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Supplement of

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

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Supplemental Methods

Composition of inputs

Litterfall input materials were collected on an annual basis by placing litterfall traps on the forest floor within six plots across each of the nine study sites across the transect for known periods of time through the entire year in 2011-2015. Within one year (2011-2012), collected materials were sorted by material type (e.g. twigs, branches, needles, cones, etc.) and pooled by their yearly average weight percentage according to mass input within each plot throughout the year. These pooled samples were analyzed for elemental composition representative of the entire year. Total litterfall input composition was determined based on litterfall elemental data by type and the input mass of each material type (including mosses, woody material, etc.) on an annual basis and averaged over the 2011-2015 period. To obtain an estimate of total inputs to the surface of the soil profile, the chemistry of the litter input was adjusted to include moss inputs based upon the L layer characterization (see below).

L layer characterization

Weight percentages of input components still identifiable in the L layer were determined through physical separation and weighing of separated components (eg. mosses, needles, twigs, etc). When complete isolation was not feasible, the percentage of components were determined through weight of the combined components and careful visual examination of their proportions. All procedures were completed by the same operator, and anything under 1% (w/w) was deemed undetectable.

End-member reconstruction

Litterfall end-member phenolic composition was determined by weighing the influence of pure end-member sources (green and brown needles, woody materials such as cones, bark, twigs, etc.) according to the same yearly average weight percentage determined from the litterfall traps collections at each site from 2011-2012, to simulate likely input materials at each site on an annual basis. Twigs, branches, cones and bark were assumed to contain the same lignin content and composition as wood, while lichens were assumed to contain no lignin. When end-member sources were not directly measured for CuO lignin phenolic composition from pure end-member sources in this study (as brown and green needles were), literature values were used (Hedges and Mann 1979; Moingt et al. 2016). This was the case for both balsam fir woody materials, and for birch woody materials. All literature values of phenolic composition were converted to nmol mgC^{-1}. When phenolic ratios were given in the literature, these values were converted directly to molar ratios; when not, these were calculated from V, S, P and C phenolic class concentrations in mg phenol gdw^{-1} utilizing average ketone, acid and aldehyde distribution detailed in Ertel and Hedges (Ertel and Hedges 1984), and converted to molar quantities or ratios. Error was propagated from these estimates utilizing the ‘propagate’ package in R, which propagates uncertainty using higher-order Taylor expansion

(Andrej-Nikolai Spiess 2018). These phenolic modeled litterfall end-members were included in the development of the LPDI to better estimate soil input materials.

Tree productivity assessment

Due to the crowded nature of the balsam fir forests in this mesic boreal region, we used rate of change in tree height as a measure of tree growth or productivity. This enabled us to largely eliminate the influence of stand dynamics, such as tree density, on the measurement of growth. Three representative trees within close proximity of each site in each of the three regions (n = 27) were felled and measured. Total height of each tree was measured from stump cut at the root collar. Measurements from the top of the tree to the first node, branch nodes indicating the lateral buds that represent the end of the years growth, was made to obtain height growth for that past year. This was continued down the tree toward the stump wherever possible occasionally checking through sections in the field between visible nodes to be sure no nodes were missed due to any damage. Once these nodes were no longer distinguishable, sectioning at 1 m intervals was applied. To normalize these measures across sites and regions we focused only on the data collected from 3 m height so that we were able to assess productivity of trees in these stands after the point they were able to freely grow unimpeded by the canopy structure. The plot of the tree height versus age for all 27 trees measured were all consistent with free growth occurring well before 3 m height. The rate of growth in m per year was estimated from the relationship between tree height and age since each tree was 3 m tall. This was further averaged at the regional level, with standard deviation of site-level slope fits assessed at the regional level (Supplemental Table 6).

Supplemental Figures and Tables

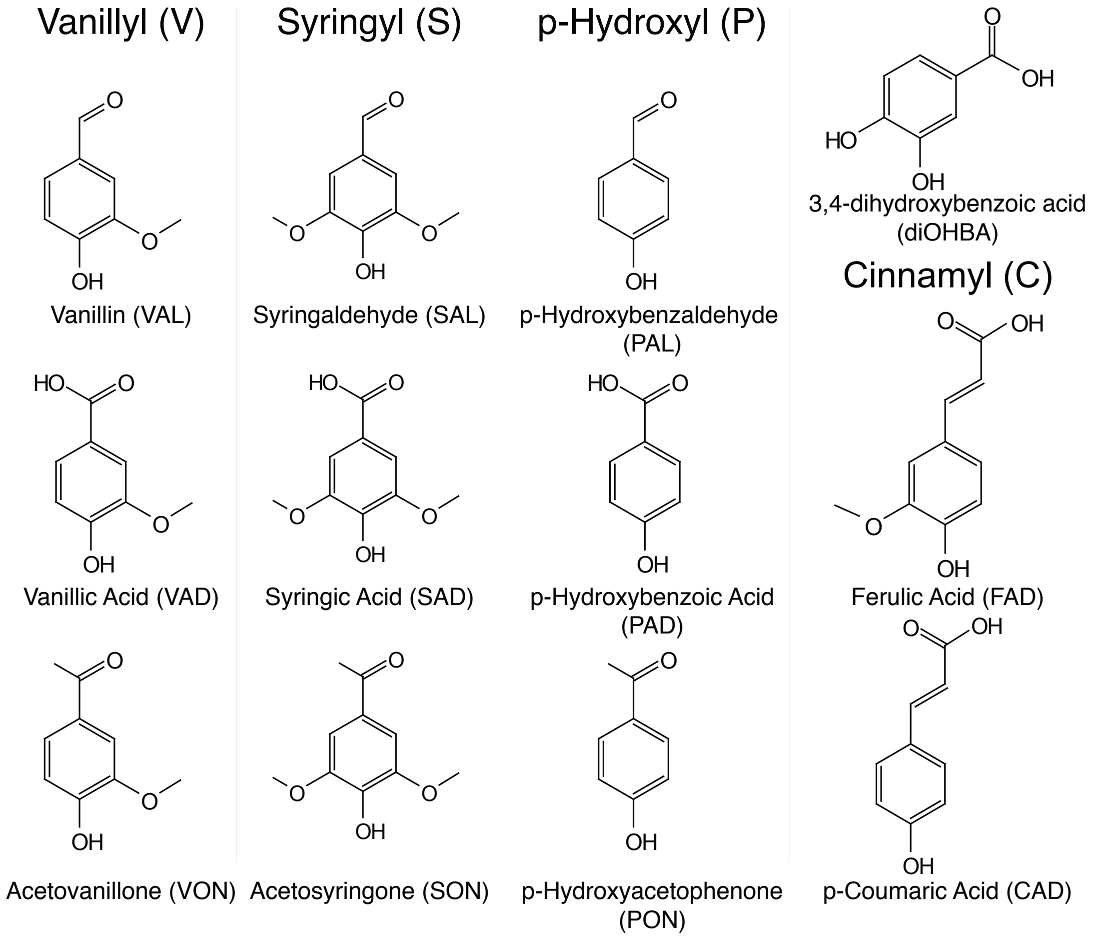

Figure S1. Twelve common CuO by-product phenols used to assess lignin composition and degradation state.

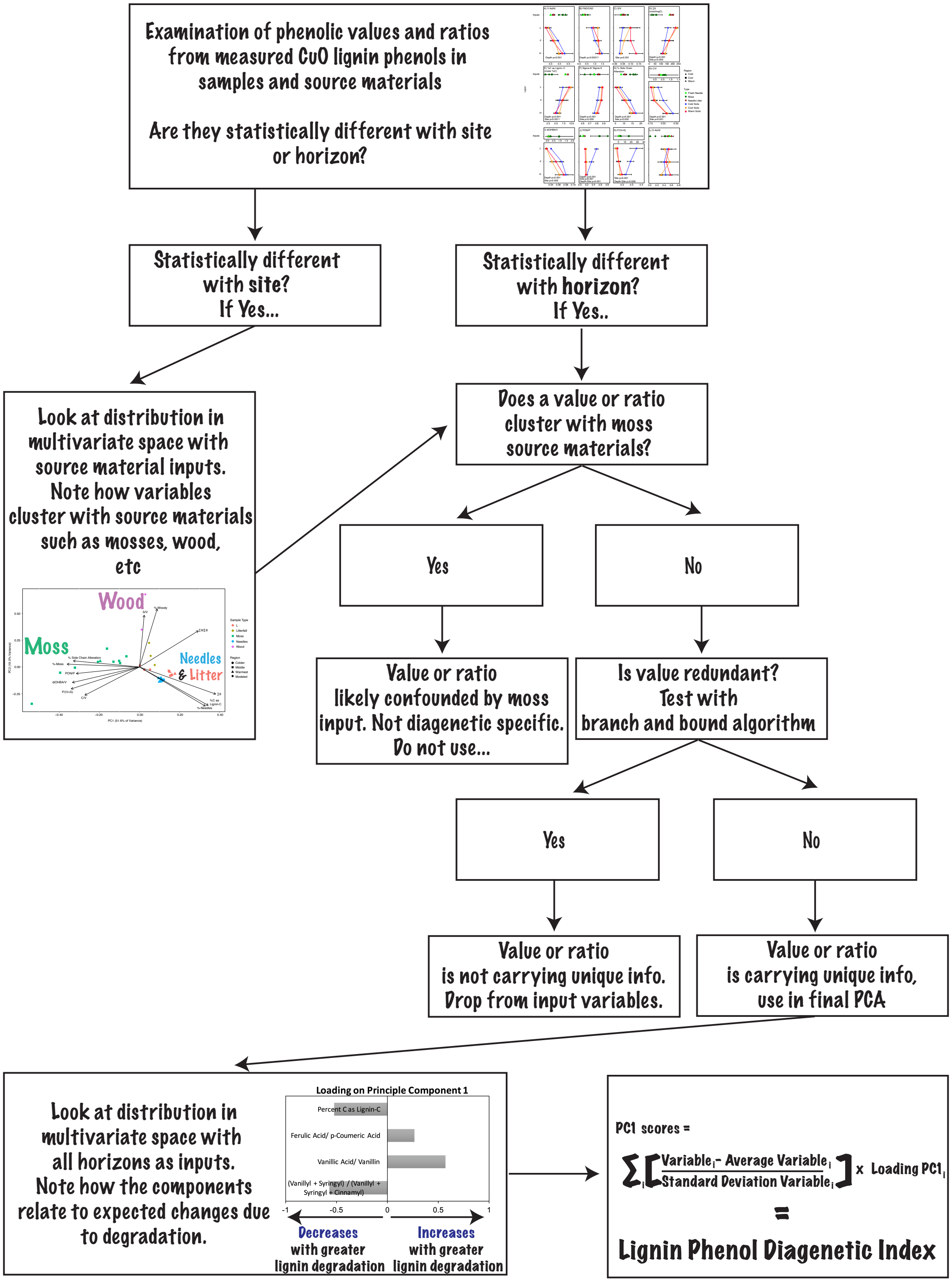

Figure S2. Flowchart of the steps taken to develop the lignin phenol diagenetic index (LPDI). Figures in the flowchart refer to Figure 1, Figure S3, and visual representations of Table S3 and S4.

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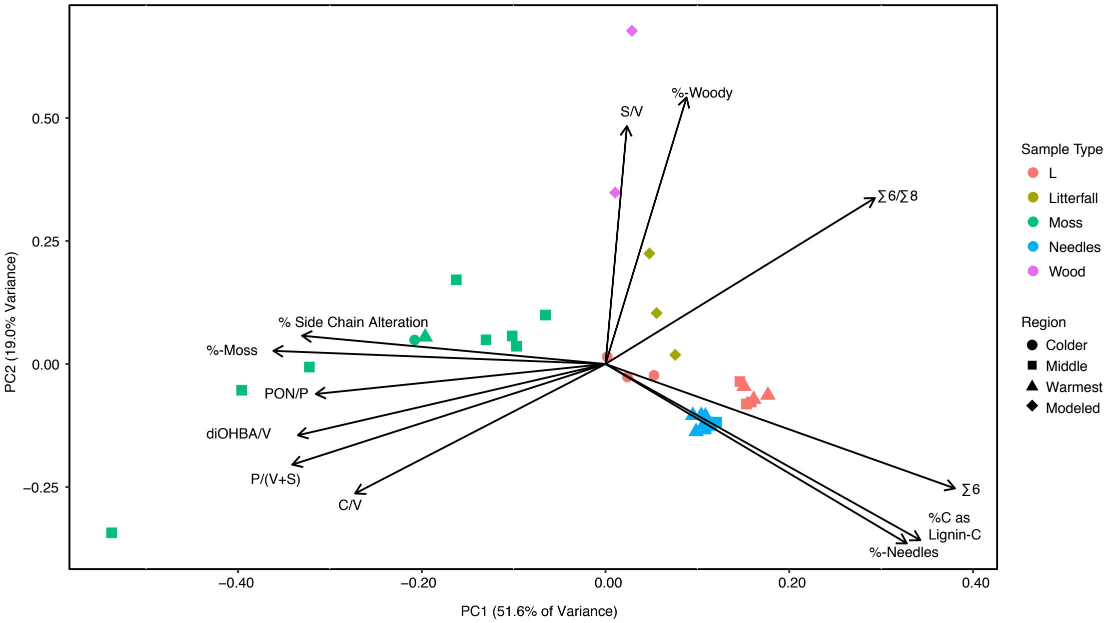

Figure S3. PCA bi-plot of source separations on the first two principle components, which cumulatively explain 70.6% of the variance in lignin phenol parameters, aimed to assess source influences on lignin content. Representative composite moss mixture samples included for the coldest and warmest site, while individual moss species were included for the middle site to illustrate the large heterogeneity in lignin content present among moss species found at these sites. Composite moss sample for middle site should be similar to the coldest site, as species composition are similar in these two forests (K. Buckeridge, pers. comm.). Needle signatures are obtained from litterfall needles from spring 2011, while litterfall signatures are modeled based on proportion of materials in litterfall traps from 2011-2012 (see supplemental methods above for details). Modeled wood input is also included (see supplemental methods above for how this was derived); expectedly the balsam fir wood endmember plots most similarly to the modeled litterfall samples and the sampled L layers, while the birch wood endmember plots uniquely. PC1 appears to differentiate between mosses and non-moss inputs, while PC2 appears to split between woody/non-woody inputs. Signatures apparently impacted by mosses include: PON/P, diOHBA/V, C/V. This is particularly interesting to note as diOHBA/V has been used to track soil inputs to aquatic systems, while C/V is generally used to differentiate between leafy and woody materials. %C as lignin-C appears to be driven by needle inputs in these systems and thus remains a relevant tracer of soil organic carbon to be tested.

Table S1. Common lignin phenol ratios and indicators of diagenesis and source.

Phenolic Index	Description	Classic interpretation	Caveats	References
Acid/Aldehyde	Used to assess side chain oxidation of the lignin macromolecule (common acid/aldehyde ratios are: Vanillic Acid/ Vanillin, $V_{ad/al}$ and Syringaldehyde/Syringic Acid, $S_{ad/al}$).	Increases with increasing degradation	Difference is solubilization, photo-reactivity between acids and aldehydes of different phenolic groups.	Ertel and Hedges 1984; Feng and Simpson 2008
Percentage Side Chain Alteration	Used to determine the percentage of side chains in the lignin macromolecule which have been altered by degradation.	Increases with increasing degradation	Not commonly used across studies. Assumes side chains linkages that form aldehydes are converted to linkages which form acids with no change in efficiency.	Ertel and Hedges 1984
∑8 (VSC)	The sum of C, S and V class phenols, used to determine lignin content.	Decreases with increasing degradation	C phenol content varies dramatically in many herbaceous tissues and C class phenols are thought to be more reactive than V or S type phenols due to their ester linkages and associations with carbohydrates.	Hedges and Mann 1979; Hedges and Weliky 1989; Opsahl and Benner 1995; Otto and Simpson 2006
∑6 (VS)	The sum of S and V class phenols used to determine lignin content.	Decreases with increasing degradation	Commonly used in marine systems instead of Σ8, as C phenols derived from aromatic amino acids are higher in proportion than from lignin in such ecosystems.	Hernes and Benner 2003
Percentage C as Lignin C (%C-Lignin C)	The percentage of carbon as lignin carbon is used to assess the total carbon that can be assigned to lignin from CuO oxidation, corrected for methodological efficiencies.	Decreases with increasing degradation	Assuming uniform methodological efficiencies. Not commonly used in soil studies.	Hedges and Weliky 1989; Benner et al. 1990b
Ferulic Acid / p-Coumaric Acid (FAD/CAD)	FAD/CAD is used to assess diagenetic state of needle inputs, as CAD is produced in much greater quantities than FAD in gymnosperm needles, while CAD is lost more rapidly from gymnosperm needles than FAD.	Decreases with increasing degradation	CAD is also thought to be more water soluble than FAD.	Hedges and Weliky 1989; Sanger et al. 1997

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Phenolic Index	Description	Classic interpretation	Caveats	References
Dihydroxybenzoic acid/Vanillyl (diOHBA/V)	diOHBA is thought to be a by-product of tannin degradation	Increases with increasing degradation	Hydroxybenzene structures are also present in mosses, potentially influencing the diOHBA/V.	Wilson et al. 1989; Goñi and Hedges 1995; Houel et al. 2006
p-Hydroxyl/ (Vanillyl + Syringyl) P/(V+S)	Increases with increasing degradation of lignin, as p-hydroxyphenols are not affected by methoxy group loss during demethoxylation.	Decreases with increasing degradation	Ratio is impacted by p-hydroxyphenol content in mosses.	Hedges and Ertel 1982; Opsahl and Benner 1995; Williams et al. 1998; Moingt et al. 2016
Cinnamyl/Vanillyl (C/V)	The ratio of the sum of cinnamyl phenols to the sum of vanillyl phenols. Non-woody materials contain higher C phenols than woody tissues, thus ratio is used to assess contribution of woody or non-woody materials	Quality indicator; lower numbers more woody tissues, higher numbers more non-woody tissues	Impacted by varying reactivity between C and V phenol classes.	Hedges and Mann 1979
Syringyl/Vanillyl (S/V)	Ratio of the sum of syringyl phenols to the sum of vanillyl phenols. Gymnosperms contain mainly V phenols, while angiosperms contain near equal mix of V and S phenols, thus ratio is used to assess major types of lignin sources; angiosperms and gymnosperms.	Quality indicator; lower numbers more influenced by gymnosperms, higher numbers more influenced by angiosperms	Also changes with degradation, as V and S type phenols have differing reactivity.	Hedges and Mann 1979; Otto and Simpson 2006
p-Hydroxyacetophenone/ p-Hydroxyl (PON/P)	PON/P has been used as a ratio of lignin vs non-lignin p-hydroxyphenol content, as PON is thought to be derived primarily from lignin content.	Increases with increasing contribution of lignin to p-hydroxyphenol content	Hydroxyphenols have many non-lignin sources, such as aromatic amino acids and mosses.	Williams et al. 1998; Goñi et al. 2000; Dittmar and Lara 2001

Table S2. Output loadings on PCs 1 & 2 for source PCA model (Supplemental Figure 2).

Variable	Loading on PC1	Loading on PC2
% Woody Material	0.08	0.52
% Needles	0.31	-0.35
% Moss	-0.34	0.03
∑6/ ∑8	0.28	0.32
∑6 (nmol/mgC)	0.36	-0.24
S/V	0.02	0.46
C/V	-0.26	-0.25
PON/P	-0.30	-0.06
diOHBA/V	-0.32	-0.14
P/(V+S)	-0.32	-0.19
% C as Lignin-C	0.33	-0.34
% Side Chain Alteration	-0.31	0.05

Table S3. Output scores on PCs 1 & 2 for Source PCA model presented in supplemental Figure 2.

Sample Type	Region	Site	Score on PC1	Score on PC2
L	Cold	Site 1 - Plot 1	0.03	0.13
L	Cold	Site 1 - Plot 2	0.75	-0.20
L	Cold	Site 1 - Plot 3	0.34	-0.23
L	Cool	Site 1- Plot 1	2.27	-0.67
L	Cool	Site 1- Plot 2	2.09	-0.31
L	Cool	Site 1- Plot 3	2.19	-0.71
L	Warm	Site 1- Plot 1	2.53	-0.55
L	Warm	Site 1- Plot 2	2.16	-0.40
L	Warm	Site 1- Plot 3	2.31	-0.62
Moss	Cold	Site 1-Composite Moss	-2.97	0.42
Moss	Cool	Site 1-Brown *Dicranum majus*	-0.93	0.86
Moss	Cool	Site 1-Green *Dicranum majus*	-2.32	1.49
Moss	Cool	Site 1- Brown *Hylocomium splendens*	-1.38	0.31
Moss	Cool	Site 1- Brown *Hylocomium splendens*	-1.86	0.43
Moss	Cool	Site 1- Green *Hylocomium splendens*	-5.66	-0.46
Moss	Cool	Site 1- Green *Hylocomium splendens*	-4.61	-0.05
Moss	Cool	Site 1- Brown *Rhytidiadelphus triquetrus*	-1.45	0.49
Moss	Cool	Site 1- Green *Rhytidiadelphus triquetrus*	-7.68	-2.98
Moss	Warm	Site 1-Composite Moss	-2.81	0.47
Needles	Cool	Site 1- Brown Balsam fir	1.62	-1.08
Needles	Cool	Site 1- Brown Balsam fir	1.65	-1.08
Needles	Cool	Site 1- Green Balsam fir	1.73	-1.02
Needles	Cool	Site 1- Green Balsam fir	1.56	-1.15

Sample Type	Region	Site	Score on PC1	Score on PC2
Needles	Warm	Site 1- Green Balsam fir	1.53	-1.07
Needles	Warm	Site 1- Green Balsam fir	1.36	-0.91
Needles	Warm	Site 1- Brown Balsam fir	1.55	-0.92
Needles	Warm	Site 1- Brown Balsam fir	1.49	-0.91
Needles	Warm	Site 1- Fresh Balsam fir	1.41	-1.19
Wood	Modeled	Balsam Fir Literature	0.15	3.02
Wood	Modeled	Birch Literature	0.41	5.88
Litterfall	Modeled-Cold	Site 1	1.08	0.16
Litterfall	Modeled-Cool	Site 1	0.79	0.90
Litterfall	Modeled-Warm	Site 1	0.68	1.95

Table S4. Output loadings on PC 1 for Lignin Phenol Degradation Index (LPDI). Variance explained by the first principle component was 64.3%.

Variable	Loading on PC1
∑6/∑8	-0.57
Ad/Al_v	0.57
FAD/CAD	0.27
% Carbon as Lignin-C	-0.53

Table S5. Average tree growth for each region as determined through growth rate curves; these were determined at each of the three sites per region, with exception of the warmest site (*), where one site was not included in this assessment. This was due to clear-cutting of the site before these measurements could be made.

Region	Number of observations per site	Average Growth $(m\ y^{-1})$	Standard Deviation
Cold	3	0.13	0.02
Cool	3	0.16	0.06
Warm*	3	0.26	0.04

Table S6. ANOVA fit results for testing LPDI ~ Depth x Region x Moss Input. Significance codes are as follows: ‘***’ 0, ‘**’ 0.001, ‘*’ 0.01, ‘.’ 0.05, ‘’ 0.1, 1.

	Df	Sum	Sq	Mean	Sq	F value Pr(>F)
Depth	2	28.589	14.295	21.974	3.48E-05	***
Region	2	2.504	1.252	1.924	0.18	
Moss_Input	1	0.58	0.58	0.891	0.36	
Depth:Region	4	1.178	0.294	0.453	0.769	
Depth:Moss_Input	2	1.663	0.831	1.278	0.307	
Residuals	15	9.758	0.651			

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