# Soil organic matter diagenetic state informs boreal forest ecosystem feedbacks to climate change

Allison. N. Myers-Pigg[1, a], Karl Kaiser[2,3], Ronald Benner[4], Susan E. Ziegler[1]

5    [1] Department of Earth Sciences, Memorial University of Newfoundland, St. John's, A1B2S7, Canada

[2] Department of Marine Science, Texas A&M University, Galveston Campus, Galveston, 77553, USA

[3] Department of Oceanography, Texas A&M University, College Station, 77840, USA

10   [4] Department of Biological Sciences and School of the Earth, Ocean and Environment, University of South Carolina, Colombia, 29208, USA

*Correspondence to:* Susan E. Ziegler (sziegler@mun.ca) and Allison Myers-Pigg (a.myerspigg@gmail.com)

[a] Current affiliation: Pacific Northwest National Laboratory, Marine and Coastal
15   Research Laboratory, 98382, U.S.A.

**Abstract**. The fate of soil organic carbon (SOC) in boreal forests is dependent on the integrative ecosystem response to climate change. For example, boreal forest productivity is often nitrogen (N) limited, and climate
20   warming can enhance N cycling and primary productivity. However, the net effect of this feedback on the SOC reservoir and its longevity with climate change remains unclear. Here, we (1) applied lignin biomarkers to assess the diagenetic alteration of SOC in boreal forest organic soils across a climate gradient; and (2) investigated the coupling of soil C and N cycling and the influence of enhanced N availability on soil C stocks along this boreal forest climate transect. The lignin diagenetic state remained constant with climate
25   warming, indicating a balance between the input and removal of lignin in these mesic boreal forests. When combined with previous knowledge of these forest ecosystems, including the diagenetic state of soil organic nitrogen and direct measures of carbon fluxes and stocks, the results indicate a coupled increase in carbon and nitrogen cycling with climate warming that supports forest productivity and maintains SOC stocks. Our observations are consistent with several lines of evidence in other biomes not limited by water availability,
30   although the mechanisms for the maintenance of SOC stocks during climate warming appear to be ecosystem dependent.

## 1 Introduction

35   Terrestrial ecosystems containing globally-relevant stores of carbon, such as boreal regions, are rapidly
responding to climate-induced change (Soja et al., 2007). Boreal forests typically act as a net carbon sink
(Vanhala et al., 2016), with 50-75% of the total carbon stock stored within soils (Scharlemann et al., 2014).
The response of soil organic carbon (SOC) stocks to climate stressors in high latitude regions is a key
uncertainty in global Earth system models (Todd-Brown et al., 2013). Climate impacts the input and

40   removal of SOC by altering aboveground vegetation (Quideau et al., 2001), litterfall inputs (Liu et al.,
2004), microbial responses (Li et al., 2012), and other soil stabilization or loss mechanisms (Barré et al.,
2017; Torn et al., 1997; Mikutta et al., 2006; Cotrufo et al., 2013).  The rate of increase in soil nitrogen (N)
availability is hypothesized to control primary production and rates of soil C accumulation (Schimel et al.,
1994; Asner, 1997; Medlyn et al., 2000; Strömgren and Linder, 2002). Productivity is often N limited in

45   temperate and boreal forests, and climate warming can enhance N cycling, primary production, soil C
inputs and stores (Melillo et al. 2011; Philben et al. 2016). However, the net effect of this feedback on
productivity and SOC stocks and its longevity with climate change remains unclear (Melillo et al., 2017;
D'Orangeville et al., 2018).  Thus, understanding the response of SOC stocks to climate requires not only
an understanding of how fluxes in and out of the soil system change with climate (Dunn et al., 2007), but

50   also how the microbial and physiochemical processing of soil organic C and N pools shift (Li et al., 2013).

Ecosystem fluxes shape the inputs and losses of soil organic matter (SOM) reservoirs on seasonal to annual
time scales, and they provide insights about the controls on SOM reservoirs. However, detecting small
differences between these large and variable fluxes is challenging and makes it difficult to determine net
changes in soil reservoirs.  Biogeochemical signatures of soil organic matter (SOM) are useful for tracing

55   the net response of SOM pools not usually discernible from the balance between soil inputs and losses at
the decadal to century timescales relevant to climate change (Billings et al. 2012). Diagenesis of SOM is
the physiochemical and biological transformation (e.g. leaching and decomposition, respectively) of the
non-living organic matter in soil that changes the original biomolecular composition of detrital inputs to
soil over time.  The accrual of these diagenetic alterations over decades to centuries is observed in the

60   composition of organic matter, and referred to as the diagenetic state of SOM  (Hedges and Prahl, 1993).  If
organic matter inputs to soil are maintained, both in terms of rate and composition, while losses are
enhanced, the diagenetic state of the SOM will increase providing evidence of net losses over time.

Estimates of the diagenetic state of SOM may be made by evaluating bulk compound class composition or
source specific molecular biomarkers. Bulk compositional measures rely on tracking the proportions of

65   more labile (e.g. carbohydrates, amino acids; Cowie and Hedges 1994) or recalcitrant classes of compounds
(e.g. lipids, pigments Harvey et al. 1986; Sun et al. 1993; Goni and Hedges 1990) relative to one another or
to total organic matter content. For example, the ratio of alkyl to o-alkyl carbon which tracks plant
carbohydrate losses and selective retention of less bio-reactive waxes with diagenesis (Preston et al., 2009)

has been applied to SOC (Baldock et al., 1997).  Increases in this ratio are often observed with soil depth

70 and have been found to be consistent with a decrease in SOC stocks observed with climate warming in dry

continental jack pine boreal forests (Norris et al., 2011).  However, the ratio of alkyl to o-alkyl C can be

complicated by shifting boreal forest vegetation inputs that develop with a warming climate (Quideau et al.,

2001; Kohl et al., 2018). Even if primary vegetation sources do not shift, changes in understory

composition can alter the soil's apparent diagenetic state as assessed through broad biogeochemical

75 signatures. For example, understory moss inputs can vary as a function of climate change altering the

proportion of alkyl to o-alkyl C delivered to boreal forest soils, preventing the use of this ratio to

understand the diagenetic state of soil carbon (Kohl et al. 2018). Source specific molecular biomarkers are

useful for determination of carbon fate in soils (e.g. Kögel-Knabner 2002; Rumpel et al. 2002; ; Otto and

Simpson 2005; Otto and Simpson 2006a Otto and Simpson 2006b), and may help to overcome these

80 challenges encountered when investigating the fate of carbon in boreal forest soils.

Molecular transformations of lignin have provided insights about warming impacts on SOC storage and

chemistry over time-scales of months to years (e.g. Feng et al. 2008), as well as over climate shifts of years

to decades represented by climosequences (e.g. Amelung et al. 1999; Guggenberger et al. 2001; Pengerud

et al. 2017). Lignin is an important component of vascular plant material (up to 30 wt%), and vascular

85 plants are the sole source of lignin to soils (Kögel-Knabner, 2002). Lignin is often used as a model

compound in climate models (Thevenot et al., 2010; Sainte-Marie et al., 2021) based on its use as an

indicator of SOC sources and processing in a variety of ecosystems (e.g. see review by Thevenot et al. 2010

for utility in soils, and Table S1 for 12 commonly used indices). Signatures of lignin phenols include

common indicators of microbial degradation and physiochemical processing, such as the ratios of phenolic

90 acids to aldehydes (e.g. vanillyl acids/ aldehydes; $V_{ad/al}$), which increase as lignin side-chain oxidation

increases (Hedges et al. 1988; See Table S1 for more detail). While certain indicators of lignin composition

are confounded by variable vegetation inputs (Williams et al., 1998), multiple lignin phenol ratios can

better account for variability due to source heterogeneity (Moingt et al., 2016). The comparison of lignin

diagenesis across systems with changing source inputs requires assessment of input and soil lignin

95 chemistry via multiple lignin phenol ratios (Simpson et al., 2008; Benner et al., 1990a, b). The diagenetic

state of lignin is a useful indicator for estimating ecosystem-level SOC responses to climate change over

various timescales if source inputs are properly constrained (Moingt et al., 2016).

Similarly, the composition of total hydrolysable amino acids (THAA) has been used to trace the diagenetic

alteration of organic N in sediments and soils (Dauwe et al. 1999; Menzel et al. 2015; Philben et al. 2016).

100 The amino acid composition of detritus is transformed by biological and physicochemical processes leading

to characteristic losses of some THAAs (e.g., glutamic acid, lysine) and enrichment or retention of others

(e.g., glycine and hydroxyproline). Amino acids comprise about half of total soil organic nitrogen (SON),

and they have been used to indicate that SON cycling in mesic boreal forest soils can increase with

warming (Philben et al., 2016). Thus, characterization of SOM composition can provide insights about

105     SOC and SON stocks that mass flux approaches cannot (Billings et al., 2012; Kane et al., 2005).
        Combining compositional approaches, for example lignin phenol and amino acid composition, enables us
        to assess the extent to which C and N cycling is coupled or decoupled in ecosystems, which is critical for
        addressing the hypothesis that stimulation of N cycling can offset C losses with climate warming by
        alleviating N limitation (Medlyn et al. 2000; Strömgren and Linder 2002).

110     In the present study, lignin biomarkers provided novel insights of SOC that are compared with previous
        measures of SON processing (Philben et al., 2016), SOC inventories and fluxes (Ziegler et al., 2017), and
        bulk and molecular-level SOM characteristics across a mesic boreal forest climate transect (Ziegler et al.,
        2017; Kohl et al., 2018). These data are used to investigate whether soil C and N cycling are coupled across
        a mesic boreal forest transect, a feedback that could be responsible for the maintenance of SOC stocks with
115     climate warming. The well-developed organic soil horizons in these forests store at least one-third of the
        total SOC stocks, serve as the primary source of SOC to the deeper, slower turnover mineral horizons in
        these forests (Ziegler et al., 2017), and are representative of a diagenetic continuum with depth (Philben et
        al., 2016; Kohl et al., 2018). Given previous observations of increased temperature sensitivity of soil
        respiration with climate warming (Podrebarac et al., 2016), the inventories of SOC in these forests might be
120     expected to decrease with warming. However, direct measurements suggest that SOC stocks are maintained
        in these forests, despite increasing ecosystem-scale C inputs and losses across the transect (Ziegler et al.,
        2017).  This apparent discrepancy highlights the need for a direct assessment of shifts in SOC diagenesis
        with climate change in these boreal forests which, unlike observations of SON diagenesis (Philben et al.,
        2018b), is complicated by shifting plant inputs. Previously measured biogeochemical indicators of SOC
125     diagenetic state, such as the ratio of alkyl to o-alkyl-C, do not exhibit a clear increase with depth across the
        climate transect. This has been attributed to declining moss inputs with climate warming in these forests
        (Philben et al., 2018a,b; Kohl et al., 2018). The decrease in the %N as amino acids observed previously,
        however, indicates an increase in the diagenetic state of soil nitrogen (Philben et al., 2016). In this study,
        the %C as lignin phenols was measured in these soils to determine if the diagenetic state of soil carbon
130     changes with depth across the climate transect.  Previous studies in other soil systems observed a decrease
        in lignin yields with depth (e.g. Guggenberger et al., 1995; Otto and Simpson, 2006b). In this study the
        application of lignin biomarkers is used to address the following hypotheses: (1) the diagenetic state of
        SOC is maintained, providing further evidence for the maintenance of SOC stocks with climate warming;
        and (2) that soil C and N cycling is coupled, thereby explaining the mechanism behind the maintenance of
135     soil C stocks along this boreal forest climate transect.

## 2 Materials and Methods

### 2.1 Field Sampling and Sample Preparation

Organic layers from humo-ferric podzols underlying similar stands of mature balsam fir (*Abies balsamea*) dominated forests located across a climosequence (Newfoundland and Labrador Boreal Ecosystem
140    Latitudinal Transect, NL-BELT) were collected in 2011 (Table 1; Ziegler et al., 2017). Sampling focused on three regions of the climosequence: the Eagle River, Salmon River and Grand Codroy regions which will be referred to herein as the Cold, Cool and Warm regions (Table 1). The three regions of similarly established forests and soils span approximately 5˚ in latitude, encompassing a 5.2˚C shift in mean annual air temperature (MAT), ~ 430 mm yr$^{-1}$ in mean annual precipitation (MAP) and ~ 180 mm yr$^{-1}$ in potential
145    evapotranspiration (PET) (Ziegler et al., 2017).

Therefore, the transect of study sites represents a temperature and precipitation gradient congruent with projected climate warming and increased precipitation expected in the region within the next century (Stocker et al., 2013). These balsam fir forests are primarily underlain by forest floor moss cover in addition to few understory plants, the most common being *Cornus canadensis* and *Trientalis borealis*.
150    Some fern cover occurs very sporadically in the Cold and Cool regions but is more common in the Warm region. The total moss cover represents the main plant difference across the study regions where lowest moss cover occurs in the Warm region (Table 1). *Pleurozium* sp. and *Hylcomium* sp. dominate the moss cover in the Cold and Cool region sites, with some additional coverage by *Ptilium* sp. and *Dicranum* sp. in the Cold region. The moss cover in the Warm region sites are dominated by *Dicranun* sp. and
155    *Rhytidiadelphus* sp.

Each of the three regions along the climosequence contained three forest sites, while at each site soil samples were collected from triplicate plots (total of n= 27 plots across the climosequence; Ziegler et al., 2017; Table 1). Samples were collected by cutting out a 20X20 cm area of the organic layer with clean serrated knife and clippers, and carefully removing from the surface of the mineral soil surface using a
160    clean trowel. Organic layers were manually separated into three horizons, L, F and H (equivalent to Oi, Oe and Oa, respectively, within the U.S. Soil Classification), and dried at 50˚C before being ground and stored for further analysis. Plant end-member samples (forest floor mosses, fresh needle foliage, needle litterfall, roots, wood) subjected to biomarker analysis were collected across one site per region, were separated visually into taxonomical groups (e.g., moss genus). Balsam fir needles were also collected in litterfall traps
165    in the Spring 2011 (litterfall), as well as roots and wood separated from one half of the organic horizons collected, and included as an additional endmember plant sources. All end-member samples were dried at 50˚C before being ground and stored for further analysis.

**Table 1.** Field site characteristics for the climate transect updated from (Ziegler et al. 2017; Kohl et al. 2018). All study sites are balsam fir (*Abies balsamea*) dominated forests with forest floor moss as the primary understory plant cover. See text for further site details.

| Region | Site | | Latitude | Longitude | MAT[a] °C | MAP[b] mm yr⁻¹ | PET[c] mm yr⁻¹ | Moss cover (%) | LFH Thickness cm | LFH Carbon Stocks kg m⁻² | LFH Nitrogen Stocks kg m⁻² |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cold | 1 | Muddy Pond | 53°33'01"N | 56°59'13"W | | | | 100 ± 4 | 9.8 | 2.75 ± 0.25 | 0.07 ± 0.01 |
| Eagle River | 2 | Sheppard's Ridge | 53°03'25"N | 56°56'02"W | 0 | 1074 | 432 | 54 ± 4 | 7.9 | 2.42 ± 0.59 | 0.07 ± 0.01 |
| | 3 | Harry's Pond | 53°35'12"N | 56°53'21"W | | | | 95 ± 1 | 7.4 | 2.21 ± 0.17 | 0.07 ± 0.01 |
| Cool | 1 | Hare Bay | 51°15'21"N | 56°8'18"W | | | | 57 ± 10 | 9.9 | 3.53 ± 0.60 | 0.11 ± 0.02 |
| Salmon River | 2 | Tuckamore | 51°9'51"N | 56°0'15"W | 2 | 1224 | 489 | 92 ± 6 | 8.7 | 2.78 ± 0.56 | 0.08 ± 0.02 |
| | 3 | Catch-a-Feeder | 51°52'1"N | 56°12'16"W | | | | 44 ± 2 | 9.7 | 3.52 ± 1.62 | 0.10 ± 0.02 |
| Warm | 1 | O'Regan's | 47°53'36"N | 59°10'28"W | | | | 42 ± 5 | 8.3 | 3.30 ± 0.52 | 0.12 ± 0.02 |
| Grand Codroy | 2 | Maple Ridge | 48°00'28"N | 58°55'14"W | 5.2 | 1505 | 608 | 39 ± 7 | 7.9 | 3.23 ± 0.19 | 0.11 ± 0.01 |
| | 3 | Slug Hill | 48°00'39"N | 58°54'16"W | | | | 40 ± 2 | 8.1 | 3.65 ± 1.01 | 0.13 ± 0.04 |

[a] Mean annual air temperature (MAT) [b] Mean annual precipitation (MAP) are climate normal from 1981-2010 from Cartwright, NL for the Colder region, Main brook, NL for the Middle region and Doyles, NL for the Warmest region from Environment Canada (2014). [c] Annual potential evaporation (PET) was calculated based on monthly temperature and precipitation (Xu and Singh 2001).

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## 2.2 Lignin Analysis

175  Samples from each of the three organic horizons (L, F and H) collected from each of the triplicate organic horizon samples collected from three plots within one site in each of the three regions (total n=27 samples) were analyzed for lignin phenols released via the cupric oxide oxidation (CuO) method (Hedges and Ertel, 1982), with modifications outlined in Kaiser and Benner (2011) and Louchouarn et al. (2010). Soil samples and the plant endmembers (n=27 moss samples, n=9 needle litter samples) were dried, homogenized and weighed (~5 mg OC)

180  into monel steel pressure vessels (Prime Focus, Inc.), along with reagents cupric oxide and ferrous ammonium sulfate and a steel ball bearing. Samples were oxidized in 2M NaOH at 155˚C for 3 hrs on a rotating rack to ensure constant sampling mixing. Samples were cooled immediately and spiked with internal standard of trans-cinnamic acid and ethyl vanillin. Sample clean-up was performed by solid phase extraction (SPE) for plant endmember materials or by liquid-liquid extraction with ethyl acetate for oxidized soil samples (Louchouarn et al., 2010; Kaiser

185  and Benner, 2011). SPE eluates or solvent extracts were dried and re-suspended in pyridine for quantification. Samples were analyzed for the twelve common phenols (Fig. S1) via GC-MS-MS (Yan and Kaiser, 2018), utilizing a six-point calibration curve. The percentage of carbon as lignin phenols was determined via methodology outlined in Benner et al. (1990a), correcting for CuO oxidation efficiency.

## 2.3 NMR

190  NMR spectra were obtained from Kohl et al. (2018). Briefly, cross-polarization magic-angle spinning solid state $^{13}$C NMR (CP-MAS $^{13}$C-NMR) was performed on the site-level scale from pooled plot-level spatial replicates (Kohl et al., 2018). NMR end-member spectra were weighted based on composite litterfall spectra, moss spectra from Kohl et al. (2018) and Douglas fir wood spectra from the literature (Preston et al., 1998). Previously identified peaks were re-integrated here as determined by the sum of integrated peaks in defined spectral regions. Regions were defined in

195  this study as in Baldock et al. (2004) : Alkyl 0-45 ppm, Methoxy/N-Alkyl 45-60 ppm, O-alkyl 60-96 ppm, Di-O-Alkyl 95-100 ppm, Aromatic 110-145 ppm, Phenolic 145-165 ppm, Amide/Carboxyl 165-215 ppm. Carboxyl was assigned as the peak at 173 ppm, and Methoxy was assigned to the peak at 56 ppm.

## 2.4 Lignin Diagenetic Index (LPDI) development and application

The development of the lignin diagenetic index for these forest ecosystems was iterative, with the initial

200  development of a purely informative PCA model for a posteriori identification of potential confounding variables due to source variability when determining diagenetic state of lignin. Based on the relationship of the individual phenol ratio with depth and or site, the source and/or processing influences on phenolic signatures were explored by principal component analysis (PCA). These included the most common phenolic indices used in the literature: $\sum6$, $\sum6{:}\sum8$, %C as lignin, C/V, S/V, $V_{Ad/Al}$, $S_{Ad/Al}$, FAD/CAD, diOHBA/V, % side chain alteration, P/(V+S), PON/P

205  (Supplemental Table 1). All data was input as a carbon normalized molar amount (nmol phenol mgC$^{-1}$) or ratio (nmol phenol nmol phenol$^{-1}$), for comparability during multivariate statistical analyses (Panetta and Gélinas, 2009). Values were processed prior to performing PCA by zero centering and scaling to unit variance for each input variable. In this model, input variables (p=12) included those variables that varied significantly with site. Pure

source or plant endmember samples (i.e., moss and needle tissues), modeled litterfall and wood samples (see
supplement for more details on how these values were derived), and the L-layers subjected to CuO oxidation were
considered as observed entities for the Source PCA model (n=33; Table S3). These include site composite samples
of the L horizon from each region (n=9), a site composite sample of moss from across both a Warm and Cold region
site as well as samples of the major genera of moss observed across the transect collected from across a site in the
Cool region including both green and lower brown tissues (n=10), two site composite samples of green and brown
balsam fir needle litter from one site in the Cool and Warm regions (n=8), a composite needle foliage sample from a
Warm region site (n=1), modelled birch and balsam fir wood from the literature (n=2), and modelled total litterfall
for each region (n=3).  Based on the information from this model, all source signatures that change significantly
with depth (a proxy for diagenesis), but do not appear to be affected by the various source inputs present at these
sites (e.g., non-vascular moss source inputs) were further considered as input variables for the diagenetic index
development via PCA. These variables (source signatures) also best retain original variance while reducing any co-
correlation's effect on overall PC loadings, and were chosen via a branch and bound algorithm (p=4; Cadima et al.,
2017). The index was then chosen as the first resulting component from this PCA analysis (on n= 30 samples), as it
best explained variation in diagenetic state while also encompassing the highest explanatory power of a single
component.

The relationship between the LPDI and $^{13}$C-NMR spectra was explored to further understand lignin diagenesis in
samples not subjected to CuO oxidation and, more broadly, the relationship between the phenols and bulk SOC
composition.

To understand whether SOC and SON processing was coupled across these forest sites the SOC diagenetic state
ascertained via the LPDI was compare with diagenetic state of SON.  We used the amino acid diagenetic index
(AADI) data retrieved from Philben et al. (2016). The data were used as published in Table 1 in Philben et al.
(2016), with the exception of the reversal of the sign of the data retrieved to allow for a more direct comparison with
the LPDI developed herein.

**2.5 Statistical Analysis**

All statistics were performed in R using R Studio, and R packages 'tidyverse' and 'ggfortify' were used for data
organization and visualization (Tang et al., 2016; Wickham, 2016; RStudio Team, 2016; R Core Team, 2018). A
threshold of α=0.05 and β= 0.20 were applied to all ANOVA results. Two-way analysis of variance (ANOVA) was
performed on all soil properties to test for the effects of organic horizon, climate region, and their interactions (n=
27, df= 2 for each). Differences between regions at each organic horizon was determined via Tukey's Honest
Significance Difference tests, when applicable.

As CuO oxidation was only conducted at one site per region, a two-way ANOVA was performed to test for the
effects of organic horizon, site and their interactions for each lignin parameter (n= 27, df= 2 for each). Thus, site
level values are reported in Figure 1, and error bars on Figure 1 indicate standard deviation for all spatial replicates

at the site level. To further understand if site-level trends were observable on the regional scale, the LPDI was

245 compared to and predicted from NMR spectra obtained at the site level (Fig. 2). Statistically significant changes
with depth are interpreted as changes with diagenesis, while significant changes by site only are considered to be
source derived. Figures 3-5 contain results from the measured and predicted LPDI values in all regions.

## 3 Results

### 3.1 Organic soil lignin composition

Twelve lignin phenol signatures (see Table S1 for definitions and common usages) were examined across the

250 diagenetic continuum, represented by horizon depth within the total O-horizon, and in response to climate warming,
represented by one site in each climate region (Fig. 1). Most phenolic signatures display significant variation with
horizon depth, a proxy for variability due to diagenesis, and climate site, a proxy for variability due to integrated
climate effects (Fig. 1). However, $V_{Ad/Al}$ and FAD/CAD increased only with depth, while S/V did not vary with
depth but increased with climate warming, and $S_{Ad/Al}$ displayed no significant trend. Varying significantly with depth

255 and site the percentage of carbon as lignin carbon (%C as lignin-C), $\sum6$, and ratio of $\sum6$ to $\sum8$ all decreased with
depth and was lowest in the Cold region site. The effect of site on the percentage of carbon as lignin carbon is
attributed to a lower L horizon value for the coldest region site (Fig. 1e). Percent side chain alteration, diOHBA/V,
and C/V all increased with depth and were generally highest in the Cold region site. Indices with *p*-hydroxyphenols–
P/(V+S) and PON/P– were the only variables that exhibited significant variations with depth-site interactions, in

260 addition to variations with depth and/or site and attributed to the decreasing values with depth observed in the Cold
region site only.

### 3.2 Lignin Phenol Diagenetic Index (LPDI) model development

The effects of source materials on phenolic signatures were considered for interpretation of lignin diagenesis in
these forests, as they derive from vegetation with highly variable phenol content (e.g., mosses vs. vascular plants;

265 Fig. S2). Input variables included the percentage of identifiable source material as woody materials, needles, or
mosses contributing to the L horizon soil, and the CuO lignin phenol indices that varied significantly with site (Fig.
1; as discussed in section 3.1): including $\sum6$, $\sum6{:}\sum8$, %C as lignin, C/V, S/V, diOHBA/V, % side chain alteration,
P/(V+S), and PON/P. Source material PCA results indicated that SOC input variables loaded in unique PC1 vs PC2
space with 70.6% of variance explained by these two components (Fig. S2). Loadings of variables were concurrent

270 with how pure plant end-member samples scored, dependent on sample type (i.e., moss, woody material, and
needles; Table S2). Therefore, loadings and scores on PC1 and PC2 were used to determine the influence of input
sources (e.g., %moss as source contributions) on lignin phenol indices assessed to identify which indices were and
were not affected by input source (Tables S2 and S3). For example, the lignin phenol indices apparently impacted
by moss inputs were PON/P, diOHBA/V, and C/V suggesting they would be the most effected by shifting

275 proportion of moss inputs across the study sites (Table 1; Kohl et al., 2018).

The diagenetic PCA model was built upon the variables identified from the above model: $V_{ad/al}$, FAD/CAD, $\sum 6:\sum 8$, and %C as Lignin-C, which all change significantly with depth (Fig. 1), but do not appear to be affected by various source inputs (e.g., non-vascular plant source inputs defined by %moss). With increasing depth, the relative ratio of
280      $Ad/Al_v$ and the ratio of cinnamyl class phenols (ferulic acid/p-coumaric acid; FAD/CAD) increased (Fig. 1) and loaded positively on PC1 (Table S4). The %C as Lignin-C (Fig. 1) decreased with depth, and the ratio of carbon-carbon and/or ether-bonded phenols to ester- and ether-bonded phenols in vascular plant materials (V+S:V+S+C or $\sum 6:\sum 8$; Fig. 1) loaded negatively on PC1 (Table S4). The first principal component explained 64.3% of the variance. The lignin phenol diagenetic index (LPDI) was defined as the first component of this PCA model, as it best
285      explained variation in lignin diagenetic state while also encompassing the highest explanatory power of a single component. Therefore, higher LPDI scores are interpreted as greater lignin diagenesis, while samples with lower scores are less altered. The results are consistent with expected declines in percentages of carbon as lignin with increasing diagenesis, and they support increasing lignin diagenesis with depth in the organic soils studied (Fig. 1).

### 3.3 Comparison of LPDI with ^{13}C NMR and assessment of LPDI across climate regions

290      The LPDI was compared with the determination of soil lignin carbon diagenesis derived from ^{13}C NMR, to validate the usage of an NMR ratio to represent the LPDI across multiple forest sites in each climate region from which O-horizon soil data are available. We created this NMR ratio to represent lignin degradation, based on previous research indicating the proportion of alkyl and carboxyl carbon increases with increasing lignin degradation in gymnosperm litter (within the operationally-defined Klason lignin fraction), while the proportion of aromatics
295      (including phenolic components) and methoxy carbon decreases with increasing lignin degradation (Zech et al., 1987). This ratio – (alkyl + carboxyl) / (aromatics + methoxy) – was used to relate the degradation of lignin observed with ^{13}C-NMR to the state of lignin diagenesis obtained from the CuO derived phenol-developed LPDI. We find good agreement between the CuO derived phenol-developed LPDI and ratio of (alkyl + carboxyl) / (aromatics + methoxy) in our soils for all regions ($n = 9$, $R^2 = 0.898$; $p < 0.001$; Fig. 2). Because of this strong linear
300      relationship, we determined that in these soils we could further estimate the LPDI for all 9 study sites along the climate transect ($n = 18$ predictions, for a total of $n=27$ observations) based on the ^{13}C-NMR spectra alone, measured in each site across all three regions (Fig. 2). The lignin phenol diagenetic state in these soils, assessed by the measured and predicted LPDI scores, increases with depth, yet is not significantly different by climate region (Fig. 3).

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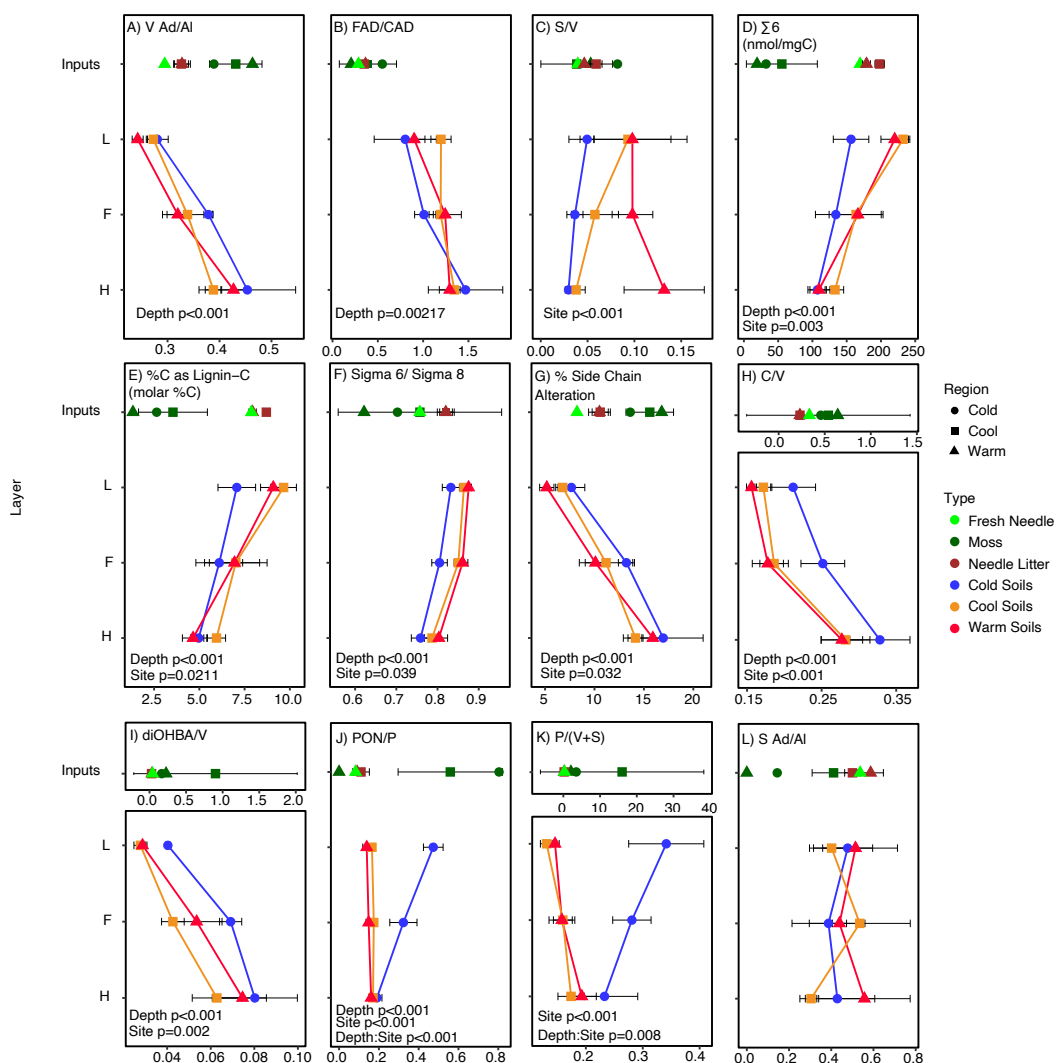
**Figure 1.** CuO measured lignin phenol content in soils and input materials at one site per climate region. Moss samples in the Warm and Cold regions are composite samples based on dominant species cover, while in the Cool region individual moss species are plotted as the average and standard deviation with equal weights to show variability in moss signatures in phenolic signatures present in these forests. Green, brown and fresh balsam fir needles from the Warm and Cool regions were also measured. The ratio of vanillic acid to vanillin ($V_{Ad/Al}$) (A) and the ratio of ferulic acid to p-coumaric acid (FAD/CAD) (B) increase with depth, while the syringyl to vanillyl ratio (S/V) (C) is different by site. The sum of syringyl and vanillyl phenols ($\sum6$) (D), %C as lignin-C (E), the ratio of $\sum6$ to the sum of all cinnamyl, syringyl and vanillyl phenols ($\sum6:\sum8$) (F), % Side Chain Alteration (G), the ratio of cinnamyl to vanillyl (C/V) (H), the ratio of dihydroxybenzoic acid to vanillyl (diOHBA/V) (I) all vary with depth and site. The ratio of p-hydroxyacetophenone to p-hydroxyl (PON/P) (J) varied with depth, site, and exhibited a depth x site interaction, while the ratio of p-hydroxyl to sum of vanillyl and syringyl phenols (P/(V+S)) (K) varied with site and exhibited a depth x site interaction. The ratio of syringyl acids to aldehydes ($S_{ad/al}$) (L) shows no trend with depth or site. Alpha level was set to 0.05, anything over this threshold was not reported on the figure. For clarity the labels and units (if applicable) for data presented are included in the figure plot subtitles rather than with each axis. Labels without units represent unitless ratios or percentages.

**3.4 Using combined biomarkers to assess soil C and N cycling**

Lignin and amino acids represent important components of soil C and N, respectively, and the LPDI and AADI
325 (Philben et al., 2016) indicate variability in lignin and amino acid compositions due to increasing alteration with
depth. Thus, the ratios of these indices can be used to explore the relative diagenetic state of these compound
classes, and to some extent SOC relative to SON as a whole (Fig. 4). Because increased diagenesis expressed via the
AADI is represented in the literature as a negative loading on its PC1 (Dauwe et al., 1999; Philben et al., 2016), in
opposition to the LPDI used here, the sign of AADI is reversed in this representation for ease of comparison. The
330 LPDI / (-AADI) is similar and near a value of one in all regions (Fig. 4). This ratio also does not change
significantly with depth (Figure 4), indicating that despite increased diagenetic state of lignin with depth (Fig. 3) the
relative degree of processing of lignin and protein (i.e., amino acids) remains similar with depth and climate
warming in these forest soils. The coupling between the LPDI and the AADI with depth and a climate region is in
contrast to the decoupling observed between the LPDI and the alkyl to O-alkyl C ratio (A/O-A), a common NMR
335 indicator of SOC diagenetic state (Baldock et al. 1997) (Fig. 5). The greatest discrepancy between the LPDI and
A/O-A is observed in Cold and Cool regions soils where moss cover (Table 1) and moss inputs (assessed via moss
detritus measured in L horizon soils) are greatest.
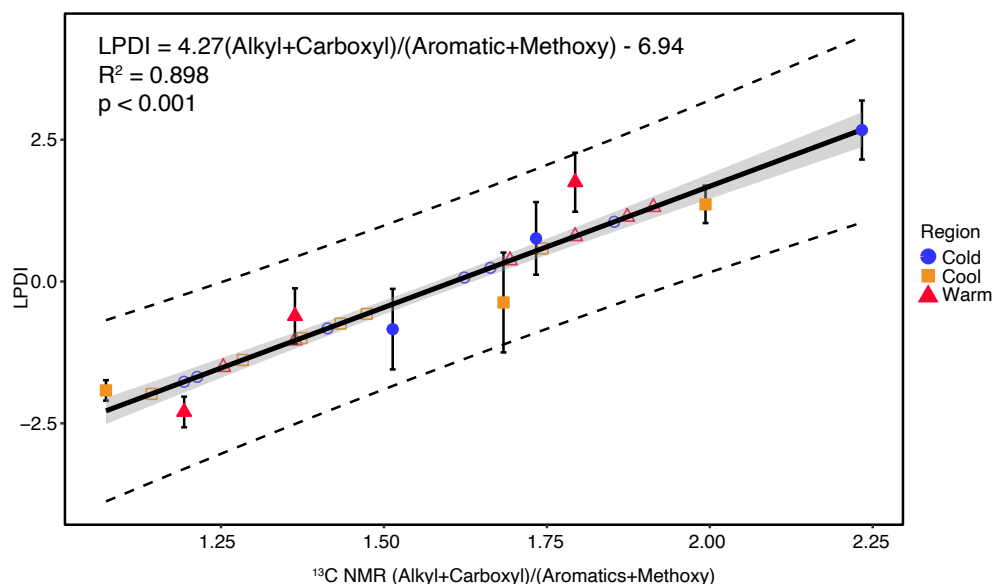
**Figure 2.** Lignin Phenol Diagenesis Index (LPDI) versus the CP-MAS $^{13}$C-NMR Alkyl+Carboxyl/
340 Aromatics+Methoxy ratio for all regions and all organic layers (including predicted values of LPDI from $^{13}$C-NMR
ratio, which are given in open symbols and fall on the prediction line). Including confidence of fit (grey shading)
and confidence of prediction (dotted line) at 95%. Standard error of predicted values falls within the confidence of
fit (grey shading), while standard deviation of measured values are calculated from observed plot level variation
within each site and are depicted with error bars.

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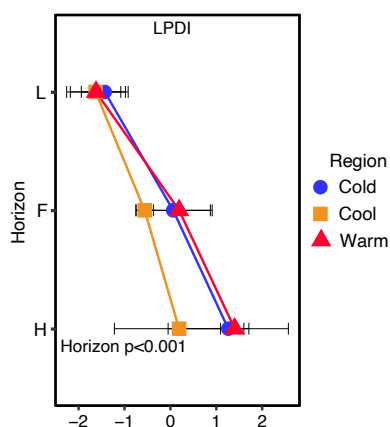
**Figure 3.** The Lignin Phenol Diagenetic Index (LPDI) increases with organic horizon depth. The actual p-value for horizon (H), region (R) or horizon x region (HXR) significance is reported with significant values (α=0.05) in bold. This ratio is unitless.

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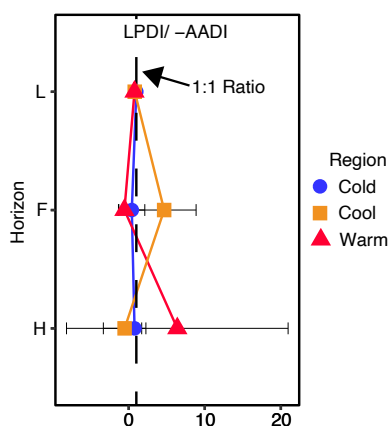
**Figure 4.** Ratio of the lignin phenol diagenetic index (LPDI) to the inverse sign of the total hydrolysable amino acid diagenetic index (-AADI) with depth in all regions averaged. Ratios do not change significantly among regions at α=0.05, indicating that the relative rates of lignin and amino acid diagenesis are similar across all regions and with

355   depth. Post-hoc tests revealed no significant difference between depth and/or site at the regional level. This ratio is unitless.
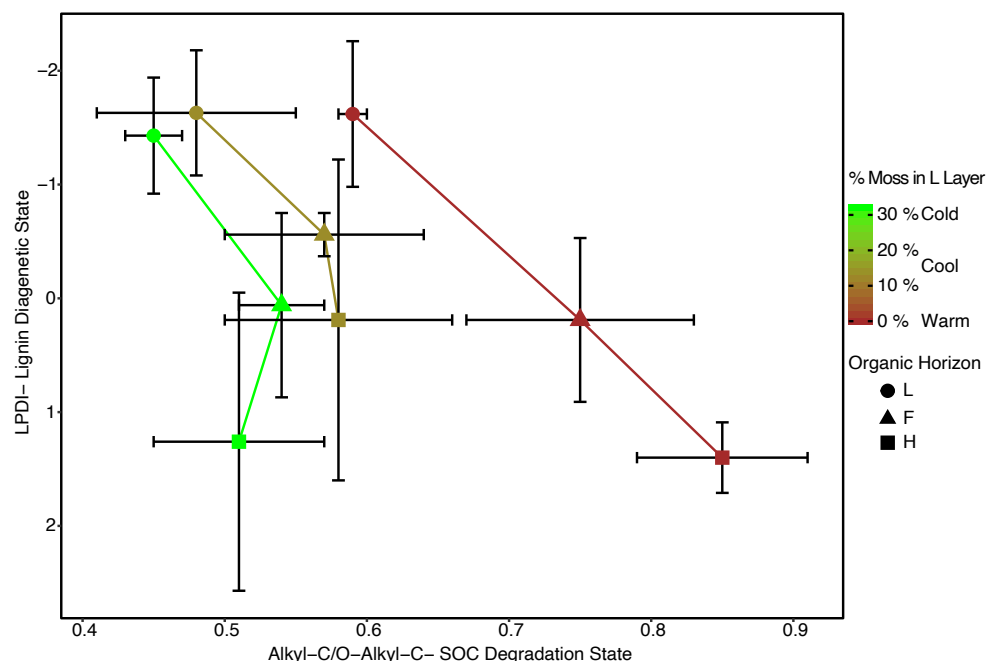
**Figure 5.** Lignin diagenetic state as observed through the lignin phenol diagenetic index (LPDI) is decoupled from
SOC diagenetic state as assessed through the ratio of Alkyl-C to O-Alkyl-C (Alkyl-C/O-Alkyl-C) across climate
region. This discrepancy appears to be related to the percentage of identifiable moss detritus in the L horizons
(depicted with color scaling). Values are given as the site average in each region with error bars representing the
standard deviation (n=3).

**4 Discussion**

The maintenance of SOC stocks despite increased soil C losses and inputs with climate warming in these mesic
boreal forests suggests inputs are keeping pace with losses (Ziegler et al., 2017). We hypothesized that increased soil
inputs result from productivity supported by enhanced N cycling (Melillo et al., 2011; Philben et al., 2016). If soil C
stocks are being maintained via new inputs, we expect soil C and N cycling are coupled across these forests in
association with climate warming.  However, the annual variability and difficulty in capturing all ecosystem fluxes
prevent us from directly linking C and N cycling over decadal time scales. Therefore, we couple previous
information with additional measures of SOM diagenetic state in these forests across the climate transect.  The
diagenetic state of SOM assessed via lignin phenol and amino acid (THAA) composition provides the net effect of
these processes on both SOC and SON reservoirs.

Shifts in non-vascular to vascular plant inputs with climate observed in these and other boreal forests (Kohl et al.,
2018) meant that we had to carefully separate biogeochemical indicators of SOC source from those signifying
diagenetic alteration. Though these shifts in non-vascular moss inputs have little impact on THAA and their use in
tracking SON diagenetic state (Philben et al., 2018b), they do impact some SOC chemical indicators. For example,

the increase in the alkyl to o-alkyl ratio observed with climate warming is consistent with the decreasing moss

380    contribution to soils with warming, and is therefore not indicative of an increase in SOC diagenetic state in the

warmer, wetter forests (Kohl et al., 2018). This trend may be due to a slow turnover of structural carbohydrates

within moss cells walls (Hobbie et al., 2000; Philben et al., 2018b; Turetsky et al., 2008), thereby affecting the

utility of this ratio to trace SOC diagenetic shifts in these systems.  However, the lignin phenol diagenetic index

(LPDI) developed and applied here enabled the assessment of SOC diagenetic state and the relative differences in

385    soil carbon dynamics among these forests despite the shifts in non-vascular to vascular plant inputs with climate

warming.  This also enabled us to evaluate the coupling of SOC and SON cycling in these forests and as a function

of climate warming.

### 4.1 Evaluation of lignin phenol signatures of SOC diagenetic state in mesic boreal forests

The amount of lignin in these soils (as %C as lignin-C) decreases with organic horizon depth at all sites, a

390    relationship which has also been observed in other soil systems (e.g., Guggenberger et al., 1995; Otto and Simpson,

2006b). The same tree species (balsam fir) is dominant across the forests of this transect, but we observed that

commonly used lignin parameters, such as source-sensitive ratios (C/V and S/V), are affected by variable vascular to

non-vascular understory inputs to these forest soils. These ratios are commonly used in soils to assess diagenetic

state (e.g., Otto and Simpson, 2006), yet they are not valid to assess diagenetic in these soils due to the over-printing

395    of source-related shifts in these forests. The PCA representation simplifies and integrates the description of observed

variability of lignin phenol diagenetic state from multiple indices allowing for ease of comparability within a given

dataset and adaptation for development and implementation in other environments. The combination of the lignin

phenol biomarker and NMR approaches (Simpson et al., 2008; Benner et al., 1990a, b) with the measured and

predicted LPDI scores indicates a robust signature of SOC diagenetic state.

400    Future studies are needed to determine the applicability of the relationship between the lignin biomarker and NMR

data for other coniferous-dominated forests. Furthermore, new LPDI loadings and scores should be derived from a

comprehensive training dataset when developing this approach for different systems. For example, the THAA-DI

approach originally developed for marine sediments (Dauwe et al., 1999) was successfully adapted for soils (Philben

et al., 2016) and dissolved organic matter (Davis et al., 2009; Peter et al., 2013). Testing the impact of mosses on

405    this index revealed no significant difference in the LPDI by region, moss input, or soil depth (data not shown). We

observed minimal moss impacts on this index, which could be useful for interpretation of phenolic composition in

areas with variable moss inputs to SOM.

### 4.2 Consistent lignin diagenetic state indicates balance between inputs and losses with climate warming

The lack of change in lignin diagenetic state across these boreal forest sites, despite the +5.2°C MAT of climate

410    warming, contrasts with the increase in the diagenetic state of lignin observed over 14 months of experimental

warming in a temperate forest (Feng et al., 2008). This may be due to a lack of additional ecosystem responses to

warming (e.g., enhanced soil inputs; Melillo et al., 2011) that were not captured over the shorter time scale. Climate warming impacts on ecosystem properties, such as altered litter inputs (Pisani et al., 2016) and shifts in climate conditions such as MAP (Duboc et al., 2014; Pisani et al., 2014), can serve as drivers of lignin decomposition and its

415     diagenetic state. This likely explains the lack of clear trends in lignin diagenetic state with increasing MAT on a continental scale (Amelung et al., 1999; Pisani et al., 2014); including a decrease in the degradative state of lignin across ~2 ˚C MAT of climate warming (Otto and Simpson, 2006b). The MAP increases with MAT and PET in the mesic boreal forests we studied and is maintained throughout the transect, representing a scenario where water limitations on enhanced productivity with warming are not likely (d'Orangeville et al., 2016). Thus, the maintenance

420     of soil lignin diagenetic state across climates gradients indicates increased inputs are keeping pace with losses over decadal time scales. This is consistent with experimental evidence of a similar balance between input and processing of lignin in temperature forest soils where the maintenance of organic carbon content and lignin composition was observed despite increased litter inputs experimentally added over several decades (Lajtha et al., 2014; Pisani et al., 2016).

**4.3 Coupled SOC and SON cycling indicate enhanced N availability supports increased productivity and**
425     **maintain SOC with climate warming**

Increases in nitrogen availability and litter inputs (Philben et al., 2016; Kohl et al., 2018) in these forest soils appear to sustain the balance between input and processing losses during climate warming. This is supported by the coupled diagenesis of SOC and SON as revealed here by comparison of lignin and amino acid diagenetic indices (i.e., the

430     LPDI/AADI). The similarity of diagenetic state observed in the soils across this transect is supported by multiple independent lines of evidence. Increased N availability, observed as an increase in soil fluxes (Ziegler et al., 2017; Kohl et al., 2018) and maintenance of amino acid diagenetic state (Philben et al., 2016), occurs with increased inputs and losses of C from soils and the maintenance of SOC stocks with climate warming (Ziegler et al., 2017). The lack of change in SOC diagenetic state observed in this study provides the missing evidence required for understanding

435     the maintenance of soil C stocks with climate warming. Increased soil organic nitrogen cycling (Philben et al., 2016, 2018a) enhanced forest productivity (Table S5), and thereby increased litter inputs to the soil. Th SOC and SON diagenetic indicators coupled with flux and stock assessments provided the net result of plant-soil interactions, which gradually respond to climate change and are not easily observed in experiments (Melillo et al., 2011, 2017) or by monitoring of ecosystem fluxes alone.

440     The sustained SOC stock with climate warming observed here contrasts with SOC dynamics in more water-limited boreal forest soils in western North America (Norris et al., 2011; Kane et al., 2005), where SOC stocks decline with climate warming. This highlights the heterogeneity of boreal forest responses to climate warming. The similarity of diagenetic state among major compound classes with depth or climate would not necessarily hold true in soils under non-steady state conditions resulting from responses to environmental change. Soil pools acclimating to increased

445     temperature, for example, display different periods of soil carbon retention and loss over multiple decades (Melillo et al., 2017). On decadal-centennial timescales, however, the net result of plant-soil interactions on soil fluxes are

likely adjusted to such changes in climate and can therefore reach a new steady state. The conservation of soil carbon stocks via increased forest productivity observed in this study region is consistent with the continuity of forest productivity expected in this region, as revealed in decadal tree ring records (D'Orangeville et al., 2016;

450   Charney et al., 2016). In fact, SOC stocks in mineral soils across boreal forests with increasing MAT and MAP appear to increase due to enhanced soil inputs as a result of increases in net primary production (NPP) (Callesen et al., 2003). In the forest sites studied here, surface mineral SOC stocks appears similar, and likely maintained by the 3-fold increase in dissolved organic carbon (DOC) inputs to these mineral soils with warming (Ziegler et al., 2017).

**4.4 Conclusion**

455   Our observation of maintained SOC stocks with climate warming are consistent with those in other biomes (Giardina et al., 2014; Sistla et al., 2013) not limited by water availability, although the mechanisms for the maintenance of SOC with warming are seemingly ecosystem-dependent. It appears the coupled increase in biogeochemical cycling of N and C maintains a balance between soil input and loss processes resulting from increased forest productivity with climate warming. This balance could markedly shift as other factors begin to limit

460   forest productivity (e.g., trace nutrients, water) with further climate change, or affect forest nutrient allocation (e.g. forest age or compositional change). Further application of the approach presented here will lead to a greater understanding of the limits of ecosystem-climate feedbacks and assist in reducing the large uncertainty in land-atmospheric carbon exchange thwarting current Earth system models for climate prediction.

465   *Data availability.* All data not included in the paper in tables and the Supplement are available on figshare at:
       https://figshare.com/s/bd211ffdb1d2d5f4b7ea

       *Supplement* can be found at this link.

       *Author contributions.* AM-P and SEZ designed the study with input from KK and RB. Site identification, design

470   and sampling was led by SEZ and supported by collaborators acknowledged below. AM-P conducted sample analyses with significant input from KK. AM-P conducted data analyses and construction of figures and tables with input from SEZ and further input from KK and RB on more finalized version. AM-P prepared the paper with significant input from SEZ and editing from KK and RB.

       *Competing interests.* The authors declare that they have no conflict of interest.

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