Multifactor effects on the mycorrhizal symbiosis

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Objectives and Hypotheses

The overall purpose of our investigation is to examine the interactive effects of both eCO_2 and eO_3 on the growth and biomass allocation of the mycorrhizal association in order to improve the scientific basis for predicting or detecting effects of simultaneous factors on this important symbiotic process of terrestrial ecosystems.

Primary Hypotheses

- The amount of photosynthate allocated to belowground structures determines the amount of mycorrhizal fungus biomass.

Primary Hypotheses

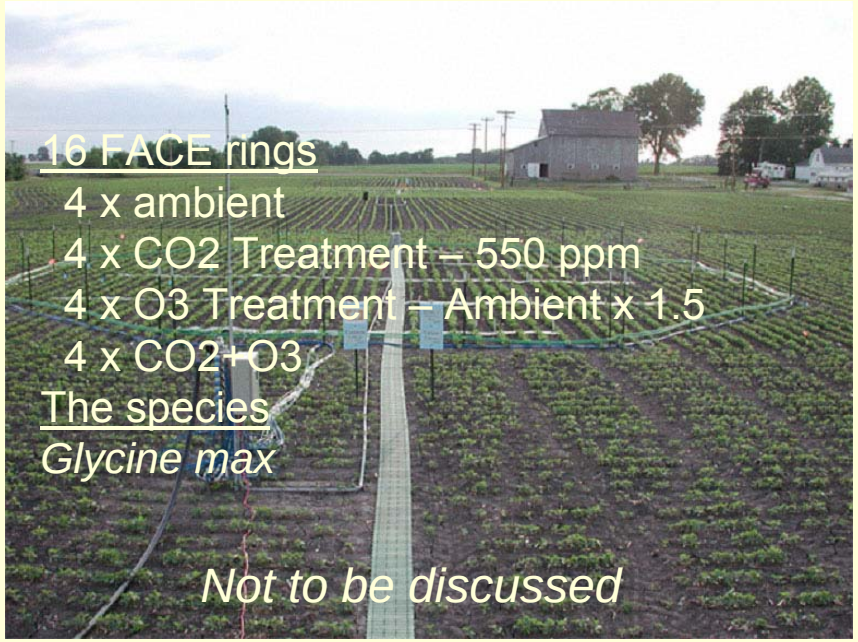
- The allocation of the fungus to internal growth versus external hyphal growth is determined by the interactions and feedbacks between carbohydrate supply to the root and soil nutrient supply rate.

Primary Hypotheses

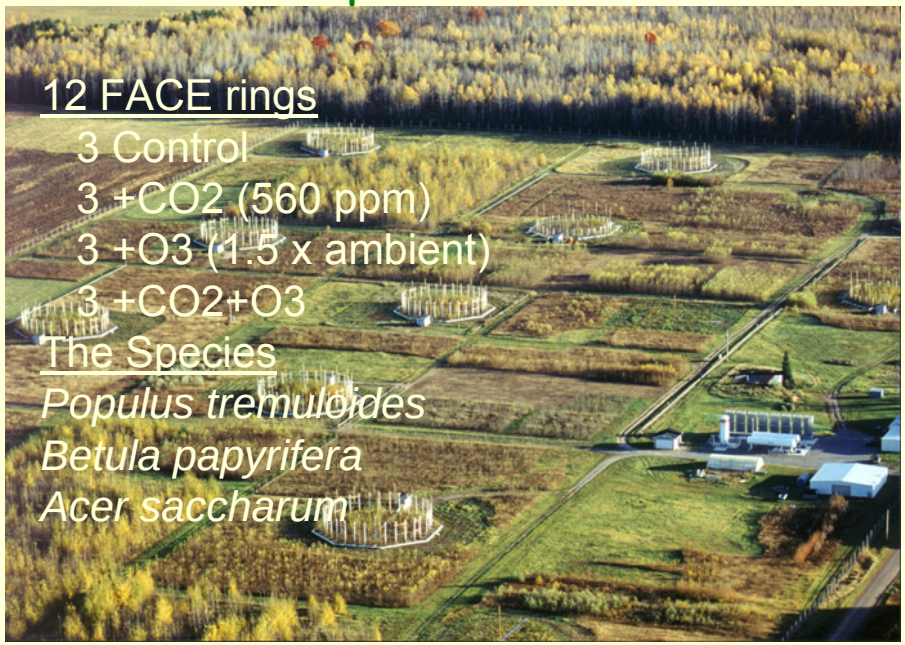
- Mycorrhizal fungi associated with hosts showing symptoms of decline due to the *e*O_3 fumigation will demonstrate depleted energy reserves that could, in turn, lead to a loss of active mycorrhizal fungus biomass.

Experimental Design
- facilities -

SoyFACE



AspenFACE



Experimental design
- methodology -

- A primary need for integrating lower-scale mechanisms associated with mycorrhizas with higher-level ecosystem processes is the ability to determine fungal biomass.
  - The methods for quantifying AMF and EcM traditionally have been based around microscopic procedures.
    - These methods are inadequate for identification and do not readily scale up to biomass.
  - Recent advancements with molecular tools offers promise for improvement in both of these areas.
- *A major goal of this project is to develop better quantitative measures of mycorrhizas.*

Experimental design
- methodology -

Quantification of mycorrhizal fungi in roots

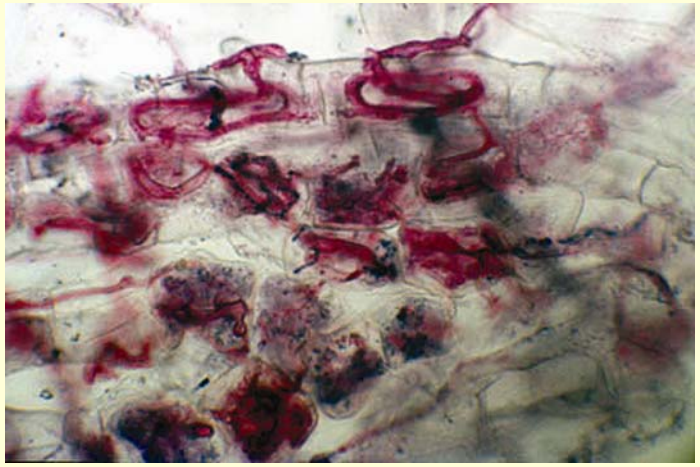
- *Colonization of fine roots (microscope - staining)*
  - Root length colonized (AMF)
  - Root tips colonized (EcM)
- *Chitin in roots (mid range FTIR-ATR)*
  - Measurement of total fungus in root
- *Ergosterol in roots (HPLC)*
  - Physiologically active fungus - good for EcM not so for AMF

Experimental design
- methodology -

- Fatty Acids in *roots* – GC-MS
  - Phospholipid Fatty Acids (PLFA) – membrane surface area
    - 16:1ω5c – marker for AMF
    - 18:2ω6,9 – marker for EcM fungi (and other micro-eukaryotes)
  - Neutral Lipid Fatty Acids – storage lipids
  - Ratio of 16:1ω5c NLFA/PLFA (measure of storage-to-biovolume fungus)

Classical methods

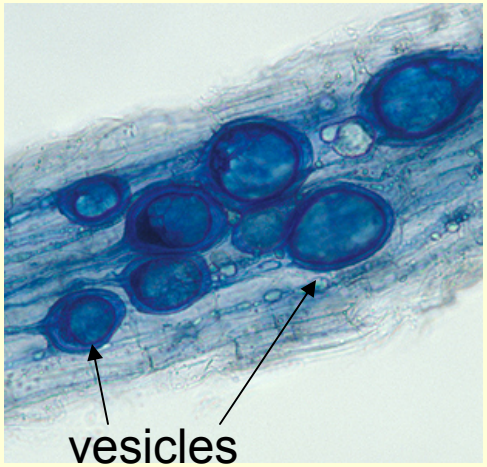
- Quantify AM fungus as a proportion of root length
  - Trypan blue - does not measure active fungus
  - Succinate dehydrogenase activity – measures active fungus
- Quantify allocation of fungus
  - Intraradical vs. extraradical allocation



AMF structures

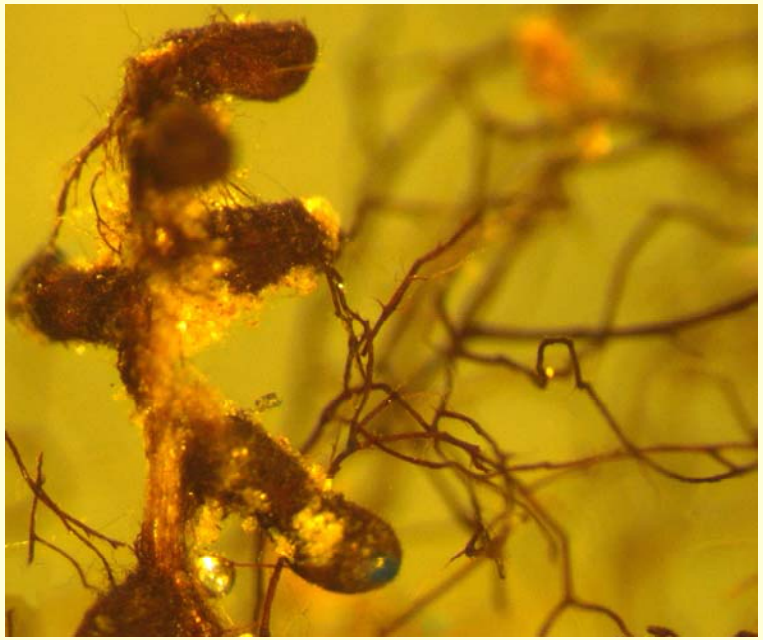
- Active uptake of phosphate associated with arbuscules and coils

- Vesicles are lipid rich storage structures of the fungus
  - Primarily the neutral lipid 16:1w5c
  - Vesicles found in Glomaceae, but not Gigasporaceae



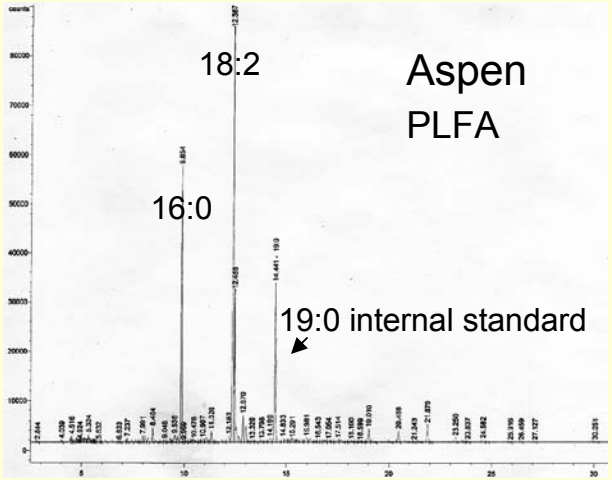
ECM fungi

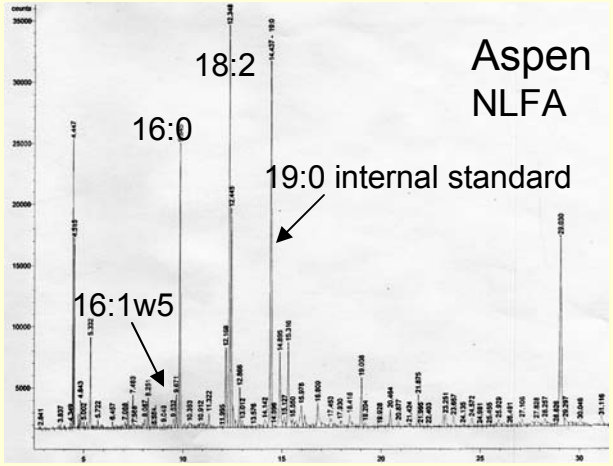
- Quantification of ECM fungi based on several approaches
  - Sporocarp surveys
  - Root morphology
    - Proportion of root tips colonized
  - Ergosterol, chitin content of roots, 18:2w6,9 NLFA
  - Hyphal in-growth bags

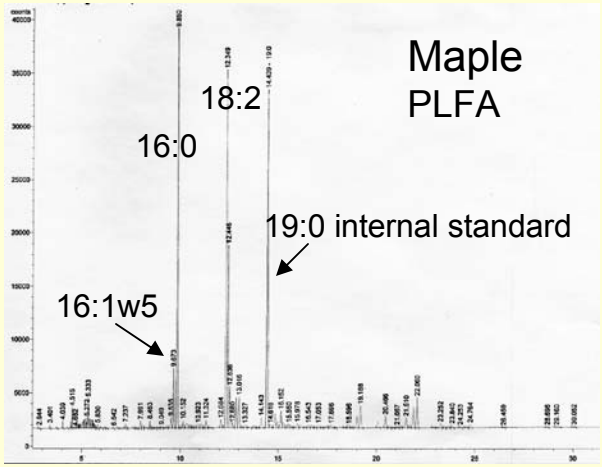


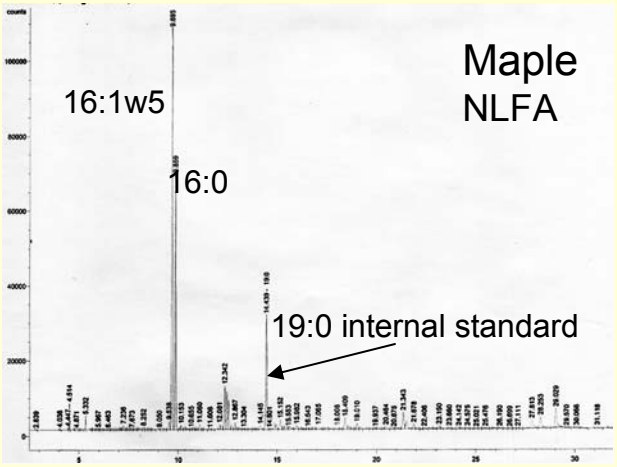
Cenococcum on aspen root tips

Use of PLFAs and NLFAs for characterizing mycorrhizae responses









GC Chromatographs

Calculation of AM fungal biomass

PLFA 16:1ω5c and AM fungal biomass

- AMF hyphae = 1.5 nmol PLFA 16:1ω5c mg^{-1} fungus as determined by hyphal isolation (Olsson et al. 1995)
- AM fungal biomass conversion to biomass C by multiplying with 0.42

$$AMF\text{-}C = [16{:}1\omega5c/1.5] \times 0.42$$

The relationship assumes that intraradical and extraradical hyphae are of similar composition – may not be true (although good first approximation)

Calculation of ECM fungal biomass

Ergosterol and ECM fungal biomass

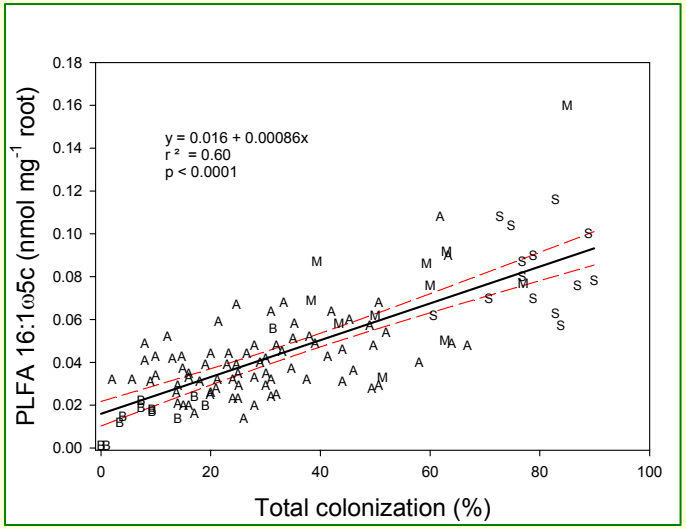
- ECM hyphae = 3.0 μg ergosterol mg^{-1} fungus (Salmanowicz & Nylund, 1988)
- biomass conversion to biomass C by multiplying with 0.42

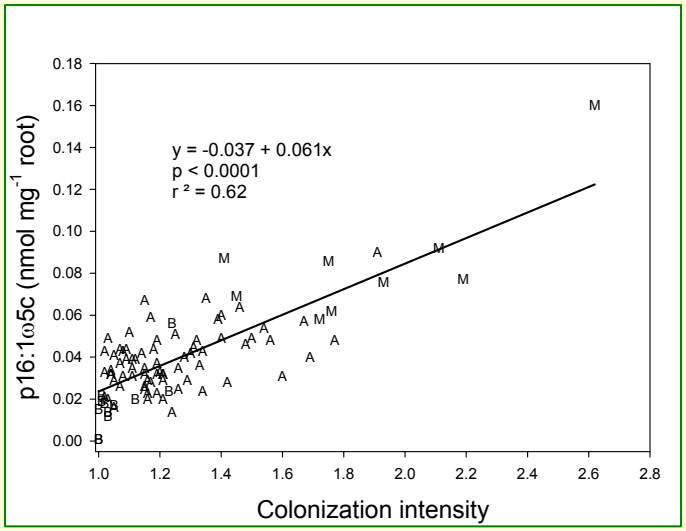
$$\text{ECM-}C = [\text{ergosterol}/3] \times 0.42$$

The relationship assumes mycelia are of similar composition (although known not be true)

Relationship between microscopic and PLFA measures

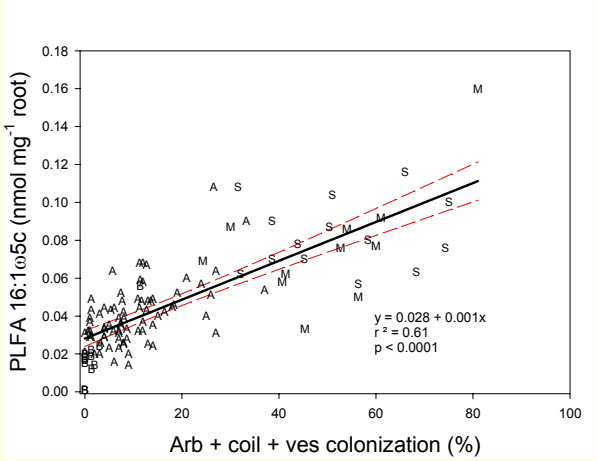
The PLFA 16:1ω5c corresponds quite well with microscopic measures of colonization.



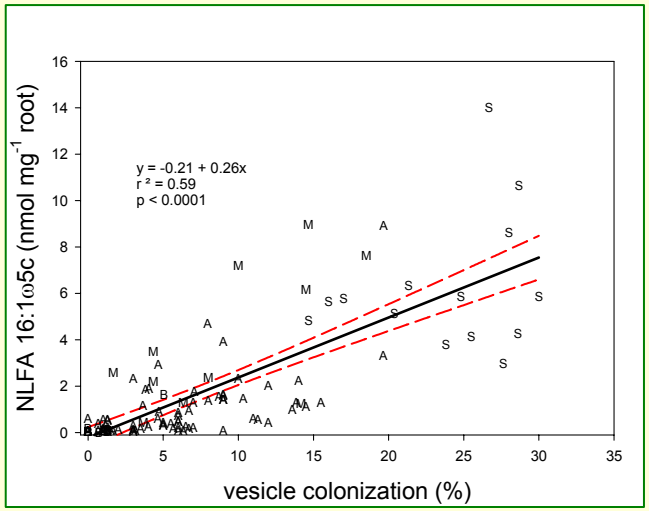


Association of AMF colonization structures with fatty acids

- There is a strong association between active structures and 16:1w5c (arbuscules, coils, vesicles).
  - Suggests these active structures may be better measures of AMF biomass than total colonization.

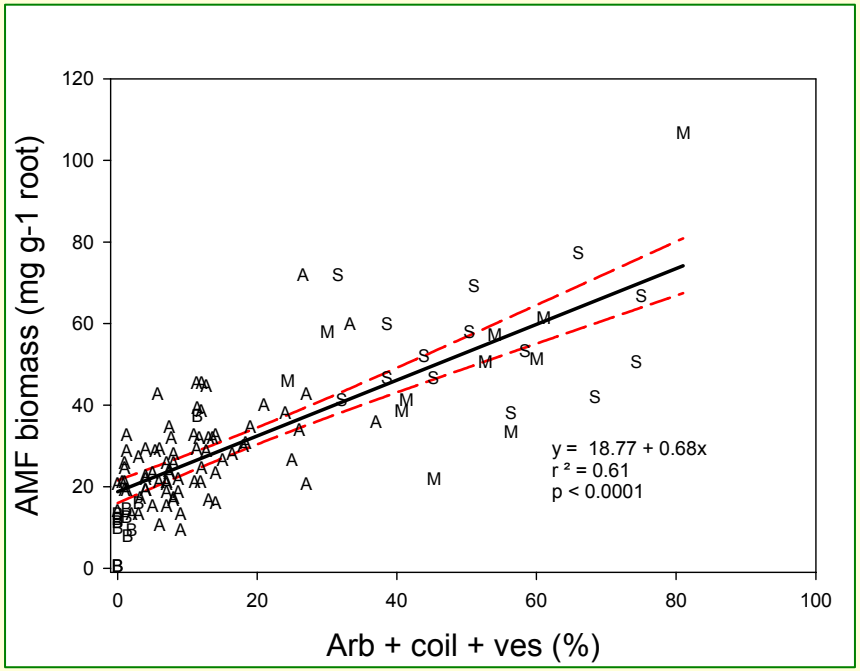


- The signature NLFA 16:1w5c is strongly associated with vesicle colonization (not all AMF taxa produce vesicles).

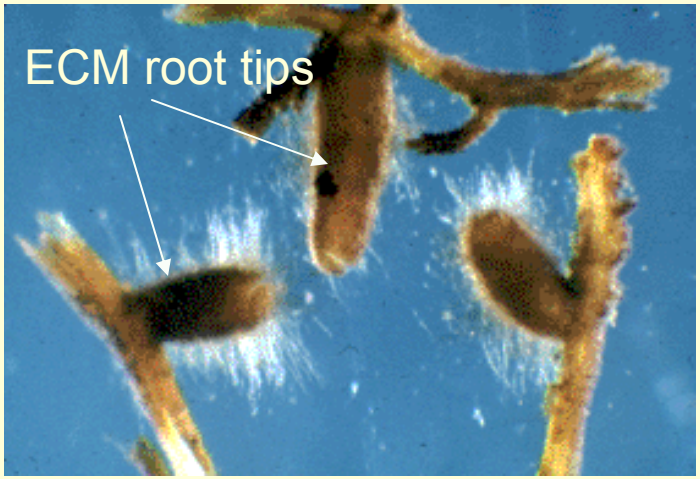


Relationship between colonization and PLFAs in terms of biomass

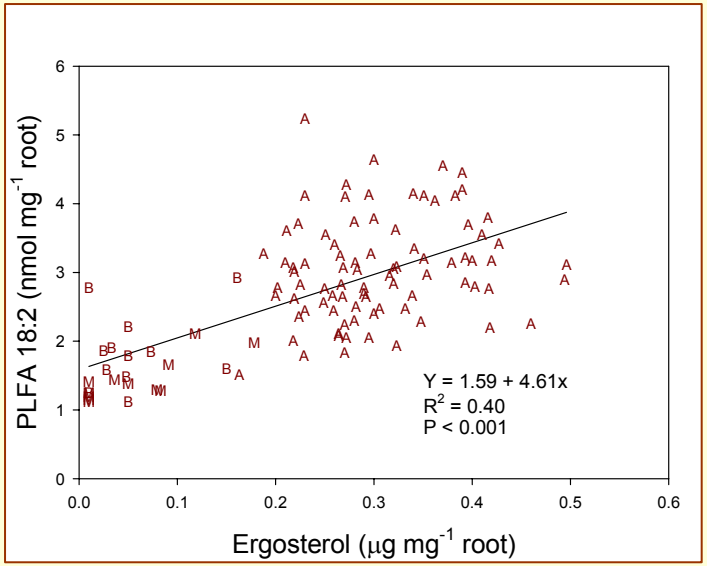
The concentration of AMF biomass in roots of aspen, birch and maple ranges from around 10 mg^{-1} g to about 118 mg^{-1} g



Association of ECM with fatty acids

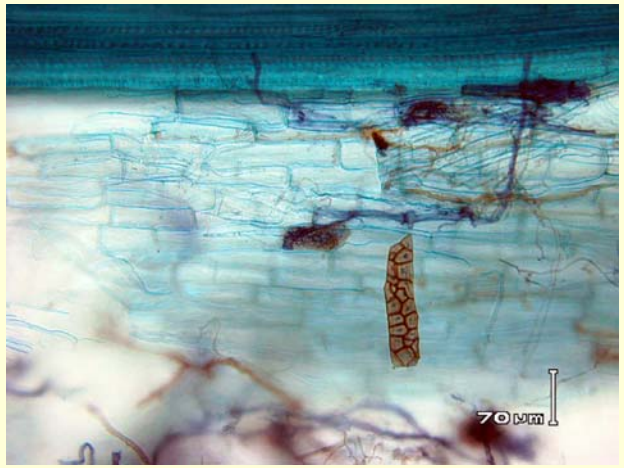


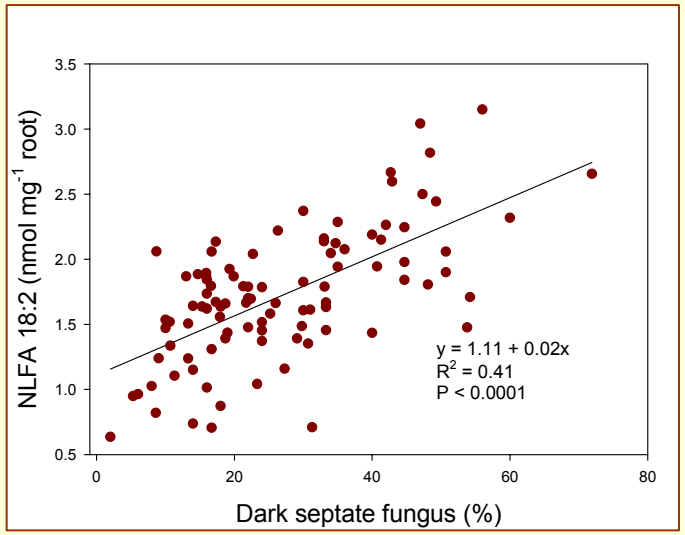
The membrane sterol ergosterol and the PLFA 18:2w6,9 are good predictors of each other.

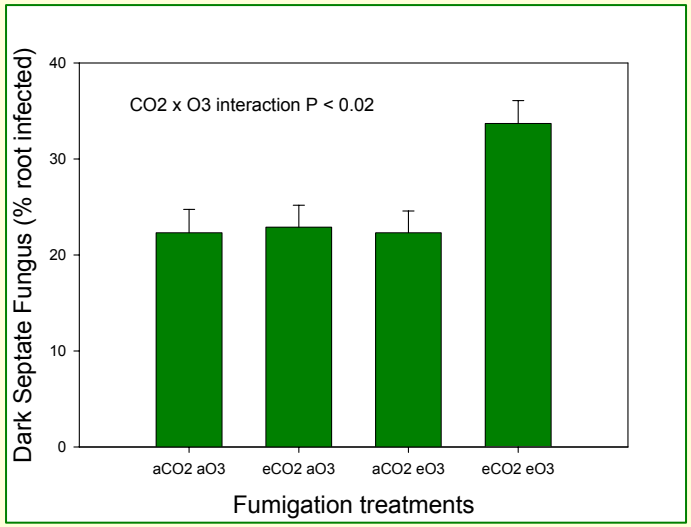


Dark septate fungus

The dark septate fungus is considered a weak mutualist or possibly a pathogen. Although common in tree roots little is know about this organism.



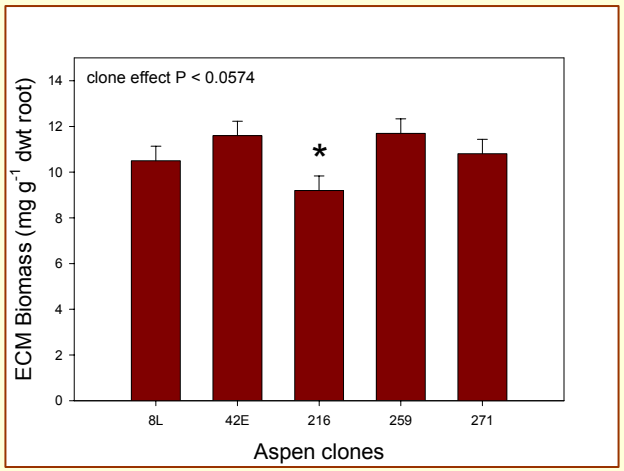


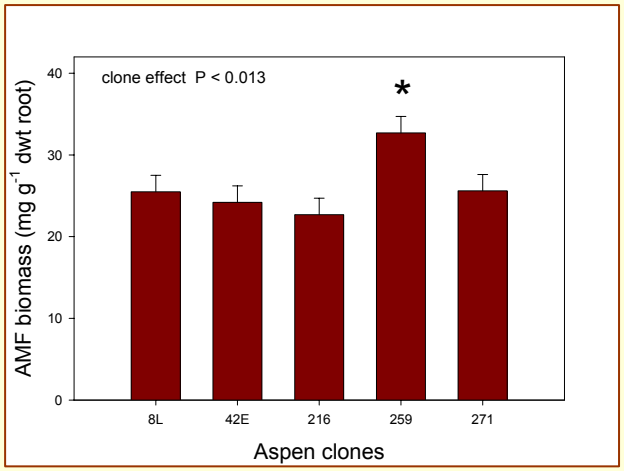


Mycorrhizal effects on Aspen Clones

- ECM biomass of the aspen clones were found to be significantly different (P <0.0574)
  - ozone sensitive clones tended to have slightly higher ECM biomass concentrations.
  - No treatment effect for ECM biomass was evident.

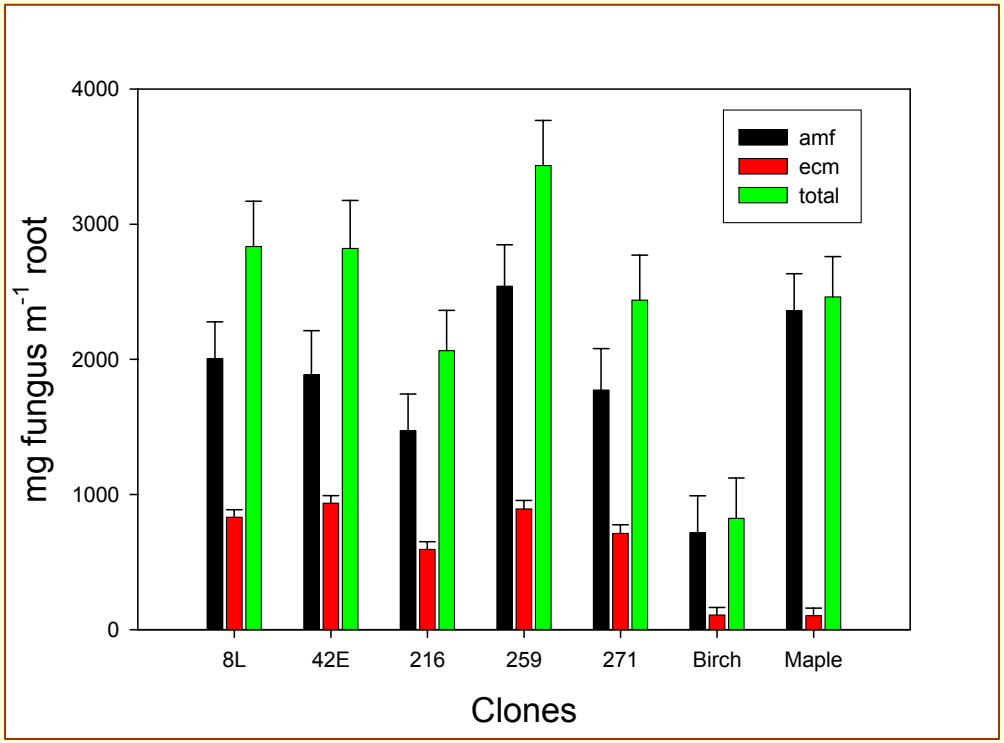
- A significant clone effect was evident for AMF biomass
  - ozone sensitive clone 259 had a significantly higher biomass concentration of AMF than the other clone root systems (P < 0.013).
  - A significant CO_2, O_3 and CO_2 x O_3 interaction were also evident for AMF biomass.





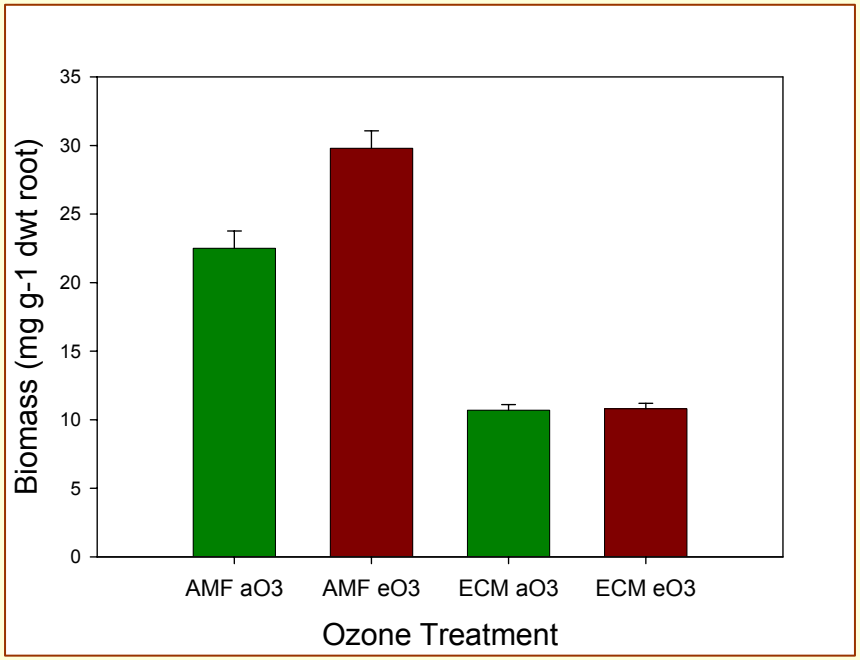
Mycorrhizal biomass

- A significant clone effect exists for the Aspen mycorrhizas.
  - AMF $P < 0.02$
  - ECM $P < 0.0091$
  - Total Myco $P < 0.0044$



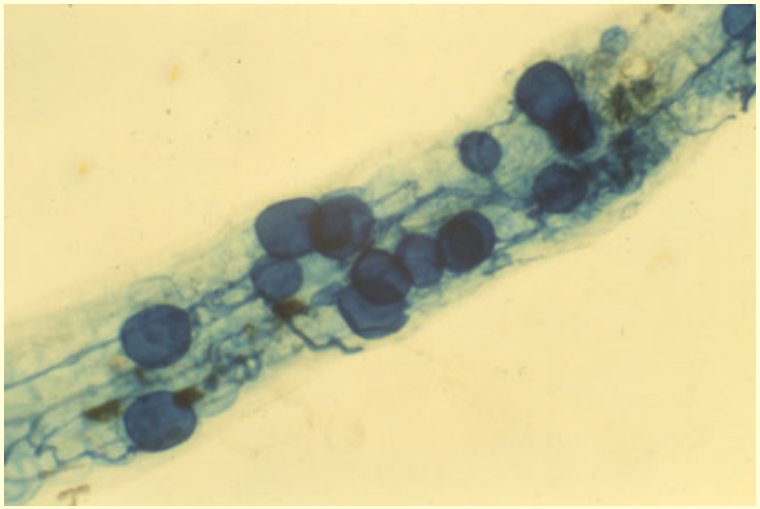
Mycorrhizal effects on Aspen Clones

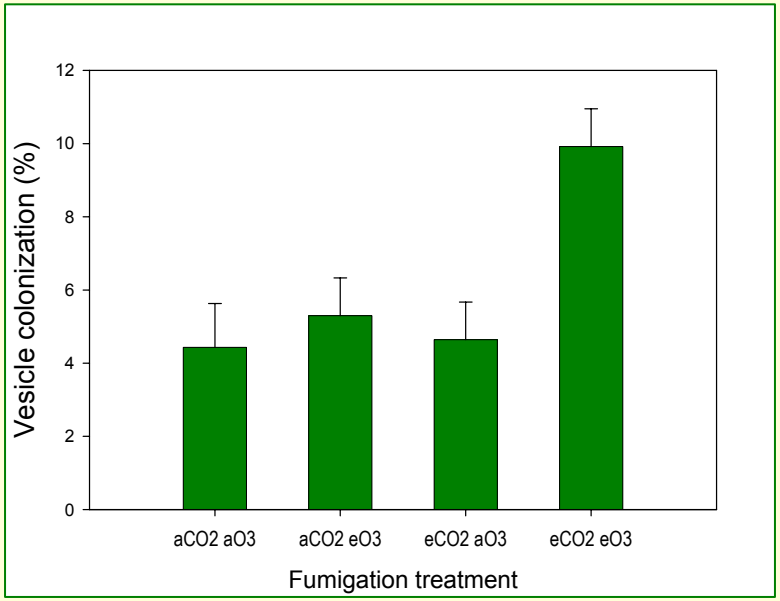
- A significant ozone effect for AMF biomass was evident for Aspen clones ($P < 0.0002$).
- No ECM biomass response was evident with O_3 fumigation.



Mycorrhizal effects on Aspen Clones

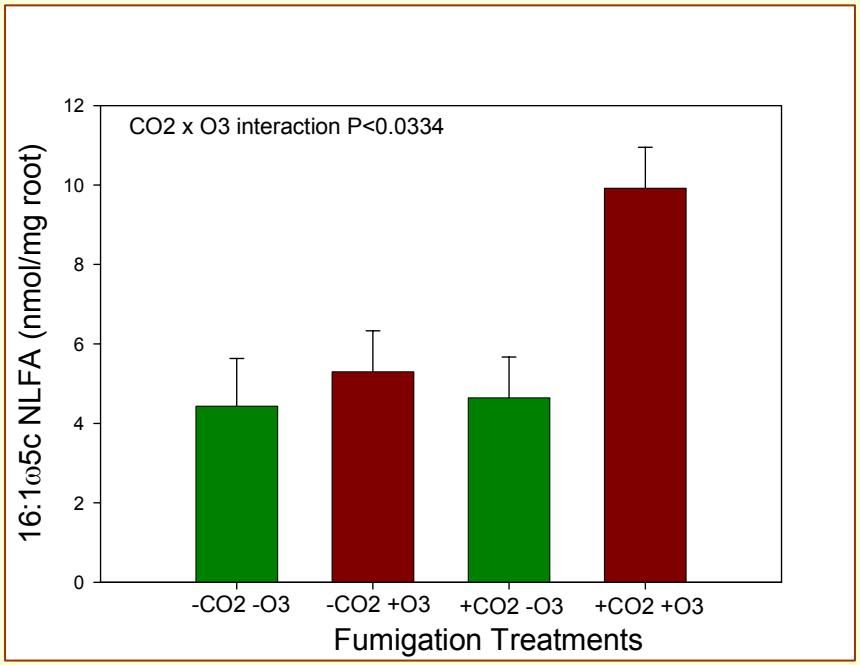
- Vesicles, the lipid storage structure of AMF was the only microscopic fungal structure that was affected by the fumigation treatments.
- A direct effect on vesicle colonization was evident for CO_2 ($P<0.0295$) and O_3 ($P<0.0083$). A CO_2 x O_3 interaction ($P<0.0483$) was also evident with the greatest increase being with elevated CO_2 and O_3.





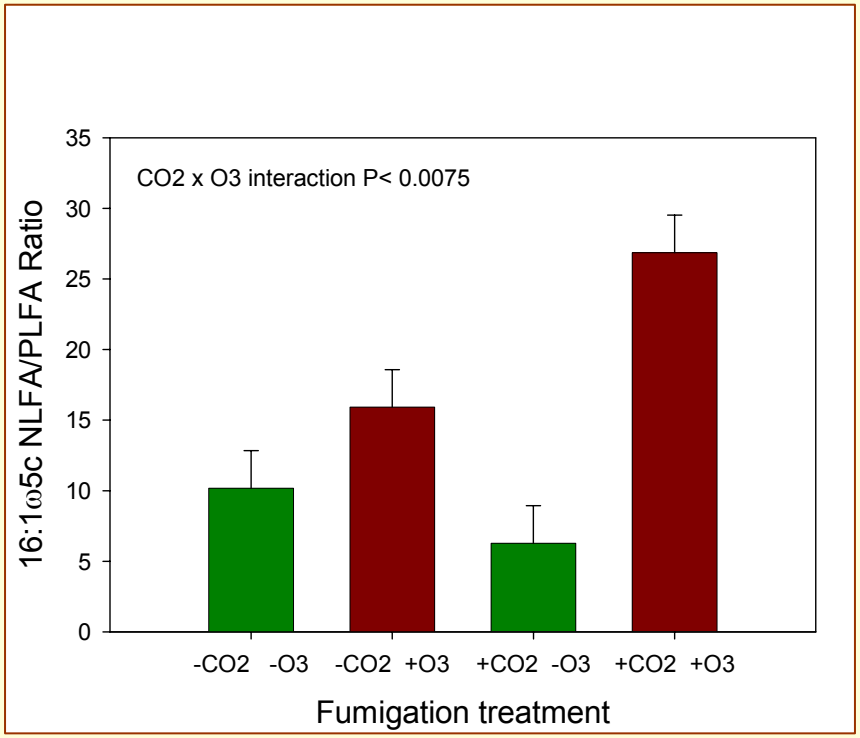
AspenFACE
Treatment effects

- Supporting the finding of increased vesicle colonization is an increase in the NLFA 16:1w5c with O_3 and the CO_2 x O_3 interaction (P< 0.0334).



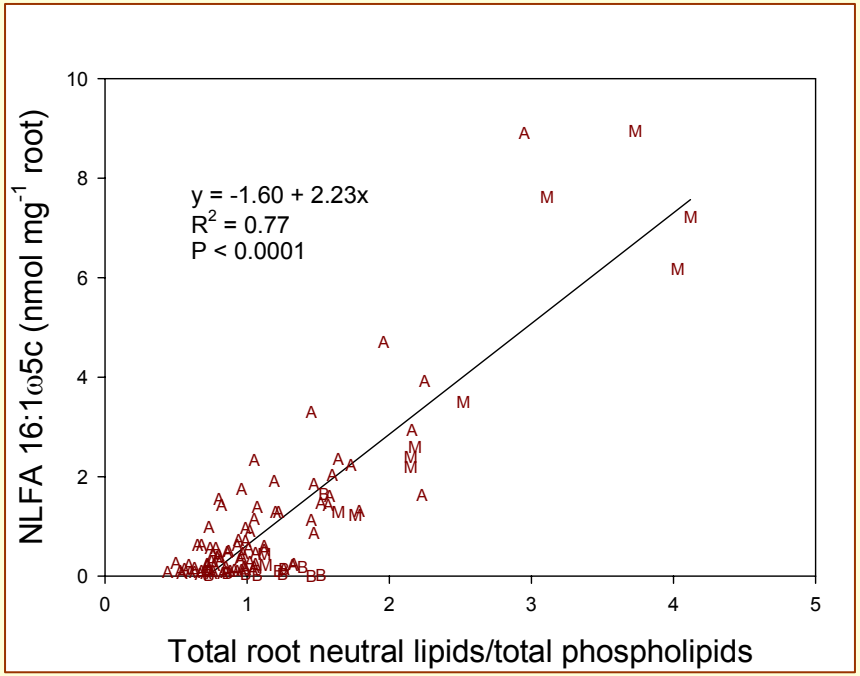
AspenFACE
Treatment effects

- The relationship of AMF lipids is best expressed as the ratio of NLFA-to-PLFA, i.e., the amount of AMF storage lipid per unit of AMF biovolume.



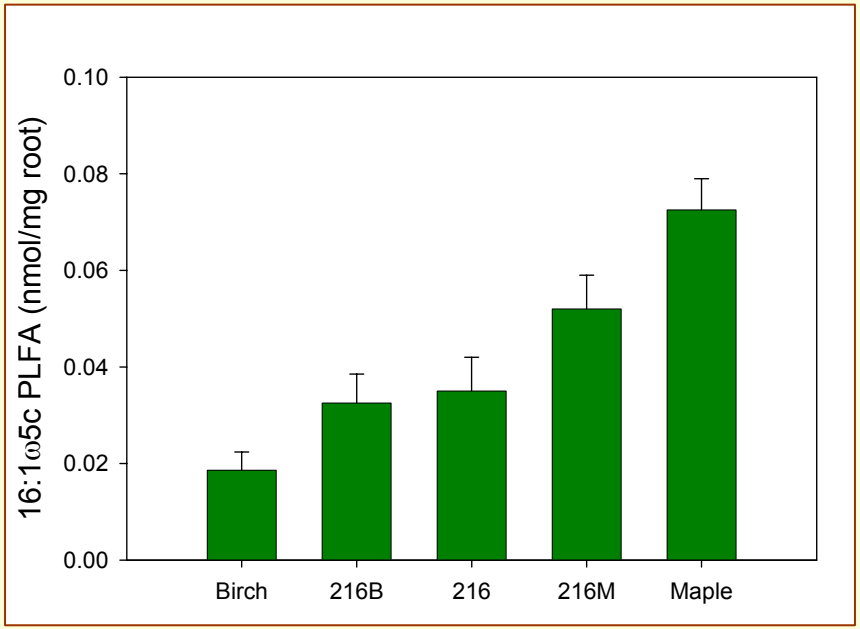
AspenFACE
Treatment effects

- The increase in root NLFA/PLFA ratio appears to be directly related to a concomitant increase in root NLFA 16:1w5c concentration, i.e., an increase in the AMF storage lipid.



The effects of having an EcM or AMF neighbor on Aspen clone 216 AMF biovolume

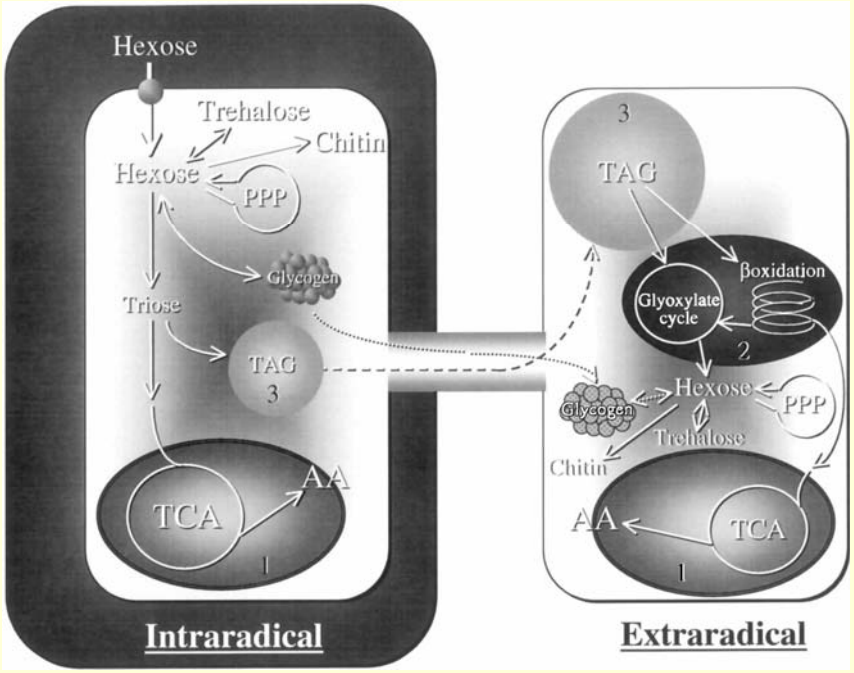
- Birch, an EcM host has quite low levels of the 16:1w5c PLFA
- Maple a highly AMF species, has quite high levels of the PLFA.
- Target aspen trees responded in a manner that one would predict
  - when a neighbor is ECM the clone has reduced PLFA levels
  - when a neighbor is AMF the clone has elevated PLFA levels
- For comparative purposes clone 216 had only other aspen clones as neighboring trees



216=Aspen clone 216B=birch neighbor, and 216M=maple neighbor.

Why high lipid content in AM fungi?

- AM fungi qualify as "oleogenic" fungi with over 25% dry wt as lipids.
  - due to spatial separation of synthesis and utilization of lipids.
    - Lipids produced by IRM and exported to ERM
    - 16:1w5c most abundant TAG
- AMF need compact form of carbon for translocation and storage.
  - Impossible for hyphae to contain carbohydrates at the caloric density equivalent to TAG



Bago et al., 2003

Ongoing experiments at Rhinelander
(collaboration with E. Lilliskov)

Quantification of external allocation of the mycorrhizal fungi

- *Buried mesh bags – 30 µm mesh*
  - *Ergosterol*
  - *Fatty Acids* - PLFAs and NLFAs
    - 16:1ω5c – AMF marker
    - 18:2ω6,9 – EcM marker
  - *Hyphal lengths*
  - *Molecular probes (E. Lilliskov)*

Bags contain 80 cm^{-3} river bottom sand (30*µm* mesh)
9 trees/ring – 3 bags/tree (aspen clone 216)
Placed in soil 7/26/02 - Harvests: May, July and October 2003

Bags at harvest - October 2003
(372 days)

Where next at AspenFACE?

- Buried bags –
  - This allows for quantification of external allocation of ECM and AMF
  - Also can be used for determining fungal composition
- Sequential root harvests
  - Determining PLFA and ergosterol on roots
  - Still working on chitin procedure (problems with standard procedures reproducibility, near FTIR seems better)
  - Work on conversions of PLFAs to biovolume and biomass
  - Follow up on neighbor effects – has relevance to real forests
  - Determine relationship for mycorrhizal biomass and tree biomass allocation