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Stories a Mushroom Told Me: ^{13}C and ^{15}N in a Pine FACE Study Reveal Fungal Functioning

Erik A. Hobbie[1], Kirsten Hofmockel[2], Linda van Diepen[3], Andrew P. Ouimette[1]
[1]ESRC-EOS, University of New Hampshire; [2]Iowa State University; [3]Department of Natural Resources, UNH

Abstract

· We used natural and tracer $\delta^{13}C$ and $\delta^{15}N$ values in a *Pinus taeda* Free Air CO_2 Enrichment (FACE) experiment to investigate the functioning of saprotrophic and ectomycorrhizal fungi in nitrogen cycling from 2004 to 2010.

· Sporocarp $\delta^{13}C$ and $\delta^{15}N$ values in ambient and elevated CO_2 plots corresponded to the range of sources from which different saprotrophic genera acquired carbon including surface litter (*Rhodocollybia* and *Mycena*), pine cones (*Baeospora*), relatively older (> 8 years) wood (*Gymnopilus* and *Pholiota*), and soil (*Ramariopsis*).

· The $\delta^{13}C$ values of ectomycorrhizal genera in ambient and elevated CO_2 plots correlated with a slope (4.3±1.2) much greater than the expected value of one, suggesting that these fungi assimilated carbon from two isotopically distinct sources, one derived from recent photosynthate and a second from organic matter (presumably protein) from litter or soil.

· Ectomycorrhizal fungi with hydrophobic ectomycorrhizae (e.g., *Cortinarius*) acquired nitrogen from deeper in the soil profile than taxa with hydrophilic ectomycorrhizae (e.g., *Russula* and *Lactarius*) that acquired nitrogen from the Oi horizon. ^{15}N enrichments relative to source nitrogen for six taxa ranged from 3-9‰; these enrichments correlate analytically with both increased allocation by fungi to hyphal development and decreased nitrogen transfer to host plants.

Approach

· In a loblolly pine plantation at Duke University, carbon dioxide was elevated by +200 ppm within 30-m rings, changing the $\delta^{13}C$ of CO_2 from -8‰ to -20‰.

· Experiment began in 1996 with final harvest in 2010.

· A pulse ^{15}N labeling experiment began in 2004.

· Isotopic signatures in fungi and other ecosystem pools collected in 2004 and 2010 were used to explore fungal functioning of both ectomycorrhizal (symbiotic) and saprotrophic fungi.

Nitrogen Isotopes

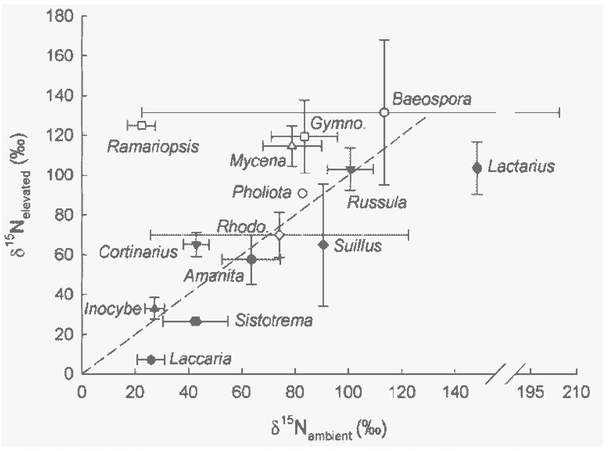

Fig. 1. High ^{15}N levels correspond to litter-inhabiting fungi, low levels to organic horizons. Nitrogen isotopes of different genera of ectomycorrhizal and saprotrophic fungi (±SE) in ambient and elevated CO_2 treatments in 2004. A 1:1 line is also shown. *Rhodo.* = *Rhodocollybia*, Gymno. = *Gymnopilus*.

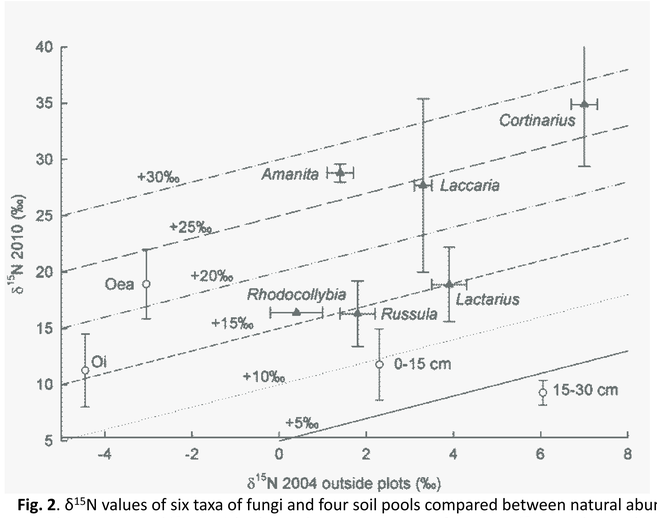

Fig. 2. $\delta^{15}N$ values of six taxa of fungi and four soil pools compared between natural abundance samples in 2004 and ^{15}N-enriched samples in 2010. Lines of constant ^{15}N enrichment between 2004 and 2010 samples are shown as dotted lines between 5‰ and 30‰. The ^{15}N enrichment of fungi relative to source nitrogen ($\varepsilon_{f\text{-}s}$) is then calculated as the ^{15}N difference (on x-axis) between fungi and source nitrogen pools along the isolines. $\varepsilon_{f\text{-}s}$ varied from 4‰ (*Rhodocollybia*) to 9‰ *(Cortinarius).*

Duke FACE site

Lactarius

Rhodocollybia

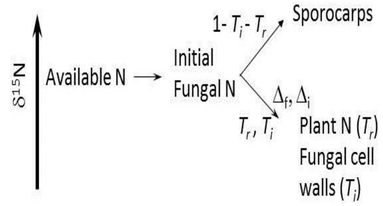

Fig. 3. Schematic of nitrogen movement and isotopic patterns in a two-pool fungal model. In this scenario, $\delta^{15}N_{sporocarp}$ is affected by the fractionation during creation of transfer compounds (Δ_f) and the proportion transferred to plants (T_r), in addition to the fractionation during creation of immobile compounds (Δ_i) and the proportion transferred to those pools (T_i).

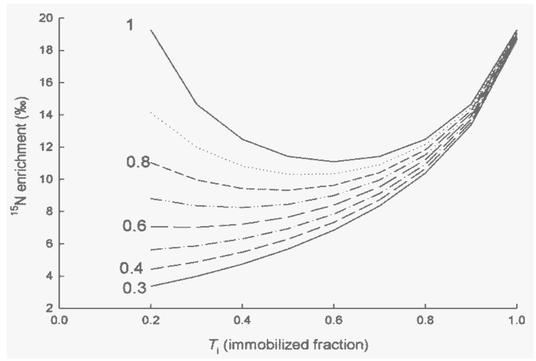

Fig. 4. The ^{15}N enrichment of sporocarps ($\varepsilon_{f\text{-}s}$) relative to the nitrogen source varies in Equation (1) as a function of T_i and of T_r, with $T_r = k \times (1 - T_i)$. k is varied from 0.3 to 1, as indicated on figure, with $\Delta_f = \Delta_i = 8‰$:

$$\delta^{15}N_{sporocarp} - \delta^{15}N_{available\ N} = -\Delta_f \ln[(1 - T_r) \times (1 - T_i)] \quad (1)$$

For realistic values of k (<0.5), higher $\varepsilon_{f\text{-}s}$ in taxa correspond to greater N sequestration and consequently greater fungal C demand.

Carbon Isotopes

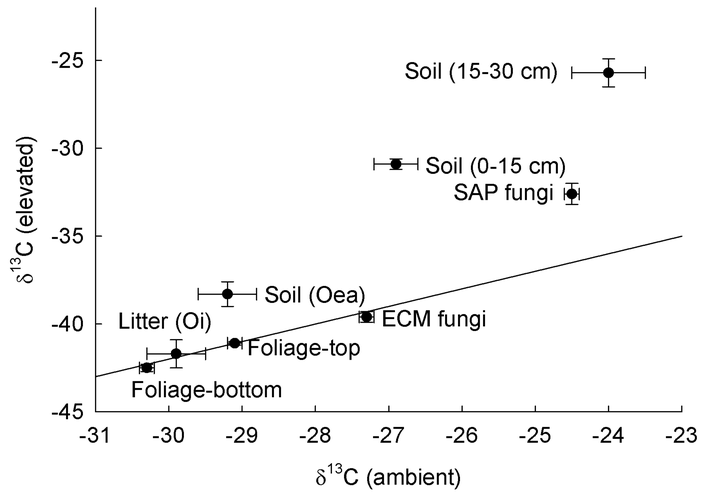

Fig. 5. Carbon isotopes of ecosystem pools (±SE) in elevated CO_2 and ambient treatments. The line fits the equation $\delta^{13}C_{elevated} = \delta^{13}C_{ambient} - 12‰$. ECM = ectomycorrhizal, SAP = saprotrophic.

Acknowledgements

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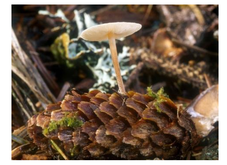


Baeospora

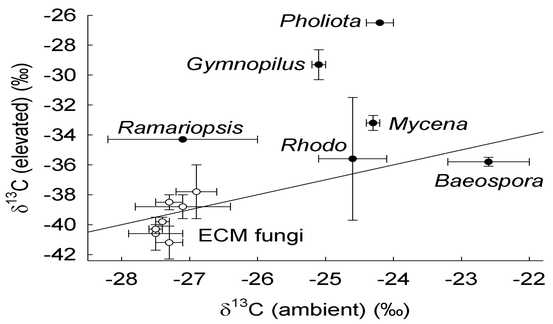
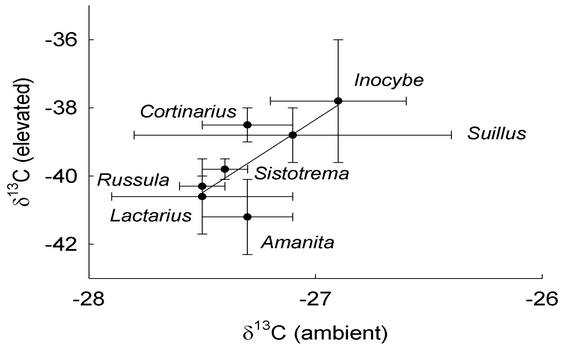


Fig. 6. Carbon isotopes of different genera of saprotrophic fungi (±SE) in elevated CO_2 (y-axis) and ambient (x-axis) treatments. Values for the putative saprobe *Ramariopis kunzei* are also included. Values for taxa of ectomycorrhizal fungi from Figure 3 are shown with clear symbols. The line fits the equation $\delta^{13}C_{elevated} = \delta^{13}C_{ambient} - 12‰$. *Rhodo* = *Rhodocollybia*.



Amanita

Fig. 7. Carbon isotopes of different genera of ectomycorrhizal fungi (±SE) in elevated CO_2 and ambient treatments. The regression line for mean values by genus between ambient and elevated treatments has a slope of 4.3±1.2, indicating an additional source of ^{13}C-enriched carbon for fungi under elevated CO_2 other than plant photosynthate.

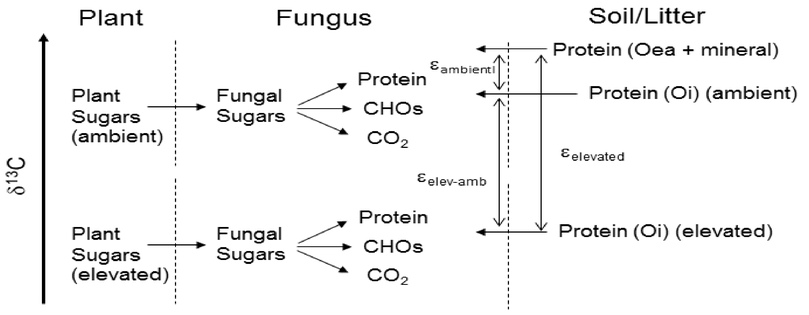


Fig. 8. Causes of carbon isotope patterns in ectomycorrhizal fungi under ambient and elevated CO_2. Fungal protein is synthesized *de novo* from fungal sugars, but a portion of fungal protein is synthesized from soil-derived organic nitrogen. Soil-derived organic nitrogen (protein) will differ in $\delta^{13}C$ depending on whether it is from ambient or elevated litter (Oi) or from deeper in the soil profile (Oea + mineral). $\varepsilon_{ambient}$ and $\varepsilon_{elevated}$ are the ^{13}C enrichment of protein derived from the Oea and mineral horizons relative to protein from the Oi horizon, in ambient and elevated CO_2 treatments, respectively. $\varepsilon_{elev\text{-}amb} = \varepsilon_{elevated} - \varepsilon_{ambient}$.

Conclusions

· $\delta^{13}C$ indicates age of saprotrophic carbon.

· $\delta^{13}C$ suggests incorporation of litter-derived or deeper soil organic N into ectomycorrhizal protein.

· Fungal $\delta^{15}N$ correlates with depth.

· $\delta^{15}N_{fungi\ -\ source}$ quantitatively correlates with proportion of immobilized N, therefore with carbon allocation (see Hobbie & Hobbie, Ecosystems, DOI: 10.1007/s10021-008-9159-7).

· Paired natural and tracer ^{15}N and ^{13}C measurements proved extremely useful, with new, quantitative insights into interpreting natural abundance and tracer isotopic patterns.