



Soil Fungal Microbiome Analysis of an Experimental Truffle Orchard in Michigan

Marc Friedman (fried208@msu.edu), Gian Maria Niccolò Benucci, Bryan Rennick and Gregory Bonito
Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing MI, USA



Introduction

Context:

- In 2016, researchers at Michigan State University established an experimental truffle orchard using four species of truffles, including two European species (*Tuber borchii* and *Tuber aestivum*) and two North American species (*Tuber canaliculatum* and *Tuber lyonii*).
- Chestnut, pine, red oak, white oak, pecan, spruce, and Douglas fir seedlings were inoculated with *Tuber* spores.
- Orchard soils were limed to pH 7.0.
- The truffle orchard was divided into four quadrants; 1) no treatment (control), 2) biochar, 3) burn, 4) burn and biochar.
- Once the root systems formed ectomycorrhizas, 320 colonized trees were planted into the soil.

Objective:

- To determine the effects of tree species and soil treatments on soil fungal communities and *Tuber* persistence & fruiting.

Approach

Experimental design:

- In 2021, soil samples were taken from the 278 trees that remained in the truffle orchard (4 cardinal points ~0.5 m from tree).
- MiSeq libraries using ITS1F and ITS4 primers were prepared from the extracted DNA, which were sequenced with Illumina MiSeq.
- Sequences were analyzed to determine if target *Tuber* species persisted in the orchard, and the impacts of tree species on *Tuber* persistence, as well as broader fungal community, 5 years after the establishment of this Michigan truffle orchard.

Results

NMDS Plots of Fungal Communities Present in Orchard

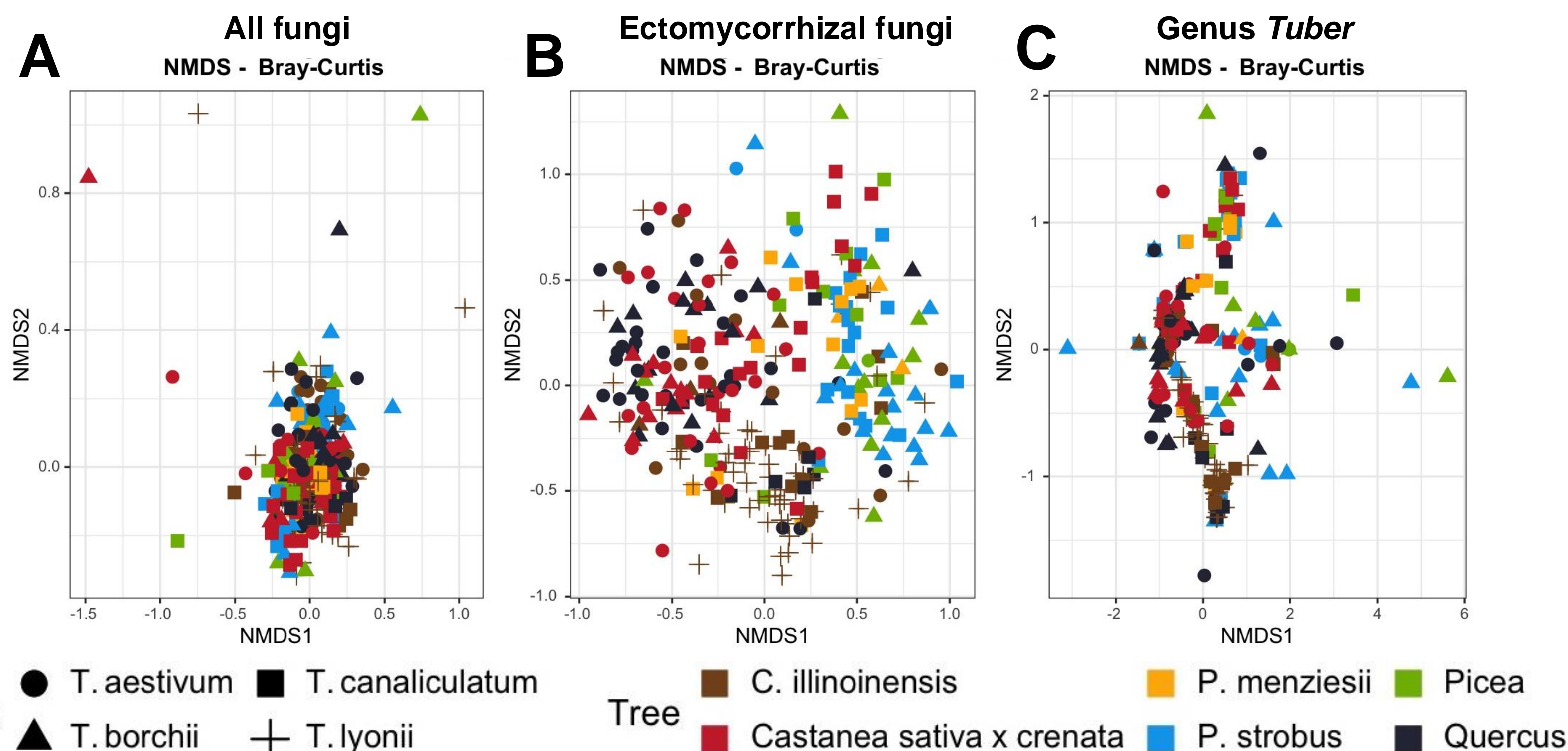


Figure 1: These nonmetric multidimensional scaling (NMDS) plots visualize the similarities between samples of multidimensional data in two dimensions. **A)** NMDS of all fungal taxa. **B)** NMDS of all ectomycorrhizal fungal taxa. **C)** NMDS of *Tuber* genus.

Results

Location of Target *Tuber* Species Detected in Orchard

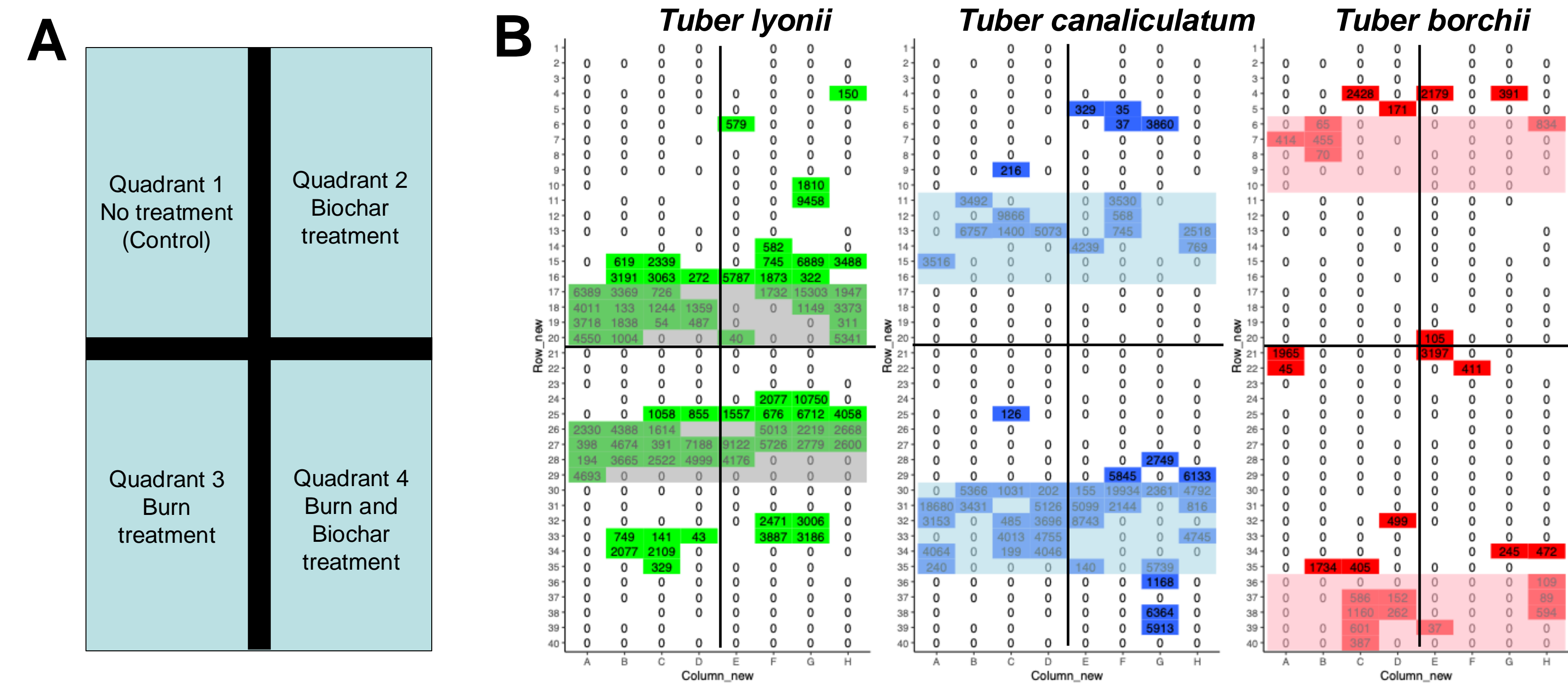


Figure 2: **A)** Soil treatments for each of the four quadrants. **B)** Heat maps depicting where three target species, *Tuber lyonii*, *Tuber canaliculatum* and *Tuber borchii*, were planted and detected in the truffle orchard.

Note: ITS1F did not amplify *Tuber aestivum*, due to group 1 introns in this species.

Relative Total Abundance of Rarefied *Tuber* Genus Data

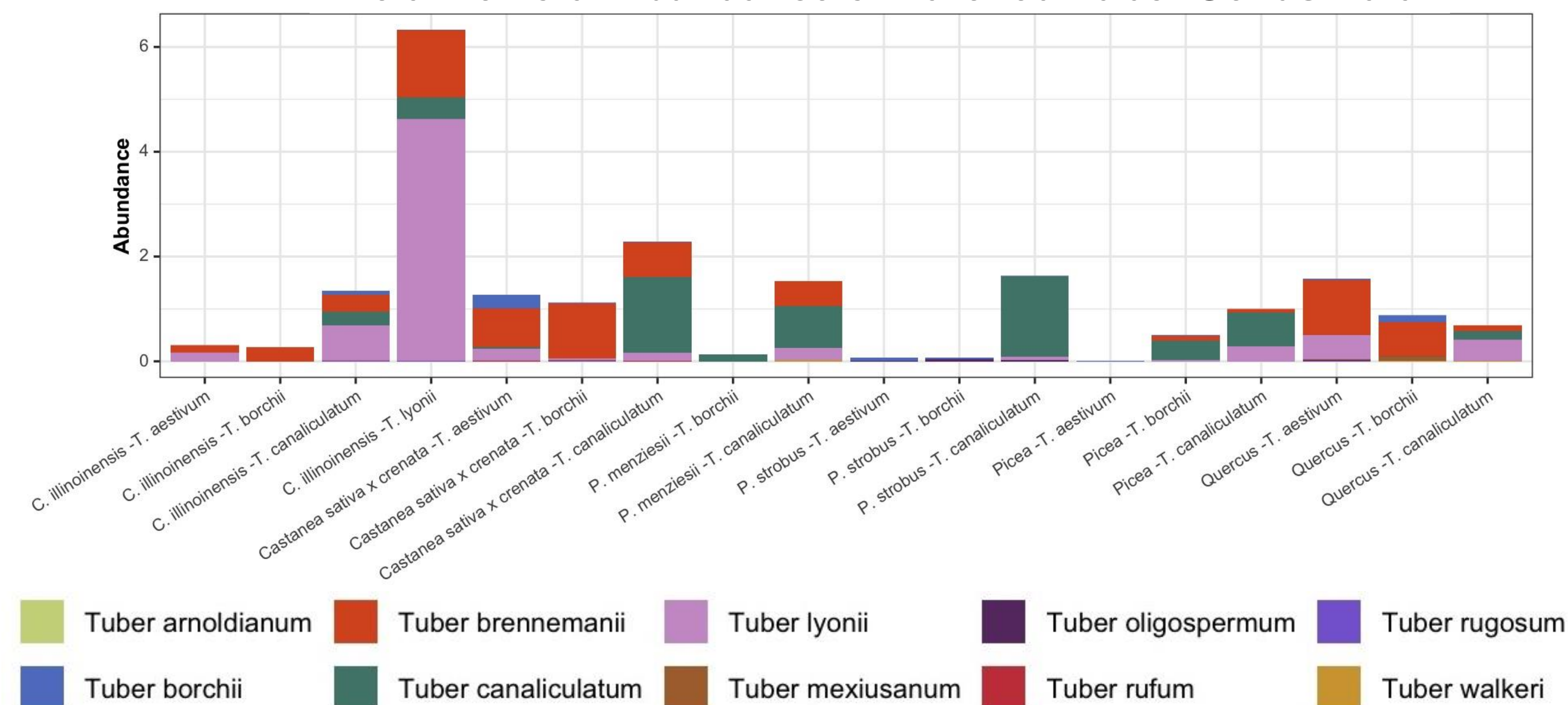


Figure 3: Ten species of *Tuber* were present, including three of the target species, one non-target European species, and six non-target North American species.

Conclusions

- Tuber borchii*, *Tuber lyonii*, and *Tuber canaliculatum* persisted on tree roots in a Michigan truffle orchard, five years after planting.
- Abundance of *Tuber* species varied between host tree species.
- Tuber canaliculatum* persisted in highest relative abundance on pine, Douglas Fir, and chestnut, and appeared to fare best with the burn treatment (17 trees with unburned, 33 trees with burned).
- Tuber borchii* did not persist well with any treatments in Michigan.
- Tuber lyonii* persisted (and fruited) on pecan, despite harsh winters.

Acknowledgments

We acknowledge Michigan State University and the Plant, Soil and Microbial Sciences Department, Project GREEN #GR23-057 (GB), NSF-DEB-1946445 (GB), NSF-GRFP Fellow ID: 2023361038 (MF), and NATGA for support. We thank Tim Brenneman, Matthew Smith, and Chuck Wilson for germplasm and helpful discussions.