

1 Responses to reviews with page and line numbers. This includes the changes.

2

3 Reviewer #1.

4

5 Specific comments by page and line number (unfortunate that it is not continuous
6 lineage)

7 1, 14 'peat depth profiles' – I think 'depth' is redundant.

8

9 Response: 1/14. 'peat depth profiles' changed to 'peat profiles'.

10

11 2, 15 I think Loisel et al. (2014) is probably the best citation to support the role of
12 peatlands being an important long-term C sink (rather than Kuhry and Vitt 1996, which is
13 rather specific) and it contains a wealth of information on C and N (but not isotopes).
14 2 29 I think aerobic decomposition has a larger effect over a shorter time scale (e.g. 80%
15 of C input lost over a few hundred years, whereas catotelmic decomposition may lose only
16 another 10% over millennia); thus the changes may occur in those first few centuries, and be
17 'stored' in the catotelm.

18 Response: Kuhry and Vitt (1996) replaced with Loisel et al. (2014).

19 2, 30 'biogeochemical processes' perhaps rather than just 'biochemical'?

20

21 Response: 'biochemical' replaced with 'biogeochemical'

22

23 3, 16 I suspect sedges are the main plant applicable to your system, but the data in Table
24 2 suggest that they play a minor role at the present time; in fact it is now a treed site, with
25 75% of photosynthesis derived from larch and spruce.....

26

27 Response: Yes, flux data indicate that sedges are not so important now; text does not need to be
28 changed.

29

30 4, 28 I think there are studies which have identified the effect of wetness (water table) on
31 vegetation $\delta^{13}\text{C}$ (you mention Loader et al. for Sphagnum), thus data on water table
32 position when plants are growing for the year of sample and preceding years for
33 evergreen foliar materials would be useful. My understanding for these continental
34 peatlands is that water table is very variable within and among years. I would think the
35 USFS gang have data on this.

36

37 Response: Our foliar data are from a single year and thus year-to-year variability in wetness or
38 water table will not be helpful for explaining this data set.

39

40 9, 6 There is considerable interest and excitement (see Larmola et al. 2014 and others)
41 about the opportunities for methanotrophic N_2 fixation in peat profiles (given that many
42 traditional N_2 fixation studies cannot get enough N into the peat profile to account for the
43 large rates of N storage), which would occur just above and below the water table and
44 once fixed, the anaerobic conditions of the peat, as the water table effectively rises as the
45 peat accumulates, may mean that this signature is retained. In the case of the hollows, this
46 would be close to the surface, whereas in the hummocks, it may be 30-40 cm beneath the

1 surface. Depending on the $\delta^{15}\text{N}$ changes based on ‘normal’ decomposition as litter turns
 2 into decomposing peat, an increase in $\delta^{15}\text{N}$, the addition of relatively $\delta^{15}\text{N}$ -enriched N
 3 might speed this transformation. Comparing hummocks and hollows (merged in Figure 3
 4 b) is there any differentiation in $\delta^{15}\text{N}$ which may be related to N_2 fixation (input of -1
 5 $\delta^{15}\text{N}$)? The average $\delta^{15}\text{N}$ value does seem to ‘bounce around’ where the water table
 6 might be located.

7
 8 Response: In Table 2, we give $\delta^{15}\text{N}$ values for the surficial Sphagnum in hummocks and
 9 hollows. They are identical, at -2.3‰, perhaps suggesting similar levels of N fixation relative to
 10 total N.

11
 12 10, 38 The role of ectomycorrhizal fungi will be dependent not only on changes in
 13 vegetation (such as from fen to bog) but also the rooting depth of the various plants –
 14 how far do spruce and larch roots penetrate at the present time, relative to water table
 15 depth?

16
 17 Response: They are quite shallow-rooted, and generally do not go below the water table. We
 18 now cite a paper on root dynamics at SPRUCE by Iversen et al. (2017) that gives relevant data.

19 The specific added text reads:

20 Because of the high water table, root distribution is quite shallow compared to upland sites
 21 (Iversen et al., 2017). 93% of fine roots were in the 0-10 cm depth, 6% at 10-20 cm depth, and
 22 only 1% at 20-30 cm depth (personal communication, A. Malhotra). The boundary between the
 23 acrotelm and catotelm at SPRUCE is around 30-40 cm depth.

24
 25
 26 Reviewer #2.

27 **Specific Comments:**

28 The initial paragraph of the Introduction uses the terms “acrotelm” and “catotelm” to describe
 29 areas of active C loss and C sinks in peatland bogs. Use of these terms could provide reference
 30 points for those in the audience unfamiliar with how peatland bog biogeochemistry changes with
 31 depth, particularly as depth explained a substantial portion of $\delta^{13}\text{C}$ and $\delta^{14}\text{C}$ patterns in this
 32 study. However, these terms are only referenced once again throughout the entire manuscript. It
 33 may be useful to match these terms with the corresponding peat coring depths in the study to
 34 provide a reference for the audience on expected areas of C loss and C storage.

35
 36 Response: The second paragraph of the introduction has been modified. It now reads: Deeper
 37 peats “in the catotelm” reflect both historic vegetation as well as accumulated effects of
 38 anaerobic fermentation occurring over thousands of years. In contrast, aerobic decomposition “in
 39 the acrotelm” alters biogeochemistry over shorter time scales.

40
 41 P.4, L. 3-9: Consider organizing the hypothesis to match the order with which they are addressed
 42 in the Discussion. This would help provide clarity if the reader refers back to the hypothesis
 43 while reading the Discussion.

44
 45 Response: The hypotheses have been reordered to reflect the order of discussion (they were
 46 simply numbered 4, 1, 2, 3 in the order in which they were discussed).

1
2 Table 4: Was there an explanation for why the core at -213 cm was older (9200±200 Age cal yr
3 BP) than the core at -225 cm (6775±260 Age cal yr BP)?

4
5 Response:

6
7 We do not have a good explanation for this. However, only 3 cores were taken at -213 cm depth,
8 whereas 11 cores were taken at -225 cm depth. Given uneven patterns of peat accumulation
9 through the bog, it is likely that the noted discrepancy reflects slower peat accumulation rates
10 (and hence older for a given depth) for the 3 cores at -213 cm versus faster peat accumulation on
11 average for the 11 cores at -225 cm depth.

12
13 **Technical Comments:**

14
15 P. 10, L. 34: Indenting of this paragraph is inconsistent with rest of the manuscript.

16
17 Response: Changed.

18
19 P. 11, L. 20: The author's name in "(Skrzypek et al., 2005, 2008)" appears to be misspelled and
20 should be changed to "Skrzypek."

21
22 Response: Changed to Skrzypek.

23
24 Figure 2: Is a plot missing a label on this figure? I only see 16 plots labeled but the caption states
25 that there are 17. The initial version of this figure had plot 2 labeled by the outlet in the first
26 photograph, but it is not visible in the new figure. Additionally, plot 2 was not listed in the
27 Groups in Table 3.

28
29 Response: Plot 2 was not sampled, thus it is not indicated on the figure. Text has been changed to
30 "16 plots" from "17 plots" to reflect this.

31
32 Table 3: Similar to the comment above, Table 3 states that plot 921 is within Groups 1 and 2, but
33 plot 921 doesn't appear anywhere else in the manuscript, including the plot coefficients of Figure
34 5.

35 Table 3: The line "δ15N model, adjusted r2 = 0.660, p<0.001" should be shifted left to begin
36 over the corresponding "Source" in the table.

37
38 Response:

39
40 Plot 921 has been changed to plot 21, the correct terminology. The heading beginning 'd15N
41 model..' has been shifted to the left.

42

1 6. Apr. 2017

2

3 **Long-term Carbon and Nitrogen Dynamics at SPRUCE Revealed through Stable Isotopes**
4 **in Peat Profiles**

5

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22

23 **Abstract.** Peatlands encode information about past vegetation dynamics, climate, and microbial
24 processes. Here, we used $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ patterns from 16 peat profiles to deduce how the
25 biogeochemistry of the Marcell S1 forested bog in northern Minnesota responded to
26 environmental and vegetation change over the past ~10,000 years. In multiple regression
27 analyses, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ correlated strongly with depth, plot location, C/N, %N, and each other.
28 Correlations with %N, %C, C/N, and the other isotope accounted for 80% of variance for $\delta^{15}\text{N}$
29 and 38% of variance for $\delta^{13}\text{C}$, reflecting N and C losses. In contrast, correlations with depth and
30 topography (hummock or hollow) reflected peatland successional history and climate. Higher
31 $\delta^{15}\text{N}$ in plots closer to uplands may reflect upland-derived DON inputs and accompanying shifts
32 in N dynamics in the lagg drainage area surrounding the bog. The Suess effect (declining $\delta^{13}\text{CO}_2$
33 since the Industrial Revolution) lowered $\delta^{13}\text{C}$ in recent surficial samples. High $\delta^{15}\text{N}$ from -35 cm
34 to -55 cm probably indicated the depth of ectomycorrhizal activity after tree colonization of the
35 peatland over the last 400 years, as confirmed by the occasional presence of wood down to -35
36 cm depth. High $\delta^{13}\text{C}$ at ~4000 years BP (-65 cm to -105 cm) could reflect a transition at that time
37 to slower rates of peat accumulation, when ^{13}C discrimination during peat decomposition may
38 increase in importance. Low $\delta^{13}\text{C}$ and high $\delta^{15}\text{N}$ at -213 cm and -225 cm (~8500 years BP)
39 corresponded to a warm period during a sedge-dominated rich fen stage. The above processes

1 appear to be the primary drivers of the observed isotopic patterns, whereas there was no clear
2 evidence for methane dynamics influencing $\delta^{13}\text{C}$ patterns.

3

4 **1 Introduction**

5

6 Carbon (C) and nitrogen (N) cycling are tightly linked (Schlesinger et al., 2011) and
7 understanding the controls of C and N turnover in boreal peatlands is fundamental to predicting
8 whether this ecosystem will continue to function as a strong C sink or switch to a source of
9 carbon dioxide (CO_2) and methane (CH_4) in response to environmental change. While 80-90% of
10 C deposited in peatlands is lost via decomposition and microbial respiration in the upper aerobic
11 layers of the acrotelm (Belyea and Malmer, 2004), the deeper anaerobic catotelm accumulates
12 recalcitrant *Sphagnum* litter and other organic matter due to low mineral nutrient availability and
13 water-logged conditions. Carbon loss from the catotelm can be 50% within the first 1700 years
14 with only an additional 15% over the next 5800 years (Loisel et al., 2014), thus making peatlands
15 an important long-term C sink.

16 Factors influencing C and N dynamics can be investigated using stable isotope
17 measurements because biochemical and physical reactions proceed faster with lighter isotopes
18 (^{12}C and ^{14}N) than with heavier isotopes (^{13}C and ^{15}N). Thus, different pools and fluxes can vary
19 in their isotopic signatures (expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Climate and foliar %N can also
20 influence the $\delta^{13}\text{C}$ of plant photosynthate by determining the relative rates of stomatal flux
21 versus fixation of CO_2 (Brooks et al., 1998; Ménot and Burns, 2001; Sparks and Ehleringer,
22 1997). Radiocarbon measurements (^{14}C) are also important in biogeochemical research, as they
23 allow dating of peat profiles and linking stable isotope patterns to specific climatic periods or
24 vegetational phases of peatland succession. The dominant factors influencing organic C and N
25 turnover can be identified by characterizing isotopic signatures of specific compounds or plant
26 components through the peat profile (Nichols et al., 2009; Gavazov et al., 2016), but interpreting
27 bulk peat signatures remains challenging. Deeper peats in the catotelm reflect both historic
28 vegetation as well as accumulated effects of anaerobic fermentation occurring over thousands of
29 years. In contrast, aerobic decomposition in the acrotelm alters biogeochemistry over shorter
30 time scales.

31 Analysis of C and N in peatland cores is a potential way to determine key
32 biogeochemical processes involved in organic matter burial and release. Cores taken through the
33 peat profile trace the trajectory of peatland succession and contain the biogeochemical
34 fingerprint of shifts in climate and vegetation states. For example, fens and bogs differ in the
35 chemical and isotopic composition of the organic peat profile, reflecting differences in their pH,
36 redox state, hydrologic inputs, and relative abundance of vascular plants compared to *Sphagnum*
37 (Vitt and Weider, 2006). Warming, drying, and increased N availability can also alter isotopic
38 composition through changes in plant community composition, with concomitant effects on C
39 and N dynamics, including enhanced production of greenhouse gases such as CO_2 , CH_4 , and

1 nitrous oxide (N₂O) (Yavitt et al., 1987; Regina et al., 1996; Bergman et al., 1999; Juutinen et
2 al., 2010).

3 Our conceptual model of C and N dynamics during peatland succession is shown in
4 Figure 1. Methanogenesis, methanotrophy, refixation of methane-derived CO₂, and plant
5 composition influence the δ¹³C of surficial layers (Ficken et al., 1998; Pancost et al., 2000), and
6 the resulting δ¹³C signal is subsequently altered further during diagenesis of these buried peat
7 soils over time. Topography could also influence δ¹³C because oxygen availability decreases
8 with increasing water depth, resulting in different levels of methanogenesis and methanotrophy
9 in hummocks versus hollows. Topography further influences δ¹³C because hummock C is older
10 than hollow C for equivalent depths below the mean bog surface. The anthropogenic addition of
11 ¹³C-depleted CO₂ to the atmosphere via the burning of fossil fuels (the Suess effect, Ehleringer et
12 al., 2000) also increases the gradient between ¹³C-depleted surficial horizons and older, ¹³C-
13 enriched deeper horizons.

14 How N dynamics will influence δ¹⁵N patterns is also shown in Figure 1. In aerobic soils,
15 uptake by mycorrhizal fungi and subsequent transfer of ¹⁵N-depleted N to host plants increases
16 the ¹⁵N divergence between deeper, ¹⁵N-enriched horizons and surficial horizons (Hobbie and
17 Ouimette, 2009), with such processes presumably not operating in *Sphagnum* and deep-rooted
18 nonmycorrhizal plants (Kohzu et al., 2003), but potentially operating in forested bogs. Nitrogen
19 transport from uplands can be considerable in the lagg drainage region surrounding a peatland
20 (Verry and Janssens, 2011), and depending on the drainage δ¹⁵N, may influence the δ¹⁵N of the
21 receiving peatland. For example, lagg drainage could contribute ¹⁵N-depleted nitrate or ¹⁵N-
22 enriched dissolved organic N (DON) (Kalbitz and Geyer, 2002), that differ isotopically from N
23 fixation (0‰) or atmospheric N inputs (Stewart et al., 1995; Högberg, 1997). In addition,
24 biogeochemical hotspots are important for N dynamics in peatlands (Hill et al., 2016). Microbial
25 processing of organic matter in soils commonly increases the δ¹⁵N and δ¹³C of the residual
26 material (Nadelhoffer and Fry, 1994), although a N loss mechanism must also be present for
27 δ¹⁵N to be affected. Such processing decreases the C/N of organic matter, since respiratory C
28 losses are generally greater than N losses.

29 Here, we used δ¹⁵N, δ¹³C, %N, and %C patterns of peat profiles, plant tissues, and fungal
30 hyphae sampled from the Spruce and Peatland Responses Under Climatic and Environmental
31 Change (SPRUCE) experimental site in northern Minnesota, USA at the Marcell S1 bog to
32 investigate potential factors influencing C and N turnover in peatlands. In addition to the
33 continuous variables of elemental concentration, isotopic signatures, and depth, nominal
34 variables included plot location, topography (hummock versus hollow), and vegetation (near
35 trees or not). We used concurrent radiocarbon measurements (Iversen et al., 2014) to link the
36 stable isotope measurements to the 11,000-year history of C and N dynamics at the SPRUCE
37 experimental site. With this combination of data, we studied how *in situ* biogeochemistry and
38 peatland succession may have influenced the isotopic profiles. We inferred the path of peatland
39 succession from a prior study of the nearby S2 bog, as given in Table 1. Successional history at
40 S1 should be similar, with the vegetation proceeding from a rich fen to a transitional fen ~5500

1 years BP, transitioning to a poor fen ~2900 years BP, and then changing to a forested bog ~400
2 years BP.

3 Isotopic patterns reflect numerous biogeochemical processes. Here, we addressed four
4 potential drivers of isotopic variation in a boreal peatland profile:

5 ~~(1) in upper peat layers, ^{13}C depletion will reflect anthropogenic declines in the $\delta^{13}\text{C}$ of~~
6 ~~atmospheric CO_2 (Suess effect);~~

7 ~~(2)~~ (1) microbial processing and biochemical composition (as inferred from %N, %C, and C/N)
8 will influence peatland $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$;

9 ~~(3)~~ (2) proximity to uplands will increase N concentrations and peat $\delta^{15}\text{N}$;

10 ~~(4)~~ (3) peatland colonization by ectomycorrhizal trees will increase peat $\delta^{15}\text{N}$ and the ^{15}N
11 enrichment between surficial and deeper peat.

12 ~~(4) in upper peat layers, ^{13}C depletion will reflect anthropogenic declines in the $\delta^{13}\text{C}$ of~~
13 ~~atmospheric CO_2 (Suess effect);~~

14
15 In addition to these drivers, we will examine the covariation of peat $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with climate
16 or vegetation through the Holocene.

17

18 2 Methods

19

20 2.1 Site description

21

22 Soil and fungal samples were collected from the SPRUCE experimental site at the 8-hectare S1
23 bog in the USDA Forest Service Marcell Experimental Forest in northern Minnesota, USA (47°
24 30.476'N, 93° 27.162'W). The bog is dominated by the trees *Picea mariana* (Mill.) Britton,
25 Sterns and Poggenb. and *Larix laricina* (Du Roi) K.Koch, ericaceous shrubs (*Ledum*
26 *groenlandicum* Oeder; *Chamaedaphne calyculata* (L.) Moench.) and *Sphagnum* mosses. Various
27 forbs and sedges are also present. The bog topography can be separated into hummocks
28 (protruding above the average water table) and depressed hollows, and divided into areas with
29 trees (*Picea* or *Larix*) or without trees. Average annual air temperature from 1961 to 2005 was
30 3.3°C with yearly mean extremes of -38°C and 30°C and average annual precipitation of 768
31 mm (Sebestyen et al., 2011). Average pH of the peat is 4.1 and average gravimetric water
32 content is 7.40 g H_2O g^{-1} dry peat (Iversen et al., 2014). The water table fluctuates about 30 cm
33 annually and water table depth can reach 1.4 m over the course of a 50-year record (Sebestyen et
34 al., 2011). The bog maintains a perched water table with a hydraulic gradient about 1 m above
35 the surrounding aquifer, which is composed of outwash sand (Verry et al., 2011). Because of the
36 high water table, root distribution is quite shallow compared to upland sites (Iversen et al., 2017).
37 93% of fine roots were in the 0-10 cm depth, 6% at 10-20 cm depth, and only 1% at 20-30 cm
38 depth (personal communication, A. Malhotra). The boundary between the acrotelm and catotelm
39 at SPRUCE is around 30-40 cm depth.

40

2.2 Procedures

Peat cores for this analysis were collected in mid-August of 2012 from locations along three boardwalks extending out into the bog beyond the lagg region (Figure 2). Surface peat (~0–30 cm) was collected using a modified hole saw, while deeper samples down to mineral soil (~30–250 cm) were collected using a Russian peat corer. Cores were taken in both hummocks and hollows, with 0 cm defined as the surface of hollows and hummock heights above that assigned positive depths. Cores were bulked and homogenized every 10 cm over the 0 to -100 cm depth, every 25 cm from -100 to -200 cm, and over the entire 50 cm increment from -200 to -250 cm (in some cases, -300 cm was reached before mineral soil was observed). Cores were sampled at 17 locations (Figure 2; the locations of experimental plots distributed across the three boardwalks) and material from 16 of these locations was used for the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and radiocarbon measurements reported here. At locations 4, 5, 6, and 7 along the southern boardwalk, separate cores were taken within 150 cm of *Picea* or *Larix* trees and in the open (no trees within 150 cm), and the distinction designated as ‘treed’ or ‘untreed’.

Peat cores were analyzed for $\Delta^{14}\text{C}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, %N, and C/N by depth increment, with the depth increment recorded as the average depth (for example, 0 to 10 cm in a hummock given as 5 cm). Peat cores were analyzed in hummocks to a depth of -10 cm and in hollows to the bottom of the core (between -200 and -300 cm). Live woody plant foliage and fine roots to -10 cm were collected in August 2012 and live *Sphagnum* in 2013.

To collect fungal hyphae, in-growth cores were constructed. Mesh (40 μm) in-growth bags (10 cm \times 10 cm) were filled with sterile sand. Bags were incubated in the field in paired hummock and hollows at six locations in the bog. In hummocks, bags were inserted at +10 to 0 cm above the adjacent hollow and in both locations from 0 to -10 cm and -10 to -20 cm below the hollow surface. Bags were installed on June 5, 2013 and recovered on September 20, 2013. Sand from in-growth bags was combined with ultrapure water and mixed at 80 rpm for 20 minutes. Suspended hyphae were removed with tweezers and the process was repeated until all hyphae were collected. Hyphal biomass was dried in the oven at 60°C for 48 hours. Of 30 in-growth samples, 20 generated enough hyphal mass for analysis. All 20 samples were treated as independent replicates in statistical analyses.

2.3 Isotopic and elemental analysis

Radiocarbon content of homogenized bulk peat was measured on the Van de Graaff FN accelerator mass spectrometer (AMS) at the Center for AMS at Lawrence Livermore National Laboratory. Peat samples were not chemically pretreated prior to ^{14}C measurement. Samples were prepared by sealed-tube combustion to CO_2 in the presence of CuO and Ag and then reduced onto Fe powder in the presence of H_2 (Vogel et al., 1984). Radiocarbon isotopic values had an average AMS precision of 2.6‰ and were corrected for mass-dependent fractionation with $\delta^{13}\text{C}$ values from analyses conducted at the Department of Geological Sciences Stable

1 Isotope Laboratory at University of California-Davis using a GVI Optima Stable Isotope Ratio
2 Mass Spectrometer. Radiocarbon values are reported here in $\Delta^{14}\text{C}$ notation corrected for ^{14}C
3 decay since 1950 (Stuiver and Polach, 1977). Calibrated ages were determined using Calib
4 (<http://calib.qub.ac.uk/calib/>) or CaliBomb (Reimer et al., 2004) with INTCAL13 (Reimer et al.,
5 2013) and Northern Hemisphere Zone 1 bomb curve extension (Hua et al., 2013) atmospheric
6 ^{14}C calibration curves. Years before present (BP) refer to years prior to 1950. For more recent
7 samples, calendar years AD may also be used.

8 These same soil samples and additional samples of hyphae and foliage were analyzed for
9 %C, %N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ at the University of New Hampshire Stable Isotope Laboratory using a
10 Costech 4010 Elemental Analyzer coupled to a Thermo Delta Plus XP IRMS. Standard
11 deviations of laboratory standards (tuna, NIST 1515, and NIST 1575a) for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
12 averaged less than 0.2%. Fine roots of the woody vascular plants were analyzed for their stable
13 isotopic composition at the Oak Ridge National Laboratory on an Integra CN mass spectrometer
14 (SerCon, Crewe, UK), using standards traceable to NIST 8547-ammonium sulfate or 8542-
15 sucrose (NIST, Gaithersburg, Maryland, USA).

16

17 **2.4 Statistical tests**

18

19 The statistical program JMP (SAS Institute, Middleton, Massachusetts, USA) was used for
20 statistical analyses. Reported values are \pm standard error, unless otherwise specified. Regression
21 models for soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were tested. Factors included in the regression model included
22 nominal variables of vegetation type (treed or non-treed), topography (hollow or hummock), and
23 plot number. Depths with only a single measurement were generally excluded, unless they were
24 very similar in depth to another value. Continuous variables included %N, %C, and isotopic
25 values.

26 To test whether plot location, proximity to trees, depth, topography, and elemental
27 concentrations influenced the carbon and N isotope patterns in peat profiles, we used multiple
28 regression analyses. Sample %C, %N, C/N, and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ were included as continuous
29 variables. Because the effects of depth or plot location on N and C dynamics are unlikely to
30 change continuously (for example, methanogenesis requires an anaerobic soil and plots at bog
31 edges may have different hydrology and N dynamics than plots in the middle of the bog, Urban
32 and Eisenreich, 1988), plot was treated as a nominal (categorical) variable in our regression
33 models. To avoid over-parameterizing the model, depth was treated as a continuous variable with
34 a cubic transformation (that is, the regression model included model depth, (model depth)², and
35 (model depth)³ as additional parameters). Stepwise regression was used and model selection
36 based on the lowest Aikike Information Criteria with a correction for sample size (AICc). Within
37 a given depth, values for radiocarbon were tested for correlations against $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the
38 slope of the regression estimated.

39

40 **3 Results**

3.1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in plants and fungal hyphae

Of the six vascular plant taxa tested, $\delta^{13}\text{C}$ of foliage varied from -30‰ in *Larix* to -28‰ in *Picea*. The $\delta^{15}\text{N}$ of plant foliage varied more widely than $\delta^{13}\text{C}$, from -8.5‰ for *Picea* to 2.5‰ for *Eriophorum*. Fine root $\delta^{13}\text{C}$ averaged $-27.4 \pm 0.3\text{‰}$ for *Larix*, $-26.9 \pm 0.1\text{‰}$ for *Picea*, and $-28.5 \pm 0.2\text{‰}$ for shrubs. Fine root $\delta^{15}\text{N}$ averaged $-4.7 \pm 0.4\text{‰}$ for *Larix*, $-4.1 \pm 0.3\text{‰}$ for *Picea*, and $-1.8 \pm 0.1\text{‰}$ for shrubs, whereas coarse roots of shrubs averaged $-3.4 \pm 0.3\text{‰}$. If we assume plant productivity patterns are similar aboveground and belowground, then the productivity-weighted average in vascular plants for $\delta^{13}\text{C}$ was -29.2‰ for foliage and -27.3‰ for roots (Table 2). Fungal hyphae from in-growth cores ($n = 20$) averaged $-26.0 \pm 0.2\text{‰}$ (se) for $\delta^{13}\text{C}$ and $-0.3 \pm 0.2\text{‰}$ for $\delta^{15}\text{N}$.

3.2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in peat profiles

Carbon isotope ($\delta^{13}\text{C}$) values of peat in the profile increased from -29‰ in the top 10 cm of hummocks and hollows to -26‰ at -112 cm and then decreased slightly at greater depths. $\delta^{13}\text{C}$ values changed most rapidly from 0 cm to -50 cm depth (Figure 3a). Nitrogen isotope values in the peat profile increased from -3‰ in hummocks above the water level to around 1‰ at -50 cm. $\delta^{15}\text{N}$ then decreased to 0‰ at -85 cm before increasing again to 1.5‰ at -200 cm. Similar to $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ changed most rapidly from 0 cm to -50 cm depth (Figure 3b).

In a stepwise regression model for $\delta^{13}\text{C}$ including $\delta^{15}\text{N}$, C/N, %N, %C, and depth as continuous variables and the vegetation type, topography, and plot sampling location as nominal variables, all factors were retained in our final model, including four terms for partitioning the 16 plots. The model explained 85% of the total variance ($n = 238$, adjusted r^2) in peat $\delta^{13}\text{C}$. The three depth terms (depth, depth squared, and depth cubed) explained 45%, %N explained 10%, C/N 11%, $\delta^{15}\text{N}$ 15%, %C explained 2%, the four plot terms explained 7%, and proximity to trees explained 3% of the variance (Table 3). Topography explained 10% of variance, with hollows lower than hummocks by 0.89‰ in $\delta^{13}\text{C}$ ($p < 0.001$). Results of the stepwise model testing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are given in Table A1.

The stepwise regression model for $\delta^{15}\text{N}$ of peat included $\delta^{13}\text{C}$ and the same variables as in the $\delta^{13}\text{C}$ regression model. This model explained 66% of the total variance (adjusted r^2), with proximity to trees and topography dropping out of the regression model. Of the explained variance, the three depth terms accounted for 8%, %N 22%, %C 7%, C/N 17%, $\delta^{13}\text{C}$ 34%, and the three plot terms 11%, (Table 3).

The influence of depth and location in our regression models on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values is plotted in Figure 4 and Figure 5, respectively. The influence of depth was negative above 0 cm (corresponding to the surface of the hollows), increased regularly from -5 cm to -25 cm, and then varied little in $\delta^{13}\text{C}$ in the deepest horizons while still increasing in $\delta^{15}\text{N}$. There was some spatial

1 patterning of values across plots, with two plots (4 and 19) near to the western upland high in
2 $\delta^{15}\text{N}$ (Figure 2 and Figure 5).

3 Although overall patterns of radiocarbon with depth were clear, radiocarbon varied
4 widely at any given depth, and correlated significantly with $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ at several depths (Table
5 4). Radiocarbon correlated positively with $\delta^{13}\text{C}$ at -162 cm, -5 cm, 5 cm, and 15 cm, and
6 correlated negatively with $\delta^{13}\text{C}$ at -35 cm and -65 cm. In contrast, radiocarbon correlated
7 positively with $\delta^{15}\text{N}$ at -65 cm, -55 cm, -45 cm, -5 cm, and 15 cm, and correlated negatively
8 with $\delta^{15}\text{N}$ at -162 cm and -25 cm. Overall patterns of $\Delta^{14}\text{C}$ with $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ are shown in Figure
9 6a and Figure 6b, respectively.

10

11 **4 Discussion**

12

13 **4.1 Potential causes of shifts in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in peat profiles**

14

15 Isotopic ratios within the profile can shift if elemental fluxes in or out of the system differ
16 isotopically from profile material (Figure 1). Loss of labile C via respiration, methanogenesis, or
17 leaching (Kolka et al., 1999) could alter the $\delta^{13}\text{C}$ of the residual material, as could inputs of ^{13}C -
18 enriched material such as roots or mycorrhizal hyphae. Similarly, changes in the $\delta^{13}\text{C}$ of
19 atmospheric CO_2 can alter the $\delta^{13}\text{C}$ of photosynthetically fixed C whereas changes in moisture,
20 temperature, or photosynthetic capacity can alter the ^{13}C discrimination between atmospheric
21 CO_2 and fixed C. For N, loss of ^{15}N -depleted material from the bulk peat via mycorrhizal transfer
22 to fine roots, direct root uptake, denitrification, or leaching of organic or inorganic N could raise
23 the $\delta^{15}\text{N}$ of the remaining soil organic matter. Inputs of N via atmospheric deposition, N fixation,
24 or transport from surrounding uplands could also influence $\delta^{15}\text{N}$ if these inputs differ isotopically
25 from peat profile values. These processes can be linked to past climate and vegetation with
26 profile radiocarbon measurements that are calibrated to calendar years. Here, we used
27 radiocarbon to indicate the potential timing of shifts in some of the primary drivers that
28 influenced C and N stable isotope patterns within the peat profiles at SPRUCE, such as the Sues
29 effect and the transition to a forested bog, but also examined processes that were not tied to a
30 particular time period, such as differences in C and N stoichiometry or proximity to uplands.

31

32 **4.2 %N, %C, and C:N stoichiometry influenced $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ patterns**

33

34 %N, %C, and C/N contributed a combined 47% and 23% of explained variance, respectively, to
35 our regression models of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. These factors reflected the biochemical and isotopic
36 composition of the original plant material, but also reflected how the chemical structure and
37 isotopic composition of plant material has altered during its slow decomposition at S1.

38 Microbially-driven C loss raises soil organic matter %N, lowers C/N, and enriches soil
39 organic matter in ^{13}C (resulting from loss of ^{13}C -depleted CO_2) (Ehleringer et al., 2000; Alewell
40 et al., 2011). The positive correlation of %N with $\delta^{13}\text{C}$ may therefore reflect an underlying

1 correlation between the accumulation of ^{13}C -enriched microbial necromass (Wallander et al.,
 2 2004) and the increased N content of the peatland organic matter. Fungal %N and $\delta^{13}\text{C}$ are
 3 positively correlated (Hobbie et al., 2012) because of the high $\delta^{13}\text{C}$ of microbially synthesized
 4 protein relative to other microbial components such as carbohydrates and lipids. In contrast, the
 5 positive correlation of C/N with $\delta^{13}\text{C}$ and negative correlation with $\delta^{15}\text{N}$ presumably reflects a
 6 legacy of buried wood, which, relative to other plant material, should be high in $\delta^{13}\text{C}$ (Trudell et
 7 al., 2004) and high in C/N. This can be seen clearly in the few samples with C/N greater than 70,
 8 which is higher than any plant tissue measured in this study. Although some *Sphagnum* taxa
 9 under pristine conditions can be very low in %N (0.22%) and $\delta^{15}\text{N}$ (-3.6‰) and high in C/N
 10 (~190) (Asada et al., 2005a), here, the presence of wood was noted seven times during laboratory
 11 examination of the 238 samples at depths from 15 cm to -35 cm, with those samples twice as
 12 high in C/N (average, 69) as other samples, and were also significantly higher in C/N in multiple
 13 regression analysis (see Table A2).

14 The negative correlation of %N with $\delta^{15}\text{N}$ indicated that either added or removed N is
 15 low in $\delta^{15}\text{N}$. One possibility for removal is that ^{15}N -depleted N has been transferred from
 16 mycorrhizal fungi to plants. Alternatively, N could be added via fixation with a $\delta^{15}\text{N}$ value of -
 17 1‰, which would lower overall $\delta^{15}\text{N}$ values deeper in the peat profile. However, we point out
 18 that only at -45 cm and -55 cm are %N and $\delta^{15}\text{N}$ significantly and negatively correlated (Table
 19 A3). At these depths, %N is about 1.8% and $\delta^{15}\text{N}$ is about 1‰ (Figures 3b, d). The value of the
 20 coefficient for %N in the $\delta^{15}\text{N}$ regression, $-2.96\text{‰} \text{ \%N}^{-1}$, implied that the perturbing N has a $\delta^{15}\text{N}$
 21 value that is $1.8\% \times 2.96\text{‰} \text{ \%}^{-1}$ less than that of 1‰, or -4.3‰, which is too low to be fixed N.
 22 The apparent ^{15}N depletion of 5.3‰ against the source N is a plausible value for ^{15}N
 23 discrimination between mycorrhizal fungi and host plants (Hobbie and Colpaert, 2003).

24 Correlations of carbon concentration with stable isotopes provide information about
 25 chemical composition or degree of processing. The negative correlation of %C with $\delta^{13}\text{C}$ is
 26 expected based on the chemical composition of ^{13}C -depleted compound classes of lignin,
 27 aromatics, and lipids, which are high in %C (Poorter et al., 1997; Hobbie et al., 2002). Initial
 28 decomposition of *Sphagnum* commonly decreases $\delta^{13}\text{C}$, as ^{13}C -enriched soluble components are
 29 leached (Asada et al., 2005b). In contrast, aromatics and lipids do not generally contain N, so the
 30 positive correlation of %C with $\delta^{15}\text{N}$ in bulk peat cannot be explained in the same manner.
 31 However, microbial processing generally enriches soils in ^{15}N (Billings and Richter 2006;
 32 Templer et al., 2007) while increasing %C, which was also true at S1 (Tfaily et al., 2014).
 33

34 **4.3 Proximity to uplands and trees increased peat $\delta^{15}\text{N}$**

35
 36 Plot-specific coefficients for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ may reflect site-specific differences in the dominance
 37 of conditions favoring ^{13}C - or ^{15}N -depleted losses during peatland development. The positive
 38 coefficients for $\delta^{15}\text{N}$ are from two plots, 4 and 19, closest to the lagg region adjacent to the
 39 western upland. This suggests that the different hydrology in the lagg has enhanced ^{15}N
 40 fractionation from N removal mechanisms such as denitrification, nitrification, or leaching of

1 DON. Alternatively, dissolved N transported from the uplands during spring thaw and melt may
 2 have provided an additional ^{15}N -enriched N source for plots 4 and 19 located near the bog edges
 3 (Figure 5). Peatland DON appears enriched in ^{15}N and ^{13}C relative to bulk peat (Broder et al.,
 4 2012), and this is presumably true for upland sources as well. In the adjacent Marcell S2 kettle
 5 bog, large N fluxes from upland locations from both surface runoff and interflow led to much
 6 larger N losses in streamflow from the lagg region ($\sim 32 \text{ kg ha}^{-1} \text{ yr}^{-1}$) than from the bog itself (2
 7 $\text{ kg ha}^{-1} \text{ yr}^{-1}$) (Urban and Eisenreich, 1988). The uplands here are dominated by ectomycorrhizal
 8 trees such as *Populus*, *Quercus*, and *Pinus*, which produce vertically stratified soil profiles with
 9 high $\delta^{15}\text{N}$ values in lower organic and mineral horizons (Hobbie and Ouimette, 2009). We
 10 therefore expect DON produced in uplands to be high in $\delta^{15}\text{N}$, which will serve as a source of
 11 ^{15}N -enriched N to lagg regions of peatlands.

12

13 **4.4 $\delta^{15}\text{N}$ patterns with depth reflected climate and vegetation**

14

15 Peatland succession and climate have been established previously at the nearby S2 bog and are
 16 summarized in Table 1. As the same climatological factors affected the S2 and S1 (SPRUCE)
 17 bogs, plant stratigraphy and isotopic patterns were probably similar, although accumulation rates
 18 are lower at S1 than at S2 (McFarlane, unpublished data).

19 In the following paragraphs, we link shifts in $\delta^{15}\text{N}$ through the profile to radiocarbon ages
 20 and the corresponding patterns in vegetation and climate at the S2 bog. The high $\delta^{15}\text{N}$ at -213 cm
 21 and -225 cm corresponded approximately to a warm period between 8000 and 9200 BP, during a
 22 sedge-rich fen stage (Verry and Janssens, 2011), with mean annual temperatures of 4-5°C. Given
 23 similar C:N ratios during this period to subsequent periods (~ 20), nitrogen losses were probably
 24 more depleted in ^{15}N than subsequent losses, that is, more losses via nitrate leaching or
 25 denitrification rather than via DON leaching. Nitrification and denitrification are higher in fens
 26 than in bogs and should change $\delta^{15}\text{N}$ patterns along the core profile as the core reflects peatland
 27 succession and climatological changes (Regina et al., 1996; Bayley et al., 2005; Wray and
 28 Bayley, 2007).

29 The S2 bog shifted from a rich fen to a transitional fen by 5600 years BP, while MAT
 30 decreased to perhaps 2°C. At the S1 bog, the negative correlation between radiocarbon and $\delta^{15}\text{N}$
 31 at -162 cm (~ 6000 years BP) suggested decreasing losses of ^{15}N -depleted N during this
 32 transition. The depth increment from -85 cm to -112 cm corresponded to a transitional fen stage
 33 3300-4800 years BP. The accompanying trough in $\delta^{15}\text{N}$ at $-400\text{‰ } \Delta^{14}\text{C}$ (4220 calibrated years
 34 BP, Figure 6b) suggested decreased sequestration of ^{15}N -enriched organic matter as nutrient
 35 availability declined during this transition.

36 The positive correlations between $\delta^{15}\text{N}$ and $\Delta^{14}\text{C}$ at -45, -55, and -65 cm (Table 4 and
 37 Figure 6b) are linked to a parallel decline in %N over these time periods, so that younger
 38 samples are lower in %N and higher in $\delta^{15}\text{N}$ than older samples, reflecting losses of ^{15}N -depleted
 39 N from the younger samples. $\delta^{15}\text{N}$ values peaked at -35 to -55 cm, and then declined above and
 40 below those depths. This pattern suggested that those depths were affected by a ^{15}N enrichment

1 process that did not affect deeper depths (that is, further in the past). The peak $\delta^{15}\text{N}$ accordingly
 2 reflected ^{15}N partitioning between surficial and deeper horizons by a new mechanism.

3 We suggest that ectomycorrhizal fungi are the most probable cause of this unusual peak
 4 in $\delta^{15}\text{N}$ at intermediate depths in the peat bog. At the nearby S2 bog, poor fen transitioned to
 5 forested bog between 1610 AD and 1864 AD, with a charcoal layer at S2 indicating that peat was
 6 consumed by fire during this period, precluding a more specific date for this transition (Verry
 7 and Janssens, 2011). This forested bog is dominated by ectomycorrhizal *Picea* and *Larix*.
 8 Transfer to plants of ^{15}N -depleted N by ectomycorrhizal fungi leads to low plant $\delta^{15}\text{N}$ and high
 9 ^{15}N enrichment of the residual N not transferred (Hobbie and Hobbie, 2008; Hobbie and
 10 Högberg, 2012). Here, the apparent influence of ectomycorrhizal uptake can be traced to ~-55
 11 cm.

12 The relatively low contribution of ectomycorrhizal and ericoid mycorrhizal fungi to total
 13 plant uptake here compared to forest sites dominated by ectomycorrhizal fungi accounted for the
 14 lower ^{15}N enrichment between surficial layers and deeper layers here (~3‰) than in forest soils,
 15 which averaged a 9.6‰ enrichment between surficial and deeper soils for ectomycorrhizal
 16 forests (reviewed in Hobbie and Ouimette, 2009). Ectomycorrhizal and ericoid mycorrhizal trees
 17 and shrubs contributed relatively little to total N uptake at the nearby Marcell S2 bog, where
 18 mosses contributed 75% of total plant uptake, herbaceous plants 13%, and mycorrhizal trees and
 19 shrubs 13% (Urban and Eisenreich, 1988). A few studies in peatlands have reported $\delta^{15}\text{N}$ values
 20 through profiles, but those values have peaked at -1‰ to 0‰ and have been in systems without
 21 ectomycorrhizal trees present (Krüger et al., 2015; Esmeijer-Liu et al., 2011).

23 **4.5 $\delta^{13}\text{C}$ patterns with depth reflected climatic factors**

24
 25 Once shifts in $\delta^{13}\text{C}$ caused by compositional shifts are removed, the remaining patterns can be
 26 explained by invoking climatic or vegetation shifts. The relatively low $\delta^{13}\text{C}$ at -213 cm and -225
 27 cm corresponded to a warm period between 8000 and 9200 BP, during a sedge-dominated rich
 28 fen stage (Verry and Janssens, 2011), with mean annual temperatures of 4-5°C. Higher
 29 temperatures in peatlands are associated with lower $\delta^{13}\text{C}$ values (Skrzypek et al., 2005, 2008).

30 Radiocarbon and $\delta^{13}\text{C}$ correlated positively at -162 cm, corresponding to a 1‰ rise in
 31 $\delta^{13}\text{C}$ which accompanied a drop in MAT to perhaps 2°C by 6000 BP. This cooling trend was also
 32 accompanied by a slight rise in precipitation, so the decreased ^{13}C discrimination could also be
 33 attributed to increased *Sphagnum* moisture (Rice and Giles, 1996), although it is difficult to
 34 distinguish between these two possible causes of ^{13}C differences (Ménot and Burns, 2001).
 35 *Sphagnum* discrimination is less with increased moisture because CO_2 diffusion is limited under
 36 wet conditions. The stratigraphy at the S2 bog indicated a vegetational shift from a rich fen to a
 37 transitional fen during this period.

38 The depth increment from -85 cm to -112 cm corresponded to a transitional fen stage
 39 3300-4800 years ago. In our regression model for $\delta^{13}\text{C}$ (Figure 4), these two depths are about
 40 0.5‰ higher in $\delta^{13}\text{C}$ than at -162 cm. The peak in $\delta^{13}\text{C}$ may reflect a phase during which sedges

1 transported methane directly to the atmosphere, thereby minimizing the re-fixation in *Sphagnum*
2 cells of ^{13}C -depleted, methanotrophic-derived carbon dioxide (Raghoebarsing et al., 2005).

4 **4.6 The Suess effect increased ^{13}C depletion in surficial peat**

6 The strong dependence of $\delta^{13}\text{C}$ on peat depth partially reflected the 1.7‰ decline in the $\delta^{13}\text{C}$ of
7 atmospheric CO_2 since 1850, with the lowest $\delta^{13}\text{C}$ values above the water table, where C is of
8 recent origin. For example, the lowest estimated values of the $\delta^{13}\text{C}$ depth coefficients ($\sim -1\%$) in
9 hummocks at 15 cm, 22 cm, and 25 cm above the mean hollow surface reflect C from the last 50
10 years (Figure 4), as confirmed by $\Delta^{14}\text{C}$ averages of 59‰, 29‰, and 52‰ for these three depths,
11 where only C influenced by ^{14}C created during thermonuclear testing should have positive $\Delta^{14}\text{C}$
12 values (Table 4). Although our sampling lacked sufficient vertical resolution to explicitly include
13 corrections for the Suess effect (e.g., as done in Esmeijer-Liu et al., 2012), the $\sim 2\%$ increase in
14 the depth coefficient of our $\delta^{13}\text{C}$ regression model from the hummocks to deeper in the profile
15 correspond well to the long-term shift in $\delta^{13}\text{C}$ of atmospheric CO_2 from pre-industrial times to
16 the present. An additional factor contributing to the higher depth coefficient could be the 1-2‰
17 higher $\delta^{13}\text{C}$ in roots than in foliage and the different input depths of foliage (surface only) and
18 roots (distributed throughout the acrotelm). The steady increase in $\delta^{13}\text{C}$ between -5 cm and -25
19 cm depth (Figure 4) presumably reflected the increasing dominance of pre-industrial C. Depths
20 of -35 cm and below all had $\Delta^{14}\text{C}$ values less than -100‰ (Table 4), indicating primarily pre-
21 bomb and pre-industrial C when the average $\delta^{13}\text{C}$ of atmospheric CO_2 was -6.5‰ (versus the
22 current value of -8.2‰). In addition, modern production of organic matter averaged -29‰ in
23 $\delta^{13}\text{C}$ (Table 2), similar to values for surficial horizons, whereas deeper horizons were between -
24 27‰ and -26‰. The Suess effect of $\sim 1.5\%$ therefore accounted for at least half of this
25 difference.

27 **5 Conclusions**

29 Although the multiple potential interactions among climate, vegetation, and soil processes made
30 definitive conclusions difficult, we identified several factors that influenced $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
31 patterns in peatland profiles, including the Suess effect, C and N stoichiometry, microbial
32 processing, proximity to uplands, and tree colonization. The potential roles of methanogenesis
33 versus respiration in influencing $\delta^{13}\text{C}$ could not be addressed using bulk samples, since ^{13}C shifts
34 were relatively small. Future measurements of deuterium isotope ratios (δD) would allow
35 separation of effects of respiration (loss of CO_2) versus methane flux on $\delta^{13}\text{C}$ patterns, since
36 methanogenesis discriminates against deuterium. Our conceptual model as given in Figure 1
37 included the Suess effect (influencing $\delta^{13}\text{C}$ patterns) and N transport from uplands (probably
38 influencing $\delta^{15}\text{N}$ patterns), but missed several additional factors that appeared to be important in
39 controlling the isotopic patterns we reported. Buried wood appeared to influence both $\delta^{13}\text{C}$ and
40 $\delta^{15}\text{N}$ directly, and the ectomycorrhizal fungi associated with tree species here created a ^{15}N -

1 enriched horizon that may be specific to forested peatlands. The model did not consider N loss
2 mechanisms and associated ^{15}N effects, nor did it consider the potential for ^{13}C differences
3 between roots and aboveground litter to influence $\delta^{13}\text{C}$ profiles. One potential way forward is to
4 put these multiple processes into quantitative models of peatland development that includes
5 vegetation succession and climatic drivers, such as the Holocene Peatland Model (Frolking et al.,
6 2010), and to adapt these models to make isotopic predictions that can be compared against data.
7 Such model-data comparisons should continue to improve our ability to interpret isotopic
8 patterns, as well as reveal areas where our model formulations are currently inadequate.

9

10 **6 Data availability**

11 The data presented in this study are available in the appendices and through the Iversen et al.
12 (2014) publication.

13 **7 Supplement link (will be included by Copernicus)**

14 The supplement related to this article is available online at
15 <http://dx.doi.org/10.3334/CDIAC/spruce.025>

16 Hofmockel, K.S., Chen, J. and Hobbie, E.A. 2016. SPRUCE S1 Bog Pretreatment Fungal
17 Hyphae Carbon and Nitrogen Concentrations and Stable Isotope Composition from In-growth
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20

21 **8 Author contributions**

22 Samples were collected by C. Iversen, P. Hansen, and K. Hofmockel and analyzed by J. Chen, E.
23 Hobbie, C. Iversen, and K. Mcfarlane. E. Hobbie prepared the manuscript with contributions
24 from all authors.

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36 to do so, for United States Government purposes. The Department of Energy will provide public
37 access to these results of federally sponsored research in accordance with the DOE Public Access
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3

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- 29

1 **11 Tables**

2 Table 1. Peatland stages at SPRUCE based on stratigraphy at the S2 watershed in the Marcell
3 National Forest, USA (Verry and Janssens, 2011).

4

5 Stage	Years BP	Climate
6	9300-7400	4-5°C, 700 mm MAT 700
7	9200-6500	Warm and dry
8 Rich fen-sedge peat	8400-5500	
9	7400-6100	4°C → 2°C, MAP 700-800 mm
10	6500-6000	Cooling trend
11	6100-3200	Stable climate
12 Transitional fen	5600-3000	
13	3200-2400	Cooled slightly
14 Open poor fen	2900-390	
15	2000-1800	Warmer and drier
16 Little Ice Age	600-150	Cooled 1°C, MAT 800 mm
17 Forested bog	384/130-now	

18

19 Table 2. Average foliar values for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, %N, and C/N (n = 7). ¹Weighted average of
20 $\delta^{13}\text{C}$ inputs is -29.2‰, based on carbon fixation measurements of different taxa (R.J. Norby,
21 personal communication). ANOVA comparing means in vascular plants used a post-hoc Tukey
22 test, with log C/N tested in place of C/N. ²Data from Tfaily et al. (2014). ³Top 10 cm only,
23 primarily *Sphagnum*.

24

25 Species	$\delta^{13}\text{C}_{\pm\text{se}}$	$\delta^{15}\text{N}_{\pm\text{se}}$	%C $\pm\text{se}$	%N $\pm\text{se}$	C/N $\pm\text{se}$	n	% C flux ¹
26 <i>Chamaedaphne</i>	-29.5±0.1 ^c	-3.8±0.4 ^c	51.30±0.42 ^a	1.50±0.04 ^b	34.53±0.88 ^{cd}	12	1.5
27 <i>Eriophorum</i>	-28.4±0.2 ^a	2.5±0.6 ^a	45.16±0.21 ^c	1.45±0.06 ^b	31.49±1.36 ^d	9	--
28 <i>Larix</i>	-30.4±0.1 ^d	-6.3±0.4 ^d	48.49±0.36 ^{bc}	0.77±0.06 ^c	65.10±5.17 ^b	7	37.0
29 <i>Ledum</i>	-29.2±0.1 ^{bc}	-5.4±0.2 ^d	51.74±0.23 ^a	1.26±0.03 ^b	41.21±1.07 ^c	13	6.6
30 <i>Picea</i>	-28.0±0.2 ^a	-8.5±0.2 ^e	49.07±0.20 ^b	0.63±0.02 ^c	79.44±3.24 ^a	11	38.3
31 <i>Smilacina</i>	-28.7±0.2 ^{ab}	0.1±0.3 ^b	47.20±0.53 ^d	2.46±0.10 ^a	19.53±0.88 ^e	12	1.5
32 ² <i>Sphagnum</i>	-29.2	-2.0	--	--	--	--	15.0
33 Hollow ³	-28.8±0.1	-2.3±0.2					
34 Hummock ³	-28.8±0.1	-2.3±0.5					

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36

1 Table 3. Stepwise regression model for explaining $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in peat profiles at SPRUCE.
 2 Plot, topography (hummock versus hollow), and nearby presence of trees are treated as nominal
 3 variables. Value = Coefficient \pm standard error; Var = % variance explained. n = 238. Depth in
 4 cm, depth-squared and depth-cubed terms are also included in the model. Plots are sequentially
 5 separated into two groups to maximize the variance explained. Plot groups are Group (1),
 6 {19&8&11&20&10&15&5 vs. 9&16&21&6&14&4&7&13&17}; Group (2), {15 vs. 5}; Group
 7 (3), {9&16&9&21&6&14&4&7 vs. 13&17}; Group (4), {14&4 vs. 7}; Group (5),
 8 {10&6&11&13&9&15 vs. 17&16&5&8&14&20&7&21&19&4}; Group (6), {13 vs. 9}; Group
 9 (7), {17&16&5&8&14&20&7&21 vs. 19&4}. Within a group, the first plots listed have the
 10 given value, the second plots listed (after vs.) have the negative of the given value.

$\delta^{13}\text{C}$ model, adjusted $r^2 = 0.846$, $p < 0.001$				$\delta^{15}\text{N}$ model, adjusted $r^2 = 0.660$, $p < 0.001$			
Source	Value \pm se	%Var	P	Source	Value \pm se	%Var	P
Intercept	-28.39 \pm 0.37	--	<0.001	Intercept	32.30 \pm 3.39	--	<0.001
$\delta^{15}\text{N}$	0.196 \pm 0.025	14.8	<0.001	$\delta^{13}\text{C}$	1.164 \pm 0.115	34.0	<0.001
%N	1.036 \pm 0.162	9.5	<0.001	%N	-2.963 \pm 0.360	22.5	<0.001
%C	-0.033 \pm 0.011	2.3	0.002	%C	0.107 \pm 0.023	7.0	<0.001
C/N	0.025 \pm 0.004	11.0	<0.001	C/N	-0.060 \pm 0.008	17.1	<0.001
Hummock	0.44 \pm 0.07	9.6	<0.001	Hummock	--	--	--
Treed	-0.12 \pm 0.05	3.5	0.023	Treed	--	--	--
Depth	-4.18 \pm 0.44e ⁻²	21.2	<0.001	Depth	-2.48 \pm 0.63e ⁻²	5.1	<0.001
Depth ²	-3.28 \pm 0.40e ⁻⁴	15.4	<0.001	Depth ²	-1.57 \pm 0.68e ⁻⁴	1.8	0.021
Depth ³	-7.03 \pm 1.21e ⁻⁷	7.9	<0.001	Depth ³	-4.89 \pm 2.27e ⁻⁷	1.6	0.032
Group (1)	-0.121 \pm 0.031	3.5	<0.001	Group (5)	-0.305 \pm 0.070	6.3	<0.001
Group (2)	0.151 \pm 0.081	1.3	0.062	Group (6)	0.280 \pm 0.188	0.7	0.137
Group (3)	-0.111 \pm 0.047	1.5	0.018	Group (7)	-0.308 \pm 0.090	3.9	<0.001
Group (4)	-0.163 \pm 0.065	1.2	0.013				

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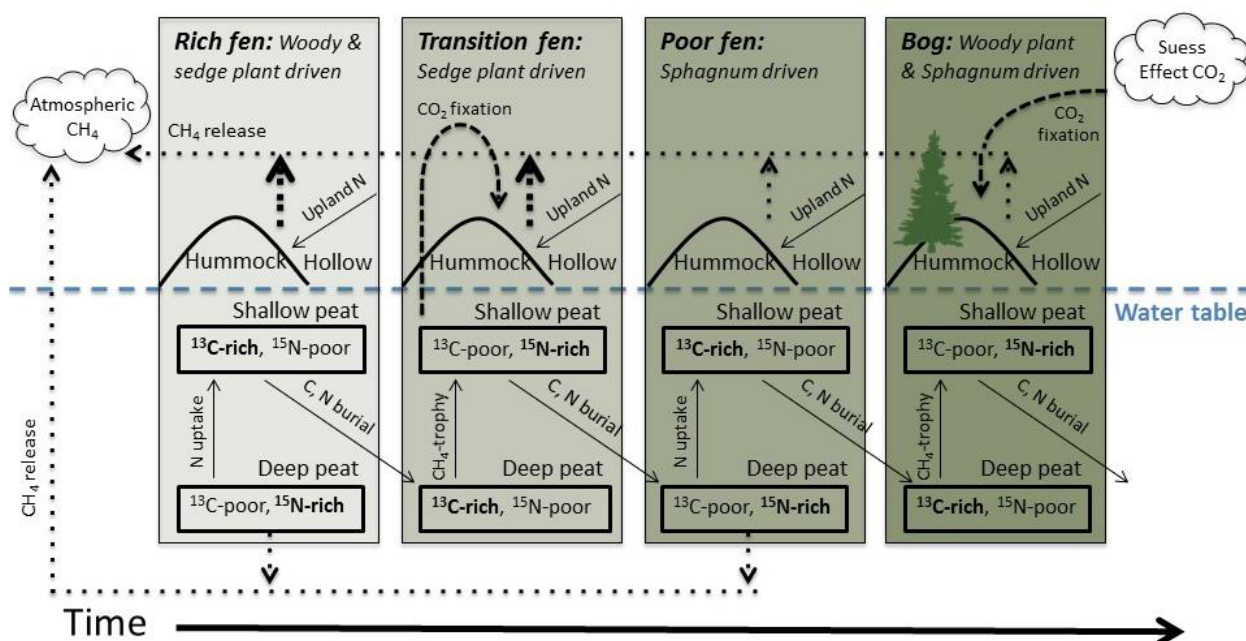
1 Table 4. Correlations between radiocarbon and stable isotopes by depth, and mean $\Delta^{14}\text{C}$ for that
 2 depth. Hummock vs hollow plots and treed vs non-treed plots were averaged together. $\delta^{13}\text{C}_{\pm\text{se}}$
 3 and $\delta^{15}\text{N}_{\pm\text{se}}$ columns reflect the shift in $\Delta^{14}\text{C}$ with a 1‰ shift in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$. Statistically
 4 significant correlations are bolded. nd = not determined.

Depth	Mean \pm se		Age (cal yr BP)	$\delta^{13}\text{C}_{\pm\text{se}}$			$\delta^{15}\text{N}_{\pm\text{se}}$		
	n	$\Delta^{14}\text{C}$ (‰)		adj. r^2	(‰)	P	adj. r^2	(‰)	P
25	3	52 \pm 5	Modern	0.881	10 \pm 3	0.157	-0.943	2 \pm 9	0.892
22	7	29 \pm 13	Modern	0.325	69 \pm 35	0.106	-0.195	2 \pm 11	0.889
15	16	59 \pm 5	Modern	0.219	13\pm6	0.039	0.221	7\pm3	0.038
5	16	115 \pm 18	Modern	0.682	74\pm13	<0.001	0.039	17 \pm 14	0.225
-5	19	71 \pm 7	Modern	0.352	33\pm10	0.004	0.293	18\pm6	0.010
-15	19	126 \pm 19	Modern	-0.053	9 \pm 30	0.762	-0.024	-16 \pm 22	0.459
-25	20	100 \pm 23	Modern	0.018	-41 \pm 35	0.261	0.414	-62\pm16	0.001
-35	18	-102 \pm 34	1580 \pm 180	0.228	-246\pm100	0.026	-0.053	-9 \pm 24	0.704
-45	18	-182 \pm 27	1730 \pm 260	-0.041	-54 \pm 95	0.575	0.570	58\pm12	<0.001
-55	18	-203 \pm 19	nd	-0.025	-63 \pm 81	0.447	0.512	52\pm12	<0.001
-65	18	-288 \pm 11	2800 \pm 600	0.486	-100\pm24	<0.001	0.340	44\pm14	0.006
-85	18	-359 \pm 7	3520 \pm 90	-0.007	-23 \pm 25	0.362	-0.049	-7 \pm 15	0.651
-113	18	-392 \pm 6	3950 \pm 90	0.081	42 \pm 27	0.134	0.016	-17 \pm 15	0.276
-163	17	-486 \pm 7	6000 \pm 500	0.713	68\pm11	<0.001	0.537	-31\pm7	<0.001
-213	3	-587 \pm 32	9200 \pm 200	-0.811	-44 \pm 136	0.801	-0.96	-11 \pm 76	0.912
-225	11	-567 \pm 12	6775 \pm 260	-0.089	27 \pm 62	0.677	-0.101	9 \pm 33	0.784

25

1 **12 Figures**

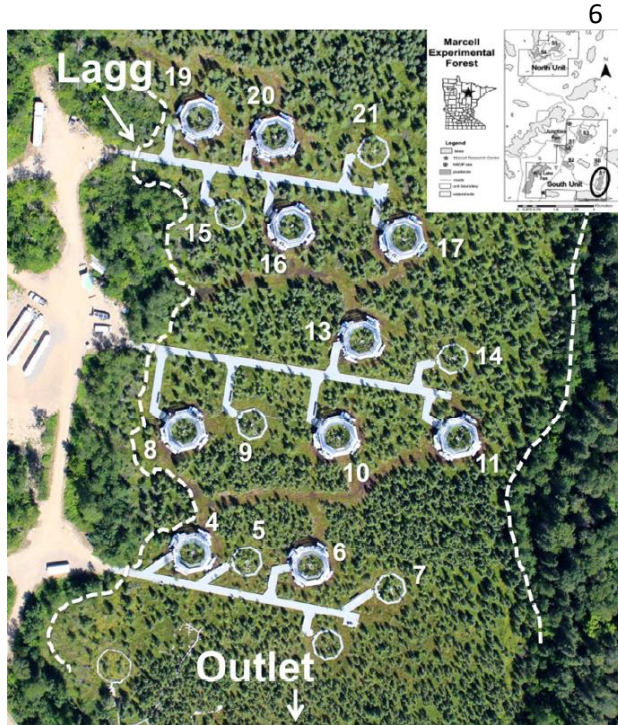
2 Figure 1. Conceptual diagram of movement of carbon (C) and nitrogen (N) during peatland
 3 development from rich fen to bog. Major processes influencing isotopic composition include
 4 methane (Me) flux from surficial and deeper layers (dotted upward arrow), methanotrophy and
 5 subsequent CO₂ recapture by *Sphagnum*, vascular plant transport of methane, N uptake by
 6 vascular plants and mycorrhizal fungi, and the sequestration of C and N over time in deeper peat.
 7 Assimilation of ¹³C-depleted CO₂ from the Sues effect influences modern peat carbon
 8 (rightmost top box); N flux from adjacent uplands influences productivity in the lagg region,
 9 peatland topography of hummocks and hollows influences methanogenesis and methanotrophy,
 10 and trees influence partitioning of nitrogen. Climate (not shown) will influence the initial $\delta^{13}\text{C}$ of
 11 fixed carbon. By rotating the figure 90° counterclockwise, the lower boxes correspond
 12 stratigraphically to the peat profile.



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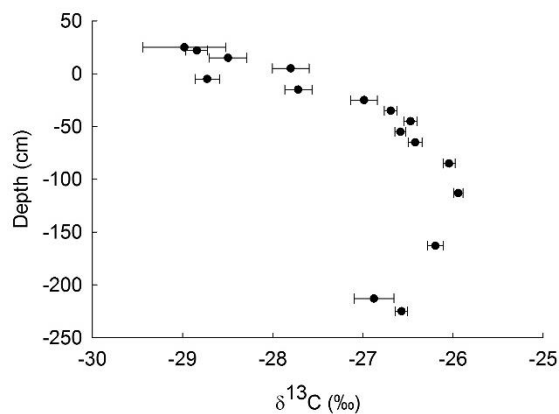
14

- 1 Figure 2. Aerial photograph of the S1 bog showing the 16 experimental plots (each 10.4 m in
- 2 diameter to the outer edge of the visible perimeter boardwalk). Plot numbers on the image
- 3 represent the plot areas within which peat was sampled. The lagg boundary is delineated with a
- 4 dashed line, and the inset indicates the location of the bog within Minnesota and the Marcell
- 5 Experimental Forest.

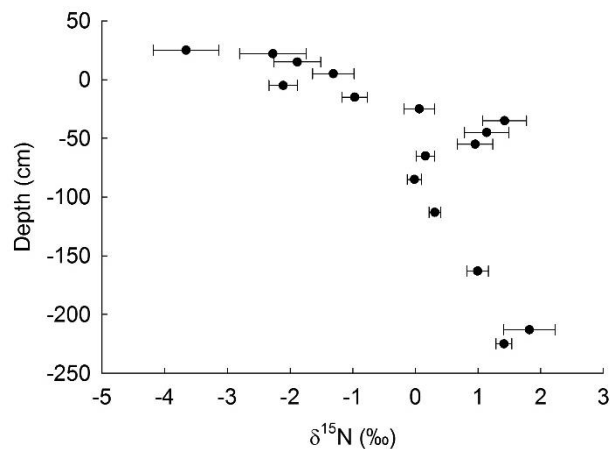


1 Figure 3. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, %N, %C, and C/N versus depth. Values (\pm se) are averaged across
 2 hummock versus hollow cores and across treed versus non-treed cores. Averages (\pm se) are given
 3 for each depth, with n given in parentheses following the depth: 25 cm (3), 22 cm (7), 15 cm
 4 (14), 5 cm (14), -5 cm (19), -15 cm (17), -25 cm (17), -35 cm (15), -45 cm (17), -55 cm (17), -65
 5 cm (17), -85 cm (18), -112 cm (17), -162 cm (17), -213 cm (3), -225 cm (8).

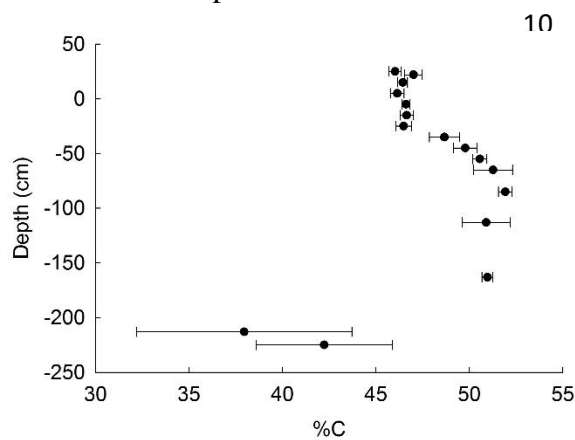
6
 7 a. $\delta^{13}\text{C}$ versus depth



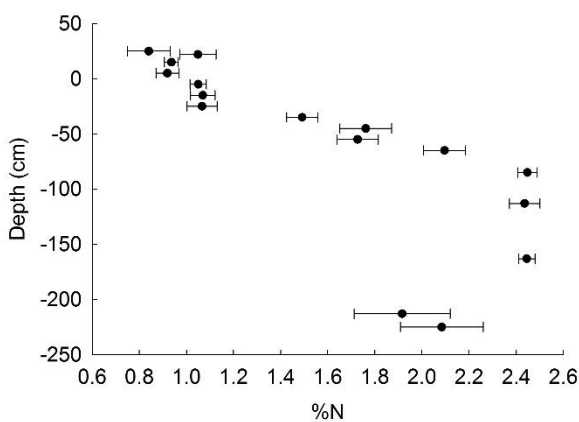
b. $\delta^{15}\text{N}$ versus depth



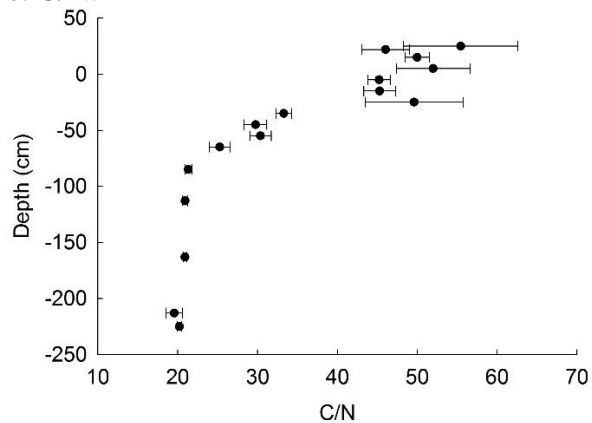
8
 9 c. %C versus depth



d. %N versus depth



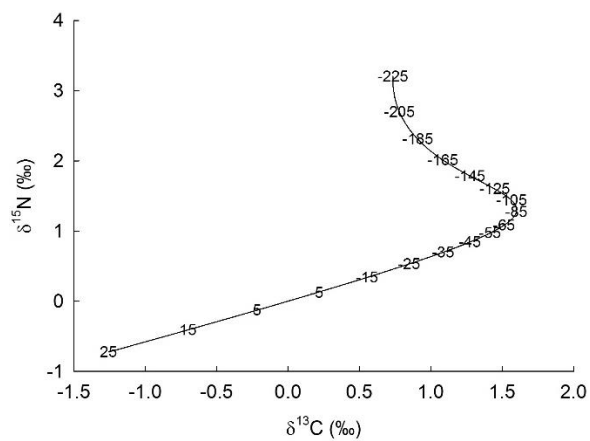
11
 12 e. C/N.



13

- 1 Figure 4. Depth coefficients of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ calculated from the three depth terms in the
- 2 regression models. The depth in cm for specific points is indicated on the line.

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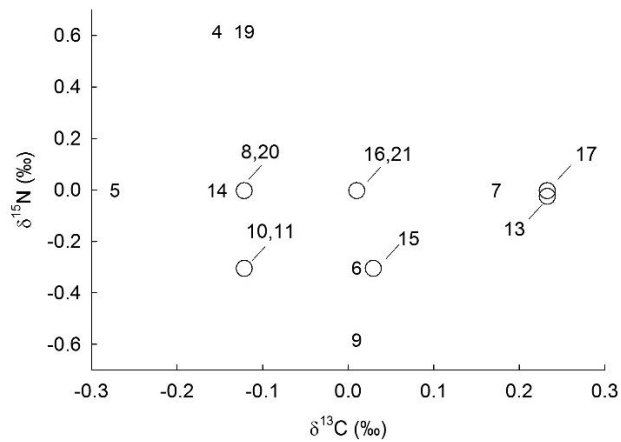


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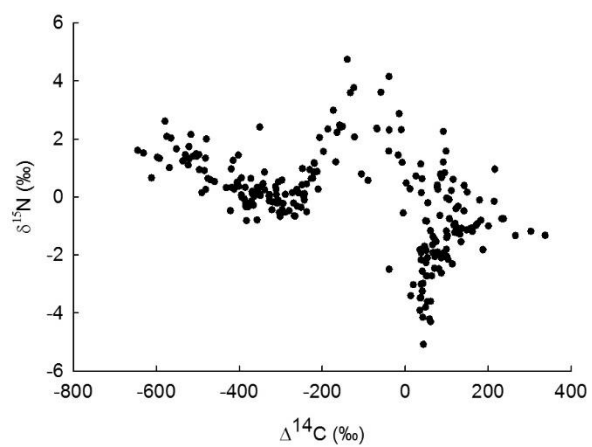
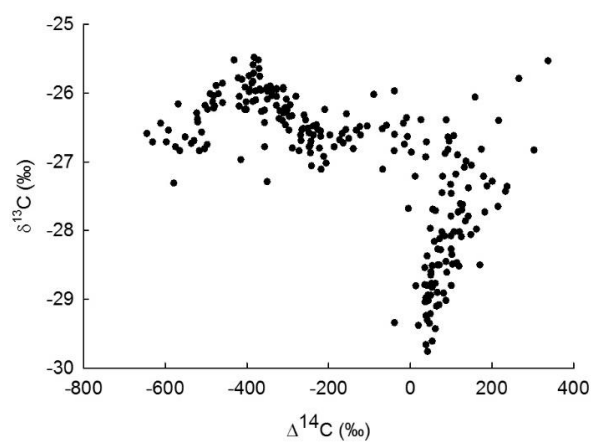
- 1 Figure 5. Plot coefficients of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from regression models in peat profiles, as calculated
- 2 from Table 1. The plot number is the symbol for the paired coefficient values. For clarity, plots
- 3 with identical or near-identical values indicated with circles.



- 4
- 5
- 6

1 Figure 6. Relationship between $\Delta^{14}\text{C}$ and a) $\delta^{13}\text{C}$; b) $\delta^{15}\text{N}$.

2 a. b.



3

4

5

- 1 **13 Appendices**
- 2