



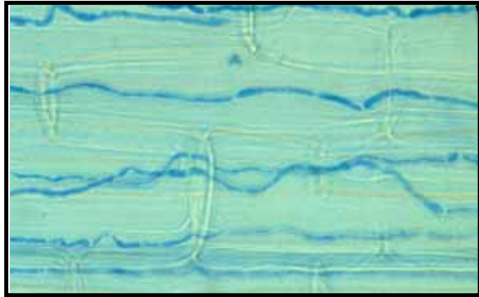
Potential for climate-induced
disruption of plant-fungal symbioses
in the Rocky Mountains

Melanie Kazenel
7 April 2016

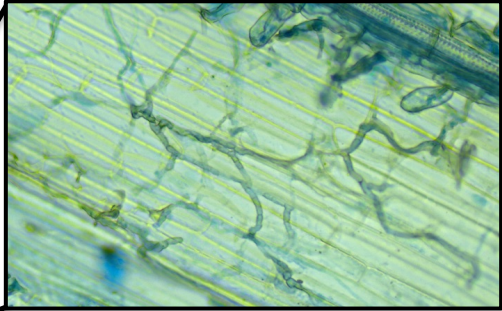


How will climate change alter
plant-symbiont interactions?

Plants and Fungal Symbionts



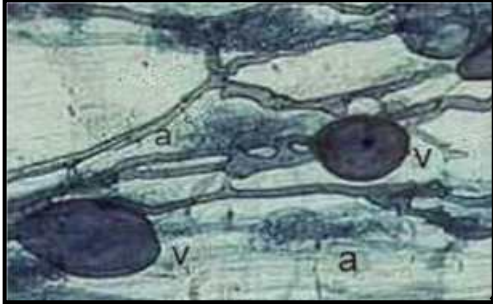
Systemic endophytes (*Epichloë* sp.)



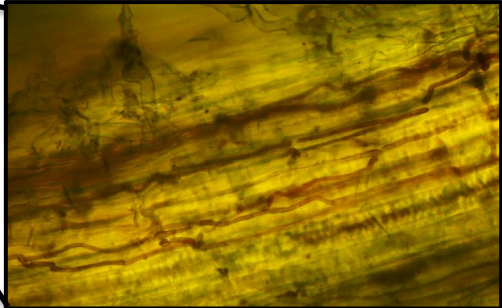
Localized foliar endophytes (LFE)



Arbuscular mycorrhizal fungi (AMF)



Dark septate endophytes (DSE)



Symbionts can mediate plant responses to climate change

AMERICAN JOURNAL OF
Botany

American Journal of Botany 100(7): 1445–1457. 2013.

SPECIAL INVITED PAPER—GLOBAL BIOLOGICAL CHANGE

**FUNGAL SYMBIONTS ALTER PLANT RESPONSES
TO GLOBAL CHANGE¹**

STEPHANIE N. KIVLIN^{2,5}, SARAH M. EMERY³, AND JENNIFER A. RUDGERS⁴

Symbionts altered plant responses to drought, N deposition, and warming

Climate change may disrupt symbioses as organisms experience range shifts

SCIENCE VOL 336 20 APRIL 2012

Recent Plant Diversity Changes on Europe's Mountain Summits

Harald Pauli,^{1*} Michael Gottfried,^{2†} Stefan Dullinger,^{2,3*} Otari Abdaladze,⁴ Maia Akhalkatsi,⁴ José Luis Benito Alonso,⁵ Gheorghe Coldea,⁶ Jan Dick,⁷ Brigitta Erschbamer,⁸ Rosa Fernández Calzado,⁹ Dany Ghosn,¹⁰ Jarle I. Holten,¹¹ Robert Kanka,¹² George Kazakis,¹⁰ Jozef Kollár,¹² Per Larsson,¹³ Pavel Moiseev,¹⁴ Dmitry Moiseev,¹⁴ Ulf Molau,¹³ Joaquín Molero Mesa,⁹ Laszlo Nagy,^{15,16} Giovanni Pelino,¹⁷ Mihai Pușcaș,¹⁸ Graziano Rossi,¹⁹ Angela Stanisci,¹⁷ Anne O. Syverhuset,¹¹ Jean-Paul Theurillat,^{20,21} Marcello Tomaselli,²² Peter Unterluggauer,⁸ Luis Villar,⁵ Pascal Vittoz,²³ Georg Grabherr¹

nature
climate change

LETTERS

PUBLISHED ONLINE: 10 JANUARY 2012 | DOI: 10.1038/NCLIMATE1329

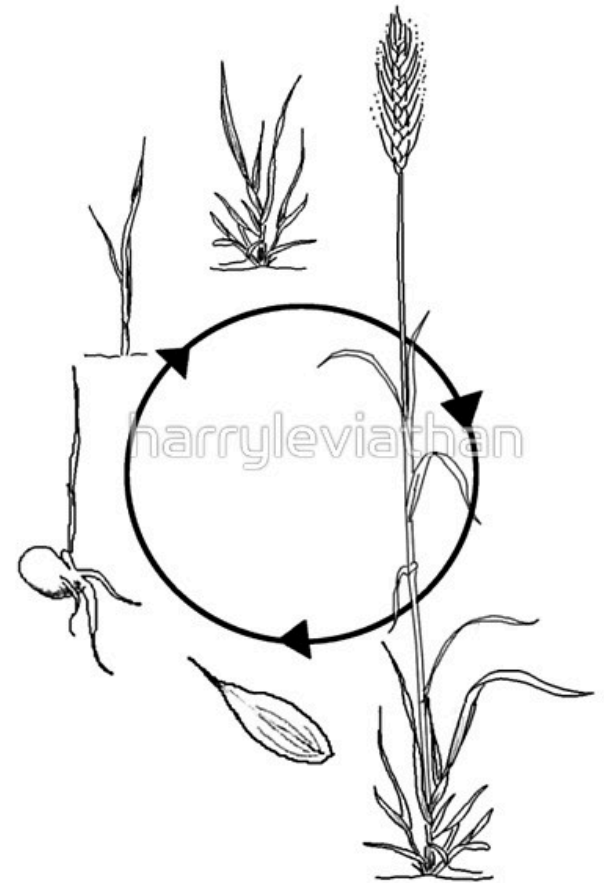
Continent-wide response of mountain vegetation to climate change

Michael Gottfried¹, Harald Pauli^{2*}, Andreas Futschik³, Maia Akhalkatsi⁴, Peter Barančok⁵, José Luis Benito Alonso⁶, Gheorghe Coldea⁷, Jan Dick⁸, Brigitta Erschbamer⁹, María Rosa Fernández Calzado¹⁰, George Kazakis¹¹, Ján Krajčí⁵, Per Larsson¹², Martin Mallaun¹³, Ottar Michelsen¹⁴, Dmitry Moiseev¹⁵, Pavel Moiseev¹⁵, Ulf Molau¹⁶, Abderrahmane Merzouki¹⁰, Laszlo Nagy^{17,18}, George Nakhutsrishvili¹⁹, Bård Pedersen²⁰, Giovanni Pelino²¹, Mihai Puscas²², Graziano Rossi²³, Angela Stanisci²¹, Jean-Paul Theurillat^{24,25}, Marcello Tomaselli²⁶, Luis Villar⁶, Pascal Vittoz²⁷, Ioannis Vogiatzakis²⁸ and Georg Grabherr²

Mechanisms for disruption of plant-symbiont interactions

Plants and symbionts may have different:

- Physiological tolerances
- Dispersal rates
- Phenological responses



Study System



Mountains

- ~25% of land area on Earth
- 50% of the human water supply
- 1/3 of terrestrial plant diversity

Grasses

- Cover 1/3 of land area (>10,000 species)
- Provide the majority of food for humans and domesticated animals
- All have mycorrhizal fungi in roots and fungi in leaves

Altitudinal Gradients



Experimental Warming



Focal questions

How do symbionts change with altitude and warming?

a) *Altitude response?*

b) *Warming response?*

c) *Are they the same?*

Warming Experiment

Rocky Mountain Biological Laboratory



Established in 1991
Warms top 15 cm of soil by $\sim 2^{\circ}\text{C}$
Dries soil by 10-20%
Extends growing season by ~ 12 days on each end

Study Species

Achnatherum lettermanii
(ACLE)



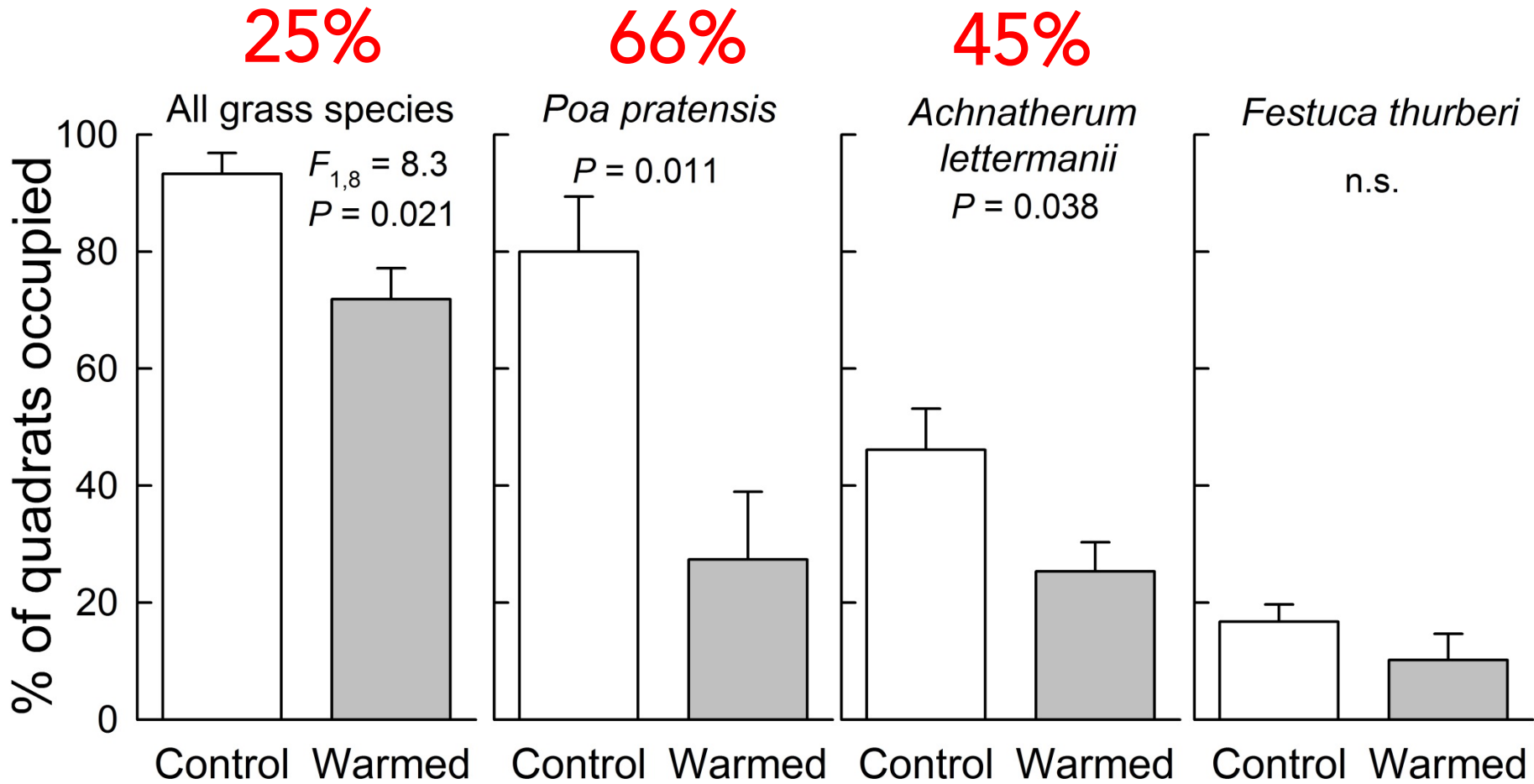
Festuca thurberi
(FETH)



Poa pratensis
(POPR)



Experimental warming reduced grasses (1991 – 2011)



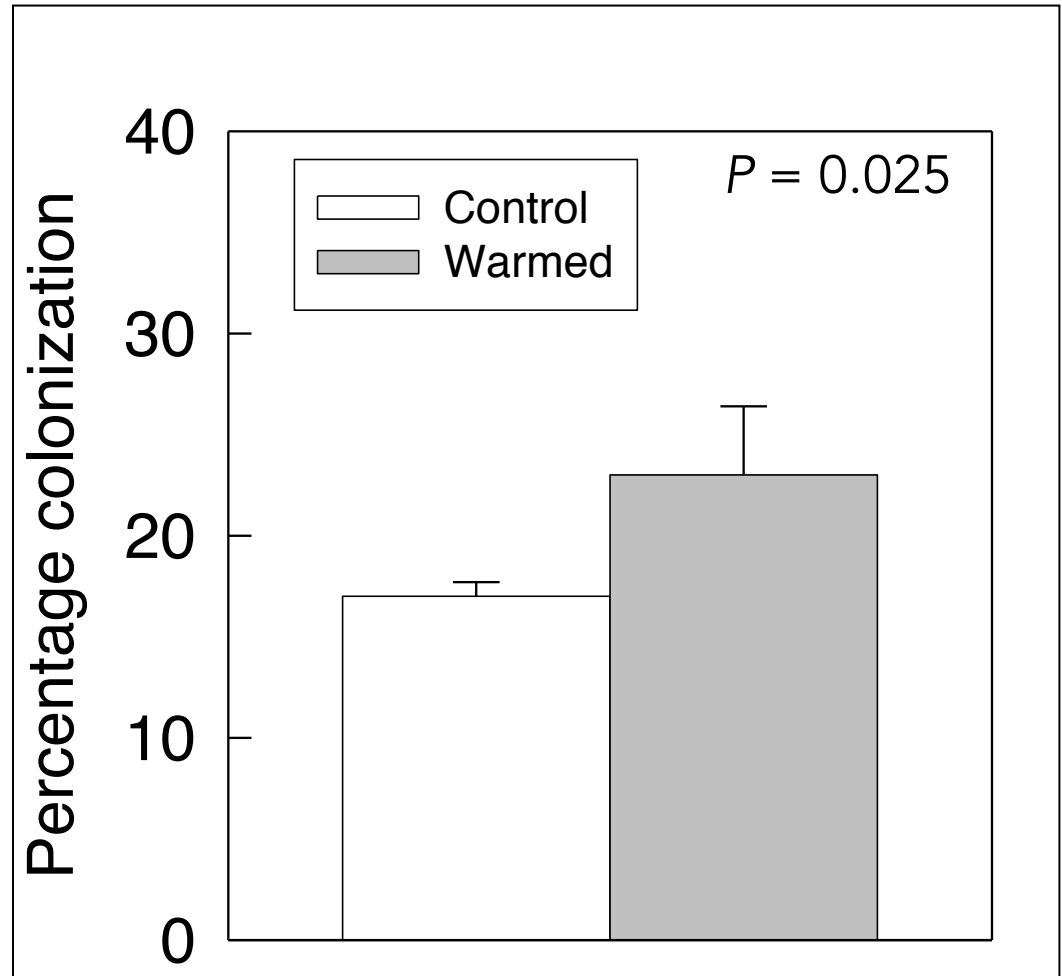
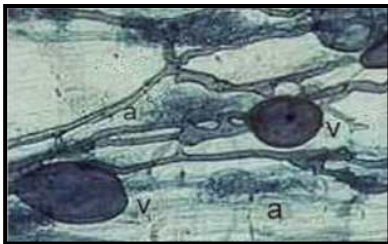
Mean % \pm s.e. of 49 (0.2m \times 0.2m) quadrats surveyed per plot
 $n = 5$ plots per warming treatment

Experimental warming increased mycorrhizal colonization of roots



A. lettermanii

Arbuscular
mycorrhizal fungi



Rudgers et al. *Ecology* (2014)

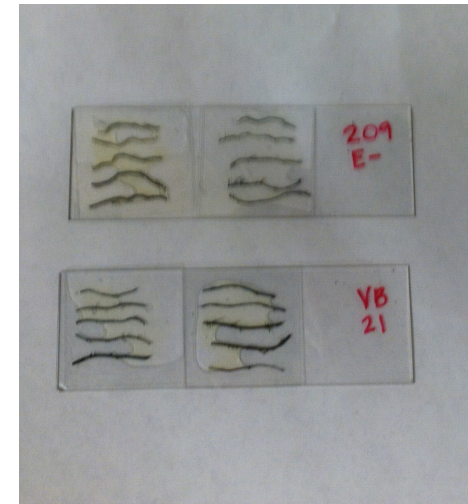
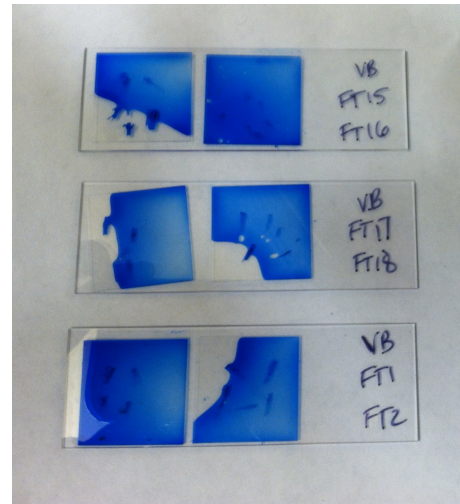
Field collection methods

- 3 focal grass species:
 - *Achnatherum lettermanii*
 - *Poa pratensis*
 - *Festuca thurberi*
- 6 individual plants collected per species per plot
- Roots and leaves (2014)
- Phenology: June and September



Laboratory methods

- Staining and microscopy → colonization



- Illumina MiSeq DNA sequencing → composition

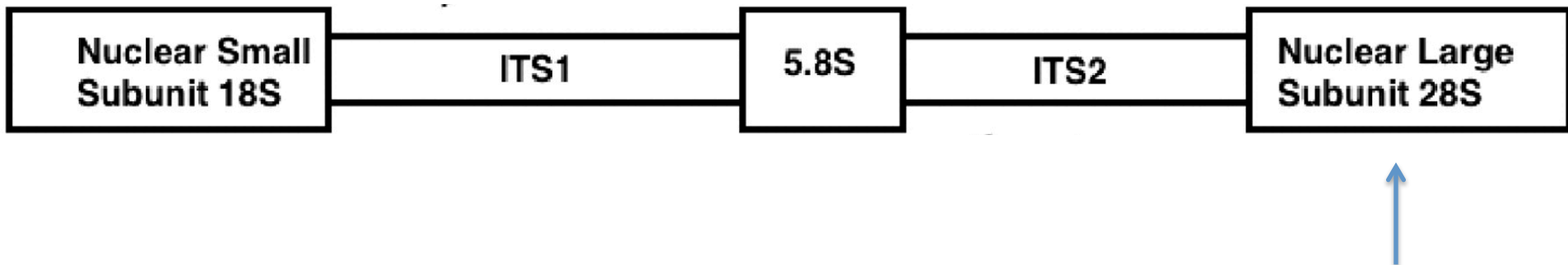


Illumina Sequencing

Paired-end sequencing of fungal nuclear ribosomal DNA using primers targeting:

- ITS2 region (for VTE, LFE, and DSE)

- ~300bp in the 28S region (for AMF) (FLR3-FLR4 primers)



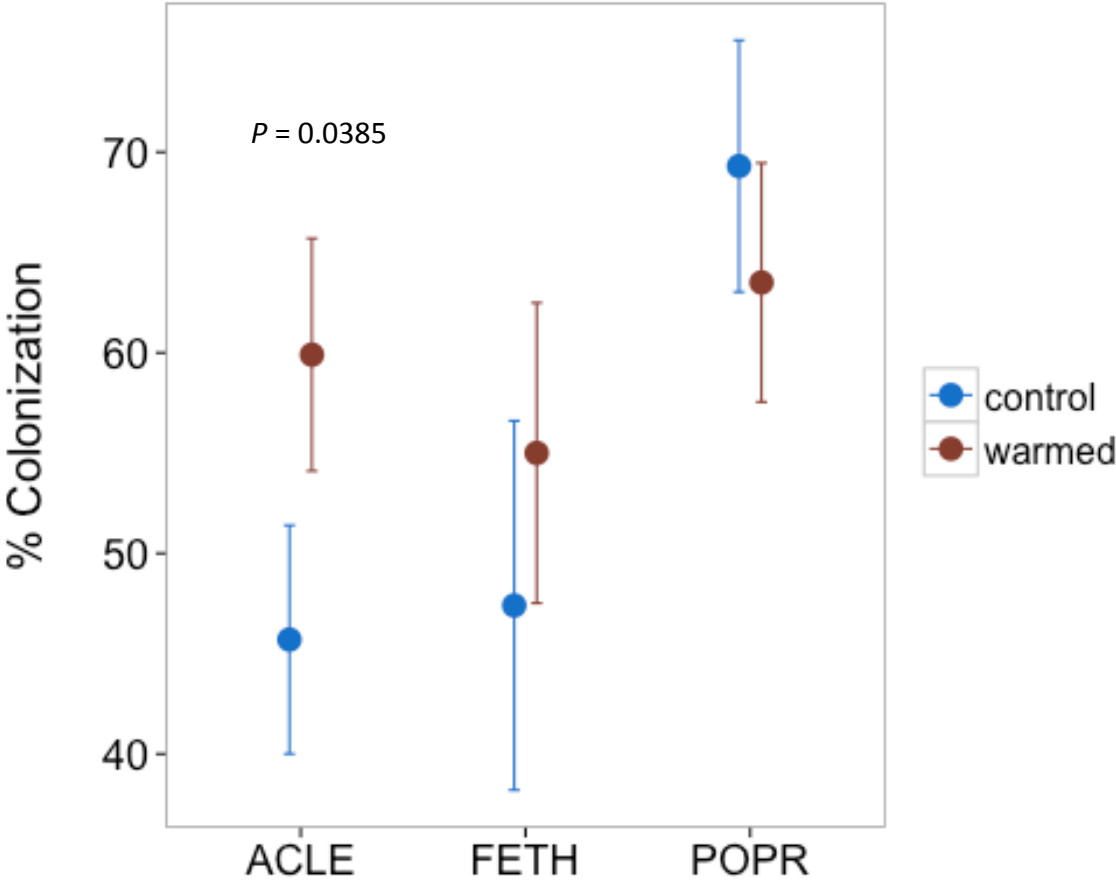
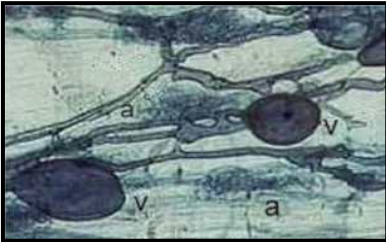
Bioinformatics

- Quality filtering in QIIME
 - Sumaclus to place similar sequences into operational taxonomic units (OTUs) at ~97% identity
 - NCBI BLAST to assign taxonomy
 - Discarded all OTUs with <97% identity to entry in database
 - Normalized data using DESeq2
 - Discarded singletons
- Conducted analyses on 802 OTUs

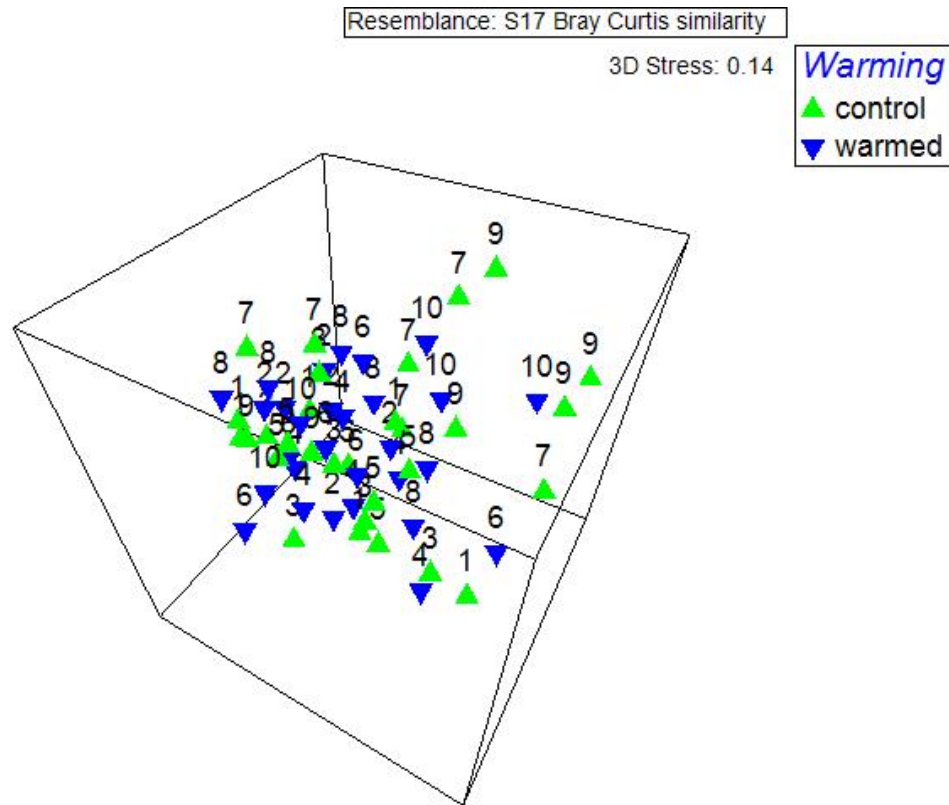
Analyses

- NMDS: to visualize OTU composition
- perMANOVA: to test how variables of interest affect OTU composition
 - Fixed effects: warming treatment, host species, sampling date
 - Random effect: block (pair of plots)
- PERMDISP: to test for dispersion within groups
- Indicator species analysis (SIMPER): to identify OTUs that contributed strongly to differences among groups

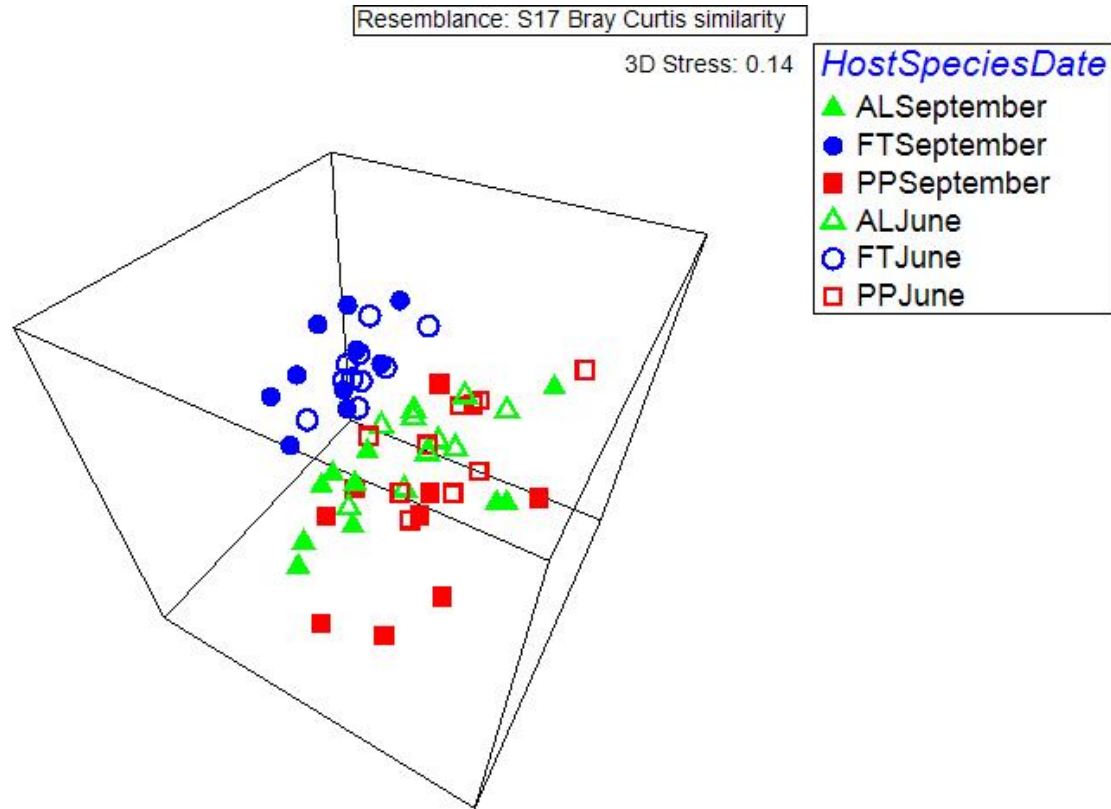
Arbuscular mycorrhizal fungi



Results: All Species



- OTU composition did not differ between warming treatments ($df = 1$, pseudo-F = 1.361, $P = 0.1391$)
- High stress value
- Spatial heterogeneity (significant effect of block)

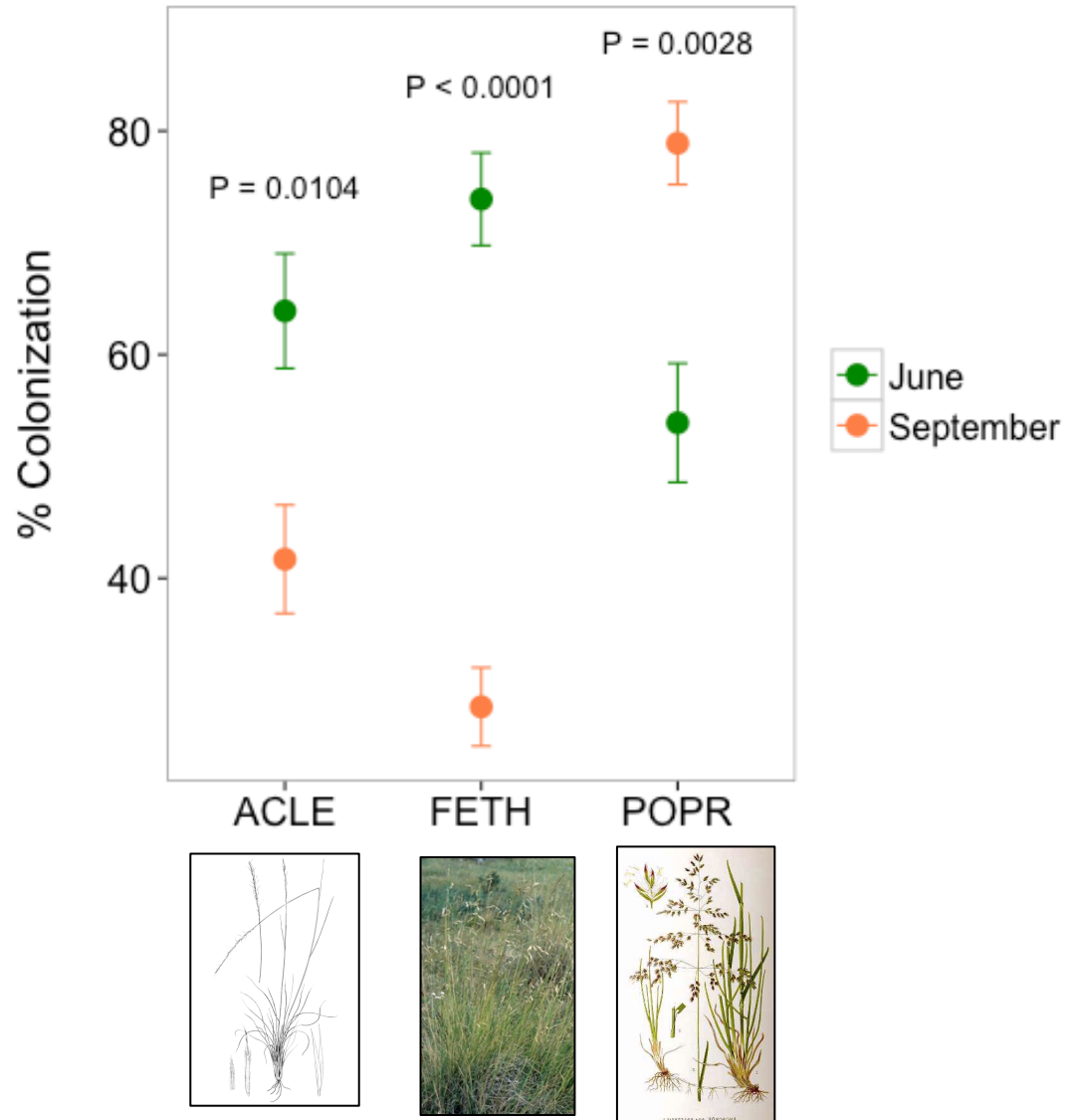
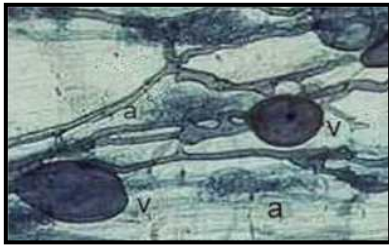


OTU composition differed between **sampling dates** ($df = 1$, pseudo-F = 2.9483, $P = 0.0009$) and among **host species** ($df = 2$, pseudo-F = 5.4469, $P = 0.0001$)

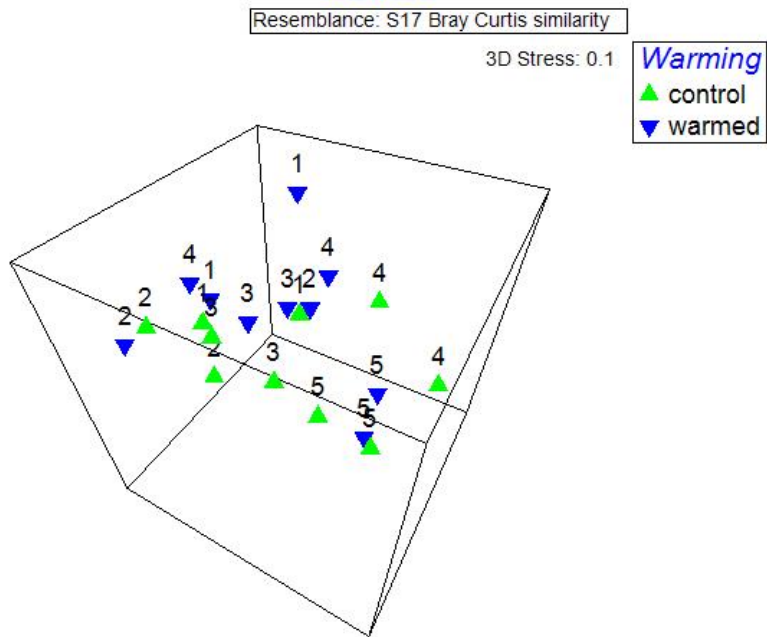
- FT differed from AL and PP
- Communities of AL and PP were significantly more dispersed relative to communities of FT (PERMDISP)

Changes in AMF colonization between June and September for all three grasses

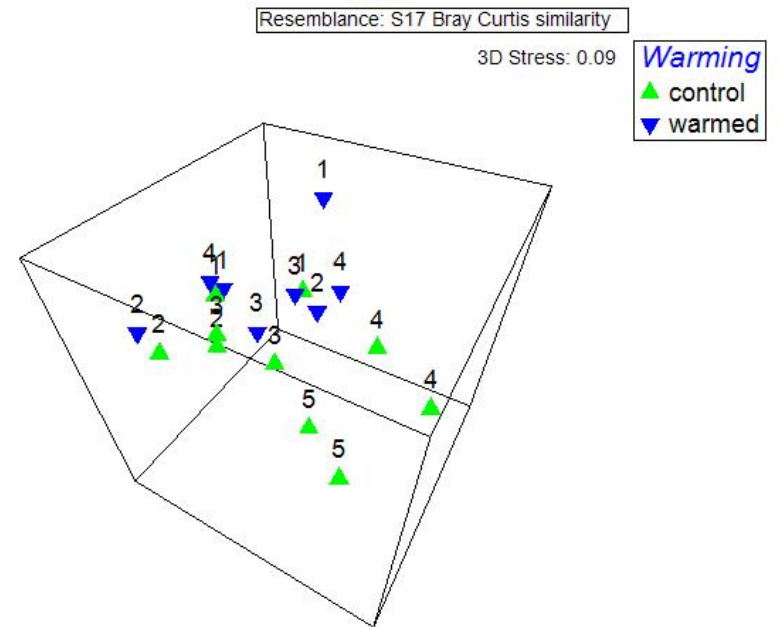
Arbuscular
mycorrhizal fungi



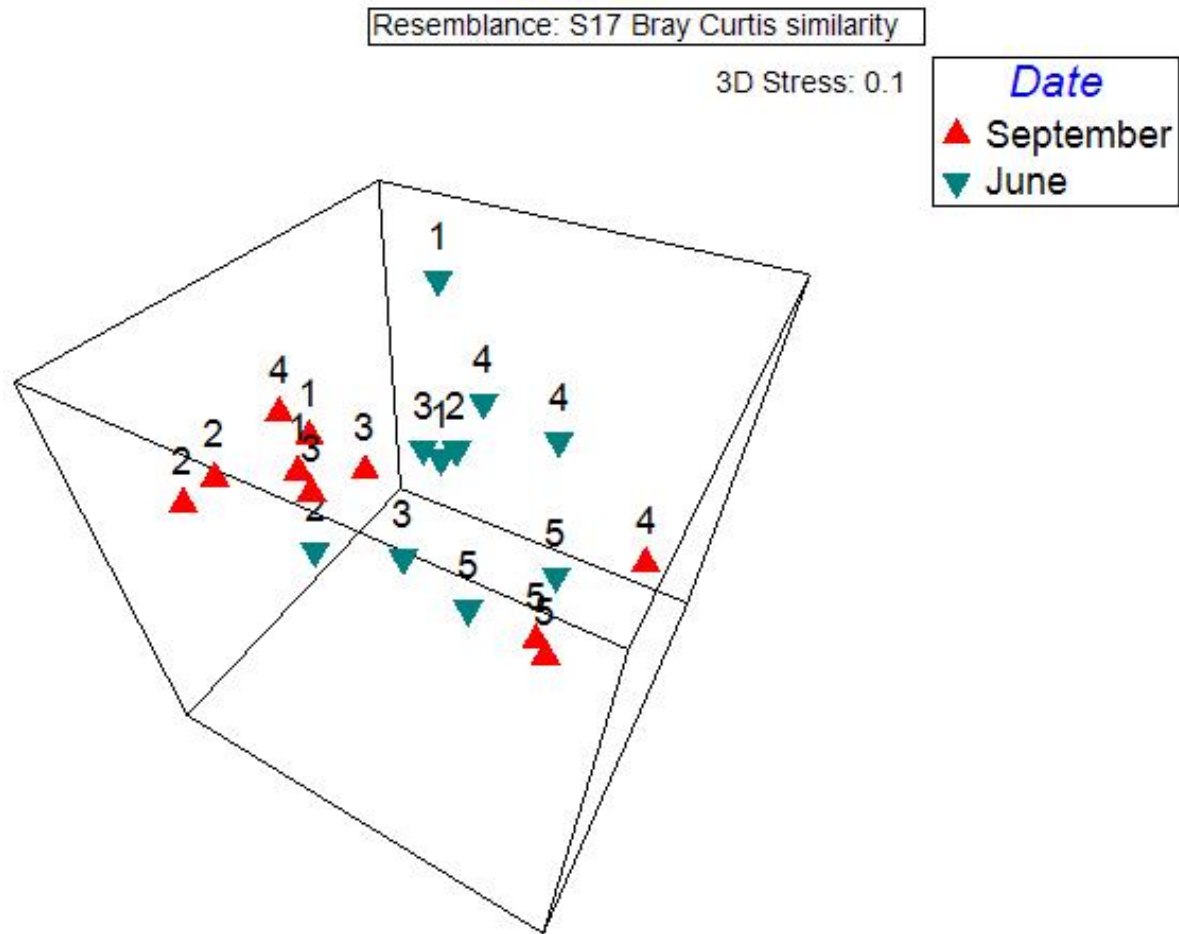
Results: *A. lettermanii*



All data

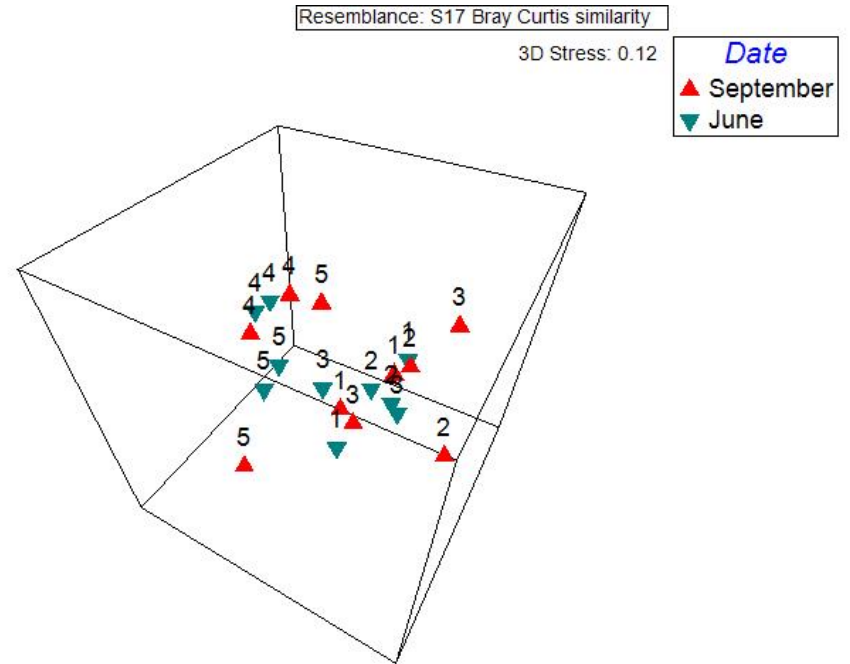
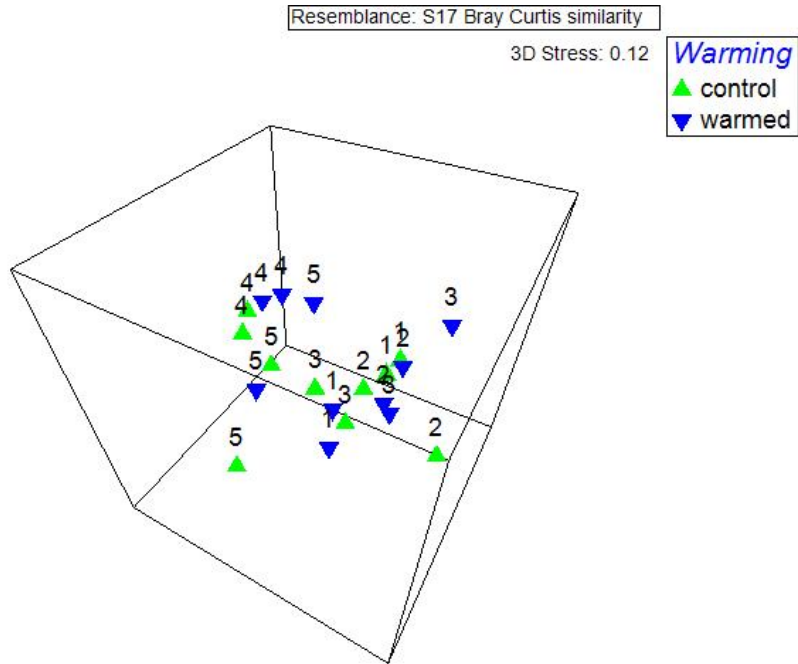


Plot 10 data removed



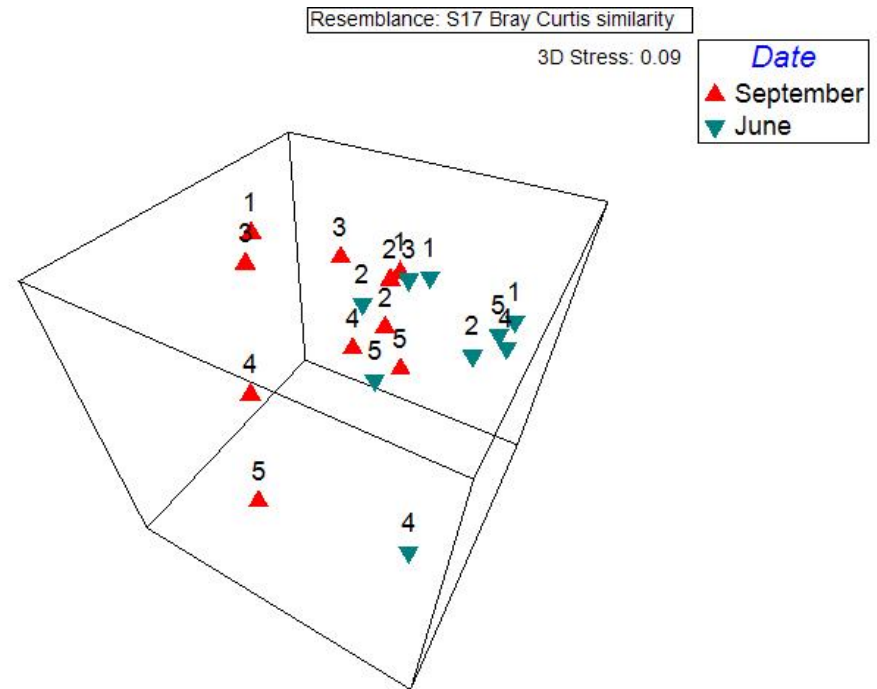
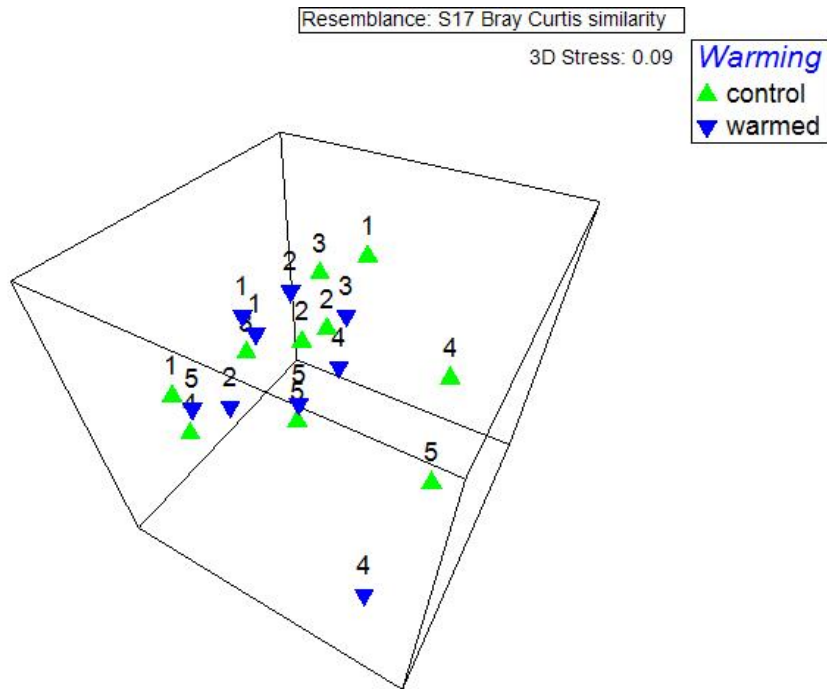
- Sampling date affected OTU composition ($df = 1$, pseudo-F = 3.1274, $P = 0.0024$)
- No difference in dispersion between two dates
- Grouping by plot

Results: *F. thurberi*



No effect of warming or
sampling date

Results: *P. pratensis*



- No effect of warming
- Effect of sampling date (df = 1, pseudo-F = 2.6595, P = 0.0065)

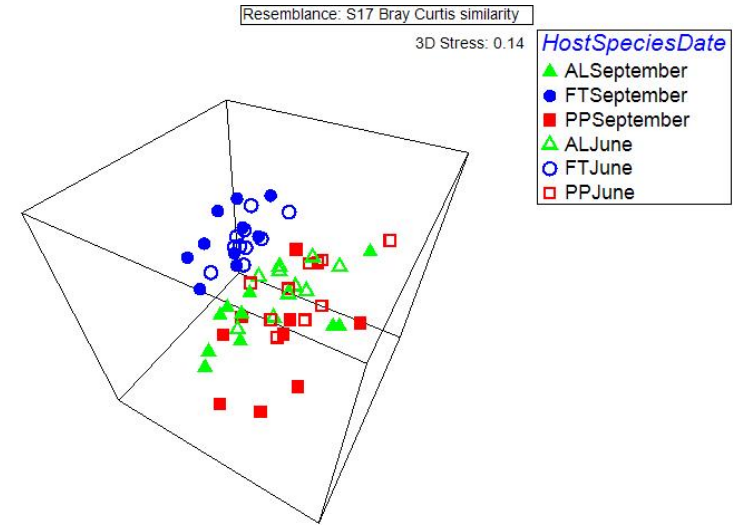
Indicator Species Analysis (SIMPER)

Control vs. warmed plots

OTU	Control Avg. Abundance	Warmed Avg. Abundance	Avg. Dissimilarity	Contribution %
OTU5	7.19	6.72	0.47	0.96
OTU4	7.52	7.14	0.42	0.86
OTU6	3.5	1.94	0.42	0.86
OTU15	3.99	4.02	0.42	0.85
OTU12	4.05	4.59	0.42	0.84

F. thurberi vs. *P. pratensis*

OTU	FETH Avg. Abundance	POPR Avg. Abundance	Avg. Dissimilarity	Contribution %
OTU15	7.17	2.36	0.53	1.02
OTU16	5.39	2.01	0.5	0.96
OTU11	4.39	8.72	0.49	0.95
OTU24	5.04	0.78	0.48	0.93
OTU12	5.96	2.72	0.47	0.91



F. thurberi vs. *A. lettermanii*

OTU	FETH Avg. Abundance	ACLE Avg. Abundance	Avg. Dissimilarity	Contribution %
OTU15	7.17	2.41	0.54	1.07
OTU5	4.81	8.68	0.53	1.05
OTU24	5.04	0.3	0.5	0.98
OTU16	5.39	1.46	0.47	0.92
OTU11	4.39	7.53	0.43	0.85

Taxonomy

OTU	Top BLAST Hit	Top BLAST Hit Details		
		Study Location	Study System	Citation
OTU16	Uncultured Glomeromycota	California, USA	Giant sequoia (<i>Sequoiadendron giganteum</i>)	Fahey et al. 2012, <i>Mycologia</i>
OTU15	Uncultured Glomus	Michigan, USA	Northern hardwood forest dominated by sugar maple (<i>Acer saccharum</i>)	van Diepen et al. 2013, <i>Applied Soil Ecology</i>
OTU24	Uncultured Glomeromycota	Qinghai-Tibetan Plateau, China	Alpine meadow	Yang et al. 2013, <i>PLOS ONE</i>
OTU11	Uncultured Glomeromycota	Hungary	Agricultural system (corn, wheat, alfalfa, barley, peas)	Magurno et al. 2014, <i>Open Journal of Ecology</i>
OTU5	Uncultured Glomeromycota	Montana, USA	Native grassland vs. system dominated by <i>Centaurea maculosa</i> (spotted knapweed)	Mummey and Rillig 2006, <i>Plant and Soil</i>
OTU12	Uncultured Glomeraceae	Czech Republic	<i>Knautia arvensis</i> (Caprifoliaceae)	Doubková et al. 2013, <i>Mycorrhiza</i>
OTU4	Uncultured Glomeromycota	Tibetan Plateau, China	Herbaceous plants	Li et al., Unpublished
OTU6	Uncultured Glomeromycota	Canada	Crested wheatgrass (<i>Agropyron cristatum</i>)	Perez et al. 2008, <i>Agriculture and Agrifood Canada</i>
OTU10	Uncultured Glomeromycota	Canada	Switchgrass (<i>Panicum virgatum</i>)	Perez et al. 2008, <i>Agriculture and Agrifood Canada</i>

Questions or comments?

