Use of phospholipid and neutral fatty acids to quantify AM fungal responses to eCO_2 and eO_3

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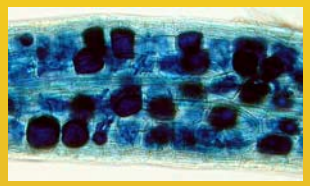
Introduction

- Growth under elevated (*e*) CO_2 favors biomass allocation to roots at the expense of shoots.
- Ozone damages leaf cells, typically increasing allocation of resources to aboveground structures.
- A significant portion of a plant's nutrients is acquired through its mycorrhizal symbiont in exchange for carbon.



Vesicles in Arbuscular Mycorrhizal Fungi (AMF)

Ectomycorrhizae (ECM) colonizing an aspen root tip

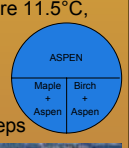
- The combined effects of eO_3 and eCO_2 on belowground allocation of plant C to the mycorrhizal symbiont are unclear.
- Fatty acid 16:1ω5c is a signature marker for AMF.
- Neutral lipid fatty acids (NLFAs) and phospholipid fatty acids (PLFAs) are associated with fungal carbon storage and cellular membranes, respectively.

Objectives

- Demonstrate the use of fatty acid 16:1ω5c for AMF quantification
- Quantify the interactive effects of eCO_2 and eO_3 on AMF biomass and storage

Study Site- FACTS II, Rhinelander, WI

- Site soils are mixed, frigid, coarse loamy Alfic Haplor-thods
- Average temperature, precipitation and degree days are 11.5°C, 833 mm/yr, and 150 days, respectively
- Circular, 30-m-diameter rings spaced 100 m apart
- Trees planted in 1997 with an equal tree density
- Split-plot, randomized, complete block design with 3 reps
  - → Factorial ambient and elevated CO_2 (360, 560 µL/L), and O_3 (36, 55 µL/L) treatments
  - → Each plot (ring) is split into 3 community types



Aerial photo of ASPENFACE, Rhinelander, WI

Sampling

2002 –Whole Tree Harvest

- One tree was harvested in each split-plot
- Root systems of selected trees were harvested over the stem using a 25.4-cm diameter corer to a depth of 25 cm
- Roots were wet-sieved, sorted by size and frozen

2003-Root Harvest

- One tree was harvested in each split-plot
- Root systems of selected trees were harvested over the stem using a 25.4-cm diameter corer to a depth of 25 cm
- Roots were wet-sieved, sorted by size and frozen

Analysis

Laboratory

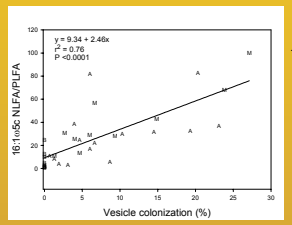
- Quantified AMF colonization in fine roots (<1 mm diameter) using trypan blue stain
- Extracted fatty acids from freeze-dried roots, followed by identification and quantification using GC/MS

Statistics

Phospholipid fatty acids were summarized using a reciprocal averaging (RA) analysis in PC-ORD. The position of each sample depends on the relative abundance of 15 signature fatty acids. The impact of different treatments on AMF was analyzed using ANOVA.

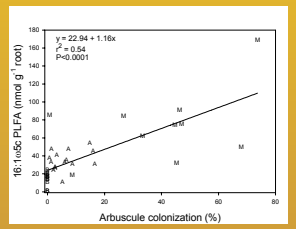
Results

1) PLFA 16:1ω5c is a good indicator of AMF in our system

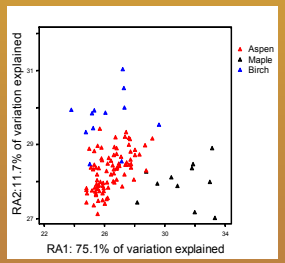


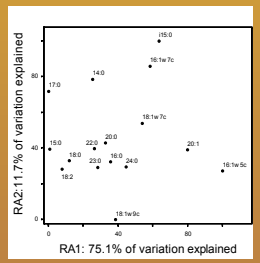
The ratio of signature lipids 16:1ω5c NLFA/PLFA in roots is strongly associated with vesicle colonization

There is a strong association between arbuscule colonization in roots and PLFA marker 16:1ω5c



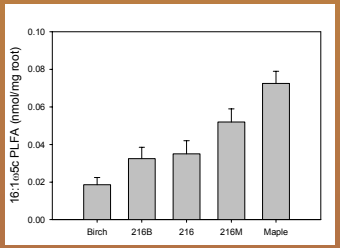
2) Tree species differ in fatty acid composition





Maple has higher relative abundance of PLFA marker 16:1ω5c

3) Neighbor identity alters fatty acid composition in roots

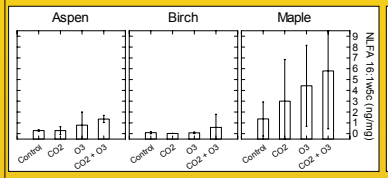


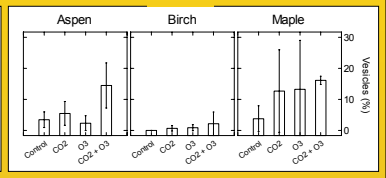
- When the neighbor is maple (exclusively AMF), the aspen has elevated PLFA 16:1ω5c levels
- When the neighbor is birch (exclusively ECM), aspen PLFA 16:1ω5c concentrations do not change

216B=aspen clone 216 next to birch
216M=aspen clone 216 next to maple

4) Treatment effects on fatty acid 16:1ω5c and colonization

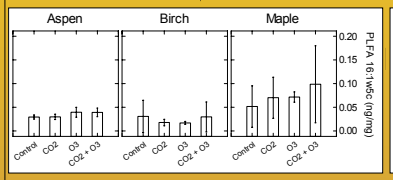
a) Storage

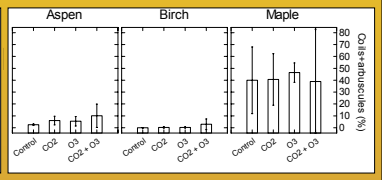




- Species has a significant effect on NLFA 16:1ω5c concentration (p<0.0001); Maple has higher NLFA 16:1ω5c concentration than aspen and birch
- Greater NLFA 16:1ω5c concentration under *e*O3 suggests an increase in allocation to storage due to stress (p≤0.0078)
- Species has a significant effect on % vesicles (p<0.0001); maple and aspen have more vesicles than birch
- More vesicles under eCO_2 (p≤0.0011) suggests greater C availability
- More vesicles under eO_3 (p≤0.0144) suggests an increase in allocation to storage due to stress

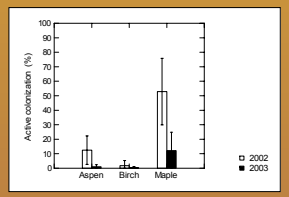
b) Active Biomass

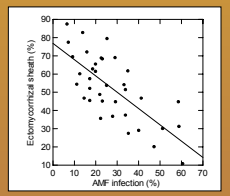




- Species has a significant effect on PLFA 16:1ω5c concentration (p<0.0001); maple has higher PLFA 16:1ω5c concentrations indicating greater AMF biomass
- No significant treatment differences in PLFA 16:1ω5c concentrations indicates no effect on AMF biomass
- Species has a significant effect on coils + arbuscules (p<0.0001); higher coils+ arbuscules in maple indicates more exchange sites for nutrients between the symbionts
- No treatment differences on coils+arbuscules indicates no effect on nutrient exchange

5) Ectomycorrhizal component of the plant community is increasing





- As aspen ages it becomes increasingly ectomycorrhizal
- Maple is exclusively AMF, but fast-growing aspen roots are collected with maple roots, diluting the AMF signal
- The negative correlation between ectomycorrhizal sheath and AMF infection (R^2=0.5161; p<0.00001) further illustrates that greater aspen root biomass dilutes the AMF signal in maple

Ongoing Work

- Establish the applicability of fatty acid 18:2ω6,9 to estimate ectomycorrhizal biomass
- In July 2002, we buried 30-µm diameter mesh bags containing 80 cm^3 of river bottom sand to accomplish the following:
  - → Assess external hyphal growth rates using 3 sequential harvests during 2003
  - → Establish the relationship between concentrations of PLFA marker 16:1ω5c and AMF hyphal biomass for this system