The role of above- and belowground interactions for plant allocation

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ABSTRACT

My dissertation builds upon decades of research on plant resource allocation, by incorporating multispecies interactions, above- and belowground, with the overarching goal of uncovering the role of a whole community in shaping plant resource allocation and change to subsequent interactions. I accomplished this goal by establishing two controlled common garden experiments where I manipulated the biotic community that a focal plant is exposed to. Using these experiments, 1) I tested whether plant neighbors alter a focal plant's resource allocation through changes to above- or belowground processes, and if these allocation changes resulted in any indirect effects on the focal plant's associated community interactions, 2) I examined the three-way relationship between a focal plant, its neighboring plants and its belowground community, with the objective of understanding how biotic context shapes a focal plant's functional diversity and its interactions with its associated belowground community, 3) I tested whether biotic interactions induce resource allocation growth-defense and growth-reproduction trade-offs in a focal plant. The findings from this dissertation show that focal plant resource allocation and trait variation are significantly affected by the whole community context. Some results were contingent on neighbor identity, such as whether plants are affected by above- versus belowground processes, others showed changes based on conspecific versus heterospecific interactions, regardless of heterospecific neighbor identity. We also provide evidence that changes to allocation and traits can result in growth-reproduction trade-offs based on the biotic context. In a time of exacerbated anthropogenic climate change, biotic interactions are changing at rates and in ways that we have yet to understand. This dissertation advocates for the need to incorporate multispecies interactions into mechanistic models to better understand plant persistence and success with these changing biotic conditions.

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INTRODUCTION

Carbon – the currency of plant resource allocation

Plant resource allocation is the dynamic process by which plants partition resources among competing plant functions (i.e., growth, reproduction, and storage), based on the availability of limiting resources and ecological interactions at play (Bazzaz et al. 1987). Since plants are not mobile, they rely on allocation strategies to optimally partition resources from carbon producing organs (sources) to competing functions such as growth and defense (carbon sinks), which requires constant regulation. Collectively, these dynamic processes ultimately determine plant fitness (Hartmann et al. 2020, Züst and Agrawal, 2017). Our understanding of plant allocation patterns is based upon frameworks dating back over half a century (Lerdau et al. 2023). Early theories described allocation tactics in relation to a plant's physical environment alone (Brouwer, 1963). Later optimal allocation theory posits that plants partition their resources based on limiting abiotic factors in a way that optimizes growth (Thornley 1972, McConnaughy and Coleman, 1999).

The inclusion of ecological interactions

Later, the importance of negative interactions such as competition and predation became defining parts of allocation theory (Poorter et al. 2012). Often likened to economics, plant carbon allocation was considered akin to cost-benefit analysis, and research sought to predict plant success based on the combined knowledge of how different plants acquire and allocate resources, along with their abiotic conditions, competition, and predation. Negative interactions were almost solely included as they were seen as limiting factors that can determine significant changes to plant allocation. More recently, the importance of positive interactions, such as mutualisms for plant carbon allocation has been highlighted (Wesselingh 2006, Pringle, 2016). Plants have evolved highly complex interactions with an array of organisms above- and belowground, resulting in some plants undergoing diverse adaptations to initiate and maintain ecological ties with other species, creatively altering their carbon usage to meet the demands of their biotic counterparts (Herrera et al. 2002, Ghazoul 2006). The vast diversity of floral forms is an example of how plants have diversified to maintain mutualistic interactions with pollinators.

Biotic interactions can also influence resource allocation strategies within the lifetime of an individual plant.

Encapsulating the community context

Given the adaptations that plants have coevolved with their biotic counterparts (Whittall and Hodges, 2007), it is clear that plant persistence is contingent not only the abiotic environment within which they are embedded, but also their biotic environment (De Deyn and Van Der Putten, 2005, Van Dam & Heil, 2011). Biotic interactions can determine how a plant allocates its resources (e.g., to growth or defense or setting seed; Zimmerman & Pyke, 1998), shape plant community composition (e.g., coexistence or competition; Guzman et al. 2019; García-Girón et al. 2020), determine basic nutrient needs (e.g., mycorrhizal interactions; Fierer et al. 2009), and as a result can also influence larger ecosystem processes such as biomass production, and carbon and nitrogen cycling (e.g., through plant-soil feedbacks [PSF], Bardgett et al. 2005). Furthermore, these myriad simultaneous interactions also mediate indirect interactions between above- and belowground organisms such as herbivores, pollinators and the soil microbiome, connecting and maintaining complex belowground (or brown) and aboveground (or green) worlds (Van Der Putten et al. 2009; Sotomayor et al. 2015, Mougi, 2020).

Since plants do not have access to unlimited resources, they must make biotic-induced trade-offs. At the expense of growth, reproduction and storage, plants provide carbohydrates to mutualistic biota, such as mycorrhizal fungi and pollinators (Pringle, 2016). For example, plants in alpine areas have been shown to decrease growth and increase flowering time, to attract pollinators, which are a scarcity in those regions (Fabbro & Korner, 2004). In return for the trade-offs, plants receive benefits from these interactions, such as defense, dispersal and pollination (Pringle, 2016, Lau and Bolin, 2024). Conversely, plants are also subject to a host of antagonistic interactions, such as competition from neighboring plants, herbivory, and pathogenic soil microbes, which can reduce plant performance or induce the production of defense compounds. Together the net consequences of these dynamic interactions determine the success of a plant population (Tao et al. 2017), local biodiversity (Valiente-Banuet et al. 2015), and are the building blocks of ecosystem processes, all of which can change when new species are introduced. Through

understanding the relative role of multispecies interactions for plant productivity, we can make better predictions about plant response to global change impacts, such as plant invasion and insect declines.

A Common Garden Approach

To disentangle complex relationships between organisms above- and belowground, this dissertation used a common garden approach. In doing so, I could factorially manipulate community members to understand how their presence or absence alters a focal plant's allocation patterns and subsequent biotic interactions.

Chapter one of this dissertation took place during the SARS-Covid 19 pandemic. I established a common garden experiment in my backyard, with *S. altissima* as the focal species, which I maintained for two growing seasons. The purpose of this experiment was to test whether plant neighbors alter a focal plant's resource allocation through above- or belowground mechanisms, and whether any induced changes affect the focal plant's associated community interactions, both directly and indirectly. I found that effects were contingent on neighbor identity, and though effects reduced with time, indirect temporal effects occurred in year two. This study contributes to a growing body of work showing how community context affects the above- and belowground interactions of a plant through plant resource allocation strategies.

Chapters two and three made use of data that I collected from a second common garden experiment, which I established on the University of Tennessee-Knoxville campus. This experiment began in May 2021, and continued for two growing seasons. The objective of the second chapter is to test how much trait variation arises in *Solidago altissima* as a result of its biotic interactions above- and belowground. Specifically, I was interested in the three-way relationship between a focal plant (*Solidago altissima*), its associated microbial community and its plant neighbors, and parsing out the direct and indirect effects of plant neighbor(s) on *S. altissima's* interactions with its belowground community. Using a trait-based approach, I found that focal plant morphology was affected by its associated belowground community more so than by plant neighbors. Furthermore, the *S. altissima* soil microbial community composition changed in polycultures through time, relative to plant neighbor monocultures, suggesting that changes to

S. altissima traits induced by plant neighbor result in different resource use, therefore indirectly altering the belowground soil community composition. These data experimentally show how multiple community interactions can determine plant traits, mediated by plasticity in plant resource allocation.

In the final data chapter of this dissertation, I switched focus from *Solidago altissima* to *Cirsium discolor*. In this study, we experimentally manipulated a focal plant's biotic community (plant neighbors and starting soil microbial community), to test how varying biotic interactions changed a focal plant's resource allocation to its leaves, and whether these changes induce different growth-defense and growth-reproduction trade-off strategies. Our focal plant, *Cirsium discolor*, was chosen because it is a biennial thistle, therefore we could test how the allocation patterns that occurred in the first year of growth influenced reproductive output in the second (and final year) of its life. We altered plant neighbor identity and richness, as well as the starting soil microbial community. In doing so, we tested how the relative effects of plant-plant and plant-soil microbial interactions altered *C. discolor* leaf allocation, and whether these cascaded to affect foliar herbivory and reproductive output. Based on resource allocation theory, we predicted that if a plant alters its resource allocation as a result of plant-plant interactions, these changes to carbon movement will cascade to affect other interactions. Similarly, plant-soil feedbacks can result in changed carbon allocation to aboveground plant functions (growth and reproduction), resulting in changes to herbivory, defense and reproductive output.

While we found no growth-defense trade-offs, which could be a result of low herbivory levels, we did find direct links between bacterial richness and herbivory, the magnitude of which changed based on plant neighbor. Our findings did uncover distinct growth-reproduction trade-offs for *C. discolor*, induced by both plant neighbors, but also to a lesser extent by the origin of the starting soil inoculum. Thus, independent of space and resource constraints, plants still faced trade-offs that significantly alter flowering quantity and quality of floral resources. And therefore, the biotic context within which a plant exists is an important determinant of allocation, further ecological interactions (such as herbivory and pollination), and potentially fitness outcomes.

Collectively, the aim of these experiments was to simultaneously examine the causes and consequences of above- and belowground communities and species interactions on focal plants. This approach yielded insights that would not be possible if I had only examined these interactions pairwise, as most studies commonly do, and clearly showed the relative importance of direct and indirect interactions on focal plant phenotype. These data indicate that ignoring community interactions can result in misleading results, which fail to identity important combined drivers of plant trait responses, mediated by variation in carbon allocation. Therefore, while traditional plant ecology studies that have studied plant phenotype and change provide important foundations for our understanding of plant resource allocation patterns, they could be missing the full picture. Lastly, this work also shows that real time feedbacks are consistently occurring within a plants growth cycle, showing the dynamic nature of plant response to diverse biotic conditions. None of these concurrent interactions are currently factored into predictive models of plant phenotype.

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CHAPTER I

PLANT NEIGHBORS DIFFERENTIALLY ALTER A FOCAL SPECIES' BIOTIC INTERACTIONS THROUGH CHANGES TO RESOURCE ALLOCATION

This chapter is accepted in Ecology, for a special feature on “pandemic pivots”. A version of this article is on a preprint server:

Turner, S. C., & Schweitzer, J. A. (2023). Plant neighbors differentially alter a focal species' biotic interactions through changes to resource allocation. *bioRxiv*, 2023-11.

Abstract

Plant resource allocation strategies are thought to be largely a consequence of changing abiotic conditions and evolutionary history. However, biotic interactions also influence how a plant allocates resources. As a result, plants mediate indirect interactions between organisms above- and belowground through resource allocation. Neighboring plants can influence plant fitness directly through competition for resources, and indirectly by altering associated community interactions (associational effects), such as pollination, herbivory and a suite of belowground interactions. Given the importance of community interactions for plant success, and the known ability for plant neighbors to change these interactions, the goal of this “pandemic project” was to understand how heterospecific plant neighbors alter plant resource allocation, whether this occurred through above- or belowground mechanisms, and if this in turn alters biotic interactions and the relationship between a focal plant and its herbivore and soil community interactions. To do so, we established a common garden experiment, manipulating plant neighbor identity and extent of interaction among neighbors (aboveground only, versus above- and belowground interactions, using customized pot types), and measured changes to a focal plant and its biotic interactions over two growing seasons. We found evidence of both neighbor effects and pot type, showing that neighbor interactions affect a focal plant through both above- and belowground processes, and how the focal plant is affected depends on neighbor identity. Though neighbors did not directly alter herbivory or most soil microbial interactions, they did alter the relationship between belowground microbial communities and a plant response trait (specific leaf area). Plant resource allocation responses were reduced with time, showing the importance of extending experiments beyond a single growing season, and is an important consideration when making predictions about plant responses to changing conditions. This study contributes to a growing

body of work showing how community contexts affect the above- and belowground interactions of a plant through plant resource allocation strategies.

Keywords: above- and belowground interactions, associational effects, Asteraceae, biotic interactions, competition, interspecific, intraspecific, plant-plant interactions, plant resource allocation, *Solidago*

Pandemic Pivot

To understand heterospecific plant neighbor associational effects on *Solidago's* above-and belowground interactions, we initially planned a common garden experiment in an old field research site, using locally collected seeds. In doing so, we could manipulate neighbor interactions and have access to a realistic source pool of associated communities. Due to travel restrictions imposed, a field experiment was not possible during the pandemic, and so we pivoted to conducting this experiment in a suburban backyard, which came with some limitations. The confines of the suburban environment significantly reduced the abundance of insects in the experiment. Therefore, despite our study species, *Solidago altissima*, being well-known for its abundance of interactions with pollinators and herbivores alike, we found no evidence of galling, one of *Solidago's* quintessential interactions, and observed low evidence of herbivory. Another constraint was the availability of space, which meant reducing replication and power in the experiment. During the first year of this experiment, the pandemic was in full swing, and we had no research facilities on campus available for use, so soil samples had to be kept frozen in a conventional household freezer (~ -18 C). Amidst the move of samples to campus, a freezer failure and freezer cleanout, one bag of samples (~10 samples) disappeared. Fortunately, two years of data compensated for this disappearance, though the mechanisms of temporal change in microbial community are hard to uncover. Two important advantages to the backyard experiment were that plant care and watering could be managed on a day-to-day basis instead of regular trips to a field setting, resulting in a very well-attended experiment. Also, we had a dog on the property, who kept the groundhog, squirrel and rabbit activity in the experiment at bay. Although, a squirrel did manage to plant a walnut seed in one of our pots over the first winter, this pot was removed from analysis. Overall, this two-year experiment, despite the constraints of

the pandemic, advances our knowledge on neighbor associational effects by showing that the processes that lead to neighbor effects are species specific, largely aboveground rather than belowground, and that effects are reduced over time.

Introduction

Resource allocation is a critical physiological process that plants undertake to distribute acquired resources, such as carbon and nitrogen, to growth, reproduction, or storage, and is primarily used to optimize fitness (i.e., the ability to successfully reproduce) under different conditions (Bazzaz et al. 1987, Monson et al. 2021, Hartmann et al. 2020). Theory predicts that a plant will allocate resources based on limiting factors (Freschet et al. 2015; Revillini et al. 2016), generally based on abiotic limitations (Tilman, 1982; Gleeson and Tilman, 1992). Therefore, if light limited, a plant would invest carbon in plant growth toward the light, if nutrient limited, a plant would increase fine root production to increase fungal mutualisms, thereby increasing nutrient uptake (Mikkelsen et al. 2008). The response of a plant to limiting factors is both environmentally (i.e., climate and other abiotic factors) and genetically based, with some plants having quick, wide-ranging responses, while other responses can be slower and more restricted (Pierce et al. 2017; Pierce & Cerabolini, 2018). Responses to limiting abiotic conditions result in individual variation in plant performance (e.g., growth, flower success, root production), which in turn can scale up to affect a plant's interaction with other organisms (Pangesti et al. 2013; Aschehoug et al. 2016). In addition to abiotic variation, plants also experience a complex web of direct and indirect interactions, which all occur simultaneously, and all affect how plants invest resources (Agrawal et al. 2006; Kos et al. 2015; Loranger et al. 2013). Insect herbivores can induce the production of volatile compounds, but this comes at a cost, reducing a plant's ability to allocate resources elsewhere (Erwin et al. 2014; Monsson et al. 2021). These changing resource allocation patterns result in organisms above- and belowground being indirectly connected through their common usage of plant resources (Wardle et al. 2004; Mulder et al. 2013; Mougi, 2020; Friman et al. 2021). For example, root herbivores indirectly interact with aboveground herbivores, mediated by plant production of volatile compounds, and this relationship differs based on genotypic plant diversity (Ohgushi, 2008; Wurst et al. 2008). As a result, plant resource allocation is a dynamic

compilation of trade-offs used to optimize survival, growth and fitness, the consequences of which affect plant-biotic interactions.

Furthermore, neighboring plants can influence plant fitness directly through resource use, and indirectly by altering associated community interactions (also known as associational effects) (Underwood et al. 2020). Direct plant interactions can result in facilitative effects (i.e., the focal plant performing better in species mixtures than in monocultures), resulting in increased plant performance. The opposite pattern can emerge either from intraspecific interactions inducing facilitation (Zhang & Tielbörger, 2019), or by plant neighbors reducing performance through competition for resources or light (Craine et al. 2013; Sauter et al. 2021). Since plant species allocate and utilize resources differently, different plant neighbors should elicit differing responses in a focal plant. For example, if a plant neighbor results in high shading, a focal species would invest more resources in growth for light. Conversely, if a plant neighbor is a strong competitor or has more belowground allocation to roots, the focal plant could increase fine root production to acquire sufficient nutrients. Therefore, plant neighbor identity can create limiting conditions, driving plant resource allocation patterns.

Neighbor effects on a focal plant's resource allocation can result in different outcomes for associated community interactions. Plant-herbivore interactions are often associated with the density of palatable plant matter (Andersson et al. 2013; Kim et al. 2015), and so if plant neighbors are not palatable to herbivores, then species mixtures would have lower levels of herbivory compared to monocultures. However, if all plants in the mixture have shared herbivore interactions, then species mixtures will experience higher herbivory. These changes to the extent of interactions can alter above- and belowground interactions between organisms – if a plant experiences higher herbivory, it could increase plant stress, thereby changing allocation to fine roots and root symbionts (top-down cascades). In addition, plant neighbors could increase belowground microbial diversity, which could alter the focal plant's ability to acquire nutrients, thereby reducing plant performance and increasing herbivory (bottom-up cascades). Thus, complex interactions among neighboring plants and diverse above- and belowground communities that associate with a plant, can all be mediated by resource allocation to various plant traits, the results of which can alter indirect linkages among community members, and

potentially, overall patterns of biodiversity. Unfortunately, empirical studies incorporating this complexity are rare.

Given the importance of biotic interactions for plant success (Pangesti et al. 2013; Tao & Roode, 2017), and the known ability for plant neighbors to change community interactions (Hamback et al. 2014; Kos et al, 2015, Underwood et al. 2015), the goal of this paper was to understand how heterospecific neighbor interactions alter a focal plant's resource allocation and response traits, their availability of associated community interactions, and whether induced changes alter the relationship between the focal plant's allocation and its associated community. The allocation measures that we quantified were above- and belowground biomass, root-to-shoot ratio and relative growth rate (RGR), while the response traits that we measured were specific leaf area (SLA), and the number of new shoots produced by the focal plant. We were specifically interested in the focal plant's associated herbivore and soil microbial (bacterial and fungal) communities. We established a two-year common garden experiment, with three common old-field Asteraceae species (focal species: *Solidago altissima,* heterospecific neighbors: *Achillea millefolium, Silybum marianum*) to test how heterospecific interactions change the focal plant's patterns of allocation (Fig. 1). We grew all plant combinations in two different pot types that separated above- and belowground interactions to test whether heterospecific neighbors altered a focal plant's allocation and subsequent interactions through above- or belowground processes. Having two years of data allowed us to analyze both years together (with year as a random effect), as well as separately, to determine if changes to allocation patterns are consistent over time. We hypothesized (Fig. 2) that: 1a) the focal plant will elicit a different resource allocation response based on the identity of a plant neighbor and 1b) that any effects will increase in year two due to resource limitation, which can increase competitive effects; 2a) in polycultures with the same abundance, we expect lower levels of herbivory, since the monocultures have a higher density of *S. altissima*, which is more palatable than either of the other two species (Felix et al. 2023). We also expected belowground interactions with heterospecific neighbor species to increase soil microbial richness and diversity, and alter microbial composition due to heterospecific plant neighbor species interacting differently with their soil microbiome; 2b) changes to plant resource allocation induced by heterospecific neighbor or pot type will result in

changes to the relationship between the focal plant's allocation and response traits, and its associated community, specifically its belowground soil community and foliar herbivory (i.e., indirect changes to associated community interactions).

Methods

Study Species

In May 2020 (during the SARS CoV-2 [COVID-19] lock-down) we set up a common garden experiment, with *Solidago altissima* (Tall Goldenrod), a ubiquitous and ecologically important native plant species in Eastern Tennessee, as a focal species. *Solidago altissima* was grown with *Achillea millefolium* (Yarrow), a naturalized species in the region (USDA 2022), as well as a non-native species, *Silybum marianum* (Milk thistle, USDA 2022) to address specific hypotheses about neighbor interactions. All three species are in the family Asteraceae, are insect pollinated, and grow in similar, disturbed, old-field habitats. *Solidago altissima* is a useful focal species for this study as it is a fast-growing perennial, that has high phenotypic diversity (Etterson et al. 2008). Research has shown the influence of interspecific genotypic diversity (i.e. neighbors) on above- and belowground biomass and other traits of *Solidago* spp. (Genung et al., 2012, 2013), suggesting that *Solidago* patterns of carbon (C) allocation and phenotype may be mediated, in part, by neighboring species.

In this experiment the focal plant, *S. altissima*, was grown from rhizomes, and the heterospecific plant neighbors, *A. millefolium* and *S. marianum* were grown from seed. Each species was grown in separate, covered trays with a generic potting soil consisting of equal parts peat, vermiculite, and perlite (Premier Pro-Mix BX) and given equal amounts of water. After five weeks, we planted the seedlings into pots in a suburban backyard, using the same Pro-Mix BX, filled to the top of the pots, leaving a lip of 3 cm to prevent any splash over effects between pots, and inoculated with a tablespoon (~15 g) of a common field soil per plant. Field soil used for the inoculation was collected using an Oakfield soil sampler at a depth of 15 cm, from Forks of the River Wildlife Management Area in Knox Co. Tennessee. Three Ziploc gallon bags of soil were collected in total, and all three were homogenized in a sterile container before application to the experiment. At the time of soil collection, the field was recently mown, and therefore the soil

was not associated with a known plant species. The purpose of this inoculum was to represent a common local soil microbial community to see if differing plant resource allocation patterns (as a result of different plant neighbors), altered the microbial community through time. The collected soil was refrigerated at 5° C until applied to the experiment (less than 10 days later). In July 2020, we collected *S. altissima* rhizomes, from the same area as the field soil inoculum, but a different section of the preserve. Rhizomes were cut to similar lengths (~ 10 cm), dipped in rooting hormone (Bonide Bontone II Rooting Powder Plant Growth Regulator), and propagated in Pro-Mix. After four weeks, we added *S. altissima* plants to the potted experiment, also inoculated with the same field soil (stored at 5° C until planting) used for heterospecific plant neighbors. By planting *S. altissima* four weeks later, we can test how the presence of heterospecific plant neighbors affects *S. altissima* establishment and patterns of carbon allocation. We ran the experiment over two growing seasons to determine if observed patterns changed through time.

Experimental design

The common garden experiment was established in a sectioned off part of the first author's backyard. The area used was a 10 m x 8 m, level area nearby a water source. Shading from the house and a tree on either side of the experiment occurred, but based on the aspect of the house, shading occurred evenly across the experiment, with some pots receiving morning shade and others receiving afternoon/evening shade. To account for this, pots were rotated once in each growing season. We found no evidence of shade affecting the data. Pots were placed atop pallets, to be slightly raised from the ground, thereby preventing fine roots from escaping the pot and interacting with a new soil community. Due to space constraints, pots were placed 0.3 m apart. We ensured plants from separate pots did not interact with one another, however, being so close together meant that herbivore effects could be an artefact of placement rather than plant neighbor in the pot. Throughout the entirety of the experiment, plants were watered equally, as needed, with tap water. Watering varied from three times a day in the height of summer year one, to only once a morning, to every few days. We added a slow-release fertilizer to the experiment once, in

the summer of year two (Osmocote Smart-Release Plant Food, NPK = 14-14-14, 1 tablespoon per pot).

We used customized, cube-shaped pots in this experiment, made from polypropylene plastic, with a length, width, and height of 0.33 m. Half of the pots had an additional sheet of polypropylene secured in the middle of the pot, dividing the pot in half; silicone adhesive was also applied to all seams to reduce water movement. This divider is both water- and airproof, thereby preventing any belowground interactions between plants on each side (but allowing aboveground interactions; hereafter called "split" pots). These pots were used in previous experiments (e.g., Genung et al. 2013), and the design was shown to not reduce total biomass compared to pots without the divider. The other half of the pots had no divider, allowing above- and belowground interactions, hereafter called "open" pots.

To address how *S. altissima* resource allocation changed based on interactions with each of the neighbor species, and whether the interactions were above- or belowground, we used a manipulative experiment (Fig. 1), planting four seedlings per pot in three combinations: 1) *S. altissima* ×*S. altissima*, 2) *S. altissima* ×*A. millefolium*, and 3) *S. altissima* ×*S. marianum*. The species combinations were planted in split pots (to impede heterospecific belowground interaction) as well as open pots (to allow heterospecific interaction above- and belowground). Thus, there were a total of six experimental treatments (3 plant neighbor treatments ×2 pot types). It should be noted that in all treatments *S. altissima* experienced intraspecific interactions, specifically, the polycultures had two *S. altissima* individuals with two heterospecific neighbor individuals, while the monocultures had four *S. altissima* individuals. Our *S. altissima* success rate was low, and as a result we had an uneven sampling design (Fig. 1B), with a total of 5-9 pots per treatment (20-36 plants per treatment). The experiment ran for two growing seasons, for a total of 16 months in the backyard.

Interpretation of treatments

There are many pathways by which a plant neighbor can alter a focal plant, either directly through resource use or through interactions with other members of the community, or indirectly through associational effects. The mechanisms for these effects can occur either aboveground,

such as competition for light, or belowground competition for space and resources (e.g. nutrients and water). Based on our experimental design, we can parse out the likely pathway through which our focal plant is affected by its heterospecific plant neighbors. For instance, in polycultures, if the focal plant allocation and response traits are significantly increased in the split versus open pots, this means that the heterospecific neighbor reduces the focal plants performance through belowground processes. Conversely, if focal performance decreases in the split pot compared to open pots, this means that the focal plant and its heterospecific neighbor have a positive belowground interaction. If there is no pot effect, then heterospecific plant neighbors do not affect the focal plant's growth through belowground processes. Furthermore, if there is no pot effect and there is a neighbor effect (i.e., heterospecific reduces focal performance regardless of pot type), we can surmise that the heterospecific neighbor effects the focal plant through aboveground processes (i.e., competition for light). No significant difference based on plant neighbor or pot type would indicate that the effect of polycultures is no greater or less than that of monocultures and that neighbor identity is not important for plant-plant interactions. Using our study design, we have identified eight possible direct pathways, pertinent to Hyp. 1a and 2a (Table 1) and five indirect pathways, pertinent to Hyp. 2b, of how heterospecific neighbors could alter a focal plant's resource allocation (Table 2).

Data Collection

We calculated the relative growth rate (RGR) of all plants from the first height measurements taken after *S. altissima* was added to the experiment, to the final height data collected before the first harvest (20 weeks). At the end of the first growing season (~five months after initiating the experiment), we removed the aboveground biomass of all individuals, which was dried at 70° C for 48 h, then weighed. Relative growth rate and aboveground biomass were used as proxies for plant performance. Pots remained uncovered over winter and were weeded as needed. At the start of the second growing season, we counted the number of new shoots that *S. altissima* produced in all pots, using mean shoot number as a proxy for plant fitness. We used height measurements taken at the beginning and end of the second growth season to calculate RGR for year two. At the end of the second growing season, we harvested both above- and belowground

biomass. The roots were washed, and all biomass was dried at 70° C for 48 h and weighed for belowground biomass. These data were used to calculate the root-to-shoot ratio (year two root mass/year two aboveground biomass). In doing so, we can test whether *S. altissima* altered allocation of resources above- and belowground based on heterospecific plant neighbor or pot type. During the second growing season, we also collected two random leaves per individual, occurring within the upper quarter of the plant (on the terminal shoot), ensuring they were fully expanded, and representative of the plant. We calculated leaf area using LeafByte (Getman-Pickering et al. 2020), after which, we dried, weighed, and calculated specific leaf area (SLA). We chose measures of allocation (biomass and relative growth rate), as well as response traits (mean number of new shoots and SLA), because the allocation measures provide information on how the focal plant changes its carbon allocation strategies based on plant interactions. The mean number of shoots provides information on potential fitness effects, as it is a response that can have reproductive consequences. Specific leaf area was measured as it provides information on plant functioning, whether the plant is conservative or acquisitive, and coupled with biomass is an important trait for identifying changes to resource allocation (Firn et al. 2019). Further, since SLA can provide information on whether plant neighbors affected leaf quality, it provides a mechanistic pathway to explain neighbor effects on a focal plant's herbivory levels (Felix et al. 2023).

To determine if heterospecific plant neighbor alters the focal plants above- and belowground interactions (Hyp. 2), we focused specifically on quantifying rates of foliar herbivory and belowground microbial communities in response to neighbor and pot. Herbivory estimates were measured near the end of the second growing season, as a proxy for accumulated herbivory, using the LeafByte app to estimate herbivory based on quantification of leaf area missing (Getman-Pickering et al. 2020). At the end of each year's growing season, we collected interspace soil between the two target *Solidago* individuals in each pot to a depth of 15 cm with an oat-field sampler. Soil samples were frozen (initially at -18° C, then moved to -80° C) until DNA was extracted for sequencing. By characterizing the belowground community, we can determine if variation in plant growth and belowground resources altered soil microbiome

diversity and composition, as well as whether there were changes to indirect relationships between herbivory rate and belowground community diversity.

DNA extractions and Illumina MiSeq sequencing

All soil samples had DNA extracted using the DNeasy PowerSoil kit, following the manufacturer's protocol (QIAGEN Inc., Germantown MD, USA). A two-step PCR approach was used for amplicon sequencing. We amplified the V3-V4 gene region of 16S rRNA, using Illumina recommended forward and reverse primers, modified with adapters for the Illumina MiSeq platform (341 F: CCTACGGGGNGGCWGCAG, 785 R: GACTACHVGGGTATCTAATCC, Klindworth et al. 2013) (Eurofins). Amplification success was confirmed by running each sample on a 2% agarose gel (Sigma-Aldrich, St. Louis, MI, USA).

Agencourt Ampure XP magentic beads were used to clean initial PCR products of any unincorporated nucleotides. We then amplified the cleaned products in a second PCR, using the Nextera XT index kit (Illumina Corporation, San Diego, CA, USA). This second-step PCR consisted of 25 ul KAPA HiFi HotStart taq (KAPA Biosystems, Wilmington, MA, USA), 5 ul each of unique combinations of Nextera XT index primers 1 and 2, and 5 ul of initial PCR product, brought up to 50 ul with PCR grade water. Agencourt Ampure XP beads were again used to purify the now indexed PCR products. These products were then quantified on a NanoDrop 1000 spectrophotometer. Amplicons were then pooled for efficiency, quality and quantity, checked on an Agilent Bioanalzyer, and diluted to 4 pM. For each run, the diluted products were combined with PhiX control DNA (Illumina Corporation, San Diego, CA, USA) at a ratio of 20 % PhiX, loaded onto a v3 600-cycle flow cell set for a paired-end read of 275 bases each, then sequenced on the Illumina MiSeq at the University of Tennessee Genomics Core (Knoxville, TN, USA).

Statistical approach

To address how *S. altissima* resource allocation and response changed based on interactions with each of the neighbor species, with and without belowground interactions (Hyp. 1, Fig.2), we measured: relative growth rate (RGR) and aboveground biomass in both years, and root:shoot in

year two. In the second year, we measured two response traits, SLA and number of new shoots produced.

We first fitted linear mixed effects models using the lme4 package in R (R Core Team, 2020).The response variables used were the measures of allocation from both years (RGR and aboveground biomass), and we tested whether they were affected by our predictors – pot type (open or split), neighbor species identity (*S. altissima*, *S. marianum*, *A. millefolium*), and the interaction between each of these groups (fixed effects), with year included as a random effect (Hyp. 1a). The Wald's test was used to identify significant differences in our model (using the Anova function in the *car* package), followed by the Dunnett's post hoc test for pairwise comparisons.

To address Hyp. 1b we analyzed each year's data separately. We built multiple regression linear models and used the "Anova" function in the *car* package in R to test for relationships between performance indicators (year one: aboveground biomass and RGR; year two: mean number of new shoots, above- and belowground biomass, root:shoot, RGR and SLA) and our predictors (Fox & Weisberg, 2019). As with Hyp. 1a, the predictors used were pot type (open or split), neighbor species identity (*S. altissima*, *S. marianum*, *A. millefolium*), and the interaction between each of these groups. When testing how aboveground biomass and RGR of the focal *S. altissima* changed based on our predictors, we also included neighbor aboveground biomass as a covariate in the initial aboveground biomass model, and neighbor RGR as a covariate in the initial RGR model. We included neighbor allocation (aboveground biomass or RGR) to determine if changes to *S. altissima* resource allocation were as a result of the identity or growth of its heterospecific neighbor. While the addition of a covariate reduces degrees of freedom, it increased the explanatory power of our models. All predictors were included in the initial model, then the "StepAIC" function in the *MASS* package in R was used to identify the most suitable predictors to keep in the model (Venables & Ripley, 2002). A new model including only suitable predictors was performed, and an Anova was used to test for significance. In the case where significant interactions occurred, we split the model to identify how interactions changed based on neighbor identity and pot type. Tukey HSD post hoc tests were used to determine significance within groups. It should be noted that *S. altissima* and *A. millefolium* are perennials, and therefore grow

new aboveground biomass each year, whereas *S. marianum* is a monocarpic biennial (sometimes acting as an annual). This was a nutrient poor experiment, to increase the competitive interactions, therefore *S. marianum* acted as an annual. Consequently, *S. marianum* individuals were no longer in the experiment in year two. However, we chose to keep those treatments in the analyses, since plant neighbors can have legacy effects. In doing so, the pots that contained *S. marianum* in the first year changed to represent the legacy effects of *S. marianum* in year two. This does, however, mean that each heterospecific treatment had to be interpreted slightly differently.

To understand if there were direct effects of heterospecific neighbors on *S. altissima* interactions above- and belowground (Hyp. 2a), we measured foliar herbivory rates in the second growing season using leaves collected for SLA and the Leafbyte app, and the soil microbial community at the end of each growing season (i.e., years 1 and 2) to assess changes in the soil microbiome due to the predictors. We standardized the herbivory estimates by leaf area. Once standardized, the data were normally distributed, and we were able to fit a linear model and use the Anova function to test for the effect of neighbor identity, pot type and their interaction on foliar herbivory.

The soil microbial community from each sample was assessed using the DADA2 pipeline to trim and merge microbial sequence reads, to run quality control checks on data, and group data into exact sequence variants (amplicon sequence variants or ASVs), which we refer to as bacterial and fungal richness. In addition, we calculated *S. altissima* associated bacterial and fungal diversity using the Shannon-Wiener Diversity Index. The Shannon-Wiener Index is the most commonly used index in community ecology and provides information on both richness and evenness. We then used linear mixed effects models and Anovas to test if bacterial and fungal richness and diversity differed based on neighbor identity, pot type, their interaction, and year as a random effect. We also used the jtools package for summary statistics (i.e., standard error and estimates). We did not have the statistical power to analyze data from each year separately. Using a principal component analysis (PcoA) with the Bray-Curtis dissimilarity index, we visualized differences in bacterial and fungal community composition, then tested whether

bacterial and fungal composition changed based on neighbor identity, pot type, and year, using PERMANOVAs (Permutational multivariate analysis of variance).

To understand if the relationship between SLA and associated communities is affected by neighbor identity or pot type (Hyp. 2b), we fit generalized linear models (with a Gaussian error distribution and log link) to test how the relationship between SLA and belowground community diversity, and SLA and herbivory changed as a result of neighbor identity and pot type. No significant relationship would indicate that our data is unable to detect a link between SLA and the associated community or an effect of neighbor identity. A significant relationship would show that plant function is affected by the associated community. If a significant relationship occurred, we further explored whether there was a relationship with plant growth (biomass and RGR) using linear models, and the jtools package to summarize model results. All analyses were conducted in R Studio (R Core Team, 2020).

Results

Effect of heterospecific plant neighbor on focal plant's allocation and response traits

Overall, we found evidence of both neighbor effects and some pot type effects, indicating variation in above- vs. belowground interactions, on *S. altissima* resource allocation. The direction of resource allocation changes tended to differ based on neighbor identity, highlighting the importance of incorporating neighbor identity when studying associational effects (Cahill, 2022; Mutz et al. 2022). Our linear mixed effect models testing Hyp. 1a showed significant pot type effects on *S. altissima* aboveground biomass and RGR (Table 3), this was driven by *S. marianum*, which reduced both aboveground biomass and RGR in open pots. Analyzing each year separately showed that *S. marianum* no longer affected biomass in year 2, when the plant was no longer present (Fig. 3). We also found that, *S. altissima* RGR increased as heterospecific neighbor RGR increased, but only without belowground contact, i.e., split pots (Hyp.1b, Appendix S1: Table S1). With the presence of belowground heterospecific interaction (open pots) this changed, and *S. altissima* had a reduced RGR with belowground heterospecific interactions, compared to conspecifics (Appendix S1: Table S1) suggesting higher competitive effects. *Solidago altissima* RGR in open pots changed depending on neighbor identity, with a

significant neighbor identity x neighbor RGR interaction in both pot types, which by year two only occurred in open pots (Appendix S1: Table S1).

In year two, root biomass, root:shoot and SLA had significant differences in the split pot treatment between *S. altissima* monocultures versus *S. altissima* planted with *S. marianum* – performing better in monocultures (i.e., higher belowground biomass and lower SLA; Fig. 3). There were no neighbor effects of *A. millefolium* on *S. altissima* root biomass or SLA Hyp. 1a, Fig. 3). We found evidence of interspecific competition, as *S. altissima* yielded higher biomass above- and belowground when planted with itself compared to the heterospecific neighbors. *Solidago altissima* mean shoot number was significantly affected by the mean number of shoots its neighbor had in open pots but not in split pots (i.e., when exposed to belowground interaction, *S. altissima* was negatively affected by the mean number of shoots that *A. millefolium* produced ($Chi^2 = 4.5124$, df = 1, P = 0.034)). While significant, this was a weak negative relationship, with a coefficient of -0.053. Further, *S. altissima* mean shoot number was significantly higher when planted with itself, or in the legacy *S. marianum* pots (no other plant interaction), compared to pots with *A. millefolium*. Interestingly, when *S. altissima* was grown with itself, irrespective of pot type, it produced significantly more shoots compared to when planted with the other neighbors ($Chi^2 = 5.891$, df = 1, P = 0.015).

Taken together, these results support our first hypothesis (1a), that *S. altissima* allocation (above- and belowground biomass and RGR) is reduced with heterospecific plant interactions, though the mechanism is not consistent across traits measured or time (1b) Further, the effect differed based on neighbor identity, with *A. millefolium* reducing biomass and the mean number of new shoots, and *S. marianum* increasing SLA and decreasing belowground allocation.

Effect of heterospecific plant neighbor on foliar herbivory and soil microbial community structure

We found limited support for hypothesis 2a. Herbivory did not differ significantly based on neighbor identity or pot type, though split pots did seem to increase variation (Fig. 3, Appendix S1: Table S1). Furthermore, both bacterial and fungal richness did not differ significantly based on neighbor identity or pot type (Fig. 4 panel A and B, Appendix S1: Table S2). Contrary to

expectations, bacterial diversity increased in conspecific split pots compared to open pots (est. = 0.35, S.E. = 0.16, p = 0.03), and bacterial diversity was significantly lower in *S. marianum* split pots compared to the conspecific split pots (est. = -0.41. S.E. = 0.21, p = 0.05), though this model did not explain a lot of the variation (fixed effect $R^2 = 0.08$, total $R^2 = 0.12$ (Fig. 4 panel C)). Heterospecific neighbors did reduce fungal diversity, though to different extents and in both cases with no pot effects (*A. millefolium*: est. = -0.26, S.E. = 0.25, p = 0.31; *S. marianum*: est. = 0.50, S.E. = 0.25, p = 0.05), shown in Figure 4, panel D. This neighbor effect explained a tenth of the variation (fixed effect $R^2 = 0.10$, total $R^2 = 0.21$), with year as a random effect. Figure 5 illustrates the results from PERMANOVA tests, showing that both bacterial and fungal community composition was mostly driven by year (bacteria: F = 15.438, P = 0.001; fungi: F = 4.8368, P = 0.001), rather than neighbor identity (bacteria: F =0.8293 , P = 0.828, fungi: F = 1.1331, P = 0.238) or pot type (bacteria: F = 1.2082, P = 0.152; fungi: F =1.407 , P = 0.078).

Linkages between plant allocation and response traits, and associated community interactions

We found no evidence of a relationship between *S. altissima* SLA and herbivory. However, we did find a negative linear relationship between SLA and bacterial richness and a quadratic relationship with bacterial diversity (Appendix SI: Figure S1). Linear models summarized in Table 4 show that bacterial diversity increases *Solidago altissima* allocation to both above- and belowground biomass (i.e. larger plants overall); and decreases SLA. However, heterospecific plant neighbors change this relationship, whereby *A. millefolium* reduces above- and belowground biomass, and increases SLA, while *S. marianum* reduces biomass and increases SLA. Fungal diversity did not have a strong relationship with SLA; however, it did have a positive effect on aboveground biomass and RGR. *Achillea millefolium* alters this relationship through above- and belowground biomass reduction, and *S. marianum* through biomass reduction and increasing in SLA (Table 4).

Discussion

The overall objective of this pandemic pivot project was to test the effects of heterospecific plant neighbors on resource allocation of a focal plant, and to determine if changes to allocation altered direct or indirect interactions among diverse plant-associated communities above- and

belowground. Plant neighbor effects have long been studied in a variety of contexts (Goldberg, 1987, Tremmel, D. C., & Bazzaz,1993, Yang et al. 2013, Kos et al. 2015, Gough 2006). While many show that plant neighbors can alter a focal plant's traits and fitness, not all extend to biotic interactions, or if they do it is pairwise interactions (Hausmann & Hawkes, 2009). We found one study that compared effects of plant neighbors on a focal plant's the above- and belowground interactions (Kos et al. 2015), though it is difficult to compare as this experiment only lasted for three months. Here, we demonstrate how heterospecific plant neighbors affect a focal plant and some of its biotic interactions, directly and indirectly, and how these effects change over two growing seasons.

Plant-plant interactions – effects on plant resource allocation

We found that heterospecific plant neighbors do indeed alter *S. altissima* resource allocation patterns, but how these changes manifest differs based on plant neighbor identity. Species-specific responses have been shown by others (Kos et al. 2015, Kim et al. 2015, Mutz et al. 2022), and are likely due to plants acclimating to differing resource-consumer relationships with their neighbors (i.e., heterospecific neighbors use resources differently, therefore the focal plant responds differently). For instance, in year one, heterospecific plant neighbors reduced *S. altissima* aboveground biomass, but by year two, when *S. marianum* was no longer in the experiment, there was no longer a neighbor effect. In contrast to classic plant competition literature, which favor belowground processes as a source of neighbor effects (Tilman 1990), we found more support for aboveground neighbor effects; for instance, aboveground biomass was driven by neighbor effects in year one. Furthermore, year two belowground biomass, root:shoot, and SLA, changed as a result of heterospecific neighbors and not pot type, suggesting that competition is not for nutrients, and instead was driven by aboveground resource needs. This shows that even in nutrient depauperate environments, such as this experiment, aboveground mechanisms (such as light and space) can drive neighbor effects. Broadly, naturally occurring *Solidago* complexes (spp. *canadensis*, *altissima* and *gigantea*) are known to dominate old field systems, with other studies demonstrating light availability as a key mechanism for this dominance (Eckberg et al. 2023). Our work substantiates the importance of light availability in

mediating plant interactions with both other plants and associated communities (Borer et al. 2014). That being said, trait responses were not consistent (Beals et al. 2023); for example, *S. altissima* produced significantly fewer shoots when planted with *A. millefolium*, particularly in open pots, showing belowground interactions impeded the focal plants' mean shoot production (i.e., a bottom-up effect), and therefore neighbor identity can change whether above- or belowground processes drive plant-plant interactions.

Effects on biotic interactions above- and belowground

Analyses showed that herbivory was not greatly affected by heterospecific neighbors nor pot type, showing that heterospecific neighbors had little direct effects on these interactions. This surprising lack of effects could either be due to the experiment being conducted in an area with low natural herbivore abundances or because the method we used only considered foliar chewing damage. *Solidago altissima* in natural systems is subject to mining, galling and tunneling by a diversity of herbivores, and a method that incorporated these types of damage would have been more appropriate. No significant difference in belowground richness could be an artefact of low sample size and effect size, and high variation within treatments, as *S. altissima* has been shown to harbor high intraspecific variation in plant-microbiome interactions (Foster et al. 2022). It is also possible that all three Asteraceae species share soil symbionts, or that changes to *S. altissima's* microbial interactions occurred within the roots instead of the associated soil (Hannula et al. 2021). There was some evidence of heterospecific neighbors reducing fungal diversity; this negative effect was more so with *S. marianum* than *A. millefolium*. Due to sample size limitations, we could not tease apart whether this occurred in both years. There was no pot type effect, indicating that the reduction in fungal diversity was a result of aboveground processes. For instance heterospecific neighbors altered aboveground biomass, which in turn altered fungal diversity.

The most notable observed changes to associated community interactions that we did find were indirect. We found indirect effects of the *S. marianum* treatment, whereby *S. marianum* reduced *S. altissima* aboveground biomass and RGR in year 1, which resulted in an increased SLA and reduced belowground biomass in year 2, thereafter altering the relationship between *S. altissima*

SLA and belowground diversity. There were no pot type effects, suggesting that this was a top-down indirect interaction, in which competition for light in year one resulted in *S. altissima* having a reduced aboveground biomass and this cascaded to affect allocation outcomes in year 2, which further changed the relationship of the focal species with both bacterial and fungal diversity.

Our results show most support for scenario 1.3 and 1.4 (Table 1) whereby most of our plant allocation measures showed neighbor effects on the focal plant with no effect on herbivore and limited effect on belowground community interactions. Some pot effects were present, however, showing heterospecific plant neighbors can have effects through belowground interactions, such as with RGR. Taken together, these intricate interactions show that *A. millefolium* has direct effects on plant aboveground allocation and shoot production but not SLA and has limited associational effects. In contrast, the invasive *S. marianum*, and its legacy effects, showed negative effects on focal allocation above- and belowground, response traits and thereby indirectly altering belowground interactions. These results show that different plant neighbors elicit differing responses, affecting a focal plant through multiple pathways. While these indirect interactions may seem cryptic, they are not without importance, as it is through a complex combination of many indirect pathways that plant's function and performance is governed.

Contextual considerations

Throughout this experiment, we uncovered demonstrable evidence of neighbor effects on resource allocation and the mechanisms thereof, however there are some important considerations when interpreting these results. We conducted this experiment during the height of the COVID-19, in a backyard, while research facilities were not accessible. As a result, we are limited in the inference we can draw from these data due to the context within which this experiment occurred. Two main limitations occurred. We were space-limited in the backyard, leading to lower replication, therefore analyses of each year separately (Hyp. 1b) had lower power. Since this common garden was established in a backyard, there is a significantly lower associated species pool than if the experiment occurred in a more natural setting. As a result, common *S. altissima* interactions were not observed, such as galling, and the presence of

goldenrod beetles. This reduction to the type of herbivory could explain the patterns observed in our experiment (i.e., no significant change to herbivory across treatments due to lower levels of herbivory throughout the system).

Conclusions and future directions

Understanding the role of neighbor interactions in shaping above- and belowground interactions is becoming increasingly important in an era of global change because communities are changing in multiple ways – such as changes to plant communities from species extinctions and introductions to alterations in plant associated arthropod and microbial communities due to changing climate and mismatches in population dynamics. Neighbor associational effects are commonplace, however, the ways in which we study them are varied. At this stage, most studies, much like ours, show individual focal plant responses to very specific interactions. However, small differences in experimental designs can lead to varied outcomes in the strength and direction of outcomes. For instance, the type of plant-soil feedback experiment (field versus greenhouse) affects plant growth response (Beals et al. 2020), as does soil inoculum preparation (Foster et al. 2022, van de Voorde et al. 2012), making it difficult to move the field toward any predictive patterns. As a result, even experimental studies using the same species are not necessarily directly comparable. To progress above- and belowground ecology, we need to design experiments in comparable ways, measure the same traits at the same stage, and measure biotic interactions in analogous ways over longer time periods. For example, it is not uncommon for greenhouse experiments to run for a single growing season, or a conditioning phase followed by a shorter-term experiment (Lepinay et al. 2018, Van Der Putten et al. 2009). While single-season studies are important for understanding initial effects of treatments, resource allocation of perennial and biennial plants is a dynamic process and over the course of two growing seasons, plant response, as we showed here, can change quite drastically. For this reason, results from single growing seasons should be taken with caution, and long-term studies need to be implemented to better understand the relationship between plant neighbor effects and plant-soil feedbacks (Bardgett et al. 2005; Beckman et al. 2022). Research has shown that traits change with ontogeny, therefore, we can expect biotic interactions to also change as a plant develops

(Garbowski et al. 2021). Therefore, to fully understand the nuances of neighbor associational effects, data collection throughout a plant's development is needed, across trophic levels. Lastly, this backyard study has shown the importance of plant-mediated indirect interactions among diverse taxa. Similar to studies such as Bezemer et al. (2005) and Pangesti et al. (2013), here we show indirect interactions among diverse groups of taxa are mediated by the resource allocation of the focal plant species. Further, we illustrate how both above- and belowground processes can shape these interactions, depending on the identity of the plant neighbor. These results have important implications for our understanding of the mechanisms through which plant resource allocation governs above- and belowground interactions (Van Dam et al. 2011). In an era of ever-increasing change, gaining a predictive understanding about how plants mediate interactions between seemingly disparate groups of species through carbon allocation is crucial to understanding drivers of community interactions.

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Author contributions

SCT and JAS conceived the idea for this experiment, SCT established, collected and analyzed data on the experiment, SCT wrote the article with feedback and assistance from JAS.

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CHAPTER II

HOW MUCH DOES COMMUNITY CONTEXT MATTER? PARTITIONING ABOVE- AND BELOWGROUND BIOTIC EFFECTS ON INDIVIDUAL PLANT PHENOTYPIC VARIATION

Abstract

While environmental conditions govern where on the landscape individuals occur, biotic interactions often determine local-scale processes. Increasingly, research has shown the importance of biotic interactions on plant traits. However, the relative influence of multiple biotic interactions on plant functional diversity is not well understood. Further, parsing the relative effects of different biotic interactions on trait expression is critical in a time of increasing environmental change, to better predict the multifaceted impacts of biodiversity loss on plant populations and community dynamics. Using a common garden experiment manipulating plant neighbor species identity and the soil microbiome, we tested how plant neighbor and belowground microbial diversity directly alter a focal plant, *Solidago altissima*, and how plant neighbors can indirectly alter *S. altissima* functional diversity through changes to the soil microbial composition. We found that plant neighbors both directly and indirectly alter *Solidago* trait expression, directly through changing aboveground functional traits, and thereby changing the relationship between *S. altissima* and its associated belowground microbial community diversity. Furthermore, *S. altissima* soil microbial community composition changes in polycultures through time, relative to plant neighbor monocultures, suggesting that changes to *S. altissima* traits induced by plant neighbor result in different resource use, therefore indirectly altering the belowground soil community composition. These data experimentally show how multiple community interactions can determine plant traits, mediated by plasticity in plant resource allocation.

Introduction

Variation is ubiquitous in the natural world, and integral to its functioning. The importance of functional trait variation (i.e., the range of plant traits that can change across biotic or abiotic environments and that can have larger effects on ecosystem processes; Violle et al. 2007) has become increasingly apparent as it serves as a source of heterogeneity to maintain population genetic diversity and enable species to better adapt to changing environmental conditions (Bolnick et al 2011; Violle et al. 2012). Plant community dynamics at local scales are largely a result of environmental filtering and biotic interactions (Gallinat & Pearse, 2021), which can give rise to individual variation in trait expression (Kraft et al. 2014). However, our knowledge of the drivers of functional diversity at local scales is sparser than at broad scales (Albert et al. 2011, Puglielli et al. 2024). Therefore, partitioning the variance that biotic interactions incur on plant trait responses is critical to understanding community structuring processes.

Two integral biotic interactions that plants partake in during their establishment are with neighboring plants and microbial communities. Plant-plant interactions can be competitive or facilitative (Hart, 2023). Neighboring plants of the same or other species can limit light and other resources available to the focal plant, resulting in focal plants' trait responses such as increased specific leaf area (SLA) or increased fine root production. On the other hand, plant neighbors can condition the soil in ways that facilitate the focal plant's growth, or cooler temperatures needed to reduce transpiration (Hischier et al. 2024). These functional responses to biotic interactions can be just as large as functional responses to abiotic effects (Le Bagousse-Pinguet et al. 2015). Plants engage in a complex web of interactions with microbial communities belowground (Bever et al. 2010, Bennett, 2012, Van der Putten et al. 2013, in 't Zandt et al. 2023), with genetic technologies advancing our understanding of their intricacies. Plant soil feedbacks (PSF) describe the reciprocal interactions between plants and soil microbial communities and soil properties. Such interactions have been widely studied in relation to agriculture, plant invasion, and eco-evolutionary dynamics (Gundale and Kardol, 2021), which have shown the importance of PSF for the maintenance of diversity through coexistence (Yacine et al. 2024).

Not only are plant-plant interactions and plant-microbe interactions important for determining a focal plant's trait expression, feedbacks between each of these sets of interactions can affect the

others (Lankau et al. 2011). For example, neighboring plants can indirectly alter a focal plant's trait expression by changing the microbial community structure in the soil, either by introducing pathogens or by exuding volatile compounds or allelopathic chemicals, thereby altering the focal plant's microbial interactions and functioning (Holt & Lawton, 1994, Stinson et al. 2006). These feedbacks produce an important and oft overlooked higher order interaction in community dynamics called trait-mediated indirect interactions (hereafter TMII), defined as instances whereby a species alters its phenotype in response to the presence of a second species which in turn cascades to affect another species (Vandermeer 1969, Werner and Peacor, 2003). Consequently, trait responses caused by one species can have indirect effects on the focal species' interactions with other community members. Multiple studies show that TMIIs are important, that trait variation inducing TMIIs is commonplace, and that the effects thereof alter community dynamics (Werner and Peacor, 2003, Utsumi et al. 2010a, Utsumi et al. 2010b, Ohgushi et al. 2012).

Testing the relationship between biotic interactions and variation in plant species traits can be complex, especially since tracking TMIIs induced by the array of organisms above- and belowground that a plant interacts with is very difficult. Further, accounting for abiotic and other confounding effects (e.g., evolutionary history) can pose a challenge. Here we make use of a common garden experiment where our focal species, *Solidago altissima*, is grown in monocultures, pairwise polycultures with two other Asteraceae, *Achillea millefolium* and *Cirsium discolor*, and multispecies polycultures with both (*A. millefolium* and *C. discolor*). These neighbor plant treatments were crossed with starting soil microbial community treatments. By controlling for abiotic conditions (i.e., soil, space, and water), we were able to uncover how a plant's initial interactions (i.e. plant-plant and plant-soil microbial) shaped the relationship between plant function and its belowground associated microbial community. Specifically, we tested how *S. altissima's* traits changed based on plant neighbor combination and soil inoculum origin. Next, we tested how *S. altissima's* associated soil microbial diversity was affected by plant neighbor combination and soil inoculum origin, as well as whether plant neighbors alter soil microbial composition and turnover. Lastly, we tested whether plant neighbors altered *S.*

altissima's functional diversity (functional richness and divergence), and whether this cascaded to affect *S. altissima's* associated microbial diversity (Fig. 6).

Methods

Plant species

In this experiment, we used three common Asteraceae species that regularly co-occur in old fields, and that vary in growth, and morphology. The focal plant in this study was *Solidago altissima* (Tall goldenrod), which we grew with *Cirsium discolor* (Field thistle) and *Achillea millefolium* (common yarrow) in factorial combinations.

Seed collection

Solidago altissima and *C. discolor* seeds were collected from ORNL National Environmental Research Park (ORNL-NERP), in early November 2020. The field site is about 1200 hectares, comprising multiple plant communities, including hardwood and old growth forests, wetlands and old fields. We collected seed from multiple patches within two geographically distinct old field areas, Freels Bend and Gallaher Bend, where *S. altissima* and *C. discolor* co-occur. The purpose of collecting seeds from multiple areas was to provide local scale genetic variation representative of the area. Previous studies from that study site identified over twenty *S. altissima* genotypes (Crutsinger et al. 2006), though knowledge on *C. discolor* in the area is sparse, as this study is the first record of it in the area. After collection, we stored the seeds at room temperature over winter before germination in April 2021. Due to difficulty locating sufficient field populations, *A. millefolium* seeds were ordered online from multiple nurseries to ensure genetic variation (nursery sources: David's Garden Seeds, Outsidepride, Eden Brothers). In April 2021, soil was collected from the same old fields where the seeds were collected. Using a soil auger and spade, we collected soil from directly underneath *S. altissima*, representing *S. altissima*-conditioned soil. We also collected soil in areas not associated with a specific plant, representing a natural old field soil. Each soil type was collected in multiple gallon-sized Ziploc bags and stored in a -4 F fridge until use. The purpose of these soils was to assess how the starting soil inoculum altered plant-plant and plant-microbial interactions. To ensure these soil

origins were indeed different, we sequenced eight samples of each origin, and results show that *S. altissima* conditioned soil has higher bacterial and fungal diversity, and different compositions than the natural old-field soil not associated with *S. altissima* (Fig. 7).

Common garden experiment

Each plant species was separately germinated in trays in the University of Tennessee greenhouse, in April 2021, using Promix germination potting mix (Promix FLX). We soaked the seeds in water overnight before planting, to encourage germination. Seedlings were watered daily, in addition to automatic misting in the greenhouse. After six weeks in the greenhouse, plants hardened for one week, by being placed outside for a few hours each day, gradually acclimatizing them to outdoor conditions. Thereafter, we transplanted representative individuals of each species into a common garden experiment on the University of Tennessee campus. Landscape fabric weed blocker was placed beneath the pots in a block design, to prevent roots from escaping the pots and thereby preventing plants from capturing resources through the pot's drain holes. The area around the blocks was mowed regularly. Treatments were planted in a fully randomized design. We used large 70-liter pots (NSIEG8000 Econo-Grip Nursery Container, Griffin Greenhouse Inc.), prepared with 'Baccto® veggie mix' potting mix. This soil type was used for its water retention properties. It should be noted that this was not sterile soil, rather this organic soil contained sedge peat and sphagnum peat moss, and as such could contain an active microbial community. All pots and treatments received the same type and amount of soil. Seedlings were planted in the last week of May 2021. Each individual plant received a tablespoon of soil inoculum during planting, so that the first soil that the plant was exposed to in the common garden experiment was live field soil.

Watering was highly contingent on temperature, and we used a flexible watering method to account for high variation in temperatures throughout the growing season. We hand watered for the first month of the experiment while the plants were young, this was done daily for the first week, then every second day, dependent upon temperature. After four weeks, a sprinkler system was established, from two spigots leading to four sprinklers, evenly spaced on the perimeter of the experiment. Plants were watered as needed throughout the experiment. A timed irrigation

system watered the plants from 5:30 am- 7:00 am daily. We varied this time, to longer periods (i.e., 5:00 am – 7:30am), during hotter days, and reduced the watering to every second or third day during colder days, as needed.

Experimental design

In all pots, we planted individuals at a fixed density (six total plants), in the following combinations: Monocultures of *S. altissima* (six individuals), pairwise polycultures of *S. altissima* X *C. discolor,* and *S. altissima* X *A. millefolium* (three individuals per species), and multispecies polycultures of *S. altissima* X *C. discolor* X *A. millefolium* (two individuals per species). These combinations were replicated in each soil inoculum origin, meaning we had one treatment where we inoculated plants with *S. altissima* conditioned soil and another where we inoculated plants with the natural old-field soil. Table S4 shows the number of replicates per treatment. In total, we had 111 pots planted with 666 individual plants.

Plant trait measurements

At the height of the growing season, in July, we measured leaf traits, specifically: specific leaf area (SLA), leaf dry matter content (LDMC), and leaf matter per area (LMA), we also measured plant height. We used these traits as proxies for plant performance, with plant height showing light acquisition and competitive ability, SLA showing plant growth strategy (fast growing acquisitive vs conservative slow growth), LMA as an indicator of stress tolerance, and LDMC as a predictor of leaf resistance to physical stress (Violle 2007, Funk et al. 2017). The way we chose leaves for trait measurement differed for each species. With *S. altissima,* we chose a fully expanded leaf within 10 cm of the terminal shoot. Since *A. millefolium* and *C. discolor* begin as rosettes, we chose the first fully expanded leaf. In the monocultures, we collected eight leaves per pot, one from each individual, and two additional randomly selected leaves. In the pairwise polycultures, we collected one leaf from each individual and an additional randomly selected leaf, totaling four leaves per species. In the multispecies polycultures, we collected a leaf from each individual and an additional random leaf per species, resulting in nine leaves collected per pot. Leaves were weighed immediately after collection to get the fresh weight (g). We then used an Epsom flatbed scanner and WinFOLIA to calculate leaf area (cm^2). After scanning, we dried

the leaves for 48 hours at 70º C, and then re-weighed them. LDMC was calculated by converting the weight to mg and dividing the fresh weight by the dry weight. SLA was calculated by dividing the leaf area by dry mass. We then calculated the mean LDMC and SLA per species per pot. We measured plant height during trait collection, as well as at the end of flowering season.

Characterizing the soil community

This study had two soil inocula, *Solidago* conditioned soil, and a natural old-field soil, we also grew monocultures of all three plant species in potting mix with no inoculum. We collected a soil sample from each pot at the end of October, before the first frost. These soil samples were taken from between two *S. altissima* individuals, to test how *S. altissima* conditioned the soil from its initial starting condition. Soil was collected using an Oakfield soil auger at a depth of 15 cm. The soil core was wiped clean with ethanol-soaked paper towels between each sample. Each sample was put in a separate Ziploc bag, in a cooler with ice packs. After collection, soil samples were stored in a -80º C freezer until DNA extraction. DNA extraction was performed using the DNeasy PowerSoil Kit, following the manufacturer's protocol (QIAGEN Inc., Germantown MD, USA), and quantified with a NanoDrop spectrophotometer. Thereafter, the University of Tennessee Genomics Core performed library preparation for the bacterial16S and fungal ITS gene regions and used a two-step PCR process to sequence the samples.

DNA extractions and Illumina NovaSeq sequencing

DNA was extracted using the DNeasy PowerSoil kit, as per the manufacturer's protocol (QIAGEN Inc., Germantown MD, USA). We used a two-step PCR approach for amplicon sequencing. The V3-V4 gene region of 16S rRNA was amplified using Illumina recommended forward and reverse primers (Klindworth et al. 2013). Amplification success was confirmed by running each sample on a 2% agarose gel (Sigma-Aldrich, St. Louis, MI, USA). Agencourt Ampure XP beads were used to clean initial PCR products of any unincorporated nucleotides. The cleaned products were then amplified in a second PCR, using the Nextera XT index kit (Illumina Corporation, San Diego, CA, USA). This second-step PCR consisted of 25 μl KAPA HiFi HotStart taq (KAPA Biosystems, Wilmington, MA, USA), 5μl each of unique combinations of Nextera XT index primers 1 and 2, and 5 μl of initial PCR product, brought up to 50 μl with

PCR grade water. Agencourt Ampure XP beads were again used to purify the now indexed PCR products. These products were then quantified on a NanoDrop 1000 spectrophotometer. Amplicons were then pooled for efficiency, quality, and quantity, checked on an Agilent Bioanalzyer, and diluted to 4 pM. For each run, the diluted products were combined with PhiX control DNA (Illumina Corporation, San Diego, CA, USA) at a ratio of 20 % PhiX, loaded onto a v3 600-cycle flow cell set for a paired-end read of 275 bases each, then sequenced on the Illumina Novaseq at the University of Tennessee Genomics Core (Knoxville, TN, USA).

Bioinformatics

We processed sequences into Amplicon Sequence Variants (ASVs) using the DADA2 16S and ITS pipeline workflow (https://benjjneb.github.io/dada2/ITS_workflow.html) in R studio (R Core Team 2020). The result from this workflow was an ASV table, with chimeras removed for both the ITS and 16S sequences. Taxonomy was assigned to the sequences using the SILVA database for 16S and the General fasta file version from April 2024, from the UNITE ITS database for ITS sequences (Abarenkov et al. 2024). ASV and Taxonomy tables for each kingdom were then merged with our experimental treatment data frame.
The ITS and 16S ASV tables were used to calculate Alpha diversity, for which we used the Hill Diversity metrics, q = 0, q = ,1 and q = 2. These represent species richness (q = 0), Shannon's diversity (q = 1) and inverse Simpson's Evenness Index (q = 2) but are conveniently scaled to be comparable across metrics.

Statistical methods

Solidago altissima individual trait variation as a result of plant neighbor and soil inoculum origin

All statistical analyses were conducted in R (R Core team, 2020). To test how individual plant traits varied based on its biotic environment, we first transformed and scaled traits, and removed outliers. Thereafter we used Generalized linear models (GLMs) to test how SLA, LDMC and LMA (response variables) changed based on plant neighbor combination, soil inoculum origin, as well as bacterial and fungal diversity (predictors). All three GLMs has the default gaussian distribution and identity link. Using a linear model with a gaussian distribution, we tested

whether plant height changed based on neighbor and soil inoculum origin. We used the rsq package to calculate the coefficient of determination (partial R^2) for each predictor in each model, this showed how much variation in the response variable is explained by the predictor variable (Zhang, 2017).

Soil microbial diversity and compositional changes as a result of plant neighbor and soil inoculum origin

To understand how microbial diversity was affected by plant neighbor and starting soil inoculum origin, we build GLMs with gaussian distributions and identity link function. Our predictors were fungal diversity and bacterial diversity at $q = 0$, $q = 1$, and $q = 2$. The response variables were plant neighbor combination and soil inoculum origin. Like with the plant trait models, we calculated partial R^2 values using the rsq package.

To assess how bacterial and fungal composition changed based on plant neighbor, we generated a Jaccard dissimilarity matrix using the vegdist function in the vegan package, then performed a Principal Coordinate Analysis (PCoA) using the pcoa function in the ape package. We used the Jaccard dissimilarity matrix, which is a presence/absence matrix instead of an abundance dissimilarity matrix (such as the Bray-Curtis dissimilarity matrix), due to the uneven number of replicates for each treatment. Thereafter, we performed a permutational multivariate analysis of variance (PERMANOVA) using the adonis function in vegan. We included belowground composition of *A. millefolium* monocultures and *C. discolor* monocultures in this analysis to see if polycultures were more similar to *S. altissima* monocultures or the heterospecific neighbors belowground composition.

Next, to assess pairwise differences in belowground composition, we used betapart to compute a distance matrix (Jaccard index), separating turnover and nestedness components (Anderson et al. 2006, Baselga & Orme, 2012). We then used the betadisper function in the vegan package to perform an analysis of multivariate homogeneity of group dispersions (i.e., the distance of each sample to the centroid of its group), using Tukey HSD post hoc test for pairwise comparisons. In doing so, we were able to see how much variance there was in the belowground community composition between pairwise groups, and how much influence neighbor identity had in shaping *S. altissima*'s belowground composition.

Solidago altissima functional diversity based on plant neighbor and its effect on microbial diversity

To quantify the functional trait diversity and test how plant neighbors changed *S. altissima* functional diversity and its relationship with belowground diversity, we used an approach created by Carmona et al. 2016 based on trait probability density (TPD), in the package funspace (Carmona et al. 2024). This package allowed us to build, analyze and plot *S. altissima's* functional trait space based on plant neighbor combinations (Carmona et al. 2024). The TPD method is a kernel density analysis that uses all sampled traits to calculate the gaussian kernel density function around each observation (Duong, 2007, Carmona et al. 2016). The data used for the PCA (i.e., the TPD function), does not use raw trait values, instead it uses the probabilities of observing combinations of trait values for a given species, which can be grouped based on treatment. In our case, we grouped our observations based on plant neighbor combination. The resulting PCA depicted observations clumping around trait combinations that were more common than others, such that multivariate peaks and valleys could be depicted in two-dimensional space. Analyzing functional traits using kernal density methods is a common approach, though more often with large data to understand broad-scale patterns. However, an advantage to this method is that it can be used across scales from individuals to across species (Carmona et al., 2016). Based on the PCA, we calculated two functional diversity measures, functional richness and divergence. Functional richness is the amount of functional trait space occupied, while functional divergence describes how much of the occupied functional space is distributed toward the edges (Carmona et al., 2019). We calculated these metrics for all trait observations altogether for overall functional richness and divergence, as well as by plant neighbor, to assess how plant neighbors changed *S. altissima* trait variation. It is worth noting that the traits that we measured are morphological traits, not direct measures of plant functioning (e.g. photosynthetic rates). Therefore, in this context, functional richness and divergence are measures of changes to trait morphology in response to our treatments (*sensu* Violle et al. 2007).

The effect of plant neighbor induced changes to functional diversity for belowground diversity

Next, to assess whether plant neighbor altered the relationship between *S. altissima* function and associated belowground diversity, we used Generalized Additive Models (GAMs). GAMs are

useful for understanding complex ecological systems, because they can be fit to multifaceted, non-linear relationships and still make interpretable predictions (Yee and Mitchell, 1991). GAMs can fit non-linear relationships by fitting smooths (also known as splines), which are flexible functions that shift their shape based on the data's distribution, unlike a regular linear regression which fits data to a linear relationship. Our predictor variable was the trait axis (the PC1 and PC2 axis of our *S. altissima* trait space), with fungal and bacterial diversity (at q= 0, q = 1, and q = 2) as the predictors. We compared how these relationships changed based on plant neighbor. GAMs were needed for these multivariate data because the trait axis encompassed peaks and valleys that required the flexibility of smooths to uncover. When interpreting GAMs, each model output provides coefficients for the parametric terms of the model (i.e. the intercept), followed by the smooth terms. The smooth terms have no coefficients, because they are built from multiple functions and therefore have many coefficients. Instead, the smooth term output provides the effective degrees of freedom, which provides information on how complex the smooth term is, for instance, an edf of 1 indicates a linear relationship, an edf of 2 is a quadratic etc. The higher the edf value, the more complex the model curve.

Results

Solidago altissima individual trait variation, as a result of plant neighbor and soil inoculum origin

Our trait GLMs (Table 4, Fig. 8) showed that traits responded differently based on the neighbor identity. *Solidago altissima* SLA showed no neighbor effects, though SLA did increase in *S. altissima* conditioned soil. The opposite occurred for *S. altissima* LMA, which was expected since it is the inverse of SLA. *Solidago altissima* LDMC did exhibit a neighbor effect, whereby it was reduced in multispecies polycultures (Est. = -0.014; p = 0.015) compared to monocultures. LDMC was also lower in *S. altissima* conditioned soil (Est. = -0.010; p = 0.006). *Solidago altissima* height was affected by both neighbor combination and inoculum origin; *S. altissima* height was lower in both pairwise polycultures with *A. millefolium* and multispecies polycultures compared to monocultures, as well as in its own soil inoculum (Table 4, Fig. 8). Based on partial R^2 values, neither neighbor nor inoculum were good predictors of *S. altissima* trait variation,

with 1.5 - 3.4% of variation explained by the predictors (Table S5). Interestingly, we found more inoculum effects than neighbor effects, and they accounted for comparable amounts of variation in *S. altissima* traits.

Soil diversity and compositional changes as a result of plant neighbor and soil inoculum origin

Next, we sought to understand how microbial diversity was affected by plant neighbor and soil inoculum origin (Table 5). Both pairwise mixtures increased *S. altissima* associated fungal diversity, but at different orders of q. When *S. altissima* was grown with *C. discolor,* fungal richness (q = 0) and Shannon's diversity (q = 1) increased. When grown with *A. millefolium*, we observed an increase in Shannon's diversity (q = 1) and inverse Simpson's diversity (q = 2) for fungi. Interestingly, the three species polycultures showed no change in fungal diversity (q = 0, q= 1, q = 2) compared to monocultures, showing that species identity was not necessarily the driver of change in the pairwise polycultures. We also observed lower fungal richness in *S. altissima* conditioned soil compared to the natural old-field inoculated treatment. The only change to bacterial diversity observed was a marginal increase in bacterial richness in *S. altissima* conditioned soil (Est. = 538.093, p = 0.074). Fungal diversity variation was best explained by plant neighbor, with partial R^2 values ranging from 5.4-9.8%, while inoculum only explained 0.5-2.3% of the variation. This showed that initial soil fungal composition was not a strong indicator of fungal diversity later in the growing season, rather plant composition had a stronger effect. Bacterial diversity was not well explained by neighbor or inoculum, with neither variable explaining more than 0.98% of the variation in bacterial diversity (Table S6).
Based on our PCoA and PERMANOVA results, we found that both fungal and bacterial composition differed significantly based on plant neighbor (Fig. 10). The betadisper pairwise comparisons revealed unexpected fungal compositional turnover results (Table 7), whereby monocultures of each species displayed no significant dispersion between them, showing that these three Asteraceae species did not condition the soil differently from one another when grown in monocultures. Furthermore, we also found no significant differences in fungal variation between the polycultures. However, our polycultures were significantly different from the

monocultures of each species. Instead of finding the polyculture fungal composition to be nested within the monocultures, as expected, we found that when grown with a heterospecific plant neighbor, *S. altissima* interacted differently with its fungal community than when grown in monocultures, but consistently differently irrespective of plant neighbor combination (Table 7). With bacterial composition, we found far less evidence of turnover between plant neighbor combinations. This was expected since earlier bacterial results showed very little variation in bacterial relative abundance across taxa (Fig. 11). The only groups that differed were *A. millefolium* monoculture from *S. altissima* planted with both heterospecific plant neighbors, and *A. millefolium* monoculture with *S. altissima* planted with *A. millefolium*. These results showed that our plant neighbors did not alter *S. altissima* associated soil microbial community through the addition of taxa (composition between monocultures did not vary), however they did change the way *S. altissima* interacted with its fungal community, leading to varying microbial compositions. This suggests that there was an indirect effect of plant neighbor on *S. altissima* through its associated soil microbial interactions.

Solidago altissima functional diversity based on plant neighbor and its effect on microbial diversity

Based on the functional trait space for the traits that we measured, principal coordinate axis 1 (PC1) explained 53.76% of the variation and principal coordinate axis 2 (PC2) explained 23.9% of the trait variation (Fig. 9). The leaf traits were largely driven by PC1 and plant height by PC2 (Table S8). Overall, the amount of trait space occupied by *S. altissima* was 87.92 (i.e. functional richness, Table S9), with high convergence in the center, and 36% of the TPD distributed toward the edges of the occupied space. *S. altissima* trait morphology varied based on plant neighbor. In monocultures, *S. altissima* occupied just over half of its overall functional trait space, in contrast, when grown in polycultures with *A. millefolium,* most of the functional space was occupied, showing high variation in trait response. Polycultures with *C. discolor,* and multispecies polycultures occupied a similar amount of functional space occupied as monocultures, although when grown with *C. discolor,* there was no longer a single peak in the TPD distribution, instead, two centroids emerged. The highest divergence was observed in multispecies monocultures,

whereby 51% of the TPD function was distributed toward the edges of the distribution. In summary, functional trait space occupied by *S. altissima* increased when grown with *A. millefolium*. *C. discolor* increases variation in where the majority of the TPD function occurs, resulting in two distinct peaks (i.e., a bimodal response to growth with *C. discolor*), and multispecies polycultures increase functional divergence. These results demonstrated that plant neighbor identity resulted in varied functional responses, and monocultures resulted in niche packing, constraining TPD functional trait space.

The effect of plant neighbor induced changes to functional diversity for belowground diversity

Based on our GAM models (Table 6, Fig. 9), we found a positive relationship between *S. altissima* functional trait space and fungal diversity, whereby fungal richness increased as functional trait space increased. This was significant for q= 0 (fungal richness) when *S. altissima* was planted with *C. discolor* and with both heterospecific plant neighbors, and for q = 2 (inverse Simpson's) when *S. altissima* was planted with *A. millefolium.* This means that changes to *S. altissima* functional trait space induced by *C. discolor,* resulted in increased fungal richness, and that the increase to *S. altissima* functional richness induced by *A. millefolium* increased dominant fungal taxa (q = 2). Based on model outputs (Table 6), the effective degrees of freedom (efd) changed considerably based on plant neighbor. There were no cases of an efd of 1, which would indicate a linear relationship. Most often we observed an edf of 2 (50% of the models), which indicates a quadratic relationship between *S. altissima* functional trait space and microbial diversity. The highest significant edf was with fungal richness as the response variable, in multispecies polycultures, with an edf of 14, implying a highly complex relationship, or more likely in this case, overfitting of the smooth terms. Bacterial diversity on the other hand appeared to decrease in multispecies polycultures. However, our GAM models for bacterial diversity (at q = 0, q = 1, and q = 2) were not significant. The lack of significant effects may have been due to the fact that the traits measured did not explain bacterial diversity well. It is also possible that no significant relationship could be identified due to the low variation in bacterial diversity seen across treatments.

Discussion

While data on the abiotic and a broad range of biotic drivers of trait variation are growing in the literature (McGill et al. 2006, Funk et al. 2017, Herz et al. 2017), few studies link biotic interactions to functional diversity (Helsen et al. 2017). Here we identify changes to the functional traits of an ecologically important perennial species, *Solidago altissima*, as a result of biotic interactions. Furthermore, we teased apart the direct and indirect mechanisms of the three-way relationship between the focal plant species, associations with the belowground microbial community, and plant neighbors.

Plant trait variation as a result of neighbor and starting soil community

We identified a bottom-up effect, whereby *S. altissima* plant traits, when analyzed individually, were more affected by the origin of the starting soil inoculum than by the plant neighbor. A negative PSF was identified whereby *S. altissima* became more acquisitive (higher SLA) with lower LDMC, LMA, and height when grown in its own soil compared to the wild field soil. Negative PSF are common and could be an evolutionary tactic to reduce intraspecific competition (Hodge and Fitter, 2013, Lekberg et al. 2018, Clark et al. 2024).
Interestingly, late season belowground diversity was more affected by plant neighbor than by soil origin, showing a top-down effect where the way in which *S. altissima* conditions its soil changes based on its plant neighbor context. Our compositional results corroborate this result, as we found evidence of functional specificity, at least with fungal composition. Monocultures of each species had no difference in fungal composition, and polycultures also had no difference between them, however, polycultures were significantly different from monocultures. Therefore, when *S. altissima* grows in polycultures, its resource use and interactions with its fungal community change such that the fungal community is altered compared to monocultures. Coupled with this, individual trait measures yielded no neighbor effects on SLA and LMA, showing little evidence of competitive effects between species. This system is more likely an intransitive competitive network, where no single species perpetually outcompetes the others, and belowground fungal communities' mediate interactions between *S. altissima* and its heterospecific neighbors, thus promoting positive coexistence (Callaway & Howard, 2007,

Lankau et al. 2011). It is also possible that other biotic interactions that we did not measure are driving changes to belowground community composition. For example, herbivory can induce the production of volatile organic compounds, which are able to indirectly alter soil microbial composition (Bardgett and Wardle, 2003, Loranger et al. 2013, Buchkowski et al. 2023).

Indirect interactions between plant neighbor and S. altissima's belowground communities

After six months, *S. altissima's* belowground community diversity was no longer contingent on the starting inoculum used; rather, this late season community was shaped by the plant neighbors that *S. altissima* interacted with. Leaf morphological trait variation increased in multispecies polycultures and *C. discolor* polycultures, resulting in increased fungal richness (q = 0). Furthermore, *Solidago altissima* had a higher functional richness when grown with *A. millefolium c*ompared to other plant combinations, though it did not increase along a specific axis. Instead, the trait space periphery increased in all directions, showing that growing with *A. millefolium* induced an array of functional responses for *S. altissima,* instead of a straightforward shift. Furthermore, *S. altissima*-associated fungal rare taxa (q = 2) were increased where *A. millefolium* reduced *S. altissima* height and LDMC. This indicated that changes to plant traits induced by plant neighbor could result in changes to fungal diversity. The GAM models confirmed what the other analyses showed: *S. altissima* height was associated with lower fungal diversity, and monocultures typically had taller plants. Therefore, changes in belowground interactions resulted in increased fungal diversity and altered composition, along with increases in functional richness.

In this study, we describe the complex relationship between a focal plant, its associated belowground community, and interactions with plant neighbors. This three-way relationship is dynamic and often seen as context dependent and based on neighbor identity (Mutz et al. 2022). However, these results showed that polycultures promoted plant functional diversity (individual variation), which was positively correlated with belowground diversity, while monocultures resulted in niche packing, and reduced belowground diversity (Helsen et al. 2017). Importantly, we also showed that the microbial community was as important as plant neighbor for *S. altissima's* morphological variation, since we observed negative PSF induced by the soil

inoculum used, as well as shifts in functional richness associated with fungal compositional change.

Our study made use of fine-scale trait-based analysis paired with belowground community analysis to illustrate how plant neighbors can shift functional trait space and to ascertain how plant-plant interactions altered the relationship between a focal plant and its belowground community. The trait-based approach we used is typically used for large-scale global datasets to understand broad patterns of trait-environment relationships (Carmona et al. 2021) but can be applied across scales (Carmona et al. 2016). Here, we are one of the first to show how it can be used to show short-term functional diversity responses based on individual trait measures. Implementing methods that can be compared across scales is needed to build better mechanistic models of trait relationships (Anderegg et al. 2018). Incorporating biotic interactions into studies is also needed to better understand their role for plant functional diversity.

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CHAPTER III

BIOTIC-INDUCED RESOURCE ALLOCATION PATTERNS RESULT IN DIFFERENT TRADE-OFFS FOR A BIENNIAL THISTLE

Abstract

Plants resource allocation is contingent on not just the abiotic environment that they are embedded in, but also their complex biotic environment. However, little is known about whether biotic-induced resource allocation strategies result in later trade-offs for a plant. The objective of this study was to test how varying biotic interactions alter a focal plant's resource allocation to leaf traits, and whether these changes induce different growth-defense and growth-reproduction trade-off strategies. We experimented on a biennial plant, *C. discolor*, to test the relative role of each studied biotic interaction (plant neighbors, starting soil inoculum, and belowground bacterial and fungal richness). We found no evidence of growth-defense trade-offs, though we did uncover direct interactions between the extent of foliar herbivory and bacterial richness. *Cirsium discolor* displayed distinct growth-reproduction trade-offs that changed based on plant neighbor and the starting soil inoculum origin, these trade-offs resulted in changes to not just the quantity of reproductive output, but also the quality (i.e., pollen nutritional value). In addition, we found evidence of indirect facilitative effects. These findings show that at an individual level, the community context results in not just resource allocation changes, but also effects that carry on throughout the lifespan of a plant.

Introduction

Plants are subject to a series of resource-based trade-offs throughout their life cycle, which ultimately determine their fitness (McGinley & Charnov, 1988, Hartmann et al. 2020). Broadly, plants fix carbon through photosynthesis, then allocation to competing plant functions, such as growth, reproduction, and secondary defense is regulated by the limited availability of resources (Bazzaz et al. 1987, Tuller et al. 2018, Hartmann et al. 2020, Züst & Agrawal, 2017). In addition, plant resource allocation is determined by biotic interactions (Züst & Agrawal, 2017, Monson et al. 2022). Traditionally, negative interactions, such as competition with other plants for the same limiting resources, and the extent of herbivory, were thought to be the domineering drivers of resource allocation (Agrawal et al. 2006, Loranger et al. 2013, Holmes et al. 2023, Daniel et al. 2024). These resource allocation trade-offs can result in fitness trade-offs for plants (McGinley & Charnov, 1988, Monson et al. 2022). For example, investing carbon into growth creates a positive feedback, since it results in increased photosynthesis and therefore increased resources (Thornley, 1972). However, growth investment comes at a cost to defense and reproduction, thereby making plants vulnerable to herbivory, and potentially decreasing fitness (Tuller et al. 2018). On the other hand, investing in secondary compounds for defense reduces resources available for growth, and therefore reduces competitive ability, and fitness (Züst & Agrawal, 2017). Investing resources into reproduction increases fitness, however it decreases competitive ability and increases vulnerability to herbivory (Obeso, 2002, Agrawal and Züst, 2017). Trade-offs are key to plant persistence, as they are drivers of genetic variation within species and specialization among species (Agrawal et al. 2006, Monson et al. 2022).

This resource-driven framework has been the center of research on plant fitness determination, and while it serves well for hypothesis generation and modeling, it has two significant shortcomings. In addition to competition and herbivory, plants interact with a vast array of other organisms above- and belowground, creating a complex web of interactions that all play some role in the movement of plant resources to various plant organs to serve different functions (Werner & Peacor, 2003, De Deyn & Van Der Putten, 2005, Sotomayor & Lortie, 2015) throughout the life cycle of an individual plant. Furthermore, not all interactions are negative; positive interactions can also result in changes to plant resource allocation, and therefore result in

different allocation trade-offs (Bertness and Callaway, 1994, Burin et al. 2024). So, while resource limitation is a key driver for plant allocation, this view diminishes the role of biotic interactions, particularly mutualisms, in the movement of carbon from "sources" to multiple "sinks" (Pringle, 2016).

Although it is known that multispecies interactions can alter plant allocation patterns (Werner and Peacor, 2003) there is limited research on how biotic context shapes plant allocation trade-offs. Much of our understanding on the role of biotic interactions for plant resource allocation has focused on pairwise interactions or negative interactions, often ignoring the role of indirect interactions that arise in multispecies communities, and positive interactions in shaping resource allocation patterns (Strauss 1991, Wootton 1994, De Deyn, 2017). For instance, plant-plant interactions can both diminish and strengthen plant-pollinator interactions (Davis et al. 2023). Furthermore, belowground microbial communities can affect plant diversity (Bennett and Cahill 2016), and plant-herbivore interactions (Tao et al. 2017). Plant soil feedbacks (PSF), can result in changing trait expression, which indirectly affects plant-plant interactions (Laliberte et al. 2015). Without accounting for these replete, whole community, interactions when modeling plant allocation patterns, we run the risk of vastly misjudging the mechanisms behind resource allocation. Better predicting plant resource allocation and fitness trade-offs requires the inclusion of multispecies positive and negative interactions (Okuyama & Bolker, 2007, Losapio, 2023).

In this study, we experimentally manipulated a focal plant's biotic community to test how varying biotic interactions changed a focal plant's resource allocation to leaf traits, and whether these changes induced different growth-defense and growth-reproduction trade-off strategies. Our focal plant, *Cirsium discolor*, was chosen because it is a biennial thistle, therefore we could test how the allocation patterns that occurred in the first year of growth influenced reproductive output in the second (and final year) of its life. We altered the plant neighbor combinations (monocultures, pairwise polycultures with *Solidago altissima*, and multispecies polycultures with *S. altissima* and *Achillea millefolium*), as well as the starting soil microbial community (inoculated with either wild old field or *S. altissima* conditioned soil). In doing so, we could test how the relative effects of plant-plant and plant-soil microbial interactions altered *C. discolor* allocation to aboveground foliar traits, and whether these allocation patterns resulted in growth-

defense and growth-reproduction trade-offs. The objective of this study was to test whether, independent of abiotic variation, biotic interactions induce resource allocation trade-offs, and the relative role of each studied biotic interaction (plant neighbors, starting soil inoculum, and community bacterial and fungal richness). Box 1 provides specific predictions.

Methods

In this experiment, we grew our focal plant, *C. discolor* with two perennial Asteraceae, *S. altissima* and *A. millefolium* in factorial combinations, at equal densities.

Life history of Cirsium discolor

Native to North America, *C. discolor* is a biennial thistle in the Asteraceae family. In their first year, *C. discolor* seedlings become a rosette. Depending on environmental conditions, they may or may not bolt in their first year of growth, typically overwintering. In the second year, *C. discolor* bolts in early summer, reaching heights ranging from 80 cm - 2 m, then produces many flowerheads with disc flowers. Flowering occurs in the height of summer and lasts for about 2-3 weeks. After flowering, the flowerheads senesce, and seeds are dispersed either by wind or seed-eating birds, such as American goldfinches (Nickell, 1951). The plants then senesce during autumn. This biennial life history strategy allowed us to test how the biotic context affected a plant throughout its lifespan, from changes to allocation in year one to reproductive output effects in year two.

Seed and soil collection

Cirsium discolor and *S. altissima* seeds were collected for this study from Oak Ridge National Lab's National Environmental Research Park (ORNL-NERP) in early November 2020. ORNL-NERP is about 1200 hectares, comprising multiple plant communities, from hardwood and old growth forests to river bluffs and wetlands to old field systems. Seeds were collected from multiple patches within two old field areas, Freels Bend and Gallaher Bend. Seeds of each species from these populations were mixed to increase genetic variation and stored at room temperature over winter before germination in April 2021. *Achillea millefolium* seeds were

ordered online from multiple nurseries to ensure genetic variation (nursery sources: David's Garden Seeds, Outsidepride, Eden Brothers).

In April 2021, soil was collected from the same old fields in which the seeds were collected. Using a soil auger and spade, we collected soil directly underneath multiple *S. altissima* patches, representing *S. altissima* conditioned soil. We also collected soil sporadically in areas not associated with a specific plant, representing wild old-field soil. To collect the *S. altissima* conditioned soil, we removed the topsoil immediately adjacent to an *S. altissima* individual and angled the auger toward the plant's roots to collect soil that is in close contact with the plant's roots. Soils from different sources (i.e., *S. altissima* and old field) were kept separately and were stored in multiple gallon-sized Ziploc bags in a -4º C fridge until use in the experiment the following month. Having soils from two origins allowed us to test whether interactions between *S. altissima* (neighbor plant) and its conditioned soil resulted in increased effects on *C. discolor* (the focal plant) trade-offs (i.e., an indirect belowground effect). Based on sequencing samples of these two soil treatments we know that the starting soils differed significantly in fungal and bacterial diversity, and composition (see Fig. 7).

Common garden experimental design

We germinated the seeds in trays using Promix germination potting mix (Promix FLX) at the University of Tennessee-Knoxville greenhouse, in April 2021, using separate trays for each species. Seedlings were watered daily, with additional moisture from misters in the greenhouse. After six weeks in the greenhouse, we hardened the plants with a few hours of sunlight each day for a week, before transplanting representative individuals of each species into a common garden experiment outside the greenhouse. This outdoor setting was a patch of greenspace amongst the university buildings. Flowering plants occur on the outskirts of this managed greenspace, so insects and birds are common during flowering periods.

We grew the experiment in large, mesocosm-like pots, that were 49.53 × 49.53 × 43.18 cm, with a holding capacity of 70 liters (NSIEG8000 Econo-Grip Nursery Container, Griffin Greenhouse Inc.), and filled with Baccto® organic veggie mix (Veggie Mix - Baccto). While this was not a sterile potting mix, we used it due to its high-water retention capabilities. Watering plants in the

experiment was highly contingent on outside air temperature, and we used a flexible watering method to account for high variation in temperatures throughout the growing season. We hand watered every two days for the first month of the experiment while the plants were young, unless temperatures rose, and the plants needed daily watering. After four weeks, we erected a sprinkler system from two spigots leading to four sprinklers, which were evenly spaced on the perimeter of the experiment. We had a timed irrigation system that watered the plants from 5:30am – 7:00am daily. We occasionally varied this time, to longer periods (i.e., 5:00 am – 7:30am), on hotter days, and reduced the watering to every second or third day during colder days as needed. The experiment continued for two years, and aboveground biomass was harvested at the end of each growing season.

We manipulated the starting biotic community, specifically the plant neighbor(s) as well as the soil inoculum in which all plants would be exposed. In all pots, we planted individuals at equal plant density, in the following combinations: monocultures of *C. discolor* (i.e., six individuals in the pot), *C. discolor* with *S. altissima* (3 individuals of each species), and *C. discolor* with both *S. altissima* and *A. millefolium* (2 individuals per species in each pot). These combinations were replicated in each soil inoculum origin, meaning we had one treatment where we inoculated plants with *S. altissima* conditioned soil and another where we inoculated plants with the wild old-field soil. To inoculate the plants, we added a tablespoon of field soil around the seedling roots while transplanting into the experimental plots, to ensure plant interactions with the field soil during the initial stages of grow in the experiment.

In total, we had three monoculture replicates in each soil inoculum. The polyculture replicates were uneven, with 11 replicates for the pairwise polyculture (with *S. altissima*) conditioned in *S. altissima* soil, and 13 reps for the pairwise polyculture (with *S. altissima*) with the wild field inoculum, and 16 replicates in each soil inoculum for the multispecies polycultures, totaling 372 individuals in 62 communities (pots). We conducted all analyses at the pot-level, using mean plant responses, mean herbivory, and percentage of flowering *C. discolor* per pot.

Data collection

To understand how the starting biotic conditions altered plant resource allocation strategies, we measured specific leaf area (SLA) and leaf dry matter content (LDMC). High SLA is associated with plants that are resource acquisitive, and a low SLA with resource conservative plants, while LDMC relates to leaf resistance to physical stress (i.e., herbivory; Felix et al. 2023, Rusch et al. 2009). In the monocultures, we collected eight leaves per pot, one from each individual, and two additional randomly selected leaves. In the pairwise polycultures, we collected four leaves per species, one from each individual and an additional randomly selected leaf. In the multispecies polycultures, we collected a leaf from each individual, and an additional random leaf per species, resulting in nine leaves collected per pot. Leaves were weighed immediately after collection to get the fresh weight in grams. We then used an Epsom flatbed scanner and WinFOLIA to calculate leaf area in cm^2. After scanning, we dried the leaves for 48 hours at 70° C, and then re-weighed them. LDMC was calculated by converting the weight to mg and dividing the fresh weight by the dry weight. SLA was calculated by dividing the leaf area by dry mass. We then calculated the mean LDMC and SLA per species per pot.

To assess growth-defense trade-offs, we measured leaf damage using NutNet's leaf damage protocol (The very easy leaf damage protocol (nutnet.org) on a subset of the plants (6 replicates of the monoculture and polyculture with *S. altissima*, and 13 reps for the multispecies polyculture, totaling 25 pots and 150 individuals). We assessed two randomly chosen leaves per plant and used the average of both leaves for the analysis.

In year two, we did not collect *C. discolor* leaf traits, as some had bolted early in the season, and once bolted, leave size and morphology changes considerably, instead we focused on reproductive output. We used two measures of reproductive output, to represent both quantity and quality. We recorded whether or not each *C. discolor* had bolted and flowered, using the percentage of flowering individuals per pot as a proxy for pot level reproductive quantity. To assess reproductive quality, we collected and measured pollen protein content from a subset of *C. discolor* flowers across treatments in the experiment. Pollen protein is a means to assess the nutritional value of pollen (Russo et al. 2019, Murray et al. 2023), and plants with higher resource availability are expected to have higher pollen protein levels. Pollen collection occurred

during dehiscence, between 10 am and 2 pm, usually in the mornings before pollinators became too active. Pollen was collected by running a sharp razor blade gently along the anthers of a flowerhead. A piece of paper with a crease in it was held underneath the flowerhead to catch the pollen before it was transferred into an Eppendorf tube. To collect sufficient pollen for the protein analysis, we pooled all pollen samples at a pot level.

To quantify pollen protein concentrations, the Braford Protein assay was employed following a slightly modified version of Vaudo et al., 2016. Briefly, pollen was weighed, added to individual test tubes, and placed in a drying oven (Quincy) for 24 hr at 36° C. The dried samples were removed and 1.5 mL of 0.1 M NaOH (Fluka) was added. The pollen grains were fractured with a Microson Ultrasonicator (Misonix Incorporated) by placing a probe into the solution for 90 s and then storing the processed samples for 24 hr at 5° C. Immediately prior to testing the pollen, solutions were centrifuged at 2,000 × g for 30 s. The Bio-Rad Protein Assay Kit (Bio-Rad Laboratories) with a bovine γ-globulin protein standard was then prepared following manufacturer's instructions. The standards were prepared in triplicate and pipetted into a sterile 96-well plate (VWR Avantor). Absorbance readings were taken at 595 nm on a SynergyHi microplate reader using Gen 5.0 software (Biotek). A 5-point calibration curve was generated to calculate protein levels ($r^2 > 97\%$).

Soil microbial community collection and processing

In October of the first year of the experiment, we collected one soil sample per pot, using an Oakfield handheld soil auger. The auger was cleaned with ethanol between each sample collection. Samples were put in a cooler with ice bricks during collection, and then placed in a -80° C freezer immediately thereafter. DNA was extracted from the samples using DNeasy PowerSoil Pro kits (QIAGEN Inc., Germantown MD, USA) and quantified with a NanoDrop spectrophotometer. We sequenced ITS and 16S gene region for fungi and bacteria using a two-step PCR process. The UTK genomics core sequenced these soil samples using an Illumina Novaseq. Once sequenced, we used the Dada2 bioinformatics pipeline to identify the amplicon sequence variants (ASVs) in each sample. Taxonomy was assigned using the UNITE ITS database for fungal and SILVA database for bacterial ASVs.

We then used the HillR package in R Studio to calculate the Hill number diversity at orders q= 0, q = 1, and q = 2. This provided a pot-level measure of soil fungal and bacterial diversity which we used in addition to soil origin (as a categorical variable), to assess whether and how much belowground community interactions altered *C. discolor* resource allocation, and whether this cascaded to effect herbivory and reproductive quantity.

Statistical approach

All analyses were conducted in RStudio (R core team, 2020). We began by fitting linear models to test how plant traits changed based on plant neighbor and soil origin (i.e., mean SLA and mean LDMC, in separate models). We transformed and scaled the plant traits to fit a linear distribution. We used a beta regression model with a logit link (package: betareg) to test how herbivory changed based on the extent of plant neighbor herbivory, plant neighbor, and soil origin. Finally, we fit a generalized linear model to test how flowering quantity changed based on plant neighbor and soil origin using the default gaussian distribution and identity link. We used these models to provide a general overview of increases and decreases in traits as a result of the biotic context.

Next, we used piecewise structural equation modeling (SEM) to uncover the effects of soil inoculum origin, plant neighbor combinations, and belowground richness on plant allocation trade-offs (Lefcheck, 2016). We created two *apriori* models, one for growth-defense trade-offs and another for growth-reproduction trade-offs (Fig. 12). The first SEM model had four components to determine growth-defense trade-off, which predicted that plant neighbor identity and soil inoculum origin changed *C. discolor* resource allocation (1) that all three effected fungal herbivory (2), then that all four affected bacterial (3) and fungal (4) richness. Soil inoculum origin was later removed since it was not significant and to increase statistical power.

The second model assessed growth reproduction trade-offs by determining whether plant neighbor identity and soil origin predicted *C. discolor* plant allocation (1), and if the number of flowering *C. discolor* per pot was determined by resource allocation, plant neighbor and soil origin (2), and whether all four of the above variables affected bacterial (3) and fungal (4) richness. We predicted that heterospecific plant neighbors would cause a growth-reproduction

trade-off by increasing SLA, resulting in a reduction in flowering and that this relationship would change, depending on the starting soil inoculum in which the plants were growing.
For both SEMs, we modeled each plant neighbor combination separately to be able to assess different responses based on plant neighbor. We also ran our models with SLA and with LDMC as the resource allocation trait, then used the model AIC scores and R^2 values to determine the better fit for the final models.
We initially included plant neighbor traits in our models (i.e., neighbor LDMC and SLA). These were removed because they were highly correlated with focal plant traits. We ran both models separately for each plant neighbor grouping, to be able to assess changes based on plant composition. The piecewise SEMs were comprised of generalized linear models (GLMs) with a gaussian distribution for the bacterial and fungal richness models, and linear models for the SLA and LDMC models with the traits scaled and log10 transformed. We modeled the percentage of flowering plants with GLMs with a Poisson distribution and identity link.
We then calculated confidence intervals for all six SEMs (three growth-defense and three growth-reproduction SEMs), by bootstrapping the model effects (1000 permutations). We used the semEFF package, which calculates nonparametric bias-corrected and accelerated confidence intervals (Efron, 1987, Murphy 2023).

Identifying trade-offs in an SEM framework

Typically, trade-offs are identified by the presence of negative correlations between two variables (Agrawal, 2020). However, since we were interested in cascading effects that led to trade-offs, we used a piecewise SEM approach to understand the biotic effects on plant allocation trade-offs. SEMs are a useful tool for this type of study, because we can parse out the relative effects of different types of interactions on leaf allocation, and determine whether this cascades to affect herbivory and reproductive output. Biotic associations can have a direct effect on herbivory and reproductive output, or they can induce a trade-off by changing leaf allocation, which in turn results in changes to herbivory and/or reproductive output. In out SEM apriori model (Fig. 12), a trait-mediated trade-off would be if plant neighbor and/or soil origin increased or decreased resource allocation, resulting in a decrease/increase in reproductive

output/herbivory. Alternatively, if resource allocation increase/decreases reproductive output/herbivory leading to an increase/decrease in soil microbial diversity. We split the model by plant neighbor to identify directionality changes of each plant neighbor (i.e., arrows from each plant neighbor is in comparison to the other plant neighbor combinations. With soil inoculum origin, we used Anova's (car package, R), to test which inoculum increased and decreased significant paths.

Pollen quality analysis

The pollen protein dataset did not have sufficient power to be included in an SEM, so we used GLMs with a Poisson distribution and the default identity link. With *C. discolor* protein content as the response, we created three separate models that tested whether the protein content changed based on *C. discolor* aboveground biomass and plant neighbor, SLA and plant neighbor, and the percentage of flowering plants and plant neighbor.

Results

Table 8 summarizes the results of our initial models. We found that both polycultures significantly increased mean SLA compared to monocultures, though only multispecies polycultures significantly decreased LDMC. There were no soil origin effects on plant traits. *Cirsium discolor* foliar herbivory increased significantly as neighbor herbivory increased, except when grown with *S. altissima* in pairwise polycultures where it was significantly reduced. We observed a soil origin effect whereby *C. discolor* herbivory was reduced in old field soil compared to *S. altissima* conditioned soil. The percentage of flowering plants was significantly reduced in pairwise polycultures with *S. altissima*, though not in multispecies polycultures, nor were there any soil origin effects. Given the significant changes to plant traits, herbivory and reproductive output based on the biotic context, we then used piecewise SEMs to uncover the causal pathways and identify if biotic-induced trade-offs occurred.

Growth-defense trade-offs

Model comparisons (SI table 9 & 10) showed that our models performed better (i.e., lower AIC scores and overall R^2 values) when using SLA in the SEMs rather than LDMC. When *C.*

discolor was grown in monocultures (Fig. 13A), we did not observe a trade-off between SLA and herbivory. Instead, we found that monocultures directly reduced SLA by 61.5% compared to polycultures, independent of soil inoculum origin, and that there was a marginally significant negative correlation between SLA and Herbivory ($p = 0.076$). Monocultures had lower SLA compared to polycultures and herbivory tended to be highest on plants with higher SLA in monocultures. Additionally, a marginally significant ($p = 0.074$) negative path between herbivory and bacterial richness occurred, so higher herbivory levels resulted in lower soil bacterial richness.

When grown in polycultures with *S. altissima* (Fig. 13B), we observed a significant increase in *C. discolor* SLA, though this did not cascade to effect herbivory. However, *C. discolor* herbivory was directly significantly reduced by *S. altissima* by 41.4%, likely because *S. altissima* is a more palatable species, resulting in more herbivory on *S. altissima* than *C. discolor*. Once again, herbivory had a negative relationship with bacterial richness, though this time significantly so.

Multispecies polycultures (Fig. 13C) had higher herbivory compared to monocultures, though no significant changes to SLA. Like the monocultures, there was a negative correlation between SLA and herbivory.

In summary, we found no trait-mediated trade-offs, and no soil inoculum origin effects. Instead, we observed a direct increase to SLA and decrease herbivory as a result *S. altissima*. The decrease in herbivory cascaded to cause a negative correlation between herbivory and bacterial richness, which was no longer observed in multispecies monocultures. Table 9 provides coefficients for each path in each model.

The bootstrapped confidence intervals showed similar results, though some effects were no longer significant. This could be because when sample sizes are small, the bootstrapping process tends to underestimate the true nominal coverage (Chernick and Labudde, 2009). Nonetheless, we consider the significant effects that remained to be the more robust effects, which in this case included herbivory being higher in monocultures, and that polycultures and bacterial richness reduced herbivory (Table 10).

Growth reproduction trade-offs

Much like the growth-defense SEMs, in our growth-reproduction SEMs monocultures reduced SLA compared to polycultures (Fig. 14A), and there was a negative path between SLA and flowering quantity, though this was neither neighbor nor soil origin induced.

When grown with *S. altissima* (Fig. 14B), we observed a trade-off whereby *S. altissima* increased *C. discolor* SLA, and reduced flowering quantity, and there was no relationship between SLA and flowering.

Multispecies polycultures (Fig. 14C) reduced SLA and flowering, and there was a negative relationship between SLA and flowering quantity.

Based on these results, flowering quantity is highest in monocultures, where SLA and herbivory is lower; *S. altissima* results in higher SLA and therefore lower flowering, but also directly reduces herbivory. While multispecies polycultures have lower SLA, but higher herbivory and lower flowering. Neither SLA nor flowering quantity had a relationship with fungal and bacterial richness, however herbivory had a negative relationship with bacterial richness, which was especially strong when herbivory was reduced (in *S. altissima* polycultures).

Figure 14D shows *C. discolor* flowering as a function of *C. discolor* SLA, with soil origin in separate panels. *C. discolor* had higher flowering and lower SLA in monocultures and multispecies polycultures compared to pairwise polycultures, resulting in trade-offs when grown with *S. altissima*. Overall, flowering was entirely explained by these biotic induced trade-offs (Table 11).

Based on the bootstrapped confidence intervals of our growth-reproduction SEMs, the significant effects that remained included that pairwise polycultures increased *C. discolor* SLA resulting in decreased in flowering, and that multispecies polycultures had the opposite trade-off whereby *C. discolor* SLA was decreased and flowering increased (Table 12).

Pollen protein assessment

We found significant neighbor effects on pollen protein content, whereby *C. discolor*'s relationship with biomass, SLA and flowering all differed based on plant neighbor combination. *Cirsium discolor* protein content had a negative relationship with aboveground biomass, except

in multispecies polycultures, where this changed to a positive relationship (Fig. 15, panel 1). Biomass was significantly higher in monocultures and multispecies polycultures compared to the other treatments (Table 13). Based on our model, the relationship between *C. discolor* protein and SLA was significantly different between both polycultures and the *C. discolor* monoculture, though there was high variation in the standard errors for these data (Fig. 15, panel 2). The relationship between *C. discolor* pollen protein and flowering per pot was not significant, though it did differ based on plant neighbor (Table 13).

Discussion

The objective of this study was to understand how biotic interactions altered plant growth defense trade-offs in year one, and growth-reproduction trade-offs in year two for a native thistle *C. discolor*. In doing so, we showed that the community context mattered, and that small changes to starting conditions can result in significantly different outcomes for the focal species.

Plant neighbor and soil microbial induced growth-defense trade-offs

We did not observe any biotic-induced growth defense trade-offs *per se*. In our system, this seemed to be due to a lack of a strong negative relationship between the leaf traits measured and herbivory (Fig. 13D). Instead, our models showed that the extent of herbivory was directly affected by biotic interactions, not indirectly through trait-mediated trade-offs. For instance, herbivory significantly increased in monocultures compared to polycultures. Furthermore, there was a negative associated between herbivory and bacterial richness in monocultures and pairwise polycultures. Trait mediated effects on herbivory have been tested by others with mixed results in the literature, some find support for herbivore-trait mediated indirect interactions (Kost and Heil, 2006, Ohgushi 2008, Utsumi et al. 2010, Li et al. 2021), while others, like ours, show direct neighbor identity effects on herbivory without indirect trait-mediated effects (Castagneyrol et al. 2017). Plant-herbivore interactions are largely regulated chemically rather than through primary resource allocation, and measuring secondary compounds could uncover plant mediated (Heil 2014, Castagneyrol et al. 2017), and indirect effects (Moreira et al. 2024). Volatile organic compounds have also been linked to soil bacterial richness (Bitas et al. 2013, Yuan et al. 2017), therefore given the importance of bacterial richness in our models, it is possible that there is a

chemical interaction accounting for trade-offs between SLA and foliar herbivory that we did not quantify. Our growth-defense SEM results are, at least in part, driven by plant neighbor-herbivore interactions (Coley et al, 1985, Wetzel et al. 2023). *Solidago altissima* is more palatable than the other species, and therefore attracted more herbivores than *C. discolor* did in pairwise polycultures (i.e., *S. altissima* experienced 15-43.5% herbivory compared to *C. discolor* with 2-13% in pairwise polycultures and up to 32% in other combinations). Another reason for the lack of trade-offs could be due to relatively low herbivory levels compared to natural field conditions, which are typically reported between 20-60% herbivory for *Cirsium* species (Suwa and Louda, 2011, Eckberg et al. 2014, Russel and Hussman, 2019). Therefore, it could be that higher herbivory levels are needed to induce a top-down trade-off. We found no effect of soil inoculum origin in our growth-defense SEM, showing that any soil microbial interactions occurring between *S. altissima* and *S. altissima* conditioned soil, versus the wild field soil community, did not indirectly influence *C. discolor* resource allocation to leaves, nor subsequent herbivory levels.

Plant neighbor and soil microbial induced growth-reproduction trade-offs

We found that *C. discolor* displayed distinct growth-reproductive trade-offs based on its biotic environment, with conservative growth and higher flowering output when grown by itself compared to polycultures with *S. altissima* alone. Interestingly, when grown with both *S. altissima* and *A. millefolium*, focal SLA increased, however this was not to the detriment of flowering, showing an inverse trade-off compared to pairwise polycultures where SLA was lower, and flowing higher. Overall, *C. discolor* performed better in multispecies polycultures compared to monocultures and pairwise polycultures, showing that negative plant interactions are reduced with three species compared to monocultures and pairwise polycultures (Aschehoug and Callaway, 2015).

Our study provides evidence of an understudied indirect facilitative effect of negative PSF on neighboring plants, whereby *S. altissima* conditioned inoculum had a direct positive effect on *C. discolor* flowering. This implies a negative PSF between *S. altissima* and its own conditioned

soil, resulting in positive effects for *C. discolor*. Plants experiencing negative PSFs in home/own soil is well established in the literature (e.g. Bever 1994 and Lekberg et al. 2018), and this is commonly attributed to the presence of pathogens, which could also be an evolutionary tactic to reduce intraspecific competition (Hodge and Fitter, 2013). It is possible that by reducing intraspecific competition, *S. altissima* inadvertently increases our focal plant's reproductive output, which supports literature suggesting that much like other resource allocation trade-offs, plants could have trade-offs between plant competition and PSF (Laliberte et al. 2015, Lekberg et al. 2018). Since there is a soil origin effect and no effect of bacterial and fungal richness, the cause of the effect is most likely due to soil microbial composition (Tedersoo et al. 2020) (Supplementary Fig. 7).

In general, plant neighbor effects were stronger than microbial effects. The pollen protein analyses showed that pollen protein content (i.e., our proxy for reproductive quality), was highest in monocultures and multispecies monocultures. These are the communities that produced more flowering plants. In monocultures, *C. discolor* had significantly lower SLA than in the other plant combinations, which not only enabled higher flowering, but also produce to flowers of higher nutritional value than other plant combinations, perhaps a response to intraspecific competition. The higher SLA in pairwise polycultures resulted in lower flowering and lower quality pollen. On the other hand, the multispecies polycultures are the goldilocks of plant combinations, having no growth-reproduction trade-offs, managing to maintain high flowering probability, and high pollen protein content. These results advocate for the need for more studies on multispecies coexistence mechanisms (Aschehoug and Callaway, 2015, Terhorst et al. 2018). Theoretically, these results show that in monocultures and in pairwise polycultures with the competitive *S. altissima*, competition is high. But the addition of *A. millefolium* to the mix alleviates competitive effects, and the addition of a third species allows the focal plant to optimize both growth and reproduction. Taken together, the growth-reproduction results show that reproductive output of this biennial thistle is highly contingent on resource use in its first year, and that biotic interactions significantly affect resource allocation.

Our findings uncover distinct growth-reproduction trade-offs for *C. discolor*, induced by both plant neighbors (Lambers et al. 2019), but also to a lesser extent by the origin of the starting soil

inoculum (Hodge et al. 2013). Thus, we show that independent of space and resource constraints, plants face allocation trade-offs, that significantly alter flowering and quality of flowering. And therefore, the biotic context within which a plant exists is an important determinant of allocation, further ecological interactions (such as herbivory and pollination), and potentially fitness outcomes. The movement of carbon is contingent not only on the availability of resources (and other abiotic factors), but also on the complex interactions with other organisms that unfold throughout a plant's life. Plant neighbor identity was a major driver of the observed patterns, likely due to species-specific resource needs (Tilman, 1990, Mutz et al. 2022). We also uncover important direct and indirect ways that microbial communities affect plant allocation trade-offs (Van Der Heijden et al. 2008).

A notable limitation of this study is that due to sample size restrictions, we studied growth-defense and growth-reproduction trade-offs independently of one another. While this study advances our understanding of the multiple pathways in which plant resource allocation is altered by biotic interactions, we fail to show connections between defense-reproduction trade-offs, which are inextricably linked (Underwood et al. 2020). Three links between pollinators and herbivores include, i) changes to plant resource allocation as a result of herbivory in a biennial's first year could result in alterations to reproductive output in year two, ii) indirect plant-microbe-herbivore interactions can result in plant-reproduction trade-offs, iii) and lastly, the diversity and abundance of herbivores could affect pollinator diversity and abundance resulting in changes to pollinator visitation and ultimately plant fitness.

Overall, this study highlights the importance of considering whole community interactions to understand plant resource allocation patterns. We show that plant neighbors can have direct effects on a focal plant's allocation strategy, which in some cases indirectly influences reproductive output and late season soil bacterial richness, while foliar herbivory is directly influenced by neighbors instead of via changes to resource allocation patterns. Resources and other abiotic factors are an important aspect in species occurrence, persistence and performance; however, they are not the full picture. Scaling down to an individual level, shows how linkages among diverse taxa shape plant allocation, trait expression and fitness.

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APPENDIX

Table 1. Direct and indirect hypotheses based on experimental design.

Based on our study design, we can interpret how heterospecific neighbors directly affect plant allocation and response traits, whether it is through above- or belowground mechanisms, and if their associated community interactions are changed. As well as indirectly through changing the relationship between plant SLA its associated community (herbivory and microbial diversity). Using this design, we can detect eight direct and five indirect possible pathways pertinent to our hypotheses.

Direct Pathways				
	Hyp. 1		**Hyp. 2**	
Scenario	**Neighbor effect on focal growth and morphology**	**Effect of belowground interaction removal (pot effect)**	**Neighbor effect on associated community interactions**	**Theoretical interpretation**
1.1	no	no	no	Plant neighbor has no effect on a focal plant or its associated community interactions
1.2	no	yes	no	Plant neighbors alter a focal plant through belowground mechanisms
1.3	yes	no	no	Plant neighbors directly affect a focal plant through aboveground mechanisms, but sufficient community diversity exists to result in no effects on associated community interactions
1.4	yes	yes	no	Plant neighbors directly affect a focal plant through belowground mechanisms, without altering associated community interactions,

Table 1. Continued

1.5	no	no	yes	Plant neighbors directly alter a focal plants community interactions but there are sufficient resources to result in no direct plant effects (facilitation/resource partitioning), through aboveground mechanisms
1.6	no	yes	yes	Plant neighbors directly alter a focal plants community interactions but there are sufficient resources to result in no direct plant effects (facilitation/resource partitioning), through belowground mechanisms
1.7	yes	no	yes	Plant neighbors alter a focal plant through direct resource use as well as directly through disruption of associated community interactions, through aboveground mechanisms
1.8	yes	yes	yes	Plant neighbors alter a focal plant through direct resource use as well as indirectly through associated community interactions, through belowground mechanisms
Indirect Pathways				
Scenario	**Relationship between focal plant and assoc. comm.**	**Pot type effect**	**Neighbor effect on relationship**	**Theoretical interpretation**
2.1	yes	yes	yes	Plant neighbors alters the relationship between a focal plant and its associated communities, through belowground mechanisms
2.2	yes	no	yes	Plant neighbors alters the relationship between a focal plant and its associated communities, through aboveground mechanisms

Table 1. Continued

2.3	yes	yes	no	Focal plant interactions with associated community are driven by belowground mechanisms and not effected by plant neighbor
2.4	yes	no	no	Focal plant interactions with associated community are driven by aboveground mechanisms and not effected by plant neighbor
2.5	no	no	no	There is no distinct relationship between plant and its associated communities, so no effect of neighbor or pot type can be detected

Table 2. Results from Anovas testing a linear mixed effects model to predict how the fixed effects of heterospecific plant neighbor and pot type (i.e., with or without belowground interactions) influence aboveground biomass (log transformed) and relative growth rate (RGR), with year as a random effect in both models.

***N* and *p* values are given. Bold types indicate significant effects ($p < 0.05$).**

Response Variables	**Predictors**	*N*	*df*	Chi^2	*p-value*
Aboveground Biomass	Pot type	77	1	0.194	**0.033**
	Neighbor		2	7.112	0.399
	Neighbor x Pot type		2	9.447	**0.020**
RGR	Pot type		1	0.194	**0.042**
	Neighbor	77	2	7.112	0.230
	Neighbor x Pot type		2	9.447	0.225

Table 3. Summaries of linear models showing the effect of heterospecific plant neighbor and pot type on the relationship between plant responses and bacterial and fungal diversity. Plant responses used included aboveground biomass year one and two (AG biomass yr 1 and yr 2), relative growth rate year one and two (RGR yr 1 and yr 2), belowground biomass (root biomass), specific leaf area (SLA), and herbivory. Values show estimates with standard error in parentheses and significant relationships are indicated accordingly: *** p < 0.001; ** p < 0.01; * p < 0.05. All continuous predictors are mean-centered and scaled by 1 standard deviation.

Bacterial Diversity							
	AG biomass yr 1	AG biomass yr 2	RGR yr 1	RGR yr 2	Root biomass	SLA	Herbivory
(Intercept)	0.74 *** (0.18)	1.65 *** (0.16)	1.25 *** (0.26)	0.55 ** (0.17)	1.75 *** (0.19)	1.48 *** (0.06)	0.03 (0.20)
Bacterial diversity	0.15 (0.09)	0.13 (0.08)	0.12 (0.13)	0.10 (0.09)	0.05 (0.10)	-0.04 (0.03)	0.09 (0.10)
Split pots	0.09 (0.17)	0.05 (0.15)	0.19 (0.24)	0.09 (0.15)	-0.14 (0.18)	-0.04 (0.05)	0.06 (0.19)
A. millefolium	0.03 (0.22)	-0.71 *** (0.19)	0.26 (0.32)	-0.06 (0.20)	-0.61 * (0.23)	0.09 (0.07)	0.23 (0.25)
S. marianum	0.08 (0.21)	-0.09 (0.19)	-0.04 (0.30)	0.14 (0.19)	-0.52 * (0.22)	0.12 (0.07)	0.28 (0.24)
N	38	38	39	39	38	39	39
R2	0.09	0.34	0.1	0.06	0.22	0.2	0.06
Fungal Diversity							
	AG biomass yr 1	AG biomass yr 2	RGR yr 1	RGR yr 2	Root biomass	SLA	Herbivory
(Intercept)	0.68 *** (0.19)	1.72 *** (0.17)	1.20 *** (0.27)	0.53 ** (0.17)	1.74 *** (0.20)	1.48 *** (0.06)	-0.01 (0.21)
Fungal diversity	0.15 (0.09)	-0.07 (0.08)	0.13 (0.13)	0.07 (0.08)	0.03 (0.10)	-0.01 (0.03)	0.10 (0.1)
Split pots	0.17 (0.16)	0.05 (0.15)	0.26 (0.24)	0.14 (0.16)	-0.11 (0.18)	-0.05 (0.06)	0.12 (0.19)

Table 3. Continued

A. millefolium	0.16	-0.73 ***	0.37	0.00	-0.58 *	0.08	0.31
	(0.23)	(0.20)	(0.33)	(0.21)	(0.24)	(0.08)	(0.25)
S. marianum	0.03	-0.22	-0.08	0.09	-0.55 *	0.15 *	0.26
	(0.20)	(0.18)	(0.29)	(0.19)	(0.21)	(0.07)	(0.22)
N	38	38	39	39	38	39	39
R2	0.1	0.31	0.1	0.05	0.22	0.16	0.07

Table 4. Summary of the coefficients for the GLMs testing how plant traits (response variables) were affected by plant neighbor combination, soil inoculum origin and bacterial and fungal richness. Significant predictors are identified in bold ($p \leq 0.05$).

Response	Predictors	Estimate	Std. Error	t value	p value
Solidago SLA	(Intercept)	174.611	5.685	30.716	**<0.001**
	Polyculture: *Achillea*	-2.606	3.816	-0.683	0.495
	Polyculture: *Cirsium*	-3.424	4.030	-0.850	0.396
	Multispecies Polyculture	3.659	3.885	0.942	0.347
	Inoculum: *Solidago*	6.166	2.477	2.490	**0.013**
	Bacterial richness (q = 0)	0.000	0.000	-0.575	0.566
	Fungal richness (q = 0)	0.007	0.004	1.899	**0.059**
Solidago LDMC	(Intercept)	0.240	0.008	29.477	**<0.001**
	Polyculture: *Achillea*	0.000	0.005	-0.030	0.976
	Polyculture: *Cirsium*	-0.001	0.006	-0.247	0.805
	Multispecies Polyculture	-0.014	0.006	-2.450	**0.015**
	Inoculum: *Solidago*	-0.010	0.004	-2.741	**0.006**
	Bacterial richness (q = 0)	0.000	0.000	1.270	0.205
	Fungal richness (q = 0)	0.000	0.000	-0.257	0.798
Solidago LMA	(Intercept)	0.006	0.000	34.209	**<0.001**
	Polyculture: *Achillea*	0.000	0.000	0.739	0.460
	Polyculture: *Cirsium*	0.000	0.000	0.727	0.468
	Multispecies Polyculture	0.000	0.000	-0.893	0.372
	Inoculum: *Solidago*	0.000	0.000	-2.541	**0.012**
	Bacterial richness (q = 0)	0.000	0.000	0.764	0.445
	Fungal richness (q = 0)	0.000	0.000	-1.755	0.080
Solidago Height	(Intercept)	3.149	0.061	51.341	**<0.001**
	Polyculture: *Achillea*	-0.181	0.068	-2.646	**0.009**
	Polyculture: *Cirsium*	-0.117	0.071	-1.640	0.102
	Multispecies Polyculture	-0.221	0.070	-3.143	**0.002**
	Inoculum: *Solidago*	-0.123	0.043	-2.857	**0.005**

Table 5. Summary of the coefficients for the GLMs testing how fungal and bacterial diversity (response variables) were affected by plant neighbor combination and soil inoculum origin. Significant predictors are identified in bold ($p \leq 0.05$).

Response	Predictors	Estimate	Std. Error	t value	p value
Fungal Richness (q = 0)	(Intercept)	933.546	50.358	18.538	**0.000**
	Polyculture: *Achillea*	71.331	56.120	1.271	0.205
	Polyculture: *Cirsium*	203.254	58.428	3.479	**0.001**
	Multispecies Polyculture	-0.284	57.665	-0.005	0.996
	Inoculum: Potting mix	-8.046	83.575	-0.096	0.923
	Inoculum: *Solidago*	-104.426	35.298	-2.958	**0.003**
Fungal Shannon's Diversity (q = 1)	(Intercept)	133.780	6.297	21.245	**0.000**
	Polyculture: *Achillea*	33.234	7.018	4.736	**0.000**
	Polyculture: *Cirsium*	22.132	7.306	3.029	**0.003**
	Multispecies Polyculture	3.206	7.211	0.445	0.657
	Inoculum: Potting mix	14.306	10.451	1.369	0.172
	Inoculum: *Solidago*	-1.012	4.414	-0.229	0.819
Fungal Inverse Simpson's Evenness (q = 2)	(Intercept)	61.347	2.570	23.872	**0.000**
	Polyculture: *Achillea*	13.384	2.864	4.673	**0.000**
	Polyculture: *Cirsium*	4.648	2.982	1.559	0.120
	Multispecies Polyculture	0.357	2.943	0.121	0.904
	Inoculum: Potting mix	3.048	4.265	0.715	0.475
	Inoculum: *Solidago*	2.605	1.801	1.446	0.149
Bacterial Richness (q = 0)	(Intercept)	5606.287	428.163	13.094	**0.000**
	Polyculture: *Achillea*	-398.448	477.152	-0.835	0.404
	Polyculture: *Cirsium*	-221.319	496.779	-0.446	0.656
	Multispecies Polyculture	-353.766	490.287	-0.722	0.471
	Inoculum: Potting mix	653.713	710.587	0.920	0.358
	Inoculum: *Solidago*	538.093	300.120	1.793	**0.074**
Bacterial Shannon's Diversity (q = 1)	(Intercept)	3824.124	299.510	12.768	**0.000**
	Polyculture: *Achillea*	-223.377	333.778	-0.669	0.504
	Polyculture: *Cirsium*	-69.155	347.508	-0.199	0.842
	Multispecies Polyculture	-151.655	342.967	-0.442	0.659
	Inoculum: Potting mix	555.642	497.072	1.118	0.264
	Inoculum: *Solidago*	367.594	209.940	1.751	0.081

Table 5. Continued

Bacterial Inverse Simpson's evenness (q = 2)	(Intercept)	2808.671	224.613	12.505	**0.000**
	Polyculture: *Achillea*	-69.582	250.312	-0.278	0.781
	Polyculture: *Cirsium*	75.103	260.608	0.288	0.773
	Multispecies Polyculture	20.959	257.203	0.081	0.935
	Inoculum: Potting mix	515.194	372.771	1.382	0.168
	Inoculum: *Solidago*	252.261	157.442	1.602	0.110

Table 6. Summary of the parametric coefficients and smooth terms for the GAMs used to test how plant neighbor changes the relationship between *Solidago* functional trait space (PC1 and PC2 as the predictor variable), and belowground diversity (predictor variables). Bold indicates significant relationships ($p \leq 0.05$).

	Response	Parametric coefficients				Approximate significance of smooth terms				
		Est.	Std. Error	T-val	Pr(>|t|)	edf	Ref. df	F-value	p-value	deviance explained
Fungal Richness	overall	961.58	17.15	56.05	<0.001	8.307	11.67	1.192	0.279	5.76
	Achillea	958.97	37.17	25.8	<0.001	3.133	4.034	0.544	0.712	3.22
	both	881.59	28.74	30.67	<0.001	14.46	19.01	2.178	**0.0083**	41
	Cirsium	1100.5	21.6	50.95	<0.001	2.011	2.022	3.465	**0.0358**	7.28
	Solidago	896.06	36.06	24.85	<0.001	2.001	2.003	1.377	0.259	3.85
Fungal Shannon's Diversity	overall	150.727	2.162	69.7	<0.001	5.26	7.421	1.86	0.0725	4.63
	Achillea	166.57	4.763	34.97	<0.001	2	2.001	2.348	0.1	3.99
	both	136.486	3.939	34.65	<0.001	8.888	12.24	1.189	0.302	21.3
	Cirsium	155.561	3.518	44.22	<0.001	4.805	6.687	1.544	0.154	14.3
	Solidago	138.212	2.485	55.61	<0.001	3.323	4.41	0.624	0.613	6.81
Fungal Inverse Simpsons	overall	67.9563	0.8916	76.22	<0.001	2.003	2.006	5.132	**0.00629**	2.68
	Achillea	75.877	2.098	36.17	<0.001	2.001	2.001	3.298	**0.0405**	5.52
	both	62.993	1.308	48.18	<0.001	5.646	7.858	0.637	0.742	9.67
	Cirsium	66.901	1.481	45.16	<0.001	3.119	4.044	1.572	0.187	8.24
	Solidago	63.231	1.158	54.59	<0.001	2.001	2.001	0.728	0.487	2.07

Table 6. Continued

Bacterial Richness	overall	5601.5	143.5	39.03	<0.001	2.039	2.078	0.087	0.938	0.07
	Achillea	5444.4	246.4	22.1	<0.001	2.006	2.013	0.114	0.896	0.21
	both	5518.8	234.7	23.52	<0.001	7.68	10.65	0.784	0.642	15.6
	Cirsium	5572.1	279.2	19.96	<0.001	2.001	2.001	1.787	0.173	3.87
	Solidago	6004	391	15.36	<0.001	5.131	7.246	1.149	0.356	16.8
Bacterial Shannon's Diversity	overall	3886	100.4	38.72	<0.001	2.042	2.084	0.096	0.931	0.07
	Achillea	3762.4	170.8	22.02	<0.001	2.005	2.01	0.121	0.889	0.22
	both	3854.4	166.5	23.14	<0.001	7.852	10.87	0.797	0.635	16.1
	Cirsium	3882.8	196.6	19.75	<0.001	2.004	2.008	1.768	0.177	3.84
	Solidago	4131.9	269.1	15.35	<0.001	5.308	7.507	1.295	0.278	18.4
Bacterial Inverse Simpsons	overall	2947.5	75.2	39.19	<0.001	2.029	2.058	0.151	0.876	0.10
	Achillea	2850	126.2	22.58	<0.001	2.002	2.004	0.153	0.859	0.27
	both	2954.5	125.9	23.47	<0.001	8.324	11.5	0.868	0.567	17.60%
	Cirsium	2971.5	149.4	19.89	<0.001	2.006	2.012	1.723	0.184	3.76
	Solidago	3064.5	197.7	15.5	<0.001	5.449	7.714	1.559	0.168	20.80

Table 7. Tukey test results for betadisper table showing pairwise differences in composition between plant combinations. Soli mon refers to *S. altissima* monocultures, Cirs mon are *C. discolor* monocultures, Achil mon are *A. millefolium* monocultures. Bold values indicate significantly different pairwise comparisons ($p \leq 0.05$), using the adjusted p-values.

Groupings	Pairwise comparisons	Fungal composition	Bacterial composition
		p.adj	p.adj
Monocultures	Cirs mon-Soli mon	0.998	1
	Achi mon-Soli mon	1	0.962
	Achi mon-Cirs mon	0.993	0.955
Multispecies polycultures compared to monocultures	Soli X Cirs X Achi-Soli mon	**0.014**	0.292
	Cirs mon-Soli X Cirs X Achi	**0.069**	0.313
	Achi mon-Soli X Cirs X Achi	**0.008**	**0.023**
C. discolor polyculture compared to monocultures	Cirs mon-Soli X Cirs	**0.02**	0.465
	Soli X Cirs-Soli mon	**0.003**	0.44
A. millefolium polycultures compared to monocultures	Achi mon-Soli X Achi	**0.029**	**0.065**
	Soli X Achi-Soli mon	**0.045**	0.509
Comparing polycultures to one another	Soli X Achi-Soli X Cirs X Achi	0.988	0.991
	Soli X Cirs X Achi-Soli X Cirs	0.949	0.999
	Soli X Achi-Soli X Cirs	0.692	1

Table 8. Summary of the initial models showing the effects of polycultures in relation to monocultures, and soil origin on *C. discolor* mean specific leaf area (SLA), mean leaf dry matter content (LDMC), foliar herbivory and flowering, with significant effects in bold ($p \leq 0.05$).

Response	Predictors	Estimate	Std. Error	z value	Pr(>|z|)
Cirsium SLA	(Intercept)	107.264	9.854	10.886	**<0.001**
	Pairwise polyculture	72.998	10.521	6.938	**<0.001**
	Multispecies polyculture	39.101	10.252	3.814	**<0.001**
	Soil origin: old field	-5.444	5.861	-0.929	0.357
Cirsium LDMC	(Intercept)	0.155	0.008	19.221	**<0.001**
	Pairwise polyculture	-0.011	0.009	-1.277	0.207
	Multispecies polyculture	-0.023	0.008	-2.734	**0.008**
	Soil origin: old field	0.003	0.005	0.550	0.585
Cirsium herbivory	(Intercept)	-2.011	0.430	-4.677	0.000
	Neighbor herbivory	0.044	0.021	2.073	**0.038**
	Pairwise polyculture	-1.236	0.326	-3.794	**<0.001**
	Multispecies polyculture	0.244	0.339	0.719	0.472
	Soil origin: old field	-0.500	0.216	-2.318	**0.020**
Cirsium flowering	(Intercept)	71.987	11.130	6.468	**<0.001**
	Pairwise polyculture	-35.321	11.865	-2.977	**0.004**
	Multispecies polyculture	2.556	11.624	0.220	0.827
	Soil origin: old field	-0.308	6.727	-0.046	0.964

Table 9. Summary table showing the coefficients for each component model of each growth-defense SEM. Shading is used to distinguish between each neighbor SEMS, beginning with the monoculture SEM, followed by the pairwise polyculture SEM, then the multispecies polyculture SEM. Significant paths are identified using bold text ($p \leq 0.05$).

Response	Predictor	DF	Crit. Value	p-value	Std. Estimate
Cirsium SLA	**Monoculture**	**22**	**-3.760**	**0.001**	**-0.615**
	Soil origin	22	1.515	0.144	0.248
Cirsium herbivory	*Cirsium* SLA	21	-1.864	0.076	-0.465
	Monoculture	21	0.049	0.962	0.012
	Soil origin	21	1.188	0.248	0.239
Bacterial richness	Monoculture	21	1.204	0.242	0.291
	Cirsium SLA	21	0.759	0.457	0.193
	Cirsium herbivory	21	-1.877	0.074	-0.403
Fungal richness	Monoculture	21	1.250	0.225	0.324
	Cirsium SLA	21	1.015	0.322	0.276
	Cirsium herbivory	21	-0.566	0.578	-0.130
Cirsium SLA	**Pairwise polyculture**	**22**	**2.585**	**0.017**	**0.480**
	Soil origin	22	0.644	0.527	0.119
Cirsium herbivory	*Cirsium* SLA	21	-1.338	0.195	-0.271
	Pairwise polyculture	21	-2.055	0.053	-0.414
	Soil origin	21	1.515	0.145	0.270
Bacterial richness	Pairwise polyculture	21	-1.107	0.281	-0.268
	Cirsium SLA	21	0.524	0.606	0.121
	Cirsium herbivory	21	-2.067	0.051	-0.479
Fungal richness	Pairwise polyculture	21	1.141	0.267	0.296
	Cirsium SLA	21	-0.041	0.968	-0.010
	Cirsium herbivory	21	-0.009	0.993	-0.002
Cirsium SLA	Multispecies polyculture	22	1.434	0.166	0.289
	Soil origin	22	1.161	0.258	0.234
Cirsium herbivory	***Cirsium* SLA**	**21**	**-3.014**	**0.007**	**-0.571**
	Multispecies polyculture	21	1.788	0.088	0.335
	Soil origin	21	1.637	0.117	0.302
Bacterial richness	Multispecies polyculture	21	0.117	0.908	0.025
	Cirsium SLA	21	0.075	0.941	0.018
	Cirsium herbivory	21	-1.689	0.106	-0.391
Fungal richness	Multispecies polyculture	21	-1.605	0.124	-0.353
	Cirsium SLA	21	0.966	0.345	0.232
	Cirsium herbivory	21	0.016	0.988	0.004

Table 10. Bootstrapped confidence intervals for each path in each model for the Growth-defense SEMs. Bold indicates significant pathways. Shading is used to distinguish between plant neighbor combination SEMs, starting with the monoculture, then pairwise polyculture, followed by multispecies polyculture SEM. Significant paths are shown in bold ($p \leq 0.05$).

Response	Predictors	Effect	Bias	Std. Err.	Lower CI	Upper CI
Cirsium SLA	(Intercept)	0	0	0	0	0
	Monocultures	**-0.613**	**-0.003**	**0.146**	**-0.837**	**-0.296**
	Soil origin	0.247	-0.023	0.136	-0.011	0.513
Cirsium herbivory	(Intercept)	0	0	0	0	0
	Cirsium SLA	-0.355	0	0.133	-0.563	0.015
	Monocultures	0.009	-0.026	0.157	-0.301	0.304
	Soil origin	0.227	0.014	0.174	-0.178	0.533
Bacterial richness	(Intercept)	0	0	0	0	0
	Monocultures	0.233	0.001	0.156	-0.048	0.542
	Cirsium SLA	0.147	0.017	0.154	-0.2	0.421
	***Cirsium* herbivory**	**-0.364**	**0.028**	**0.186**	**-0.714**	**-0.001**
Fungal richness	(Intercept)	0	0	0	0	0
	Monocultures	0.259	-0.018	0.158	-0.045	0.587
	Cirsium SLA	0.211	-0.003	0.148	-0.104	0.493
	Cirsium herbivory	-0.117	0.027	0.191	-0.498	0.26
Cirsium SLA	(Intercept)	0	0	0	0	0
	Pairwise polycultures	**0.473**	**-0.006**	**0.117**	**0.225**	**0.681**
	Soil origin	0.118	-0.021	0.172	-0.236	0.45

Table 10. Continued

Cirsium flowering	(Intercept)	0	0	0	0	0
	Cirsium SLA	-0.233	0.006	0.157	-0.522	0.072
	Pairwise polycultures	-0.358	0.017	0.167	-0.621	0.054
	Soil origin	0.264	-0.02	0.163	-0.074	0.571
Bacterial richness	(Intercept)	0	0	0	0	0
	Pairwise polycultures	-0.216	0.023	0.192	-0.608	0.143
	Cirsium SLA	0.102	-0.015	0.169	-0.237	0.43
	***Cirsium* herbivory**	**-0.403**	**0.031**	**0.169**	**-0.731**	**-0.096**
Fungal richness	(Intercept)	0	0	0	0	0
	Pairwise polycultures	0.238	0.008	0.167	-0.134	0.531
	Cirsium SLA	-0.009	-0.004	0.176	-0.345	0.368
	Cirsium herbivory	-0.002	0.019	0.209	-0.414	0.392
Cirsium SLA	(Intercept)	0	0	0	0	0
	Multispecies polycultures	0.287	-0.028	0.202	-0.14	0.65
	Soil origin	0.232	-0.025	0.189	-0.18	0.548
Cirsium flowering	(Intercept)	0	0	0	0	0
	***Cirsium* SLA**	**-0.536**	**0.042**	**0.157**	**-0.772**	**-0.197**
	Multispecies polycultures	0.318	-0.019	0.167	-0.008	0.618
	Soil origin	0.291	0	0.169	-0.072	0.579
Bacterial richness	(Intercept)	0	0	0	0	0
	Multispecies polycultures	0.023	-0.006	0.2	-0.367	0.406
	Cirsium SLA	0.015	0.008	0.196	-0.354	0.415
	Cirsium herbivory	-0.338	0.009	0.183	-0.663	0.048
Fungal richness	(Intercept)	0	0	0	0	0
	Multispecies polycultures	-0.326	0.005	0.159	-0.6	0.037
	Cirsium SLA	0.196	-0.011	0.146	-0.091	0.482
	Cirsium herbivory	0.003	0.005	0.176	-0.345	0.347

Table 11. Summary table showing the coefficients for each component model of each growth-reproduction SEM. We ran a separate model for each plant neighbor combination, using shading to distinguish them from one another, beginning with the monoculture SEM, followed by the pairwise polyculture SEM, then the multispecies polyculture SEM. Significant paths are shown in bold ($p \leq 0.05$).

Response	Predictor	DF	Crit. Value	p-value	Std. Estimate
Cirsium SLA	**Monoculture**	**59**	**-3.693**	**0.001**	**-0.433**
	Soil origin	59	0.909	0.367	0.107
Cirsium flowering	*Cirsium* SLA	58	-4.446	0.000	-0.555
	Monoculture	58	-0.843	0.403	-0.105
	Soil origin	58	0.916	0.363	0.104
Bacterial richness	Monoculture	57	-0.068	0.946	-0.010
	Soil origin	57	1.621	0.111	0.213
	Cirsium SLA	57	-0.505	0.616	-0.084
	Cirsium flowering	57	-0.236	0.814	-0.036
Fungal richness	Monoculture	57	0.863	0.392	0.125
	Soil origin	57	0.557	0.580	0.073
	Cirsium SLA	57	0.606	0.547	0.101
	Cirsium flowering	57	-0.694	0.491	-0.105
Cirsium SLA	**Pairwise polyculture**	**59**	**5.834**	**0.000**	**0.604**
	Soil origin	59	0.979	0.332	0.101
Cirsium flowering	*Cirsium* SLA	58	-1.943	0.057	-0.255
	Pairwise polyculture	**58**	**-3.219**	**0.002**	**-0.421**
	Soil origin	58	0.537	0.593	0.056
Bacterial richness	Pairwise polyculture	57	-0.291	0.772	-0.051
	Soil origin	57	1.601	0.115	0.209
	Cirsium SLA	57	-0.343	0.733	-0.058
	Cirsium flowering	57	-0.326	0.746	-0.053
Fungal richness	Pairwise polyculture	57	0.612	0.543	0.109
	Soil origin	57	0.709	0.481	0.093
	Cirsium SLA	57	-0.037	0.971	-0.006
	Cirsium flowering	57	-0.490	0.626	-0.081
Cirsium SLA	**Multispecies polyculture**	**59**	**-2.110**	**0.039**	**-0.264**
	Soil origin	59	0.683	0.497	0.086
Cirsium flowering	***Cirsium* SLA**	**58**	**-3.850**	**0.000**	**-0.412**
	Multispecies polyculture	**58**	**3.447**	**0.001**	**0.368**
	Soil origin	58	0.709	0.481	0.073

Table 11. Continued

Bacterial richness	Multispecies polyculture	57	0.436	0.665	0.064
	Soil origin	57	1.623	0.110	0.211
	Cirsium SLA	57	-0.514	0.609	-0.077
	Cirsium flowering	57	-0.390	0.698	-0.064
Fungal richness	Multispecies polyculture	57	-0.901	0.371	-0.133
	Soil origin	57	0.680	0.499	0.089
	Cirsium SLA	57	0.237	0.814	0.036
	Cirsium flowering	57	-0.352	0.726	-0.058

Table 12. Bootstrapped confidence intervals for each path in each model for the Growth-defense SEMs. Bold indicates significant pathways ($p \leq 0.05$). Shading is used to distinguish between plant neighbor combination SEMs, starting with the monoculture, then pairwise polyculture, followed by multispecies polyculture SEM.

Response	Predictors	Effect	Bias	Std. Err.	Lower CI	Upper CI
Cirsium SLA	(Intercept)	0	0	0	0	0
	Monocultures	**-0.432**	**0.009**	**0.104**	**-0.635**	**-0.228**
	Soil origin	0.106	-0.01	0.11	-0.111	0.322
Cirsium flowering	(Intercept)	0	0	0	0	0
	***Cirsium* SLA**	**-0.499**	**0.008**	**0.094**	**-0.667**	**-0.285**
	Monocultures	-0.095	0	0.099	-0.299	0.071
	Soil origin	0.103	0.001	0.11	-0.129	0.33
Bacterial richness	(Intercept)	0	0	0	0	0
	Monocultures	-0.009	0.004	0.12	-0.236	0.221
	Soil origin	0.21	-0.017	0.124	-0.054	0.442
	Cirsium SLA	-0.065	0.009	0.122	-0.341	0.158
	Cirsium flowering	-0.031	0.005	0.104	-0.24	0.163
Fungal richness	(Intercept)	0	0	0	0	0
	Monocultures	0.112	-0.007	0.092	-0.073	0.286
	Soil origin	0.072	0.002	0.128	-0.188	0.301
	Cirsium SLA	0.079	0.003	0.146	-0.22	0.336
	Cirsium flowering	-0.09	0.001	0.125	-0.317	0.158
Cirsium SLA	(Intercept)	0	0	0	0	0
	Pairwise polyculture	**0.603**	**-0.003**	**0.06**	**0.464**	**0.706**
	Soil origin	0.101	0.004	0.1	-0.119	0.272

Table 12. Continued

Cirsium flowering	(Intercept)	0	0	0	0	0
	***Cirsium* SLA**	**-0.202**	**0.007**	**0.095**	**-0.38**	**-0.009**
	Pairwise polyculture	**-0.335**	**0.01**	**0.088**	**-0.512**	**-0.165**
	Soil origin	0.056	0.003	0.106	-0.15	0.249
Bacterial richness	(Intercept)	0	0	0	0	0
	Pairwise polyculture	-0.038	0.004	0.122	-0.275	0.205
	Soil origin	0.207	-0.01	0.133	-0.049	0.46
	Cirsium SLA	-0.044	0.009	0.12	-0.297	0.16
	Cirsium flowering	-0.042	0.002	0.104	-0.237	0.164
Fungal richness	(Intercept)	0	0	0	0	0
	Pairwise polyculture	0.08	-0.002	0.125	-0.174	0.317
	Soil origin	0.092	0.004	0.131	-0.175	0.327
	Cirsium SLA	-0.005	-0.006	0.14	-0.252	0.301
	Cirsium flowering	-0.064	-0.001	0.131	-0.31	0.216
Cirsium SLA	(Intercept)	0	0	0	0	0
	Multispecies polyculture	-0.264	-0.003	0.128	-0.482	0.044
	Soil origin	0.086	-0.002	0.115	-0.142	0.305
Cirsium flowering	(Intercept)	0	0	0	0	0
	***Cirsium* SLA**	**-0.396**	**0.006**	**0.098**	**-0.59**	**-0.197**
	Multispecies polyculture	**0.355**	**-0.006**	**0.093**	**0.162**	**0.521**
	Soil origin	0.073	0.001	0.101	-0.124	0.275
Bacterial richness	(Intercept)	0	0	0	0	0
	Multispecies polyculture	0.056	-0.007	0.126	-0.18	0.303
	Soil origin	0.21	-0.017	0.125	-0.037	0.478
	Cirsium SLA	-0.066	0.005	0.117	-0.302	0.161
	Cirsium flowering	-0.05	-0.001	0.113	-0.284	0.156
Fungal richness	(Intercept)	0	0	0	0	0
	Multispecies polyculture	-0.117	0.011	0.115	-0.35	0.101
	Soil origin	0.088	0.009	0.131	-0.181	0.34
	Cirsium SLA	0.031	-0.006	0.131	-0.229	0.272
	Cirsium flowering	-0.046	-0.005	0.122	-0.267	0.201

Table 13. Table providing the summary statistics for the pollen protein analyses models, where we tested how pollen protein content (predictor) changed based on *C. discolor* aboveground biomass, mean SLA, and flowering, comparing polycultures to the monocultures. Significant paths are shown in bold ($p \leq 0.05$).

Response Variables	Estimate	Std. Error	z value	p-value	AIC
(Intercept)	6.535	0.039	168.913	< **0.001**	1983.4
C. discolor aboveground biomass	-0.002	0.000	-8.014	< **0.001**	
Polyculture: *S. altissima*	-0.767	0.039	-19.548	< **0.001**	
Polyculture: multispecies	-0.010	0.026	-0.392	0.695	
(Intercept)	6.577	0.061	107.802	< **0.001**	2017.8
C. discolor SLA	-0.003	0.000	-5.559	< **0.001**	
Polyculture: *S. altissima*	-0.408	0.038	-10.672	< **0.001**	
Polyculture: multispecies	0.133	0.024	5.586	< **0.001**	
(Intercept)	6.289	0.040	158.788	< **0.001**	2047.7
C. discolor flowering per pot	-0.001	0.000	-1.098	0.272	
Polyculture: *S. altissima*	-0.567	0.034	-16.911	< **0.001**	
Polyculture: multispecies	0.100	0.023	4.343	< **0.001**	

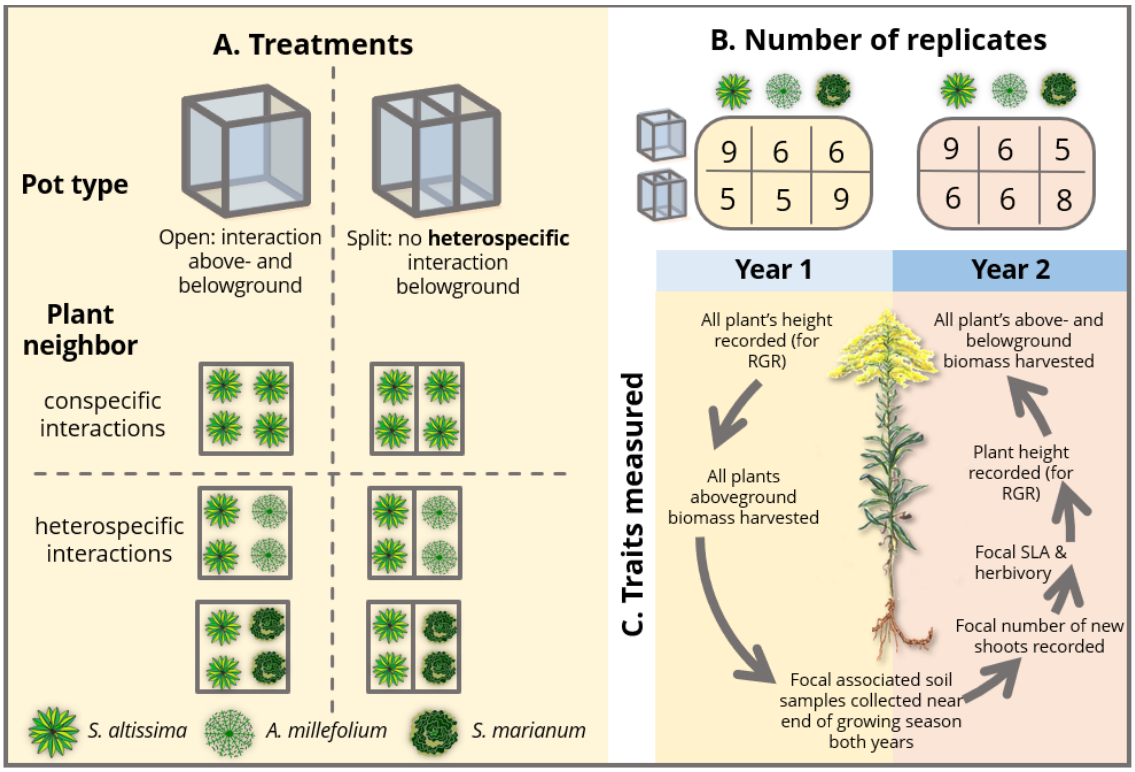


Figure 1. Schematic of the experimental design.

Panel A shows the treatments in the experiment - pot type and plant neighbor. We had two pot types, one allowing full belowground interaction (open pots), the other prevented belowground interaction with heterospecific neighbors, thereby only allowing aboveground heterospecific interactions (split pots). Two individuals of the focal species (*S. altissima*) were planted with either two *S. altissima*, *A. millefolium* or *S. marianum* individuals, in both pot types. Note that intraspecific interactions occur in all treatments. Panel B shows the number of replicates across treatments and years. Panel C shows the data collected in each year; note, relative growth rate (RGR) was calculated from the height data over time (see methods description). Original *S. altissima* watercolor painting by Shannon Bayliss, adapted for this figure.

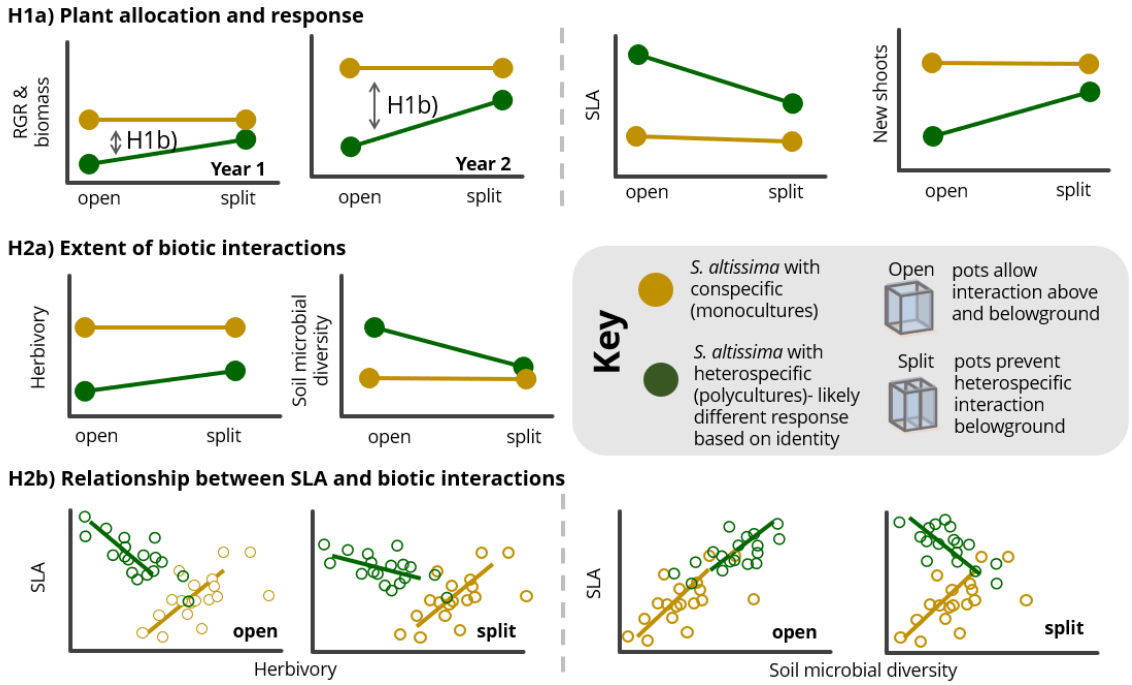


Figure 2. Schematic describing the predictions of our hypotheses

1a) tests direct changes to plant allocation, and response traits, predicting reduction in growth and fitness and increase in specific leaf area (SLA) in the presence of heterospecific plant neighbors, mediated by belowground processes. 1b) predicts that changes to allocation will differ from year to year, with a larger effect in year two as resources become limiting. Hypothesis 2a) predicts a decrease in herbivory in polycultures, and an increase in soil microbial richness and diversity in open pot polycultures, and hypothesis 2b) predicts that the relationship (both slope and direction) between SLA and herbivory, and SLA and the soil microbial community will change based on pot type and neighbor. Yellow lines denote the response of *S. altissima* with conspecific, green lines denote *S. altissima* response to heterospecifics. Though not illustrated, we do expect different plant neighbors to elicit different responses.

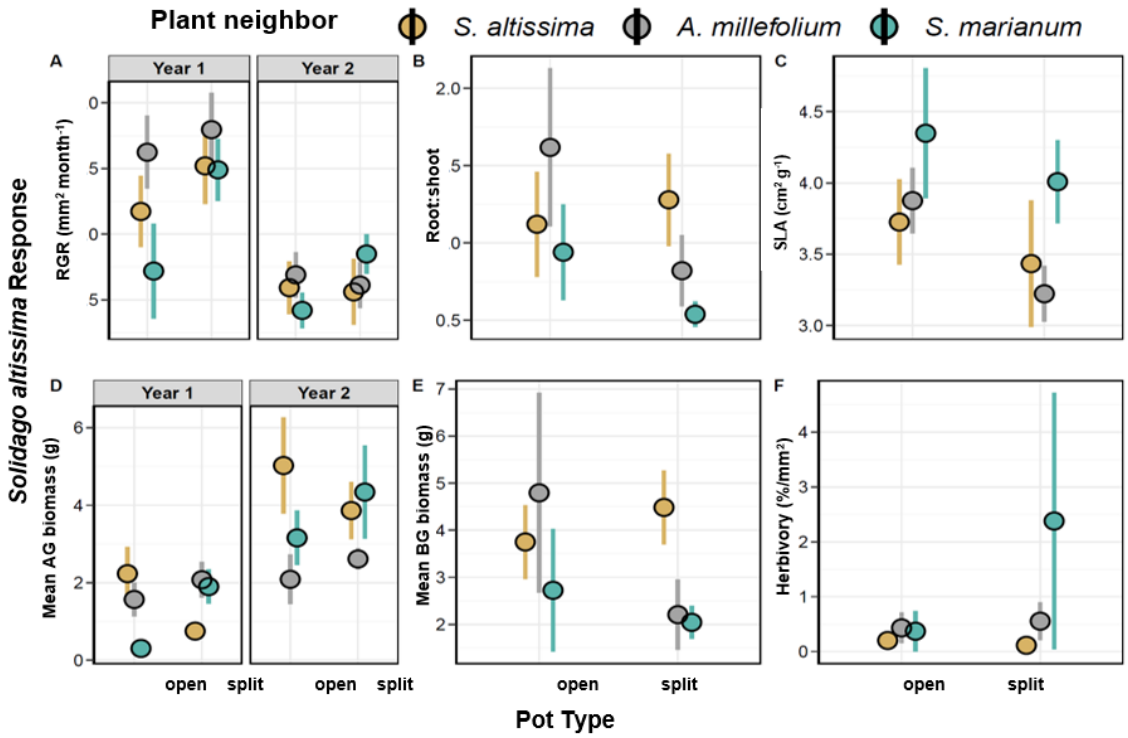


Figure 3. Mean and standard error plots showing *S. altissima* (A) relative growth rate (RGR), (B) root-to-shoot ratio (i.e., root:shoot), (C) specific leaf area (SLA), (D) mean aboveground biomass, (E) mean root biomass, and (F) foliar herbivory as a function of pot type (open or split, with or without belowground interactions, respectively), plant identity, (either with *S. altissima, A. millefolium* or *S. marianum,* differentiated by color) and year.

Note, all plots show data collected in year two, except for RGR and aboveground biomass which were collected in both years one and two.

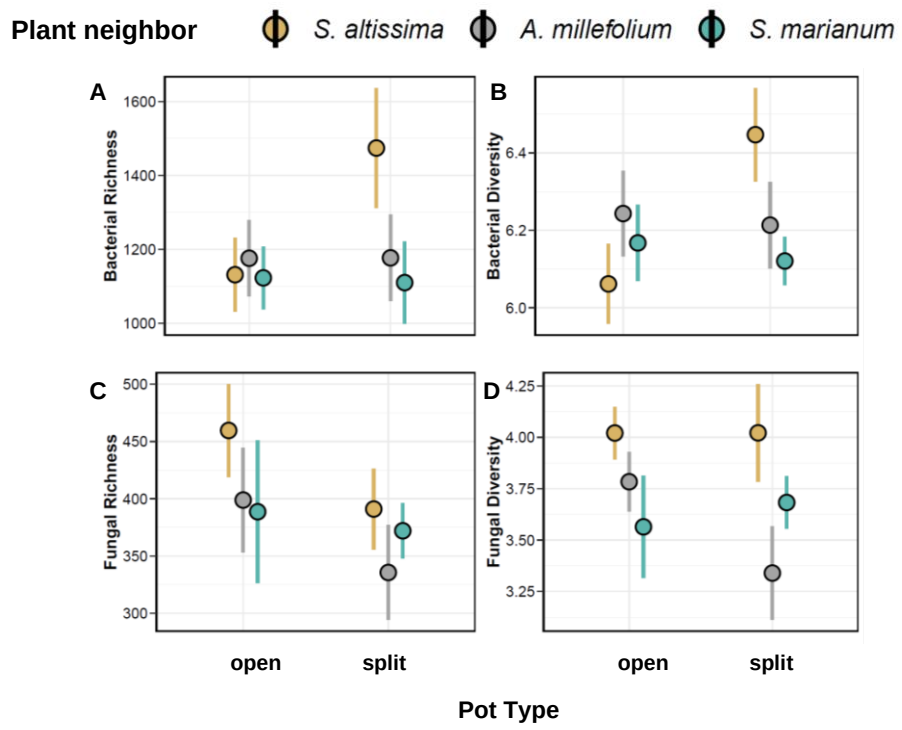


Figure 4. Mean and standard error plots showing (A) bacterial richness and (B) bacterial diversity and (C) fungal richness and (D) fungal diversity from soil associated with the focal S. altissima based on pot type (i.e., with or without belowground interactions) and plant neighbor identity.

Colors represent specific plant neighbors (gray for *A. millefolium*, yellow with *S. altissima* and blue with *S. marianum*).

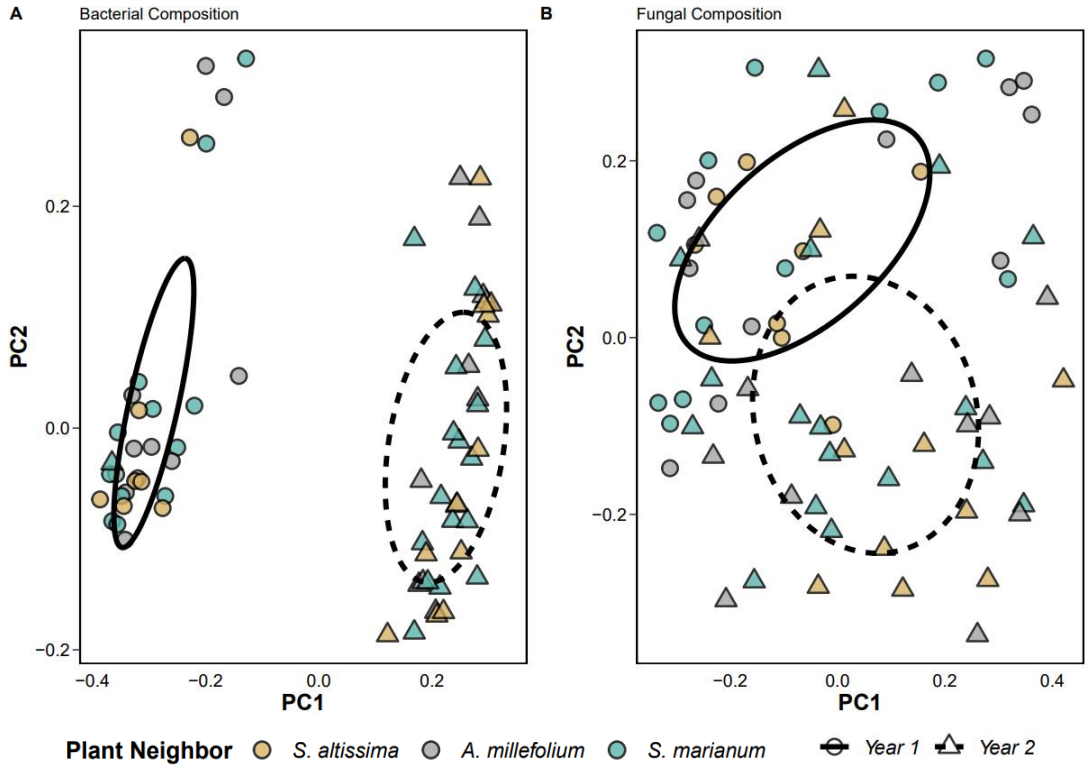


Figure 5. Biplots depicting the principal components analysis (PCoA) results, used to visualize dissimilarities in (A) bacterial community composition, and (B) fungal community composition.

Points represent the community composition of each sample (*S. altissima* associated soil from each pot), colored based on plant neighbor (gray for *A. millefolium*, yellow with *S. altissima* and blue with *S. marianum*). The 95% confidence ellipses are used to display the region where 95% of the samples are located, for each year. Year was significant for both bacterial and fungal composition, while neighbor and pot type did not alter microbial composition.

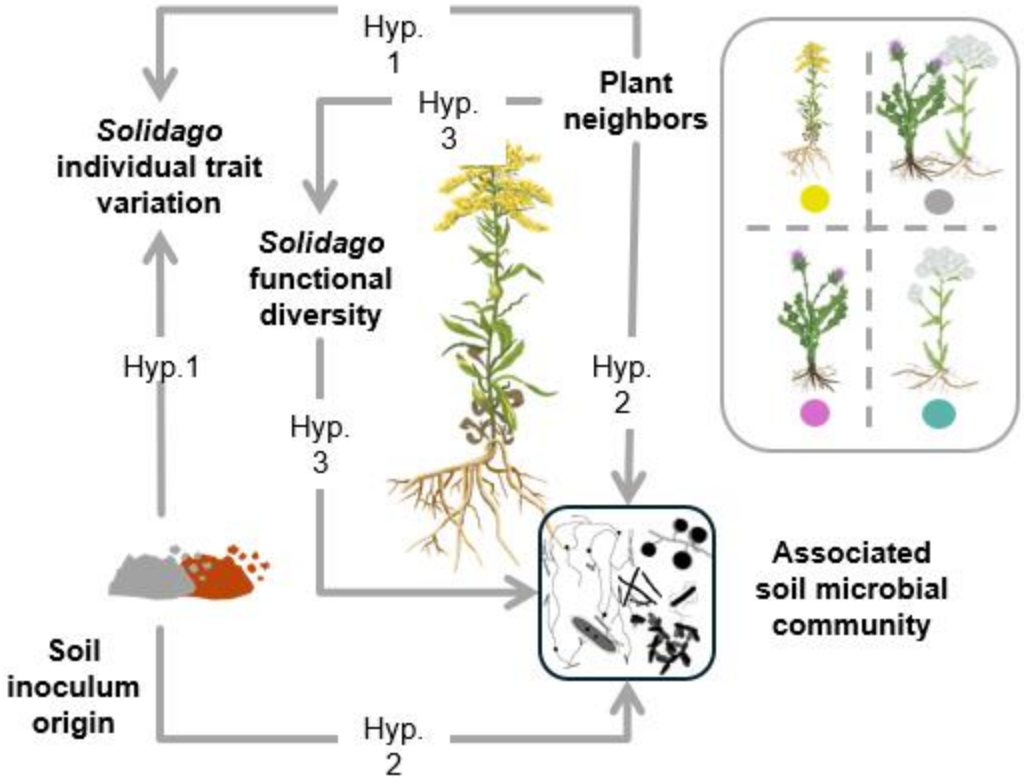


Figure 6. Conceptual figure showing the hypotheses tested in this study.

Based on the relationship between a focal plant, its associated microbial community and plant neighbors. We tested how *S. altissima's* individual plant traits changed based on plant neighbor combination and soil inoculum origin (1). Next, we tested how *S. altissima's* associated soil microbial diversity was affected by plant neighbor combination and soil inoculum origin, as well as whether plant neighbors alter soil microbial composition and turnover (2). Lastly, we tested whether plant neighbors altered *S. altissima's* functional diversity (functional richness and divergence), and whether this cascades to affect *S. altissima's* associated microbial diversity (3).

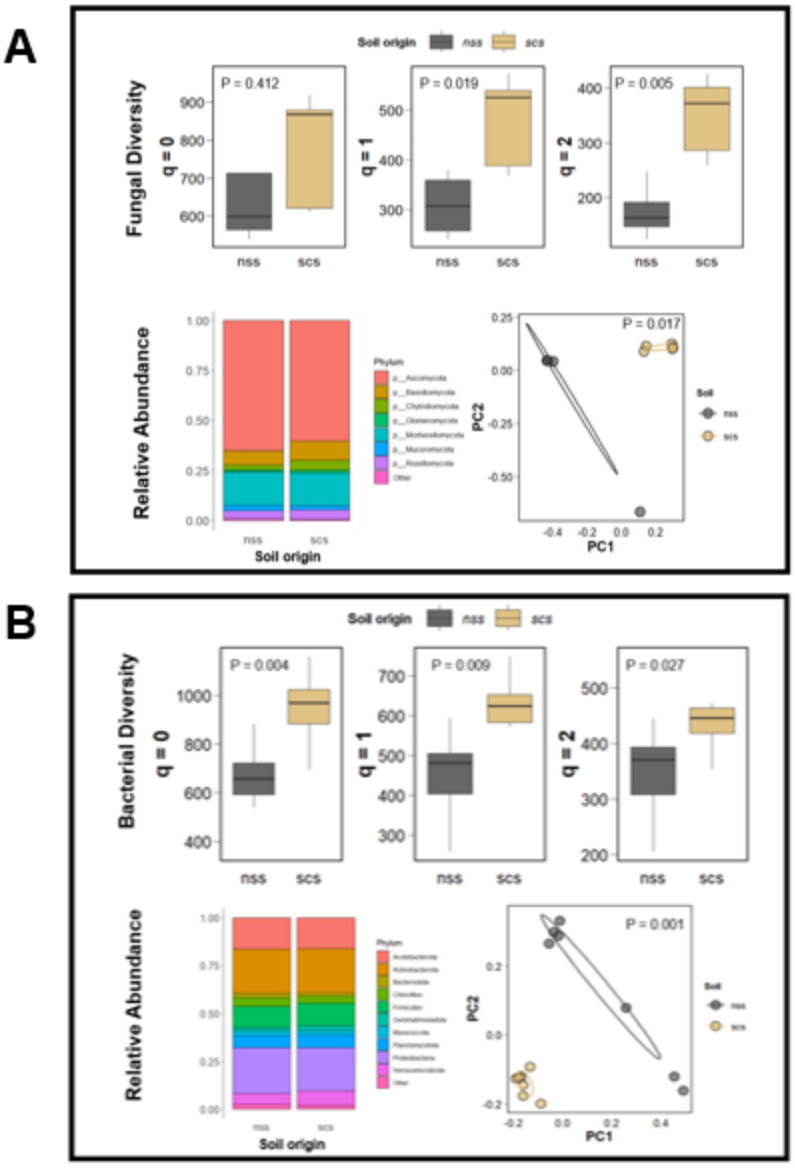


Figure 7. Results from sequencing the starting soil inoculum, which show that *S. altissima* conditioned soil had higher fungal and bacterial richness compared to the wild old field soil inoculum. In addition, the soil inoculum origins had different bacterial and fungal composition from one another.

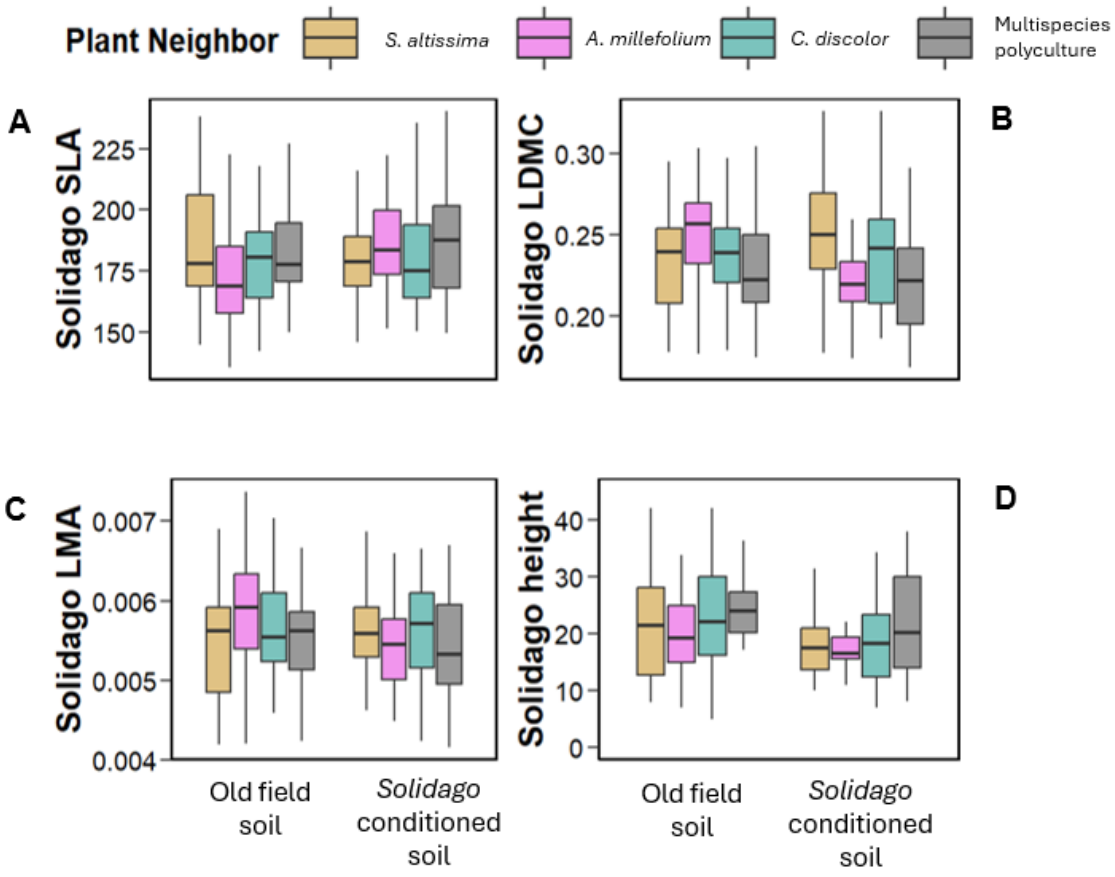
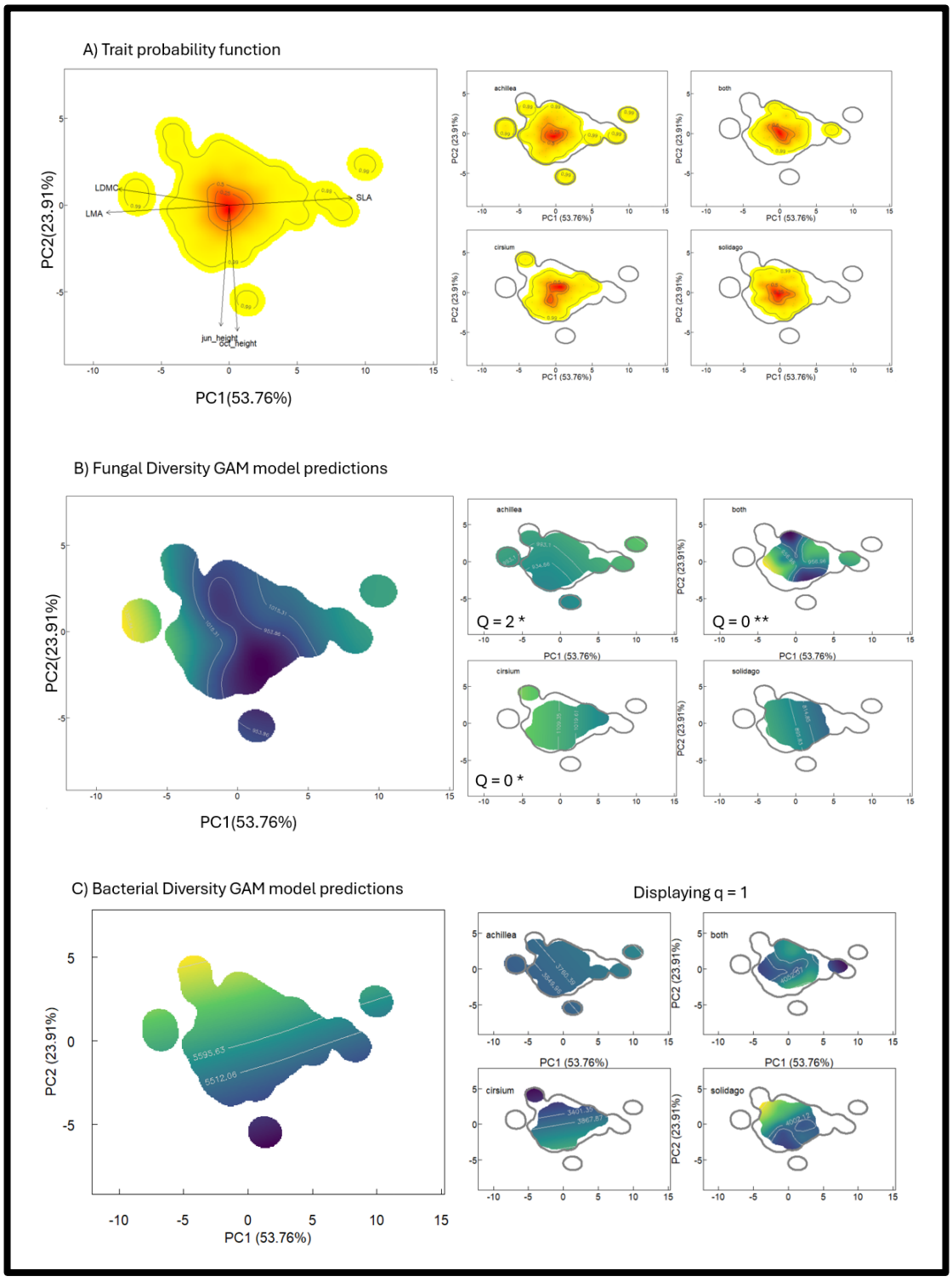


Figure 8. Boxplots showing *S. altissima* trait response to plant neighbor and soil inoculum origin treatments, colored based on plant neighbor. Traits measured include A) specific leaf area (SLA), B) leaf dry matter content (LDMC), C) leaf matter per area (LMA), and D) plant height.

Figure 9. A) Principal components analysis (PCA) plots made using funspace() objects, depicting: A) Functional trait space of S. altissima traits measured, B) a heatmap depicting how fungal richness is distributed within *S. altissima's* functional trait space, C) a heatmap depicting how bacterial richness is distributed within *S. altissima's* functional trait space

A) Principal components analysis (PCA) plots made using funspace() objects, depicting: a) Functional trait space of *S. altissima* traits measured, defined in a PCA, with the color showing the probabilistic distribution of trait combinations, whereby red indicates high probability of traits density and yellow indicating low probability. Contour lines show the 0.99, 0.50 and 0.25 quantiles of the probability distribution. Our output shows a single hotspot for *S. altissima* in trait space. The variance explained by each component and the loadings of the original traits are also shown. This trait space is then depicted in PCA plots using funspace() showing the *S. altissima* functional trait space change based on the plant combination treatment.
B) a heatmap depicting how fungal richness is distributed within *S. altissima's* functional trait space, here we see an increase in fungal richness toward the left of *S. altissima's* functional space. The contour lines show the quantiles for fungal richness (i.e., 0.25 quantile = 953.86; 0.50 = 1015.31, and 0.99 = 1355.84). Fungal Richness (q = 0) displayed for each grouping within *S. altissima* functional trait space. Significant models for *S. altissima* grown when planted with both heterospecific neighbor species, and when planted with *C. discolor*. Further, at q = 2 fungal diversity has a significant relationship with *S. altissima* ITV (q = 2: overweighs abundant ASVs – the inverse Simpsons evenness index.
C) a heatmap depicting how bacterial richness is distributed within *S. altissima's* functional trait space, here we see bacterial richness is highest in monocultures compared to polycultures. Bacterial diversity (q = 1) is depicted for each plant grouping.


A) Trait probability function
LDMC
LMA
SLA
jun_height
oct_height
PC2(23.91%)
PC1(53.76%)
achillea
both
cirsium
solidago
B) Fungal Diversity GAM model predictions
Q = 2 *
Q = 0 **
Q = 0 *
C) Bacterial Diversity GAM model predictions
Displaying q = 1

A) Fungal composition **B) Bacterial composition**

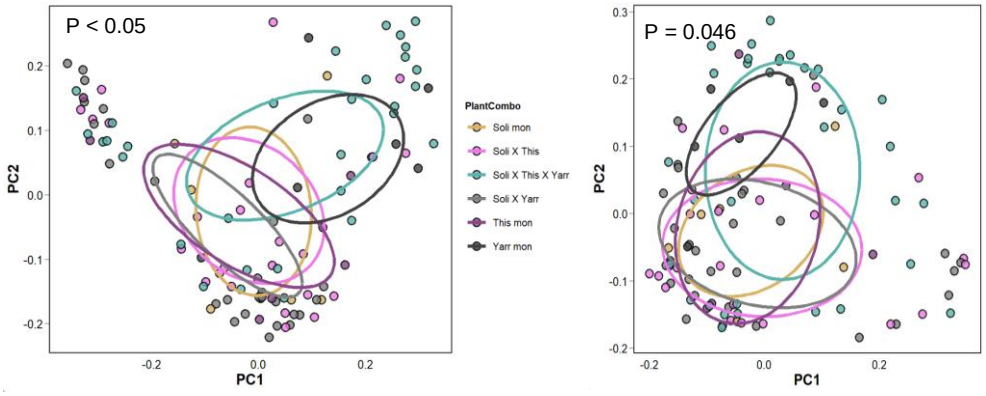


Figure 10. Principal components analysis (PCoAs) showing fungal (A) and bacterial (B) composition, for which both PERMANOVAs (Permutational multivariate analysis of variance) show significant differences based on plant combinations.

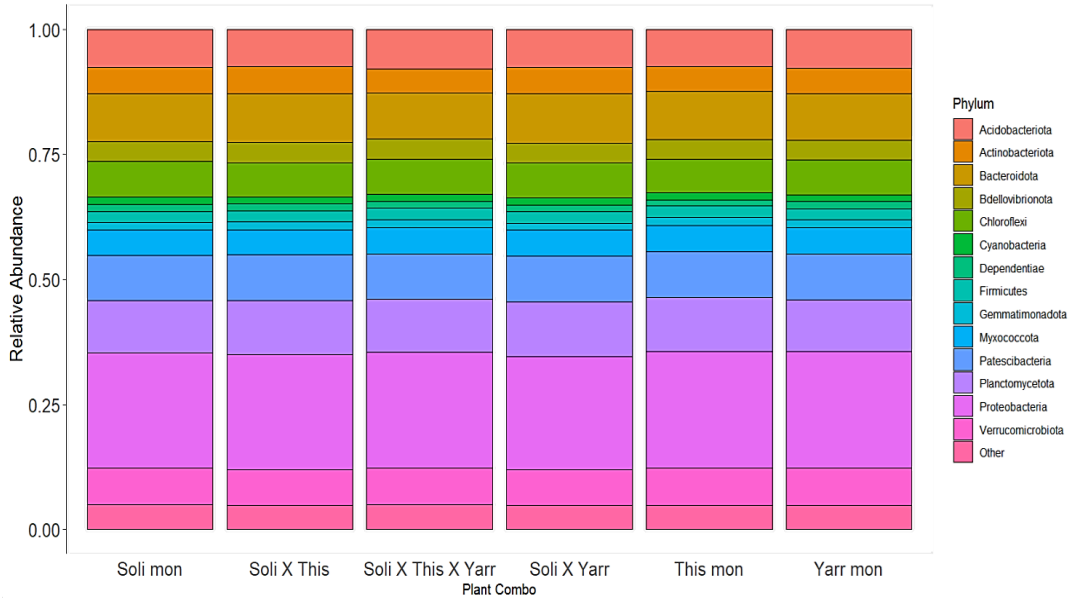
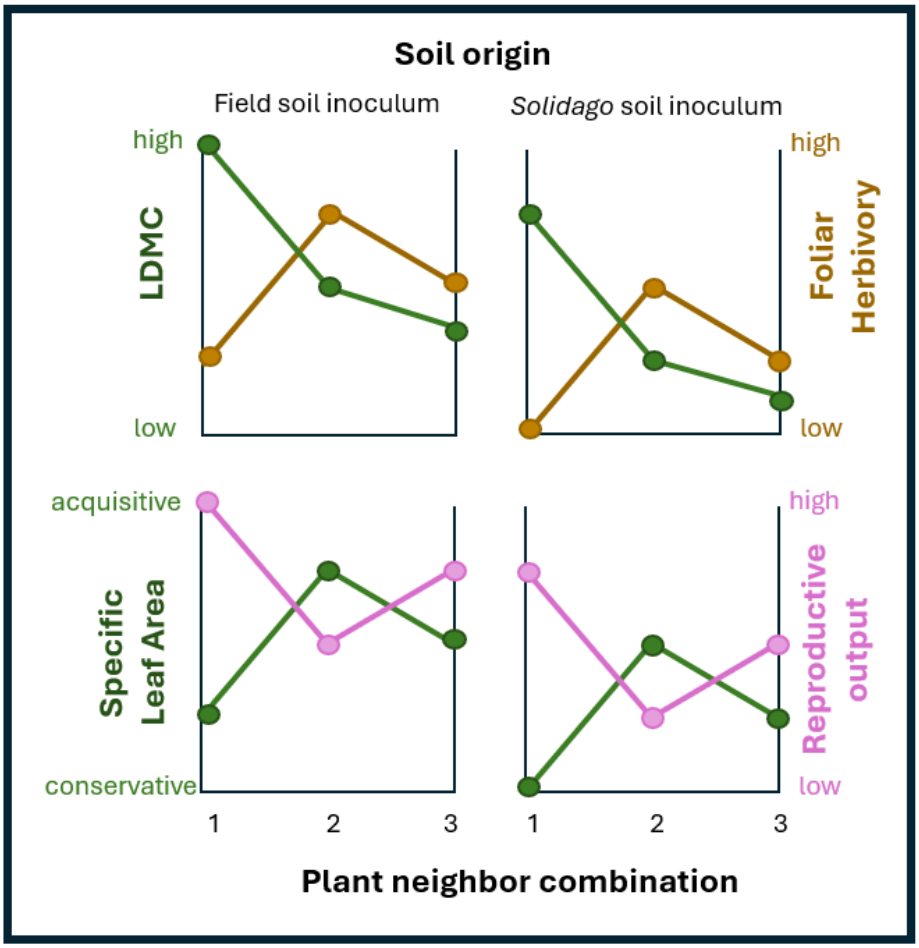


Figure 11. Chart showing relative abundance of bacterial phyla based on plant combination. Combinations include, *S. altissima* monocultures (Soli mon), pairwise polycultures with *C. discolor* (Soli X This), multispecies polycultures (Soli X This X Yarr), pairwise polycultures with *A. millefolium* (Soli X Yarr), *C. discolor* monocultures (This mon), and *A. millefolium* monocultures (Yarr mon).


Soil origin
Field soil inoculum
Solidago soil inoculum
high
low
LDMC
high
low
Foliar Herbivory
acquisitive
conservative
Specific Leaf Area
high
low
Reproductive output
1
2
3
1
2
3
Plant neighbor combination

Box 1. Trait-mediated Trade-off hypotheses.

A) Based on the causal paths in our *apriori* models (Fig. 1), we expect the following trade-offs to occur. The graphs show predictions of trait responses X herbivory and trait X reproductive output using leaf dry matter content (LDMC), and specific leaf area (SLA), interacting with plant neighbor combination (monocultures (1), pairwise polyculture with *S. altissima* (2), and multispecies polycultures (3) and soil inoculum origin. LDMC and SLA typically have a negative relationship. Based on this assumption, we predict higher LDMC in monocultures and pairwise polycultures, which are competitive scenarios, making growth becomes acquisitive. We predict herbivory to be higher on plants with low LDMC (and therefore higher water content). If these predictions are true, then the multispecies polyculture could harbor the highest herbivory levels. If plant-herbivore interactions are not driven by water content, it is also possible that herbivory will be highest in monoculture pots, which have the highest density of *C. discolor*. B) For the growth-reproduction tradeoffs, we predict high growth and low reproductive output in the monocultures due to all species competing for the same resources; still high acquisition and low reproductive output with the pairwise polyculture, though less so than the monoculture due to resource partitioning, then we expect conservative growth and higher reproductive output in the multispecies polyculture as a result of lower competition. We expect to see changes to these trade-off patterns based on soil inoculum origin. We expect the *S. altissima* conditioned soil to result in changes to S. altissima, which indirectly alter *C. discolor* resource allocation. We expect changes to the nutritional value of the floral output too, whereby plants with a higher SLA will have reduced investment in flowers and their protein content, and plants with lower SLA will have higher reproductive quantity and quality.

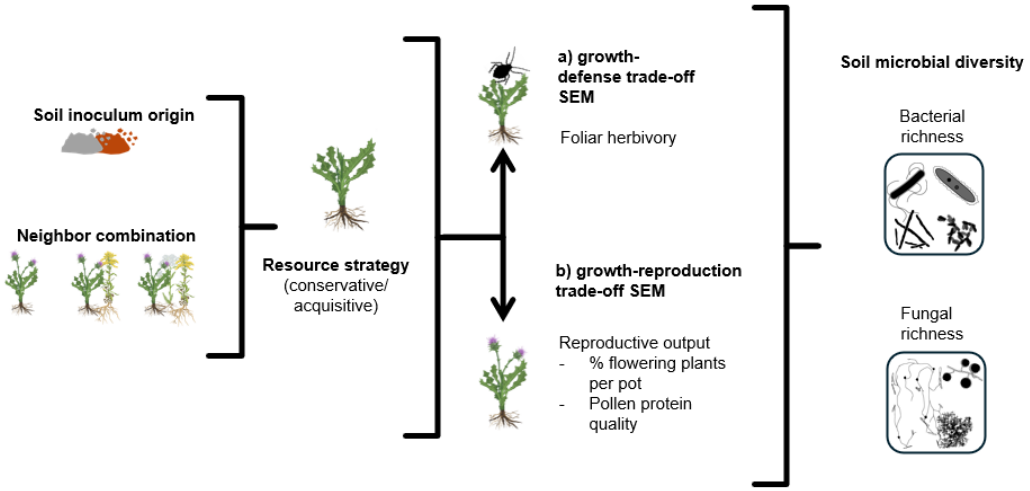


Figure 12. *Apriori* models for both the growth-defense and growth-reproduction SEMs.

These models show the expectation that *C. discolor* leaf allocation is contingent on the origin of the soil inoculum used, plant neighbor combination, bacterial richness, and fungal richness during the experiment, the resulting leaf allocation in addition to the aforementioned biotic factors will result in changes to a) foliar herbivory and b) reproductive output. We also included an indirect path, whereby plant neighbor combination affects bacterial and fungal richness, resulting in cascading effects on plant allocation, herbivory and reproductive output.

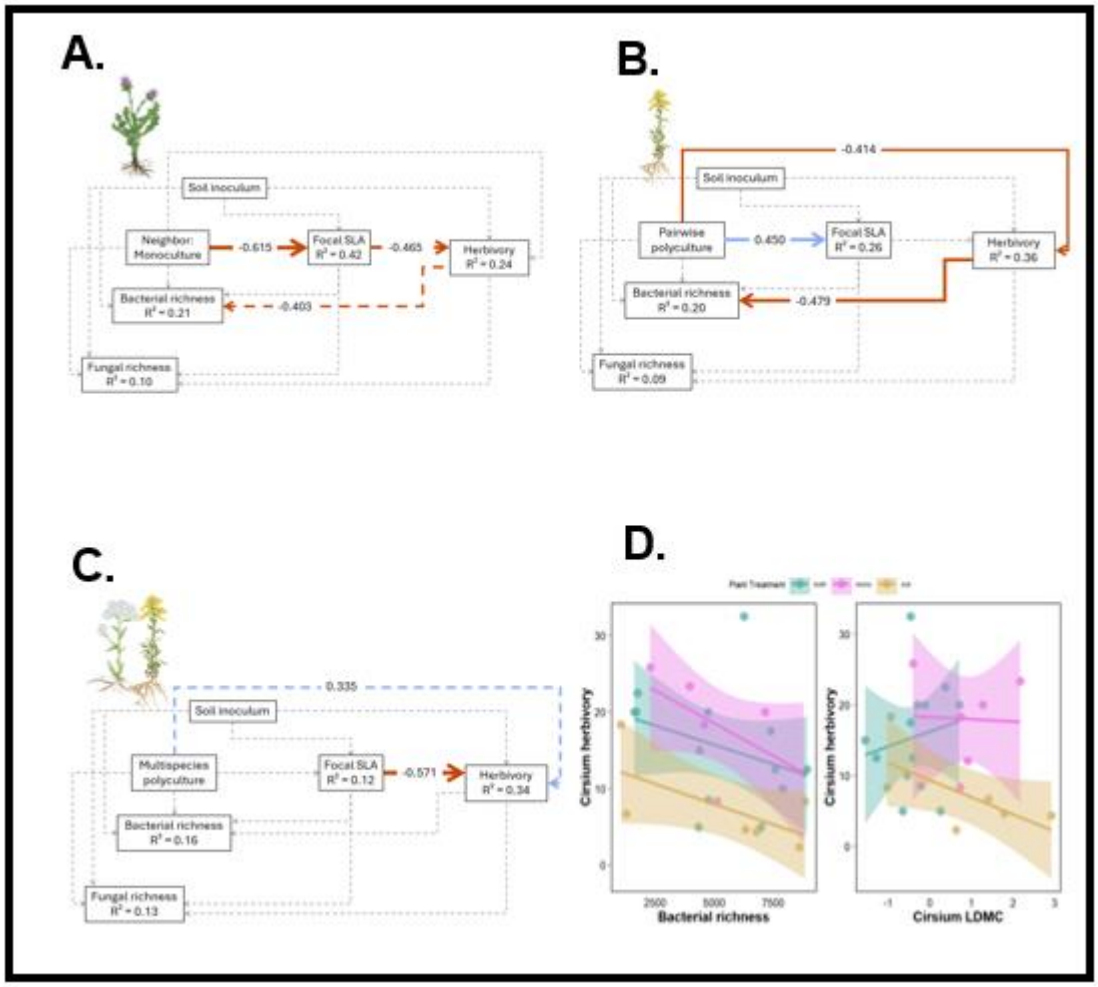


Figure 13. Results of the growth-defense trade-off structural equation models

Results of the growth-defense piecewise structural equation models (SEMs). Solid lines refer to statistically significant relationships ($p \leq 0.05$) and dashed refer to non-significant relationships. Blue lines are positive, and orange are negative relationships. Individual R^2 values are provided in the boxes. Panel A shows SEM model in monocultures, B shows SEM results with *S. altissima* as the plant neighbor, and C shows SEM results when planted with both *S. altissima* and *A. millefolium*. Panel D has two scatterplots showing how the relationship between herbivory and bacterial richness changes based on plant neighbor (D1), and how the relationship between herbivory and leaf dry matter content (LDMC) change based on plant neighbor (D2).

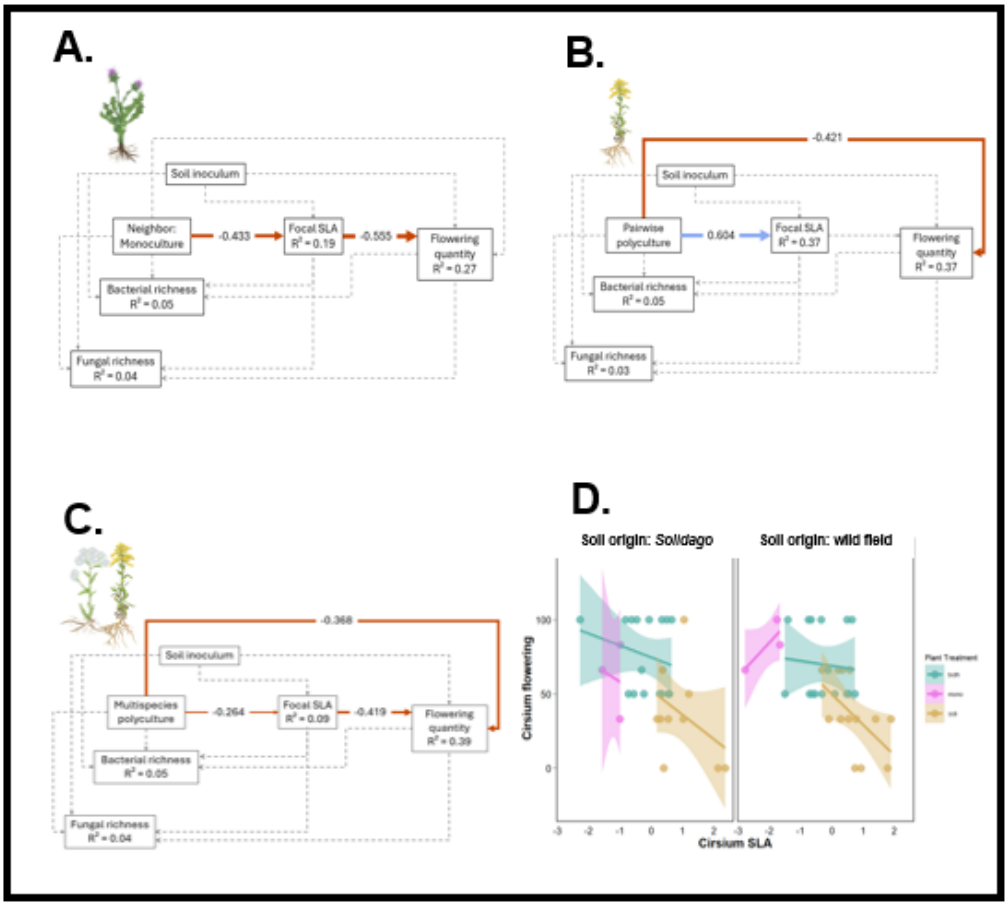


Figure 14. Results of the growth-reproduction trade-off structural equation models

Results of the tested piecewise structural equation models (SEMs) for growth-reproduction trade-offs. Solid lines refer to statistically significant relationships ($p \leq 0.05$) and dashed refer to non-significant relationships. Blue lines are positive, and orange are negative relationships. Individual R^2 values are provided in the boxes. Based on our models, all three biotic contexts result in a trade-off with neighbor increasing *C. discolor* specific leaf area (SLA), resulting in a decrease in flowering. We modeled each neighbor context in separate piecewise SEMs, here we show the growth-reproductive SEM when *C. discolor* is planted in monocultures (panel A), in pairwise polycultures with *S. altissima* (panel B), and multispecies polycultures (panel C). Panel D shows the relationship between *C. discolor* flowering and *C. discolor* SLA based on plant neighbor and separated based on soil inoculum origin.

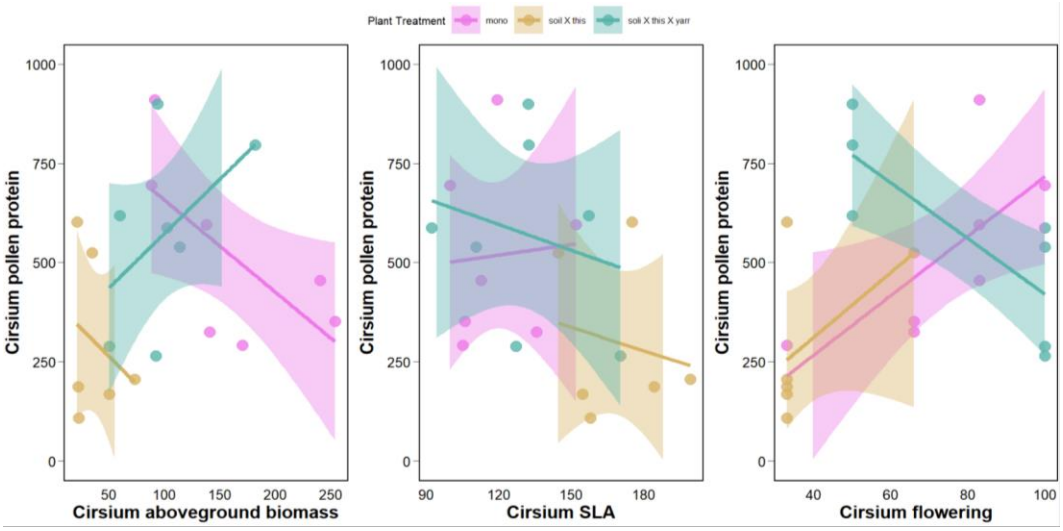


Figure 15. Pollen protein content results

Scatterplots showing *C. discolor* pollen protein content as a function of aboveground biomass (a), *C. discolor* specific leaf area (SLA) (b), and flowering output (c), colored based on plant neighbor context, with monocultures in pink, pairwise polycultures with *S. altissima* in yellow, and multispecies polycultures in teal.

Supplementary information

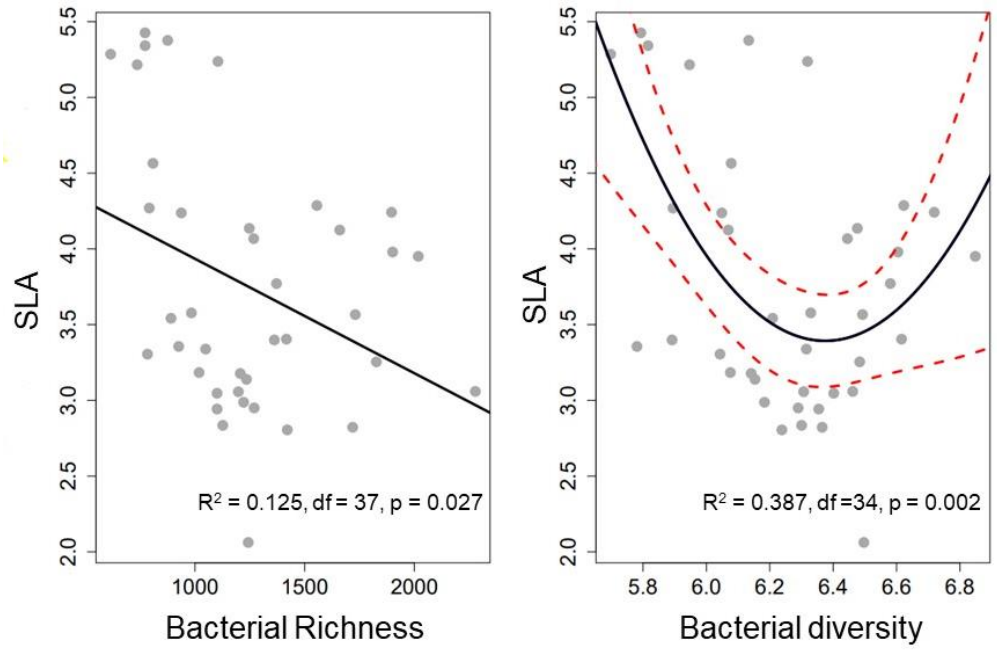


Figure S1. Scatterplots showing the relationship between specific leaf area (SLA) and bacterial richness and diveristy.

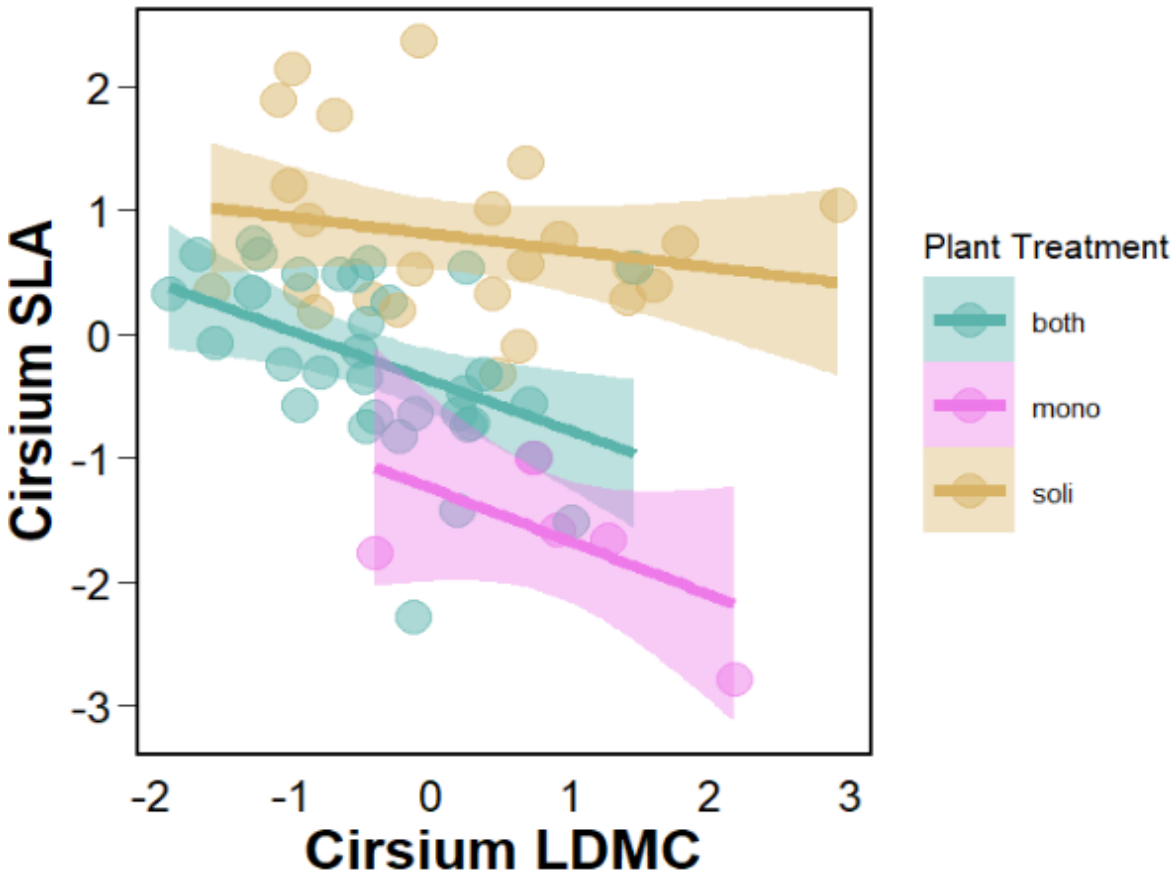


Figure S12. Results showing the negatice relationship between *C. discolor* specific leaf area (SLA) and leaf dry matter content (LDMC).

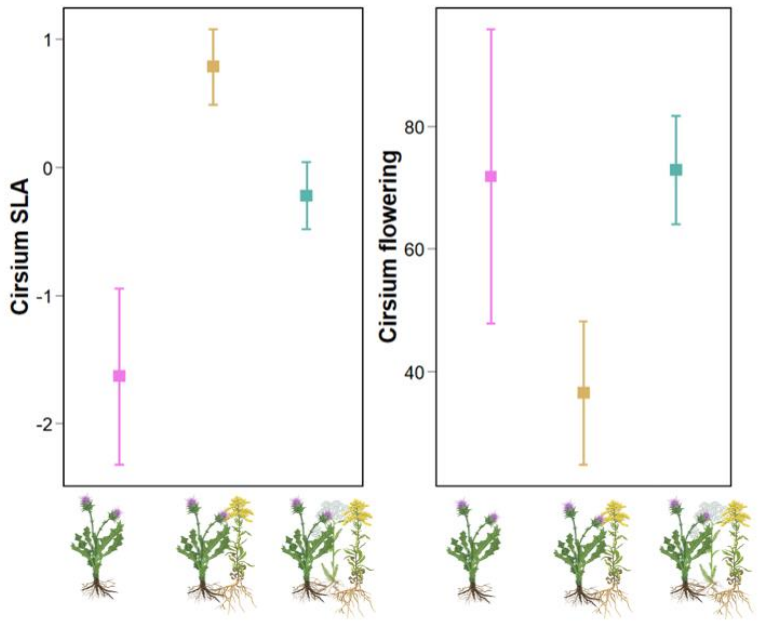


Figure S3. Mean and confidence intervals of *Cirsium discolor* specific leaf area (SLA) and flowering as a function of plant neighbor, monocultures in pink, polycultures with *S. altissima* in yellow and polycultures with both *S. altissima* and *A. millefolium* in teal.

Table S1. Results from Anovas (car package, R) testing linear models and generalized linear models fit to predict how *S. altissima* plant traits changed as a result of plant neighbor, pot type and their interaction. Bold types indicate significant effects ($p < 0.05$).

Year	Response Variables	Predictors	Open Pots (full interaction)				Split pots (no belowground interaction)			
			N	*df*	*F-value*	*p-value*	*N*	*df*	*F-value*	*p-value*
Year 1	Log aboveground biomass	Neighbor	43	2	4.697	**0.015**	39	2	4.981	**0.012**
	RGR	Neighbor RGR	43	1	0.056	0.815	43	1	16.747	**>0.001**
		Neighbor		2	0.773	0.469		2	0.383	0.685
		Neighbor RGR x Neighbor		2	3.853	**0.03**		2	9.607	**0.001**
Year 2	Log aboveground biomass	Neighbor	20	2	0.209	0.814	20	2	1.281	0.303
	RGR	Neighbor RGR	20	1	1.259	0.281	20	1	0.575	0.461
		Neighbor		2	0.009	0.991		2	1.165	0.34
		Neighbor RGR x Neighbor		1	4.456	**0.053**		2	0	1
	Log belowground biomass	Neighbor	20	2	0.273	0.765	20	2	4.147	**0.034**
	Root:Shoot	Neighbor	20	2	0.485	0.624	20	2	3.649	**0.048**

Table S1. Continued.

Year 2	SLA	Neighbor	20	2	1.274	0.31	20	2	3.292	0.067
		Root:shoot		1	2.047	0.175		1	3.478	0.083
		Neighbor x Root:shoot		2	1.547	0.247		2	1.3239	0.297
	Number of shoots	Neighbor shoots	22	1	4.512	**0.034**	18	1	0.021	0.885
	Herbivory	Neighbor	20	2	0.027	0.974	20	2	0.178	0.838

Table S2. Results from Anovas (car package, R) testing linear mixed effects models fit to predict the effect of pot type (i.e., with or without belowground interactions) and plant neighbor on *S. altissima* associated soil microbial communities (bacterial and fungal richness and diversity), with year as a random effect. Bold typed numbers indicate significant effects ($p < 0.05$).

Response Variables	Predictors	*N*	*df*	*Chi²*	*p-value*
Bacterial Richness	Pot Type	69	1	0.6236	0.4297
	Neighbor		2	1.9283	0.3813
	Neighbor x Pot type		2	2.5555	0.2787
Bacterial Diversity	Pot Type	69	1	0.4344	0.5099
	Neighbor		2	1.0769	0.5837
	Neighbor x Pot type		2	4.4780	0.1066
Fungal Richness	Pot Type	70	1	1.4440	0.2295
	Neighbor		2	1.7031	0.4268
	Neighbor x Pot type		2	0.4224	0.8096
Fungal Diversity	Pot Type	70	1	0.7157	0.39757
	Neighbor		2	5.3581	0.06863
	Neighbor x Pot type		2	2.4551	0.29301

Table S3. Table showing the effect size calculated for each model, along with the power of the model. We also included the sample size needed to get a power of 0.8.

Hypothesis	Dependent variable	Data				Required for power = 0.8	
		total sample size	effect size	sig. level	power	N per group	total sample size
1a	biomass year 1	40	0.28	0.05	0.49	44	132
	RGR year 1	40	0.25	0.05	0.40	54	162
	biomass year 2	40	0.30	0.05	0.36	38	114
	RGR year 2	40	0.34	0.05	0.47	29	87
	SLA	40	0.50	0.05	0.81	14	42
	root biom	40	0.41	0.05	0.62	21	63
	root:shoot	40	0.11	0.05	0.09	275	825
	number of new shoots	40	0.62	0.05	0.96	35	105
1b	biomass - both years	80	0.26	0.05	0.53	48	144
	RGR - - both years	80	0.31	0.05	0.69	35	105

Table S3. continued

2a	herbivory	40	1.02	0.05	1.00	5	15
	bacterial richness	69	0.26	0.05	0.44	49	147
	bacterial diversity	69	0.15	0.05	0.17	140	420
	fungal richness	70	0.37	0.05	0.76	25	75
	fungal diversity	70	0.39	0.05	0.81	22	66
2b	Bacterial diversity X above biomass	39	0.52	0.05	0.96	25	
	Fungal diversity X above biomass	39	0.45	0.05	0.92	29	
	Bacterial diversity X SLA	40	0.25	0.05	0.67	52	
	Fungal diversity X SLA	40	0.19	0.05	0.52	69	

Table S4. Column 1 contains the soil treatments (inoculated with soil from under a *Solidago* vs non-*Solidago* soil), the rest of the columns are the plant combinations, where 'mon' refers to monoculture. The numbers are the numbers of pots within each treatment, totaling 111 pots. Each pot has 6 individuals growing in them, totaling 666 individuals. The monocultures contain six of one species; polycultures with two species contain three individuals of each species and the polycultures containing all three species have two individuals of each species.

	Plant combinations						**Total**
Soil Inoculum	Soli mon	This mon	Achi mon	Soli X Cirs	Soli X Achi	Soli X Cirs X Achi	
Solidago conditioned soil	3	3	3	10	12	16	47
Non-*Solidago* soil	3	3	3	14	16	16	55
Potting mix	3	3	3				9
Total							111

Table S5. Summary of the partial R^2 values for plant trait models. Traits were measured on the focal plant, *S. altissima,* and included specific leaf area (SLA), leaf dry matter content (LDMC), leaf matter per area (LMA), and plant height.

Model response Variable	Partial R^2			
	Neighbor	Inoculum	Bacterial richness	Fungal richness
Solidago SLA	1.708%	1.866%	0.101%	1.094%
Solidago LDMC	3.437%	2.253%	0.492%	0.020%
Solidago LMA	1.558%	1.941%	0.179%	0.936%
Solidago Height	3.124%	2.291%		

Table S6. Summary of the partial R^2 for plant neighbor and inoculum for models testing how belowground richness changes based on plant neighbor and soil inocula treatments.

Model Response Variable	partial R^2	
	neighbor	inoculum
Fungal Richness (q = 0)	5.489%	2.380%
Fungal Shannon's Diversity (q = 1)	9.483%	0.578%
Fungal Inverse Simpsons (q = 2)	9.845%	0.607%
Bacterial Richness (q = 0)	0.217%	0.940%
Bacterial Shannon's Diversity (q = 1)	0.157%	0.975%
Bacterial Inverse Simpsons (q = 2)	0.142%	0.989%

Table S7. Table summarizing the functional indexes measured – functional richness and divergence for all traits measured, as well as for traits grouped based on plant combination treatment.

Grouping	Threshold (%)	Functional Richness	Functional Divergence
All	99.9	87.92	0.36
Polyculture: *Achillea*	99.9	84.2	0.44
Polyculture: *Cirsium*	99.9	53.99	0.40
Polyculture: Both	99.9	45.95	0.51
Solidago monocultures	99.9	46.05	0.42

Table S8. Table summarizing the percentage of variance of each *S. altissima* trait measured, that is explained by the first two principal components or PCs (Comp. 1 and Comp. 2) and summed across PCs (Overall explained).

Trait	Comp.1	Comp. 2	Overall explained
SLA	95.13	0.21	95.34
LMA	95.13	0.21	95.34
LDMC	77.66	1.02	78.68
Plant height (Jun)	0.46	56.81	57.27
Plant height (Oct)	0.42	61.33	61.75

Table S9. Growth-defense structural equation model comparison testing which trait modeled better, comparing specific leaf area (SLA) and leaf dry matter content (LDMC). Based on variance explained (R^2 and the lower corrected AIC scores), I opted to use SLA for the final flowering SEMs.

Plant neighbor	SLA			LDMC		
	Response	R^2	AICc	Response	R^2	AICc
Monoculture	SLA	0.42	1074.935	LDMC	0.07	1092.376
	Herbivory	0.24		Herbivory	0.25	
	Bacterial richness (q = 0)	0.21		Bacterial richness (q = 0)	0.25	
	Fungal richness (q = 0)	0.1		Fungal richness (q = 0)	0.05	
Pairwise polyculture	SLA	0.26	1076.675	LDMC	0.13	1088.172
	Herbivory	0.36		Herbivory	0.33	
	Bacterial richness (q = 0)	0.2		Bacterial richness (q = 0)	0.21	
	Fungal richness (q = 0)	0.09		Fungal richness (q = 0)	0.09	
Multispecies polyculture	SLA	0.12	1082.122	LDMC	0.36	1087.281
	Herbivory	0.34		Herbivory	0.11	
	Bacterial richness (q = 0)	0.16		Bacterial richness (q = 0)	0.19	
	Fungal richness (q = 0)	0.13		Fungal richness (q = 0)	0.12	

Table S10. Flowering structural equation model comparison testing which trait modeled better, comparing specific leaf area (SLA) and leaf dry matter content (LDMC). Based on variance explained (R^2 and the lower corrected AIC scores, I opted to use SLA for the final flowering SEMs.

Plant neighbor	SLA			LDMC		
	Response	R^2	AICc	Response	R^2	AICc
Monoculture	SLA	0.19	2806.792	LDMC	0.06	2831.883
	Flowering per pot	0.27		Flowering per pot	0.07	
	Bacterial richness (q = 0)	0.05		Bacterial richness (q = 0)	0.04	
	Fungal richness (q = 0)	0.04		Fungal richness (q = 0)	0.04	
Pairwise polyculture	SLA	0.37	2782.319	LDMC	0.04	2811.13
	Flowering per pot	0.37		Flowering per pot	0.34	
	Bacterial richness (q = 0)	0.05		Bacterial richness (q = 0)	0.05	
	Fungal richness (q = 0)	0.03		Fungal richness (q = 0)	0.04	
multispecies polyculture	SLA	0.08	2804.101	LDMC	0.14	2813.785
	Flowering per pot	0.39		Flowering per pot	0.23	
	Bacterial richness (q = 0)	0.05		Bacterial richness (q = 0)	0.05	
	Fungal richness (q = 0)	0.04		Fungal richness (q = 0)	0.04	

CONCLUSION

My dissertation makes use of traditional plant resource allocation theory, coupled with modern sequencing techniques to understand the multifaceted role of biotic interactions for plant resource allocation, functioning and ultimately fitness. My findings illustrate that plant neighbors affect a focal plant through both above- and belowground processes, and that the direct and indirect effects are sometimes contingent on neighbor identity. Next, I showed how plant neighbors directly and indirectly affect plant functional diversity and plant-fungal interactions. We also provide evidence of functional specificity in plant-fungal interactions. Lastly, I identified biotic-induced changes to leaf-traits, which resulted in growth-reproductive trade-offs for a biennial thistle. In this chapter, we also show how a negative PSF experienced by a plant neighbor can indirectly facilitate a focal plant. Collectively, the findings of this dissertation show that independent of abiotic and space constraints, biotic interactions can have significant effects how a focal plant allocates resources, functional trait variation, and even proxies for fitness, such as the number of shoots produced and floral output. Studying the role of multiple groups of organisms for plant resource allocation in unison is not common, but this dissertation shows that by incorporating multiple interactions, we can better understand the regulatory changes that a plant undergoes throughout the growing season, and how the biotic context alters the outcomes of plant resource allocation pathways.

Meaningful measures of biotic interactions

The study of direct and indirect interactions, and particularly multispecies interactions in ecology has fallen in and out of favor over the past few decades. Part of the issue is that there are so many types of interactions, and so many ways to study them, from observational and behavioral studies to passive collection of species occurrence data, to advanced sequencing techniques, and tracking of isotopes and secondary compounds. This complexity makes it difficult to create experiments that are applicable to natural systems and makes it difficult to compare studies and systems. In this dissertation, we show how using plant traits and morphology coupled with evidence of interactions (i.e., foliar herbivory, presence of particular microbes), is a promising way to elucidate complex relationships between seemingly disparate organism above- and belowground.

Moreover, we show that context can be ascribed to species interactions by using biotic gradients to study them.

By using biotic gradients, we can go beyond case-study examples of above-and belowground model systems and toward a mechanistic understanding of how changing interactions affect plant functioning and cascade to affect further interactions. We need this type of knowledge to be able to better predict how species will fare in changing climates. Climatic predictions can help with species occurrence, but local scale information on biotic interactions will enable us to better predict species persistence.

VITA

Sophia Carmel Turner is an Irish-born to Rhodesian parents. Her formative years were spent in Ireland and Zimbabwe, with most of her education in South Africa, where she graduated from Rhenish Girls' High School in 2011. She studied Nature Conservation through the University of South Africa. During her third year of tertiary education, she began an internship at the DSI-NRF Center of Excellence for Invasion Biology (CIB), for their science outreach project, Iimbovane. Sophia remained working for Iimbovane as an outreach educator from 2014- 2019. From 2017-2018, she also completed a MSc in Conservation Ecology through Stellenbosch University, funded by the CIB, on mountain plant invasions. Sophia joined the Department of Ecology and Evolutionary Biology at the University of Tennessee in August 2019, and completed her dissertation in July 2024.