Tracing recent photosynthate at SPRUCE into mushrooms and berries: preliminary isotopic results

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Introduction

- Mycorrhizal fungi supply nutrients to their host plants in return for plant-derived sugars, whereas saprotrophic fungi dominate decomposition in surficial horizons.
- By increasing plant carbon fixation and the availability of soil nutrients, elevated CO_2 and temperature could alter interactions among plants and fungi.
- At the SPRUCE site (Fig. 1), ^{13}C-depleted CO_2 was added to elevate CO_2 concentrations within chambers that spanned a 0-9 °C warming gradient, thereby providing a tracer of short-term C dynamics.
- Plant and fungal $\delta^{15}N$ patterns are informative of preferred N forms, plant-mycorrhizal N and C transfers, and depth of N acquisition.
- Here, we investigated controls over $\delta^{15}N$, $\delta^{13}C$, and C/N patterns in berries and mushrooms (caps vs stipes) from SPRUCE harvested ~2 months after the initiation of elevated CO_2.
- Hypothesized controls included both treatment effects (e.g., elevated CO_2 or temperature) and isotopic partitioning among different functional groups of organisms or among different compound classes (e.g., ^{15}N- and ^{13}C-enriched protein, ^{15}N-depleted chitin, ^{13}C-depleted lipids).

Methods

- Plots were visited August 30-31, 2016 to harvest mushrooms and berries. Both chambered and unchambered plots were sampled.
- Mushrooms were identified to genus and species in the field, photos of mushrooms taken, and classified as either ectomycorrhizal or saprotrophic.
- Mushrooms were separated into caps and stipes (stems). Samples were then dried and ground prior to analysis for $\delta^{15}N$, $\delta^{13}C$, %N, and %C at the University of New Hampshire.
- Bulk $\delta^{13}C$ and $\delta^{15}N$ patterns were analyzed using multiple regression with CO_2, warming, genus, and sample type (plant, ectomycorrhizal fungi, and saprotrophic fungi) as independent variables.
- The isotopic difference between caps and stipes ($\delta^{13}C_{c\text{-}st}$ and $\delta^{15}N_{c\text{-}st}$) were correlated against differences of %N, %C, and C/N between caps and stipes.

Results and Discussion

- High C/N of berries under elevated CO_2 may reflect increased N limitation or enhanced photosynthesis, whereas low C/N at +2.25 °C may reflect increased N availability or decreased sugar accumulation and export in leaves (Fig. 2a and Table 1).
- *Vaccinium* berries and saprotrophic fungi were very low in $\delta^{15}N$ (Fig. 2b) relative to worldwide published results (Fig. 3 and Mayor et al. 2009).
- Low $\delta^{15}N$ in *Vaccinium* corresponded with shallow rooting depth (relative to *Maianthemum*) and high transfer of ^{15}N-depleted N from mycorrhizal fungi (Fig. 2b).
- Saprotrophic fungi (mostly *Tephrocybe*) were lower in $\delta^{13}C$ than ectomycorrhizal fungi (mostly *Lactarius*) under ambient CO_2, presumably because ^{13}C-depleted moss was a saprotrophic C source (Fig. 2c).
- Low $\delta^{13}C$ in elevated CO_2 plots for berries and ectomycorrhizal fungi reflected incorporation of recent, ^{13}C-depleted CO_2 by plants (Fig. 2c).
- High temperatures lowered ectomycorrhizal $\delta^{13}C$, presumably reflecting increased inputs of ^{13}C-depleted CO_2 into these treatments.
- In multiple regression analyses, $\delta^{13}C$ correlated strongly with CO_2 treatment, with the organism type, and with the interaction of these two factors (respectively, 41%, 10%, and 40% of variance). In addition, organism type and CO_2 both interacted significantly with temperature (Table 2). The model explained 97% of variance for $\delta^{13}C$.
- The $\delta^{15}N_{cap\text{-}stipe}$ was 1.1‰ lower in chambered treatments than in unchambered (Fig. 4). This may reflect increased availability of ^{15}N-depleted surficial N caused by disturbance during chamber establishment (Table 3).
- ^{13}C differences between caps and stipes were primarily driven by differences in %N, presumably reflecting protein content (Table 3 and Fig. 5). The negative effect of ectomycorrhizal status on $\delta^{13}C_{c\text{-}st}$ differed from a previous study in coniferous forests (Hobbie et al. 2012), possibly because of the small levels here of ^{13}C-enriched cellulose from woody plants to supply saprotrophic fungi.

Conclusions

- Rapid assimilation of ^{13}C-depleted CO_2 into ectomycorrhizal mushrooms and berries validated using these tissues as short-term integrators of recent photosynthesis.
- Low $\delta^{13}C$ values with elevated CO_2 and negative effects of warming on $\delta^{13}C$ reflected treatment effects of greater photosynthesis with higher CO_2 and warmer temperatures.
- $\delta^{15}N$ primarily reflected species-specific traits and the isolation of ^{15}N-enriched N in the catotelm.
- C and N balance in berries responded to temperature and CO_2 treatments and may prove a useful response integrator in the future.

References

Craine et al. 2009: Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist* 183: 980-992.
Hobbie et al. 2012: Controls of isotopic patterns in saprotrophic and ectomycorrhizal fungi. *Soil Biology & Biochemistry* 48: 60-68.
Mayor et al. 2009: Elucidating the nutritional dynamics of fungi using stable isotopes. *Ecology Letters* 12171-183.

Acknowledgements

This material is based upon work supported in part by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725. DOE grant ER65430 to Iowa State University also supported this work. We thank Shin Ugawa for assistance during fieldwork and thank Peter Kennedy and Chris Fernandez for supplying additional fungal specimens.

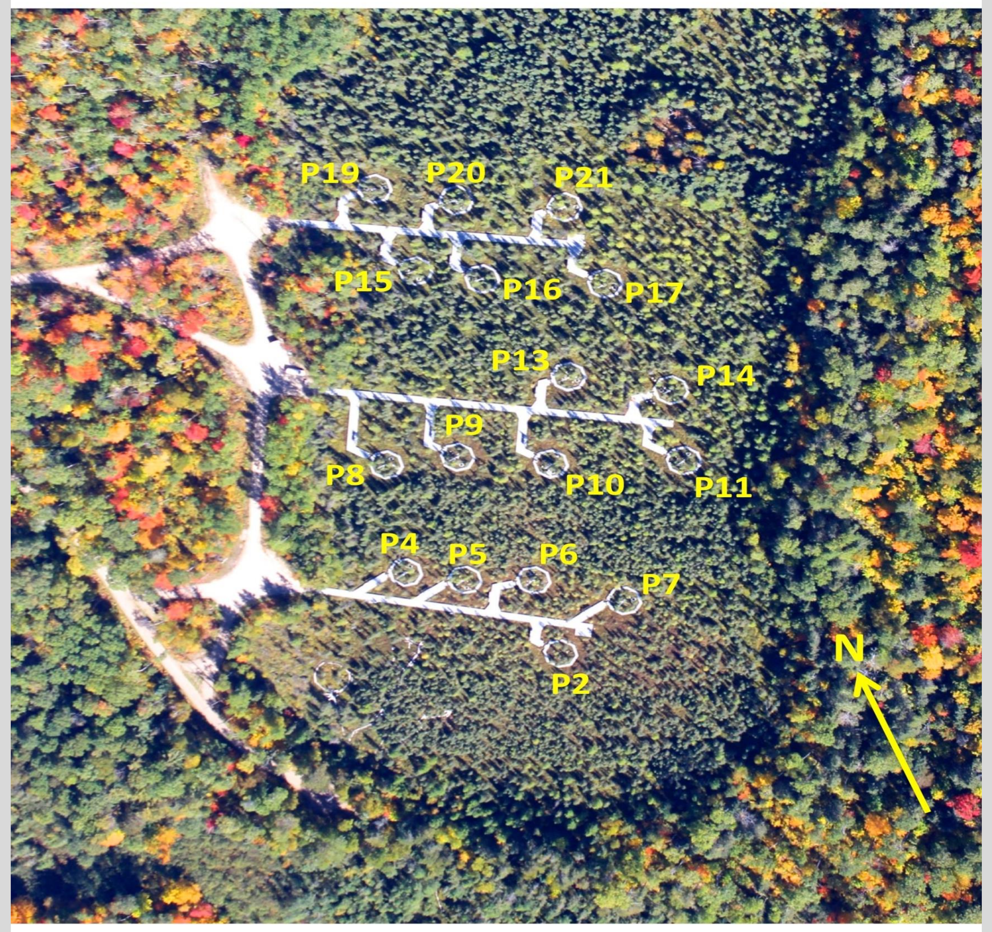

Figure 1. Aerial photograph of the S1 bog (23 September 2014) showing the 17 experimental plots (each 10.4 m in diameter to the outer edge of the visible perimeter boardwalk).

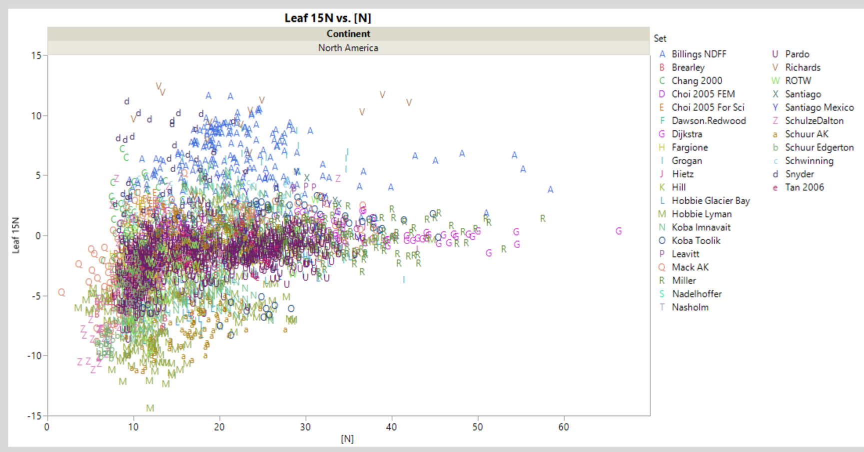

Figure 3. Foliar $\delta^{15}N$ versus %N in North America from a worldwide survey (Craine et al. 2009).

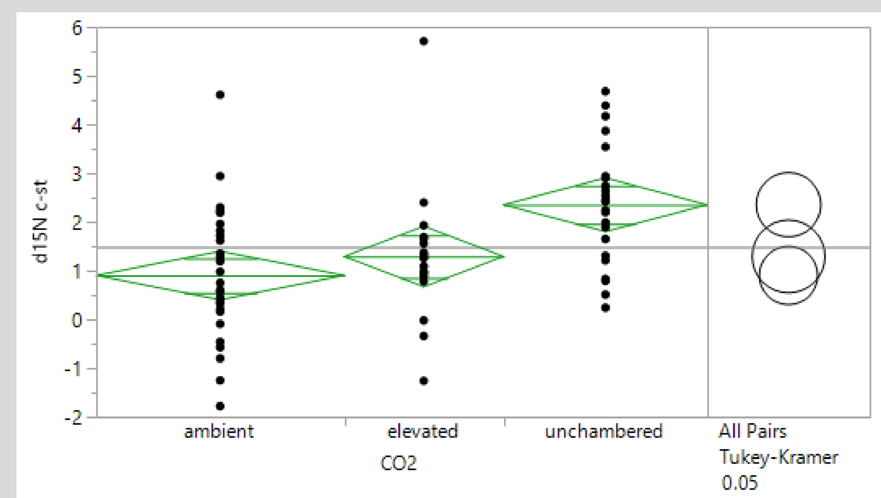

Figure 4. Unchambered treatments were higher in $\delta^{15}N_{c\text{-}st}$ than chambered treatments by Tukey post hoc ANOVA.

Figure 2. Mean±se values by CO_2 treatment, temperature treatment, and functional type for C/N (Fig. 2a), $\delta^{15}N$ (Fig. 2b), and $\delta^{13}C$ (Fig. 2c).

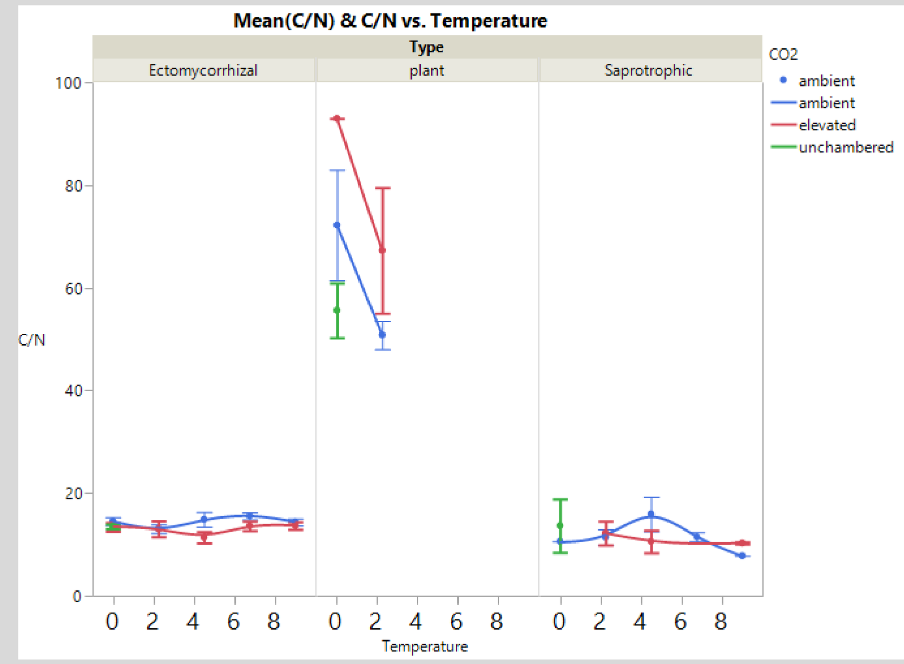

Figure 2a C/N patterns.

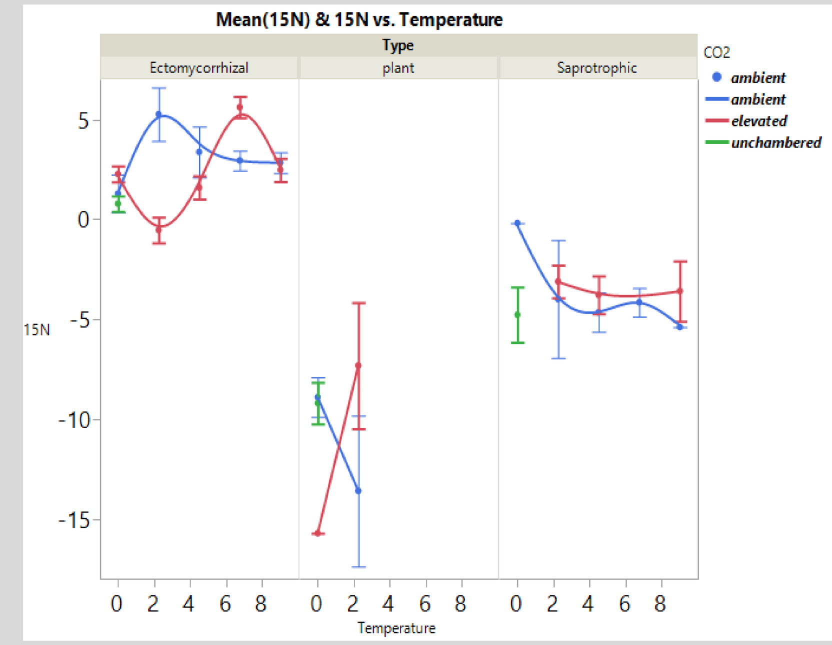

Figure 2b. $\delta^{15}N$ patterns.

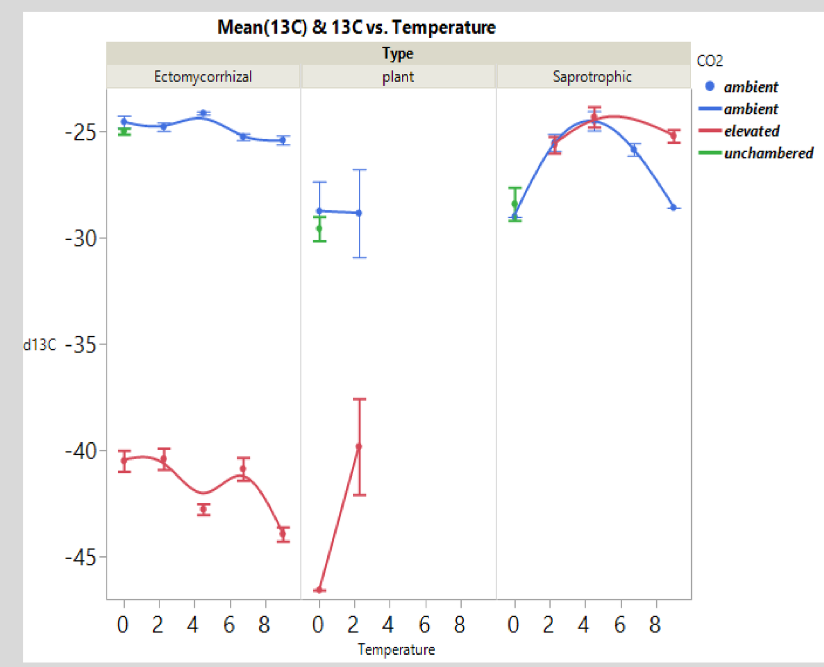

Figure 2c. $\delta^{13}C$ patterns

Table 1. C/N of *Vaccinium oxycoccus* and *V. uliginosum* berries are affected by CO_2, temperature, and species. %Var = % of explained variance.
Adjusted r^2 = 0.585, p = 0.0154, n = 14

Term	Value±se	P	%Var
Intercept	66.31±4.76	**<0.0001**	
CO_2	--	**0.0206**	46.75
Ambient	3.17±4.31	0.481	
Elevated	14.37±5.51	**0.0282**	
Unchambered	-17.54		
Temperature	-8.74±3.75	**0.0446**	20.61
V. oxycoccus	10.29±3.51	**0.0166**	32.64

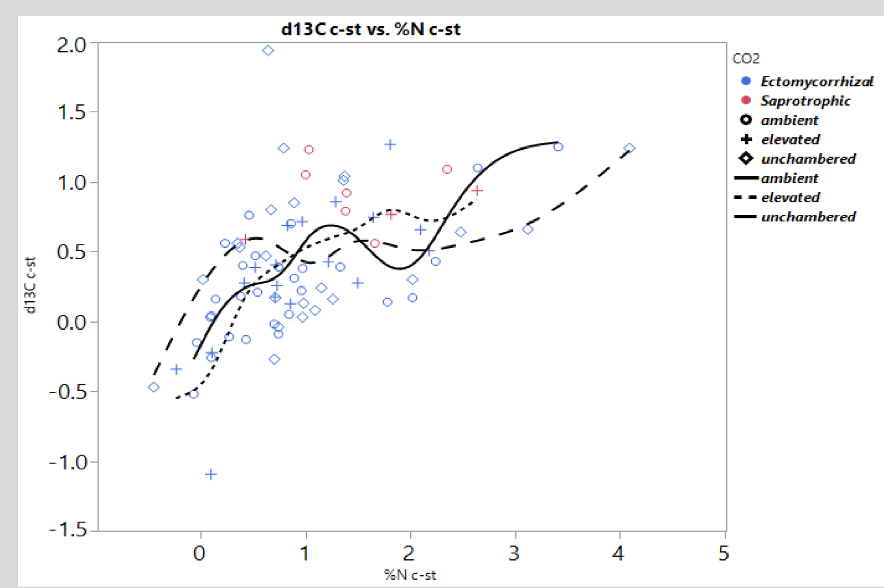

Figure 5. The differences between caps and stipes in %N and $\delta^{13}C$ are strongly correlated (see Table 3).

Table 2. Multiple regressions of $\delta^{13}C$ for mushrooms and berries. %Var = % of explained variance. Treatment mean for temperature was +2.93 °C. Adjusted r^2 = 0.966, p < 0.0001, n = 94. Ecm = ectomycorrhizal.

Term	%Var	Value±se	P
Intercept		-30.2±0.2	<0.0001
CO_2	41.5	--	<0.0001
Ambient		4.39±0.46	<0.0001
Elevated		-5.22±0.38	<0.0001
Unchambered		0.84	--
Type	10.5	--	<0.0001
Ectomycorrhizal		-1.21±0.34	0.0004
Plant		-1.63±0.63	0.0105
Saprotrophic fungi		2.84	--
Type x CO_2 (4)	46.2	--	<0.0001
Ecm x ambient		2.24±0.24	<0.0001
Ecm x elevated		-4.58±0.29	<0.0001
Ecm x unchambered		2.34	--
Plant x ambient		1.13±0.37	0.0024
Plant x elevated		-3.21±0.46	<0.0001
plant x unchambered		2.09	--
CO_2 x temperature (2)	0.9	--	0.0011
Ambient x temperature		0.41±0.15	0.0069
Elevated x temperature		0.18±0.15	0.2533
Unchambered x temperature		-0.58	--
Type x temperature (2)	0.9	--	0.0017
Ecm x temperature		-0.52±0.15	0.0005
Plant x temperature		0.81±0.28	0.0047
Saprotrophic x temperature		-0.29	

Table 3. Stepwise multiple regressions of the isotopic differences between caps and stipes in mushrooms ($\delta^{13}C_{c\text{-}st}$ and $\delta^{15}N_{c\text{-}st}$). Models minimizing values of the Akaike Information Criteria (AICc) are shown. Mushroom type is either ectomycorrhizal (Ecm) or saprotrophic (Sap), and CO_2 level is either ambient (a), elevated (e), or unchambered (un). %Var = % of explained variance. n = 79.

$\delta^{13}C_{c\text{-}st}$, adj. r^2 =0.454, p < 0.0001

Term	Value±se	%Var	P
Intercept	0.46±0.14	--	0.0014
$\delta^{15}N_{c\text{-}st}$	0.06±0.03	12.1	**0.0324**
$\%C_{c\text{-}st}$	-0.06±0.02	16.4	**0.0132**
$\%N_{c\text{-}st}$	0.27±0.05	72.2	**<0.0001**
Temperature	-0.02±0.01	7.4	0.0913
Type{Ecm-Sap}	-0.20±0.07	20.3	**0.0060**

$\delta^{15}N_{c\text{-}st}$, adj. r^2 =0.291, p < 0.0001

Term	Value±se	%Var	P
Intercept	9.62±3.04	--	0.0023
$\delta^{13}C_{c\text{-}st}$	0.66±0.39	8.0	0.0968
$\%C_{c\text{-}st}$	0.19±0.09	12.6	**0.0391**
$C/N_{c\text{-}st}$	-8.57±3.08	22.1	**0.0068**
log $C/N_{c\text{-}st}$	-12.18±4.43	21.5	**0.0075**
Type{Ecm-Sap}	0.46±0.27	8.4	0.0894
CO_2{a&e-un}	-0.54±0.17	27.4	**0.0027**