < 0.05$.

activities exhibited an increase with warming of more than ten times (about 1341 nmol activity h^{-1} g_{SOM}^{-1}) on day 5, and a twofold increase (about 524 nmol activity h^{-1} g_{SOM}^{-1}) in the cooler site soils with warming on day 120, while labile C acquisition enzyme activities exhibited significant increase with warming of 1.5 times (about 1001 nmol activity h^{-1} g_{SOM}^{-1}) activities in cooler soils on day 120. More northern, cooler soils (SR) tended to exhibit significantly higher EEA than south warmer soils (GC) on average on day 5 or 120 (Figure 5).

3.5. Site Influence on Warming-Induced Changes in Measured Variables

[26] As indicated above, warming usually induced positive changes in respiration and enzymatic activities. Further, warming-induced increases in some variables exhibited significant differences between sites such that positive changes with warming at south site (GC) were consistently larger than at the north site (SR). These include respiration rate in the Oea treatment (day 16 and 61) and in the Oi + Oea treatment (day 79), the δ^{13}C of respired CO_2-C in the Oea treatment (day 32), and in the Oi + Oea treatment (day 120), and EEA associated with labile C acquisition in the Oi + Oea treatments (day 5). The warming-induced changes in other variables did not show significant differences between sites on any date or treatments (Oea, or Oi + Oea) during the incubation. These variables include cumulative respiration, microbial biomass and oxidative enzyme activities (phenol oxidase and peroxidase).

4. Discussion

[27] Both incubation data and profile SOM characteristics are consistent with enhanced turnover rates of relatively slower-turnover SOM with warmer temperatures. As with many studies reporting observational data describing soil profiles, we cannot conclusively determine the dominant processes governing their values. However, the more detailed incubation data offer insight into the microbial mechanisms likely important in determining O horizon decay responses to temperature and the relative losses of fresh (Oi) versus more humified material (Oea) with warming. Further, these results offer insight into mineral profile SOM dynamics as well by providing mechanisms for changes in input materials and microbial processes with warming relevant to the mineral profiles in these soils. Relating the O horizon responses to laboratory warming to the C:N and isotopic depth distributions in the field represents a first step toward assessing the potential relevancy of shorter-term processes to the longer-term timescales relevant for SOM with relatively long mean residence times. Here, we discuss profile trends and incubation data, and then discuss the degree to which patterns in these data can assist in the development of new insights into how climate warming may influence SOM transformations and fate in these forests.

4.1. Profile Trends Across the Latitudinal Gradient

[28] Contrasting soil C:N, δ^{15}N, δ^{13}C and radiocarbon ages across the latitudinal gradient represented by our sites suggest pronounced differences in SOM dynamics with

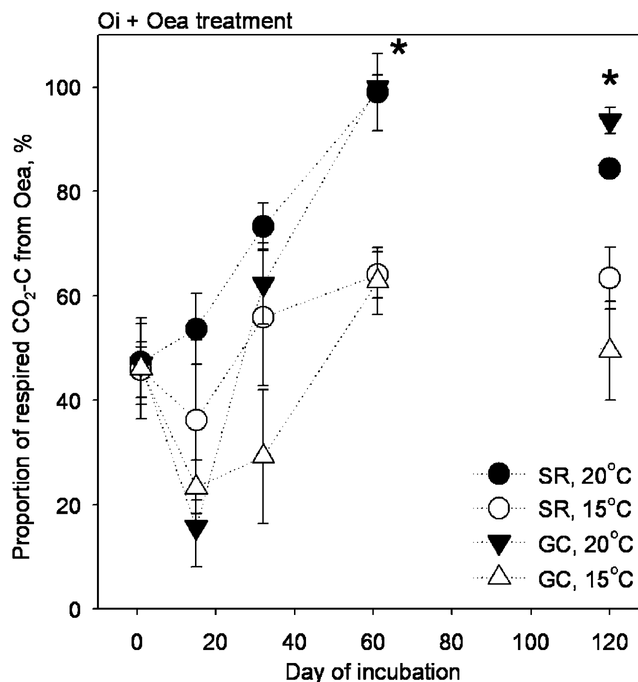

Figure 4. Percent (%) of respired CO_2 derived from the Oea material in Oi + Oea treatments at Salmon River (SR) and Grand Codroy (GC). Asterisks denote significant temperature effects at p value < 0.05. The data for day 5 are presented on a later date on the x axis in order to distinguish them from day 1.

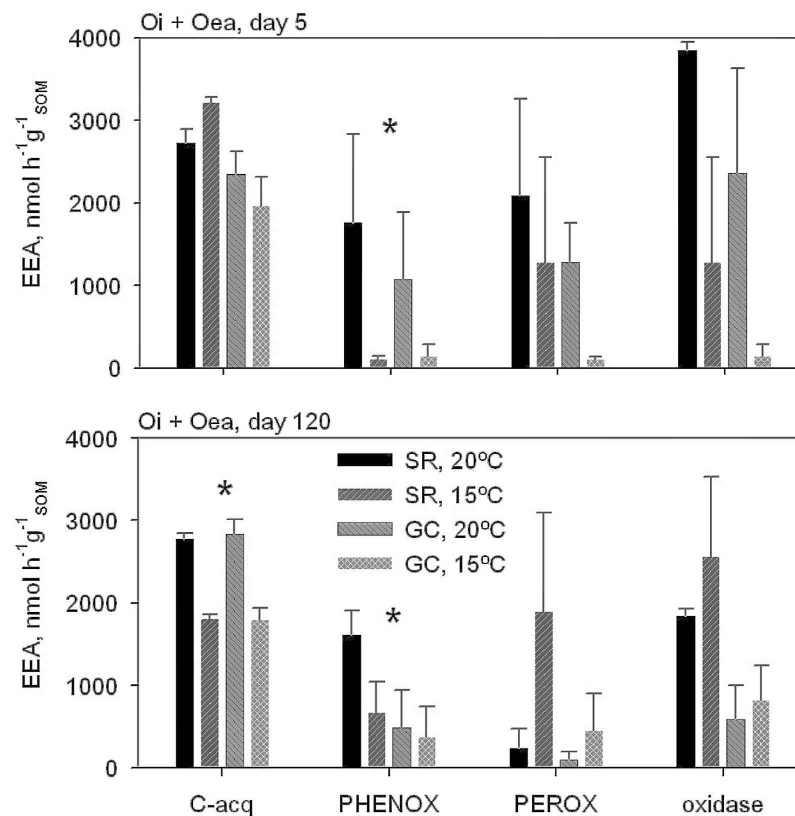

Figure 5. Extracellular enzyme activities (EEA, nmol h^{-1} g_{SOM}^{-1}) in the Oi + Oea incubations on days 5 and 120. C-acq denotes the sum of α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), cellobiohydrolase (CBH), and β-1,4-xylosidase (BXYL), four enzymes associated with relatively labile C acquisition; PHE is phenolic oxidase; PER is peroxidase; and oxidase is the sum of PHE and PER. Each bar represents a mean with a standard error (n = 3). Asterisks denote significant temperature effect at p value < 0.05 based on two-way analysis of variance (ANOVA).

changing climate. The lower soil C:N ratios at the warmer site, in spite of higher litter C:N ratios, are consistent with a greater degree of microbial cycling and processing of SOM in the warmer environment [*Rice and Tenore*, 1981; *Melillo et al.*, 1984; *Hedges et al.*, 1994]. These trends parallel those observed in δ^{15}N and δ^{13}C patterns at the sites. Relative ^{15}N and ^{13}C enrichment in organic and mineral soil, particularly when paired with similar values in needle inputs, is consistent with greater microbial processing of these materials [*Natelhoffer and Fry*, 1988; *Billings and Richter*, 2006].

[29] Though radiocarbon signatures in Oi and Oea horizons do not exhibit clear patterns of age with depth, nor site differences in these horizons, the average younger age (~500 years younger) of mineral SOC at the warmer, southern site suggests differences in profile C dynamics between this and the northern, cooler site. The radiocarbon age of soil profiles reflects both C inputs and outputs. Initial characterization of inputs at these sites indicates that soil profiles at the warmer site receive approximately 1.3 times the annual foliar litterfall observed at the northern site (0.37 versus 0.28 kg m^{-2} yr^{-1}, X. Zhu et al. unpublished data, 2011), suggesting that greater input rates of relatively "young" C may drive, in part, differences in mineral profile radiocarbon signatures. However, additional observations suggest that differences in the radiocarbon age observed in the mineral profiles at these sites are reflective of different patterns of SOC losses as well. For example, we observed consistently shallower O horizons (10 cm versus 15 cm on average) and lower estimates of total SOC in the O horizon (3.3 versus 4.3 kg m^{-2}) and the top 10 cm of mineral material (2.5 versus 4.7 kg m^{-2}) at the warmer site in spite of greater litterfall inputs. Though mineral profile estimates may be confounded by rock content, our observations of SOC pool differences in both horizons with latitude are consistent with SOC estimates in other boreal forests spanning MAT differences [*Vogel et al.*, 2008; *Kane and Vogel*, 2009]. Further, root biomass is about 8.1% and 6.6% of bulk soil weight in organic horizons, compared to 0.37% and 0.43% of bulk soil weight in mineral profiles of the cooler and warmer sites, respectively (J. Laganière, unpublished data, 2011), suggesting that roots are less of an input to the mineral profiles than to the O horizons at both sites. We do not know rates of root or dissolved organic matter (DOM) input into these mineral profiles from upper organic horizons, but greater litterfall rates in conjunction with lower SOC pool sizes at the warmer site, together with low root biomass in mineral relative to O horizons at both sites, suggest that observed radiocarbon age differences between these mineral profiles convey important information about differences in patterns of C losses. Though not by any means conclusive, these radiocarbon data are consistent with greater relative

losses of 'old' SOC at the warmer, southern site compared to the more northern site. We further explore this idea below when we discuss these data in conjunction with incubation data.

4.2. O Horizon Transformations During Laboratory Incubations

[30] During the incubation, warming elevated the proportion of respired CO_2 and leached DOC derived from indigenous, more humified SOM in comparison to the fresher Oi material. This is consistent with the relative ^{13}C-enrichment of respired CO_2-C with warming in the incubated Oea subhorizons with no Oi replacements, and leached DOC released from both treatments, given that older, more recalcitrant SOC is usually ^{13}C enriched [*Fessenden and Ehleringer*, 2002]. These results collectively imply that microbial communities in these O horizons shift their substrate choice to more humified material to a greater extent with warming than to relatively labile litter material. If humified SOM experiences relatively greater mineralization to CO_2 and transformation to DOC with warming than more labile material, we would expect relatively greater increases in the activities of enzymes responsible for that material's breakdown. Indeed, we observed such EEA responses in our study, with the largest increases with warming in phenol oxidase activities, an exoenzyme that plays an active role in more humified SOC degradation [*Kirk and Farrell*, 1987; *Sinsabaugh*, 2010]. These increases with warming were significantly larger than those observed for the hydrolytic enzymes linked to labile C decay (100% versus 55%). Given the slower-turnover nature of more humified material relative to fresh litterfall [*Trumbore*, 2009], these results are suggestive of a biochemical response to warming that is at least qualitatively similar to that predicted by the Arrhenius relationship.

[31] The observed increases in exoenzymatic activities with warming were not driven by altered biomass pool size. Though some studies have showed that warming can increase microbial biomass C [*Bell et al.*, 2010; *Zhang et al.*, 2010], warming did not influence this parameter in our incubation. Given the lack of biomass changes with warming, altered EEA likely was driven by changes in ecophysiological properties within the microbial community that influenced EEA per unit biomass. Alternatively, community composition may have changed with warming such that the relative abundances of organisms with contrasting EEA responses to warming were altered. Isotopic signatures and relative abundances of phospholipid fatty acids extracted from the same incubated soils imply that warming enhanced fungal use of more humified SOM relative to the added litter (S. E. Ziegler et al., unpublished data, 2011), suggesting that both ecophysiological and structural features of the soil microbial communities were influenced by warming. Future work exploring the relative abundance of the laccase gene, associated with the expression of phenol oxidase [*Lauber et al.*, 2009], as well as other related functional genes may help inform us on what level (gene and/or community) microbial community structure and function may be altered with warming.

4.3. Linking Profile Characteristics to Incubation Data

[32] The differences we observed between O horizons subjected to different laboratory temperature regimes are consistent in some ways with differences between profile characteristics of the two sites. Most simplistically, we observed greater microbial processing rates with warming during the incubation, which are congruent with the decreased C:N and increased δ^{13}C and δ^{15}N within the soil profiles at the warmer site relative to the cooler site. Greater insight is possible when we consider the incubation data in more detail. The ^{13}C enrichment of DOC leachates, the similarity between leachate DOC concentrations, and greater relative releases of ^{13}C enriched CO_2 with warming all have implications for C transfer between horizons in these soils in situ. These data suggest that though the amount of DOC leached into the mineral profile may not vary with temperature, its composition changes, becoming increasingly dominated by material derived from relatively humified organic matter with warming. This, in turn, is consistent with the greater δ^{13}C signature of mineral profile SOC at the warmer, southern site (Figure 2), given that mineral horizon SOM is largely derived from compounds transported from the O horizon [*Dawson et al.*, 1978], particularly in soils such as these that exhibit extremely limited root development in the mineral horizons.

[33] It is more challenging to associate processes observed during O horizon incubations with the radiocarbon profile data. In addition to the obvious issue of differences in timescales between drivers of incubation and profile data, age differences between fresh Oi and more humified Oea subhorizons could not be resolved using radiocarbon (Figure 2). However, we know from incubation data that microbial communities in these O horizons appear to access a greater proportion of humified Oea material with warming, at least on relatively short timescales and after labile Oi input. In the mineral profile, the observed large differences in mean SOC radiocarbon age primarily reflect the net results of O horizon processing, subsequent transport of O horizon DOC to mineral horizons, and processing within the mineral profile itself. As described above, there are a number of potential explanations for the younger mean age of mineral SOC at the warmer site, but the relative lack of root growth into the mineral profile at these sites and our observations of litterfall inputs, O horizon thicknesses, and SOC content in multiple horizons provide constraints on possible scenarios. The most parsimonious scenario, consistent with the incubation observations and mean radiocarbon age differences in the mineral profile, is greater microbial acquisition of (1) a disproportionately larger fraction of more humified O horizon material, (2) associated increases in export of DOC derived from that material, and (3) potentially greater relative mineralization of "old" SOM within the mineral profile at the warmer site. If robust, this scenario has important implications for the warming of soils in these forests, as it implies that more humified and relatively older mineral SOC is relatively more susceptible to decay with warming, a conclusion mimicked on much shorter timescales by our O horizon incubation data.

[34] The incubation data also revealed differences between sites in microbial activity and Oea mineralization relevant to our understanding of the potential impact of warming in these forests over longer timescales. At a given temperature (15°C or 20°C), higher-latitude O horizon materials exhibited greater microbial biomass and oxidative EEA than lower-latitude O horizons. In contrast, with laboratory

warming lower-latitude O horizon material exhibited a greater relative and absolute response than higher-latitude O horizon material in terms of changes in the proportion of humified SOM mineralized and EEA associated with labile C acquisition. These differences between site incubation data suggest that microbial communities native to the more southern soils were better able to profit from the enhanced access to substrates provided by the temperature increase. These data are consistent with profile characteristics, given the frequency at which microorganisms from the more southern soils experience this warmer temperature. Here it remains unclear what physiological or community structural mechanisms are responsible for these communities' differences in abilities to respond to laboratory warming.

5. Conclusions

[35] This study offers empirical evidence derived from soil profile characteristics and laboratory incubations qualitatively consistent with predictions of enzyme kinetics with warming. Extrapolated over a longer timescale, the relatively greater microbial processing rates of humified SOM with warming during the short-term incubation are congruent with the elemental, stable isotopic, and radiocarbon patterns of soil profiles at the warmer site relative to the cooler site. We thus suggest that climate warming may influence soil microbial communities and their enzymatic dynamics such that relative losses of humified SOM in these mesic boreal soils may be disproportionately enhanced, particularly in the lower-latitude soils. Further, though quantifying spatially and temporally heterogeneous processes is inherently challenging, we suggest that linking microbially mediated mechanisms elucidated in laboratory warming studies with observations of profile characteristics across sites represents an important first step in linking biogeochemical processes operating on diverse timescales.

[36] **Acknowledgments.** This research was supported by the Humber River Basin Project, a research initiative of the provincial government of Newfoundland and Labrador, Canada, Memorial University of Newfoundland, and the Newfoundland and Labrador Department of Education, and by a grant from the National Science Foundation (0950095). We thank Martin Moroni, Darrel Harris and Kate Edwards for site identification and establishment as well as their forest knowledge, Doug Piercey for making the site map, Nameer Baker, Allison King and Lisa Tiemann for their laboratory assistance, and the Ecosystems Research Group at University of Kansas (KUERG) for insightful discussions.

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