

# Effects of Various Soil Microbiomes on Native and Invasive Plants

Eric Wang

**Abstract—** Many nature reserves use exclosures to preserve pockets of native plant biodiversity. These exclosures typically have a high proportion of native plants, as there are few invasive species to harm them, resulting in different plant-soil feedbacks (PSFs) within and outside of exclosures. PSFs alter the soil microbiome and have lasting effects on plant community composition; as such, they have important implications for natural ecosystem conservation. For this case study, three native and two invasive plant species were grown in soil inoculated with microbiomes collected from inside and outside of ten exclosures. Analyses of their biomass revealed that native species performed better in the soil microbiome from the exclosures, while invasive species' growth was not significantly impacted by the different microbiomes. This research provides new insights into plants and the soil microbiome in the context of conservation and has important implications on the protection of natural ecosystems.

## I. INTRODUCTION

The soil microbiome consists of billions of microorganisms living in a complex, underground ecosystem. In relation to their interactions with nearby plants, soil microorganisms can generally be categorized into three groups: enemies, which negatively affect plants; mutualists, which positively affect plants; and decomposers, whose main roles are in nutrient cycles [1]. Taken altogether, the balance of these three groups has significant effects on plant growth and development. Plants can take advantage of these effects by altering the soil microbial community composition to their benefit. For instance, in the “cry for help” phenomenon, plants release various organic chemicals through their roots to recruit certain microorganisms that help them resist environmental stressors, such as drought [2]. These changes are short-term, but plants can also have lasting effects on the soil microbiome. Known as plant-soil feedbacks (PSFs), these changes last long after the original plant is gone and have important influences on plant community assemblage [3].

Plants have species-specific feedback effects, which contribute to differing fitness levels among plant species. In the context of conservation, it is hypothesized that non-native plants may exploit PSFs to outcompete native plants and become invasive. Meisner et al. (2014) recently conducted a meta-analysis of native and non-native PSFs, which revealed that non-native species typically had fewer self-negative PSFs; this may be an important contributor to their invasiveness, which warrants further research into how these PSFs affect the spread of invasive species [5].

Invasive species management is key to preserving native biodiversity and healthy natural ecosystems [6]. To combat the spread of invasive plant species, many nature reserves use

exclosures, fenced-in regions meant to protect native biodiversity from herbivores and the spread of invasive species. One study in Europe found that protected areas such as these had lower invasive richness inside compared to outside despite being a suitable habitat for them [7]. A recent survey of the exclosures at the South Mountain Reservation, located in Essex County, NJ, similarly found that exclosures typically had higher native plant coverage than outside the exclosures [8].

This case study analyzes how the soil microbiome inside and outside of the exclosures at the South Mountain Reservation differ in their effects on native and invasive plant species. It seeks to determine whether the PSFs resulting from the differing native plant cover inside and outside exclosures influence how the soil microbiome affects different plant species.

## II. METHODS

Soil samples were collected from both inside and outside of ten randomly sampled exclosures in the southeast portion of the South Mountain Reservation. The soil microbiomes of each site were then transferred to a sterilized potting mix of potting soil (80% (v/v)) and topsoil (12.5% (v/v)) through direct inoculation at a rate of 7.5% (v/v), which transfers the soil microbiome while only minimally altering abiotic conditions. [9] A sterile control was included using a sterile inoculant. Pots were incubated overnight at ambient temperature (~27 °C) prior to planting.

The experiment consisted of 3 blocks and 21 treatments. Two of the blocks had individual plants grown in each pot, while the third block had larger pots with two individuals from each species per pot. Plants were kept in a grow tent under 600 W metal-halide lamps providing approximately 220  $\mu\text{mol}$  light quanta  $\text{m}^{-2}\text{s}^{-1}$  at plant level with a photoperiod of 16:8 hours (day:night). Ambient temperature was 30°C day/22°C night and relative humidity was 50-70%.

Five plant species were chosen based on their family and their relative conservation priorities at the reservation[8]. Three species were native (*Solidago flexicaulis* (Asteraceae), *Calamagrostis canadensis* (Poaceae), and *Geranium maculatum* (Geraniaceae)) and two species were invasive (*Artemisia vulgaris* (Asteraceae) and *Miscanthus sinensis* (Poaceae)). Seeds were surface-sterilized with a solution of 2.75% NaClO and 0.005% Tween20 for 1 minute, then germinated on a moist paper towel medium. Seedlings that died within the first two weeks were replaced, though seedling mortality was low (~1.2%). Plants were watered daily for the first 4 weeks, then every other day for the remainder of the experiment. Plants were allowed to grow for 7 weeks total.

Fresh biomass was then measured as total aboveground biomass; dry biomass was measured by drying the plants.

All statistical analyses were performed using R version 4.1.0 [10]. Biomass data from the smaller pots were analyzed with linear mixed effects models using the *lme4* package with plant species, microbiome origin (inside/outside), and their interaction as fixed effects and a random effect of block. Separate models were also generated for each plant species. Data from the larger pots were analyzed using the total native and total invasive biomasses per pot. Statistical significance was tested using F-tests with the *anova* function in the package *lmerTest*.

### III. RESULTS AND DISCUSSION

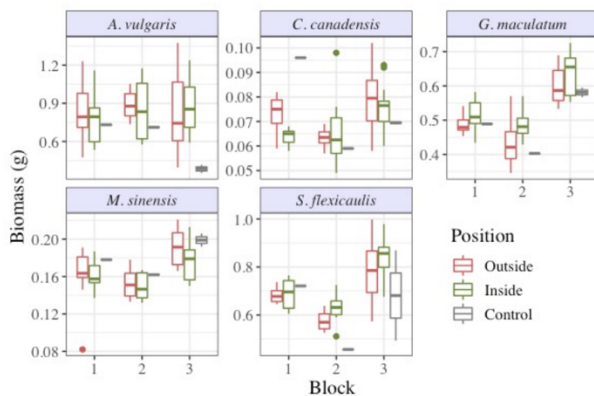


Fig. 1 Box plots of biomass separated by species, block, and position

Species	F	p
Overall (Species Position)	$F_{1,37} = 1.627$	0.1627
<i>A. vulgaris</i>	$F_{1,37} = 0.3985$	0.5317
<i>C. canadensis</i>	$F_{1,37} = 2.0298$	0.1626
<i>G. maculatum</i>	$F_{1,37} = 6.545$	<b>0.01475</b>
<i>M. sinensis</i>	$F_{1,37} = 1.0684$	0.3082
<i>S. flexicaulis</i>	$F_{1,37} = 3.4441$	0.07146
Total Native Biomass	$F_1 = 5.42$	<b>0.03177</b>
Total Invasive Biomass	$F_1 = 0.2017$	0.6587

Table 1. Effects of microbiome origin on biomass

In general, plant biomass did not differ significantly based on whether the soil microbiome originated from inside or outside of an enclosure. However, responses to changes in microbiome origin were species-specific: the effect was statistically significant for *G. maculatum* ( $F_{1,37} = 6.5450$ ,  $p = 0.01475$ ) and trended towards significance for *S. flexicaulis* ( $F_{1,37} = 3.4441$ ,  $p = 0.07146$ ). Biomass when grown in soil microbiomes from within enclosures was approximately 8% higher than outside for *G. maculatum* and 5% higher for *S. flexicaulis*. Microbiome origin also had a statistically significant effect on total native biomass ( $F_1 = 5.42$ ,  $p = 0.03177$ ) but not on total invasive biomass ( $F_1 = 0.2017$ ,  $p = 0.6587$ ).

Overall, these results are generally consistent with prior research. The differing microbiome origins did not have a significant effect on invasive species, while native species tended to be more positively affected when the soil microbiome originated from within an enclosure. This suggests that the detrimental PSFs caused by invasive species outside of enclosures outweigh the harm caused by self-negative PSFs of native species within the enclosures. In addition, it suggests that invasive species are not significantly affected by either the positive or negative PSFs that typically hinder native plants. The effects of microbiome origin also varied with plant life form, consistent with the previously mentioned meta-analysis by Meisner et al. (2014). Microbiome origin had a significant effect on native forbs (*G. maculatum* and *S. flexicaulis*) than the grasses (*C. canadensis* and *M. sinensis*), suggesting that native forbs may be more negatively impacted by invasive PSFs than grasses.

### IV. CONCLUSION

This study addresses the previous literature gap on the effects of enclosures and PSFs on the soil microbiome. Future research could focus on analyzing other nature reserves and using a wider variety of species to better understand how the soil microbiomes of enclosures affect native and invasive plant species. In short, these findings can be used to guide conservationists in designing methods to prevent the destruction of native biodiversity and serve as a starting point for further research into the field.

### V. ACKNOWLEDGEMENTS

I would like to acknowledge my science research teacher, Dr. Arrigoni, and my mentor, Dr. Howard from Indiana University, for all of their help with my research. I would also like to thank the South Mountain Conservancy for their help with collecting soil samples at the reservation.

### VI. REFERENCES

- [1] Van der Putten, W. H., Bradford, M. A., Pernilla Brinkman, E., Voorde, T. F. J., & Veen, G. F. (2016). Where, when and how plant–soil feedback matters in a changing world. *Functional Ecology*, 30(7), 1109–1121. <https://doi.org/10/f8wg5t>
- [2] Rodriguez, R., & Durán, P. (2020). Natural Holobiome Engineering by Using Native Extreme Microbiome to Counteract the Climate Change Effects. *Frontiers in Bioengineering and Biotechnology*, 8, 568. <https://doi.org/10.3389/fbioe.2020.00568>
- [3] van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N., Van de Voorde, T. F. J., & Wardle, D. A. (2013). Plant–soil feedbacks: The past, the present and future challenges. *Journal of Ecology*, 101(2), 265–276. <https://doi.org/10/f24cwq>
- [4] Meisner, A., Gera Hol, W. H., de Boer, W., Krumins, J. A., Wardle, D. A., & van der Putten, W. H. (2014). Plant–soil feedbacks of exotic plant species across life forms: A meta-analysis. *Biological Invasions*, 16(12), 2551–2561. <https://doi.org/10/f2484n>
- [5] Adams, S. N., Jennings, S., & Warnock, N. (2020). Plant invasion depresses native species richness, but control of invasive species does little to restore it. *Plant Ecology & Diversity*, 13(3–4), 257–266. <https://doi.org/10/gj2m44>
- [6] Gallardo, B., Aldridge, D. C., González-Moreno, P., Pergl, J., Pizarro, M., Pyšek, P., Thuiller, W., Yesson, C., & Vilà, M. (2017). Protected areas offer

refuge from invasive species spreading under climate change. *Global Change Biology*, 23(12), 5331–5343. <https://doi.org/10/cbh8>

[7] Van Clef, M. (2014). South Mountain Reservation Forest Regeneration Site Evaluation Report. [https://www.somocon.org/wordpress/wp-content/uploads/2015/03/SMR\\_Forest\\_Regeneration\\_Site\\_Evaluation\\_Report\\_2014\\_12\\_02.pdf](https://www.somocon.org/wordpress/wp-content/uploads/2015/03/SMR_Forest_Regeneration_Site_Evaluation_Report_2014_12_02.pdf)

[8] Howard, M. M., Bell, T. H., & Kao-Kniffin, J. (2017). Soil microbiome transfer method affects microbiome composition, including dominant microorganisms, in a novel environment. *FEMS Microbiology Letters*, 364(11). <https://doi.org/10.1093/femsle/fix092>