MAKING SENSE OF SOIL MICROBIOME COMPLEXITY FOR PLANT AND ECOSYSTEM FUNCTION IN A CHANGING WORLD

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DEDICATION

I dedicate this dissertation to my parents Kim and Dexter Beals and to my grandmother Marion Snyder. To my parents, thank you for your endless and unwavering support and encouragement of my passion to pursue science. To Nana, thank you for setting such a wonderful example of what it means to march to the beat of one's own drum and for always asking me how the microbes are doing.

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ABSTRACT

Soils contain the highest biodiversity on Earth. While the importance of the soil microbiome for larger-scale ecological phenomena such as nutrient and carbon cycling, plant growth and plant community dynamics is well-established, the fundamental question of the ecological and evolutionary function of this immense belowground microbial diversity for plant and ecosystem function still remains a great challenge in microbial ecology research. The objective of this dissertation is to understand how the importance of soil microbial community composition for plant and ecosystem function and how changes to soil microbial community composition from climate change-induced disturbance events, specifically fire, influence plant and ecosystem function. Specifically, I examine: (1) the relative importance of microbial taxa and functional genes within complex natural soil microbiomes for plant phenotypic variation in a common herbaceous plant (Solidago); (2) the trends in responses of plant-soil microbial interactions to multiple gradients of environmental stress and disturbance; (3) the effect of fire-induced changes to soil microbial community composition on the restoration success of a common tree species (Quercus velutina); and (4) the effect of fire-induced changes to composition of microbial carbon use efficiency on carbon cycling. The findings of this dissertation suggest that a small proportion of a complex soil microbial community may be important for plant variation in plant traits, and that the importance of soil microbial community composition varies among plant trait and plant species; that there is high variability in how plant-soil interactions respond to climate change-induced stressors and disturbances; and that shifts in microbial community membership can influence plant function and specific ecosystem-level processes. These ideas have broad implications for predicting and managing plant communities and ecosystems under changing environmental conditions because they show that changes in soil microbial community composition can yield significant shifts in plant and ecosystem function.

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INTRODUCTION

Soils contain the highest biodiversity on Earth. It is estimated that one gram of soil may contain as many as one million species of bacteria (Torvisk et al. 2002; Tringe et al. 2005) and as much as 200 meters of fungal hyphae (Leake et al. 1997). The importance of the soil microbiome for larger-scale ecological phenomena such as nutrient and carbon cycling, plant growth and plant community dynamics is well-established (Bardgett & van der Putten, 2014; Van Der Heijden et al., 2008). Yet, the fundamental question of the ecological and evolutionary effects of this immense belowground microbial diversity for plant and ecosystem function still remains a great challenge in microbial ecology research.

Findings from plant soil linkages and feedback research over the past nearly 30 years have shown that plant traits vary in response to different bulk soil microbiome inoculation treatments (Bever, 1994; Bever et al., 1997; Ehrenfeld et al., 2005; Mangan et al., 2010; Putten et al., 2013). Bulk soil microbiome inoculations have been found to alter multiple plant physiological (Friesen et al., 2011; Kannenberg & Phillips, 2017; Lau & Lennon, 2011; Zhu et al., 2016), reproductive (Lau & Lennon, 2011, 2012), and phenological traits (Panke-Buisse et al., 2015; Wagner et al., 2014) in addition to commonly-studied traits of above- and belowground biomass. This suggests that collectively, networks of soil microbial taxa can influence plant function. Concomitantly, single-inoculation studies have also found that individual microbial taxa have specific functional importance for plant traits (Ali et al., 2009; Knief et al., 2012; Mendes et al., 2014; Mishra et al., 2009; Narula et al., 2011; Niu et al., 2018; Ofek-Lalzar et al., 2014; Vílchez et al., 2016). Additionally, a recent review has documented an increasing wave of studies identifying putative keystone soil microbial taxa for plant productivity (Banerjee et al., 2018). While all of these findings have significantly advanced our understanding of the role of soil microbial communities on plant function, the relative importance of soil microbial compositional complexity for the plant phenotype spectrum is still unclear. To pinpoint the important individual microbial taxa and microbial functions within complex microbial communities, research is needed that examines the role of soil microbial community composition on multiple plant traits simultaneously.

At a broader level, microbes are essential to large-scale ecosystem processes. For example, collectively, soil microbes drive carbon cycling through the decomposition of organic carbon and the subsequent respiration of carbon gases into the atmosphere (Bradford et al., 2019; Castellano et al., 2015; Cotrufo et al., 2013). A growing body of research has also documented linkages

between nutrient transformation processes and individual microbial taxa (Banerjee et al., 2016; Ding et al., 2015; Li et al., 2017; Zhou et al., 2011). Importantly, however, soil microbial mediation of plant and ecosystem functions will change with climate change-induced disturbances and stressors. For example, soil microbes are the leading drivers of soil organic carbon cycling (SOC). Changes in soil carbon stocks due to variation in microbial decomposition rates of SOC will have consequences for production of greenhouse gases such as carbon dioxide and methane at the global scale. In addition, soil microbial mediated processes, such as SOC decomposition, are expected to vary by the type of climate change disturbance or stressor (Jansson & Hofmockel, 2020). As one example of a type of disturbance exacerbated by climate change, increases in fire frequency and severity (Barbero et al., 2015; Moritz et al., 2012) are altering both soil microbial communities (Dooley & Treseder, 2012; Pressler et al., 2019) and the rate at which carbon is released into the atmosphere (Nave et al., 2011; Pellegrini et al., 2020). Yet, it is still relatively unclear how fire-induced changes to soil microbial community composition will affect carbon cycling dynamics. Collectively, these findings emphasize that investigations into the role of soil microbial mediation of plant and ecosystem function under climate change scenarios are lacking compared to research that has been conducted on relationships of soil microbiomes and plant and ecosystem processes under ambient environmental conditions. Directed research in this area will enable more nuanced and accurate predictions of how plant communities and ecosystems will function under changing environments. Identifying these relationships will also enable more effective management of ecosystems and their plant communities in the face of climate change.

My dissertation bridges these knowledge gaps by addressing the following questions: 1) What is the role of soil microbial community composition for plant and ecosystem function? And 2) How do variations in soil microbial community composition from climate change-induced disturbance events influence plant and ecosystem function? I used a combination of field observations, glasshouse experiments, genomic analysis, and literature review to distill the complexity of soil microbial communities and identify the key microbial taxa and their respective contributions for plant and ecosystem function.

In Chapter I, I used field observations, amplicon sequencing, shotgun metagenomic sequencing, and a microbiome inoculation glasshouse experiment to identify specific microbial taxa and/or functional genes within complex natural soil microbiomes that are associated with shifts in a range of diverse traits of three species of Solidago (goldenrod). I found that soil

microbial mediation of plant phenotype can be associated with a small number of microbial taxa and that the importance of soil microbial mediation varies among plant traits and plant species. This chapter illustrates that it is possible to identify important individual microbial taxa from complex soil microbial communities. In Chapter II, I conducted a meta-analysis to identify trends in responses of plant-soil interactions to environmental gradients of stress and disturbance. I found that there is wide variation in how plants respond to environmental-induced changes to soil microbial communities and that this variability is caused by the dearth of studies that examine plant-soil interactions under climate change scenarios. This chapter shows that research in the field of plant-soil interactions needs to focus on how environmental stress and disturbance (including fire) alters soil microbial communities and how these environmental-induced changes to soil biota subsequently influence plant function. Based on the meta-analysis findings, I conducted an empirical study in Chapter III in which I used amplicon sequencing on field-collected soil and a microbiome inoculation glasshouse experiment to investigate how alterations in soil microbial community composition from a fire disturbance in Great Smoky Mountains National Park, TN, can impact the restoration success of a foundational tree species (Quercus velutina, black oak). I found that fire disturbance altered soil fungal communities more than bacterial communities, and that fire particularly reduced the diversity and abundance of plant pathogenic fungi. Importantly, oak seedlings benefited from a reduction in pathogenic fungi in the soil more so than to changes to the overall fungal community. Work from this chapter demonstrates that soil microbial groups will likely differ in their response to environmental disturbance and that plant function may benefit or be hindered by environmental disturbance depending on which microbial taxa or functions are impacted by the disturbance. Lastly in Chapter IV, I used the same fire disturbance system in Great Smoky Mountains NP and a structured conceptual framework (Hall et al., 2018) to explicitly identify how disturbance-induced changes to soil microbial community composition influenced ecosystem function. I conducted amplicon sequencing and measured soil abiotic properties to assess the composition of soil microbial communities and ecosystem functions associated with carbon cycling. I found that fire disturbance altered the composition of microbial carbon use efficiency such that burned soils were comprised of more taxa that thrive under low-resource conditions compared to unburned soils, and that this change in composition was driven by bacteria rather than by fungi. I found that these in microbial carbon use efficiency composition were associated with variation in carbon-degrading enzyme activity, an important component of carbon

cycling. This chapter illustrates that environmental disturbance can alter soil microbial community composition in functionally-relevant ways and that this shift in community composition can be important for ecosystem-level processes.

These four chapters overall address a range of questions to ascertain the role of soil microbial diversity on plant function and ecosystem processes, and how these interactions between soil microbial communities and aboveground functions may change in a changing world with more frequent disturbances, such as fire. Ultimately, my dissertation takes a novel approach to making sense of the broader significance of the hyper-diversity of soil microbial communities by targeting the most important components of soil microbial communities for plant and ecosystem function in a global-change context.

REFERENCES

Ali, B., Sabri, A. N., Ljung, K., & Hasnain, S. (2009). Auxin production by plant associated bacteria: Impact on endogenous IAA content and growth of Triticum aestivum L. Letters in Applied Microbiology, 48(5), 542–547. https://doi.org/10.1111/j.1472-765X.2009.02565.x

Banerjee, S., Kirkby, C. A., Schmutter, D., Bissett, A., Kirkegaard, J. A., & Richardson, A. E. (2016). Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biology and Biochemistry, 97, 188–198. https://doi.org/10.1016/j.soilbio.2016.03.017

Banerjee, S., Schlaeppi, K., & van der Heijden, M. G. A. (2018). Keystone taxa as drivers of microbiome structure and functioning. Nature Reviews Microbiology, 16(9), 567–576. https://doi.org/10.1038/s41579-018-0024-1

Barbero, R., Abatzoglou, J. T., Larkin, N. K., Kolden, C. A., & Stocks, B. (2015). Climate change presents increased potential for very large fires in the contiguous United States. International Journal of Wildland Fire, 24(7), 892–899. https://doi.org/10.1071/WF15083

Bardgett, R. D., & van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. Nature, 515(7528), 505–511. https://doi.org/10.1038/nature13855

Bever, J. D. (1994). Feeback between Plants and Their Soil Communities in an Old Field Community. Ecology, 75(7), 1965–1977. https://doi.org/10.2307/1941601

Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach. Journal of Ecology, 85(5), 561–573. JSTOR. https://doi.org/10.2307/2960528

Bradford, M. A., Carey, C. J., Atwood, L., Bossio, D., Fenichel, E. P., Gennet, S., Fargione, J., Fisher, J. R. B., Fuller, E., Kane, D. A., Lehmann, J., Oldfield, E. E., Ordway, E. M., Rudek, J., Sanderman, J., & Wood, S. A. (2019). Soil carbon science for policy and practice. Nature Sustainability, 2(12), 1070–1072. https://doi.org/10.1038/s41893-019-0431-y

Castellano, M. J., Mueller, K. E., Olk, D. C., Sawyer, J. E., & Six, J. (2015). Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology, 21(9), 3200–3209. https://doi.org/10.1111/gcb.12982

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology, 19(4), 988–995. https://doi.org/10.1111/gcb.12113

Ding, J., Zhang, Y., Wang, M., Sun, X., Cong, J., Deng, Y., Lu, H., Yuan, T., Van Nostrand, J. D., Li, D., Zhou, J., & Yang, Y. (2015). Soil organic matter quantity and quality shape microbial community compositions of subtropical broadleaved forests. Molecular Ecology, 24(20), 5175–5185. https://doi.org/10.1111/mec.13384

Dooley, S. R., & Treseder, K. K. (2012). The effect of fire on microbial biomass: A meta-analysis of field studies. Biogeochemistry, 109(1), 49–61. https://doi.org/10.1007/s10533-011-9633-8

Ehrenfeld, J. G., Ravit, B., & Elgersma, K. (2005). Feedback in the Plant-Soil System. Annual Review of Environment and Resources, 30(1), 75–115. https://doi.org/10.1146/annurev.energy.30.050504.144212

Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., & Martinez-Romero, E. (2011). Microbially Mediated Plant Functional Traits. Annual Review of Ecology, Evolution, and Systematics, 42(1), 23–46. https://doi.org/10.1146/annurev-ecolsys-102710-145039

Hall, E. K., Bernhardt, E. S., Bier, R. L., Bradford, M. A., Boot, C. M., Cotner, J. B., del Giorgio, P. A., Evans, S. E., Graham, E. B., Jones, S. E., Lennon, J. T., Locey, K. J., Nemergut, D., Osborne, B. B., Rocca, J. D., Schimel, J. P., Waldrop, M. P., & Wallenstein, M. D. (2018). Understanding how microbiomes influence the systems they inhabit. Nature Microbiology, 3(9), 977–982. https://doi.org/10.1038/s41564-018-0201-z

Jansson, J. K., & Hofmockel, K. S. (2020). Soil microbiomes and climate change. Nature Reviews Microbiology, 18. https://doi.org/10.1038/s41579-019-0265-7

Kannenberg, S. A., & Phillips, R. P. (2017). Soil microbial communities buffer physiological responses to drought stress in three hardwood species. Oecologia, 183(3), 631–641. https://doi.org/10.1007/s00442-016-3783-2

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., von Mering, C., & Vorholt, J. A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. The ISME Journal, 6(7), 1378–1390. https://doi.org/10.1038/ismej.2011.192

Lau, J. A., & Lennon, J. T. (2011). Evolutionary ecology of plant–microbe interactions: Soil microbial structure alters selection on plant traits. New Phytologist, 192(1), 215–224. https://doi.org/10.1111/j.1469-8137.2011.03790.x

Lau, J. A., & Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences, 109(35), 14058–14062. https://doi.org/10.1073/pnas.1202319109

Li, F., Chen, L., Zhang, J., Yin, J., & Huang, S. (2017). Bacterial Community Structure after Long-term Organic and Inorganic Fertilization Reveals Important Associations between Soil Nutrients and Specific Taxa Involved in Nutrient Transformations. Frontiers in Microbiology, 8. https://www.frontiersin.org/article/10.3389/fmicb.2017.00187

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010). Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature, 466(7307), 752–755. https://doi.org/10.1038/nature09273

Mendes, L. W., Kuramae, E. E., Navarrete, A. A., van Veen, J. A., & Tsai, S. M. (2014). Taxonomical and functional microbial community selection in soybean rhizosphere. The ISME Journal, 8(8), 1577–1587. https://doi.org/10.1038/ismej.2014.17

Mishra, P. K., Mishra, S., Selvakumar, G., Kundu, S., & Gupta, H. S. (2009). Enhanced soybean (Glycine max L.) plant growth and nodulation by Bradyrhizobium japonicum-SB1 in presence of Bacillus thuringiensis-KR1. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 59(2), 189–196. https://doi.org/10.1080/09064710802040558

Moritz, M. A., Parisien, M.-A., Batllori, E., Krawchuk, M. A., Dorn, J. V., Ganz, D. J., & Hayhoe, K. (2012). Climate change and disruptions to global fire activity. Ecosphere, 3(6), art49. https://doi.org/10.1890/ES11-00345.1

Narula, N., Deubel, A., Gans, W., Behl, R. K., & Merbach, W. (2011). Paranodules and colonization of wheat roots by phytohormone producing bacteria in soil. Plant, Soil and Environment, 52(No. 3), 119–129. https://doi.org/10.17221/3355-PSE

Nave, L. E., Vance, E. D., Swanston, C. W., & Curtis, P. S. (2011). Fire effects on temperate forest soil C and N storage. Ecological Applications, 21(4), 1189–1201. https://doi.org/10.1890/10-0660.1

Niu, X., Song, L., Xiao, Y., & Ge, W. (2018). Drought-Tolerant Plant Growth-Promoting Rhizobacteria Associated with Foxtail Millet in a Semi-arid Agroecosystem and Their Potential in Alleviating Drought Stress. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.02580

Ofek-Lalzar, M., Sela, N., Goldman-Voronov, M., Green, S. J., Hadar, Y., & Minz, D. (2014). Niche and host-associated functional signatures of the root surface microbiome. Nature Communications, 5(1), 1–9. https://doi.org/10.1038/ncomms5950

Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E., & Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. The ISME Journal, 9(4), 980–989. https://doi.org/10.1038/ismej.2014.196

Pellegrini, A. F. A., Hobbie, S. E., Reich, P. B., Jumpponen, A., Brookshire, E. N. J., Caprio, A. C., Coetsee, C., & Jackson, R. B. (2020). Repeated fire shifts carbon and nitrogen cycling by changing plant inputs and soil decomposition across ecosystems. Ecological Monographs, 90(4), e01409. https://doi.org/10.1002/ecm.1409

Pressler, Y., Moore, J. C., & Cotrufo, M. F. (2019). Belowground community responses to fire: Meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos, 128(3), 309–327. https://doi.org/10.1111/oik.05738

Putten, W. H. van der, 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., Voorde, T. F. J. V. de, & Wardle, D. A. (2013). Plant–soil feedbacks: The past, the present and future challenges. Journal of Ecology, 101(2), 265–276. https://doi.org/10.1111/1365-2745.12054

Van Der Heijden, M. G. A., Bardgett, R. D., & Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11(3), 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Vílchez, J. I., García-Fontana, C., Román-Naranjo, D., González-López, J., & Manzanera, M. (2016). Plant Drought Tolerance Enhancement by Trehalose Production of Desiccation-Tolerant Microorganisms. Frontiers in Microbiology, 7. https://doi.org/10.3389/fmicb.2016.01577

Wagner, M. R., Lundberg, D. S., Coleman-Derr, D., Tringe, S. G., Dangl, J. L., & Mitchell-Olds, T. (2014). Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild Arabidopsis relative. Ecology Letters, 17(6), 717–726. https://doi.org/10.1111/ele.12276

Zhou, J., Deng, Y., Luo, F., He, Z., & Yang, Y. (2011). Phylogenetic Molecular Ecological Network of Soil Microbial Communities in Response to Elevated CO2. MBio, 2(4), e00122-11. https://doi.org/10.1128/mBio.00122-11

Zhu, X., Song, F., Liu, S., & Liu, F. (2016). Arbuscular mycorrhiza improve growth, nitrogen uptake, and nitrogen use efficiency in wheat grown under elevated CO2. Mycorrhiza, 26(2), 133–140. https://doi.org/10.1007/s00572-015-0654-3

CHAPTER I: CONDITIONALITY OF SOIL MICROBIAL MEDIATION OF PLANT PHENOTYPE

Abstract

While distinct soil microbiomes and individual soil microbial taxa can alter particular plant traits under highly controlled conditions, little is known about the role of particular microbial taxa and microbial functions within complex soil microbial communities for mediating plant phenotypes or if the strength of microbial mediation of plant phenotype varies among plant species or plant phenotypic traits. Examining how the plant phenotype spectrum is influenced by the taxonomic and functional composition of complex soil microbial communities allows for a more accurate understanding of the biotic environmental drivers of plant phenotype. Using rhizosphere soil collected from field sites, we conducted a microbiome transfer glasshouse experiment to test the hypothesis that the taxonomic and functional composition of different soil microbiomes would differentially shift growth, physiological or reproductive phenotypes of three Solidago species. We found that soil microbiome inoculations influenced Solidago growth traits more than physiological and reproductive traits. We found that root growth of one of the Solidago species was negatively correlated with 77% of the indicator bacterial and fungal taxa from one of the soil microbiome treatments. Soil microbial mediation of plant phenotype varies by plant traits, is not universal across plant species, and can be associated with a small number of microbial taxa. This study illustrates that specific microbial taxa within a soil microbiome are associated with shifts in plant phenotype by pinpointing important individual microbial taxa from complex field soil microbial communities.

Introduction

In the past 10-15 years, numerous and diverse relationships discovered between plants and the soil microbiome have shifted the long-established paradigm of plant phenotype as the sole product of interactions between a plant's genes (G) and the abiotic environment (E) (i.e., G x E interactions; Clausen et al., 1948; Conner & Hartl, 2004) to that of a 'holobiont' interpretation (G x G x E interactions), in which microbes at the root-soil interface serve as a reservoir of additional genes and functions for the host plant (Bordenstein & Theis, 2015; Theis et al., 2016; Vandenkoornhuyse et al., 2015; Zilber-Rosenberg & Rosenberg, 2008). Multiple avenues of research have informed this updated perspective. At a broad scale, the existence of plant-soil feedbacks, in which soil conditioned from one plant species can promote or inhibit the growth of other conspecific or

heterospecific plants (Bever, 1994; Bever et al., 1997; Ehrenfeld et al., 2005; Mangan et al., 2010; Putten et al., 2013), has established that individual soil microbes collectively, referred to as a microbiome, can influence plant phenotype. The mechanisms that underlie plant-soil feedbacks are largely attributed to the fact that phenotypically distinct plants can differentially alter both the abiotic and microbial composition of the surrounding soil, in part due to differences in organic matter turnover, and root chemical exudates that in turn favor distinct communities of microbes (Hu et al., 2018; Jones et al., 2019). The identification of core rhizosphere microbiome members for a diversity of plant taxa (Schweitzer et al., 2008; Lundberg et al., 2012; Colin et al., 2017; Yeoh et al., 2017; Fitzpatrick et al., 2018; Hugoni et al., 2018; Timm et al., 2018; Lasa et al., 2019; Singer et al., 2019) provides further evidence for the ability of different plants to recruit particular soil microbes. At a fine scale, single inoculation studies—mostly targeted towards crop health and production—have determined that particular individual soil microbial taxa can modify plant traits, such as growth phenotypes or flowering phenology. In isolation, bacterial genera such as Microbacterium, Pseudomonas, and Enterobacter, for example, can increase desiccation tolerance in some crop varieties by stimulating trehalose production (Vílchez et al., 2016; Niu et al., 2018). Similarly, plant growth promoting rhizobacteria assist in nutrient acquisition of nitrogen, phosphate, potassium, and iron by producing various phytohormones (Ali et al., 2009; Mishra et al., 2009; Ofek-Lalzar et al., 2014). Moreover, whole microbiome soil inoculation studies have documented microbial mediation of a diversity of plant phenotypes including physiological traits such as photosynthesis (Friesen et al., 2011; Lau and Lennon, 2011; Zhu et al., 2016; Kannenberg and Phillips, 2017), phenological traits such as flowering time (Wagner et al., 2014; Panke-Buisse et al., 2015) and reproductive traits such as fruit (Lau and Lennon, 2011, 2012) and flower production (Lau and Lennon, 2012).

While findings from these research fields show that both isolated individual microbial taxa and diverse soil microbial communities can influence plant function, pinpointing the important individual microbial taxa and functions within complex soil microbial communities remains a challenge. Identifying significant individuals or functions within complex microbial communities is crucial for advancing ecology of natural ecosystems because plants in natural landscapes interact simultaneously with a multitude of beneficial, benign, and pathogenic microbes (Morris et al., 2007; Zolla et al., 2013; Putten et al., 2016). Beneficial or deleterious effects from individual taxa may be enhanced or suppressed by interactions with other nearby microbial members. Examining

how the taxonomic and functional composition of soil microbial communities affects plant phenotype will allow for a more accurate understanding of the surrounding biotic environmental drivers of plant phenotype.

The goal of this study was to identify the strength of soil microbial mediation for different plant phenotypes, the consistency of these relationships among plant species, and identify specific soil microbial taxa and/or functions in complex field soil communities that are associated with particular plant phenotypes. Using field soils associated with three phenotypically distinct Solidago species, we conducted a glasshouse experiment and inoculated three Solidago species in separate treatments of each field-collected soil and microbiome. We tested the following hypotheses: 1) Soil microbiome source inoculation will differentially alter phenotypes of three Solidago species; 2) Soil microbiome source is associated with distinct taxonomic and/or functional soil microbial communities; 3) Specific microbial taxa and/or microbial functions are associated with particular Solidago phenotypes. Shifts in Solidago phenotypes between microbiome source treatments would indicate that plant traits are influenced by variation in microbial taxonomic and/or functional composition. Variation in response to microbiome treatments among traits would indicate conditional effects of microbial mediation of plant phenotype. Variation in response to microbiome treatments among Solidago species would indicate conditional effects of microbial mediation of plant phylogeny. Correlations between specific microbial taxa and/or microbial functions and particular Solidago phenotypes would provide evidence for the importance of individual taxonomic or functional components within a microbiome for influencing plant phenotype.

Materials and Methods

Study system

Solidago species are a model system for this study because they commonly occur across North America, with 120 species native to the United States (Semple, 2016) that grow in variable habitats, with different morphologies and phenotypes. We chose to use S. caesia, S. flexicaulis, and S. gigantea in this study because they were the most abundant Solidago species found across our sampling range (northeastern TN) and vary in evolutionary history, leaf, stem, and flower morphology and habitat preference. Solidago caesia and S. flexicaulis grow in woodlands and

belong to the Glomerulifloraea subgroup of Solidago (Semple, 2016). Solidago gigantea grows in meadows and fields and belongs to the Triplinerviae subgroup (Semple, 2016). Furthermore, previous work has found evidence for the influence of interspecific and genotypic diversity on above- and belowground biomass of S. altissima and S. gigantea (Genung et al., 2012, 2013), suggesting that some Solidago phenotypes may be mediated in part by modifications of soil biota from neighboring Solidago species.

Preliminary field surveys

To assess differences in plant phenotypes among the three Solidago species, we conducted field surveys of three geographically distinct populations of each species, all located throughout northeastern Tennessee, U.S.A. In May 2017, we measured stem height, stem base diameter, specific leaf area (SLA), and stomatal density of 15 randomly selected putative genotypes of each species (S. caesia, S. flexicaulis, and S. gigantea) in northeastern TN for a total of 45 individuals per species (Figure 1a; all tables and figures are in Appendix 1 at the end of this chapter). The field survey confirmed that the three species vary in this suite of growth and physiological phenotypes (see Supplementary Material; Table S1).

Soil collection and processing

To assess Hypothesis 2 that the soil microbiome sources have distinct taxonomic and/or functional microbial communities, we collected rhizosphere soil from each genotype in the field surveys by collecting soil attached to the roots of each plant (Figure 1b). We pooled individual soil samples by field site to represent an average belowground microbiome of three soil sources (n = 3 sites per soil source). While we tried to collect soil microbes that were only associated with the rhizosphere soil of each plant species, it is likely that we also captured microbes that are representative of surrounding non-rhizosphere soil. Due to this fact and that some climatic and edaphic soil characteristics including mean annual temperature, soil organic matter content, and soil bulk density slightly varied among the three groups of Solidago species sites (Table S2), we refer to the three groups of sites as soil microbiome sources rather than soils associated with each Solidago species. Soil samples were transported to the laboratory on ice and stored at 0°C until analysis at the University of Tennessee, Knoxville, TN, U.S.A. A 2 g subsample of soil from each field site was stored at -80°C for molecular analysis. We assessed the taxonomic community composition

of the soils using high-throughput amplicon sequencing of the V3-V4 region of the 16S rRNA gene (16S) and the ITS2 region of the internal transcribed spacer gene regions for bacteria and fungi, respectively. Detailed methods are described in Molecular Protocols and Sequencing Methods of the Supplementary Material.

We performed all amplicon sequence processing using the DADA2 platform (Callahan et al., 2016). For 16S sequences, primers were removed prior to the DADA2 pipeline using the cutadapt function in conda. Samples were normalized for sampling depth with a variance stabilizing transformation with the DESeq2 package (Love et al., 2014). We chose this method over the common practice of rarefaction because rarefaction results in loss of data by using the lowest sampling depth and it inflates variances across samples (McMurdie and Holmes, 2014). Taxonomy of ASVs was assigned using the RDP (Wang et al., 2007) and UNITE (Abarenkov et al., 2010) databases for bacteria and fungi, respectively. After processing, we had 16,245 bacterial and 2,565 fungal ASVs, respectively. Additionally, we assigned fungal ASVs to functional guilds using the FUNGuild database (Nguyen et al., 2016). For analyses, we assigned taxa to one of seven broad functional guilds: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi, endophytic fungi, plant pathogenic fungi, saprotrophic fungi, and "other." We considered only FUNGuild assignments with a confidence ranking of "highly probable" or "probable." Unassigned taxa were excluded from further guild-based analyses. Of the 2,565 fungal ASVs, 1,741 were assigned to a fungal guild. Of those assigned, we used the 1,328 ASVs that had a confidence ranking of "highly probable" or "probable."

We assessed functional community composition with shotgun metagenomic sequencing, as detailed in Methods S1 of the Supplementary Materials. Sequences retrieved from shotgun metagenomic sequencing were assigned to KEGG (Kyoto Encyclopedia of Genes and Genomes) ortholog numbers using the MG-RAST online annotation tool. KEGG orthologs assign genes to microbial complexes, functional sets, and metabolic pathways and are a common tool used to describe functional attributes of microbes (Ortiz-Álvarez et al., 2018; Sorensen et al., 2019). KEGG ortholog numbers were matched to hierarchical KEGG pathways.

Glasshouse experiment

To assess Hypotheses 1 and 3 that plant phenotypes are in part mediated by the taxonomic and/or functional composition of soil microbial communities and that the strength of microbial mediation

varies among plant species and phenotypic traits, we conducted a glasshouse experiment and grew S. caesia, S. flexicaulis, and S. gigantea in factorial soil inoculum treatments of each microbiome source. Seeds of each Solidago species were purchased from separate nurseries to account for intraspecific variation in plant response to soil microbes (S. caesia: Ernst Conservation Seeds, Meadville, PA; NorthCreek Nurseries, Landenburg, PA; Michigan Wildflower Farm, Portland, MI; S. flexicaulis: Ernst Conservation Seeds, Prairie Moon Nurseries, Winona, MI; Minnesota Native Landscapes, Ostego, MN; S. gigantea: Prairie Moon Nurseries, Minnesota Native Landscapes). Seeds were refrigerated at 4°C prior to sowing, and then were sown by population into a commercial peat moss-based, non-mycorrhizal potting mix (Premier Promix BX, containing perlite, vermiculite, and limestone). A subset of Solidago seeds did not withstand surface sterilization trials, so we did not surface sterilize the seeds used in the experiment. While it is possible that any seed-borne microbes may have impacted plant phenotype, all plants were grown in all soil treatments and exposed to the same glasshouse conditions, such that any effect of seed-borne microbes on plant phenotype should be equally distributed across treatment categories.

After approximately three weeks of growth, 54 similar-sized seedlings of each population were individually transplanted into half-gallon circular pots into soil inoculum treatments which consisted of factorial combinations of microbiome source (Microbiome source 1 vs. Microbiome source 2 vs. Microbiome source 3) (Figure 1c). Furthermore, since soils from each field site of each microbiome source were kept separate, seeds were planted into three sites of Microbiome source 1, three sites of Microbiome source 2, and three sites of Microbiome source 3. Each pot was inoculated with 2 teaspoons of field soil (< 1% of the total pot volume) to reduce effects of variation in soil nutrients on plant phenotypic responses (Troelstra et al., 2001). In total, 243 pots were established: 3 Solidago species x 3 seed populations x 3 microbiome sources (Microbiome source 1, Microbiome source 2, Microbiome source 3) x 3 field soil sites x 3 replicates = 243 total pots). Pots were randomly positioned in the glasshouse based on random number assignments. All plants were treated monthly for thrips and whiteflies throughout the experiment (0.5 tsp/gal Avid 0.15 EC insecticide, 0.5 tsp/gal AzaGuard insecticide). Plants were equally watered from above, as needed (approximately 4 days/week), and allowed to grow for 5 months in a glasshouse at the University of Tennessee.

A suite of plant phenotypes was measured during and post-experiment. Stem height and stem diameter were measured every two weeks for the first two months of growth, then at 13 weeks

and at the termination of the experiment at 20 weeks. Relative growth rates were calculated from these data. For each individual plant, timing of flower bud formation (hereafter referred to as "flower bud break") and flowering were monitored with daily surveys by recording the day of the appearance of the first distinguishable flower bud and first open flower, respectively. Prior to termination of the experiment, an average of four healthy and mature leaves were randomly selected per plant, scanned using WinFOLIA software (Regent Instruments Inc.), oven-dried at 70°C for 72 hours (Pérez-Harguindeguy et al., 2016), and weighed to calculate specific leaf area (cm^2/g) (SLA). After five months of growth and regular watering, each individual was harvested and separated into shoot and root biomass and inflorescence biomass. Shoot and root tissue was weighed after 48 hours of oven-drying at 60°C. Prior to drying, roots were carefully rinsed over 2 and 0.5 mm sieves to remove lingering soil and collect all fine roots.

Statistical Analyses

In the field survey, we analyzed differences in Solidago phenotypes using linear mixed-effects models with the lmer function in the lme4 package (Bates et al., 2014). We built separate mixed-effects models for each phenotype (stem height, stem diameter, SLA, and stomatal density) using Solidago species as the fixed effect and population as the random effect. When necessary, all data were transformed to conform to normality before analysis. To test Hypothesis 1 that phenotypes of each Solidago species differ when grown in soils inoculated with microbial communities associated with a different microbiome source, we built linear mixed effects models with the lmer function in the lme4 package. First, to identify traits most important to growth, physiology, and reproduction and to reduce Type I error, we tested for correlations between the ten phenotypes measured from the glasshouse experiment (relative growth rate in stem height, stem diameter at maturity, shoot biomass, root biomass, total biomass, root to shoot ratio, SLA, flower bud break, days to flower, inflorescence biomass) using the cor.test function. We chose to exclude stem diameter, total biomass, and root to shoot ratio from the analysis because they were all significantly correlated with two other growth phenotypes, shoot and root biomass (Table S5). We also chose to exclude days to flower and inflorescence biomass from the analysis because the experiment ended before the majority of S. gigantea individuals flowered. Relative growth rate, shoot biomass, root biomass, SLA, and flower bud break were included in the analysis.

Multiple models were used to assess Hypothesis 1. Separate models were built for the five phenotypes (relative growth rate, shoot biomass, root biomass, SLA, and timing of flower bud formation). When necessary, all data was transformed to conform to normality before analysis. First, to test that differences in soil microbial community composition have a general effect on plant phenotypes regardless of plant species, we built linear mixed effects models with microbiome source as a fixed effect and Solidago species, seed population, and field soil site as random effects. Then to identify interspecific variation in response to microbial community composition we separated the dataset by each Solidago species and built individual linear mixed effects models for each Solidago species with microbiome source as a fixed effect and seed population and field soil site as random effects. For all models, we used the Anova function to calculate ANOVA tables using Type II sums of squares, with significance assessed for each fixed effect using Wald X^2 statistics. If any of the fixed effects were significant, we conducted post hoc Tukey contrasts using the TukeyHSD function.

To test Hypothesis 2 that each microbiome source is associated with distinct taxonomic and/or functional soil microbial communities, we took multiple approaches. First, we assessed microbial diversity across microbiome source by calculating hill numbers based on ASV counts and unique KEGG identities using the hill_div function in the hilldiv package (Alberdi and Gilbert, 2019). Hill numbers serve as effective numbers of diversity that provide more intuitive estimates of diversity compared to traditional diversity indices based on entropy (Chao et al., 2014). We calculated hill numbers for all orders of diversity at $q = 0$, $q = 1$, and $q = 2$, and tested for significant differences in hill numbers between microbiome source at each order of diversity using the div_test function in the hilldiv package. A diversity order $q = 0$ provides raw richness by weighting rare taxa the same as abundant taxa and thus not accounting for species' abundances. A diversity order $q = 1$ weights ASVs by their abundance but without disproportionately favoring abundant taxa. A diversity order $q = 2$ overweighs abundant ASVs.

Second, we created Bray-Curtis distance matrices for microbial taxonomic and functional composition of the nine field soils. To assess variation in community composition of bacteria, fungi, and KEGGs across microbiome source, we conducted PERMANOVA analysis with 9,999 permutations using the adonis function in the vegan package (Oksanen et al., 2019). Prior to conducting PERMANOVA we confirmed homogeneity of dispersion across microbiome source with the betadisper function in the vegan package. We then performed a distance-based

redundancy analysis (db-RDA) using the dbrda function in the vegan package to assign variation in composition of bacteria, fungi, and KEGGs to microbiome source and geographic location. We conducted three individual db-RDAs for bacteria, fungi, and KEGG composition. We used the anova.cca function in the vegan package to assess the cumulative significance of microbiome source and geographic location on community composition. We partitioned the variation in composition with respect to microbiome source and geographic location using the varpart function in the vegan package. To visualize composition of bacteria, fungi, and KEGGs among soil origin, we used principal coordinate analysis (PCoA) for ordination based on the Bray-Curtis distance matrices.

We then performed indicator species analysis with the multipatt function in the indicspecies package (Cáceres and Legendre, 2009) to identify particular bacteria, fungi, and KEGGs that are uniquely highly associated with each microbiome source. Because the FUNGuild data set contained a high amount of zero counts, we built individual zero-inflated models for each fungal guild using the glmmTMB function in the glmmTMB package (Brooks et al., 2017). We specified microbiome source as the fixed effect, count total per soil sample (i.e. site) as the random effect, zi formula as soil origin, and family as poisson. For all models, we used the Anova function in the car package (Fox et al., 2013) to calculate analysis of variance (ANOVA) tables using Type II sums of squares, with significance assessed for microbiome source using Wald X^2 statistics. If the effect of microbiome source was significant, we conducted post hoc Tukey contrasts using the emmeans function in the emmeans package (Lenth et al., 2020) and the cld function in the multcomp package (Hothorn et al., 2008).

To test Hypothesis 3 that specific microbes and/or microbial functions are associated with particular Solidago phenotypes, we assessed the effect of variation in microbial indicator taxa composition on Solidago phenotypes that responded to microbiome source treatment. Since no KEGG identities were identified as indicators across the three microbiome sources, subsequent analyses were conducted only with bacterial and fungal indicator taxa. Using a db-RDA, we assigned variation in composition of bacterial and fungal indicator taxa to the three microbiome sources and geographic location. We then extracted the axes scores from the db-RDA model. For each phenotype, we built a linear model that included the two axes (CAP1, CAP2) from the db-RDA model as fixed effects. A significant relationship between db-RDA axes and plant phenotypes would indicate that differences in the community of bacterial and fungal indicator taxa

associated with each microbiome source are associated with shifts in plant phenotype. To pinpoint individual bacterial and fungal indicator taxa that may be associated with particular plant phenotypes, we built linear models to test for correlations between the relative abundance of each bacterial and fungal indicator taxon and each phenotype that showed significant responses to the axes of variation from the indicator species db-RDA model.

All analyses were performed in R (R Core, 2020). Boxplot, and linear regression figures were made with the ggplot2 package (Wickham, 2016). Ordination figures were made with the phyloseq package (McMurdie and Holmes, 2013). Heatmap figures were made with the Heatplus (Ploner, 2020) and gplots (Warnes et al., 2020) packages. Individuals figures were aggregated with the patchwork package (Pedersen, 2020).

Results

Plant phenotype responses to soil microbiome sources (glasshouse experiment)

While the three Solidago species overall varied significantly in relative growth rate, shoot and root biomass, and flower bud break, only root biomass differed by microbiome source ($\chi^2 = 6.14$, $p = 0.04$) (Table 1). Among all three Solidago species, there was 29% greater root biomass production when plants were grown in inoculum from microbiome source 1 relative to microbiome source 3 (Tukey post hoc: $p = 0.05$) (Figure 2a). In partial support of Hypothesis 1, the species-specific models showed that phenotypic responses to microbiome source inoculum varied by Solidago species and by phenotype. Solidago caesia shoot biomass differed among microbiome source treatments, whereas no S. flexicaulis or S. gigantea phenotypes differed among microbiome source treatments (Table 2). Solidago caesia produced 8.9% more shoot biomass when grown in inoculum from microbiome source 2 relative to microbiome source 3 (Tukey post hoc: $p = 0.09$) (Figure 2b), indicating that different soil microbiomes can shift plant traits.

Community composition among soil microbiome sources

Across the three soil microbiome sources, we identified over 16,000 bacterial and 2,500 fungal ASVs. Taxonomic and functional diversity of soil microbial communities did not vary by microbiome source at any order of diversity (Tables S3, S4). In partial support of Hypothesis 2, whole microbiomes did not differ in taxonomic or genetic pathway composition among the

microbiome sources, but distinct indicator taxa were identified for each microbiome source. PERMANOVA analysis revealed that taxonomic and functional composition of soil microbial communities did not vary significantly by microbiome source (Bacteria: $F = 1.04$, $p = 0.37$; Fungi: $F = 1.10$, $p = 0.18$; KEGGs: $F = 0.92$, $p = 0.55$) (Figure 3). The db-RDA revealed that microbiome source and geographic location cumulatively accounted for less than 10% of variation in bacteria, fungi, and KEGG composition (Bacteria: adj. $R^2 = -0.013$; Fungi: adj. $R^2 = 0.053$; KEGGs: adj. $R^2 = -0.068$). Microbiome source accounted for less than 5% of variation in bacteria, fungi, and KEGG composition (Bacteria: adj. $R^2 = 0.011$; Fungi: adj. $R^2 = 0.025$; KEGGs: adj. $R^2 = -0.021$). Similarly, geographic location also accounted for less than 5% of variation in bacteria, fungi, and KEGG composition (Bacteria: adj. $R^2 = 0.026$; Fungi: adj. $R^2 = 0.033$; KEGGs: adj. $R^2 = -0.02$).

Indicator species analysis identified significant bacteria and fungi indicator taxa for each microbiome source. In total, 77 bacterial ASVs (out of 16,245 detected; 0.5%) and eight fungal ASVs (out of 2,565 detected; 0.3%) were identified as indicator taxa among the three microbiome sources (Figure 4, Tables S6, S7). Twenty-nine bacterial ASVs were uniquely shared among microbiome sources 1 and 2, whereas microbiome source 3 uniquely shared only six bacterial ASVs with either microbiome source 1 or 2. Fungal guilds were assigned to approximately 68% of the fungal ASVs. Of those assigned to a guild, approximately 76% had a confidence ranking of "probable" or "highly probable." Out of the five fungal guilds (arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi, endophytic fungi, and plant pathogenic fungi), ericoid mycorrhizal fungi ($\chi^2 = 11.29$, $p = 0.004$) and endophytic fungi ($\chi^2 = 20.14$, $p < 0.0001$) differed significantly among the microbiome sources (Table S8). Both microbiome sources 1 and 2 had approximately 1.5- and 2-fold greater abundance of ericoid mycorrhizal fungi and endophytic fungi, respectively, than microbiome source 3 (Figures 5d, e).

KEGG composition overall did not differ among the three microbiome sources, indicating functional redundancy among soil microbial communities. Of the 122 pathways identified, less than a quarter accounted for more than 1% of relative abundance of all KEGGs among the three origins (Table S9). Of this subset, 70% were pathways involved in metabolism of either energy (in the form of nitrogen, methane, sulfur, and oxidative phosphorylation), amino acids, carbohydrates, or lipids. The most abundant pathways across the three soil origins were two pathways for ATP-binding cassette (ABC) transporters, which accounted for 20% of the relative abundance. No KEGG pathways were detected as indicators among the microbiome sources.

Correlations between soil microbiome composition and plant phenotypes

In support of Hypothesis 3, individual microbial taxa were associated with specific Solidago phenotypes. We only examined shoot biomass of S. caesia as it was the only phenotype that responded to microbiome source treatments. Axes of variation in composition of the bacteria indicator taxa were significantly correlated with S. caesia shoot biomass (Axis CAP1: $F = 7.63$, $p = 0.03$). Relative abundance of 77% (20 out of 26) of the bacterial and fungal indicator taxa of microbiome source 3 were significantly negatively correlated with S. caesia shoot biomass when S. caesia was grown in inoculum of microbiome source 3 (Figure 6, Table S10). Although S. caesia produced more shoot biomass when grown in inoculum of microbiome source 2 compared to that of microbiome source 3 (Figure 2b), none of the eight bacterial indicator taxa or the one fungal indicator taxon of microbiome source 2 were significantly positively correlated with S. caesia shoot biomass.

Discussion

Identifying ways in which the taxonomic and functional composition of the soil microbiome influences plant phenotype is a central challenge for understanding the overall importance of complex soil microbial communities on plant function, as well as how changes to soil microbial communities may in turn affect plant function. While recent studies have explored the importance of both whole soil microbiomes and individual soil microbial taxa on particular plant phenotypes, it is also crucial to understand if and how particular taxa and functions within complex soil microbial communities influence a broad spectrum of plant phenotypes and if these relationships are consistent across multiple plant species. In this study we compared the taxonomic and functional composition of rhizosphere soil microbial communities from three phenotypically distinct and naturally occurring Solidago species and sites (referred to as soil microbiome source above) and conducted a microbiome transfer glasshouse experiment to test for plant phenotypic shifts in response to field soil inoculum. We found that soil microbiome taxonomic variation can shift some plant phenotypes and that this response varied by plant species, with some species more responsive to microbial taxonomic variation than others. Specifically, we identified indicator bacteria and fungi associated with each microbiome source, some of which were correlated with shifts in plant growth responses. We found that microbiome source altered growth traits for only one of the three Solidago species. Lastly, we found that plant growth traits were more likely to be

influenced by variation in soil microbial communities than physiological or reproductive traits. Together, these findings show that soil microbial mediation of plant phenotype 1) varies by plant traits, 2) is not consistent across plant species, and 3) can be influenced, in part, by a small number of microbial taxa.

Soil microbial mediation of plant phenotype varies by plant phenotype and plant species

We found that soil microbial communities can shift some plant phenotypes, but that the strength of microbially-mediated phenotypic plasticity varies by plant phenotype and by plant species. Across all three Solidago species, plants grown in inocula from microbiome source 1 produced more root biomass compared to plants grown in inocula from microbiome source 3. While no S. flexicaulis or S. gigantea phenotypes responded to microbiome source treatments, S. caesia produced more shoot biomass in microbiome source 2 inocula relative to microbiome source 3 inocula. Although we found that the amount of soil plant pathogenic fungi was similar across all three soil origins, we did find that microbiome sources 1 and 2 had significantly greater amounts of endophytic and ericoid mycorrhizal fungi than microbiome source 3. A difference in plant-beneficial fungi may in part account for the greater growth of S. caesia in microbial communities of microbiome source 2 relative to microbial communities of microbiome source 3.

Despite the breadth of research on plant-soil biota relationships, the role of soil microbial communities on plant phenotype is heavily limited by the scarcity of knowledge of these relationships on traits such as physiology and reproduction. Of over 70 recent studies that have examined plant phenotype in response to unmanipulated and sterilized soil microbial treatments, only four studies have examined traits related to physiology (Mursinoff and Tack, 2017; Hahn et al., 2018; Siefert et al., 2018; Xi et al., 2018) and two have examined reproductive traits (Bauer and Flory, 2011; Dudenhöffer et al., 2018). In this study we found that neither SLA nor timing of flower bud formation responded to soil microbiome origin treatments, indicating that not all plant phenotypes are in part mediated by soil microbes. However, further examination of soil microbial mediation across plant phenotypes is needed to reveal if the findings here represent a general trend that microbial mediation is stronger for growth traits than for physiological or reproductive traits.

Soil microbial mediation of plant phenotype can be influenced in part by small number of microbial taxa.

Despite similarity in overall microbiome composition among the three soil microbiome sources, we identified specific bacterial and fungal indicator taxa of each microbiome source. In general, Proteobacteria taxa were more highly abundant in microbiome sources 1 and 2 than in microbiome source 3, whereas Acidobacteria taxa were more highly abundant in microbiome source 3 than in microbiome sources 1 and 2. Within the Proteobacteria phylum, indicator taxa of microbiome sources 1 and 2 spanned a larger diversity of taxonomic orders including Rhizobiales, Rhodospirillales, Burkholderiales, and Xanthomonadales relative to those of microbiome source 3 which comprised the orders of Rhizobiales and Myxococcales. These findings highlight the importance of taxonomic resolution when assessing the role of the soil microbiome on plant phenotype as root-associated soil is known to contain some of the highest microbial biodiversity on Earth (Curtis et al., 2002; Buée et al., 2009; Berendsen et al., 2012). In this study, we identified over 16,000 bacterial ASVs and over 2,500 fungal ASVs among the three microbiome sources. These findings suggest that identifying microbial differences among focal groups may require focusing on specific indicator taxa that have high affiliation with the plant species or field site rather than overall microbiome composition.

We found that differences in indicator taxa among separate soil microbial communities may contribute to shifts in plant phenotype even though this relationship is likely not consistent across plant species. We found that Solidago caesia produced significantly less vegetative biomass in inocula of microbiome source 3 compared to inocula of microbiome source 2, and that the relative abundance of 77% of the indicator taxa of microbiome source 3 were correlated with decreases in S. caesia shoot production. These indicator bacteria included mostly members of the Acidobacteria and Actinobacteria phyla in addition to members of the Bacteriodetes, Firmicutes, and Proteobacteria phyla. Indicator fungi included members of the Ascomycota and Chytridiomycota phyla. Despite greater shoot production in microbiome source 2 inocula, none of the microbiome source 2 indicator taxa were correlated with positive shifts of S. caesia shoot production.

Although these indicator taxa account for a very small proportion of the total microbial communities identified in this study, it is notable that out of the thousands of ASVs identified in microbiome source 2, twenty bacterial and fungal ASVs explained on average 7% of the variation

in root biomass when S. caesia was grown in inocula of microbiome source 2. This suggests that individual soil microbial taxa may be involved, in part, with mediating some plant traits. This study demonstrates the utility of this approach of examining correlations between individual microbes and plant traits. Identifying individual microbial taxa that are associated with shifts in plant traits can pinpoint particular microbial taxa to target for further experiments that test causative mechanisms of plant trait variation. While the correlative relationships we identified in this study are context-specific to these particular microbial taxa and plant species, the approach used here can be applied to any plant-microbial system.

While we did not assess microbial dormancy in this study, it likely plays a significant role in soil microbial mediation of plant phenotype. Dormancy, in which individuals undergo a temporary reduced state of metabolic activity, has long been hypothesized to be widespread among microorganisms because it allows them to cope with environmental variability, particularly when conditions are unfavorable (Stevenson, 1977; Lau and Lennon, 2011). Differentiating between active and dormant microbial taxa requires examining the active ribosomal RNA in addition to the total ribosomal DNA. Since we only used rDNA-based techniques in this study, our inferences are limited to microbial taxa that are potentially active. However, despite the fact that the indicator taxa accounted for a very small amount of the diversity of each microbiome source, evidence suggests that rare taxa may confer particular importance within a microbiome. A previous study examining proportions of rRNA to rDNA in temperate lakes found that rare taxa had a higher probability of being metabolically active than common taxa (Jones & Lennon, 2010). Combined with the observation that soil microbiome diversity is primarily comprised of rare taxa (Elshahed et al., 2008), our findings and those from Jones and Lennon (2010) highlight the importance of examining how less abundant rare taxa within soil microbial communities may influence plant phenotype. Other microbial interactions within the soil environment also likely influenced the plant trait variation we observed, such as differences in microbial growth rates, differences in decomposition via extracellular enzymes, and changes to the microbial communities due to conditioning from the plants. However, testing these mechanisms was outside of the scope of this study.

In this study, we found high similarity in soil microbial function (i.e. KEGG pathway composition) among the three microbiome sources, suggesting functional redundancy in which the absence of one or more microbial species does not greatly affect the functioning of the whole

microbial community because the same functions are fulfilled by many different taxa (Fernández et al., 1999; Louca et al., 2016, 2018). Functional redundancy is widespread in microbial systems (Tringe et al., 2005; Allison and Martiny, 2008; Bezemer et al., 2010; Martiny et al., 2013; Nelson et al., 2016). The relatively small number of known functions associated with soil microbial communities indicates that much is still unknown about microbial functional genes that may translate to physiological differences among microbial taxa. While the metagenomic methods we employed in this study can be used to infer potential microbial functions, other molecular approaches such as metatranscriptomics can identify particular microbial genes that are being actively transcribed (Moran, 2009; Carvalhais et al., 2012; Damon et al., 2012), and in doing so provide a more accurate representation of the microbial functions that characterize a particular soil microbiome.

Conclusions

Soil microbes represent a largely overlooked but often important biotic factor for influencing plant phenotype. This is the first study, to our knowledge, to examine how taxonomic and functional gene composition of complex soil microbial communities influence a suite of multiple plant phenotypes across multiple plant species. Our study shows that soil microbiomes and specific taxa within complex soil microbial communities can alter some plant phenotypes, but that not all plant species, even those belonging to the same genus, will respond to soil microbial communities in the same manner. Thus, the belowground biotic environment is just one of a host of important biotic factors that can mediate plant phenotype, in addition to plant genetic background and abiotic environmental variation. While the findings from this study are founded in ecology theory, identifying the nuances of relationships between soil microbes and plant phenotype has wide scale applications. Substantial efforts to engineer core rhizosphere microbiomes to optimize plant production signify the need to identify functional linkages between soil microbial communities and plants (Bakker et al., 2012; Busby et al., 2017; Wallenstein, 2017; Qiu et al., 2019). While soil biota may not be a universal solution to enhance some plant phenotypes, specific microbial taxa may be harnessed to improve plant growth and plant tolerance to adverse environmental conditions. This study illustrates that it is possible to identify specific microbial taxa within a complex soil microbial community that are associated with shifts in some plant phenotypes.

REFERENCES

Abarenkov, K., Nilsson, R. H., Larsson, K.-H., Alexander, I. J., Eberhardt, U., Erland, S., et al. (2010). The UNITE database for molecular identification of fungi – recent updates and future perspectives. New Phytologist 186, 281–285. doi:10.1111/j.1469-8137.2009.03160.x.

Alberdi, A., and Gilbert, M. T. P. (2019). hilldiv: an R package for the integral analysis of diversity based on Hill numbers. bioRxiv, 545665. doi:10.1101/545665.

Ali, B., Sabri, A. N., Ljung, K., and Hasnain, S. (2009). Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of Triticum aestivum L. Letters in Applied Microbiology 48, 542–547. doi:10.1111/j.1472-765X.2009.02565.x.

Allison, S. D., and Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. PNAS 105, 11512–11519. doi:10.1073/pnas.0801925105.

Bakker, M. G., Manter, D. K., Sheflin, A. M., Weir, T. L., and Vivanco, J. M. (2012). Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant Soil 360, 1–13. doi:10.1007/s11104-012-1361-x.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting Linear Mixed-Effects Models using lme4. arXiv:1406.5823 [stat]. Available at: http://arxiv.org/abs/1406.5823 [Accessed April 23, 2020].

Bauer, J. T., and Flory, S. L. (2011). Suppression of the Woodland Herb Senna hebecarpa by the Invasive Grass Microstegium vimineum. amid 165, 105–115. doi:10.1674/0003-0031-165.1.105.

Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. Trends in Plant Science 17, 478–486. doi:10.1016/j.tplants.2012.04.001.

Bever, J. D. (1994). Feeback between Plants and Their Soil Communities in an Old Field Community. Ecology 75, 1965–1977. doi:10.2307/1941601.

Bever, J. D., Westover, K. M., and Antonovics, J. (1997). Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach. Journal of Ecology 85, 561–573. doi:10.2307/2960528.

Bezemer, T. M., Fountain, M. T., Barea, J. M., Christensen, S., Dekker, S. C., Duyts, H., et al. (2010). Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. Ecology 91, 3027–3036. doi:10.1890/09-2198.1.

Bordenstein, S. R., and Theis, K. R. (2015). Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. PLOS Biology 13, e1002226. doi:10.1371/journal.pbio.1002226.

Brooks, M., E., Kristensen, K., Benthem, K., J. ,van, Magnusson, A., Berg, C., W., Nielsen, A., et al. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal 9, 378. doi:10.32614/RJ-2017-066.

Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R. H., Uroz, S., et al. (2009). 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytologist 184, 449–456. doi:10.1111/j.1469-8137.2009.03003.x.

Busby, P. E., Soman, C., Wagner, M. R., Friesen, M. L., Kremer, J., Bennett, A., et al. (2017). Research priorities for harnessing plant microbiomes in sustainable agriculture. PLOS Biology 15, e2001793. doi:10.1371/journal.pbio.2001793.

Cáceres, M. D., and Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574. doi:10.1890/08-1823.1.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13, 581–583. doi:10.1038/nmeth.3869.

Carvalhais, L. C., Dennis, P. G., Tyson, G. W., and Schenk, P. M. (2012). Application of metatranscriptomics to soil environments. Journal of Microbiological Methods 91, 246–251. doi:10.1016/j.mimet.2012.08.011.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., et al. (2014). Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological Monographs 84, 45–67. doi:10.1890/13-0133.1.

Clausen, J., Keck, D. D., and Hiesey, W. M. (1948). Experimental studies on the nature of species. III. Environresponses of climatic races of Achillea. Experimental studies on the nature of species. III. Environresponses of climatic races of Achillea. Available at: https://www.cabdirect.org/cabdirect/abstract/19491603367 [Accessed March 23, 2020].

Colin, Y., Nicolitch, O., Van Nostrand, J. D., Zhou, J. Z., Turpault, M.-P., and Uroz, S. (2017). Taxonomic and functional shifts in the beech rhizosphere microbiome across a natural soil toposequence. Scientific Reports 7, 9604. doi:10.1038/s41598-017-07639-1.

Conner, J. K., and Hartl, D. L. (2004). A primer of ecological genetics. Sinauer Associates Incorporated.

Curtis, T. P., Sloan, W. T., and Scannell, J. W. (2002). Estimating prokaryotic diversity and its limits. Proceedings of the National Academy of Sciences 99, 10494–10499. doi:10.1073/pnas.142680199.

Damon, C., Lehembre, F., Oger-Desfeux, C., Luis, P., Ranger, J., Fraissinet-Tachet, L., et al. (2012). Metatranscriptomics Reveals the Diversity of Genes Expressed by Eukaryotes in Forest Soils. PLOS ONE 7, e28967. doi:10.1371/journal.pone.0028967.

Dudenhöffer, J.-H., Ebeling, A., Klein, A.-M., and Wagg, C. (2018). Beyond biomass: Soil feedbacks are transient over plant life stages and alter fitness. Journal of Ecology 106, 230–241. doi:10.1111/1365-2745.12870.

Ehrenfeld, J. G., Ravit, B., and Elgersma, K. (2005). Feedback in the Plant-Soil System. Annual Review of Environment and Resources 30, 75–115. doi:10.1146/annurev.energy.30.050504.144212.

Elshahed, M. S., Youssef, N. H., Spain, A. M., Sheik, C., Najar, F. Z., Sukharnikov, L. O., et al. (2008). Novelty and Uniqueness Patterns of Rare Members of the Soil Biosphere. AEM 74, 5422–5428. doi:10.1128/AEM.00410-08.

Fernández, A., Huang, S., Seston, S., Xing, J., Hickey, R., Criddle, C., et al. (1999). How Stable Is Stable? Function versus Community Composition. Appl. Environ. Microbiol. 65, 3697–3704. doi:10.1128/AEM.65.8.3697-3704.1999.

Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M., and Johnson, M. T. J. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. PNAS 115, E1157–E1165. doi:10.1073/pnas.1717617115.

Fox, J., Friendly, M., and Weisberg, S. (2013). Hypothesis Tests for Multivariate Linear Models Using the car Package. The R Journal 5, 39. doi:10.32614/RJ-2013-004.

Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., and Martinez-Romero, E. (2011). Microbially Mediated Plant Functional Traits. Annual Review of Ecology, Evolution, and Systematics 42, 23–46. doi:10.1146/annurev-ecolsys-102710-145039.

Genung, M. A., Bailey, J. K., and Schweitzer, J. A. (2012). Welcome to the neighbourhood: interspecific genotype by genotype interactions in Solidago influence above- and belowground biomass and associated communities. Ecology Letters 15, 65–73. doi:10.1111/j.1461-0248.2011.01710.x.

Genung, M. A., Bailey, J. K., and Schweitzer, J. A. (2013). The Afterlife of Interspecific Indirect Genetic Effects: Genotype Interactions Alter Litter Quality with Consequences for Decomposition and Nutrient Dynamics. PLoS One 8. doi:10.1371/journal.pone.0053718.

Hahn, P. G., Bullington, L., Larkin, B., LaFlamme, K., Maron, J. L., and Lekberg, Y. (2018). Effects of Short- and Long-Term Variation in Resource Conditions on Soil Fungal Communities and Plant Responses to Soil Biota. Front. Plant Sci. 9. doi:10.3389/fpls.2018.01605.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous Inference in General Parametric Models. Biometrical Journal 50, 346–363. doi:10.1002/bimj.200810425.

Hu, L., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., et al. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nature Communications 9, 2738. doi:10.1038/s41467-018-05122-7.

Hugoni, M., Luis, P., Guyonnet, J., and Haichar, F. el Z. (2018). Plant host habitat and root exudates shape fungal diversity. Mycorrhiza 28, 451–463. doi:10.1007/s00572-018-0857-5.

Jones, P., Garcia, B. J., Furches, A., Tuskan, G. A., and Jacobson, D. (2019). Plant Host-Associated Mechanisms for Microbial Selection. Front. Plant Sci. 10. doi:10.3389/fpls.2019.00862.

Jones, S. E., and Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. Proceedings of the National Academy of Sciences 107, 5881–5886. doi:10.1073/pnas.0912765107.

Kannenberg, S. A., and Phillips, R. P. (2017). Soil microbial communities buffer physiological responses to drought stress in three hardwood species. Oecologia 183, 631–641. doi:10.1007/s00442-016-3783-2.

Lasa, A. V., Fernández-González, A. J., Villadas, P. J., Toro, N., and Fernández-López, M. (2019). Metabarcoding reveals that rhizospheric microbiota of Quercus pyrenaica is composed by a relatively small number of bacterial taxa highly abundant. Scientific Reports 9, 1695. doi:10.1038/s41598-018-38123-z.

Lau, J. A., and Lennon, J. T. (2011). Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits. New Phytologist 192, 215–224. doi:10.1111/j.1469-8137.2011.03790.x.

Lau, J. A., and Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. PNAS 109, 14058–14062. doi:10.1073/pnas.1202319109.

Lenth, R., Buerkner, P., Herve, M., Love, J., Riebl, H., and Singmann, H. (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means. Available at: https://CRAN.R-project.org/package=emmeans [Accessed September 9, 2020].

Louca, S., Jacques, S. M. S., Pires, A. P. F., Leal, J. S., Srivastava, D. S., Parfrey, L. W., et al. (2016). High taxonomic variability despite stable functional structure across microbial communities. Nature Ecology & Evolution 1, 1–12. doi:10.1038/s41559-016-0015.

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O'Connor, M. I., et al. (2018). Function and functional redundancy in microbial systems. Nature Ecology & Evolution 2, 936–943. doi:10.1038/s41559-018-0519-1.

Love, M., Anders, S., and Huber, W. (2014). Differential analysis of count data–the DESeq2 package. Genome Biol 15, 10–1186.

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., et al. (2012). Defining the core Arabidopsis thaliana root microbiome. Nature 488, 86–90. doi:10.1038/nature11237.

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., et al. (2010). Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466, 752–755. doi:10.1038/nature09273.

Martiny, A. C., Treseder, K., and Pusch, G. (2013). Phylogenetic conservatism of functional traits in microorganisms. The ISME Journal 7, 830–838. doi:10.1038/ismej.2012.160.

McMurdie, P. J., and Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. doi:10.1371/journal.pone.0061217.

McMurdie, P. J., and Holmes, S. (2014). Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLOS Computational Biology 10, e1003531. doi:10.1371/journal.pcbi.1003531.

Mishra, P. K., Mishra, S., Selvakumar, G., Kundu, S., and Gupta, H. S. (2009). Enhanced soybean (Glycine max L.) plant growth and nodulation by Bradyrhizobium japonicum-SB1 in presence of Bacillus thuringiensis-KR1. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 59, 189–196. doi:10.1080/09064710802040558.

Moran, M. A. (2009). Metatranscriptomics: Eavesdropping on Complex Microbial Communities. Microbe Magazine 4, 329–335. doi:10.1128/microbe.4.329.1.

Morris, W. F., Hufbauer, R. A., Agrawal, A. A., Bever, J. D., Borowicz, V. A., Gilbert, G. S., et al. (2007). Direct and Interactive Effects of Enemies and Mutualists on Plant Performance: A Meta-Analysis. Ecology 88, 1021–1029. doi:10.1890/06-0442.

Mursinoff, S., and Tack, A. J. M. (2017). Spatial variation in soil biota mediates plant adaptation to a foliar pathogen. New Phytologist 214, 644–654. doi:10.1111/nph.14402.

Nelson, M. B., Martiny, A. C., and Martiny, J. B. H. (2016). Global biogeography of microbial nitrogen-cycling traits in soil. PNAS 113, 8033–8040. doi:10.1073/pnas.1601070113.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., et al. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248. doi:10.1016/j.funeco.2015.06.006.

Niu, X., Song, L., Xiao, Y., and Ge, W. (2018). Drought-Tolerant Plant Growth-Promoting Rhizobacteria Associated with Foxtail Millet in a Semi-arid Agroecosystem and Their Potential in Alleviating Drought Stress. Front. Microbiol. 8. doi:10.3389/fmicb.2017.02580.

Ofek-Lalzar, M., Sela, N., Goldman-Voronov, M., Green, S. J., Hadar, Y., and Minz, D. (2014). Niche and host-associated functional signatures of the root surface microbiome. Nature Communications 5, 1–9. doi:10.1038/ncomms5950.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., and McGlinn, D. (2019). vegan: Community Ecology Package. R package version 2.5–6. 2019.

Ortiz-Álvarez, R., Fierer, N., de los Ríos, A., Casamayor, E. O., and Barberán, A. (2018). Consistent changes in the taxonomic structure and functional attributes of bacterial communities during primary succession. The ISME Journal 12, 1658–1667. doi:10.1038/s41396-018-0076-2.

Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E., and Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. The ISME Journal 9, 980–989. doi:10.1038/ismej.2014.196.

Pedersen, T. L. (2020). patchwork: The Composer of Plots. Available at: https://CRAN.R-project.org/package=patchwork [Accessed February 15, 2021].

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al. (2016). Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 64, 715–716. doi:10.1071/bt12225_co.

Ploner, A. (2020). Heatplus: Heatmaps with row and/or column covariates and colored clusters.

Putten, W. H. van der, Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., et al. (2013). Plant–soil feedbacks: the past, the present and future challenges. Journal of Ecology 101, 265–276. doi:10.1111/1365-2745.12054.

Putten, W. H. van der, Bradford, M. A., Brinkman, E. P., Voorde, T. F. J. van de, and Veen, G. F. (2016). Where, when and how plant–soil feedback matters in a changing world. Functional Ecology 30, 1109–1121. doi:10.1111/1365-2435.12657.

Qiu, Z., Egidi, E., Liu, H., Kaur, S., and Singh, B. K. (2019). New frontiers in agriculture productivity: Optimised microbial inoculants and in situ microbiome engineering. Biotechnology Advances 37, 107371. doi:10.1016/j.biotechadv.2019.03.010.

R Core (2020). R: a language and environment for statistical computing (version 3.5. 3)[software].

Schweitzer, J. A., Bailey, J. K., Fischer, D. G., LeRoy, C. J., Lonsdorf, E. V., Whitham, T. G., et al. (2008). Plant–Soil–Microorganism Interactions: Heritable Relationship Between Plant Genotype and Associated Soil Microorganisms. Ecology 89, 773–781. doi:10.1890/07-0337.1.

Semple, J. (2016). (11) (PDF) An intuitive phylogeny and summary of chromosome number variation in the goldenrod genus Solidago (Asteraceae: Astereae). ResearchGate. Available

at:
https://www.researchgate.net/publication/303366007_An_intuitive_phylogeny_and_summary_of_chromosome_number_variation_in_the_goldenrod_genus_Solidago_Asteraceae_Astereae [Accessed March 24, 2020].

Siefert, A., Zillig, K. W., Friesen, M. L., and Strauss, S. Y. (2018). Soil microbial communities alter conspecific and congeneric competition consistent with patterns of field coexistence in three Trifolium congeners. Journal of Ecology 106, 1876–1891. doi:10.1111/1365-2745.13042.

Singer, E., Bonnette, J., Kenaley, S. C., Woyke, T., and Juenger, T. E. (2019). Plant compartment and genetic variation drive microbiome composition in switchgrass roots. Environmental Microbiology Reports 11, 185–195. doi:10.1111/1758-2229.12727.

Sorensen, J. W., Dunivin, T. K., Tobin, T. C., and Shade, A. (2019). Ecological selection for small microbial genomes along a temperate-to-thermal soil gradient. Nat Microbiol 4, 55–61. doi:10.1038/s41564-018-0276-6.

Stevenson, L. H. (1977). A case for bacterial dormancy in aquatic systems. Microb Ecol 4, 127–133. doi:10.1007/BF02014283.

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., et al. (2016). Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. mSystems 1. doi:10.1128/mSystems.00028-16.

Timm, C. M., Carter, K. R., Carrell, A. A., Jun, S.-R., Jawdy, S. S., Vélez, J. M., et al. (2018). Abiotic Stresses Shift Belowground Populus-Associated Bacteria Toward a Core Stress Microbiome. mSystems 3. doi:10.1128/mSystems.00070-17.

Tringe, S. G., Mering, C. von, Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., et al. (2005). Comparative Metagenomics of Microbial Communities. Science 308, 554–557. doi:10.1126/science.1107851.

Troelstra, S. R., Wagenaar, R., Smant, W., and Peters, B. a. M. (2001). Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. New Phytologist 150, 697–706. doi:10.1046/j.1469-8137.2001.00133.x.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. New Phytologist 206, 1196–1206.

Vílchez, J. I., García-Fontana, C., Román-Naranjo, D., González-López, J., and Manzanera, M. (2016). Plant Drought Tolerance Enhancement by Trehalose Production of Desiccation-Tolerant Microorganisms. Front. Microbiol. 7. doi:10.3389/fmicb.2016.01577.

Wagner, M. R., Lundberg, D. S., Coleman-Derr, D., Tringe, S. G., Dangl, J. L., and Mitchell-Olds, T. (2014). Natural soil microbes alter flowering phenology and the intensity of selection

on flowering time in a wild Arabidopsis relative. Ecology Letters 17, 717–726. doi:10.1111/ele.12276.

Wallenstein, M. D. (2017). Managing and manipulating the rhizosphere microbiome for plant health: A systems approach. Rhizosphere 3, 230–232. doi:10.1016/j.rhisph.2017.04.004.

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Appl. Environ. Microbiol. 73, 5261–5267. doi:10.1128/AEM.00062-07.

Warnes, G., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A., et al. (2020). gplots: Various R Programming Tools for Plotting Data.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer.

Xi, N., Chu, C., and Bloor, J. M. G. (2018). Plant drought resistance is mediated by soil microbial community structure and soil-plant feedbacks in a savanna tree species. Environmental and Experimental Botany 155, 695–701. doi:10.1016/j.envexpbot.2018.08.013.

Yeoh, Y. K., Dennis, P. G., Paungfoo-Lonhienne, C., Weber, L., Brackin, R., Ragan, M. A., et al. (2017). Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. Nature Communications 8, 215. doi:10.1038/s41467-017-00262-8.

Zhu, X., Song, F., Liu, S., and Liu, F. (2016). Arbuscular mycorrhiza improve growth, nitrogen uptake, and nitrogen use efficiency in wheat grown under elevated CO2. Mycorrhiza 26, 133–140. doi:10.1007/s00572-015-0654-3.

Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS microbiology reviews 32, 723–735.

Zolla, G., Badri, D. V., Bakker, M. G., Manter, D. K., and Vivanco, J. M. (2013). Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. Applied Soil Ecology 68, 1–9. doi:10.1016/j.apsoil.2013.03.007.

APPENDIX

Table 1. ANOVA results showing the effect of microbiome source on Solidago phenotypes measured from the glasshouse experiment. Solidago species, seed population, and soil inoculum field site were included as random effects in the models. Statistically significant results are shown in bold.

		Plant growth						Plant physiology		Plant phenology	
		RGR		Shoot		Root		SLA		flower bud break	
Effect	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Microbiome source	2	1.65	0.44	4.38	0.11	6.14	0.046	3.05	0.22	0.37	0.83

Table 2. ANOVA results showing the effect of microbiome source on the phenotypes of each Solidago species measured from the glasshouse experiment. Seed population and soil inoculum field site were included as random effects in the models. Statistically significant results are shown in bold.

			Plant growth						Plant physiology		Plant phenology	
			RGR		Shoot		Root		SLA		flower bud break	
Solidago species	Effect	df	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
S. caesia	Microbiome source	2	0.99	0.61	5.65	0.06	3.34	0.19	1.53	0.46	1.88	0.39
S. flexicaulis	Microbiome source	2	1.35	0.51	0.95	0.62	2.74	0.25	1.6	0.45	1.16	0.56
S. gigantea	Microbiome source	2	2.51	0.28	0.65	0.72	1.14	0.56	4.52	0.1	3.37	0.180

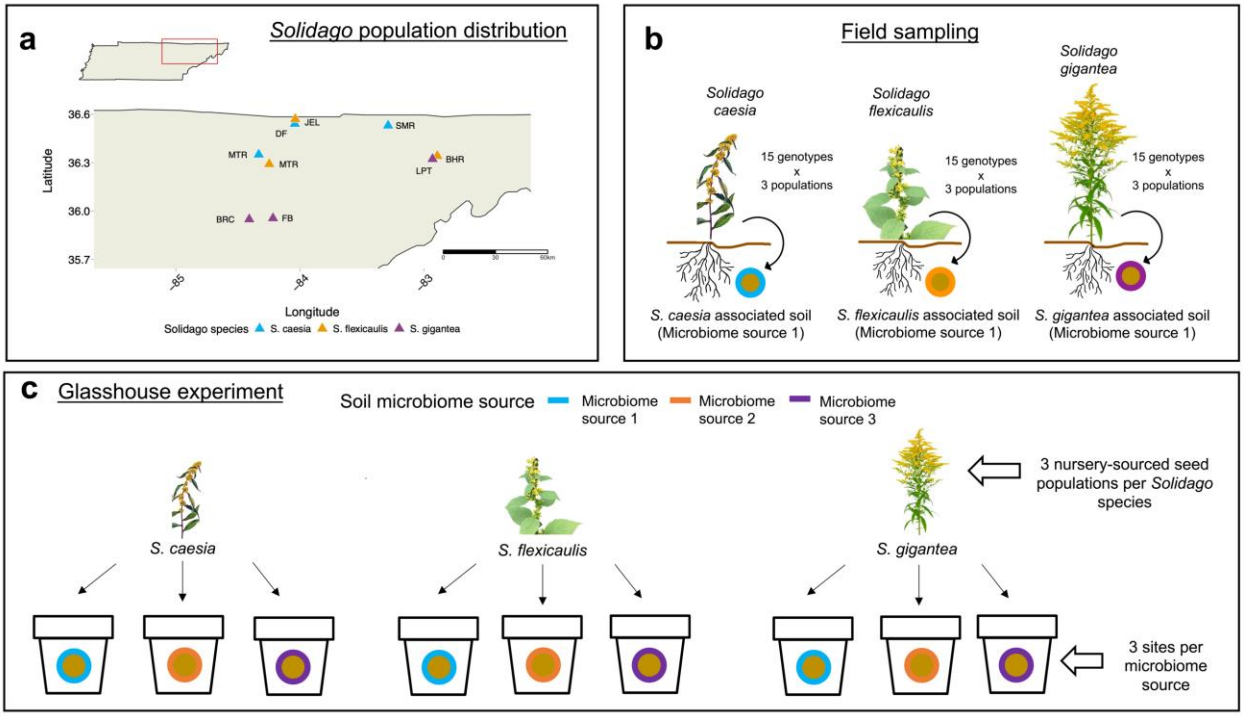

Figure 1. Solidago population locations, field sampling design, and experiment design for soil inoculation experiment. (a) Field sampling was conducted for three distinct natural field populations of Solidago gigantea, S. caesia, and S. flexicaulis in northeastern TN, U.S.A. (b) Growth and physiology phenotypes were measured from 15 putative genotypes at each population for each Solidago species. Rhizosphere soil (S. caesia-associated, S. flexicaulis-associated, and S. gigantea-associated) was collected from beneath each genotype and pooled at the site level. (c) Nursery-sourced seeds of S. caesia, S. flexicaulis, and S. gigantea and field soil were used in a 5-month glasshouse inoculation experiment. Seedlings of each species x nursery population were grown in separate treatments of soil containing inoculum from microbiome sources 1, 2, and 3 collected from the corresponding field sites. (N = 3 Solidago species x 3 seed populations x 3 microbiome sources x 3 field soil sites x 3 replicates = 243 total pots).

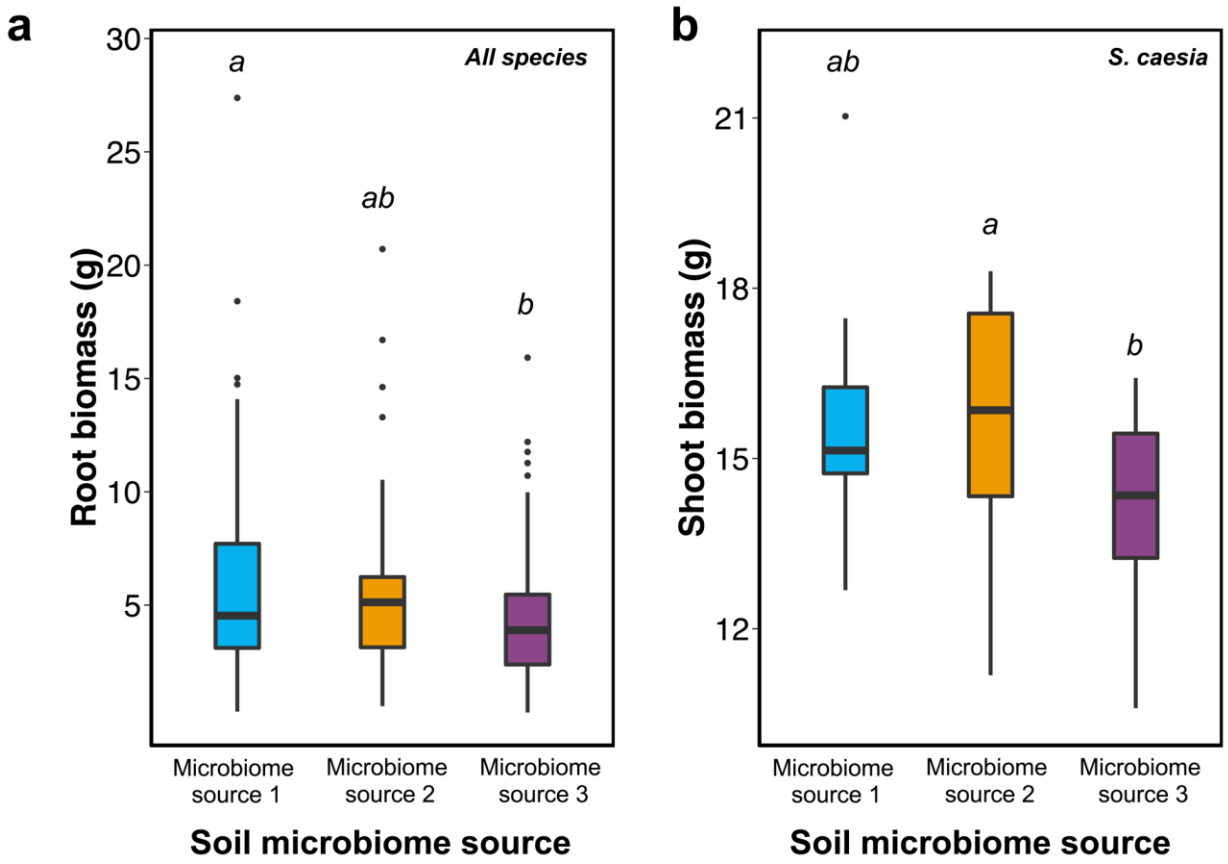

Figure 2. (a) Root biomass response of all Solidago species (S. caesia, S. flexicaulis, S. gigantea) and (b) shoot biomass response of S. caesia to treatments of microbiome source. Bars that do not share letters are significantly different from one other ($\alpha < 0.05$).

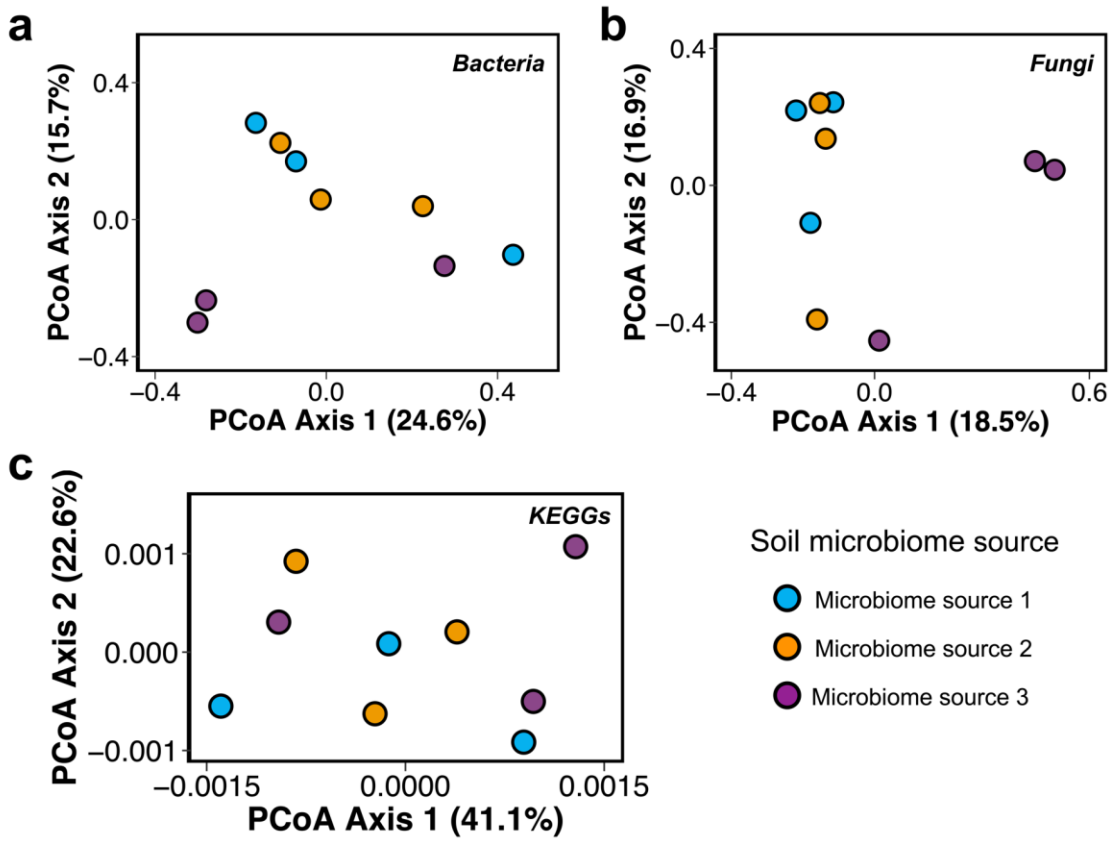

Figure 3. Composition of (a) bacteria, (b) fungi, and (c) KEGG pathways among the three microbiome sources. Each data point represents a field site.

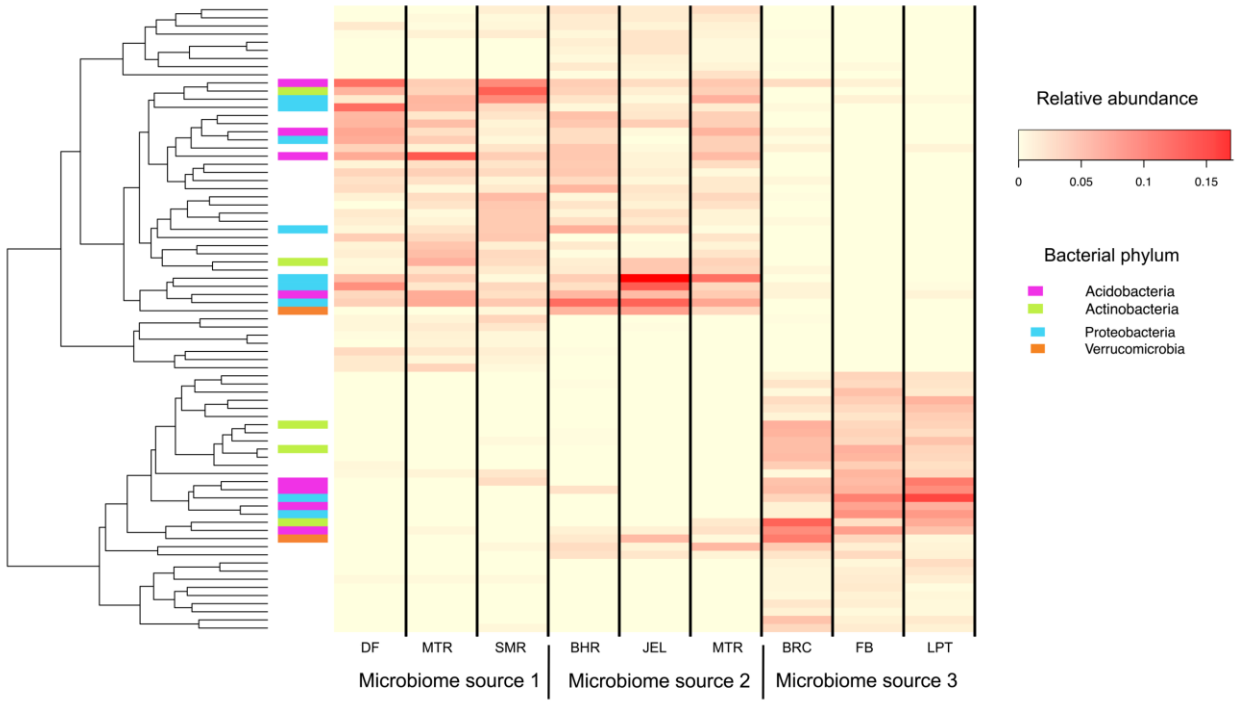

Figure 4. Heatmap of relative abundance of the 77 bacterial ASVs produced from Indicator Species analysis across the three microbiome sources. Rows represent individual ASVs. Columns represent soil from individual field sites. Taxa of each microbiome source with relative abundance 0.05 (5%) or greater are color coded by phylum.

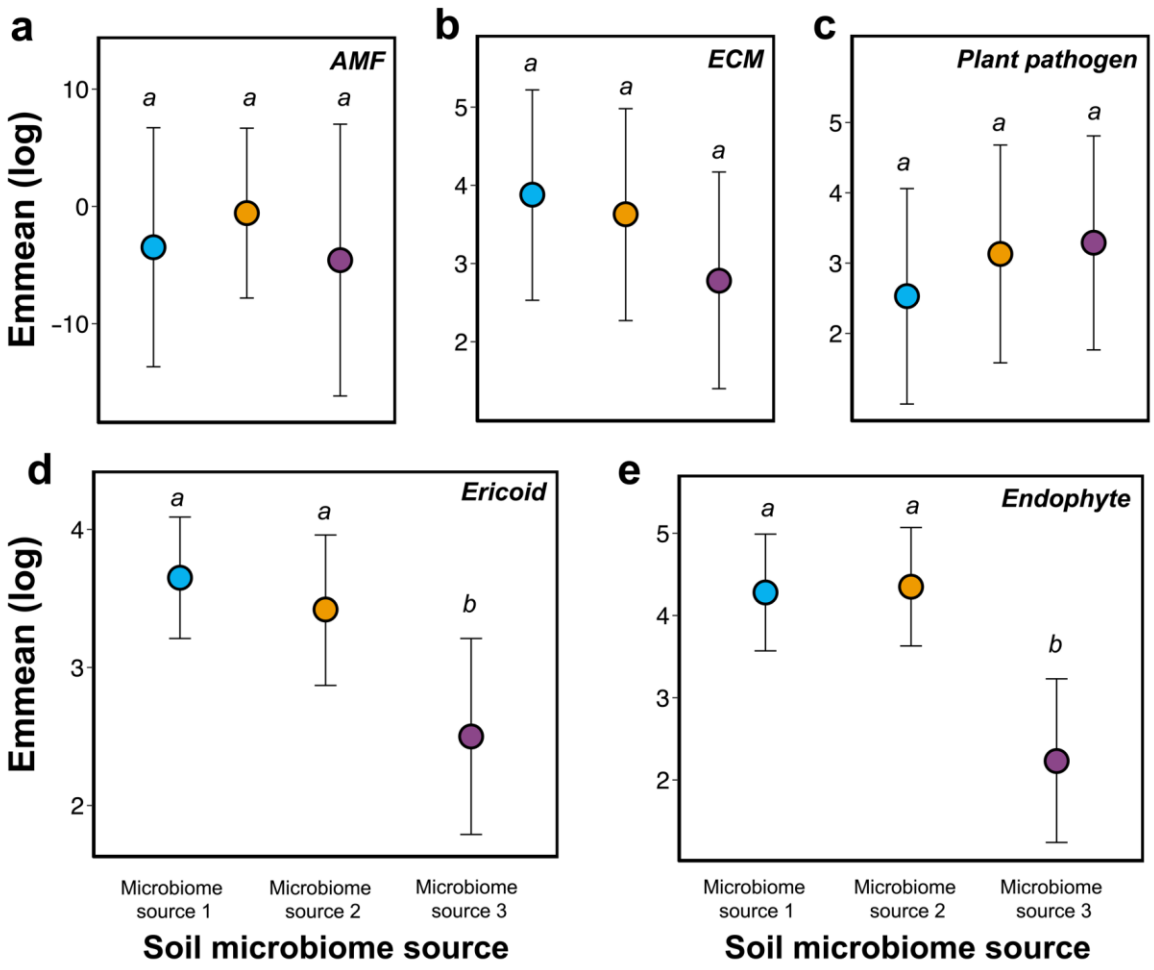

Figure 5. Mean abundance of (a) arbuscular mycorrhizal fungi (AMF), (b) ectomycorrhizal fungi (ECM), (c) plant pathogenic fungi, (d) ericoid mycorrhizal fungi, and (e) endophytic fungi. Emmeans are reported on the log scale. Data shown are pooled across samples (i.e. field sites). Data that do not share letters are significantly different from one other ($\alpha < 0.05$).

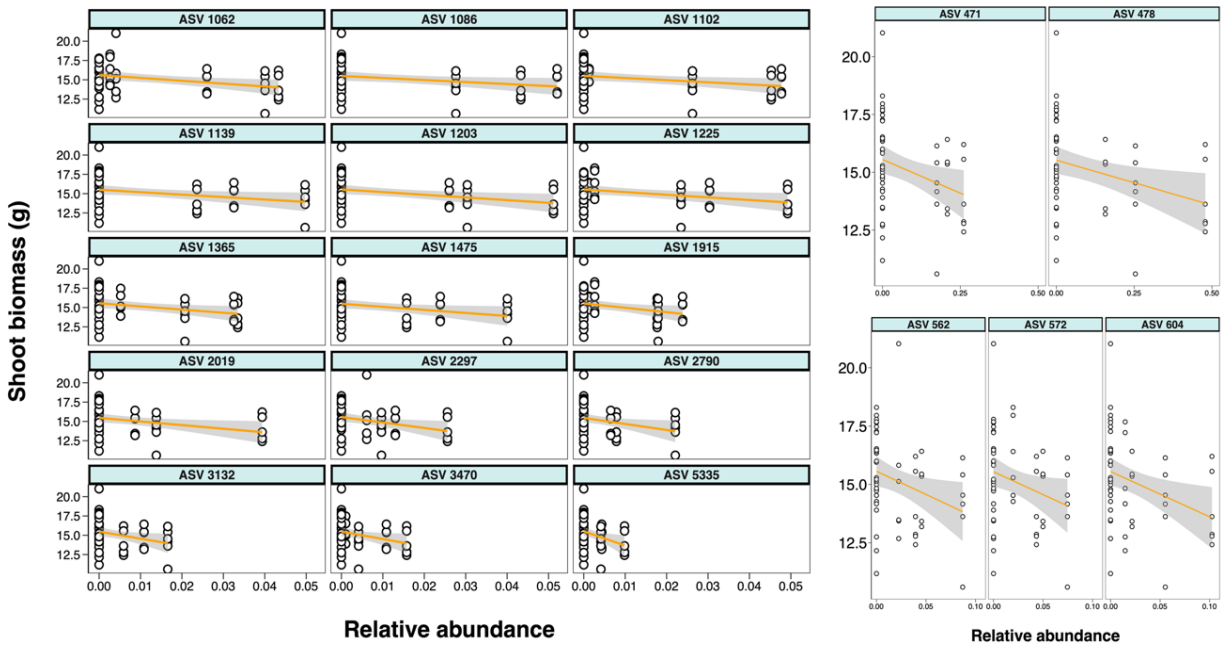

Figure 6. Negative correlations between the relative abundance of indicator taxa of microbiome source 3 and S. caesia shoot biomass. Indicators comprise bacterial and fungal taxa. S. caesia shoot biomass was negatively correlated with the relative abundance of 20 out of the 29 indicator taxa of microbiome source 3.

CHAPTER II: PREDICTING PLANT-SOIL FEEDBACK IN THE FIELD: META-ANALYSIS REVEALS THAT COMPETITION AND ENVIRONMENTAL STRESS DIFFERENTIALLY INFLUENCE PSF

This chapter was originally published by Kendall K. Beals, Jessica A.M. Moore, Stephanie N. Kivlin, Shannon L.J. Bayliss, Candice Y. Lumibao, Leigh C. Moorhead, Megan Patel, Jennifer L. Summers, Ian M. Ware, Joseph K. Bailey, and Jennifer A. Schweitzer:

Beals, Moore, et al. (2020). "Predicting plant-soil feedback in the field: meta-analysis reveals that competition and environmental stress differentially influence PSF." Frontiers in Ecology and Evolution, 8:191. http://doi.org/10.3389/fevo.2020.00191

KKB, JAMM, SLJB, CYL, LCM, MP, JLS, and IMW screened all potential papers and coded data for papers that were included in the meta-analysis, curated by KKB and JAMM. KKB and JAMM ran all initial analyses and made figures. SNK guided data analysis. KKB ran all final analyses and made all final figures. KKB, JAMM, JAS, and JKB coordinated writing, to which all authors contributed. Ideas were conceived of by all authors.

Abstract

Past research on plant-soil feedbacks (PSF), largely undertaken in highly controlled greenhouse conditions, has established that plant species differentially alter abiotic and biotic soil conditions that in turn affect growth of other conspecific and heterospecific individuals in that soil. Yet, whether feedbacks under controlled greenhouse conditions reflect feedbacks in natural environments where plants are exposed to a range of abiotic and biotic pressures is still unresolved. To address how environmental context affects PSF, we conducted a meta-analysis of previously published studies that examined plant growth responses to multiple forms of competition, stress, and disturbance across various PSF methodology. We asked the following questions: (1) Can competition, stress, and disturbance alter the direction and/or strength of PSF? (2) Do particular types of competition, stress, or disturbance affect the direction and/or strength of PSF more than others? and (3) Do methods of conducting PSF research (i.e., greenhouse vs. field experiments and whether the source of soil inoculum conditioning is from the field vs. greenhouse) affect plant growth responses to PSF or competition, stress, and disturbance, or their interactions? We discovered four patterns that may be predictive of what future PSF studies conducted under more realistic conditions might reveal. First, relatively little is known about how PSF responds to environmental stress and disturbance compared to plant-plant competition. Second, specific types of competition enhanced negative effects of soil microbes on plant growth, and specific environmental stressors enhanced positive effects of soil microbes on plant growth. Third, whether PSF experiments are conducted in the field or greenhouse can change plant growth responses. And, fourth, how the soil conditioning phase is conducted can change plant growth responses. With more detail than previously shown,

these results confirm that environmental context writ large can change plant growth responses in PSF experiments. These data should aid in theory and predictions for conservation and restoration applications by showing the relative importance of competition, stress, and disturbance in PSF studies over time. Lastly, these data demonstrate how variation in experimental methods can alter interpretation and conclusions of PSF studies.

Introduction

The past twenty-five years of research on plant-soil feedback (PSF), largely under highly controlled conditions, has established that plant species differentially alter abiotic and biotic soil conditions that in turn affect growth of other conspecific and heterospecific individuals in that soil (Bever, 1994; Bever et al., 1997; Wardle et al., 2004; Ehrenfeld et al., 2005; Kulmatiski and Kardol, 2008; Mangan et al., 2010; Putten et al., 2013). The broad conclusion from this research is that positive and negative PSF can shape community composition and ecosystem functioning by driving patterns of plant diversity, succession, and invasion (Bever et al., 1997; Mills and Bever, 1998; Klironomos, 2002; Reynolds et al., 2003; Mangan et al., 2010; Johnson et al., 2012; Van Der Heijden et al., 2018). Yet most PSF experiments are rarely conducted under the environmental conditions where large-scale ecological and evolutionary mechanisms occur, that is, in the field. Various findings indicate differences in PSF between greenhouse and field experiments (Putten et al., 2016; Schittko et al., 2016; Florianová and Münzbergová, 2018; Heinze and Joshi, 2018; Kivlin et al., 2018; Forero et al., 2019) which suggest that inferences from greenhouse studies may not accurately represent how PSF functions on the landscape (Kulmatiski and Kardol, 2008; Putten et al., 2013, 2016; Smith-Ramesh and Reynolds, 2017; Crawford et al., 2019; De Long et al., 2019). While greenhouse experiments have the advantage of maintaining control over non-focal variables and are crucial for confirming and falsifying mechanisms, recent field based PSF research has demonstrated that thoughtful field study designs can generate tractable and interesting results (Long et al., 2019). By not accounting for the surrounding environmental matrix in which plant-soil interactions occur, it is difficult to accurately predict community composition and productivity based on the plant species that are either inhibited by negative PSF or those that persist by positive PSF.

Competition, stress, and disturbance are common environmental pressures that occur across the landscape. To cope with these pressures, plants can differentially allocate resources to

growth, reproduction, or maintenance. Selection for each of these strategies depends on tradeoffs for traits that allow tolerance to competition, stress, or disturbance events. This phenomenon, known as the competition-stress-disturbance (C-S-D) hypothesis (Grime, 1977), predicts that competitive plants thrive in ecosystems with high competition because of evolved traits such as large size and extensive fine roots. Likewise, traits such as high stem and root storage of resources allow stress-tolerant plants to thrive under highly stressful conditions, while disturbance-resilient plants thrive in frequently or intensely disturbed ecosystems due to evolved traits such as fast growth rates and fast reproduction. Changes in plant traits in response to these environmental pressures may subsequently impact plant-soil interactions by altering soil biota and chemistry. Although the C-S-D hypothesis is well-studied, (Herms and Mattson, 1992; Reich, 2014; Rosado and Mattos, 2017), it does not directly describe the role of plant-soil interactions in plant response to the biotic and abiotic environment (which C-S-D represents) that may enhance or impede a plant's ability to cope with competition, stress, or disturbance. For example, when accounting for soil microbial dynamics under well-watered vs. drought-stress conditions, a greenhouse study (Lau and Lennon, 2012) found that Brassica rapa fitness increased under drought when focal plants were grown with "dry-adapted" soil microorganisms. As such, examining PSF across environmental gradients of competition, stress, or disturbance will provide insight into plant-soil dynamics that influence plant species' persistence or decline under more realistic field conditions.

Competition is a prominent driver of plant fitness, community composition, and coexistence (Tilman, 1982; Callaway and Walker, 1997; Chesson, 2000; Aschehoug et al., 2016) and may influence the strength and direction of PSF in several ways. While competition reduces resource availability, a plant may benefit from its "home" microbiome and thus exhibit positive PSF if soil mutualists increase the availability of limiting resources (Bessler et al., 2012). Conversely, the phenomenon of reduced survival and density of conspecific seedlings near mature or parent conspecifics (Burkey, 1994; Bell et al., 2006; Mangan et al., 2010; Rolhauser et al., 2011; Reinhart et al., 2012; Comita et al., 2014) suggests that intraspecific competition may promote negative PSF due to accumulation of host-specific soil pathogens. The relative importance of competition and PSF on plant performance was examined in a recent meta-analysis (Lekberg et al. 2018). Distinguishing between both inter- vs. intraspecific and low vs. high-density competition and across multiple PSF treatments Lekberg et al. (Lekberg et al., 2018) found antagonistic interactive effects of competition and PSF. In other words, the combined effect of competition and

PSF was greater than the individual effects of PSF or competition alone, often leading to highly reduced plant growth and demonstrating how biotic interactions can alter PSF outcomes. Plant growth responses to PSF are likely also modified by plant responses to changes in the abiotic environment, although this was not tested by Lekberg et al. (Lekberg et al., 2018).

As human-induced global change accelerates, stress (defined here as prolonged or continuous environmental pressures) and disturbance events defined here as sudden, temporally constrained changes in the environment) (Hillebrand and Kunze, 2020) are predicted to rapidly increase (Allen et al., 2010; Dai, 2013; Barbero et al., 2015). These types of environmental pressures will likely have additionally profound effects on plant distributions and plant-soil interactions. Increasing evidence suggests that plant response to environmental stressors including drought (Lau and Lennon, 2012; Vílchez et al., 2016; Kannenberg and Phillips, 2017), herbivory (Badri et al., 2013), and salt tolerance (Qin et al., 2016) can be mediated in part by interactions with soil biota.

As with competition, environmental stress or disturbance can alter resource availability (e.g., soil nutrients, light, water). Fertilization is often considered a benefit to plant growth, however there is evidence that it can cause an imbalance in PSF. A surplus of nutrients from nutrient deposition could promote positive PSF if plant defense against pathogens is enhanced with high resource availability (Smith-Ramesh and Reynolds, 2017). Positive PSF may also occur under low-resource stress if plants rely on host-specific soil microbes for limiting nutrients (Reynolds et al., 2003; Revillini et al., 2016). Alternatively, nutrient inputs in excess of plant growth demands can shift the balance of plant-microbe interactions and prompt dissociations between plants and their home soil biota (Wallenda and Kottke 1998; Treseder & Allen 2002; Revillini et al. 2016). Nutrient inputs from fertilizer applications can lead to limitation of other micronutrients (Whalen et al. 2018) and changes in soil chemistry (Erisman et al. 2013) that modify a plant's association with the soil microbiome. Negative PSF could also occur if soil pathogens thrive under resource-rich conditions (Hersh et al., 2012; Spear et al., 2015).

Other types of environmental stressors like aboveground herbivory could promote negative PSF if grazed plants are less able to defend from soil pathogens that are more abundant in conspecific-conditioned soil (Smith-Ramesh and Reynolds, 2017). Negative PSF under herbivory may also occur if grazed plants reduce carbon allocation to roots and thus reduce the ability to support host-specific microbial mutualists (Smith-Ramesh and Reynolds, 2017). Alternatively,

positive PSF could occur if grazed plants allocate growth to roots and thus support host-specific microbial mutualists (Smith-Ramesh and Reynolds, 2017). Disturbance events such as fire could promote either negative or positive PSF depending on how soil microbes respond to fire disturbance. Fire has generally been found to decrease microbial biomass and diversity (Dooley and Treseder, 2012; Pressler et al., 2019; Whitman et al., 2019) due to heat-sterilization of microbes and loss of soil carbon valuable for soil microbes (Dooley and Treseder, 2012). Negative PSF could occur if abundance of host-specific mutualists declines from fire, whereas positive PSF could occur if fire reduces soil pathogen abundance. However, it is possible that increases in ash deposition following fire could stimulate microbial growth by increasing availability of soil inorganic nitrogen and alleviating nutrient limitation (Rau et al., 2008; Schafer and Mack, 2010; Dooley and Treseder, 2012). In this scenario, negative or positive PSF could occur depending on whether host-specific mutualists or pathogens thrive from nutrient availability.

Predicting PSF accurately on the landscape also requires examining the methods under which plant growth response is measured and the methods that are used to condition the soil microbiome. PSFs have been found to vary between greenhouse and field experiments. An experiment by Schittko et al. (2016) for example, found that the majority of plant species in an experiment exhibited positive PSF under controlled greenhouse conditions, but did not exhibit any significant PSF under natural field conditions. A meta-analysis of the PSF literature seems to corroborate this observation—experiments conducted in the greenhouse often produce larger effect sizes in PSF than those conducted in the field (Kulmatiski et al., 2008; Forero et al., 2019). This broad pattern likely suggests that the true importance of PSF on the landscape is different than what is measured in greenhouse studies. Importantly, experiment location informs interpretation of the specific mechanisms of PSF. Field experiments testing PSF allow for understanding how a natural environment influences soil effects on plants, whereas PSF experiments conducted in the greenhouse do not provide this level of understanding (Smith-Ramesh and Reynolds, 2017).

Similarly, how the soil microbiome is conditioned can greatly influence plant growth. An important distinction between greenhouse-conditioned soil inoculum and field-conditioned soil inoculum is that greenhouse-conditioned soil contains primarily only microbes associated with the focal conditioning plant(s), whereas field-conditioned soil contains microbes associated with site-specific edaphic characteristics in addition to microbes associated with the focal conditioning

plant(s). Subsequent differences in microbial composition can alter plant growth. For example, the perennial forb Centaurea maculosa exhibited negative PSF with field-conditioned soil inoculum and positive PSF with greenhouse-conditioned soil inoculum (Callaway et al., 2004). In this instance, greenhouse-conditioned soil could result in the accumulation of microbial symbionts or the loss of pathogens compared to field-conditioned soil (Kulmatiski et al., 2008). Similar to the role of experiment location, soil conditioning source can also inform interpretation of the specific mechanisms of PSF. Using soil conditioned by focal plants in the greenhouse limits understanding how the environmental context of a natural field environment influences plant effects on soil (Smith-Ramesh and Reynolds, 2017).

Several recent papers have specifically called for empirical studies that measure PSF under manipulations of abiotic and biotic variation (Putten et al., 2013, 2016; Smith-Ramesh and Reynolds, 2017; Lekberg et al., 2018; Crawford et al., 2019). However, no concerted effort has been made to assess the current extent and relative importance of PSF across environmental gradients of competition, stress, or disturbance. We expanded upon a recent effort (Lekberg et al., 2018) that analyzed the interaction between PSF and plant competition. Lekberg et al. found that PSF varied when plants were exposed to competitors or not. We build on that study by examining PSF when plants are exposed to environmental stress or disturbance, to leverage our understanding of plant C-S-D strategies in making predictions of PSF. We also investigated how PSF responses to C-S-D may vary in greenhouse and field conditions to further develop PSF predictive frameworks. Using data from 300 independent manipulations from 76 publications, we examined plant growth responses to multiple forms of competition, stress, and disturbance across a range of PSF methods to identify trends in plant-soil interactions across biotic and abiotic environments and experimental conditions. To understand the relative importance of environmental context as well as common methods for PSF, we addressed the following questions to better understand and predict what PSF outcomes might be expected under realistic field conditions: 1) Can competition, stress, and disturbance alter the direction and/or strength of PSF? 2) Do particular types of competition, stress, and disturbance affect the direction and/or strength of PSF more than others? 3) Do methods of conducting PSF research (i.e., greenhouse vs. field experiments and whether the source of soil conditioning inoculum is from the field vs. greenhouse) affect plant growth responses to PSF or competition, stress, and disturbance, or the interaction of both?

Materials and Methods

Paper selection

We collected previously published, peer-reviewed data of PSF in response to experimental manipulations of competition from studies used in a recent meta-analysis (Lekberg et al., 2018) supplemented with a search conducted in Web of Science for more recent competition studies as well as stress or disturbance. We searched through April 2019 for publications that crossed PSF experiments with one of the environmental manipulations of competition, stress, or disturbance using the following search terms "plant-soil feedback*" OR "PSF" AND "competi*"; "plant-soil feedback*" OR "PSF" AND "stress" OR "drought" OR "herbiv*" OR "precipitation" OR "temperature" OR "salinity" OR "light" OR "fertiliz*" OR "enrichment"; "plant-soil feedback*" OR "PSF" AND "disturbance" OR "mining" OR "mine tailings" OR "wind" OR "hurricane" OR "tornado" OR "fire" OR "grazing" OR "agriculture." We identified additional publications not found in our initial Web of Science search by searching for studies that had cited publications included in our initial data set. Several publications were included twice in our data set because they measured PSF under multiple manipulations of competition, stress, or disturbance (Larios and Suding, 2015; Shivega and Aldrich-Wolfe, 2017; Yu et al., 2017; Hawkins and Crawford, 2018; Zhao et al., 2018), or under more than one stress or disturbance level (Heinze et al., 2016; Valliere and Allen, 2016).

We screened publications for studies that included 1) soil treatment methods indicative of a manipulative PSF experimental design (as detailed below), 2) plant growth responses to soil treatments, specifically, either aboveground biomass or plant height, 3) factorial design of PSF treatments crossed with some manipulation of either competition, stress, or disturbance, where the experiments were undertaken in the field or in the greenhouse and 4) measures of mean, error, and sample size for plant growth in all treatments. We did not have any criteria for the length of the study. We excluded one publication (Brandt et al., 2015) and experiments from several publications (Coykendall and Houseman, 2014; Maron et al., 2016; Zuppinger-Dingley et al., 2016) from the Lekberg et al. (2018) data set because of the absence of a full factorial design. We found nine additional PSF x competition publications that were not included in the Lekberg et al. (2018) meta-analysis (de la Peña et al., 2010; Chen et al., 2012; Zhang et al., 2014; Chung and Rudgers, 2016; Bezemer et al., 2018; Hawkins and Crawford, 2018; Xue et al., 2018; Zhao et al., 2018; Lozano et al., 2019). Soil feedback manipulations for PSF experimental design were

conducted in three ways in the included publications: 1) soil conditioned from the focal species ("home" soil) or from a heterospecific species ("away" soil) (hereafter, home-conditioned vs away-conditioned), 2) live soil (majority of soil biota active; hereafter referred to as "active soil") or sterilized soil (majority of soil biota absent inactive; hereafter referred to as "inactive soil"), or 3) soil untreated or treated with fungicide (non-fungicide treated soil hereafter referred to as "diverse soil"). All studies that included treatments of untreated vs. fungicide soil or treatments of live vs. sterilized soil used soil that had been conditioned by plants. Publications that measured competition included one or two types of competition treatments, either the number of plants differed between treatments and the focal plant grew alone or with other plants (here referred to as "alone-together"). In other instances, competition was quantified as equal number of plants between treatments and the focal plant was exposed to either intra- or interspecific competition (here referred to as "interspecific-intraspecific"). The soil treatments were then added factorially, either directly as field-conditioned inocula (at various quantities and according to treatments) or as a second phase conditioned soil from a two-part design in which plants were initially grown in pots in a greenhouse conditioning phase using a field soil inoculum. Soil conditioned from the first phase was then used as the inoculum in the PSF phase of the experiment (see Kulmatiski et al., 2008 for details of these designs).

Data collection

Mean values and measures of plant growth were collected from text and tables in the main publication and/or supplemental information. We used GraphClick (Arizona Software) to extract mean and standard error values from figures when raw data was not provided. If not provided, standard deviations were back calculated from standard errors and sample sizes ($SD = SE \times \sqrt{n}$). In cases where data was not clearly available in the publication, we contacted the authors. We excluded two publications (Medina-Roldán et al., 2012; Kaisermann et al., 2017) for which we received no response from authors, and therefore could not include these studies. Some studies measured the performance of multiple focal species, and thus included multiple experiments. Some studies contained multiple trials within an experiment in which a focal species was examined under multiple treatments (i.e. multiple home/away soils, multiple competitors, or multiple levels of stress or disturbance).

For each record in our dataset, we recorded the type of soil feedback manipulation (as described above) and environmental manipulation (competition manipulation described above). Stress manipulations consisted of drought, fertilization (as a representation of nutrient deposition), grazing/herbivory, shade (light availability), mining, and temperature. Disturbance manipulations consisted of fire and tornado. We differentiated stress manipulations as those that represented prolonged or continuous environmental pressures experienced by focal plant(s) and disturbance manipulations as those that represent sudden, temporally constrained changes in the environment (Hillebrand and Kunze, 2020).

We recorded duration of the feedback experiment and multiple descriptors of the focal plant. We also recorded the location of where the soil was collected, as well as the source of inoculum conditioning phase (i.e., collected directly from the field = conditioned in the field, or collected from a training phase in pots under controlled conditions = conditioned in the greenhouse). Field-conditioned soil represents microbiota associated with specific plant species in the field as well as microbiota associated with the edaphic conditions of that site (i.e. pH, nutrient levels, soil moisture), whereas greenhouse-conditioned soil represents mostly microbiota associated with the plants that were used to condition the inoculum. For each record we also recorded experiment location. Experiment location was defined as greenhouse if the plant growth response to soil phase was conducted in the greenhouse. Location was defined as field if the response phase was conducted in a natural field environment. Extracted data of all publications included in the dataset are available Table S1 of the Supplementary Material.

Effect size calculations

We conducted an interaction meta-analysis to assess effects of PSF, competition, stress, and disturbance across PSF methods on plant growth using the relative interaction intensity (RII) as the effect size metric (Armas et al., 2004). We preferred this metric over the log response ratio – a widely used metric in ecological meta-analyses – because unlike the log response ratio, RII is bounded between -1 and 1, and therefore symmetrical around zero, and it can be calculated if plant growth is zero in control groups. Following Armas et al. (2004), RII is calculated as:

$$\frac{\text{treatment} - \text{control}}{\text{treatment} + \text{control}}$$

We therefore calculated the effect of PSF manipulation as:

$$\frac{\bar{Y}_{c,f} - \bar{Y}_{c,n}}{\bar{Y}_{c,f} + \bar{Y}_{c,n}},$$

And the effect of competition, stress, or disturbance (i.e., C-S-D) manipulation as:

$$\frac{\bar{Y}_{t,n} - \bar{Y}_{c,n}}{\bar{Y}_{t,n} + \bar{Y}_{c,n}}.$$

To quantify the combined effect of PSF with C-S-D, we followed the calculation in Kivlin et al. (Kivlin et al., 2013) modified from Armas et al. (2004) for a two-factor RII. The interactive effect of soil feedback and competition, stress, or disturbance can be described as the effect of PSF when competition, stress, or disturbance is present compared to the effect of PSF when competition, stress, or disturbance is absent. This was calculated as:

$$\frac{\bar{Y}_{t,f} - \bar{Y}_{t,n}}{\bar{Y}_{t,f} + \bar{Y}_{t,n}} - \frac{\bar{Y}_{c,f} - \bar{Y}_{c,n}}{\bar{Y}_{c,f} + \bar{Y}_{c,n}}$$

In these equations, $\bar{Y}$ is the mean plant growth for t = treatment or c = control for the competition, or stress, or disturbance treatment, and f = soil feedback imposed (away-conditioned soil, live soil, or non-fungicide treated soil) or n = no soil feedback imposed (home-conditioned soil, sterilized soil, or fungicide-treated soil). To calculate the 95% confidence intervals around the means for each record, variance was weighted by the sample size (n) and calculated using the standard deviation (s). For each record, we followed the calculations used in Kivlin et al. (2013) to calculate variance. Variance for the main effect of PSF was calculated as:

$$\text{PSF Vi} = \frac{s_{c,f}^2}{n_{c,f}\bar{Y}_{c,f}^2} + \frac{s_{c,n}^2}{n_{c,n}\bar{Y}_{c,n}^2},$$

and the variance of the main effect of competition, stress or disturbance as:

$$\text{C– S– D Vi} = \frac{s_{t,n}^2}{n_{t,n}\bar{Y}_{t,n}^2} + \frac{s_{c,n}^2}{n_{c,n}\bar{Y}_{c,n}^2},$$

Variance for the interaction of PSF and competition, stress or disturbance was calculated as:

$$\text{Int Vi} = \frac{s_{c,n}^2}{n_{c,n}\bar{Y}_{c,n}^2} + \frac{s_{t,n}^2}{n_{t,n}\bar{Y}_{t,n}^2} + \frac{s_{c,f}^2}{n_{c,f}\bar{Y}_{c,f}^2} + \frac{s_{t,f}^2}{n_{t,f}\bar{Y}_{t,f}^2}.$$

An RII_{PSF} significantly greater than zero indicates that away-conditioned, active, or diverse soil enhances plant growth. An RII_{PSF} significantly less than zero indicates that away-conditioned, active, or diverse soil inhibits plant growth. A RII_{PSF} not significantly different from zero indicates

that away-conditioned, active, or diverse soil does not alter plant growth. An RII_{CSD} significantly greater than zero indicates plant growth is enhanced by competition, stress, or disturbance, whereas an RII_{CSD} significantly less than zero indicates plant growth is inhibited by competition, stress, or disturbance. An RII_{CSD} not significantly different from zero indicates that competition, stress, or disturbance does not alter plant growth. An RII_{Int} significantly greater than zero indicates a synergistic effect such that away-conditioned, active, or diverse soil enhances plant growth under competition, stress, or disturbance. An RII_{Int} significantly less than zero indicates an antagonistic effect such that away-conditioned, active, or diverse soil inhibits plant growth under competition, stress, or disturbance. An RII_{Int} not significantly different from zero indicates that the interactive effects of PSF and competition, stress, or disturbance are neutral. Specifically, a neutral RII_{Int} indicates that away-conditioned, active, or diverse soil does not influence plant growth under competition, stress, or disturbance.

Data analysis

We used the rma.mv function in the metafor package (Viechtbauer, 2010) in R 3.5.2 for all analyses. In all analyses, we separated the dataset by competition studies, stress studies, and disturbance studies and thus ran competition, stress, and disturbance models separately. Prior to analyses to assess our questions, we first identified the individual effects of PSF and C-S-D by building separate multivariate mixed effects models using RII_{PSF} and RII_{CSD} as response variables and PSF Vi and CSD Vi as the variance. These models tested for the main effects of PSF and C-S-D averaged across soil feedback manipulations and across competition, stress, disturbance manipulations. Thus, we tested for differences in plant growth in response to types of soil feedback and types of competition, stress, disturbance by including soil manipulation and C-S-D manipulation as moderators in separate models. A moderator in the rma.mv function is analogous to a fixed effect in an ANOVA model (Viechtbauer, 2010) and allows the model to calculate the effect size of specific levels of a factor.

To address the first question of whether competition, stress, and/or disturbance alters the direction and/or strength of PSF, we built multivariate mixed effects models using RII_{Int} as the response variable and as Int Vi as the variance to identify the main interactive effect of PSF x C-S-D (averaged across soil feedback manipulations and across competition, stress, disturbance manipulations). To address the second question of whether particular types of competition, stress,

and disturbance affect the direction and/or strength of PSF more than others, we built separate multivariate mixed effects RII_{Int} models that included C-S-D manipulation as a moderator. To address the third question of whether methods of conducting PSF research alter the individual effects of PSF, or C-S-D, or the interactive effect of PSF x C-S-D, we built additional RII_{Int} mixed effects models using experiment location (field vs. greenhouse) and soil inoculum conditioning source (field-conditioned vs. greenhouse-conditioned) as moderators.

We included plant species as a random effect in all models because species are not independent and past evolutionary history may affect plant response regardless of treatment (Wooliver et al., 2017). Including plant species as a random effect also allows us to make comparisons across studies. To further account for phylogenetic variation of plant growth responses, we replicated the analysis using plant family as a random effect. Results did not vary between the species and family analyses. While it is likely that the strength of PSF varies with plant ontogeny (Kardol et al., 2013), the included studies lacked sufficient replication in experiment duration to use duration as another random effect. For all models we performed post-hoc tests using the linearHypothesis function in the car package (Fox et al., 2013) to test whether effect sizes differed from one another. We report QM as the test statistic for moderator coefficients of the rma.mv models. We considered results to be significantly different from zero if $\alpha \leq 0.05$.

Results

Distribution of studies

Using the selection criteria, we identified 300 studies from 76 publications that measured plant growth in response to different soil PSF methods in a specific environmental context (competition, stress, or disturbance). Of these, 199 studies (43 publications) measured PSF with competition, 95 studies measured PSF with stress (34 publications), and 5 studies measured PSF with disturbance (2 publications). The majority of studies were conducted in the greenhouse and 86% of all studies used grasses or forbs as the focal plant species. Field experiments comprised only 9% of all studies (29 studies from 7 publications); 26 field studies focused on competition (6 publications), and 3 field studies focused on stress (1 publication).

Main effects of PSF, competition, stress, and disturbance on plant growth

In general, plant growth responses to the main effects of PSF, stress, and disturbance were neutral and the response to competition was negative (Figure 7a; all figures are in Appendix 2 at the end of this chapter). PSF had a generally neutral effect on plant growth (Figure 7a; $p = 0.28$; Table S2, row 3). However, the direction of PSF varied by type of soil feedback manipulation (Figure 8a; QM = 28.43, $p < 0.0001$; Table S2, rows 5-7). Active (i.e. live) soil reduced plant growth compared to sterile soil ($p < 0.0001$; Table S2, row 6), and the effect of PSF was neutral in studies that tested home vs. away-conditioned soil ($p = 0.28$; Table S2, row 7) or in those that tested diverse vs. nondiverse soil (i.e. untreated vs. fungicide treated soil) ($p = 0.97$; Table S2, row 5). Post-hoc analysis revealed that plant growth was reduced more in live vs sterile soil manipulations than in home vs away soil manipulations ($\chi^2 = 26.84$, $p < 0.0001$; Table S2, row 92) or untreated vs. fungicide manipulations ($\chi^2 = 3.97$, $p = 0.05$; Table S2, row 90). PSF was similarly neutral in studies that tested home vs away soil manipulations and untreated vs. fungicide manipulations ($\chi^2 = 0.20$, $p = 0.66$; Table S2, row 91).

Plant growth responses to competition, stress, and disturbance were variable and depended on the type of competition, stress, or disturbance imposed. Competition in general significantly reduced plant growth (Fig. 7a; $p < 0.0001$; Table S2, row 32), and the effect of plants grown together with a competitor was nearly 150% greater than the effect of inter- vs. intraspecific competition on plant growth (Fig 8a; $\chi^2 = 51.78$, $p < 0.0001$; Table S2, row 113). Generally, stress had marginally negative effects on plant growth (Fig 7A; $p = 0.22$; Table S2, row 56), and plant growth varied among different types of stress manipulations (QM = 94.94, $p < 0.0001$; Table S2, rows 58-63). As expected, drought (Fig 8a; $p = 0.0001$; Table S2 row 58) and shade (Fig 8a; $p < 0.0001$; Table S2, row 61) greatly reduced plant growth, and plant growth was reduced similarly under drought and shade ($\chi^2 = 2.66$, $p = 0.20$; Table S2, row 132). Fertilization, on the other hand, increased plant growth (Fig 8a; $p = 0.0003$; Table S2, row 59). Increases in plant growth in response to fertilization were significantly different than reductions in plant growth in response to drought ($\chi^2 = 29.65$, $p < 0.0001$; Table S2, row 130) and shade ($\chi^2 = 70.51$, $p < 0.0001$; Table S2, row 137). Disturbance in general had no effect on plant growth (Fig. 7a; $p = 0.94$; Table S2, row 85). Disturbance caused by fire or tornado did not significantly affect plant growth ($p > 0.05$ for

both; Table S2, rows 87-88) and plants responded similarly to these two disturbances (χ^2 = 1.86, p = 0.17; Table S2, row 164).

Do competition, stress, and/or disturbance alter the direction of PSF?

The strength of PSF was modified by plant-plant competition and environmental stress, but not disturbance. There was no general trend of competition affecting the outcome of PSF (Fig 7b; p = 0.21; Table S2, row 166), but specific types of competition interacted with specific soil feedback manipulations differently (QM = 8.22, p = 0.22; Table S2, rows 174-179). Interspecific competition reduced plant growth compared to intraspecific competition to a greater degree when plants were grown in away-conditioned soil (p = 0.04; Table S2, row 179). Interspecific competition affected plant growth similarly relative to intraspecific competition when plants were grown in active soils (p = 0.42; Table S2, row 178). Interspecific competition also affected plant growth similarly relative to intraspecific competition when plants were grown in diverse soils (p = 0.96; Table S2, row 177). Post-hoc analysis showed that interspecific competition effects in away-conditioned soil were marginally different than active soil (χ^2 = 3.62, p = 0.06; Table S2, row 234) and similar to diverse soils (χ^2 = 0.43, p = 0.51; Table S2, row 233). The effect of away-conditioned (p = 0.82; Table S2, row 176), active (p = 0.25; Table S2, row 175), and diverse soils (p = 0.36; Table S2, row 174) was similar when plants were grown together with a competitor compared to growing alone.

There was a general synergistic effect of PSF and environmental stress (Fig 7b; p = 0.03; Table S2, row 193), yet this trend was driven by the effect of drought stress which enhanced the effect of PSF (Fig 8b; QM = 19.06, p = 0.0002; Table S2, row 195). All other stressors had no effect on plant growth when in combination with PSF: fertilization (Fig 8b; p = 0.12; Table S2, row 196), grazing/herbivory (Fig 8b; p = 0.18; Table S2, row 197), shade (Fig 8b; p = 0.63; Table S2, row 198), mining (Fig 8b; p = 0.34; Table S2, row 199), and temperature (Fig 8b; p = 0.98; Table S2, row 200). Post-hoc analysis showed that the effect of drought on PSF was significantly greater than fertilization (χ^2 = 8.50, p = 0.004; Table S2, row 252), shade (χ^2 = 10.01, p = 0.001; Table S2, row 254), and temperature stress (χ^2 = 4.60, p = 0.03; Table S2, row 256). Drought effects on PSF were similar to grazing/herbivory (χ^2 = 2.62, p = 0.11; Table S2, row 253) and mining χ^2 = 0.07, p = 0.79; Table S2, row 255), which were also positive but not statistically

significant. All other environmental stressors had similar neutral effects on PSF ($p > 0.05$ for all other post-hoc comparisons). We were unable to analyze effects of different environmental stressors on different PSF soil manipulations due to lack of sufficient studies. There was no general effect of PSF with environmental disturbance (Fig 7b; $p = 0.80$; Table S2, row 217), and there were no disturbance-specific differences (QM = 0.45, $p = 0.80$; Table S2, rows 219-220). Plant-soils feedbacks were not modified by fire (Fig 8b; $p = 0.61$; Table S2, row 219) or tornados (Fig 8b; $p = 0.66$; Table S2, row 220). These disturbances had similar neutral effects on PSFs ($\chi^2 = 0.40$, $p = 0.53$; Table S2, row 278). Disturbance effects on different PSF soil manipulations could not be further differentiated due to the low number of studies.

Do the effects of PSF and C-S-D vary between methods used to conduct PSF research?

Whether the experiment was conducted in the field or greenhouse altered the effects of competition and stress on plant growth, but not the effect of PSF (Fig 9a). There was no difference in the effects of away-conditioned vs home-conditioned soils ($\chi^2 = 0.24$, $p = 0.62$; Table S2, row 103) or diverse vs non-diverse soils ($\chi^2 = 2.61$, $p = 0.11$; Table S2, row 105) on plant growth between studies where the feedback phase occurred in the field or greenhouse. Studies that used live and sterile soils as the soil feedback manipulation were only conducted in the greenhouse. Conversely, competition reduced plant growth when plants were grown in the field (Fig 9a; $p < 0.001$; Table S2, row 42) and the greenhouse (Fig 9a; $p < 0.001$; Table S2, row 43), but the effect was 138% stronger in field experiments than greenhouse experiments ($\chi^2 = 48.21$, $p < 0.001$; Table S2, row 122). This trend was driven by alone/together competition studies because inter/intra-specific competition studies were only conducted in the greenhouse. Only grazing/herbivory stress was conducted in both greenhouse and field settings, and thus it was the only stressor we could analyze between experiment location. Grazing/herbivory stress enhanced plant growth in the greenhouse (Fig 9a; $p < 0.001$; Table S2, row 71), whereas its effects on plant growth were neutral in field experiments (Fig 9a; $p = 0.48$; Table S2, row 70). Grazing/herbivory stress enhanced plant growth over 300% more in greenhouse experiments than in field experiments (Fig 9a; $\chi^2 = 11.31$, $p < 0.001$; Table S2, row 157). Disturbance studies were only tested in greenhouse conditions.

The interactions between PSF and competition were neutral in both field and greenhouse experiments (Fig 9b; QM = 2.52, $p = 0.28$; Table S2, rows 181-182) and post-hoc analysis showed

that these effects were similar between field and greenhouse experiments (χ^2 = 0.95, p = 0.33; Table S2, row 245). Contrary to the finding that the main effect of grazing/herbivory was only positive in greenhouse experiments, there was a synergistic effect of PSF and grazing/herbivory only in field experiments (Fig 9b; p = 0.04; Table S2, row 202). In other words, away-conditioned, active or diverse soil enhanced plant growth under grazing/herbivory in field experiments. However, post-hoc analysis showed that the interaction of PSF and grazing/herbivory in field experiments was not significantly different than the neutral effect of PSF and grazing/herbivory in greenhouse experiments (χ^2 = 2.34, p = 0.13; Table S2, row 268). Disturbance interactions with PSFs were only tested in greenhouse conditions.

In general, soil inoculum conditioning source modified the effect of PSF on plant growth (Fig. 10a; QM = 34.93, p < 0.0001; Table S2, rows 23-24). Away-conditioned, active, or diverse soil reduced plant growth when soil inoculum was conditioned in the field (Fig 10a; p < 0.0001; Table S2, row 23) and enhanced plant growth when soil inoculum was conditioned in the greenhouse (Fig 10a; p = 0.007; Table S2, row 24). The various soil feedback manipulations also responded differently depending on the source of the conditioning phase. Active soil reduced plant growth when conditioned in the field (p = 0.03; Table S2, row 26) but had no effect on plant growth when conditioned in the greenhouse (p = 0.40; Table S2, row 27). The effects of away-conditioned soil on plant growth did not vary between field-conditioned (p = 0.20; Table S2, row 29) or greenhouse-conditioned soil (p = 0.50; Table S2, row 30).

The interaction of PSF and competition was similarly neutral between experiments that used field-conditioned and greenhouse-conditioned inoculum (Fig 10b; χ^2 = 0.04, p = 0.84; Table S2, row 247). In general, the interaction of PSF and environmental stress was slightly synergistic when soil inoculum was conditioned in the field (Fig 10b; QM = 4.92, p = 0.08; Table S2, row 205). This trend was likely driven by drought, fertilization, and grazing/herbivory studies in which the average effects of PSF and each of these stressors was positive, though not statistically significant. However, post-hoc analysis showed that the interaction of PSF and stress with field-conditioned soil inoculum was not significantly different than the neutral effect of PSF and stress with greenhouse-conditioned soil inoculum (χ^2 = 0.52, p = 0.47; Table S2, row 270). Conversely, the interaction of PSF and mining stress was slightly synergistic when soil was conditioned in the greenhouse (QM = 4.73, p = 0.09; Table S2, row 215). Away-conditioned, active, or diverse soil

enhanced plant growth 300% more under mining stress when soil inoculum was conditioned in the greenhouse rather than conditioned in the field ($\chi^2 = 3.81$, $p = 0.05$; Table S2, row 276). Studies that manipulated temperature were not included in this analysis as these studies only included greenhouse-conditioned soil.

Discussion

In general, our meta-analysis revealed important patterns that may aid predictions of PSF under natural or field conditions where plant-plant competition and environmental stressors are common. The results of this meta-analysis suggest that soil microbes may generally reduce plant growth where plant-plant competition for resources occurs and may enhance plant growth under drought stress conditions. These results also highlight an important research gap in examining PSF under environmental disturbance. Additionally, this meta-analysis suggests that where PSF experiments are conducted (field or greenhouse) affects the outcome of the study – especially when plants are competing. The nature of the soil conditioning manipulation (live vs sterile, home vs away, or fungicide application) can change plant growth responses, and again this is most evident when plants are competing for limiting resources. Our meta-analysis corroborates and expands upon Lekberg et al. (2018) to confirm that environmental context writ large can alter plant growth responses in PSF experiments, at least in grass and forb species with which the majority of PSF studies have been conducted.

Plant competition, and specific environmental stressors alter direction and magnitude of PSF

Competition shapes resource availability (Tilman, 1982, 1990) which can subsequently affect PSF, and the data to date show that PSF is often more examined in response to plant-plant competition than to environmental stress and disturbance. While growing next to a plant compared to growing alone more greatly reduced plant growth than when plants were growing with conspecifics or heterospecifics, types of competition reduced plant growth differently depending on the type of soil feedback imposed. When focal plants were grown in away-conditioned soil, interspecific competition reduced plant growth more than intraspecific competition. This likely indicates that interspecific competition either enhanced negative effects of non-host associated soil biota on plant growth or reduced beneficial effects of non-host associated soil biota on plant growth. When focal plants were grown in active or diverse soil, interspecific competition reduced plant growth to a similar degree to that of intraspecific competition. This could suggest that

taxonomic composition of the soil microbiome is more important than microbial diversity for reducing plant growth under interspecific competition. Conversely, in alone vs. together competition studies, the effect of away-conditioned, active, and diverse soil was similar. This may suggest that just the presence of a soil microbiome is influential for inhibiting plant growth when a plant is in close proximity to a competitor.

While the frequency of environmental stress and disturbance are increasingly occurring in terrestrial landscapes, these data from 76 publications suggest that stress and disturbance can have both negative and positive effects on plant growth individually (e.g., drought and shade reduce growth but fertilization increases growth) but when combined with PSF, lead to few synergistic or antagonistic effects. While the combined effect of PSF and environmental stress was generally synergistic, the trend was driven by drought stress studies. Away-conditioned, active, or diverse soil enhanced plant growth under drought conditions. Though the exact mechanisms to explain this trend remain unclear, soil microbes may facilitate plant growth under low-resource stress if plants rely on microbes for acquiring limiting nutrients (Reynolds et al., 2003; Revillini et al., 2016). Although this meta-analysis found that the combined effect of PSF and grazing/herbivory was on average synergistic but not statistically significant, grazing/herbivory could potentially induce benefits of the soil microbiome to plant growth if plants allocated resources to roots and subsequently supported microbial mutualists (Smith-Ramesh and Reynolds, 2017).

This meta-analysis found that the combined effects of PSF and environmental disturbance were neutral for both fire and tornado disturbance treatments. It is unclear, however, whether this is a true pattern across fire and tornado disturbance or across many types of environmental disturbance because this dataset only includes five studies from two publications (Nagendra and Peterson, 2016; Senior et al., 2018). Soil microbiomes and their interactions with plants are likely to be affected by drastic landscape changes brought about by environmental stress and disturbance, yet this meta-analysis shows that there is not yet enough data to predict how PSF responds to environmentally disruptive events. This gap of knowledge highlights the necessity of PSF experiments to incorporate manipulations that are representative of environmental variation under global climate change (Putten et al., 2016; Long et al., 2019).

Experiment location and soil conditioning source affect the outcomes of PSF research

Comparisons of field vs. greenhouse studies showed that PSF did not vary by experiment location. Competition had a more negative effect when experiments were conducted in the field rather than in the greenhouse. However, the combined effects of PSF and competition were similarly neutral between field and greenhouse experiments. Grazing/herbivory only increased plant growth in greenhouse conditions. The combined effect of PSF and grazing/herbivory was synergistic in field conditions and neutral in greenhouse conditions, though further analyses indicated that the interactive effects of PSF and grazing/herbivory were not significantly different between field and greenhouse experiments.

It is important to note that only 9% of these studies were conducted in the field, suggesting that we have little inference for the strength or direction that environmental variation will demonstrate when interacting with PSF to affect plant growth. The few interactive effects shown here both support and contradict studies showing that PSF can differ between greenhouse and field experiments in different environments (Putten et al., 2016; Schittko et al., 2016; Florianová and Münzbergová, 2018; Heinze and Joshi, 2018; Kivlin et al., 2018) demonstrating how little is understood about the function of PSF in nature (Forero et al., 2019).

PSF varied by soil inoculum conditioning source. Away-conditioned, active, or diverse soil reduced plant growth if soil was conditioned in the field and enhanced plant growth if soil was conditioned in the greenhouse. The combined effect of PSF and competition was similarly neutral between studies that used field-conditioned and greenhouse-conditioned soil inoculum. The combined effect of PSF and stress was slightly synergistic when soil inoculum was conditioned in the field, though this was not significantly different than the neutral effect of PSF and stress with greenhouse-conditioned soil inoculum. The combined effect of PSF and mining stress was slightly synergistic when soil was conditioned in the greenhouse. Away-conditioned, active, or diverse soil enhanced plant growth 300% more under mining stress when soil inoculum was conditioned in the greenhouse rather than in the field. While it is difficult to identify detailed mechanisms, the trend from this meta-analysis of increased plant growth in greenhouse-conditioned soil relative to field-conditioned soil may be due to lower microbial diversity in greenhouse-conditioned soil. Specifically, greenhouse-conditioned soil may contain lower abundance of pathogens than field-conditioned soil (Callaway et al., 2004; Kulmatiski et al., 2008).

These results demonstrate that there are multiple methodological approaches that both allow us to infer how field studies may respond and to show how variation in methods can change interpretation of results. The finding that effects of PSF can differ between greenhouse and field experiments provides justification for pairing PSF experiments in the greenhouse with those conducted in the field, when possible (Smith-Ramesh and Reynolds, 2017). Additionally, that the source of inoculum for PSF experiments can also mediate interactive effects show that careful interpretation is required of studies that use each method (Smith-Ramesh and Reynolds, 2017). Studies that use field-conditioned inoculum for example, should be mindful to infer that focal plants are responding to microbes associated with the focal conditioning plant(s) in addition to microbes associated with site-specific soil characteristics. Focal plants in studies that use greenhouse-conditioned inoculum, on the other hand, are responding primarily to microbes associated with the focal conditioning plant(s). Moreover, it should be noted that 86% of the focal plants examined in this meta-analysis are grasses and forbs. While, overall, we did not see mean differences in effect sizes among plant functional groups (data not shown – see results in Table S2), this result is related to by the reduced power to detect an effect due to low sample sizes of trees and shrubs that have been studied to date.

An important distinction between greenhouse-conditioned soil inoculum and field-conditioned soil inoculum is that greenhouse-conditioned soil contains primarily only microbes associated with the focal conditioning plant(s), whereas field-conditioned soil contains microbes associated with site-specific edaphic characteristics in addition to microbes associated with the focal conditioning plant(s).

Conclusions

Environmental context writ large can change plant growth responses in PSF experiments, a conclusion supported by our analyses comparing the interactive effects of PSF with a range of competition, stress and disturbance types relative to their individual effects. Our results have direct application in ecological conservation and restoration because we show that positive PSF is synergistically enhanced by drought and stress. Additionally, our study suggests that field experiments may yield different responses than greenhouse experiments. Data from these 76 studies show the need for more research on PSF across environmental stresses and disturbances (i.e., two-thirds of these studies were conducted on competition) and the need for increased

representation of a wide diversity of focal plant species, because the majority of PSF studies were conducted with grasses and forbs. The lack of studies investigating true gradients of stress and disturbance (i.e., multiple experimental levels, rather than presence-absence of different types of stress or disturbance) indicate that we know very little about how PSF effects will respond to stress and disturbance on the landscape. Our meta-analysis enables future research into plant community dynamics in a changing world. There is, therefore, much empirical work to look forward to.

REFERENCES

Alexander, J.M., Diez, J.M. and Levine, J.M. (2015). Novel competitors shape species responses to climate change. Nature 525:515-518. doi: 10.1038/nature14952.

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., Mcdowell, N., Vennetier, M., et al. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manag. 259, 660–684. doi:10.1016/j.foreco.2009.09.001.

Armas, C., Ordiales, R., and Pugnaire, F. I. (2004). Measuring Plant Interactions: A New Comparative Index. Ecology 85, 2682–2686. doi:10.1890/03-0650.

Aschehoug, E. T., Brooker, R., Atwater, D. Z., Maron, J. L., and Callaway, R. M. (2016). The Mechanisms and Consequences of Interspecific Competition Among Plants. Annu. Rev. Ecol. Evol. Syst. 47, 263–281. doi:10.1146/annurev-ecolsys-121415-032123.

Badri, D. V., Zolla, G., Bakker, M. G., Manter, D. K., and Vivanco, J. M. (2013). Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytol. 198, 264–273. doi:10.1111/nph.12124.

Barbero, R., Abatzoglou, J. T., Larkin, N. K., Kolden, C. A., and Stocks, B. (2015). Climate change presents increased potential for very large fires in the contiguous United States. Int. J. Wildland Fire 24, 892–899. doi:10.1071/WF15083.

Bell, T., Freckleton, R. P., and Lewis, O. T. (2006). Plant pathogens drive density-dependent seedling mortality in a tropical tree. Ecol. Lett. 9, 569–574. doi:10.1111/j.1461-0248.2006.00905.x.

Bessler, H., Oelmann, Y., Roscher, C., Buchmann, N., Scherer-Lorenzen, M., Schulze, E.-D., et al. (2012). Nitrogen uptake by grassland communities: contribution of N2 fixation, facilitation, complementarity, and species dominance. Plant Soil 358, 301–322. doi:10.1007/s11104-012-1181-z.

Bever, J. D. (1994). Feeback between Plants and Their Soil Communities in an Old Field Community. Ecology 75, 1965–1977. doi:10.2307/1941601.

Bever, J. D., Westover, K. M., and Antonovics, J. (1997). Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach. J. Ecol. 85, 561–573. doi:10.2307/2960528.

Bezemer, T. M., Jing, J., Bakx-Schotman, J. M. T., and Bijleveld, E.-J. (2018). Plant competition alters the temporal dynamics of plant-soil feedbacks. J. Ecol. 106, 2287–2300. doi:10.1111/1365-2745.12999.

Brandt, A. J., Leahy, S. C., Zimmerman, N. M., and Burns, J. H. (2015). Plant trait expression responds to establishment timing. Oecologia 178, 525–536. doi:10.1007/s00442-014-3216-z.

Burkey, T. V. (1994). Tropical tree species diversity: a test of the Janzen-Connell model. Oecologia 97, 533–540. doi:10.1007/BF00325893.

Callaway, R. M., Thelen, G. C., Barth, S., Ramsey, P. W., and Gannon, J. E. (2004). Soil Fungi Alter Interactions Between the Invader Centaurea Maculosa and North American Natives. Ecology 85, 1062–1071. doi:10.1890/02-0775.

Callaway, R. M., and Walker, L. R. (1997). Competition and Facilitation: A Synthetic Approach to Interactions in Plant Communities. Ecology 78, 1958–1965. doi:10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2.

Chen, H., Wang, R. Q., Ge, X. L., Zhang, J., Du, N., Wang, W., et al. (2012). Competition and soil fungi affect the physiological and growth traits of an alien and a native tree species. Photosynthetica 50, 77–85. doi:10.1007/s11099-012-0013-y.

Chesson, P. (2000). General Theory of Competitive Coexistence in Spatially-Varying Environments. Theor. Popul. Biol. 58, 211–237. doi:10.1006/tpbi.2000.1486.

Chung, Y. A., and Rudgers, J. A. (2016). Plant–soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. Proc. R. Soc. B Biol. Sci. 283, 20160608. doi:10.1098/rspb.2016.0608.

Comita, L. S., Queenborough, S. A., Murphy, S. J., Eck, J. L., Xu, K., Krishnadas, M., et al. (2014). Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. J. Ecol., 845–856. doi:10.1111/1365-2745.12232@10.1111/(ISSN)1365-2745.Plant-pathogensJEC2018.

Coykendall, K. E., and Houseman, G. R. (2014). Lespedeza cuneata invasion alters soils facilitating its own growth. Biol. Invasions 16, 1735–1742. doi:10.1007/s10530-013-0623-8.

Crawford, K. M., Bauer, J. T., Comita, L. S., Eppinga, M. B., Johnson, D. J., Mangan, S. A., et al. (2019). When and where plant-soil feedback may promote plant coexistence: a meta-analysis. Ecol. Lett. 22, 1274–1284. doi:10.1111/ele.13278.

Dai, A. (2013). Increasing drought under global warming in observations and models. Nat. Clim. Change 3, 52–58. doi:10.1038/nclimate1633.

de la Peña, E., de Clercq, N., Bonte, D., Roiloa, S., Rodríguez-Echeverría, S., and Freitas, H. (2010). Plant-soil feedback as a mechanism of invasion by Carpobrotus edulis. Biol. Invasions 12, 3637–3648. doi:10.1007/s10530-010-9756-1.

De Long, J. R., Heinen, R., Steinauer, K., Hannula, S. E., Huberty, M., Jongen, R., et al. (2019). Taking plant–soil feedbacks to the field in a temperate grassland. Basic Appl. Ecol. 40, 30–42. doi:10.1016/j.baae.2019.08.001.

De Long, J. R., Fry, E. L., Veen, G. F., and Kardol, P. (2019b). Why are plant–soil feedbacks so

unpredictable, and what to do about it? Funct. Ecol. doi:10.1111/1365-2435.13232.

Dooley, S. R., and Treseder, K. K. (2012). The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49–61. doi:10.1007/s10533-011-9633-8.

Ehrenfeld, J. G., Ravit, B., and Elgersma, K. (2005). Feedback in the Plant-Soil System. Annu. Rev. Environ. Resour. 30, 75–115. doi:10.1146/annurev.energy.30.050504.144212.

Erisman, J. W., J. N. Galloway, S. Seitzinger, A. Bleeker, N. B. Dise, A. R. Petrescu, A. M. Leach, and W. de Vries. 2013. Consequences of human modification of the global nitrogen cycle. Philosophical Transactions of the Royal Society B: Biological Sciences 368:20130116.

Florianová, A., and Münzbergová, Z. (2018). The intensity of intraspecific plant-soil feedbacks in alien Impatiens species depends on the environment. Perspect. Plant Ecol. Evol. Syst. 32, 56–64. doi:10.1016/j.ppees.2018.04.004.

Forero, L. E., Grenzer, J., Heinze, J., Schittko, C., and Kulmatiski, A. (2019). Greenhouse- and Field-Measured Plant-Soil Feedbacks Are Not Correlated. Front. Environ. Sci. 7. doi:10.3389/fenvs.2019.00184.

Fox, J., Friendly, M., and Weisberg, S. (2013). Hypothesis Tests for Multivariate Linear Models Using the car Package. R J. 5, 39. doi:10.32614/RJ-2013-004.

Grime, J. P. (1977). Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. Am. Nat. 111, 1169–1194. doi:10.1086/283244.

Hawkins, A. P., and Crawford, K. M. (2018). Interactions between plants and soil microbes may alter the relative importance of intraspecific and interspecific plant competition in a changing climate. AoB PLANTS 10. doi:10.1093/aobpla/ply039.

Heinze, J., and Joshi, J. (2018). Plant–soil feedback effects can be masked by aboveground herbivory under natural field conditions. Oecologia 186, 235–246. doi:10.1007/s00442-017-3997-y.

Heinze, J., Sitte, M., Schindhelm, A., Wright, J., and Joshi, J. (2016). Plant-soil feedbacks: a comparative study on the relative importance of soil feedbacks in the greenhouse versus the field. Oecologia 181, 559–569. doi:10.1007/s00442-016-3591-8.

Herms, D. A., and Mattson, W. J. (1992). The Dilemma of Plants: To Grow or Defend. Q. Rev. Biol. 67, 283–335. doi:10.1086/417659.

Hersh, M. H., Vilgalys, R., and Clark, J. S. (2012). Evaluating the impacts of multiple generalist fungal pathogens on temperate tree seedling survival. Ecology 93, 511–520. doi:10.1890/11-0598.1.

Hillebrand, H., and Kunze, C. (2020). Meta-analysis on pulse disturbances reveals differences in functional and compositional recovery across ecosystems. Ecol. Lett. 23, 575–585. doi:10.1111/ele.13457.

Johnson, D. J., Beaulieu, W. T., Bever, J. D., and Clay, K. (2012). Conspecific Negative Density Dependence and Forest Diversity. Science 336, 904–907. doi:10.1126/science.1220269.

Kaisermann, A., Vries, F. T. de, Griffiths, R. I., and Bardgett, R. D. (2017). Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. New Phytol. 215, 1413–1424. doi:10.1111/nph.14661.

Kannenberg, S. A., and Phillips, R. P. (2017). Soil microbial communities buffer physiological responses to drought stress in three hardwood species. Oecologia 183, 631–641. doi:10.1007/s00442-016-3783-2.

Kardol, P., Deyn, G. B. D., Laliberté, E., Mariotte, P., and Hawkes, C. V. (2013). Biotic plant–soil feedbacks across temporal scales. J. Ecol. 101, 309–315. doi:10.1111/1365-2745.12046.

Kivlin, S. N., Bedoya, R., and Hawkes, C. V. (2018). Heterogeneity in arbuscular mycorrhizal fungal communities may contribute to inconsistent plant-soil feedback in a Neotropical forest. Plant Soil 432, 29–44. doi:10.1007/s11104-018-3777-4.

Kivlin, S. N., Emery, S. M., and Rudgers, J. A. (2013). Fungal symbionts alter plant responses to global change. Am. J. Bot. 100, 1445–1457. doi:10.3732/ajb.1200558.

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417, 67–70. doi:10.1038/417067a.

Kulmatiski, A., Beard, K. H., Stevens, J. R., and Cobbold, S. M. (2008). Plant–soil feedbacks: a meta-analytical review. Ecol. Lett. 11, 980–992. doi:10.1111/j.1461-0248.2008.01209.x.

Kulmatiski, A., and Kardol, P. (2008). "Getting Plant—Soil Feedbacks out of the Greenhouse: Experimental and Conceptual Approaches," in Progress in Botany Progress in Botany., eds. U. Lüttge, W. Beyschlag, and J. Murata (Berlin, Heidelberg: Springer), 449–472. doi:10.1007/978-3-540-72954-9_18.

Larios, L., and Suding, K. N. (2015). Competition and soil resource environment alter plant–soil feedbacks for native and exotic grasses. AoB PLANTS 7. doi:10.1093/aobpla/plu077.

Lau, J. A., and Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc. Natl. Acad. Sci. 109, 14058–14062. doi:10.1073/pnas.1202319109.

Lekberg, Y., Bever, J. D., Bunn, R. A., Callaway, R. M., Hart, M. M., Kivlin, S. N., et al. (2018). Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for coexistence. Ecol. Lett. 21, 1268–1281. doi:10.1111/ele.13093.

Long, J. R. D., Fry, E. L., Veen, G. F., and Kardol, P. (2019). Why are plant–soil feedbacks so unpredictable, and what to do about it? Funct. Ecol. 33, 118–128. doi:10.1111/1365-2435.13232.

Lozano, Y. M., Hortal, S., Armas, C., and Pugnaire, F. I. (2019). Soil micro-organisms and competitive ability of a tussock grass species in a dry ecosystem. J. Ecol. 107, 1215–1225. doi:10.1111/1365-2745.13104.

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., et al. (2010). Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466, 752–755. doi:10.1038/nature09273.

Maron, J. L., Smith, A. L., Ortega, Y. K., Pearson, D. E., and Callaway, R. M. (2016). Negative plant-soil feedbacks increase with plant abundance, and are unchanged by competition. Ecology 97, 2055–2063. doi:10.1002/ecy.1431.

Medina-Roldán, E., Paz-Ferreiro, J., and Bardgett, R. D. (2012). Grazing-induced effects on soil properties modify plant competitive interactions in semi-natural mountain grasslands. Oecologia 170, 159–169. doi:10.1007/s00442-012-2287-y.

Mills, K. E., and Bever, J. D. (1998). Maintenance of Diversity Within Plant Communities: Soil Pathogens as Agents of Negative Feedback. Ecology 79, 1595–1601. doi:10.1890/0012-9658(1998)079[1595:MODWPC]2.0.CO;2.

Nagendra, U. J., and Peterson, C. J. (2016). Plant-soil feedbacks differ in intact and tornado-damaged areas of the southern Appalachian mountains, USA. Plant Soil 402, 103–116. doi:10.1007/s11104-015-2766-0.

Pressler, Y., Moore, J. C., and Cotrufo, M. F. (2019). Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos 128, 309–327. doi:10.1111/oik.05738.

Putten, W. H. van der, Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., et al. (2013). Plant–soil feedbacks: the past, the present and future challenges. J. Ecol. 101, 265–276. doi:10.1111/1365-2745.12054.

Putten, W. H. van der, Bradford, M. A., Brinkman, E. P., Voorde, T. F. J. van de, and Veen, G. F. (2016). Where, when and how plant–soil feedback matters in a changing world. Funct. Ecol. 30, 1109–1121. doi:10.1111/1365-2435.12657.

Qin, Y., Druzhinina, I. S., Pan, X., and Yuan, Z. (2016). Microbially Mediated Plant Salt Tolerance and Microbiome-based Solutions for Saline Agriculture. Biotechnol. Adv. 34, 1245–1259. doi:10.1016/j.biotechadv.2016.08.005.

Rau, B. M., Chambers, J. C., Blank, R. R., and Johnson, D. W. (2008). Prescribed Fire, Soil, and Plants: Burn Effects and Interactions in the Central Great Basin. Rangel. Ecol. Manag. 61, 169–181. doi:10.2111/07-037.1.

Reich, P. B. (2014). The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. J. Ecol. 102, 275–301. doi:10.1111/1365-2745.12211.

Reinhart, K. O., Johnson, D., and Clay, K. (2012). Conspecific Plant-Soil Feedbacks of Temperate Tree Species in the Southern Appalachians, USA. PLoS ONE 7. doi:10.1371/journal.pone.0040680.

Revillini, D., Gehring, C. A., and Johnson, N. C. (2016). The role of locally adapted mycorrhizas and rhizobacteria in plant–soil feedback systems. Funct. Ecol. 30, 1086–1098. doi:10.1111/1365-2435.12668.

Reynolds, H. L., Packer, A., Bever, J. D., and Clay, K. (2003). Grassroots Ecology: Plant–Microbe–Soil Interactions as Drivers of Plant Community Structure and Dynamics. Ecology 84, 2281–2291. doi:10.1890/02-0298.

Rolhauser, A. G., Chaneton, E. J., and Batista, W. B. (2011). Influence of conspecific and heterospecific adults on riparian tree species establishment during encroachment of a humid palm savanna. Oecologia 167, 141–148. doi:10.1007/s00442-011-1957-5.

Rosado, B. H. P., and Mattos, E. A. de (2017). On the relative importance of CSR ecological strategies and integrative traits to explain species dominance at local scales. Funct. Ecol. 31, 1969–1974. doi:10.1111/1365-2435.12894.

Schafer, J. L., and Mack, M. C. (2010). Short-term effects of fire on soil and plant nutrients in palmetto flatwoods. Plant Soil 334, 433–447. doi:10.1007/s11104-010-0394-2.

Schittko, C., Runge, C., Strupp, M., Wolff, S., and Wurst, S. (2016). No evidence that plant–soil feedback effects of native and invasive plant species under glasshouse conditions are reflected in the field. J. Ecol. 104, 1243–1249. doi:10.1111/1365-2745.12603.

Senior, J. K., O’Reilly-Wapstra, J. M., Schweitzer, J. A., Bailey, J. K., and Potts, B. M. (2018). Forest fire may disrupt plant–microbial feedbacks. Plant Ecol. 219, 497–504. doi:10.1007/s11258-018-0811-9.

Shivega, W. G., and Aldrich-Wolfe, L. (2017). Native plants fare better against an introduced competitor with native microbes and lower nitrogen availability. AoB PLANTS 9. doi:10.1093/aobpla/plx004.

Smith-Ramesh, L. M., and Reynolds, H. L. (2017). The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. J. Veg. Sci. 28, 484–494. doi:10.1111/jvs.12519.

Spear, E. R., Coley, P. D., and Kursar, T. A. (2015). Do pathogens limit the distributions of tropical trees across a rainfall gradient? J. Ecol. 103, 165–174. doi:10.1111/1365-2745.12339.

Tilman, D. (1982). Resource Competition and Community Structure. Princeton University Press.

Tilman, D. (1990). Constraints and Tradeoffs: Toward a Predictive Theory of Competition and Succession. Oikos 58, 3–15. doi:10.2307/3565355.

Treseder, K. K., and Allen, M. F. (2002). Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytol. 155, 507–515. doi:10.1046/j.1469-8137.2002.00470.x.

Valliere, J. M., and Allen, E. B. (2016). Interactive effects of nitrogen deposition and drought-stress on plant-soil feedbacks of Artemisia californica seedlings. Plant Soil 403, 277–290. doi:10.1007/s11104-015-2776-y.

Van Der Heijden, M. G. A., Bardgett, R. D., and Van Straalen, N. M. (2018). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett., 296–310. doi:10.1111/j.1461-0248.2007.01139.x@10.1111/(ISSN)1461-0248.year-of-biodiversity.

Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. J. Stat. Softw. 36, 1–48.

Vílchez, J. I., García-Fontana, C., Román-Naranjo, D., González-López, J., and Manzanera, M. (2016). Plant Drought Tolerance Enhancement by Trehalose Production of Desiccation-Tolerant Microorganisms. Front. Microbiol. 7. doi:10.3389/fmicb.2016.01577.

Wallenda, T., and Kottke, I. 1998. Nitrogen deposition and ectomycorrhizas. New Phytologist 139:169-187.

Whalen, E. D., R. G. Smith, A. S. Grandy, and S. D. Frey. 2018. Manganese limitation as a mechanism for reduced decomposition in soils under atmospheric nitrogen deposition. Soil Biology and Biochemistry 127:252-263.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., Putten, W. H. van der, and Wall, D. H. (2004). Ecological Linkages Between Aboveground and Belowground Biota. Science 304, 1629–1633. doi:10.1126/science.1094875.

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K., and Parisien, M.-A. (2019). Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. Soil Biol. Biochem. 138, 107571. doi:10.1016/j.soilbio.2019.107571.

Wooliver, R. C., Marion, Z. H., Peterson, C. R., Potts, B. M., Senior, J. K., Bailey, J. K., et al. (2017). Phylogeny is a powerful tool for predicting plant biomass responses to nitrogen enrichment. Ecology 98, 2120–2132. doi:10.1002/ecy.1896.

Xue, W., Bezemer, T. M., and Berendse, F. (2018). Density-dependency and plant-soil feedback: former plant abundance influences competitive interactions between two grassland plant species through plant-soil feedbacks. Plant Soil 428, 441–452. doi:10.1007/s11104-018-3690-x.

Yu, L., Song, M., Lei, Y., Duan, B., Berninger, F., Korpelainen, H., et al. (2017). Effects of phosphorus availability on later stages of primary succession in Gongga Mountain glacier retreat area. Environ. Exp. Bot. 141, 103–112. doi:10.1016/j.envexpbot.2017.07.010.

Zhang, Q., Sun, Q., Koide, R. T., Peng, Z., Zhou, J., Gu, X., et al. (2014). Arbuscular Mycorrhizal Fungal Mediation of Plant-Plant Interactions in a Marshland Plant Community. Sci. World J. doi:https://doi.org/10.1155/2014/923610.

Zhao, N., Gao, S., Ren, H., Yang, X., Sun, Z., Wang, J., et al. (2018). Competition alters plant–soil feedbacks of two species in the Inner Mongolia Steppe, China. Plant Soil 429, 425–436. doi:10.1007/s11104-018-3703-9.

Zuppinger-Dingley, D., Flynn, D. F. B., Deyn, G. B. D., Petermann, J. S., and Schmid, B. (2016). Plant selection and soil legacy enhance long-term biodiversity effects. Ecology 97, 918–928. doi:10.1890/15-0599.1.

APPENDIX

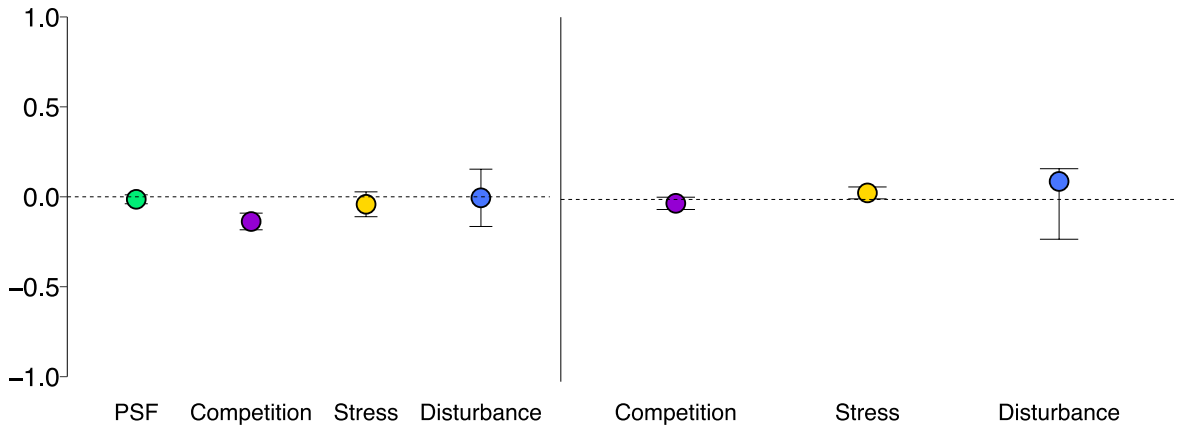

Figure 7. (a) Main effects of PSF, competition, stress, and disturbance and (b) interaction effects of PSF x competition, PSF x stress, and PSF x disturbance on plant growth. Competition main effects are averaged across alone/together and interspecific/intraspecific competition manipulations. Stress main effects are averaged across all stress manipulations (drought, fertilization, grazing/herbivory, mining, shade, temperature). Disturbance main effects are averaged across fire and tornado disturbance manipulations. PSF main effects are averaged across all soil manipulations (i.e., live/sterile, away/home, untreated/fungicide). Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

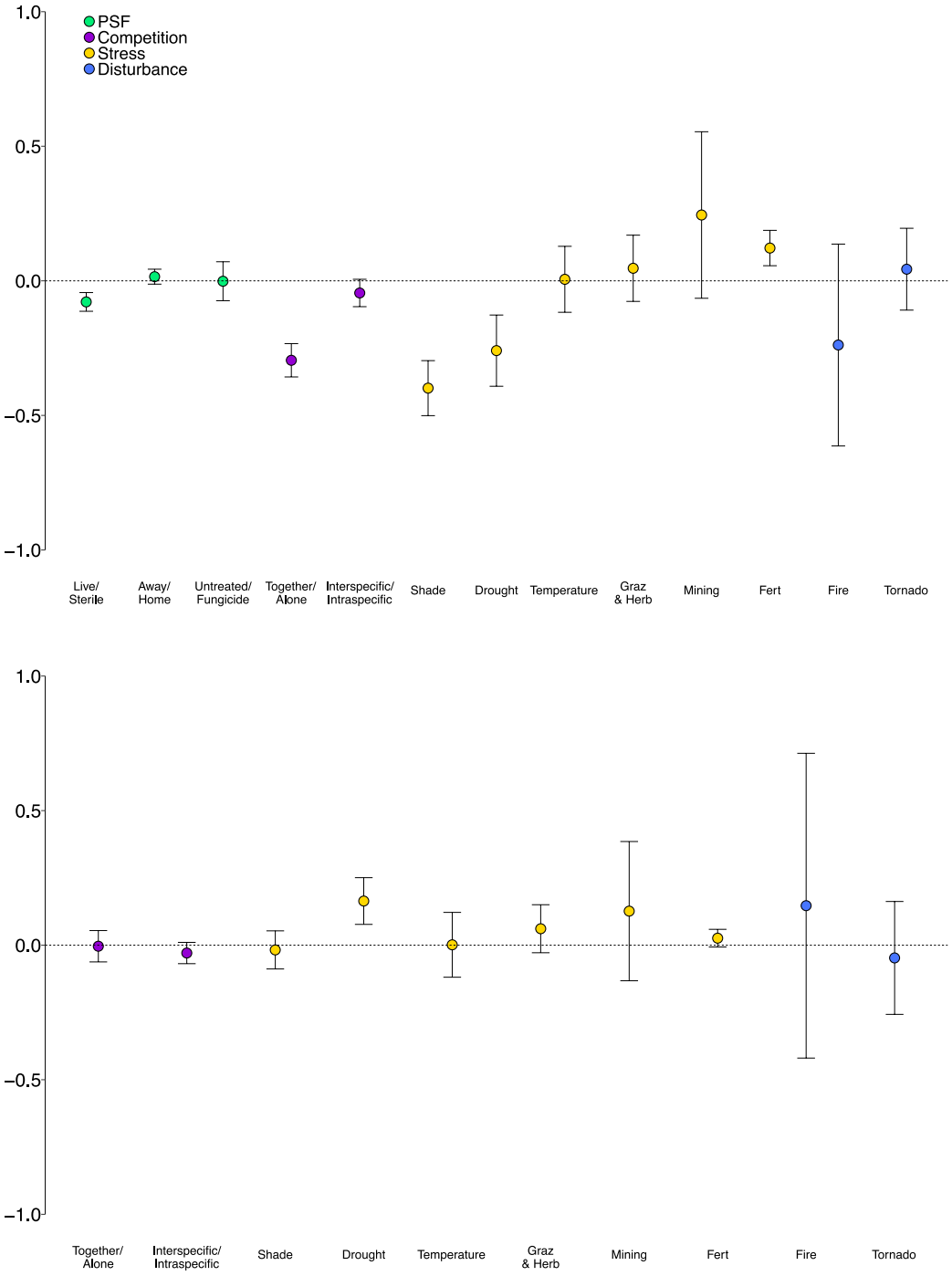

Figure 8. (a) Main effects of PSF, competition, stress, and disturbance manipulations and (b) interaction effects of PSF x competition, PSF x stress, and PSF x disturbance manipulations on plant growth, averaged across soil manipulations (live/sterile, away/home, untreated/fungicide). Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

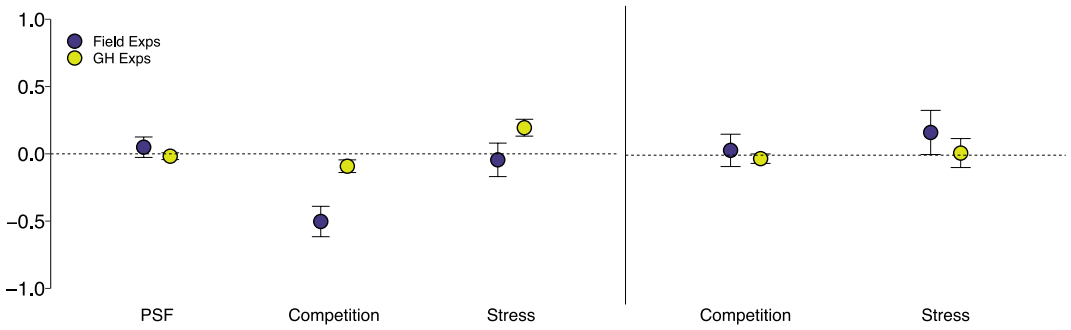

Figure 9. (a) Main effects of PSF, competition, stress, and disturbance and (b) interaction effects of PSF x competition and PSF x stress between field and greenhouse experiments. Interaction effects of PSF x competition and PSF x stress are averaged across soil manipulations (live/sterile, away/home, untreated/fungicide) and across competition and stress manipulations. Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

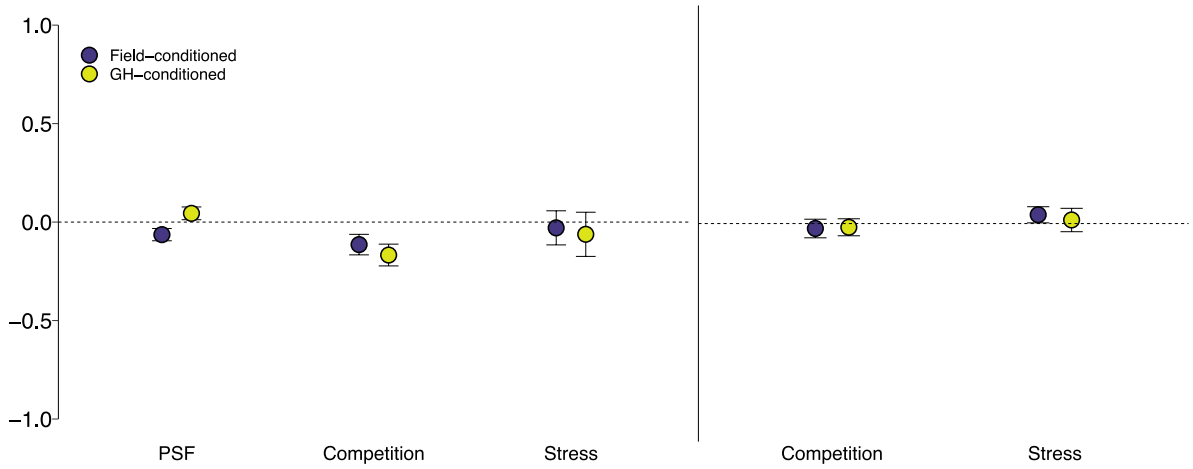

Figure 10. (a) Main effects of PSF, competition, stress, and disturbance and (b) interaction effects of PSF x competition and PSF x stress between field-conditioned and greenhouse-conditioned soil inoculum. Interaction effects of PSF x competition and PSF x stress between field-conditioned and greenhouse-conditioned soil inoculum. Interaction effects of PSF x competition and PSF x stress are averaged across soil manipulations (live/sterile, away/home, untreated/fungicide) and across competition and stress manipulations. Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

CHAPTER III: BELOWGROUND MECHANISMS FOR OAK REGENERATION: INTERACTIONS AMONG FIRE, SOIL MICROBES, AND PLANT COMMUNITY ALTER OAK SEEDLING GROWTH

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JAS designed the study. KKB and ATS conducted fieldwork. ATS conducted lab benchwork. AES led the glasshouse experiment and data collection. KKB analyzed the data and led writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

It has been firmly established that oak regeneration benefits from prescribed burning and reduced competition with fire-intolerant tree species. Despite recommendations for research on the role of the microsite environment for oak regeneration, very little is known about the interacting effects of fire, soil, and the surrounding plant community on oak establishment. We collected undisturbed and burned soil in the aftermath of a wildfire in Great Smoky Mountains National Park and used amplicon sequencing to identify differences in composition of bacterial and fungal communities between unburned and burned soils. To assess the effects of plant community, fire-induced shifts in soil microbial communities, and their interaction we conducted a glasshouse experiment and grew Quercus velutina seedlings in factorial treatments of plant neighbor (oak vs. pine seedling) and soil burn status (unburned vs. burned soil). Fire reduced the diversity of plant pathogenic and saprotrophic fungi and reduced the relative abundance of plant pathogenic fungi. Fire did not affect soil bacterial communities. Shifts in soil fungal community composition enhanced oak seedling root growth, but the effect of the soil microbiome was mediated by plant neighbor interactions. Seedling root growth was negatively correlated with diversity of pathogenic fungi. Root growth was enhanced in burned soil relative to unburned soil, but only when growing with a pine seedling neighbor as opposed to an oak seedling neighbor. Results from this study show that interactions between soil microbes and nearby plants can in part mediate oak seedling growth. As such, nuanced decisions that consider the ecological interactions of the microsite environment are needed to achieve desired outcomes for oak regeneration.

Introduction

Prescribed fire is the leading restoration tool for regenerating oak-dominated forests in the United States (Abrams, 1992; Brose et al., 2001, 2013) primarily because Quercus species have fire-adapted morphological and physiological traits (Reich & Hinckley, 1980; Abrams, 1992, 1990) and because frequent fire reduces other plant competitors that are less tolerant of fire (Wang et al., 2005; Nowacki & Abrams, 2008; Arthur et al., 2012). Prescribed burning can be implemented at different life stages of oak forest regeneration to target specific restoration outcomes (Arthur et al., 2012; Brose, 2014). For example, seedbed preparation burning is used to make the forest floor environment more hospitable for oak seedlings to establish, while release burning is used at a later growth stage to remove non-oak saplings and free young oaks from competition with mesophytic species (Brose, 2014).

While targeted prescribed burning methods such as these are beneficial, facilitating oak growth in the seedling development phase, in which oak seedlings are highly vulnerable to many environmental factors, likely requires more nuanced management practices targeted toward the forest floor environment. It is well-understood that high light availability, reduced herbivory, and reduced competition with other tree and shrub species is needed for successful oak seedling maturation (Arthur et al., 2012; Brose, 2014). What is less understood is the role of the soil environment, and specifically the interacting effects of fire, soil, and the plant community on oak seedling development, despite that recommendations for improvements to oak regeneration management have called for investigations on the role of the microsite environment (Arthur et al., 2012; Brose, 2014; Brose et al., 2013; Taylor & Midgley, 2018). For example, recent work by Taylor and Midgely (2018) on soil abiotic and biotic responses to prescribed burning in an oak-dominated forest concluded that "assessing the direct effects of burned soil on oak regeneration is necessary to disentangle other burning effects, such as increased light availability, from soil effects on plant community composition" (Taylor & Midgley, 2018).

Soil microbial communities can be altered by burning, and these changes may be critical for oak seedling establishment. Fire can alter soil microbial communities through heat sterilization (Certini, 2005), changes to soil temperature and moisture (Treseder et al., 2004), changes to soil carbon and changes to inorganic nitrogen (Certini, 2005; Peay et al., 2009; Knelman et al., 2015). Fire generally reduces soil microbial diversity and abundance (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019; Sáenz de Miera et al., 2020), but soil fungi and bacteria often

respond differently to fire. Some studies have documented greater decreases in soil fungi compared to bacteria (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019), in part because fungi are more sensitive to alkaline soils than bacteria (Bárcenas-Moreno et al., 2011; Rousk et al., 2010) and ash deposition from fire often increases soil pH (Certini, 2005; Peay et al., 2009; Knelman et al., 2015). Declines in mycorrhizal fungi may be greater than those of free-living soil fungi under certain scenarios, such as high severity fires, in which fire removes the respective plant hosts (Fultz et al., 2016).

Disentangling how the interactions between plant competitors and soil microbes affect oak seedling growth is crucial for improving oak regeneration after fire. The few studies that have examined the effects of fire on soil microbial communities in oak-dominated ecosystems have found mixed results. Wildfire reduced soil bacterial and fungal diversity in an oak-pine forest in western North Carolina (Huffman & Madritch, 2018), while prescribed burning in oak-dominated forests in Illinois resulted in increases in microbial biomass N but no changes in microbial biomass C (Taylor & Midgley, 2018). In contrast, prescribed burning conducted in an oak-dominated forest in Turkey yielded reduced microbial biomass C in burned plots (Akburak et al., 2018). Even less is known about how alterations to soil microbial communities from fire subsequently influence oak seedling development, and no research to our knowledge has examined this in conjunction with plant competition. Neighboring plants can influence how soil microbes interact with a focal plant. For instance, oaks frequently grow with pine species that are also fire dependent and some pine species are capable of transferring nutrients like nitrogen to nearby oak species through ectomycorrhizal networks (He et al., 2006).

The goal of this study was to identify the relative importance of fire-induced changes to soil microbial communities, intra- vs. interspecific plant competition, and the interaction of these two factors on oak seedling growth. We used amplicon sequencing to assess the diversity and composition of soil microbial communities collected from soil that had been burned by a wildfire in Great Smoky Mountains National Park (GSMNP) in 2016. Using this soil, we conducted a glasshouse experiment and grew black oak Quercus velutina seedlings in factorial treatments of soil inoculum (unburned vs. burned) and plant neighbor (black oak seedling vs. loblolly pine Pinus taeda seedling). Based on trends in the literature, we hypothesized that burned soils would harbor reduced diversity and abundance of bacteria and fungi relative to unburned soils (Hypothesis 1). We then hypothesized that reductions in soil microbial diversity and/or abundance caused by fire

would influence oak seedling growth in one of two outcomes: either low microbial diversity and/or abundance in burned soil would enhance seedling growth or it would inhibit seedling growth (Hypothesis 2). Lastly, we hypothesized that oak seedling growth response to fire-induced changes to the soil microbiome would be influenced by the nearby plant community (Hypothesis 3). Based on personal observations that severely burned sites in our study system contained very few oak seedlings but many pine seedlings, we suspected that oak seedlings were unable to successfully colonize burned sites because they were outcompeted by pine seedlings. From these hypotheses and personal field observations, we predicted two possible outcomes in our glasshouse experiment (Figure 11; all tables and figures are in Appendix 3 at the end of the chapter). If low microbial diversity and/or abundance of burned soil inhibits oak seedling growth, we expected oak seedling growth to be greatest when grown in unburned soil with an oak neighbor, followed by burned soil with an oak neighbor or unburned soil with a pine neighbor, and growth to be lowest when grown in burned soil with a pine neighbor. If low microbial diversity and/or abundance of burned soil enhances oak seedling growth, we expected oak seedling growth to be greatest when grown in burned soil with an oak neighbor, followed by unburned soil with an oak neighbor or burned soil with a pine neighbor, and growth to be lowest when grown in unburned soil with a pine neighbor.

Materials and Methods

Study system

Field soil used in this study was collected from areas impacted by the Chimney Tops 2 (CT2) wildfire that occurred in the Great Smoky Mountains National Park (GSMNP) and in Gatlinburg, TN in November and December of 2016. The fire created a patchy gradient of burn severity within the fire perimeter in which some areas were unburned, some were low-moderately burned, and some areas were severely burned. To establish soil treatments representative of unburned and burned soils, we used a GIS fire severity raster map with a 30 m resolution generated by the US Forest Service Remote Sensing Application Center to randomly select candidate field sites that were severely burned and unburned. We chose to use severely burned sites to represent "burned" sites and we excluded low-moderately burned sites because they varied greatly in vegetation cover post-fire upon visual inspection. Soils in GSMNP are mostly loamy soils with a mixture of channery soil and fine sandy loam (Brown et al., 2019). We verified this by gathering soil texture classification from the Natural Resources Conservation Service (NRCS) Web Soil

Survey database using the coordinates of each site (Table 3). We identified three 90 m^2 blocks within each burn status classification that were located within 300 m from roads and park trails for ease of accessibility for a total of seven sites (3 unburned sites and 4 burned sites). Because three of the burned sites were located in close proximity to one another, we included an additional burned site that was geographically distinct from the others. Sites were verified for differences in burn damage using the satellite-derived normalized difference vegetation index (NDVI) which quantifies the density of plant growth in a given area by measuring the intensity of light reflected by vegetation (Cihlar et al., 1991). To account for variation in canopy cover across sites before the Chimney Tops 2 fire, we calculated the delta NDVI (dNDVI) for each site, which is the difference in NDVI before and after the wildfire. We did this to accurately quantify only the difference in canopy cover due to damage from the wildfire. While we were not able to acquire ground measurements of burn, it has been shown that satellite-derived metrics of burn strongly correlate with ground measurements of burn (Cocke et al., 2005). Significant difference in dNDVI between the unburned and burned sites confirmed that the burned sites were in fact significantly damaged from the wildfire relative to the unburned sites ($\chi^2 = 36.3$, $p < 0.0001$) (Table 3).

Soil collection and processing

To assess changes to microbial communities in unburned and burned sites, we collected soil from each site in May 2019, approximately 2.5 years post-fire. Soils were collected by sampling from 10 random points within each site using a trowel to collect the top ~15 cm of mineral soil. Trowels were cleaned with ethanol between each site to reduce the possibility of microbial cross contamination. Following field collection, soil pH of each site was measured on air-dried soils in 0.01 M of $CaCl_2$ in the laboratory. An additional 2 g subsample of soil from each site was stored at -80°C for assessment of microbial DNA. We used the Qiagen DNEasy PowerSoil DNA Isolation kit (Qiagen, Venlo, Netherlands) to extract DNA from 0.25 g of soil. DNA was eluted in 50 ul of buffer, and DNA concentration and quality were measured on a NanoDrop 2000 spectrophotometer (Thermo Scientific). Libraries were prepped using a two-step Polymerase Chain Reaction (PCR) approach with a mixture of 341F and 785R Illumina primers (Klindworth et al., 2013) to characterize bacterial 16S gene regions, and a mixture of 5.8S-FUN and ITS4-FUN primers to characterize fungal ITS2 gene regions (Taylor et al., 2016). Illumina-specific adapters were added to each forward and reverse primer to make them compatible with Illumina Nextera

XT indexes. PCR for 16S rRNA and ITS2 were performed separately. The initial PCR consisted of 2x KAPA HiFi HotStart ReadyMix Taq (Roche, Indianapolis, Indiana, USA), 1.5 um of each forward and reverse primer per sample, and 2.5 ul DNA. A negative control of only nuclease-free water was included in the PCR and sequencing. The initial 16S PCR consisted of 3 min at 95°C, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 sec, with a final extension at 72°C for 5 min. The initial ITS2 PCR consisted of 2 min at 96°C, followed by 30 cycles of 94°C for 30 s, 58°C for 40 s, 72°C for 2 min, with a final extension at 72°C for 10 min. Successful PCR amplification was confirmed by running 5 ul of PCR product alongside 5 ul of DNA ladder (GeneRuler 1kb Plus DNA ladder 0.1 ug/ul, Thermo Scientific) on a 1.5% agarose gel. The PCR product was purified with AMPure XP beads (Agencourt, Beverly, Massachusetts, USA). Nextera XT indexes were then ligated to the PCR products with a second PCR of 95°C for 3 min, followed by 8 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. The PCR products were purified again using AMPure XP beads. Samples were quantified on a NanoDrop 2000 spectrophotometer. Final PCR product size and concentration were confirmed on a Bioanalyzer. Amplicon sequencing was performed on the Illumina MiSeq platform at the University of Tennessee Genomics Core facility (Knoxville, TN, U.S.), using a 4 pM amplicon concentration including a 20% PhiX spike loaded onto a v3 600-cycle flow cell set for a paired-end read of 275 bases each.

All microbial genomic processing including primer removal was completed using a DADA2 pipeline (Callahan et al., 2016). Due to the variation in sequencing depth among samples, samples were normalized with a variance stabilizing transformation with the DESeq2 package (Love et al., 2014). We chose this method over the common practice of rarefaction because rarefaction results in loss of data by using the lowest sampling depth and it inflates variances across samples (McMurdie & Holmes, 2014). Taxonomy of amplicon sequence variants (ASVs) was assigned using the RDP (Q. Wang et al., 2007) and UNITE (Abarenkov et al., 2010) databases for bacteria and fungi, respectively. After processing the fungal sequences into ASVs, we assigned ASVs to a fungal functional guild using the FUNGuild database of fungal taxa with known or suspected ecological functions (Nguyen et al., 2016). All fungal guilds were assigned at the genus level. We refined taxa to one of seven functional guilds: arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi, endophytic fungi, plant pathogenic fungi, saprotrophic fungi, and “other” (including animal pathogens, fungal parasites, epiphytes, lichen-

forming, dung-inhabiting, etc.). For analysis we only included FUNGuild assignments with a confidence score of "probable" or "highly probable."

Glasshouse experiment

To address hypotheses that oak seedling growth is mediated by both fire-induced changes to the soil microbial communities, type of plant competition, and the interaction between these two factors (Hypotheses 2 and 3; Figure 11), we conducted a reciprocal glasshouse experiment with factorial soil burn status x plant neighbor treatments. We chose to use Q. velutina and P. taeda for this study because both species occur in GSMNP at low-mid elevation (260-760 m. above sea level), which is similar elevation range to the sites included in this study and because both species had high seed availability from a commercial vendor. Acorns of Q. velutina and seeds of Pinus taeda were purchased from Sheffield's Seed Co. (Locke NY), and refrigerated at 4ºC prior to sowing. Acorns and seeds were sown into a commercial peat moss-based, non-mycorrhizal potting mix (Premier Promix BX, containing perlite, vermiculite, and limestone). Once emerged, oak and pine seedlings were transplanted into half gallon pots into eight treatments which consisted of factorial combinations of soil burn status (unburned soil, burned soil) and plant neighbor (oak or pine). Each pot was filled with a 5:1 ratio of Premier Promix BX potting mix:play sand and inoculated with 5.5 tablespoons of field soil (3% of total soil volume). We used a small amount of field soil to reduce any effects of variation in soil nutrients between inocula treatments, a common practice in soil inoculation experiments (Troelstra et al., 2001). Although the amount of field soil inoculum is very small relative to the volume of potting mix, it has been repeatedly shown that a small amount of microbial inoculum can induce plant responses (Lau & Lennon, 2011, 2012; Panke-Buisse et al., 2015). To confirm the effect of a field soil microbiome on plant function, we also included a control treatment in which pots contained only potting mix and no field soil. In total, 60 pots were established ([3 unburned field sites x 2 plant neighbor treatments x 3 replicates] + [4 burned field sites x 2 plant neighbor treatments x 3 replicates] + [2 plant neighbor treatments x 9 potting soil only controls]). To avoid positional effects, pots were randomly positioned in the glasshouse. Plants were equally watered from above, as needed (approximately 4 days/week), and allowed to grow for 4 months in a glasshouse at the University of Tennessee, Knoxville, TN, U.S.A.

A suite of plant phenotypes was measured throughout the duration of the experiment and post-experiment. Stem height was measured every two weeks and relative growth rates were calculated from these data. Prior to termination of the experiment, two terminal, mature leaves were randomly selected per oak plant, scanned using WinFOLIA software (Regent Instruments Inc.), oven-dried at 70°C for 72 hours (Pérez-Harguindeguy et al., 2016), and weighed to calculate specific leaf area (SLA; cm^2/g). In addition, we measured leaf chlorophyll content of one randomly selected mature top leaf for each oak using a CCM-300 chlorophyll content meter (Opti-Sciences). At 17 weeks, each individual was harvested and separated into above- and belowground biomass. Above- and belowground tissue was weighed after 48 hours of oven-drying at 60°C. Prior to drying, roots were carefully rinsed over 2 and 0.5 mm sieves to remove soil and collect all fine roots. We also measured average specific root length (SRL) for each individual using a digital scanner and WinRhizo software (Regent Instruments Inc.) prior to drying. Root to shoot ratio was calculated by dividing each individual's belowground biomass by aboveground biomass.

Statistical Analyses

To test Hypothesis 1 (Figure 11) that fire reduces soil microbial diversity, we calculated hill numbers based on ASV counts using the hill_div function in the hilldiv package (Alberdi & Gilbert, 2019). Hill numbers serve as effective numbers of diversity that provide more intuitive estimates of diversity compared to traditional diversity indices based on entropy (Chao et al., 2014). This method allows for separate estimates of raw richness and diversity that accounts for species evenness. We calculated hill numbers for all orders of diversity at $q = 0$, $q = 1$, and $q = 2$. A diversity order $q = 0$ provides raw richness by weighting rare taxa the same as abundant taxa. A diversity order $q = 1$ weights ASVs by their frequency without disproportionately favoring either rare or abundant taxa. A diversity order $q = 2$ overweighs abundant ASVs. For each order of q, we used the div_test function in the hilldiv package to test for differences in bacterial and fungal diversity between unburned and burned soils. We also assessed diversity within each fungal guild by further parsing the fungal ASV dataset into separate fungal guild datasets. We then calculated the relative abundance of each fungal guild among unburned and burned soils by dividing the sum of ASV counts of each fungal guild for each site by the total number of all ASV counts for each field site. We built linear models for each fungal guild using the lm function in the stats package in base R. We specified burn status as the fixed effect. We used the Anova function in the car

package (Fox et al., 2013) to calculate analysis of variance (ANOVA) tables using Type II sums of squares. When necessary, relative abundance was logit transformed to conform to normality prior to running models.

To test Hypotheses 2 and 3 (Figure 11) that oak seedling growth is influenced by fire-induced changes to soil microbial communities, type of plant competition, and the interaction of these two factors, we used the lmer function in the lme4 package (Chao et al., 2014) to build linear mixed effects models with soil burn status, plant neighbor, and soil burn status x plant neighbor as fixed effects and soil inoculum field site as a random effect. For all analyses, separate models were built for each of the six oak seedling phenotypes (relative growth rate, shoot biomass, root biomass, SRL, SLA, leaf chlorophyll content). When necessary, phenotype data was transformed to conform to normality before analysis. For all models, we used the Anova function in the car package (Fox et al., 2013) to calculate analysis of variance (ANOVA) tables using Type II sums of squares, with significance assessed for each fixed effect using Wald X^2 statistics. For phenotypes in which the effect of soil burn status was significant we then built separate linear mixed effects models to specifically test if differences in overall microbial diversity, fungal guild diversity, and relative abundance of fungal guilds influenced oak seedling phenotypes. For the diversity models, we included hill number at each order of diversity as the fixed effect and plant neighbor and soil inoculum field site as random effects. Separate models were built for each order of diversity. For the relative abundance models, we included fungal guild proportion (%) as the fixed effect and plant neighbor and soil inoculum field site as random effects. When significant, we calculated model fit (R^2) with the r.squaredGLMM function in the MuMIn package (Nakagawa & Schielzeth, 2013). If the effect of soil burn status x plant neighbor was significant, we conducted post hoc Tukey contrasts using the TukeyHSD function. All analyses were performed in R (R Team, 2020). Boxplots were made with the ggplot2 package (Wickham, 2016). Ordination figures were made with the phyloseq package (McMurdie & Holmes, 2013). Multi-panel figures were compiled with the patchwork package (Pedersen 2020).

Results

Overall, we found support for two of our three hypotheses. Fire reduced species diversity of some components of the soil microbiome, and these microbial responses were associated with

differential oak seedling growth, but only when oak seedlings were grown with a heterospecific seedling neighbor.

Sequencing output and fungal community composition

After processing, we had 2,912 ASVs of the total 134,465 bacterial sequencing reads and 1,657 ASVs of the total 190,297 fungal sequencing reads. Of the 1,657 fungal ASVs, 842 were assigned to a fungal guild, and the rest were unassigned. Of those assigned to a guild, we used the 616 ASVs that had a confidence ranking of "highly probable" or "probable." The majority of ASVs were assigned as saprotrophic fungi (65%), followed by "Other" (11.5%), ectomycorrhizal fungi (6.7%), arbuscular mycorrhizal fungi (6.2%), plant pathogenic fungi (6%), endophytic fungi (4.1%), and ericoid mycorrhizal fungi (0.5%). We excluded the ASVs assigned as ericoid mycorrhizal fungi and "Other" from further analysis because the dataset only contained 3 ericoid ASVs and the "Other" category was comprised of fungi that could not be grouped with any of the other six guilds and were thus not applicable to our system.

Soil microbiome response to wildfire

Soil microbial diversity was reduced in burned soils relative to unburned soils. However, this was only true for fungal communities and not for bacterial communities 2.5 years post-fire, providing partial support for Hypothesis 1. Unburned soils contained nearly three times as many fungal ASVs on average as burned soils at order of diversity q0 ($t = -5.79$, $p = 0.01$) (Table 4, Figure 12b). Contrary to the majority of findings, burned sites in this study were lower in soil pH relative to unburned soils ($\chi^2 = 26.03$, $p < 0.0001$) (Table 3). As such, sensitivity of soil fungi to wildfire in these sites is likely due to other edaphic factors rather than soil pH. The overall fungal diversity did not significantly differ at orders of diversity q1 or q2 (Table 4, Figure 12b), indicating that rare fungal taxa likely drove differences in diversity caused by fire. Bacterial diversity did not significantly differ between unburned and burned soils at any order of diversity (Table 4, Figure 12a). Among the six fungal guilds, unburned and burned soils differed in the diversity of plant pathogenic and saprotrophic fungi. Unburned soil contained 2.4 times greater diversity of plant pathogenic fungi and 2.2 times greater diversity of saprotrophic fungi than burned soil at q0 (Table 4, Figure 12c). Unburned soil also contained 2.6 and 2.4 times greater diversity of plant pathogenic fungi than burned soil at q1 and q2, respectively (Table 4, Figure 12c). Diversity of saprotrophic

fungi did not differ between unburned and burned soils at q1 or q2 (Table 4). Additionally, unburned soil harbored 2.5 times greater relative abundance of plant pathogenic fungi than burned soil (Table 5, Figure 12d). The proportion of saprotrophic fungi was similar among unburned and burned soil (Table 5). Diversity and relative abundance of arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and endophytic fungi were similar among unburned and burned soils (Table 5).

Effect of fire-induced changes to soil microbial communities on oak seedling success

While oak seedling growth did respond to soil burn status, oak growth was enhanced in burned soil relative to unburned soil, contrary to our hypothesis (Hyp 2). Seedlings produced 25% more root biomass when grown in soils containing burned soil inoculum compared to soils containing unburned soil inoculum ($\chi^2 = 4.10$, $p = 0.04$; Table 7, Figure 13a). Differences in overall fungal diversity caused by burn did not significantly affect oak seedling root biomass ($\chi^2 = 1.79$, $p = 0.18$). However, root biomass was significantly correlated with fire-induced changes to diversity of plant pathogenic fungi. Oak seedling root biomass was negatively correlated with plant pathogenic fungi at diversity q1 ($\chi^2 = 3.95$, $p = 0.05$; $R^2 = 0.11$) and q2 ($\chi^2 = 5.64$, $p = 0.02$; $R^2 = 0.13$; Table 6; Figure 13c, d), but not correlated with relative abundance of plant pathogenic fungi (Table 6; Figure 13b). Diversity q0 of saprotrophic fungi was not correlated with oak seedling root biomass (Table 6). Shoot biomass, relative growth rate, specific root length, specific leaf area, and leaf chlorophyll content were not affected by soil burn status (Table 7).

Interactive effect of soil microbiome and plant neighbor on oak seedling success

In support of Hypothesis 3, soil burn status and plant neighbor had a slight interactive effect on oak seedling growth ($\chi^2 = 3.43$, $p = 0.06$; Table 7). The benefit of a burned-associated soil microbiome on oak root biomass was only present when oak seedlings were growing with pines. Oak seedlings grown in soils containing burned soil inoculum produced 39% more roots when growing with pine seedlings compared to growing with other oak seedlings (Figure 14c). The individual effect of plant neighbor was also significant. Across all soil inocula treatments, seedlings produced vegetative biomass more than twice as quickly ($\chi^2 = 5.63$, $p = 0.02$; Table 7, Figure 14a) and produced 20% more root biomass ($\chi^2 = 7.06$, $p = 0.008$; Table 7, Figure 14b) when grown with a pine seedling compared to another oak seedling. The significant interactive effect on

root biomass shows that belowground growth was driven by the combination of a pine neighbor and fire-induced changes to the soil fungal community (Figure 14c).

Discussion

Fire alters community composition of soil fungi rather than soil bacteria

We found that fire reduced soil fungal diversity, and that bacterial diversity was largely unaffected by fire. Since burned sites in this study were more acidic than unburned sites, it is likely that reductions in soil fungal diversity in this system are not due to soil pH. Although we cannot pinpoint the specific causes of soil fungal sensitivity to wildfire in this system, soil heating and drying caused by fire can negatively impact soil fungal communities because fungi in general are more heat-sensitive than bacteria (Bárcenas-Moreno & Bååth, 2009). Our overall findings, however, of greater reductions in soil fungi relative to soil bacteria in response to fire are consistent with other studies (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019). In our study, differences in overall fungal diversity are likely driven by rare taxa because unburned and burned soils only differed in diversity at order q0, and not at q1 or q2. This suggests that unburned soil has a greater number of rare fungal species compared to burned soil. A previous study that examined response of soil microbial communities to the CT2 wildfire also found that fungal richness was reduced in severely burned sites relative to unburned sites, but did not find changes in fungal species evenness with fire (Brown et al., 2019). As soil microbiome diversity is primarily comprised of rare taxa (Elshahed et al., 2008) our finding of only significant differences in number of rare species is not uncommon.

More specifically, we found that fire reduced the diversity of plant pathogenic fungi. Burned soil had significantly fewer fungal pathogen taxa than unburned soil. This was true for both rare and abundant taxa as unburned and burned soils differed for all diversity orders of q. Fire also affected the proportion of plant pathogenic fungi as the relative abundance was 2.5-fold greater in unburned soil relative to burned soil. While Brown et al. (2019) did not find differences in the relative abundance of plant pathogens between unburned and burned sites, their findings reflect soil fungal responses within six months after the CT2 wildfire whereas here we show fungal responses 2.5 years after the wildfire. We found that fire also reduced the diversity of saprotrophic fungi, but only by reducing the number of rare taxa. However, fire did not affect the proportion of saprotrophic fungi as the relative abundance of saprotrophic fungi was similar among unburned

and burned soils. Other studies have found reductions in saprotrophic fungi after fire (Kaye et al., 2005; Brown et al., 2019; Day et al., 2019). Our finding that fire reduced saprotrophic raw richness (q0), but not species' evenness (q1 and 2) is similar to a previous study that found a decline in saprotroph richness with increasing burn severity, but no effect of fire on saprotroph species evenness (Day et al., 2019). Removal of organic carbon in upper layers of soil after fire and subsequent losses of lignin-rich litter may cause reductions in saprotrophs (Kaye et al., 2005). Overall losses of litter may correspond to lower litter diversity and a subsequent decrease in saprotroph species diversity.

In our study, fire did not affect mycorrhizal communities, as the diversity and relative abundance of both arbuscular- and ectomycorrhizal fungi were similar among unburned and burned soils. Our findings contradict a previous study that found reductions in ectomycorrhizal fungi from the CT2 fire (Brown et al., 2019). It is important to note, however, that these differences again may be due to time since the fire as the soil used in our study was sampled 2.5 years after the CT2 fire whereas the previous study sampled soil less than six months after the fire. Although a recent meta-analysis found that fire overall reduces richness of mycorrhizal fungi (Dove & Hart, 2017), a lack of response to fire in mycorrhizal fungi has been documented elsewhere, in a Canadian boreal forest system (Whitman et al., 2019).

Fire-induced changes to soil fungal diversity alter oak seedling growth

In our glasshouse experiment we found that reductions in diversity of soil fungi caused by fire likely altered oak seedling growth. Seedlings grown in burned soil inocula produced significantly more root biomass than those grown in unburned soil inocula, and root biomass was negatively correlated with diversity of plant pathogenic fungi. Although saprotroph richness (q0) was reduced in burned soil and saprotrophs comprised over half of the fungal community compared to plant pathogenic fungi which only accounted for 6% of the fungal community, plant pathogen diversity was more important for oak seedling root growth than saprotroph diversity.

Specifically, increases in seedling root biomass in burned soil inocula relative to unburned soil inocula were likely driven by the abundant plant pathogen species rather than the rare species as significant correlations between plant pathogen diversity and root biomass were found at diversity orders q1 and q2 which account for species' evenness, but not at q0 which estimates raw richness. Plant pathogen relative abundance, however, was not correlated with seedling root

biomass. These findings suggest that seedling root growth likely benefits from the lack of particular abundant plant pathogen species rather than the total number of plant pathogen individuals in the soil. These results are concurrent with the well-documented ecological phenomenon of enemy release from soil biota (Agrawal et al., 2005; Mangan et al., 2010; Comita et al., 2014).

Interestingly, a previous study of a similar experimental design found the near opposite outcome. Using soil and alder and spruce seeds collected from arctic tundra in Alaska, Hewitt and colleagues (2016) found a higher proportion of plant pathogenic fungi with increasing soil burn severity and found that tree seedling biomass declined when inoculated with burned soil inocula in a growth chamber experiment (Hewitt et al., 2016). While it is unclear why the abundance of plant pathogens is reduced by fire in one system and enhanced by fire in another, it is evident that tree seedling growth is inhibited in part by pathogenic fungi. Furthermore, our findings and those of Hewitt et al. (2016) show that fire can indirectly affect tree seedling success via soil microbial communities.

Fire and soil fungi effects on oak seedling growth influenced by plant species interactions

Our finding that oak seedlings produced more root biomass and produced aboveground biomass faster when growing with a pine seedling rather than another oak seedling contradicts our hypothesis (Hyp 2) that pine seedlings hinder oak seedling growth. This finding is, however, consistent with root resource partitioning. Belowground niche separation is a common mechanism for plant species coexistence. Vertical resource partitioning has been most notably demonstrated in savanna ecosystems with trees and grasses and is known as the two-layer hypothesis in which plant species with shallow root systems, such as grasses, take advantage of resources in the topsoil and plant species with deep roots such as trees primarily access resources in the subsoil (Ward et al., 2013). Oaks and pines also exhibit this ecological interaction, largely because of specialization in different water management strategies. Oaks are anisohydric and keep their stomata open which allows for high leaf gas exchange and the ability to photosynthesize under a wide range of moisture conditions (Poulos et al., 2020). Oaks also have a deep root system that compensates for this "riskier" water management strategy by allowing access to water in the deep soil layers (Williams & Snyder, 2003). Pines, in contrast, are isohydric and tightly regulate their stomata, which allows for drought avoidance, but an inability to photosynthesize and grow under low moisture conditions

(Poulos et al., 2020). Pines have a shallow root system that allows access to water in the upper soil layers (Williams & Snyder, 2003). While we were not able to quantify and compare root niche space among oak and pine seedlings, we visually noticed stark differences in root structure between the oak and pine seedlings while harvesting plant tissue from the glasshouse experiment. All oak seedlings had a long taproot and pine seedlings had a much shallower root system. We did not measure soil moisture in the glasshouse experiment, but our findings likely indicate soil moisture partitioning as oak seedlings that were grown with a pine seedling neighbor were likely better able to mine the deeper soil layers compared to seedlings that were competing for the same soil space with another oak seedling. On the landscape, neighboring pine seedlings may benefit oak seedling establishment due to root niche partitioning. Other belowground interactions between oaks and pines may also facilitate oak success. For example, some research has found that some pine species are capable of transferring nutrients like nitrogen to nearby oak species through ectomycorrhizal networks (Poulos et al., 2020).

Lastly, we found that the effects of soil pathogenic fungi on oak seedling growth were mediated by the plant neighbor interactions. The soil enemy release advantage was only present when oak seedlings were growing with a pine neighbor. Similarly, the advantage of growing with a pine neighbor only occurred in soil containing burned soil inocula with reduced fungal pathogen diversity. Our finding contradicts our hypothesis (Hyp 3) that oak seedling growth would differ between all soil burn status and plant community treatment combinations, when in fact treatments of oak neighbor-unburned soil inoculum, oak neighbor-burned soil inoculum, and pine neighbor-unburned soil inoculum all yielded similarly low root biomass compared to the combination of pine neighbor and burned soil inoculum.

These findings could be caused by multiple belowground interactions. It is possible that spatial separation of roots between seedlings somehow further enhances the benefit of the low-pathogen soil inoculum. Although our study did not test specific mechanisms like this, other research has found associations between root architecture and soil microbes. A study by Ulbrich et al. found that the relative abundance of certain fungal taxa was correlated to root length (Ulbrich et al., 2021). It is also possible that oak seedlings benefit from the combination of the low-pathogen inoculum and a pine-associated microbial community. It is well-established that soil microbial communities vary among plant species. As such, oak seedlings may benefit from pine-associated microbial communities that likely contain fewer oak-specific microbial pathogens. While our

study is limited in mechanistic inference, other research has found benefits from the combination of interspecific plant competition and soil microbial communities. A recent study by Fitzpatrick et al. (2019), for example, documented an increase in plant fitness-related traits when a focal plant was undergoing interspecific competition and in contact with a soil microbiome.

Conclusions and management implications

This study shows that successful oak regeneration on the landscape may require understanding the effects of ground-level ecological interactions in addition to the direct impact of fire on oak seedling establishment and growth. Despite thoroughly documented evidence for the importance of soil microbes on plant growth and for the effect of fire on soil microbial communities, there has been limited research investigating the role of fire on plant-soil interactions (Senior et al., 2018). This is the first study, to our knowledge, to examine the individual and interactive effects of fire, soil microbial communities, and plant species interactions on oak restoration. Results from this study enhance our understanding of some of the belowground mechanisms for oak seedling success post-fire. Here we show that not all microbial groups respond equally to fire, with pathogenic and saprotrophic fungi showing the most sensitivity in these sites. We show that fire may alleviate oak seedlings from many interactions with soil enemies by reducing the abundance of particularly harmful pathogenic fungi. Moreover, this release from harmful soil biota is facilitated under root niche partitioning scenarios in which oak seedlings are growing in proximity with plants that have different root architectures.

Expanding on previous findings that prescribed burning in oak-dominated forests can alter soil nutrients (Taylor & Midgley, 2018), this study shows that fire effects on soil biotic conditions may also affect trajectories of oak regeneration. Additionally, our findings that interactions between soil microbes and the surrounding plant community can interact to alter oak seedling growth emphasize that nuanced decisions are needed to achieve desired outcomes for oak regeneration depending on the context of the microsite environment (Arthur et al., 2012; Brose, 2014; Brose et al., 2013; Taylor & Midgley, 2018). Specifically, oak seedling success may benefit in scenarios in which prescribed burning reduces the soil pathogen load or may be hindered if burning increases soil pathogens, as has been documented previously (Hewitt et al., 2016). While reducing competition with mesophytic plant species is important, oak seedlings may also benefit from growing in a heterogenous plant community that facilitates a diversity of rooting strategies.

It is unknown how a glasshouse experiment like this one translates to the field. Further field studies are needed to directly test if these relationships are upheld on the landscape and to determine how site-specific environmental variation may influence interactions between soil microbial communities and plant neighbors and their subsequent effects on oak seedling growth. For example, while our study only examined the presence or absence of fire, soil microbial response will likely vary with burn severity, intensity, and frequency (Taylor & Midgley, 2018). This study underscores that fire is not a one-size-fits-all solution for oak regeneration. Accurate management of oak seedling establishment will also require consideration of the ecological interactions of the microsite environment, including those among soil microbial communities and the surrounding plant community.

REFERENCES

Abarenkov, K., Nilsson, R. H., Larsson, K.-H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U., & Kõljalg, U. (2010). The UNITE database for molecular identification of fungi – recent updates and future perspectives. New Phytologist, 186(2), 281–285. https://doi.org/10.1111/j.1469-8137.2009.03160.x

Abrams, M. D. (1990). Adaptations and responses to drought in Quercus species of North America. Tree Physiology, 7(1-2-3–4), 227–238. https://doi.org/10.1093/treephys/7.1-2-3-4.227

Abrams, M. D. (1992). Fire and the Development of Oak Forests. BioScience, 42(5), 346–353. https://doi.org/10.2307/1311781

Agrawal, A. A., Kotanen, P. M., Mitchell, C. E., Power, A. G., Godsoe, W., & Klironomos, J. (2005). Enemy Release? An Experiment with Congeneric Plant Pairs and Diverse Above- and Belowground Enemies. Ecology, 86(11), 2979–2989. https://doi.org/10.1890/05-0219

Akburak, S., Son, Y., Makineci, E., & Çakir, M. (2018). Impacts of low-intensity prescribed fire on microbial and chemical soil properties in a Quercus frainetto forest. Journal of Forestry Research, 29(3), 687–696. https://doi.org/10.1007/s11676-017-0486-4

Alberdi, A., & Gilbert, M. T. P. (2019). hilldiv: An R package for the integral analysis of diversity based on Hill numbers. BioRxiv, 545665. https://doi.org/10.1101/545665

Arthur, M. A., Alexander, H. D., Dey, D. C., Schweitzer, C. J., & Loftis, D. L. (2012). Refining the Oak-Fire Hypothesis for Management of Oak-Dominated Forests of the Eastern United States. Journal of Forestry, 110(5), 257–266. https://doi.org/10.5849/jof.11-080

Bárcenas-Moreno, G., & Bååth, E. (2009). Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. Soil Biology and Biochemistry, 41(12), 2517–2526. https://doi.org/10.1016/j.soilbio.2009.09.010

Bárcenas-Moreno, G., García-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., & Bååth, E. (2011). Soil microbial recolonisation after a fire in a Mediterranean forest. Biology and Fertility of Soils, 47(3), 261–272. https://doi.org/10.1007/s00374-010-0532-2

Brose, P. (2014). Development of Prescribed Fire as a Silvicultural Tool for the Upland Oak Forests of the Eastern United States. Journal of Forestry, 112(5), 525–533. https://doi.org/10.5849/jof.13-088

Brose, P., Dey, D. C., Phillips, R. J., & Waldrop, T. A. (2013). A Meta-Analysis of the Fire-Oak Hypothesis: Does Prescribed Burning Promote Oak Reproduction in Eastern North America? Forest Science, 59(3), 322–334. https://doi.org/10.5849/forsci.12-039

Brose, P., Schuler, T., van Lear, D., & Berst, J. (2001). Bringing Fire Back: The Changing Regimes of the Appalachian Mixed-Oak Forests. Journal of Forestry, 99(11), 30–35. https://doi.org/10.1093/jof/99.11.30

Brown, S. P., Veach, A. M., Horton, J. L., Ford, E., Jumpponen, A., & Baird, R. (2019). Context dependent fungal and bacterial soil community shifts in response to recent wildfires in the Southern Appalachian Mountains. Forest Ecology and Management, 451, 117520. https://doi.org/10.1016/j.foreco.2019.117520

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

Certini, G. (2005). Effects of fire on properties of forest soils: A review.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., & Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. Ecological Monographs, 84(1), 45–67. https://doi.org/10.1890/13-0133.1

Cihlar, J., St.-Laurent, L., & Dyer, J. A. (1991). Relation between the normalized difference vegetation index and ecological variables. Remote Sensing of Environment, 35(2), 279–298. https://doi.org/10.1016/0034-4257(91)90018-2

Cocke, A., Fulé, P., & Crouse, J. (2005). Comparison of burn severity using Difference Normalized Burn Ratio and ground data. International Journal of Wildland Fire, 14, 189–198.

Comita, L. S., Queenborough, S. A., Murphy, S. J., Eck, J. L., Xu, K., Krishnadas, M., Beckman, N., & Zhu, Y. (2014). Testing predictions of the Janzen–Connell hypothesis: A meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. Journal of Ecology, 845–856. https://doi.org/10.1111/1365-2745.12232@10.1111/(ISSN)1365-2745.Plant-pathogensJEC2018

Day, N. J., Dunfield, K. E., Johnstone, J. F., Mack, M. C., Turetsky, M. R., Walker, X. J., White, A. L., & Baltzer, J. L. (2019). Wildfire severity reduces richness and alters composition of soil fungal communities in boreal forests of western Canada. Global Change Biology, 25(7), 2310–2324. https://doi.org/10.1111/gcb.14641

Dooley, S. R., & Treseder, K. K. (2012). The effect of fire on microbial biomass: A meta-analysis of field studies. Biogeochemistry, 109(1), 49–61. https://doi.org/10.1007/s10533-011-9633-8

Dove, N. C., & Hart, S. C. (2017). Fire Reduces Fungal Species Richness and In Situ Mycorrhizal Colonization: A Meta-Analysis. Fire Ecology, 13(2), 37–65. https://doi.org/10.4996/fireecology.130237746

Elshahed, M. S., Youssef, N. H., Spain, A. M., Sheik, C., Najar, F. Z., Sukharnikov, L. O., Roe, B. A., Davis, J. P., Schloss, P. D., Bailey, V. L., & Krumholz, L. R. (2008). Novelty and Uniqueness Patterns of Rare Members of the Soil Biosphere. Applied and Environmental Microbiology, 74(17), 5422–5428. https://doi.org/10.1128/AEM.00410-08

Fitzpatrick, C. R., Mustafa, Z., & Viliunas, J. (2019). Soil microbes alter plant fitness under competition and drought. Journal of Evolutionary Biology, 32(5), 438–450. https://doi.org/10.1111/jeb.13426

Fox, J., Friendly, M., & Weisberg, S. (2013). Hypothesis Tests for Multivariate Linear Models Using the car Package. The R Journal, 5(1), 39. https://doi.org/10.32614/RJ-2013-004

Fultz, L. M., Moore-Kucera, J., Dathe, J., Davinic, M., Perry, G., Wester, D., Schwilk, D. W., & Rideout-Hanzak, S. (2016). Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. Applied Soil Ecology, 99, 118–128. https://doi.org/10.1016/j.apsoil.2015.10.023

He, X., Bledsoe, C. S., Zasoski, R. J., Southworth, D., & Horwath, W. R. (2006). Rapid nitrogen transfer from ectomycorrhizal pines to adjacent ectomycorrhizal and arbuscular mycorrhizal plants in a California oak woodland. New Phytologist, 170(1), 143–151. https://doi.org/10.1111/j.1469-8137.2006.01648.x

Hewitt, R. E., Hollingsworth, T. N., Stuart Chapin III, F., & Lee Taylor, D. (2016). Fire-severity effects on plant–fungal interactions after a novel tundra wildfire disturbance: Implications for arctic shrub and tree migration. BMC Ecology, 16(1), 25. https://doi.org/10.1186/s12898-016-0075-y

Huffman, M. S., & Madritch, M. D. (2018). Soil microbial response following wildfires in thermic oak-pine forests. Biology and Fertility of Soils, 54(8), 985–997. https://doi.org/10.1007/s00374-018-1322-5

Kaye, J. P., Hart, S. C., Fulé, P. Z., Covington, W. W., Moore, M. M., & Kaye, M. W. (2005). Initial Carbon, Nitrogen, and Phosphorus Fluxes Following Ponderosa Pine Restoration Treatments. Ecological Applications, 15(5), 1581–1593. https://doi.org/10.1890/04-0868

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research, 41(1), e1–e1. https://doi.org/10.1093/nar/gks808

Knelman, J. E., Graham, E. B., Trahan, N. A., Schmidt, S. K., & Nemergut, D. R. (2015). Fire severity shapes plant colonization effects on bacterial community structure, microbial biomass, and soil enzyme activity in secondary succession of a burned forest. Soil Biology and Biochemistry, 90, 161–168. https://doi.org/10.1016/j.soilbio.2015.08.004

Lau, J. A., & Lennon, J. T. (2011). Evolutionary ecology of plant–microbe interactions: Soil microbial structure alters selection on plant traits. New Phytologist, 192(1), 215–224. https://doi.org/10.1111/j.1469-8137.2011.03790.x

Lau, J. A., & Lennon, J. T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences, 109(35), 14058–14062. https://doi.org/10.1073/pnas.1202319109

Love, M., Anders, S., & Huber, W. (2014). Differential analysis of count data–the DESeq2 package. Genome Biol, 15(550), 10–1186.

Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010). Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature, 466(7307), 752–755. https://doi.org/10.1038/nature09273

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE, 8(4), e61217. https://doi.org/10.1371/journal.pone.0061217

McMurdie, P. J., & Holmes, S. (2014). Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLOS Computational Biology, 10(4), e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution, 4(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Neff, J. C., Harden, J. W., & Gleixner, G. (2011). Fire effects on soil organic matter content, composition, and nutrients in boreal interior Alaska. Canadian Journal of Forest Research. https://doi.org/10.1139/x05-154

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology, 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nowacki, G. J., & Abrams, M. D. (2008). The Demise of Fire and "Mesophication" of Forests in the Eastern United States. BioScience, 58(2), 123–138. https://doi.org/10.1641/B580207

Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E., & Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. The ISME Journal, 9(4), 980–989. https://doi.org/10.1038/ismej.2014.196

Peay, K. G., Garbelotto, M., & Bruns, T. D. (2009). Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing Pinus muricata seedlings. Journal of Ecology, 97(3), 537–547. https://doi.org/10.1111/j.1365-2745.2009.01489.x

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S., Cornwell, W. K., Craine, J. M., Gurvich, D. E., Urcelay, C., Veneklaas, E. J., Reich, P. B., Poorter, L., Wright, I. J., Ray, P., Enrico, L., Pausas, J. G., Vos, A. C. de, … Cornelissen, J. H. C. (2016). Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany, 64(8), 715–716. https://doi.org/10.1071/bt12225_co

Poulos, H. M., Barton, A. M., Berlyn, G. P., Schwilk, D. W., Faires, C. E., & McCurdy, W. C. (2020). Differences in leaf physiology among juvenile pines and oaks following high-severity wildfire in an Arizona Sky Island Mountain range. Forest Ecology and Management, 457, 117704. https://doi.org/10.1016/j.foreco.2019.117704

Pressler, Y., Moore, J. C., & Cotrufo, M. F. (2019). Belowground community responses to fire: Meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos, 128(3), 309–327. https://doi.org/10.1111/oik.05738

R Core. (2020). R: A language and environment for statistical computing (version 3.5. 3)[software].

Reich, P. B., & Hinckley, T. M. (1980). Water Relations: Soil Fertility, and Plant Nutrient Composition of a Pygmy Oak Ecosystem. Ecology, 61(2), 400–416. https://doi.org/10.2307/1935197

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal, 4(10), 1340–1351. https://doi.org/10.1038/ismej.2010.58

Sáenz de Miera, L. E., Pinto, R., Gutierrez-Gonzalez, J. J., Calvo, L., & Ansola, G. (2020). Wildfire effects on diversity and composition in soil bacterial communities. Science of The Total Environment, 726, 138636. https://doi.org/10.1016/j.scitotenv.2020.138636

Santín, C., Doerr, S. H., Preston, C. M., & González-Rodríguez, G. (2015). Pyrogenic organic matter production from wildfires: A missing sink in the global carbon cycle. Global Change Biology, 21(4), 1621–1633. https://doi.org/10.1111/gcb.12800

Senior, J. K., O'Reilly-Wapstra, J. M., Schweitzer, J. A., Bailey, J. K., & Potts, B. M. (2018). Forest fire may disrupt plant–microbial feedbacks. Plant Ecology, 219(5), 497–504. https://doi.org/10.1007/s11258-018-0811-9

Taylor, D. L., Walters, W. A., Lennon, N. J., Bochicchio, J., Krohn, A., Caporaso, J. G., & Pennanen, T. (2016). Accurate Estimation of Fungal Diversity and Abundance through Improved Lineage-Specific Primers Optimized for Illumina Amplicon Sequencing. Applied and Environmental Microbiology, 82(24), 7217–7226. https://doi.org/10.1128/AEM.02576-16

Taylor, Q. A., & Midgley, M. G. (2018). Prescription side effects: Long-term, high-frequency controlled burning enhances nitrogen availability in an Illinois oak-dominated forest. Forest Ecology and Management, 411, 82–89. https://doi.org/10.1016/j.foreco.2017.12.041

Treseder, K. K., Mack, M. C., & Cross, A. (2004). Relationships Among Fires, Fungi, and Soil Dynamics in Alaskan Boreal Forests. Ecological Applications, 14(6), 1826–1838. https://doi.org/10.1890/03-5133

Troelstra, S. R., Wagenaar, R., Smant, W., & Peters, B. a. M. (2001). Interpretation of bioassays in the study of interactions between soil organisms and plants: Involvement of nutrient factors. New Phytologist, 150(3), 697–706. https://doi.org/10.1046/j.1469-8137.2001.00133.x

Ulbrich, T. C., Friesen, M. L., Roley, S. S., Tiemann, L. K., & Evans, S. E. (2021). Intraspecific Variability in Root Traits and Edaphic Conditions Influence Soil Microbiomes Across 12 Switchgrass Cultivars. Phytobiomes Journal, 5(1), 108–120. https://doi.org/10.1094/PBIOMES-12-19-0069-FI

Waldrop, M. P., & Harden, J. W. (2008). Interactive effects of wildfire and permafrost on microbial communities and soil processes in an Alaskan black spruce forest. Global Change Biology, 14(11), 2591–2602. https://doi.org/10.1111/j.1365-2486.2008.01661.x

Wang, G. G., Lear, D. H. V., & Bauerle, W. L. (2005). Effects of prescribed fires on first-year establishment of white oak (Quercus alba L.) seedlings in the Upper Piedmont of South Carolina, USA. Forest Ecology and Management, 213(1), 328–337. https://doi.org/10.1016/j.foreco.2005.03.049

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology, 73(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Ward, D., Wiegand, K., & Getzin, S. (2013). Walter's two-layer hypothesis revisited: Back to the roots! Oecologia, 172(3), 617–630. https://doi.org/10.1007/s00442-012-2538-y

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K., & Parisien, M.-A. (2019). Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. Soil Biology and Biochemistry, 138, 107571. https://doi.org/10.1016/j.soilbio.2019.107571

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer.

Williams, D., & Snyder, K. (2003). Responses of woody plants to heterogeneity of soil water in arid and semiarid environments. In Changing precipitation regimes and terrestrial ecosystems: A North American perspective. (pp. 28–46). University of Arizona.

APPENDIX

Table 3. Site characteristics of the seven field soil collection sites within Great Smoky Mountains NP. Soil texture classification was gathered from the NRCS Web Soil Survey database. Soil pH was measured in the laboratory. dNDVI represents delta normalized difference vegetation index which quantifies the density of plant growth in a given area by measuring the intensity of light reflected by vegetation.

Burn status	Site	Lat (dd)	Long (dd)	Elev (m)	Soil texture	Soil pH	dNDVI
Unburned	BGB	35.6809845	-83.5275497	517.09	fine sandy loam	5.09	0.0736
	PL441	35.7051697	-83.5240479	407.03	gravelly sand	5.67	0.0730
	RS441	35.7013168	-83.5252075	428.68	gravelly sand	5.44	0.0405
Burned	RGB	35.6829185	-83.5126953	628.82	sandy clay loam	4.34	0.2025
	FCM	35.6972008	-83.5328674	547.41	channery silt loam	4.20	0.2961
	SCM	35.6972008	-83.5328674	547.41	fine sandy loam	4.35	0.2961
	LCM	35.6972008	-83.5328674	547.41	fine sandy loam	4.23	0.2204

Table 4. T-test of the effect of soil burn status (unburned vs. burned) on ASV diversity of soil bacteria, fungi overall, and specific fungal trophic guilds. Diversity was calculated using hill numbers which represent effective numbers of diversity. A diversity order q = 0 provides raw richness by weighting rare taxa the same as abundant taxa. A diversity order q = 1 weights ASVs by their frequency without disproportionately favoring either rare or abundant taxa. A diversity order q = 2 overweighs abundant ASVs. Presented as t statistics, p-values, and directionality of response (where significant). Statistically significant results are shown in bold. AMF = arbuscular mycorrhizal fungi; ECM = ectomycorrhizal fungi.

	q0		q1		q2		
Microbial group	t value	p-value	t value	p-value	t value	p-value	Directionality
Bacteria	2.26	0.073	1.42	0.220	0.35	0.750	--
Fungi (overall)	-5.79	0.011	-3.08	0.077	-2.99	0.070	Decrease with burn at q0 only
AMF	-1.50	0.200	-1.34	0.240	-0.99	0.390	--
ECM	0.77	0.480	1.24	0.300	1.32	0.250	--
Endophyte	-2.40	0.063	-1.08	0.340	-0.95	0.400	--
Plant pathogen	-5.47	0.003	-7.83	0.001	-6.48	0.002	Decrease with burn at q0, q1, q2
Saprotroph	-3.28	0.048	-2.50	0.077	-1.50	0.220	Decrease with burn at q0 only

Table 5. One-way ANOVA of the effect of soil burn status (unburned vs. burned) on relative abundance of each fungal trophic guild. Presented as F statistics, p-values, and directionality of response (where significant). Statistically significant results are shown in bold. AMF = arbuscular mycorrhizal fungi; ECM = ectomycorrhizal fungi.

Fungal trophic guild	F value	p-value	Directionality
AMF	4.74	0.082	--
ECM	1.03	0.356	--
Endophyte	0.08	0.790	--
Plant pathogen	10.97	0.021	Decrease with burn
Saprotroph	0.03	0.859	--

Table 6. One-way ANOVA of the effects of plant pathogenic fungi ASV diversity, plant pathogenic fungi relative abundance, and saprotrophic fungi ASV diversity on oak seedling root biomass. Presented as Chi-squared statistics, p-values, and directionality of root biomass response (where significant). Statistically significant results are shown in bold. Diversity was calculated using hill numbers which represent effective numbers of diversity.

		Root biomass		
Fungal trophic guild	Effect	χ^2	p-value	Directionality
Plant pathogen	Hill numbers (q0)	1.46	0.23	--
	Hill numbers (q1)	3.95	0.05	Decrease with increasing diversity
	Hill numbers (q2)	5.64	0.02	Decrease with increasing diversity
	Relative abundance	0.52	0.47	--
Saprotroph	Hill numbers (q0)	0.68	0.41	--
	Hill numbers (q1)	0.19	0.67	--
	Hill numbers (q2)	0.05	0.83	--

Table 7. Two-way ANOVA of the effects of soil burn status, plant neighbor, and their interaction on oak seedling phenotypes. Presented as Chi-squared statistics and p-values (p-values reported as 0.06 were rounded to the nearest hundredth decimal place). Statistically significant results are shown in bold. Shoot = aboveground biomass; Root = belowground biomass; RGR = relative growth rate; SRL = specific root length; SLA = specific leaf area; CHL = leaf chlorophyll content.

	Effect	χ^2	p-value
Shoot	Soil burn status	0.39	0.53
	Plant neighbor	2.59	0.11
	Soil burn x plant neighbor	0.63	0.43
Root	Soil burn status	3.66	0.06
	Plant neighbor	6.57	0.01
	Soil burn x plant neighbor	3.43	0.06
RGR	Soil burn status	0.24	0.62
	Plant neighbor	5.66	0.02
	Soil burn x plant neighbor	1.27	0.26
SRL	Soil burn status	0.32	0.57
	Plant neighbor	0.90	0.34
	Soil burn x plant neighbor	0.31	0.58
SLA	Soil burn status	1.46	0.23
	Plant neighbor	0.38	0.54
	Soil burn x plant neighbor	2.05	0.15
CHL	Soil burn status	1.62	0.20
	Plant neighbor	0.05	0.82
	Soil burn x plant neighbor	0.006	0.94

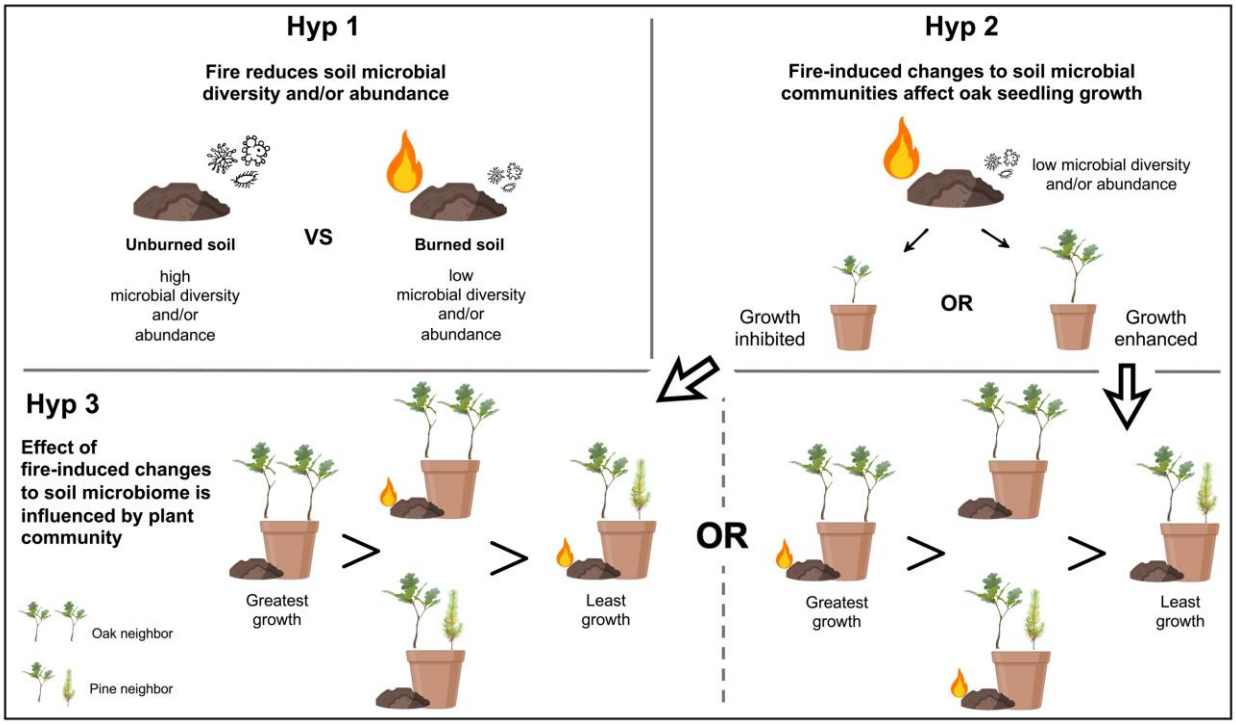

Figure 11. Diagram of study hypotheses. The objective of this study was to identify the relative importance of fire-induced changes to soil microbial communities, type of plant competition, and the interaction of these two factors on oak seedling growth. Hypothesis 1) burned soils contain reduced bacterial and fungal diversity and/or abundance relative to unburned soils. Hypothesis 2) reductions in soil microbial diversity and/or abundance caused by fire either inhibit or enhance oak seedling growth. Hypothesis 3) oak seedling growth response to fire-induced changes to the soil microbiome is influenced by the plant community. If low microbial diversity and/or abundance of burned soil inhibits oak seedling growth, seedling growth would be greatest when grown in unburned soil with an oak neighbor, followed by burned soil with an oak neighbor or unburned soil with a pine neighbor, and growth would be lowest when grown in burned soil with a pine neighbor. If low microbial diversity and/or abundance of burned soil enhances oak seedling growth, seedling growth would be greatest when grown in burned soil with an oak neighbor, followed by unburned soil with an oak neighbor or burned soil with a pine neighbor, and growth would be lowest when grown in unburned soil with a pine neighbor.

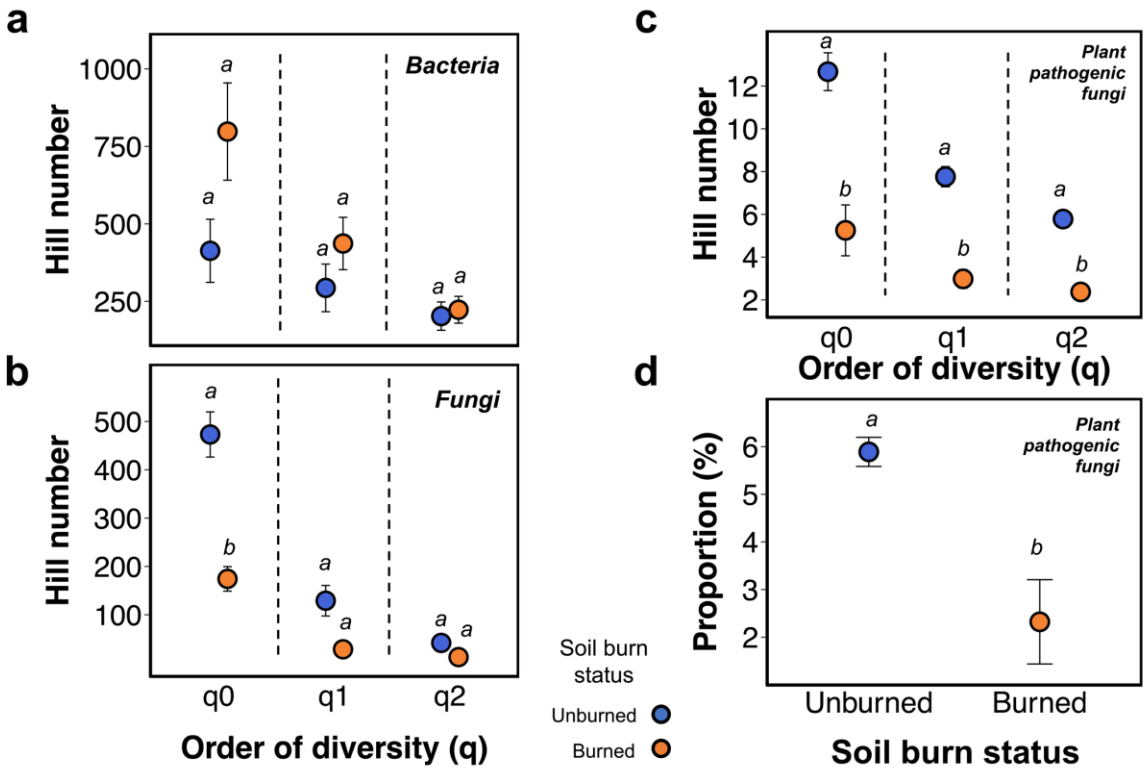

Figure 12. ASV diversity of unburned and burned soils for (a) bacteria, (b) overall fungi, and (c) plant pathogenic fungi at various orders of q. (d) Relative abundance of plant pathogenic fungi in unburned and burned soils. Data are pooled across field sites. Data are pooled across field sites. Data points that do not share letters are significantly different from each other ($\alpha < 0.05$).

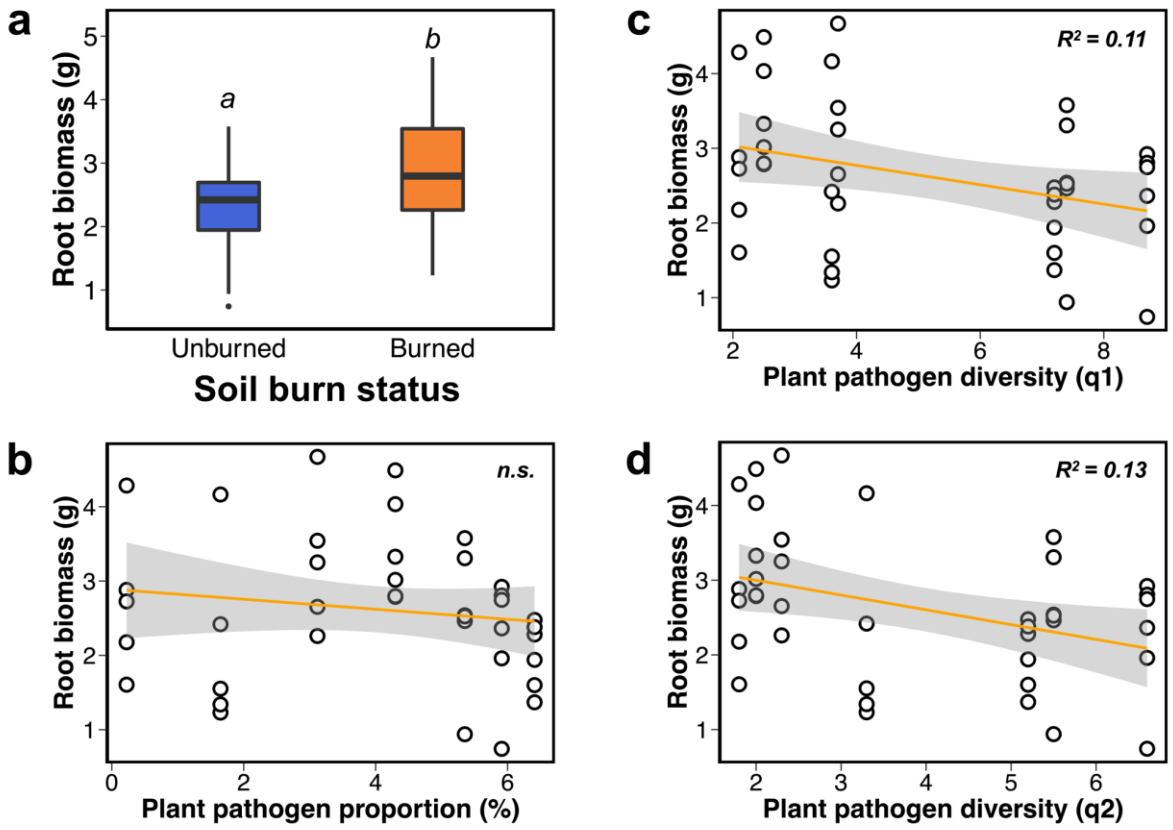

Figure 13. (a) Oak seedling root biomass when grown in treatments of unburned and burned soil inocula. Data are pooled across plant neighbor treatments. (b) Correlation of relative abundance of plant pathogenic fungi (displayed as proportion) and oak seedling root biomass. (c) Correlation of plant pathogenic fungal diversity (q1) and oak seedling root biomass. (d) Correlation of plant pathogenic fungal diversity (q2) and oak seedling root biomass. Different letters in figure a show that data points are significantly different from each other ($\alpha < 0.05$). Solid lines in figures b-d show predicted average values obtained using generalized linear mixed models with 95% confidence intervals represented by the shaded area. For all figures, root biomass data were pooled across plant neighbor treatments.

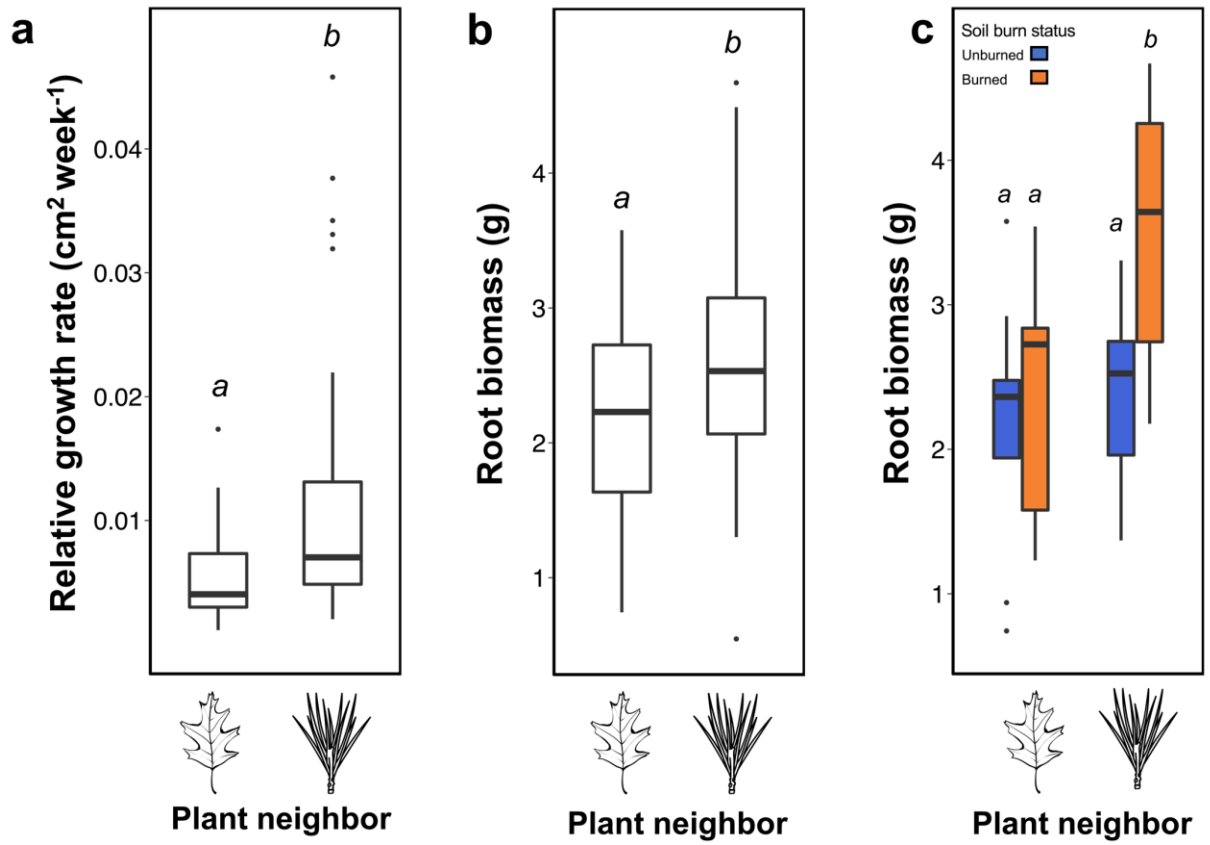

Figure 14. (a) Oak seedling relative growth rate and (b) root biomass when grown in treatments of oak or pine seedling neighbor. (c) Response of oak seedling root biomass to interactive effect of soil inoculum burn status and plant neighbor. Data are pooled across soil inoculum treatments in figures a, b. Data points that do not share letters are significantly different from each other (Tukey test $p < 0.05$).

CHAPTER IV: FIRE-INDUCED SHIFTS IN SOIL MICROBIAL COMMUNITY COMPOSITION INFLUENCE RATES OF CARBON DEGRADATION

Abstract

Across the globe, fire events are becoming more frequent with climate change, which can have important consequences for soil communities and carbon cycling. The objective of this study was to identify the importance of fire-induced changes on microbial community composition on carbon cycling. To do this we sampled soil that had been burned by a wildfire in Great Smoky Mountains National Park (GSMNP), TN, relative to unburned sites. We conducted amplicon sequencing and measured soil abiotic characteristics to assess the composition of soil microbial communities and ecosystem functions associated with carbon cycling to address the following hypotheses: 1) Fire shifts microbial community composition to a community comprised of taxa that thrive under low-resource conditions; and 2) Post-fire shifts in community membership of taxa with different carbon use efficiencies (CUE) are associated with differences in microbial community properties and carbon cycling processes. We found that fire reduced the amount of soil carbon and nitrogen, and five of the seven microbial CUE membership metrics varied among burned and unburned soils and/or with fire-induced changes in soil carbon and nitrogen. Burned soils were generally comprised of more high microbial CUE taxa compared to unburned soils, though this trend was mostly driven by oligotrophic bacteria rather than by high CUE fungal taxa. These difference in microbial CUE composition were associated with variation in carbon-degrading enzyme activity, but not microbial biomass carbon. These results suggest that 1) fire disturbance alters soil microbial community composition in functionally-relevant ways, and 2) that a shift in microbial community composition can be important at the ecosystem scale.

Introduction

Across the globe, fire events are becoming more frequent with climate change and long-standing practices of fire suppression (Barbero et al., 2015; Flannigan et al., 2009; Moritz et al., 2012), which can have important consequences for soil microbial communities and carbon cycling (Jansson & Hofmockel, 2020). Fire alters many biotic and abiotic aspects of ecosystems and can alter soil microbial composition through a variety of mechanisms including changes to the quantity and quality of substrates necessary for microbial growth such as carbon and inorganic nitrogen (Certini, 2005; Knelman et al., 2015; Peay et al., 2009), as well changes to soil pH (Certini, 2005), moisture and temperature (Treseder et al., 2004). Fire can reduce the amount of soil organic carbon that is available to microbes by combustion of plant and litter biomass and subsequent

volatilization of carbon in plant biomass (Baird et al., 1999; Muqaddas et al., 2015; Pellegrini et al., 2014). A recent meta-analysis found that across a variety of ecosystem types, repeated burning reduced soil carbon and nitrogen by 12% in burned sites; a change that persisted over multiple decades (Pellegrini et al., 2018).

Microbes release carbon as carbon dioxide and methane by consuming and subsequently decomposing organic carbon inputs from plants (Bradford et al., 2013; Castellano et al., 2015; Cotrufo et al., 2013). Microbes stabilize carbon when some of the inputs are converted into microbial residues, including metabolites and dead microbial biomass which then adhere to mineral soil surfaces and become resistant to decomposition (Bradford et al., 2013; Castellano et al., 2015; Cotrufo et al., 2013). This balance between microbial carbon respiration and carbon stabilization determines whether soil serves as a source or sink of carbon. Therefore, developing an accurate understanding of how carbon cycling will change with changing fire regimes requires close examination of soil microbial responses to more frequent and higher severity fires.

Knowledge has been amassing on how soil microbial communities and individual microbial taxa respond to fire. At a coarse scale, fire generally reduces microbial abundance and diversity (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019; Sáenz de Miera et al., 2020) and some studies document greater decreases in soil fungi compared to bacteria (Beals et al., 2022; Dooley & Treseder, 2012; Pressler et al., 2019). Declines in mycorrhizal fungi, in particular, may be common when fire removes obligate plant partners (Fultz et al., 2016). At a finer scale, specific bacterial and fungal taxa have been identified as particularly sensitive or tolerant of fire disturbance. Some studies report decreases in bacterial phyla like Acidobacteria (Mikita-Barbato et al., 2015; Sáenz de Miera et al., 2020; Xiang et al., 2014), Alpha- and Deltaproteobacteria (Xiang et al., 2014), Verrucomicrobia, and Chloroflexi in burned soils and increases in Actinobacteria (Mikita-Barbato et al., 2015; Sáenz de Miera et al., 2020) and Betaproteobacteria (Sáenz de Miera et al., 2020; Xiang et al., 2014) in burned soils. In some environments, the fungal genus Trechispora (Mikita-Barbato et al., 2015) was sensitive to fire whereas the genera Gelasinospora, Lactarius, Leptodontidium, Sphaerosporella (Mikita-Barbato et al., 2015), Penicillium, and Fusicladium (Whitman et al., 2019) were abundant in burned soils. While it is important to understand how soil microbial community composition changes with fire, it is also crucial to understand the broader significance of these composition changes for the larger ecosystem. Despite an increase in effort to examine soil microbial response to fire, it is still

relatively unclear how fire-induced changes to soil microbial community composition relate to microbial functions associated with carbon cycling. This is a crucial knowledge gap to fill because as fire alters the presence and abundance of soil microbes, it is likely that changes in community composition would affect how soil microbes process carbon.

Research that integrates microbial ecology and ecosystem science often infers ecosystem processes from microbial composition characteristics without explicitly testing that the relationships exist (Bier et al., 2015; Rocca et al., 2015). To address this, a recent conceptual framework has been proposed to link microbial community composition more effectively and empirically to system-level processes (Hall et al., 2018). This framework identifies three distinct groups of microbial characteristics: 1) microbial membership: the taxonomic and/or functional identities of microbes; 2) microbial community properties: an integrated characteristic of the microbial community that has the potential to predict a particular microbial process; 3) microbial processes: the collective metabolisms of a microbial community that contribute to changes in pools or fluxes of elements or compounds (Hall et al., 2018). By testing for relationships between these three groups, this framework makes it possible to demonstrate that microbial-driven processes, such as enzyme activity or organic carbon mineralization, are directly influenced by aggregate properties of microbial communities (such as microbial biomass carbon), which in turn are influenced by the membership (i.e., composition) of microbiomes and environmental factors. This conceptual framework has recently been applied to assess the importance of soil microbial composition on ecosystem functions in the context of forest disturbance (Osburn et al., 2021), but has not yet been extended to fire disturbances.

Microbial community membership can provide useful information for microbial contributions to ecosystem function. In the context of carbon cycling, microbial taxonomic identities can be used as composition metrics that are representative of microbial carbon use efficiency (CUE). Carbon use efficiency is the amount of carbon that is stored in microbial biomass relative to the amount of carbon that is respired by microbes (Geyer et al., 2016). High microbial CUE is often associated with relatively slow carbon cycling and therefore carbon stabilization in soil, whereas low microbial CUE is often associated with relatively fast carbon cycling and subsequent carbon release from soil. Different groups of microbes have been integrated into this CUE framework based on whether the life history strategy of a microbial group or taxon exhibits a slow-growing or fast-growing strategy (Andrews & Harris, 1986). Oligotrophic bacteria, for

example, are slow-growing and thrive under low-resource conditions, and copiotrophic bacteria are fast-growing and thrive under high-resource conditions. Bacteria belonging to the phyla Acidobacteria (Fierer et al., 2007; Strickland et al., 2009; Zechmeister-Boltenstern et al., 2015), Actinobacteria (Fierer et al., 2007; Strickland et al., 2009; Zechmeister-Boltenstern et al., 2015), and Verrucomicrobia (Ho et al., 2017) have been identified as oligotrophic, whereas Proteobacteria and Bacteroidetes have been identified as copiotrophic Fierer et al., 2007; Strickland et al., 2009; Zechmeister-Boltenstern et al., 2015). Fungi tend to grow slower than bacteria because they are often larger in size, have longer turnover times, and have higher resource use efficiency (Bailey et al., 2002; Six et al., 2006; Waring et al., 2013). More specifically, fungal functional types also differ in CUE. Ericoid and ectomycorrhizal fungi, for example, have been found to be associated with high soil carbon storage (Averill et al., 2014; Clemmensen et al., 2015). This variation in CUE based on phylogenetic variation in life history strategies can be used as an important predictor of how fire disturbances lead to changes in carbon cycling.

The objective of this study was to identify the importance of fire-induced changes to microbial community composition for carbon cycling. To do this, we sampled soil that had been burned by a wildfire in Great Smoky Mountains National Park (GSMNP), TN. We used amplicon sequencing and measured soil abiotic characteristics to assess the composition of soil microbial communities and ecosystem functions associated with carbon cycling, respectively. We focused on two primary questions: 1) How does fire affect soil microbial membership of CUE potential? 2) How do fire-induced changes to soil microbial membership of CUE potential influence microbial community properties and processes associated with carbon cycling? Based on trends in the literature that fire generally reduces total soil carbon and nitrogen (Pellegrini et al., 2018), we hypothesized the following (Fig. 15): 1) Fire shifts microbial community composition to a community comprised of taxa that thrive in low-resource conditions and that have potentially high CUE. Specifically, we predicted that burned soils would have higher oligotroph:copiotroph bacterial ratios, higher fungi:bacteria ratios, higher relative abundance of ecto- and ericoid mycorrhizal fungi and greater proportion of oligotrophic indicator taxa relative to unburned soils. 2) Shifts in microbial membership of microbial CUE potential are associated with differences in microbial community properties and processes of carbon cycling.

Materials and Methods

Study system

To conduct this study, we collected field soil from areas impacted by the Chimney Tops 2 (CT2) wildfire that occurred in the Great Smoky Mountains National Park and in Gatlinburg, TN in November, and December of 2016. Within the fire perimeter, we identified four 90 m² blocks within unburned and heavily burned areas that were located within 300 m from roads and park trails for ease of accessibility for a total of eight sites (4 unburned sites and 4 burned sites). A detailed description of the methods we used to select the field sites is described in a previous study using many of these same field sites (Beals et al., 2022). We verified differences in burn damage among our selected unburned and burned sites using the satellite-derived normalized difference vegetation index (NDVI) which quantifies the density of plant growth in a given area by measuring the intensity of light reflected by vegetation (Cihlar et al., 1991). To quantify only the difference in canopy cover due to damage from the wildfire and not for variation in canopy cover among the sites pre-fire, we calculated delta NDVI (dNDVI) for each site, i.e., the difference in NDVI before and after the wildfire. We were not able to acquire ground measurements of burn, however it has been shown that satellite-derived metrics of burn strongly correlate with ground measurements of burn (Cocke et al., 2005). We confirmed that unburned and burned sites did significantly vary in dNDVI ($F = 22.2$, $p < 0.0001$) (Table S1).

Soil sampling and processing

To assess fire-induced changes to soil microbial community composition and soil abiotic characteristics, we collected soil from each site in October 2018, approximately 2 years post-fire. Soils were collected by sampling from 10 random points within each site using a trowel to collect the top 10-15 cm of mineral soil. Trowels were cleaned with ethanol between each site to reduce the possibility of microbial cross contamination. Following field collection, we measured gravimetric water content and soil pH for all samples. For each sample, we dried a subset of soil in a drying oven at 105º C for 48 h. Gravimetric water content was measured by subtracting the weight of oven-dried soil from the weight of fresh soil and dividing that difference by the weight of oven-dried soil. To measure soil pH, we added approximately 5 g of fresh soil to 50 mL plastic centrifuge tubes. We added 10 mL 0.01 M $CaCl_2$ solution to achieve a 1:2 weight:volume ratio. The pH of the slurry was then measured using a pH meter. To provide a representation of a

microbial community property and microbial process, we measured microbial biomass carbon (MBC) and multiple hydrolytic carbon-degrading enzymes, respectively, on all samples. MBC was measured using a modified chloroform fumigation-K_2SO_4 extraction method and carbon content was estimated by calculating the difference between fumigated and non-fumigated samples of soil from each site (Fierer & Schimel, 2003). We measured enzyme activity for the following enzymes that indicate microbial carbon degradation: cellobiohydrolase (CBH; degrades cellulose), β-glucosidase (BG; degrades cellulose), α-glucosidase (AG; degrades starch), N-acetyl-β-D-glucosaminidase (NAG; degrades chitin). We followed the protocol for enzyme assays described in Saiya-Cork et al. (Saiya-Cork et al., 2002). Approximately 2 g of field-wet soil was blended in a sodium acetate buffer with a pH of 5 for 2 min. and incubated with the respective fluorescently-labeled substrates for 1 hour. Fluorescence was measured at 365 nm excitation and 450 nm emission. All soil processing was conducted at the University of Tennessee, Knoxville, TN.

Sequencing preparation

An additional 2 g subsample of soil from each site was stored at -80°C for subsequent characterization of microbial communities. We used the Qiagen DNEasy PowerSoil DNA Isolation kit (Qiagen, Venlo, Netherlands) to extract DNA from 0.25 g of soil. DNA was eluted in 50 ul of buffer, and DNA concentration and quality were measured on a NanoDrop 2000 spectrophotometer (Thermo Scientific). Libraries were prepped using a two-step Polymerase Chain Reaction (PCR) approach with a mixture of 341F and 785R Illumina primers to characterize bacterial 16S gene regions, and a mixture of 5.8S-FUN and ITS4-FUN primers to characterize fungal ITS2 gene regions (Taylor et al., 2016). PCR for 16S rRNA and ITS2 were performed separately. Amplicon sequencing was performed on the Illumina MiSeq platform at the University of Tennessee Genomics Core facility (Knoxville, TN). Detailed methods for PCR and sequencing are described in Beals et al. (2022).

Bioinformatics

All processing of 16S and ITS2 sequences including primer removal was completed using a customized DADA2 pipeline (Callahan et al., 2016). Taxonomy of amplicon sequence variants (ASVs) was assigned using the RDP (Wang et al., 2007) and UNITE (Abarenkov et al., 2010) databases for bacteria and fungi, respectively. We assigned bacterial ASVs as either oligotrophic

(high potential microbial CUE) or copiotrophic (low potential microbial CUE) based on previous findings that the phyla Acidobacteria (Fierer et al., 2007; Strickland et al., 2009; Zechmeister-Boltenstern et al., 2015), Actinobacteria (Fierer et al., 2007; Strickland et al., 2009; Zechmeister-Boltenstern et al., 2015), and Verrucomicrobia (Ho et al., 2017) have been identified as oligotrophic, and the phylum Bacteroidetes (Fierer et al., 2007; Strickland et al., 2009; Zechmeister-Boltenstern et al., 2015), and the class Betaproteobacteria have been identified as copiotrophic (Fierer et al., 2007; Strickland et al., 2009; Zechmeister-Boltenstern et al., 2015). We assigned fungal ASVs to one of seven functional guilds using the FUNGuild database of fungal taxa with known or suspected ecological functions (Nguyen et al., 2016): arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (ECM), ericoid mycorrhizal fungi (ERM), endophytic fungi, plant pathogenic fungi, saprotrophic fungi, and "other" (including animal pathogens, fungal parasites, epiphytes, lichen-forming, dung-inhabiting, etc.). After processing, we had 6,074 bacterial ASVs and 2,590 fungal ASVs. Fifty-six percent of fungal ASVs (1,440 ASVs) were assigned to a fungal guild. The majority of ASVs were assigned as saprotrophic fungi (54.4%), followed by plant pathogenic fungi (10%), "other" (9.0%), ECM (12.6%), AMF (7.1%), endophytic fungi (5.6%), and ERM (1.2%). For the purposes of this study, we focused on ECM and ERM because of their role in affecting soil carbon cycling.

Statistical analysis

All analyses were conducted in R (R Core, 2020). Prior to testing our hypotheses, we used the lm function in the stats package in R to confirm that fire altered the amount of soil carbon and nitrogen. For these models we specified burn status as the fixed effect. We built additional linear models to also assess if MBC and carbon-degrading enzyme rates were altered by fire and/or fire-induced changes to soil carbon and nitrogen. For the first set of MBC and enzyme models we specified burn status as the fixed effect, and we specified soil carbon and soil nitrogen as the fixed effects in the second and third sets of models, respectively. For all models we used the Anova function in the car package (Fox et al., 2013) to calculate one-way analysis of variance (ANOVA) using Type II sums of squares. We created Bray-Curtis distance matrices using the relative abundance of each ASV within each sample. We then assessed variation in community composition of bacterial and fungal communities using PERMANOVA analysis with 10,000 permutations using the adonis function in the vegan package (Oksanen et al., 2019). Prior to

conducting PERMANOVA, we confirmed homogeneity of dispersion among burn status treatments with the betadisper function in the vegan package. We then performed a distance-based redundancy analysis (db-RDA) using the dbrda function in the vegan package to assign variation in composition of bacteria and fungi to burn status and edaphic variables (soil C, soil N, soil pH). We used the anova.cca function in the vegan package to assess the cumulative significance of burn status and edaphic variables on community composition. We partitioned the variation in composition with respect to burn status and edaphic variables using the varpart function in the vegan package. To visualize composition of bacterial and fungal communities among burned and unburned soils, we used principal coordinate analysis (PCoA) for ordination based on the Bray-Curtis distance matrices.

Prior to analysis, we also generated the following microbial membership metrics to assess composition of microbial CUE: 1) ratios of the richness and relative abundance of oligotrophic to copiotrophic bacteria, referred to respectively as O:C (richness) and O:C (abundance); 2) relative abundance of oligotrophic bacteria and high CUE fungal indicator taxa; 3) ratios of the richness and relative abundance of fungi to bacteria, referred to respectively as F:B (richness) and F:B (abundance); 4) relative abundance of ERM; 5) relative abundance of ECM. Ratios of the richness for each sample were calculated by summing all ASVs that had a non-zero sequence count and were identified as high microbial CUE taxa or low microbial CUE taxa. We then divided the high microbial CUE ASV sum by the low microbial CUE ASV sum. Ratios of the abundance were calculated by summing all sequence counts for all ASVs that were identified as high microbial CUE taxa or low microbial CUE taxa, and then subsequently dividing the high microbial CUE ASV sum by the low microbial CUE ASV sum. To identify particular bacterial and fungal taxa that were uniquely highly associated with burned and unburned soil, we performed indicator species analysis using volcano plots to visualize differential representation of ASVs among the soil burn treatments. To do this, we first calculated the log2 fold change in relative abundance from burned to unburned soils for each bacterial and fungal ASV. We used t-tests to identify the ASVs which differed significantly in relative abundance between burned and unburned soils with a significance threshold of $p < 0.055$. We then constructed separate volcano plots for all bacterial and all fungal ASVs to visualize the magnitude of log2 fold change vs. statistical significance, in which each data point in the volcano plot represented the mean log2 fold change and accompanying p-value of the relative abundance of a single ASV across all four burned soil sites

and all four unburned soil sites. ASVs with a log2 fold change ≥ 2 and a p-value ≤ 0.055 were identified as over-represented in burned soil and therefore indicators of burned soil. ASVs with a log2 fold change ≤ -2 and a p-value ≤ 0.055 were identified as under-represented in burned soil and therefore indicators of unburned soil. We then used the multipatt function in the indicspecies package (Cáceres & Legendre, 2009) to verify that the taxa we identified with the volcano plot method were strong indicators. From this dataset we selected the ASVs that were high microbial CUE taxa. Finally, we calculated the relative abundance of high microbial CUE indicator ASV sequences compared to all ASV sequences.

To test Hypothesis 1 that microbial communities in burned soils contain more taxa that have potentially high CUE relative to unburned soils (Fig. 15), we built linear models for each microbial membership metric using the lm function in the stats package. We specified burn status as the fixed effect. We used the Anova function in the car package (Fox et al., 2013) to calculate one-way analysis of variance (ANOVA) using Type II sums of squares. To further elucidate if differences in microbial CUE composition between burned and unburned soils were influenced by fire-induced changes to soil carbon and/or nitrogen, we conducted separate linear models in which soil carbon and soil nitrogen were specified as the fixed effect in each model. When necessary, response variables were transformed to conform to normality prior to running models.

To test Hypothesis 2 that shifts in microbial membership of microbial CUE potential are associated with differences in microbial community properties and processes of carbon cycling (Fig. 15), we used Pearson correlation to identify correlations between the microbial membership metrics and MBC and the four carbon-degrading enzymes, respectively. To determine the relative importance of microbial membership metrics for explaining variation in MBC and carbon-degrading enzyme rates, we conducted multiple subset regression using the bestglm function in the leaps package (Lumley, 2020). For each multiple regression model, we specified the family as gaussian, the information criterion as AIC, and the method as exhaustive. For MBC and each enzyme, we identified the regression model that had the best-supported subset of microbial membership metrics based on AIC. For all analyses, $p \leq 0.05$ was considered statistically significant and $p < 0.1$ was considered marginally significant. All figures were made with the ggplot2 package (Wickham, 2016), and multi-panel figures were compiled with the patchwork package (Pedersen, 2020).

Results

Overall, we found partial support for our hypotheses. Fire reduced the amount of soil carbon and nitrogen, and five of the seven microbial CUE membership metrics varied among burned and unburned soils and/or with fire-induced changes in soil carbon and nitrogen. Burned soils were generally composed of more potentially high microbial CUE taxa compared to unburned soils, though this trend was mostly driven by oligotrophic bacteria rather than by high CUE fungal taxa. These difference in microbial potential CUE composition were associated with variation in carbon-degrading enzyme activity, but not MBC.

Fire-induced changes to soil nutrients, MBC, soil enzyme activity, and overall microbial community composition

As expected, burned soils contained on average 1.8 and 4.2 times less soil carbon and nitrogen, respectively compared to unburned soils (soil carbon: $F = 24.2$, $p = 0.003$; soil nitrogen: $F = 94.0$, $p < 0.0001$; Table S2; Fig. 16). On average, BG and AG enzyme activity were 3.3 times and 2.1 times slower in burned soils relative to unburned soils, respectively (Tables S2, 8). MBC and rates of CBH and NAG enzymes were similar between burned and unburned soils (Tables S2, 8). MBC and all enzymes except CBH varied with changes in soil carbon (Table 8). MBC, enzyme activity rates of BG, AG, and NAG were significantly positively correlated with soil carbon. BG and AG enzyme activity was also positively correlated with changes in soil nitrogen (Table 8).

PERMANOVA analysis revealed that bacterial and fungal communities varied significantly by burn status (Bacteria: $F = 3.20$, $p = 0.03$; Fungi: $F = 3.04$, $p = 0.03$) (Fig. S1). The db-RDA revealed that burn status and edaphic variables cumulatively accounted for 39% and 33% of variation in bacterial and fungal composition, respectively (Bacteria: adj. $R^2 = 0.39$; Fungi: adj. $R^2 = 0.33$). Burn status accounted for 24% and 23% of variation in bacterial and fungal composition, respectively (Bacteria: adj. $R^2 = 0.24$; Fungi: adj. $R^2 = 0.23$). Edaphic variables of soil carbon, soil nitrogen, and soil pH cumulatively accounted for 29% and 28% of variation in bacterial and fungal composition, respectively (Bacteria: adj. $R^2 = 0.29$; Fungi: adj. $R^2 = 0.28$).

Composition of microbial CUE potential in response to fire and soil nutrients

In partial support of Hyp 1, some of the microbial membership metrics were affected by fire and subsequent changes to soil carbon and nitrogen. Ratios of the richness and abundance of oligotrophic to copiotrophic bacteria varied significantly among burned and unburned soils and

also varied with changes in soil carbon and nitrogen. Burned soils contained on average 2.3 times greater O:C (richness) ($F = 7.1$, $p = 0.04$; Table 9; Fig. 17a) and 3.3 times greater O:C (abundance) ($F = 9.4$, $p = 0.02$; Table 9; Fig. 17d) than unburned soils. O:C (richness) decreased with increases in soil carbon ($F = 24.5$, $p = 0.003$; Table 9; Fig. 17b) and soil nitrogen ($F = 9.7$, $p = 0.02$; Table 9; Fig. 17c). O:C (abundance) also decreased with increases in soil carbon ($F = 23.9$, $p = 0.003$; Table 9; Fig. 17e) and soil N ($F = 11.5$, $p = 0.02$; Table 9; Fig. 17f). Our indicator species analysis identified 62 bacterial indicators (Fig. 18a) and 9 fungal indicators that were uniquely highly associated with either burned or unburned soils. Since there were very few fungal taxa identified as indicators, we proceeded with just the bacterial indicators in the subsequent analyses to test for the effect of fire on the relative abundance of high CUE indicator taxa. Forty-two percent of the bacterial indicators were identified as oligotrophic. The relative abundance of oligotrophic bacteria indicator sequence counts strongly varied with fire and changes in soil carbon and nitrogen. Burned soils contained on average 3.8 times greater relative abundance of oligotrophic bacteria indicator sequence counts than unburned soil ($F = 43.8$, $p = 0.0006$; Table 9; Fig. 18b). The relative abundance of oligotrophic bacteria indicator sequence counts decreased with increases in soil carbon ($F = 45.7$, $p = 0.0005$; Table 9; Fig. 18c) and nitrogen ($F = 61.8$, $p = 0.0002$; Table 9; Fig. 18c).

The effect of fire on the ratio of the richness of fungi to bacteria was marginally significant ($F = 4.3$, $p = 0.09$; Table 9), with F:B (richness) slightly lower in burned soils compared to unburned soils (Fig. 19a). On the other hand, the ratio of the abundance of fungi to bacteria was similar among burned and unburned soils ($F = 0.5$, $p = 0.52$; Table 9; Fig. 19b). Neither F:B (richness) nor F:B (abundance) varied with changes in soil carbon or nitrogen (Table 9). The relative abundance of ERM did vary significantly with fire and soil carbon and nitrogen, but the relative abundance of ECM did not. Burned soils contained 32 times greater relative abundance of ERM sequence counts than unburned soils ($F = 11.0$, $p = 0.02$; Table 9; Fig. 20a). The relative abundance of ERM sequence counts also decreased with increases in soil carbon ($F = 5.1$, $p = 0.06$; Table 9; Fig. 20b) and nitrogen ($F = 11.6$, $p = 0.01$; Table 9; Fig. 20c). Conversely, the relative abundance of ECM sequence counts did not vary among burned and unburned soils (Table 9; Fig. 20d) or with changes in soil carbon or nitrogen (Table 9; Figs. 20e, f).

Influence of microbial membership on MBC and soil enzyme activity

In partial support of Hyp 2, activity of all four carbon-degrading enzymes was significantly correlated with at least one microbial membership metric, whereas MBC was not correlated with any membership metrics. O:C (richness), O:C (abundance), and relative abundance of oligotrophic bacteria indicator taxa were all significantly negatively correlated with activity of BG, AG, and NAG (Fig. 21a). F:B richness was significantly positively correlated with the activity of CBH, BG, and AG (Fig. 21a).

Multiple subsets regression and model selection produced similar results to the Pearson's correlation tests. For this component of the analysis, we only included the microbial membership metrics that varied significantly in response to burn status and/or fire-induced changes to soil carbon and nitrogen which were O:C (richness), O:C (abundance), relative abundance of oligotrophic bacteria indicator taxa, F:B (richness), and relative abundance of ERM. Of the four membership metrics included in the best model for CBH activity, F:B (richness) was identified as the most important variable for explaining variation in CBH activity (Table 10). Of the four membership metrics included in the best model for BG activity, O:C (richness) was identified as the most important variable for explaining variation in BG activity (Table 10). Only O:C (richness) and O:C (abundance) were included in the best model for NAG activity, and both were identified as important for explaining variation in NAG activity (Table 10).

Discussion

Fire-induced changes to soil nutrients alter some soil enzyme activity

As expected, we found that fire reduced soil resource availability for microbes. The CT2 wildfire in this study greatly reduced soil carbon and nitrogen, which supports previous findings of trends across multiple ecosystems that soil carbon and nitrogen decrease with burning (Pellegrini et al., 2018). We found that burned soils also had reduced rates of starch (AG) degradation and reduced rates of one form of cellulose (BG) degradation. Although MBC did not vary between burned and unburned soils, MBC was associated with fire-induced reductions in soil carbon. Low soil carbon corresponded to low amounts of MBC. Although we did not detect differences in MBC from fire itself, associations between fire-induced reductions in soil carbon and decrease in MBC are similar to previous findings from two meta-analyses that document

reductions in MBC from fire (Dooley & Treseder, 2012; Holden & Treseder, 2013). Similarly, rates of starch and one form of cellulose degradation decreased with reductions in soil carbon and nitrogen. Rates of chitin (NAG) degradation also decreased with reductions in soil carbon. Our findings of enzyme activity responses to fire corroborate previous findings that report reductions in enzyme rates from fire (Boerner et al., 2008; Knelman et al., 2017; Pellegrini et al., 2020).

Fire shifts microbial community composition to more potentially high CUE taxa, mostly driven by bacteria

We found that fire generally shifted microbial community composition to a community comprised of more potentially high CUE taxa, as we had predicted. However, this shift was driven mostly by the bacterial communities rather than by the fungal communities. Fire and reductions in soil carbon and nitrogen from fire increased the diversity and abundance of oligotrophic bacteria relative to copiotrophic bacteria, as well as the relative abundance of oligotrophic indicator taxa.

Ratios of fungi to bacteria were largely unaffected by fire or by reductions in soil carbon and nitrogen from fire, although burned soils contained slightly fewer fungal species relative to bacterial species when compared to unburned soils, contrary to our prediction. However, this finding may not be surprising considering that fungi are typically more sensitive to fire compared to bacteria (Dooley & Treseder, 2012; Fultz et al., 2016; Pressler et al., 2019). Additionally, our findings are in line with a previous study that also examined the responses of soil microbial communities responded to the CT2 wildfire and documented reduced fungal richness in burned sites compared to unburned sites (Brown et al., 2019). Similar to our results, Brown et al. (2019) found no effect of the CT2 wildfire on fungal abundance.

Fire and reductions in soil carbon and nitrogen from fire increased the relative abundance of ericoid mycorrhizal fungi, but not the relative abundance of ectomycorrhizal fungi. This pattern could be due in part to the surrounding plant communities. Ericoid mycorrhizal fungi exclusively associate with plants belonging to the Ericaceae (heath) family. In Great Smoky Mountains NP, the most common Ericaceae plants are Kalmia sp. (mountain-laurel) and Rhododendron sp. (azalea). Plant surveys among these same unburned and burned sites conducted after the CT2 fire have found that Kalmia sp. are highly abundant in the burned sites and nearly absent in the unburned sites (Hubert et al. in prep). As such, it is unclear whether the abundance of ERM in these burned soils is due to CUE of ERM or due to the presence of plant partners.

Composition shifts in microbial CUE potential important for carbon-degradation rates

Soils contain more carbon than vegetation and the atmosphere combined (Lal, 2004; Lehmann & Kleber, 2015) and the vast network of belowground microbial communities are the main drivers of soil organic carbon cycling, despite only generally accounting for 1-2% of total soil organic matter mass (Xu et al., 2013). As such, even a small change in soil carbon stocks can cause large variations in the atmospheric concentrations of greenhouse gases such as carbon dioxide and methane (Jansson & Hofmockel, 2020). Here we found that changes in composition of microbial CUE potential from fire were correlated with variation in broader microbial functions. Richness and abundance of oligotrophic bacteria relative to copiotrophic bacteria were negatively correlated with degradation rates of starch, chitin, and one form of cellulose (BG). As richness and abundance of oligotrophic bacteria increased, degradation rates of starch, chitin, and cellulose decreased. This pattern is understandable considering that oligotrophic bacteria are slow-growing in comparison to copiotrophic bacteria, and as such would degrade carbon at slower rates. While this study is one of the first to document this pattern in a fire disturbance context, it has been previously found that fast-growing:slow-growing bacterial ratios were important for explaining variation in carbon mineralization rates (Osburn et al., 2021). Conversely, richness of fungi relative to bacteria was positively correlated with rates of starch and cellulose degradation. As fungal richness increased, starch and cellulose degradation rates increased. This finding contradicts previous work that documented decreased hydrolytic enzyme activity in response to fire-induced reductions in soil fungal abundance (Holden et al., 2013).

Building upon previous findings that microbial decomposition rates often decrease after fire (Holden et al., 2015; Pellegrini et al., 2020; Smith et al., 2008), the findings put forth here in our study suggests that changing fire regimes may alter microbial community composition in ways that impact carbon cycling. Increases in fire frequency and fire severity may promote bacterial communities that are dominated by C-stabilizing (i.e., oligotrophic) taxa due to reductions in soil carbon and nitrogen after fire. This indicates the potential for burned soils to stabilize carbon after fire disturbance, but our findings here are representative of short-term (2 yrs) soil microbial responses to fire. Currently it is uncertain whether a greater proportion of slow-growing, carbon-stabilizing bacteria in soil will sequester enough carbon over time to notably counteract the amount of carbon that is lost to the atmosphere from fire via soil organic carbon combustion. The findings

here prompt next steps in this area of research that should examine if shifts in microbial CUE composition persist over the long-term.

Conclusions

As one of the first studies to examine the importance of fire-induced changes to microbial community composition for carbon cycling, we show that fire disturbance alters soil microbial community composition in functionally-relevant ways, and that a shift in microbial community composition can be important for broader microbial functions. In this study we found that fire-induced changes to microbial CUE composition were associated with variation in microbially-mediated carbon degradation rates. This study demonstrates that alterations to microbial community composition are relevant at the ecosystem level. Specifically, understanding microbial diversity can provide a greater understanding of how microbes contribute to ecosystem functions.

REFERENCES

Abarenkov, K., Nilsson, R. H., Larsson, K.-H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U., & Kõljalg, U. (2010). The UNITE database for molecular identification of fungi – recent updates and future perspectives. New Phytologist, 186(2), 281–285. https://doi.org/10.1111/j.1469-8137.2009.03160.x

Andrews, J. H., & Harris, R. F. (1986). R- and K-Selection and Microbial Ecology. In K. C. Marshall (Ed.), Advances in Microbial Ecology (pp. 99–147). Springer US. https://doi.org/10.1007/978-1-4757-0611-6_3

Averill, C., Turner, B. L., & Finzi, A. C. (2014). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature, 505(7484), 543–545. https://doi.org/10.1038/nature12901

Bailey, V. L., Smith, J. L., & Bolton, H. (2002). Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology and Biochemistry, 34(7), 997–1007. https://doi.org/10.1016/S0038-0717(02)00033-0

Baird, M., Zabowski, D., & Everett, R. L. (1999). Wildfire effects on carbon and nitrogen in inland coniferous forests. Plant and Soil, 209(2), 233–243. https://doi.org/10.1023/A:1004602408717

Barbero, R., Abatzoglou, J. T., Larkin, N. K., Kolden, C. A., & Stocks, B. (2015). Climate change presents increased potential for very large fires in the contiguous United States. International Journal of Wildland Fire, 24(7), 892–899. https://doi.org/10.1071/WF15083

Beals, K. K., Scearce, A. E., Swystun, A. T., & Schweitzer, J. A. (2022). Belowground mechanisms for oak regeneration: Interactions among fire, soil microbes, and plant community alter oak seedling growth. Forest Ecology and Management, 503, 119774. https://doi.org/10.1016/j.foreco.2021.119774

Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T., Nemergut, D. R., Osborne, B. B., Ruiz-González, C., Schimel, J. P., Waldrop, M. P., & Wallenstein, M. D. (2015). Linking microbial community structure and microbial processes: An empirical and conceptual overview. FEMS Microbiology Ecology, 91(10), fiv113. https://doi.org/10.1093/femsec/fiv113

Boerner, R. E. J., Giai, C., Huang, J., & Miesel, J. R. (2008). Initial effects of fire and mechanical thinning on soil enzyme activity and nitrogen transformations in eight North American forest ecosystems. Soil Biology and Biochemistry, 40(12), 3076–3085. https://doi.org/10.1016/j.soilbio.2008.09.008

Bradford, M. A., Keiser, A. D., Davies, C. A., Mersmann, C. A., & Strickland, M. S. (2013). Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. Biogeochemistry, 113(1), 271–281. https://doi.org/10.1007/s10533-012-9822-0

Brown, S. P., Veach, A. M., Horton, J. L., Ford, E., Jumpponen, A., & Baird, R. (2019). Context dependent fungal and bacterial soil community shifts in response to recent wildfires in the Southern Appalachian Mountains. Forest Ecology and Management, 451, 117520. https://doi.org/10.1016/j.foreco.2019.117520

Cáceres, M. D., & Legendre, P. (2009). Associations between species and groups of sites: Indices and statistical inference. Ecology, 90(12), 3566–3574. https://doi.org/10.1890/08-1823.1

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

Castellano, M. J., Mueller, K. E., Olk, D. C., Sawyer, J. E., & Six, J. (2015). Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology, 21(9), 3200–3209. https://doi.org/10.1111/gcb.12982

Certini, G. (2005). Effects of fire on properties of forest soils: A review.

Cihlar, J., St.-Laurent, L., & Dyer, J. A. (1991). Relation between the normalized difference vegetation index and ecological variables. Remote Sensing of Environment, 35(2), 279–298. https://doi.org/10.1016/0034-4257(91)90018-2

Clemmensen, K. E., Finlay, R. D., Dahlberg, A., Stenlid, J., Wardle, D. A., & Lindahl, B. D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytologist, 205(4), 1525–1536. https://doi.org/10.1111/nph.13208

Cocke, A., Fulé, P., & Crouse, J. (2005). Comparison of burn severity using Difference Normalized Burn Ratio and ground data. International Journal of Wildland Fire, 14, 189–198.

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology, 19(4), 988–995. https://doi.org/10.1111/gcb.12113

Dooley, S. R., & Treseder, K. K. (2012). The effect of fire on microbial biomass: A meta-analysis of field studies. Biogeochemistry, 109(1), 49–61. https://doi.org/10.1007/s10533-011-9633-8

Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an Ecological Classification of Soil Bacteria. Ecology, 88(6), 1354–1364. https://doi.org/10.1890/05-1839

Fierer, N., & Schimel, J. P. (2003). A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid Rewetting of a Dry Soil. Soil Science Society of America Journal, 67(3), 798–805. https://doi.org/10.2136/sssaj2003.7980

Flannigan, M. D., Krawchuk, M. A., Groot, W. J. de, Wotton, B. M., & Gowman, L. M. (2009). Implications of changing climate for global wildland fire. International Journal of Wildland Fire, 18(5), 483–507. https://doi.org/10.1071/WF08187

Fox, J., Friendly, M., & Weisberg, S. (2013). Hypothesis Tests for Multivariate Linear Models Using the car Package. The R Journal, 5(1), 39. https://doi.org/10.32614/RJ-2013-004

Fultz, L. M., Moore-Kucera, J., Dathe, J., Davinic, M., Perry, G., Wester, D., Schwilk, D. W., & Rideout-Hanzak, S. (2016). Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. Applied Soil Ecology, 99, 118–128. https://doi.org/10.1016/j.apsoil.2015.10.023

Geyer, K. M., Kyker-Snowman, E., Grandy, A. S., & Frey, S. D. (2016). Microbial carbon use efficiency: Accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry, 127(2), 173–188. https://doi.org/10.1007/s10533-016-0191-y

Hall, E. K., Bernhardt, E. S., Bier, R. L., Bradford, M. A., Boot, C. M., Cotner, J. B., del Giorgio, P. A., Evans, S. E., Graham, E. B., Jones, S. E., Lennon, J. T., Locey, K. J., Nemergut, D., Osborne, B. B., Rocca, J. D., Schimel, J. P., Waldrop, M. P., & Wallenstein, M. D. (2018). Understanding how microbiomes influence the systems they inhabit. Nature Microbiology, 3(9), 977–982. https://doi.org/10.1038/s41564-018-0201-z

Ho, A., Di Lonardo, D. P., & Bodelier, P. L. E. (2017). Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiology Ecology, 93(3), fix006. https://doi.org/10.1093/femsec/fix006

Holden, S. R., Berhe, A. A., & Treseder, K. K. (2015). Decreases in soil moisture and organic matter quality suppress microbial decomposition following a boreal forest fire. Soil Biology and Biochemistry, 87, 1–9. https://doi.org/10.1016/j.soilbio.2015.04.005

Holden, S. R., Gutierrez, A., & Treseder, K. K. (2013). Changes in Soil Fungal Communities, Extracellular Enzyme Activities, and Litter Decomposition Across a Fire Chronosequence in Alaskan Boreal Forests. Ecosystems, 16(1), 34–46. https://doi.org/10.1007/s10021-012-9594-3

Holden, S., & Treseder, K. (2013). A meta-analysis of soil microbial biomass responses to forest disturbances. Frontiers in Microbiology, 4. https://www.frontiersin.org/article/10.3389/fmicb.2013.00163

Jansson, J. K., & Hofmockel, K. S. (2020). Soil microbiomes and climate change. Nature Reviews Microbiology, 18. https://doi.org/10.1038/s41579-019-0265-7

Knelman, J. E., Graham, E. B., Ferrenberg, S., Lecoeuvre, A., Labrado, A., Darcy, J. L., Nemergut, D. R., & Schmidt, S. K. (2017). Rapid Shifts in Soil Nutrients and Decomposition Enzyme Activity in Early Succession Following Forest Fire. Forests, 8(9), 347. https://doi.org/10.3390/f8090347

Knelman, J. E., Graham, E. B., Trahan, N. A., Schmidt, S. K., & Nemergut, D. R. (2015). Fire severity shapes plant colonization effects on bacterial community structure, microbial biomass, and soil enzyme activity in secondary succession of a burned forest. Soil Biology and Biochemistry, 90, 161–168. https://doi.org/10.1016/j.soilbio.2015.08.004

Lal, R. (2004). Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science, 304(5677), 1623–1627. https://doi.org/10.1126/science.1097396

Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. Nature, 528(7580), 60–68. https://doi.org/10.1038/nature16069

Lumley, T. (2020). leaps: Regression Subset Selection.

Mikita-Barbato, R. A., Kelly, J. J., & Tate, R. L. (2015). Wildfire effects on the properties and microbial community structure of organic horizon soils in the New Jersey Pinelands. Soil Biology and Biochemistry, 86, 67–76. https://doi.org/10.1016/j.soilbio.2015.03.021

Moritz, M. A., Parisien, M.-A., Batllori, E., Krawchuk, M. A., Dorn, J. V., Ganz, D. J., & Hayhoe, K. (2012). Climate change and disruptions to global fire activity. Ecosphere, 3(6), art49. https://doi.org/10.1890/ES11-00345.1

Muqaddas, B., Zhou, X., Lewis, T., Wild, C., & Chen, C. (2015). Long-term frequent prescribed fire decreases surface soil carbon and nitrogen pools in a wet sclerophyll forest of Southeast Queensland, Australia. Science of The Total Environment, 536, 39–47. https://doi.org/10.1016/j.scitotenv.2015.07.023

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology, 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., & McGlinn, D. (2019). vegan: Community Ecology Package. R package version 2.5–6. 2019.

Osburn, E. D., Badgley, B. D., Strahm, B. D., Aylward, F. O., & Barrett, J. E. (2021). Emergent properties of microbial communities drive accelerated biogeochemical cycling in disturbed temperate forests. Ecology, 102(12), e03553. https://doi.org/10.1002/ecy.3553

Peay, K. G., Garbelotto, M., & Bruns, T. D. (2009). Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing Pinus muricata seedlings. Journal of Ecology, 97(3), 537–547. https://doi.org/10.1111/j.1365-2745.2009.01489.x

Pedersen, T. L. (2020). patchwork: The Composer of Plots (1.1.1) [Computer software]. https://CRAN.R-project.org/package=patchwork

Pellegrini, A. F. A., Ahlström, A., Hobbie, S. E., Reich, P. B., Nieradzik, L. P., Staver, A. C., Scharenbroch, B. C., Jumpponen, A., Anderegg, W. R. L., Randerson, J. T., & Jackson, R.

B. (2018). Fire frequency drives decadal changes in soil carbon and nitrogen and ecosystem productivity. Nature, 553(7687), 194–198. https://doi.org/10.1038/nature24668

Pellegrini, A. F. A., Hobbie, S. E., Reich, P. B., Jumpponen, A., Brookshire, E. N. J., Caprio, A. C., Coetsee, C., & Jackson, R. B. (2020). Repeated fire shifts carbon and nitrogen cycling by changing plant inputs and soil decomposition across ecosystems. Ecological Monographs, 90(4), e01409. https://doi.org/10.1002/ecm.1409

Pellegrini, A. F. A., Hoffmann, W. A., & Franco, A. C. (2014). Carbon accumulation and nitrogen pool recovery during transitions from savanna to forest in central Brazil. Ecology, 95(2), 342–352. https://doi.org/10.1890/13-0290.1

Pressler, Y., Moore, J. C., & Cotrufo, M. F. (2019). Belowground community responses to fire: Meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos, 128(3), 309–327. https://doi.org/10.1111/oik.05738

R Core. (2020). R: A language and environment for statistical computing (version 3.5. 3)[software].

Rocca, J. D., Hall, E. K., Lennon, J. T., Evans, S. E., Waldrop, M. P., Cotner, J. B., Nemergut, D. R., Graham, E. B., & Wallenstein, M. D. (2015). Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. The ISME Journal, 9(8), 1693–1699. https://doi.org/10.1038/ismej.2014.252

Sáenz de Miera, L. E., Pinto, R., Gutierrez-Gonzalez, J. J., Calvo, L., & Ansola, G. (2020). Wildfire effects on diversity and composition in soil bacterial communities. Science of The Total Environment, 726, 138636. https://doi.org/10.1016/j.scitotenv.2020.138636

Saiya-Cork, K. R., Sinsabaugh, R. L., & Zak, D. R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry, 34(9), 1309–1315. https://doi.org/10.1016/S0038-0717(02)00074-3

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006). Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. Soil Science Society of America Journal, 70(2), 555–569. https://doi.org/10.2136/sssaj2004.0347

Smith, N. R., Kishchuk, B. E., & Mohn, W. W. (2008). Effects of Wildfire and Harvest Disturbances on Forest Soil Bacterial Communities. Applied and Environmental Microbiology, 74(1), 216–224. https://doi.org/10.1128/AEM.01355-07

Strickland, M. S., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Testing the functional significance of microbial community composition. Ecology, 90(2), 441–451. https://doi.org/10.1890/08-0296.1

Taylor, D. L., Walters, W. A., Lennon, N. J., Bochicchio, J., Krohn, A., Caporaso, J. G., & Pennanen, T. (2016). Accurate Estimation of Fungal Diversity and Abundance through Improved Lineage-Specific Primers Optimized for Illumina Amplicon Sequencing.

Applied and Environmental Microbiology, 82(24), 7217–7226. https://doi.org/10.1128/AEM.02576-16

Treseder, K. K., Mack, M. C., & Cross, A. (2004). Relationships Among Fires, Fungi, and Soil Dynamics in Alaskan Boreal Forests. Ecological Applications, 14(6), 1826–1838. https://doi.org/10.1890/03-5133

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology, 73(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Waring, B. G., Averill, C., & Hawkes, C. V. (2013). Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: Insights from meta-analysis and theoretical models. Ecology Letters, 16(7), 887–894. https://doi.org/10.1111/ele.12125

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K., & Parisien, M.-A. (2019). Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient. Soil Biology and Biochemistry, 138, 107571. https://doi.org/10.1016/j.soilbio.2019.107571

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer.

Xiang, X., Shi, Y., Yang, J., Kong, J., Lin, X., Zhang, H., Zeng, J., & Chu, H. (2014). Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. Scientific Reports, 4(1), 1–8. https://doi.org/10.1038/srep03829

Xu, X., Thornton, P. E., & Post, W. M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Global Ecology and Biogeography, 22(6), 737–749. https://doi.org/10.1111/geb.12029

Zechmeister-Boltenstern, S., Keiblinger, K. M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., & Wanek, W. (2015). The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecological Monographs, 85(2), 133–155. https://doi.org/10.1890/14-0777.1

APPENDIX

Table 8. Results for separate one-way ANOVAs of the effect of burn status (burned vs. unburned), soil carbon (C), and soil nitrogen (N) on microbial biomass carbon (MBC) and C-degrading enzyme rates. CBH = cellobiohydrolase; BG = β-glucosidase; AG = α-glucosidase; NAG = N-acetyl-β-D-glucosaminidase. Asterisks indicate statistically significant differences at $p \leq 0.05$.

	MBC		CBH		BG		AG		NAG	
	F	p	F	p	F	p	F	p	F	p
Burn status	3.04	0.14	1.4	0.29	6.88	0.04*	8.61	0.03*	2.88	0.14
Soil C	6.67	0.05*	1.58	0.26	9.4	0.02*	8	0.03*	10.08	0.02*
Soil N	2.41	0.18	1.08	0.35	6.51	0.04*	6.4	0.04*	2.66	0.15

Table 9. Results for separate one-way ANOVAs of the effect of burn status (burned vs. unburned), soil carbon (C), and soil nitrogen (N) on microbial membership metrics. O:C (rich) = ratio of the richness of oligotrophic to copiotrophic bacteria; O:C (abund) = ratio of the relative abundance of oligotrophic to copiotrophic bacteria; Oligo indicator abund = relative abundance of oligotrophic indicator bacteria; F:B (rich) = ratio of the richness of fungi to bacteria; F:B (abund) = ratio of the relative abundance of fungi to bacteria; ECM abund = relative abundance of ectomycorrhizal fungi; ERM abund = relative abundance of ericoid mycorrhizal fungi. Statistically significant results are indicated by * $p < 0.05$, † $p < 0.1$.

	O:C (rich)		O:C (abund)		Oligo indicator abund		F:B (rich)		F:B (abund)		ECM abund		ERM abund	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Burn status	7.05	0.038*	9.41	0.022*	43.82	0.0006*	4.26	0.085 †	0.46	0.52	0.07	0.81	10.98	0.016*
Soil C	24.53	0.003*	23.86	0.003*	45.73	0.0005*	1.83	0.23	0.03	0.87	0.36	0.57	5.12	0.06 †
Soil N	9.69	0.021*	11.52	0.015*	61.75	0.0002*	3.74	0.1	0.18	0.68	0.14	0.72	11.56	0.014*

Table 10. Best-supported multiple regression models for microbial biomass carbon (MBC) and each carbon-degrading enzyme with accompanying adjusted R^2 values and AIC scores. CBH = cellobiohydrolase; BG = β-glucosidase; AG = α-glucosidase; NAG = N-acetyl-β-D-glucosaminidase; O:C (richness) = ratio of the richness of oligotrophic to copiotrophic bacteria; O:C (abundance) = ratio of the relative abundance of oligotrophic to copiotrophic bacteria; Oligo indicator abundance = relative abundance of oligotrophic indicator bacteria; F:B (richness) = ratio of the richness of fungi to bacteria; F:B (abundance) = ratio of the relative abundance of fungi to bacteria; ECM abundance = relative abundance of ectomycorrhizal fungi; ERM abundance = relative abundance of ericoid mycorrhizal fungi. Statistically significant results are indicated by * $p < 0.05$, † $p < 0.1$.

Variable	Predictor variables included	Adj. R^2	AIC	Predictor variable	p
MBC	O:C (richness)	0.82	51.8	O:C (richness)	0.18
	O:C (abundance)			O:C (abundance)	0.3
	Oligo indicator abundance			Oligo indicator abundance	0.55
	F:B (richness)			F:B (rich)	0.21
	ERM abundance			ERM abundance	0.36
CBH	O:C (richness)	0.84	93.2	O:C (richness)	0.13
	O:C (abundance)			O:C (abundance)	0.27
	F:B (richness)			F:B (richness)	0.05 †
	ERM abundance			ERM abundance	0.22
BG	O:C (richness)	0.81	142.2	O:C (richness)	0.09 †
	O:C (abundance)			O:C (abundance)	0.13
	Oligo indicator abundance			Oligo indicator abundance	0.38
	F:B (richness)			F:B (richness)	0.11
AG	O:C (richness)	0.69	126.2	O:C (richness)	0.19
	O:C (abundance)			O:C (abundance)	0.16
	Oligo indicator abundance			Oligo indicator abundance	0.21
	F:B (richness)			F:B (richness)	0.16
NAG	O:C (richness)	0.89	132.4	O:C (richness)	0.008*
	O:C (abundance)			O:C (abundance)	0.04*

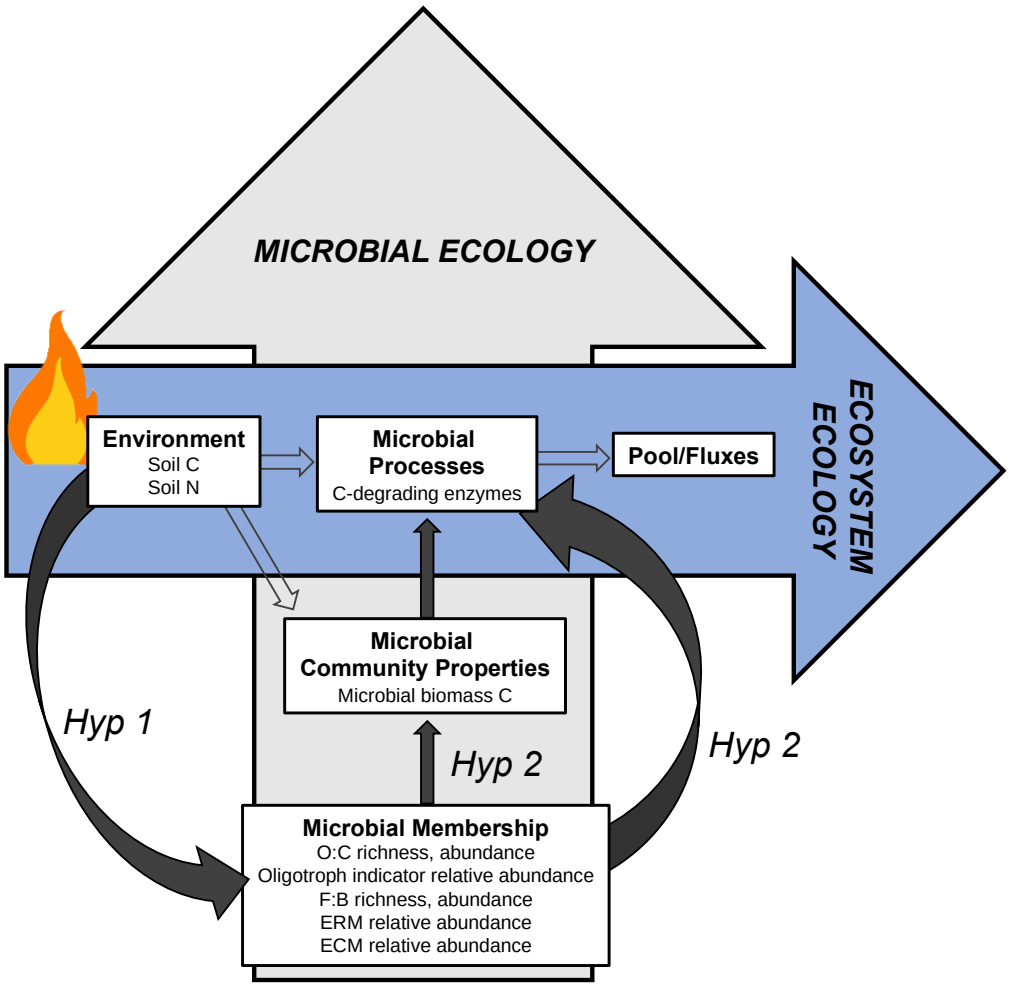

Figure 15. Conceptual framework of the study objective of identifying the importance of fire-induced changes on microbial community composition for carbon cycling. Dark arrows indicate the relationships tested in this study's hypotheses: Hyp 1) Fire shifts microbial community composition to a community comprised of more high CUE taxa that thrive under low-resource conditions. Specifically, we predict that burned soils will have higher oligotroph:copiotroph bacterial ratios, greater proportion of oligotrophic indicator taxa relative to unburned soils, higher fungi:bacteria ratios, and higher relative abundance of ecto- and ericoid mycorrhizal fungi. Hyp 2) Shifts in membership of microbial CUE are associated with differences in microbial community properties and processes of carbon cycling. Specifically, we predict that a community of higher CUE taxa in burned soil will have greater microbial biomass carbon and faster rates of carbon-degrading enzymes than a community of lower CUE taxa in unburned soil.

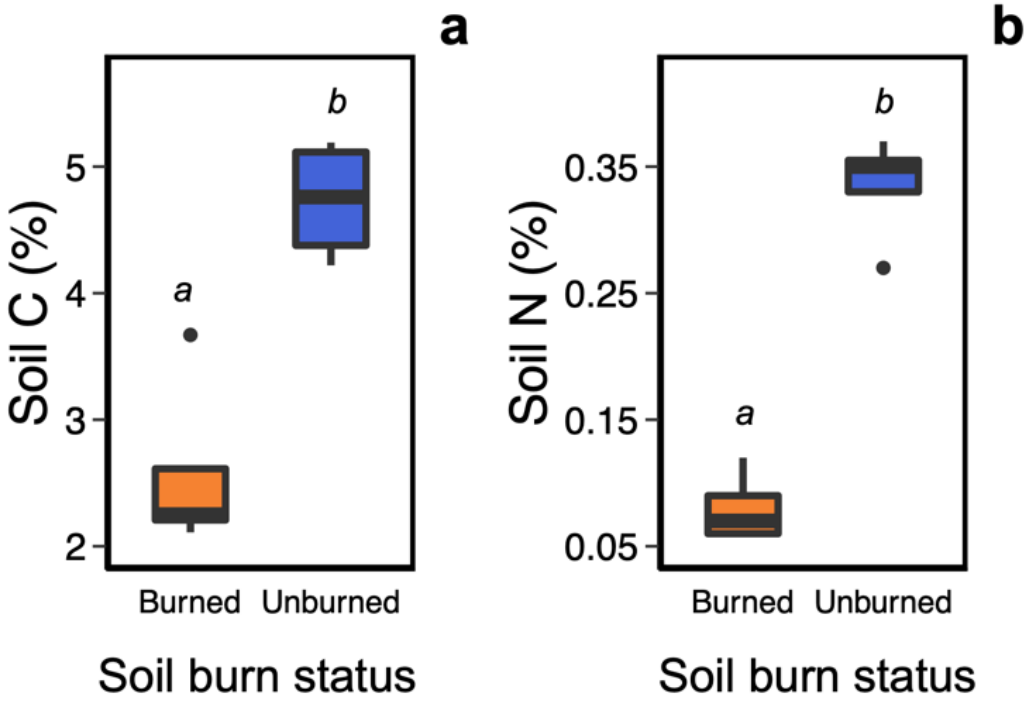

Figure 16. Soil carbon (C) and nitrogen (N) significantly differed between burned and unburned soils. (a) Burned soils contained on average 1.8 times less soil carbon than unburned soils ($F = 24.2$, $p = 0.003$). (b) Burned soils contained on average 4.2 times less soil nitrogen than unburned soils ($F = 94.0$, $p < 0.0001$). Data points that do not share letters are significantly different from each other ($p < 0.05$).

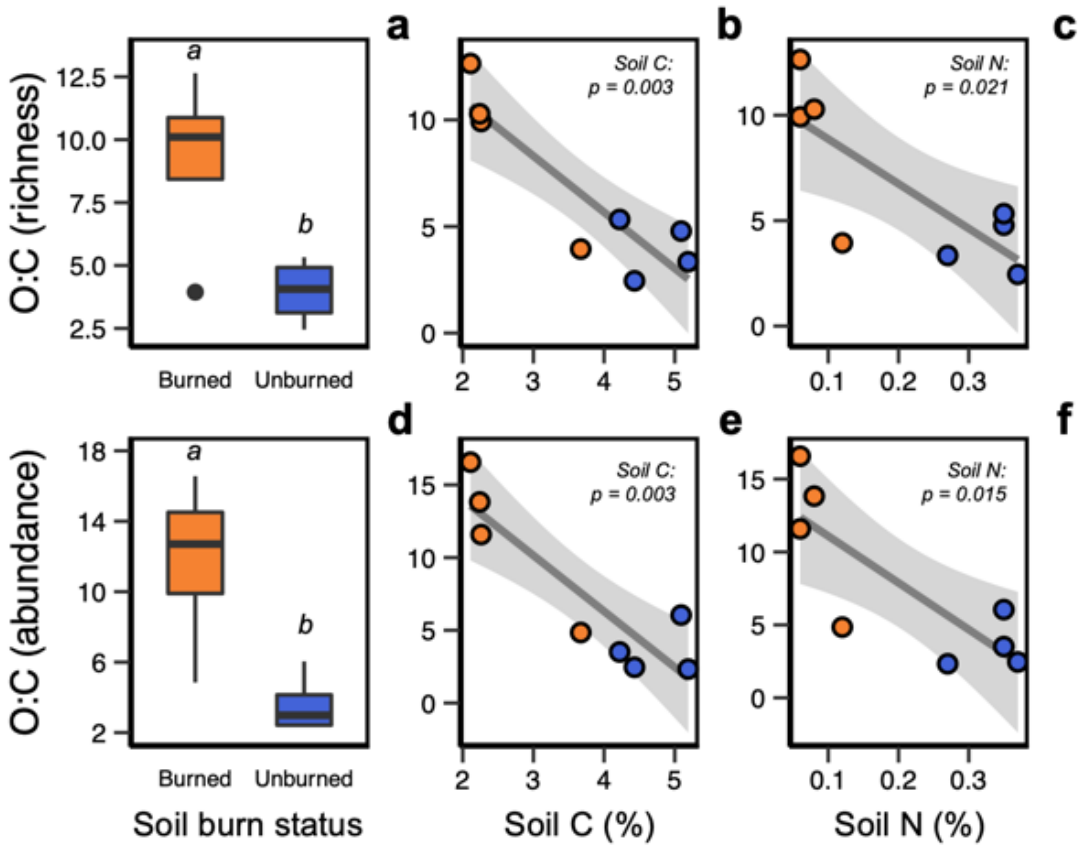

Figure 17. (a) Burned soils contained on average 2.3 times greater oligotrophic:copiotrophic bacterial richness ($F = 7.1$, $p = 0.04$) and (d) 3.3 times greater oligotrophic:copiotrophic bacterial abundance ($F = 9.4$, $p = 0.02$) than unburned soils. O:C richness decreased with increases in soil carbon (b) and soil nitrogen (c). O:C abundance also decreased with increases in soil carbon (e) and soil nitrogen (f). In all figures, orange represents burned soils and blue represents unburned soils. Data points that do not share letters are significantly different from each other ($p < 0.05$).

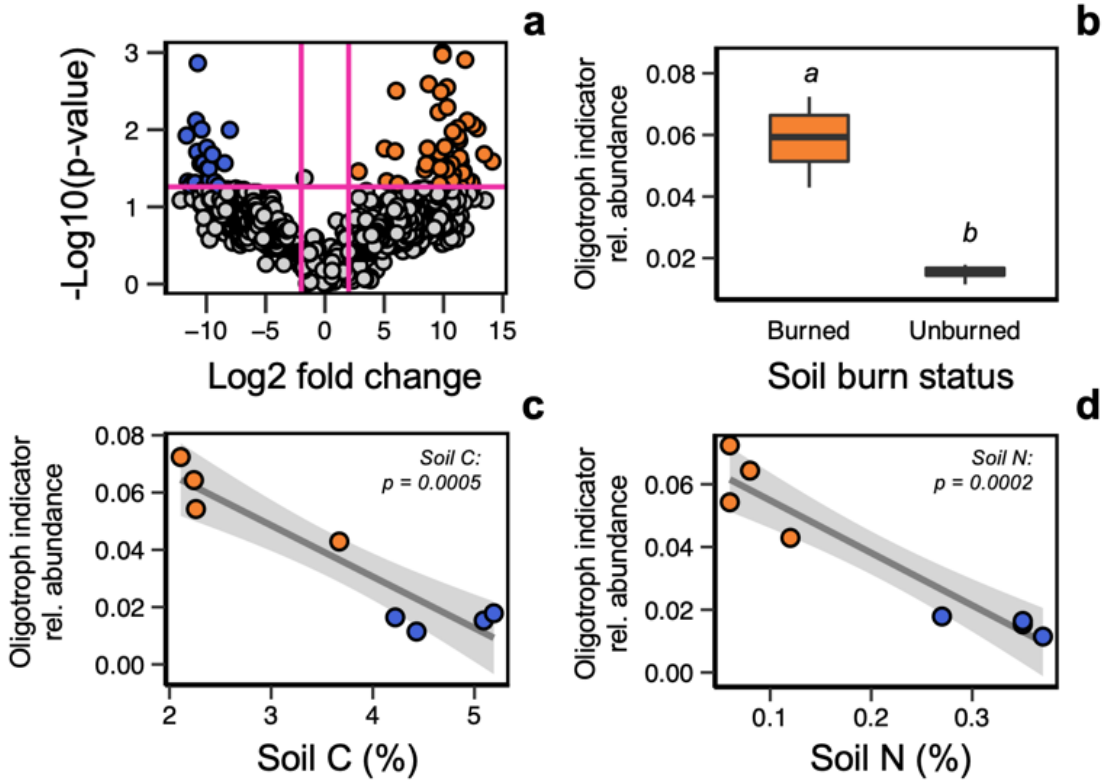

Figure 18. (a) Indicator species analysis identified 62 bacterial indicators among burned and unburned soils. (b) Burned soils contained on average 3.8 times greater relative abundance of oligotrophic bacterial indicator sequence counts than unburned soil ($F = 43.8$, $p = 0.0006$). The relative abundance of oligotrophic bacterial indicator sequence counts decreased with increases in soil carbon (c) and nitrogen (d). In all figures, orange represents burned soils and blue represents unburned soils. Data points that do not share letters are significantly different from each other ($p < 0.05$).

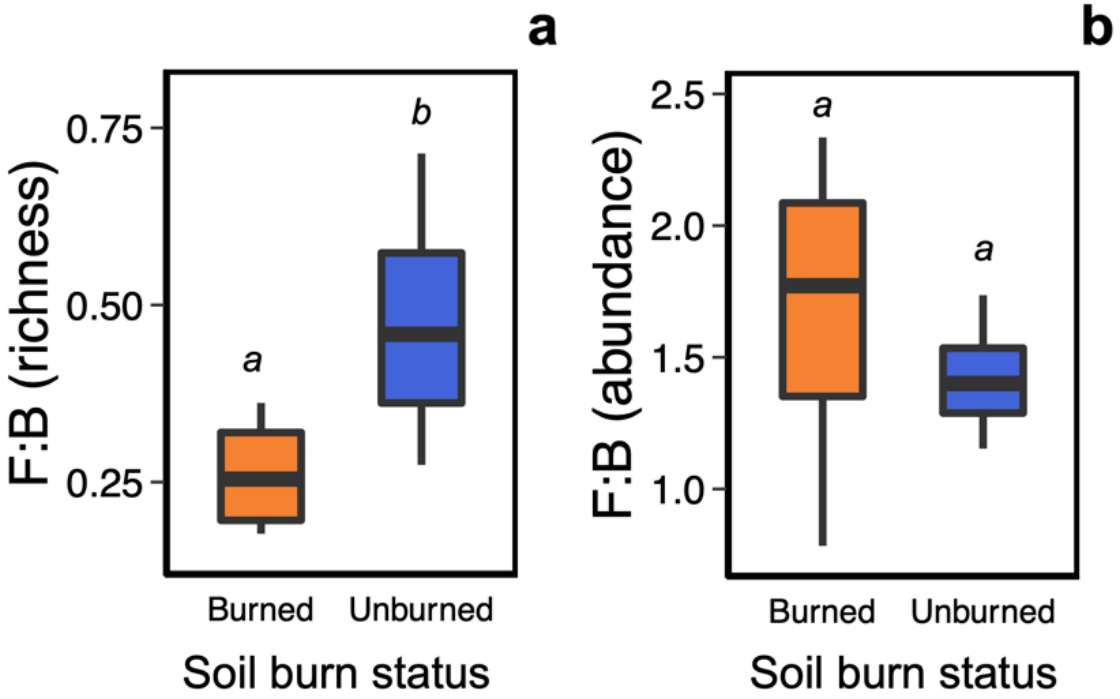

Figure 19. (a) Fungi:bacteria richness was slightly lower in burned soils compared to unburned soils ($F = 4.3$, $p = 0.09$). (b) F:B abundance was similar among burned and unburned soils ($F = 0.5$, $p = 0.52$). Data points that do not share letters are significantly different from each other ($p < 0.1$).

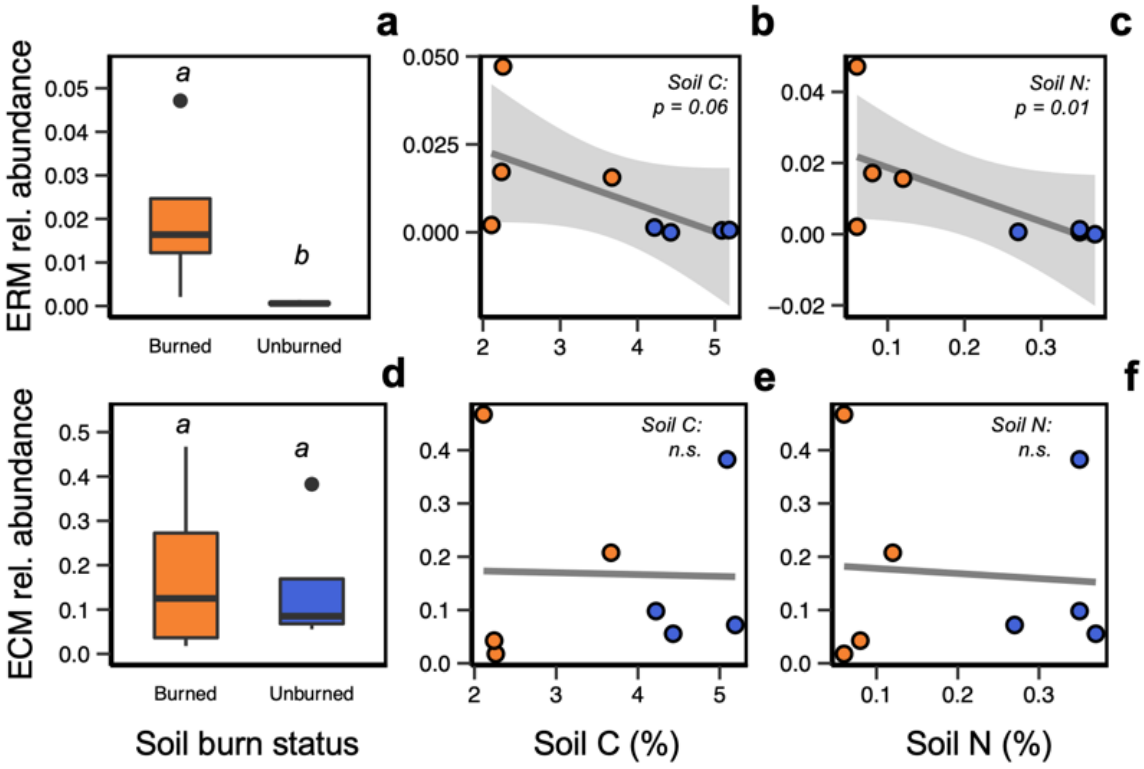

Figure 20. (a) Burned soils contained 32 times greater relative abundance of ERM sequence counts than unburned soils ($F = 11.0$, $p = 0.02$). The relative abundance of ERM sequence counts decreased with increasing soil carbon (b) and nitrogen (c). The relative abundance of ECM sequence counts did not vary among burned and unburned soils (d) or with changes in soil carbon (e) or nitrogen (f). Orange represents burned soils and blue represents unburned soils. In all figures, orange represents burned soils and blue represents unburned soils. Data points that do not share letters are significantly different from each other ($p < 0.05$).

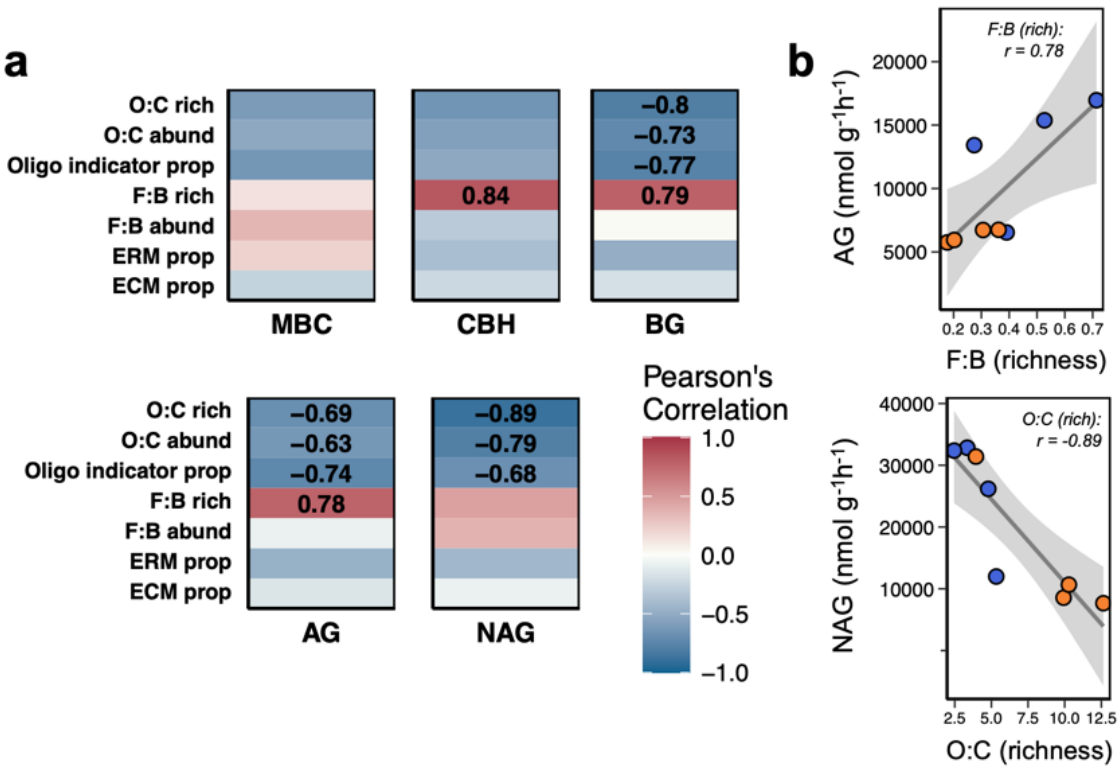

Figure 21. (a) Pearson's correlation coefficients between microbial membership metrics and microbial community property (microbial biomass carbon: MBC) and microbial processes (carbon-degrading enzyme rates: CBH, BG, AG, NAG). Only statistically significant correlation values are displayed ($p < 0.1$). (b) Visualization of correlation trends between fungi:bacteria richness and AG activity and between oligotrophic:copiotrophic bacterial richness and NAG activity. AG activity is significantly positively correlated with F:B richness and NAG activity is significantly negatively correlated with O:C richness. Rates of enzyme activities are nanomoles of substrate per gram of soil per hour. Orange represents burned soils and blue represents unburned soils.

CONCLUSION

Soil microbial communities are some of the most biodiverse communities on Earth and are the unseen foundation of plant dynamics and ecosystem functions yet targeting the importance of belowground microbial complexity for plant and ecosystem function is a great challenge for ecological science. This is partly due to the fact that the advances in genomic sequencing produce a deluge of microbial data that can be very difficult to find meaning from. My dissertation stimulates progress in this area by exploring how relevance of soil microbial community composition for plant traits and ecosystem processes can be distilled from complex natural soil microbiomes.

Using a combined empirical and bioinformatic approach, my work reveals four major insights that have advanced the scientific understanding of the role of soil microbiome complexity for plant and ecosystem function. First, a small proportion of a complex soil microbiome can be associated with changes in plant traits (Chapter I). Using indicator species analysis, I identified that changes in growth of a widespread Solidago species were associated with soil bacterial and fungal taxa that represented just 0.2% of the total bacterial and fungal communities. This work shows that it is possible to pinpoint microbial taxa within complex soil microbial communities that are potentially important for plant function and that specific taxa could be harnessed to alter plant growth. Second, my meta-analysis on the response of plant-soil feedbacks to environmental gradients revealed that there is high variability in how soil microbiome-plant interactions respond to environmental disturbances, and as such, research that examines these interactions under environmental disturbance needs to be prioritized (Chapter II). This work reveals a significant gap in our scientific understanding: as the impacts of climate change are ubiquitous, much more research should be directed toward examining soil microbiome-plant interactions under climate change-induced disturbances.

Third, changes to particular microbial functional groups from fire disturbance can influence plant success on the landscape (Chapter III). By examining soil microbiome-plant interactions in an oak restoration context, this work showed that fire disturbance may alleviate some plants from interactions with soil microbial enemies by reducing diversity of plant pathogenic fungi in soil. These findings illustrate that effective restoration can be enhanced by considering how management practices influence belowground microbial communities. Fourth, fire-induced shifts

in microbial community composition can be important for ecosystem processes (Chapter IV). Using a previously established conceptual framework from the scientific literature, I demonstrated that it is feasible to link fire-induced changes to community composition of soil microbial life history strategies, such as potential for carbon use efficiency, to variation in ecosystem processes, such as carbon degradation. Broadly, this work shows that environmental disturbance can alter soil microbial community composition in ways that are functionally relevant at the ecosystem scale. In an applied context, the findings from this work could motivate further research of using microbial membership metrics to enhance accuracy of earth system model predictions. Collectively, the work of my dissertation shows that indicator taxa and functional groups within complex soil microbial communities are important at multiple ecological scales: individual plant functions, plant success on the landscape, and ecosystem function.

VITA

Kendall Kathleen Beals grew up in Chapel Hill, North Carolina and graduated from Dickinson College in 2013 with a Bachelor of Science degree in Biology. From there, Kendall worked for several ecology laboratories that led her to work in a wide array of ecosystems including the tropical rainforests of Australia and Gabon, the deserts of the southwestern U.S., and the temperate rainforests of Alaska. During this time Kendall developed a keen interest in understanding the relationships between soil microbial communities and plants. To pursue this area of research, Kendall returned to her Southeastern U.S. roots to begin a PhD at the University of Tennessee in 2016 with Dr. Jennifer Schweitzer. She received multiple grants during this time, including those from the American Genetic Association, the Torrey Botanical Society, and the University of Tennessee. To date, Kendall has authored and co-authored 8 peer-reviewed articles. She has received several awards from the University of Tennessee, including the Cokkinias-Fite Award for Outstanding Scholarly Achievement by a Graduate Student, the Sandy Echternacht Award for Outstanding Graduate Student based on Excellence in Research, and the Chancellor's Honors Award of Extraordinary Professional Promise. During the latter years of her PhD training, Kendall served as a co-organizer of the Women in Soil Ecology networking group that provides professional development opportunities for early-career women soil scientists around the world. Kendall will graduate from the University of Tennessee in 2022 with a doctorate in Ecology and Evolutionary Biology. Following graduation, she will work as a postdoctoral researcher at San Diego State University focusing on soil microbial ecology in restoration grasslands.