

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

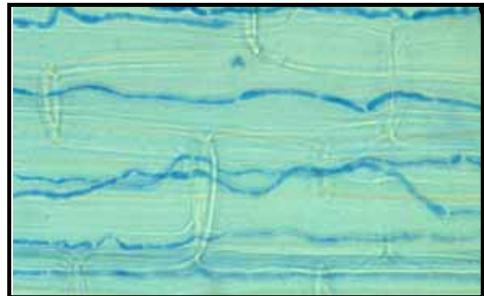
Melanie Kazenel

7 April 2016

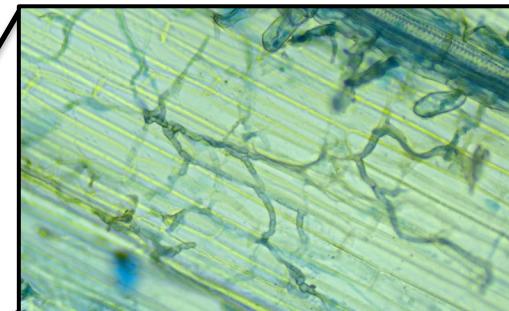


How will climate change alter  
plant-symbiont interactions?

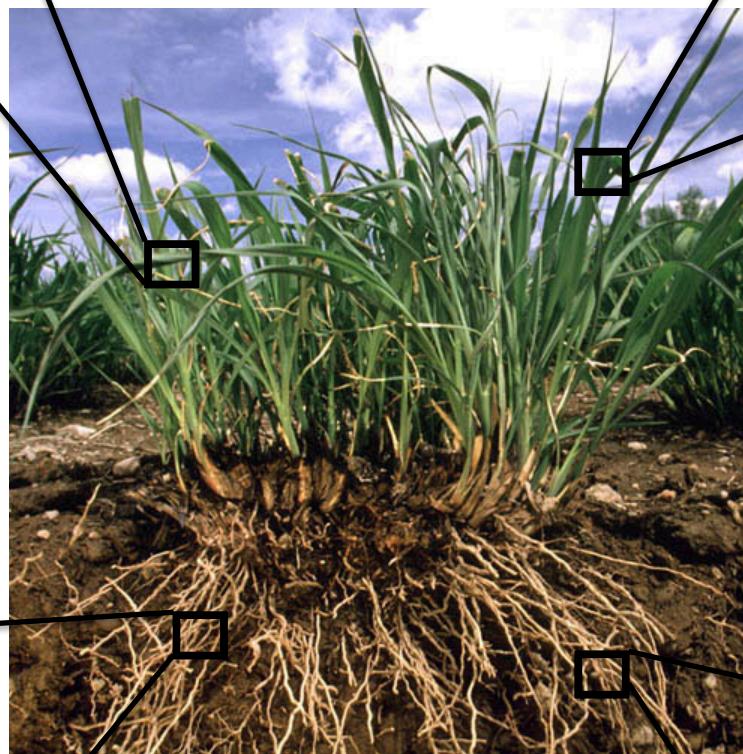
# Plants and Fungal Symbionts



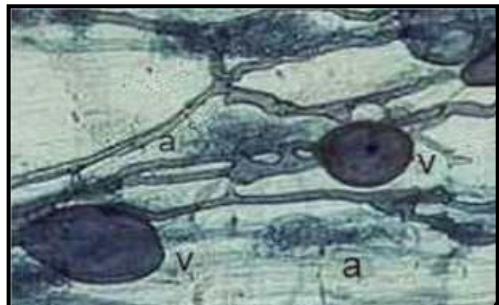
Systemic  
endophytes  
(*Epichloë* sp.)



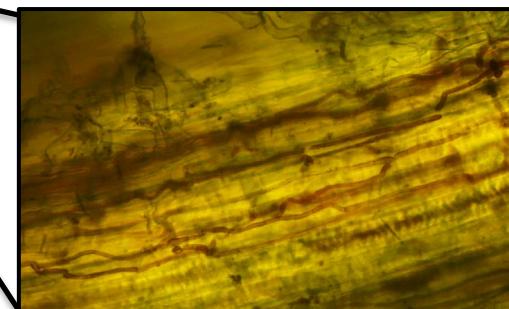
Localized foliar  
endophytes (LFE)



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 CHANGE<sup>1</sup>

STEPHANIE N. KIVLIN<sup>2,5</sup>, SARAH M. EMERY<sup>3</sup>, AND JENNIFER A. RUDGERS<sup>4</sup>

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

nature  
climate change

LETTERS

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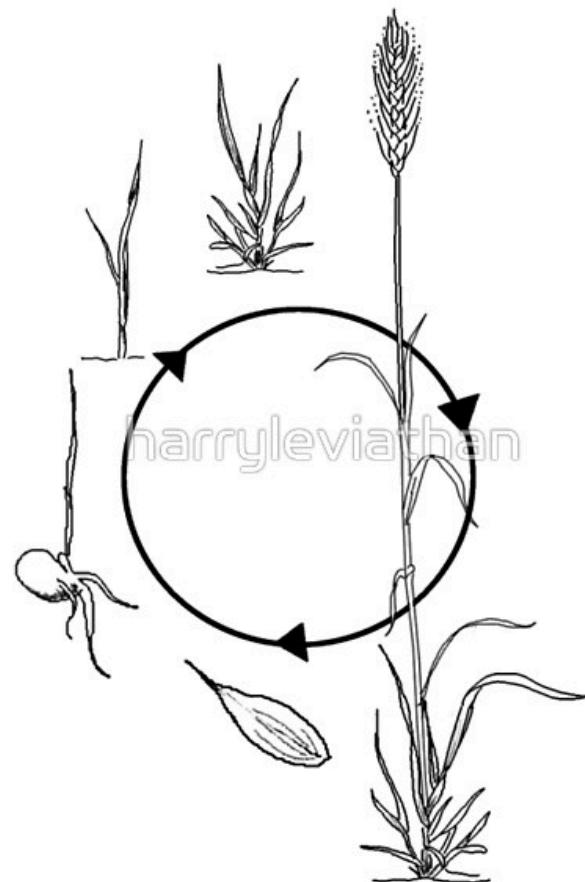
## Continent-wide response of mountain vegetation to climate change

Michael Gottfried<sup>1</sup>, Harald Pauli<sup>2\*</sup>, Andreas Futschik<sup>3</sup>, Maia Akhalkatsi<sup>4</sup>, Peter Barančok<sup>5</sup>, José Luis Benito Alonso<sup>6</sup>, Gheorghe Coldea<sup>7</sup>, Jan Dick<sup>8</sup>, Brigitta Erschbamer<sup>9</sup>, María Rosa Fernández Calzado<sup>10</sup>, George Kazakis<sup>11</sup>, Ján Krajčí<sup>5</sup>, Per Larsson<sup>12</sup>, Martin Mallaun<sup>13</sup>, Ottar Michelsen<sup>14</sup>, Dmitry Moiseev<sup>15</sup>, Pavel Moiseev<sup>15</sup>, Ulf Molau<sup>16</sup>, Abderrahmane Merzouki<sup>10</sup>, Laszlo Nagy<sup>17,18</sup>, George Nakhutsrishvili<sup>19</sup>, Bård Pedersen<sup>20</sup>, Giovanni Pelino<sup>21</sup>, Mihai Puscas<sup>22</sup>, Graziano Rossi<sup>23</sup>, Angela Stanisci<sup>21</sup>, Jean-Paul Theurillat<sup>24,25</sup>, Marcello Tomaselli<sup>26</sup>, Luis Villar<sup>6</sup>, Pascal Vittoz<sup>27</sup>, Ioannis Vogiatzakis<sup>28</sup> and Georg Grabberr<sup>2</sup>

# Mechanisms for disruption of plant-symbiont interactions

Plants and symbionts may have different:

- Physiological tolerances
- Dispersal rates
- Phenological responses



# Study System



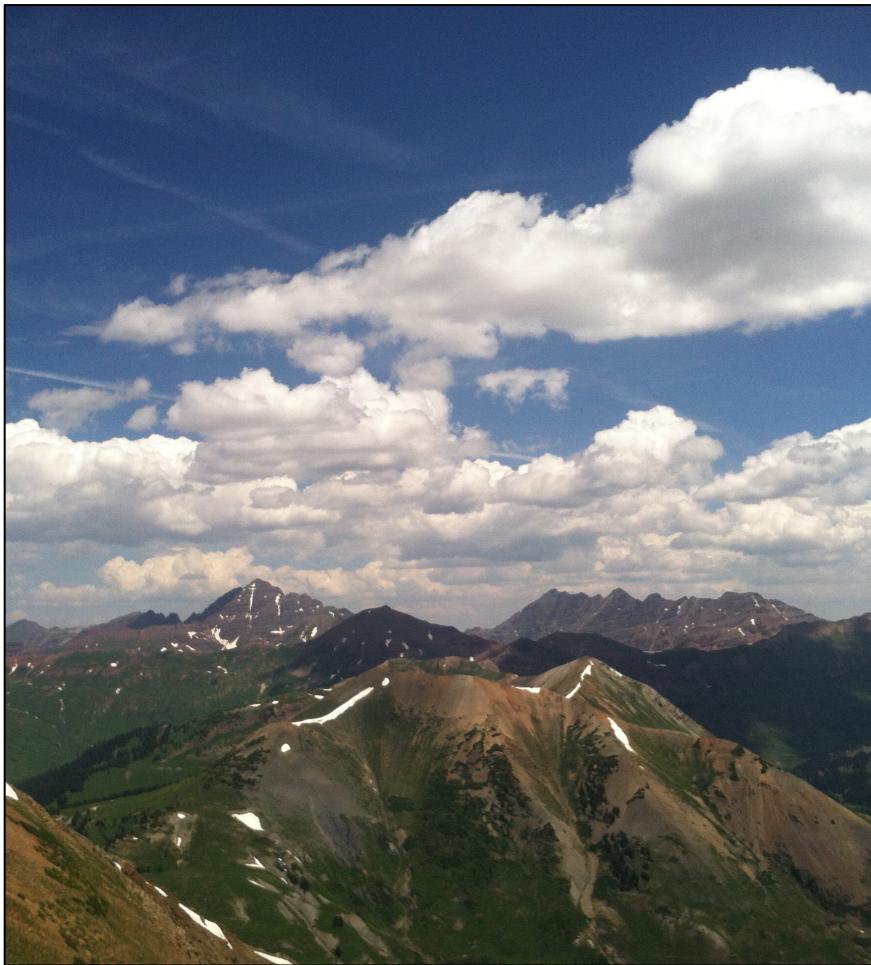
## Mountains

- ~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



Photo: Mary Ellen Harte

# 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



Photo: Mary Ellen Harte

Established in 1991  
Warms top 15 cm of soil by ~2°C  
Dries soil by 10-20%  
Extends growing season by ~12 days on each end

# Study Species

*Achnatherum lettermanii*  
(ACLE)



*Festuca thurberi*  
(FETH)

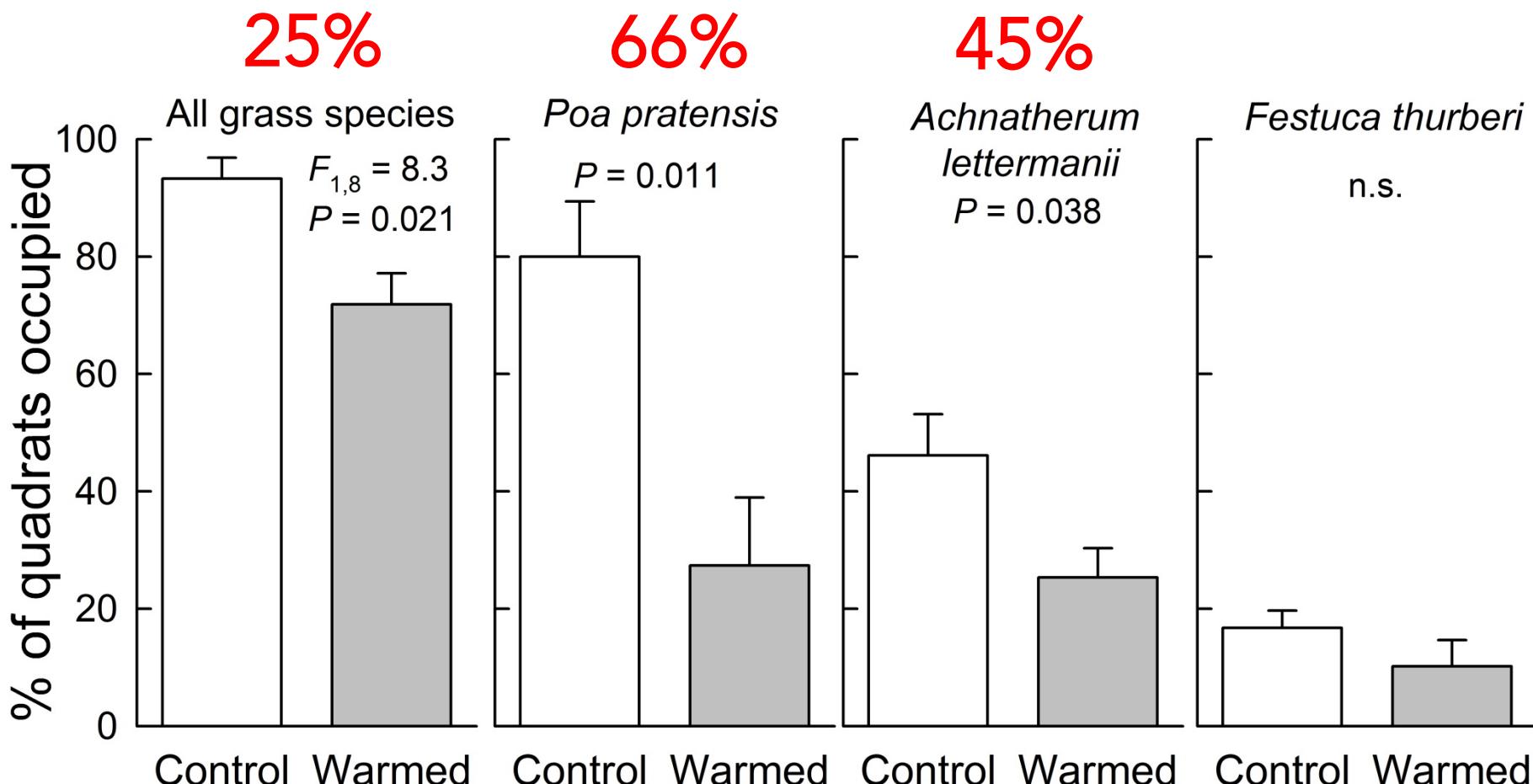


*Poa pratensis*  
(POPR)



ÅNGSGRÖE, POA PRATENSIS L.

# Experimental warming reduced grasses (1991 – 2011)



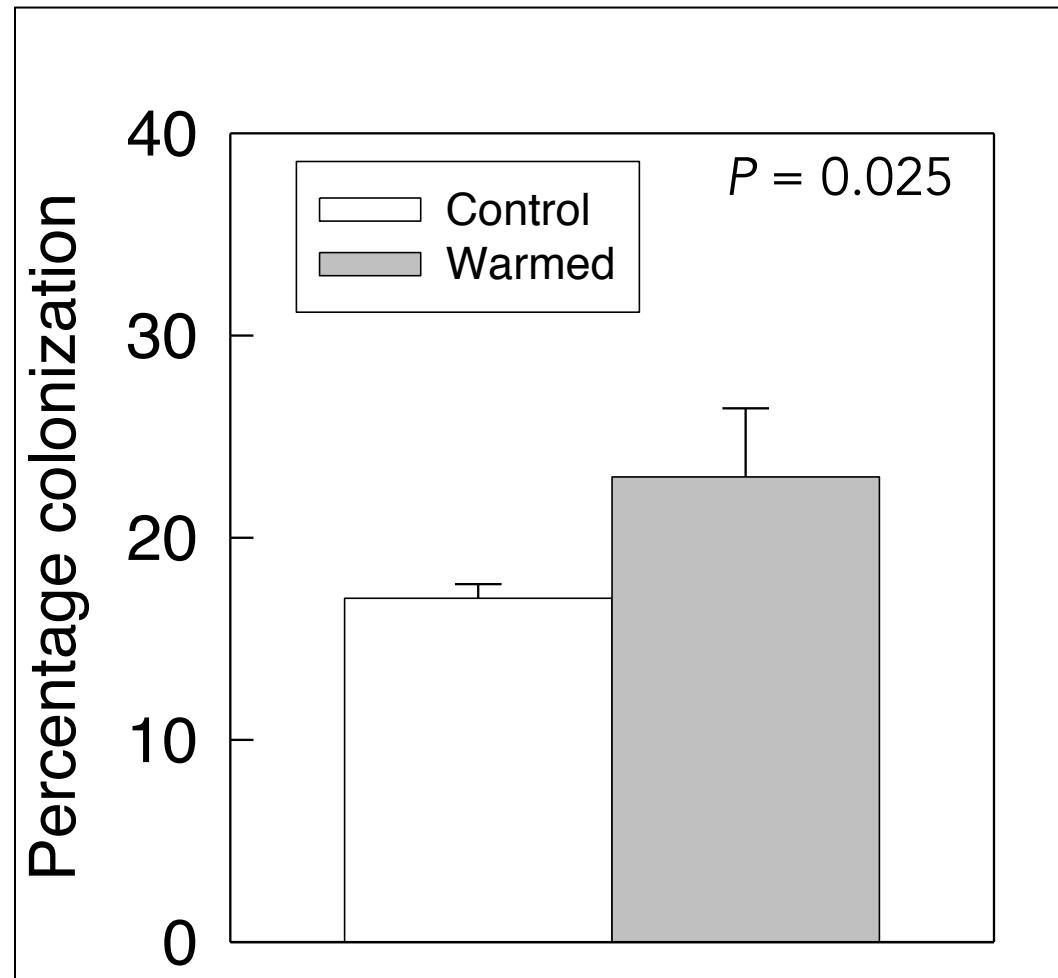
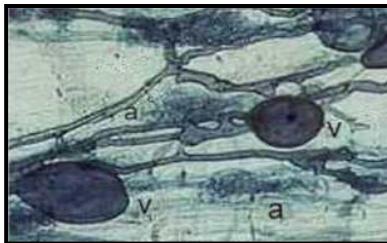
Mean %  $\pm$  s.e. of 49 (0.2m  $\times$  0.2m) quadrats surveyed per plot  
n = 5 plots per warming treatment

# Experimental warming increased mycorrhizal colonization of roots



*A. lettermanii*

Arbuscular  
mycorrhizal fungi



# Field collection methods

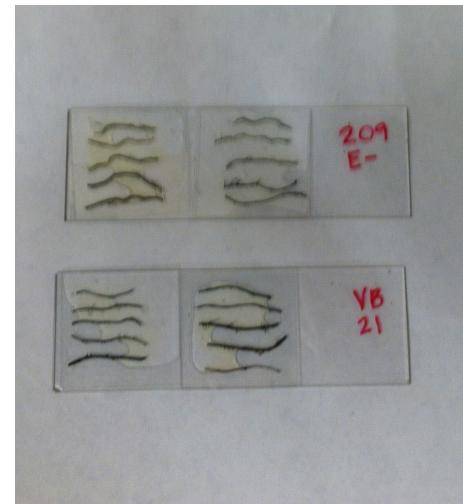
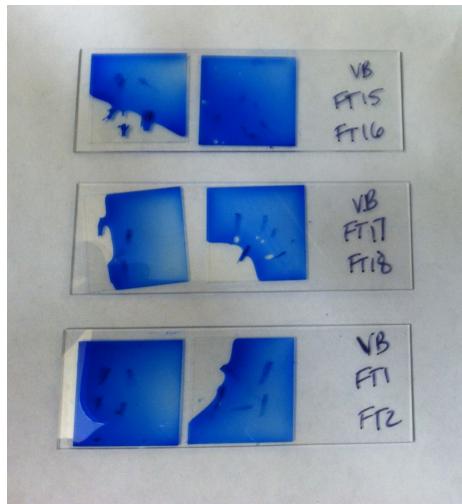
- 3 focal grass species:
  - *Achnatherum lettermanii*
  - *Poa pratensis*
  - *Festuca thurberi*
- 6 individual plants collected per species per plot
- Roots and leaves (2014)
- Phenology: June and September



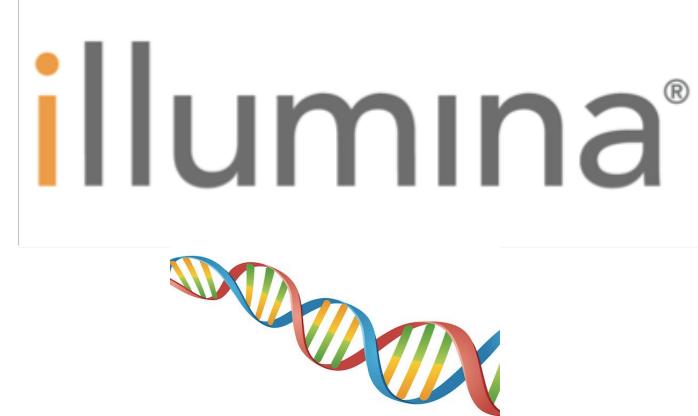
Photo: Noah Whiteman

# Laboratory methods

- Staining and microscopy → colonization



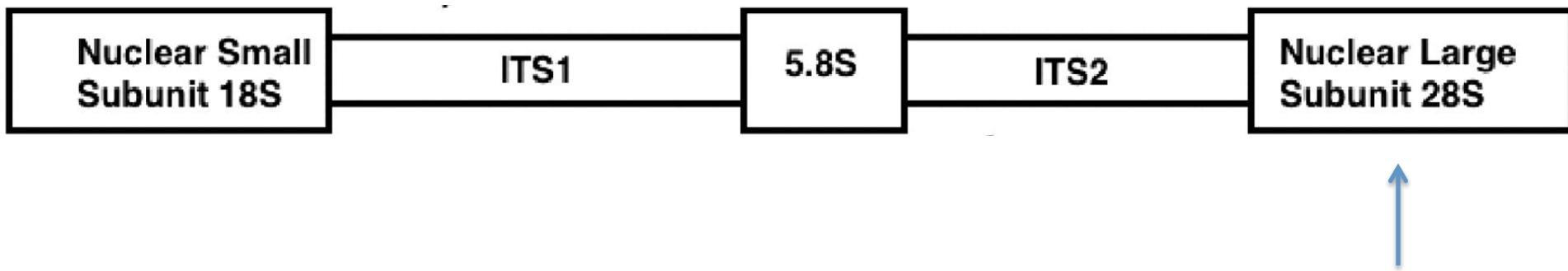
- Illumina MiSeq DNA sequencing → composition



# 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

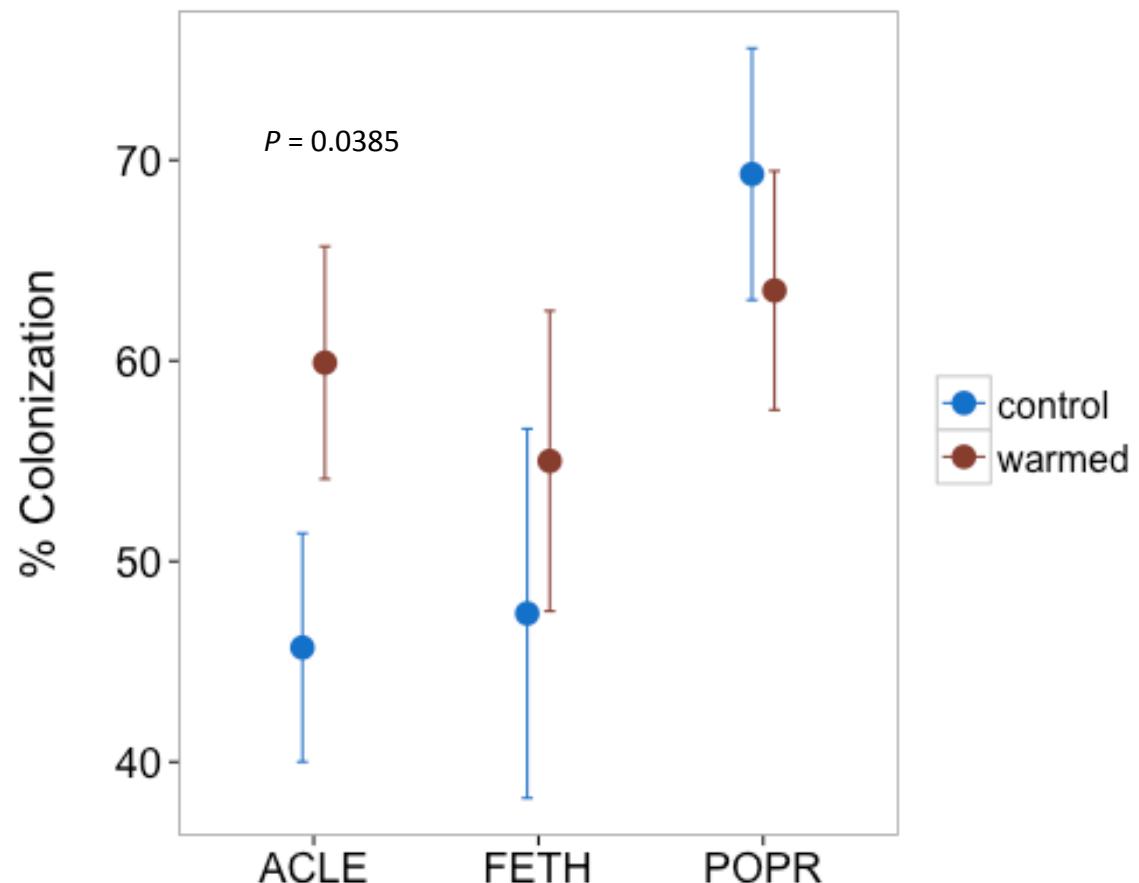
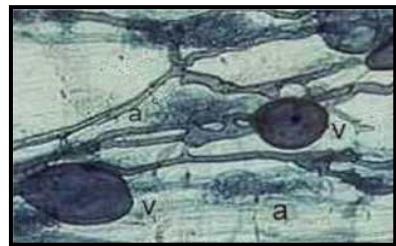
- Quality filtering in QIIME
- Sumaclust to place similar sequences into operational taxonomic units (OTUs) at ~97% identity
- NCBI BLAST to assign taxonomy
  - Discarded all OTUs with <97% identity to entry in database
- Normalized data using DESeq2
- Discarded singletons

→ Conducted analyses on 802 OTUs

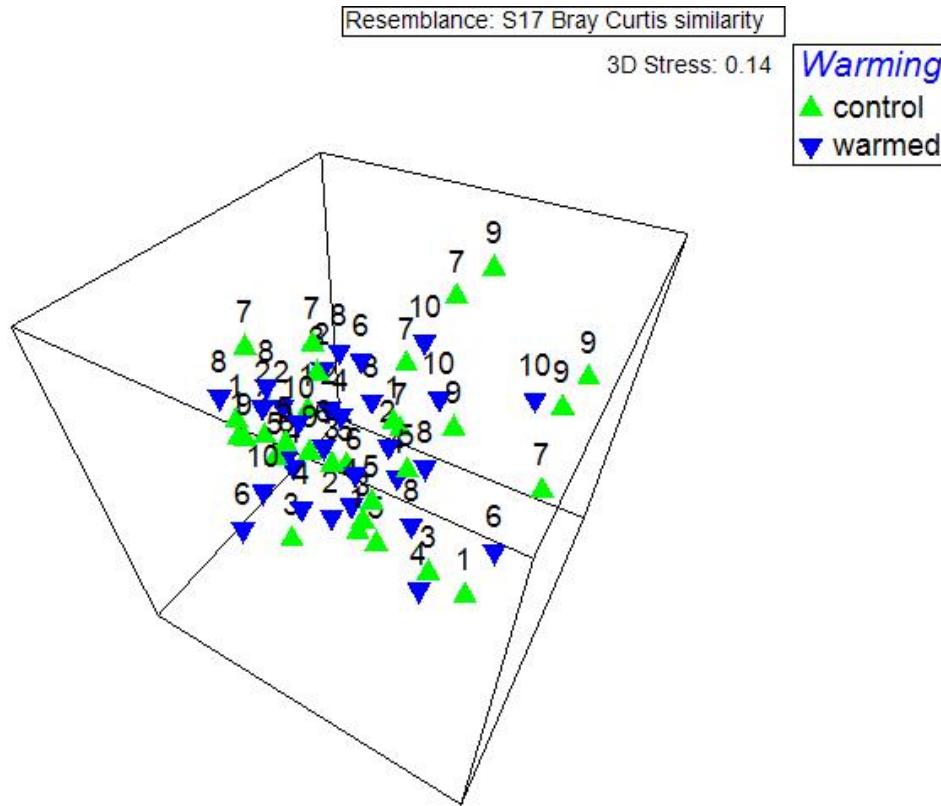
# Analyses

- 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

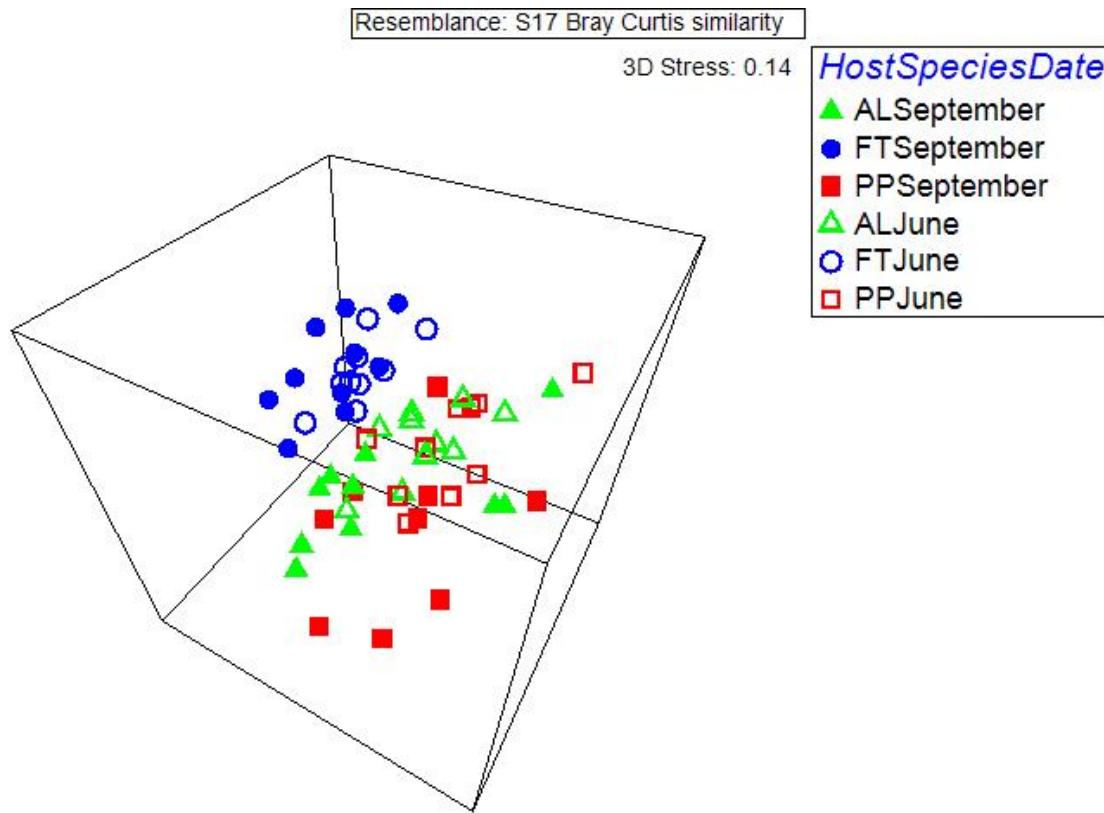
# Arbuscular mycorrhizal fungi



# Results: All Species



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

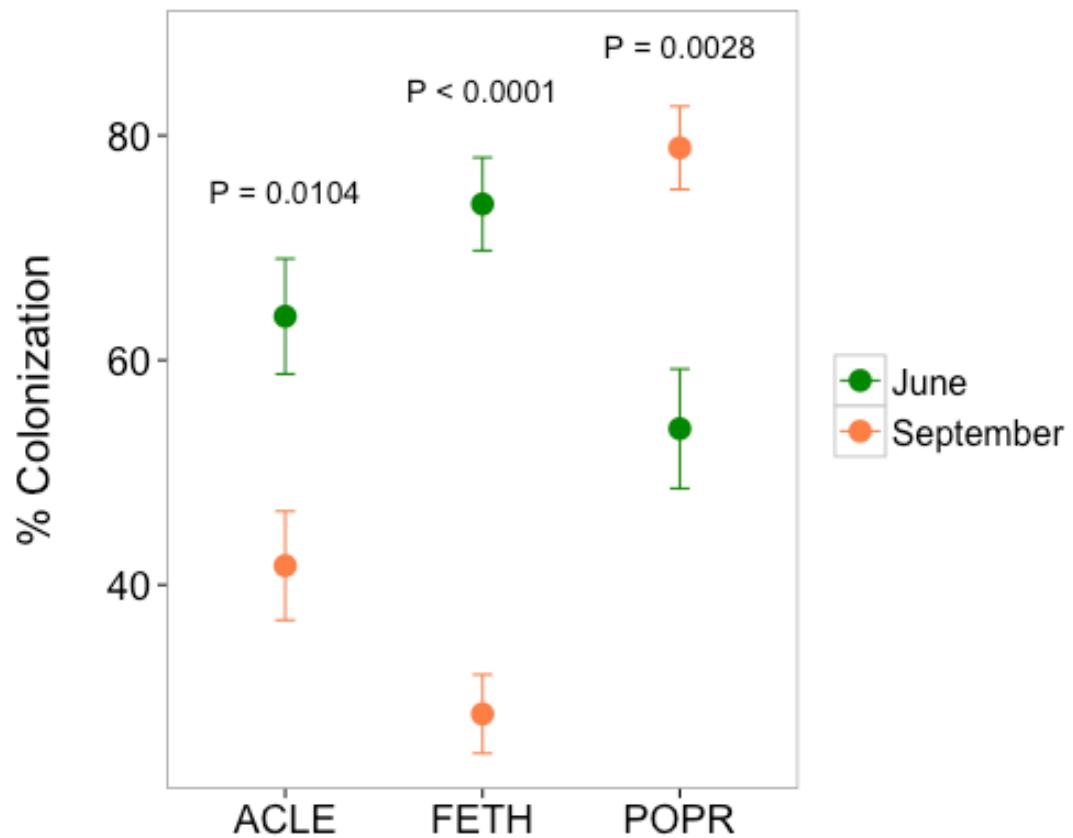
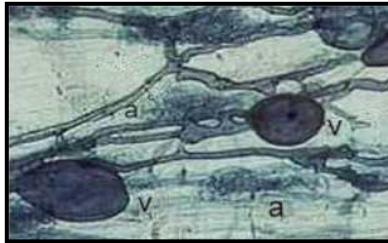


OTU composition differed between **sampling dates** ( $df = 1$ ,  $\text{pseudo-}F = 2.9483$ ,  $P = 0.0009$ ) and among **host species** ( $df = 2$ ,  $\text{pseudo-}F = 5.4469$ ,  $P = 0.0001$ )

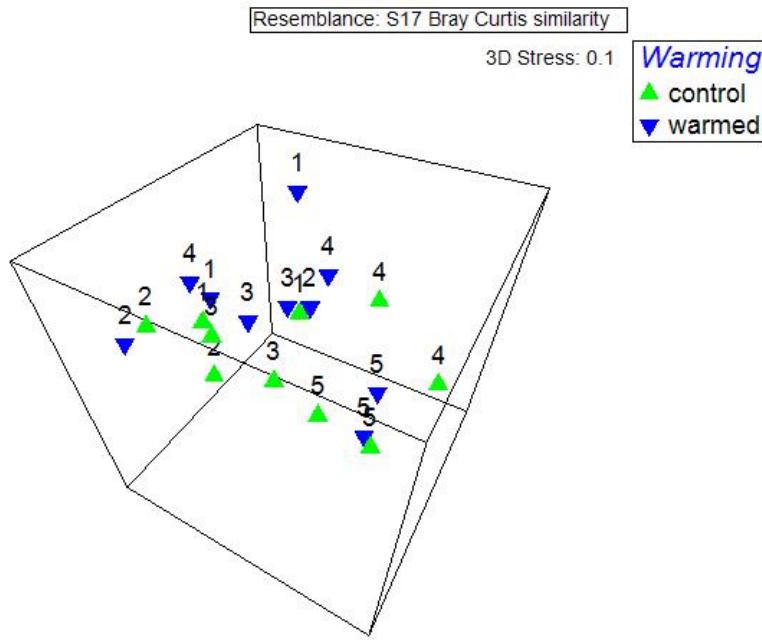
- FT differed from AL and PP
- Communities of AL and PP were significantly more dispersed relative to communities of FT (PERMDISP)

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

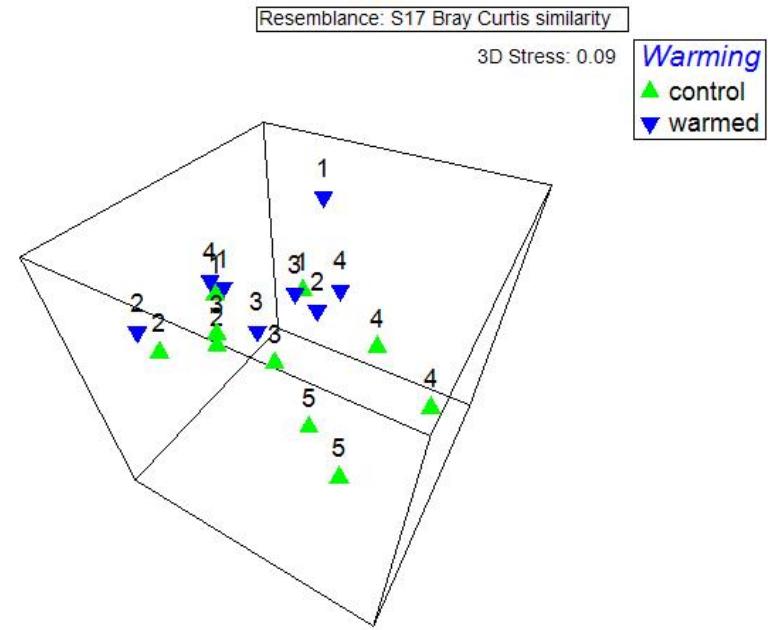
Arbuscular  
mycorrhizal fungi



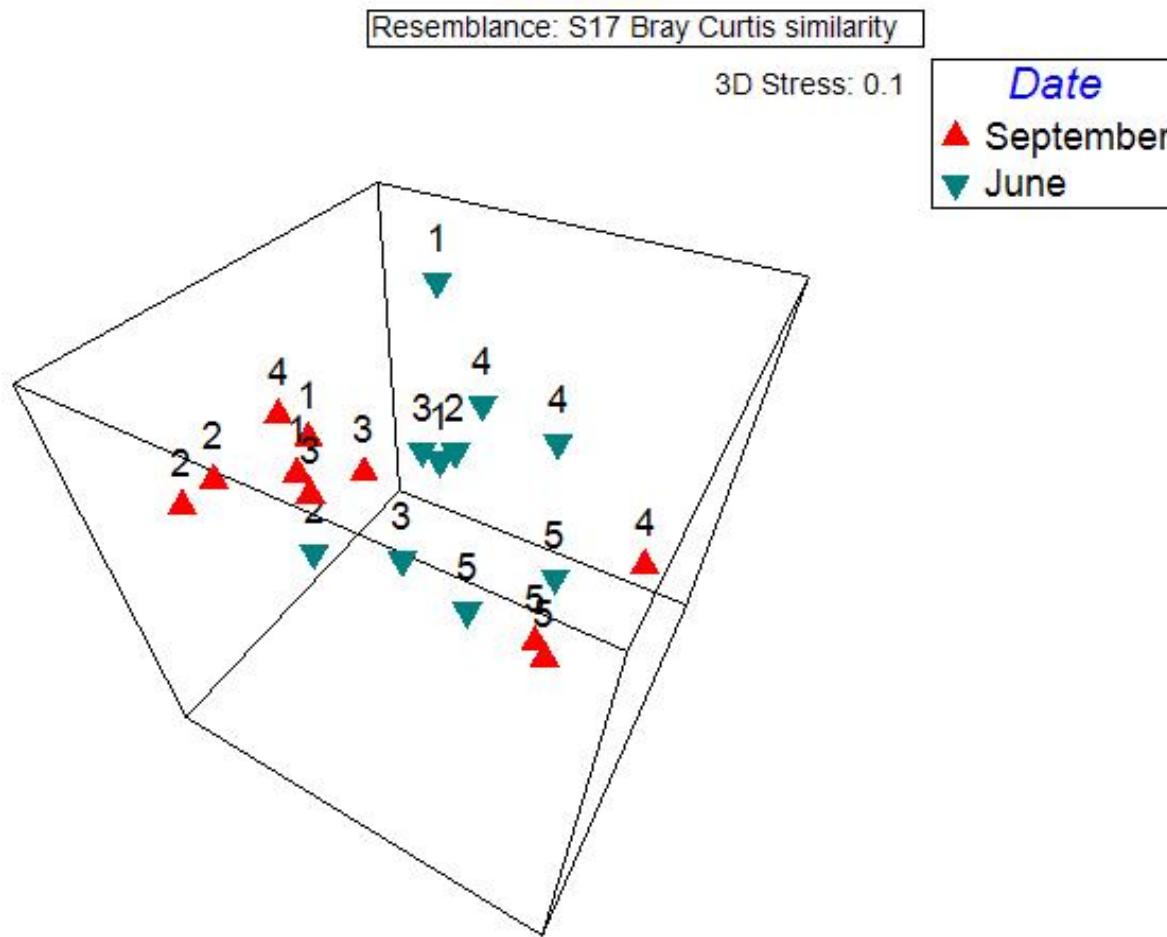
# Results: *A. lettermanii*



All data

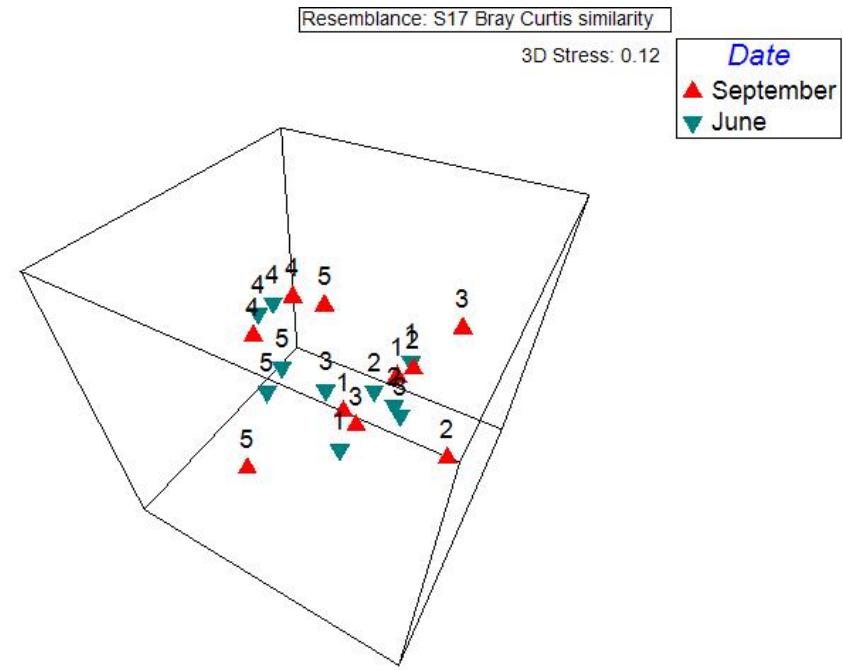
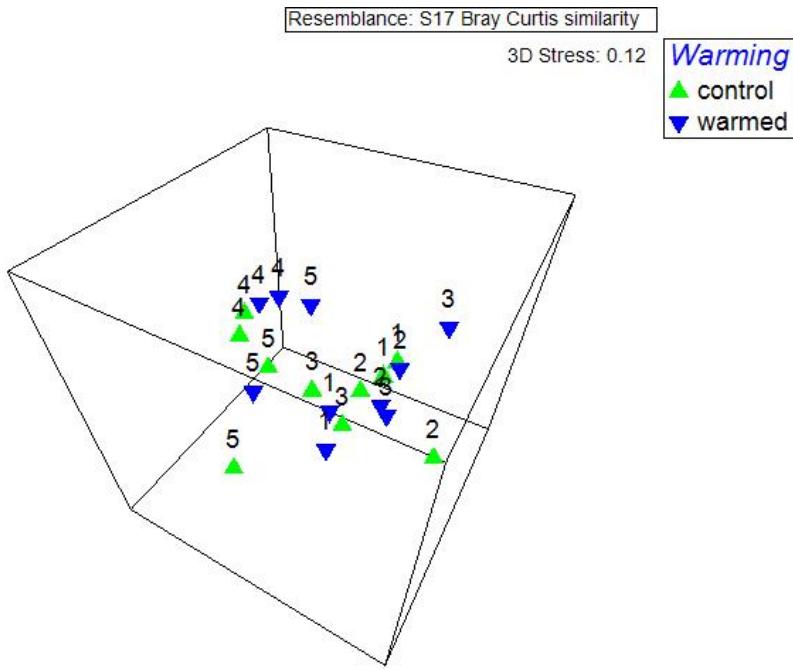


Plot 10 data  
removed



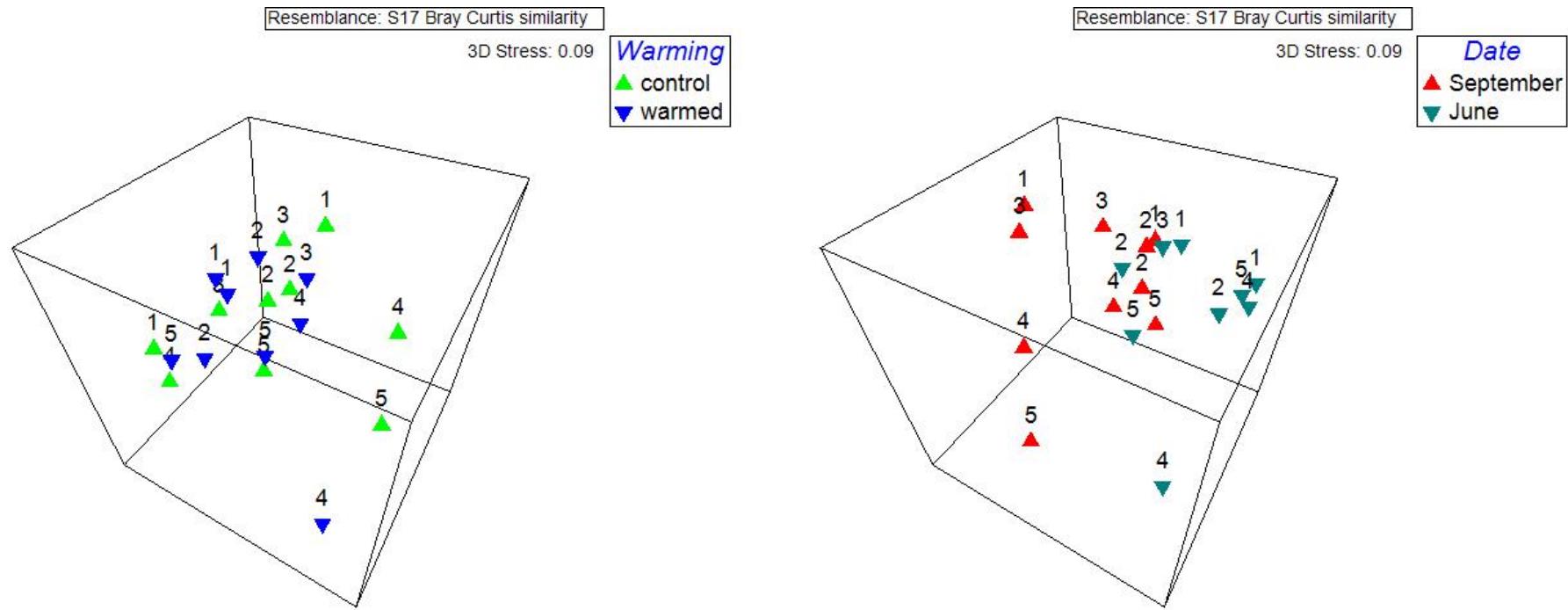
- Sampling date affected OTU composition ( $\text{df} = 1$ ,  $\text{pseudo-F} = 3.1274$ ,  $P = 0.0024$ )
- No difference in dispersion between two dates
- Grouping by plot

# 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		Warmed	
			Avg.	Avg.
	Abundance	Abundance	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*

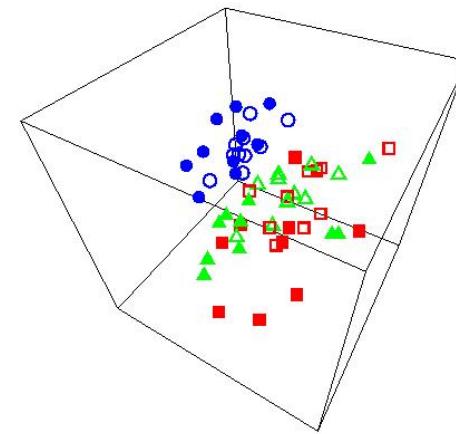
OTU	FETH		POPR		Contribution
	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	%	
OTU15	7.17	2.36	0.53	1.02	
OTU16	5.39	2.01	0.5	0.96	
OTU11	4.39	8.72	0.49	0.95	
OTU24	5.04	0.78	0.48	0.93	
OTU12	5.96	2.72	0.47	0.91	

Resemblance: S17 Bray Curtis similarity

3D Stress: 0.14

HostSpeciesDate

- ▲ ALSeptember
- FTSeptember
- PPSeptember
- △ ALJune
- FTJune
- PPJune



## *F. thurberi* vs. *A. lettermanii*

OTU	FETH		ACLE		Contribution
	Avg. Abundance	Avg. Abundance	Avg. Dissimilarity	%	
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 Details			
	Top BLAST Hit	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?

