

Mycorrhizal Quantification for Prairie Chronosequence



Nicole Ann Aragon
Washington State University



Introduction

- Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with a variety of plants
- Mycorrhizal roots are those which have a symbiotic relationship with fungi in the soil
- Despite their importance, few studies show the amount of external hyphae that are present in the soil (Sylvia 1992; Miller and Jastrow 1994)



Methods for Quantifying AMF Abundance

- Fatty Acid Analysis
- Hyphal Extraction
- Colonization



Fatty Acid Analysis

- Lipids extracted from freeze-dried soil and roots in a single-phase mixture of chloroform, methanol and water
- Phospholipids (PLFAs) separated from other lipid fractions using silicic acid column chromatography
- Fatty acids quantified and identified by a simultaneously run qualitative standard
- Signature for AMF is 16:1w5c

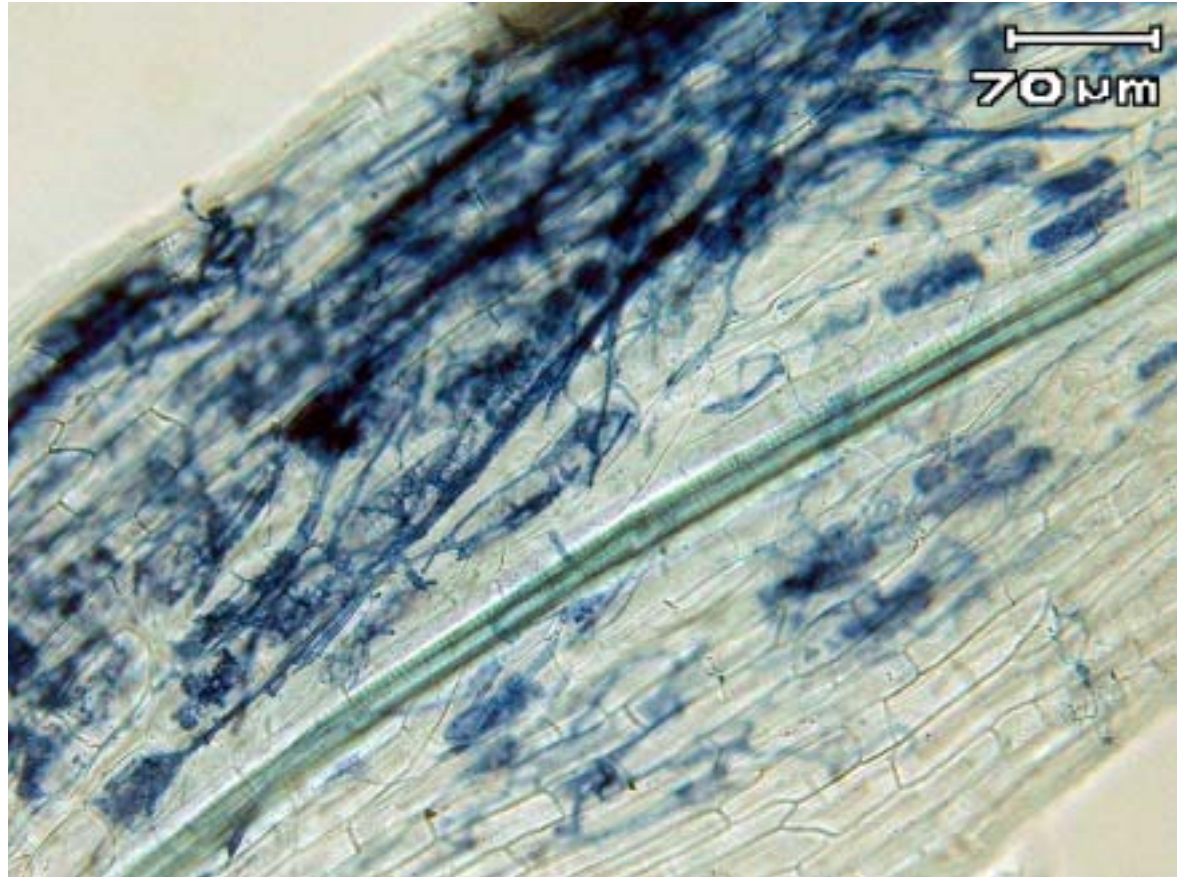
Hyphal Extraction

- Soils diluted and sonicated to break up aggregates
- Centrifuged to separate out heavier organic matter
- Stained in Trypan blue
- Filtered onto a white cellulose nitrate filter
- Quantified by visual scoring on a grid



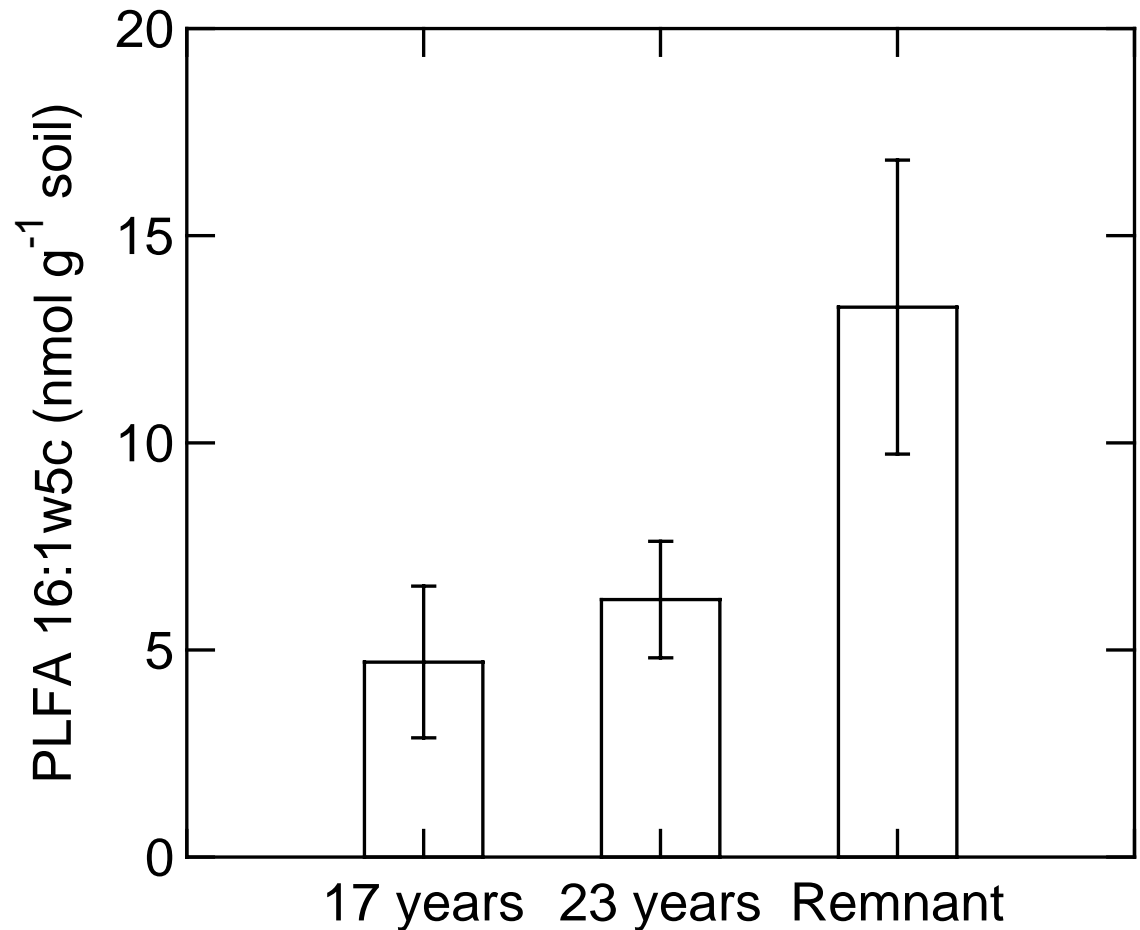
Colonization

- Root colonization determined by scoring slides with roots based on the fungal structures present (coils, hyphae etc.) and the percentage of cover.

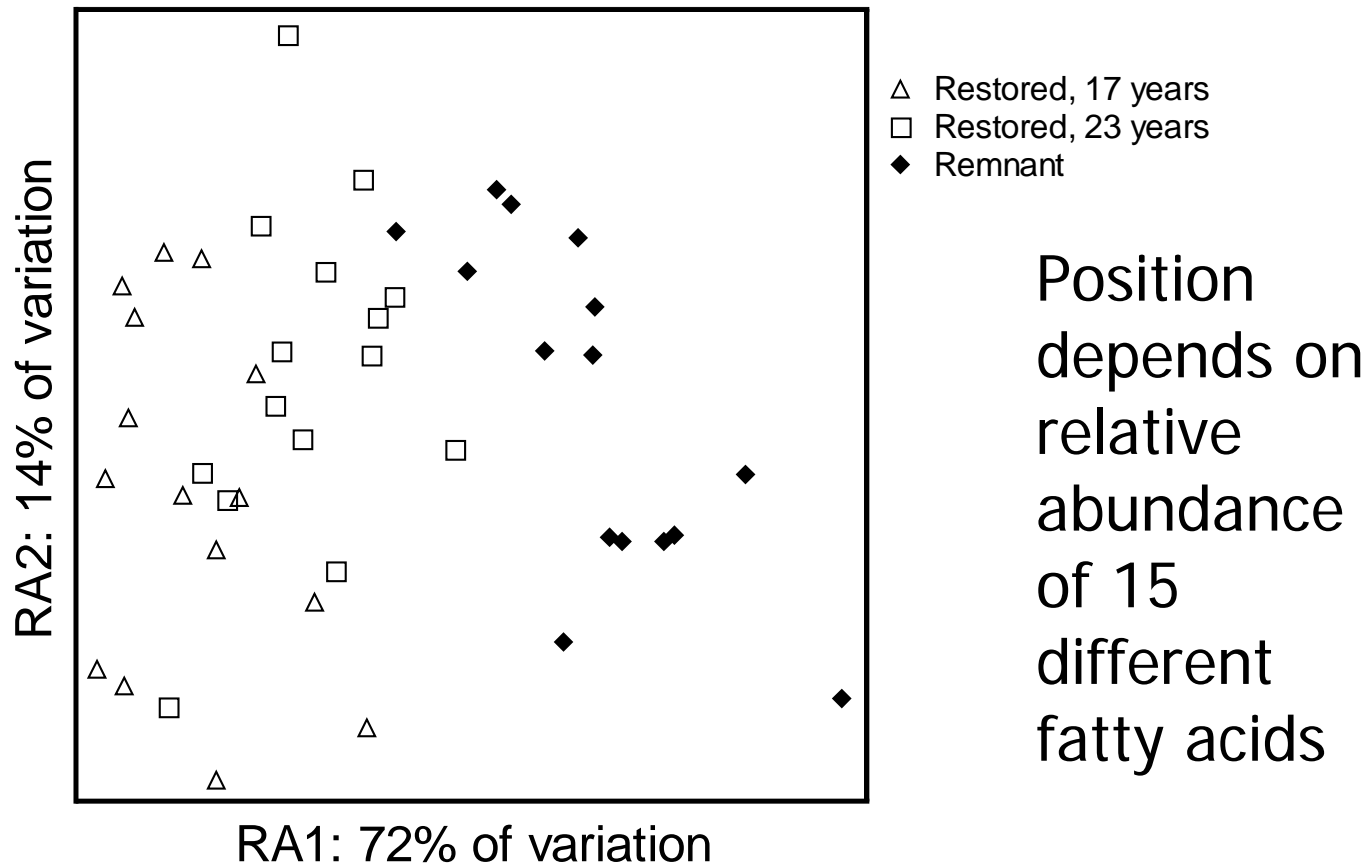


Previous Study

- Previous studies showed increase in AMF with prairie succession using fatty acid analysis

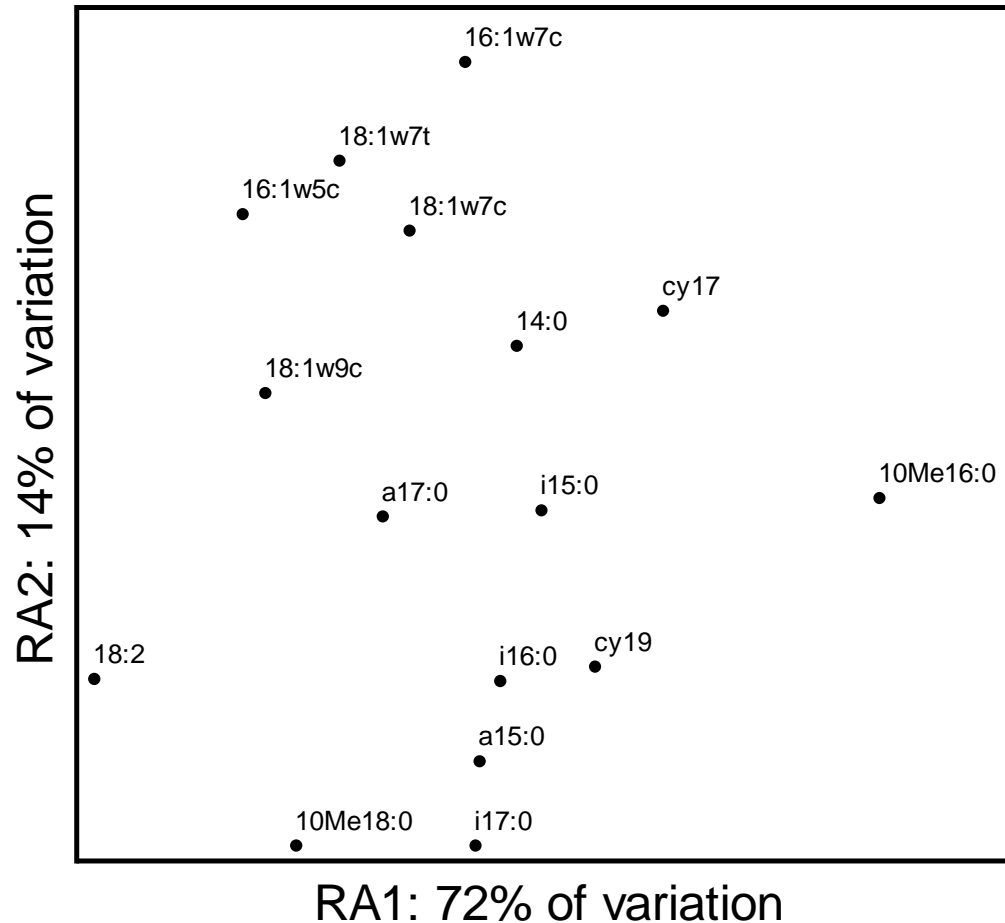


Ordination of Fatty Acid Composition



Fatty Acid Composition

- Most abundant area for each fatty acid





Question

- Is fatty acid analysis a valid method for identifying fungal biomass present in a community?



Alternative Methods Used

- Hyphal Extraction
- Colonization

Samples

- Collected from the National Environmental Research Park at Fermi National Accelerator Laboratory
- Soil cores were taken to a depth of 10cm



Samples

- Samples were taken from:
 - 17 year post reconstruction plot
 - 23 year post reconstruction plot
 - Remnant prairie plot

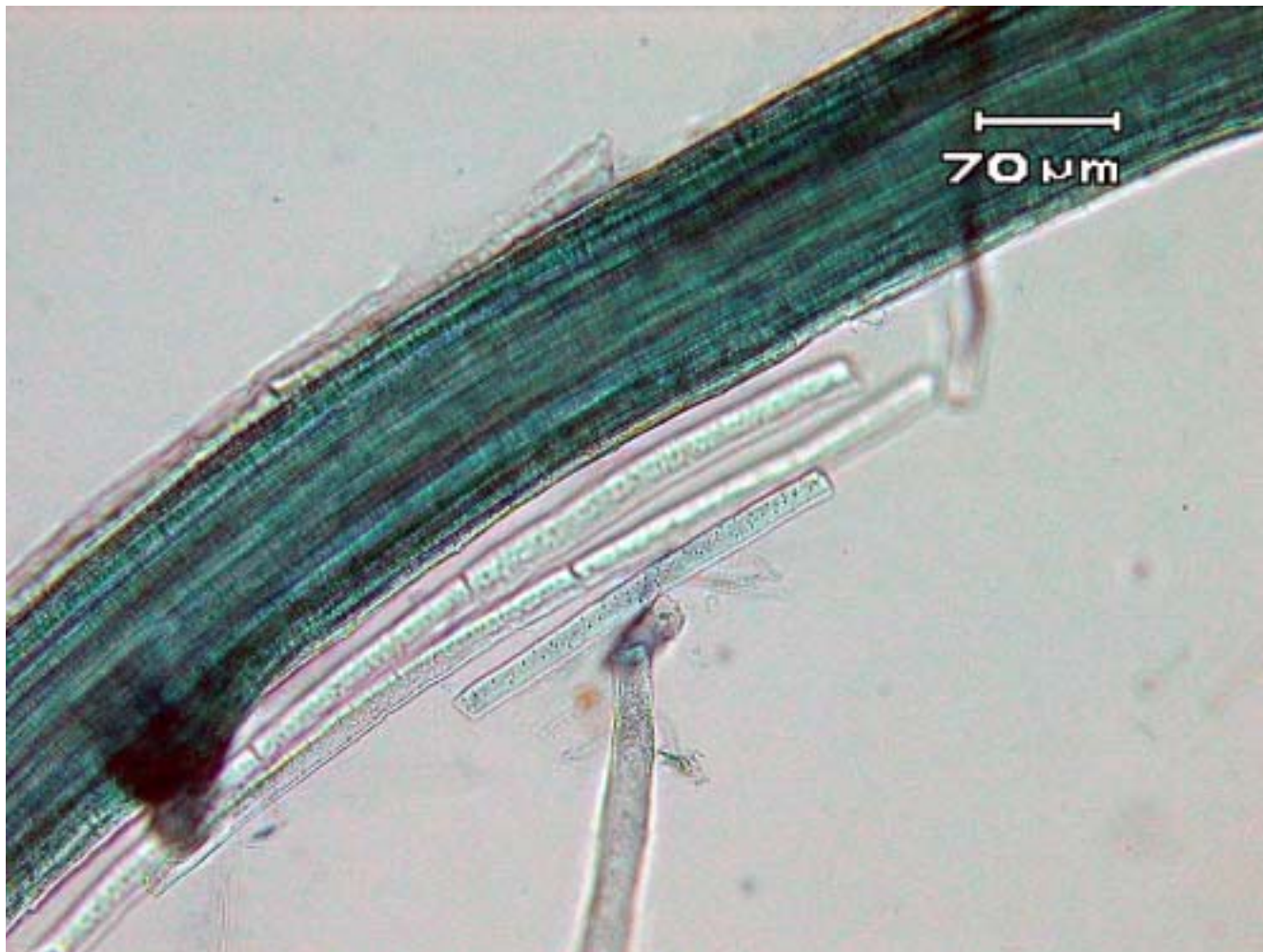




The Staining Process

- Roots are cleared by soaking in KOH solution
 - Over cleared roots may disintegrate
 - Under cleared roots have cell contents which obscure mycorrhizal structures
- Time variances
 - Fine roots need less time than thicker, tougher roots
 - Root mixtures had a variety of species
 - Roots removed from KOH based on appearance

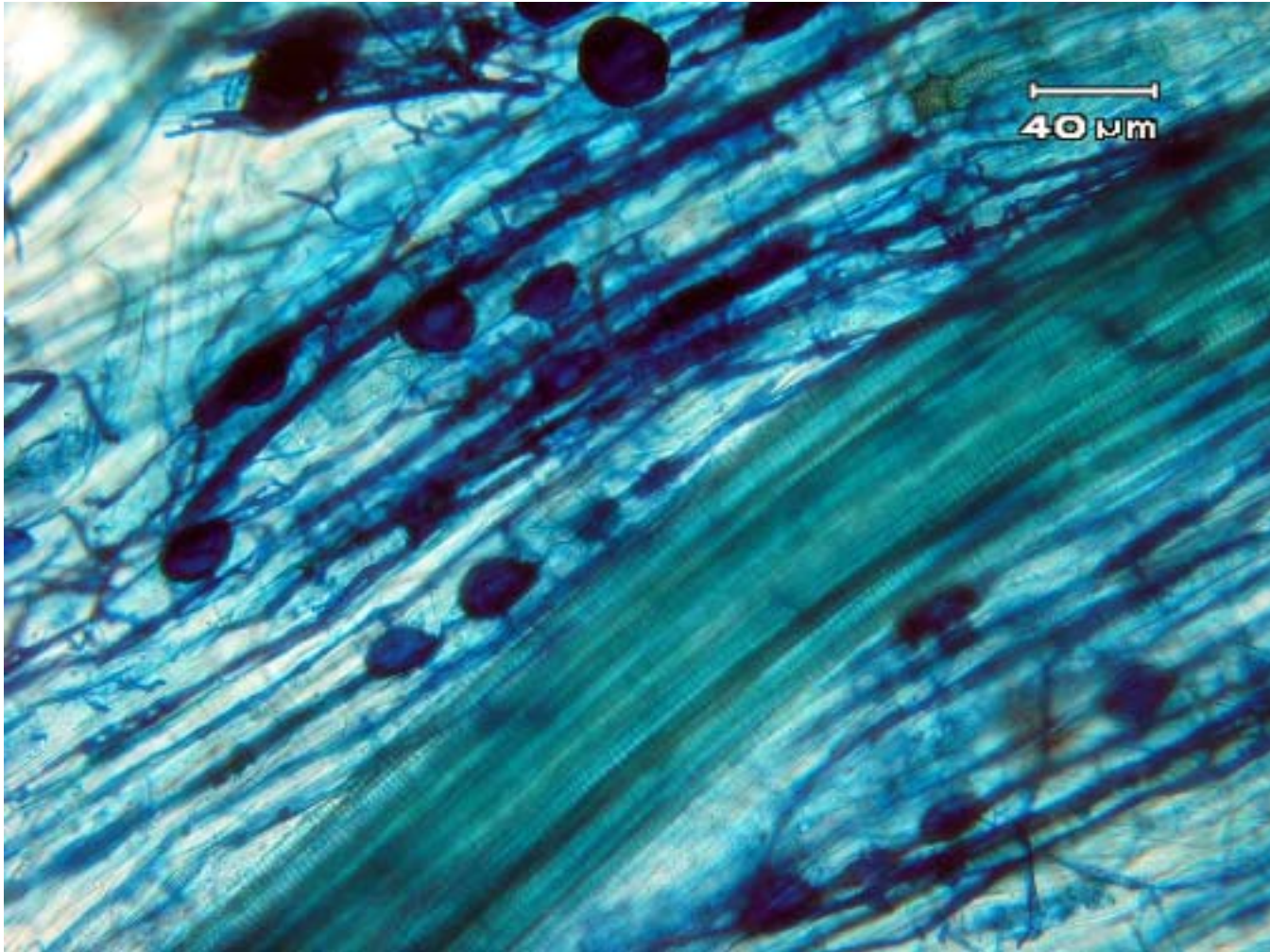
Over Cleared



Under Cleared

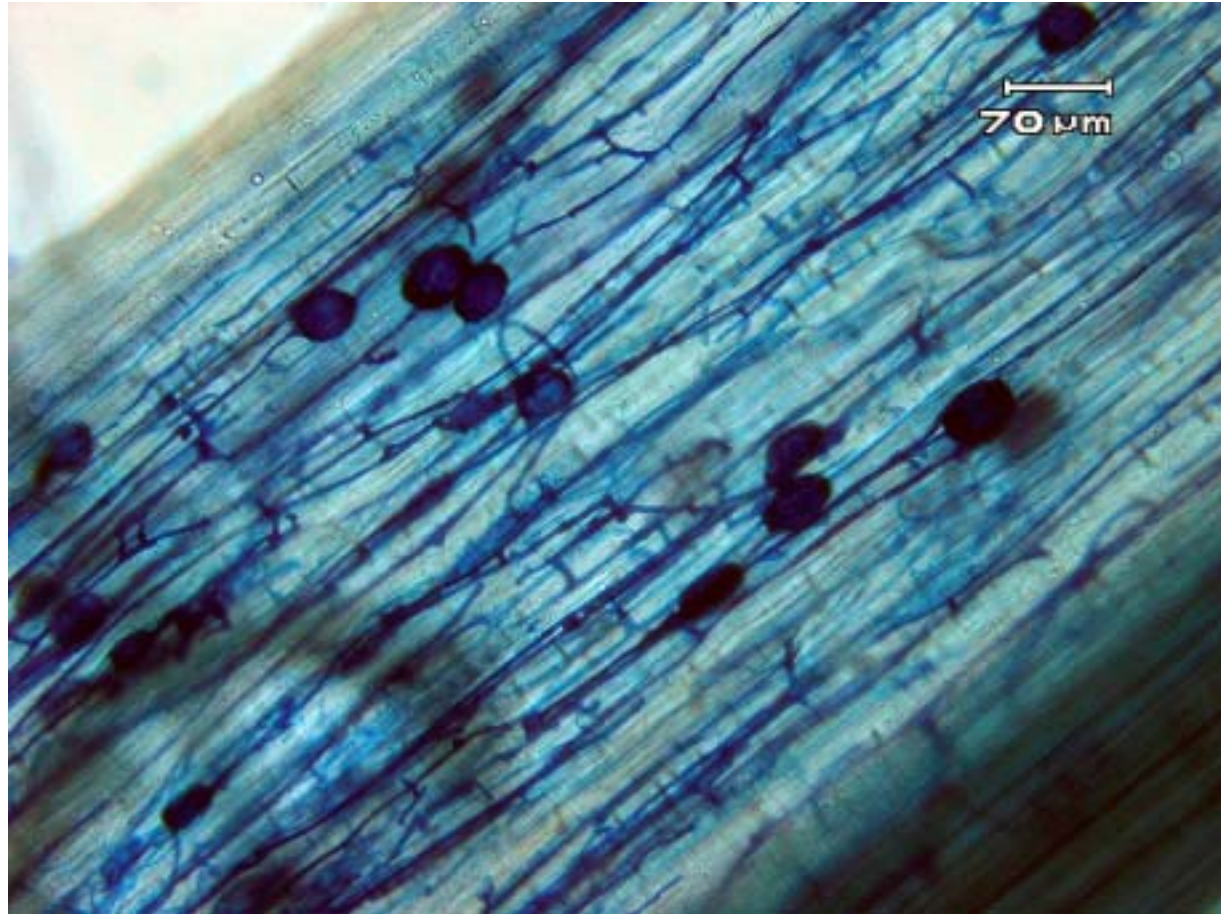


Correctly Cleared



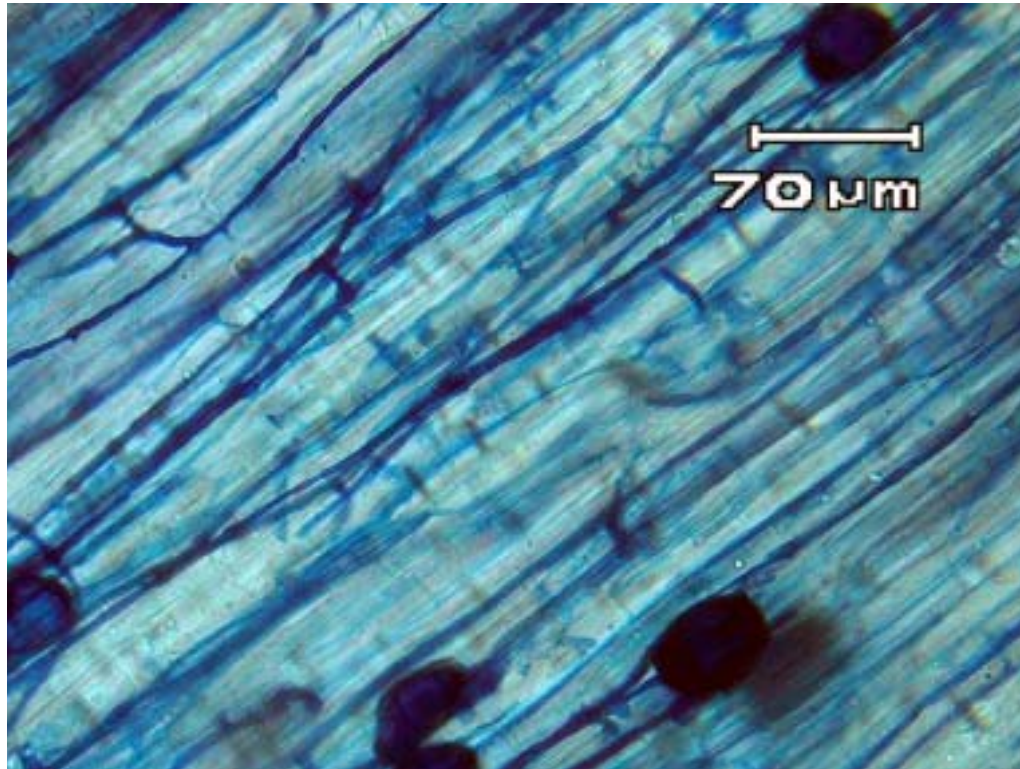
Structures for Colonization

- Hyphae
- Vesicles
- Coils
- Arbuscules



Hyphae

- Filamentous bodies that branch out and increase total root surface area

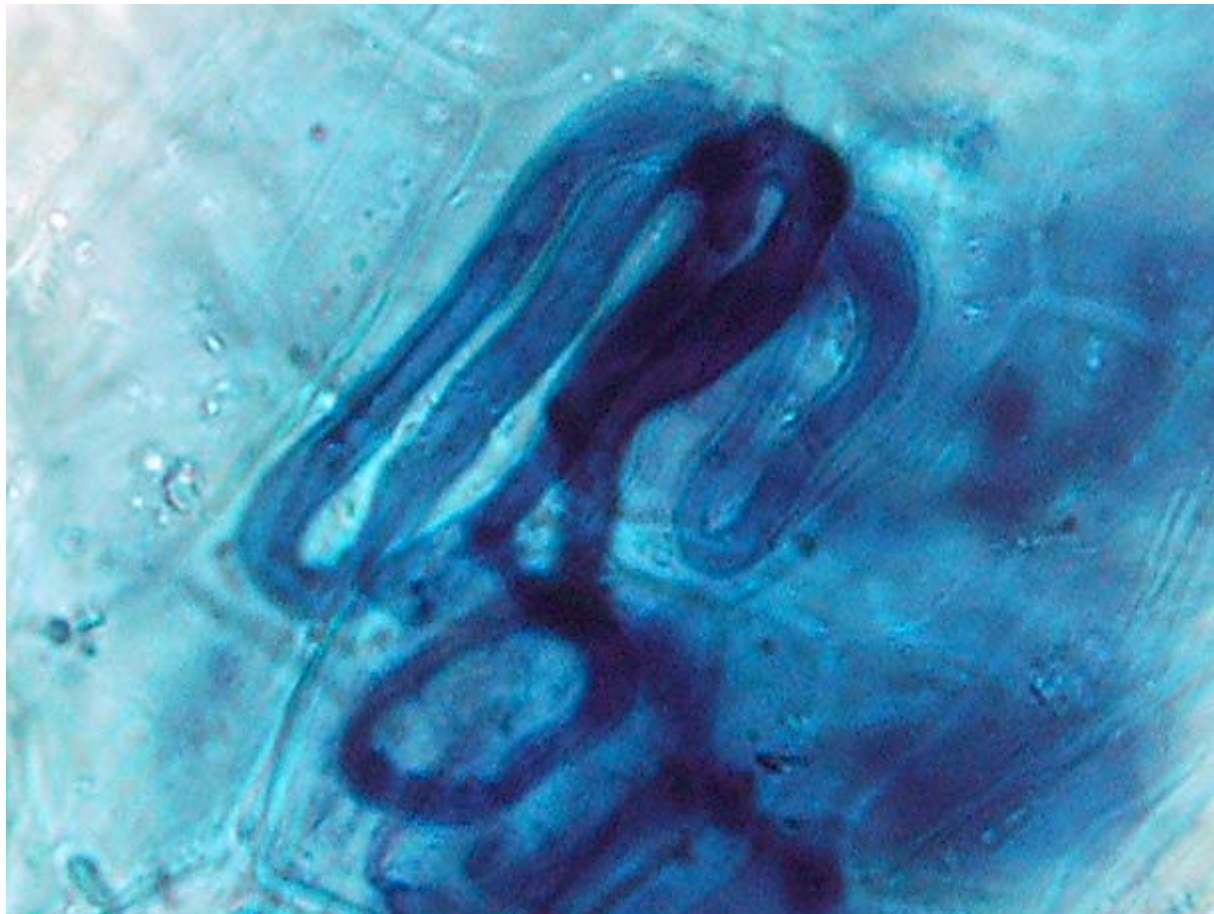


Vesicles

- Vesicles are hyphal swellings that are utilized for storage

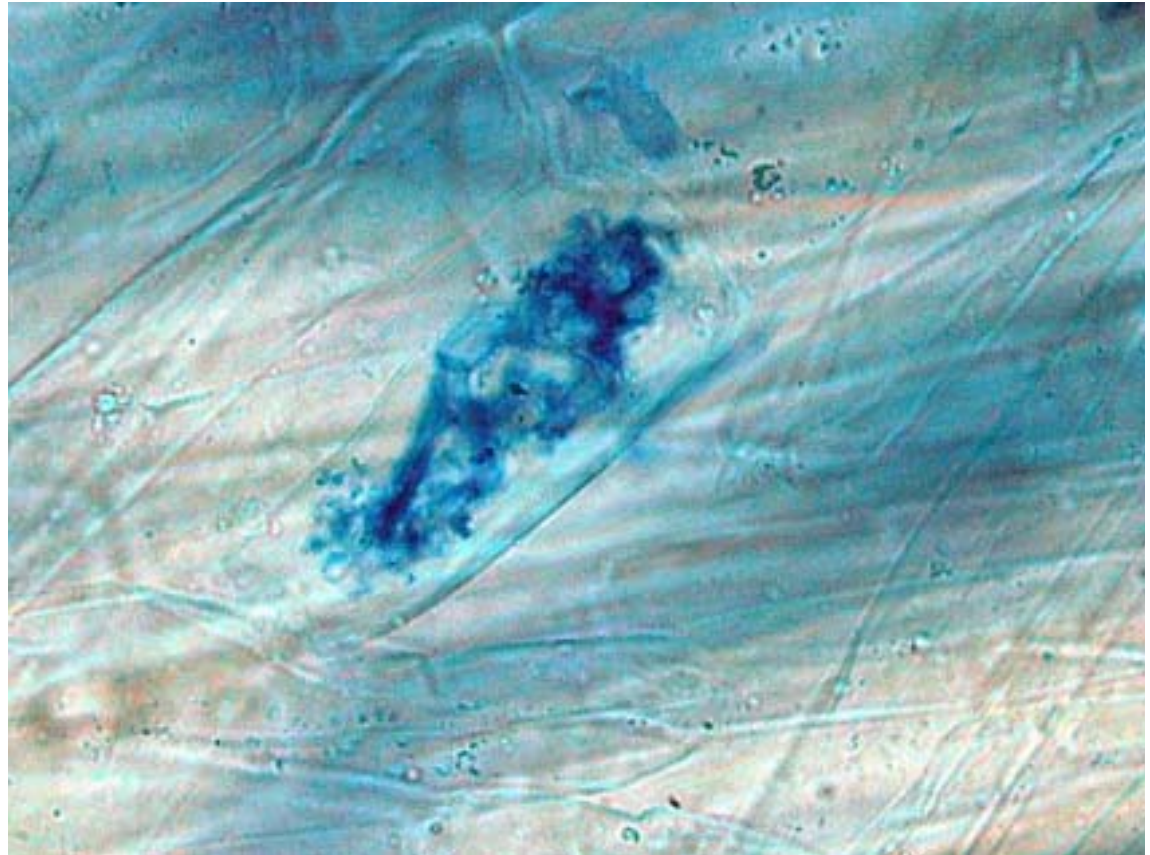


Coils



Arbuscules

- Not always present, but the main distinguishing feature for AMF fungi



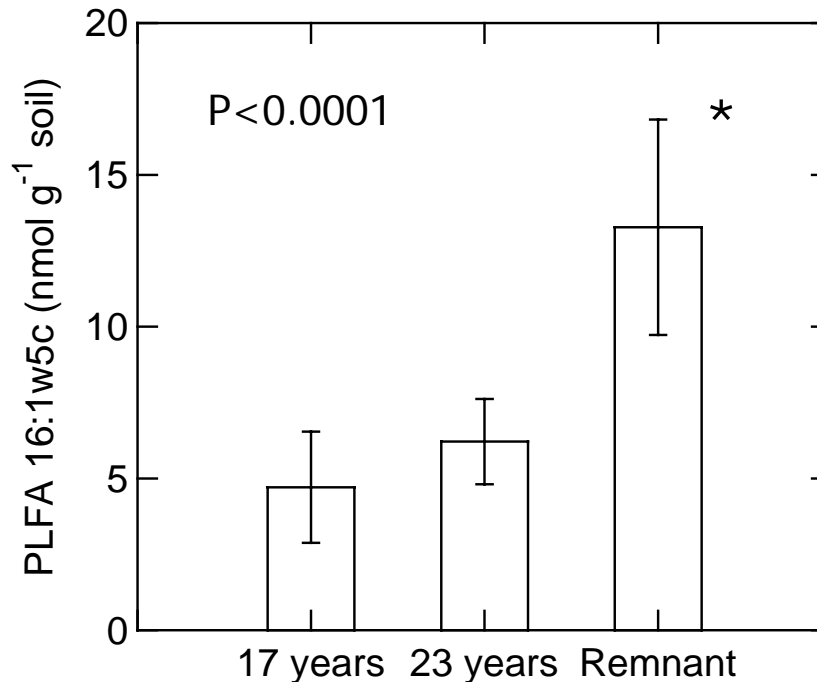


Results

Quantified and analyzed for percent infection and hyphal length.

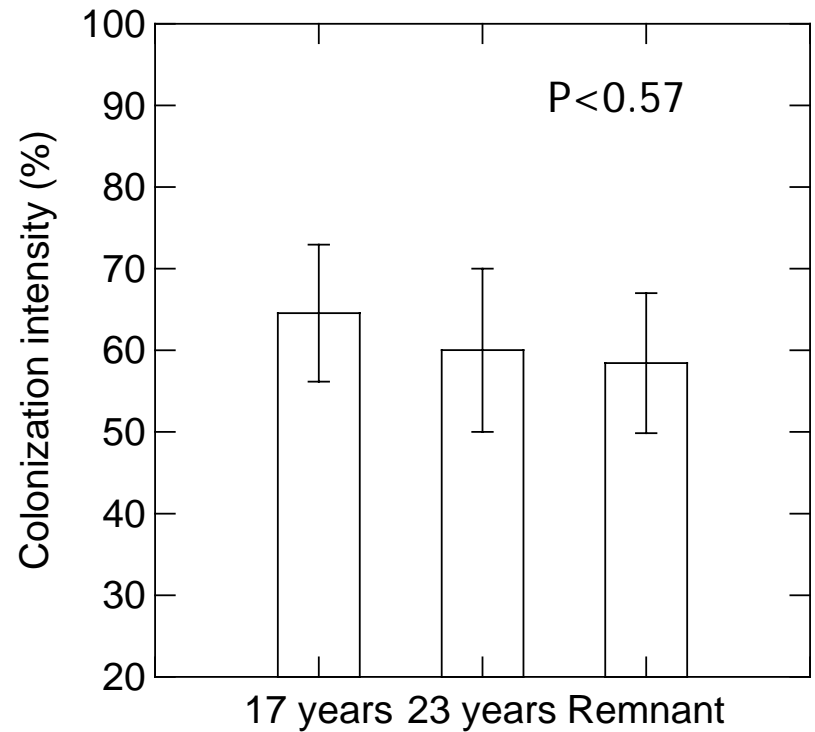
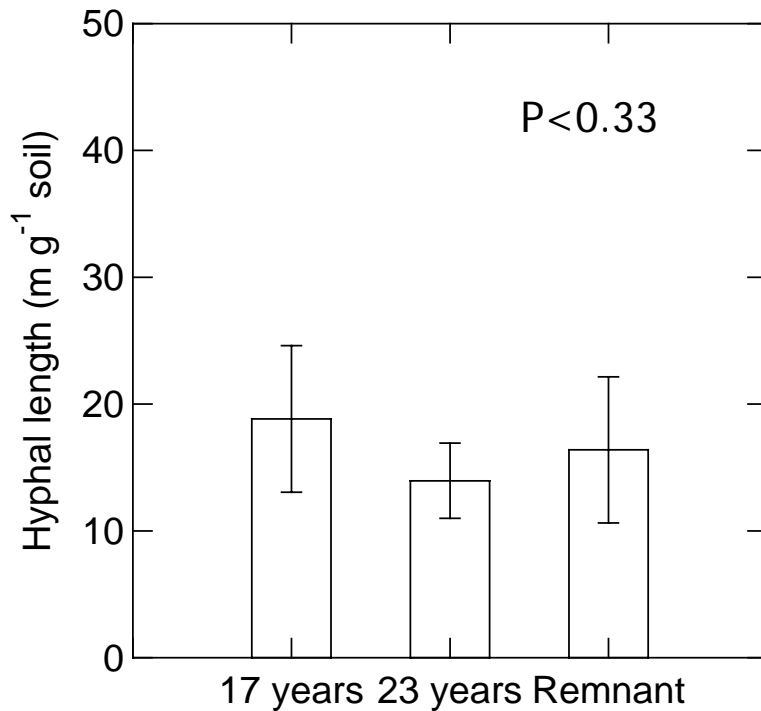
Results

- We hypothesized that our results would correspond to the phospholipid fatty acid analysis
 - Significant differences in site



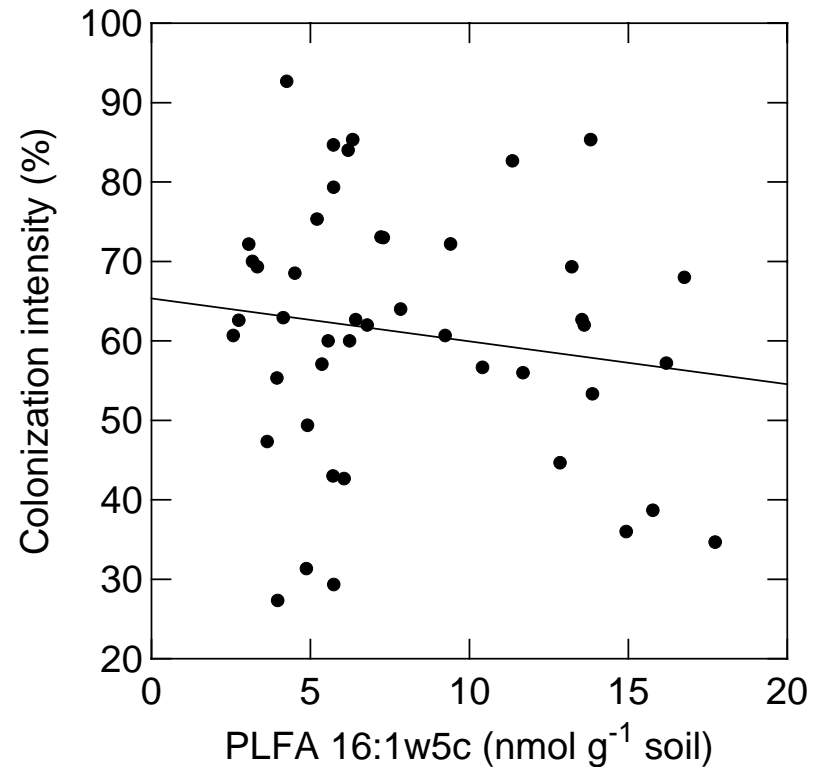
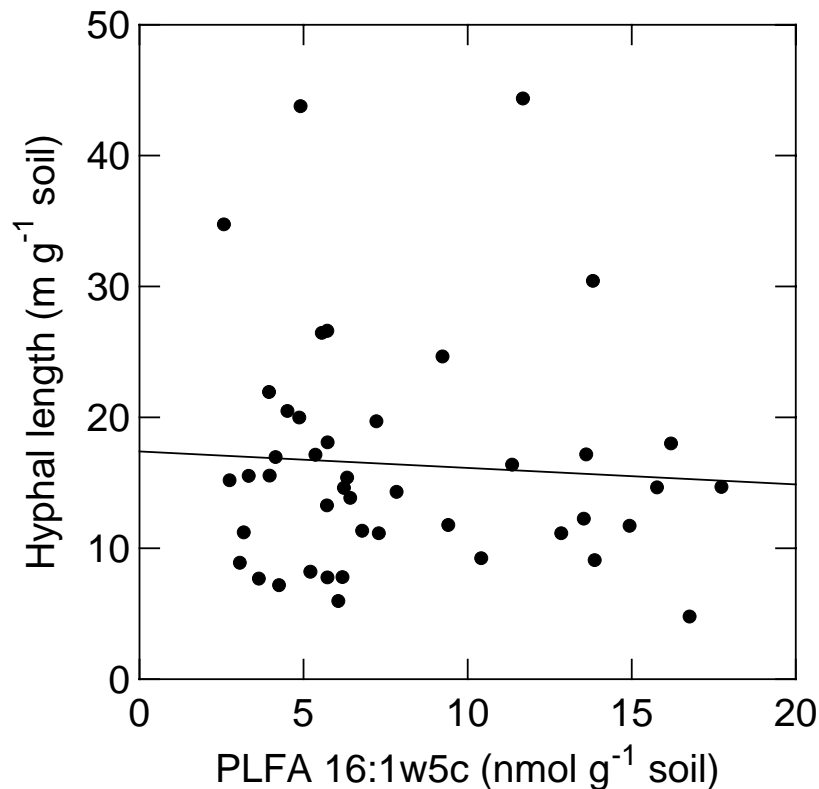
Our results

- No significant differences in site



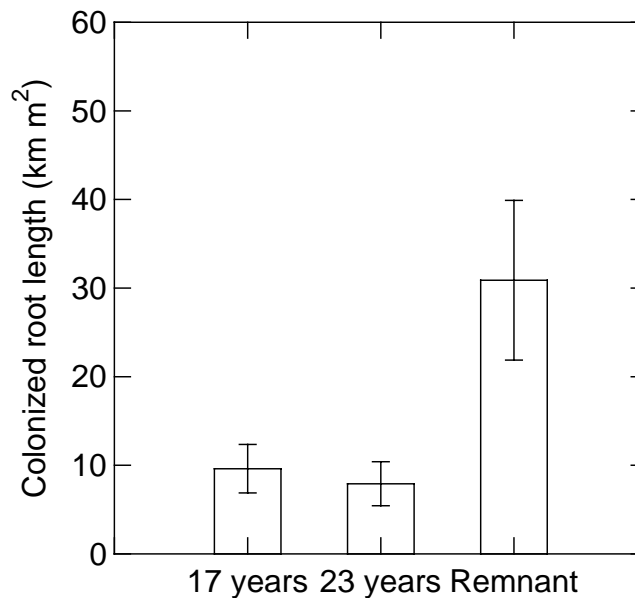
Comparison of the Two Studies

- Is there a relationship between AMF measured using fatty acid 16:1w5c and using hyphal length and colonization?



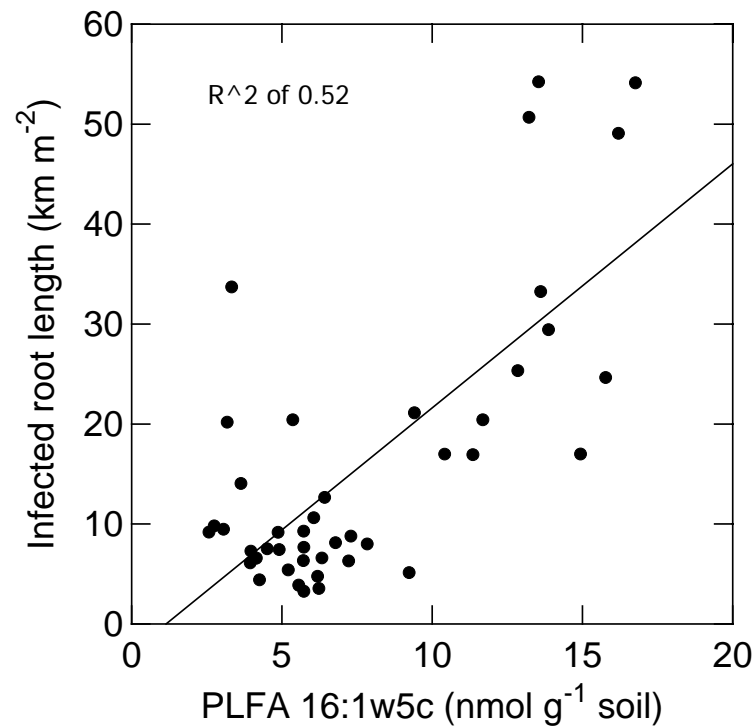
Explanation

- Perhaps the fatty acid analysis results more to do with total colonized root than just percent infection.
- How does colonized root length vary with site?



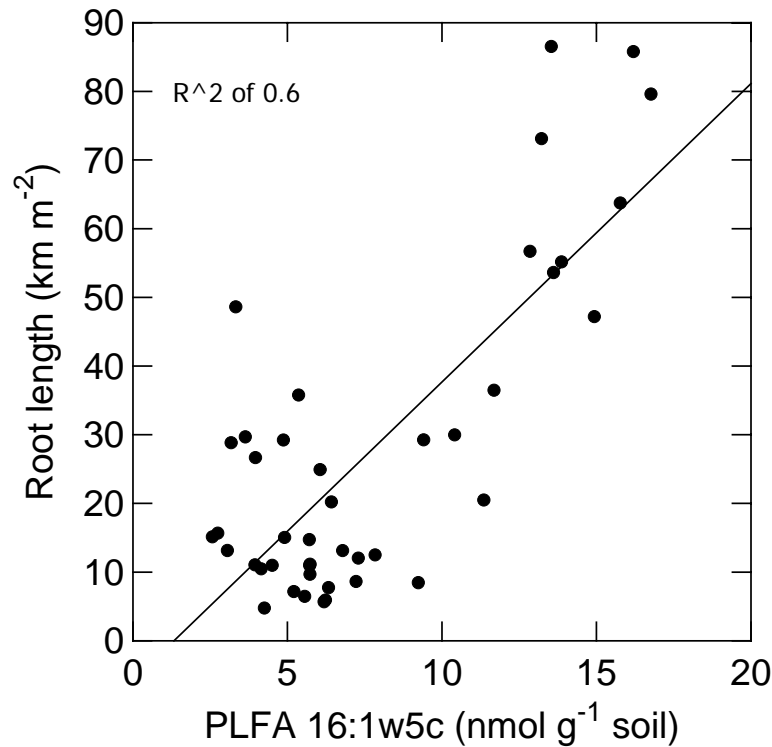
Analysis

- Is there a linear relationship between AMF assessed using fatty acid, and colonized root length?



Analysis

- BUT Maybe the relationship has nothing to do with our measures for colonization? Maybe the relationship is just as strong when only root length is considered?





Relationship?

- Our relationship is actually weakened when we consider percent root colonized and fatty acid analysis vs. root length and fatty acid analysis.
 - R^2 of 0.52 for % colonized
 - R^2 of 0.6 for root length



Possible Errors

- The fatty acid analysis counted living biomass only while the hyphal extraction and colonization counted both living and dead
- Extraction efficiency
 - Significantly more biomass in remnant prairie
 - Root fragments not included in samples
- Aging sonicator
- Centrifuge settings



Continuation

- A follow up on extraction efficiency is being conducted



Acknowledgments

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