

Factors shaping the distribution, abundance, and diversity of temperate forest plants

By

Jared J. Beck

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The dissertation is approved by the following members of the Final Oral Committee:

Donald M. Waller, Professor, retired, Botany and Chair

Thomas J. Givnish, Professor, Botany

Bret Larget, Professor, Botany and Statistics

Monica G. Turner, Professor, Integrative Biology

Anthony R. Ives, Professor, Integrative Biology

To all things natural, wild, and free

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"Of what avail are forty freedoms without a blank spot on the map?" – Aldo Leopold

Statement of author contributions

Chapter 1. J. Beck designed the study, analyzed the data, and wrote the manuscript. D. Li, S. Johnson, and D. Rogers collected data and contributed to manuscript revisions. D. Waller and T. Givnish secured funding to collect data, helped design the research, and contributed substantially to manuscript revisions. K. Cameron and K. Sytsma helped secure funding to support data collection and contributed to manuscript revisions.

Chapter 2. J. Beck designed and conducted the study, analyzed the data, and drafted the manuscript. A. Salvi and R. Henderson collected data and contributed to manuscript revisions. D. Waller secured funding for the vegetation surveys and helped revise the manuscript.

Chapter 3. J. Beck designed the study, collected data, analyzed the data, and led the writing of the manuscript. T. Givnish conceived the theoretical foundation for this study and contributed substantially to manuscript revisions.

Chapter 4. J. Beck designed the study, conducted the experiment, analyzed the data, and wrote the manuscript.

Abstract

Factors shaping the distribution, abundance, and diversity of temperate forest plants

Jared J. Beck

Under the supervision of Professor Donald M. Waller

At the University of Wisconsin-Madison

More than a century after they emerged as central themes in ecology, community assembly and species coexistence remain rich areas of inquiry. Recent advances in coexistence theory and the development of new and powerful quantitative techniques have offered new insights into the varied processes shaping ecological communities. Here, I elucidate the processes driving community assembly in understory temperate forest plant communities. I first investigated how environmental conditions, functional traits, and phylogenetic relationships act together to influence plant distributions. Traits mediated plant distributions along environmental gradients, as expected, while phylogenetic relationships and niche differences had little effect on co-occurrence patterns. These findings reaffirm the importance of trait-mediated environmental sorting in determining plant distributions but call into question common assumptions about the roles of local niche differentiation and evolutionary history in plant community assembly.

To build on this first project, I studied how two congener herbs, *Hylodesmum glutinosum* and *H. nudiflorum* manage to co-exist despite many similarities. Their broad-scale distributions reflect certain differences in morphology and physiology but there was little evidence of resource partitioning. Instead, patterns of co-occurrence and key demographic differences suggest equalizing processes that minimize competitive differences and life history tradeoffs promote local co-occurrence.

I next examined local distributions of three forest herbs to understand the role of fine-scale environmental heterogeneity in structuring local plant distributions. These distributions matched *a priori* predictions based on differences in these species' morphology and physiology. Strong patterns of intraspecific spatial aggregation and heterospecific segregation coupled with limited dispersal capacity suggest these differential responses to fine-scale environmental variation could promote local coexistence among these species.

Finally, I conducted an experiment to examine how plant-soil interactions influence herb growth. The effect of soil biota varied considerably among species providing evidence for both mutualistic and antagonistic interactions. These findings support the idea that plant-soil feedbacks play important but previously underappreciated roles in structuring herbaceous plant communities. Taken as a whole, my research illustrates how a diverse set of processes, operating at different rates and spatial scales, differentially affect various plant species to shape the overall distribution, abundance, and diversity of temperate forest herbs.

Introduction

For more than 100 years, ecologists have endeavored to understand the ecological processes shaping the distribution, abundance, and diversity of species (Clements 1916, Gleason 1926, Gause 1932, Whittaker 1956, MacArthur 1958, Curtis 1959, Hutchinson 1959, MacArthur and Wilson 1967, Tilman 1982, Weiher and Keddy 1999, Hubbell 2001, Vellend 2016). Recent advances in coexistence theory (Chesson 2000a, Mayfield and Levine 2010, HilleRisLambers et al. 2012, Chesson 2018), the development of new and powerful quantitative techniques (Ives and Helmus 2011, Pollock et al. 2012, 2014, Jamil et al. 2013, Li and Ives 2017, Li et al. 2017, Miller et al. 2019), and growing recognition of the diverse processes capable of shaping plant communities (Silvertown 2004, Ehrenfeld et al. 2005, Adler et al. 2006, Dybzinski and Tilman 2007, Angert et al. 2009, Bagchi et al. 2014, Godoy et al. 2014, Bever et al. 2015, Kraft et al. 2015b, Levine et al. 2017) have driven a burgeoning interest in community assembly over the past 20 years. Despite these advances, elucidating the ecological processes responsible for shaping ecological communities and maintaining species diversity remains a rich area of inquiry. My PhD research strives to improve our understanding of factors shaping the distribution, abundance, and diversity of temperate forest plants.

Modern coexistence theory establishes a useful conceptual frame for understanding the diverse array of processes that shape ecological communities (Mayfield and Levine 2010, HilleRisLambers et al. 2012, Kraft et al. 2015a). At its core, this theory posits that species coexistence and patterns of biological diversity depend on the relative importance of stabilizing processes, which increase the strength intraspecific competition relative to interspecific competition, and relative fitness differences (or fitness inequalities), which describe differences in competitive ability or population growth rates among species (Chesson 2000a). Stabilizing processes are characterized by negative density-dependent population growth and include mechanisms such as complementary resource use (Tilman 1982), differential species' responses to spatial or temporal environmental variation (Chesson and Warner 1981, Chesson 2000b, Angert et al. 2009), and density-dependent effects of predators or pathogens on particular species (Janzen 1970, Connell 1971). Meanwhile, relative fitness differences can reflect differences in resource use or

other mechanisms that promote competitive exclusion. Long-term coexistence is possible when one or more of these stabilizing processes become sufficiently strong to overcome fitness inequalities (Chesson 2000a). Coexistence is also theoretically possible when there are no relative fitness differences (i.e. species are ecologically and demographically equivalent *sensu* Hubbell 2001), but such equalizing processes alone are not sufficient for long-term coexistence (Chesson 2000a, Mayfield and Levine 2010).

This theoretical foundation laid the groundwork for advances in community assembly theory (HilleRisLambers et al. 2012). Many ecologists now cite a heuristic model of community assembly in which communities are assembled via a hierarchy of nested processes. According to this model, regional species pools are shaped by climate, historical contingencies, biogeography, evolution, and other processes operating at broad spatial scales and over long periods of time (Ricklefs 1987, HilleRisLambers et al. 2012, Cornell and Harrison 2014). From this regional species pool, individuals then disperse and sort into local communities according to relative fitness differences (Weiher and Keddy 1999, HilleRisLambers et al. 2012). This environmental sorting can reflect different physiological tolerances that filter out organisms unable to tolerate certain abiotic conditions or competitive hierarchies which favor organisms with traits that allow them to successfully compete for resources given local biotic and abiotic conditions (Kraft et al. 2015a). Finally, after this environmental sorting occurs, biotic interactions come into play to shape the final species composition and diversity of local communities. Stabilizing processes, and particularly complementary resource use, are often assumed to operate at these fine spatial scales (Weiher and Keddy 1999, Silvertown 2004, Silvertown et al. 2006, HilleRisLambers et al. 2012, Scherrer et al. 2019). This hierarchical model of community assembly has now been extended to incorporate evolutionary history to make predictions about how phylogenetic relationships could influence ecological communities (Webb et al. 2002, Cavender-Bares et al. 2009). Historical processes of evolution and biogeography interact with climate and continent-scale environmental variation to determine which lineages exist within the regional species pool (Cornell and Harrison 2014). Because functional traits are often phylogenetically conserved, related species tend to respond to environmental variation in similar ways (the β -niche *sensu* Ackerly et al. 2006 and Silvertown et al. 2006). Within local

communities, close relatives should then segregate unless evolution has led to niche differentiation that stabilizes local coexistence (the α -niche – see Webb 2002, Silvertown et al. 2006).

Despite the appeal of these heuristic models, relatively few empirical studies have investigated the potential for such scale-dependent processes to drive community assembly. The primary goal of my PhD work was to evaluate these predictions from contemporary community assembly theory and examine how a diverse array of ecological processes shape the distribution, abundance, and diversity of herbaceous plants in temperate forests. In my dissertation, I employ observational studies, experiments, legacy data sets documenting the distribution of plant species, and a broad suite of quantitative tools to gain insights into the diverse processes driving herbaceous community assembly. In Chapter 1, I investigate how environmental conditions, functional traits, and phylogenetic relationships influence plant distributions and co-occurrence patterns using an extensive data set describing the distribution of 139 plant species across 259 forest stands. Building on these community-wide analyses, I conduct a more detailed study of two congeners, *Hylodesmum glutinosum* and *H. nudiflorum*, in Chapter 2 to elucidate how morphological, physiological, and demographic characteristics shape plant distributions and co-occurrence patterns across spatial scales. In Chapter 3, I characterize how fine-scale environmental heterogeneity influences the local distribution of three spring-flowering forest herbs and explore the potential for this environmental variation to promote local coexistence via spatial niche partitioning. Finally, in Chapter 4 I conducted an experiment to examine how interactions between forest herbs and soil microorganisms affect plant growth in nine common forest herbs and the potential for plant-soil feedbacks to promote coexistence among herbaceous plant species.

Study system

My PhD research focuses on herbaceous plant communities within temperate forests. These forests cover approximately 1 billion hectares of the earth's terrestrial surface and support a diverse assemblage of life (Olson et al. 2001, Tyrrell et al. 2012). Herbaceous taxa comprise more than 80 percent of the plant species found in temperate forests (Gilliam 2007). Many forest herbs are long-lived,

reproduce vegetatively, and share other life history characteristics that adapt them to forest understories (Bierzzychudek 1982). However, these species also exhibit great variation in other ecological characteristics that adapt them to the particular and diverse environments they occupy (Whigham 2004, Gilliam 2014). At broad spatial scales, the distribution and abundance of understory herbaceous species vary in response to numerous biotic and abiotic factors along environmental gradients (Whittaker 1956, Curtis 1959, Leach and Givnish 1999, Gilbert and Lechowicz 2004, Amatangelo et al. 2014, Beatty 2014, Burton et al. 2014, Peet et al. 2014, Gilliam et al. 2016). Differential plant responses to these broad environmental gradients reflect physiological tradeoffs and strategies adapted for different environmental conditions (Givnish 1982, 1987, 1995, Amatangelo et al. 2014, Neufeld and Young 2014). Understory plant species also respond sensitively to environmental variation at finer spatial scales. Within forest stands, herbaceous plant species often exhibit highly heterogeneous spatial distributions that reflect, in part, fine-scale environmental variation in microtopography, light levels, soil fertility, soil depth, etc. (Struik and Curtis 1962, Anderson et al. 1969, Bratton 1976, Thompson 1980, Beatty 1984, Crozier and Boerner 1984, Vellend et al. 2000, Burton et al. 2011, Sabatini et al. 2014, Chudomelová et al. 2017, Catella et al. 2019).

My research includes broad-scale (among site) analyses of plant distributions and co-occurrence patterns for 139 plant species that occur regularly in Wisconsin forests (Chapter 1). I also apply more focused and nuanced analyses to better understand the distributions and abundance of 13 focal herbaceous species (Chapters 2, 3, and 4). These species are relatively common and occur widely across temperate forests in eastern North America. Chapter 2 focuses on two congeneric forest herbs, *Hyloidesmum glutinosum* and *H. nudiflorum* (Fabaceae) widely distributed in oak forests and woodlands in eastern North America. Chapter 3 focuses on three spring-flowering herbs: *Anemone acutiloba* (Ranunculaceae), *Sanguinaria canadensis* (Papaveraceae), and *Trillium flexipes* (Melanthiaceae). In Chapter 4, I focus on nine understory species: *Ageratina altissima* (Asteraceae), *Aquilegia canadensis* (Ranunculaceae), *Elymus hystrix* (Poaceae), *Geranium maculatum* (Geraniaceae), *H. glutinosum* (Fabaceae), *Polemonium reptans* (Polemoniaceae), *Polygonum virginianum* (Polygonaceae), *Solidago flexicaulis* (Asteraceae), and

Thalictrum dioicum (Ranunculaceae) chosen to represent the diverse characteristics and lineages present among forest herbs.

The rich plant diversity of temperate forests and the availability of survey data make these an ideal system for studying mechanisms of community assembly. However, widespread ecological change driven primarily by human activities has substantially impaired the capacity of these ecosystems to maintain biological diversity. Ongoing habitat loss and fragmentation (Vellend 2003, Honnay et al. 2005, Rogers et al. 2009, McCune and Vellend 2013, Johnson et al. 2016), anthropogenic climate change (Kucharik et al. 2010, De Frenne et al. 2013, Ash et al. 2017, Li and Waller 2017), altered trophic interactions (Rooney and Waller 2003, Rooney et al. 2004, Wiegmann and Waller 2006, Alverson et al. 2016), altered disturbance regimes (Rogers et al. 2008, Johnson and Waller 2013, Paulson et al. 2016, Meunier et al. 2019), and other drivers of ecological change threaten the long-term persistence of many plant and animal species. Efforts to identify these drivers, estimate the pace of ecological change, and forecast community responses, however, have been impeded by the scarcity of historical baseline data (Magnuson 1990, Vellend et al. 2013), sampling effects that (Beck et al. 2018), and an incomplete understanding of the ecological processes structuring communities (HilleRisLambers et al. 2012). Thus, an improved understanding of community assembly is essential for forecasting community responses to drivers of ecological change and informing conservation and restoration efforts.

Study area

My research focuses on forests within Wisconsin (USA). Here an exceptional legacy of ecological research has laid the foundation for understanding the ecology of forest herbs as well as our understanding of plant community assembly (Curtis 1959). Extensive surveys conducted throughout the state in the 1940s and 1950s by John T. Curtis and his colleagues described the distribution of plant species in relation to environmental conditions. This research provided fundamental insights into the ecology of forest herbs and the nature of ecological communities. Most notably, Curtis' research provided

unequivocal support for the individualistic model of communities (Gleason 1926) in which communities change gradually as individual species respond mostly independently to gradients in environmental conditions rather than together and in concert as integrated units (Clements 1916). These researchers also pioneered numerous quantitative techniques anticipating modern multivariate statistics (Bray and Curtis 1957, Curtis 1959) and spatial statistics (Struik and Curtis 1962).

In Chapter 1, I use extensive resurveys conducted at Curtis' original study sites between 2000 and 2012 to assess drivers of community composition in 259 forest stands distributed across Wisconsin (and the western Upper Peninsula of Michigan). These stands represent four community types with distinct differences in species composition and environmental conditions (Curtis 1959). Northern Upland Forests (NUF) include a range of forest types occurring in northern Wisconsin including coniferous and mixed-hardwood forests dominated by Sugar Maple (*Acer saccharum*), Eastern Hemlock (*Tsuga canadensis*), and White Pine (*Pinus strobus*) (Curtis 1959, Rooney et al. 2004). Pine barrens (PB) occur on sandy, well-drained soils with low soil fertility (Curtis 1959, Li and Waller 2015). Such conditions favor xeric tree species such as jack pine (*Pinus banksiana*) and northern pin oak (*Quercus ellipsoidalis*) and recurrent fires. The PB sites surveyed occurred in the central sand plains of Wisconsin (Li and Waller 2015). Southern Upland Forests (SUF) include sites in southern Wisconsin that range from oak-hickory forests on relatively sandy, low fertility soils to maple-basswood forests on more fertile soils with greater water holding capacity (Curtis 1959, Rogers et al. 2008). Southern Lowland Forests (SLF) include periodically flooded forests adjacent to rivers and lakes (Curtis 1959, Johnson and Waller 2013, Johnson et al. 2016). Dominant canopy trees included silver maple (*Acer saccharum*), elms (*Ulmus* spp.), willows (*Salix* spp.), and poplars (*Populus* spp.) but which species dominated the canopy varied greatly among sites (Johnson and Waller 2013, Johnson et al. 2013, 2016).

Chapters 2, 3, and 4 rely on my own fieldwork in upland forests within the Baraboo Hills (Sauk County, WI, USA – see Fig. 2). This 580 km² region is defined by remnants of an ancient mountain range composed of uplifted Precambrian quartzite that rises roughly 200 m above the surrounding area in south-central WI (Lange 1990). The Baraboo Hills and surrounding landscape are a regional biodiversity

hotspot. Despite covering less than 0.5% of Wisconsin's total area, nearly half of Wisconsin's 2300 plant species occur within the Baraboo Hills (Lange 1998). With over 200 km² of forest and 28 distinct ecological communities, this area constitutes the most intact forest ecosystem in southern Wisconsin making it a regional conservation priority. Forest types include oak savannas and woodlands, bedrock glades with sparse tree coverage, extensive blocks of oak forest, more mesic maple-basswood forests, pine forests on exposed ridges and outcrops, and hemlock-hardwood forests typically restricted to northern latitudes in sheltered stream gorges.

Chapter 1. Asymmetric pegs in square holes? Functional and phylogenetic determinants of plant community assembly in temperate forests

In attempting to explain species distributions and co-occurrence patterns, many ecologists now reference a hierarchical model of community assembly (Weiher and Keddy 1999, HilleRisLambers et al. 2012, Kraft et al. 2015a). Individuals from the regional species pool sort into local communities based on their physiology and ability to compete successfully for resource within a local community. This results in co-occurrence among functionally similar species (Kraft et al. 2015a). At finer scales within those local communities, biotic interactions (namely niche differentiation shape local plant distributions and community favoring coexistence among functionally dissimilar species with complementary resource usage. If relevant functional traits are phylogenetically conserved, close relatives should respond similarly to environmental gradients but disassociate at finer scales (Webb et al. 2002, Silvertown et al. 2006, Kraft et al. 2007, Cavender-Bares et al. 2009). Despite the appeal of this hierarchical model and its ability to explain community assembly in several systems, further studies in a broad range of systems are needed to establish its generality and elucidate potential shortcomings. We sought to examine whether scale-dependent effects of environmental sorting and niche differentiation could explain patterns of plant community assembly in the herbaceous plant community of temperate forests. Using Bayesian phylogenetic linear mixed effects models (Ives and Helmus 2011, Ives et al. 2018), we examined how environmental conditions, functional traits, and phylogenetic relationships influenced the distribution and

co-occurrence of 139 herbaceous plant species across 259 forest sites in Wisconsin (USA). Consistent with previous research and predictions from community assembly theory, we found strong evidence that functional traits including leaf height, specific leaf area, leaf C:N, and seed mass vary systematically along gradients in climate, edaphic conditions, and light. Although closely related species responded similarly to certain environmental gradients, this tendency was relatively weak. There was no evidence that functionally similar or phylogenetically related species segregated either among sites or among microsites within forest sites. This finding contradicts predictions from conventional community assembly theory and calls into question the extent to which niche differentiation and evolutionary history influence local community dynamics. We conclude that strong environmental sorting coupled with the varied biogeographic history of forest herbs and individualistic responses to environmental variation may preclude the evolution of highly refined local niches, increasing the importance of equalizing processes in local community dynamics.

Chapter 2. Coexistence revisited: Can we integrate demographic, physiological, and distributional data to understand patterns of co-occurrence among close relatives?

Coexistence among closely related species poses an ecological conundrum regarding the role of convergence and divergence in plant community assembly. As a result of their shared evolutionary history, close relatives are expected to share morphological and physiological traits that influence their responses to environmental variation (β -niche *sensu* Silvertown et al. 2006). These shared characteristics should favor coexistence within suitable habitats. However, the co-occurrence of ecologically similar species can also result in these species competing strongly for the same resources. As a result, close relatives may segregate as a result of competitive exclusion or evolve niche differences that stabilize local coexistence (α -niche *sensu* Silvertown et al. 2006). To test these predictions and elucidate the ecological processes shaping plant distributions across spatial scales, we conducted a multi-faceted study of two congeneric forest herbs that co-occur regularly, *Hylodesmum glutinosum* and *H. nudiflorum* (Fabaceae). This study integrated data characterizing comparative morphology and physiology, plant distributions

along broad-scale environmental gradients as well as finer-scale species distributions, and local demographic turnover in these congeners to gain insights into the ecological processes favoring frequent co-occurrence. Differences in leaf height, leaf chemistry, and water use efficiency among these *Hylodesmum* species reflect their respective distributions along environmental gradients across the landscape. The ability of *H. glutinosum* to hold its leaves higher and regulate its water balance more tightly contribute to its tolerance of a broader set of ecological conditions and ability to compete in open habitats with more light. Surprisingly, the observed distribution of *H. nudiflorum* fell entirely within *H. glutinosum*'s ecological distribution with both species co-occurring in shady woodlands where *H. glutinosum*'s competitive advantage declines. This suggests equalizing processes that minimize differences in competitive ability between species are responsible for widespread co-occurrence of *Hylodesmum* species. *Hylodesmum nudiflorum*, however, allocates proportionally more to reproduction and less to growth and defense than its congener which results in greater rates of local recruitment and mortality. We surmise equalizing processes that minimize competitive differences within moderately shaded woodlands and the potential for life history tradeoffs that stabilize local coexistence could explain the frequency of local co-occurrence between these congeners. Our findings provide insights into the diverse ecological processes that influence plant distributions and co-occurrence patterns as well as the spatial scales at which the different processes may operate.

Chapter 3. Fine-scale environmental heterogeneity and spatial niche partitioning among spring-flowering forest herbs

Environmental variation helps shape the distribution and diversity of plant species across spatial scales (Stein et al. 2014). In species-rich communities like the herbaceous plant communities of temperate forests, fine-scale environmental variation may promote local coexistence by allowing species to spatially partition microsites within habitats (Bratton 1976, Beatty 1984, 2014). The potential for fine-scale environmental heterogeneity to maintain local plant diversity, however, depends on the strength of local species-environment association as well as the extent to which differential responses to local

environmental variation, the spatial scale of environmental variation, and patterns of seed dispersal allow species to segregate spatially from one another (Amarasekare 2003, Snyder and Chesson 2004, Leibold et al. 2004, Lundholm 2009, Hart et al. 2017). We examined the local distribution of three spring-flowering forest herbs (*Anemone acutiloba*, *Sanguinaria canadensis*, and *Trillium flexipes*) to test whether local plant distributions emerge from predictable species-environment relationships and assess whether the spatial distribution of plants is compatible with spatial niche partitioning. Consistent with our *a priori* predictions based on interspecific differences in morphology and physiology, *A. acutiloba* occupied microsites with shallower soil and was found in closer proximity to trees than either *S. canadensis* or *T. flexipes*. *Anemone*'s less substantial root system, shorter leaf height, and low nutrient demands should allow it to thrive in the nutrient-poor microsites with shallow soils adjacent to trees but make it a poor competitor in deeper soils with greater nutrient availability. Here the larger-statured *S. canadensis* and *T. flexipes* should have a competitive advantage (Givnish 1982, 1987). Examining intraspecific and interspecific spatial associations revealed strong evidence of intraspecific aggregation and interspecific segregation. Moreover, the spatial scales of intraspecific aggregation, variation in soil depth, and expected dispersal capacity all matched closely suggesting that limited dispersal could reinforce (not disrupt) local species-environment relationships. Our findings support the hypothesis that fine-scale environmental heterogeneity and spatial niche partitioning promote local coexistence among temperate forest herbs (Bratton 1976, Beatty 1984, 2014, Catella et al. 2019).

Chapter 4. Variation in plant-soil feedbacks among temperate forest herbs

Increasingly, ecologists recognize that interactions between plants and soil microorganisms play a fundamental role in shaping the composition and diversity of plant communities (Klironomos 2002, Reynolds et al. 2003, Ehrenfeld et al. 2005, Bever et al. 2010, 2015, Mangan et al. 2010, Van der Putten et al. 2013, Bennett et al. 2017). Both positive plant-soil feedbacks (PSFs) resulting from mutualistic interactions that promote plant growth and negative PSFs caused by antagonistic interactions that depress plant growth (Ehrenfeld et al. 2005, Van der Putten et al. 2013, Bennett and Klironomos 2019) influence

plant community dynamics. Empirical studies suggest that negative PSFs are more common (Kulmatiski et al. 2008, Petermann et al. 2008, Fitzsimons and Miller 2010) and that these negative PSFs may play an important role in maintaining plant diversity (Mills and Bever 1998, Mordecai 2011, Bever et al. 2015). Despite the prevalence of PSFs and increasing recognition of their importance in plant communities, little is known about plant-soil interactions in temperate forests or their potential to maintain local plant diversity (Shannon et al. 2012, 2014, Comita et al. 2014, Smith and Reynolds 2015). I conducted a 2x2 factorial experiment to investigate the prevalence and strength of PSFs among nine understory forest herbs. For this experiment, I grew seedlings of each focal species in pasteurized and unpasteurized soils that had been trained by either conspecific plants or heterospecific plants. After letting these plants grow, I quantified biomass production to learn how plant growth depended on exposure to soil microorganisms (pasteurized vs. unpasteurized) and the origin of soil biota (conspecific vs. heterospecific). If specialized pathogens accumulate locally, plants grown in unpasteurized, conspecific-trained soil should fare worse than plants grown in pasteurized and/or heterospecific-trained soils. In fact, there was considerable variation in the direction and strength of plant-soil feedbacks among species. *Thalictrum dioicum*, *Elymus hystrix*, *Solidago flexicaulis*, and *Polemonium reptans* all exhibited reduced growth in unpasteurized soils, suggesting microbial antagonists. *Thalictrum dioicum* seedlings grown in conspecific, unpasteurized soil accumulated 30% less biomass than seedlings grown in heterospecific, unpasteurized soil suggesting that host-specific microbes restrict seedling growth (consistent with the Janzen-Connell hypothesis). In contrast, four species that often form dense clusters showed no significant response to experimental treatments manipulating soil biota. These results suggest plant-soil feedbacks can play important and often unappreciated roles in shaping local plant distributions and thus species diversity within temperate forests.

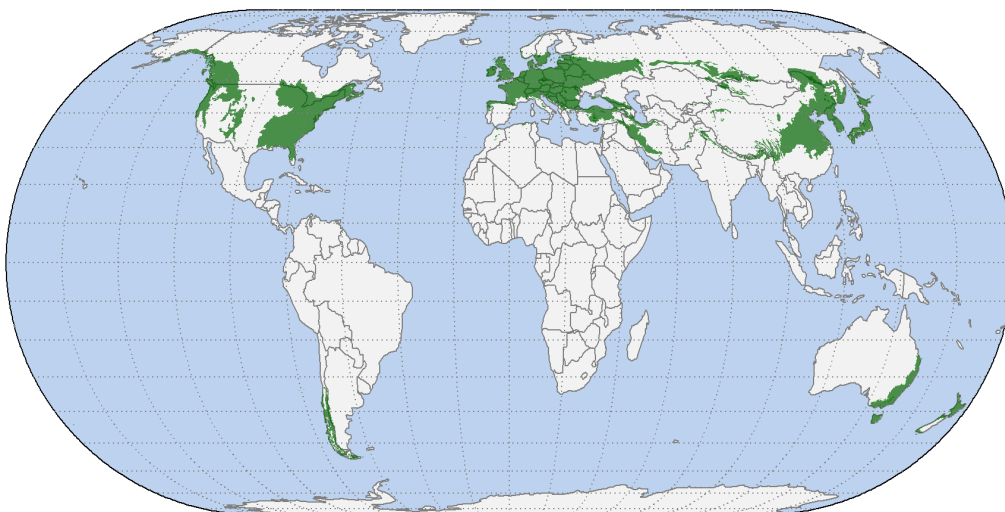


Figure 1. Map illustrating the global extent of temperate forests. Green regions depict the location of temperate forest biomes. Geospatial data obtained from the World Wildlife Fund's terrestrial ecoregions of the world map (Olson et al. 2001).

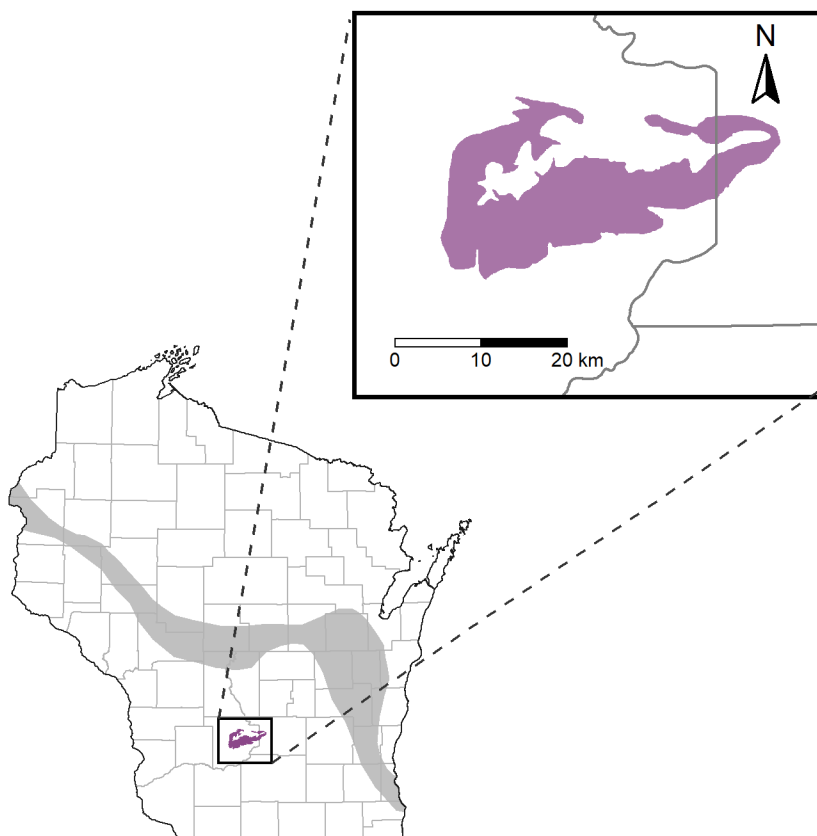


Figure 2. Map of Wisconsin (USA). The grey band illustrates the location of a pronounced floristic tension zone which delineates the range limits of plant species with northern and southern affinities (Curtis 1959). The purple area depicts the location of the Baraboo Hills (Lange 1990).

References

- Ackerly DD, Schilck DW, Webb CO. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. *Ecology* 87:S50-61.
- Adler, P. B., J. HilleRisLambers, P. C. Kyriakidis, Q. Guan, and J. M. Levine. 2006. Climate variability has a stabilizing effect on the coexistence of prairie grasses. *Proceedings of the National Academy of Sciences* 103:12793–8.
- Alverson, W. S., D. M. Waller, and S. L. Solheim. 2016. Forests too deer: Edge effects in northern Wisconsin. *Conservation Biology* 2:348–358.
- Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: A synthesis. *Ecology Letters* 6:1109–1122.
- Amatangelo, K., S. Johnson, D. Rogers, and D. M. Waller. 2014. Trait-environment relationships remain strong despite 50 years of trait compositional change in temperate forests. *Ecology* 95:1780–1791.
- Anderson, A. R. C., O. L. Loucks, and A. M. Swain. 1969. Herbaceous response to canopy cover, light intensity, and throughfall precipitation in coniferous forests. *Ecology* 50:255–263.
- Angert, A. L., T. E. Huxman, P. Chesson, and D. L. Venable. 2009. Functional tradeoffs determine species coexistence via the storage effect. *Proceedings of the National Academy of Sciences* 106:11641–11645.
- Ash, J. D., T. J. Givnish, and D. M. Waller. 2017. Tracking lags in historical plant species' shifts in relation to regional climate change. *Global Change Biology* 23:1305-1315.
- Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton, and O. T. Lewis. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* 506:85–88.
- Beatty, S. W. 1984. Influence of microtopography and canopy species on spatial patterns of forest understory plants. *Ecology* 65:1406–1419.
- Beatty, S. W. 2014. Habitat Heterogeneity and the Maintenance of Species in Understory Communities. Pages 215–232 *in* F. S. Gilliam, editor. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Beck, J. J., B. Larget, and D. M. Waller. 2018. Phantom species: Adjusting estimates of colonization and extinction for pseudo-turnover. *Oikos* 127: 1605–1618.
- Bennett, J. A., and J. Klironomos. 2019. Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytologist* 222:91–96.

- Bennett, J. A., H. Maherali, K. O. Reinhart, Y. Lekberg, M. M. Hart, and J. Klironomos. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* 355:181–184.
- Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett, and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology and Evolution* 25:468–478.
- Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics* 46:305–325.
- Bierzychudek, P. 1982. Life histories and demography of shade-tolerant temperate forest herbs: A review. *New Phytologist* 90:757–776.
- Bratton, S. P. 1976. Resource division in an understory herb community: Responses to temporal and microtopographic gradients. *The American Naturalist* 110:679–693.
- Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* 27:326–349.
- Burton, J. I., D. J. Mladenoff, M. K. Clayton, and J. A. Forrester. 2011. The roles of environmental filtering and colonization in the fine-scale spatial patterning of ground-layer plant communities in north temperate deciduous forests. *Journal of Ecology* 99:764–776.
- Burton, J. I., D. J. Mladenoff, J. A. Forrester, and M. K. Clayton. 2014. Experimentally linking disturbance, resources and productivity to diversity in forest ground-layer plant communities. *Journal of Ecology* 102:1634–1648.
- Catella, S. A., S. R. Eysenbach, and K. C. Abbott. 2019. Novel insights into how the mean and heterogeneity of abiotic conditions together shape forb species richness patterns in the Allegheny plateau ecoregion. *Ecology and Evolution*:12374–12389.
- Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. *Ecology letters* 12:693–715.
- Chesson, P. 2000a. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics* 31:343–358.
- Chesson, P. 2000b. General theory of competitive coexistence in spatially-varying environments. *Theoretical population biology* 58:211–37.
- Chesson, P. 2018. Updates on mechanisms of maintenance of species diversity. *Journal of Ecology* 106:1773–1794.

- Chesson, P. L., and R. R. Warner. 1981. Environmental variability promotes coexistence in lottery competitive systems. *The American naturalist* 117:923–943.
- Chudomelová, M., D. Zelený, and C. F. Li. 2017. Contrasting patterns of fine-scale herb layer species composition in temperate forests. *Acta Oecologica* 80:24–31.
- Clements, F. E. 1916. *Plant Succession: An Analysis of the Development of Vegetation*. Carnegie Institution of Washington, Washington D.C.
- Comita, L. S., S. A. Queenborough, S. J. Murphy, J. L. Eck, K. Xu, M. Krishnadas, N. Beckman, and Y. Zhu. 2014. Testing predictions of the Janzen-Connell hypothesis: A meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology* 102:845–856.
- Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298–312 *in* den B. PJ and G. GR, editors. *Dynamics of Populations*. The Netherlands: Centre for Agricultural Publishing and Documentation.
- Cornell, H. V, and S. P. Harrison. 2014. What are species pools and when are they important? *Annu. Rev. Ecol. Evol. Syst* 45:45–67.
- Crozier, C. R., and R. E. J. Boerner. 1984. Correlation of understory herb distribution patterns with microhabitats under different tree species in a mixed mesophytic forest. *Oecologia* 62:337–343.
- Curtis, J. T. 1959. *The Vegetation of Wisconsin: An Ordination of Plant Communities*. The University of Wisconsin Press, Madison, WI.
- Dybzinski, R., and D. Tilman. 2007. Resource use patterns predict long-term outcomes of plant competition for nutrients and light. *The American Naturalist* 170:305–318.
- Ehrenfeld, J. G., B. Ravit, and K. Elgersma. 2005. Feedback in the plant-soil system. *Annual Review of Environment and Resources* 30:75–115.
- Fitzsimons, M. S., and R. M. Miller. 2010. The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. *American Journal of Botany* 97:1937–1943.
- De Frenne, P., F. Rodríguez-Sánchez, D. A. Coomes, L. Baeten, G. Verstraeten, M. Vellend, M. Bernhardt-Römermann, C. D. Brown, J. Brunet, J. Cornelis, G. M. Decocq, H. Dierschke, O. Eriksson, F. S. Gilliam, R. Hédli, T. Heinken, M. Hermy, P. Hommel, M. A. Jenkins, D. L. Kelly, K. J. Kirby, F. J. G. Mitchell, T. Naaf, M. Newman, G. Peterken, P. Petřík, J. Schultz, G. Sonnier, H. Van Calster, D. M. Waller, G.-R. Walther, P. S. White, K. D. Woods, M. Wulf, B. J. Graae, and K. Verheyen. 2013. Microclimate moderates plant responses to macroclimate warming. *Proceedings of the National Academy of Sciences* 110:18561–5.

- Gause, G. 1932. Experimental studies on the struggle for existence. *Journal of Experimental Biology* 9:389–402.
- Gilbert, B., and M. J. Lechowicz. 2004. Neutrality, niches, and dispersal in a temperate forest understory. *Proceedings of the National Academy of Sciences* 101:7651–7656.
- Gilliam, F. S. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. *BioScience* 57:845.
- Gilliam, F. S., editor. 2014. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press.
- Gilliam, F. S., N. T. Welch, A. H. Phillips, J. H. Billmyer, W. T. Peterjohn, Z. K. Fowler, C. A. Walter, M. B. Burnham, J. D. May, and M. B. Adams. 2016. Twenty-five-year response of the herbaceous layer of a temperate hardwood forest to elevated nitrogen deposition. *Ecosphere* 7:1–16.
- Givnish, T. J. 1982. On the adaptive significance of leaf height in forest herbs. *American Naturalist* 120:353–381.
- Givnish, T. J. 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106:131–160.
- Givnish, T. J. 1995. Plant stems: biomechanical adaptation for energy capture and influence on species distributions. Pages 3–49 *Plant stems: Physiology and functional morphology*.
- Gleason, H. 1926. The individualistic concept of the plant association. *Bulletin of the Torrey Botanical Club* 53:7–26.
- Godoy, O., N. J. B. Kraft, and J. M. Levine. 2014. Phylogenetic relatedness and the determinants of competitive outcomes. *Ecology letters* 17:836–44.
- Hart, S. P., J. Usinowicz, and J. M. Levine. 2017. The spatial scales of species coexistence. *Nature Ecology and Evolution* 1:1066–1073.
- HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking community assembly through the lens of coexistence theory. *Annual Review of Ecology, Evolution, and Systematics* 43:120830113150004.
- Honnay, O., H. Jacquemyn, B. Bossuyt, and M. Hermy. 2005. Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. *New Phytologist* 166:723–736.
- Hubbell, S. P. 2001. *The Unified Neutral Theory of Island Biogeography and Biodiversity*. Princeton University Press.

- Hutchinson, G. E. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? *The American Naturalist* 93:145–159.
- Ives, A., and M. Helmus. 2011. Generalized linear mixed models for phylogenetic analyses of community structure. *Ecological Monographs* 81:511–525.
- Ives, A. R., M. R. Helmus, R. Dinnage, and D. Li. 2018. phyr: Phylogenetic Analysis with R. R package.
- Jamil, T., W. A. Ozinga, M. Kleyer, and C. J. F. Ter Braak. 2013. Selecting traits that explain species-environment relationships: A generalized linear mixed model approach. *Journal of Vegetation Science* 24:988–1000.
- Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. *The American Naturalist* 104:501–528.
- Johnson, S. E., K. L. Amatangelo, P. A. Townsend, and D. M. Waller. 2016. Large, connected floodplain forests prone to flooding best sustain plant diversity. *Ecology* 97:3019–3030.
- Johnson, S. E., E. L. Mudrak, and D. M. Waller. 2013. Local increases in diversity accompany community homogenization in floodplain forest understories. *Journal of Vegetation Science* 25:885–896.
- Johnson, S. E., and D. M. Waller. 2013. Influence of dam regulation on 55-year canopy shifts in riparian forests. *Canadian Journal of Forest Research* 43:159–170.
- Klironomos, J. N. 2002. Feedback with soil biota contributes to plants rarity and invasiveness in communities. *Nature* 417:67–69.
- Kraft, N. J. B., P. B. Adler, O. Godoy, E. C. James, S. Fuller, and J. M. Levine. 2015a. Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology* 29:592–599.
- Kraft, N. J. B., W. K. Cornwell, C. O. Webb, and D. D. Ackerly. 2007. Trait evolution, community assembly, and the phylogenetic structure of ecological communities. *The American naturalist* 170:271–83.
- Kraft, N. J. B., O. Godoy, and J. M. Levine. 2015b. Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences* 112:797–802.
- Kucharik, C. J., S. P. Serbin, S. Vavrus, E. J. Hopkins, and M. M. Motew. 2010. Patterns of Climate Change Across Wisconsin From 1950 to 2006. *Physical Geography* 31:1–28.
- Kulmatiski, A., K. H. Beard, J. R. Stevens, and S. M. Cobbold. 2008. Plant-soil feedbacks: A meta-analytical review. *Ecology Letters* 11:980–992.

- Lange, K. I. 1990. A postglacial vegetational history of Sauk County and Caledonia Township, Columbia County, south central Wisconsin. Wisconsin Department of Natural Resources Technical Bulletin.
- Lange, K. I. 1998. Flora of Sauk County and Caledonia Township, Columbia County, South Scentral Wisconsin. Wisconsin Department of Natural Resources Technical Bulletin.
- Leach, M. K., and T. J. Givnish. 1999. Gradients in the composition , structure , and diversity of remnant oak savannas in southern Wisconsin. *Ecological Monographs* 69:353–374.
- Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7:601–613.
- Levine, J. M., J. Bascompte, P. B. Adler, and S. Allesina. 2017. Beyond pairwise mechanisms of species coexistence in complex communities. *Nature* 546:56–64.
- Li, D., and A. R. Ives. 2017. The statistical need to include phylogeny in trait-based analyses of community composition. *Methods in Ecology and Evolution*.
- Li, D., A. R. Ives, and D. M. Waller. 2017. Can functional traits account for phylogenetic signal in community composition? *New Phytologist* 214:607–618.
- Li, D., and D. Waller. 2017. Fire exclusion and climate change interact to affect long-term changes in functional composition. *Diversity and Distributions* 23:496–506.
- Li, D., and D. M. Waller. 2015. Drivers of observed biotic homogenization in pine barrens of central Wisconsin. *Ecology* 96:1030–1041.
- Lundholm, J. T. 2009. Plant species diversity and environmental heterogeneity: Spatial scale and competing hypotheses. *Journal of Vegetation Science* 20:377–391.
- Macarthur, R. H. 1958. Population ecology of some warblers of northeastern coniferous forests. *Ecology* 39:599–619.
- MacArthur, R., and E. Wilson. 1967. *The Theory of Island Biogeography*. Princeton University Press, Princeton, NJ.
- Magnuson, J. J. 1990. Long-term ecological research and the invisible present. *BioScience* 40:495–501.
- Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. MacK, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* 466:752–755.
- Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic

- structure of communities. *Ecology letters* 13:1085–93.
- McCune, J. L., and M. Vellend. 2013. Gains in native species promote biotic homogenization over four decades in a human-dominated landscape. *Journal of Ecology* 101:1542–1551.
- Meunier, J., N. S. Holoubek, and M. Sebasky. 2019. Fire regime characteristics in relation to physiography at local and landscape scales in Lake States pine forests. *Forest Ecology and Management* 454:117651.
- Miller, J. E. D., E. I. Damschen, and A. R. Ives. 2019. Functional traits and community composition: A comparison among community-weighted means, weighted correlations, and multilevel models. *Methods in Ecology and Evolution* 10:415–425.
- Mills, K. E., and J. D. Bever. 1998. Maintenance of diversity within plant communities: Soil pathogens as agents of negative feedback. *Ecology* 79:1595–1601.
- Mordecai, E. 2011. Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. *Ecological Monographs* 81:429–441.
- Neufeld, H. S., and D. R. Young. 2014. Ecophysiology of the herbaceous layer in temperate deciduous forests. Pages 35–96 *in* F. S. Gilliam, editor. *The herbaceous layer in forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Olson, D. M., E. Dinerstein, E. D. Wikramanayake, N. D. Burgess, G. V. N. Powell, E. C. Underwood, J. A. D’amico, I. Itoua, H. E. Strand, J. C. Morrison, C. J. Loucks, T. F. Allnutt, T. H. Ricketts, Y. Kura, J. F. Lamoreux, W. W. Wettengel, P. Hedao, and K. R. Kassem. 2001. Terrestrial ecoregions of the world: A new map of life on earth. *BioScience* 51:933.
- Paulson, A. K., S. Sanders, J. Kirschbaum, and D. M. Waller. 2016. Post-settlement ecological changes in the forests of the Great Lakes National Parks. *Ecosphere* 7:1–20.
- Peet, R. K., K. A. Palmquist, and S. M. Tessel. 2014. Herbaceous layer species richness of southeastern forests and woodlands: Patterns and causes. Pages 255–276 *in* F. S. Gilliam, editor. *The Herbaceous Layer in forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, J. S. Petermann, A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89:2399–2406.
- Pollock, L. J., W. K. Morris, and P. A. Vesk. 2012. The role of functional traits in species distributions revealed through a hierarchical model. *Ecography* 35:716–725.
- Pollock, L. J., R. Tingley, W. K. Morris, N. Golding, R. B. O’Hara, K. M. Parris, P. A. Vesk, and M. A. McCarthy. 2014. Understanding co-occurrence by modelling species simultaneously with a Joint

- Species Distribution Model (JSDM). *Methods in Ecology and Evolution* 5:397–406.
- Van der Putten, W. H., R. D. Bardgett, J. D. Bever, T. M. Bezemer, B. B. Casper, T. Fukami, P. Kardol, J. N. Klironomos, A. Kulmatiski, J. A. Schweitzer, K. N. Suding, T. F. J. Van de Vooorde, and D. A. Wardle. 2013. Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology* 101:265–276.
- Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* 84:2281–2291.
- Ricklefs, R. E. 1987. Community diversity: relative roles of local and regional processes. *Science* 235:167–71.
- Rogers, D. A., T. P. Rooney, T. J. Hawbaker, V. C. Radeloff, and D. M. Waller. 2009. Paying the extinction debt in southern Wisconsin forest understories. *Conservation Biology* 23:1497–1506.
- Rogers, D. A., T. P. Rooney, D. Olson, and D. M. Waller. 2008. Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. *Ecology* 89:2482–2492.
- Rooney, T. P., and D. M. Waller. 2003. Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest Ecology and Management* 181:165–176.
- Rooney, T. P., S. M. Wiegmann, D. A. Rogers, and D. M. Waller. 2004. Biotic impoverishment and homogenization in unfragmented forest understory communities. *Conservation Biology* 18:787–798.
- Sabatini, F. M., B. Jimenez-Alfaro, S. Burrascano, and C. Blasi. 2014. Drivers of herb-layer species diversity in two unmanaged temperate forests in northern Spain. *Community Ecology* 15:113–54–64.
- Scherrer, D., H. K. Mod, J. Pottier, A. Litsios-Dubuis, L. Pellissier, P. Vittoz, L. Götzenberger, M. Zobel, and A. Guisan. 2019. Disentangling the processes driving plant assemblages in mountain grasslands across spatial scales and environmental gradients. *Journal of Ecology* 107:265–278.
- Shannon, S., S. L. Flory, and H. L. Reynolds. 2012. Competitive context alters plant-soil feedback in an experimental woodland community. *Oecologia* 169:235–243.
- Shannon, S. M., J. T. Bauer, W. E. Anderson, and H. L. Reynolds. 2014. Plant-soil feedbacks between invasive shrubs and native forest understory species lead to shifts in the abundance of mycorrhizal fungi. *Plant and Soil* 382:317–328.
- Silvertown, J. 2004. Plant coexistence and the niche. *Trends in Ecology and Evolution* 19:605–611.
- Silvertown, J., M. Dodd, D. Gowing, C. Lawson, and K. McConway. 2006. Phylogeny and the hierarchical organization of plant diversity. *Ecology* 87:39–49.

- Smith, L. M., and H. L. Reynolds. 2015. Plant-soil feedbacks shift from negative to positive with decreasing light in forest understory species. *Ecology* 96:2523–2532.
- Snyder, R. E., and P. Chesson. 2004. How the spatial scales of dispersal, competition, and environmental heterogeneity interact to affect coexistence. *American Naturalist* 164:633–650.
- Stein, A., K. Gerstner, and H. Kreft. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters* 17:866–880.
- Struik, G. J., and J. T. Curtis. 1962. Herb distributions in an *Acer saccharum* forest. *American Midland Naturalist* 68:285–296.
- Thompson, J. N. 1980. Treefalls and colonization patterns of temperate forest herbs. *American Midland Naturalist* 104:176.
- Tilman, D. 1982. *Resource Competition and Community Structure*. Princeton University Press.
- Tyrrell, M. L., J. Ross, and M. Kelty. 2012. Carbon dynamics in the temperate forest. Pages 77–107 in M. S. Ashton, M. L. Tyrrell, D. Spalding, and B. Gentry, editors. *Managing Forest Carbon in a Changing Climate*. Springer.
- Vellend, M. 2003. Habitat loss inhibits recovery of plant diversity as forests regrow. *Ecology* 84:1158–1164.
- Vellend, M. 2016. *The Theory of Ecological Communities*. Princeton University Press, Princeton, NJ.
- Vellend, M., C. D. Brown, H. M. Kharouba, J. L. Mccune, and I. H. Myers-Smith. 2013. Historical ecology: Using unconventional data sources to test for effects of global environmental change. *American Journal of Botany* 100:1294–1305.
- Vellend, M., M. J. Lechowicz, and M. J. Waterway. 2000. Environmental distribution of four *Carex* species (Cyperaceae) in an old-growth forest. *American Journal of Botany* 87:1507–1516.
- Webb, C. O., D. D. Ackerly, M. A. McPeck, and M. J. Donoghue. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* 33:475–505.
- Weiher, E., and P. A. Keddy, editors. 1999. *Ecological Assembly Rules: Perspectives, Advances, Retreats*. Cambridge University Press, Cambridge, United Kingdom.
- Whigham, D. E. 2004. Ecology of woodland herbs in temperate deciduous forests. *Annual Review of Ecology and Systematics* 35:583–621.
- Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. *Ecological Monographs* 26:1–80.

Wiegmann, S. M., and D. M. Waller. 2006. Fifty years of change in northern upland forest understories: Identity and traits of “winner” and “loser” plant species. *Biological Conservation* 129:109–123.

**Chapter 1. Asymmetrical pegs in square holes? Functional and phylogenetic
determinants of plant community assembly in temperate forests**

Jared J. Beck^{1,5}, Daijiang Li², Dave Rogers³, Sarah Johnson⁴, Kenneth Sytsma¹, Ken Cameron¹, Thomas J. Givnish¹, and Donald M. Waller¹

¹Department of Botany, University of Wisconsin-Madison, Madison, WI (USA)

²Department of Wildlife Ecology & Conservation, University of Florida, Gainesville, FL (USA)

³Department of Biological Sciences, University of Wisconsin-Parkside, Kenosha, WI (USA)

⁴Department of Biology, Northland College, Ashland, WI (USA)

⁵Corresponding author Email: jared.j.beck@gmail.com

ABSTRACT

Despite recent advances in community assembly theory, uncertainties remain concerning the ecological processes shaping species distributions and the role of evolutionary history in structuring ecological communities. Closely related species often share important ecological characteristics as a result of their shared evolutionary history. If local environmental conditions favor particular traits, we then expect functionally similar and phylogenetically related species to co-occur. However, ecologically similar species are expected to compete strongly leading to competitive exclusion. This limiting similarity should favor coexistence among functionally dissimilar (or unrelated) species. Given these divergent predictions, we must rely on empirical studies of ecological convergence and divergence to elucidate the ecological processes driving community assembly at various spatial scales. Here, we combined extensive data on environmental conditions with phylogenetic, functional trait, and species occurrence data for 139 herbaceous plant species across 259 forest stands in Wisconsin (USA). Bayesian phylogenetic linear mixed effects models (PGLMMs) allow us to characterize how functional traits and phylogenetic relationships influence plant distributions along environmental gradients and how functional and phylogenetic similarity affect species co-occurrence. Leaf height, specific leaf area, leaf C:N, and seed mass strongly mediate plant responses to gradients in edaphic conditions, light, and climate. This reaffirms the importance of environmental sorting for structuring broad-scale distributions in temperate forest plants. Closely related and functional similar species, however, are no less likely to co-occur. This absence of functional or phylogenetic repulsion undermines common assumptions about the role of phylogenetic relatedness in plant community assembly and the importance of local niche differentiation. We conclude that nuanced patterns of niche evolution within and among clades coupled with strong environmental sorting increases the importance of equalizing processes and relatively weak stabilizing mechanisms within local communities.

KEYWORDS: phylogeny, functional traits, coexistence, Wisconsin, environmental filtering, phylogenetic scale, community assembly, resource partitioning

INTRODUCTION

Disentangling the complex interplay of biotic and abiotic factors that structure ecological communities has been a central focus in ecology for more than a century (Clements 1916, Gleason 1926, Curtis 1959, MacArthur and Levins 1967, Tilman 1982, Weiher and Keddy 1999, Vellend 2016). Over the past 20 years, conceptual developments in coexistence theory coupled with methodological advances have stimulated renewed interest in community assembly (Chesson 2000a, 2018, Cavender-Bares et al. 2009, Weiher and Freund 2011, HilleRisLambers et al. 2012). Much of this recent work examines how functional traits (Westoby and Wright 2006, McGill et al. 2006, Funk et al. 2017) and/or phylogenetic relationships (Webb et al. 2002, Mayfield et al. 2009, Vamosi et al. 2009, Cavender-Bares et al. 2009, Pavoine et al. 2011) affect community assembly. For example, environmental sorting should favor those species best able to compete for resources under particular environmental conditions leading to co-occurrence among functionally similar species (Webb et al. 2002, Cornwell and Ackerly 2009a, Kraft and Ackerly 2010, Kunstler et al. 2012, Kraft et al. 2015a). Trait similarities among species often reflect their shared evolutionary history and phylogenetic niche conservatism (Losos 2008, Wiens et al. 2010). Alternatively, similarities could reflect evolutionary convergence on morphological or physiological characteristics that confer a competitive advantage under particular conditions (Webb et al. 2002, Cavender-Bares et al. 2006, Swenson et al. 2006, 2007). In either case, we expect strong trait-environment relationships and thus co-occurrence among functionally similar (and potentially related) species. In contrast to this ecological convergence caused by environmental sorting, long-term coexistence is thought to depend on resource partitioning and other niche differences that promote and stabilize co-occurrence among ecologically divergent species (Weiher and Keddy 1999, Chesson 2000a, 2018, Mayfield and Levine 2010, Kraft et al. 2015b). Limiting similarity should lead ecologically similar species to exclude each other. This includes close relatives if traits are phylogenetically conserved. Thus, we expect greater co-occurrence of related species when evolution has favored niche diversification within those clades (Mayfield and Levine 2010)

Advancing our understanding of community assembly now hinges on reconciling these conflicting predictions about ecological convergence vs. niche differentiation within communities including the role of evolutionary history in shaping ecological communities (Cavender-Bares et al. 2009, Mayfield and Levine 2010, Weiher and Freund 2011, HilleRisLambers et al. 2012). Many ecologists now favor a hierarchical model of community assembly in which ecological communities emerge from a set of nested processes operating at different spatial scales. This heuristic model asserts that local communities form as individuals from the regional species pool disperse and sort into communities based on their traits and ability to successfully compete within the local environmental context (Weiher and Keddy 1999, HilleRisLambers et al. 2012, Kraft et al. 2015a). At finer scales within these communities, biotic interactions such as competition and resource partitioning further shape local species composition and community dynamics (Weiher and Keddy 1999, Silvertown et al. 2006, HilleRisLambers et al. 2012). While spatial scale-dependence appears to resolve the tension between ecological convergence and divergence in certain systems (Cavender-Bares et al. 2004, Cavender-Bares et al. 2006, 2018, Kraft and Ackerly 2010, Cavender-Bares 2018, Scherrer et al. 2019), the broader applicability of this hierarchical model of community assembly needs to be tested.

Here, we seek to improve our understanding of the processes affecting community assembly by exploring how species trait values and phylogenetic relationships affect their distributions along environmental gradients and patterns of ecological convergence and divergence. We work with extensive data from a large set of temperate forest plant communities focusing on the herbaceous species that comprise more than 80% of the plant species present (Gilliam 2007). Forest herbs share some general characteristics well-adapted to forest understory conditions but also differ greatly in morphological and physiological characteristics in ways that reflect the diverse habitats they occupy (Bierzychudek 1982, Whigham 2004, Gilliam 2014). Herbs respond sensitively to edaphic conditions and light availability, for example (Curtis 1959, Givnish 1987, Gilliam 2014). Species turnover along these environmental gradients reflect physiological tradeoffs suggesting that environmental sorting and competitive interactions often act to structure herb distributions at both local scales (Beck and Givnish, *in review*,

Bratton 1976, Beatty 1984, Gilbert and Lechowicz 2004, Catella et al. 2019) and across landscapes (Curtis 1959, Amatangelo et al. 2014, Beatty 2014, Peet et al. 2014). Still, the potential for trait differences to stabilize local coexistence among forest herbs has received little attention and we lack a comprehensive understanding of how phylogenetic relationships affect species' patterns of co-occurrence.

In this study, we integrate extensive data on forest plant community composition, environmental conditions, plant functional traits, and a high-resolution molecular phylogeny to investigate the ecological processes structuring these forest plant communities. We specifically ask: (1) To what extent do functional traits mediate plant responses to environmental variation? (2) Do shared responses to environmental variation lead to co-occurrence among closely related species (phylogenetic attraction)? (3) Are functionally similar or closely related species less likely to co-occur within either sites or microsites as a result of limiting similarity or niche diversification?

METHODS

Study region

We focus on 259 forest stands distributed across Wisconsin (and the western Upper Peninsula of Michigan – Fig. 1). These stands represent four community types with distinct differences in species composition and environmental conditions (Curtis 1959). Northern Upland Forests (NUF) include a range of forest types occurring in northern Wisconsin including coniferous and mixed-hardwood forests dominated by Sugar Maple (*Acer saccharum*), Eastern Hemlock (*Tsuga canadensis*), and White Pine (*Pinus strobus*) (Curtis 1959, Rooney et al. 2004). Pine barrens (PB) occur on sandy, well-drained soils with low soil fertility (Curtis 1959, Li and Waller 2015). Such conditions favor xeric tree species such as jack pine (*Pinus banksiana*) and northern pin oak (*Quercus ellipsoidalis*) and recurrent fires. The PB sites were surveyed were located in the central sand plains of Wisconsin (Li and Waller 2015). Southern Upland Forests (SUF) include sites in southern Wisconsin that range from oak-hickory forests on relatively sandy, low fertility soils to maple-basswood forests on more fertile soils with greater water holding capacity (Curtis 1959, Rogers et al. 2008). Southern Lowland Forests (SLF) include periodically

flooded forests adjacent to rivers and lakes (Curtis 1959, Johnson and Waller 2013, Johnson et al. 2016). Dominant canopy trees include silver maple (*Acer saccharium*), elms (*Ulmus* spp.), willows (*Salix* spp.), and poplars (*Populus* spp.) but dominant canopy species vary greatly among sites (Johnson and Waller 2013, Johnson et al. 2013, 2016).

Vegetation sampling

To characterize plant distributions and co-occurrence patterns, we employ extensive understory plant community surveys conducted throughout Wisconsin between 2000 and 2012 (Rooney et al. 2004, Wiegmann and Waller 2006, Rogers et al. 2008, 2009, Waller et al. 2012, Johnson and Waller 2013, Johnson et al. 2013, 2016, Li and Waller 2015). The forest stands included in this analysis were originally surveyed between 1946 and 1958 (Curtis 1959). These sites were relocated and resurveyed using similar but more intensive methods (Waller et al. 2012). Here we use the post-2000 resurvey data when sampling was more intensive and the vegetation could be linked directly to contemporary data on environmental conditions and plant traits. Despite clear shifts in species composition over the 50+ year intervals (see citations above), species-environment and trait-environment relationships were maintained (Amatangelo et al. 2014). During the resurveys, the presence of all vascular plant species was scored within many replicate 1x1m quadrats (Table 1; see Waller et al. 2012 and citations above for details on field methods). We analyzed plant distributions and co-occurrence patterns for the 139 plant species present in at least 5 stands and for which we had complete functional trait data (Fig. 2). These focal species represent 77% of all site-level occurrences and 84% of quadrat-level occurrences across the 259 forest sites.

Environmental data

We selected a suite of trait and environmental predictors expected to influence herbaceous plant distributions (Table 1). We chose to analyze effects of four environmental predictor variables: soil Calcium content (log-transformed Ca ppm), soil sand content (percent), average tree basal area (cm²), and Precip:PET. Soil samples were taken during contemporary surveys of all stands and analyzed for soil pH,

soil organic matter content, soil silt, sand, and clay content (%), and concentrations of leading cation nutrients (calcium: Ca, potassium: K, and magnesium: Mg). Many soil variables were intercorrelated with one another so we selected soil Calcium and soil sand to represent the two primary sources of edaphic variation (soil fertility and soil texture respectively; see Fig. S1 for PCA of soil variables). In our analyses, soil Calcium was log-transformed to improve the distribution of residuals.

Although we lacked direct measurements of light levels within these stands, we obtained a proxy for light by estimating the average basal area of trees within each forest site. Young, recently disturbed forests have low basal area and greater light availability in their understories than more mature forests with higher basal area. Trees >10 cm DBH were sampled from each forest site (Waller et al. 2012).

We characterized climate variation likely to influence broad-scale plant distributions using an inverse measure of aridity (Fig. S2): mean annual precipitation divided by potential evapotranspiration (hereafter Precip:PET –<http://www.cgiar-csi.org>). PET, which measures how quickly water is lost to the atmosphere by evapotranspiration, was calculated using monthly WORLDCLIM climate data (Trabucco et al. 2008, Zomer et al. 2008). These data were derived from 1 km resolution historical climate data from 1970 to 2000 (Fick and Hijmans 2017). Soil Calcium and Precip:PET were moderately correlated ($r = -0.49$, $N = 259$, $P < 0.001$) but exploratory analyses suggested that this collinearity did not affect our inferences.

Functional traits

We analyzed effects of four functional traits expected to influence plant distributions and competitive ability (Westoby 1998, Westoby et al. 2002, Westoby and Wright 2006): leaf height, specific leaf area (SLA), leaf C:N, and seed mass (Table 1). Our data set contained several non-seed plants (ferns, lycophytes, and horsetails). Although these species lack true seeds, we assigned the minimum seed mass in the data set to all non-seed plants given their functionally analogous diaspores. We supplemented our own data on seed mass from field-collected plants in Wisconsin with estimates for an additional 12 species from the Kew Botanic Garden Seed Information Database (2020). We log-transformed seed mass

and leaf height to incorporate their large ranges and improve residual distributions. SLA and leaf C:N were moderately correlated ($r = -0.54$, $N = 139$, $P < 0.001$) but exploratory analyses suggested that multicollinearity did not affect our inferences.

Phylogeny

We used a molecular phylogeny assembled specifically for use with the regional flora of Wisconsin (Fig. S4) (Spalink et al. 2018). This phylogeny includes over 2300 species and was assembled using DNA sequences from seven chloroplast genes (matK, rbcL, atpB, atpF-atpH, ndhF, rpl32, and trnH-psbA). Phylogenetic methods are detailed in Spalink et al. (2018). We subset this regional phylogeny to obtain a tree for the 139 focal species analyzed in this study.

Data analysis

We formulated three sets of Bayesian phylogenetic generalized linear mixed-effects models (PGLMM) to examine how species distributions and co-occurrence patterns were influenced by environmental conditions, functional traits, and phylogenetic relationships (Ives and Helmus 2011, Li and Ives 2017, Ives et al. 2018). The first and second set of PGLMMs characterize plant distributions among forest sites while the third set focuses on plant co-occurrences within sites. For the first and second set, we visually inspected species-environment and trait-environment relationships, checking for non-monotonic (e.g., unimodal) relationships. Species distributions relative to Precip:PET were all monotonic but many species responded unimodally to soil fertility (calcium), soil texture (% sand), and tree basal area. Among traits, only seed mass appeared to respond unimodally to soil calcium and basal area. We included these quadratic terms in our models analyzing how species were distributed among sites. The probability of species occurrence within each site was modeled using a binomial distribution. We analyzed presence/absence rather than any index of abundance at these sites in order to facilitate model convergence. The first PGLMM followed the general form:

$$\mu_i = P(Y_i = 1)$$

$$\begin{aligned} \mu_i = \text{logit}^{-1} & (\beta_0 + a_{\text{site}[i]} + c_{\text{spp}[i]} + d_{\text{spp.phy}[i]} + (\beta_1 + f_{\text{spp}[i]} + g_{\text{spp.phy}[i]}) * \text{env}_{\text{site}[i]} \\ & + (\beta_2 + h_{\text{spp}[i]} + i_{\text{spp.phy}[i]}) * \text{env}_{\text{site}[i]}^2 + \beta_3 * \text{trait}_{\text{spp}[i]} + \beta_4 * \text{trait}_{\text{spp}[i]} \\ & * \text{env}_{\text{site}[i]} + \beta_5 * \text{trait}_{\text{spp}[i]} * \text{env}_{\text{site}[i]}^2) \end{aligned}$$

$$a \sim \text{Gaussian}(0, \sigma_{\text{site}}^2 I_m)$$

$$c \sim \text{Gaussian}(0, \sigma_{\text{spp}}^2 I_n)$$

$$d \sim \text{Gaussian}(0, \sigma_{\text{spp}}^2 \Sigma_{\text{phy}})$$

$$f \sim \text{Gaussian}(0, \sigma_{\text{spp}}^2 I_n)$$

$$g \sim \text{Gaussian}(0, \sigma_{\text{spp}}^2 \Sigma_{\text{phy}})$$

$$h \sim \text{Gaussian}(0, \sigma_{\text{spp}}^2 I_n)$$

$$i \sim \text{Gaussian}(0, \sigma_{\text{spp}}^2 \Sigma_{\text{phy}})$$

Where β_0 specifies the average probability of occurrence across all species, β_1 allows the probability of occurrence to vary according to an environmental predictor, β_2 accounts for non-monotonic species-environment responses, β_3 allows the average probability of plant occurrence to vary with the value of a trait, β_4 quantifies trait-environment interactions in which plant responses to environmental variation depend on trait differences, and β_5 accounts for unimodal trait-environment associations. a is a random effect that allows the overall probability of occurrence to vary among forest sites, c allows the probability of occurrence to vary among species (independent of phylogenetic relationships), d allows the probability of occurrence to vary among species such that there is phylogenetic covariance in species abundance, f allows species to respond differently to environmental variation (independent of phylogeny and traits), g allows for phylogenetic covariance in species responses to the environment, h allows for unimodal species responses to an environmental predictor, and i accounts for phylogenetic covariance in unimodal species-environment relationships. All quantitative predictor variables were centered and scaled to unit variance to aid model convergence and facilitate comparison among predictors (Gelman and Hill 2007).

For quadratic terms, we first centered the predictor by subtracting its mean and then squared the centered estimates to minimize correlation between linear and quadratic predictors for the same environmental variable. Fixed effects were evaluated using 95% credible intervals. To assess random effects, we fit models with and without each random effect and compared the Deviance Information Criterion (DIC) of the two models. When the differences in Deviance Information Criterion (Δ DIC) was greater than 5, we concluded that including the random effect substantially improved model performance (Spiegelhalter et al. 2002).

The second set of PGLMMs examine patterns of functional and phylogenetic repulsion in plant co-occurrences among forest stands. Computational limitations prevented us from modeling stand-scale functional and phylogenetic repulsion using the full data set so we split the full data set into two independent subsets consisting of 129 and 130 sites (Subset 1 and Subset 2, respectively) and fit similar PGLMMs to the model described above. These models included the same environmental and functional trait predictors as set one while also retaining random effects for variation in the probability of occurrence across sites (term *a*), across species (term *c*), and across species such that the probability of occurrence was similar among close relatives (term *d*). In set two, however, we compared this model to PGLMMs that included covariance matrices specifying either functional or phylogenetic repulsion (Ives and Helmus 2011, Ives et al. 2018). To obtain the phylogenetic repulsion matrix, we scaled the phylogenetic covariance matrix so that its determinant equaled 1 and then found its inverse. We then calculated the Kronecker product of the resulting species covariance matrix and a diagonal site matrix to obtain the covariance matrix blocked by site specifying phylogenetic repulsion within forest stands (Ives and Helmus 2011). The functional repulsion matrix used functional traits to generate a dissimilarity matrix based on Gower distances. Traits used to calculate this dissimilarity matrix included leaf height, SLA, leaf C:N, seed mass, longevity (short-lived vs. long-lived), pollination syndrome (biotic vs. abiotic), and bloom season (spring, summer, or fall). We then used a hierarchical clustering algorithm to produce a trait dendrogram and functional covariance matrix (Fig. S5). As with the phylogenetic repulsion matrix, we scaled the functional covariance matrix to obtain a determinant of 1, found its inverse, and then calculated

the Kronecker product to obtain the functional repulsion matrix blocked by site used in our model. We assessed the strength of functional and phylogenetic repulsion by comparing DIC values from the PGLMMs that included repulsion matrices to models without these repulsion terms.

The final set of PGLMMs tested the strength of functional and phylogenetic repulsion at finer spatial scales by examining species co-occurrence patterns among the individual 1x1 m² quadrats within each stand. These models were formulated as described above but included only trait (not environmental) predictors. For each site, we fit three different PGLMMs. All three included a suite of trait predictors (leaf height, SLA, leaf C:N, and seed mass) and random effects allowing variation in the probability of occurrence among quadrats, the probability of occurrence among species, and the probability of species occurrence conditioned on phylogenetic relatedness (analogous to the random effects *a*, *c*, and *d* described above). The second model added a covariance matrix specifying functional repulsion while the third model added one specifying phylogenetic repulsion. Comparing DIC values from the second and third models to the first PGLMM allows us to evaluate the strength of functional and phylogenetic repulsion within forest stands. All analyses were performed in R 3.5.3 (R Core Team 2019). We fit PGLMMs using the *communityPGLMM()* function within the ‘phyr’ package (Ives et al. 2018) and generated figures using ‘ggplot2’ (Wickham 2009).

RESULTS

Herbaceous plant distributions reflect variation in environmental conditions, functional traits, and often their interactions (Fig. 3). Overall, probabilities of species occurrence decreased with the Precip:PET of forest sites and increased with plant SLA. Across species, probabilities of occurrence first increased, then declined, in response to increases in soil fertility (Ca here), soil sand, and average tree basal area. The probabilities of occurrence also tended to increase with seed mass (though the credible interval just overlapped zero). Leaf C:N and leaf height did not appear to affect overall plant occurrences.

Traits mediate plant responses to edaphic conditions in these forest communities. As soil fertility (Ca) increased, one finds species that are taller with reduced leaf C:N. More fertile sites also tended to

support species with higher SLA (but again the credible interval overlapped with zero). Seed mass did not appear to respond to soil fertility. Sites with sandier soils tended to support species with high leaf C:N and smaller seeds. Leaf height or SLA do not appear to mediate plant responses to soil texture. Plant species with low C:N ratios occurred more commonly in shadier forests (with higher basal area). Leaf height and SLA did not affect these responses to tree basal area. The occurrence of large-seeded plants peaked at intermediate basal area. As the ratio of precipitation to potential evapotranspiration increased, mean leaf heights declined and mean leaf C:N ratios increased. Plants with greater SLA occurred more commonly in sites with high Precip:PET (though credible intervals again touched zero). Seed mass does not appear to associate with any gradient in climate conditions.

After accounting for the effects of all eight trait and environmental predictors, we tested how residual variation can be attributed to sites, species, and phylogenetic relationships (Fig. 4). Probabilities of species occurrence vary considerably among plant species and forest sites independent of functional traits and environmental conditions (Fig. 4). Herbaceous species occurrences responded to soil fertility, soil sand, and Precip:PET in ways that were independent of their functional traits. Many species responded unimodally to soil fertility, but different species reached peak occurrences at different points. Closely related species tended to have similar probabilities of occurrence. Distributions of related species also tended to respond similarly to Precip:PET. However, overall levels of phylogenetic covariance in plant responses to environmental conditions were weak explaining little residual variation.

We found little evidence for functional or phylogenetic repulsion in species co-occurrence patterns either among sites (Table S3) or among quadrats within sites (Fig. 5). Among sites, there was no indication that functionally similar species were segregated (Δ DIC values for the independent subsets were 1.08 and 1.43). Similarly, we found no evidence that closely related species were less likely to co-occur within the same forest sites (Δ DIC = 0.53 and 1.35). Examining species co-occurrence patterns among quadrats within forest stands revealed some variation but similarly weak evidence of local functional and phylogenetic repulsion (Fig. 5). Species distributions in just 12 of 259 sites showed

evidence ($\Delta\text{DIC} > 5$) that functionally similar species disassociate at the quadrat-scale (Fig. 5a).

Meanwhile, closely related species were segregated in just 13 sites (Fig. 5b).

DISCUSSION

We characterized how 139 temperate forest herbs were distributed across 259 sites to gain insights into the ecological processes structuring understory plant communities. Consistent with previous studies from temperate forests and other ecosystems, we found strong evidence that traits mediate plant responses to environmental gradients in soil fertility, soil texture, disturbance, and climate (Ordoñez et al. 2009, Pollock et al. 2012, Jamil et al. 2013, Amatangelo et al. 2014). Greater soil fertility increases the probability of encountering taller plant species, reflecting in part how tall-statured plants often occur in fertile lowlands where greater leaf height confers a competitive advantage (Menges and Waller 1983). Likewise, sparse plant coverage in pine barrens and nutrient poor upland sites reduces the advantage of growing taller, favoring short stature (Givnish 1982). Sites with greater soil fertility also supported fewer nutrient conservative species (plants with high leaf C:N) while these plants occurred frequently in sites with sandy soil. These trait-environment relationships reflect interspecific differences in resource demands and probably increased allocation to defenses in resource-poor environments (Mooney and Gulmon 1982, Coley et al. 1985, Chapin et al. 1993).

The trait-environment relationships we observed in temperate forests are largely consistent theoretical predictions and previous empirical studies (Givnish 1982, 1987, Westoby 1998, Ordoñez et al. 2009, Amatangelo et al. 2014, Neufeld and Young 2014). These findings support the idea that trait-mediated environmental sorting plays a strong role in assembling plant communities along environmental gradients (Weiher and Keddy 1999, Kunstler et al. 2012, Kraft et al. 2015a).

Shadier and more mature forests (higher mean basal area) favored species with high leaf N (i.e., low C:N ratio). This is expected as higher leaf N enhances photosynthetic capacity in shady environments (Givnish 1987, Wright et al. 2004, Neufeld and Young 2014, Reich 2014). We also expected high basal area forests to favor species with high SLA but no environmental variable affected this trait. This may

reflect widespread mesification in temperate forests where broad reductions in understory light may favor shade-tolerant, high SLA species (Nowacki and Abrams 2008, Rogers et al. 2008, Li and Waller 2015). Alternatively, SLA may have responded to more fine-scale local variation in understory light availability within these stands.

Seed mass was associated with both forest maturity and soil sand content. Small-seeded plants were most abundant in sandy soils while seed mass peaked at intermediate basal area. These distributions likely reflect how small-seeded plants are favored in disturbed, sparsely vegetated environments with little competition (Westoby 1998, Westoby and Wright 2006). The declines in seed size observed within mature forests could reflect the greater occurrence of species reliant on ants to disperse seeds or low competition within shady forests because of sparse herb coverage. Sites with higher ratios of precipitation to PET tended to support more short-statured and nutrient-conservative species. This may reflect how high leaching in such sites depletes soil nutrients throughout the soil profile (beyond the surface soils sampled to measure soil fertility). The strong North-South gradient in Precip:PET that exists among our sites (Fig. S2) means that the relationships found between traits and Precip:PET could also reflect latitudinal gradients in temperature, seasonality, or some other climatic factor (Curtis 1959, Amatangelo et al. 2014).

In contrast to this strong evidence that plant functional traits mediate species' responses to environmental variation, we found much weaker evidence that phylogenetic relationships affect plant distributions along environmental gradients and virtually no evidence that these affect patterns of species co-occurrence. Closely related species often occurred at similar frequencies and tended to respond similarly to variation in climate and soil texture. Related species responded less similarly to gradients in other conditions. These results suggest that conserved characters lead closely related species to respond similarly to some environmental gradients but that relying on phylogenetic relationships alone would be misleading (Swenson and Enquist 2009).

Among our focal species, different clades have often converged on characteristics that confer a competitive advantage in certain environments (Givnish 1987). While shared ecological characteristics

can favor co-occurrence in suitable habitats, competition is expected discourage co-occurrence for closely related species at the spatial scale where competition occurs (Cavender-Bares et al. 2009). Here, we found no evidence that close relatives segregate (phylogenetic repulsion) at the scale of either sites or 1m² quadrats (where plants likely compete directly for resources). These weak phylogenetic influences contradict common assumptions about how phylogenetic relatedness affects plant distributions and co-occurrence patterns (Webb et al. 2002, Cornwell and Ackerly 2009b, Vamosi et al. 2009, Cavender-Bares et al. 2009, Kraft and Ackerly 2010, Mayfield and Levine 2010). Phylogenetic relatedness is often used as a surrogate for functional similarity under the assumption that ecologically relevant traits are conserved within evolutionary lineages (Webb et al. 2002, Wiens et al. 2010). Our findings indicate that evolutionary history plays a nuanced role in assembling understory forest plant communities. This casts doubt on simple heuristic models that ignore complex patterns of trait evolution and biogeographic history present in these diverse clades (Swenson and Enquist 2009). Phylogenetic patterns tend to be stronger at broad scales (reflecting biogeographic trends that differentiate lineages) and within species-rich clades where strong ecological interactions can drive patterns of niche evolution (Cavender-Bares et al. 2006, 2018, Swenson et al. 2006, 2007, Helmus et al. 2007, Cavender-Bares 2018). Phylogenetic relationships may have less utility for explaining community assembly at local or regional scales involving a broad range of taxa from diverse clades such as in our study.

Taken together, our findings support the idea that environmental sorting strongly affects the assembly of these forest plant communities while undermining support for the idea that competitive and other biotic interactions strongly drive community assembly. Several heuristic models of community assembly predict that after sorting into environments, biotic interactions (e.g., niche differences associated with complementary resource use) shape the composition and dynamics of local communities (Weiher and Keddy 1999, Cavender-Bares et al. 2009, HilleRisLambers et al. 2012). Our analyses failed to support this prediction in that little evidence of functional or phylogenetic repulsion among species emerged. What might account for this lack of support for niche differentiation? Possibly, phylogenetic relationships and the traits included in our analyses of co-occurrence failed to capture actual

complementary ecological strategies that exist and function to stabilize local coexistence. It is also possible that static co-occurrence patterns obscure such resource partitioning. However, there is little evidence for complementary resource usage among temperate forest herbs (Amarasekare 2003, Silvertown 2004, Costanza et al. 2011, Beatty 2014, Catella et al. 2019). In contrast, differential plant responses to environmental heterogeneity are known to influence local plant distributions (Beatty 2014). Fine-scale variation in microtopography; soil depth, fertility, and moisture; and light availability all influence plant distributions and co-occurrence patterns within forest sites (Beck and Givnish, *in review*, Struik and Curtis 1962, Bratton 1976, Thompson 1980, Beatty 1984, 2014, Crozier and Boerner 1984). Environmental heterogeneity occurring within sites but at spatial scales coarser than 1m² might also explain the lack of functional or phylogenetic repulsion. Previous extensive studies of fine-scale (quadrat-scale) co-occurrence patterns at these sites revealed primarily weak interspecific associations that were independent of traits or phylogenetic relationships (Li and Waller 2016). This suggests that local plant distributions seldom reflect strong, deterministic processes in these forest communities.

The lack of evidence for stabilizing niche differences and generally weak interspecific associations could also reflect a predominance of stochastic over deterministic forces acting locally within communities as expected if the species present share roughly equal fitness. Chase (2014) notes that environmental sorting should generate local communities composed of species well-equipped to compete for resources within that given environment, reducing relative fitness differences among co-occurring species. In contrast to stabilizing processes that promote coexistence by reducing interspecific competition relative to intraspecific competition, equalizing processes reduce absolute fitness differences among species (Chesson 2000a, Mayfield and Levine 2010). Under such circumstances, rates of competitive exclusion decline, increasing the relative importance of local dispersal and stochastic demographic events. Although species clearly differ ecologically, this would lead local community dynamics to resemble neutral communities driven by ecological drift (Hubbell 2001, Chase 2014). Such equalizing processes also set the stage for other relatively weak processes to affect local plant distributions and dynamics. This might allow differential responses to subtle spatial or temporal

environmental variation (Chesson and Warner 1981, Chesson 2000a, 2000b, Adler et al. 2006, Angert et al. 2009), demographic differences and life-history tradeoffs (Beck et al., *in preparation*, Grubb 1977, Shmida and Ellner 1984), or localized plant-soil feedbacks (Beck, *in review*, Smith and Reynolds 2015) to favor local coexistence. Several recent studies have investigated the potential for scale-dependent processes to shape plant community assembly but these mostly focused on the potential for complementary resource use (Maire et al. 2012, Gross et al. 2013, Scherrer et al. 2019). To gain further insights into scale-dependent drivers of plant community assembly, we should consider a full range of potentially stabilizing processes.

Our study incorporated environmental, trait, and phylogenetic data to illuminate how these factors affect the distribution and co-occurrence of temperate forest herbs. Our results re-affirm how trait-mediated environmental sorting and competitive hierarchies shape plant distributions along environmental gradients (Weiher and Keddy 1999, HilleRisLambers et al. 2012). This echoes century-old concepts that emphasize how plant species sort individualistically into communities according to their relative fitness in a local environment (Gleason 1926, Whittaker 1956, Curtis 1959). In contrast, the absence of strong functional or phylogenetic repulsion among co-occurring species in our study seems out of line with some predictions from community assembly theory (Weiher and Keddy 1999, Silvertown et al. 2006, Cavender-Bares et al. 2009, HilleRisLambers et al. 2012). This lack of conspicuous niche differentiation among locally co-occurring plants could reflect local environmental heterogeneity within sites coupled with equalizing processes that drive stochastic assembly within relatively homogenous environments. Diffuse and unpredictable species interactions arising from varied biogeographic histories and individualistic responses to environmental variation may preclude the evolution of highly refined niche differences (the α -niche *sensu* Silvertown et al. 2006). This would allow species to fit themselves into ecological communities as “asymmetrical pegs in square holes” (Janzen 1985) if species occupy niche space within local communities opportunistically rather than via deterministic assembly rules. This might account for finding weak evidence of resource partitioning among plants in the absence of spatial or temporal environmental heterogeneity (Amarasekare 2003, Silvertown 2004). Future studies of

community dynamics should seek to assess these mechanisms rigorously to further refine our understanding of the forces driving community assembly across communities.

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Table 1. Description of environmental and trait predictors used in our phylogenetic generalized linear mixed effects models.

Predictor	Description
<i>Environment</i>	
Soil calcium (ppm)	Measure of soil fertility, correlates with organic matter content, concentration of other leading cations, and organic matter content
Soil sand content (percent)	Reflects soil water holding capacity, correlated with other measures of soil texture and soil pH
Average tree basal area (cm ²)	Primarily reflects stand age and disturbance history, forests with smaller basal area will tend to be recently disturbed forests with potentially greater light availability to understory plants
Precip:PET	Ratio of mean annual precipitation to potential evapotranspiration (PET); a lower Precip:PET ratio indicates locations where we expect increased water stress
<i>Traits</i>	
Leaf height (cm)	Average distance from ground to tallest leaf; plants with greater leaf height tend to be better competitors for light
Specific leaf area (SLA, cm ² /g)	Specific leaf area; species with high SLA (broad, thin leaves) achieve greater photosynthetic returns in low light environments.
Leaf C:N	Ratio of carbon (%) to nitrogen (%) in plant leaves; reflects growth-defense tradeoffs. Plants with high C:N ratios allocate a greater portion of resources to defense.
Seed mass (mg)	Mass of seed; reflects differences in dispersal and seedling competitive ability. Small seeded plants tend to have greater dispersal capacity, but seedling have fewer nutrients available and are weak competitors.

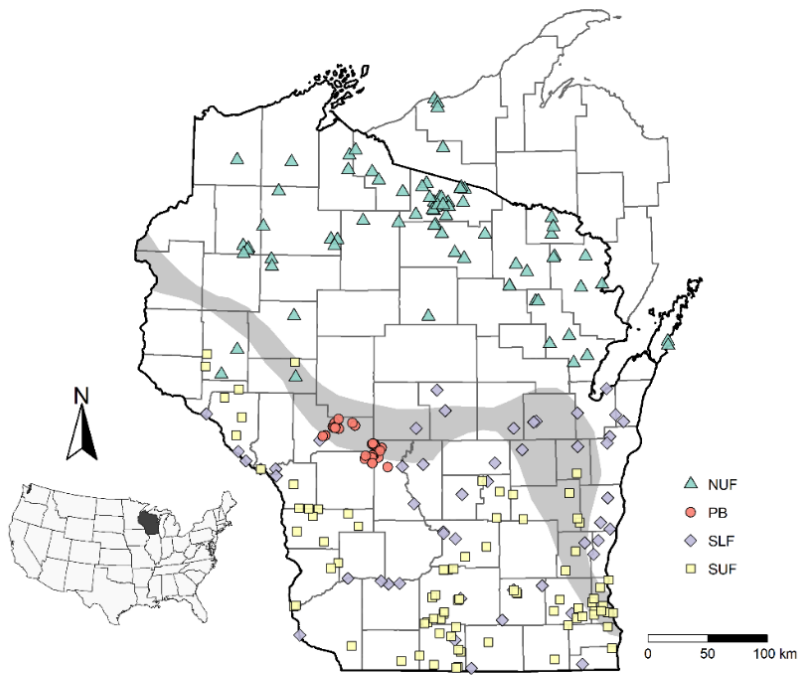


Figure 1. Map of study sites in Wisconsin, USA. Vegetation surveys were conducted at 259 forest stands across the state of Wisconsin between 2000 and 2012. Community classification follows Curtis (1959): blue triangles – Northern Upland Forests (NUF), red circles – Pine Barrens (PB), purple diamonds – Southern Lowland Forests (SLF), and yellow squares – Southern Upland Forests (SUF).

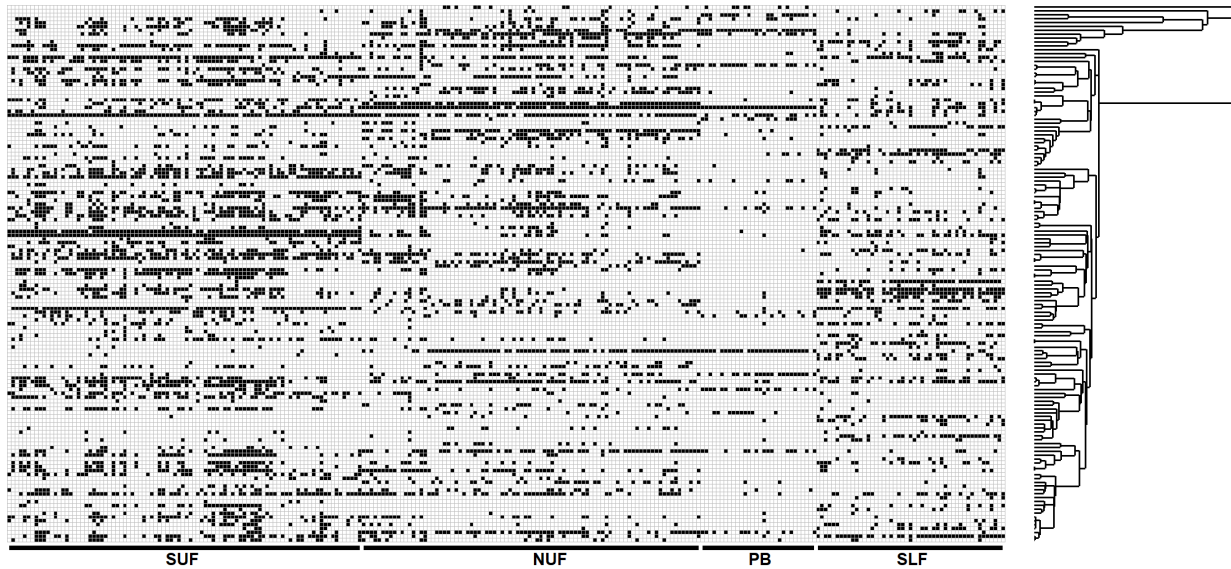


Figure 2. Heatmap illustrating distributional and co-occurrence pattern among 139 herbaceous species (rows) distributed across 259 study sites (columns) in Wisconsin (USA). Filled cells represent sites in which a particular species was present. The tree illustrates phylogenetic relationships among herbaceous plant species. Black bars and labels correspond to community types (Curtis 1959): SUF – Southern Upland Forest, NUF – Northern Upland Forest, PB – Pine Barrens, SLF – Southern Lowland Forest.

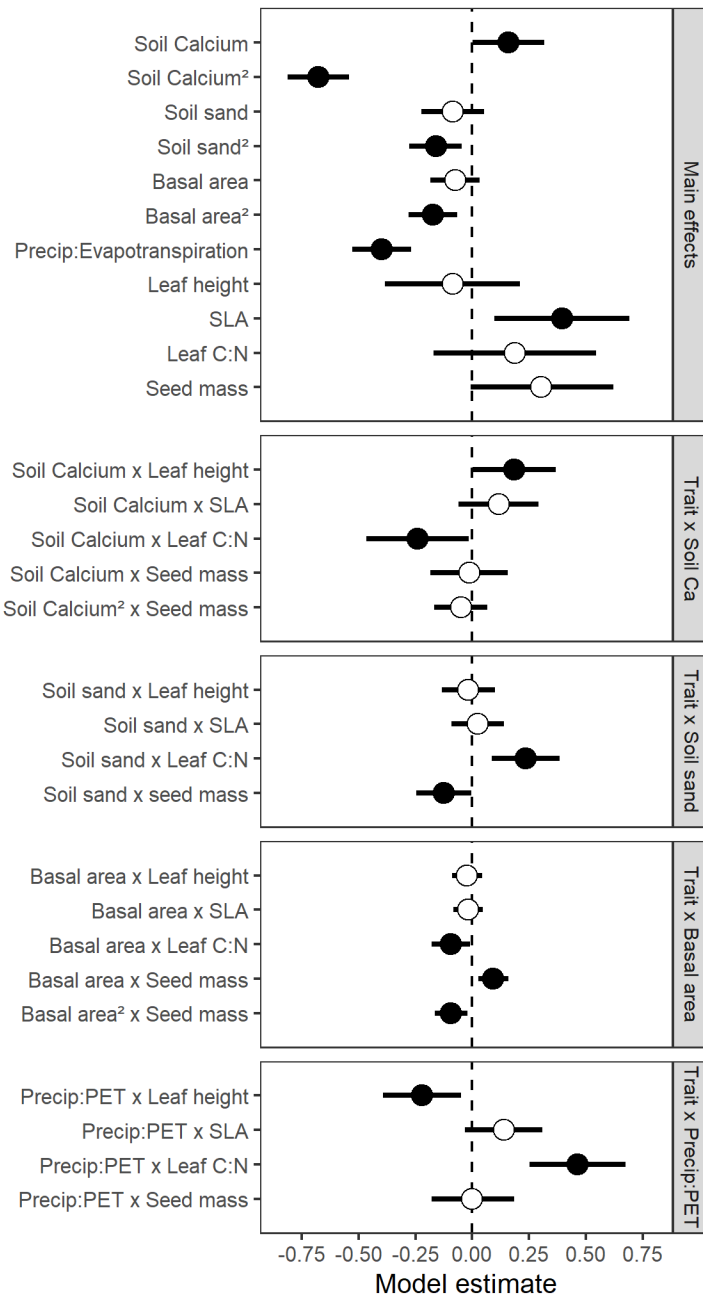


Figure 3. Estimated PGLMM coefficients for the effect of trait and environmental predictors on the probability of plant species occurrence. Top panel depicts main effects of predictors while the bottom four panels illustrate estimates for trait-environment interactions. Positive estimates indicate a positive relationship between predictors and the probability of occurrence. Solid circles indicate estimates that are different from zero (95% credible interval do not overlap with zero).

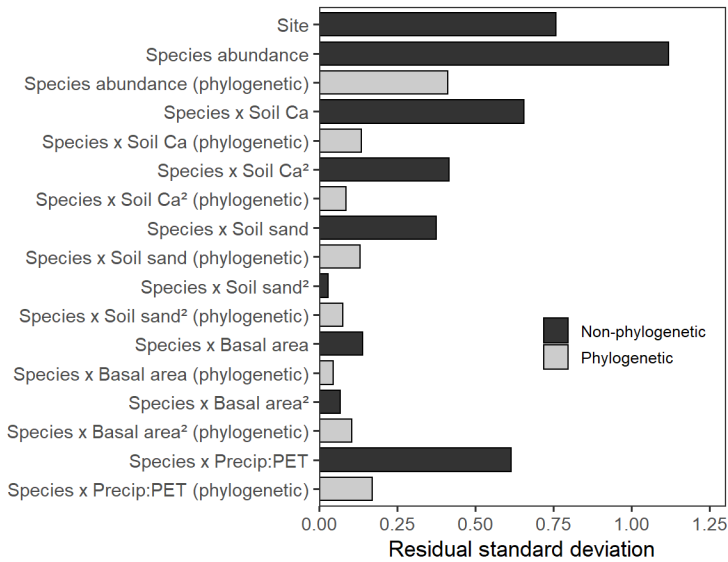


Figure 4. Estimated standard deviation for residual phylogenetic and non-phylogenetic effects in PGLMM. Dark grey bars represent standard deviation estimates for non-phylogenetic random effects while light grey bars represent standard deviation estimates for random effects specifying phylogenetic covariance among species.

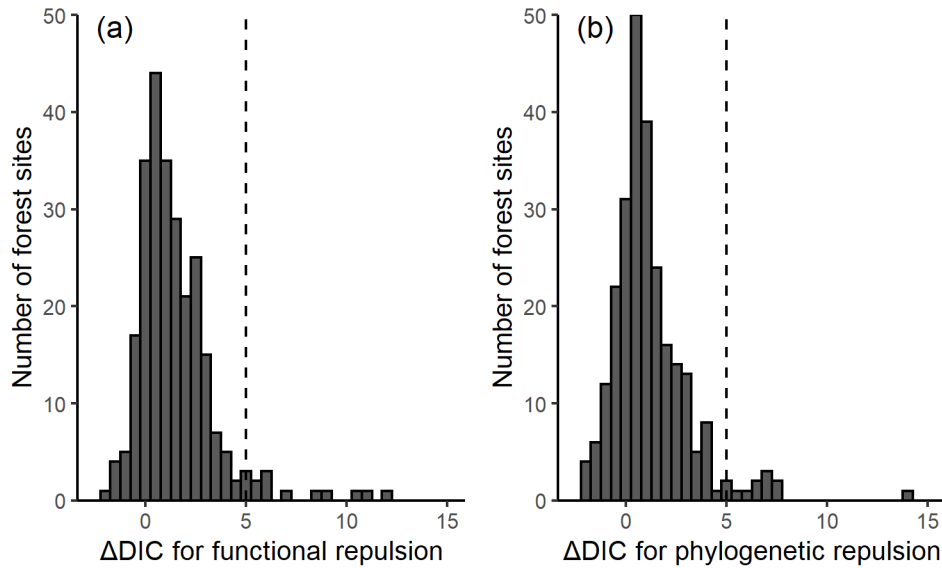


Figure 5. Patterns of functional (a) and phylogenetic (b) repulsion at the quadrat scale. These histograms display Δ DIC values (the differences in DIC values between PGLMMs with and without repulsion covariance matrices) for all 259 forest sites. Only Δ DIC values >5 (vertical dashed line) provide evidence of functional or phylogenetic repulsion (spatial segregation among species within sites).

LITERATURE CITED

- Adler, P. B., J. HilleRisLambers, P. C. Kyriakidis, Q. Guan, and J. M. Levine. 2006. Climate variability has a stabilizing effect on the coexistence of prairie grasses. *Proceedings of the National Academy of Sciences* 103:12793–8.
- Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: A synthesis. *Ecology Letters* 6:1109–1122.
- Amatangelo, K., S. Johnson, D. Rogers, and D. M. Waller. 2014. Trait-environment relationships remain strong despite 50 years of trait compositional change in temperate forests. *Ecology* 95:1780–1791.
- Angert, A. L., T. E. Huxman, P. Chesson, and D. L. Venable. 2009. Functional tradeoffs determine species coexistence via the storage effect. *Proceedings of the National Academy of Sciences* 106:11641–11645.
- Beatty, S. W. 1984. Influence of microtopography and canopy species on spatial patterns of forest understory plants. *Ecology* 65:1406–1419.
- Beatty, S. W. 2014. Habitat Heterogeneity and the Maintenance of Species in Understory Communities. Pages 215–232 *in* F. S. Gilliam, editor. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Beck, J. J. Variation in the strength of plant-soil feedbacks among temperate forest herbs. *In review*.
- Beck, J. J., and T. J. Givnish. Fine-scale environmental heterogeneity and spatial niche partitioning among spring-flowering forest herbs. *In review*.
- Beck, J. J., A. Salvi, R. A. Henderson, and D. M. Waller. Coexistence revisited: Can we integrate demographic, physiological, and distributional data to understand patterns of co-occurrence among close relatives? *In preparation*.
- Bierzychudek, P. 1982. Life histories and demography of shade-tolerant temperate forest herbs: A review. *New Phytologist* 90:757–776.
- Bratton, S. P. 1976. Resource division in an understory herb community: Responses to temporal and microtopographic gradients. *The American Naturalist* 110:679–693.
- Catella, S. A., S. R. Eysenbach, and K. C. Abbott. 2019. Novel insights into how the mean and heterogeneity of abiotic conditions together shape forb species richness patterns in the Allegheny plateau ecoregion. *Ecology and Evolution*:12374–12389.
- Cavender-Bares, J. 2018. Diversification, adaptation, and community assembly of the American oaks (*Quercus*), a model clade for integrating ecology and evolution. *New Phytologist*.

- Cavender-Bares, J., A. Keen, and B. Miles. 2006. Phylogenetic structure of Floridian plant communities depends on taxonomic and spatial scale. *Ecology* 87:109–122.
- Cavender-Bares, J., S. Kothari, J. E. Meireles, M. A. Kaproth, P. S. Manos, and A. L. Hipp. 2018. The role of diversification in community assembly of the oaks (*Quercus* L.) across the continental U.S. *American Journal of Botany* 105:565–586.
- Cavender-Bares, J., K. H. Kozak, P. V. a Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. *Ecology letters* 12:693–715.
- Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic Overdispersion in Floridian Oak Communities. *The American Naturalist* 163:823–843.
- Chapin, F. S., K. Autumn, and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. *The American Naturalist* 142:S78–S92.
- Chase, J. M. 2014. Spatial scale resolves the niche versus neutral theory debate. *Journal of Vegetation Science* 25:319–322.
- Chesson, P. 2000a. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics* 31:343–358.
- Chesson, P. 2000b. General theory of competitive coexistence in spatially-varying environments. *Theoretical population biology* 58:211–37.
- Chesson, P. 2018. Updates on mechanisms of maintenance of species diversity. *Journal of Ecology* 106:1773–1794.
- Chesson, P. L., and R. R. Warner. 1981. Environmental variability promotes coexistence in lottery competitive systems. *The American naturalist* 117:923–943.
- Clements, F. E. 1916. *Plant Succession: An Analysis of the Development of Vegetation*. Carnegie Institution of Washington, Washington D.C.
- Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895–899.
- Cornwell, W. K., and D. D. Ackerly. 2009a. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs* 79:109–126.
- Cornwell, W. K., and D. D. Ackerly. 2009b. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecological Monographs* 79:109–126.
- Costanza, J. K., A. Moody, and R. K. Peet. 2011. Multi-scale environmental heterogeneity as a predictor

- of plant species richness. *Landscape Ecology* 26:851–864.
- Crozier, C. R., and R. E. J. Boerner. 1984. Correlation of understory herb distribution patterns with microhabitats under different tree species in a mixed mesophytic forest. *Oecologia* 62:337–343.
- Curtis, J. T. 1959. *The Vegetation of Wisconsin: An Ordination of Plant Communities*. The University of Wisconsin Press, Madison, WI.
- Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37:4302–4315.
- Funk, J. L., J. E. Larson, G. M. Ames, B. J. Butterfield, J. Cavender-Bares, J. Firn, D. C. Laughlin, A. E. Sutton-Grier, L. Williams, and J. Wright. 2017. Revisiting the Holy Grail: Using plant functional traits to understand ecological processes. *Biological Reviews* 92:1156–1173.
- Gelman, A., and J. Hill. 2007. *Data analysis using regression and multilevel/hierarchical models*. Cambridge University Press, New York, New York, USA.
- Gilbert, B., and M. J. Lechowicz. 2004. Neutrality, niches, and dispersal in a temperate forest understory. *Proceedings of the National Academy of Sciences* 101:7651–7656.
- Gilliam, F. S. 2007. The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. *BioScience* 57:845.
- Gilliam, F. S., editor. 2014. *The herbaceous layer in forests of eastern North America*. Oxford University Press.
- Givnish, T. J. 1982. On the adaptive significance of leaf height in forest herbs. *American Naturalist* 120:353–381.
- Givnish, T. J. 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106:131–160.
- Gleason, H. 1926. The Individualistic Concept of the Plant Association. *Bulletin of the Torrey Botanical Club* 53:7–26.
- Gross, N., L. Börger, S. I. Soriano-Morales, Y. Le Bagousse-Pinguet, J. L. Quero, M. García-Gómez, E. Valencia-Gómez, and F. T. Maestre. 2013. Uncovering multiscale effects of aridity and biotic interactions on the functional structure of Mediterranean shrublands. *Journal of Ecology* 101:637–649.
- Grubb, P. J. 1977. The maintenance of species richness in plant communities: The importance of the regeneration niche. *Biological Reviews* 52:107–145.

- Helmus, M. R., K. Savage, M. W. Diebel, J. T. Maxted, and A. R. Ives. 2007. Separating the determinants of phylogenetic community structure. *Ecology Letters* 10:917–925.
- HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking Community Assembly Through the Lens of Coexistence Theory. *Annual Review of Ecology, Evolution, and Systematics* 43:120830113150004.
- Hubbell, S. P. 2001. *The Unified Neutral Theory of Island Biogeography and Biodiversity*. Princeton University Press.
- Ives, A., and M. Helmus. 2011. Generalized linear mixed models for phylogenetic analyses of community structure. *Ecological Monographs* 81:511–525.
- Ives, A. R., M. R. Helmus, R. Dinnage, and D. Li. 2018. *phyr*: Phylogenetic Analysis with R. R package.
- Jamil, T., W. A. Ozinga, M. Kleyer, and C. J. F. Ter Braak. 2013. Selecting traits that explain species-environment relationships: A generalized linear mixed model approach. *Journal of Vegetation Science* 24:988–1000.
- Janzen, D. H. 1985. On Ecological Fitting. *Oikos* 45:308.
- Johnson, S. E., K. L. Amatangelo, P. A. Townsend, and D. M. Waller. 2016. Large, connected floodplain forests prone to flooding best sustain plant diversity. *Ecology* 97:3019–3030.
- Johnson, S. E., E. L. Mudrak, and D. M. Waller. 2013. Local increases in diversity accompany community homogenization in floodplain forest understories. *Journal of Vegetation Science* 25:885–896.
- Johnson, S. E., and D. M. Waller. 2013. Influence of dam regulation on 55-year canopy shifts in riparian forests. *Canadian Journal of Forest Research* 43:159–170.
- Kraft, N. J. B., and D. D. Ackerly. 2010. Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecological Monographs* 80:401–422.
- Kraft, N. J. B., P. B. Adler, O. Godoy, E. C. James, S. Fuller, and J. M. Levine. 2015a. Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology* 29:592–599.
- Kraft, N. J. B., O. Godoy, and J. M. Levine. 2015b. Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences* 112:797–802.
- Kunstler, G., S. Lavergne, B. Courbaud, W. Thuiller, G. Vieilledent, N. E. Zimmermann, J. Kattge, and D. a Coomes. 2012. Competitive interactions between forest trees are driven by species' trait hierarchy, not phylogenetic or functional similarity: implications for forest community assembly. *Ecology letters* 15:831–40.

- Li, D., and A. R. Ives. 2017. The statistical need to include phylogeny in trait-based analyses of community composition. *Methods in Ecology and Evolution*.
- Li, D., and D. M. Waller. 2015. Drivers of observed biotic homogenization in pine barrens of central Wisconsin. *Ecology* 96:1030–1041.
- Li, D., and D. M. Waller. 2016. Long-term shifts in the patterns and underlying processes of plant associations in Wisconsin forests. *Global Ecology and Biogeography*:1–11.
- Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology letters* 11:995–1003.
- Macarthur, R. H., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist* 101:377–385.
- Maire, V., N. Gross, L. Börger, R. Proulx, C. Wirth, L. da S. Pontes, J. F. Soussana, and F. Louault. 2012. Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytologist* 196:497–509.
- Mayfield, M. M., M. F. Boni, and D. D. Ackerly. 2009. Traits, habitats, and clades: Identifying traits of potential importance to environmental filtering. *American Naturalist* 174.
- Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology letters* 13:1085–93.
- McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in ecology & evolution* 21:178–85.
- Menges, E. S., and D. M. Waller. 1983. Plant Strategies in Relation to Elevation and Light in Floodplain Herbs. *American Naturalist* 122:454–473.
- Mooney, H. A., and S. L. Gulmon. 1982. Constraints on leaf structure and function in reference to herbivory. *BioScience* 32:198–206.
- Neufeld, H. S., and D. R. Young. 2014. Ecophysiology of the herbaceous layer in temperate deciduous forests. Pages 35–96 *in* F. S. Gilliam, editor. *The herbaceous layer in forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Nowacki, G. J., and M. D. Abrams. 2008. The demise of fire and “mesophication” of forests in the Eastern United States. *BioScience* 58:123–138.
- Ordoñez, J. C., P. M. Van Bodegom, J. P. M. Witte, I. J. Wright, P. B. Reich, and R. Aerts. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography* 18:137–149.

- Pavoine, S., E. Vela, S. Gachet, G. De Bélair, and M. B. Bonsall. 2011. Linking patterns in phylogeny, traits, abiotic variables and space: A novel approach to linking environmental filtering and plant community assembly. *Journal of Ecology* 99:165–175.
- Peet, R. K., K. A. Palmquist, and S. M. Tessel. 2014. Herbaceous layer species richness of southeastern forests and woodlands: Patterns and causes. Pages 255–276 in F. S. Gilliam, editor. *The Herbaceous Layer in forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Pollock, L. J., W. K. Morris, and P. A. Vesk. 2012. The role of functional traits in species distributions revealed through a hierarchical model. *Ecography* 35:716–725.
- Reich, P. B. 2014. The world-wide “fast-slow” plant economics spectrum: A traits manifesto. *Journal of Ecology* 102:275–301.
- Rogers, D. A., T. P. Rooney, T. J. Hawbaker, V. C. Radeloff, and D. M. Waller. 2009. Paying the extinction debt in southern Wisconsin forest understories. *Conservation Biology* 23:1497–1506.
- Rogers, D. A., T. P. Rooney, D. Olson, and D. M. Waller. 2008. Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. *Ecology* 89:2482–2492.
- Rooney, T. P., S. M. Wiegmann, D. A. Rogers, and D. M. Waller. 2004. Biotic impoverishment and homogenization in unfragmented forest understory communities. *Conservation Biology* 18:787–798.
- Royal Botanic Gardens Kew. 2020. Seed Information Database (SID). Version 7.1. Available from: <http://data.kew.org/sid/> (March 2020)
- Scherrer, D., H. K. Mod, J. Pottier, A. Litsios-Dubuis, L. Pellissier, P. Vittoz, L. Götzenberger, M. Zobel, and A. Guisan. 2019. Disentangling the processes driving plant assemblages in mountain grasslands across spatial scales and environmental gradients. *Journal of Ecology* 107:265–278.
- Shmida, A., and S. Ellner. 1984. Coexistence of plant species with similar niches. *Vegetatio* 58:29–55.
- Silvertown, J. 2004. Plant coexistence and the niche. *Trends in Ecology and Evolution* 19:605–611.
- Silvertown, J., M. Dodd, D. Gowing, C. Lawson, and K. McConway. 2006. Phylogeny and the hierarchical organization of plant diversity. *Ecology* 87:39–49.
- Smith, L. M., and H. L. Reynolds. 2015. Plant-soil feedbacks shift from negative to positive with decreasing light in forest understory species. *Ecology* 96:2523–2532.
- Spalink, D., R. Kriebel, P. Li, M. C. Pace, B. T. Drew, J. G. Zaborsky, J. Rose, C. P. Drummond, M. A. Feist, W. S. Alverson, D. M. Waller, K. M. Cameron, T. J. Givnish, and K. J. Sytsma. 2018. Spatial phylogenetics reveals evolutionary constraints on the assembly of a large regional flora. *American Journal of Botany* 105:1938–1950.

- Spiegelhalter, D. J., N. G. Best, B. P. Carlin, and A. Van Der Linde. 2002. Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society. Series B: Statistical Methodology* 64:583–616.
- Struik, G. J., and J. T. Curtis. 1962. Herb distributions in an *Acer saccharum* forest. *American Midland Naturalist* 68:285–296.
- Swenson, N. G., and B. J. Enquist. 2009. Opposing assembly mechanisms in a Neotropical dry forest: Implications for phylogenetic and functional community ecology. *Ecology* 90:2161–2170.
- Swenson, N. G., B. J. Enquist, J. Pither, J. Thompson, and J. K. Zimmerman. 2006. The problem and promise of scale dependency in community phylogenetics. *Ecology* 87:2418–2424.
- Swenson, N. G., B. J. Enquist, J. Thompson, and J. K. Zimmerman. 2007. The influence of spatial and size scale on phylogenetic relatedness in tropical forest communities. *Ecology* 88:1770–1780.
- R Core Team, 2019. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Thompson, J. N. 1980. Treefalls and Colonization Patterns of Temperate Forest Herbs. *American Midland Naturalist* 104:176.
- Tilman, D. 1982. *Resource Competition and Community Structure*. Princeton University Press.
- Trabucco, A., R. J. Zomer, D. A. Bossio, O. van Straaten, and L. V. Verchot. 2008. Climate change mitigation through afforestation/reforestation: A global analysis of hydrologic impacts with four case studies. *Agriculture, Ecosystems and Environment* 126:81–97.
- Vamosi, S. M., S. B. Heard, J. C. Vamosi, and C. O. Webb. 2009. Emerging patterns in the comparative analysis of phylogenetic community structure. *Molecular Ecology* 18:572–592.
- Vellend, M. 2016. *The Theory of Ecological Communities*. Princeton University Press, Princeton, NJ.
- Waller, D. M., K. Amatangelo, S. Johnson, and D. Rogers. 2012. Wisconsin Vegetation Database – plant community survey and resurvey data from the Wisconsin Plant Ecology Laboratory. *Biodiversity & Ecology* 4:255–264.
- Webb, C. O., D. D. Ackerly, M. A. McPeck, and M. J. Donoghue. 2002. Phylogenies and Community Ecology. *Annual Review of Ecology and Systematics* 33:475–505.
- Weiher, E., and D. Freund. 2011. Advances, challenges and a developing synthesis of ecological community assembly theory. ... of the Royal ... 366:2403–13.
- Weiher, E., and P. A. Keddy, editors. 1999. *Ecological Assembly Rules: Perspectives, Advances,*

Retreats. Cambridge University Press, Cambridge, United Kingdom.

Westoby, M. 1998. A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil* 199:213–227.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. PLANT ECOLOGICAL STRATEGIES: Some Leading Dimensions of Variation Between Species. *Annual Review of Ecology and Systematics* 33:125–159.

Westoby, M., and I. J. Wright. 2006. Land-plant ecology on the basis of functional traits. *Trends in Ecology and Evolution* 21:261–268.

Whigham, D. E. 2004. Ecology of woodland herbs in temperate deciduous forests. *Annual Review of Ecology and Systematics* 35:583–621.

Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. *Ecological Monographs* 26:1–80.

Wickham, H. 2009. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York, USA.

Wiegmann, S. M., and D. M. Waller. 2006. Fifty years of change in northern upland forest understories: Identity and traits of “winner” and “loser” plant species. *Biological Conservation* 129:109–123.

Wiens, J. J., D. D. Ackerly, A. P. Allen, B. L. Anacker, L. B. Buckley, H. V. Cornell, E. I. Damschen, T. Jonathan Davies, J.-A. Grytnes, S. P. Harrison, B. a Hawkins, R. D. Holt, C. M. McCain, and P. R. Stephens. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology letters* 13:1310–24.

Wright, I., P. Reich, M. Westoby, and D. Ackerly. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.

Zomer, R. J., A. Trabucco, D. A. Bossio, and L. V. Verchot. 2008. Climate change mitigation: A spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agriculture, Ecosystems and Environment* 126:67–80.

APPENDIX S1. Supplemental materials for Chapter 1.

Table S1. Summary of vegetation data sets used in analysis of community composition. Year of the vegetation surveys, the number of sites surveyed (range if applicable), the number of 1 x 1 meter quadrats sampled per site, and the number of taxa included in our analysis (139 unique taxa).

Community type	Abbreviation	Survey year	No. sites	No. quadrats/site	No. taxa
Pine Barrens	PB	2012	30	50	49
Northern upland forest	NUF	2000-04	88	80-120 (40-120)	109
Southern lowland forest	SLF	2007-08	49	42 (39-42)	109
Southern upland forest	SUF	2002-03	92	80 (40-403)	115

Table S2. Summary of random effects for PGLMM describing distribution of 139 herabecous plant species across 259 temperate forest stands. We fit PLGMMs with and without the focal terms and quantified the difference in DIC (Δ DIC). A Δ DIC greater than 5 indicates that including the random effect substantially improved models of species distributions.

Model term	Δ DIC
Site	
Species abundance	
Species abundance (phylogenetic)	
Species x Soil Calcium	
Species x Soil Calcium (phylogenetic)	
Species x Soil Calcium ²	
Species x Soil Calcium ² (phylogenetic)	
Species x Soil sand	
Species x Soil sand (phylogenetic)	
Species x Soil sand ²	
Species x Soil sand ² (phylogenetic)	
Species x Basal area	
Species x Basal area (phylogenetic)	
Species x Basal area ²	
Species x Basal area ² (phylogenetic)	
Species x Precip:PET	
Species x Precip:PET (phylogenetic)	

Table S3. Summary information for site-level models quantifying the strength of functional and phylogenetic repulsion in species co-occurrence patterns. We fit PGLMMs for two independent sets of forest sites (Subset 1 and Subset 2 respectively) and compared models with and without covariance matrices specifying site-level functional and phylogenetic repulsion. The table includes standard deviation estimates quantifying the amount of residual variation attributed to functional and phylogenetic repulsion as well as the difference in DIC (Δ DIC) between PGLMMs with and without each repulsion covariance matrix. A Δ DIC greater than 5 indicates that including the covariance matrix substantially improved models of species distributions and co-occurrence patterns.

Model term	Subset 1		Subset 2	
	Standard Deviation	ΔDIC	Standard Deviation	ΔDIC
Functional repulsion	0.012	1.56	0.012	1.45
Phylogenetic repulsion	0.019	1.35	0.014	0.53

Table S4. Phylogenetic signal in functional traits

Trait	Pagel's λ	P-value	Blomberg's K	P-value
Leaf height	0.520	0.013	0.054	0.016
SLA	0.666	0.003	0.051	0.061
Leaf C:N	0.824	<0.001	0.094	0.002
Seed mass	0.982	<0.001	0.953	0.001

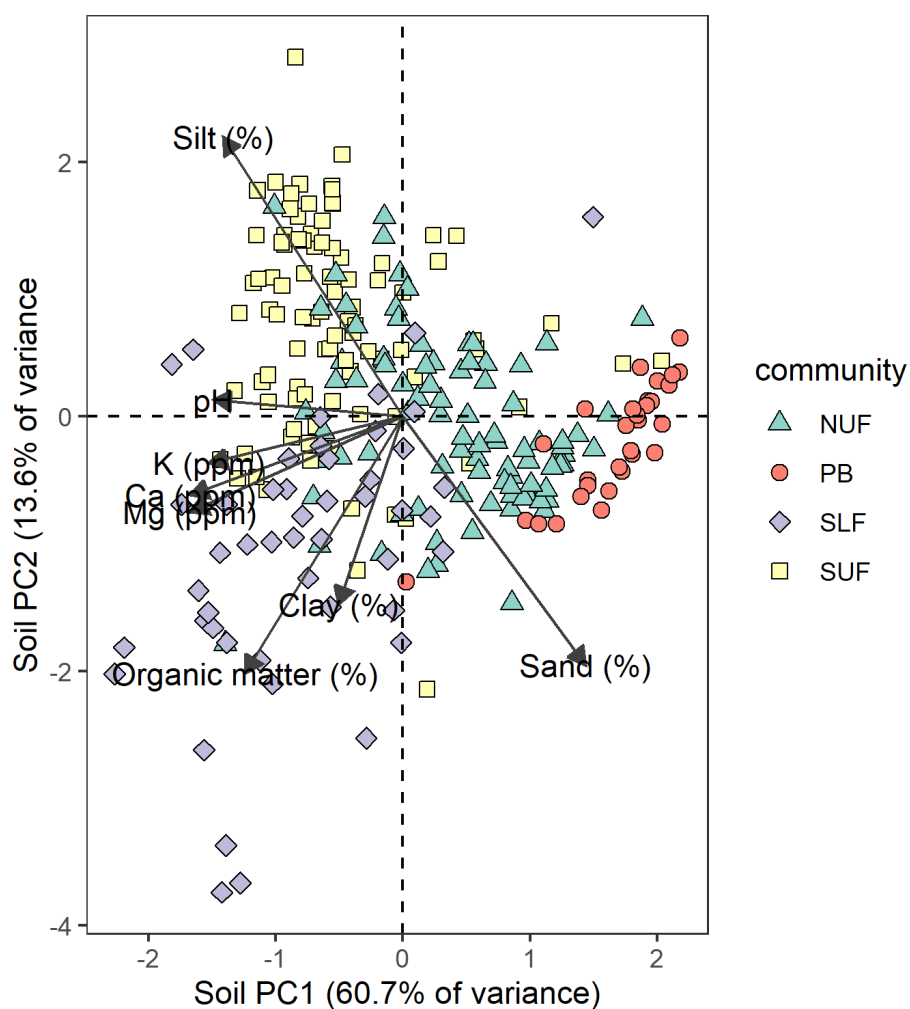


Figure S1. PCA of environmental variables results of principal components analysis illustrating variation in soil characteristics among study sites and community types. We log-transformed soil organic matter content and cation concentrations to improve the linearity of relationships to soil variables. The first principal component (PC1) of the soil PCA accounts for 60.7% of the variance in soil characteristics – primarily the gradient from sandy, nutrient-poor sites to more fertile soils with more silt and clay. The second principal component (PC2) accounts for 13.6% of the variation and is strongly associated with differences in soil organic matter and clay and silt content.

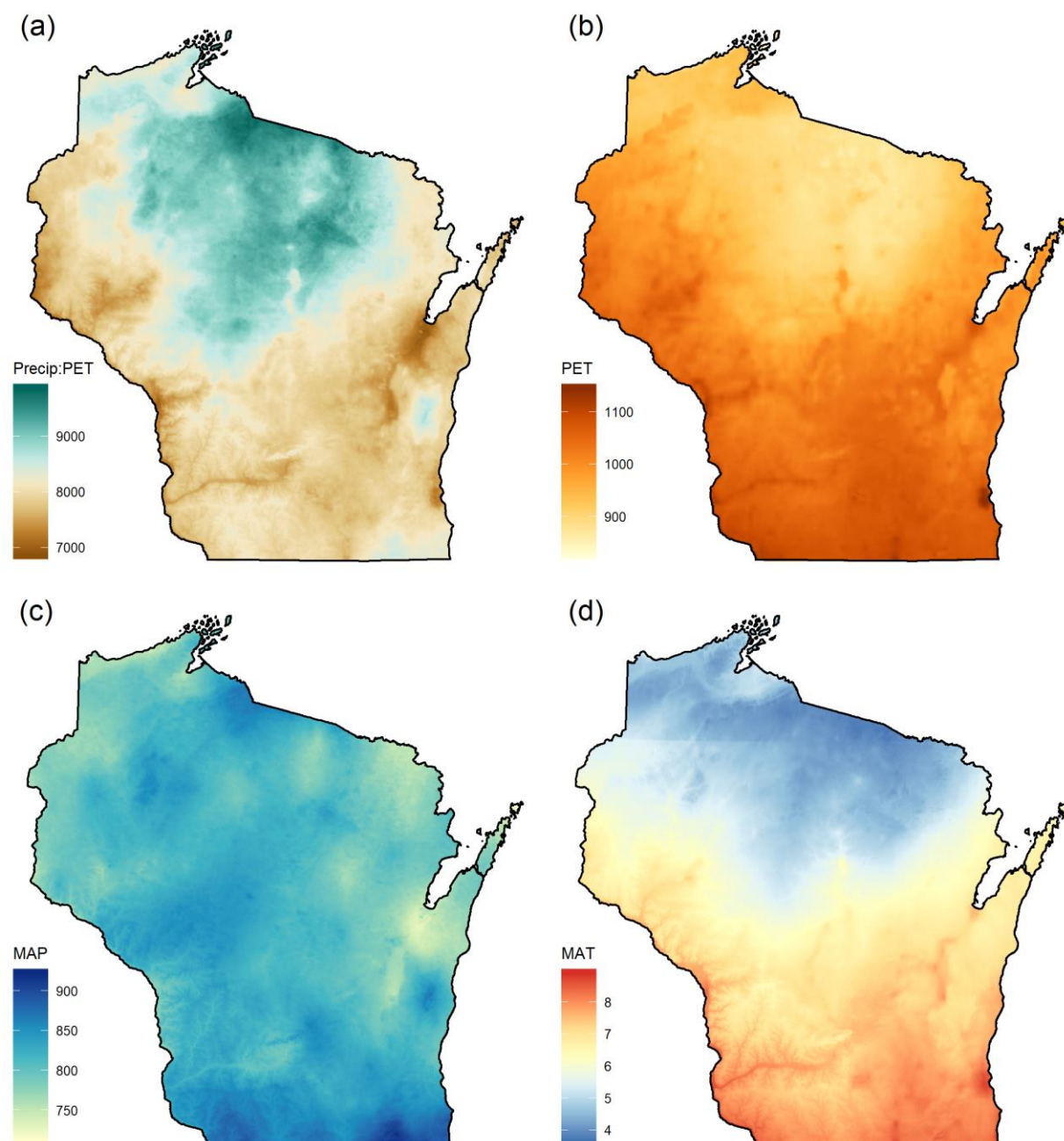
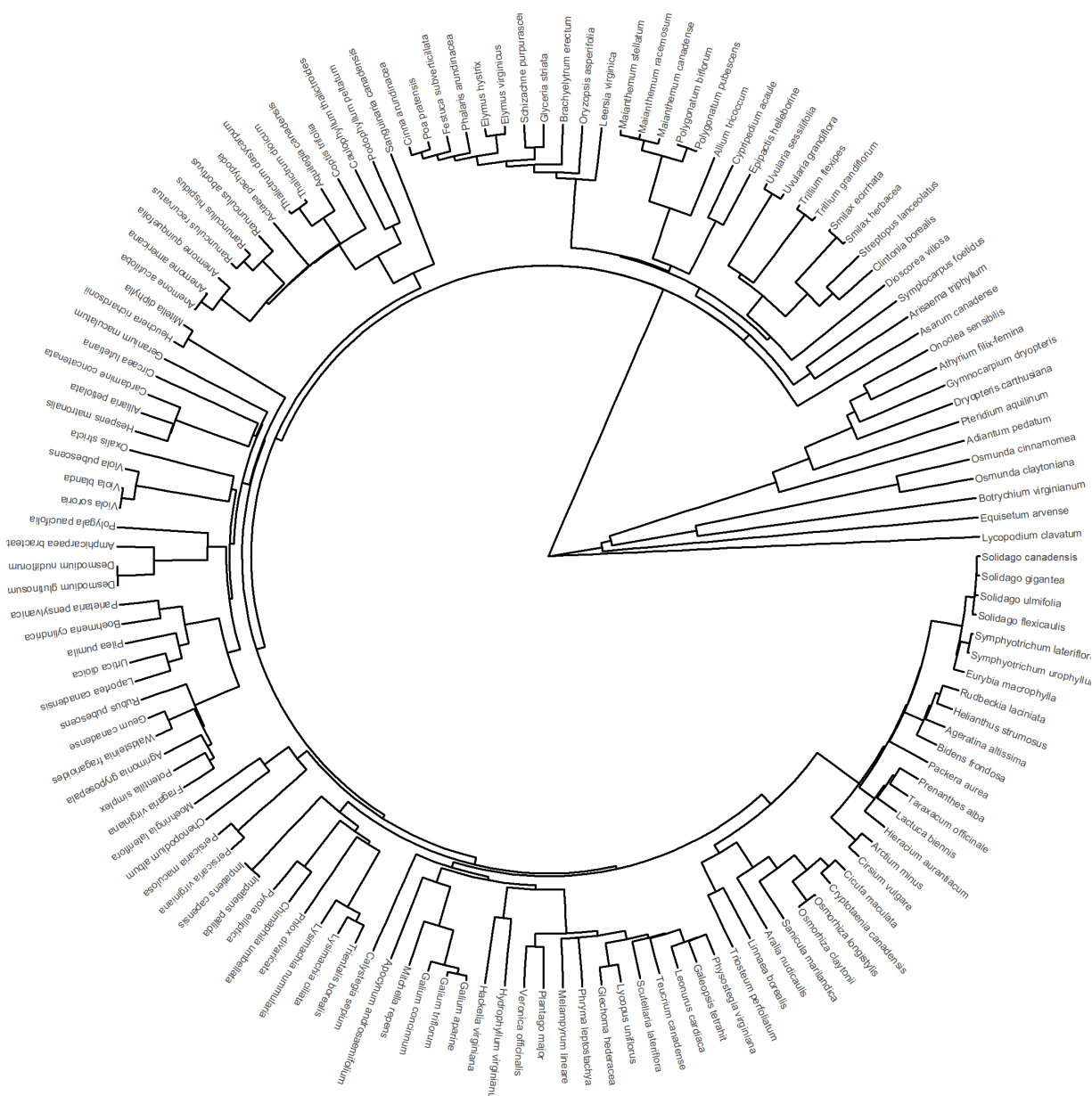


Figure S2. Variation in climate variables across the study area (Wisconsin, USA) including: (a) Precip:PET – ratio of mean annual precipitation to potential evapotranspiration, (b) PET – potential evapotranspiration, (c) MAP – mean annual precipitation (in mm), and (d) MAT – mean annual temperature (°C). These data are derived from 1 km resolution WORLDCLIM climate data from 1970 – 2000.



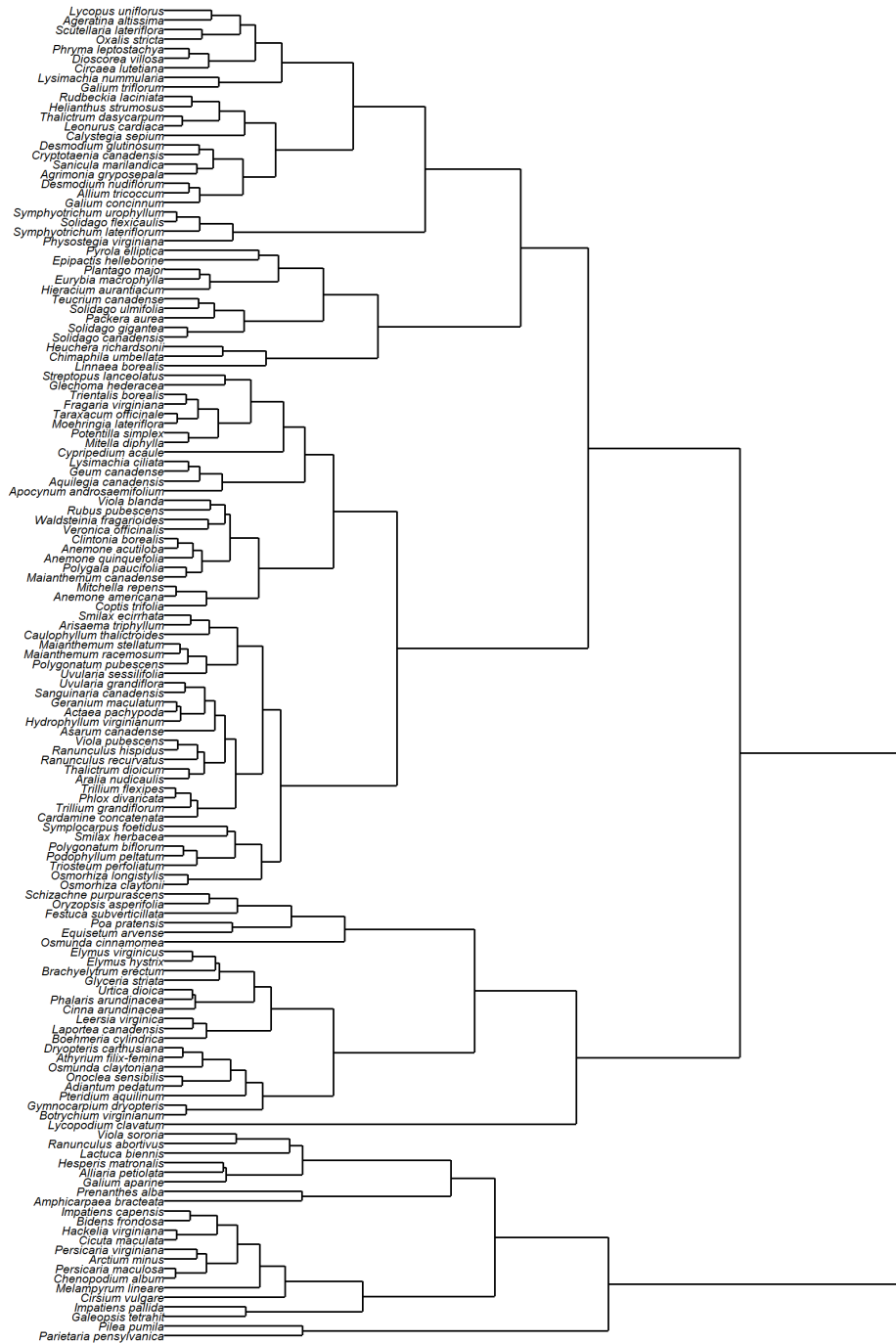


Figure S4. Functional dendrogram illustrating patterns of trait similarity among the 139 focal herb species. The dendrogram was produced using hierarchical clustering algorithm and trait values for leaf height (log-transformed), SLA, leaf C:N, seed mass (log-transformed), species longevity (short-lived versus long-lived), pollination syndrome (biotic versus abiotic), and bloom season (spring, summer, or fall).

Chapter 2. Coexistence revisited: Can we integrate demographic, physiological, and distributional data to understand patterns of co-occurrence among close relatives?

Jared J. Beck¹, Amanda Salvi¹, Richard Henderson² and Donald M. Waller¹

¹Department of Botany, University of Wisconsin-Madison, 430 Lincoln Drive Madison, WI, 53706, USA

²Wisconsin Department of Natural Resources, Office of Applied Science, Science Operations Center,
2801 Progress Road, Madison, WI 53715, USA

ABSTRACT:

Closely related species often share physiological and morphological characteristics reflecting their shared evolutionary history. These similarities suggest that related species should co-occur in similar environments, but classical niche models predict that ecologically similar species that compete strongly will tend to competitively exclude each other. To help resolve continuing uncertainty over the ecological processes driving community assembly and species coexistence, we investigated the demography, comparative physiology, and spatial distributions of two closely related and commonly co-occurring forest herbs, *Hylodesmum glutinosum* and *H. nudiflorum*. These species differ in leaf height, leaf chemistry, and water regulation. The ability of *H. glutinosum* to hold its leaves higher and regulate its water balance more tightly contribute to its tolerance of a broader set of ecological conditions and ability to compete in open habitats with more light. Surprisingly, the observed distribution of *H. nudiflorum* fell entirely within *H. glutinosum*'s ecological distribution with both species co-occurring in shady woodlands where *H. glutinosum*'s competitive advantage declines. This suggests equalizing processes that minimize differences in competitive ability between species are responsible for widespread co-occurrence of *Hylodesmum* species. *Hylodesmum nudiflorum*, however, allocates proportionally more to reproduction and less to growth and defense than its congener which results in greater rates of local recruitment and mortality. Given these demographic differences and the lack of spatial niche segregation, we infer that life-history tradeoffs (differences in the 'regeneration niche') could stabilize local coexistence. Such equalizing processes and demographic trade-offs may commonly facilitate local coexistence among plant species within temperate forest and other plant communities.

KEYWORDS: *Hylodesmum*, *Desmodium*, coexistence, equalizing, demography, light response curves, leaf height, temperate forest understory.

INTRODUCTION

Coexistence among closely related species presents an ecological dilemma. Close relatives often share a suite of traits adapted to particular environmental conditions (Cavender-Bares et al. 2009, Wiens et al. 2010). If environmental conditions act to filter out species poorly adapted to compete within a local environment, we expect species with similar morphological and physiological traits to co-occur within suitable habitats (Mayfield and Levine 2010, Kunstler et al. 2012, Kraft et al. 2014). Yet, species exhibiting too much ecological similarity are expected to compete more strongly with one another leading to competitive exclusion (Gause 1932, MacArthur and Levins 1967, Chesson 2000, Mayfield and Levine 2010). Conventional niche theory holds that closely related species must either exhibit sufficient niche differences to allow local coexistence ('limiting similarity') or segregate spatially as a result of competition or divergent environmental preferences (Hutchinson 1961, MacArthur and Levins 1967, Mayfield and Levine 2010, HilleRisLambers et al. 2012, Godoy et al. 2014, Kraft et al. 2015b). These conflicting predictions regarding co-occurrence among close relatives reflect broader uncertainty about the processes responsible for structuring ecological communities, including how niche conservatism and ecological diversification shape ecological communities, as well as the spatial scales at which those processes operate (Mayfield and Levine 2010, HilleRisLambers et al. 2012, Hart et al. 2017).

Efforts to reconcile the tension between niche conservatism and ecological diversification among close relatives have provided insights into the ecological processes responsible for species coexistence (MacArthur 1958, Hutchinson 1961, Cavender-Bares et al. 2009, HilleRisLambers et al. 2012, Godoy et al. 2014, Weber and Strauss 2016). Many previous studies focus on species-rich clades that have undergone adaptive radiations including the North American oaks (Cavender-Bares et al. 2004, Cavender-Bares 2018, Cavender-Bares et al. 2018), sedges in the genus *Carex* (Bell et al. 2000, Vellend et al. 2000), and Hawaiian lobeliads (Givnish et al. 2004, 2009, Montgomery and Givnish 2008, Givnish and Montgomery 2014). These studies confirm that many physiological and morphological characteristics are evolutionarily conserved within clades. However, close relatives often differ in key niche-structuring traits in ways that allow them to segregate spatially, partitioning habitats at scales of meters to many

kilometers. Spatial resource partitioning resulting from niche diversification appears to be a common mechanism to facilitate coexistence among close relatives within species-rich clades. However, other ecological processes can also promote species coexistence (Shmida and Ellner 1984, Tilman 1994, Chesson 2000, 2018, Weber and Strauss 2016). For example, competition-colonization tradeoffs (Levins and Culver 1971, Horn and MacArthur 1972, Platt 1985, Levine and Rees 2002, Turnbull et al. 2004), multitrophic interactions (Caswell 1978, Bradshaw and Holzapfel 1983), density-dependent effects of pathogens or predators (Connell 1971, Janzen 1971, Mordecai 2011, Bever et al. 2015), and ecologically neutral dynamics driven by dispersal and drift (Hubbell 2001) can all promote coexistence. The extent to which these other processes influence patterns of coexistence among close relatives remains unclear.

Examining the ecological processes structuring species distributions and the spatial scales at which those processes operate is especially important for developing an improved understanding of coexistence and community assembly in species-rich communities. For example, herbaceous plants constitute approximately 80 percent of the plant species found in temperate forests (Gilliam 2007). In these forests, 50-100 herbaceous species often coexist within an area of 1 ha, and 10-20 species may co-occur within 1m² (Rogers et al. 2008, Peet et al. 2014). Empirical studies suggest environmental heterogeneity and spatial resource partitioning play an important role in maintaining herb diversity and structuring plant distributions (Bell et al. 2000, Beatty 2014, Catella et al. 2019). Understory plants respond sensitively to environmental variation at multiple spatial scales (Beck and Givnish, *in review*, Whittaker 1956, Curtis 1959, Struik and Curtis 1962, Bratton 1976, Beatty 1984, Gilbert and Lechowicz 2004, Burton et al. 2011, Peet et al. 2014). At broad scales, the distribution and abundance of herbs vary along environmental gradients of understory light availability and edaphic conditions (Curtis 1959, Amatangelo et al. 2014). At finer spatial scales, environmental heterogeneity caused by microtopographic variation (Beatty 1984, Peterson et al. 1990), canopy gaps (Thompson 1980), and variation in soil depth (Beck and Givnish, *in review*, Bratton 1976) can influence the local distribution of herbaceous species and promote coexistence within forest stands. Nevertheless, local coexistence among species with similar responses to environmental variation suggests ecological processes unrelated to environmental

heterogeneity also influence local community dynamics. Targeted studies examining the comparative ecology of closely related species could reveal important insights into plant coexistence and community assembly.

To gain insights into the ecological processes structuring understory plant communities in temperate forests, we investigated the comparative physiology, distributions, and demography of two congeneric forest herbs: *Hylodesmum glutinosum* and *H. nudiflorum* (Fabaceae). These sister taxa are widely distributed across temperate forests in eastern North America and exhibit broadly overlapping ranges. *Hylodesmum glutinosum* and *H. nudiflorum* co-occur regularly in oak woodlands. In a previous study, Smith (1975) found *H. glutinosum* to be a superior competitor by showing that seedlings of both species experienced increased mortality near *H. glutinosum* adults in a reciprocal seedling transplant experiment. These competitive differences plus widespread co-occurrences of *Hylodesmum* congeners thus provide a useful system for studying the ecology and coexistence of closely related species. Here, we integrate data on the comparative morphology and physiology of co-occurring *Hylodesmum* individuals, their distributions at several spatial scales, and local population dynamics to investigate the mechanisms whereby these herbaceous plant species coexist.

METHODS

Study species

Our study focused two legume species (*Hylodesmum glutinosum* L. and *H. nudiflorum*) distributed widely across North America. Recent molecular phylogenies confirm that *H. glutinosum* and *H. nudiflorum* are sister taxa (Stefanović et al. 2010, Li et al. 2019). Both species range from eastern North America to west of the Mississippi River. *Hylodesmum nudiflorum* reaches its western range limit just west of the Mississippi River while the range of *H. glutinosum* extends further west into the great plains. *Hylodesmum glutinosum* can be found across a broad range of habitats including wooded edges, stream banks, semi-open microsites in woodlands and savannas, and disturbed areas to closed canopy oak forests. *Hylodesmum nudiflorum* is more narrowly distributed in forests and woodlands. Across their

broadly overlapping ranges, *H. glutinosum* and *H. nudiflorum* co-occur regularly within oak woodlands and oak-hickory forests (Smith 1975, Voss and Reznicek 2012).

The congeners exhibit many ecological and morphological similarities but *Hylodesmum nudiflorum* tends to be smaller and more delicate. Both species produce a whorl of trifoliate leaves, and above this, *H. glutinosum* produces a flowering stalk that extends the main stem. In contrast, *H. nudiflorum*'s inflorescence forks out laterally from the main stem at or below ground-level producing a distinct flowering stalk typically lacking leaves (Voss and Reznicek 2012). Both species produce similar numbers of seeds and depend primarily on seedlings for recruitment (Smith 1975).

Study sites

We studied 94 upland forest stands in southern Wisconsin, USA first surveyed by J.T. Curtis and his students in the late 1940's and early 1950s (Curtis, 1959) then resurveyed in detail 50-60 years later (Rogers et al., 2008; Fig. 1). We also surveyed the Olson Oak Woods State Natural Area (Dane County, WI) in more detail in order to characterize the micro-distribution of *Hylodesmum* species along environmental gradients and assess co-occurrence patterns (see below). Our studies of the comparative physiology and fine-scale spatial distribution of *Hylodesmum* occurred at three forest stands in the Baraboo Hills (Fig. 1), the largest contiguous forest ecosystem in southern Wisconsin. The region supports more than 1300 plant species and is considered a regional conservation priority (Lange 1998). Across the Baraboo Hills, both *Hylodesmum* species are commonly in oak woodlands and co-occur regularly, often in forest stands situated on ridge tops or south-facing slopes with *Quercus alba* as the dominant canopy species (*Beck, personal observation*). Our comparative physiological work and fine-scale mapping focused on three locations in Sauk County, WI: Hemlock Draw State Natural Area (SNA), Natural Bridge and Rockshelter SNA, and a parcel owned by the University of Wisconsin-Madison Botany Department (Botany Farm - Fig. 1). These sites were separated by 2-3 km and differ in edaphic characteristics, but none were glaciated during the last Wisconsin glaciation.

Plants studied at the Botany Farm were distributed along a ridgetop that sloped gently to the south. A steep ravine was located ~25 m northeast. Soil at this site is classified as well-drained silt loams and bedrock consists of Baraboo quartzite conglomerate. Canopy trees consisted mainly of *Quercus alba* and *Q. rubra* with some *Acer saccharum* and *A. rubrum*. The sub-canopy largely consisted of *A. saccharum* and with some *Hammamelis virginiana*. Plants studied at Hemlock Draw SNA were distributed on a moderate southwest slope. Soils consisted of moderately well-drained silt loam over quartzite bedrock. Canopy trees were almost all *Q. alba* with several *Q. rubra* and *A. rubrum*. The subcanopy included *A. rubrum* and *H. virginiana*. Plants at the Natural Bridge and Rockshelter SNA site were distributed on south-facing ridge that sloped gently to the south over sandstone bedrock. Soils here are classified as excessively well-drained sandy loams. Canopy trees included mostly *Q. alba* with some *A. rubrum*. *Acer rubrum* was abundant in the sub-canopy.

Comparative physiology

To investigate the comparative physiology of co-occurring *Hylodesmum* congeners, we haphazardly selected 24 individuals of each species across the three focal sites (8 plants per species per site). When choosing focal plants, we selected congeneric pairs close to one another (within 5 m) to minimize the potential effects of environmental heterogeneity on physiological traits. We then recorded the leaf span (maximum horizontal distance spanned by the leaf canopy), leaf height (vertical distance between ground and whorl of leaves), and reproductive status of each individual (flowering versus vegetative) before quantifying its photosynthetic light-response curves using a portable gas exchange system (LI-6400, Li-Cor, Inc., Lincoln, NE, USA). We made all measurements between 0900 and 1500 h on the youngest fully developed leaf, and the largest of the three leaflets. We maintained leaf temperature, relative humidity, and cuvette CO₂ concentration at near ambient conditions (23.8 ± 1.5 °C, $59 \pm 7.7\%$ relative humidity, and 400 ppm, respectively). We measured net assimilation (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) at 11 different light levels ($\mu\text{mol m}^{-2} \text{s}^{-1}$): 1500, 1250, 1000, 800, 600, 400, 200, 100, 50, 20, 0. Before starting these measurements, the leaf was acclimated in the chamber for 10-15 min to achieve stable gas

exchange. We also allowed the chamber to equilibrate for at least 3 minutes before measurements at each light level. After completing the light-response curve, the measured leaf was collected, scanned and measured for leaf area, dried at 75°C for 24 h, and weighed for dry mass. Leaf area and dry mass was used to calculate SLA (cm g^{-1}), which was then used to convert area-based photosynthetic rates ($\mu\text{mol m}^{-2} \text{s}^{-1}$) to mass-based photosynthetic rates ($\mu\text{mol g}^{-1} \text{s}^{-1}$). We fit three-parameter Michaelis-Menten models to the observed photosynthetic rates of *Hylodesmum* individuals (Givnish et al. 2004):

$$A_i = \frac{A_{\max} * I}{I + k} - R$$

Where A_{\max} is maximum assimilation rate, I is the observed light intensity, k is light intensity required to half-saturate assimilation, and R is the rate of dark respiration. After weighing dried leaf samples, leaves were ground individually into a fine homogenous powder and sent to the central Appalachians Stable Isotope Facility to measure percent leaf nitrogen, the ratio of carbon to nitrogen, and $\delta^{13}\text{C}$. During photosynthesis, enzymes discriminate against the heavier C isotope, so plants that have smaller rates of gas exchange and thereby have smaller concentrations of CO_2 inside leaf mesophyll assimilate more ^{13}C into their tissue. Thus greater concentrations of $\delta^{13}\text{C}$ are associated with more stringent water regulation (O’Leary 1988, Farquhar et al. 1989). We used t-tests to test for differences in maximum photosynthetic rates (A_{\max}), the light intensity required to half-saturate photosynthesis (k), respirations rates (R), leaf C, leaf N, leaf carbon-to-nitrogen ratios (C:N), and $\delta^{13}\text{C}$.

Broad-scale plant distributions along environmental gradients

To assess broad-scale species distributions, we characterized the distributions of both *Hylodesmum* species along environmental gradients among the 94 upland forest stands surveyed in southern Wisconsin. Originally surveyed during the 1950s (Curtis 1959), these sites were relocated and resurveyed between 2002-2004 (Rogers et al. 2008, Waller et al. 2012). Here, we focus on analyzing modern *Hylodesmum* distributions as limited sampling in the 1950s handicaps our ability to robustly infer distributions for relatively uncommon species like *H. nudiflorum* (Beck et al. 2018). Within each stand,

we scored the presence/absence of all vascular plant species in 80 1x1m quadrats spaced 10-15 m apart (see Rogers et al. 2008 and Waller et al. 2012 for more details). We also characterized contemporary environmental (edaphic) conditions at each site by sampling soils for analyses of their texture (percent sand, silt, and clay) and chemical characteristics (pH, % organic matter by mass, total nitrogen, calcium, potassium, and magnesium). We performed principal components analysis (PCA) to examine covariance among soil characteristics. The first PCA axis (soil PC1) accounted for 50.5% of the total variation, primarily by reflecting differences in soil fertility. The second PCA axis (PC2) accounted for an additional 20.3% of the total variation and corresponded largely to differences in soil texture (Fig. S1). To model plant distributions, we also included soil Calcium and soil silt content as predictors to aid in biological interpretation. Substituting site scores for PC1 and PC2 yielded qualitatively similar inferences. Given the large number of zeros in our data set (species absent from a site) and high observed variance (greater than binomial), we modeled *Hylodesmum* distributions using a zero-inflated beta-binomial regression model. This model included species identity (*H. glutinosum* or *H. nudiflorum*), soil Calcium concentration (reflecting soil fertility), and soil silt content (reflecting texture) to predict *Hylodesmum* abundance (proportion of quadrats occupied). We also included the interaction between all environmental predictors and species identity. These environment x species interaction terms reflect any differential responses between the two species to environmental conditions. Environmental variables were centered and scaled to unit variance to facilitate model fitting and comparisons. We included site as a random effect. We inspected raw data and model residuals for evidence of non-linearity but found no evidence of non-monotonic relationships.

Local environmental distributions

To examine how *Hylodesmum* was distributed at a finer scale, we surveyed the Olson Oak Woods State Natural Area between 1997 and 1998 (Dane County, WI, USA). We scored the presence/absence and cover of all vascular plant species across 2360 0.5 x 1.0 m quadrats spaced 15 – 20 m apart. This topographically and edaphically heterogeneous site encompasses 78 hectares and supports an oak forest dominated by White Oak (*Quercus alba*) and Black Oak (*Quercus velutina*). Internal valleys with richer

soils also support *Q. rubra*, *A. saccharum*, *O. virginiana*, *Ulmus spp.*, and other more mesic tree species. In contrast, the exposed ridgetops over sandstone support oak savanna or prairie vegetation.

To characterize how *Hylodesmum* distributions varied with competitive context, we calculated community-weighted mean leaf height for plants species occupying each quadrat:

$$CWM(leaf\ height)_i = \sum_{j=1}^{S_i} w_{ij} * h_j$$

Where w_{ij} is the weight (relative cover) of species j in quadrat i and h_j is the average leaf height of species j . This index of community leaf height reflects variation in the competitive environment caused by light availability, an important limiting resource for understory plants in temperate forests and woodlands (Thompson 1980, Givnish 1982, 1987, 1995, Whigham 2004). In sunlit microsites, competition for light favors woody shrubs and herbaceous plants with greater leaf height (Givnish 1982). We fit a logistic regression model to predict the probability of occurrence of both *Hylodesmum* species within individual quadrats. Predictor variables in this model included species identity, CWM(leaf height), CWM(leaf height)², and the interaction of species and each environmental covariate. We included the quadratic term to account for unimodal relationships between community leaf height and the probability of occurrence. Significant interactions between species identity and other predictors indicate differential responses to environmental conditions between *Hylodesmum* congeners.

Fine-scale spatial associations

During August 2018 and 2019, we established mapping plots at the same three focal sites where comparative physiological data were collected. Plot locations were chosen to be representative of the surrounding forest structure and vegetation, avoid recent disturbances, and include sufficient numbers of both *H. glutinosum* and *H. nudiflorum* individuals. The Botany Farm plot was 25x25m and mapped in both 2018 and 2019. The Hemlock Draw and Natural Bridge plots were 20x20m as they supported substantially higher plant densities. These plots were mapped only in 2019 (Table S4; Fig. S3). Within mapping plots, we flagged all *Hylodesmum* individuals and established a grid system to obtain precise

spatial coordinates (~10 cm precision). We then mapped all *Hylodesmum* individuals recording the species identity, reproductive status, leaf height, and leaf span. We binned all plants into two size classes based on their leaf span and the observed size distribution of reproductive plants. The threshold for adults (potentially reproductive individuals) was defined by the minimum leaf span observed for flowering plants of a given species (Fig. S2; 18 cm for *H. glutinosum* versus 12 cm for *H. nudiflorum*). All individuals falling below this leaf span threshold were classified as juveniles.

We used pair correlation functions (PCFs) to describe spatial associations within and between species. PCFs quantify the probability of observing a pair of spatial events (plant occurrences) at a particular distance divided by the probability of observing a pair of events at that distance if events are distributed completely at random with respect to one another, $g(r)$ (Wiegand and Moloney 2004). For all analyses, we used inhomogeneous pair correlation functions, $g_{inhom}(r)$, which allow the density of individuals to vary in space independent of proximity to events via a non-stationary Poisson process (Wiegand and Moloney 2004, 2014). For each PCF, we calculated a simulation envelope (based on 499 iterations) for expected $g_{inhom}(r)$ values under complete spatial randomness. Observed $g_{inhom}(r)$ values that fall outside the simulation envelope represent statistically significant deviations from spatial randomness (Wiegand and Moloney 2014). To summarize spatial associations across mapping plots, we calculated a weighted average of the estimated PCFs to obtain a pooled $g_{inhom}(r)$ function:

$$\bar{g}_{inhom}(r) = \frac{1}{\sum_{i=1}^m w_i} \sum_{i=1}^m w_i \cdot g_{inhom}(r)_i$$

Where w_i is the relative weight for mapping plot i and $g_{inhom}(r)_i$ is the estimated PCF for mapping plot i . For univariate PCFs, we calculated weights for each function as: $w_i = n_{x,i}^2 / A_i^{J-1}$. Where $n_{x,i}$ is the number of points for replicate function i and A_i is the area encompassed by function i . For bivariate PCFs, we calculated weighted averages as: $w_i = n_{x,i} \cdot n_{y,i} / A_i^{J-1}$. Where $n_{x,i}$ is the number of points for replicate function i and point type x , and $n_{y,i}$ is the number of points for replicate function i and point type y (Bagchi and Illian 2015).

Demographic turnover

We estimated annual rates of demographic turnover for each species using mapped plant data from 2018 and 2019 at the Botany Farm. Plot corners were permanently marked so mapping data from 2018 and 2019 utilize the same reference points to establish the coordinate system. We employed the same high-precision mapping techniques in both years. Although individual plants were not permanently marked, we relied on data about plant size, pairwise distances, and spatial maps to infer the identity of individual plants. We followed several rules to infer the identity of individual plants: for a pair of plants mapped in different years to be considered the same individual, their map locations had to be within 25cm, they needed to display similar leaf spans (relative to neighboring plants), and plants had to retain a similar spatial arrangement relative to neighboring plants. These rules may underestimate recruitment rates (given that we conservatively assume which plants are the same individual), but should provide reasonable estimates of mortality. As we are most interested in comparing rates of demographic turnover between *Hylodesmum* species, these inferred rates of mortality and recruitment are suitable. In this study, we compare the relative number of deaths (individuals present in 2018 but absent in 2019), new recruits (individuals absent in 2018 but present in 2019), and persisting plants (individuals present in both 2018 and 2019) between species using a Chi-square test of independence.

All analyses were performed using R 3.5.1 (R Core Team 2019). The zero-inflated beta-binomial regression models were fit using the *glmmTMB* function in the ‘glmmTMB’ package (Brooks et al. 2017). Univariate PCFs describing intraspecific spatial associations without regard to age class were performed using the *pcfinhom* function and bivariate PCFs describing interspecific spatial associations and associations between species or age classes were performed using the *pcfcross.inhom* function within the ‘spatstat’ package (Baddeley 2005). The package ‘ggplot2’ was used for generating figures (Wickham 2009).

RESULTS

Comparative physiology

Our comparative analyses revealed several morphological and physiological differences between co-occurring *H. glutinosum* and *H. nudiflorum* (Fig. 2; Table S1). The most conspicuous differences between species relate to size. *Hylodesmum glutinosum* had consistently greater leaf height than *H. nudiflorum* (Fig. 2b, c), even after controlling for differences in leaf span (Fig. 2b; ANCOVA: $F_{1,2362} = 248$, $P < 0.001$). The difference in height between *H. glutinosum* and *H. nudiflorum* increased as the leaf span of plants grew larger (Fig. 2b; $F_{1,2362} = 72$, $P < 0.001$). Among reproductive plants (Fig. 2b), *H. glutinosum* was on average 13 cm taller (28 versus 15 cm) than *H. nudiflorum* (t-test: $t = 13.7$, $df = 69$, $P < 0.001$). There was no difference between species in specific leaf area (Fig. 2d; $t = 0.63$, $df = 38$, $P = 0.531$).

The congeners also differed in leaf chemistry suggesting a difference in physiology. The concentration of $\delta^{13}\text{C}$ was 5.4% greater in *H. glutinosum* ($t = 6.66$, $df = 46$, $P < 0.001$). Meanwhile, *H. nudiflorum* exhibited greater leaf C ($t = 3.11$, $df = 44.7$, $P = 0.003$) and leaf N (Fig. 2h; $t = 2.8$, $df = 43.4$, $P = 0.007$). Despite increased C and N concentrations in *H. nudiflorum*, leaf C:N ratios were 6.2% greater in *H. glutinosum* (Fig. 2g; $t = 2.05$, $df = 45.9$, $P = 0.046$). Plant photosynthetic responses to light availability were nearly identical (Fig. 2f). *Hylodesmum nudiflorum* exhibited slightly (but not significantly) greater rates of maximum photosynthesis than *H. glutinosum* (3.50 versus 3.13 $\mu\text{mol g}^{-1} \text{s}^{-1}$, $t = 1.36$, $df = 35.3$, $P = 0.18$). Similarly, we found no differences in the amount of light required to half-saturate photosynthesis ($t = 1.32$, $df = 34.7$, $P = 0.196$) or respiration rates ($t = 0.19$, $df = 43$, $P = 0.849$).

Plant distributions along environmental gradients and co-occurrence patterns

Our broad-scale analysis of *Hylodesmum* distributions reveals that *H. glutinosum* is more abundant and occupied a broader set of sites (56 sites vs. just 17 for *H. nudiflorum*) and range of ecological conditions than *H. nudiflorum*. *Hylodesmum nudiflorum* occurred in 29 percent of the sites occupied by *H. glutinosum* while *H. glutinosum* was found in nearly all (94 percent) of the sites occupied by *H. nudiflorum*. Both *Hylodesmum* species declined in local abundance as soil Ca increased, but the abundance of *H. nudiflorum* declined more rapidly (Table S2; Figure 3a; Species x Soil Ca interaction: χ^2

= 9.67, $P = 0.002$). These congeners differed in their response to soil fertility (Figure 3b). *Hylodesmum glutinosum* abundance declined slightly as soil silt content increased (and sand content decreased) while *H. nudiflorum* abundance tended to increase as soil silt content increased (Species x Soil Silt interaction: $\chi^2 = 5.48$, $P = 0.019$).

Distributions of *Hylodesmum* within Olson Oak Woods State Natural Area reveal that both *Hylodesmum* species respond similarly to local competitive environments. In general, *H. glutinosum* was distributed more broadly, occurring in 379 quadrats versus 73 for *H. nudiflorum* (and co-occurring in 25). Thus, although *H. nudiflorum* occurred in just 6.6 percent of the quadrats with *H. glutinosum*, *H. glutinosum* was found in 34.2 percent of the quadrats with *H. nudiflorum*. Both species occurred most often in quadrats occupied by plant species with intermediate leaf heights resulting in a unimodal relationship between abundance and community-weighted leaf height (Table S3; Fig. 4). However, communities where *H. glutinosum* reached its peak probability of occurrence had slightly greater leaf heights (41.5 cm) than *H. nudiflorum* (37.5 cm).

Fine-scale spatial associations

At fine scales, *Hylodesmum* species show contagious patterns of distribution in that conspecific individuals co-occurred within 1m more often than expected if individuals were distributed at random (Fig. 5a, d). Conspecific individuals generally became segregated beyond 2m (disassociated relative to a random pattern). This local aggregation of conspecifics reflects spatial associations between juveniles and adults as juveniles of both *Hylodesmum* species cluster near adult conspecifics (Fig. 5c, f). This aggregation mostly occurs within 0.5m but decays at distances above 1m. *Hylodesmum glutinosum* tended to form regularly spaced clusters leading to more variable spatial associations beyond 2 m (Figs. 5c, S3). In contrast to the strong patterns of aggregation within species, pairs of plants belonging to different species are largely segregate from one another (Figs. 5b, e, S3).

Local population turnover

Annual rates of demographic turnover (2018-2019) at the Botany Farm were 18.4 percent lower in *H. glutinosum* than *H. nudiflorum* (51.1% versus 69.5%; Table 1; Fig. 6). This difference is biologically and statistically significant (Chi-square Test of Independence: $\chi^2 = 37.054$, $df = 2$, $P < 0.001$). Greater annual demographic turnover in *H. nudiflorum* was driven by higher rates of both recruitment and mortality with greater mortality in *H. nudiflorum* contributing more to this difference (31.2% in *H. glutinosum* vs. 60.3% in *H. nudiflorum*). Among plants mapped in 2019, 37.2% of *H. glutinosum* individuals were new recruits compared to 43.2% of *H. nudiflorum*.

DISCUSSION

Our goal in integrating demographic, physiological, and distributional data for two co-occurring forest herbs – *H. glutinosum* and *H. nudiflorum* – was to better understand the ecological processes influencing the distribution and coexistence of these temperate forest herbs and to characterize the spatial scales at which these processes act. We uncovered subtle morphological and physiological differences between *Hylodesmum* congeners that help shape their distributional differences. *Hylodesmum glutinosum* was consistently a taller, more robust plant than *H. nudiflorum*. It appears to be more conservative in how it uses water (showing greater $\delta^{13}\text{C}$) and to invest more in structure and defense (greater leaf C:N). These traits contribute to its ability to occupy a broad range of ecological conditions including habitats with increased light availability. Sunlit microsites within temperate forests favor taller herbaceous plants that are better equipped to compete for light among higher densities of competitors (Givnish 1982). In contrast, *H. nudiflorum*'s comparatively small stature, greater nutrient requirements, and less stringent water use may restrict its growth to shadier microsites and more fertile soils that hold more water. Thus, taller *H. glutinosum* should outcompete *H. nudiflorum* in sun-lit patches where it can compete more effectively against other tall plants. These inferences concur with results from a previous study investigating competition between *H. glutinosum* and *H. nudiflorum* that demonstrated *H. glutinosum* to be a superior competitor (Smith 1975). Although Smith (1975) surmised that *H. glutinosum* should

eventually displace *H. nudiflorum*, the widespread co-occurrence of *Hylodesmum* species across the landscape suggests the operation of some mechanism(s) that allow these species to coexist.

Although more complex dynamics driven by intransitive competition or indirect interactions could also play a role (Gallien et al. 2017, Levine et al. 2017), our data point to three potential mechanisms that could foster local coexistence between these two species of *Hylodesmum*. First, equalizing processes often play an important role in favoring local patterns of coexistence (Chesson 2000, Mayfield and Levine 2010, Chase 2014). *Hylodesmum glutinosum* and *H. nudiflorum* often co-occur in habitats where environmental conditions limit *H. glutinosum*'s competitive advantage. While we have demonstrated that these *Hylodesmum* species are not ecologically or demographically equivalent (as assumed by neutral theory - Hubbell, 2001), their difference in competitive ability is reduced in shady microsites where reduced plant coverage diminishes the competitive advantage associated with increased leaf height (Givnish 1982). This in combination with reduced water stress should slow the rate at which competitive exclusion can occur. Under such conditions, local community dynamics will be more strongly influenced by dispersal and demographic processes. These equalizing processes that minimize competitive differences between species could thus set the stage for other weak ecological processes to stabilize local coexistence, as discussed next (Adler et al. 2007).

Second, local dispersal from individuals of both species will automatically generate more intra- than inter-specific competition. Local dispersal of juvenile plants from conspecific adults generates fine-scale clusters and patterns of recruitment in both species of *Hylodesmum* (Figs. 5 and 6). *Hylodesmum*'s seed pods are covered in hooked hairs to cling to passing vertebrates giving them the potential to be dispersed long distances. Nevertheless, seeds rarely disperse more than 0.5m away from their maternal plant without a suitable dispersal vector. As a consequence, seedlings compete strongly with their mothers and siblings for resources. Given that *H. glutinosum* is a superior competitor and inhibits the growth and survival of seedlings to a greater extent than *H. nudiflorum* (Smith 1975), this strong spatial structure probably increases intraspecific competition more for *H. glutinosum* than for *H. nudiflorum*. This decline could be enough to slow local population growth rates within *H. glutinosum*.

Life history tradeoffs associated with longevity and fecundity can also promote local coexistence (Shmida and Ellner 1984, Amarasekare et al. 2004, Kneitel and Chase 2004). Several lines of evidence suggest that such life history tradeoffs influence local *Hyloidesmum* population dynamics. First, our analyses of demographic turnover revealed higher annual rates of recruitment and especially mortality in *H. nudiflorum* than *H. glutinosum*. Although *H. glutinosum* exhibits higher survivorship, *H. nudiflorum* appears to invest more heavily in reproduction, perhaps reducing its longevity. More data are needed to quantify such differences in reproductive allocation and fecundity as we noted no difference in annual flowering rates in our study (18.8% of *H. glutinosum* adults versus 16.3% of *H. nudiflorum* adults). However, the smaller size of *H. nudiflorum* suggests that this species may be investing proportionally more resources in reproduction. In parallel with this idea are our observations of lower leaf C:N ratios and reduced $\delta^{13}\text{C}$ in *H. nudiflorum* suggesting that this species invests fewer resources in structure, defense, and water conservation. These demographic and functional differences are consistent with a fecundity-longevity life history tradeoff between these two species great enough to prevent the superior competitor, *H. glutinosum*, from competitively excluding *H. nudiflorum* in shady habitats (Smith 1975, Shmida and Ellner 1984). Most studies of competition-colonization tradeoffs in plants focus on seed traits and dispersal (Levine and Rees 2002, Turnbull et al. 2004), but faster demographic turnover and increased reproductive allocation could increase colonization rates even when dispersal syndromes are similar.

Detailed studies of the ecology of closely related species have offered fundamental insights into community assembly processes and the factors promoting species coexistence (Macarthur 1958, Bell et al. 2000, Cavender-Bares et al. 2004, Helmus et al. 2007). In contrast to the conspicuous diversification in traits and niche partitioning observed within species-rich clades (e.g, North American *Carex* - Bell et al., 2000; Vellend et al., 2000), oaks -Cavender-Bares, 2018; Cavender-Bares et al., 2018; Cavender-Bares et al., 2004), and Hawaiian lobeliads (Givnish et al. 2004, 2009, Montgomery and Givnish 2008, Givnish and Montgomery 2014), our study uncovered a more nuanced set of factors influencing the distribution and coexistence of closely related species. The patterns and processes described here are nonetheless

relevant for understanding community assembly and species coexistence in both temperate forests and more generally.

Environmental heterogeneity and spatial niche partitioning play important roles in determining plant distributions and promoting coexistence (Amarasekare 2003, Silvertown 2004, Adler et al. 2010, HilleRisLambers et al. 2012, Beatty 2014, Stein et al. 2014). In temperate forests, plant species often segregate along environmental gradients into local communities that reflect their physiological characteristics and thus competitive prowess within a given environmental context (Givnish 1987, Amatangelo et al. 2014). This leads guilds of functionally similar species with shared habitat affinities to co-occur under environmental conditions to which they are well-adapted (Givnish 1982, 1987, 1995, Whigham 2004, HilleRisLambers et al. 2012, Amatangelo et al. 2014, Kraft et al. 2015a). Local plant distributions and community dynamics may also reflect fine-scale environmental heterogeneity (Beck and Givnish, *in review*, Struik and Curtis 1962, Bratton 1976, Thompson 1980, Beatty 1984). The degree to which such local heterogeneity can sustain spatially structured communities, however, is limited to cases where there are sufficiently strong competitive differences to prevent dispersal from scrambling local species-environment associations. In the presence of disturbance, herbivory, disease, and other forms of ecological and demographic stochasticity, local communities formed by species with subtle trait or niche differences may become nearly neutral (Chesson 2000, Hubbell 2001, Mayfield and Levine 2010). The increased importance of equalizing processes that minimize interspecific competitive differences sets the stage for weaker localized processes to operate. These include life history trade-offs like those demonstrated here (Shmida and Ellner 1984) as well as plant-soil feedbacks (Beck, *in review*, Smith and Reynolds 2015). We follow Chase (2014) here in arguing that equalizing (or neutral) processes should operate more strongly on local community dynamics. We emphasize, however, that species need not be ecologically equivalent for equalizing processes to affect species distributions and coexistence at local scales.

Examining the physiology, distributions, and demography of two *Hylodesmum* species that regularly co-occur revealed insights into the ecological processes underpinning their distributions and

potential mechanisms promoting local co-occurrence. Despite morphological and physiological differences that may influence how these species respond to environmental variation, these congeners did not segregate into different habitats. Instead, the species co-occurred in microsites where *H. glutinosum*'s competitive advantage is diminished by environmental conditions. Under these conditions, we surmise life history trade-offs and differences in their regeneration niche could stabilize local coexistence (Grubb 1977). Although further research is needed to fully investigate the role of equalizing processes, local dispersal, and life history tradeoffs in local coexistence, this study illustrates the utility of integrating physiological, distributional, and demographic data to gain insights into the ecological processes structuring species distributions and co-occurrence patterns as well as how those processes may vary spatial scale.

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Table 1. Summary of annual population turnover between 2018 and 2019 within 625 m² plot at Botany Farm. Total plant counts for each year as well as the number of individuals that persisted, the number of new recruits in 2019, and the number of plants that died between 2018 and 2019 are provided. The turnover rates was calculated as: $(\text{Recruits} + \text{Deaths})/(\text{Persisting} + \text{Recruits} + \text{Deaths})$. See Fig. 6 for a map of plant locations.

Species	Count 2018	Count 2019	Persisting	Recruits	Deaths	Turnover
<i>Hylodesmum glutinosum</i>	157	172	108	64	49	51.1%
<i>Hylodesmum nudiflorum</i>	317	224	126	96	191	69.5%

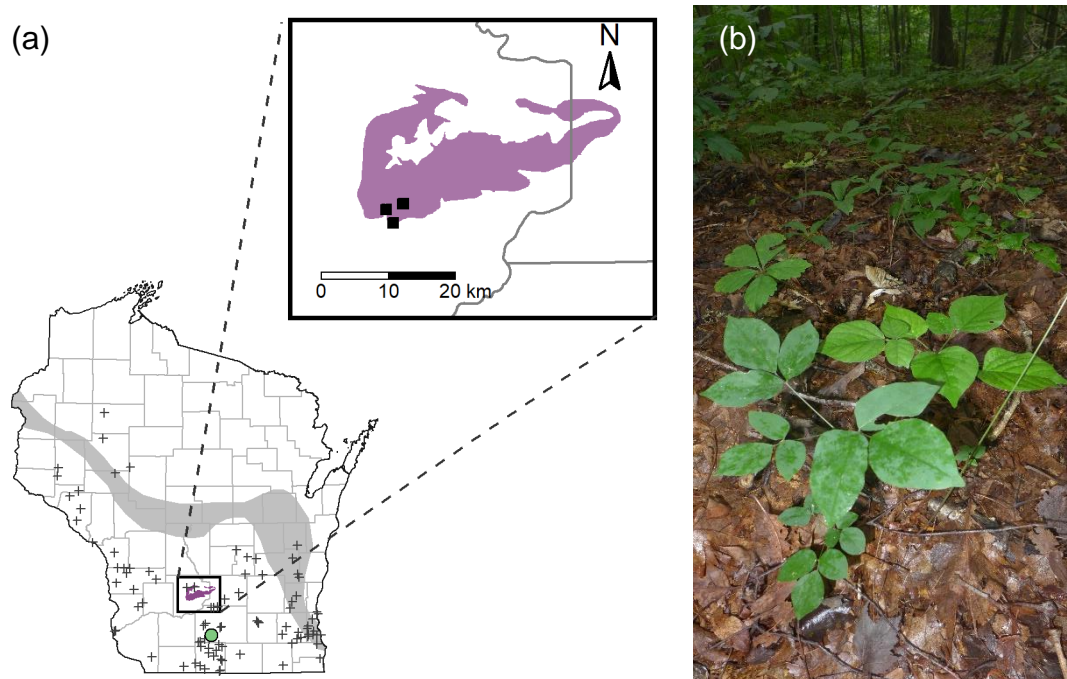


Figure 1. Map of study sites (a) and photograph of co-occurring *Hylodesmum* plants (b). Grey crosses depict the location of 94 forest stands surveyed in southern Wisconsin and the green circle illustrates the location of Olson Oak Woods State Natural Area (Dane County, WI). The grey band represents the location of a pronounced floristic tension zone (Curtis 1959). The inset includes an enlarged map of plot locations in the Baraboo Hills (delineated by purple region). The photographs illustrate co-occurring *H. nudiflorum* (left) and *H. glutinosum* (right) at Hemlock Draw State Natural Area (Sauk County, WI). Photography by J. Beck.

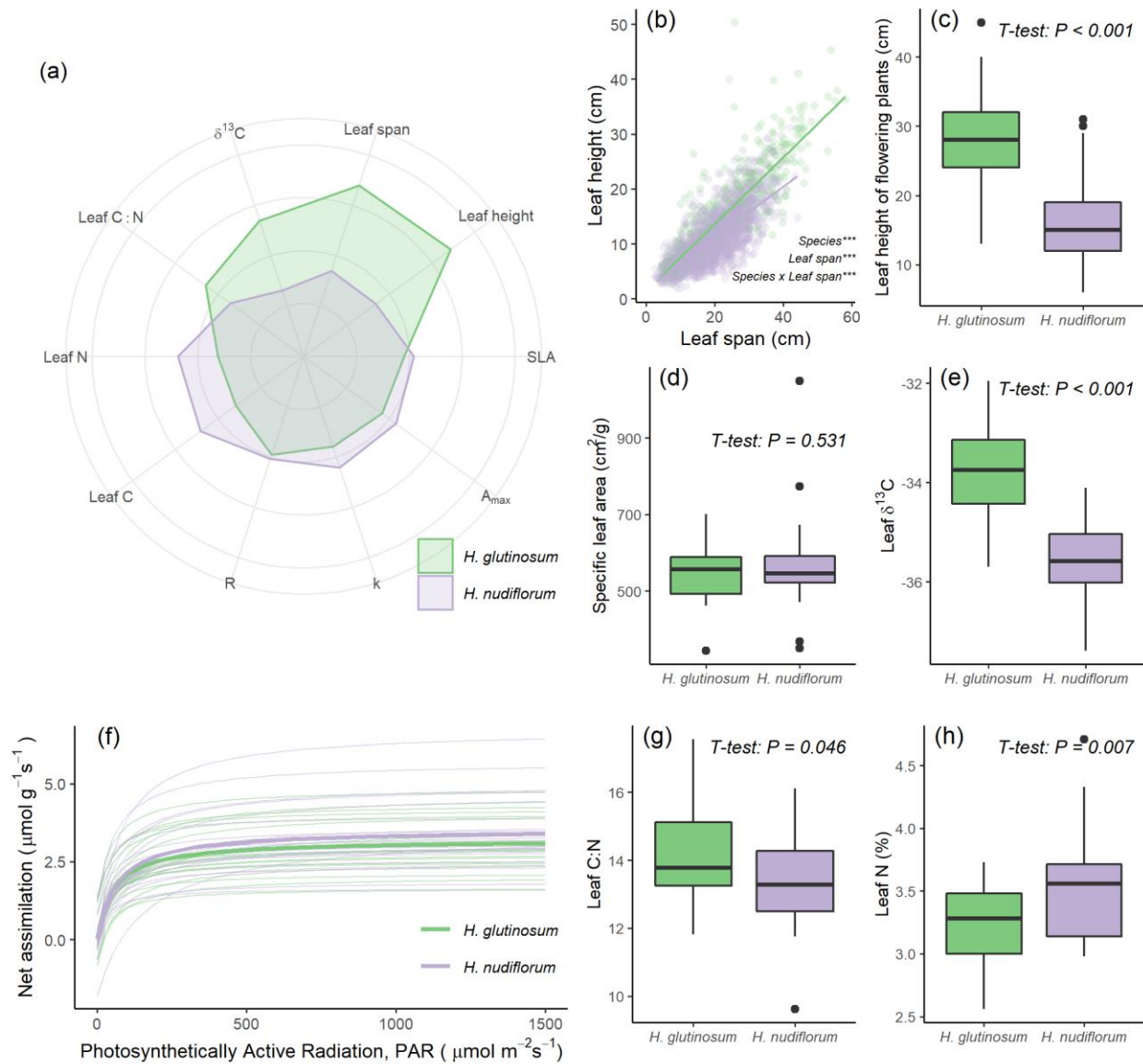


Figure 2. Comparative physiology and morphology of co-occurring *Hylodesmum* species. (a) Summary of morphological and physiological trait distributions; (b) similar relationships between leaf height and leaf span (rosette diameter) in the two species; and differences in distributions for: (c) leaf height at the time of flowering, (d) specific leaf area, (e) water use efficiency (as quantified by $\delta^{13}\text{C}$ isotopes), (g) leaf C:N ratios, and (h) leaf Nitrogen (%). (f) Light response curves quantifying how photosynthetic rates increase in response to photosynthetically active radiation. Throughout, *H. glutinosum* is represented by green and *H. nudiflorum* by purple.

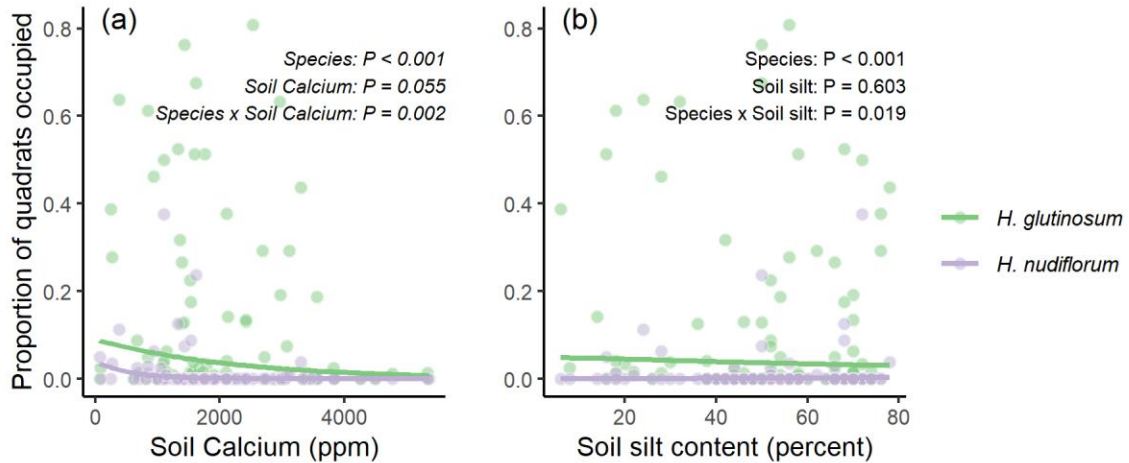


Figure 3. Broad-scale ecological distributions for *Hyloidesmum* across 94 forest stands in southern Wisconsin in relation to gradients in soil richness (estimated by soil calcium – a) and soil texture (estimated by levels of silt in the soil – b). Points represent the proportion of quadrats occupied at each site. Lines represent marginal predictions from a zero-inflated beta-binomial regression model for each species (green – *H. glutinosum*; purple – *H. nudiflorum*). We report P-values from likelihood ratio tests evaluating the effects of species identity, environmental conditions, and their interaction on local plant abundance. See Table S2 for more detailed statistical results.

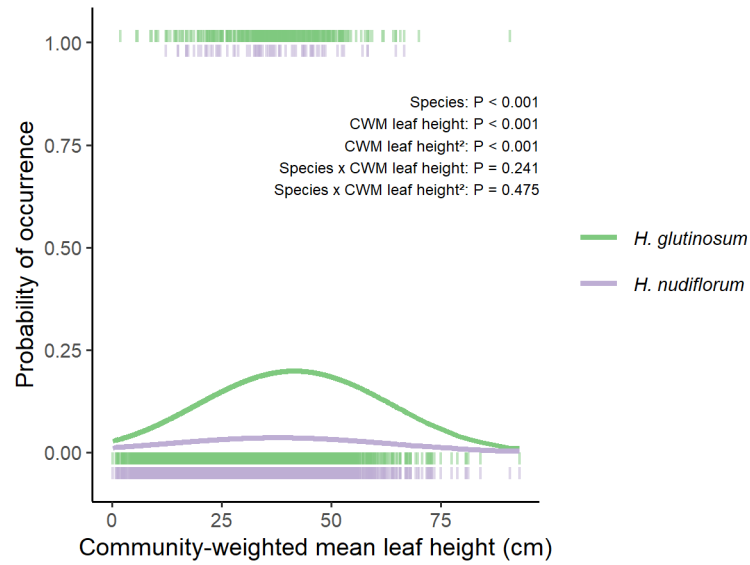


Figure 4. Distributions of both *Hylodesmum* species in relation to local leaf height in Olson Oak Woods State Natural Area. Vertical tick marks represent observed presences (top) and absences (bottom). Lines reflect the predicted probability of occurrence for each species derived from the fitted logistic regression model. We report P-values from likelihood ratio tests evaluating the effect of species identity, community-weighted mean leaf height (including quadratic term to account for unimodal relationship), and interactions between species identify and community-weighted mean leaf height on the probability of plant occurrence within quadrats. See Table S3 for more detailed statistical results.

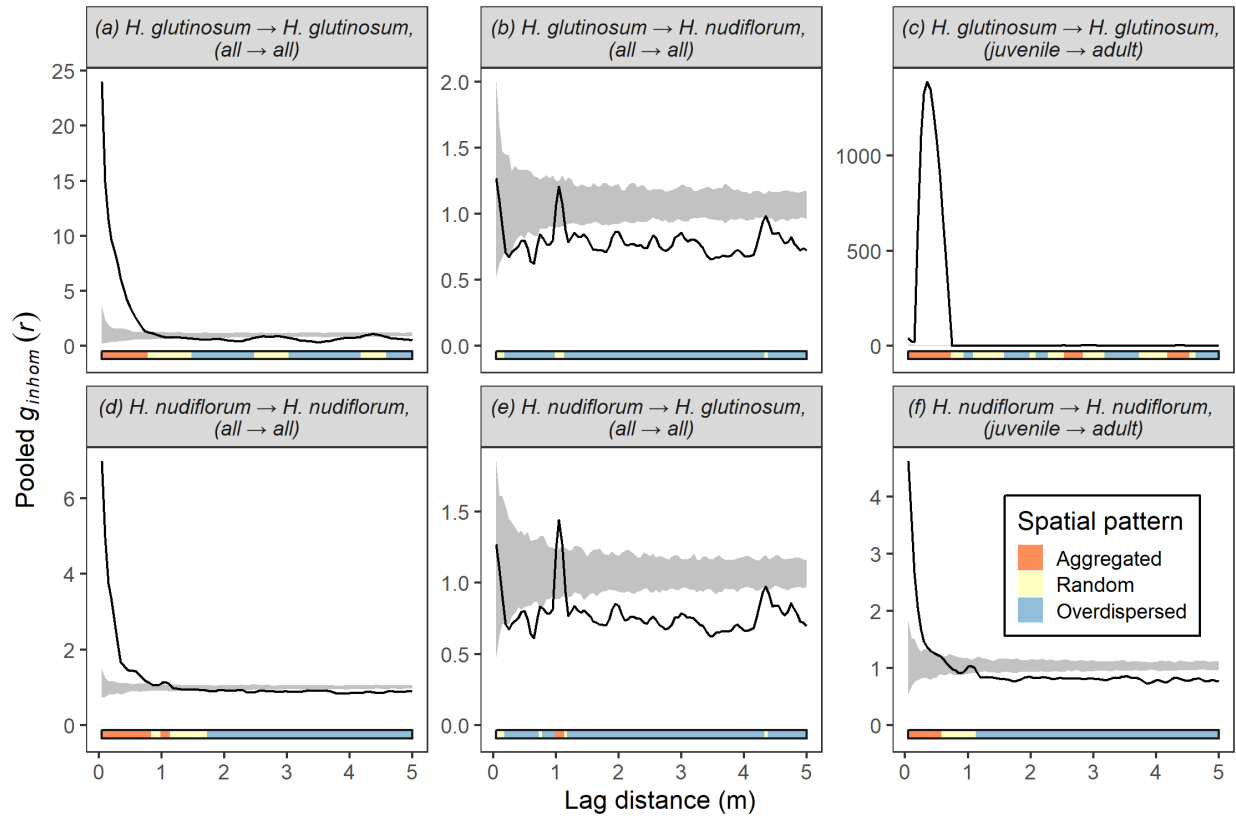


Figure 5. Pooled Pair Correlation Functions (PCFs) describing how interspecific and intraspecific spatial associations, $g_{inhom}(r)$, vary with distance between plant pairs (lag distance). Panels illustrate intraspecific spatial associations within *H. glutinosum* and *H. nudiflorum* (panels a and d), interspecific spatial associations (b and e), and spatial associations between juvenile and adult plants of the same species (c and f). The black line illustrates how the pooled pair correlation function, $g_{inhom}(r)$, varies with distance while the grey ribbon represents a pooled simulation envelope for $g_{inhom}(r)$ under complete spatial randomness. Aggregated spatial patterns occur when the probability of occurrence exceeds that expected under spatial randomness while segregated patterns occur when the probability of occurrence falls below that expected under complete spatial randomness.

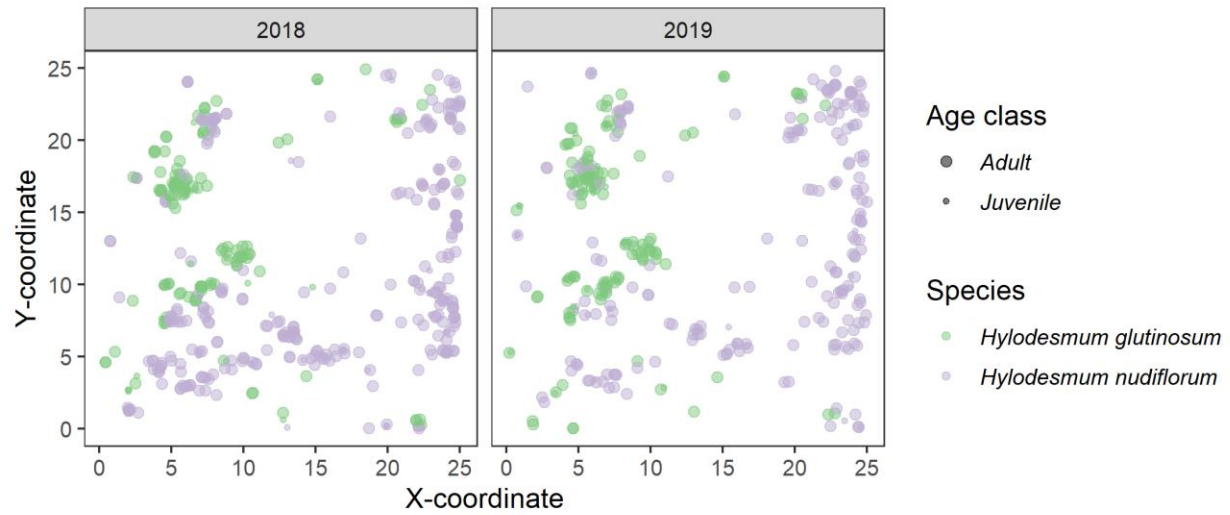


Figure 6. Map illustrating local distribution of *Hylodesmum* individuals and patterns of annual demographic turnover between 2018 and 2019 at the Botany Farm. See Table S3 for statistical summary of annual demographic turnover.

LITERATURE CITED

- Adler, P. B., S. P. Ellner, and J. M. Levine. 2010. Coexistence of perennial plants: An embarrassment of niches. *Ecology Letters* 13:1019–1029.
- Adler, P. B., J. Hillerislambers, and J. M. Levine. 2007. A niche for neutrality. *Ecology letters* 10:95–104.
- Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: A synthesis. *Ecology Letters* 6:1109–1122.
- Amarasekare, P., M. F. Hoopes, N. Mouquet, and M. Holyoak. 2004. Mechanisms of coexistence in competitive metacommunities. *American Naturalist* 164:310–326.
- Amatangelo, K., S. Johnson, D. Rogers, and D. M. Waller. 2014. Trait-environment relationships remain strong despite 50 years of trait compositional change in temperate forests. *Ecology* 95:1780–1791.
- Baddeley, A. 2005. spatstat: An R Package for Analyzing Spatial Point Patterns . *J. Stat. Soft.* 12:1–42.
- Bagchi, R., and J. B. Illian. 2015. A method for analysing replicated point patterns in ecology. *Methods in Ecology and Evolution* 6:482–490.
- Beatty, S. W. 1984. Influence of microtopography and canopy species on spatial patterns of forest understory plants. *Ecology* 65:1406–1419.
- Beatty, S. W. 2014. Habitat Heterogeneity and the Maintenance of Species in Understory Communities. Pages 215–232 *in* F. S. Gilliam, editor. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Beck, J. J. Variation in the strength of plant-soil feedbacks among temperate forest herbs. *In review*.
- Beck, J. J., and T. J. Givnish. Fine-scale environmental heterogeneity and spatial niche partitioning among spring-flowering forest herbs. *In review*
- Beck, J. J., B. Large, and D. M. Waller. 2018. Phantom species: Adjusting estimates of colonization and extinction for pseudo-turnover. *Oikos*.
- Bell, G., M. J. Lechowicz, and M. J. Waterway. 2000. Environmental heterogeneity and species diversity of forest sedges. *Journal of Ecology* 88:67–87.
- Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics* 46:305–325.
- Bradshaw, W. E., and C. M. Holzapfel. 1983. Predator-mediated, non-equilibrium coexistence of tree-hole mosquitoes in southeastern North America. *Oecologia* 57:239–256.

- Bratton, S. P. 1976. Resource division in an understory herb community: Responses to temporal and microtopographic gradients. *The American Naturalist* 110:679–693.
- Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9:378–400.
- Burton, J. I., D. J. Mladenoff, M. K. Clayton, and J. A. Forrester. 2011. The roles of environmental filtering and colonization in the fine-scale spatial patterning of ground-layer plant communities in north temperate deciduous forests. *Journal of Ecology* 99:764–776.
- Caswell, H. 1978. Predator-mediated coexistence: A nonequilibrium model. *The American Naturalist* 112:127–154.
- Catella, S. A., S. R. Eysenbach, and K. C. Abbott. 2019. Novel insights into how the mean and heterogeneity of abiotic conditions together shape forb species richness patterns in the Allegheny plateau ecoregion. *Ecology and Evolution*:12374–12389.
- Cavender-Bares, J. 2018. Diversification, adaptation, and community assembly of the American oaks (*Quercus*), a model clade for integrating ecology and evolution. *New Phytologist*.
- Cavender-Bares, J., S. Kothari, J. E. Meireles, M. A. Kaproth, P. S. Manos, and A. L. Hipp. 2018. The role of diversification in community assembly of the oaks (*Quercus* L.) across the continental U.S. *American Journal of Botany* 105:565–586.
- Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. *Ecology letters* 12:693–715.
- Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. *The American Naturalist* 163:823–843.
- Chase, J. M. 2014. Spatial scale resolves the niche versus neutral theory debate. *Journal of Vegetation Science* 25:319–322.
- Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics* 31:343–358.
- Chesson, P. 2018. Updates on mechanisms of maintenance of species diversity. *Journal of Ecology* 106:1773–1794.
- Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298–312 *in* den B. PJ and G. GR, editors. *Dynamics of Populations*. The Netherlands: Centre for Agricultural Publishing and Documentation.

- Curtis, J. T. 1959. *The Vegetation of Wisconsin: An Ordination of Plant Communities*. The University of Wisconsin Press, Madison, WI.
- Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:503–537.
- Gallien, L., N. E. Zimmermann, J. M. Levine, and P. B. Adler. 2017. The effects of intransitive competition on coexistence. *Ecology Letters* 20:791–800.
- Gause, G. 1932. Experimental studies on the struggle for existence. *Journal of Experimental Biology* 9:389–402.
- Gilbert, B., and M. J. Lechowicz. 2004. Neutrality, niches, and dispersal in a temperate forest understory. *Proceedings of the National Academy of Sciences* 101:7651–7656.
- Gilliam, F. S. 2007. The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. *BioScience* 57:845.
- Givnish, T. J. 1982. On the adaptive significance of leaf height in forest herbs. *American Naturalist* 120:353–381.
- Givnish, T. J. 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106:131–160.
- Givnish, T. J. 1995. Plant stems: biomechanical adaptation for energy capture and influence on species distributions. Pages 3–49 *Plant stems: Physiology and functional morphology*.
- Givnish, T. J., K. C. Millam, A. R. Mast, T. B. Paterson, T. J. Theim, A. L. Hipp, J. M. Henss, J. F. Smith, K. R. Wood, and K. J. Sytsma. 2009. Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society B: Biological Sciences* 276:407–416.
- Givnish, T. J., R. A. Montgomery, and G. Goldstein. 2004. Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: Light regimes, static light responses, and whole-plant compensation points. *American Journal of Botany* 91:228–246.
- Givnish, T. J., and R. A. Montgomery. 2014. Common-garden studies on adaptive radiation of photosynthetic physiology among Hawaiian lobeliads. *Proceedings of the Royal Society B* 281:20132944.
- Godoy, O., N. J. B. Kraft, and J. M. Levine. 2014. Phylogenetic relatedness and the determinants of competitive outcomes. *Ecology letters* 17:836–44.
- Grubb, P. J. 1977. The maintenance of species richness in plant communities: The importance of the

- regeneration niche. *Biological Reviews* 52:107–145.
- Hart, S. P., J. Usinowicz, and J. M. Levine. 2017. The spatial scales of species coexistence. *Nature Ecology and Evolution* 1:1066–1073.
- Helmus, M. R., K. Savage, M. W. Diebel, J. T. Maxted, and A. R. Ives. 2007. Separating the determinants of phylogenetic community structure. *Ecology Letters* 10:917–925.
- HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking community assembly through the lens of coexistence theory. *Annual Review of Ecology, Evolution, and Systematics* 43:120830113150004.
- Horn, H. S., and R. H. MacArthur. 1972. Competition among fugitive species in a harlequin environment. *Ecology* 53:749–752.
- Hubbell, S. P. 2001. *The Unified Neutral Theory of Island Biogeography and Biodiversity*. Princeton University Press.
- Hutchinson, G. E. 1961. The paradox of the plankton. *American Naturalist* 95:137–145.
- Janzen, D. 1971. Seed predation by animals. *Annual review of ecology and systematics* 2:465–492.
- Kneitel, J. M., and J. M. Chase. 2004. Trade-offs in community ecology: Linking spatial scales and species coexistence. *Ecology Letters* 7:69–80.
- Kraft, N. J. B., P. B. Adler, O. Godoy, E. C. James, S. Fuller, and J. M. Levine. 2015a. Community assembly, coexistence and the environmental filtering metaphor. *Functional Ecology* 29:592–599.
- Kraft, N. J. B., P. B. Adler, O. Godoy, E. James, S. Fuller, and J. M. Levine. 2014. Community assembly, coexistence, and the environmental filtering metaphor. *Functional Ecology*:n/a-n/a.
- Kraft, N. J. B., O. Godoy, and J. M. Levine. 2015b. Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences* 112:797–802.
- Kunstler, G., S. Lavergne, B. Courbaud, W. Thuiller, G. Vieilledent, N. E. Zimmermann, J. Kattge, and D. A. Coomes. 2012. Competitive interactions between forest trees are driven by species' trait hierarchy, not phylogenetic or functional similarity: Implications for forest community assembly. *Ecology letters* 15:831–40.
- Lange, K. I. 1998. *Flora of Sauk County and Caledonia Township, Columbia County, south central Wisconsin*. Madison, WI.
- Levine, J. M., J. Bascompte, P. B. Adler, and S. Allesina. 2017. Beyond pairwise mechanisms of species coexistence in complex communities. *Nature* 546:56–64.

- Levine, J. M., and M. Rees. 2002. Coexistence and relative abundance in annual plant assemblages: the roles of competition and colonization. *The American Naturalist* 160:452–67.
- Levins, R., and D. Culver. 1971. Regional coexistence of species and competition between rare species. *Proceedings of the National Academy of Sciences* 68:1246–1248.
- Li, H. C., X. L. Zhao, X. F. Gao, and B. Xu. 2019. Molecular phylogeny of the genus *Hylodesmum* (Fabaceae). *Phytotaxa* 403:221–229.
- Macarthur, R. H. 1958. Population ecology of some warblers of northeastern coniferous forests. *Ecology* 39:599–619.
- Macarthur, R. H., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist* 101:377–385.
- Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecology Letters* 13:1085–93.
- Montgomery, R. A., and T. J. Givnish. 2008. Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: Dynamic photosynthetic responses. *Oecologia* 155:455–467.
- Mordecai, E. 2011. Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. *Ecological Monographs* 81:429–441.
- O’Leary, M. H. 1988. Carbon isotopes in photosynthesis. *BioScience* 38:328–336.
- Peet, R. K., K. A. Palmquist, and S. M. Tessel. 2014. Herbaceous layer species richness of southeastern forests and woodlands: Patterns and causes. Pages 255–276 in F. S. Gilliam, editor. *The Herbaceous Layer in forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Peterson, C. J., W. P. Carson, B. C. McCarthy, S. T. A. Pickett, C. J. Peterson, W. P. Carson, B. C. McCarthy, S. T. A. Pickett, and S. T. A. Microsite. 1990. Microsite variation and soil dynamics within newly created treefall pits and mounds. *Oikos* 58:39–46.
- Platt, W. J. 1985. An experimental study of competition among fugitive prairie plants. *Ecology* 66:708–720.
- Rogers, D. A., T. P. Rooney, D. Olson, and D. M. Waller. 2008. Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. *Ecology* 89:2482–2492.
- Shmida, A., and S. Ellner. 1984. Coexistence of plant species with similar niches. *Vegetatio* 58:29–55.
- Silvertown, J. 2004. Plant coexistence and the niche. *Trends in Ecology and Evolution* 19:605–611.

- Smith, L. M., and H. L. Reynolds. 2015. Plant-soil feedbacks shift from negative to positive with decreasing light in forest understory species. *Ecology* 96:2523–2532.
- Smith, W. G. 1975. Dynamics of pure and mixed populations of *Desmodium glutinosum* and *D. nudiflorum* in natural oak-forest communities. *American Midland Naturalist* 94:99–107.
- Stefanović, S., B. E. Pfeil, J. D. Palmer, and J. J. Doyle. 2010. Relationships among phaseoloid legumes based on sequences from eight chloroplast regions. *Systematic Botany* 34:115–128.
- Stein, A., K. Gerstner, and H. Kreft. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters* 17:866–880.
- Struik, G. J., and J. T. Curtis. 1962. Herb distributions in an *Acer saccharum* forest. *American Midland Naturalist* 68:285–296.
- R Core Team. 2019. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Thompson, J. N. 1980. Treefalls and colonization patterns of temperate forest herbs. *American Midland Naturalist* 104:176.
- Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. *Ecology* 75:2–16.
- Turnbull, L. A., D. Coomes, A. Hector, and M. Rees. 2004. Seed mass and the competition/colonization trade-off: Competitive interactions and spatial patterns in a guild of annual plants. *Journal of Ecology* 92:97–109.
- Vellend, M., M. J. Lechowicz, and M. J. Waterway. 2000. Environmental distribution of four *Carex* species (Cyperaceae) in an old-growth forest. *American Journal of Botany* 87:1507–1516.
- Voss, E. G., and A. A. Reznicek. 2012. *Field Manual of Michigan Flora*. The University of Michigan Press, Ann Arbor, MI.
- Waller, D. M., K. Amatangelo, S. Johnson, and D. Rogers. 2012. Wisconsin Vegetation Database – plant community survey and resurvey data from the Wisconsin Plant Ecology Laboratory. *Biodiversity & Ecology* 4:255–264.
- Weber, M. G., and S. Y. Strauss. 2016. Coexistence in Close Relatives: Beyond Competition and Reproductive Isolation in Sister Taxa. *Annual Review of Ecology, Evolution, and Systematics* 47:359–381.
- Whigham, D. E. 2004. Ecology of woodland herbs in temperate deciduous forests. *Annual Review of Ecology and Systematics* 35:583–621.

- Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. *Ecological Monographs* 26:1–80.
- Wickham, H. 2009. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York, USA.
- Wiegand, T., and K. A. Moloney. 2004. Rings, circles, and null-models for point pattern analysis in ecology. *Oikos* 104:209–229.
- Wiegand, T., and K. A. Moloney. 2014. *Handbook of Spatial Point-Pattern Analysis in Ecology*. Chapman and Hall/CRC Press, Boca Raton, FL.
- Wiens, J. J., D. D. Ackerly, A. P. Allen, B. L. Anacker, L. B. Buckley, H. V. Cornell, E. I. Damschen, T. Jonathan Davies, J.-A. Grytnes, S. P. Harrison, B. A. Hawkins, R. D. Holt, C. M. McCain, and P. R. Stephens. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology letters* 13:1310–24.

APPENDIX S2. Supplemental materials for Chapter 2.

Table S1. Summary of comparative morphological and physiological data for *Hylodesmum glutinosum* and *H. nudiflorum*.

Trait	<i>H. glutinosum</i>	<i>H. nudiflorum</i>	P-value
Light response curves			
A _{max} (maximum photosynthetic rate)	3.13	3.50	0.181
k (light require to half saturate photosynthesis)	55.76	64.26	0.196
R (respiration rate)	-0.06	-0.03	0.849
Specific leaf area (cm ² /g)	548.28	568.33	0.531
Leaf $\delta^{13}\text{C}$	-33.81	-35.62	<0.001
Leaf C (%)	45.18	46.67	0.003
Leaf N (%)	3.23	3.54	0.007
Leaf C:N	14.15	13.33	0.046
Leaf height of flowering plants (cm)	27.89	15.78	<0.001

Table S2. Summary of zero-inflated beta-binomial regression models for assessing broad-scale *Hylodesmum* distributions along environmental gradients from field vegetation data. Test statistics and P-values are presented from likelihood ratio tests for the effect of species identity (Species), soil Calcium concentrations (ppm), soil silt content (%), and the interaction of soil variables with species identity. Significant interactions imply that the response of local plant abundance to environmental variables varies between *Hylodesmum* species. See Figure 3 for graphical depiction of species distributional patterns.

Model term	χ^2	P-value
Species	47.854	<0.001
Soil Calcium	3.689	0.055
Soil Silt Content	0.271	0.603
Species x Soil Calcium	9.673	0.002
Species x Soil Silt Content	5.484	0.019

Table S3. Summary of logistic regression model examining *Hylodesmum* distributions within Olson Oak Woods State Natural Area. Test statistics and P-values are presented from likelihood ratio tests for the effect of species identity, community-weighted mean leaf height, and the interaction of species identity and community-weighted mean leaf height. We included a quadratic term to account for the unimodal relationship between the probability of *Hylodesmum* occurrence and community-weighted mean leaf height. Significant interactions indicate that the two *Hylodesmum* species differ in their response to community-weighted mean leaf height. See Fig. 4 for corresponding graphical depictions of these species distributional patterns.

Model term	χ^2	P-value
Species	253.406	<0.001
CWM(leaf height)	76.383	<0.001
CWM(leaf height) ²	58.019	<0.001
Species x CWM(leaf height)	0.809	0.368
Species x CWM(leaf height) ²	0.133	0.715

Table S4. Summary information for mapping locations

Location	Year	Plot dimensions (m)	Total plants	<i>H. glutinosum</i>	<i>H. nudiflorum</i>
Botany Farm	2018	25 x 25	474	157	317
Botany Farm	2019	25 x 25	396	172	224
Natural Bridge	2019	20 x 20	821	140	681
Hemlock Draw	2019	20 x 20	1153	149	1004

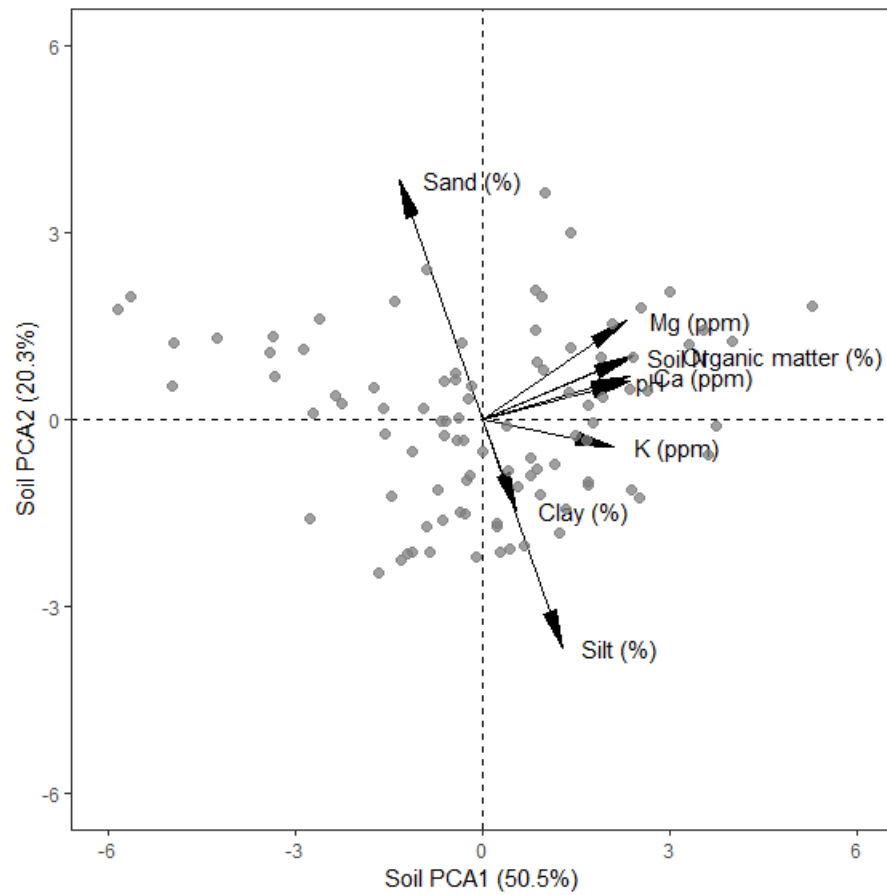


Figure S1. Principal Components Analysis of soil characteristics across 94 forest stands in southern Wisconsin.

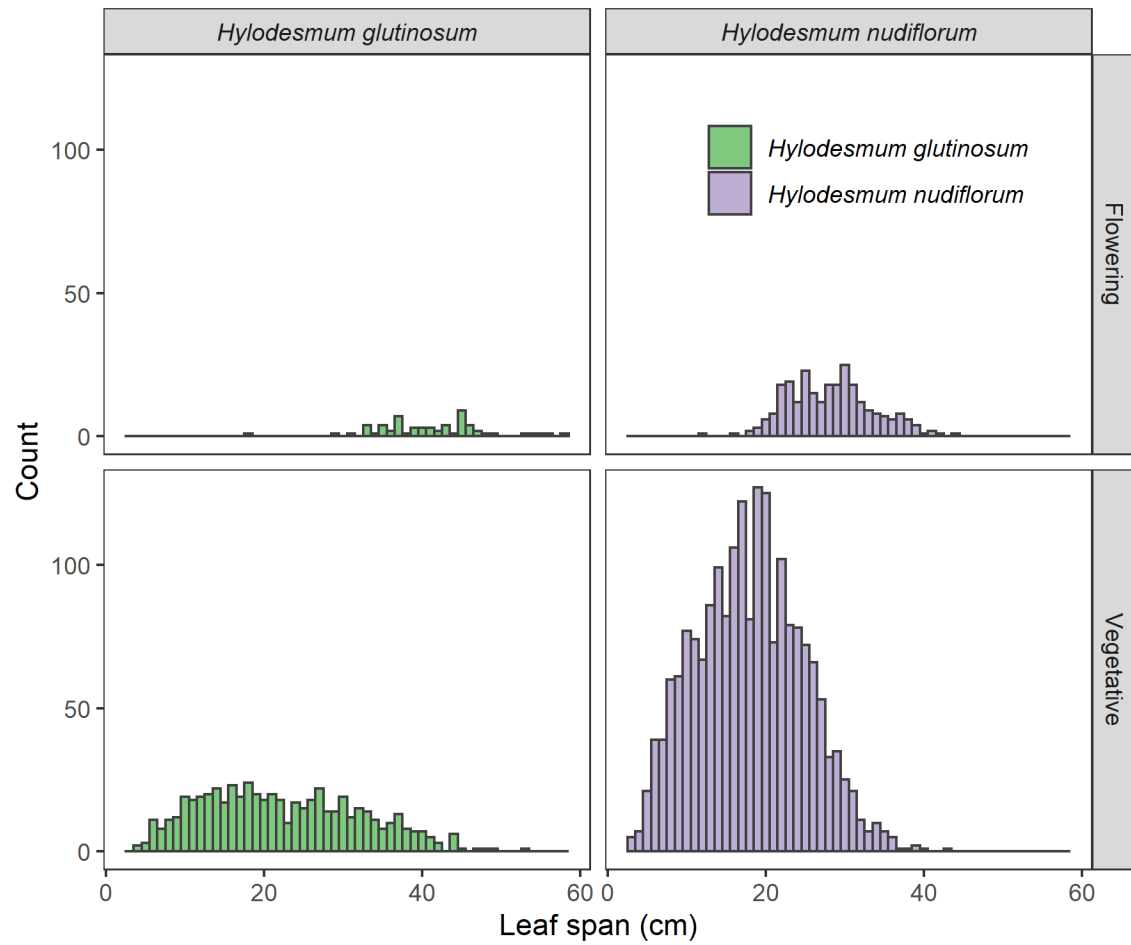


Figure S2. Observed size distribution (leaf span) of vegetative and flowering *H. glutinosum* and *H. nudiflorum*.

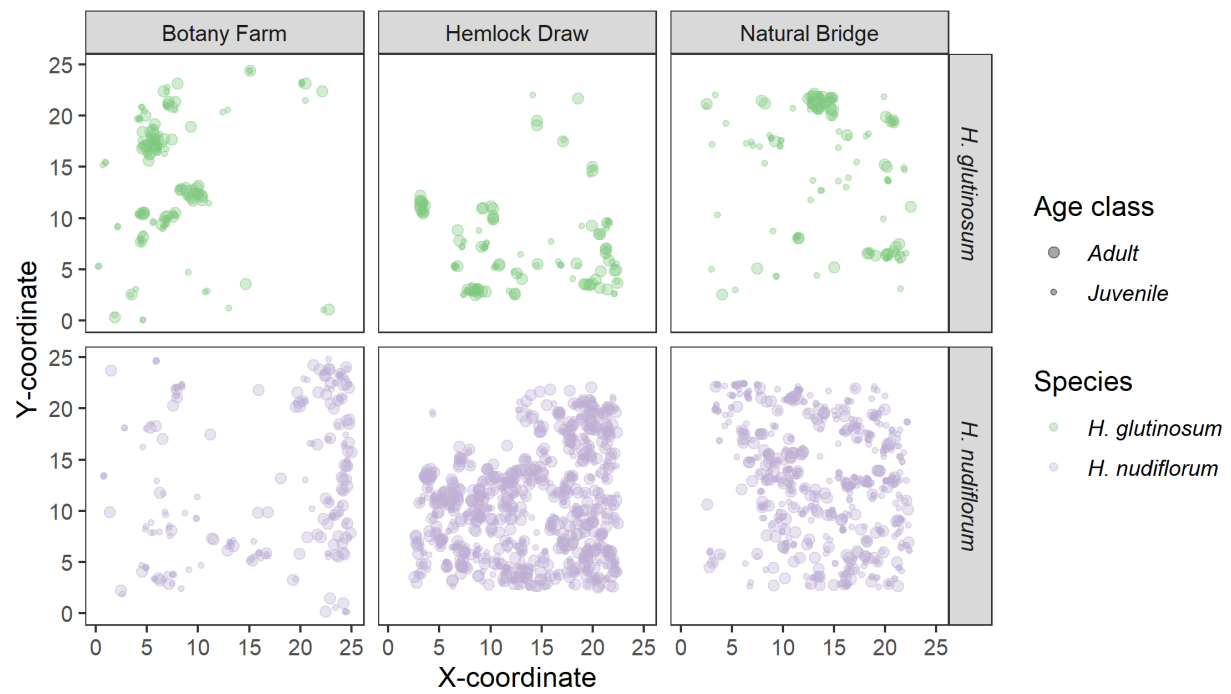


Figure S3. Maps illustrating the fine-scale spatial distribution of co-occurring plants at each focal site.

Chapter 3. Fine-scale environmental heterogeneity and spatial niche partitioning among spring-flowering forest herbs

Jared J. Beck and Thomas J. Givnish

Department of Botany

University of Wisconsin-Madison

430 Lincoln Drive, Madison, WI 53706

ABSTRACT

Environmental heterogeneity shapes plant distributions and diversity at several spatial scales. In temperate deciduous forests, fine-scale environmental variation may promote local coexistence among herbaceous species by allowing plants to spatially partition microsites within forest stands. Here we argue that shallow soils, low soil fertility, and reduced soil water holding capacity near tree boles should favor short, shallow-rooted, evergreen species like *Anemone acutiloba* with low nutrient requirements. Farther from trees, richer, deeper soils should favor taller, deeper-rooted herbs with greater nutrient demands such as *Sanguinaria canadensis* and *Trillium flexipes*. We tested these hypotheses by mapping the local distribution of all *Anemone*, *Sanguinaria*, and *Trillium* individuals within a 50 x 50 m forest plot (767 total plants) and examining variation in the distribution of these species with respect to soil depth and proximity to neighboring trees. Distributional differences among species were consistent with our predictions based on plant physiology. *Anemone acutiloba* was found in shallower soil microsites and closer to trees than either *Sanguinaria* or *Trillium*. *Sanguinaria canadensis* and *Trillium* occurred essentially at random with respect to trees whereas *Anemone* was clustered near tree bases. Within all three species, individual plants were spatially aggregated within 2 m, but spatially segregated from individuals of the other species beyond 2 m. The scale of spatial dependence in soil depth, the scale of intraspecific aggregation, and expected dispersal capacity all aligned closely suggesting that local species-environment associations could facilitate coexistence. These results demonstrate how fine-scale environmental heterogeneity coupled with phenological and physiological differences likely contribute to spatial niche partitioning among spring-flowering forest herbs and highlight the importance of fine-scale environmental variation in maintaining high local plant diversity within temperate forests.

KEYWORDS: environmental heterogeneity; coexistence; temperate forest; soil depth; understory

INTRODUCTION

Environmental heterogeneity plays an important role in determining plant distributions and maintaining species diversity across a range of spatial scales (Ricklefs 1977, Amarasekare 2003, Silvertown 2004, Krefth and Jetz 2007, Bartels and Chen 2010, Costanza et al. 2011, Stein et al. 2014). At broad spatial scales, plant distributions vary with climate, edaphic conditions, and other abiotic factors (Krefth and Jetz 2007). This environmental variation is positively associated with species richness and has been implicated as an important factor maintaining regional diversity (Ricklefs 1977, Krefth and Jetz 2007, Stein et al. 2014). Environmental heterogeneity can also influence plant distributions at finer scales (Lundholm 2009, Bartels and Chen 2010, Costanza et al. 2011, Catella et al. 2019). Yet, few empirical studies carefully consider the physiological differences and competitive interactions underlying differential species responses to local environmental heterogeneity (Silvertown 2004) or the spatial scales at which this local heterogeneity could facilitate coexistence (Amarasekare 2003, Kneitel and Chase 2004, Lundholm 2009, Tamme et al. 2010, Hart et al. 2017).

Terrestrial plant species compete for a limited set of finite resources including water, light, and a suite of macronutrients (Silvertown 2004). While interspecific tradeoffs in resource acquisition and use are expected to stabilize coexistence when differential resource limitation increases intraspecific competition relative to interspecific competition (Tilman 1982, Chesson 2000a), there is somewhat modest evidence that plants partition resources in the absence of environmental variation and it remains unclear whether there are enough limiting resources or sufficiently strong tradeoffs to maintain high local plant diversity (Goldberg and Barton 1992, Silvertown 2004, Dybzinski and Tilman 2007, Adler et al. 2010, Kraft et al. 2015). In contrast, there is ample evidence that differential plant responses to spatial (Amarasekare 2003, Silvertown 2004, Bartels and Chen 2010, Stein et al. 2014) and temporal (Chesson and Warner 1981, Levine and Rees 2004, Adler et al. 2006, Angert et al. 2009) environmental variation can facilitate coexistence. The potential for spatial heterogeneity to promote coexistence, however, depends critically on the scale of heterogeneity relative to the dispersal capacity of competing plants and how strongly plant performance varies with the environment (Chesson 2000b, Snyder and Chesson 2004).

An improved understanding of the physiological characteristics underlying differential plant responses to environmental variation and careful examination of local plant distributions are important for assessing potential for fine-scale environmental heterogeneity to maintain local plant diversity in natural communities (Kneitel and Chase 2004, Silvertown 2004, Lundholm 2009, HilleRisLambers et al. 2012, Hart et al. 2017). Specifically, examining whether fine-scale species-environment associations are predictable and conform to theoretical expectation based on physiological differences can provide insights into the processes shaping local plant distributions. Likewise, evaluating intraspecific and interspecific spatial associations in natural plant communities can elucidate whether the net effect of competitive interactions, environmental sorting, and dispersal reinforces interspecific spatial segregation (an important prerequisite for spatial variation to facilitate coexistence) or will tend to scramble species-environment relationships.

Characterizing the potential for fine-scale environmental heterogeneity to structure local plant distributions and promote coexistence is especially important in plant communities with high local plant diversity. One intriguing case involves the coexistence of large numbers of herbaceous plants in temperate deciduous forests. Herbaceous taxa comprise approximately 80% of plant species in such forests (Gilliam 2007). Between 50 and 100 understory species regularly coexist within a single hectare, and 10-15 such species may co-occur within 1 m² (Gilliam 2007, Rogers et al. 2008, Peet et al. 2014). Environmental variation shapes the distribution of herbaceous species in temperate deciduous forests at multiple spatial scales (Gilbert and Lechowicz 2004, Bartels and Chen 2010, Costanza et al. 2011, Beatty 2014, Peet et al. 2014). At broad spatial scales, the distribution and abundance of understory herbaceous species vary in response to numerous biotic and abiotic factors along environmental gradients (Whittaker 1956, Curtis 1959, Leach and Givnish 1999, Gilbert and Lechowicz 2004, Amatangelo et al. 2014, Beatty 2014, Burton et al. 2014, Peet et al. 2014, Gilliam et al. 2016). Differential plant responses to these broad environmental gradients reflect physiological tradeoffs and strategies adapted for different environmental conditions (Givnish 1982, 1987, 1995, Amatangelo et al. 2014, Neufeld and Young 2014).

Understory plant species also respond sensitively to environmental variation at finer spatial scales. Within forest stands, herbaceous plant species often exhibit highly heterogeneous spatial distributions and these local distributions reflect, in part, fine-scale variation in the environment (Struik and Curtis 1962, Anderson et al. 1969, Burton et al. 2011, Sabatini et al. 2014, Chudomelová et al. 2017, Catella et al. 2019). For example, fallen trees create light gaps in otherwise closed canopy forests which favor the growth of certain herbaceous species (Thompson 1980). Treefalls can also create microtopographic heterogeneity that influences the distribution of distribution of herbaceous species independent of light availability (Beatty 1984, Beatty and Stone 1986, Moore and Vankat 1986, Peterson et al. 1990, Scheller and Mladenoff 2002, Graves et al. 2006). Trees overhead can influence the distribution of understory plant species by modifying the physical environment and modulating the flow of resources through forest ecosystems (Augusto et al. 2003, Barbier et al. 2008). Variation in how much light trees transmit light to the forest floor (Canham and Burbank 1994), the seasonal timing of light availability (Lopez et al. 2008), rates and nutrient concentrations of stem-flow (Crozier and Boerner 1984), and differences in resource uptake (e.g., due to shallower roots) and litter characteristics among tree species (Finzi et al. 1998) can all affect the distribution and growth of understory plants.

Linking local plant distributions to niche differences requires considering plant distributions relative to underlying environmental variation as well as differences in plant morphology and physiology. In a classic study of within-stand distributions of forest herbs, Bratton (1976) found that herb species composition varied with proximity to canopy trees as well as exposed bedrock and boulders. Bratton (1976) argued that soil depth and plant rooting depth helped determine species distributions. Herbs with shallow, fibrous root systems were more abundant on or near boulders and the bases of trees, whereas plants with more substantial bulbs and rhizomes and deeper roots were physically unable to establish in microsites with shallow soil. Yet soil depth is not the only factor that varies near tree trunks. Increased stem flow can wash away soil from around the trunk, especially fine particles that contribute to moisture and nutrient supply. Decreased soil depth due to large shallow tree roots also restrict soil moisture and nutrient supplies; combined with the lower light levels near the trunk, this should result in sparser herb

cover. Finally, in cool or cold temperate forests, snow melts earlier from areas immediately adjacent to tree boles, extending the potential growing season for understory herbs there (Vellend et al. 2017). Consequently, we would expect herbs with shallower roots, leaves held closer to the ground (Givnish 1982, 1987, 1995), and a greater tendency toward the evergreen habit – here associated with poor soils and a longer growing season (Givnish 2002) – in species growing near tree boles compared with those growing further away. These considerations provide testable insights into the fine-scale distribution of forest herbs and some ecological processes responsible for maintaining understory plant diversity (Collins et al. 1984).

Here we describe the local distribution of three common, spring-flowering forest herbs: *Anemone acutiloba*, *Sanguinaria canadensis*, and *Trillium flexipes*. Although these species commonly co-occur in North American forests and share ecological characteristics typical of temperate forest herbs, the three focal species differ with respect to several key physiological characteristics (Table 1). We address three primary questions: (1) Does fine-scale environmental heterogeneity influence their local distributions, (2) Are differential responses to local environmental variation linked to interspecific phenotypic differences in accord with theory, and (3) Do these forest herbs exhibit interspecific spatial segregation within forest stands? Differences in rooting depth, photosynthetic phenology, leaf height, and resource requirements should influence the local distribution of plants and cause fine-scale spatial segregation. We hypothesized that *Anemone* (with evergreen leaves, short stature, shallow fibrous roots, and low nutrient requirements) should be found in shallow soils and/or closer to trees than the taller, more deeply rooted, spring- and summer-active *Sanguinaria* and *Trillium*, which might competitively exclude *Anemone* from deeper soils and microsites further from trees.

MATERIALS AND METHODS

Study site – We conducted studies at McGilvra Woods State Natural Area (Sauk County, WI USA), a 26-ha forest stand managed by the Wisconsin Department of Natural Resources (Appendix S1: Fig. S1). The stand is located within the Baraboo Hills, a 216 km² region marked by remnants of an ancient mountain

range composed of uplifted Precambrian quartzite that rises roughly 200 m above the surrounding area in south-central WI. This area is considered a regional conservation priority that encompasses the largest tract of contiguous forest in southern Wisconsin and supports more than 1300 plant species (Lange 1998). McGilvra Woods lies near the center of the Baraboo Range. The forest stand slopes gently to the northwest (2-6% slope within study plot). Soils consist of Fayette and La Farge silt loams derived from loess deposits. McGilvra Woods is dominated by *Acer saccharum* (40% of tree basal area in our study plot; Table S1) with *Quercus rubra* (22%), *Tilia Americana* (17%), *Fraxinus americana* (10%), *Carya cordiformis* (5%), *Quercus alba* (4%) *Prunus serotina*, (1%), and *Ulmus americana* (<1%) as subdominant canopy trees. The understory includes a rich diversity of herbaceous plant species including several spring ephemerals (*Cardamine concatenata*, *C. douglasii*, *Claytonia virginica*, *Erythronium albidum*, *E. americanum*) and early-summer species (*Actaea rubra*, *Enemion biternatum*, *Hydrophyllum virginianum*, *Mitella diphylla*, *Phlox divaricata*, *Thalictrum dioicum*, *Uvularia grandiflora*).

Our study focused on three species common to forests throughout eastern North America: *Anemone acutiloba* DC., *Sanguinaria canadensis* L., and *Trillium flexipes* Raf – hereafter, *Anemone*, *Sanguinaria*, and *Trillium*. All three focal species possess elaisomes and are primarily dispersed by ants (Pudlo et al. 1980, Smith et al. 1989). However, these species differ in leaf phenology, stature, nutrient requirements, and other relevant physiological characteristics (Table 1; Appendix 1: Fig. S2). *Anemone* is relatively short-statured (leaves held < 15 cm above ground) and has evergreen leaves which senesce after one year. This species has relatively low nutrient demands (2.1% leaf N content, Table 1) and possesses a shallow, fibrous root system. *Sanguinaria* flowers early in the spring, and leafs out early for an early-summer species, with leaves unfolding into a fully umbrella-like form 15-35 cm tall around the time of canopy closure in mid-May and persisting through mid-summer. Compared with *Anemone*, *Sanguinaria* has extensive fibrous roots that extend from a thickened belowground rhizome, taller leaf height (typically 15-35 cm), and – based on larger leaves with higher nutrient concentrations – higher nutrient demands (2.9% leaf N content). *Trillium* also flowers in spring and is an early-summer species. Its leaves emerge a bit later than *Sanguinaria* and are taller (25-50 cm). *Trillium* has greater nutrient demands (3.9%

leaf N content) and possesses a thickened rhizome and a more extensive system of fibrous roots growing from the rhizome.

Field methods – In May 2019, we mapped the distribution of all *Anemone*, *Sanguinaria*, and *Trillium* within a 50 x 50 m plot. This focal plot was embedded within a larger 1 ha forest monitoring plot in which all trees greater than 1 cm DBH are permanently marked, mapped, and identified to species (Appendix S1: Fig. S3). The 50 x 50 m focal plot included 110 trees of 8 species (Appendix S1: Table S1). After delineating plot boundaries, we systematically searched the plot for the focal herb species and temporarily marked the location of each plant (Fig. 1). For the purposes of this study, we considered all stems greater than 10 cm apart and not obviously connected to nearby stems by rhizomes as separate individuals for mapping. We then split the plot into 5 m wide strips, re-searched each segment to ensure no plants were missed, and mapped the location of each individual plant within the plot with ca. 10 cm precision. In addition to mapping focal plants, we used a 2.6 mm diameter metal wire to probe the soil at the base of each stem and estimate soil depth (to nearest cm, maximum of 50 cm). We also measured the distance from each focal individual to the nearest live or dead tree stem (0.1 m precision). Finally, we measured soil depth every 2.5 m along eleven 50-m East-West transects spaced 5 m apart to obtain a reference distribution of background soil depths within the plot.

Data analysis – To test whether the focal species tended to segregate into different environmental microsites, we analyzed differences in their distributions with respect to soil depth and proximity to neighboring trees. We used a spatial ANOVA to test for differences in mean soil depth among focal species while accounting for non-independence of residuals caused by spatial autocorrelation (Zuur et al. 2009). This model included observed soil depths for all focal species as well as the reference sample of background soil depths allowing us to test for differences among species as well as differences between focal species and the soil depth expected if plant species occur at random with respect the background environmental conditions. We examined spatial dependence in soil depth using semivariogram (Bivand et

al. 2013). Exponential, Gaussian, and spherical correlation structures provided similar fits for the spatial dependence among residuals so we used an exponential correlation structure in our spatial ANOVA to account for spatial dependencies in soil depth within the plot (Zuur et al. 2009). To examine whether species were distributed differently with respect to trees, we used an ANOVA to test for variation among focal species in their proximity to the nearest tree. In this model, we did not include a term for residual spatial covariance because the dependent variable, distance to nearest tree, is itself a spatial measurement.

We next explored spatial associations among conspecific individuals, between pairs of focal species, and between each focal species and neighboring trees using pair correlation functions (PCFs). PCFs describe the probability of observing a pair of points at a particular distance divided by the expected probability under complete spatial randomness, $g(r)$ (Wiegand and Moloney 2014). Whereas the related K- and L-functions rely on cumulative point densities at successively larger radii to infer spatial patterns (thus patterns at a given radius depend on point densities at smaller radii), spatial patterns inferred from PCFs do not necessarily exhibit spatial dependence across scales and thus lend themselves to more straightforward interpretation (Wiegand and Moloney 2004). For all analyses, we used inhomogeneous pair correlation functions, $g_{inhom}(r)$, which allow the density of individuals to vary in space independent of proximity to events via a non-stationary Poisson process (Wiegand and Moloney 2014). We applied Ripley's isotropic edge correction to reduce estimation bias caused by edge effects. This method weights the influence of spatial events on estimated PCFs according to their proximity to the plot boundary (Wiegand and Moloney 2004, 2014, Baddeley 2005). In the first set of point pattern analyses, we explored how the observed density of individuals belonging to a given focal species varied with proximity to conspecific individuals. In the second set of spatial analyses, we investigated how the probability of encountering individuals of a given focal species varied with proximity to heterospecific individuals for each pair of species. Finally, we characterized how the probability of encountering individuals belonging to a given focal species varied with proximity to neighboring trees. For each PCF, we calculated a simulation envelope (499 iterations) for expected $g_{inhom}(r)$ values under complete spatial randomness.

Observed $g_{inhom}(r)$ values that fall outside the simulation envelope can be interpreted as statistically significant deviations from spatial randomness (Wiegand and Moloney 2014).

All analyses were performed using R 3.5.3 (R Core Team 2019). Spatial linear models were performed using the *gls* function in the ‘nlme’ package (Pinheiro et al. 2018) and semivariograms were fit using the ‘gstat’ package (Pebesma 2004). PCFs describing conspecific spatial associations were performed using the *pcfinhom* function and PCFs describing heterospecific spatial associations were performed using the *pcfcross.inhom* function within the ‘spatstat’ package (Baddeley 2005). The package ‘ggplot2’ was used for generating figures (Wickham 2009).

RESULTS

The 50 x 50 m study plot at McGilvra Woods State Natural Area contained 201 individuals of *Anemone acutiloba*, 133 of *Sanguinaria canadensis*, and 433 of *Trillium flexipes*. While the density of *Trillium* was fairly consistent across the plot, *Sanguinaria* and *Anemone* were more patchily distributed (Fig. 1). These distributional differences among focal species reflected variation in soil depth and proximity to neighboring trees (Fig. 2). *Anemone* occurred more frequently at shallow soil depths compared to *Sanguinaria*, *Trillium*, and the background distribution of soil depth within the plot (Fig. 2a; Spatial ANOVA: $F_{3,993} = 45.799$, $P < 0.001$). Only 21% of *Anemone* individuals occurred at soil depths greater than 50 cm while 56% of *Sanguinaria* and 66% of *Trillium* were located in microsites with soil depths greater than 50 cm. *Anemone* was restricted to shallower soils than the reference distribution of soil depths within the plot (27.75 cm +/- 1.21 standard error versus 42.47 cm +/- 0.97 on average for *Anemone* and background respectively). We found no evidence that the soil depth distributions of *Trillium* (41.60 cm +/- 0.84 on average) and *Sanguinaria* (39.76 cm +/- 1.42) differed from one another. On average, *Sanguinaria* was found in slightly shallower soil than expected from the reference distribution. We found no difference in soil depth between *Trillium* and the background distribution of soil depth within the plot. Accounting for spatial covariance in soil depth ANOVAs improved the model (Likelihood ratio test comparing model with and without spatial structure: $\chi^2 = 43.607$, $P < 0.001$). The

semivariogram of soil depth reveals highly localized spatial dependence (range of spatial dependence is 1.72 m) and relatively weak spatial structure that accounts for just 23.1 percent of the semivariance (Fig. 3).

The three focal species also differed in proximity to neighboring trees (Fig. 2b; ANOVA: $F_{2,764} = 27.870$, $P < 0.001$). *Anemone* occurred in closer proximity to neighboring trees (on average 1.22 m \pm 0.08 standard error) than either *Sanguinaria* (1.77 m \pm 0.09) or *Trillium* (1.91 m \pm 0.05). In the plot, 81% of *Anemone* were located within 2 m of a tree, compared to just 61% of *Sanguinaria* and 56 percent of *Trillium*. There was no significant difference between *Sanguinaria* and *Trillium* in distance to nearest tree. Soil depth increased with distance to the nearest tree within the plot ($r = 0.44$, $P < 0.001$), though proximity to neighboring trees explained only 19.7% of the variance in soil depth (Appendix S1: Fig. S4).

In our analysis of spatial point patterns, all three focal species exhibited conspecific aggregation at fine spatial scales within 2 m (Fig. 3a, b, c). The probability of observing a conspecific individual within 2 m of a given focal individual were far greater than would be expected if plants were distributed randomly in space. Additionally, each focal species tended to form clusters distributed regularly at larger spatial scales leading to a lower probability of observing conspecific pairs beyond 8 meters than would be expected if plants were randomly distributed. *Anemone* exhibited particularly strong spatial clustering within 2 meters and consistent segregation beyond 4 meters. In general, heterospecific individuals occurred at random with respect to one another at small distances (< 2 m) but exhibited spatial segregation at distances greater than ~ 3 m (Fig. 3e, f, g). *Anemone* and *Trillium* were more likely to co-occur within 0.5 m than would be expected if species were distributed at random with respect to one another but interspecific pairs otherwise co-occurred at random within 2 m. At distances greater than 3 m, all three focal species co-occurred less often than expected from spatially random distributions reflecting the tendency for species to form conspecific clumps. *Trillium* and *Sanguinaria* occurred at random with respect to one another within 2 meters but were spatially segregated at distances greater than 2 meters (Fig. 3f). Our spatial analysis further illustrated clear differences in the distribution of focal species in relation to trees. *Anemone* occurred more frequently near the base of trees (within 1 m) than expected if

plants were distributed randomly in space (Fig. 3g; Appendix S1: Fig. S5). Meanwhile, *Sanguinaria* and *Trillium* occurred essentially at random with respect to trees in the plot (Fig. 3h, i).

DISCUSSION

Our study documented differences in the local spatial distribution of three herbaceous plant species common to North American temperate forests that were consistent with fine-scale spatial niche partitioning. The local distribution of *Anemone acutiloba*, *Sanguinaria canadensis*, and *Trillium flexipes* reflected variation in soil depth and proximity to neighboring trees. *Anemone* occupied shallower soils and was found in closer proximity to trees than *Sanguinaria* or *Trillium*. These differential responses to environmental variation resulted in spatial segregation among species, an important prerequisite for environmental heterogeneity to facilitate local coexistence (Chesson 2000b, Amarasekare 2003). Moreover, the microsites occupied by each focal species were consistent with our predictions based on interspecific differences in plant stature, nutrient requirements, and accompanying physiological traits suggesting local species-environment distributions result from environmental sorting and competition. *Anemone* – a short-statured evergreen species with low photosynthetic rates, low nutrient demands, and shallow root system – occurred more commonly in microsites with shallow soils and near canopy trees. Meanwhile, *Sanguinaria* and *Trillium* – relatively large-statured early summer species with higher photosynthetic rates, greater nutrient demands, and more substantive root systems – were distributed across a broader range of soil depths and did not cluster near canopy trees.

Although previous research has demonstrated how environmental heterogeneity influences local herb distributions within temperate forests (Struik and Curtis 1962, Bratton 1976, Hicks 1980, Beatty 1984, 2014, Crozier and Boerner 1984, Vellend et al. 2000, Frelich et al. 2003, Burton et al. 2011, McIntosh et al. 2016), our study links differential species' responses to local environmental variation to interspecific differences in leaf phenology, leaf stature, and rooting depth. Bratton (1976) previously argued that rooting depth constrained the distribution of herbaceous species with more substantial belowground organs to deeper soils. Meanwhile, herbs with fibrous roots and small rhizomes were more

commonly found in shallow soil microsites near the base of trees, boulders, or exposed rocky substrate. The distribution of *Anemone*, *Sanguinaria*, and *Trillium* are consistent with these predictions. However, other physiological differences accompany this variation in rooting depth (Givnish 1987, Uemura 1994, Neufeld and Young 2014). Evergreen herbs like *Anemone* often have shallow rooting systems, shorter stature, lower photosynthetic rates, and lower nutrient demands (Givnish 1987). Furthermore, as noted previously, soil depth is just one of several environmental factors that influences plant growth and competition near the base of trees. These microsites are often nutrient poor either as a result of reduced soil volume or leaching caused by stemflow (Barbier et al. 2008). Increased stem flow washes away fine particles at the base of tree trunks which are important for retaining moisture and soil nutrients.

Furthermore, early snowmelt and the formation of thaw circles may affect plant distributions by extending the growing season near the base of trees (Veblen et al. 1977, Vellend et al. 2017). As dark tree boles absorb sunlight in early spring, radiant heat melts snow and accelerates soil thaw near the tree base. The additional days of unobstructed light and access to belowground water should provide an important energetic boost for evergreen plants, such as *Anemone* growing near the base of trees. Spring ephemerals could similarly benefit from an extended photosynthetic season (Vellend et al. 2017), though they have greater nutrient demands than evergreen herbs and may be inhibited by leaching near tree boles. In contrast, early thaw should have less influence on early summer species such as *Sanguinaria* and *Trillium* whose leaves emerge later in the spring and have a long photosynthetic season. The greater height of *Sanguinaria* and *Trillium*, however, should be advantageous in microsites with greater herb coverage on deeper soils further from tree bases, where they competitively exclude short evergreen species with low rates of photosynthesis (see Givnish 1982, Givnish 1987, 1995, and Introduction). Shorter, shallowly rooting evergreens should be better able to tolerate thin soils near tree boles, and such microsites might become refuges from taller competitors. Other relatively small-statured plants with insubstantial root systems that exhibit similar a similar distribution to *A. acutiloba* include *Mitella diphylla* (J. Beck, *personal observation*), *Micranthes virginiensis* (T. Givnish, *personal observation*), and *Sedum ternatum* (Bratton 1976). Physiological integration among rooting depth, leaf height, nutrient requirements, plant

hydraulics, and photosynthetic phenology coupled with covariance among multiple environmental factors makes it difficult to disentangle which factors constrain local herb distributions in an observational study (Givnish 1982, 1987, 1995, Uemura 1994, Lapointe 2001, Rothstein and Zak 2001, Neufeld and Young 2014).

Past studies exploring the context-dependent advantages of particular physiological attributes suggest competitive interactions mediate how species sort spatially in heterogeneous habitats (Givnish 1982, 1987, 1995). As expected, we found evergreen *Anemone* restricted to thin soils near tree boles (Fig. 2; Fig. 3g; Appendix 1: Fig. S5). In contrast, *Sanguinaria* and *Trillium* occur at random with respect to trees and deviate little from the background distribution of soil depths within the focal forest stand (Fig. 2; Fig. 3h, i). With strong interspecific competition, we expected to observe spatial segregation among species at the scale they are competing for resources such as light, water, and soil resources (< 1 m). However, we did not observe heterospecific spatial segregation within 1 m (Fig. 3d, e, f). The lack of spatial segregation at this scale may be attributed to several factors such as reduced power to detect spatial associations at small distances, the relatively slow pace of competitive exclusion among perennial herbs, or weak spatial structure in soil depth. Tree roots and submerged, decomposing woody debris accounted for much of the variation in soil depth leading to heterogeneous soil depths that varied considerably over short distances (Fig. 3). Nevertheless, the spatial segregation among focal species beyond 2 m and environmental distributions are consistent with our predictions and provide evidence of local spatial niche partitioning.

The tendency of our focal species to cluster spatially likely reflects, at least in part, patterns of seed dispersal. All three species exhibited conspecific aggregation within ~2 m (Fig. 3a, b, c). Given that soil depth within the plot exhibited relatively weak spatial structure, patterns of seed dispersal likely contribute to conspecific spatial aggregation. All three focal species possess elaiosomes and are primarily dispersed by ants (Pudlo et al. 1980, Smith et al. 1989). Dispersal in myrmecorous species tends to occur at relatively short distances, often less than 1 m (Smith et al. 1989, Kalisz et al. 1999). Limited dispersal likely reinforces fine-scale conspecific aggregation and the tendency for species to segregate and form

regularly spaced clumps at coarser scales. Notably, the observed scale of spatial dependence in soil depth (1.72 m; Fig. 3), the spatial scale of intraspecific aggregation (0.75 – 1.59 m; Fig. 4), and expected dispersal distances (1-2 m) all align closely in our study. More extensive dispersal has the potential to undermine the stabilizing effects of environmental heterogeneity on species coexistence if rates of dispersal outpace the competitive interactions that sort species according to local environmental variation (Snyder and Chesson 2004, Leibold et al. 2004).

Niche-based processes predicated on environmental heterogeneity and spatial resource partitioning are often invoked to explain local patterns of species diversity in temperate forests and many other plant communities (Chesson 2000a, Amarasekare 2003, Silvertown 2004, HilleRisLambers et al. 2012, Stein et al. 2014). Nevertheless, uncertainties about the scale of environmental heterogeneity relative to dispersal capacity and whether physiological tradeoffs are sufficiently strong to maintain local species-environment associations cloud how important a role fine-scale environmental variation plays in maintaining local plant diversity (Lundholm 2009, Tamme et al. 2010, Beatty 2014). This observational study of herbaceous plant distributions in a temperate forest illustrates how characterizing the spatial structure of environmental variation and empirical patterns of aggregation and segregation in relation to dispersal capacity is critical for assessing the potential for environmental heterogeneity to drive fine-scale spatial niche partitioning in plant communities. We show that local species-environment relationships are predictable based on physiological characteristics and that heterospecific individuals remain spatially segregated as environmental sorting, competition, and dispersal play out at local scales. These findings are consistent with spatial niche partitioning and provide insights into how fine-scale environmental heterogeneity, physiological differences, and local dispersal structure local plant distributions among temperate forest herbs (Bratton 1976, Amarasekare 2003, Silvertown 2004, Beatty 2014). This study advances our understanding of how environmental heterogeneity could maintain high local plant diversity in temperate forest understories (Bratton 1976, Beatty 1984, 2014, Bell et al. 2000, Vellend et al. 2000, Peet et al. 2014, Catella et al. 2019), the spatial scale at which forest herbs can partition habitat (Amarasekare 2003, Hart et al. 2017), and, more generally, points to the importance of spatial resource

partitioning in promoting local plant diversity (Amarasekare 2003, Silvertown 2004, Lundholm 2009, Bartels and Chen 2010, Stein et al. 2014).

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Table 1. Comparison of relevant ecological and physiological characteristics among focal forest plant species. Mean leaf height (cm) and leaf N content (percent by mass) derived from the UW-Madison Plant Ecology Laboratory trait database (Amatangelo et al. 2014, Sonnier et al. 2014).

	Focal species		
	<i>Anemone acutiloba</i>	<i>Sanguinaria canadensis</i>	<i>Trillium flexipes</i>
Family	Ranunculaceae	Papaveraceae	Melanthiaceae
Flowering phenology	Early spring (March-May)	Early spring (March-May)	Early summer (May-June)
Leaf (photosynthetic) phenology	One-year evergreen (retains leaves for one full year)	Early Summer (leaves emerge during flowering period but open in May and persist through mid-summer)	Early summer (leaves emerge in May and persist through mid-summer)
Rooting physiology	Shallow, fibrous root system	Deeper fibrous roots extending from thickened rhizome	Deeper fibrous roots extending from thickened rhizome
Leaf height (mean)	< 15 cm (11.9)	15-35 cm (25.3)	25-50 cm (32.1)
Nutrient demands (leaf N percent by mass)	Low (2.1%)	Intermediate (2.9%)	High (3.9%)
Seed dispersal mode	Ant (elaiosomes)	Ant (elaiosomes)	Ant (elaiosomes)

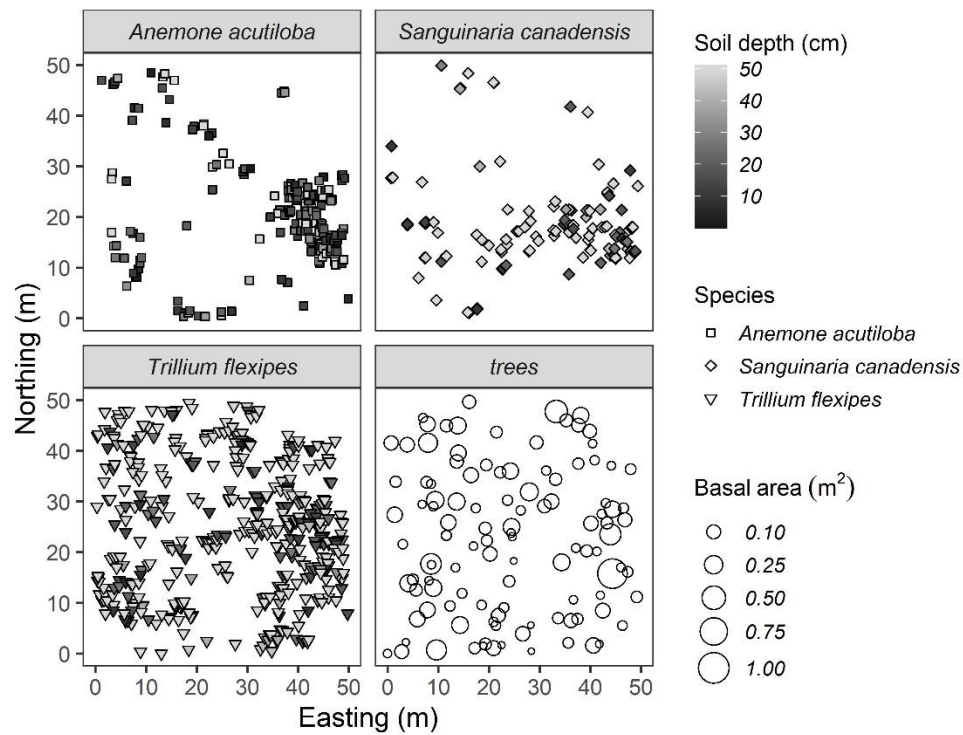


Figure 1. Map illustrating the spatial distribution of *Anemone acutiloba*, *Sanguinaria canadensis*, *Trillium flexipes*, and trees greater than 1 cm DBH within a 50 x 50 meter plot at McGilvra Woods State Natural Area (Sauk County, WI USA). Each point represents the location of a focal herb or tree. The Easting and Northing coordinates measure the distance in meters from the origin (southwest corner) of the plot. Herb points are shaded according to the measured soil depth and the size of tree circles is proportional to its basal area.

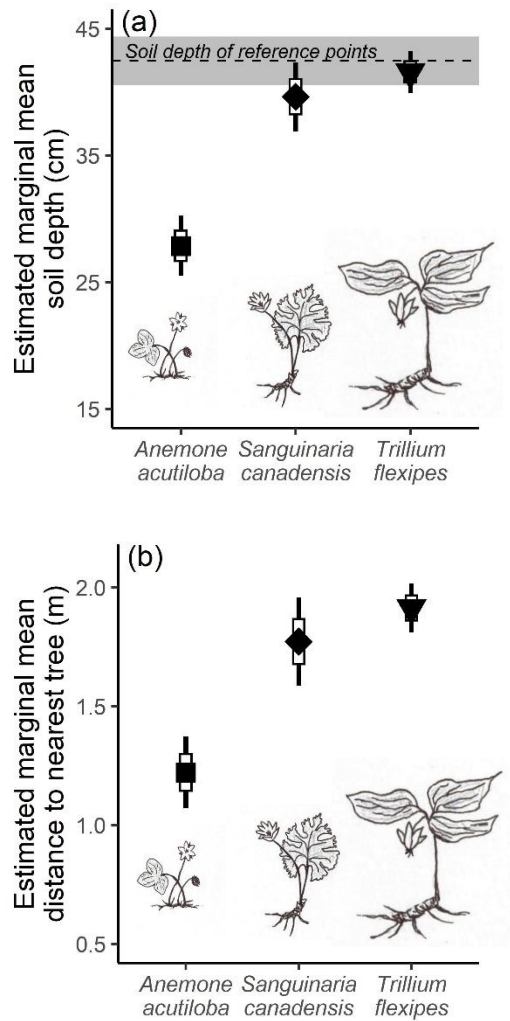


Figure 2. Estimated marginal means for (a) soil depth and (b) proximity to nearest tree for each focal species. The statistical model examining comparative soil depth accounted for spatial covariance. Black bars represent 95% confidence intervals and white bars represent one standard error of the estimated marginal mean. The dashed line represents the estimated marginal mean of soil depth for reference points within the focal plot and the grey shaded area illustrates a 95% confidence interval for the reference points. Illustrations courtesy of M. Persche.

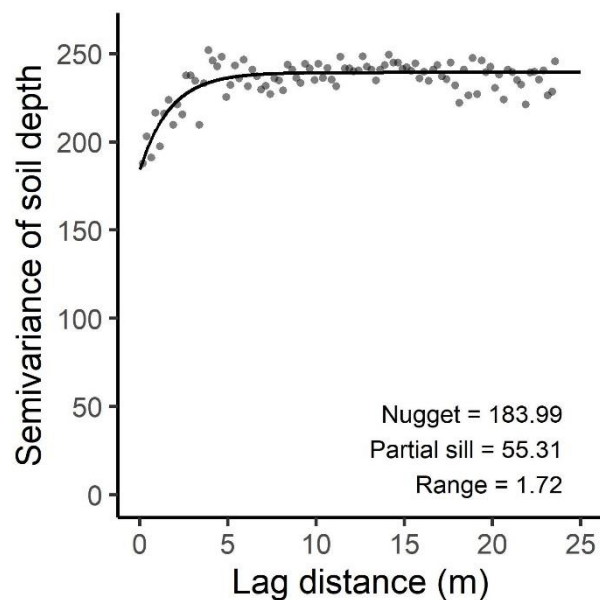


Figure 3. Semivariogram illustrating the spatial dependency of soil depth within the study plot at McGilvra Woods State Natural Area (Sauk County, WI). Points represent empirical estimates of semivariance versus the distance between samples and the line represents the predicted semivariance from an exponential model. Text describes the estimated nugget, partial sill (sill – nugget), and range for the semivariogram model.

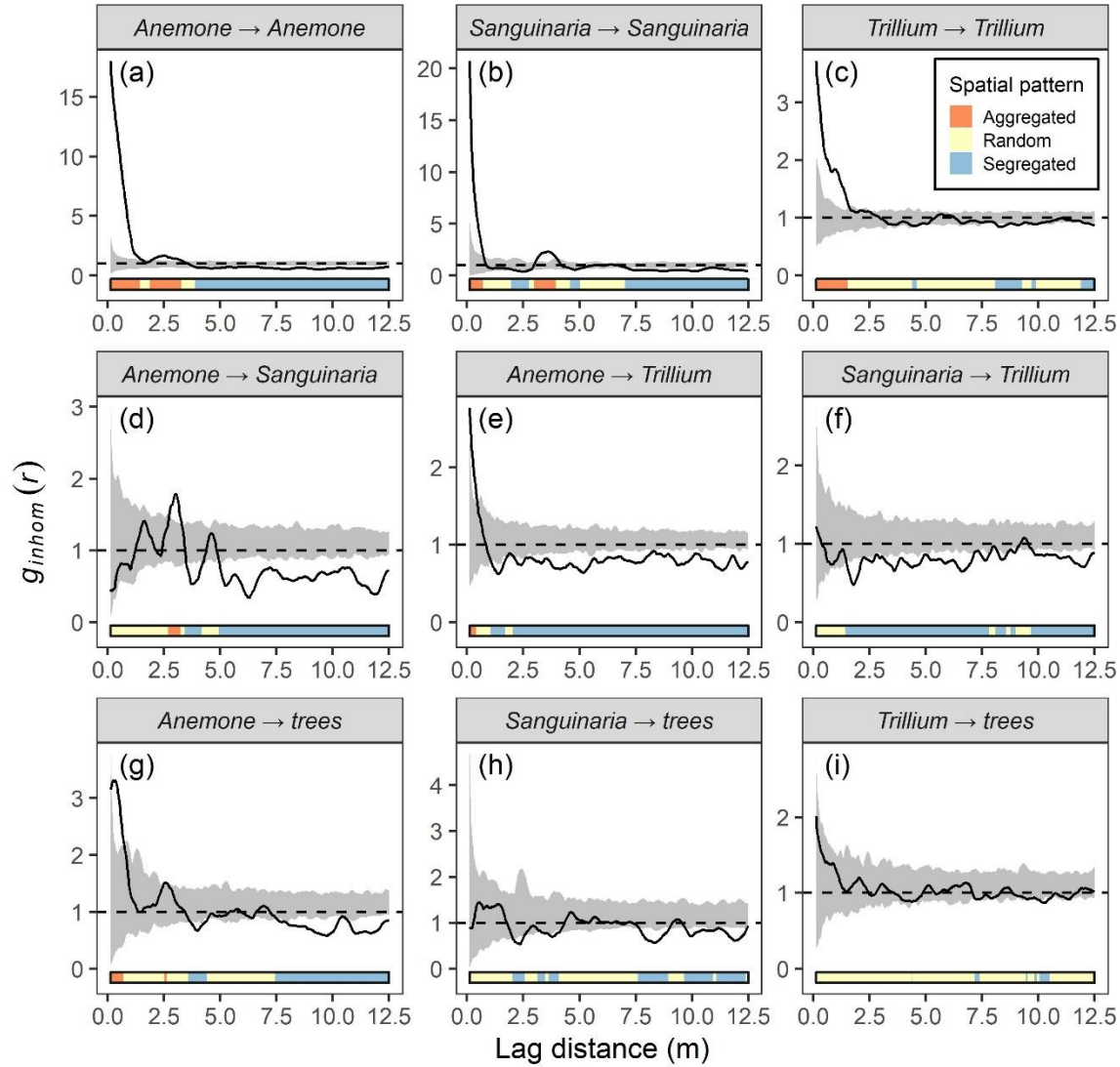


Figure 4. Pair correlation functions describing how the probability of observing a focal individual varied with proximity to conspecific individuals (a, b, c), heterospecific individuals for each pair of focal species (d, e, f), and neighboring trees (g, h, i). The black line illustrates how the observed pair correlation function, $g_{inhom}(r)$, varies with distance while the grey ribbon represents a simulation envelope for $g_{inhom}(r)$ under complete spatial randomness. Aggregated spatial patterns occur when the probability of occurrence exceeds that expected under spatial randomness while segregated patterns occur when the probability of occurrence falls below that expected under complete spatial randomness.

LITERATURE CITED

- Adler, P. B., S. P. Ellner, and J. M. Levine. 2010. Coexistence of perennial plants: An embarrassment of niches. *Ecology Letters* 13:1019–1029.
- Adler, P. B., J. HilleRisLambers, P. C. Kyriakidis, Q. Guan, and J. M. Levine. 2006. Climate variability has a stabilizing effect on the coexistence of prairie grasses. *Proceedings of the National Academy of Sciences* 103:12793–8.
- Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: A synthesis. *Ecology Letters* 6:1109–1122.
- Amatangelo, K., S. Johnson, D. Rogers, and D. M. Waller. 2014. Trait-environment relationships remain strong despite 50 years of trait compositional change in temperate forests. *Ecology* 95:1780–1791.
- Anderson, A. R. C., O. L. Loucks, and A. M. Swain. 1969. Herbaceous response to canopy cover, light intensity, and throughfall precipitation in coniferous forests. *Ecology* 50:255–263.
- Angert, A. L., T. E. Huxman, P. Chesson, and D. L. Venable. 2009. Functional tradeoffs determine species coexistence via the storage effect. *Proceedings of the National Academy of Sciences* 106:11641–11645.
- Augusto, L., J. Dupouey, and J. Ranger. 2003. Effects of tree species on understory vegetation and environmental conditions in temperate forests. *Annals of Forest Science* 60:823–831.
- Baddeley, A. 2005. spatstat: An R Package for Analyzing Spatial Point Patterns. *J. Stat. Soft.* 12:1–42.
- Barbier, S., F. Gosselin, and P. Balandier. 2008. Influence of tree species on understory vegetation diversity and mechanisms involved – A critical review for temperate and boreal forests. *Forest Ecology and Management* 254:1–15.
- Bartels, S. F., and H. Y. H. Chen. 2010. Is understory plant diversity driven by resource quantity or resource heterogeneity? *Ecology* 91:1931–1938.
- Beatty, S. W. 1984. Influence of microtopography and canopy species on spatial patterns of forest understory plants. *Ecology* 65:1406–1419.
- Beatty, S. W. 2014. Habitat Heterogeneity and the Maintenance of Species in Understory Communities. Pages 215–232 in F. S. Gilliam, editor. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Beatty, S. W., and E. L. Stone. 1986. The variety of soil microsites created by tree falls. *Canadian Journal of Forest Research* 16:539–548.

- Bell, G., M. J. Lechowicz, and M. J. Waterway. 2000. Environmental heterogeneity and species diversity of forest sedges. *Journal of Ecology* 88:67–87.
- Bivand, R. S., E. Pebesma, and V. Gómez-Rubio. 2013. *Applied Spatial Data Analysis with R*. Page Applied Spatial Data Analysis with R: Second Edition. Springer.
- Bratton, S. P. 1976. Resource division in an understory herb community: Responses to temporal and microtopographic gradients. *The American Naturalist* 110:679–693.
- Burton, J. I., D. J. Mladenoff, M. K. Clayton, and J. A. Forrester. 2011. The roles of environmental filtering and colonization in the fine-scale spatial patterning of ground-layer plant communities in north temperate deciduous forests. *Journal of Ecology* 99:764–776.
- Burton, J. I., D. J. Mladenoff, J. A. Forrester, and M. K. Clayton. 2014. Experimentally linking disturbance, resources and productivity to diversity in forest ground-layer plant communities. *Journal of Ecology* 102:1634–1648.
- Canham, C. D., and D. H. Burbank. 1994. Causes and consequences of resource heterogeneity in forests: Interspecific variation in light transmission by canopy trees. *Canadian Journal of Forest Research* 24:337–349.
- Catella, S. A., S. R. Eysenbach, and K. C. Abbott. 2019. Novel insights into how the mean and heterogeneity of abiotic conditions together shape forb species richness patterns in the Allegheny plateau ecoregion. *Ecology and Evolution*:12374–12389.
- Chesson, P. 2000a. Mechanisms of maintenance of species diversity. *Annual Review of Ecology, Evolution, and Systematics* 31:343–358.
- Chesson, P. 2000b. General theory of competitive coexistence in spatially-varying environments. *Theoretical population biology* 58:211–37.
- Chesson, P. L., and R. R. Warner. 1981. Environmental variability promotes coexistence in lottery competitive systems. *The American naturalist* 117:923–943.
- Chudomelová, M., D. Zelený, and C. F. Li. 2017. Contrasting patterns of fine-scale herb layer species composition in temperate forests. *Acta Oecologica* 80:24–31.
- Collins, B. S., L. S. Ferrara, and H. L. Motto. 1984. Coincidence of spring herb distribution and flowering with tree bases in a New Jersey Piedmont forest. *Bulletin of the Torrey Botanical Club* 111:301.
- Costanza, J. K., A. Moody, and R. K. Peet. 2011. Multi-scale environmental heterogeneity as a predictor of plant species richness. *Landscape Ecology* 26:851–864.
- Crozier, C. R., and R. E. J. Boerner. 1984. Correlation of understory herb distribution patterns with

- microhabitats under different tree species in a mixed mesophytic forest. *Oecologia* 62:337–343.
- Curtis, J. T. 1959. *The Vegetation of Wisconsin: An Ordination of Plant Communities*. The University of Wisconsin Press, Madison, WI.
- Dybzinski, R., and D. Tilman. 2007. Resource use patterns predict long-term outcomes of plant competition for nutrients and light. *American Naturalist* 170:305–318.
- Finzi, A. C., N. Van Breemen, and C. D. Canham. 1998. Canopy tree-soil interactions within temperate forests: Species effects on soil carbon and nitrogen. *Ecological Applications* 8:440–446.
- Frelich, L. E., J. L. Machado, and P. B. Reich. 2003. Fine-scale environmental variation and structure of understorey plant communities in two old-growth pine forests. *Journal of Ecology* 91:283–293.
- Gilbert, B., and M. J. Lechowicz. 2004. Neutrality, niches, and dispersal in a temperate forest understory. *Proceedings of the National Academy of Sciences* 101:7651–7656.
- Gilliam, F. S. 2007. The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. *BioScience* 57:845.
- Gilliam, F. S., N. T. Welch, A. H. Phillips, J. H. Billmyer, W. T. Peterjohn, Z. K. Fowler, C. A. Walter, M. B. Burnham, J. D. May, and M. B. Adams. 2016. Twenty-five-year response of the herbaceous layer of a temperate hardwood forest to elevated nitrogen deposition. *Ecosphere* 7:1–16.
- Givnish, T. J. 1982. On the adaptive significance of leaf height in forest herbs. *American Naturalist* 120:353–381.
- Givnish, T. J. 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytologist* 106:131–160.
- Givnish, T. J. 1995. Plant stems: biomechanical adaptation for energy capture and influence on species distributions. Pages 3–49 *Plant stems: Physiology and functional morphology*.
- Givnish, T. J. 2002. Adaptive significance of evergreen vs. deciduous leaves: Solving the triple paradox. *Silva Fennica* 36:703–743.
- Goldberg, D. E., and A. M. Barton. 1992. Patterns and consequences of interspecific competition in natural communities: A review of field experiments with plants. *The American Naturalist* 139:771–801.
- Graves, J. H., R. K. Peet, and P. S. White. 2006. The influence of carbon-nutrient balance on herb and woody plant abundance in temperate forest understories. *Journal of Vegetation Science* 17:217.
- Hart, S. P., J. Usinowicz, and J. M. Levine. 2017. The spatial scales of species coexistence. *Nature*

- Ecology and Evolution 1:1066–1073.
- Hicks, D. J. 1980. Intrastand distribution patterns of southern Appalachian cove forest herbaceous species. *The American Midland Naturalist* 104:209–223.
- HilleRisLambers, J., P. B. Adler, W. S. Harpole, J. M. Levine, and M. M. Mayfield. 2012. Rethinking community assembly through the lens of Coexistence theory. *Annual Review of Ecology, Evolution, and Systematics* 43:120830113150004.
- Kalisz, S., F. M. Hanzawa, S. J. Tonsor, D. A. Thiede, and S. Voigt. 1999. Ant-mediated seed dispersal alters pattern of relatedness in a population of *Trillium grandiflorum*. *Ecology* 80:2620–2634.
- Kneitel, J. M., and J. M. Chase. 2004. Trade-offs in community ecology: Linking spatial scales and species coexistence. *Ecology Letters* 7:69–80.
- Kraft, N. J. B., O. Godoy, and J. M. Levine. 2015. Plant functional traits and the multidimensional nature of species coexistence. *Proceedings of the National Academy of Sciences* 112:797–802.
- Kreft, H., and W. Jetz. 2007. Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences* 104:5925–5930.
- Lange, K. I. 1998. Flora of Sauk County and Caledonia Township, Columbia County, south central Wisconsin. Madison, WI.
- Lapointe, L. 2001. How phenology influences physiology in deciduous forest spring ephemerals. *Physiologia Plantarum* 113:151–157.
- Leach, M. K., and T. J. Givnish. 1999. Gradients in the composition, structure, and diversity of remnant oak savannas in southern Wisconsin. *Ecological Monographs* 69:353–374.
- Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7:601–613.
- Levine, J. M., and M. Rees. 2004. Effects of temporal variability on rare plant persistence in annual systems. *The American naturalist* 164:350–63.
- Lopez, O. R., K. Farris-Lopez, R. A. Montgomery, and T. J. Givnish. 2008. Leaf phenology in relation to canopy closure in southern Appalachian trees. *American Journal of Botany* 95:1395–1407.
- Lundholm, J. T. 2009. Plant species diversity and environmental heterogeneity: Spatial scale and competing hypotheses. *Journal of Vegetation Science* 20:377–391.
- McIntosh, A. C. S., S. E. Macdonald, and S. A. Quideau. 2016. Understory plant community composition

- is associated with fine-scale above- and below-ground resource heterogeneity in mature lodgepole pine (*Pinus contorta*) forests. *PLoS ONE* 11:1–17.
- Moore, M. R., and J. L. Vankat. 1986. Responses of the herb layer to the gap dynamics of a mature beech-maple forest. *American Midland Naturalist* 115:336–347.
- Neufeld, H. S., and D. R. Young. 2014. Ecophysiology of the herbaceous layer in temperate deciduous forests. Pages 35–96 in F. S. Gilliam, editor. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Pebesma, E. J. 2004. Multivariable geostatistics in S: The gstat package. *Computers and Geosciences* 30:683–691.
- Peet, R. K., K. A. Palmquist, and S. M. Tessel. 2014. Herbaceous layer species richness of southeastern forests and woodlands: Patterns and causes. Pages 255–276 in F. S. Gilliam, editor. *The Herbaceous Layer in forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Peterson, C. J., W. P. Carson, B. C. McCarthy, S. T. A. Pickett, C. J. Peterson, W. P. Carson, B. C. McCarthy, S. T. A. Pickett, and S. T. A. Microsite. 1990. Microsite variation and soil dynamics within newly created treefall pits and mounds. *Oikos* 58:39–46.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. Core Team. 2018. nlme: Linear and nonlinear mixed effects models. R package.
- Pudlo, R. J., A. J. Beattie, and D. C. Culver. 1980. Population consequences of changes in an ant-seed mutualism in *Sanguinaria canadensis*. *Oecologia* 46:32–37.
- R Core Team. 2019. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ricklefs, R. E. 1977. Environmental heterogeneity and plant species diversity: A hypothesis. *The American Naturalist* 111:376–381.
- Rogers, D. A., T. P. Rooney, D. Olson, and D. M. Waller. 2008. Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. *Ecology* 89:2482–2492.
- Rothstein, D. E., and D. R. Zak. 2001. Photosynthetic adaptation and acclimation to exploit seasonal periods of direct irradiance in three temperate, deciduous-forest herbs. *Functional Ecology* 15:722–731.
- Sabatini, F. M., B. Jimenez-Alfaro, S. Burrascano, and C. Blasi. 2014. Drivers of herb-layer species diversity in two unmanaged temperate forests in northern Spain. *Community Ecology* 15:113–54–64.

- Scheller, R. M., and D. J. Mladenoff. 2002. Understory species patterns and diversity in old-growth and managed northern hardwood forests. *Ecological Applications* 12:1329–1343.
- Silvertown, J. 2004. Plant coexistence and the niche. *Trends in Ecology and Evolution* 19:605–611.
- Smith, B. H., P. D. Forman, and A. E. Boyd. 1989. Spatial patterns of seed dispersal and predation of two myrmecochorous forest herbs. *Ecology* 70:1649–1656.
- Snyder, R. E., and P. Chesson. 2004. How the spatial scales of dispersal, competition, and environmental heterogeneity interact to affect coexistence. *American Naturalist* 164:633–650.
- Sonnier, G., S. E. Johnson, K. L. Amatangelo, D. A. Rogers, and D. M. Waller. 2014. Is taxonomic homogenization linked to functional homogenization in temperate forests? *Global Ecology and Biogeography* 23:894–902.
- Stein, A., K. Gerstner, and H. Kreft. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecology Letters* 17:866–880.
- Struik, G. J., and J. T. Curtis. 1962. Herb distributions in an *Acer saccharum* forest. *American Midland Naturalist* 68:285–296.
- Tamme, R., I. Hiiesalu, L. Laanisto, R. Szava-Kovats, and M. Pärtel. 2010. Environmental heterogeneity, species diversity and co-existence at different spatial scales. *Journal of Vegetation Science* 21:796–801.
- Thompson, J. N. 1980. Treefalls and Colonization Patterns of Temperate Forest Herbs. *American Midland Naturalist* 104:176.
- Tilman, D. 1982. *Resource Competition and Community Structure*. Princeton University Press.
- Uemura, S. 1994. Patterns of leaf phenology in forest understory. *Canadian Journal of Botany* 72:409–414.
- Veblen, T. T., D. H. Ashton, F. M. Schlegel, and A. T. Veblen. 1977. Distribution and dominance of species in the understorey of a mixed evergreen-deciduous *Nothofagus* forest in south-central Chile. *Journal of Ecology* 65:815–830.
- Vellend, M., M. J. Lechowicz, and M. J. Waterway. 2000. Environmental distribution of four *Carex* species (Cyperaceae) in an old-growth forest. *American Journal of Botany* 87:1507–1516.
- Vellend, M., A. B. Young, G. Letendre, and S. Rivest. 2017. Thaw circles around tree trunks provide spring ephemeral plants with a big head start on the growing season. *Ecology* 98:3224–3226.
- Whittaker, R. H. 1956. Vegetation of the Great Smoky Mountains. *Ecological Monographs* 26:1–80.

- Wickham, H. 2009. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York, USA.
- Wiegand, T., and K. A. Moloney. 2004. Rings, circles, and null-models for point pattern analysis in ecology. *Oikos* 104:209–229.
- Wiegand, T., and K. A. Moloney. 2014. *Handbook of Spatial Point-Pattern Analysis in Ecology*. Chapman and Hall/CRC Press, Boca Raton, FL.
- Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York, NY, USA.

APPENDIX S3. Supplemental materials for Chapter 3.

Table S1. Summary of tree composition within the 50 x 50 m study plot. The 50 x 50 m plot contained 110 trees greater than 1 cm diameter at breast height (DBH at 1.3 m) representing eight species. Table includes the total number of individuals within the study plot, the cumulative basal area (at 1.3 m) for each tree species, and the relative basal area.

Species	No. trees	Basal area (m ²)	Relative basal area
<i>Acer saccharum</i>	79	3.92	0.40
<i>Quercus rubra</i>	5	2.12	0.22
<i>Tilia americana</i>	10	1.67	0.17
<i>Fraxinus americana</i>	7	0.99	0.10
<i>Carya cordiformis</i>	5	0.50	0.05
<i>Quercus alba</i>	1	0.35	0.04
<i>Prunus serotina</i>	1	0.12	0.01
<i>Ulmus americana</i>	2	0.07	<0.01

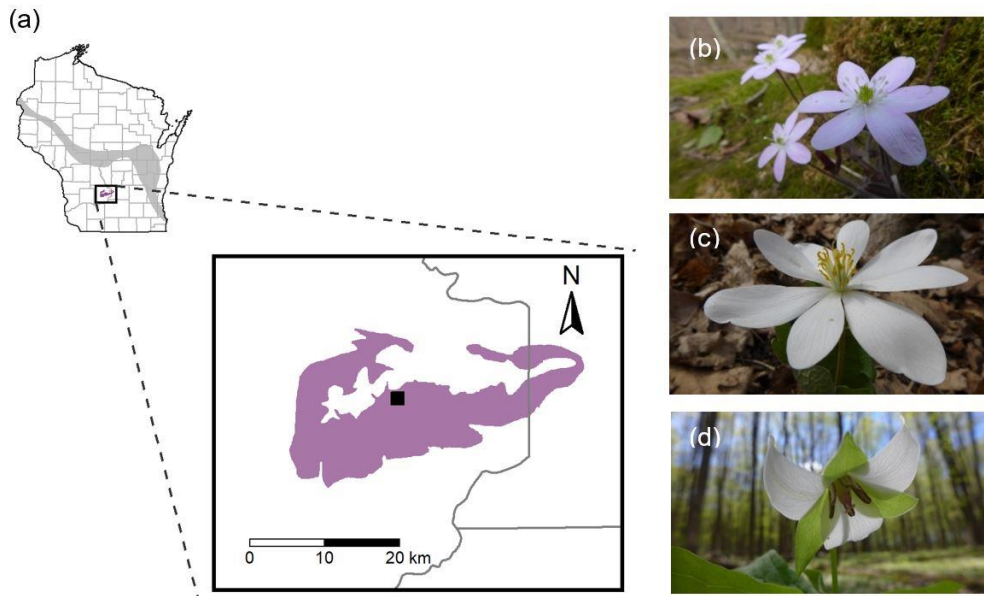


Figure S1. Map illustrating the location of the study site (a), McGilvra Woods State Natural Area (Sauk County, Wisconsin, USA) and photographs of *Anemone acutiloba*, *Sanguinaria canadensis*, and *Trillium flexipes* (b, c, and d respectively). The grey band illustrates the location of a pronounced floristic tension zone (Curtis 1959). In the map inset, the black square depicts the location of our study plot (McGilvra Woods State Natural Area) and the purple region delineates the area encompassed by the Baraboo Hills. Photographs by J. Beck.

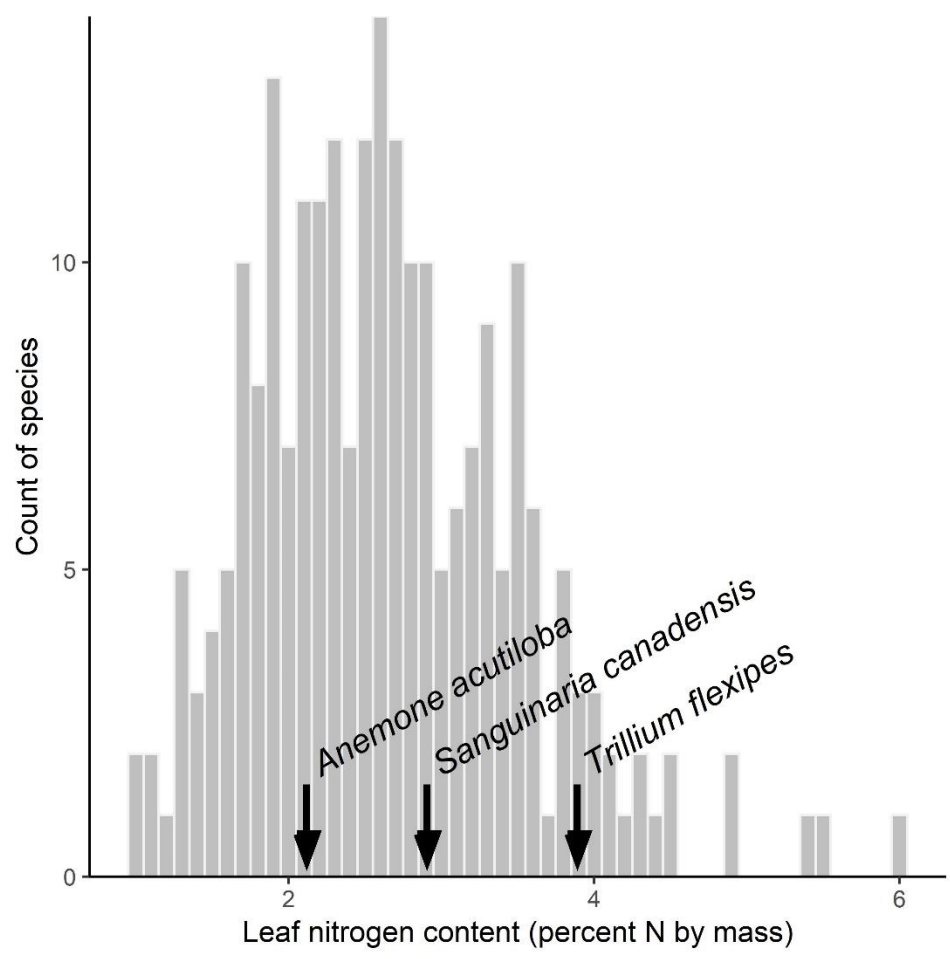


Figure S2. Histogram of leaf nitrogen content for 242 understory forest herbs. Data derived from the University of Wisconsin-Madison Plant Ecology Laboratory trait database. From the distribution of leaf nitrogen (N) contents, we classify *Anemone acutiloba* (28th percentile) as low nutrient requirements, *Sanguinaria canadensis* (66th percentile) as intermediate nutrient requirements, and *Trillium flexipes* (92nd percentile) as high nutrient requirements.

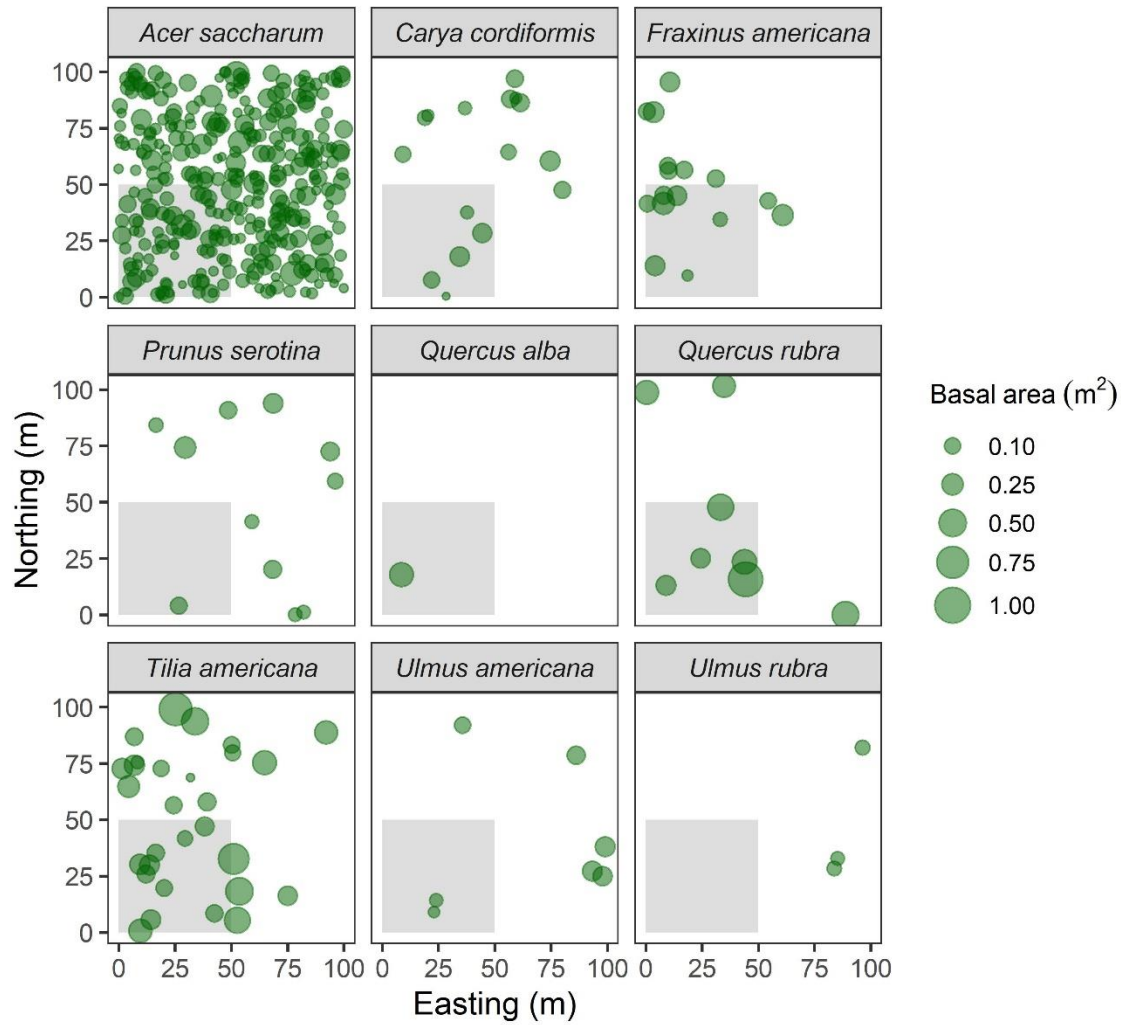


Figure S3. Map of tree distributions within the 1 hectare forest monitoring plot at McGilvra Woods State Natural Area. The grey box illustrates the location the 50 x 50 m subset of the larger 1 ha plot in which herbaceous stems were mapped. Each circle represents a single tree and circle diameters are proportional to the basal area of the trees.

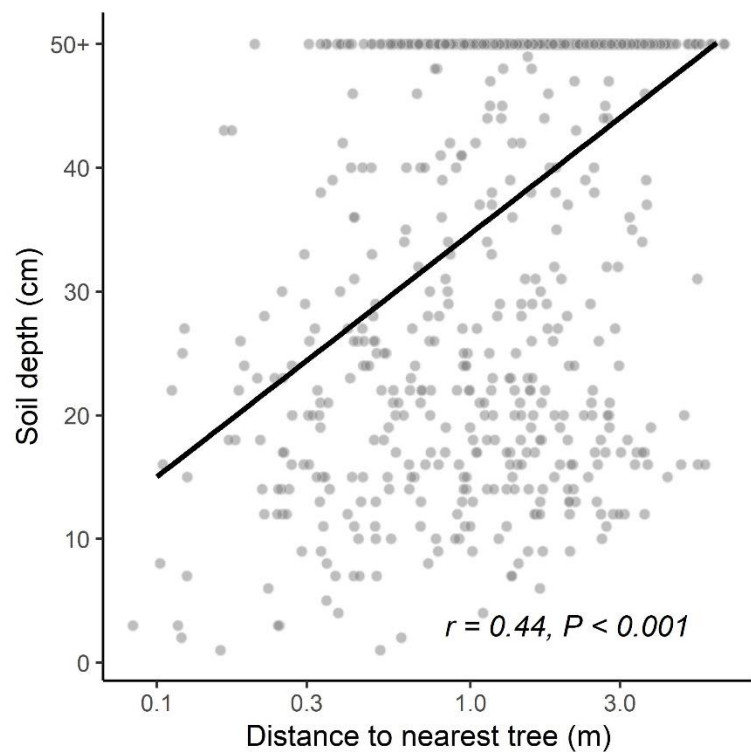


Figure S4. Scatterplot illustrating the relationship between proximity to trees (log-transformed) and soil depth within the study plot at McGilvra Woods State Natural Area.



Figure S5. Photograph illustrating the tendency for *Anemone acutiloba* to cluster near the base of large trees. *Anemone* frequently occupied the shallow soil pocket between root buttresses. Photograph taken within the mapping plot at McGilivra Woods State Natural Area (Sauk County, Wisconsin, USA). Photograph by J. Beck.

Chapter 4. Variation in plant-soil feedbacks among temperate forest herbs

Jared J. Beck¹

¹Department of Botany
University of Wisconsin-Madison
430 Lincoln Drive
Madison, WI 53706

ABSTRACT

Herbaceous plants comprise more than 80% of plant species found in temperate forests, but the ecological processes maintaining local herb diversity are not fully understood. Antagonistic interactions between plants and soil biota promote species diversity in many plant communities. The potential for plant-soil feedbacks to facilitate coexistence among temperate forest herbs has not been investigated.

Methods: To investigate how plant-soil feedbacks affect the growth herbaceous forest plants, I grew seedlings of nine species in the greenhouse in soil that had supported either conspecific or heterospecific plants. This treatment was crossed with a subsequent soil treatment in which half of the soils were pasteurized to eliminate soil microbial communities. If specialized pathogens accumulate locally, plants grown in unpasteurized, conspecific-trained soil should fair worse than plants grown in pasteurized and/or heterospecific-trained soils. The direction and strength of plant-soil feedbacks varied considerably among species. *Thalictrum dioicum*, *Elymus hystrix*, *Solidago flexicaulis*, and *Polemonium reptans* all exhibited reduced growth in unpasteurized soils, suggesting microbial antagonists. *Thalictrum dioicum* seedlings grown in conspecific, unpasteurized soil accumulated 30% less biomass than seedlings grown in heterospecific, unpasteurized soil suggesting that host-specific microbial restrict seedling growth (consistent with the Janzen-Connell hypothesis). In contrast, four species that often form dense clusters showed no significant response to experimental treatments manipulating soil biota. Differences in the strength of plant-soil feedbacks among herbaceous plants play an important but previously underappreciated role in shaping the local abundance, spatial distribution, and diversity of temperate forest herbs.

KEYWORDS: plant-soil feedbacks; Janzen-Connell effects; temperate forests; Wisconsin; coexistence; forest herbs; understory; *Thalictrum dioicum*; *Elymus hystrix*; *Solidago flexicaulis*

INTRODUCTION

Plant-soil interactions play a fundamental role in shaping the composition and diversity of plant communities (Ehrenfeld et al. 2005, Bever et al. 2010). In addition to responding sensitively to variation in soil texture, soil fertility, and the community of microorganisms present in soil, plants modify both abiotic and biotic soil properties in ways that influence the growth and survival of other plants (Reynolds et al. 2003, Bennett and Klironomos 2019). Positive plant-soil feedbacks occur when plants alter biotic and abiotic soil properties in ways that promote the growth and survival of other, often conspecific, neighboring plants (Bever et al. 1997, Van der Putten et al. 2013). These positive feedbacks can result from mutualistic relationships with microorganisms or the creation of favorable environmental conditions that promote the survival and growth of conspecific individuals (Bennett and Klironomos 2019). While positive feedbacks have been observed in some plant communities, negative plant-soil feedbacks appear to be far more common (Kulmatiski et al. 2008). Negative plant-soil feedbacks are the product of plant-soil interactions that are detrimental to the growth and survival of plants, often impairing the growth conspecific individuals (Bever et al. 1997, Van der Putten et al. 2013). These negative feedbacks may result from the depletion of soil nutrients that limit the growth of conspecific plants, or from the accumulation of greater densities of pathogens or herbivores that inhibit conspecific growth or survival (Ehrenfeld et al. 2005, Mordecai 2011, Bever et al. 2015, Liu et al. 2015, Bennett and Klironomos 2019).

Density-dependent growth and mortality caused by pathogens has been implicated as a potentially important mechanism facilitating coexistence and maintaining species diversity in many plant communities (Janzen 1970, Connell 1971, Wright 2002, Mordecai 2011, Terborgh 2012, Bever et al. 2015, Ke and Wan 2019). The accumulation of host-specific pathogens at high population densities or near conspecific adults can cause increased mortality among conspecific individuals or alter competitive dynamics in ways that stabilize multi-species coexistence (Janzen 1970, Connell 1971, Bonanomi et al. 2005, Bever et al. 2015, Ke and Wan 2019). Natural enemies may play a particularly important role in tropical forests (Givnish 1999, Wright 2002, Terborgh 2012, Comita et al. 2014, LaManna et al. 2017) where pathogenic fungi and other soil pathogens cause density-dependent growth and mortality in many

species (Bell et al. 2006, Bagchi et al. 2010, 2014, Maron et al. 2016, Johnson et al. 2017). Negative plant-soil feedbacks are also prevalent among herbaceous plants in temperate grasslands (Klironomos 2002, Petermann et al. 2008, Fitzsimons and Miller 2010, Reinhart 2012) and temperate forest trees (Packer and Clay 2000, Johnson et al. 2014, Bennett et al. 2017, Lankau and Keymer 2018). Despite growing recognition that interactions with soil biota influence the composition and diversity of many plant communities, little is known about the prevalence of plant-soil feedbacks among herbaceous understory species in temperate forests .

Herbaceous taxa represent more than 80 percent of plant species in temperate forests with high densities and diversities of these herbs co-occurring within small areas (Gilliam 2007, Peet et al. 2014). Resource partitioning and environmental heterogeneity are the most widely cited mechanisms facilitating coexistence among forest herbs (Bell et al. 2000, Bartels and Chen 2010, Beatty 2014, Catella et al. 2019). Forest herb distributions vary in response to environmental variation at a variety of spatial scales (Curtis 1959, Struik and Curtis 1962, Beatty 2014, Peet et al. 2014). At local scales, differential plant responses to fine-scale variation in soil depth (Bratton, 1976; Beck and Givnish, *in review*), soil fertility and soil moisture (Collins et al. 1984, Crozier and Boerner 1984, Vellend et al. 2000), microtopography (Beatty 1984, Peterson et al. 1990), light availability (Anderson et al. 1969, Thompson 1980), and other abiotic factors can promote coexistence via spatial resource partitioning. Yet, spatial resource partitioning cannot account for coexistence among many functionally similar species at small spatial scales (Gilliam 2007, Beatty 2014, Peet et al. 2014). Potential interactions among herbaceous plants and soil biota could shape many understory community dynamics and potentially promote local coexistence, but these have received little attention in temperate forests (Whigham 2004, Comita et al. 2014, Gilliam 2014). We propose that such interactions, especially antagonistic interactions with soil pathogens, play an important but previously unappreciated role in the maintenance of herbaceous plant diversity in temperate forests.

Apart from the prevalence of negative plant-soil feedbacks in other temperate ecosystems (Klironomos 2002, Bell et al. 2006, Kulmatiski et al. 2008, Petermann et al. 2008, Fitzsimons and Miller 2010), host-specific pathogens appear to be common among the temperate forest herbs. Empirical studies

have documented evidence of negative plant-soil feedbacks among a handful understory plant species (Shannon et al. 2012, Smith and Reynolds 2015, Ma et al. 2019). Host-specific pathogens, often fungal, have been described for numerous temperate herbs (Tiffany et al. 1984). In some cases, different strains of the same fungal pathogen differentially infect cryptic variations of the same species (Parker 1985, Kartzinel et al. 2016). These infections, in turn, can affect the growth and survival of forest herbs (Wennstrom and Ericson 1994). Plant-soil interactions also mediate the success of species' invasions in temperate forest understories (Shannon et al. 2012, 2014, Smith and Reynolds 2012). For example, *Alliaria petiolata*'s capacity to disrupt mycorrhizal associations contributes to its ability to successfully invades temperate forest understories and displace native herbs and tree seedlings (Prati and Bossdorf 2004, Burke 2008, Wolfe et al. 2008, Lankau 2011). These studies suggest plant-soil interactions influence understory community dynamics but that we need more thorough investigations to assess the prevalence and strength of plant-soil feedbacks among temperate forest herbs.

To explore the potential for plant-soil feedbacks to facilitate coexistence among temperate forest herbs, I conducted a greenhouse experiment designed to assess the direction and strength of plant-soil feedbacks among nine herbaceous plant species common to North American temperate deciduous forests. Seedlings of each focal species were grown in soils trained by conspecific and heterospecific plants, and then either pasteurized or left unpasteurized. If antagonistic interactions between plants and soil microbes promote herbaceous plant diversity, we expected to observe reduced seedling growth and increased mortality when exposed to soil microbial communities trained by conspecific plants (Fig. S1a). In contrast, if mutualistic interactions are more important, we expected greater seedling growth in unpasteurized soils (Fig. S1d) or conspecific, unpasteurized soils if host-specific mutualists are responsible for the improved seedling performance (Fig. S1c).

MATERIALS AND METHODS

Focal species – We selected nine native herbaceous plant species common to woodlands and forests of eastern and central North America to represent a variety of evolutionary lineages, life histories,

and physiological characteristics (Table 1; Bierzychudek, 1982; Whigham, 2004). In fall 2018 and early 2019, we conducted a greenhouse experiment to characterize the strength and prevalence of plant-soil feedbacks among these nine focal species (Pernilla Brinkman et al. 2010). In the first phase of the experiment, we conditioned field-collected soil by allowing microbial communities to acclimate to host plant species. In the second phase of the experiment, we treated these soils to eliminate or retain soil microbial communities then used these conditioned soils as substrates to examine plant-soil feedbacks.

Conditioning phase – We obtained seeds from Prairie Moon Nursery (Winona, Minnesota, USA) and followed their directions for germinating seeds. Seeds of seven species received a 60-day cold-moist stratification treatment in sterile sand before being sown in germination media. One other species required 15-days of cold-moist stratification. The last species required no germination treatment. Approximately one week after germination, when true leaves began to emerge, we transplanted seedlings into sterilized 0.5 L pots filled with field-collected soil to begin the soil conditioning phase of the experiment. We collected field soil from several forested stands within the Baraboo Hills, the largest contiguous complex of forested habitats in southern Wisconsin and a regional biodiversity hotspot supporting >1300 vascular plant species (Lange 1998). Field-collected soils were silt loams. Forest stands where the soil was collected were dominated by oaks (*Quercus rubra* and *Quercus alba*) and maples (*Acer rubrum* and *Acer saccharum*) with *Prunus serotina*, *Carya cordiformis*, *Carya ovata*, *Fraxinus americana*, *Tilia americana*, *Populus grandidentata*, and *Ostrya virginiana* as the primary subdominant trees. Most of the field soil was collected haphazardly but we also collected targeted soil samples immediately adjacent to each focal species. Field-collected soils were stored at 4°C for 4-6 weeks before starting soil conditioning. We then homogenized the haphazardly collected field soil samples and filled pots. Finally, we added targeted soil from conspecifics to the pots for all focal species. Seedlings were watered daily and allowed to grow for 2-3 months (70-96 days).

Experiment phase – After the soil-conditioning period, we harvested plants, divided the trained soil from each pot in half, and randomly selected one subset to be pasteurized using an autoclave. Pasteurized and

unpasteurized soil pairs from the conditioning pots were used as inoculum in the plant-soil feedback experiment. This “split-pot” design allows us to account for variation in soil characteristics among training pots unrelated to the sterilization treatment (e.g. soil fertility, texture, moisture, etc.). Soil subsets assigned to the pasteurization treatment were autoclaved for 45 minutes at 121°C. We monitored temperature in a subset of autoclaved soil samples. Soil temperatures in all monitored samples reached at least 100°C for at least 30 minutes. This combination of temperature and duration is sufficient to kill nearly all soil microorganisms, though some of the most hardy soil organisms may survive (Baker and Roistacher 1957).

For the plant-soil feedback experiment, we stratified seeds of our focal species following the protocols outlined for the conditioning phase. We then sowed seeds in a germination media before transplanting seedlings into 0.5 L pots filled with 95% pasteurized fill soil and 5% experimental inoculum (pasteurized and unpasteurized soil pairs from the conditioning phase). The fill soil serves as a common background medium for the experiment and reduces the potential influence of differences in soil characteristics other than microbial composition (i.e. fertility, soil texture, or the presence of phytotoxic compounds). Fill soil was autoclaved for 90 minutes at 121°C.

We transplanted seedlings of each focal species into pots with inoculum derived from conspecific- and heterospecific-trained soils that had been pasteurized or left unpasteurized. For each focal species, we selected three heterospecific species to serve as training hosts (Table S1). Throughout the experiment, paired experimental pots by using pasteurized and unpasteurized inoculum from the same conditioning pot and kept track of the inoculum origin. This allows us to statistically account for non-independence among soil inoculum originating from the same conditioning pots due to differences in soil fertility, texture, or chemistry (Pernilla Brinkman et al. 2010). For each focal species, we aimed for 60 conspecific-trained soils (30 pasteurized, 30 unpasteurized) and 90 heterospecific trained soils (15 pasteurized, 15 unpasteurized for 3 different training hosts). However, realized sample sizes were constrained by seed germination and space constraints (Table S2). Heterospecific trained soils included

inoculant trained by three different plant species varying in phylogenetic relatedness of the focal seedlings. In the greenhouse, the position of experimental pots was randomized within focal species.

At the end of the experiment, we clipped and harvested aboveground biomass for all plants approximately two months (47-77 days) after seeds were transplanted. For a random subset of experimental plants representing each focal species, we harvested both aboveground and belowground biomass. Roots were washed using a fine sieve. All harvested plant material was placed in paper bags and dried in a drying oven at 75°C for 72 hours before being weighed.

Data analysis – We analyzed plant performance (aboveground biomass production) for each species using a linear mixed effects model. Fixed effects included exposure to soil microorganisms (pasteurized versus unpasteurized), training host (conspecific versus heterospecific), and the interaction between these factors. We accounted for the split-pot design by including a random effect for training pot. This approach accounts for the non-independence among experimental pots with soil inoculum from the same training pot. We also included the number of days between transplanting and biomass harvest as a random effect for species that were harvested over multiple days. To illustrate variation in plant-soil feedbacks among focal species, we extracted the estimated marginal mean of differences in biomass produced between soil treatments and calculated the log response ratio comparing biomass production in unpasteurized soil versus pasteurized soil: $\ln(\text{unpasteurized}/\text{pasteurized})$. This metric quantifies the generalized effect of soil microorganisms on plant growth. Negative values reflect a net reduction in plant growth in the presence of soil microorganisms while positive values suggest soil microbes benefit plant growth. Log response ratios facilitate comparisons among species that differ in biomass production (Pernilla Brinkman et al. 2010). We also compared the difference in plant biomass produced in conspecific, unpasteurized soil versus heterospecific, unpasteurized soil:

$\ln(\text{conspecific}_{\text{unpasteurized}}/\text{heterospecific}_{\text{unpasteurized}})$. This metric reflects how specialized any plant-soil interactions are with negative values (pathogens reducing seedling growth in conspecific-trained soils) suggesting host-specific effects while positive values reflect presumed host-specific mutualisms that promote seedling growth.

To compare relative aboveground-belowground biomass allocation among species, we calculated the log response ratio of aboveground to belowground biomass production for each plant:

$\ln(\text{aboveground}/\text{belowground})$. We then performed an ANOVA to test for differences in relative allocation among species. We performed all analyses using R 3.5.3 (R Core Team, 2019). We fit mixed models using the ‘lme4’ package (Bates et al. 2015) and generated figures using ‘ggplot2’ (Wickham 2009).

RESULTS

Aboveground biomass was strongly correlated with total plant biomass across all focal species (Pearson correlation: $r > 0.71$ and $P < 0.001$ for all species; Table S3; Fig. S2), confirming that aboveground biomass provides a suitable proxy for total plant growth and fitness. Above- and belowground plant biomasses were also strongly correlated across the focal species ($r > 0.63$ and $P < 0.002$; Table S3 S4; Fig. S3). Above-ground biomass production varied substantially among focal species. Biomass production was greatest for *Ageratina altissima* (mean of 2.60 g \pm 0.06 standard error), followed by *Elymus hystrix* (1.63 \pm 0.03), *Solidago flexicaulis* (1.51 \pm 0.05), *Polygonum virginianum* (1.43 \pm 0.09), *Geranium maculatum* (1.17 \pm 0.06), *Aquilegia canadensis* (1.15 \pm 0.04), *Polemonium reptans* (1.13 \pm 0.03), *Thalictrum dioicum* (0.67 \pm 0.03), and *Hylodesmum glutinosum* (0.24 \pm 0.01).

The effects of manipulating soil biota on plant growth also varied among species (Fig. 2). In four of the nine focal species, plants grown in unpasteurized soil performed worse than plants grown in pasteurized soils (Table 2; Fig. 3a). Seedling biomass in unpasteurized soils declined by 20.2% in *T. dioicum* (ANOVA: $F_{1,136.5} = 8.300$, $P = 0.005$), 8.6% in *E. hystrix* ($F_{1,126.7} = 7.35$, $P = 0.008$), 12.3% in *S. flexicaulis* ($F_{1,136.2} = 4.739$, $P = 0.031$), and tended to decline (9.7%) in *P. reptans* ($F_{1,108.4} = 2.892$, $P = 0.092$). Unpasteurized soils also depressed biomass in *P. virginianum* (by 14.9%), but this was not significant ($F_{1,56.6} = 1.525$, $P = 0.222$). Aboveground biomass in *A. altissima* remained similar in pasteurized and unpasteurized soils. Seedling biomass for *H. glutinosum* (+15.3%), *A. canadensis*

(+4.6%), and *G. maculatum* (+12.7%) tended to be greater in unpasteurized soil, though not significantly greater (all $P > 0.150$; Table 2).

The origin of soil inoculum also affected plant growth in unpasteurized soils (Fig. 3b). In *Thalictrum dioicum*, conspecific soil inoculant with intact soil biota depressed seedling biomass by 30.0% relative to heterospecific soils (also unpasteurized). Similar but smaller depressions occurred in *Elymus hystrix* (-8.5%), *Polygonum virginianum* (-16.8%), and *Polemonium reptans* (-5.0%), though confidence intervals overlapped zero. In contrast, conspecific, unpasteurized soils tended to enhance seedling growth in *Ageratina altissima* (+9.2%) and *Geranium maculatum* (+25.0%), and to a lesser extent *A. canadensis* (+4.2%) and *H. glutinosum* (+7.3%).

Relative above- vs. belowground allocation varied significantly among focal species (ANOVA: $F_{8,278} = 21.695$, $P < 0.001$). *A. altissima*, *P. virginianum*, and *T. dioicum* invested heavily in aboveground biomass whereas *E. hystrix* and *G. maculatum* slightly favored aboveground allocation. In contrast, *H. glutinosum* and *P. reptans* invested more resources belowground (Fig. 4) while *S. flexicaulis* and *A. canadensis* allocated biomass roughly equally between aboveground and belowground growth. As noted above, these differences in above- vs. belowground allocation did not eliminate the strong correlation between them ($r > 0.63$, $P < 0.002$).

DISCUSSION

Interactions between plants and soil microorganisms play an important role in structuring the composition and diversity of plant communities (Reynolds et al. 2003, Ehrenfeld et al. 2005, Kulmatiski et al. 2008, Bever et al. 2010). Nevertheless, plant-soil interactions, including the negative plant-soil feedbacks that could facilitate coexistence and promote diversity, have received little attention in the herbaceous plant communities (Whigham, 2004; Comita et al., 2014; Gilliam, 2014; but see Smith and Reynolds 2015). We characterized plant-soil feedbacks among nine herbaceous plant species common in North American temperate forests. The strength and direction of plant-soil feedbacks varied widely among these species but four of the nine exhibited reduced growth in unpasteurized soils suggesting

antagonistic interactions with soil biota are common (Fig. 2, Fig. 3a). Our findings are consistent with previous empirical studies documenting the prevalence of negative plant-soil feedbacks in other plant communities (Klironomos 2002, Kulmatiski et al. 2008, Petermann et al. 2008, Fitzsimons and Miller 2010, Mangan et al. 2010, Reinhart 2012, Maron et al. 2016, Teste et al. 2017). The Janzen-Connell hypothesis predicts that host-specific pathogens should inhibit the growth of individuals near conspecific adults, acting in a density-dependent way to foster diversity (Janzen 1970, Connell 1971, Bever et al. 2015). Patterns of seedling growth in *T. dioicum* and, to a lesser extent, *E. hystrix* are consistent with this hypothesis (Fig. 3b). *Polemonium reptans* and *P. virginianum* also tended to accumulate less biomass in conspecific-trained soil, though confidence intervals overlapped with zero. Smith and Reynolds (2015) also found evidence of conspecific inhibition for *E. hystrix* and two other understory forest species. These results suggest that localized host-specific pathogens could facilitate coexistence among many herbaceous species (as also noted by Bonanomi et al., 2005; Bever et al., 2010, 2015; Mordecai, 2011).

Despite the presence of negative plant-soil feedbacks sufficient to affect the growth of several forest herbs, we observed that *A. altissima*, *A. canadensis*, and *H. glutinosum* are apparently unaffected by the biotic soil community. The lack of treatment effects for these species could reflect several factors and does not necessarily demonstrate that plant-soil interactions are unimportant. Our method to test plant-soil interactions quantifies only the net effect of soil microbes (Ehrenfeld et al. 2005, Kulmatiski et al. 2008, Pernilla Brinkman et al. 2010, Van der Putten et al. 2013). Because pasteurizing soil eliminates both pathogens and mutualists, it may obscure complex plant-soil interactions that could affect plant growth and survival. Most conspicuously, the majority of forest herbs associate with mycorrhizal fungi and exchange carbon-rich compounds for mineral nutrients (Whigham 2004). These associations can benefit plant growth and may offset the negative effect of pathogens (Whigham 2004, Burke 2012, Burke et al. 2018). Plant-soil feedbacks could also vary among life stages or in different environmental contexts (Smith-Ramesh and Reynolds 2017). Although seedlings are generally expected to respond more sensitively to soil microbes than adult plants, little is known about the effects of soil microbes on seeds or seed germination (Nelson 2018). Seeds are exposed to a diverse assortment of microorganisms while

dormant in the soil. Though rarely quantified, these interactions could generate positive or negative plant-soil feedbacks distinct from those exhibited by seedlings (Kirkpatrick and Bazzaz 1979, Burns and Strauss 2011, Nelson 2018). Furthermore, soil fertility, light availability, and soil moisture may mediate the effects of soil microbes on plant growth and mortality (Givnish 1999, Shannon et al. 2012, Larios and Suding 2015, Smith and Reynolds 2015). Fungal communities vary seasonally and among years in response to environmental variation, especially soil moisture (Burke 2015). Moreover, a handful of empirical studies demonstrate that the strength and net effect of plant-soil interactions on plant growth may depend on environmental conditions such as light, water, or nutrient availability (Smith and Reynolds 2015). Consequently, soil biota, plant-soil feedbacks, and the influence of plant-soil interactions on plant fitness could vary both spatially and temporally in plant communities (Brandt et al. 2013). Soil communities and the nature of plant-soil interactions may be especially heterogeneous in temperate forest understories. In contrast to trees, which possess extensive root systems and greater biomass, the rhizosphere of forest herbs is comparatively small and herbs often occur at low densities. As a result, forest herbs likely influence soil communities at much finer scales than do trees potentially leading to heterogeneity in soil biota and plant-soil interactions at finer spatial scales within forest stands. Likewise, plant-soil interactions may be heterogeneous at larger spatial scales due to geographic variation in microbial communities and localized co-evolution between plants and microbes (Lankau and Keymer 2018).

The considerable variation we observed in the responses of various herbaceous taxa to soil microbiota parallels previous research documenting variation in plant-soil feedbacks among temperate forest trees (Bennett et al. 2017), grassland plant species (Klironomos 2002, Fitzsimons and Miller 2010, Maron et al. 2016), and plant species in other communities (Kulmatiski et al. 2008, Mangan et al. 2010, Reinhart 2012, Teste et al. 2017). This variation has important implications for the distribution, abundance, and diversity of plants in all these ecosystems. In both temperate grasslands (Klironomos 2002) and tropical forests (Mangan et al. 2010), the strength of plant-soil feedbacks is associated with the relative abundance of plant species in the community. The stronger negative plant-soil feedbacks

observed in many rare species suggests that their susceptibility to pathogens may contribute to their rarity. All nine of the focal species studied here are relatively common. It is noteworthy, however, that *A. altissima*, *G. maculatum*, and *H. glutinosum* – all species exhibiting neutral to positive plant-soil feedbacks – tend to form dense, monospecific patches and all but *H. glutinosum* will spread aggressively via vegetative reproduction (J. Beck, *personal observation*). Smith and Reynolds (2015) similarly noted that two monoculture-forming understory plants (*Asarum canadense* and the invasive *Euonymus fortunei*) exhibited neutral plant-soil feedbacks. In contrast, the species with strongly negative plant-soil feedbacks in our study (e.g. *Thalictrum dioicum*, *Elymus hystrix*, *Polemonium reptans*, *Polygonum virginianum*) exhibit patchy, rather sparse spatial distributions and rarely form dense, monospecific clusters (J. Beck, *personal observation*; also see Smith and Reynolds 2015). There have been very few investigations of distance- or density-dependence among temperate forest herbs so it is unclear whether the local spatial distribution of forest herbs reflects Janzen-Connell effects and elevated mortality near adult conspecifics (Bever et al. 1997, Comita et al. 2014). However, temperate forest tree species that exhibit negative plant-soil feedbacks, such as *Prunus serotina*, rarely cluster and tend to occur more regularly than expected if distributed spatially at random (Packer and Clay, 2000; Johnson, Clay, et al., 2017; J. Beck, *unpublished data*). Future investigation into fine-scale spatial distributions forest herbs as well as spatial patterns of recruitment and mortality, especially in relation to plant-soil feedbacks, may provide valuable insights into how plant-soil interactions shape the local distribution of understory forest plants.

Temperate forest herbs coexist at high local densities, with a dozen or more species able to co-occur within one square meter (Rogers et al. 2008, Peet et al. 2014). This diversity is maintained, in part, by environmental heterogeneity which allows species to spatially partition resources within forest stands (Bratton, 1976; Beatty, 1984, 2014; Beck and Givnish, *in review*). There is little evidence, however, regarding whether forest herbs partition resources in the absence of spatial heterogeneity (Beatty, 2014; Catella et al., 2019; Beck and Givnish, *in review*). Negative plant-soil feedbacks and the density-dependent effects of pathogens could be especially important for stabilizing coexistence among

physiologically similar species that respond similarly to environmental variation and within microsites with little environmental heterogeneity. While future research is clearly needed to fully understand plant-soil feedbacks and their influence in the understory plant community of temperate forests, this study adds to a growing body of knowledge about how plant-soil interactions shape plant community composition and diversity across an array of ecological communities (Ehrenfeld et al. 2005, Kulmatiski et al. 2008, Mordecai 2011, Bever et al. 2015). Plant-soil interactions clearly play an important, but previously underappreciated, role in structuring the local distribution, abundance, and diversity of temperate forest herbs.

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Table 1. List of focal species included in the plant-soil feedback experiment and relevant ecological characteristics including flowering phenology, photosynthetic phenology, and mode of seed dispersal.

Species	Family	Flowering phenology	Leaf phenology	Dispersal mode
<i>Ageratina altissima</i> (L.) R.M. King & H. Rob.	Asteraceae	July - October	Late summer	Wind-dispersed
<i>Aquilegia Canadensis</i> L.	Ranunculaceae	May - July	Early summer	Ballistic
<i>Elymus hystrix</i> L.	Poaceae	July - September	Late summer	Gravity
<i>Geranium maculatum</i> L.	Geraniaceae	May - June	Early summer	Ballistic
<i>Hylodesmum glutinosum</i> (Muhl. ex Willd.) H. Ohashi & R.R. Mill	Fabaceae	June - August	Late summer	Epizoochorous
<i>Polemonium reptans</i> L.	Polemoniaceae	May - July	Early summer	Gravity
<i>Polygonum virginianum</i> L.	Polygonaceae	July - September	Late summer	Ballistic
<i>Solidago flexicaulis</i> L.	Asteraceae	August - October	Late summer	Wind-dispersed
<i>Thalictrum dioicum</i> L.	Ranunculaceae	April - June	Early summer	Gravity

Table 2. Summary of statistical models examining the effects of experimental treatments on aboveground biomass production. For each species, we report F statistics, degrees of freedom (numerator, demoninator), and P-values for the effect of soil treatment (pasteurized versus unpasteurized soil inoculant), host treatment (conspecific versus heterospecific training host), and their interaction. We used Kenward-Roger approximations for the denominator degrees of freedom. Significant effects are bolded, marginally significant effects are italicized.

Focal species	Soil treatment			Host treatment			Soil x Host treatment		
	F	df	P	F	df	P	F	df	P
<i>Ageratina altissima</i>	0.003	1,139.9	0.954	0.168	1,20.6	0.686	1.658	1,136.6	0.200
<i>Aquilegia canadensis</i>	0.497	1,68.7	0.483	0.039	1,24.5	0.844	0.156	1,73.0	0.695
<i>Elymus hystrix</i>	7.354	1,126.7	0.008	1.470	1,21.2	0.239	1.083	1,125.0	0.300
<i>Geranium maculatum</i>	1.645	1,42.1	0.207	0.306	1,21.7	0.586	2.962	<i>1,41.8</i>	<i>0.093</i>
<i>Hylodesmum glutinosum</i>	2.094	1,87.9	0.152	2.303	1,27.6	0.141	0.597	1,84.6	0.442
<i>Polemonium reptans</i>	2.892	<i>1,108.4</i>	<i>0.092</i>	0.209	1,30.7	0.651	0.089	1,108.4	0.766
<i>Polygonum virginianum</i>	1.525	1,56.6	0.222	1.280	1,20.9	0.271	0.045	1,52.4	0.833
<i>Solidago flexicaulis</i>	4.739	1,136.2	0.031	0.895	1,36.9	0.350	1.145	1,134.9	0.287
<i>Thalictrum dioicum</i>	8.300	1,136.5	0.005	4.584	1,34.5	0.039	2.083	1,136.2	0.151

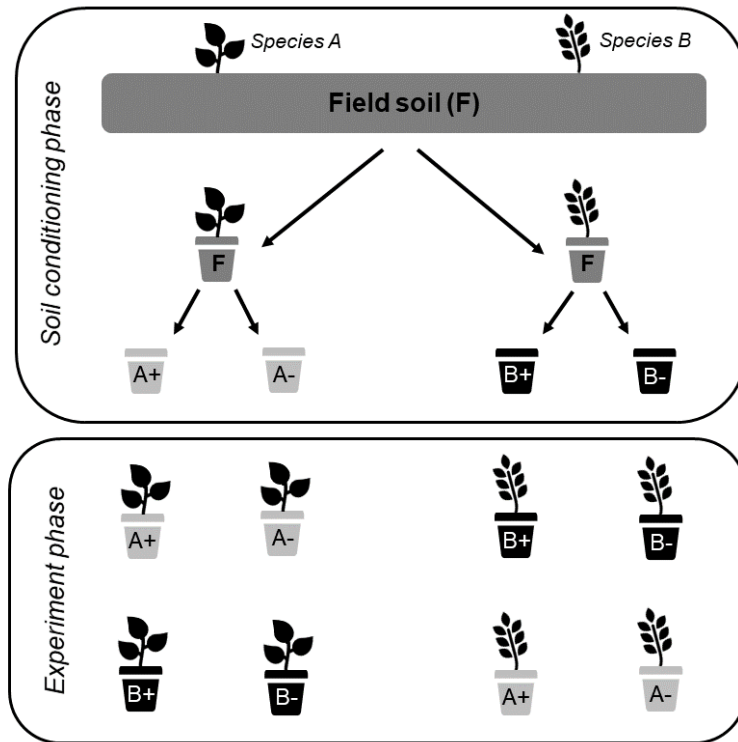


Figure 1. Diagram illustrating simplified experimental design for two hypothetical focal species (species A and B). Field soil (F) was collected from forest stands in which focal species were present. During the soil conditioning phase of the experiment (top panel), each focal species was grown in field soil within training pots for two months (20-30 training pots for each focal species). Soil from each training pot was then divided equally. Half of the soil from each training pot was pasteurized using an autoclave (microbes absent, -) and the other half left unpasteurized (microbes present, +). During the experiment phase (bottom panel), we grew each focal species in a common soil medium inoculated with soil from the conditioning phase. Experimental treatments included soil inoculum from different training hosts (conspecific- versus heterospecific-trained soils) and with different exposure to soil microbes (pasteurized versus unpasteurized).

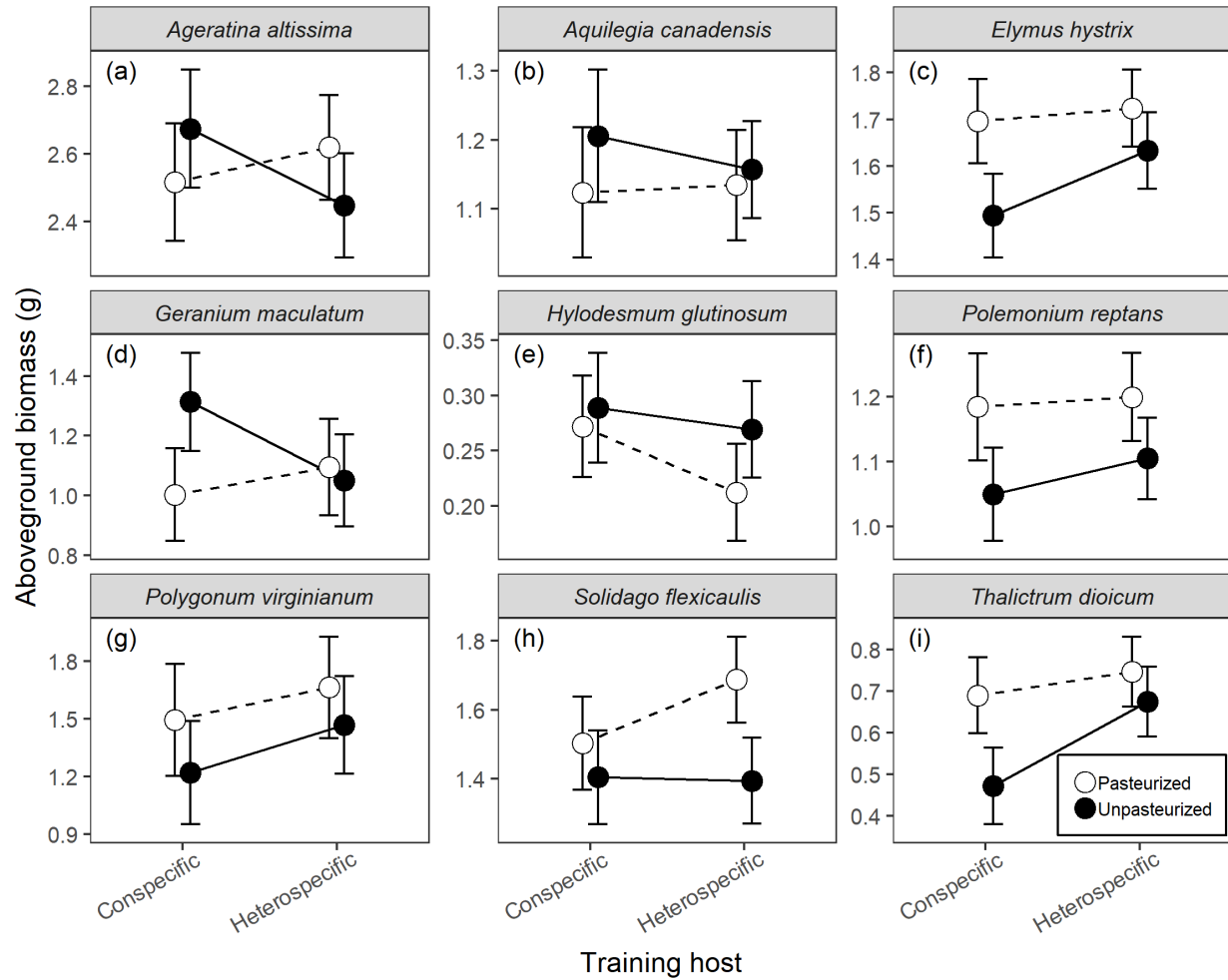


Figure 2. Estimated marginal means of aboveground biomass (in grams) for each focal species across the four experimental treatments. Pasteurized soils are represented by open circles and dashed lines while unpasteurized soils are represented by closed circles and solid lines. Error bars represent one standard error of the marginal means.

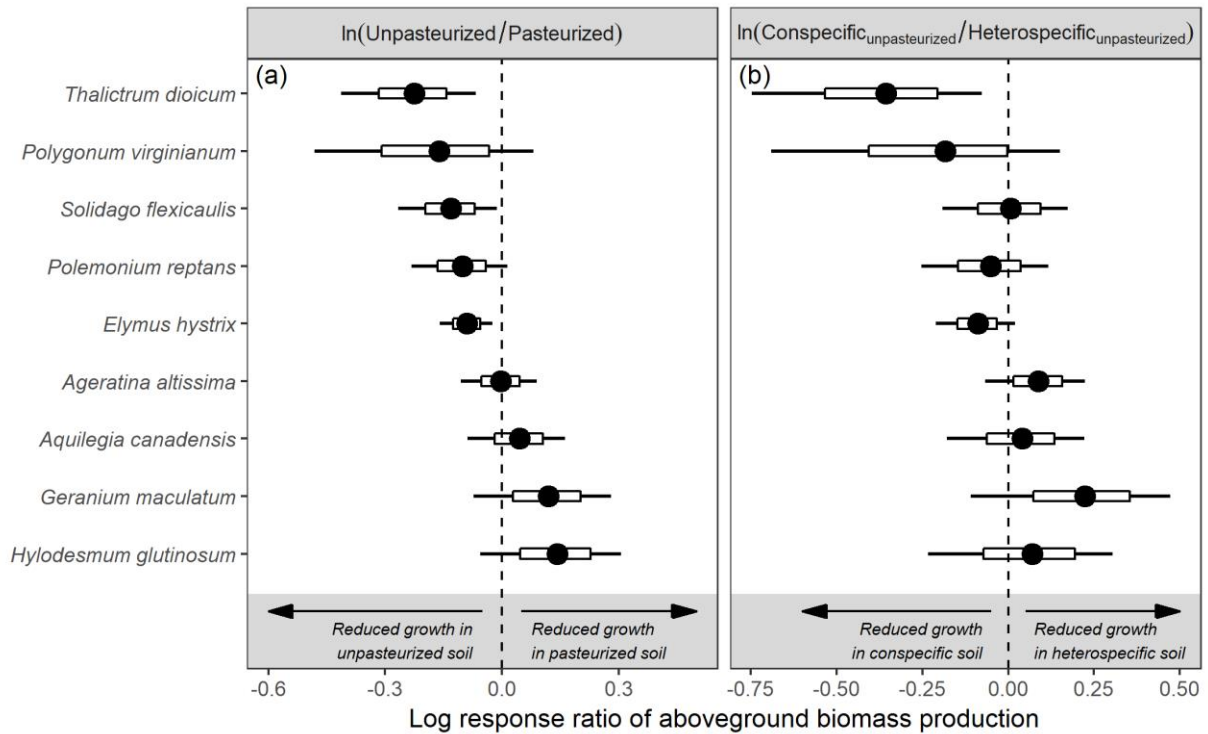


Figure 3. Variation in the strength of plant-soil feedbacks among species illustrated for (a) differences in aboveground biomass production between pasteurized and unpasteurized soil treatments and (b) differences in biomass production between conspecific- and heterospecific-trained soil inocula that were not pasteurized. In (a), negative values indicate reduced growth in unpasteurized soil compared to pasteurized soil. In (b), negative values indicate reduced growth in unpasteurized, conspecific-trained soil relative to unpasteurized, heterospecific-trained soil. Points represent the estimated log response ratio of aboveground biomass production in pasteurized versus unpasteurized soils (a) or conspecific_{unpasteurized} versus heterospecific_{unpasteurized} soils (b), white bars indicate \pm one standard error, and black lines represent a 95 percent confidence interval for this log response ratio.

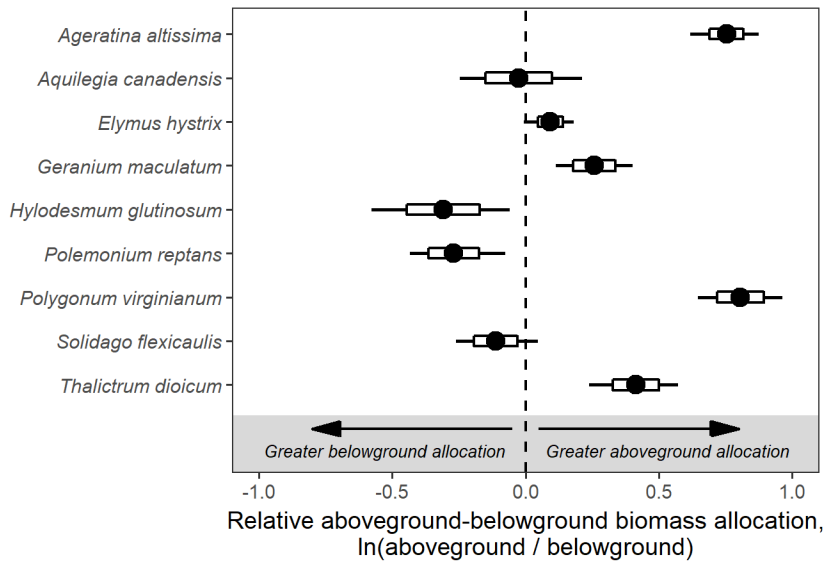


Figure 4. Variation in relative aboveground-belowground biomass allocation among focal species in our greenhouse experiment. Relative biomass allocation was quantified as the logarithm of the ratio of aboveground biomass to belowground biomass production. White bars indicate mean \pm one standard error of the mean and black bars represent 95% confidence intervals. The dashed line represents equal investment in aboveground and belowground biomass.

LITERATURE CITED

- Anderson, A. R. C., O. L. Loucks, and A. M. Swain. 1969. Herbaceous response to canopy cover, light intensity, and throughfall precipitation in coniferous forests. *Ecology* 50:255–263.
- Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton, and O. T. Lewis. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* 506:85–88.
- Bagchi, R., T. Swinfield, R. E. Gallery, O. T. Lewis, S. Gripenberg, L. Narayan, and R. P. Freckleton. 2010. Testing the Janzen-Connell mechanism: Pathogens cause overcompensating density dependence in a tropical tree. *Ecology Letters* 13:1262–1269.
- Baker, K. F., and C. N. Roistacher. 1957. Principles of Heat Treatment of Soil. Pages 138–161 in K. F. Baker, editor. *The U.C. System for Producing Healthy Container Grown Plants, Manual 23*. University of California, Division of Agricultural Sciences, Agricultural Experiment Station, Extension Service, Berkeley, CA.
- Bartels, S. F., and H. Y. H. Chen. 2010. Is understory plant diversity driven by resource quantity or resource heterogeneity? *Ecology* 91:1931–1938.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Beatty, S. W. 1984. Influence of microtopography and canopy species on spatial patterns of forest understory plants. *Ecology* 65:1406–1419.
- Beatty, S. W. 2014. Habitat Heterogeneity and the Maintenance of Species in Understory Communities. Pages 215–232 in F. S. Gilliam, editor. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Beck, J. J., and T. J. Givnish. Fine-scale environmental heterogeneity and spatial niche partitioning among spring-flowering forest herbs. *In review*.
- Bell, G., M. J. Lechowicz, and M. J. Waterway. 2000. Environmental heterogeneity and species diversity of forest sedges. *Journal of Ecology* 88:67–87.
- Bell, T., R. P. Freckleton, and O. T. Lewis. 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters* 9:569–574.
- Bennett, J. A., and J. Klironomos. 2019. Mechanisms of plant–soil feedback: Interactions among biotic and abiotic drivers. *New Phytologist* 222:91–96.
- Bennett, J. A., H. Maherali, K. O. Reinhart, Y. Lekberg, M. M. Hart, and J. Klironomos. 2017. Plant-soil

- feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* 355:181–184.
- Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett, and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology and Evolution* 25:468–478.
- Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics* 46:305–325.
- Bever, J., K. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: The utility of the feedback approach. *Journal of Ecology* 85:561–573.
- Bierzychudek, P. 1982. Life histories and demography of shade-tolerant temperate forest herbs: A review. *New Phytologist* 90:757–776.
- Bonanomi, G., F. Giannino, and S. Mazzoleni. 2005. Negative plant-soil feedback and species coexistence. *Oikos* 111:311–321.
- Brandt, A. J., H. De Kroon, H. L. Reynolds, and J. H. Burns. 2013. Soil heterogeneity generated by plant-soil feedbacks has implications for species recruitment and coexistence. *Journal of Ecology* 101:277–286.
- Bratton, S. P. 1976. Resource division in an understory herb community: Responses to temporal and microtopographic gradients. *The American Naturalist* 110:679–693.
- Burke, D. J. 2008. Effects of *Alliaria petiolata* (garlic mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest. *American Journal of Botany* 95:1416–1425.
- Burke, D. J. 2012. Shared mycorrhizal networks of forest herbs: Does the presence of conspecific and heterospecific adult plants affect seedling growth and nutrient acquisition? *Botany* 90:1048–1057.
- Burke, D. J. 2015. Effects of annual and interannual environmental variability on soil fungi associated with an old-growth, temperate hardwood forest. *FEMS Microbiology Ecology* 91:1–13.
- Burke, D. J., M. K. Klenkar, and J. S. Medeiros. 2018. Mycorrhizal network connections, water reduction, and neighboring plant species differentially impact seedling performance of two forest wildflowers. *International Journal of Plant Sciences* 179:314–324.
- Burns, J. H., and S. Y. Strauss. 2011. More closely related species are more ecologically similar in an experimental test. *Proceedings of the National Academy of Sciences* 108:5302–5307.
- Catella, S. A., S. R. Eysenbach, and K. C. Abbott. 2019. Novel insights into how the mean and

heterogeneity of abiotic conditions together shape forb species richness patterns in the Allegheny plateau ecoregion. *Ecology and Evolution*:12374–12389.

Collins, B. S., L. S. Ferrara, and H. L. Motto. 1984. Coincidence of spring herb distribution and flowering with tree bases in a New Jersey Piedmont forest. *Bulletin of the Torrey Botanical Club* 111:301.

Comita, L. S., S. A. Queenborough, S. J. Murphy, J. L. Eck, K. Xu, M. Krishnadas, N. Beckman, and Y. Zhu. 2014. Testing predictions of the Janzen-Connell hypothesis: A meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology* 102:845–856.

Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Pages 298–312 *in* den B. PJ and G. GR, editors. *Dynamics of Populations*. The Netherlands: Centre for Agricultural Publishing and Documentation.

Crozier, C. R., and R. E. J. Boerner. 1984. Correlation of understory herb distribution patterns with microhabitats under different tree species in a mixed mesophytic forest. *Oecologia* 62:337–343.

Curtis, J. T. 1959. *The Vegetation of Wisconsin: An Ordination of Plant Communities*. The University of Wisconsin Press, Madison, WI.

Ehrenfeld, J. G., B. Ravit, and K. Elgersma. 2005. Feedback in the plant-soil system. *Annual Review of Environment and Resources* 30:75–115.

Fitzsimons, M. S., and R. M. Miller. 2010. The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. *American Journal of Botany* 97:1937–1943.

Gilliam, F. S. 2007. The Ecological Significance of the Herbaceous Layer in Temperate Forest Ecosystems. *BioScience* 57:845.

Gilliam, F. S., editor. 2014. *The Herbaceous Layer in Forests of Eastern North America*. Oxford University Press.

Givnish, T. J. 1999. On the causes of gradients in tropical tree species diversity. *Journal of Ecology* 87:193–210.

Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. *The American Naturalist* 104:501–528.

Johnson, D. J., N. A. Bourg, R. Howe, W. J. Mcshea, D. J. Johnson, N. A. Bourg, R. Howe, W. J. Mcshea, A. Wolf, and K. Clay. 2014. Conspecific negative density-dependent mortality and the structure of temperate forests. *Ecology* 95:2493–2503.

Johnson, D. J., K. Clay, and R. P. Phillips. 2018. Mycorrhizal associations and the spatial structure of an

- old-growth forest community. *Oecologia* 186:195–204.
- Johnson, D. J., R. Condit, S. P. Hubbell, and L. S. Comita. 2017. Abiotic niche partitioning and negative density dependence drive tree seedling survival in a tropical forest. *Proceedings of the Royal Society B* 284:1–8.
- Kartzinel, R. Y., D. Spalink, D. M. Waller, and T. J. Givnish. 2016. Divergence and isolation of cryptic sympatric taxa within the annual legume *Amphicarpaea bracteata*. *Ecology and Evolution* 6:3367–3379.
- Ke, P. J., and J. Wan. 2019. Effects of soil microbes on plant competition: a perspective from modern coexistence theory. *Ecological Monographs* 0:1–17.
- Kirkpatrick, B. L., and F. A. Bazzaz. 1979. Influence of certain fungi on seed germination and seedling survival of four colonizing annuals. *Journal of Applied Ecology* 16:515.
- Klironomos, J. N. 2002. Feedback with soil biota contributes to plants rarity and invasiveness in communities. *Nature* 417:67–69.
- Kulmatiski, A., K. H. Beard, J. R. Stevens, and S. M. Cobbold. 2008. Plant-soil feedbacks: A meta-analytical review. *Ecology Letters* 11:980–992.
- LaManna, J. A., S. A. Mangan, A. Alonso, N. A. Bourg, W. Y. Brockelman, S. Bunyavejchewin, L. Chang, J. Chiang, G. B. Chuyong, K. Clay, R. Condit, S. Cordell, S. J. Davies, T. J. Furniss, C. P. Giardina, I. A. U. N. Gunatilleke, C. V. S. Gunatilleke, F. He, R. W. Howe, S. P. Hubbell, C.-F. Hsieh, F. M. Inman-Narahari, D. Janík, D. J. Johnson, D. Kenfack, L. Korte, K. Král, A. J. Larson, J. A. Lutz, S. M. McMahon, W. J. McShea, H. R. Memiaghe, A. Nathalang, V. Novotny, P. S. Ong, D. A. Orwig, R. Ostertag, G. G. Parker, R. P. Phillips, L. Sack, I.-F. Sun, J. S. Tello, D. W. Thomas, B. L. Turner, D. M. Vela Díaz, T. Vrška, G. D. Weiblen, A. Wolf, S. Yap, and J. A. Myers. 2017. Plant diversity increases with the strength of negative density dependence at the global scale. *Science* 356:1389–1392.
- Lange, K. I. 1998. Flora of Sauk County and Caledonia Township, Columbia County, south central Wisconsin. Madison, WI.
- Lankau, R. A. 2011. Resistance and recovery of soil microbial communities in the face of *Alliaria petiolata* invasions. *New Phytologist* 189:536–548.
- Lankau, R. A., and D. P. Keymer. 2018. Simultaneous adaptation and maladaptation of tree populations to local rhizosphere microbial communities at different taxonomic scales. *New Phytologist* 217:1267–1278.
- Larios, L., and K. N. Suding. 2015. Competition and soil resource environment alter plant-soil feedbacks

- for native and exotic grasses. *AoB PLANTS* 7:1–9.
- Liu, Y., S. Fang, P. Chesson, and F. He. 2015. The effect of soil-borne pathogens depends on the abundance of host tree species. *Nature Communications* 6:4–10.
- Ma, S., P. De Frenne, S. Wasof, J. Brunet, S. A. O. Cousins, G. Decocq, A. Kolb, I. Lemke, J. Liira, T. Naaf, A. Orczewska, J. Plue, M. Wulf, and K. Verheyen. 2019. Plant–soil feedbacks of forest understorey plants transplanted in nonlocal soils along a latitudinal gradient. *Plant Biology* 21:677–687.
- Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. MacK, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* 466:752–755.
- Maron, J. L., A. L. Smith, Y. K. Ortega, D. E. Pearson, and R. M. Callaway. 2016. Negative plant-soil feedbacks increase with plant abundance, and are unchanged by competition. *Ecology* 97:2055–2063.
- Mordeciai, E. 2011. Pathogen impacts on plant communities: unifying theory, concepts, and empirical work. *Ecological Monographs* 81:429–441.
- Nelson, E. B. 2018. The seed microbiome: Origins, interactions, and impacts. *Plant and Soil* 422:7–34.
- Packer, A., and K. Clay. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature* 404:278–281.
- Parker, M. A. 1985. Local population differentiation for compatability in an annual legume and its host-specific fungal pathogen. *Evolution* 39:713–723.
- Peet, R. K., K. A. Palmquist, and S. M. Tessel. 2014. Herbaceous layer species richness of southeastern forests and woodlands: Patterns and causes. Pages 255–276 in F. S. Gilliam, editor. *The Herbaceous Layer in forests of Eastern North America*. Oxford University Press, New York, NY, USA.
- Pernilla Brinkman, E., W. H. Van der Putten, E. J. Bakker, and K. J. F. Verhoeven. 2010. Plant-soil feedback: Experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology* 98:1063–1073.
- Petermann, J. S., A. J. F. Fergus, L. A. Turnbull, J. S. Petermann, A. J. F. Fergus, L. A. Turnbull, and B. Schmid. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89:2399–2406.
- Peterson, C. J., W. P. Carson, B. C. McCarthy, S. T. A. Pickett, C. J. Peterson, W. P. Carson, B. C. McCarthy, S. T. A. Pickett, and S. T. A. Microsite. 1990. Microsite variation and soil dynamics within newly created treefall pits and mounds. *Oikos* 58:39–46.

- Prati, D., and O. Bossdorf. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *American Journal of Botany* 91:285–288.
- Van der Putten, W. H., R. D. Bardgett, J. D. Bever, T. M. Bezemer, B. B. Casper, T. Fukami, P. Kardol, J. N. Klironomos, A. Kulmatiski, J. A. Schweitzer, K. N. Suding, T. F. J. Van de Voorde, and D. A. Wardle. 2013. Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology* 101:265–276.
- R Core Team. 2019. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Reinhart, K. O. 2012. The organization of plant communities: Negative plant-soil feedbacks and semiarid grasslands. *Ecology* 93:2377–2385.
- Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* 84:2281–2291.
- Rogers, D. A., T. P. Rooney, D. Olson, and D. M. Waller. 2008. Shifts in southern Wisconsin forest canopy and understory richness, composition, and heterogeneity. *Ecology* 89:2482–2492.
- Shannon, S., S. L. Flory, and H. L. Reynolds. 2012. Competitive context alters plant-soil feedback in an experimental woodland community. *Oecologia* 169:235–243.
- Shannon, S. M., J. T. Bauer, W. E. Anderson, and H. L. Reynolds. 2014. Plant-soil feedbacks between invasive shrubs and native forest understory species lead to shifts in the abundance of mycorrhizal fungi. *Plant and Soil* 382:317–328.
- Smith-Ramesh, L. M., and H. L. Reynolds. 2017. The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. *Journal of Vegetation Science* 28:484–494.
- Smith, L. M., and H. L. Reynolds. 2012. Positive plant-soil feedback may drive dominance of a woodland invader, *Euonymus fortunei*. *Plant Ecology* 213:853–860.
- Smith, L. M., and H. L. Reynolds. 2015. Plant-soil feedbacks shift from negative to positive with decreasing light in forest understory species. *Ecology* 96:2523–2532.
- Struik, G. J., and J. T. Curtis. 1962. Herb distributions in an *Acer saccharum* forest. *American Midland Naturalist* 68:285–296.
- Terborgh, J. 2012. Enemies maintain hyperdiverse tropical forests. *The American Naturalist* 179:303–314.
- Teste, F. P., P. Kardol, B. L. Turner, D. A. Wardle, G. Zemunik, M. Renton, and E. Laliberté. 2017.

- Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science* 355:173–176.
- Thompson, J. N. 1980. Treefalls and colonization patterns of temperate forest herbs. *American Midland Naturalist* 104:176.
- Tiffany, L. H., G. Knaphus, L. H. Tiffany, and G. Knaphus. 1984. The plant rusts (Uredinales) of the driftless area of northeastern Iowa. *Proceedings of the Iowa Academy of Sciences* 91.
- Vellend, M., M. J. Lechowicz, and M. J. Waterway. 2000. Environmental distribution of four *Carex* species (Cyperaceae) in an old-growth forest. *American Journal of Botany* 87:1507–1516.
- Wennstrom, A., and L. Ericson. 1994. The effect of the systemic smut *Urocystis carcinodes* on the long-lived herb *Actaea spicata*. *Oikos* 71:111–118.
- Whigham, D. E. 2004. Ecology of woodland herbs in temperate deciduous forests. *Annual Review of Ecology and Systematics* 35:583–621.
- Wickham, H. 2009. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York, USA.
- Wolfe, B. E., V. L. Rodgers, K. A. Stinson, and A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology* 96:777–783.
- Wright, J. S. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* 130:1–14.

APPENDIX S4. Supplemental materials for Chapter 4.

Table S1. Table of heterospecific training hosts used for each focal species. Each focal species was assigned three heterospecific training hosts in the plant-soil feedback experiment.

Focal species	Training host #1	Training host #2	Training host #3
<i>Ageratina altissima</i>	<i>P. reptans</i>	<i>P. virginianum</i>	<i>S. flexicaulis</i>
<i>Aquilegia canadensis</i>	<i>P. reptans</i>	<i>S. flexicaulis</i>	<i>T. dioicum</i>
<i>Elymus hystrix</i>	<i>A. canadensis</i>	<i>H. glutinosum</i>	<i>S. flexicaulis</i>
<i>Geranium maculatum</i>	<i>E. hystrix</i>	<i>H. glutinosum</i>	<i>P. virginianum</i>
<i>Hylodesmum glutinosum</i>	<i>E. hystrix</i>	<i>G. maculatum</i>	<i>P. virginianum</i>
<i>Polemonium reptans</i>	<i>A. altissima</i>	<i>A. canadensis</i>	<i>T. dioicum</i>
<i>Polygonum virginianum</i>	<i>A. altissima</i>	<i>E. hystrix</i>	<i>H. glutinosum</i>
<i>Solidago flexicaulis</i>	<i>A. altissima</i>	<i>E. hystrix</i>	<i>P. reptans</i>
<i>Thalictrum dioicum</i>	<i>A. canadensis</i>	<i>H. glutinosum</i>	<i>S. flexicaulis</i>

Table S2. Table of sample sizes for each focal species and experimental treatment. List of focal species included in the plant-soil feedback experiment and the number of transplanted seedlings within each treatment for which we sampled aboveground biomass. We sampled aboveground biomass for a total of 1035 seedlings.

Species	Conspecific- unpasteurized	Conspecific- pasteurized	Heterospecific- unpasteurized	Heterospecific -pasteurized
<i>Ageratina altissima</i>	31	31	44	43
<i>Aquilegia canadensis</i>	17	18	31	23
<i>Elymus hystrix</i>	30	30	45	45
<i>Geranium maculatum</i>	13	17	20	15
<i>Hylodesmum glutinosum</i>	15	23	32	33
<i>Polemonium reptans</i>	29	23	35	32
<i>Polygonum virginianum</i>	16	14	21	19
<i>Solidago flexicaulis</i>	29	30	43	43
<i>Thalictrum dioicum</i>	28	29	43	45

Table S3. Table of correlation statistics between aboveground, belowground, and total biomass production. Correlations between aboveground and belowground biomass production (left columns) as well as aboveground and total biomass production (right columns) for each focal species. Table includes sample sizes for the subset of experimental plants for which both above and belowground biomass was measured (n), the Pearson's correlation coefficient (r) describing the relationship between log-transformed belowground biomass and aboveground biomass, and the P-value for the correlation. See Figs. S1 and S2 for scatterplots.

Focal species	Aboveground biomass vs. (ln) Belowground biomass			Aboveground biomass vs. Total biomass		
	n	r	P-value	n	r	P-value
<i>Ageratina altissima</i>	60	0.63	< 0.001	60	0.73	< 0.001
<i>Aquilegia canadensis</i>	19	0.77	< 0.001	19	0.81	< 0.001
<i>Elymus hystrix</i>	60	0.64	< 0.001	60	0.71	< 0.001
<i>Geranium maculatum</i>	19	0.92	< 0.001	19	0.95	< 0.001
<i>Hylodesmum glutinosum</i>	17	0.72	0.001	17	0.93	< 0.001
<i>Polemonium reptans</i>	20	0.66	0.001	20	0.78	< 0.001
<i>Polygonum virginianum</i>	15	0.83	< 0.001	15	0.99	< 0.001
<i>Solidago flexicaulis</i>	60	0.70	< 0.001	60	0.75	< 0.001
<i>Thalictrum dioicum</i>	17	0.91	< 0.001	17	0.95	< 0.001

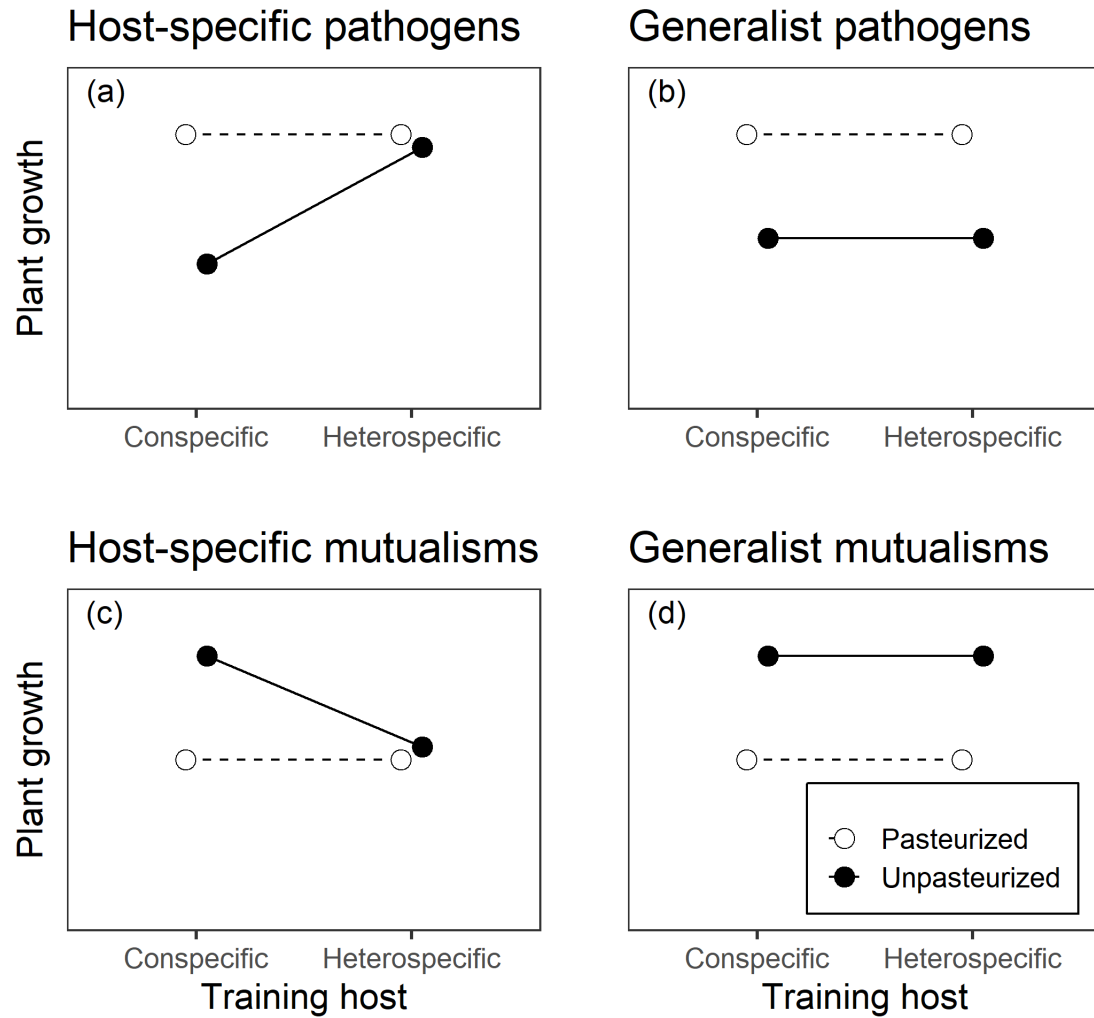


Figure S1. Conceptual diagram illustrating potential outcomes of plant-soil feedback experiment. The conceptual figures depict expected patterns of plant growth if (a) host-specific pathogens reduce plant growth, (b) generalist pathogens depress plant growth, (c) host-specific mutualists promote seedling growth, and (d) generalized mutualists increase plant growth.

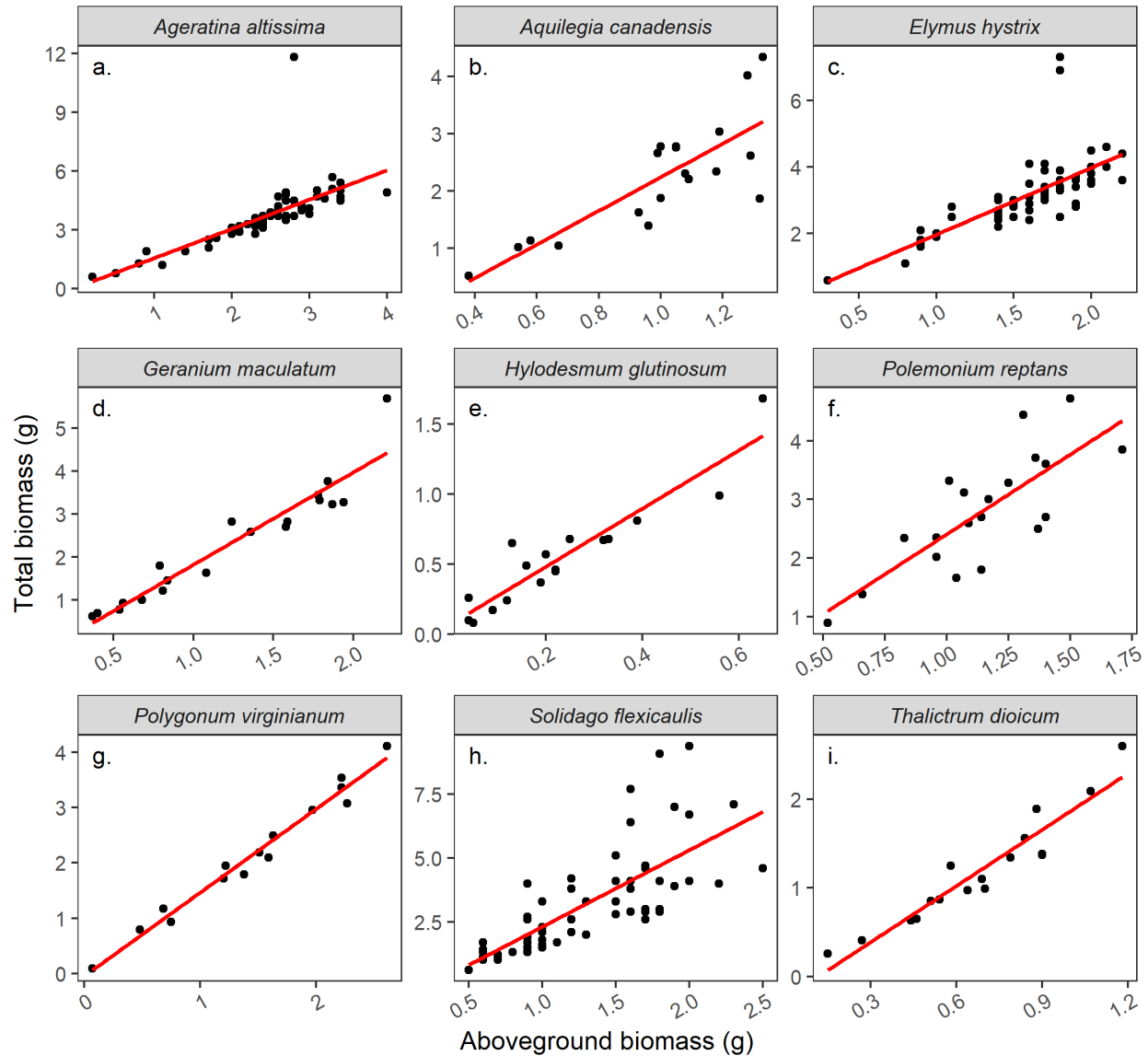


Figure S2. Correlation between aboveground and total biomass production. Scatterplots depict the relationship between aboveground and total biomass for the nine focal species included in our plant-soil feedback experiment. See Appendix S4 for correlation statistics.

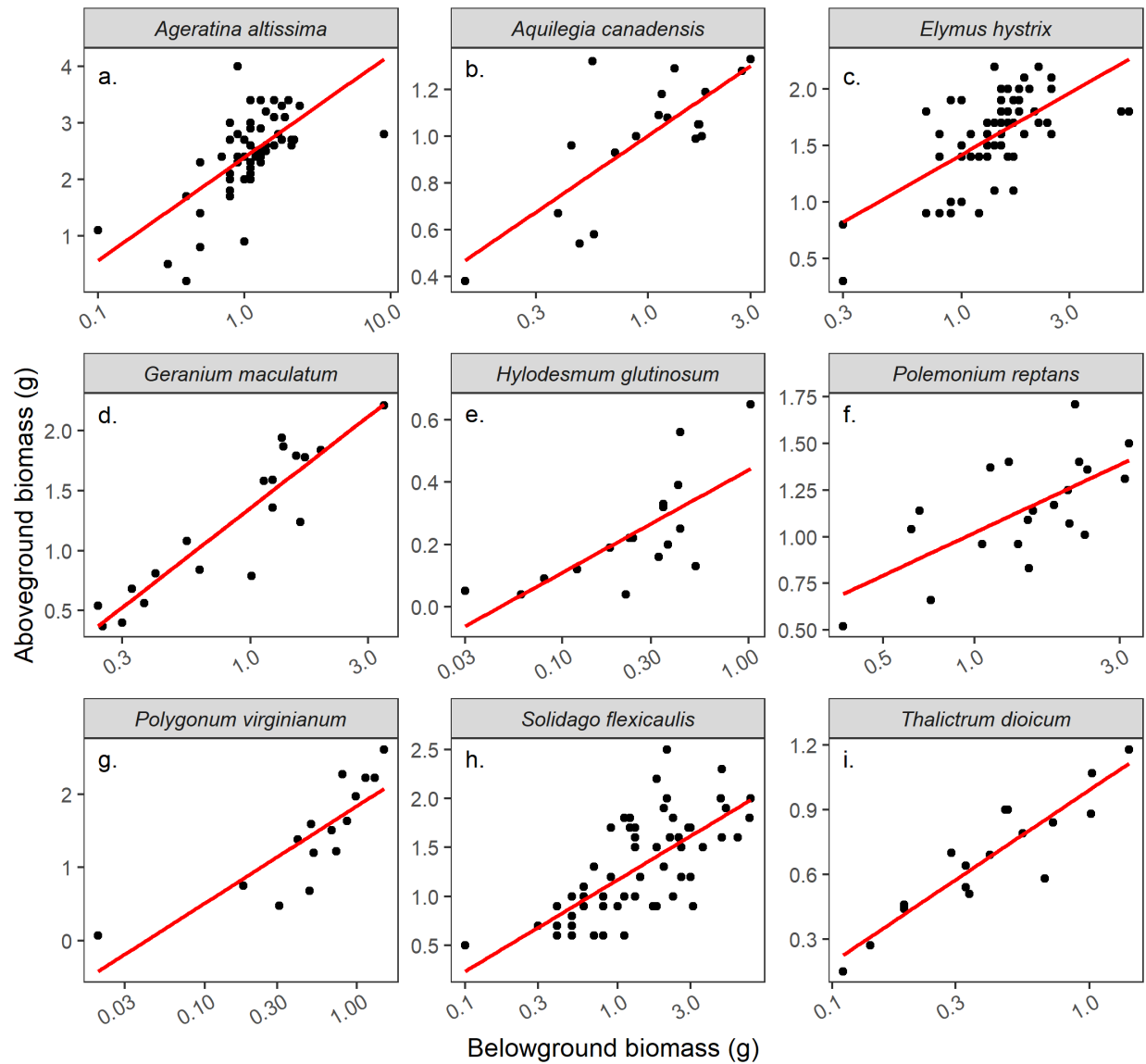


Figure S3. Correlation between aboveground and belowground biomass allocation. Scatterplots depict the relationship between aboveground and belowground biomass for the nine focal species included in our plant-soil feedback experiment. See Appendix S4 for correlation statistics.

“We are all asymmetrical pegs in square holes” – Daniel Janzen