THE INFLUENCE HERBACEOUS VEGETATION ON ECTOMYCORRHIZAL ROOT COLONIZATION AND NUTRIENT UPTAKE

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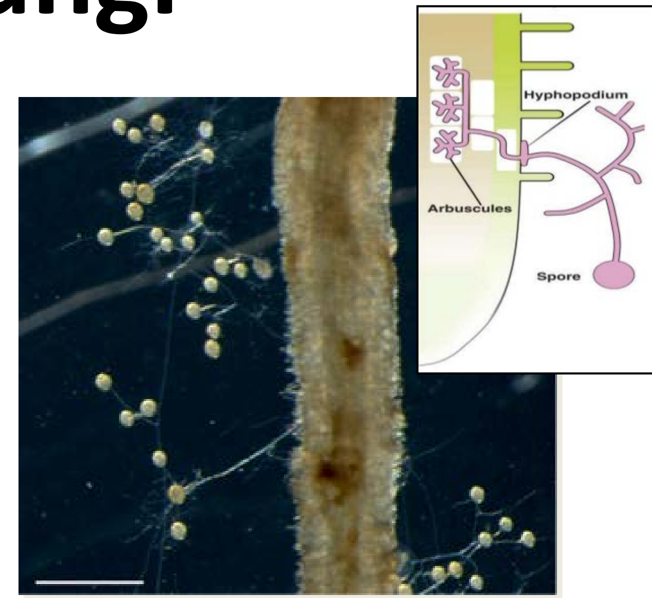
Mycorrhizal Fungi

Mutualistic to both:

- Plant receives water and nutrients
- Fungi receives ecological niche and carbohydrates

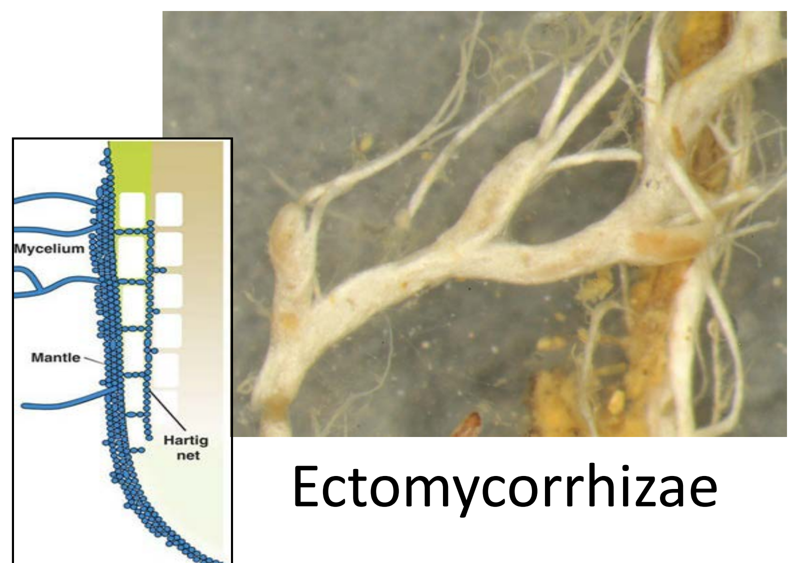


Endomycorrhizae

Prolific in natural systems

Two Distinct Types:

1. Endomycorrhizae
2. Ectomycorrhizae



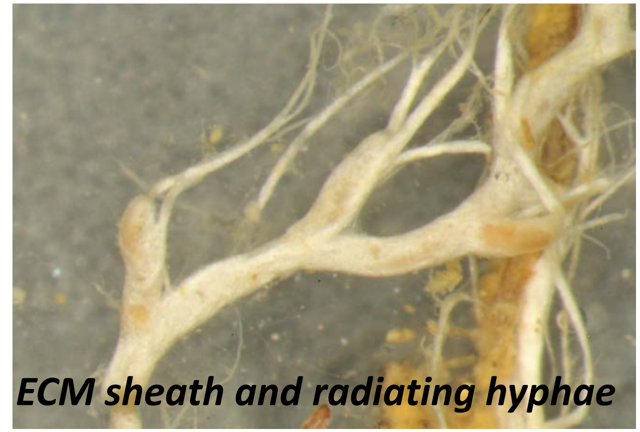
Ectomycorrhizae

Ectomycorrhizae (ECM)

Beneficial Attributes:

- Greater access to water and nutrients
- Tolerance of heavy metals
- Protection from disease
- Provides networks to established trees

Marx 1972; Simard et al. 1996; Cairney and Chambers 1997; Walker et el. 2004; Nara 2005; Bauman et al. 2012



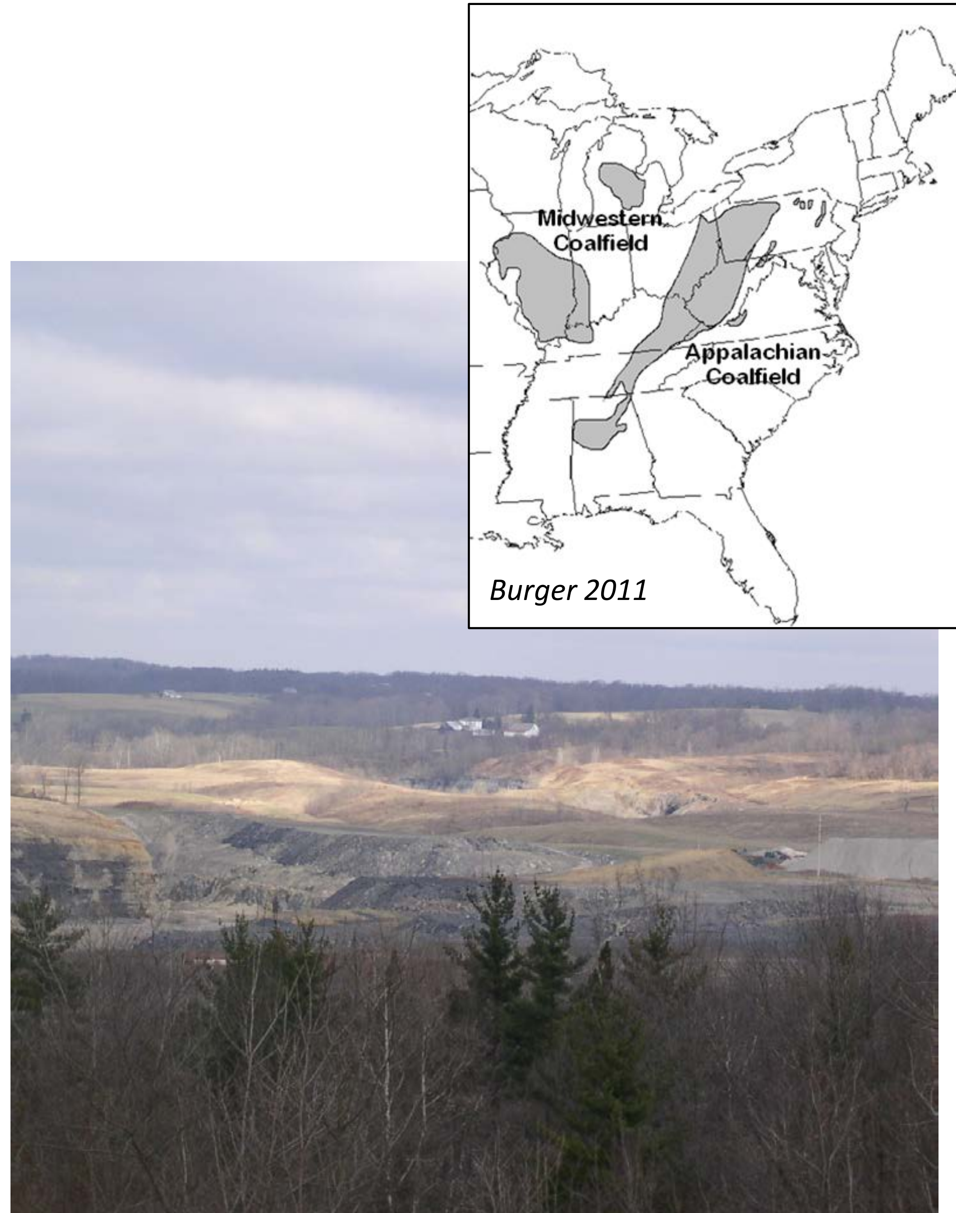
ECM sheath and radiating hyphae

Photo credit: Smith and Read 1997

Coal Mine Reclamation in Appalachian

Forestry Reclamation Approach:

- Appropriate substrate
- Loose soils
- Proper ground cover
- Proper planting methods using a valuable tree species



Burger 2011

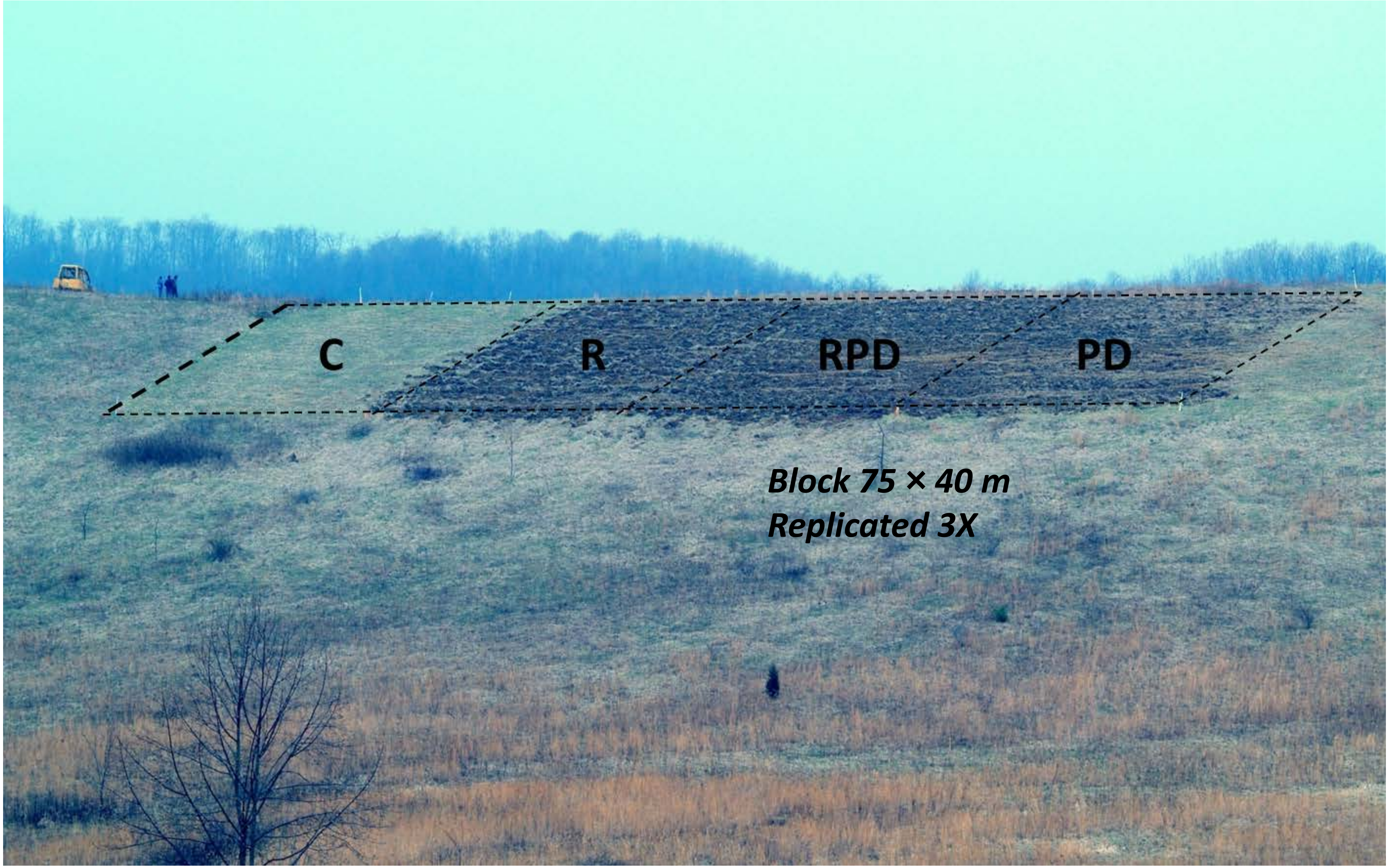
Angel et al. 2005; Burger et al. 2005; Groninger et al. 2007; Zipper et al. 2011; Franklin et al. 2012

American Chestnut in Restoration

American chestnut is an ECM tree host, therefore, it is important to create a planting environment for both the tree and its fungal symbiont

Former Surface Mine Land

Field Testing Planting Methods



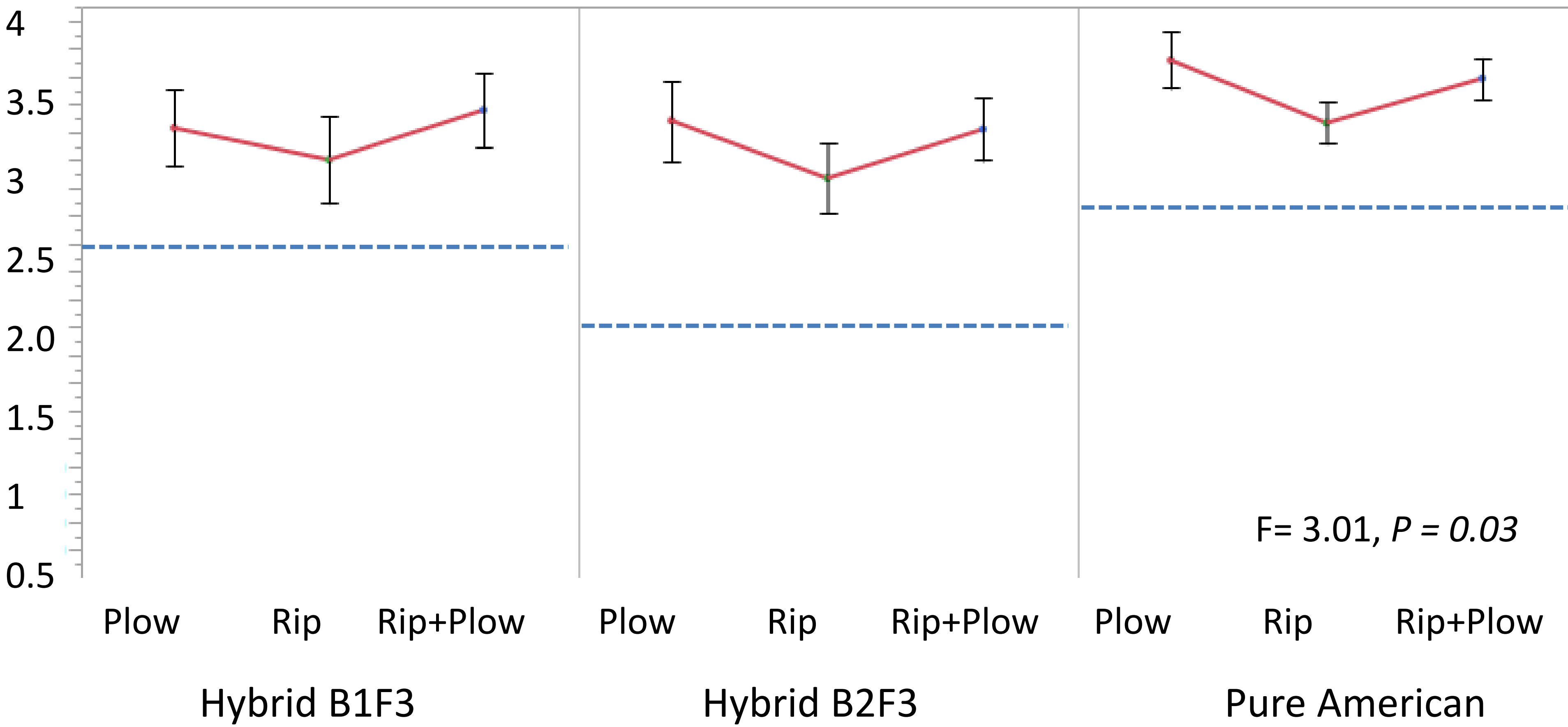
Primary groundcover after 5 years

Species name	Common Name	% Cover
Poa pratensis L.	Kentucky Bluegrass	23.2
Lespedeza cuneata (Dumont) G. Don	Chinese Lespedeza	16.3
Solidago canadensis L.	Canada Golden Rod	10.8
Rudbeckia hirta L.	Black Eyed Susan	10.7
Festuca arundinacea Schreb.	Tall Fescue	6.5
Achillea millefolium L.	Yarrow	4.1

- 34 species were documented across treatments this study
- Five plant species made up 70% of the vegetation sampled
- The two most abundant herbaceous plants were reclamation species
- No difference in vegetation across treatment plots
- One very interesting plant found in vegetation sample…

Five-year-old chestnut hybrids

Chestnut Height (m) After 10 Years



Pure American chestnuts were taller (3.5 m) than the hybrids (3.1 m; F= 3.01, *P = 0.03).*

ECM Survey Methods

Seedlings were sampled non-destructively by trenching dormant trees, morphotyped and fungal DNA was sequenced

Field Sample

Quantify

Describe and Voucher

Methods: Metals in Soils

Element analyzed	Drip line mean (ppm)	Drip line 95% CI	Site reference	County ave (ppm)	Ohio soil (ppm)
Ag	0.45	[0.30, 0.74]	0.24	<1	0.25
Al	11,172	[9,909, 13,261]	9983.4	NA	7,685
As	10.62**	[8.68, 11.07]	12.78	7.9	5.72
Cd	0.21**	[0.13, 0.24]	0.27	0.2	0.507
Cu	26.0	[21.60, 32.25]	23.38	16	12
Mn	767.84	[644.5, 915.7]	732.68	459	459
Pb	14.37	[12.70, 15.94]	14.54	28	16.2
Se	3.55*	[2.05, 4.25]	4.71	0.3	0.25
Zn	75.07	[64.35, 92.0]	66.18	65	42.7

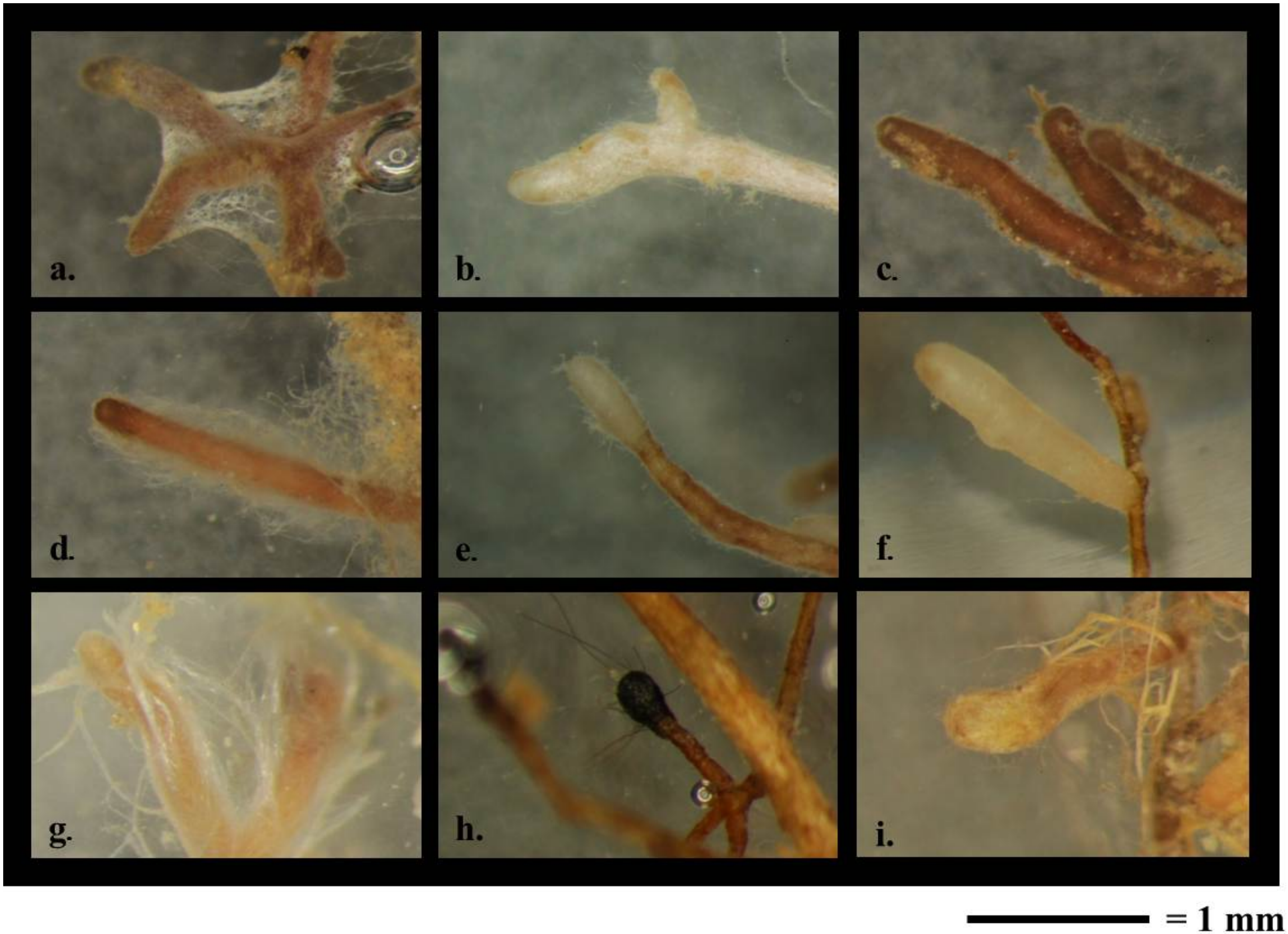
Mean concentrations of metals in drip line soils compared with average background metal concentration in county and state soils

Methods: Metals in Tissue

Chestnut foliage (ppm)	95% CI	Chestnut floral (ppm)	95% CI	Range in plant tissues
0.15	[0.06, 0.24]	0.11	[0, 0.22]	0.05–1.5
74.83	[58.96, 90.71]	41.70	[17.47, 65.90]	30–250
BDL	—	BDL	—	0.009–1.5
BDL	—	BDL	—	0.1–2.4
0.91	[−0.5, 2.34]	23.90	[14.35, 33.38]	4–15
965.18	[823.7, 1106.7]	662.08	[544.75, 779.41]	20–400
0.48	[0–0.97]	0.37	[−0.21, 0.95]	0.1–10
2.48	[1.36, 3.6]	7.12	[5.50, 8.74]	<10
64.79	[29.19, 100.39]	58.0	[14.51, 101.39]	27–100

Mean concentrations of metals in foliage, flowers, and nuts of chestnuts and compared with ranges of metals in plant tissue (ppm) as reported in the literature

ECM Morphotypes



Community Composition Among Years

ECM on 2-yr-old	%	ECM on 6 -yr-old	%
Hebeloma sp.1	31	*Cortinarius* sp. 1	44
Hebeloma sp. 2	20	*Cenococcum* sp.	20
Cortinarius sp. 1	16	*Cortinarius* sp. 3	10
Scleroderma sp. 1	9	*Scleroderma* sp. 1	6
Thelephora sp.	7	*Cortinarius* sp. 4	4
Unknown ECM 2	4	*Russula* sp.	3
Hebeloma sp. 3	3	*Scleroderma* sp. 2	3
Laccaria sp.	3	*Thelephora* sp.	2
Unknown ECM 1	2	*Inocybe* sp.	2
Scleroderma sp. 2	1	*Cortinarius* sp. 2	2
Cortinarius sp. 2	1	Unknown 1	1
Pisolithus sp.	1	*Sebacinales* sp.	< 1
Tomentella sp.	< 1	*Laccaria* sp	< 1
Cenococcum sp.	< 1	*Tomentella* sp.	< 1
Thelephoraceae	< 1	*Lactarius* sp.	< 1
		Cantherellaceae	< 1
Average Root Colonization	44%	Average Root Colonization	58%

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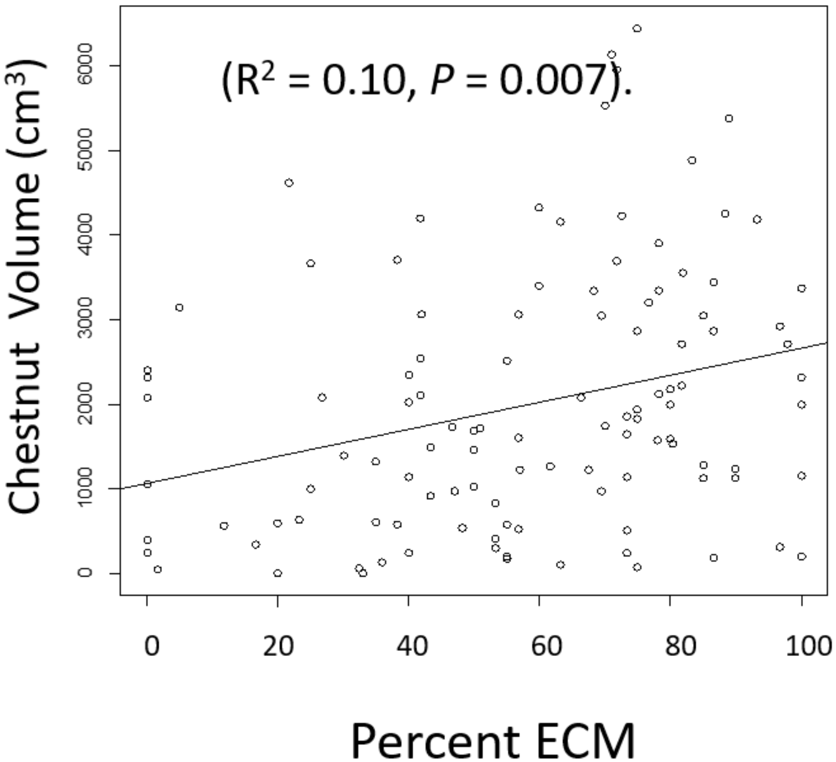
Laccaria sp.	3	*Thelephora* sp.	2
Unknown ECM 1	2	*Inocybe* sp.	2
Scleroderma sp. 2	1	*Cortinarius* sp. 2	2
Cortinarius sp. 2	1	Unknown 1	1
Pisolithus sp.	1	*Sebacinales* sp.	< 1
Tomentella sp.	< 1	*Laccaria* sp	< 1
Cenococcum sp.	< 1	*Tomentella* sp.	< 1
Thelephoraceae	< 1	*Lactarius* sp.	< 1
		Cantherellaceae	< 1
Average Root Colonization	44%	Average Root Colonization	58%

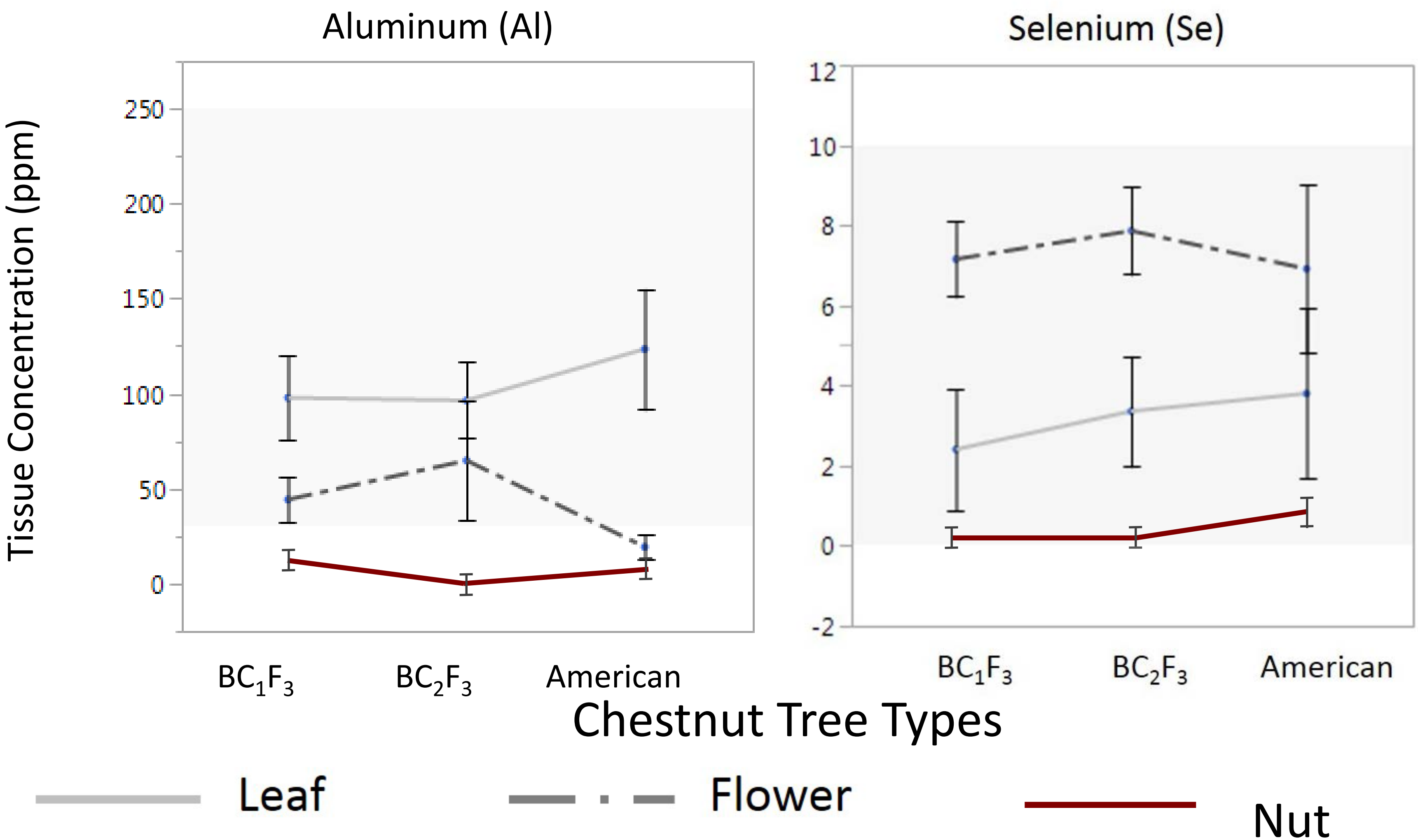
DNA sequencing confirmed 22 sequences and ECM colonization increases over time

Plant and ECM Interactions



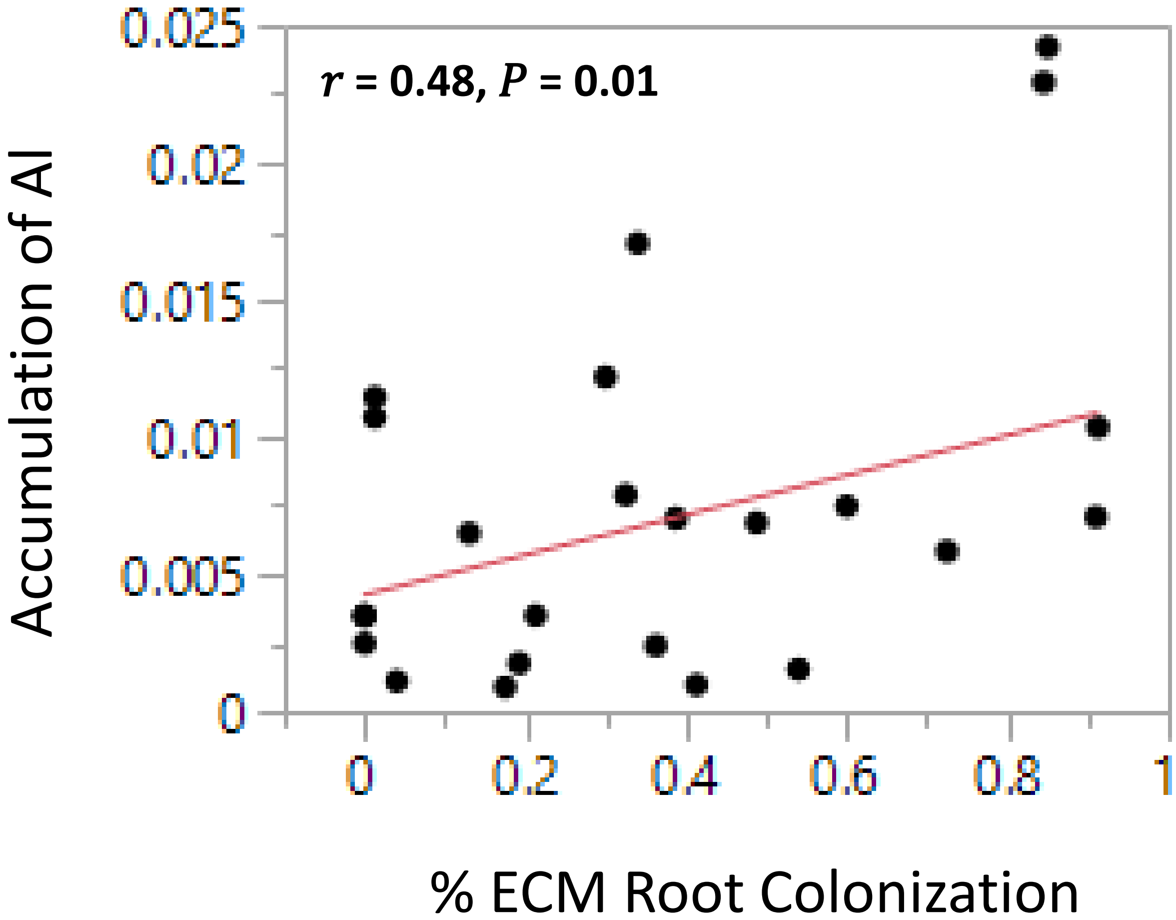
It is not clear whether ECM activity was the driver of plant growth, or if growth contributed to ECM colonization.
Both are strong indicators of healthy tree establishment.

Micronutrients and Metals in Tissue



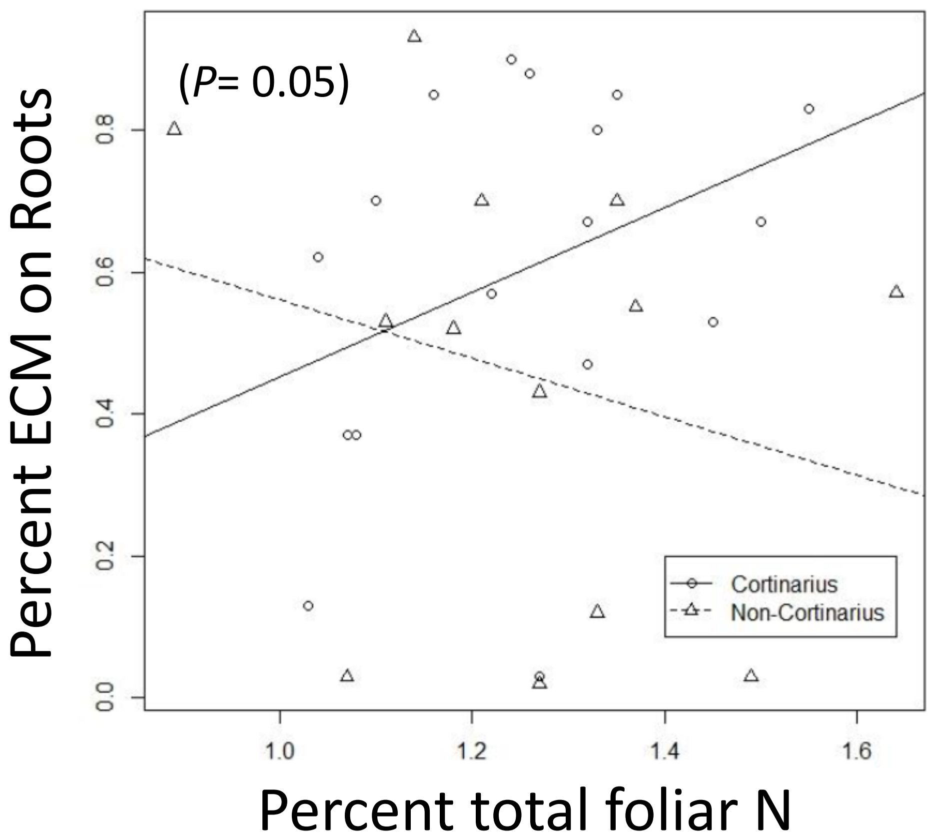
Metals in normal to low levels, nut tissue safe for consumption

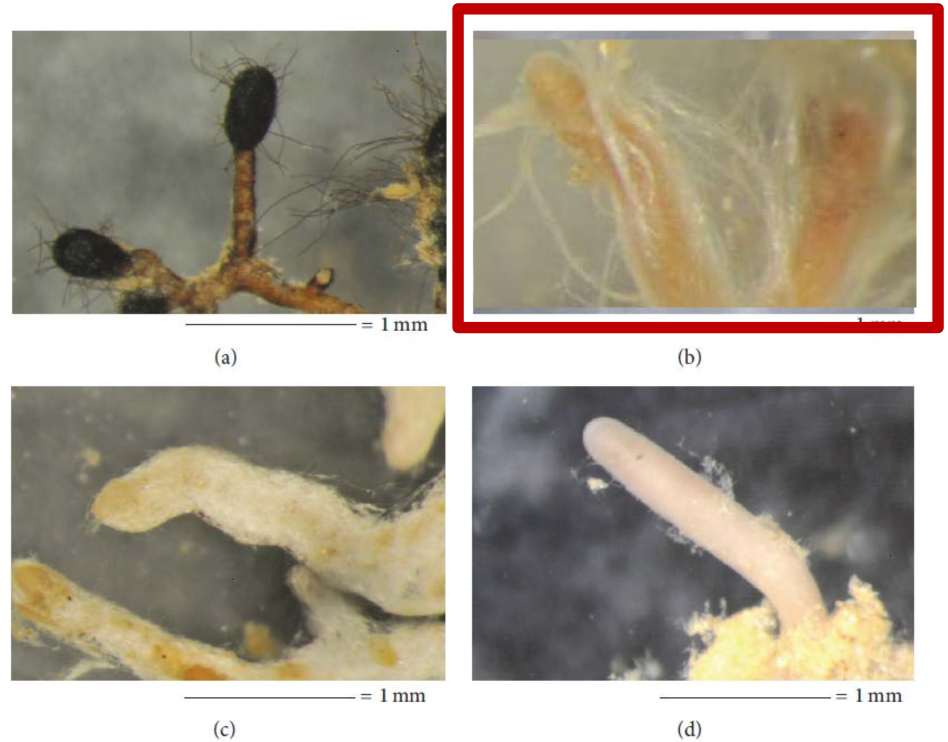
Nutrient Dynamics and ECM



Of the metals, % ECM on roots was significantly correlated to aluminum

Macronutrients





A positive correlation existed between *Cortinarius* ECM colonization and foliar nitrogen in seedling tissue

Eastern Tennessee- Site Differences

Low Groundcover diversity

Medium groundcover diversity

High groundcover diversity

The objective of this study is to investigate the plant cover communities' influence on ECM root colonization, species composition and nutrient uptake

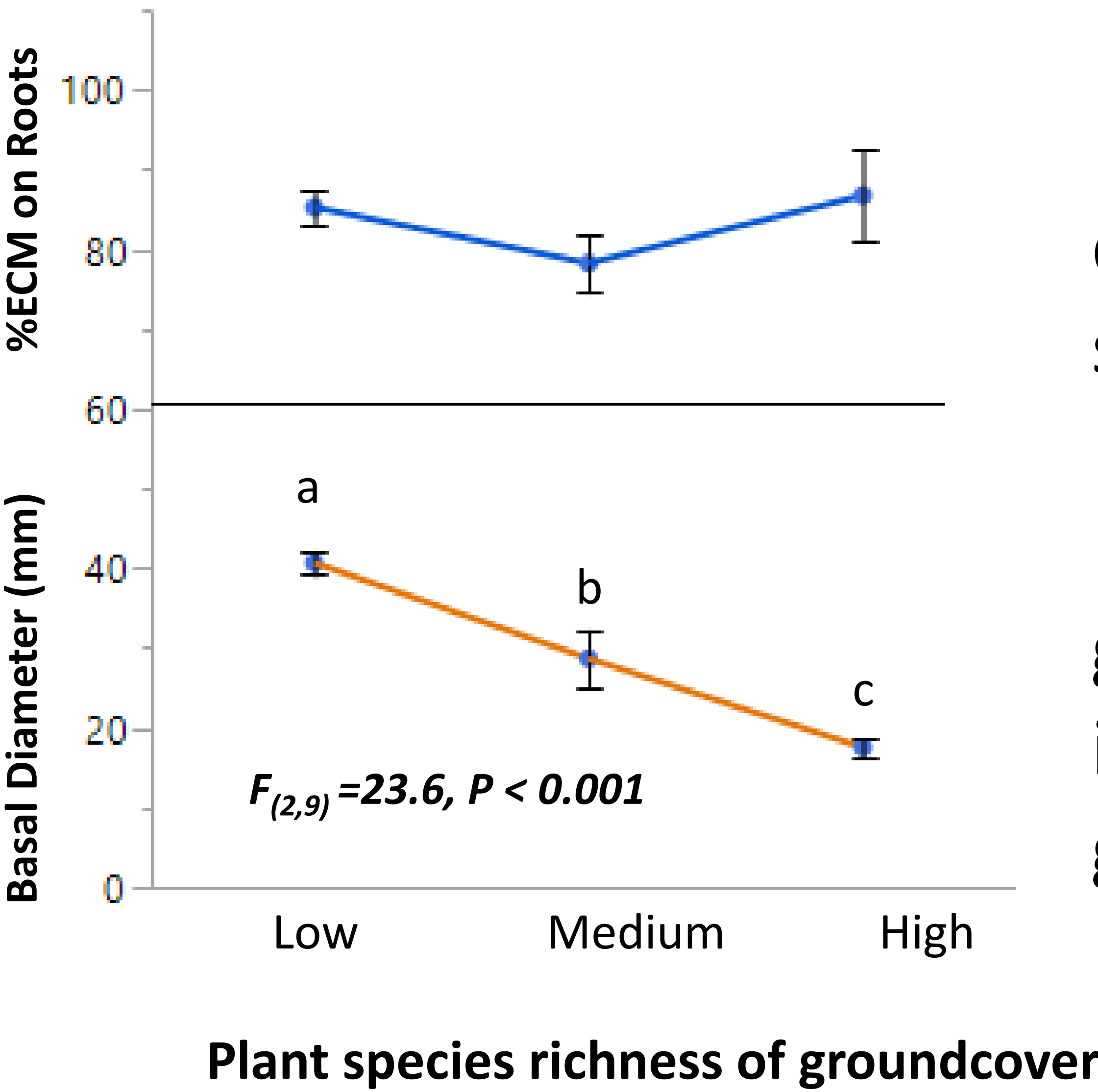
Eastern Tennessee- Site Differences

Reclamation Methods:

These sites were reclaimed in 2009 using end-dumping

2016-2017 - ECM, soil nutrient and metal analysis was the same as the Ohio study

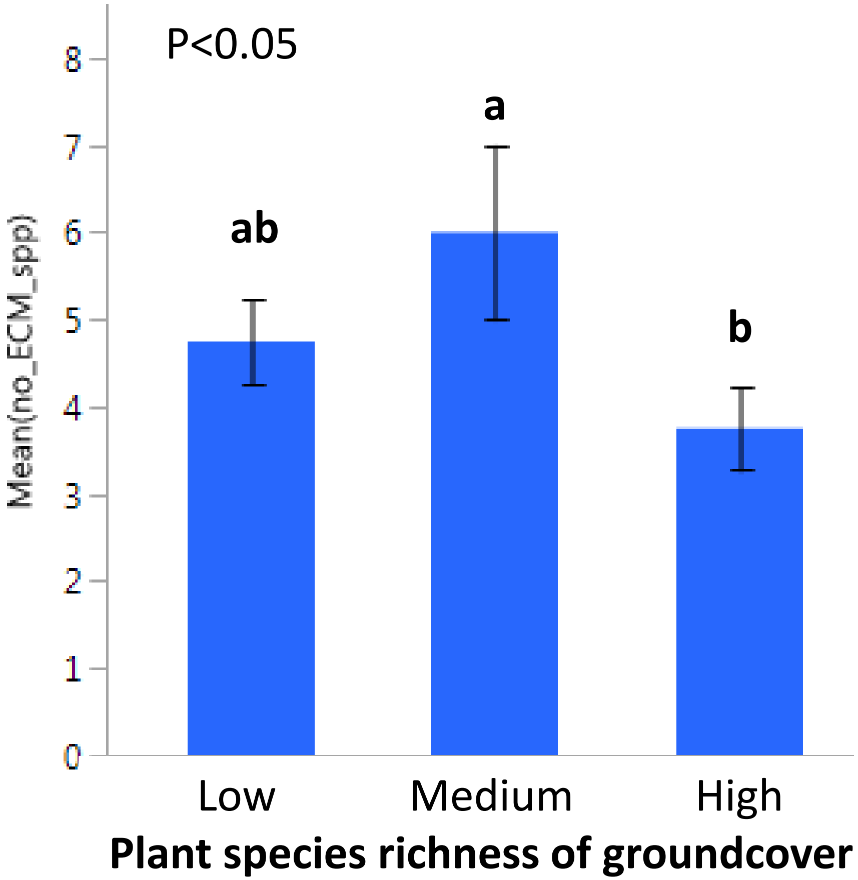
Comparing ECM and Growth



ECM colonization did not differ among sites

However, chestnut growth was higher in the low diverse groundcover plots

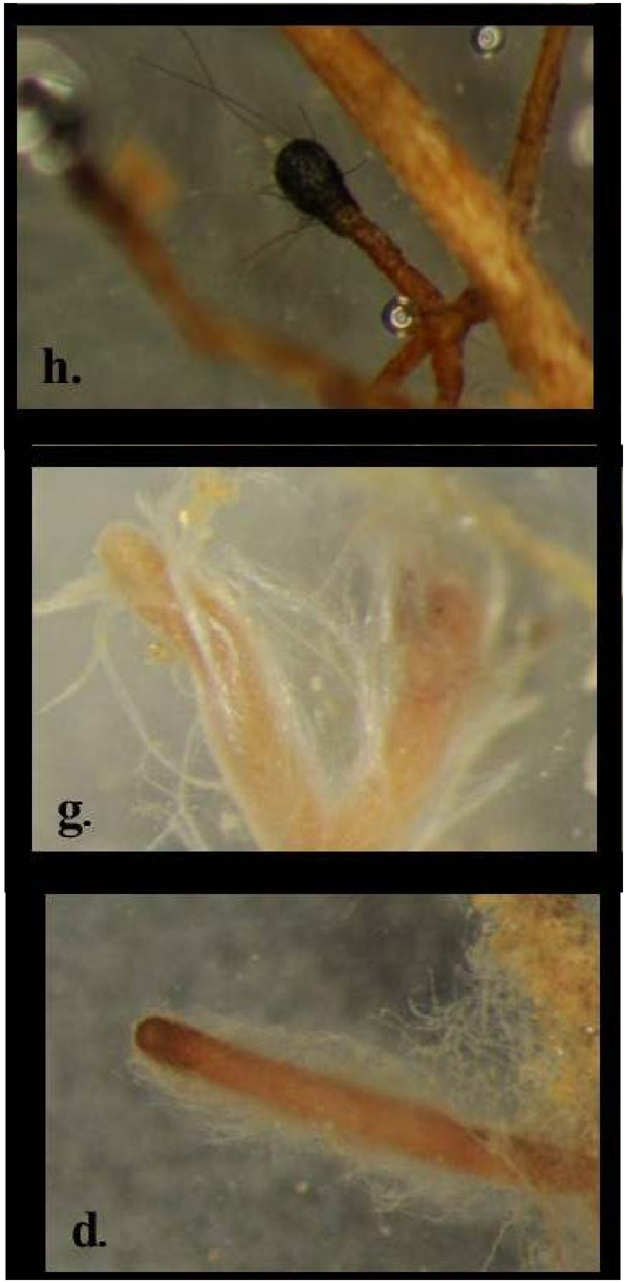
ECM Colonization and Species Richness



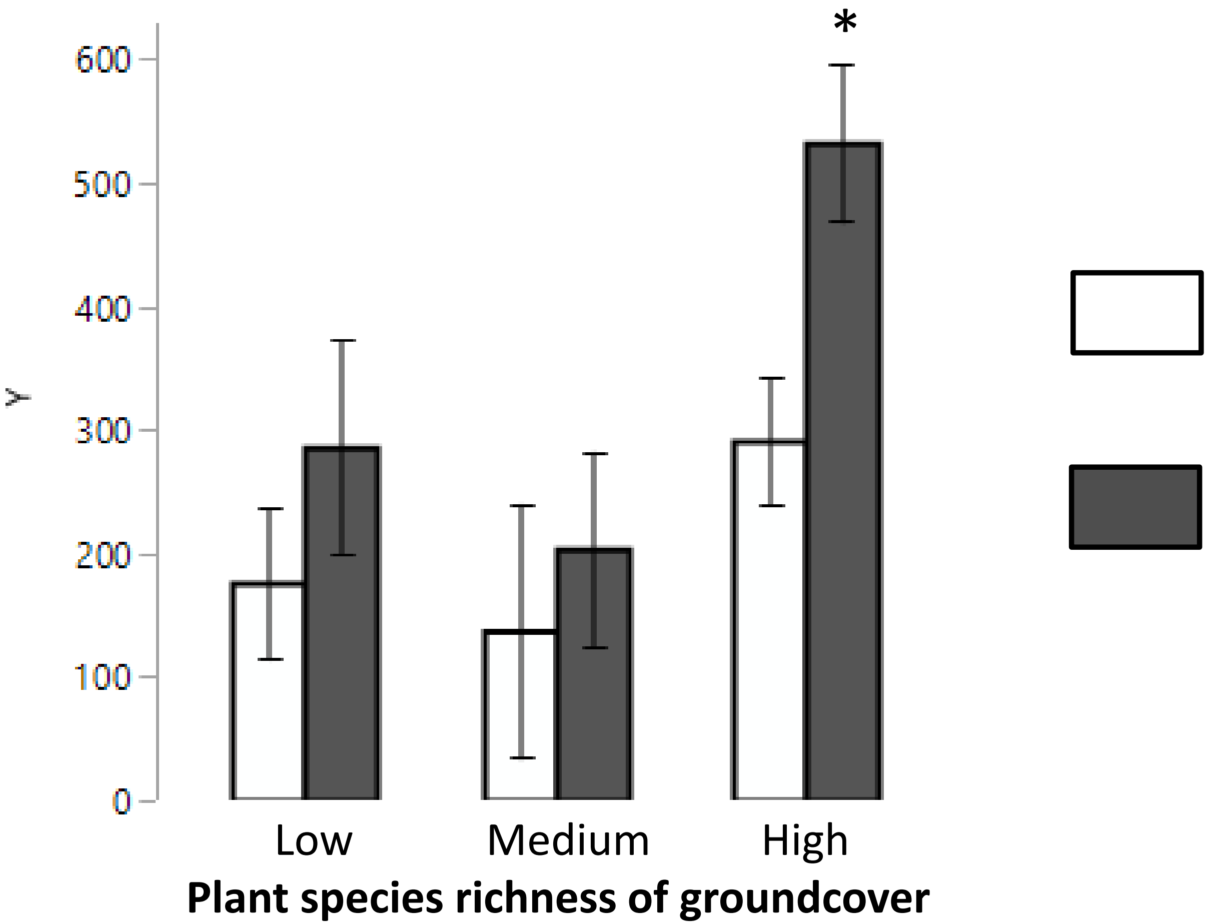
There were more ECM species in the plots with medium groundcover species, when compared to plots with higher groundcover species

20 ECM sequences

Code	ECM Genus species	Proportion
Cen	*Cenococcum geophilum*	*39.3*
Cort1	*Cortinarius decipiens*	*13.4*
Cort2	*Cortinarius vernus*	*1.7*
Cort3	*Cortinarius balaustinus*	*8.0*
Cort4	*Cortinarius sp.*	*0.2*
Heb1	*Hebeloma arenosum*	*0.6*
Heb2	*Hebeloma vaccinum*	*8.7*
Helo	*Helotiaceae*	*1.6*
Ino1	*Inocybe cincinnata*	*0.3*
Ino2	*Inocybe leucoloma*	*3.1*
Ino3	*Inocybe malenconii*	*0.2*
Pis	*Pisolithus arhizus*	*1.0*
Rus	*Russula pectinatoides*	*2.0*
Scl	*Scleroderma areolatum*	*3.6*
Thel	*Thelephora terrestris*	*0.3*
Tom	*Tomentella*	*3.5*
Tuber	*Tuber canaliculatum*	*1.6*
UNK1	*Unknown ECM 1*	*1.8*
UNK2	*Unknown ECM 2*	*7.1*
UNK3	*Unknown ECM 3*	*2.0*

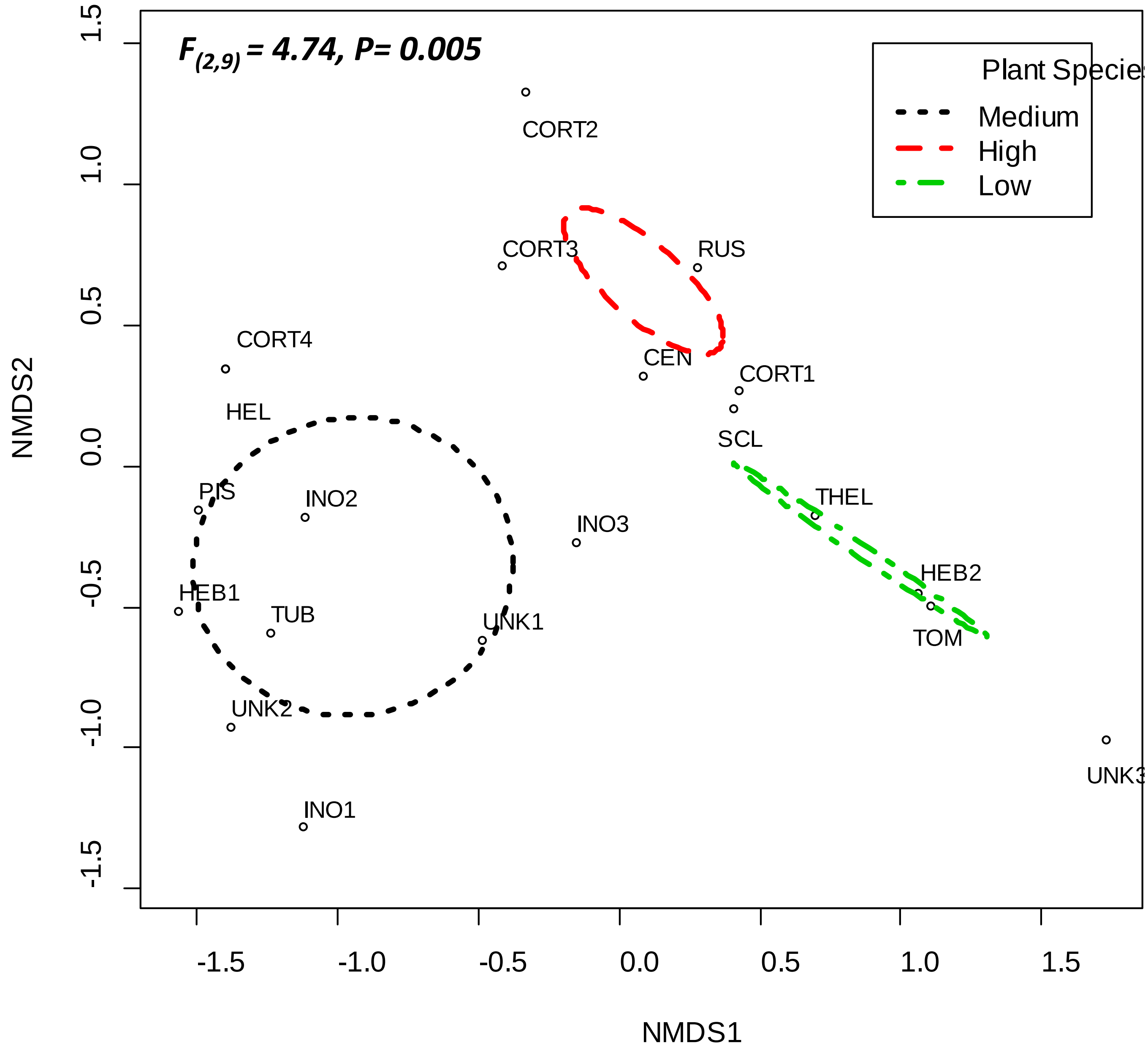


Dominant ECM Species

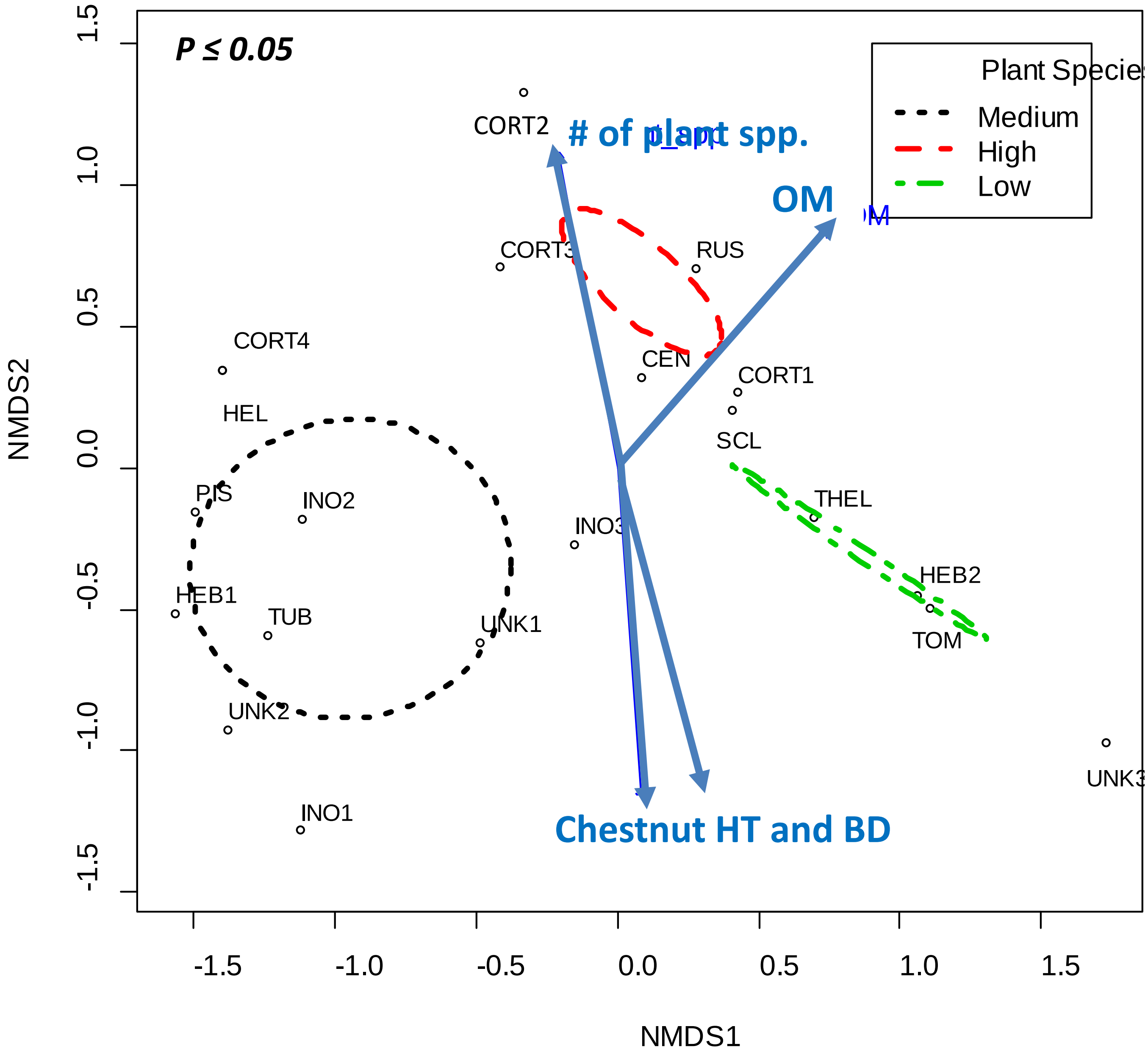


Cenococcum sp. significantly abundant in the plots with higher groundcover species diversity ($F_{(2,9)} = 4.94$, $P = 0.03$)

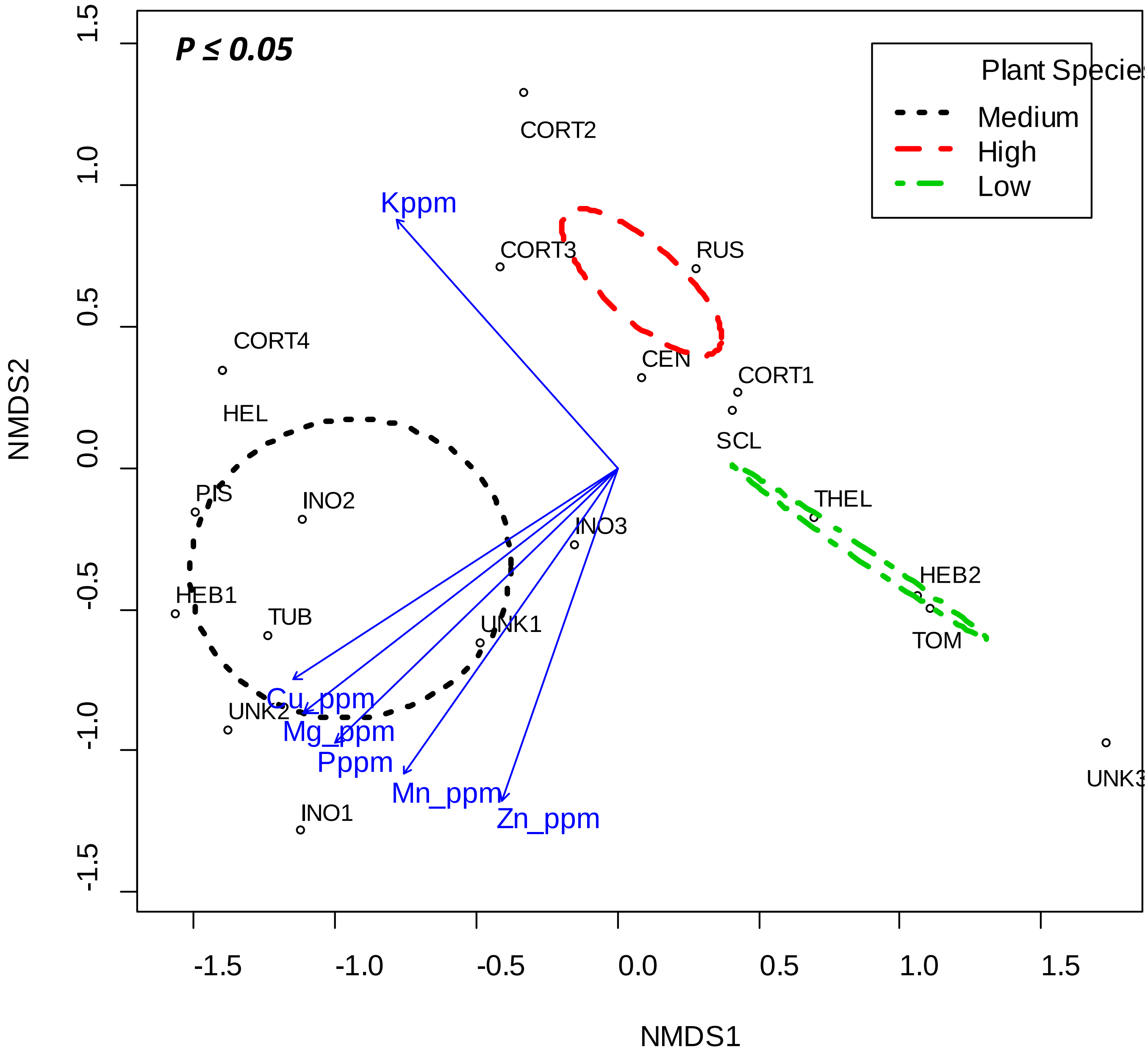
ECM Community Composition per Site



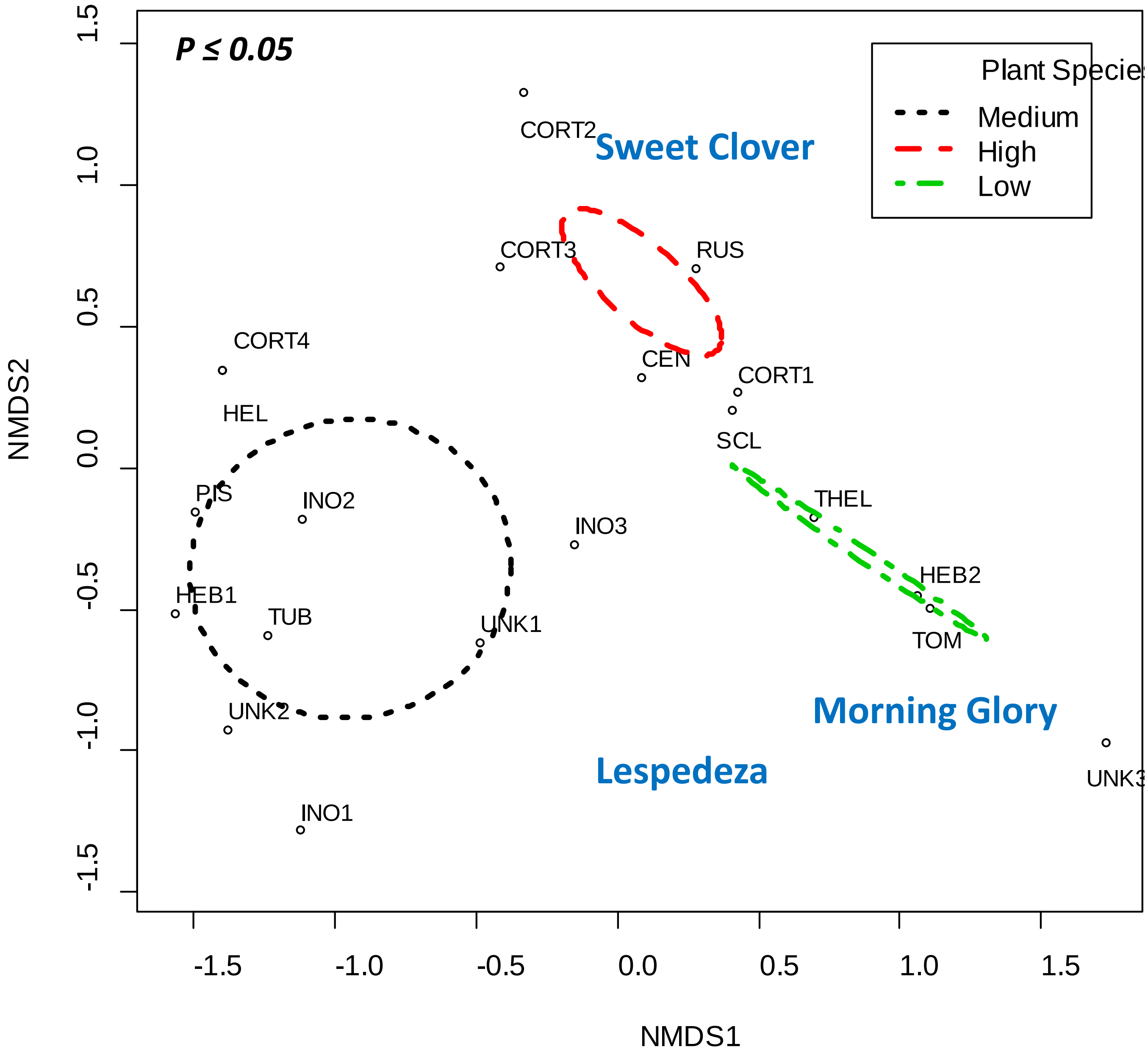
Site variables that were correlated to ECM Community



Site variables that were correlated to ECM Community



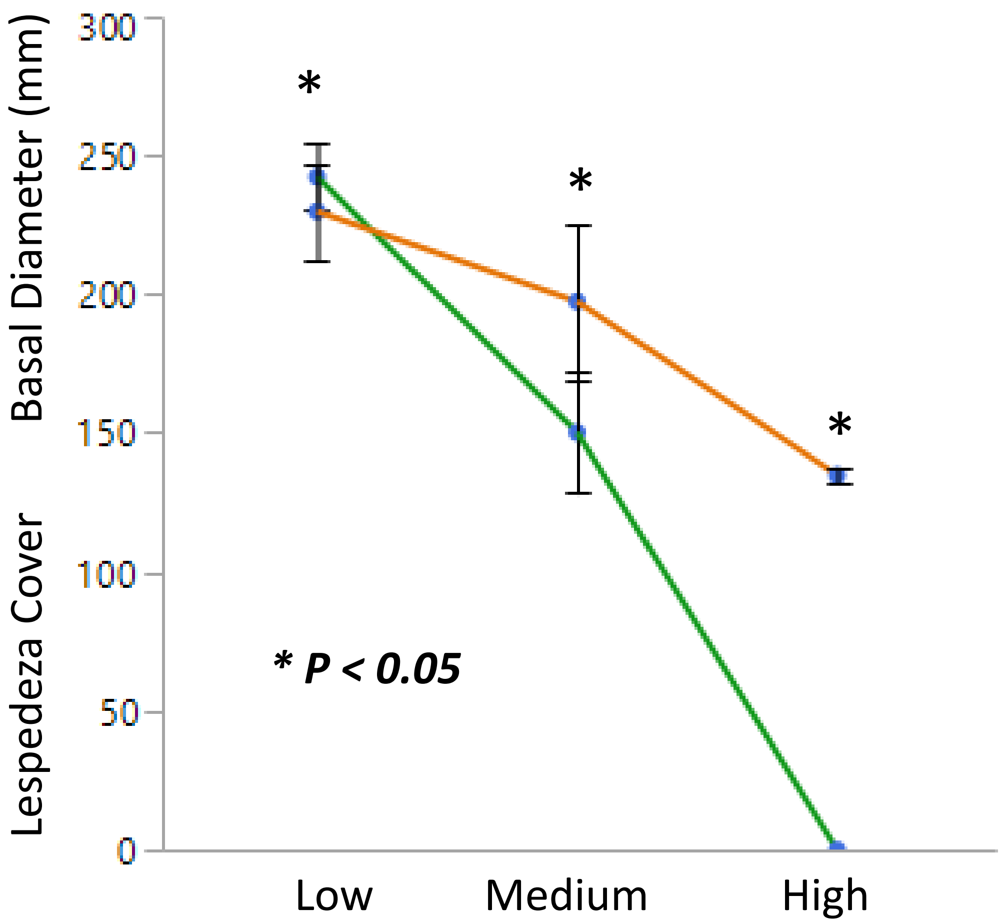
Plant species influencing site



Dominant Plant Species per Site

As Lespedeza cover increased in the plots, groundcover species richness decreased

Comparing Growth and Lespedeza



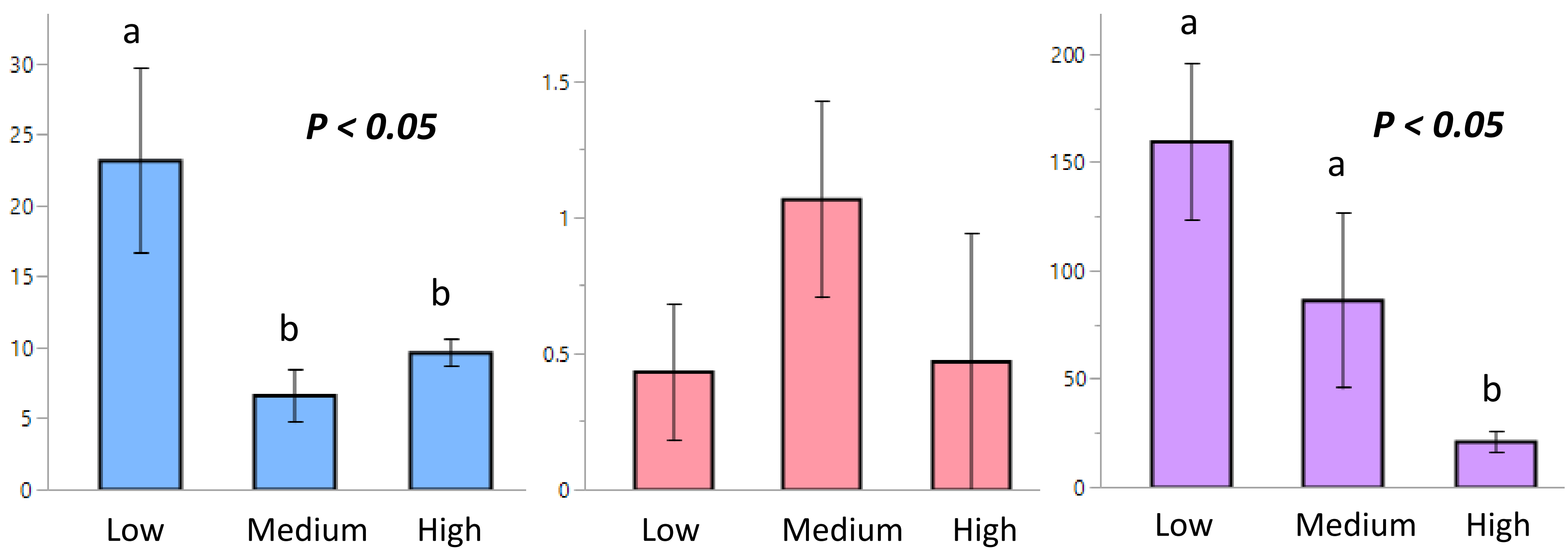
Plant species richness of groundcover

Chestnut growth increases with Lespedeza

As Lespedeza increases, plant species richness decreases

Metals in Leaf Tissue

Aluminum **Copper** **Manganese**



Plant species richness of groundcover

Metals in low levels with nut tissue safe for consumption

Conclusion

Chestnut had similar abundant ECM, however, *Cortinarius* was most abundant in the Ohio site that was reclaimed by ripping, where *Cenococcum* was most abundant in Tennessee, where sites were end-dumped, and most abundant on the driest site

- ECM selected by the most limiting resource?

When compaction is mitigated or avoided, Lespedeza may produce shade that reduces temperature, add organic matter, and enrich soils that facilitate seedling growth. Current work is analyzing N uptake by the tree.

-Is there an ECM species that chestnut shares with Lespedeza that could further facilitate chestnut's growth on reclaimed sites?


Thank you!