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Climate mediates geographic patterns in ecoevolutionary plant-soil dynamics

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I am submitting herewith a dissertation written by Ian Michael Ware entitled "Climate mediates geographic patterns in ecoevolutionary plant-soil dynamics." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

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Climate mediates geographic patterns in eco-evolutionary plant-soil dynamics

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Ian Michael Ware
May 2019

DEDICATION

I dedicate this dissertation to my wonderful family.

To Blair and Lillian, my strong, sharp, and hilarious women, thank you for the endless love and adventures.

To my parents, thank you for all the love and support, and showing me the wonders of the natural world.

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ABSTRACT

Unifying ecosystem ecology and evolutionary biology promises a more complete understanding of the processes that link different levels of biological organization across space and time. Feedbacks across levels of organization link theory associated with eco-evolutionary dynamics, niche construction, and the geographic mosaic theory of co-evolution. The work presented in this dissertation directly extends the integration of eco-evolutionary dynamics by 1) highlighting our current knowledge of eco-evolutionary feedbacks in ecosystems, to provide an improved synthesis and foundation for understanding the interplay between biodiversity and ecosystem function through an eco-evolutionary lens; 2) examining the hypothesis that climate-driven evolution of plant traits will have downstream consequences for associated soil microbiomes and ecosystem function across the landscape; and 3) examining genetically-based plant-soil feedback at the landscape scale to understand how variation in climate, soil microbiome function, and tree-driven soil conditioning interact to influence phenotypic variation in bud break phenology. The findings from this dissertation provides evidence that understanding the natural variation in genetic components of both above- and belowground portions of the plant-soil linkage are important for predicting patterns of divergence in ecosystem function in a warmer world. Cumulatively, this dissertation extends the field of eco-evolutionary dynamics by highlighting the interplay between ecology and evolution that governs the expression of phenotypes, patterns of community composition, and divergence in ecosystem function at spatial scales rarely appreciated.

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INTRODUCTION

Plant responses to global change are being well documented. Changing climates, for example, are a) increasing the frequency of mortality (Gitlin et al. 2006, Van Mantgem et al. 2009, Anderegg et al. 2013), b) influencing migration (Fei et al. 2017), and c) driving evolutionary and plastic responses in plant populations (reviewed in Franks, Weber, and Aitken 2017). Cumulatively, these responses will likely have major implications for biodiversity and ecosystem function on the landscape (Anderegg et al. 2013). If we are to accurately explore and predict the consequences of climate change, we must continue to link field and experimental observations to ensure we can provide real world context to the patterns and processes we observe. Using elevational gradients as a space-for-time substitution is a prominent experimental approach for gaining inference on *in situ* plant responses of individuals, populations, communities, and ecosystems to climatic gradients (Fukami & Wardle 2005, Körner 2007). However, studies using elevation as a climate change proxy often do not address how intraspecific variation, potential evolutionary responses, or range position (i.e., leading edge, continuous, or trailing edge) may influence the patterns of plant responses detected. Additionally, studies to date have limited inference and replication by only sampling one or two elevation gradients per study, per system, or through time (see Pfennigwerth et al. 2017). Addressing these limitations by further developing both field observations and experiments at appropriate scales will further our understanding of how interacting environmental gradients influence plant responses across such gradients and ultimately to climate change scenarios.

One such approach integrates perspectives from population genetics, global change biology, and ecosystem ecology by comparing individual populations, and their associated communities and ecosystems, across the geographic extent of a species' distribution. Modern plant distributions inherently include genetic differentiation in plant traits shaped by climatic history, geographic structure, gene flow, demographic processes, and ecological interactions through time. For example, plant species spanning large altitudinal, latitudinal, or longitudinal gradients likely experience drastically different climates and biotic communities across their geographic extent, especially populations that occur along the leading and trailing range edges (Hampe & Jump 2011, Woolbright et al. 2014). For example, dominant, forest trees with large geographical ranges can experience a high abiotic and biotic environmental variation, resulting in large differences in quantitative trait variation and population genetic differentiation across the species' extent (Evans et al. 2016). This geographic variation in of quantitative trait variation, climate, and biotic interactions, and ecosystem characteristics will interact to influence the ecological and evolutionary dynamics on the landscape (Thompson 2009, Hendry 2017), but manifest in complex ways that can make parsing these interacting effects difficult (Kinnison et al. 2015, Ware et al. 2019).

Variation in or the advancement of phenology (i.e., the timing of life history events such as leaf out of flowering) is generally thought to be driven by climatic factors such as temperature and light and thus, it is a useful tool for understanding the patterns and consequences of climate change. As such, phenological change in response to shifting environmental conditions is now well documented in natural populations, communities, and ecosystems globally (Walther et al. 2002, Parmesan 2006, Cleland et al. 2007, Walther et al. 2010). Phenological plant traits, such a leaf bud break or flowering, are complex functional traits tightly related to plant net primary productivity, reproduction, and important biotic interactions such as timing of pollination, herbivory, plant-microbe interactions, all of which can influence overall plant fitness, growth performance, and adaptation to varying conditions (Strauss et al. 1996, Strauss et al. 2002, Wagner et al. 2014). For example, spring emergence of foliar tissue (i.e., bud break phenology) initiates the growing season and represents a significant driver of ecosystem productivity, soil resource acquisition, and carbon dynamics (Nord & Lynch 2009; Polgar & Primack 2011; Richardson et al. 2009, 2010).

Plants can also exert local influence on their soils by modifying distinct physical, chemical, and biotic environments that are a response to, and consequence of, functional plant traits (Laland et al. 1999) and that varies by environment. Soil conditioning by plants is largely driven by inputs of leaf litter, root turnover, or exudates that modify distinct chemical and nutrient pools belowground. Plant genetic variation in tissue and exudate chemistry can lead to unique conditioning and selection of plant-associated soil microbial communities (Schweitzer et al. 2008, Hartmann et al. 2009, Hu et al. 2018) A series of recent studies showed that changes to soil communities drove plant adaptations in novel environments (Lau and Lennon 2012), and that the evolutionary changes in plants subsequently affected soil communities (ter Horst et al. 2014). Further, plant genotypic variation was recently found to affect belowground ecosystem processes across large spatial scales (Madritch et al. 2014), adding to a small, but growing, body of important work demonstrating the importance of plant-soil linkages at the landscape level. However, the landscape-level evolutionary consequences of soil gradients on plant populations and how they interact with strong climate gradients is poorly understood, and such information will be critical for understanding the ecological and evolutionary effects of climate warming on the complex interactions that occur belowground.

The major themes for my dissertation include, the role of feedbacks in linking evolution and ecosystem ecology across terrestrial ecosystems, variation in tree-driven conditioning of associated soil microbial communities and soil chemistry, the importance of tree-associated soil microbes in mediating range-wide bud break phenology in a foundation tree species, and the role of climate-driven reduction of genetic variation in plant phenology alters soil communities and nutrient pools. To explore these themes, I have been examining geographic variation and interactions in plant phenotypes, plant population genetic differentiation, soil microbiomes, and soil nutrient pools across the range of a single species, *Populus angustifolia* James. The goal of these chapters is to document evolution in the context of contemporary above- and belowground ecological interactions to accurately understand responses to climate warming. In dominant or foundation tree species, intraspecific variation in phenotypes is a key force in governing biodiversity, community structure, and ecosystem function, and thus is important to include in modeling the effects of climate change in natural and experimental systems (Whitham et al. 2006, Bailey et al. 2014, Van Nuland et al. 2016). Previous work in this system highlights the importance of including plant-soil-microbe linkages to understand how plant populations may persist under predicted climate change scenarios. Further, replicated sampling across larger scales (i.e. latitudinal, longitudinal, or range-wide studies), will allow a better picture of the amount of intraspecific variation that exists and begin exploring how ecological dynamics shift along landscape-level climatic gradients.

In the individual chapters below, I show how intraspecific variation in leaf bud break phenology and productivity is structured and influenced by interacting abiotic and biotic environments by using a combination of *in situ* observations and greenhouse common garden experiments with 17 distinct *Populus angustifolia* populations sampled across its geographic range. Results from these studies will demonstrate the importance of 1) integrating ecosystems ecology with evolutionary biology, 2) the role biotic interactions in mediating variation in phenology, and 3) the downstream consequences of intraspecific variation in a foundation tree species on ecosystems.

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CHAPTER I

FEEDBACKS LINK ECOSYSTEM ECOLOGY AND EVOLUTION ACROSS SPATIAL AND TEMPORAL SCALES: EMPIRICAL EVIDENCE AND FUTURE DIRECTIONS

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I.M.W., J.K.B., and J.A.S. participated in the developing the conceptual framework. I.M.W. performed initial literature review and wrote initial manuscript draft. All authors discussed the provided context for conceptual framework and made significant contributions to revisions.

Abstract

1. Unifying ecosystem ecology and evolutionary biology promises a more complete understanding of the processes that link different levels of biological organization across space and time. Feedbacks across levels of organization link theory associated with eco-evolutionary dynamics, niche construction, and the geographic mosaic theory of co-evolution.

2. We describe a conceptual model, which builds upon previous work that shows how feedback among different levels of biological organization can link ecosystem and evolutionary processes over space and time. We provide empirical examples across terrestrial and aquatic systems that indicate broad generality of the conceptual framework and discuss its macroevolutionary consequences.

3. Our conceptual model is based on three premises: genetically-based species interactions can vary spatially and temporally from positive to neutral (i.e., no net feedback) to negative and drive evolutionary change; this evolutionary change can drive divergence in niche construction and ecosystem function; and lastly, such ecosystem-level effects can reinforce spatiotemporal variation in evolutionary dynamics. Just as evolution can alter ecosystem function locally and across the landscape differently, variation in ecosystem processes can drive evolution locally and across the landscape differently.

4. By highlighting our current knowledge of eco-evolutionary feedbacks in ecosystems, as well as information gaps, we provide a foundation for understanding the interplay between biodiversity and ecosystem function through an eco-evolutionary lens.

Introduction

A critical challenge in biology is to understand the reciprocal interactions (hereafter, ***feedbacks***) between ecological and evolutionary processes. Evolution in a focal organism can alter processes occurring at the population, community, and ecosystem-level, which in turn can feed back to alter subsequent evolution of the focal organism

(Hendry, 2017). This eco-evolutionary (eco-evo) feedback occurs because evolution can shift phenotypic variation in populations, which can alter species interactions and ecosystem processes, such as energy flow and nutrient cycling (Lindeman, 1942; Jones, Lawton, & Shackak, 1994). Further, these changes to energy flow and nutrient cycling can vary spatially and temporally in strength and direction, can persist for generations via ***legacy effects***, and can feed back to shape future species interactions. Numerous examples suggest that eco-evo dynamics are ubiquitous, however, detecting them remains challenging because the net result of interacting feedbacks could range from positive to negative, and cumulatively appear neutral (Schweitzer et al., 2014; Kinnison et al., 2015). Further, the drivers and consequences of variation in eco-evo feedbacks across levels of biological organization, spatial scales, and time are unclear. Here, we integrate the temporal dynamics of ***niche construction*** theory (NCT), the spatial scale of ***geographic mosaic theory of coevolution*** (GMT), and energy and nutrient dynamics of classic ecosystems ecology (circa Jenny, 1941; Lindeman, 1942; Chapin, Matson, & Vitousek, 2012), to provide a conceptual framework to link ecosystem ecology and evolution (terminology defined in **Box 1.1**). The framework presented here builds upon previous work (Post & Palkovacs, 2009; Matthews et al., 2014; Van Nuland et al., 2016) in three significant ways. First, we expand a conceptual model, showing three levels of feedback that incorporate temporal and spatial scales that are explicitly related to ***ecosystem state factors*** and the legacy effects of past species interactions. Second, we provide some empirical examples across multiple terrestrial and aquatic systems that indicate generality of this conceptual framework. Third, we examine potential macroevolutionary consequences of this conceptual framework.

Niche construction is the process by which organisms modify and create their own niche, or those of other, interacting organisms (Odling-Smee et al., 2003). Niche construction varies across populations due to variation in phenotypes that directly or indirectly affect environments through genetically-based species interactions. ***Ecosystem processes*** cumulatively represent niche construction because they control the input, loss, and transfer of materials and energy to and from the ecosystem, are mediated by phenotypic interactions within communities, and can reinforce patterns of phenotypic interactions on the landscape (Genung et al., 2013); all of which can alter patterns selection. For example, ecosystem processes such as energy flow across trophic levels and nutrient cycling (e.g. decomposition rates in soils, nitrogen and phosphorous immobilization in soils) in terrestrial ecosystems occur over short to long timescales and can shift in response to genetic variation in interacting phenotypes (Bailey et al., 2014; Schweitzer et al., 2014). Interactions among phenotypes (and underlying genotypes), therefore, play an integral role in niche construction by altering communities and ecosystems in ways that produce legacy effects and long-term changes in ecosystem pools over time (Bailey et al., 2009). Complementing NCT, the GMT explores the same genetically-based interactions driving niche construction but along landscape-level environmental gradients such as climate, other organisms, relief, and parent material (i.e., ecosystem state factors; Benkman, 1999; Brodie et al., 2002; Chapin et al., 2012; Parchman et al., 2016; Van Nuland, Bailey, & Schweitzer, 2017). Geographic variation in genetically-based species interactions, gene flow, and natural selection lead to different evolutionary outcomes across spatial scales (e.g., **co-**

evolutionary hotspots and coldspots; *sensu* Thompson, 1998, 2005). However, ecosystem-level consequences and their potential feedbacks to population dynamics are rarely considered as a consequence of co-evolutionary interactions across spatial scales. Integrating these two established bodies of theory may help our understanding of how different feedbacks emerge across multiple levels of biological organization, further linking population, community, and ecosystem processes across space and time.

Extending a Conceptual Framework

We use an eco-evolutionary framework to show synthesis between NCT with GMT by incorporating feedbacks from multiple levels of organization to link scales of space and time (modified from Van Nuland et al., 2016; **Fig. 1.1,** *All figures and tables for this chapter are located in this chapter's Appendix*). This builds upon and reconciles previous work in community and ecosystem genetics (Whitham et al., 2006; Bailey et al., 2009; Schweitzer et al., 2012; Des Roches et al., 2018), eco-evolutionary dynamics (Post & Palkovacs, 2009; Genung et al., 2011; Matthews et al., 2014; Hendry, 2017), niche construction theory (Kylafis & Loreau, 2008; Odling-Smee et al., 2013), and the geographic mosaic theory of co-evolution (Thompson 1998). Together, these fields show how genetically-based species interactions at different scales generate reciprocal feedbacks that may drive ecological (including ecosystem) functions through time, as well as alter evolutionary processes across the landscape. This synthesis shows the common elements in these areas of study can be merged theoretically and empirically to quantify when and how genetically-based feedbacks can occur among species across space and time to influence both ecosystem and evolutionary processes.

In the simplest case, our conceptual model shows two interacting species (co)evolving through time and along abiotic gradients of ecosystem state factors (space) (Fig. 1.1: *FB1*, genotype x genotype [GxG] interactions and reciprocal selection; Janzen, 1980). The boxes in *FB1* represent heritable trait variation for each interactor (i.e., individual traits, trait covariances, or community-level trait matrices). The evolutionary consequences of *FB1* can vary within and among communities and geographically, ranging from non-evolving interactions to tightly coevolving interactions (Thompson, 2005). On this spectrum, the majority of eco-evolutionary interactions are likely the product of diffuse (co)evolution, and this heterogeneity in GxG interactions along abiotic gradients will produce variation in the strength and reciprocity of selection across the landscape. The same genetically-based species interactions drive variation in energy flow and nutrient cycling through processes such as trophic interactions, decomposition, and nutrient transformation (i.e., ecosystem engineering). Niche construction occurs when the abiotic environmental variation (i.e., energy and nutrients) that is the result of species interactions over time (i.e., legacy effects) feeds back to affect contemporary population and community dynamics in the next generation (*FB2*). For example, genetically-based species interactions lead to changes in trophic dynamics and nutrient cycling influencing the processes that build a niche (alteration of physical or chemical conditions) and change natural selection across temporal scales. Because the cumulative effects of *FB1* and *FB2* (i.e., species interactions, their ecosystem effects, and feedbacks) can vary geographically in strength and direction along environmental gradients representative of ecosystem state factors, the conceptual model directly integrates the temporal dynamics of NCT with the spatial scale of the

GMT. Finally, NCT and GMT can be further integrated by the cumulative effects of past species interactions (*FB3*). Feedback 3 starts with the original abiotic gradient of ecosystem state factors that underlie *FB1* and *FB2*. Feedback 3 is driven by and can change due to the slow alteration of the ***ecosystem pool*** from the legacy effects of *FB1* (Vitousek, 2004; Wooliver et al., 2016, 2018; Van Nuland et al., 2019; Fig. 1.1). The ecosystem pool is composed of the abiotic factors (e.g., nutrient pools, pH) which change over time and vary across spatial scales and are affected by the initial conditions, climate and other factors which were present when the eco-evo dynamics "started" (e.g., ***abiotic origin***). The historical ecosystem pool can change through time due to gradual effects of past species interactions related to *FB1* and *FB2*, as the ecosystem pool and the historical ecosystem pool covary (Vitousek, 2004; Van Nuland et al., 2019). The eco-evo feedback at this scale can be thought of as ***historical contingency*** because contemporary interactions are contingent on the ecosystem-wide effects of prior interactions that vary geographically (Van Nuland et al., 2016, 2017, Senior et al., 2018). Importantly, the interactions that constitute *FB1* (Figure 1.1) are not restricted to populations of different species but can also occur among individuals within a population (e.g., Turcotte et al., 2011), or among populations (e.g., assortative mating). Second, the diffuse nature of coevolving ecological interactions on the landscape is a product of gene flow among populations, spatial structure of genetically-based interactions (e.g., regional species pools), and the reciprocity of selection in those genetically-based interactions. This diffuse (co)evolutionary dynamic gives rise to a geographic mosaic of "hotspots" and "coldspots" of coevolution (Thompson, 2005); however, we know little about how variation in *FB1* might shape the functions of ecosystems (Bailey et al., 2014; Schweitzer et al., 2018.)

Empirical evidence of these points furthers our understanding of eco-evolutionary dynamics as well as the genetic and environmental factors that determine phenotypes over time and space. We use three examples from terrestrial and aquatic systems to demonstrate the broad applicability of these concepts and which of the three feedbacks from Fig. 1 have been demonstrated empirically to date. Further, we identify and explore research frontiers to demonstrate paths forward in understanding how eco-evo feedbacks link population, community and ecosystem-level processes across space and time.

Evidence of Eco-evolutionary Feedbacks Across Terrestrial and Aquatic Systems

Ecosystem effects of evolution in plant-herbivore interactions.

Evolution resulting from plant-herbivore interactions is likely to shape ecosystems when the genetic variation mediating the interaction is correlated with both fitness and ecosystem-level effects. Direct and indirect evidence of the co-evolutionary dynamic of plant-herbivore interactions (**Fig. 1.2**: *FB1*) exists, but empirical evidence of ecosystem feedbacks resulting from plant-herbivore interactions (*FB2*) remain scarce. However, studies investigating the ecological importance of genetic variation in plants and herbivores provide compelling evidence that contemporary evolution as a result of plant-herbivore interactions can have ecosystem-level effects (Fig. 1.2: ecosystem engineering side of *FB2*). For example, Classen et al. (2007, 2013) demonstrated that piñon pine (*Pinus edulis*) susceptibility to a scale insect herbivore is correlated with plant traits that increased nitrogen (N) cycling through litter decomposition but reduced N and carbon (C) accumulation in soil over decades. Additionally, selective consumption of particular plants (e.g. Belovsky & Slade, 2000; Bailey et al., 2004, Yang & Gratton, 2014), induction of defense compounds (Schweitzer et al., 2005; Katayama et al., 2013), herbivore genetics (Kant et al., 2008; Turley & Johnson, 2015; Zytynska et al., 2016) and differences in the quality of insect herbivore excretions can influence soil N availability and even feedback to influence plant production (Kagata & Ohgushi, 2013). Finally, interacting organisms from different trophic groups (e.g., predators [Schmitz et al., 2008], or soil microorganisms [Pineda et al., 2013]), could indirectly mediate the ecosystem effects of herbivory (Utsumi, 2011). Taken together, these studies suggest that genetic variation mediating the interaction between plants and herbivores can exhibit feedbacks to ecosystem processes.

Growing empirical evidence is showing ecosystem effects resulting from evolution in plant-herbivore interactions. For example, replicate populations of *Oenothera biennis* (a North American biennial forb) exposed to either ambient or reduced herbivory diverged in genotypic compositions after 5 years (Agrawal et al., 2012). Using the same experimental evolution study, Fitzpatrick et al. (2015) found significant effects of both the direction and magnitude of plant evolution on litter decomposition and soil N mineralization rates occurred within experimental plots. Evidence of evolutionary divergence on *O. biennis* seedling performance in soil collected from each of the experimental plots was found, suggestive of an eco-evo feedback (Fig. 1.2: *FB2*). From the herbivore side, although a long history of investigating contemporary evolution exists (especially in an agricultural context; Via, 1990; Gould, 1991), the effects of herbivore evolution on ecosystem processes are unclear. The evolution of traits which increase herbivore population growth would be expected to increase plant consumption. This could result in increased herbivore-derived resources or induced plant defenses, both of which could have ecosystem effects (Yang & Gratton, 2014). In the green peach aphid, Turcotte et al. (2011) found that evolution of increased population growth rates did not affect host plant biomass, while Turley and Johnson (2015) found negative effects dependent on host plant species. These few studies provide direct evidence that evolution resulting from plant-herbivore interactions can have ecosystem-level consequences. Even less is known about how landscape-level variation in the strength of plant-herbivore interactions may drive eco-evo feedback (Fig. 2: *FB3*). The necessary ingredients for *FB3* exist: geographic clines in both heritable plant defense traits (e.g. Anstett et al., 2015) and the strength of plant-herbivore interactions (e.g. Benkman, 1999; Pennings & Silliman, 2005). Thus, landscape-level variation in eco-evo feedback due to plant-herbivore interactions are likely common. Our conceptual approach highlights a way forward for examining how plant-herbivore interactions (*FB1*) shape ecosystem pools and processes that may feedback (*FB2*) to influence ecosystem-level evolutionary effects at landscape scales (*FB3*).

Ecosystem consequences of evolution in aquatic systems.

Eco-evolutionary feedbacks in aquatic ecosystems have been studied primarily through the perspectives of trophic interactions and nutrient recycling (Post & Palkovacs, 2009; Matthews et al., 2011b; Schoener, 2011). The presence of eco-evo feedbacks in aquatic microcosms is now incontrovertible with evidence for eco-evo effects outside of lab experiments for a wide variety of aquatic taxa, including zooplankton (Matthews et al., 2011a; Miner et al., 2012), aquatic macroinvertebrates (Ousterhout et al., 2018), amphibians (Reinhardt et al., 2013; Urban, 2013), and fishes (Carlson et al., 2011; Fryxell & Palkovacs, 2017; Tuckett et al., 2017; Auer et al., 2018). Here we detail evidence for feedbacks in three fish study systems – alewife, guppies, and threespine stickleback in the context of Fig. 1.1.

It is clear that species interactions and trophic position can drive eco-evo feedback in multiple fish systems (Fig. 1.3: *FB1*; Palkovacs & Post, 2008, Post et al., 2008). For example, the evolution of freshwater resident alewife populations shapes the seasonality of predation on zooplankton communities. Year-round predation reduces zooplankton body size, thereby creating an eco-evo feedback that selects for smaller alewife gape and gill raker spacing (Palkovacs & Post, 2008). The ecological effects of alewife divergence also have impacts on the evolution of alewife prey (Walsh & Post, 2011), competitors (Huss et al., 2014), and predators (Brodersen et al., 2015). In guppy populations, fish predators increase mortality rates and decrease guppy densities (Fig. 1.3: *FB1a*; Reznick et al., 1990; Reznick et al., 1996). These ecological changes shape guppy feeding traits (Palkovacs et al., 2011; Zandonà et al., 2011), which in turn alter invertebrate and periphyton abundances (Fig. 1.3: *FB1b*; Palkovacs et al., 2009; Bassar et al., 2010). Changes in guppy density and resource availability appear to underlie the evolution of guppy life history traits, including age and size at maturity (Bassar et al., 2013). This change in body size, in turn, alters nutrient recycling rates (Fig. 1.3: *FB2*; El-Sabaawi et al., 2015). In stickleback populations, fish predators specialized on either pelagic or littoral prey reshape prey community structure through alternative feeding preferences (Harmon et al., 2009; Des Roches et al., 2013). Stickleback specialized on either stream or lake habitats reduce their favored prey, causing a negative eco-evo feedback in mesocosms that favors the alternative type (Matthews et al., 2016). Further, a recent mesocosm experiment showed how patterns of phenotypic variation between lakes can lead to differential prey depletion and ecosystem modification, feeding back into selection regimes (Best et al., 2017). Similarly, several studies have shown how environmental context, such as nutrient environment, influences *FB1* (see Declerck et al., 2015; Tuckett et al., 2017; Brunner et al., 2017). The presence of sticklebacks has been shown to influence aquatic food webs and ecosystem pools (see Limberger et al., 2018), and if similar ecological and ecosystem dynamics could feedback to have evolutionary ramifications across trophic levels and vary geographically (similar to Best et al., 2017), *FB3* may arise. As with plant-herbivore interactions, little information currently exists regarding how *FB1* and *FB2* may vary across environmental gradients to generate variation in ecosystem pools and processes that may drive and reinforce *FB3*. Understanding how the legacy effects of *FB1* and *FB2* influence *FB3* represents an important future research challenge in aquatic systems.

***Ecosystem consequences of evolution in plant-soil feedbacks*.**

Plants alter the soils in which they grow, and evidence that these modifications can feed back to influence the same or different plants represents a rich and growing mechanism for a variety of ecological phenomena (Schweitzer et al., 2012; Van der Putten et al., 2016). Within *Populus* (and many other plant taxa), population-level approaches show evidence that tree genotypes condition and host distinct microbial communities (**Fig. 1.4A**: *FB1*), and differentially influence soil nutrient dynamics (Schweitzer et al., 2004; Schweitzer et al., 2008; Cregger et al., 2018), which can feed back to influence plant productivity and performance (Fig. 1.4B; *FB1* & *FB2*). Further, as plant-driven soil nutrient conditioning increases so does the strength of plant-soil feedback, which demonstrates that *FB1* and *FB2* are related and vary geographically, directly linking populations, communities, and ecosystems (Fig. 1.4C, Van Nuland, Bailey, & Schweitzer 2017). When this occurs over long time periods across environmental gradients (Fig. 1.1: *FB3*), ecosystem processes can drive population-level divergence. The best observational example for *FB3* is evidenced by the long-term soil nutrient gradient across the Hawaiian Islands in which divergent populations of *Metrosideros polymorpha* resulted from differences in litter traits that accelerated or slowed nutrient cycling depending on their position along a soil fertility gradient (Treseder & Vitousek, 2001; Vitousek, 2004). However, Van Nuland et al. (this issue) provide further direct evidence integrating *FB's 1-3* across a landscape-level soil fertility gradient.

Together, these empirical results in both terrestrial and aquatic systems show unequivocally that the ecosystem consequences of genetic-based species interactions and niche construction vary (or will likely vary) across spatial environments. They provide evidence for multiple types of feedback, primarily through the evolution of trophic interactions. Although the means and specific evolutionary mechanisms differ among plants and herbivores, among predators and prey, and with both trophic and non-trophic interactions among plants, microorganisms and soils, feedbacks over time connect populations, communities, and ecosystem pools and processes across space.

Conclusions and Future Directions

Here, an expanded conceptual framework links ecosystem ecology and evolution by integrating genetically-based species interactions, NCT, and GMT to highlight feedbacks over time and space. To date, empirical and theoretical evidence shows that feedbacks from multiple levels of organization can vary in strength and reciprocity and may be mediated by both direct and indirect interactions and by the environmental context in which such interactions take place. The examples outlined above show that genetically-based species interactions (*FB1*) have consequences for both ecosystem and evolutionary processes. Though there is little direct evidence of *FB2* in the plant-herbivore example, the aquatic predator-prey-producer and plant-soil-microbe examples show patterns of ecosystem-level effects that directly alter evolutionary processes. Limited empirical information currently exists for *FB3* in both plant-herbivore interactions and aquatic trophic dynamics, but we show direct evidence that ecosystem-level effects of plant-soil-microbe interactions vary geographically, reinforcing *FB1* and *FB2* and providing examples of context dependency in feedbacks supporting this conceptual framework.

As outlined above, growing empirical evidence is beginning to clarify linkages between evolving trophic dynamics and differences in nutrient cycling rates. In all three systems, improving knowledge on environmental context (*FB3*) is critical for understanding the strength and reciprocity of ecological and evolutionary dynamics in experimental (and natural) settings. For example, eutrophication levels are being included as treatments in many aquatic studies to examine how ecosystem-level differences and environmental context may mediate eco-evo dynamics (Declerck et al., 2015; Tuckett et al., 2017; Brunner et al., 2017). In terrestrial systems, growing evidence shows the importance of ecosystem legacy effects in plant-herbivore and plant-soil interactions (e.g., Fitzpatrick et al., 2015; Pregitzer et al., 2010, Van Nuland et al., 2017, 2019 *this issue*). Further integrating large-scale, geographic approaches with ecosystem perspectives (including ecosystem state factors) will likely improve our understanding of how the interplay among phenotypes, trophic dynamics, and environmental context influences both ecosystem and evolutionary processes in aquatic and terrestrial ecosystems.

Demonstrating *FB's 1-3* at macroevolutionary scales will improve understanding of how feedbacks across time and space (Fig. 1.1) have long-term consequences for patterns of biodiversity (Weber et al., 2017). For example, Wooliver et al. (2017) showed that *Eucalyptus* spp. species differ in their capacities to use N for growth. Such evolutionary divergence across species was found to be associated with both genetic variation in root function and soil N levels in their home ranges, whereby species occurring in higher nutrient soils have evolved greater specific root length and nutrient use capacities. This demonstrates that soil N has been a strong selective agent for plant function in this plant group that can in turn drive soil nutrient pools. Further, coevolution with root symbionts seem to play a key role in driving nutrient use within the Tasmanian eucalypts (Wooliver et al., 2018). Overall, this work in plant-soil interactions demonstrates that natural variation in soil nutrient pools can drive feedbacks between plants and their soil microbial communities that vary from positive to negative depending on environmental context (GMT) and are phylogenetically based. From a plant-herbivore perspective, considering that plants have faced herbivory since their colonization of terrestrial Earth over 400 MYA (Labandeira, 2007), this ancient interaction was likely responsible for the development of complex food webs (Olson, 1966; Sues & Reisz, 1998) and are also attributed with giving rise to the rich arsenal of physiological, chemical, and mechanical plant defenses found today as well as the diversification of numerous plant and animal lineages (Becerra et al., 2009; Futuyama & Agrawal, 2009; Wiens, Lapoint, & Whiteman, 2015). Thus, using recently developed phylogenetic tools and other comparative approaches to demonstrate the role of NCT and GMT across landscapes will be important to demonstrate the concepts in Fig. 1.1 and their consequences in terrestrial ecosystems.

Our comparative approach highlighted, conceptually and empirically, how feedbacks can link ecosystem ecology and evolution by merging concepts of NCT and GMT over time and space. We showed multiple examples of genetically-based species interactions (*FB1*) and how these interactions lead to niche construction over time, altering physical or chemical conditions to impact natural selection (*FB2*). In a plant-soil system we showed how *FB's* 1 and 2 can be examined across gradients and spatial scales (*FB3*) that may overall have macroevolutionary consequences. Overall this approach indicates similarities and linkages among independent lines of research/theory (NCT and GMT), highlights research gaps and reveals many testable hypotheses. Testing these will progress the synthesis showing the ecological and evolutionary relationships between patterns of biodiversity and ecosystem function. Exciting work awaits.

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Appendix

Table 1.1 Definitions of terms

Term	Definition
Eco-evolutionary feedback	Feedback describes a sequence of interactions in which the result of a process affects the conditions that initially generate the process / eco-evolutionary feedbacks are the cyclical interaction between ecology and evolution such that changes in ecological interactions drive evolutionary change in organismal traits that, in turn, alter the form of ecological interactions, and so forth.
Genetically-based species interactions	Genetically-based phenotypic interactions within populations or among species.
Phenotype	The physical appearance or biochemical characteristic of an organism as a result of the interaction of its genotype and the environment.
Population	A group of organisms of one species that interbreed and live in the same place at the same time.
Community	An association of interacting species that live in a particular area.
Ecosystem	Ecological system consisting of all the organisms in an area and the physical environment with which they interact.
Ecosystem pool	Quantity of energy, material, or nutrients in an ecosystem compartment.
Ecosystem state factors	Independent variables that control the characteristics of ecosystems (climate, parent material, topography, potential biota, time; sensu Jenny 1941).
Ecosystem processes	Inputs or losses of materials and energy to and from the ecosystem and the transfers of these substances among components of the ecosystem; ecosystem processes include decomposition, production, nutrient cycling, and fluxes of nutrients and energy.
Ecosystem engineering	Modifications to the environment by a species that affects resource availability for another species.
Niche construction	The process whereby organisms actively modify their own and each other’s evolutionary niches.
Legacy effects	The phenotypic effects of an organism that extends beyond the life of the organism // an indirect effect that persists for a long time period in the absence of the causal species, or after this species has ceased the causal activity.
Ecological inheritance	The persistence of environmental modifications by a species over multiple generations to influence the evolution of that or other species.
Abiotic origin	The initial conditions, climate and other factors which were present when the eco-evo dynamics “started”.
Historical contingency	The evolutionary effects of the ecosystem pool which remains constant over ecological timescales but change over longer time periods due to gradual accumulation of changes (i.e., abiotic and biotic environmental effects) happening every generation.
Environmental context	The biophysical environment consisting of biotic and abiotic components surrounding a population or interacting populations.
Coevolutionary hotspots	Interactions are subject to reciprocal selection only within some local communities. These coevolutionary hotspots are embedded in a broader matrix of coevolutionary coldspots, where local selection is nonreciprocal.
Geographical mosaic hypothesis	States that because species interactions vary geographically, a mosaic of population genetic structure will result that leads to different evolutionary trajectories.

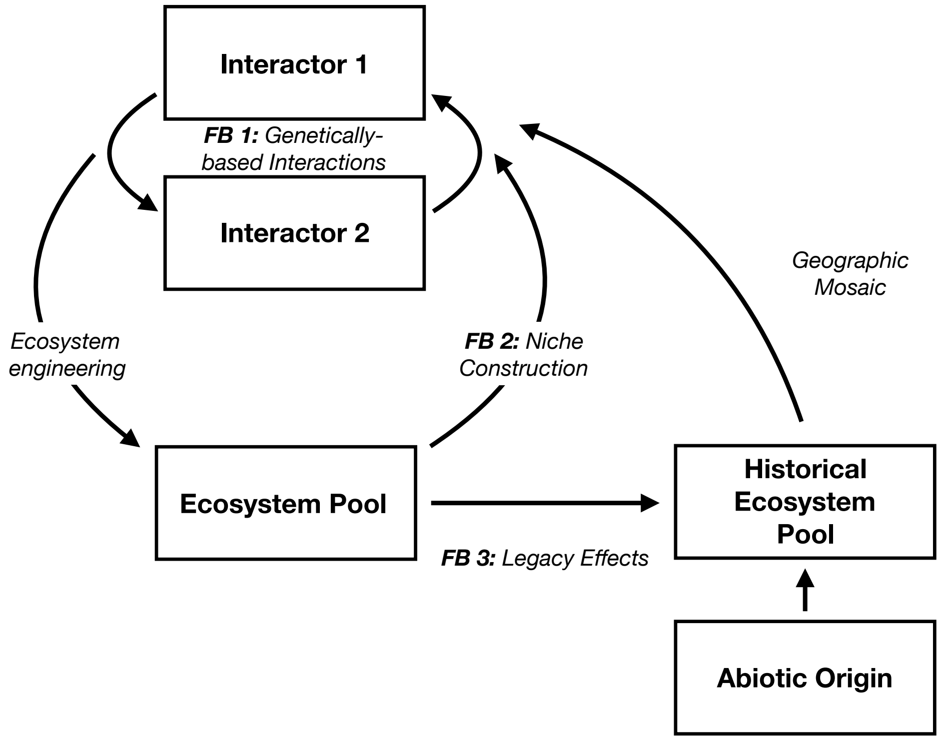

Figure 1.1 A conceptual illustration of interacting feedbacks linking genetically-based interactions, niche construction, ecosystem dynamics, and the geographic mosaic of coevolution. **Individual components within each feedback are defined conceptually to express how each feedback operates. FB1** represents reciprocal, genetically-based interactions. **FB2** shows how these genetically-based interactions can influence ecosystem engineering and niche construction. **FB3** shows how historical contingency of past ecosystem-level feedbacks (i.e., temporal dynamics inherent to niche construction) and underlying abiotic origin (e.g., climatic gradients) can interact and feedback to influence strength and reciprocity of genetically-based interactions in FB1 (i.e., integrating NCT and the GMT).

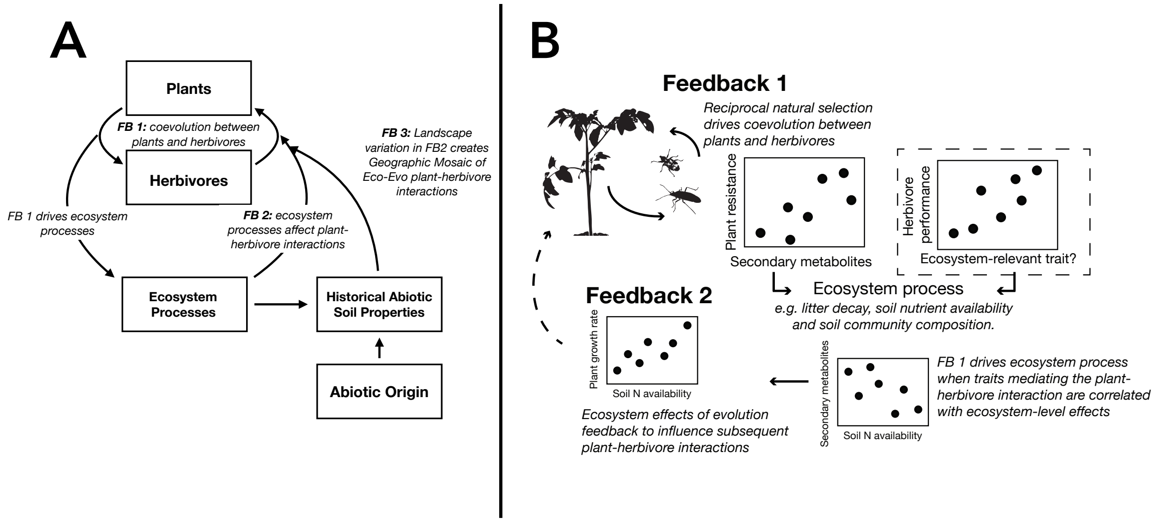

Figure 1.2 A conceptual illustration of the various feedbacks occurring between plants, herbivores and ecosystems. Feedback 1 (Panel **A**: FB1) is the coevolution between plants and herbivores caused by reciprocal natural selection. FB1 can alter ecosystem processes because heritable traits mediating plant-herbivore interactions are often correlated with ecosystem-level effects (Panel **B**: secondary metabolite production in plants alters soil N availability; e.g. Schweitzer et al., 2004). The ecosystem-level effects of plant defense evolution can feed back to influence subsequent plant-herbivore interactions, due to the effects of altered ecosystem processes on plant performance (Panels **A** and **B**: FB2; e.g., Fitzpatrick et al., 2015). In addition to local ecosystem processes plant-herbivore interactions, and the feedbacks they initiate, are affected by variation in the abiotic and biotic environment across the landscape (Panel **A**: FB3). Edaphic features, climate and biotic factors such as community composition and over longer timescales, the regional rates of speciation and extinction, cause this landscape-level variation in the environment. Dashed lines highlight current knowledge gaps in this system.

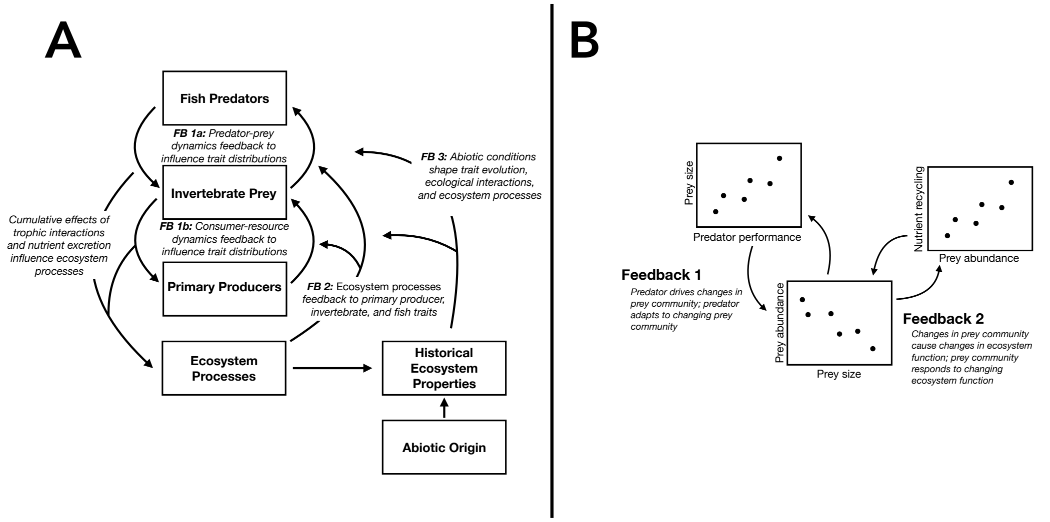

Figure 1.3 A conceptual illustration of the various feedbacks occurring between fish predators, invertebrate prey, and primary producers in aquatic ecosystems. Feedback 1a and 1b (Panel **A**: FB1a & 1b) represent the evolutionary interactions driven by predator-prey and consumer-resource dynamics. Cumulative effects of the trophic interactions and nutrient excretions represented in FB1 can alter ecosystem processes, and feedback to influence changes to aquatic prey community (see Panel **A**: FB2; Panel **B** FB 1 and 2**)**. Hypothetical data in Panel **B** representative of findings from in the Trinidadian guppy system (**FB1a**: Reznick et al., 1990; Reznick et al., 1996, Palkovacs et al., 2011; Zandonà et al., 2011; **FB1b**: Palkovacs et al., 2009; Bassar et al., 2010, 2013; **FB2**: El-Sabaawi et al., 2015).

Figure 1.4 A conceptual illustration of various feedbacks occurring between *Populus angustifolia*, tree-associated soil microbiome, and local to landscape-level environmental context. Feedback 1 (Panel **A**: FB1) is the genetically-based plant-soil feedback between plant traits and soil microbiome. Feedback 1 can alter ecosystem processes as both plants and soil microbes directly (Panel **C**: FB1 and FB2, Van Nuland et al., 2017) alters soil nitrogen pools, soil carbon pools, and soil pH, which in turn reinforces geographic variation in plant-soil feedback (Panel **A**: FB2). Feedback 3 (Panel **A**: FB3) is exhibited by showing geographic variation in existing PSF (FB1) across home and away soil inoculation treatments (Panel **B**, FB1 and FB3: Schweitzer et al., 2018), likely driven by differences in abiotic and biotic environmental context. Panel **C** shows how plant-soil feedbacks (PSF) are related to the strength of soil conditioning across elevation gradients. The effect of (a) soil carbon (C) and (b) soil nitrogen (N) conditioning (i.e., the standardized difference between conditioned and unconditioned soil locations, ***ecosystem engineering*** **(*FB2*)** positively relates to feedback effects (*FB1* & *FB2*) for interior trees, but not edge trees (geographic variation resulting from *FB3*). Soil pH conditioning (c) did not predict interior or edge PSF. Solid lines depict significant regressions with grey areas representing 95% confidence interval, dashed lines represent insignificant regressions.

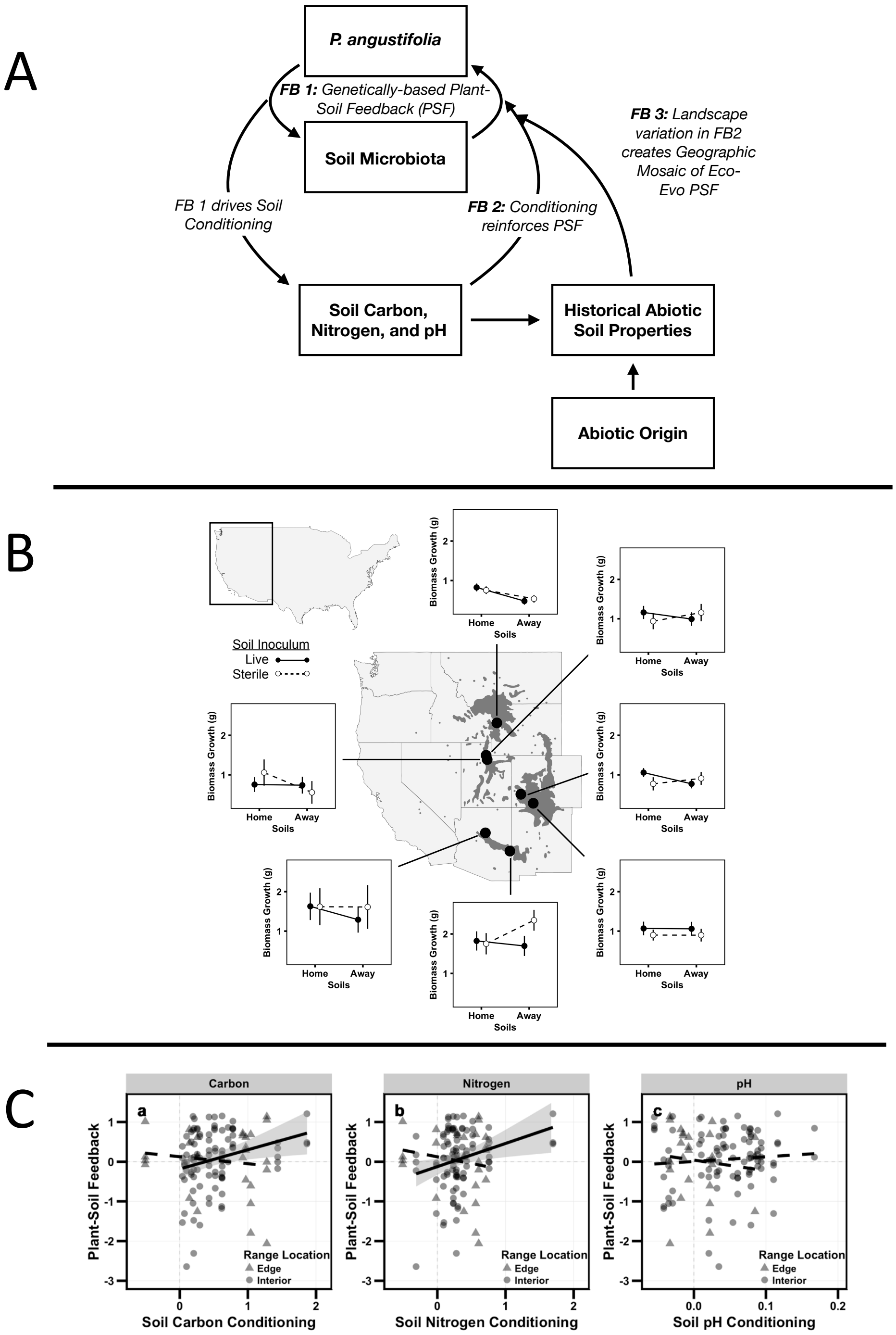
A
P. angustifolia
FB 1: Genetically-based Plant-Soil Feedback (PSF)
Soil Microbiota
FB 1 drives Soil Conditioning
FB 2: Conditioning reinforces PSF
FB 3: Landscape variation in FB2 creates Geographic Mosaic of Eco-Evo PSF
Soil Carbon, Nitrogen, and pH
Historical Abiotic Soil Properties
Abiotic Origin
B
Soil Inoculum
Live
Sterile
Biomass Growth (g)
Home
Away
Soils
C
Carbon
Nitrogen
pH
a
b
c
Plant-Soil Feedback
Range Location
Edge
Interior
Soil Carbon Conditioning
Soil Nitrogen Conditioning
Soil pH Conditioning

Figure 1.4

CHAPTER II

CLIMATE-DRIVEN REDUCTION OF GENETIC VARIATION IN PLANT PHENOLOGY ALTERS SOIL COMMUNITIES AND NUTRIENT POOLS

This chapter was originally published by Ian M. Ware, Michael E. Van Nuland, Jennifer A. Schweitzer, Zamin Yang, Christopher W. Schadt, Lindsay C. Sidak-Loftis, Nathan E. Stone, Joseph, D. Busch, David M. Wagner, and Joseph K. Bailey:

Ian M. Ware et al. (2019) Climate-driven reduction of genetic variation in plant phenology alters soil communities and nutrient pools. *Global Change Biology* VOL:PAGE# **https://doi.org/10.1111/gcb.14553**

I.M.W., J.K.B., and J.A.S. participated in the study design. I.M.W. performed field work and initial sample and data collection. I.M.W., M.E.V.N, J.A.S., Z.Y., C.W.S., L.C.S.-L., N.E.S., J.D.B., D.M.W. and J.K.B. collected data. I.M.W. performed statistical analyses and all authors discussed the results. I.M.W. wrote initial manuscript draft, and all authors made significant contributions to revisions.

Abstract

We examined the hypothesis that climate-driven evolution of plant traits will influence associated soil microbiomes and ecosystem function across the landscape. Using a foundation tree species, *Populus angustifolia*, observational and common garden approaches, and a base population genetic collection that spans 17 river systems in the western United States, from AZ to MT, we show that: 1) as mean annual temperature (MAT) increases, genetic and phenotypic variation for bud break phenology decline; 2) soil microbiomes, soil nitrogen (N), and soil carbon (C) vary in response to MAT and conditioning by trees; and 3) with losses of genetic variation due to warming, population-level regulation of community and ecosystem functions strengthen. These results demonstrate a relationship between the potential evolutionary response of populations and subsequent shifts in ecosystem function along a large temperature gradient.

Keywords: climate, genetic divergence, phenology, intraspecific variation, ecosystem dynamics, *Populus*

Introduction

Understanding how climate change may drive evolution in plant traits that lead to shifts in community structure and ecosystem function remains a frontier in modern ecology and will become increasingly important as global environments continue to warm (Bailey et al. 2014; Gilman et al. 2010; Lavergne et al. 2010; Woolbright et al. 2014). The lack of long-term, well replicated data at spatial scales relevant to projected climate change scenarios make it difficult to produce comprehensive studies and predictions about inherently linked ecological and evolutionary responses across multiple levels of organization (i.e., from genes to ecosystems). However, existing plant populations that are the result of past climate-driven range shifts can be useful for understanding the consequences of climate change events because they persist in suitable extant habitat patches that are potentially near the edge of their climate threshold. As a result, they are often locally adapted to the environmental conditions in which they persist because of natural selection, genetic drift, and minimal gene flow among populations (reviewed in Rehm et al. 2015; Woolbright et al. 2014). Maladaptation can occur due to continual gene flow from more central populations or Allee effects if population size reaches a critical minimum threshold (Angert & Schemske 2007; Kirkpatrick & Barton 1997; Sexton et al. 2009). However, there is ample evidence documenting ecological and evolutionary changes in natural systems responding to modern climate change scenarios (Lustenhouwer et al. 2017; Parmesan 2006; Walther 2010). Thus, investigating patterns of intraspecific genetic variation across populations could prove important for predicting adaptive responses to future changes in climate. Plant phenological traits show strong genetic differentiation along natural climatic gradients associated with latitudinal and elevational clines (Kooyers et al 2015; Peterson, Doak, & Morris 2017; Rohde et al. 2011; Wadgymar, Daws, & Anderson 2017), often resulting in locally adapted ecotypes (Aitken et al. 2008 and references therein). However, abiotic and biotic soil characteristics are gaining appreciation as ecologically important factors in predicting variation in a variety of phenology traits. For example, differences in soil fertilization treatments, soil chemistry, and soil microbial communities have been directly linked to changes in phenological trait variation (Arend, Gessler, & Schaub 2016; Sigurdsson 2001; Wagner et al. 2014). Further, spring emergence of foliar tissue (i.e., bud break phenology) initiates the growing season and represents a significant driver of ecosystem productivity, soil resource acquisition, and carbon dynamics (Nord & Lynch 2009; Polgar & Primack 2011; Richardson et al. 2009, 2010). Together, the individuals, populations, and communities that persist along environmental gradients, above- and belowground, are central to understanding how climate drives evolution of functional plant traits, affects species interactions, and may have ecosystem-level consequences (Hampe & Jump 2011; Kawecki 2008; Woolbright et al. 2014).

The effects of intraspecific variation on ecological processes have been detected across scales and diverse taxonomic groups (Des Roches et al. 2017). Genetic variation in plants traits can have direct consequences for associated community structure and ecosystem functions (reviewed in Hughes et al. 2008; Schweitzer et al. 2012; Van Nuland et al. 2016; Whitham et al. 2006). Landscape-level plant genetic variation has been linked with above- and belowground invertebrate community composition (Andrew & Hughes 2007; Fitzpatrick et al. 2017; Pratt et al. 2016), as well as soil nutrient availability (Fischer et al. 2010; Schweitzer et al. 2011). Further exploring natural variation in plant-soil-microbe linkages at landscape scales is fundamental to understanding how climate warming may drive the evolution of plant traits that subsequently alter soil communities and microbially-mediated ecosystem processes (van der Putten et al. 2016; Wardle et al. 2004).

Soil gradients of chemical, physical, or biological properties, play a key role in determining plant fitness, growth, and adaptation (Brady, Kruckeburg, & Bradshaw 2005), variation in plant traits related to resource allocation (Treseder & Vitousek 2001), as well as plant community structure and geographic distribution (Kardol, Bezemer, & van der Putten 2006; Kardol et al. 2007; Reinhart & Callaway 2006). For example, soil characteristics along substrate age and fertility gradients created by Hawaiian lava flows have been shown to reduce plant growth and alter community composition as fertility increases and declines as soils age (Crews et al. 1995; Kitayama & Mueller-Dombois 1995; Vitousek 2004). Plants also exert local influence on their soils by conditioning distinct physical, chemical, and biotic environments in response to functional plant traits (Laland, Odling-Smee, & Feldman 1999). For example, a series of recent studies showed that soil communities drove plant adaptations in novel environments (Lau & Lennon 2012), and the evolutionary changes in plants subsequently altered soil community composition (terHorst, Lennon, & Lau 2014). Further, plant genotypic variation can affect variation in belowground ecosystem processes across large spatial scales (Fischer et al. 2010; Madritch et al. 2014; Van Nuland et al. 2017), adding to a small body of important work demonstrating plant-soil linkages at the landscape level.

To understand how climate gradients influence the evolution of functional plant traits and alter ecosystem function, we used both field observations and a common garden composed of 583 replicated genotypes from 17 rivers systems across ~80% of the distribution of a dominant riparian forest tree, *Populus angustifolia*. We collected plants for a common garden and measured site soil characteristics beneath trees and in adjacent unconditioned interspaces for all 17 observed field populations (**Fig. 2.1**, *All figures and tables for this chapter are located in this chapter's Appendix*). These 17 tree populations span 10.4°C, which is more than double the 4°C predicted global temperature shift in the next century (IPCC 2013; Seager et al. 2007)*. Populus angustifolia* has been steadily expanding northward since the last glacial maximum leaving the southern populations isolated as climate relicts (Evans et al. 2015), making them good analogues and natural laboratories for examining the potential impacts of future climatic conditions (Woolbright et al. 2014). Further, within *Populus*, population-level approaches show evidence that tree genotypes condition and host distinct soil microbial communities and differentially influence soil nutrient dynamics (Schweitzer et al., 2004; Schweitzer et al., 2008), which can feed back to influence plant productivity and performance. For example, Pregitzer et al. (2010) found that when seedlings from randomly collected *Populus angustifolia* genetic families were planted into soils that were conditioned by various *Populus* species*, P. angustifolia* seedlings grown in their own soils were twice as likely to survive and had the highest genetic variation in performance traits, even though *P. angustifolia* soils were less fertile overall. Together, the use of prior knowledge in the *Populus* system, landscape-level field surveys, an experimental common garden, and structural equation modeling (SEM) provide a comprehensive means of addressing the ecological and evolutionary relationships among temperature, population-level genetic variation in foliar phenology and the control trees exert on their associated soil microbial communities and nutrient pools. Specifically, we hypothesized that as temperatures increase, there is phenotypic differentiation and reductions in population-level genetic variation in foliar phenology which results in a change in the control trees exert on their associated soils. We addressed this overarching hypothesis with three hypotheses: 1) Genetically-based plant traits vary along climatic gradients; 2) Population-level soil-conditioning effects of *P. angustifolia* are related to climate; and 3) Soil conditioning is, in part, driven by climate and reductions in genetic variation of foliar phenology. Here we show how ecosystems can experience altered function along a landscape-level temperature gradient because of evolutionary divergence in plant traits.

Materials and Methods

Study species and Site Selection.

Populus angustifolia James is a dominant tree species distributed throughout high elevation riparian zones (900 to 2500 m) along the Rocky Mountains from southern

Alberta, through the intermountain United States, and into northern Mexico (Cooke & Rood 2007). Contemporary migration and population expansion are believed to be present in northern and central *P. angustifolia* populations, leading to increasing geographic isolation, increasing population age, and a reduction in population size in southern populations (Evans et al. 2015). Further, individual populations (i.e., rivers) function as distinct genetic populations since gene flow among geographically separate forest stands is greatly reduced by geographic barriers, climatic factors, and the obligate riparian nature of *P. angustifolia* (Evans et al. 2015). During May and June 2012, 17 distinct *P. angustifolia* populations were surveyed collectively from three different genetic provenances (Arizona, Eastern, and Northern/Wasatch Clusters; Evans et al. 2013) across a gradient of ~1700 km latitude from southeastern Arizona to south central Montana. All trees used in the study were geolocated in the field, and 18 bioclimatic traits were determined for the collection sites along each river (QGIS; Hijmans et al. 2005). See the Supplemental Information (S) for further info on climatic data parameters. To capture the range of genetic variation that occurred in each population, we identified and sampled from 3-5 collection sites within each population: the highest and lowest elevation site with *P. angustifolia* trees and variable intermediate locations (1-3) within each river riparian area. Twenty-five terminal shoot cuttings (~20 cm) were collected from each genotype (*n*=582 total genotypes). Cuttings were planted in general potting mix and allowed to root for four months. Each surviving cutting was transplanted to individual plastic 6.4 x 36 cm pots (D60, Stuewe and Sons Inc, Tangent, Oregon, USA). All transplanted cuttings were randomized using a random number generator on the bench tops to remove any microsite variation in light and temperature within the greenhouse. Site-level details and collections are described in depth in the SI.

***Experimental greenhouse common garden*.**

To understand how climatic gradients might influence the evolution of plant phenotypes (*Hypothesis 1*), 20 cm stem cuttings were grown in a common greenhouse environment to minimize environmental effects and examine the genetic basis of functional plant phenotypes (Kreyling et al. 2014; Vitasse et al. 2009). Saplings grew for two years (quadrupling in growth) in ambient light with weekly water and monthly fertilizer during growing season for maintenance (a water soluble, balanced 20-20-20 of N, P, K). Ultra-Pure Oil Horticultural Miticide/Insecticide/ Fungicide treatments were applied before bud break, after leaf senescence, and as needed to control fungal and pest outbreaks. The greenhouse common garden is located at the University of Tennessee in a climate-controlled glass greenhouse programmed to mimic seasonal changes in temperature. Two to four replicate saplings were selected at random from each surviving genotype to measure multiple plant traits associated with plant growth. In 2014, foliar bud break phenology (*n*=1,032 total plants) was measured every 48 h until all trees had flushed by recording bud break as the ordinal day when new leaves unfurl during spring emergence and represents the onset and ultimately the total accumulation of annual aboveground biomass production (Richardson et al. 2009, 2010). In 2014, before leaf senescence, internode diameter (mm) and shoot length (mm) was measured on the longest stem to provide an estimate of annual growth. In 2016, total aboveground biomass was estimated using new measurements of height (mm) and basal diameter (mm) of replicated genotypes in the same greenhouse common garden. Aboveground biomass estimations are a representation of cumulative growth and productivity throughout the life of the tree. To estimate aboveground biomass (g), we established an allometric equation using six different *P. angustifolia* genotypes that were collected at three time periods (June 2012, 2013, and 2014) and grown in the same greenhouse common garden environment. We measured height and basal stem diameter from these six plants in September 2014 before the aboveground portion was dried at 72° C for 48 h and dry biomass was measured. We calculated plant cross sectional areas from basal stem diameter measurements (Cross sectional area = $\pi(0.5^*\text{diameter})^2$), and multiplied area by plant height to quantify total stem volume (mm^3). We then used a linear regression to test the relationship between stem volume and aboveground biomass. Stem volume predicted more than 98% of the variation in aboveground biomass. As a result, we created the following allometric equation: Aboveground biomass (g) = (stem volume (mm^3) * 0.41899) - 2.40137 (method in Van Nuland, Bailey, & Schweitzer 2017).

Soil collection.

To understand the ecosystem-level consequences of climate driven variation in plant-soil linkages across the populations (*Hypotheses 2 & 3*), paired conditioned (i.e., tree associated, that trees have influenced or changed) and unconditioned (without the immediate influence or change by trees) soils were collected for each genotype across the range of *P. angustifolia* at the same time cuttings were collected. Trees condition (i.e., change) soils by altering microclimate, contributing organic matter in the form of leaf litter, roots and root exudates that collectively can structure soil biotic communities and alter soil physical and chemical properties (Hobbie 1992; Wardle 2004). To separate the conditioning effects of *P. angustifolia* from underlying site differences, tree-conditioned soils were collected at the base of each trunk (within 0.25 m) and unconditioned interspace soils were collected from a paired random location away from the tree canopy, approximately five meters from the trunk and consistently outside the drip line of each tree canopy. Interspace soils were not collected directly beneath another plant, although we cannot say that other plant species had not influenced interspace soils over time. Soil samples were collected with a 2.5 cm diameter oatfield soil core to a vertical depth of 15 cm, placed in a plastic bag, transported cold from the field and stored at 4° C in the lab until analysis (within three months); sub-samples of all soils for microbial analyses were frozen immediately and stored at -80° C until DNA extraction. Field fresh soil was sieved to 2 mm and then sub-sampled and preserved for various analyses. A 2:1 slurry of deionized water and soil (20 mL: 10 g) was combined for soil pH analysis (Denver Instruments, New York, NY, USA). Another oven-dried soil subsample (oven dried at 105° C for 48 h) was measured for total soil C and N using an elemental analyzer (Flash EA1112 Elemental Analyzer, Thermo Electron S.p.A, Rodano Italy). Soil DNA was extracted from a 0.25 g frozen sub-sample of each soil by using the Power Soil DNA isolation kit (MoBio, Carlsbad, CA USA) according to the manufacturer's instructions. Quantitative PCR reactions to assess bacterial and fungal abundance in each soil sample were performed after Castro et al. (2010) in 96-well plates on a CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA USA); technique described in depth in the SI.

Statistical Analyses

Genetically-based plant traits vary along climatic gradients (Hypothesis 1).

To address hypothesis 1, we used a common garden and a model selection approach to determine which abiotic gradients (climatic and edaphic) are correlated with genetic clines in bud break phenology and subsequent plant growth. Significant correlations between abiotic parameters and genetically based traits provide evidence of local adaptation to the environmental parameter (Aitken et al. 2008; Primack & Kang 1989). The following predictors were used in multiple linear regressions to predict variation in bud break phenology and aboveground biomass in a greenhouse common garden: mean annual temperature (MAT), annual precipitation (AP), latitude, longitude, interspace soil nitrogen, and interspace soil pH. Elevation was excluded from our model selection approach as it is strongly correlated with MAT (r = -.56), therefore MAT was included as it represents the more biologically relevant gradient. Latitude was included in the analysis as a proxy for photoperiod. Further, latitude and longitude were included to best account for geographic distances between populations. Model selection based on minimum AICc scores was used to identify the most parsimonious subset of abiotic predictor variables that explain variation in trait variation. Stepwise model selection was conducted using the MASS R package. Once the most parsimonious subset was determined, we included those abiotic predictor variables in a linear mixed effects model. For this model, collection site was used as a random effect to control for unmeasured environmental variation, and relatedness of subpopulations within each population. To determine any trait correlation between phenology and biomass in the greenhouse common garden, a linear mixed effects model was constructed with plant traits as fixed effects and site included as a random effect. Linear mixed effects model was conducted using the lme4 package in R. Distance-based Redundancy Analysis (dbRDA, vegan R package) was used to account for geographic distance, as well as confirm and reinforce our multiple regression approach. Moran's I was used to test for spatial autocorrelation between the continuous environmental variables (ape R package, method further described in SI). This statistical approach provides a conservative depiction of landscape-level relationships between plant traits and abiotic gradients.

In order to test whether genetic clines in bud break phenology and biomass are due to underlying population structure, we included neutral genetic variance as a fixed effect in the multiple regression framework described above (see Kooyers et al. 2015). This approach is robust and directly integrates phenotypic and genotypic data into a single analytical approach. First, we used a principle component analysis on microsatellite data (from 270 tree genotypes) using the vegan R package. Principle component axes one and two (hereafter referred to as genetic PC 1 and PC 2) represent neutral genetic variance and account for underlying population genetic structure. If the effects of environmental variables remain significant after including genetic PC effects, then environment-trait correlations are consistent with adaptive trait differentiation. In contrast, if environment-trait correlations become non-significant, neutral and demographic processes are important in any trait differentiation on the landscape. DNA extraction and genotyping are described in detail in supplemental information.

To estimate quantitative genetic variation within observed populations and estimate the possibility for selection, broad-sense heritability (H^2_B) using plant clonal replicates was determined for plant traits in the common garden using the following equations: $H^2_B = V_G / V_P$ (Conner & Hartl 2004). Broad-sense heritability estimates are defined as the proportion of phenotypic variance (V_P) attributable to all genetic variance (V_G) components. Broad-sense heritability can range between zero and one, estimates near or equal to zero mean there is little or no genetic variation in the trait, where estimates closer to or equal to one mean all variation in the phenotype is related to genotype. As a caveat, estimates of broad-sense heritability are relative to the focal populations in the particular environmental context at which the estimates are observed, and greenhouse-measured estimates could overestimate heritability compared to field-measured estimates. Similarly, broad-sense heritability may overestimate heritability in outcrossing species. However, these estimates of within-population genetic variation of phenotypes are a conservative estimate to further explore genetic clines in plant traits along landscape-level environmental gradients and are known to be good estimates of adaptive potential (Reed & Frankham 2007). Additionally, we calculated the genetic coefficient of variation, (CV_G) to estimate evolvability using the methods in Houle (1992): $CV_G = 100 \times V_G/\bar{x}$, where $\bar{x}$ is the population mean trait value. CV_G estimates were determined to further describe patterns of genetic variation among populations and support heritability estimates. To determine if estimates of broad-sense heritability and evolvability vary geographically, field measured environmental parameters were used to predict differences in genetic variation estimates among tree populations. Determining environmental drivers of within-population genetic variation will add further evidence to the selective agents driving evolution in plant traits.

Population-level soil conditioning effects of P. angustifolia are related to climate (Hypothesis 2).

To address this hypothesis, we tested whether soil environments and soil conditioning effects vary by population and explored potential environmental drivers of soil conditioning variation. Our paired soil collections allow for pairwise comparisons to separate the conditioning effects of *P. angustifolia* from the baseline, ambient, environment (i.e., outside the direct influence of *P. angustifolia* trees). Individual estimates of the relative abundances of soil fungi, soil bacteria, and the ratio of fungi to bacteria were standardized per unit soil carbon (henceforth soil F:B/C) to remove variation attributed to landscape-level variation in soil carbon (Powers 1990; Schweitzer et al. 2004). A linear mixed effects model was used to determine landscape-level differences in abiotic and biotic characteristics between tree-conditioned and interspace soil samples across observed tree populations, with collection site included as a random effect, and tree/interspace as a fixed effect. Including collection site as a random effect in the model above accounts for site-level variation in parent material, topography, and substrate geology. Percent change between paired tree and interspace soil samples were determined to provide an estimate of the conditioning each tree and tree population exerted on its associated soil environment. Percent change estimates were standardized by field-measured tree diameter at breast height (DBH) to account for variation in tree size within each population. Genetically-based variation in tree DBH is related to plant-soil interactions and reinforces soil nutrient feedback in *P. angustifolia*

(Van Nuland et al. 2019). A linear mixed effects model was used to determine population-level differences between the percent change of soil conditioning for nitrogen and carbon pools. Collection site was included in the model as a random effect, and population as a fixed effect. Tree-driven percent change in soil nutrients was averaged to determine the mean population-level differences in soil conditioning. The values were averaged within a population to allow for direct comparisons with the available WorldClim climate data, which are at larger scales than individual, paired tree-interspace comparisons.

Using ANCOVA, we examined the relationship between tree soil characteristics and interspace soil characteristics by population. Detection of a significant interspace soil characteristic by population effect would suggest that the drivers of soil conditioning vary by population. If the full model yields a significant interaction effect, individual models were used to determine *beta coefficients* for each population to further explore tree-interspace soil relationships. Site was included as a random effect to account for microsite variation and site-level heterogeneity in soil nutrients. Mixed effects ANCOVA was conducted using the lme4 R package. Individual linear models were used to examine the direct relationship between tree-conditioned and interspace abiotic and biotic soil environments for each tree population (e.g., *tree-conditioned soil C ~ interspace soil C* + *Error*). Interspace soil traits, representing baseline local soil conditions, were used as independent variables predicting tree-conditioned soil traits. *Beta coefficients* (*ß* values range from -1 to 1) were determined within each tree population, showing directional relationships between tree-conditioned and interspace soils. If a model yields a strong, positive correlation between tree-conditioned and interspace soils (*ß* closer to +1), soils beneath trees reflect baseline conditions as tree-conditioned and interspace soil nutrient pools follow similar directions (even if the magnitudes are different). In contrast, if a model yields a weak or no relationship (*ß* closer to 0) population-level conditioning effects are less related to surrounding interspace soil environments (even if the magnitudes are different). *Beta coefficients* and 95% confidence intervals were determined to be able to compare population-level clines in how trees condition soils given their baseline conditions (i.e., are they related to interspace conditions or not).

We regressed the *ß coefficients* with the percent change estimates of tree conditioning to determine the relationship between these variables to aid in interpretation of final results. Together these estimates of tree-interspace relationship and soil conditioning effects provide an informative and useful description of how trees in each population alter their soil. To determine if the relationship between tree-associated and interspace soil environments, represented here by *ß* coefficients, varied climatically, population-level variation in *ß* coefficients were correlated with population-level climatic means. A significant relationship between the *ß* coefficients and climate would highlight if trees respond similarly to baseline conditions predictably (vs. if their responses do not reflect interspace soils) and if that relationship varies geographically.

Soil conditioning is, in part, driven by climate-driven reductions in genetic variation of bud break phenology (Hypothesis 3).

To investigate the plant-soil network linking tree quantitative genetics, environmental gradients, and soil conditioning, we used a regression framework to identify potential correlations between estimates of plant genetic variation and tree-driven soil conditioning. Individual relationships between plant genetic variation and patterns in soil conditioning were examined to address if reductions in intraspecific genetic variation may contribute to changes in tree conditioning and ecosystem function at landscape scales. Specifically, within-population genetic variation was used to predict variation in both the relationship (i.e., unrelated [low] to correlated [high] β *coefficient* estimates) and percent change (i.e., population-level mean differences in tree vs. interspace soils) of soil nutrient conditioning. Once we established plant-soil linkages with our individual regression framework, we tested the nature of the entire plant-soil system using the lavaan package in R for developing structural equation models (SEMs, Rosseel 2012). Relationships between microbial conditioning and soil conditioning parameters were also assessed to provide further insight into the hypothetical plant-soil linkages and any potential feedbacks.

To directly test the structure of variance within the plant-soil system in a single hypothetical framework, a full SEM model (i.e., with all hypothetical connections) and each subsequent reduced model were developed to investigate potential strength and significance of the relationships within the plant-soil network. Model selection based on minimum AIC scores was used to identify the most parsimonious network. SEMs, such as the one described below, provide a very general and convenient framework for regression analysis and allow for simple conceptualizations to understand direct and indirect relationships between multiple variables in a network (Grace et al. 2006). Individual relationships and the direction of relationships are based on the regression framework described above. All analyses were performed in R (R Core Team 2016)

Results

Genetically-based plant traits vary along climatic gradients.

We found significant genetically-based, phenotypic differences among populations in mean foliar bud break phenology and aboveground biomass (environmental means in Table S2.1, model results in Table S2.2). Mean population-level bud break phenology varied by up to 28-days across the species range, and aboveground biomass varied up to 69% among all observed populations (Fig. S2.1e,f). Genetically-based variation in bud break phenology and aboveground biomass are negatively correlated (**Fig. 2.2e**, Table S3), indicating that populations with earlier mean bud break dates had higher aboveground biomass (g). Consistent with expectation, there was also significant among site variation in abiotic factors including a 10.4°C range in mean annual temperature and 67.3 cm range in mean annual precipitation; mean bud break phenology and aboveground biomass were correlated with MAT (Fig. 2.2a,d) and latitude (**Table 2.1**). We also found that within-population genetic variation for bud break phenology was negatively correlated with mean annual temperature (Fig. 2.2b**,** $X^2_{(1,16)}$=7.402, p=0.006, *n*=17) indicating that foliar bud break phenology was earlier in warm populations and genetic variance in those populations was lower. There was also a negative relationship between MAT and the genetic coefficient of variation indicating that bud break evolvability declined as MAT increased (Fig. 2.2c, $X^2_{(1,16)}$=11.37, p=0.0007, *n*=17). dbRDA model results further confirmed bud break phenology is related to climate and latitude (Table S2.9). To account for demography and population structure, dimensionality in microsatellite genetic data was reduced using PCA. Genetic

PC1 (92% total variance explained) and PC2 (3.6% total variance explained) were included as fixed effects in our multiple regression approach. Including genetic PC effects in our multiple regression framework did not alter the significant correlations between MAT and Latitude on bud break phenology and aboveground biomass. Importantly, neither genetic PC effect was found to be significant in explaining any trait variation. Together, these results support the hypothesis that bud break phenology is evolving along temperature and latitude (a photoperiod proxy) gradients.

Population-level soil conditioning effects are related to climate.

Across all populations in interspace soils there was 140% difference in total soil carbon (Fig. S.21a); and 125% difference in total soil N (Fig. S2.1b; Table S2.2). There was also significant variation in the soil microbial community as measured by qPCR of the relative abundance of fungi: bacteria per unit (Fig. S2.1c), soil fungal relative abundance per unit C, soil bacterial relative abundance per unit C, and soil pH (Table S2.2). Consistent with the expectation that individual trees can differently condition their associated soils, when averaged across the geographic distribution of *P. angustifolia* total soil N ($X^2_{(1,60)}$=29.43, p<<0.0001, *n*=542) and total soil C ($X^2_{(1,60)}$=43.977, p<<0.0001, *n*=542) were 23% and 20% higher, respectively, in tree-associated soils relative to adjacent interspace soils. Soil pH was also significantly higher in tree-associated soils ($X^2_{(1,60)}$=56.705, p<<0.0001, *n*=532). Similarly, the ratio of the relative abundance of fungi to bacteria per unit soil C in tree-associated soils (tree F:B/C) was significantly different than interspace soils ($X^2_{(1,54)}$=14.516, p=0.0001, *n*=310), with a higher fungal relative abundance per unit carbon in interspace soils ($X^2_{(1,54)}$=17.299, p<<0.0001, *n*=310). Overall, we find, on average, tree-driven soil conditioning increases N and C by 23% and 20%, respectively. Percent change in soil conditioning by trees varied by 109 and 188% (for N and C), and there were significant differences among populations (N: $X^2_{(1,16)}$=34.25, p=0.005, *n*=17; C: $X^2_{(1,16)}$=.89, p<<0.0001, *n*=17). These results show that soil conditioning effects can be detected across large abiotic and biotic gradients (i.e., a 1,700 km gradient).

To understand how the relationship between tree-associated soils and interspace soils may vary across the climatic gradients spanning the observed sites, we used ANCOVA and individual linear models to determine the population-level *ß coefficients*. A significant interspace soil characteristic by population effect was determined for individual soil C, N, and F:B/C models (Table S2.4). Individual model results and *ß coefficients* (with associated confidence intervals) are displayed in supplemental information (Table S2.5). Soil C conditioning (*ß coefficient* values) is negatively correlated with soil C percent change ($X^2_{(1,16)}$=8.401, p=0.003, *n*=17). No relationship was detected between soil N conditioning and soil N percent change ($X^2_{(1,16)}$=1.825, p=0.17, *n*=17). For both soil N and C conditioning, *ß coefficient* values were negatively correlated with population-level mean annual temperature (**Fig. 2.3**; soil N: $X^2_{(1,16)}$=5.33, p=0.036, *n*=17; soil C: $X^2_{(1,16)}$=10.82, p=0.001, *n*=17), indicating that as sites warm tree conditioning does not follow the baseline soil conditions, instead being influenced by other unknown factors and that could reflect a stronger tree influence. We found no predictable pattern in the relationship between unconditioned interspace and tree conditioned soil fungi:bacteria along climatic gradients (i.e., *ß* coefficients are not correlated with population-level mean annual temperature; $X^2_{(1,14)}$=1.05, p=0.31, *n*=15).

Alternatively, we found that the percent change in soil N and C were positively correlated with population-level mean annual temperature (Fig. 2.3d,e; soil N: $X^2_{(1,16)}$=4.17, p=0.04; soil C: $X^2_{(1,16)}$=5.09, p=0.023, *n*=17). Together, the tree-driven percent change of soil conditioning and *ß coefficient* datasets show that there is variation in the magnitude of tree conditioning among populations, that varies along a landscape-level temperature gradient.

Soil conditioning is, in part, driven by climate-driven reductions in genetic variation of bud break phenology.

Our results were consistent with the hypothesis that climate-driven evolution of bud break phenology has altered the linkage between genetically-based plant traits, soil microbiomes, and soil N. Population-level genetic variation in foliar bud break phenology are positively correlated with the *beta coefficients* (the strength of correlation between tree soils and interspace soils). Specifically, genetic variation in bud break phenology is significantly positively correlated to the *beta coefficients* of soil N conditioning (**Fig. 2.4a**: $X^2_{(1,16)}$=11.04, p=0.0008, *n*=17), and the *beta coefficients* related to soil microbial conditioning (Fig. 2.4e, $X^2_{(1,14)}$=6.435, p=0.011, *n*=15).

Further, variation in the *beta coefficients* of soil N conditioning is positively correlated with the *beta coefficients* of soil C conditioning (Fig. 2.4c: $X^2_{(1,16)}$=17.211, p<0.0001, *n*=17). Similarly, population-level percent change estimates of soil N are positively correlated with percent change in soil C ($X^2_{(1,16)}$=41.49, p<0.0001, *n*=17). While percent change estimates were not correlated with population-level genetic variation, percent change in both soil N and soil C is positively correlated with genetically-based differences in average aboveground biomass (g) estimates (soil N: $X^2_{(1,16)}$=6.71, p=0.009, *n*=17; soil C: $X^2_{(1,16)}$=11.03 p=0.0008, *n*=17).

To integrate these findings in a single hypothetical framework, we used a structural equation model (SEM) to confirm the hypothetical network where climate alters the landscape-level plant-soil-microbe linkage (see Fig. 2.4d, Table 2.2, Table S2.8). Similar to the relationship described above, mean annual temperature is negatively correlated with population-level estimates of genetic variation in bud break phenology, explaining 45% of the variation in estimates of genetic variation. We also found that population-level estimates of genetic variation in bud break phenology are positively correlated with soil N *beta coefficients* (R^2=28%). Soil N *beta coefficients* are positively correlated with soil C *beta coefficients* and predicted 48% of the variation in soil C *beta coefficients*. The SEM also shows a positive relationship between soil C *beta coefficients* and soil microbial *beta coefficients*, with soil C *beta coefficients* explaining 45% of the variation in soil microbial *beta coefficients*. Lastly, the SEM yielded no significant relationship between estimate of genetic variation in bud bread phenology and soil microbial *beta coefficients*. Standardized effects reported in Table 2.2 below represent standardized regression coefficients and provide detail on the strength and direction of the individual relationships and are comparable across all other relationships within Fig. 2.4d. Together, these results support the overarching hypothesis that as temperatures increase, population-level genetic variation of phenology declines, and changes the relationships between baseline, interspace soils and tree-conditioned soils, reflecting the importance of tree influence on soils in warmer environments.

Discussion

Across the western US, our results suggest that climate-driven evolutionary change can have consequences for patterns of tree-driven conditioning of soil microbiomes and overall ecosystem function. Our landscape-level study of *P. angustifolia*, coupled with mechanistic experiments and an SEM model show that the relationship between plants and soil N and C vary with increasing temperature along a large latitudinal gradient. First, at the landscape scale, we found that within-population variation in foliar bud break phenology declines in warmer environments. Second, our results indicate that as temperatures increase, the correlation between tree-conditioned soils and interspace soils disappears; as MAT increases, trees become more important to soil nutrient pools (i.e., trees are conditioning soils independent of the local soil conditions; Figs. 2.3a, 2.4d). Lastly, we show that as population-level mean aboveground biomass increases, so does the magnitude of tree-driven soil conditioning (Fig. 2.4f, g). Together these results suggest that climate-driven evolutionary change can have consequences for patterns of tree-driven soil conditioning and overall ecosystem function.

Genetically-based plant traits vary along climatic gradients.

Natural plant populations evolve in complex environments shaped by both abiotic and biotic factors. Plant adaptation to stressful abiotic conditions is one of the primary mechanisms of persistence on the landscape (Davis, Shaw, & Etterson 2005; Gitlin et al. 2006; Ikeda et al. 2014). Modern plant distributions inherently include genetic differentiation in plant functional traits shaped by geographic structure, gene flow, demographic processes, and ecological interactions through time. Using geographic distributions of contemporary tree populations is a powerful approach to explore both the abiotic and biotic interactions that influence plant adaptation. We describe populations of a northward expanding tree species across its distribution to examine changes in quantitative trait variation along environmental gradients and show that adaptation along climatic environments is occurring for *P. angustifolia* (Capon et al. 2013; Fischer et al. 2014). We found that genetic clines in bud break phenology and aboveground biomass, measured in a greenhouse common garden, are correlated with landscape-level temperature gradients (Fig. 2.2a,d). Similarly, we also show within-population genetic variation for bud break phenology declines along the same landscape-level temperature gradient, which suggests populations in warmer environments have reduced adaptive potential and evolvability (Fig. 2.2b,c). Together, these results highlight strong climate-driven patterns of phenotypic variation in wild populations of *P. angustifolia* across this species distribution, extending previous studies on the relationship between climate and phenology (Lustenhouwer et al. 2017).

Temperature and photoperiod are considered the main drivers of foliar bud break phenology, with water availability important in warmer, xeric systems (Badeck et al. 2004; Korner & Basler 2010; Parmesan 2006). Our data show similar responses, as MAT and latitude (a photoperiod proxy) are correlated with genetic clines in bud break phenology and aboveground biomass. Recent studies show that variation in soil microbial communities may also influence plant phenology (*sensu* Friesen et al. 2011; Kannenberg & Phillips 2017; Panke-Buisse et al. 2015; Wagner et al. 2014), suggesting that the ultimate mechanism for the patterns we show here is yet to be unraveled. For example, Wagner et al. (2014) show *Boechera stricta* flowering time is sensitive to soil microbes and soil abiotic characteristics, and variation in the soil microbial community influenced selection on flowering time. Environmental and ecological context influences how the evolution of phenology may alter species interactions, or the ecosystem processes they support. Future work providing such context will have broad implications for our understanding of the consequences of climate change.

Population-level soil conditioning effects are related to climate.

Predicting ecosystem-level responses to changing climates remains a challenging frontier and goal (Pecl et al. 2017; Walther 2010). Understanding how the magnitude and linkage of plants on soil nutrient dynamics may vary within and among populations on the landscape is largely unexplored. On average across the landscape, there is a 23% and 20% difference in soil N and C, respectively, between tree-conditioned and interspace soils, and this result is comparable to what has been found when comparing the effects of elevated CO_2 to ambient conditions on labile soil C (~14%; Dijkstra, Hobbie, & Reich 2006) and between grasslands and forests in the top 20 cm of soil (~16%; Jobbagy & Jackson 2000). We find evidence of variation in the strength of tree conditioning effects as population-level estimates of total soil N and C conditioning vary from 109-188%, respectively, and large differences among populations in soil microbial communities, at least in the fungal to bacterial ratios. These results indicate that tree-driven differences in soil microbial communities may lead to differences in their functional effects on soil nutrient pools (Allison & Martiny 2008; Bardgett & van der Putten 2014). For example, recent studies show that climate change can directly and indirectly alter relative abundances, composition, and function of soil communities, because microbial community members possess phylogenetic differences in metabolic activity, physiology, and environmental tolerance (Amend et al. 2016; Castro et al. 2010; Whitaker et al. 2014). Microbial communities contribute strongly to the regulation of C and N dynamics and shifts in microbial abundance and composition will likely influence soil nutrient availability, net primary productivity, and soil C storage (Wardle et al. 2004, van der Heijden, Bardgett, & van Straalen 2008). Further, population-level variation in the relationship between tree-conditioned and interspace soils (i.e., *beta coefficient* estimates) and the strength of tree conditioning (i.e., percent change estimates) are related to landscape-level variation in mean annual temperature (Fig. 2.3). Specifically, we see the relationship between tree-conditioned and interspace soils decline, and the strength of conditioning increase as mean annual temperature increases. Together, these results suggest exploring the drivers of range-wide genetic and phenotypic variation in tree populations is important to our understanding of how biotic and abiotic environments interact to influence the degree of plant-soil conditioning and impact on ecosystem function. Strong climate-driven differences in genetically-based plant phenotypes, soil microbial communities, and soil nutrients, as shown in this study, highlight how individual components of plant-soil-microbe linkages may change in warming ecosystems.

Climate-driven reductions in intraspecific genetic variation alter the linkage between plant phenotypes and ecosystems.

It is important to understand the role of biotic interactions in the expression of adaptive phenotypes, the ecosystem processes they support, and their feedbacks to future generations if we are to understand and accurately predict the consequences of climate change (Afkami, McIntyre, & Strauss 2014; Johnson et al. 2010; Schweitzer et al. 2014; Van Nuland et al. 2016; Van Nuland, Bailey, & Schweitzer 2017). Rapidly changing climates will impact the distribution of plant phenotypes as well as the soil microbiome to influence plant-soil-microbe interactions, create geographic differences in community structure and ecosystem function, and potentially lead to ecosystem change by altering biodiversity and nutrient cycling (van der Putten 2012; Whitham et al. 2006; Woolbright et al. 2014). Our results indicate that genetic by environment interactions are important for understanding the ecosystem consequences of climate change. Consistent with the hypothesis that intraspecific genetic variation in plant phenotypes related to soil conditioning may vary in response to temperature, we show that as genetic variation for bud break phenology declines: 1) tree-conditioned and interspace soil N becomes increasingly unrelated, driving reductions in soil C (Fig. 2.4a,b,d); and 2) soil microbial communities in tree conditioned soils are less related to soil microbial communities in interspace soils (Fig. 2.4e). The correlations between genetic variation and soil *beta coefficients* suggest that reduction in population-level genetic variation alters the mechanism by which trees condition soils. Genetic clines in bud break phenology and aboveground biomass are correlated (Fig. 2.2e), indicating that genetically-based differences in productivity are, in part, a consequence of a longer growing season due to earlier phenology. Therefore, the correlations between percent change in soil N and soil C and aboveground biomass provide a mechanism for how tree-driven soil conditioning is related to changes in genetic variation in phenology and productivity on the landscape. Together, these results suggest geographic variation in plant-microbe linkages may interact to influence the magnitude of tree effects on soil nutrient pools (Fig. 2.4d,e).

As described above, we show that the interaction of genetic, biotic, and abiotic factors is important for understanding the consequences of climate change at the landscape scale. Consistent with this conclusion, results from our structural equation model further support the hypothesis that climate-driven reduction of genetic variation in phenology alters the linkage between plants and soils. This is an important result as it builds upon the regression approach described above to account for the covariance among variables in the model and provides a genetically-based network to begin teasing apart intertwined C and N dynamics above- and belowground. Cumulatively, our findings provide evidence that understanding the natural variation in genetic components of both above- and belowground portions of the plant-soil linkage are important for predicting patterns of divergence in ecosystem function in a warmer world (Johnson et al. 2010; Kannenberg & Phillips 2017; Van Nuland, Bailey, & Schweitzer 2017).

In conclusion, we show how climate-driven reduction of genetic variation in plant traits decouples above- and belowground linkages. These results show that climate can drive the evolution of a foundation tree species to influence ecosystem function, represented here by the landscape-level variation in plant-soil linkages altering soil nutrient pools. These results strongly suggest a call for research on range shift dynamics that move beyond population-level approaches to understand how genetically-based species interactions vary along abiotic gradients of global change to affect ecosystem function.

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Appendix

Table 2.1 Results from multiple regression and stepwise model selection of site abiotic factors predicting variation in average bud break time (i.e., foliar phenology) and average aboveground biomass when grown in a common greenhouse environment. Site is treated as a random effect.

Factors	**DF**	X^2	**Pr(>X^2)**
***Response**: Bud Break Time*			
Latitude	1	6.24	**0.012**
Mean Annual Temperature	1	9.62	**0.002**
Residuals	59		
***Response**: Aboveground Biomass*			
Latitude	1	13.9	**0.0001**
Mean Annual Temperature	1	15.6	**<0.0001**
Residuals	59		

Table 2.2 Climate-driven effects on plant-soil linkages generated from final structural equation model (SEM; library(lavaan)). Standardized effects are reflected in the arrow widths in Fig. 4d. Backwards model selection was used to identify the reduced and most parsimonious network. Model selection was based on minimum AIC score. Full model results are displayed in Table S8. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Regressions	**Estimate**	**SE**	**Std. Effect**
Bud break H^2_B ← MAT	-0.039**	0.011	-0.674
Soil N Conditioning ← Bud break H^2_B	0.887*	0.377	0.532
Soil C Conditioning ← Soil N Conditioning	0.795***	0.221	0.693
Soil Microbial Conditioning ← Soil C Conditioning	0.912*	0.392	0.496
Soil Microbial Conditioning ← Bud break H^2_B	1.068	0.750	0.304

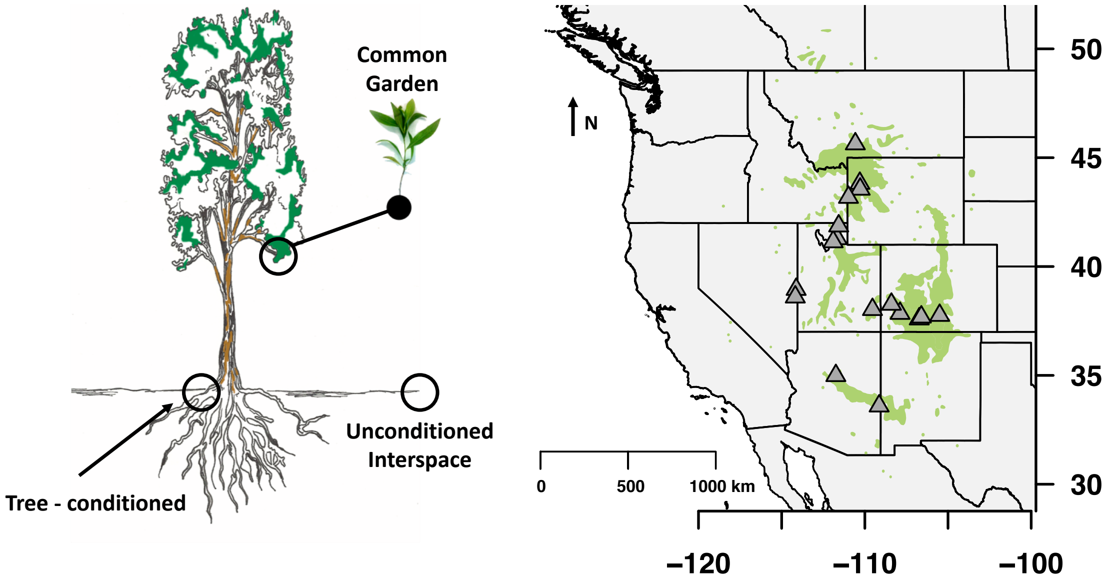

Figure 2.1 Geographic distribution of the current range of *Populus angustifolia* and collection sites. Map shows the idealized geographic distribution (in green) of *P. angustifolia* as well as the sampled populations where tree cuttings and soils were collected, along individual rivers in the western U.S. Inset conceptual diagram describes field locations for soil collections. Paired soil samples were collected from tree-conditioned soils and adjacent unconditioned interspaces, outside of the influence of trees, to determine both the relationship between tree-conditioned and interspace soils as well as the percent change due to tree-conditioning differences. Interspace soils were collected approximately five meters from the trunk and consistently outside the drip line of each tree canopy. Tree cuttings, which clonally replicate individual genotypes, were established in an experimental common garden.

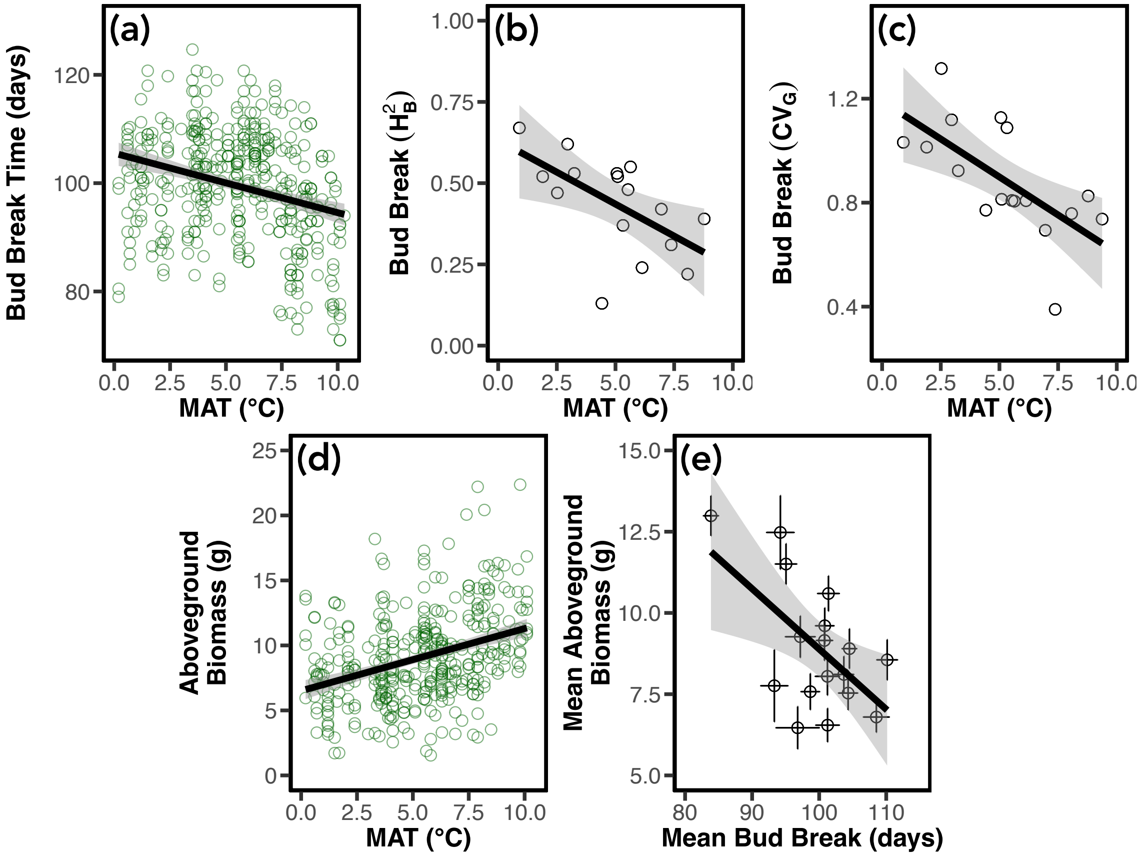

Figure 2.2 Mean annual temperature is a selective agent on foliar bud break phenology and aboveground biomass. Plant traits were measured in a greenhouse common garden. Panel **(a)** shows genetic variation in average bud break time (ordinal days) is correlated with mean annual temperature (°C, MAT, *n*=397). Panel **(b)** shows as significant negative relationship between broad-sense heritability estimates for foliar bud break time and MAT (°C, *n*=17). Panel **(c)** show a significant negative relationship between the genetic coefficient of variation and MAT (°C, *n*=17). Panel **(d)** shows genetic variation in aboveground biomass is correlated with mean annual temperature (°C, MAT, *n*=376). Panel **(e)** shows relationship between genetically-based variation in mean above ground biomass (g) and mean bud break day (*n*=17). Error bars represent ± 1 standard error of the mean.

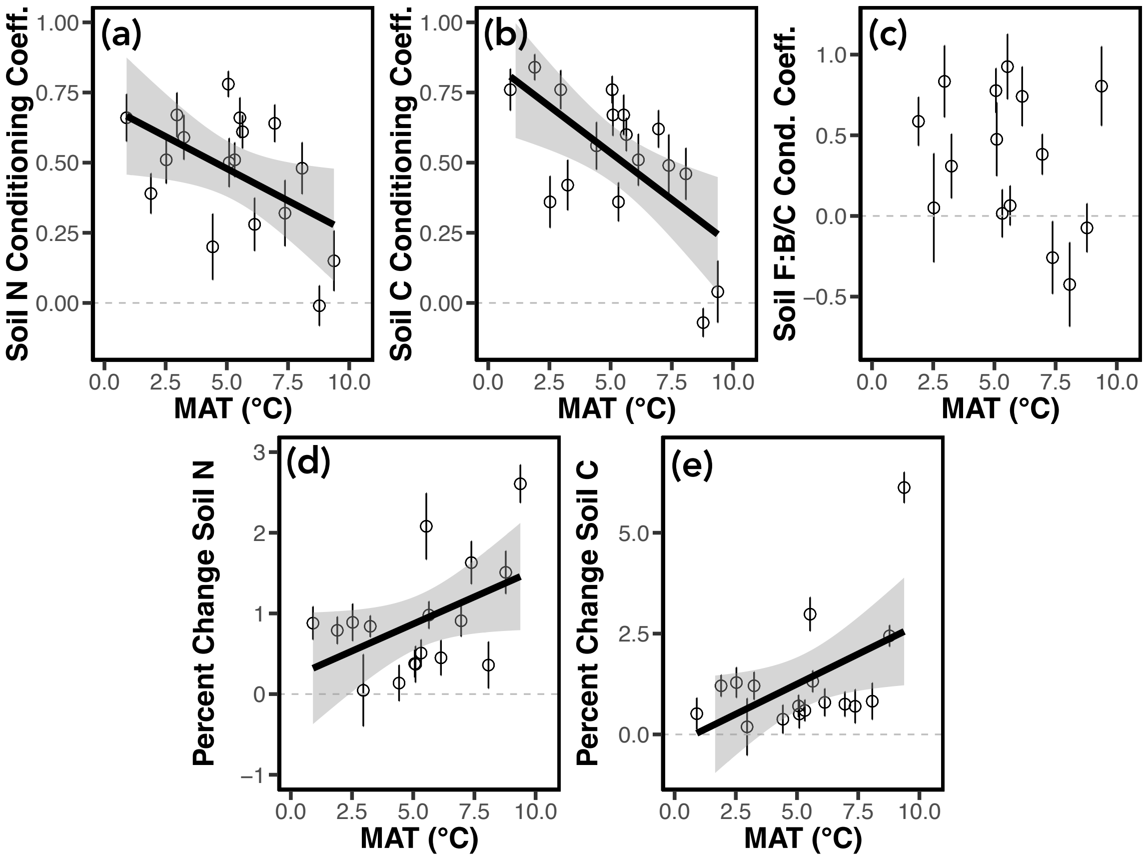

Figure 2.3 Landscape-level field variation between trees and interspace soils is correlated with mean annual temperature (MAT). Panel **(a)** shows the relationship between variation in soil nitrogen (N) conditioning and mean annual temperature (°C, MAT, *n*=17). Panel **(b)** shows relationship between variation in soil carbon (C) conditioning and mean annual temperature (°C, MAT, *n*=17). Panel **(c)** shows the absence of relationship between variation in soil microbe conditioning and mean annual temperature (°C, MAT, *n*=15). Panel **(d)** shows relationship between population-level mean percent change in soil N and mean annual temperature (°C, MAT, *n*=17). Panel **(e)** shows the relationship between population-level mean percent change in soil C and mean annual temperature (°C, MAT, *n*=17). Error bars in a, b, & c represent ± 1 standard error around beta coefficient estimates. Error bars in Panels d & e represent ± 1 standard error of the mean.

Figure 2.4 The reduction of population-level genetic variation alters the relationship between tree-conditioned soils and interspaces and increases the magnitude of soil nutrient conditioning. Panel **(a)** shows the relationship between population-level genetic variation in foliar bud break and soil nitrogen (N) conditioning (*n*=17). Panel **(b)** shows the lack of relationship between population-level genetic variation in bud break and soil carbon (C) conditioning (*n*=17). Panel **(c)** shows the relationship between the *beta coefficients* of soil c conditioning and soil n conditioning (*n*=17). Panel **(d)** shows the structural equation model. bold, black arrows represent the final, reduced model. size of the bolded, black arrows corresponds with standardized regression coefficients. numbers in parentheses represent the variation explained (R^2) for each associated component of the SEM. Grey, dotted lines represent insignificant correlations from more complex SEMs that were removed after model selection approach. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Panel **(e)** shows the relationship between population-level genetic variation in bud break and soil microbial conditioning (*n*=17). High *beta coefficient* estimates (*ß* closer to +1) represent a relationship whereby soils beneath trees are correlated to baseline conditions in those populations. In contrast, lower *beta coefficient* estimates (*ß* closer to 0) show population-level conditioning effects are unrelated to interspace soils. Panels **(f)** and **(g)** show the relationships between aboveground biomass (g) and the percent change in soil N and soil C due to tree-driven soil conditioning (i.e., tree % - interspace %) / interspace %); *n*=17). Error bars in each figure represent ± 1 standard error around beta coefficient estimates.

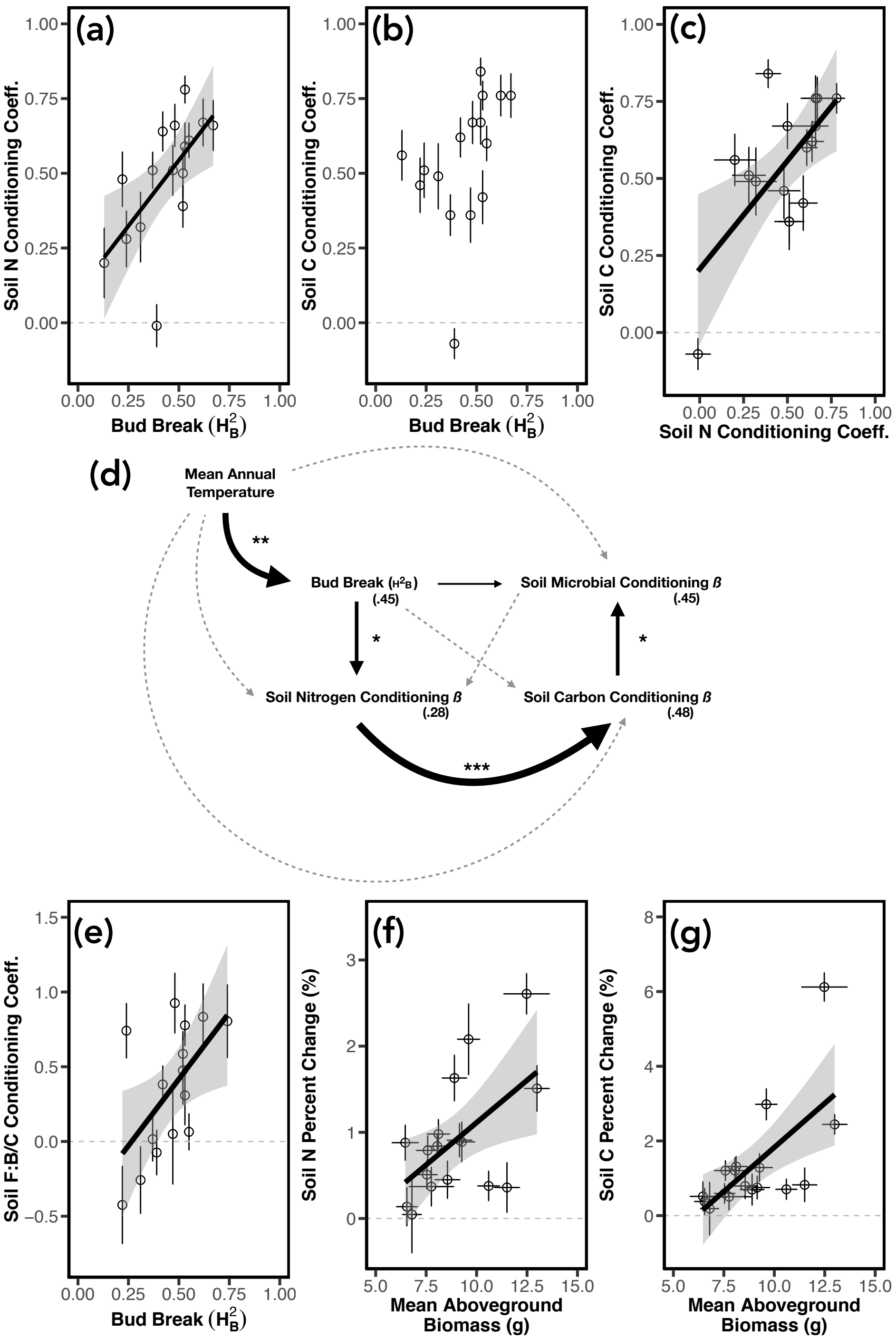
(a)
Soil N Conditioning Coeff.
Bud Break (H^2_B)
(b)
Soil C Conditioning Coeff.
Bud Break (H^2_B)
(c)
Soil C Conditioning Coeff.
Soil N Conditioning Coeff.
(d)
Mean Annual Temperature
**
Bud Break (H^2_B)
(.45)
Soil Microbial Conditioning β
(.45)
*
*
Soil Nitrogen Conditioning β
(.28)
Soil Carbon Conditioning β
(.48)
***
(e)
Soil F:B/C Conditioning Coeff.
Bud Break (H^2_B)
(f)
Soil N Percent Change (%)
Mean Aboveground Biomass (g)
(g)
Soil C Percent Change (%)
Mean Aboveground Biomass (g)

Figure 2.4

CHAPTER III

TREE-ASSOCIATED SOIL MICROBIOME MEDIATES RANGE-WIDE BUD BREAK PHENOLOGY OF A FOUNDATION TREE SPECIES

Abstract

Identifying the role of the soil microbiome in plant responses to climate change is a significant research challenge and frontier. For example, phenological events are critical for plant responses to climate change, persistence on the landscape, and for the timing of associated species interactions. In plant-soil research, there is evidence that soil microbiomes may be important, but complex, influencers to phenotypic variation in traits such as phenology. However, integrating spatial scales relevant to climate change with plant genetics and soil microbial ecology is difficult and makes appropriate studies of broad inference rare. Building upon previous research in this system showing 1) evolution of bud break phenology of *Populus angustifolia* and correlated shifts in the tree-associated soil microbiome along landscape-level climatic gradients, as well as a strong effect of plant conditioning on soil microbial communities, we established a greenhouse soil inoculation experiment with *Populus angustifolia* trees and associated soil microbiomes to explore how plant genetics, soil microbiomes, and plant and soil climatic origins interact to influence plant phenological traits. We specifically hypothesized that tree-conditioned soil microbiomes collected along strong climatic gradients will predictably alter the timing of leaf-out phenology. Our results show that geographic-driven variation in the tree-conditioned soil microbiome results in functional differences for plant phenology. We provide evidence that geographic, climatic, and plant genetic factors influence variation in the soil microbiome, and tree-associated soil microbiomes mediate plant phenology at landscape scales. This study begins to further develop frameworks necessary to help partition the effects of climate, plant genetics, and biotic interactions on plant phenotypes and adaptation, so that we can then begin to provide context on what phenotypes and combination of interactions may allow for survival, persistence, and adaptation in a warming world.

Introduction

Variation in or advancement of phenology is generally thought to be evidence of variation in climatic factors and their effects on plants; and is thus a useful tool for understanding the patterns and consequences of climate change. Advances in plant phenology are particularly worrisome for several reasons: 1) the consequences of interspecific mismatches in the timing of interactions may become more frequent; 2) The effects of advanced phenology on ecosystem processes and services are poorly understood but could be significant; and 3) communities and ecosystems could begin to disassemble. Therefore, understanding the factors that determine the timing of phenological events is critical. Phenology in plants is a complex trait that is widely considered to be driven by the interaction of climate and genetics (Epperson 2003, Howe et al. 2003, Aitken et al. 2008, Evans et al. 2016). Much research and even citizen science programs are focused on quantifying the genetic and environmental controls on phenological cycles. However, environmental variation in biotic interactions that exists on the landscape may also affect patterns of phenology and remains understudied. This is particularly true when scales relevant to climate change are considered (e.g., Woolbright et al. 2014, Lankau et al. 2015, but see Van Nuland et al. 2017, 2019, Ware et al. 2019a).

Spanning a plant's geographic range, plant hosts and associated microbial communities may experience similar climate gradients and environmental constraints.

Understanding the potential role biotic environmental variation, and in particular the soil microbiome, has in determining plant phenotypes, and phenology specifically, is a major frontier in ecology. It has been shown that soil microbes can mediate plant functional traits (reviewed in Freisen et al. 2011). For example, Wagner et al. (2014) showed that soil microbiomes can have a strong effect on the timing flowering in *Boechera stricta*. Similarly, we know soil microbial symbionts and communities have been shown to confer drought tolerance and fitness advantages in response to environmental stress (Yang et al. 2009, Zolla et al. 2013, Gehring et al. 2017, Fitzpatrick et al. 2019). While these studies reveal the importance of soil microbial communities on plant traits, the generality of microbial function across populations and species, plant responses to soil communities, and how these interactions may change as a consequence of global change is still largely unknown (e.g., Woolbright et al. 2014, Lankau et al. 2015). Integrating above- and belowground perspectives regarding the climatic gradients shaping biological interactions may prove critical in how we understand phenotypic variation on the landscape, forecast the potential for local adaptation to future climatic scenarios, as well as how we develop data to populate species distribution models and range shift projections. Further, since plant hosts and associated microbial communities are experiencing the same climatic gradients, it is critical to partition the effects of plant genetics, climate and soil microbial communities on plant phenotypes such as phenology in order to have broad inference at the landscape level.

Here we are building upon previous research in this system showing climate driven reduction of genetic variation for bud break phenology that alters soil microbiomes and soil nutrient pools under *Populus angustifolia* across the western United States (Ware et al. 2019a). We sought to understand how climate, plant genetics and soil microbial communities interact to influence bud break phenology. Our overarching hypothesis was that soil microbes collected across the geographic range of *Populus angustifolia* along a mean annual temperature gradient predictably affect plant phenology in a common environment. Based upon a correlation between bud break phenology in the greenhouse and tree conditioned, but not unconditioned, soil in the field, we specifically hypothesized that: 1) soil microbiomes vary along a MAT gradient; 2) soil microbiomes respond differently under trees than adjacent interspaces; and 3) tree-conditioned soil microbiomes function differently to alter the timing of leaf-out phenology in a reciprocal soil inoculation experiment. Our results show that across the western US, there are population level differences in how soil microbiomes respond to plant genetic variation, consistent with other work linking genetic variation in plants to biodiversity and ecosystem function; and climate-driven variation in the tree conditioned soil microbiome mediates the range-wide phenological clock of foundation tree species, *P. angustifolia* by up to 10 days. Importantly, consistent with the observational data, when trees were planted with warm or cool soil microbiomes, they woke up 6 days earlier or 4 days later, respectively. These results provide a strong challenge to any general framework on the function of soil microbial communities. They also indicate that using tree species identity to infer soil community structure or function is equally tenuous.

Methods

***Study species and Site Selection*.**

Populus angustifolia James is a dominant tree species distributed throughout high elevation riparian zones (900 to 2500 m) along the Rocky Mountains from southern Alberta, through the intermountain United States, and into northern Mexico (Cooke & Rood 2007). Contemporary migration and population expansion are believed to be present in northern and central *P. angustifolia* populations, while increasing geographic isolation, increasing population age, and a reduction in population size are important in southern populations (Evans et al. 2015). Further, individual populations (i.e., rivers) function as distinct genetic populations since gene flow among geographically separate forest stands is greatly reduced by geographic barriers of the mountain landscape, climatic factors across the range, and the obligate riparian nature of *P. angustifolia* (Evans et al. 2015). During May and June 2012, 17 distinct *P. angustifolia* populations were surveyed collectively from three different genetic provenances (Arizona, Eastern, and Northern/Wasatch Clusters; Evans et al. 2013) across a gradient of ~1700 km latitude from southeastern Arizona to south central Montana. All trees used in the study were geolocated in the field, and 18 bioclimatic traits were determined for the collection sites along each river (QGIS; Hijmans et al. 2005). To capture the range of genetic variation that occurred in each population, we identified and sampled from 3-5 collection sites within each population: these included the highest and lowest elevation site with *P. angustifolia* trees and variable intermediate locations (1-3) within each river riparian area. Twenty-five terminal shoot cuttings (~20 cm) were collected from each genotype (*n*=582 total genotypes). Cuttings were planted in general potting mix and allowed to root for four months. Each surviving cutting was transplanted to individual plastic 6.4 x 36 cm pots (D60, Stuewe and Sons Inc, Tangent, Oregon, USA). All transplanted cuttings were randomized using a random number generator with regard to position on the bench tops to remove any microsite variation in light and temperature within the greenhouse.

***Characterizing abiotic and biotic components of the soil environment*.**

To understand the range wide variation in the *P. angustifolia* associated soil environment, paired conditioned (i.e., tree associated) and unconditioned soils were collected for each tree surveyed across the range of *P. angustifolia* at the same time cuttings were collected. To separate the conditioning effects of *P. angustifolia* from underlying site differences, tree-conditioned soils were collected at the base of each trunk (within 0.25 m) and unconditioned interspace soils were collected from a random location out from the tree canopy, no less than five meters from the trunk and consistently outside of the drip line of each tree canopy. Soil samples were collected with a 2.5 cm diameter Oatfield soil core to a vertical depth of 15 cm, placed in a plastic bag, transported cold from the field and stored at 4°C in the lab until analysis. Field collected wet soils were sieved to 2 mm and then sub-sampled and preserved for various analyses. A 2:1 slurry of deionized water and soil (20 mL: 10 g) was combined for soil pH analysis (Denver Instruments, New York, NY, USA). Another oven-dried soil subsample (oven dried at 105° C for 48 h) was measured for total soil C and N using an elemental analyzer (Flash EA1112 Elemental Analyzer, Thermo Electron S.p.A, Rodano Italy). Soil DNA was extracted from a 0.25 g frozen sub-sample of each sieved soil by using the Power Soil DNA isolation kit (MoBio, Carlsbad, CA USA) according to the manufacturer's instructions. Quantitative PCR reactions to assess bacterial and fungal abundance in each field soil sample were performed after Castro et al. (Castro et al. 2010) in 96-well plates on a CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA USA).

Sample preparation for iTag sequencing.

Samples were amplified for the 16S v4 region using primers 515F/806R, and for the ITS2 region using primers ITS9F/ITS4R. Samples were sent to the Department of Energy Joint Genome Institute for sequencing on an Illumina MiSeq (2x300bp; Illumina Inc., San Diego, CA). The resultant demultiplexed samples underwent initial pre-processing using BBTools. Specifically, adapters were trimmed and contaminants were filtered from reads using BBDuk. Paired-end reads were then merged with BBmerge before further processing.

***Experimental greenhouse common garden*.**

To understand how abiotic and biotic gradients might influence the genetic variation plant phenotypes, 20 cm stem cuttings were grown in a common greenhouse environment to minimize environmental effects and examine the genetic basis of functional plant phenotypes (Kreyling et al. 2014, Vitasse et al. 2009). Saplings grew for two years (quadrupling in growth) in ambient light with weekly water and monthly fertilizer during growing season for maintenance (a water soluble, balanced 20-20-20 of N, P, K). Ultra-Pure Oil Horticultural Miticide/Insecticide/ Fungicide treatments were applied before bud break, after leaf senescence, and as needed to control fungal and pest outbreaks. The greenhouse common garden is located at the University of Tennessee in a climate-controlled glass greenhouse programmed to mimic seasonal changes in temperature. Two to four replicate saplings were selected at random from each surviving genotype to measure multiple plant traits associated with plant growth. In 2014, foliar bud break phenology (n=1,032 total plants) was measured every 48 h until all trees had flushed by recording bud break as the ordinal day when new leaves unfurl during spring emergence and represents the onset and ultimately the total accumulation of annual aboveground biomass production (Richardson et al. 2009, 2010). In 2014, before leaf senescence, internode diameter (mm) and shoot length (mm) was measured on the longest stem to provide an estimate of annual growth. In 2016, total aboveground biomass was estimated using new measurements of height (mm) and basal diameter (mm) of replicated genotypes in the same greenhouse common garden. Aboveground biomass estimations are a representation of cumulative growth and productivity throughout the life of the tree. To estimate aboveground biomass (g), we established an allometric equation using six different *P. angustifolia* genotypes that were collected at three time periods (June 2012, 2013, and 2014) and grown in the same greenhouse common garden environment. We measured height and basal stem diameter from these six plants in September 2014 before the aboveground portion was dried at 72° C for 48 h and dry biomass was measured. We calculated plant cross sectional areas from basal stem diameter measurements (Cross sectional area = $\pi(0.5*\text{diameter})^2$), and multiplied area by plant height to quantify total stem volume (mm^3). We then used a linear regression to test the relationship between stem volume and aboveground biomass. Stem volume predicted more than 98% of the variation in aboveground biomass. As a result, we created the following allometric equation: Aboveground biomass (g) = (stem volume (mm^3) * 0.41899) - 2.40137 (method in Van Nuland, Bailey, & Schweitzer 2017).

Soil inoculation experimental design.

To address if plant genetics, soil biota, and soil climatic origin interact to influence plant phenology, we established a completely randomized greenhouse soil inoculation experiment. Tree-conditioned soils were re-collected from 10 *Populus angustifolia* populations in May 2015 (**Figure 3.1A**, *All figures and tables for this chapter are located in this chapter's Appendix*). Based on mean annual temperature (MAT), the five warmest and five coolest populations from the original 2012 survey and collection, were included in the inoculation experiment to span the landscape-level temperature gradient (See **Table 3.1**). For each of the 10 populations sampled, we surveyed 5 tree genotypes and collected tree-associated soils from each of the 5 genotypes. Replicates of each genotype collected were established in a greenhouse common garden. Replicated genotypes were randomized and grown as described above in ***Experimental greenhouse common garden*** methods. A sub-sample of each soil sample collected was sterilized using gamma-irradiation (exposed to radioisotope cobalt 60 radiaiton field and irradiated at approximately 25-30 kGy; STERIS Corporation; Spartanburg, SC), to specifically test the influence of the soil microbiome on the phenotypes expressed in the common garden trial. Each replicated genotype was inoculated with live and sterile soil from their home soil, their home and away climate (warm v. cool) soil. For example, one genotype replicate from a warm population was inoculated with live (i.e., microbes present) and another replicate of the same genotype was inoculated with sterile soil from its true home soil, live and sterile soil from a random warm population's soil, and live and sterile soil from a random cool population's soil. This was replicated for all genotypes and populations (~250 trees survived at ambient greenhouse temperature).

Data Processing and Statistical Approach

Landscape-level relationship between soil microbiome and bud break phenology.

To explore the linkage between bud break phenology and tree-associated soil microbes at the landscape scale, a linear mixed effects model was constructed with bud break phenology included as the response variable, the log-transformed ratio of fungi to bacteria from tree-associated soil was included as the independent variable, and population included as a random effect. A similar model was constructed to test for a relationship with interspace (i.e., unconditioned soils) soil microbes, where bud break phenology was included as the response variable, the ratio of log-transformed soil bacteria abundance to fungi abundance from interspace soil was included as the independent variable, and population was included as a random effect. Again, including population as a random effect removes the variation attributable to differences in soil type and nutrient differences across the landscape.

Tree-driven conditioning of soil microbial community.

We processed iTags to identify amplicon sequence variants (ASVs) using DADA2 version 1.6.0 (Callahan et al. 2016). Reads were truncated to 280bp to remove low-quality nucleotides at the tails, and quality filtered by removing phiX contamination and allowing a maximum of 1 expected errors (maxEE = 1). A parametric error model was learned from the data and identical sequences were deprelicated before ASVs were identified and an ASV table (analogous to an OTU table) was constructed. This workflow was performed for each of the three plates, resulting in three ASV tables. Because DADA2 identifies ASVs (rather than clustering OTUs based on similarity), the three ASV tables were merged into a single table from which chimeras were removed. Taxonomy was assigned for each unique ASV using the RDP trainset 16 (16S) and the UNITE 28/06/2017 general release (ITS2). In total, 48,686 prokaryotic and 50,630 fungal ASVs were identified from 147 samples. To focus on the most prevalent taxa, we filtered bacterial ASVs not seen more than three times in at least 10% of samples, and fungal ASVs not seen more than 3 times in at least 5% of samples. This resulted in a total of [3,486] bacterial ASVs and [2,523] fungal ASVs used to analyze soil microbial community variation and taxonomic composition. Archaea accounted for less than 1% of the filtered ASVs (26 out of 3,486 ASVs). This is expected given the 16S primers JGI used for sequencing are not good at recovering much Archaea taxa.

To understand if trees directly influence soil microbial communities, we calculated community turnover between tree and interspace samples. Turnover was calculated for both bacterial (16S) and fungal (ITS2) communities using diversity orders q=0-2 [**q=0**, S (Richness); **q=1**, exp(H') (exponential of Shannon's Index); **q=2**, 1/γ (reciprocal of Simpson's γ)] (Hill numbers, Ma 2018). Using Hill numbers allows for comparison of microbial communities among important groups and along environmental gradients, while accounting for rare community members (e.g., **q=2**, 1/γ (reciprocal of Simpson's γ)). Tree-interspace pairs were rarefied to the sample with the lowest number of reads. Relative abundances for each community member were determined, and beta diversity was calculated for each order of q (0-2) using the 'vegetarian' R package. A t-test was used to test whether estimates of turnover differed from 0. A turnover = 0 would mean that tree and interspace communities were identical, and a turnover = 1 would mean tree and interspace communities did not share any community members. A generalized linear model was used to explore among population-level variation in both bacterial and fungal community turnover for each order of q (glm() function).

Drivers of soil microbial community composition and dissimilarity.

To identify the environmental drivers of soil microbial community composition at the landscape-scale, we used distance-based redundancy analysis in the vegan() R package. Individual dbRDAs were completed separately for tree-associated bacteria, interspace bacteria, tree-associated fungi, and interspace fungi. Jaccard distance was used to determine dissimilarity among samples, and dissimilarity matrices were included in the dbRDA as the response variable. Biotic and abiotic environmental variables were included in each dbRDA model including: latitude, longitude, mean annual temperature, annual precipitation, total soil C, total soil N, field DBH, field SLA, field Foliar C:N, common garden genetic clines in bud break phenology, and common garden genetic clines in aboveground biomass. Including field and common garden traits will allow for exploring the importance of genetic variation in phenology and productivity in determining soil microbial community composition. The same model was run for each of the four community matrices (i.e., tree-associated bacteria, interspace bacteria, tree-associated fungi, and interspace fungi). This approach allows us to understand if plant traits are of any importance to interspace soil microbial communities, providing further evidence that trees are conditioning their associated soil microbial community. To compliment dbRDA, we explored soil bacterial and fungal community dissimilarity using generalized dissimilarity modeling (GDM; Ferrier 2007, Fitzpatrick et al. 2013). GDM models biological variation as a function of environment and geography using distance matrices – specifically by relating dissimilarity in species composition. The same variables used in the dbRDA were included in individual GDMs for tree-associated bacteria and tree-associated fungi. If plant traits are determined to be unimportant in the dbRDA framework, they will be excluded from GDM models for interspace bacteria and interspace fungi. Similar to dbRDA, the GDMs will identify significant drivers of community dissimilarity, but will also provide a better picture of where community dissimilarity changes and allow for comparisons among individual predictors. All analyses were performed in R (R Core Team 2016).

Soil microbiome and climate origin effects on bud break phenology.

To test how soil microbiome origin influenced bud break phenology, a fully factorial linear mixed effects model was constructed (lme4 package in R) with plant climatic origin (i.e., from warm or cool populations), live/sterile (i.e., with and without microbes), soil climatic origin (i.e., from warm or cool habitats), and subsequent interactions included as fixed effects. Population was included as a random effect, to account for population-level variance in plant traits. If the full model were to yield any significant interaction terms, the model would be reduced to specifically examine individual fixed effects. To examine the effects of soil microbes and soil climatic origin of plant phenology in a continuous framework, the difference in the tree's climatic origin and the soil inoculum's climatic origin were determined to provide a 'temperature transfer distance' and will be referred to as Δ°C hereafter. Mortality within the experiment was recorded and analyzed using a Chi-Squared test.

Results

***Landscape-level relationship between soil microbiome and bud break phenology*.**

Observationally, there was a significant relationship between the landscape level patterns of bud break phenology in the greenhouse and the tree-associated soil fungi to bacteria ratio (Figure 1B; $X^2_{(1,13)}$=4.245, Pr(>X^2)=0.039). Plants in soils from cool sites were breaking bud later than those in soils from warm sites. Interestingly, there was no relationship between bud break phenology and soil fungi:bacteria found in interspace soils (Pr(>X^2)=0.864; **Figure 3.1B inset**). It was also apparent that plants in soils from cool sites were breaking bud later than those in soils from warm sites. These results raised two questions: 1) do soil microbiomes respond to tree conditioning to affect phenology; and 2) do microbial communities collected along a landscape-level MAT gradient vary in functional effects on bud break?

Tree conditioning of soil microbial communities and drivers of soil microbial community composition.

To understand if trees directly influence soil microbial communities, we calculated community turnover between tree and interspace samples. Soil microbial communities respond strongly to conditioning by trees when compared to adjacent paired interspace microbiomes. Independent one-sample t-tests indicated that each estimate of soil microbial community turnover (q=0-2) for both soil bacteria and soil fungal communities, was significantly different than zero (Figure 3.2A & B, **Table 3.4**). Our turnover estimates suggest that soil bacterial communities underneath trees are on average 33-46% different than paired interspace bacterial communities across the diversity orders (q=0-2, Figure 3.2A). Similarly, soil fungal communities underneath trees are on average 46-57% different than paired interspace fungal communities across the diversity orders (q=0-2, Figure 3.2B). This objectively describes the degree tree-associated soil microbial communities are different from interspace soil microbial communities, while accounting for rare community members (e.g., **q=2**, 1/γ (reciprocal of Simpson's γ). Total soil C, total soil N, and soil pH also varied between tree and interspace soil samples (Figure 3.2C, **Table 3.5**). There were also significant differences among populations, indicating that the soil microbial response to tree conditioning varied geographically (Figure 3.3, Table 3.6); results that are potentially consistent with different soil microbial functions across the landscape.

In order to understand the potential relationship between bud break phenology and soil microbial community dynamics, we used distance-based redundancy analysis (dbRDA) to test the hypothesis that tree-associated soil microbiomes were responding differently to their environment relative to interspace soil microbiomes. We found that genetic clines in bud break phenology and abiotic environmental factors explain significant variation in tree-associated bacterial and fungal communities (**Table 3.7**). Consistent with our initial observation that there was no relationship between bud break phenology and interspace soils, only abiotic predictors explained significant variation in interspace soil microbial community composition. Similarly, generalized dissimilarity models show variation in plant phenotypes, tree-associated soil chemistry, and environmental gradients are important in driving community dissimilarity in tree-associated soil bacterial and fungal communities (Figure 3.4A & C, Table 3.8). Likewise, geography, interspace soil chemistry, and climate are the most important drivers of interspace soil bacterial and fungal communities (Figure 3.4B & D, Table 3.8). These results clearly show that tree-associated soil microbial communities are responding to genetic variation in plant phenotypes, geographic location, tree-conditioned soil chemistry, and climatic gradients across the range of *P. angustifolia* in the western US.

Soil microbial feedback to bud break phenology.

To understand whether tree conditioned soil microbial communities collected from warm and cool sites have different functional effects on bud break phenology, we established a greenhouse soil inoculation experiment to test how soil biota and soil climatic origin interact to influence the expression of plant phenotypes. Our full model results showed a significant effect of plant climatic origin and significant interaction between live/sterile soil microbiomes and soil climatic origin (i.e., warm and cool habitats) (**Table 3.2**). Since a significant interaction was detected, we developed a reduced model including live/sterile as a fixed effect and population as a random effect for both warm and cool soil origins. We determined that adding live microbial inoculations from warm habitats advanced bud break phenology by ~7 days across all populations when compared to sterile inoculations (Figure 3.1C, Table 3.2). Further, live microbial inoculations from cool habitats resulted in bud break phenology that was ~5 days later, compared to sterile inoculations (Figure 3.1C, Table 3.2). To understand this result in a more continuous framework, we show that for every 1 degree Δ°C (i.e., the difference in temperature between the plants origin and the soils they are planted in) in the live soil treatment there is advancement of bud break phenology in the greenhouse by 1 day. The sterile soil treatment shows no significant pattern (Figure 3.1D, **Table 3.3**). Mortality patterns were not statistically different among live and sterile inoculation treatments ($X^2 = 0.22$, df = 1, p= 0.6376). Together, these results provide evidence that soil biota and soil climatic origin interact to mediate the expression of bud break phenology across large spatial scales and genetic backgrounds and suggest an adaptive relationship between trees and tree-conditioned soil microbiomes.

Discussion

The scale at which the linkage and feedback between genes and ecosystems exists is far greater than previously appreciated. Our results clearly demonstrate that soil microbiomes and nutrient pools vary in response to inputs by plants at the tree level, among populations and across the western United States. Our results also demonstrate that variation in the soil microbiome due to conditioning by trees can feedback to affect patterns of geographic patterns of bud break phenology. Together, these results effectively synthesize community and ecosystem genetics (see Schweitzer et al. 2004, 2008, Johnson & Agrawal 2005, Crutsinger et al. 2006, Barbour et al. 2009, Fitzpatrick et al. 2015, 2017; synthesized & reviewed in: Whitham et al 2003, 2006, Bailey et al. 2009, Crutsinger 2016) with eco-evolutionary dynamics (Hendry 2017, Van Nuland et al. 2016, Ware et al. 2019b) at landscape scales.

Geographic variation in biotic interactions is difficult to study but clearly important to the functioning of ecosystems (Walther et al. 2010). Our results are consistent with the hypotheses that there is geographic variation in the soil microbiome, soil microbiomes respond to tree conditioning, and tree-conditioned microbial communities function differently along a MAT gradient. Across the western US, MAT and precipitation are important determinants of soil microbiome community composition irrespective of the input of trees (Fig. 1B; Table 7). Additionally, the soil microbiome also varies in response to input by trees when averaged across all trees (Fig. 1B, 2A,B), and when grouped by tree population (Fig. 3). These findings challenge the notion that one can predict the soil microbiome by using the tree community because soil interspace communities are different. These findings also show that plant populations condition soils differently, suggesting that using plant species may not be as useful for predicting belowground communities as previously thought (i.e., there is clearly variation within species that is important to belowground communities). Finally, we provide evidence of a tree conditioned soil microbial feedback on bud break phenology, suggesting that there is geographic variation in soil microbiome function. Evidence of geographic functional variation in the soil microbial community mediating variation in bud break phenology is a paradigm shifting perspective on the drivers of plant phenology. The effect of soil biota on bud break phenology (~14-17 days on average; Fig. 3.1D) is about 50-61% of the total quantitative difference due to genetics across the range of *P. angustifolia* (~28 days; Ware et al. 2019a), and is two to three times greater than historical phenological changes in trees over the last half century and modeled future predictions (5-9.2 days; Menzel 2000, 2003; Morin et al. 2010). Further, the rates of evolutionary response or range shifts in tree species may be outpaced by contemporary (i.e., 20th century) climatic change (Loarie et al. 2009, Alberto et al. 2013, Bertrand et al. 2011, Renwick & Rocca 2015). Since soil microbial communities will respond quicker to changes in climate than trees due to obvious differences in generation times and dispersal abilities (Elena & Lenski 2003, Finlay 2002), soil microbial mediation of plant phenotypes may represent a mechanism for plant adaption and persistence in future climatic scenarios (Lau and Lennon 2012, Fitzpatrick et al. 2019). Cumulatively, these results highlight the importance of building on current climate-centric models to include plant genetic variation and plant-soil-microbiome interactions. Developing such eco-evolutionary dynamic approaches is pivotal if we are to accurately provide geographic context to climate-driven responses from genes to ecosystems (Van Nuland et al. 2016, Van Nuland et al. 2017, Ware et al. 2019a, Ware et al. 2019b).

Conclusions and Frontiers

Together these results highlight a powerful observational and experimental platform to examine eco-evolutionary plant-soil microbiome feedbacks at a scale relevant to climate change. The results may have far reaching implications for sustaining forest ecosystems under global change. While we do not believe the soil microbiome is a panacea, a 14-17 day phenological buffer to climate warming is remarkable. Whether or not these results represent a pattern of local adaptation is still questionable, but soil microbiomes respond to population-level differences in tree conditioning and trees respond differently to tree conditioned soil microbiomes across the western US, a pattern that is consistent with coadaptation. If we can further develop frameworks that help partition the effects of climate, plant genetics, biotic interactions and ecosystem function on population dynamics and adaptation, identifying the phenotypes and interactions that allow for survival, persistence, and adaptation in a warming world may be possible.

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Appendix

Table 3.1 Site characteristics of populations sampled across the range of *Populus angustifolia* for soil inoculation experiment. Climatic and edaphic characteristics are represented by mean population-level values for each river sampled. The 10 populations are a subset of 17 populations surveyed in 2012 (Ware et al. 2019a). Soil type was extracted from GIS layers published in Zobler (1999). MAT represents mean annual temperature of surveyed populations in degrees Celsius. AP represents annual precipitation of surveyed populations in centimeters (cm). Mean population-level total soil carbon (C) and nitrogen (N) are displayed as percentages.

River	**Latitude**	**MAT (°C)**	**AP (cm)**	**Soil C (%)**	**Soil N (%)**	**Soil Type**
Oak Creek, AZ	35.1435	9.4	57.0	2.99	0.18	Luvic Kastanozem
Blue River, AZ	33.6677	8.8	47.2	1.47	0.09	Luvic Kastanozem
Indian Creek, UT	37.9460	8.1	37.5	2.52	0.16	Calcic Yermosol
Lexington Creek, NV	38.8604	7.1	35.4	7.01	0.29	Luvic Yermosol
Snake Creek, NV	38.9212	7.1	34.8	7.39	0.28	Luvic Yermosol
Dolores River, CO	37.6713	3.2	66.8	3.42	0.22	Eutric Regosol
Shoshone River, WY	44.4365	3.0	45.9	3.19	0.18	Albic Luvisol
Rio Grande, CO	37.5748	2.0	60.6	3.48	0.20	Albic Luvisol
Snake River, WY	43.5855	1.9	51.4	3.63	0.17	Albic Luvisol
Gros Ventre River, WY	43.5884	0.9	51.1	6.36	0.29	Albic Luvisol

Table 3.2 Soil microbes mediate plant phenology. Linear mixed effects model results exploring the response of bud break phenology (Julian days) to soil inoculation treatments (results represented in Figure 1C). Significant predictors are in bold.

***Response**: Julian Day (full model)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Live/Sterile	1	4.621	0.031
Soil Source habitat class	1	6.820	**0.009**
L/S * Soil Source habitat class	1	9.6765	**0.001**
***Response**: Julian Day (within warm habitat class)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Live/Sterile	1	5.190	**0.039**
***Response**: Julian Day (within cool habitat class)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Live/Sterile	1	5.415	**0.019**
***Response**: Julian Day (within live inoculated)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Soil Source habitat class	1	7.556	**0.005**
***Response**: Julian Day (within sterile inoculated)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Soil Source habitat class	1	2.72	0.11

Table 3.3 Biotic interaction Temperature Distance (Δ°C) is related to plant phenology. Linear mixed effects model results exploring the relationship between Δ°C and bud break phenology (Julian days). Significant predictors are in bold.

***Response**: Julian Day (full model)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Temperature transfer (Δ°C)	1	5.761	**0.016**
Live/Sterile	1	0.0001	0.992
Temperature transfer (Δ°C) * Live/Sterile	1	10.775	**0.0010**
***Response**: Julian Day (within live inoculated)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Temperature transfer (Δ°C)	1	7.257	**0.007**
***Response**: Julian Day (within sterile inoculated)*			
Factors	**DF**	***X^2***	**Pr(>*X^2*)**
Temperature transfer (Δ°C)	1	2.631	0.11

Table 3.4 Tree-driven conditioning of soil microbial communities. One-sample t-test results testing the hypothesis that true mean community turnover is greater than zero across diversity orders (q=0-2). Means and 95% confidence intervals displayed in Figure 3.2A and 3.2B.

Data	**DF**	**t**	**Pr(>t)**
Bacterial mean turnover (q=0)	128	40.401	<0.0001
Bacterial mean turnover (q=1)	128	27.317	<0.0001
Bacterial mean turnover (q=2)	128	19.91	<0.0001
Fungal mean turnover (q=0)	127	50.29	<0.0001
Fungal mean turnover (q=1)	127	34.91	<0.0001
Fungal mean turnover (q=2)	127	28.161	<0.0001

Table 3.5 Tree-driven conditioning of soil chemistry. One sample t-test results testing the hypothesis that the tree conditioning effect (T-IS) on total soil C, total soil N, and soil pH is greater than zero. Results displayed in Figure 3.2C.

Data	**DF**	***t***	**Pr(>t)**
Soil C Difference (T-IS)	573	8.71	<0.0001
Soil N Difference (T-IS)	567	7.32	<0.0001
Soil pH Difference (T-IS)	535	*8.66*	<0.0001

Table 3.6 Population-level variation in bacterial and fungal community turnover. Generalized linear model results show population-level differences in community turnover for each order of q (0-2, *Hill numbers*; Z. Ma (2018)).

Response:	*Bacterial Community Turnover*			*Fungal Community Turnover*		
Factor	**DF**	**X^2**	**Pr(>X^2)**	**DF**	**X^2**	**Pr(>X^2)**
Population (q=0)	14	33.2	**0.002**	14	30.5	**0.007**
Population (q=1)	14	30.8	**0.005**	14	24.6	**0.039**
Population (q=2)	14	24.8	**0.03**	14	24.4	**0.041**

Table 3.7 Drivers of soil microbial community composition. Distance-based redundancy analysis (dbRDA) shows plant phenotypic variation and abiotic environmental variation influence tree-associated soil bacterial and fungal communities. Only abiotic environmental variation explained significant variation in interspace soil bacterial and fungal communities. Significant predictors are in bold. (F) denotes field measured plant traits, and (GH) denotes genetic variation in plant traits measured in the greenhouse common garden. Constrained axes for model predicting tree bacterial community explained 24.7% of the variation in community composition (dbRDA1: 27.5%; dbRDA2: 13%). Constrained axes for model predicting interspace bacterial community explained 24.27% of the variation in community composition (dbRDA1: 26%; dbRDA2: 16%). Constrained axes for model predicting tree fungal community explained 22.99% of the variation in community composition (dbRDA1: 19%; dbRDA2: 12.5%). Constrained axes for model predicting interspace fungal community explained 24.3% of the variation in community composition (dbRDA1: 16.6%; dbRDA2: 13%).

Response:		*Tree Bacterial Community*			*Interspace Bacterial Community*			*Tree Fungal Community*			*Interspace Fungal Community*	
Factor	**DF**	**X^2**	**Pr(>X^2)**	**DF**	**X^2**	**Pr(>X^2)**	**DF**	**X^2**	**Pr(>X^2)**	**DF**	**X^2**	**Pr(>X^2)**
Latitude	1	7.06	**0.007**	1	21.8	**<0.0001**	1	32.47	**<0.0001**	1	115.9	**<0.0001**
Longitude	1	4.77	**0.02**	1	9.78	**0.002**	1	0.11	0.73	1	11.76	**0.0006**
Annual Precip.	1	5.00	**0.03**	1	24.29	**<0.0001**	1	12.97	**0.0003**	1	72.9	**<0.0001**
MAT	1	0.71	0.39	1	8.32	**0.004**	1	15.19	**<0.0001**	1	24.02	**<0.0001**
Soil C	1	3.74	0.053	1	0.56	0.45	1	4.39	**0.03**	1	2.23	0.13
Soil N	1	0.05	0.81	1	4.34	**0.037**	1	12.96	**0.0003**	1	0.85	0.35
Soil pH	1	2.82	0.093	1	0.939	0.33	1	0.14	0.71	1	9.9	**0.002**
DBH (F)	1	0.004	0.95	1	1.85	0.17	1	7.21	**0.007**	1	0.03	0.86
Foliar C:N (F)	1	13.72	**0.0002**	1	0.0004	0.98	1	24.7	**<0.0001**	1	0.21	0.64
Bud Break (GH)	1	4.77	**0.03**	1	0.26	0.61	1	12.6	**0.0003**	1	1.19	0.27

Table 3.8 Relative importance of predictor variables for soil microbiome diversity. Relative importance determined by summing the coefficients of the I-splines from GDM models. The most important predictor for tree and interspace soil bacterial and soil fungal communities is shown in bold. Predictors found to be not significant are indicated by dashes. (GH) denotes greenhouse-measured, genetically-based plant trait variation; (F) denotes field-measured plant traits. This table is visually represented in Figure 3.4.

Gradient	**Tree Bacterial Community**	**Interspace Bacterial Community**	**Tree Fungal Community**	**Interspace Fungal Community**
Annual Precipitation	0.022	**0.511**	0.182	0.29
Bud Break (GH)	0.10	--	0.09	--
DBH (F)	**0.29**	--	0.124	--
Elevation	--	0.053	0.023	0.188
Foliar C:N (F)	0.21	--	0.104	--
Geographic Location	0.116	0.133	**0.656**	0.56
Mean Ann. Temp.	0.234	0.141	0.255	0.262
Specific Leaf Area (F)	0.002	--	0.11	--
Soil C	0.191	0.04	--	0.121
Soil N	0.182	0.36	0.11	**0.61**
Soil pH	0.22	0.117	0.12	0.058

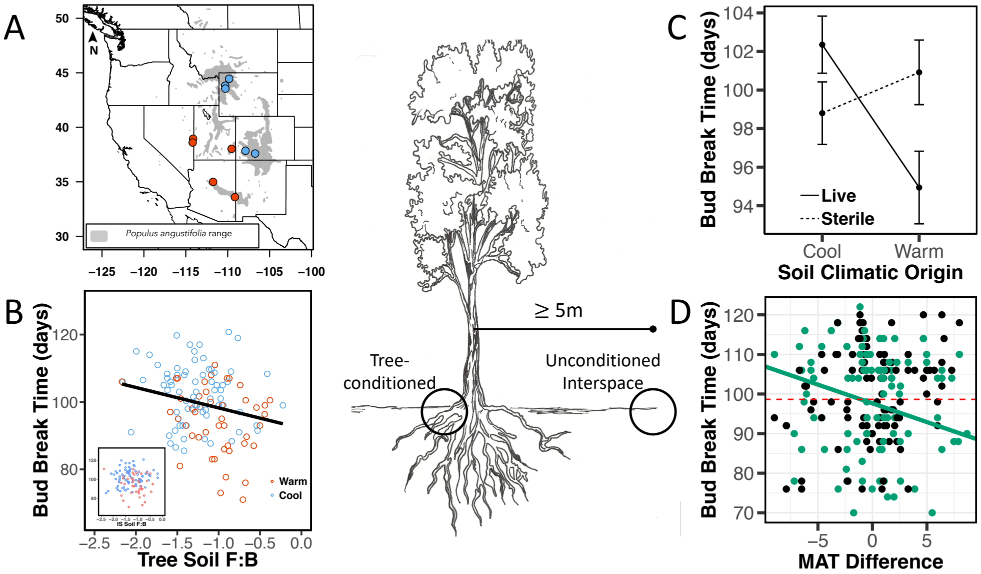
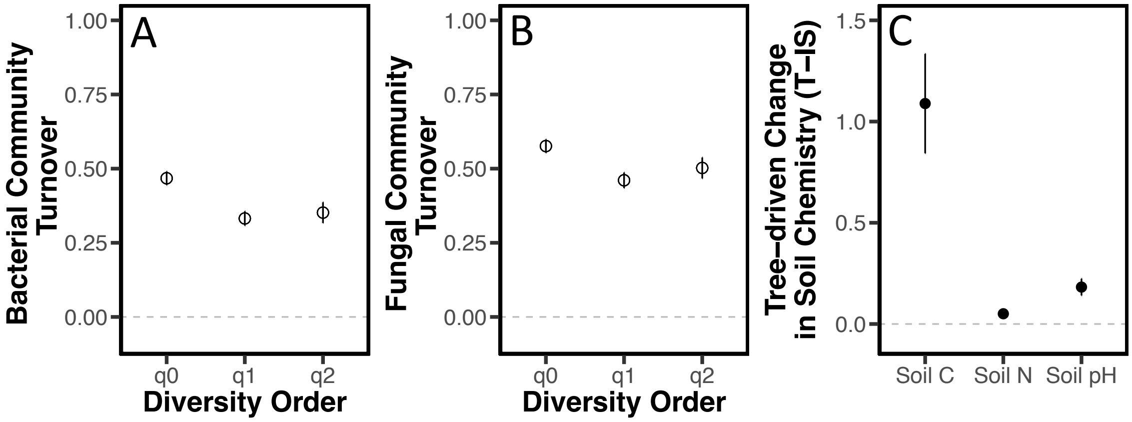


Figure 3.1 Geographic distribution of *Populus angustifolia* and soil inoculation experiment. Panel **A** shows idealized distribution of *P. angustifolia* and 2015 collection sites. Red symbols represent the five warm sites, and blue symbols represent cool sites. Panel **B** presents a hypothetical relationship between tree-associated soil microbial communities (i.e., qPCR based relative abundances of fungi:bacteria) and the genetic cline in bud break phenology measured in an experimental common garden. Inset figure shows a lack of relationship between interspace soil microbial communities and the genetic cline in bud break phenology. Panel **C** is a reaction norm depicting soil inoculation effects on bud break phenology (Julian days). Error bars represent +/- one standard error from the mean. Panel **D** shows the difference in soil microbiome climatic origin and tree genotype climatic origin (i.e., Δ℃) predicts variation in the timing of bud break (green line). Dotted red line represents experimental mean phenology.



q=0, *S (Richness);* ***q=1***, *exp(H') (exponential of Shannon's Index);* ***q=2***, *1/γ (reciprocal of Simpson's γ)*

Figure 3.2 Tree-driven differences in soil microbial communities and soil chemistry. Panel **A** shows mean bacterial community turnover for Diversity orders q=0-2. Panel **B** mean fungal community turnover for diversity orders q=0-2. Panel **C** shows mean differences in soil C, soil N, and soil pH between each tree-interspace pair total soil C between tree and interspace soils. Error bars in each panel represent 95% confidence interval of the mean.

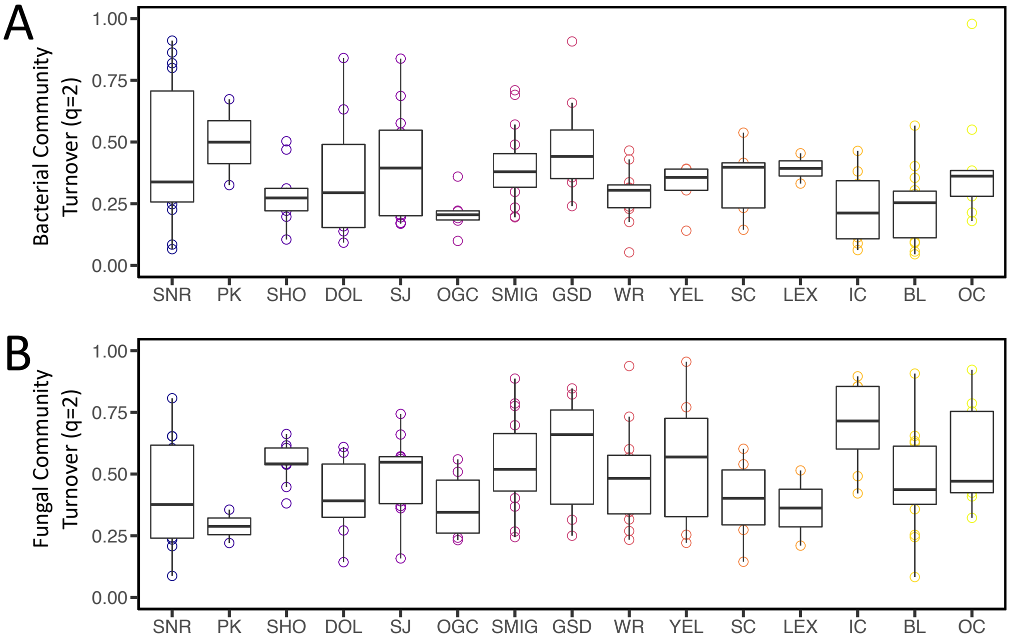


Figure 3.3 Population-level variation in tree-driven conditioning of soil microbial communities. Panel **A** shows population-level differences in bacterial community turnover (q=2; 1/*γ* (reciprocal of Simpson's *γ*)). Panel **B** shows population-level differences in fungal community turnover (q=2). Populations are arranged from coolest to warmest.

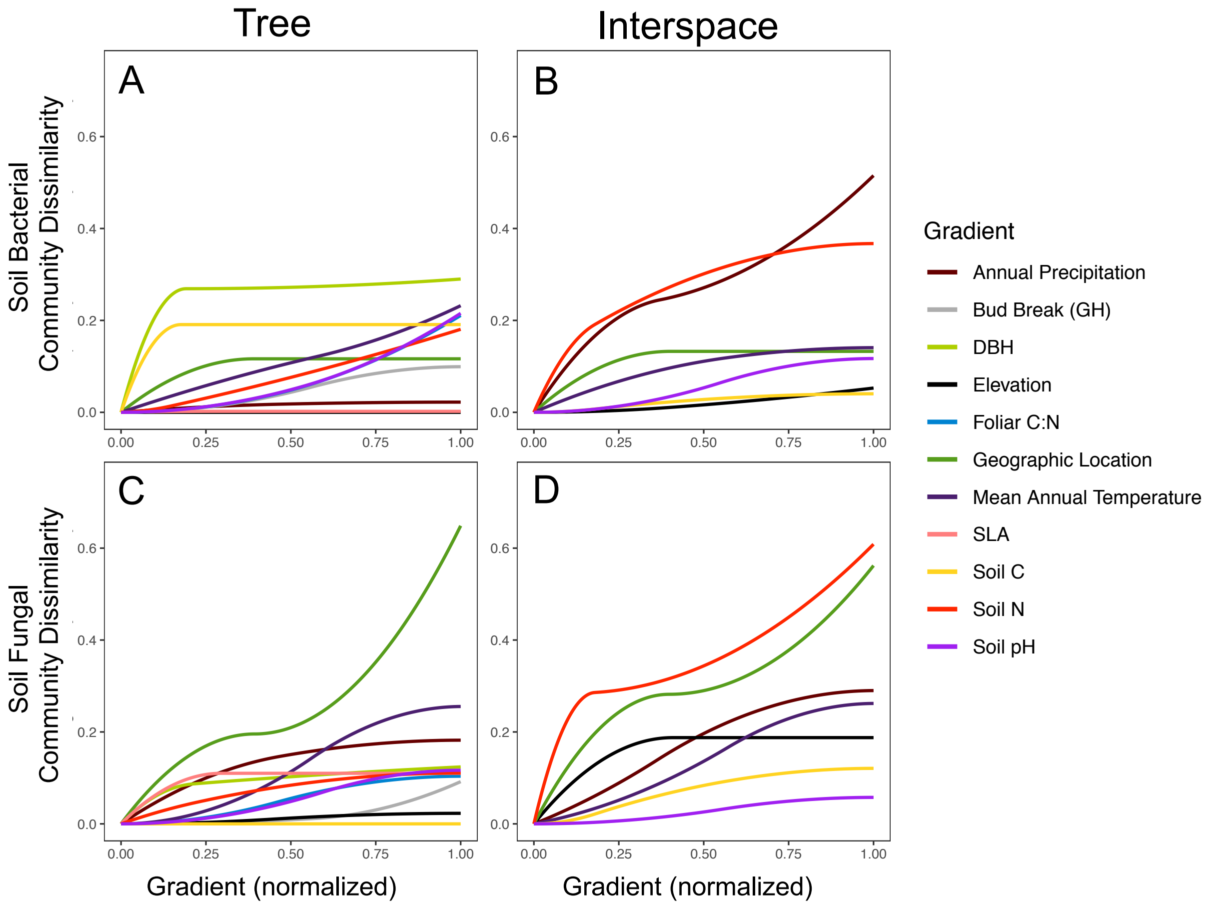


Figure 3.4 Landscape-level variation in climate, edaphic characteristics, and plant phenotypes drive turnover in soil bacterial and fungal communities. Panel **A** shows the plotted generalized dissimilarity model (GDM) for tree soil bacterial community dissimilarity. Panel **B** shows the plotted GDM for interspace soil bacterial community dissimilarity. Panel **C** shows the plotted GDM for tree soil fungal community dissimilarity. Panel **D** shows the plotted GDM for interspace soil fungal community dissimilarity. X-axes are normalized to allow for comparison of biotic and abiotic environmental gradients.

CONCLUSION

Plant populations are an inherent and pivotal component of almost all terrestrial communities and ecosystems. Yet the influence of among population variation in determining patterns of biodiversity and divergence in ecosystem function at geographic scales is rarely considered. Plants alter the soils in which they grow, and evidence that these modifications can feed back to influence the same or different plants represents a rich and growing mechanism for a variety of ecological phenomena. Genetically-based phenotypic variation in plant chemistry, morphology, and physiology structures belowground communities and regulates soil processes. Because the effects of plants on soil biotic and abiotic environments are the result of phenotypic variation, soils modified by plants create a link among genes, soil communities, and ecosystem function. Building upon foundational work in the fields of community and ecosystem genetics and eco-evolutionary dynamics, my work for this dissertation sought to investigate the abiotic gradients that drive variation and divergence in intraspecific genetic variation of a dominant tree species, and explore the community and ecosystem ramifications and feedbacks related to patterns of genetic variation in phenology and productivity at the landscape scale.

There are several major findings from my dissertation research that advance the field. First, plant phenology and productivity have evolved on the landscape in response to climatic gradients, and this evolution in response to warmer temperatures has reduced within-population genetic variation and evolvability on the landscape. These findings provide evidence that tree populations have diverged on the landscape in response to climatic gradients and suggest that modern climate change will likely have evolutionary consequences in natural systems. Second, climatic, plant genetic, and edaphic variation influences tree-associated soil microbiome community composition across the geographic extent of *P. angustifolia*. These changes in soil microbial community composition lead to a microbial feedback which mediated and reinforced range-wide patterns in bud break phenology. These results highlight that soil microbial mediation of plant phenotypes can be detected at landscape scales and suggest that soil microbial communities have diverged in their function in response to climatic, phenotypic, and edaphic variation. Third, climate-driven reduction in genetic variation alters the linkage between populations, communities, and ecosystems. The reduction of population-level genetic variation alters the relationship between tree-conditioned soils and interspace soil, which is driven by increases in the magnitude of soil nutrient conditioning in warmer, more productive populations. By analyzing geographic variation in climate, soil microbiomes and nutrient pools, and population-level genetic variation and evolvability, my work provides evidence that landscape-level climatic gradients has altered how plants and soils interact to impact plant phenology, productivity, soil nutrient pools, and carbon storage. Together, these results highlight that exploring how the eco-evolutionary dynamics of plant-microbe-soil interactions change in response to climatic variation is critical for understanding the mechanisms driving adaptation across scales and levels of biological organization (i.e., from genes to ecosystems).

Evolution meets ecology at the population level, and evolutionary divergence among populations is in part determined by both the ecological structure of species interactions and geographic variation in the eco-evolutionary outcomes of such genetically-based interactions. My work advances the field of eco-evolutionary dynamics by integrating population-level approaches with ecosystem ecology to improve our understanding of the variation in genetically-based phenotypes across scales that feed back to influence community structure, niche construction, and ecosystem-level evolutionary dynamics. An eco-evolutionary approach may be essential to linking the two artificially separated fields of ecosystem ecology and evolution by further exploring variation and feedback within and among individual ecosystem state factors described in Chapter I. This integration will also be critical to understanding how ecosystems may change as plant phenotypes and species distributions change along existing environmental gradients on the landscape.

VITA

Ian M. Ware was born in Memphis, TN and his teeth were cut on camping trips and musical snobbery. He left the bucolic stillness in eastern Shelby County to attend the University of Tennessee, Knoxville in hopes to find meaning in this grand simulation. Unsurprisingly, he developed great interest in plant ecology and conservation biology. He earned a Bachelor of Science degree in the Department of Ecology and Evolutionary Biology in 2009, and in 2010 moved to northern Utah to further his education at Utah State University with Dr. Peter Adler. While in Utah, he surveyed mesa top grass-shrublands in the remote Henry Mountains of southern Utah. His work in the Henry's sought to alleviate a human-wildlife conflict generated by an introduced herd of American Bison by providing descriptions of rangeland heath and forage plant species abundance and diversity. He completed his Master's degree in Wildland Resources, and headed back to the University of Tennessee, Knoxville to pursue a Ph.D. with Dr. Joe Bailey and Dr. Jen Schweitzer. The work for his Ph.D. sent him to explore the riparian forests of the western United States and cemented those ecosystems as his favorite natural system. The observations of which are described *ad nauseum* in the chapters above. To date, Ian has coauthored 7 publications, mentored more than twenty undergraduate ecologists, and won several awards for his efforts. Wherever Ian is today, I'm sure he'd be happy to talk more about his favorite music, latest adventures, and of course the interface of ecology and evolution.