

# How does climate change affect endophytic fungi?

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## Introduction

- Plant-associated microorganisms can have major effects on plant health (e.g., growth, pathogen defense)
- Climate change can affect plant microbiomes
  - Ex: Elevated CO<sub>2</sub> concentration in the atmosphere increased populations of nitrogen-fixing rhizobia bacteria in white clover roots (Grover et al. 2015)
- It is unknown how climate change will affect aboveground plant-associated microorganisms, such as foliar endophytic fungi
  - Foliar endophytic fungi are microbial colonizers of all plant species sampled to date

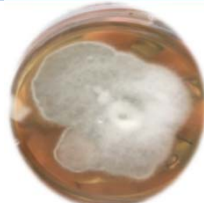
## Hypothesis

- We hypothesize that **increased CO<sub>2</sub> concentrations will increase species richness and diversity in endophytic fungal communities**, because increased plant photosynthesis under elevated CO<sub>2</sub> will increase the resources available to the fungi

## Methods

### 1 Collect plant samples

- Soybean (*Glycine max*) leaf samples were collected from 3 ambient plots and 3 elevated CO<sub>2</sub> plots at the SoyFACE research facility. 15 samples were taken from each plot (total N=90).



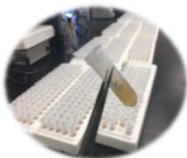
### 2 Cut & surface-sterilize

- Leaves were cut into 0.8cm x 0.8cm pieces and further cut into 16 2mm x 2mm pieces
- Placed in tea strainers and rinsed in ethanol, bleach, and water



### 3 Culture fungi

- Individual leaf segments were placed into tubes containing malt extract agar (called slants)
- 1,440 slants were made containing ambient and enriched CO<sub>2</sub> plant samples

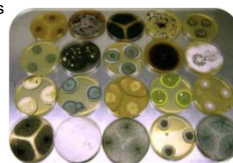


### 4 Subculture fungi

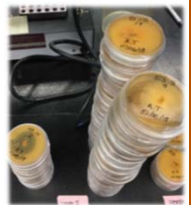
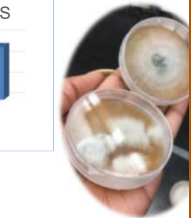
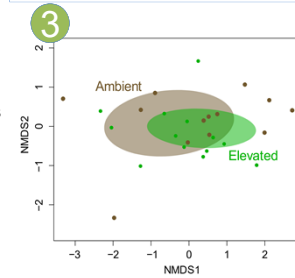
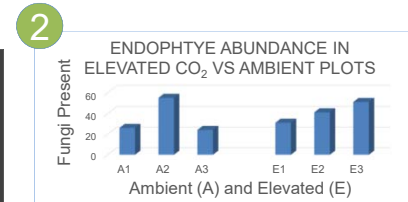
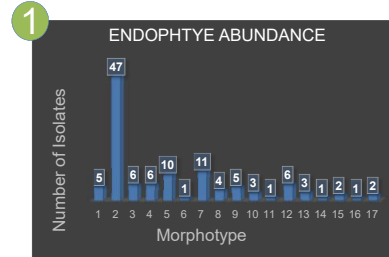
#### Morphotyping

#### DNA extraction

- Fungi growing in slants were transferred to agar plates
- Fungi were classified into morphotypes to be compared and evaluated before DNA extraction to find if there is species diversity, richness, and composition of endophyte communities



## Results



1. We have identified 17 fungal morphotypes isolated from soybean leaves
2. Endophyte species richness (abundance) and diversity (not pictured) were not affected by elevated CO<sub>2</sub> concentrations
3. Endophyte community composition was significantly affected by elevated CO<sub>2</sub> (p = 0.006)

### Specific endophytes of interest:

- Morphotype 2 (the most common morphotype) was isolated 39 times from elevated CO<sub>2</sub> plots, but only 9 times from ambient plots
- Morphotypes 4 and 9 also showed greater isolation rate from elevated CO<sub>2</sub> plots than ambient plots, while Morphotypes 11, 16, and 6 were only found in elevated plots.

## Conclusions

- Elevated CO<sub>2</sub> affects the composition of communities of fungi colonizing soybean leaves, but not their overall abundance and diversity

## Future Work

- Ongoing DNA extraction will confirm morphotype assignment and assign species names to fungi
- Endophytes isolated from elevated CO<sub>2</sub> and ambient plots will be re-inoculated onto soybean hosts to test for their different effects on soybean health and growth
- Our work informs engineering of plant microbiomes that could help protect plants against the effects of climate change

## References

Olbrich et al. 2010. "Ozone fumigation reduces leaf infestation following natural and artificial inoculation by the endophytic fungus *apiognomonina errabunda* of adult European trees." Environmental Pollution.

Hewitt et al. 2016 "N-fixation in legumes - An assessment of the potential threat posed by ozone pollution." Environmental Pollution.

Grover et al. 2015 "Elevated CO<sub>2</sub>: plant associated microorganisms and carbon sequestration". Applied Soil Ecology.

## Acknowledgments

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