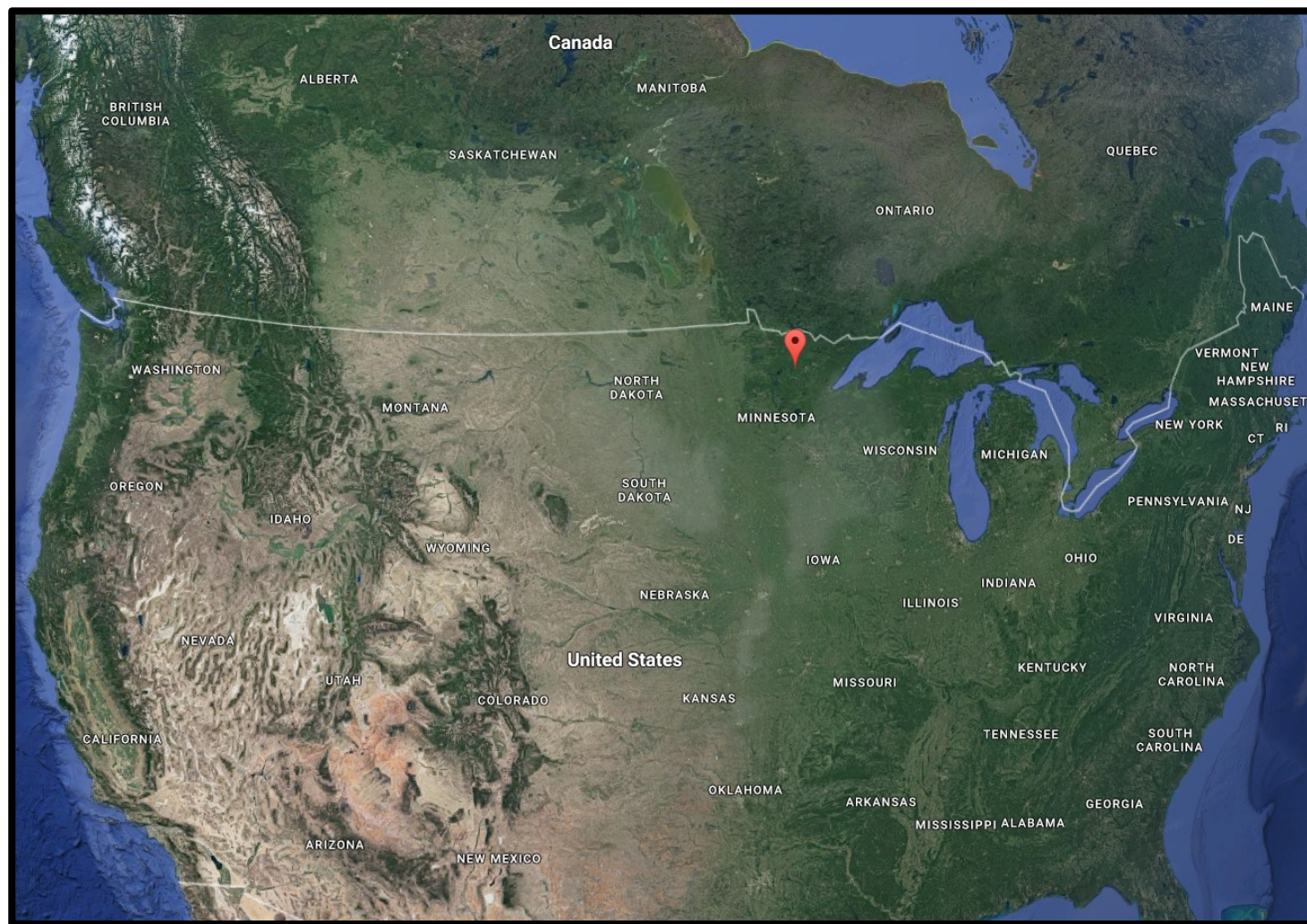


Using radiocarbon and stable isotopes to infer vegetation responses to experimental warming and elevated CO₂ at a southern boreal peatland



Erik A. Hobbie, Avni Malhotra, Jen Peters, Karis J. McFarlane, Jeffrey Warren, Rachel M. Wilson, Jeffrey P. Chanton, Dave Weston, Soren Weber, Verity Salmon, Richard J. Norby, Paul Hanson

Spruce and Peatland Responses Under Changing Environments



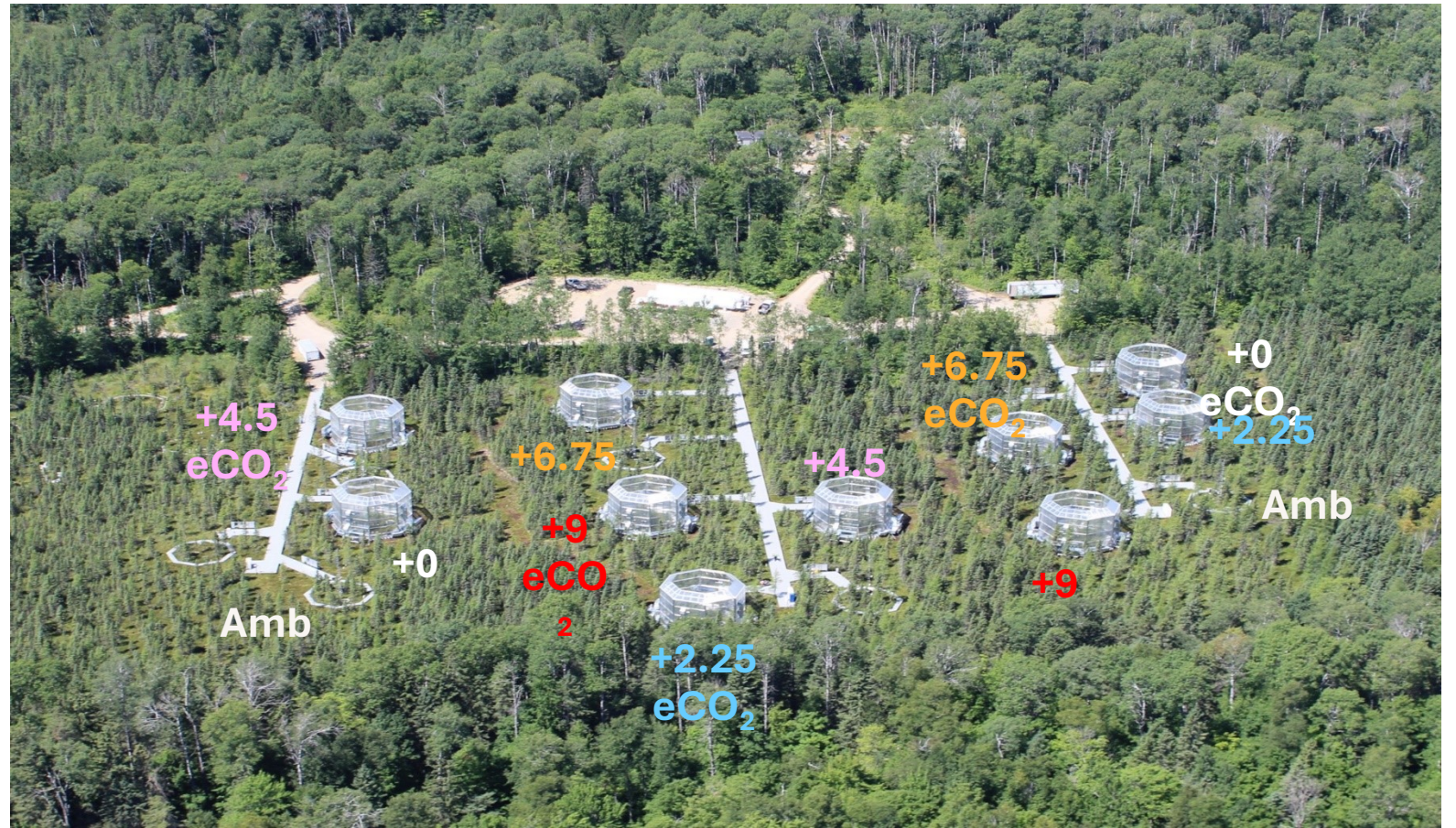
SPRUCE Project Setting – S1-Bog at the Marcell Experimental Forest, Minnesota, USA

Experimental set up

Treatments:

- + 0°C
- + 2.25°C
- + 4.5°C
- + 6.75°C
- + 9°C

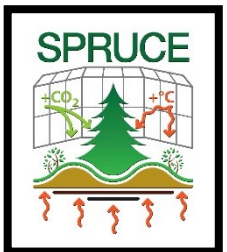
eCO₂ ~ 900 ppm CO₂



- 5 temperature treatments duplicated at elevated CO₂ concentrations
- Ten 12-m diameter enclosures
- Ecosystem scale
- Aboveground and **belowground** warming (*what depth?*)
- Continuous full year experimental operation for 10 years
- Added CO₂ differs in $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ from atmospheric and soil-respired CO₂

Sampling and Analysis

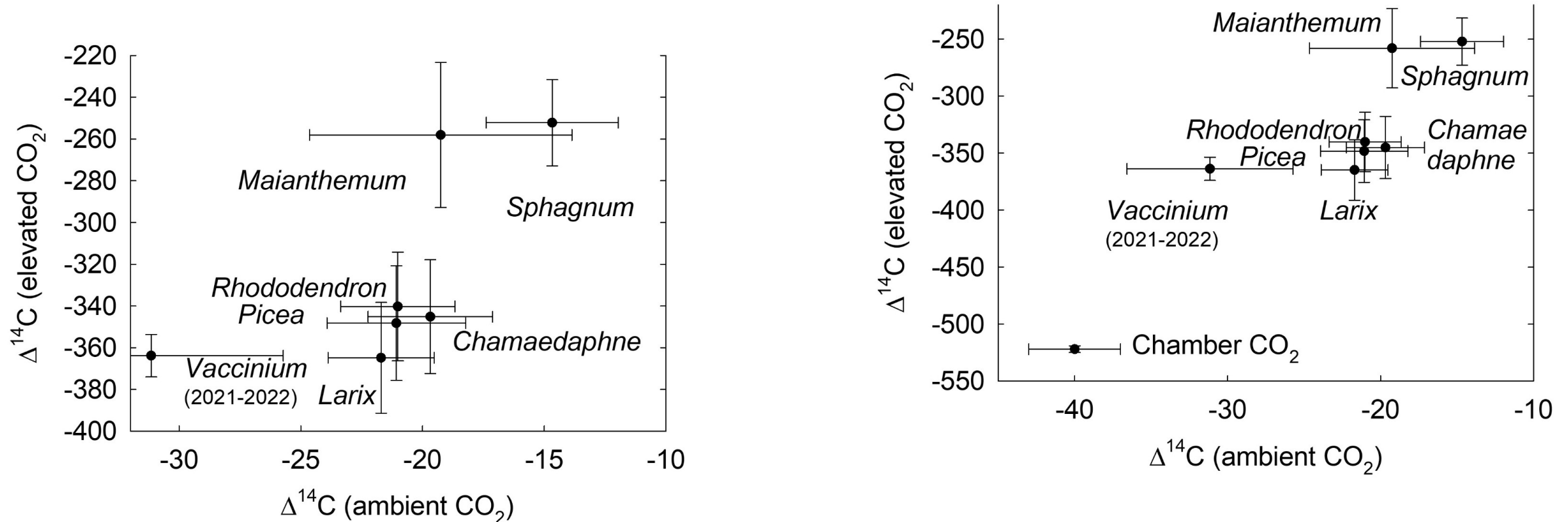
- Foliage was sampled 2016-2022 for the dominant plant species, including *Sphagnum* spp. and *Maianthemum trifolium* (ground veg), *Picea* and *Larix* (trees), and *Chamaedaphnae*, *Vaccinium*, and *Rhododendron* (shrubs).
- Samples were analyzed for $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$. In 2021 and 2022, samples were also analyzed for $\delta^{15}\text{N}$. Here, only current-year foliage is discussed.
- Data were analyzed using stepwise regressions, with model selection based on the minimal value of the Bayesian Information Criterion (BIC).
- Factors included species (taxon), CO_2 level, growing season temperature at 2 m, year, and 2nd-order interactions among these variables. $\Delta^{14}\text{C}$ was included as a factor in regressions on $\delta^{13}\text{C}$.



Observations & Hypotheses

- Because of added fossil fuel-derived C, chamber atmosphere CO₂ differs greatly in $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ between ambient & elevated CO₂ treatments.
- Peat-respired C should have similar $\delta^{13}\text{C}$, $\Delta^{14}\text{C}$ in ambient & elevated CO₂ treatments.
- In numerous studies, *Sphagnum* incorporated some peat-respired CO₂.
- Other ground vegetation (here, *Maianthemum*) should also incorporate peat-respired CO₂.
- In open-top chambers, peat C incorporation may increase: ground veg > shrubs > trees.
- Warming increases peat C turnover at SPRUCE (CO₂ release).
- Warming should therefore increase peat C incorporation by vegetation.
- Increased peat C turnover with warming should also increase N release/availability.
- Mobilized deep peat N is high in $\delta^{15}\text{N}$, shallow peat N is not.
- *Maianthemum* is suspected to have aerenchyma and has deeper roots than other taxa.
- Taxa accessing deep N should increase more than other taxa in $\delta^{15}\text{N}$.

Radiocarbon in chamber air and taxon foliage under ambient and elevated CO₂



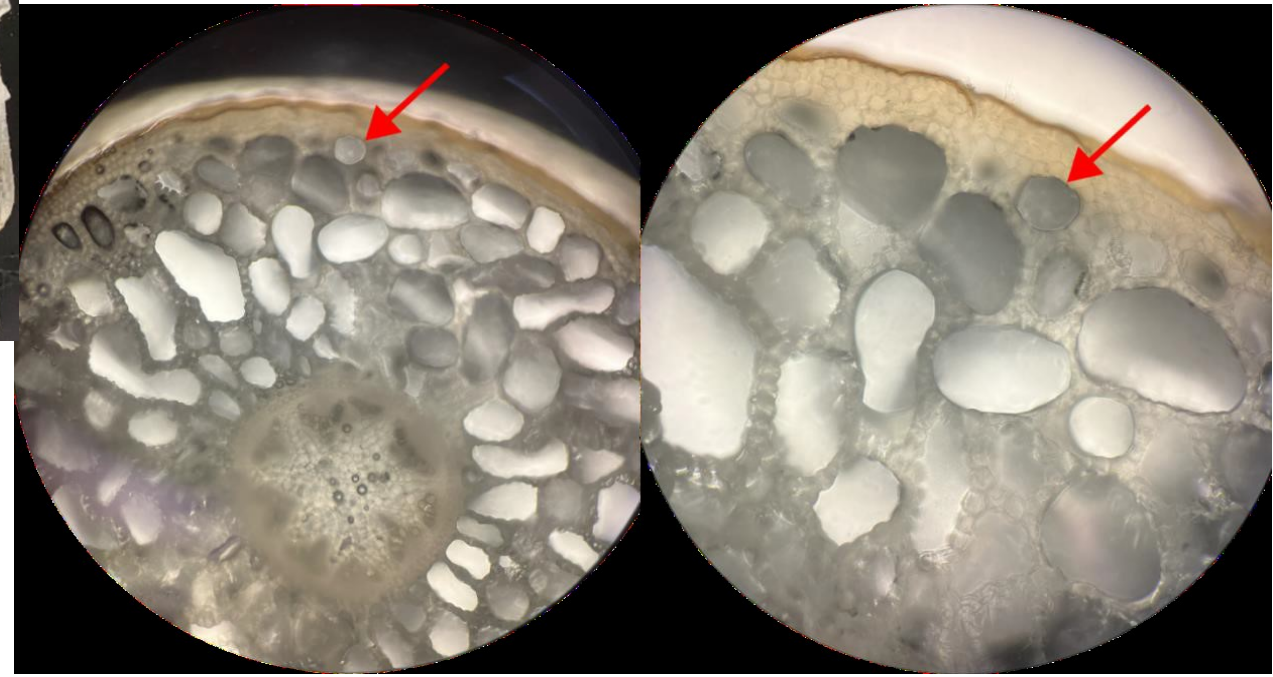
The $\Delta^{14}\text{C}$ of foliage of seven taxa under ambient and elevated CO₂, averaged across all years and temperature treatments, $\pm$ se. Values in ‰. Higher values indicate increasing contribution of sources other than chamber CO₂ (indicated).

Maianthemum trifolium has aerenchyma



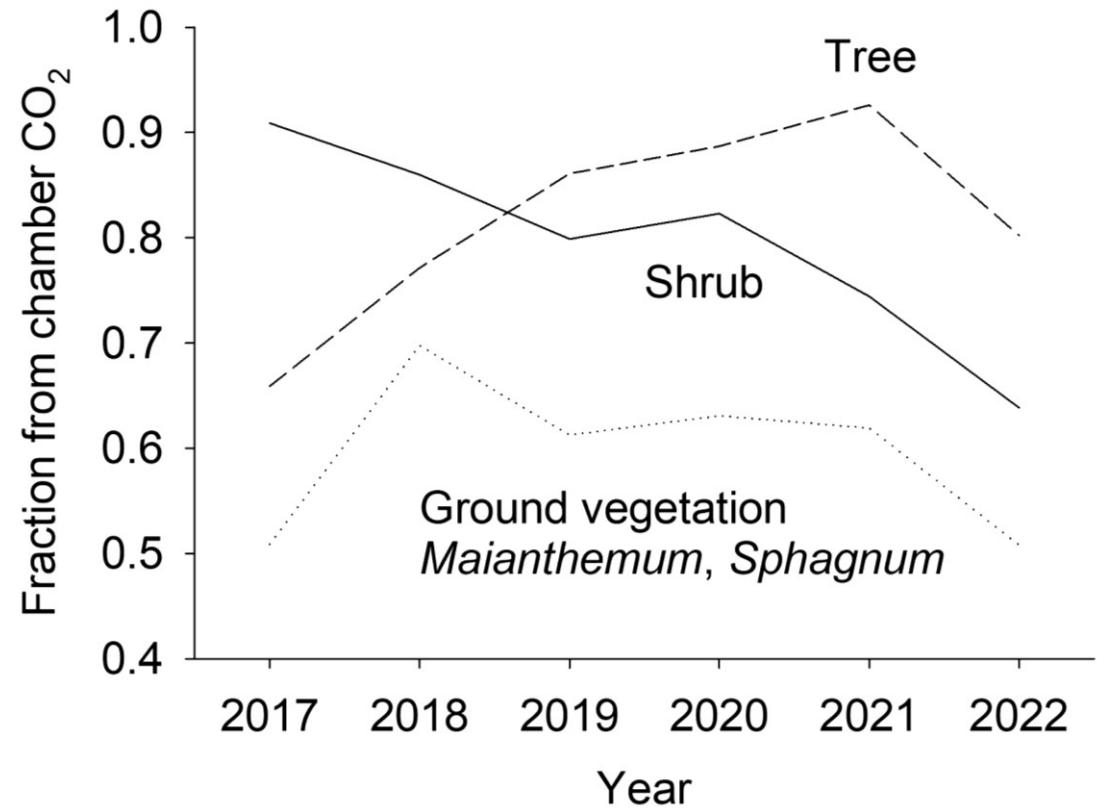
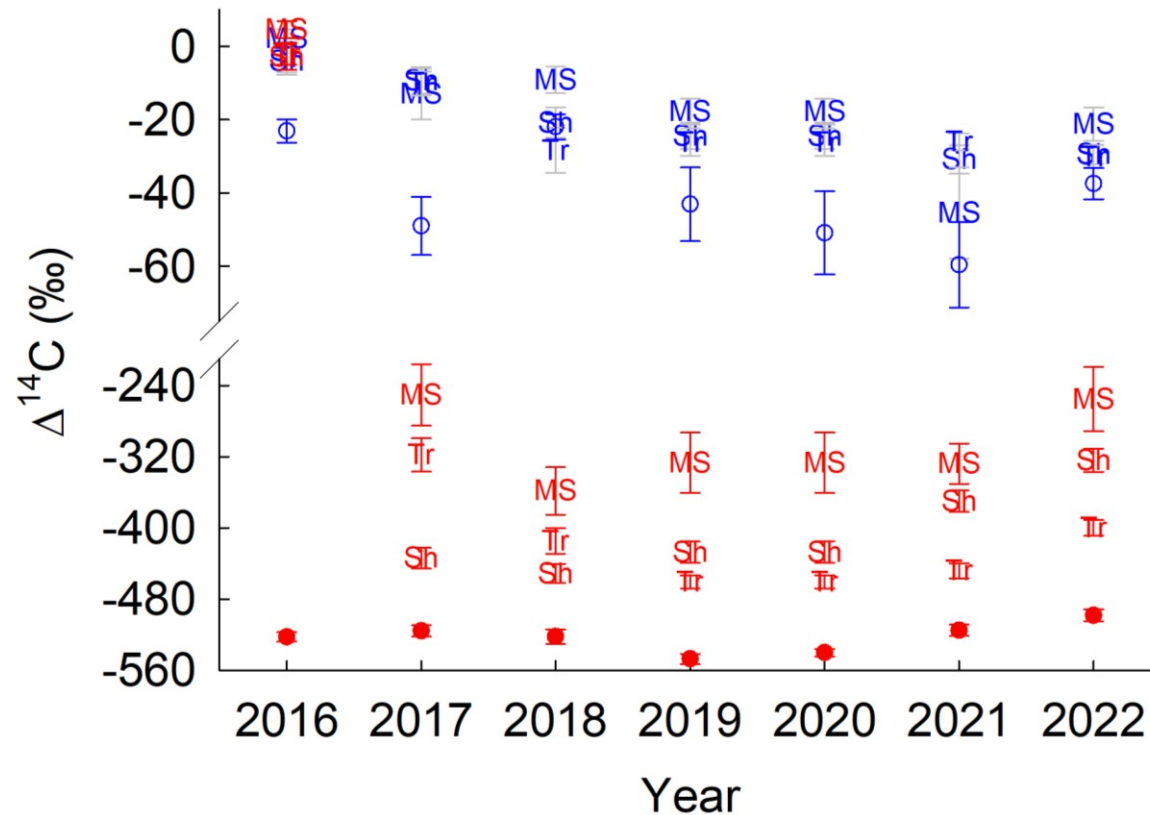
A.

B.



Maianthemum trifolium root cross-section A. at 100x magnification and B. 200x. Arrow points to the same cavity in each panel to orient the reader. Photos by Soren Weber.

Estimating fraction of foliar C from chamber CO₂



Fractional incorporation of foliar C from chamber CO_2 ($\text{FolC}_{\text{atmfr}}$)

$$\text{FolC}_{\text{atmfr}} = (\Delta^{14}\text{C}_{\text{FolAmb}} - \Delta^{14}\text{C}_{\text{FolElev}}) / (\Delta^{14}\text{C}_{\text{CO2Amb}} - \Delta^{14}\text{C}_{\text{CO2Elev}})$$

$\text{FolC}_{\text{atmfr}} = 82 \pm 4\%$ (trees), $80 \pm 4\%$ (shrubs), and $60 \pm 3\%$ (ground vegetation)

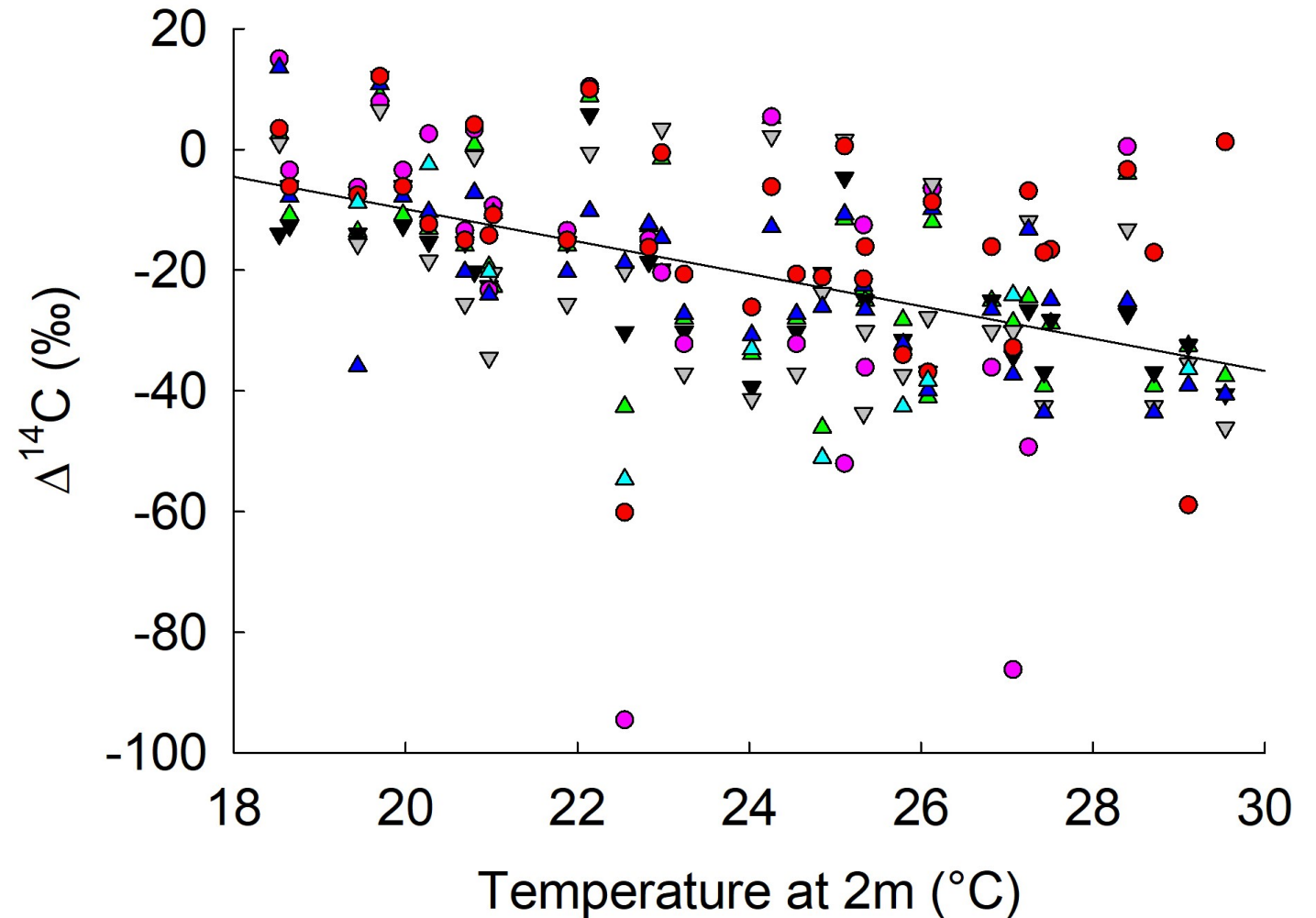
a. ^{14}C signatures of trees (Tr), shrubs (Sh), ground vegetation (MS), and atmospheric CO_2 (circles) by year under elevated and ambient CO_2 . Ambient, blue symbols; elevated, red symbols. b. Yearly fraction of plant C for three types derived from chamber CO_2 .

Foliar $\Delta^{14}\text{C}$ in ambient plots correlated with temperature

Colors indicate taxa. *Chamaedaphne*, green;
Larix, black; *Maianthemum*, magenta;
Picea, grey; *Rhododendron*, blue;
Sphagnum, red; *Vaccinium*, light blue.

Slope: $-2.68 \pm 0.33\text{‰ C}^{-1}$

(adjusted $r^2 = 0.239$, $P < 0.0001$, $n = 207$)



Estimating the temperature sensitivity of peat C

Regression on foliar $\Delta^{14}\text{C}$. Nonwoody: *Sphagnum*, *Maianthemum*

Temperature influenced foliar radiocarbon in 4 terms. .

temperature coefficient (Tc, Δ shift per degree Celsius shift during the active season):

	Ambient CO ₂	Elevated CO ₂ ,
woody species	$-2.89 \pm 1.03\text{‰ dC}^{-1}$	$-0.30 \pm 1.03\text{‰}^{\circ} \text{ dC}^{-1}$
Nonwoody	$-3.01 \pm 1.84\text{‰ dC}^{-1}$	$10.69 \pm 1.84\text{‰}^{\circ} \text{ dC}^{-1}$

These temperature coefficients were applied to Equation (2):

$$\text{FolC}_{\text{atmfr}} \text{ dC}^{-1} = (\text{Tc}_{\text{Amb}} - \text{Tc}_{\text{Elev}})/(\Delta^{14}\text{C}_{\text{CO2Amb}} - \Delta^{14}\text{C}_{\text{CO2Elev}})$$

Sphagnum and *Maianthemum* increased incorporation of non-atmospheric C by 2.44% per $^{\circ} \text{ dC}$ of warming.

We then divided the slope $-2.69\text{‰}^{\circ} \text{ dC}^{-1}$ from Figure 4 by the fractional incorporation of $0.0244^{\circ} \text{ dC}^{-1}$ to estimate that the $\Delta^{14}\text{C}$ of the secondary C source was $-2.69\text{‰}^{\circ} \text{ C}^{-1}/0.0244^{\circ} \text{ C}^{-1}$, or -110‰.

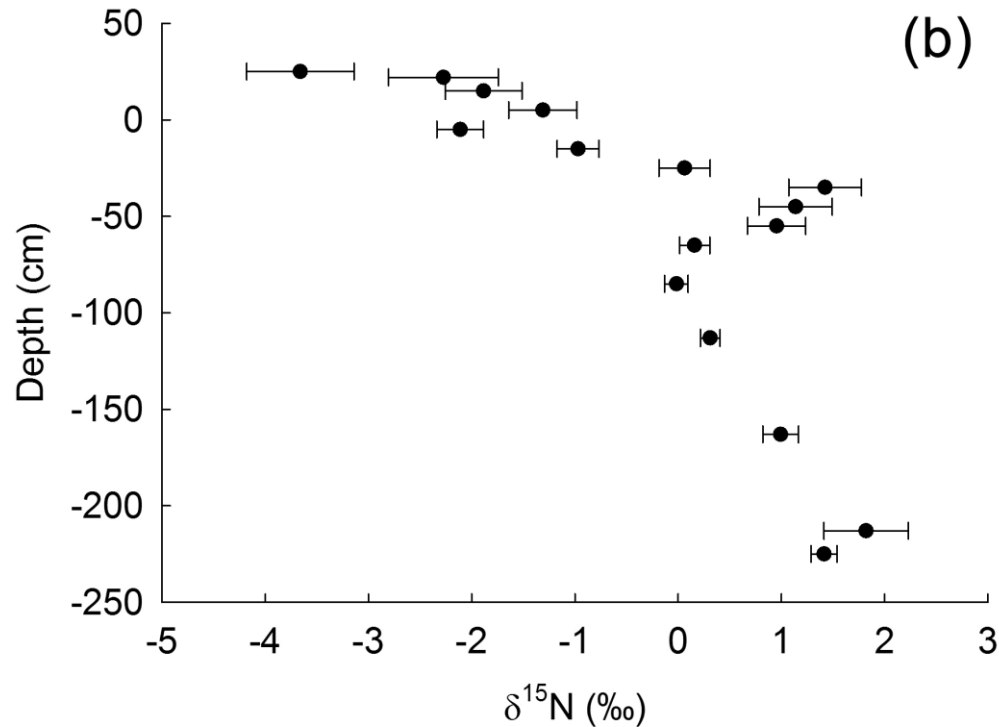
Temperature sensitivity of plant N isotopes

Stepwise regression on $\delta^{15}\text{N}$ signatures on plant foliage (2021-2022). Adjusted $r^2 = 0.741$, $n = 131$. All values in ‰. VIF = variance inflation factor. Temp = Temperature at 2 m height for the growing season. Rh = *Rhododendron*; Ch = *Chamaedaphne*.

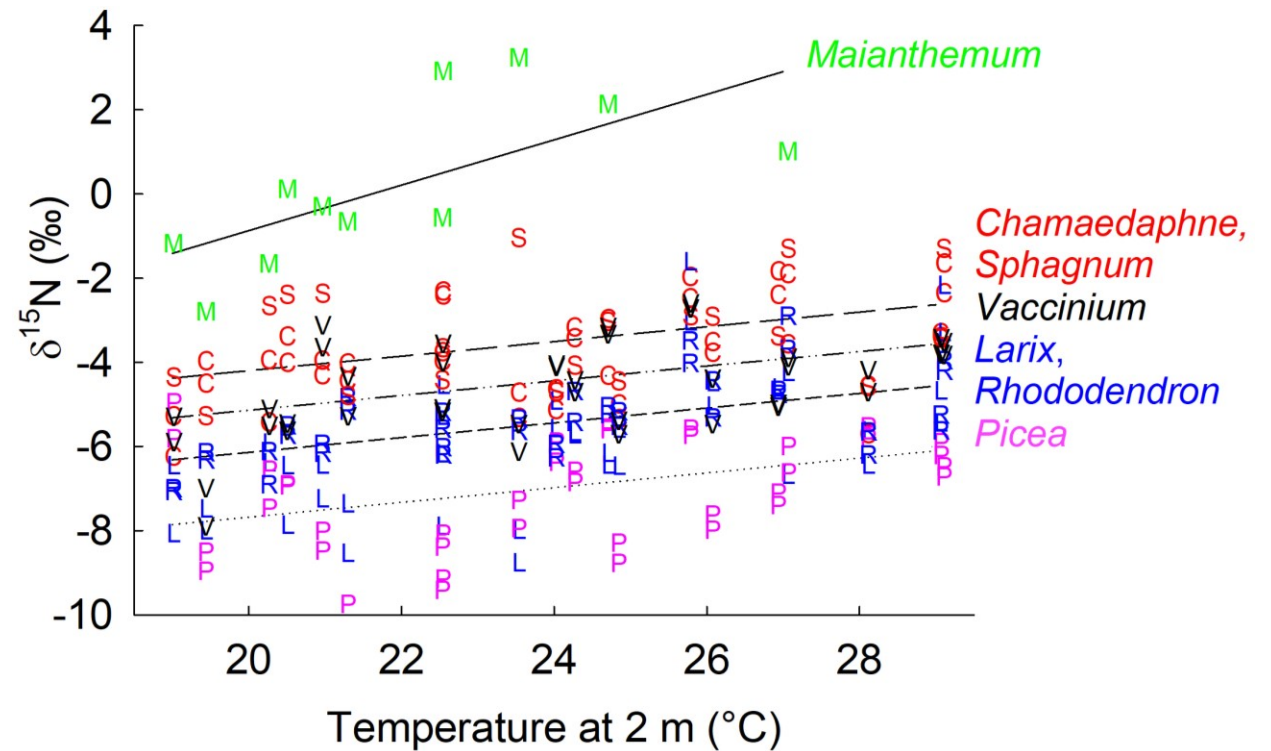
#	Term	%Var	Estimate $\pm$ se	Prob> t	VIF
0	Intercept	--	-6.87 $\pm$ 0.78	< 0.0001	--
1	Temperature (2 m height)	7.49	0.21 $\pm$ 0.03	< 0.0001	1.06
2	Species (<i>Maianthemum</i> – Other)	38.41	3.16 $\pm$ 0.23	< 0.0001	1.60
3	(<i>Picea</i> , <i>Larix</i> , Rh – <i>Vacc</i> , Ch, <i>Sph</i>)	20.37	-1.12 $\pm$ 0.11	< 0.0001	1.13
4	(<i>Picea</i> – <i>Larix</i> , <i>Rhododendron</i>)	4.87	-0.77 $\pm$ 0.16	< 0.0001	1.07
5	(<i>Vacc</i> – <i>Chamaedaphne</i> , <i>Sphag</i>)	1.81	-0.47 $\pm$ 0.16	0.0032	1.07
6	(Temp - 23.8) $\times$ (<i>Maia</i> – Other + 0.83)	1.12	0.18 $\pm$ 0.08	0.0195	1.54

Soil N turnover increases with warming

Foliar $\delta^{15}\text{N}$ of *Maianthemum* increases more than other taxa with rising temperatures, reflecting increased access to deeper N with aerenchyma. Symbols reflect the first letter of the genus and are colored differently if statistically distinct.

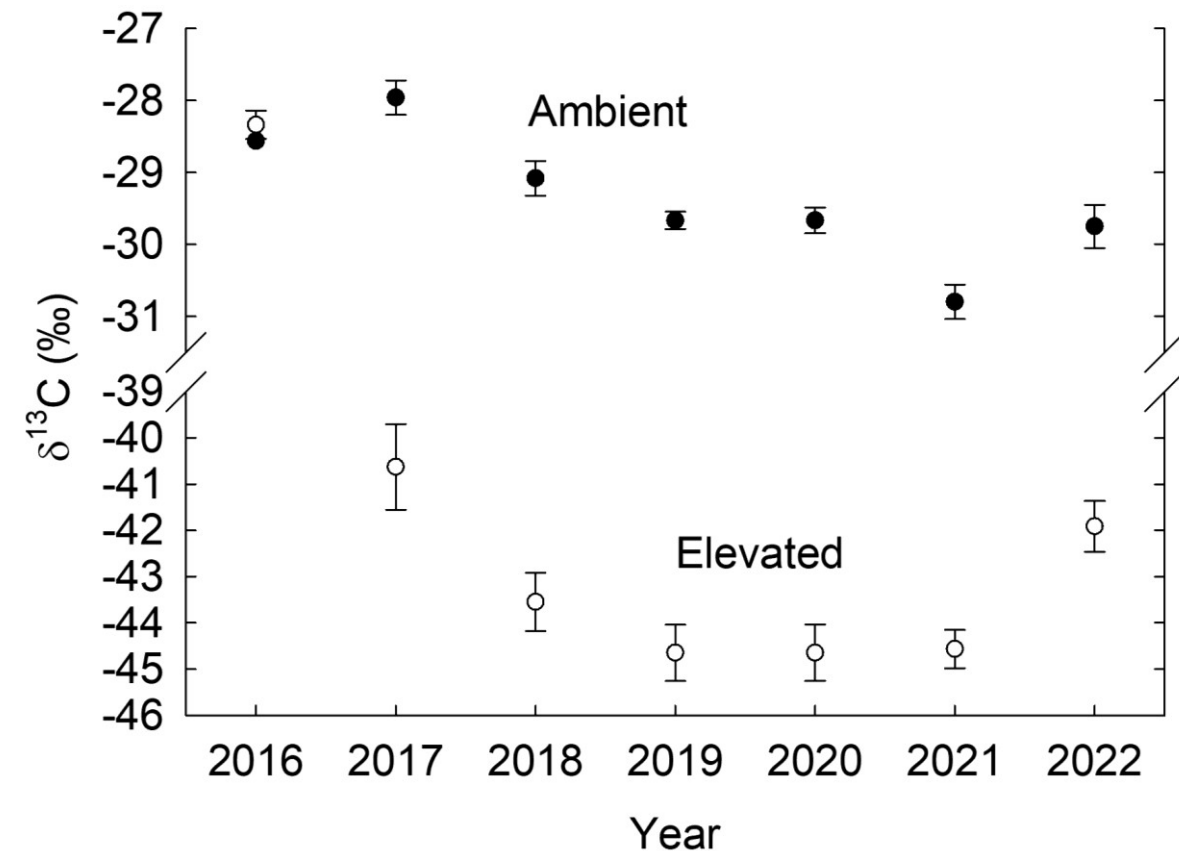
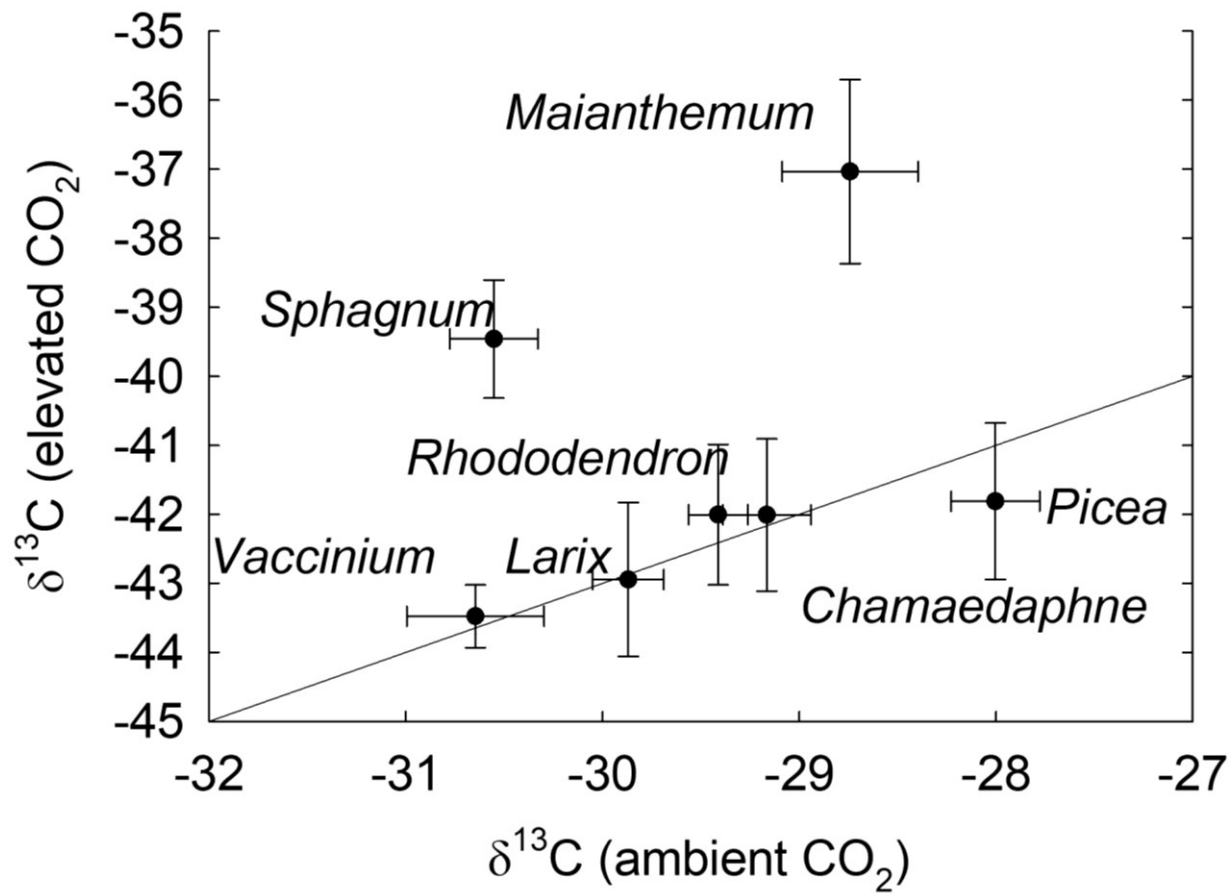


a. Peat $\delta^{15}\text{N}$ with depth (Hobbie et al. 2017)

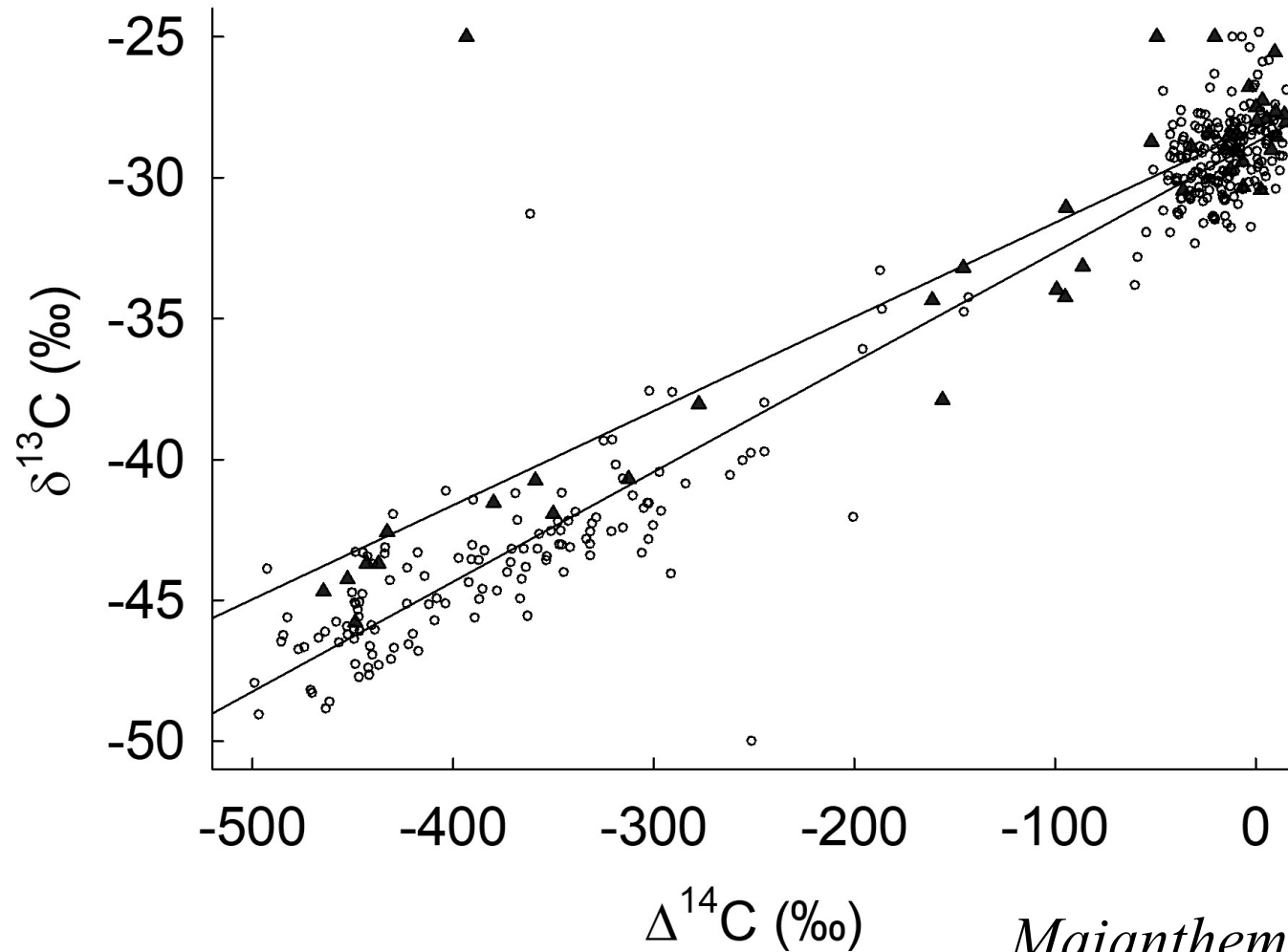


b. Foliar $\delta^{15}\text{N}$ by taxon with warming.

Stable C isotopes indicate C sources differ for ground vegetation



Foliar $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ correlation differ in *Maianthemum*



Coefficient of foliar $\Delta^{14}\text{C}$ with foliar $\delta^{13}\text{C}$:

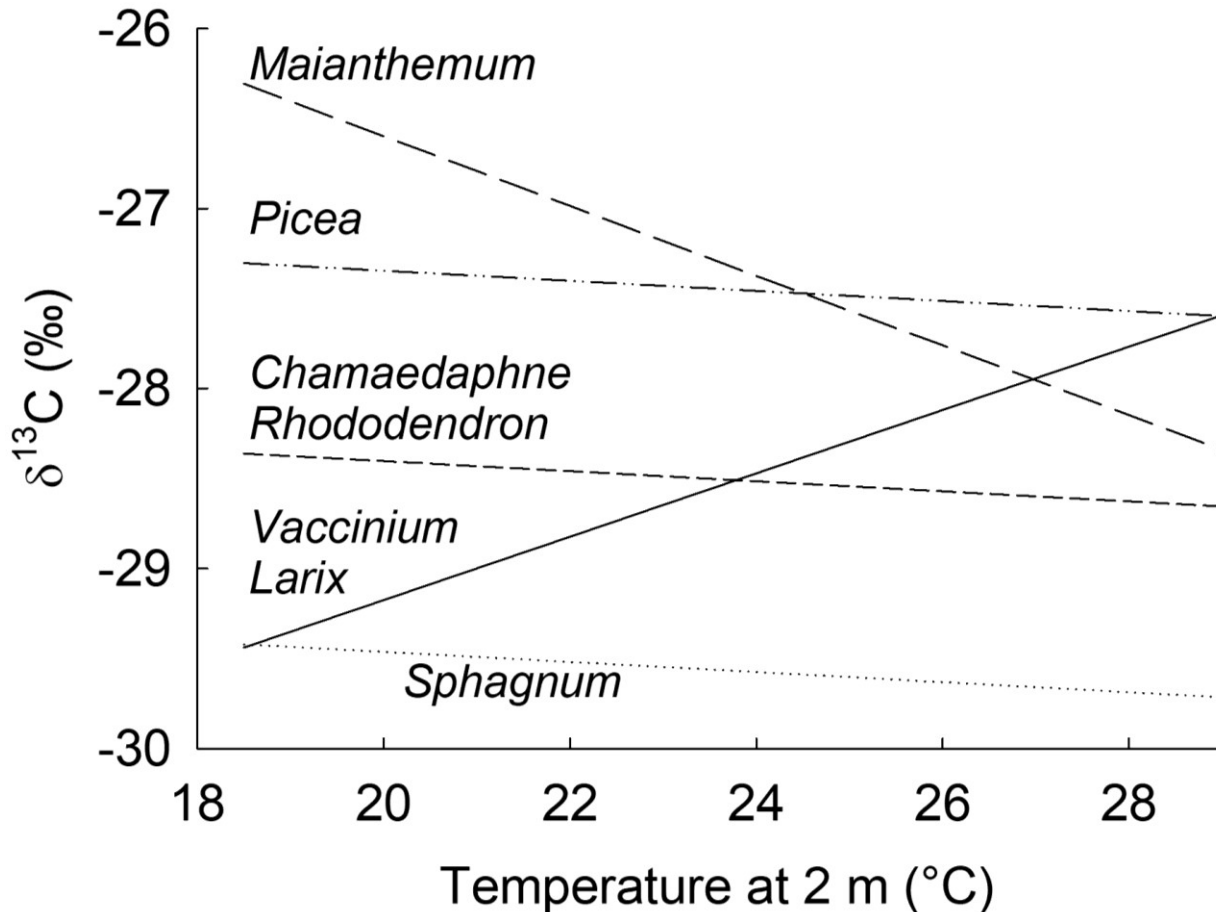
Maianthemum 0.0335 ± 0.0013

Other taxa 0.0390 ± 0.0005

Maianthemum assimilated some CO_2 differing in $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ from other plants.

Maianthemum, black triangles; other taxa, clear circles.

Temperature influences foliar $\delta^{13}\text{C}$



Regression model predictions of the effect of taxon and temperature on $\delta^{13}\text{C}$.
Note: model accounted for radiocarbon effect on $\delta^{13}\text{C}$.

Summary

- Secondary sources of CO₂ were 18-20% (trees, shrubs) & 40% (ground veg) of photosynthesis
- No clear evidence that methanotrophy influenced *Sphagnum*. (*Maianth.* = *Sphag.*)
- Peat C turnover increased with rising temperature.
- *Maianthemum* shown to have aerenchyma.
- *Maianthemum* captured deep N released with peat warming & turnover.
- Age of secondary C source ~1000 years, 30-40 cm deep on average.
- *Maianthemum* or other aerenchymatous plants could be used as sensors of peat turnover
- Future work: Finish nitrogen isotopes (just 2 years now).
- Test natural temperature gradients for shifts in $\delta^{13}\text{C}$, $\Delta^{14}\text{C}$, and $\delta^{15}\text{N}$ of different peatland plants.

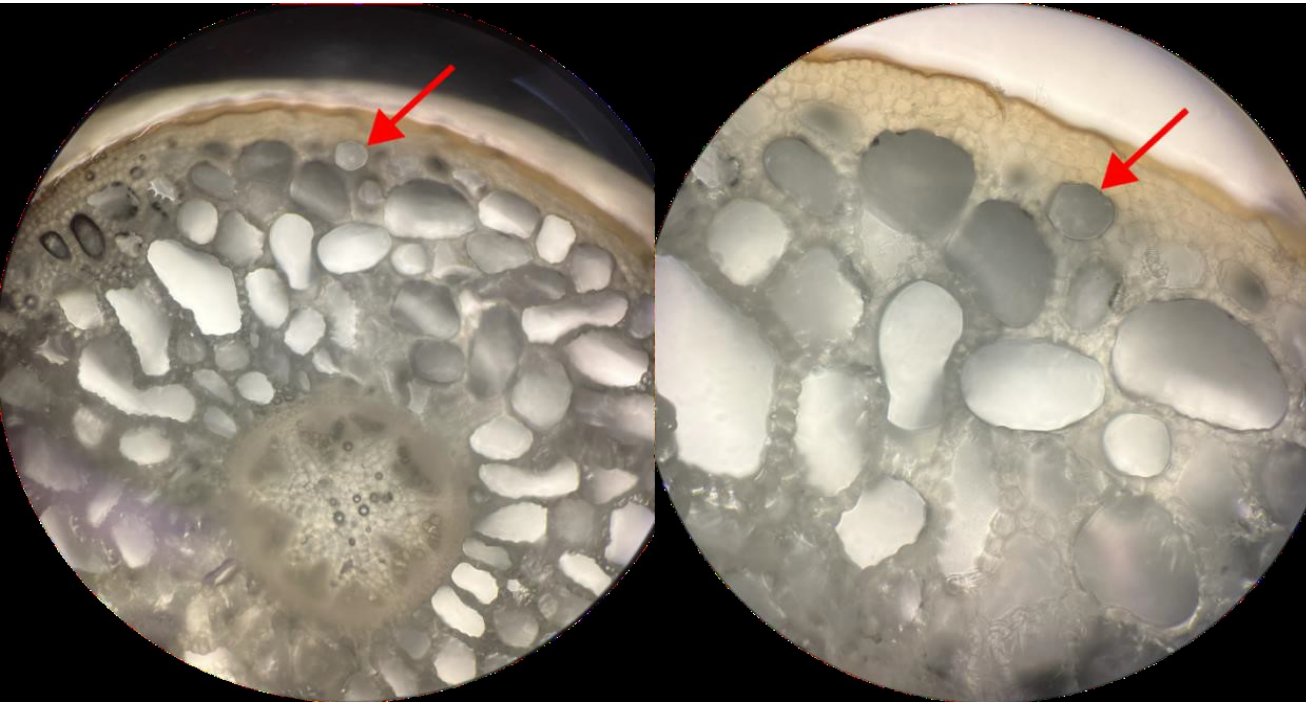
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I thank Avni Malhotra and Soren Weber for several of the slides.



Supporting Information Table S1. Stepwise regression on $\Delta^{14}\text{C}$ signatures in plant tissues. Amb = ambient CO_2 treatments. AcTemp = Active season temperature (DoY 122-244) at 2 m height. Woody = Trees, shrubs. Nonwoody = *Maianthemum*, *Sphagnum*. Year = 20xx. Adjusted $r^2 = 0.956$, $n = 415$. All values in ‰. VIF = variance inflation factor. Var = variance explained by a variable. Y = year. Tr = tree, Shr = shrub.

# Term	%Var	Value $\pm$ se	P-value	VIF
0 Intercept	--	-87.4 ± 2.9	< 0.0001	--
1 CO_2 [Amb]	66.56	151.5 ± 1.9	< 0.0001	1.0
2 Species (Woody – Nonwoody)	1.35	-23.6 ± 2.1	< 0.0001	1.0
3 Year (16 – rest)	13.04	97.1 ± 2.8	< 0.0001	1.0
4 Year (17, 22 – 18, 19, 20, 21)	0.74	18.1 ± 2.2	< 0.0001	1.0
5 Amb · Year (16 – rest + 0.72)	10.15	-85.5 ± 2.8	< 0.0001	1.0
6 Amb · Year (17, 22 – 18, 19, 20, 21 + 0.26)	0.62	-16.8 ± 2.2	< 0.0001	1.0
7 Amb · (Woody – Nonwoody - 0.41)	1.17	22.1 ± 2.1	< 0.0001	1.0
8 (Woody – Nonwoody - 0.41) · Y (16 – rest + 0.72)	0.19	-12.3 ± 3.0	< 0.0001	1.0
9 (Tr – Shr + 0.06) · Y (17, 22 – 18, 19, 20, 21 + 0.26)	0.08	7.04 ± 2.59	0.0069	1.0
10 (Tree – Shrub + 0.06) · Y (17 – 22 + 0.01)	0.35	-23.8 ± 4.2	< 0.0001	1.0
11 (AcTemp – 23.8) · Year (16 – rest + 0.72)	0.04	1.80 ± 0.90	0.0452	1.0
12 (AcTemp – 23.8) · Amb	0.26	-3.03 ± 0.62	< 0.0001	1.0
13 (AcTemp – 23.8) · (Woody – Nonwoody - 0.41)	0.20	-2.90 ± 0.68	< 0.0001	1.0
14 (AcTemp – 23.8) · Amb · (Woody – Nonwoody - 0.41)	0.20	2.91 ± 0.68	0.0002	1.0
15 Amb · (Woody – Nonwoody - 0.41) · Y (16 – rest + 0.72)	0.21	-13.1 ± 3.0	< 0.0001	1.0
16 Amb · (Tr – Shr + 0.06) · Y (17/22 – 18/19/20/21 + 0.26)	0.07	-6.45 ± 2.59	0.0132	1.0
17 Amb · (Tree – Shrub + 0.06) · Y (17 – 22 + 0.01)	0.35	-24.1 ± 4.2	< 0.0001	1.0

Supporting Information Table S3. Stepwise regression on $\delta^{13}\text{C}$ signatures on plant foliage. Adjusted $r^2 = 0.954$, $n = 415$. All values in ‰. VIF = variance inflation factor. Temperature at 2 m height for the growing season.

# Term	%Var	Estimate $\pm$ se	P-value	VIF
0 Intercept	--	-27.92 $\pm$ 0.15	< 0.0001	--
1 $\Delta^{14}\text{C}$	93.34	0.0383 $\pm$ 0.0004	< 0.0001	1.1
8 ($\Delta^{14}\text{C} + 171.7$) $\cdot$ (Other – <i>Maianthemum</i> - 0.75)	0.18	0.0027 $\pm$ 0.0007	0.0001	1.1
2 Species (<i>Maianthemum</i> – Other)	0.26	0.591 $\pm$ 0.126	< 0.0001	1.1
3 Species (<i>Picea</i> – <i>Sphagnum</i>)	0.70	1.06 $\pm$ 0.14	< 0.0001	1.0
4 Year (2017 – 2018, 2019, 2020, 2021, 2022)	0.27	0.53 $\pm$ 0.11	< 0.0001	1.1
5 Year (2018, 2022 – 2019, 2020, 2021)	0.21	0.41 $\pm$ 0.10	< 0.0001	1.1
6 Year (2019, 2020 – 2021)	0.10	0.36 $\pm$ 0.12	0.0039	1.1
7 Year (2018 – 2022)	0.10	0.44 $\pm$ 0.15	0.0038	1.0
9 (Other – <i>Maianthemum</i> - 0.75) $\cdot$ (Temp - 23.78)	0.08	0.111 $\pm$ 0.042	0.0091	1.1
10 (<i>Larix</i> , <i>Vaccinium</i> – <i>Sphagnum</i> , <i>Chamaedaphne</i> , <i>Picea</i> , <i>Rhododendron</i> + 0.45) $\cdot$ (Temp - 23.78)	0.13	0.102 $\pm$ 0.031	0.0009	1.0