ECOLOGICAL AND EVOLUTIONARY DYNAMICS OF PLANT-SOIL FEEDBACKS: INFLUENCES ON EVOLUTION AND RANGE DYNAMICS

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ABSTRACT

Plants interact with, modify, and are affected by their soil environments. Though plant-soil interactions are well known to be important and active regulators of ecosystem function and community structure, much less is known about how these interactions affect plant evolution. The primary goal of my dissertation was to examine plant-soil interactions under a range of ecological and evolutionary contexts to better understand patterns of biodiversity, ecosystem function, and whole system responses to environmental change. Taking such an eco-evolutionary perspective allows for a holistic understanding of the causes and consequences of complex abiotic and biotic interactions that link ecosystem ecology and evolution.

In my first chapter, I reviewed what is known about genetic interactions between plants, soils, and soil communities, and in doing so, identified a new mechanism for how genetically based plant-soil feedbacks might emerge at large scales. In my second chapter, I used field observations and multiple experimental approaches to test whether soil N acts as a selective gradient on plant phenotypes, if soil microbial communities mediate the selective pressure, and whether plant genetic variation impacts soil N pools. In my third chapter, I developed climate and soil ecological niche models, combined with a new double quantile regression approach, to tests how traits are adapted or plastic at critical environmental limits. Finally, my fourth chapter examined how plant-soil interactions and feedbacks at landscape scales may influence range dynamics and associated ecosystem processes as species move upwards towards higher elevations with rising temperatures. Overall, my dissertation sought to bring an evolutionary perspective to ecosystem ecology research by investigating the genetic mechanisms and outcomes of plant-soil interactions.

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INTRODUCTION

Plants interact with, modify, and are affected by their soil environments. For example, most tree species on Earth form symbioses with soil fungi by trading some of their photosynthesis products for soil nutrients recovered by their fungal partners (Smith and Read 2008), and this partnership may have supported terrestrial colonization by ancient plants. Tropical and temperate forest diversity is partly maintained by soil pathogens that accumulate beneath parent trees over time, negatively affecting the establishment of the same species' seedlings, thereby promoting species co-occurrence (i.e., the Janzen-Connell effect; Packer and Clay 2000, Mangan et al. 2010). Early successional plants condition soil communities that help drive the overall trajectory of succession by favoring the growth of later successional plants (Kardol et al. 2006). These examples highlight plant-soil interactions as important and active regulators of ecosystem function and community structure. The primary goal of my dissertation was to examine plant-soil interactions under a range of ecological and evolutionary contexts to better understand patterns of biodiversity, ecosystem function, and whole system responses to environmental change. Taking such an eco-evolutionary perspective allows for a holistic understanding of the causes and consequences of complex abiotic and biotic interactions that link ecosystem ecology and evolution.

Although research on plant-soil interactions has improved our understanding of many population, community, and ecosystem processes, much less is known about how these interactions affect plant evolution. This represents a major frontier towards unifying ecology and evolution, which is critical to advance our understanding of how biological diversity and energy flow are connected in communities and ecosystems. While the gap between ecosystem ecology and evolution remains relatively large (Matthews et al. 2014), one way to begin integrating these research areas is by examining how genetic feedbacks can occur between plants and soils (i.e., "plant-soil feedback"). In the first chapter of my dissertation, I reviewed what is known about genetic interactions between plants, soils, and soil communities (Van Nuland et al. 2016). In doing so, I revealed a new mechanism for how genetically based plant-soil feedbacks might emerge across large scale, namely, that landscape-level feedbacks may be common and driven by soil dynamics (e.g., nutrient pools and fluxes) that are influenced by genetic interactions between plants and soil communities.

Variation in soil nitrogen (N) influences plant distributions, fitness, and functional traits, but few studies have shown that phenotypic responses to soil N are adaptive. An evolutionary response to soil nutrient variation may impact plant traits that, in turn, alter soil N conditions. This could lead to large-scale nutrient-mediated feedbacks that change the quantitative genetic differences among populations. Moreover, few studies have simultaneously explored the scale at which soil nutrient gradients may influence plant evolutionary processes or how genetic variation then influences local soil processes. In my second chapter, I used field observations and multiple experimental approaches to test whether soil N acts as a selective gradient on plant phenotypes, if soil microbial communities mediate the selective force, and whether plant genetic variation impacts soil N pools. This work expands our understanding of the role of soils in plant trait evolution, and the reciprocal effects that trait evolution has on soils.

Plant range limits reflect the environmental constraints (both abiotic and biotic) that determine where populations are no longer self-sustaining (i.e., the realized niche). Species distribution models (SDMs) visually describe geographical variation of species' realized niches, typically using only climate data. However, biogeochemical gradients can influence the spatial

distribution of plants at large scales (Laliberté et al. 2013), and recent work shows how SDM accuracy is improved with soil information (Beauregard and de Blois 2014). While SDMs are useful for visualizing correlations between environmental conditions and species occurrences, they are not designed to test mechanisms of those relationships. For instance, a plant species' distribution may fall between a certain range of precipitation values (thus, precipitation may be an important factor for the accuracy and precision of an SDM), but few niche models are designed to test why or how precipitation values outside this range lead to population declines. Plant functional traits link inherently correlational SDMs and a mechanistic understanding of range limits. This is because plant traits related to their growth and fitness are formed, in part, by abiotic environments that are used to parameterize niche models. The expression of functional trait variation at regional and global scales, therefore, reflects the underlying physical constraints applied by abiotic environments (before biotic interactions mediate phenotypic expression at local scales). As a result, plant trait variation should be able to predict the environmental range of a species, identifying range limits where climate and soil environments occur that, beyond which, are unsuitable for sustained population growth (Stahl et al. 2014). In my third chapter, I developed climate and soil SDMs to compliment a new double quantile regression approach that tests how traits are adapted or plastic at environmental range limits.

Although species distributions and range limits are shaped by a complex set of abiotic and biotic forces, evidence has begun to emerge that plant-soil-biotic interactions could play a significant role in defining range limits. As plants move to new soil environments, their fitness will vary depending on the net impact of previously conditioned soil communities that may impact plant survival and performance. For instance, the degree to which plants modify the diversity and activity of soil communities at higher elevations could influence upward range shifts through these feedback effects. Most examples describing how PSF relate to plant range shifts use latitude gradients and make comparisons among species, even though plants generally have much shorter distances to travel in elevation relative to latitude to track the same 1°C temperature change. Within-species variation can also have important consequences for understanding the ecological and evolutionary consequences of feedbacks in plant-soil systems. Consequently, the importance of intraspecific PSF in determining range shift responses to climate warming could be greater and more immediate along elevation gradients relative to latitude gradients. My fourth chapter examined how plant-soil feedbacks at landscape scales could influence range dynamics and associated ecosystem processes within a single species.

Overall, my dissertation sought to bring an evolutionary perspective to ecosystem ecology research by investigating the genetic mechanisms and outcomes of plant-soil interactions. Using a combination of literature review, field observations, common garden experiments, local adaptation studies, next-generation sequencing, and ecological niche models, my research examined the causes and consequences of plant-soil-biotic interactions that influence ecological and evolutionary dynamics. Because foundation species that occur across large environmental gradients are most likely to show the greatest effects of evolution on communities and ecosystems, and divergent environments strongly shape adaptive evolution (Matthews et al. 2014), I use plant-soil interactions in *Populus angustifolia* (James) and riparian ecosystems across the Rocky Mountains. This species occurs across large climate and soil gradients, and seminal research using *Populus* describes how genetic variation impacts phenotypes, above- and belowground community, and soil nutrient processes. Moreover, these riparian areas are predicted to experience significant warming (2-6 °C) over the next 50 years,

and understanding how the species' range dynamics will respond as environments change is critical to maintain the diversity and function of these important habitats.

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CHAPTER I
PLANT-SOIL FEEDBACKS: CONNECTING ECOSYSTEM ECOLOGY AND EVOLUTION

This chapter was originally published by Michael E. Van Nuland, Rachel C. Wooliver, Alix A. Pfennigwerth, Quentin D. Read, Ian M. Ware, Liam Mueller, James A. Fordyce, Jennifer A. Schweitzer and Joseph K. Bailey:

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Abstract

While an appreciation of plant-soil feedbacks (PSF) continues to expand for community and ecosystem ecology, the eco-evolutionary mechanisms and consequences of such feedbacks remain largely unknown or untested. Determining the cause and effect of plant phenotypes is central for understanding these eco-evolutionary dynamics since phenotypes respond to soil selective gradients that are, in turn, modified by plant traits. Genetic variation in plant phenotypes can change soil processes and biotic communities; oppositely, soil gradients and microbial communities can influence the expression and evolution of plant phenotypes. Although these processes represent the two halves of genetic based PSF, research in these areas has developed independently from one another. Greater connectivity between research on ecosystem consequences of plant genetic variation and soil selective gradients that drive plant phenotypic evolution will create novel and important opportunities to link ecology and evolution in natural systems. Papers in this special feature build on the inherent ecological and evolutionary processes involved in plant-soil feedbacks, outlining many ways to identify and test mechanisms that connect ecosystem ecology and evolution.

Introduction

Plant-soil feedbacks have important evolutionary implications because they are associated with changes in plant fitness (van der Putten 1997), which might ultimately affect genetic divergence among populations, adaptive or contemporary evolution, and diversification (Bailey et al. 2014; Schweitzer et al. 2014). 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. By exuding root compounds such as hormones, phenolics, sugars, and organic acids (reviewed in Bais et al. 2006), and contributing organic matter such as leaf and root litter (Vivanco & Austin 2008), plants shape soil biotic communities that use these plant products as energy sources. Plant interactions with soil biota (bacteria, fungi, archea, viruses, and macro- and microarthropods) in turn alter the physical and chemical properties of soils that, together with soil biota, positively or negatively affect the fitness and phenotype of the same or different plants (Ehrenfeld, Ravit & Elgersma 2005; Kulmatiski et al. 2008; Miki 2012). These effects of past soil changes that influence plant performance and fitness are known as plant-soil feedbacks (PSF; Bever 1994). Such feedbacks have been shown to facilitate or slow the spread of invasive species (Kourtev et al. 2002; Wolfe & Klironomos 2005; Levine et al. 2006; Reinhart & Callway 2006; Nijjer, Rogers & Siemann 2007; Batten, Scow & Espeland 2008), direct plant community succession (Kardol, Bezemer & van der Putten 2006), and underlie abundance and co-existence patterns within plant communities (Klironomos et al. 2002; Kulmatiski et al. 2008; Mangan et al. 2010; van der Putten et al. 2013). While an appreciation of PSF continues to expand for community and ecosystem ecology, much less work has focused on the evolutionary mechanisms and consequences of PSF.

Understanding the evolutionary dynamics of PSF relies on identifying the cause and effect of plant phenotypes since 1) they are the material upon which natural selection acts and 2) they largely control plant-soil relationships. For instance, soil metal toxicity is an important selective agent for plants on abandoned mine sites or harsh serpentine soils that leads to locally adapted populations with phenotypes of increased tolerance of aluminum (Al) and magnesium (Mg) (Whitaker 1954; Shaw 1989; Brady, Kruckeberg & Bradshaw 2005; Anacker 2014). However, soils are hyper-diverse with living organisms, and biotic interactions with soil microbes create genetic environments that also impact phenotypes. Such is the case when plant growth-promoting rhizobacteria in the soil environment produce phytohormones that direct plant root growth, mycorrhizal development of roots, or root nodule formation for symbiotic nitrogen (N) fixation (Dighton 2014). These interactions can result in phenotypic shifts due to plasticity and may not always result in relatively fast reciprocal selection (Agrawal 2001; Fordyce 2006), but here we focus on the selective consequences of plant-microbe interactions. While soils and microbes can shape the underlying genetic variation and expression of plant phenotypes, plants actively change belowground processes and determine the nature of above-belowground relationships (Kardol et al. 2015). For example, Ke et al. (2015) modeled how the effect of plant traits (e.g., litter decomposability) on PSF direction and strength depend on the relative abundance of certain soil biota groups (e.g., decomposers, mutualists, pathogens). As a broader example, litter decomposability is an important phenotype determining soil nutrient status through plant-litter-nutrient feedbacks (Hobbie 1992, 2015). With the potential for feedback loops to exist between plants, microbial communities, and soil environments, understanding the genetic basis of these interactions is essential for PSF research to begin incorporating and demonstrating evolutionary concepts.

Genetic variation in plants can affect soil microbial communities, and, in turn, these communities affect genetically based plant traits. For example, when seedlings from 20 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 (Pregitzer et al. 2010). A related study using P. angustifolia genotypes found that positive feedback effects on plant performance traits were related to conditioned soil communities (Smith et al. 2012). If PSF comprise genetic interactions between plants and soil communities, then not only could these feedbacks drive genetic changes in plants and alter biodiversity patterns (Vitousek 2004, Bailey et al. 2014), but the feedbacks themselves might evolve across different environments (Schweitzer et al. 2014). Because (i) soil microbial communities respond to plant phenotypes, (ii) plants respond to variation in soil microbial communities, (iii) plant-microbial interactions drive nutrient cycling and (iv) these interactions vary and evolve along environmental gradients that are critical to ecosystem function and evolutionary processes, the field of PSF is an excellent opportunity for placing ecosystem ecology into an evolutionary framework and vice-versa.

Building upon a growing literature and a previous special feature highlighting the ecological mechanisms and consequences of PSF (van der Putten et al. 2013), papers in this special feature demonstrate or suggest many of the evolutionary mechanisms and consequences of PSF at a broad scale. Topics in this special feature include the evolutionary role of PSF in biodiversity and ecosystem function (Evans et al. 2016; terHorst & Zee 2016), local adaptation of soil microbial communities and their reciprocal effects on plant phenotypes (Revillini, Gehring & Johnson 2016; Herrera Paredes & Lebeis 2016), phylogenetic responses of plants to

soil N, demonstrating large scale evolutionary effects of plant-soil linkages (Wooliver et al. 2016), and finally, the applications and frontiers of PSF under land use and environmental change (de la Pena et al. 2016; van der Putten et al. 2016). Using the genes-to-ecosystems concepts expressed throughout the special feature, the goal of this paper is to highlight how PSF research bridges ecological and evolutionary concepts to place ecosystem ecology into an evolutionary framework.

Mechanisms that link ecosystems and evolution via PSF

Genetic variation and ecosystem function are linked in terrestrial systems (Schweitzer et al. 2004; Crutsinger, Souza & Sanders 2008; Hughes et al. 2008; Bailey et al. 2009; Fitzpatrick et al. 2015; terHorst & Zee 2016). The link between genes and ecosystems is fundamentally related to genetically based species interactions and the energy flow or nutrient cycling that emerges from these interactions (Whitham et al. 2003, 2006; Shuster et al. 2006; Bailey et al. 2014; Schweitzer et al. 2014). Here, we define genetically based interactions between plants and soil microbial communities as Genotype X Genotype interactions (GxG), where genetically based plant phenotypes interact with phylogenetically diverse soil microbial communities. Although soil communities harbor an immense level of diversity, conceptually referring to them as a single genetic unit is a first step for our purposes of exploring genetic links above- and belowground. Below, we outline how PSF research provides an important platform to identify and test mechanisms that connect ecology and evolution through these plant-soil genetic linkages.

Belowground consequences of aboveground genetic variation

The effect of plant genes can reach to higher levels of communities and ecosystems (Dawkins 1982; Whitham et al. 2003, 2006). This is a critical element to the evolutionary mechanisms of PSF, whereby GxG interactions change the soil environment, and those changes ultimately affect plant fitness and phenotypes. In this way, GxG interactions structure communities (Goodnight 1990a, b; Brodie 2005; Shuster et al. 2006; Allan et al. 2012) and drive ecosystem level change (Whitham et al. 2003, 2006; Lojewski et al. 2009, 2012; Genung et al. 2011, 2013; Miki 2012; Schweitzer et al. 2014). This has been shown repeatedly for plants across many terrestrial (and aquatic) systems (reviewed in Bailey et al. 2009; Schweitzer et al. 2012; Bailey et al. 2014; Matthews et al. 2014). Genetically based phenotypic variation in plant chemistry, morphology, and physiology structures belowground communities and regulates soil processes (reviewed in Schweitzer et al. 2012; Table 1.1). Plants vary in soil nutrient uptake and use (Wooliver et al. 2016), affecting the quantity and quality of root and leaf litter (e.g., lignin content and foliar C to N ratio). These differences lead to well-documented effects on litter arthropod assemblages, soil fungal and bacterial community composition, and ultimately carbon (C) and N mineralization (Zinke 1962; Hobbie 1992; Bardgett 2005; Chapman et al. 2006; Bardgett & Wardle 2010; Gorman et al. 2013). At finer scales, species modify local soil conditions in the vicinity of their highly variable root environments through the exudation of hormones, sugars, phenolics, and amino acids (Bardgett & van der Putten 2014). Root exudates structure rhizosphere communities by providing carbohydrate sources and functioning as signaling molecules (Chaparro et al. 2013;

Table 1.1. Evidence that plant genetic effects influence abiotic and biotic soil properties

Abiotic

Plant genetic effect	Soil response	Reference
Genotype, genotypic diversity, population, species	Litter decomposition	Treseder & Vitousek 2001; Schweitzer et al. 2004, 2005; Madritch & Hunter 2005; Crutsinger, Sanders & Classen 2009; Genung et al. 2013; Fitzpatrick et al. 2015; Hobbie 2015
Genotype, genotypic diversity, species	Litter nutrient release	Madritch & Hunter 2002, 2005; Madritch, Donaldson & Lindroth 2006; Silfer et al. 2007; Schweitzer et al. 2011a; Genung et al. 2013
Genotype, species	Soil nitrogen/carbon	Hobbie 1992; Pregitzer et al. 2013
Genotype, genotypic diversity, population, provenance	Nitrogen availability	Fischer et al. 2010; Schweitzer et al. 2011b

Biotic

Plant genetic effect	Soil response	Reference
Genotype, species	Microbial nutrient pools	Schweitzer et al. 2008; Pregitzer et al. 2010
Genotype, population	Extracellular enzyme activity	Schweitzer et al. 2008; Madritch, Greene & Lindroth 2009
Genotype	Net nitrogen mineralization	Schweitzer et al. 2011a
Genotype	Soil C respiration and efflux	Fischer et al. 2007; Lojewski et al. 2012; Fitzpatrick et al. 2015
Genotype, population, species	Microbial community composition	Schweitzer et al. 2008; Aleklett et al. 2015

Dighton 2014; Herrera Paredes & Lebeis 2016). Plant control on soil and root microbiota has been observed under highly controlled greenhouse, common garden, and agricultural settings (Marschner & Yang 2001; Schweitzer et al. 2008a; Lundberg et al. 2012), and recently in wild plant species growing in close proximity in the field (Aleklett et al. 2015). Even in diverse tropical forests, taxonomic and phylogenetic metrics of tree composition are correlated with soil bacterial and fungal composition (Barberan et al. 2015). Belowground microbial activity (e.g., decomposition, nutrient cycling, predator-prey dynamics) changes soil physico-chemical properties such as the type and concentration of inorganic nutrients, pH, or water holding capacity (Ehrenfeld et al. 2005; Bardgett 2005; van der Putten et al. 2016), all of which can feedback to impact plant performance. Since the expression of genes determine plant phenotypes, and the effects of plants on soil biotic and abiotic environments are the result of phenotypic variation, soils modified by plants create a link between genes, soil communities, and ecosystem function that generate PSF with evolutionary consequences (Whitham et al. 2006; Schweitzer et al. 2008b, 2014; Genung et al. 2013; Bailey et al. 2014).

Aboveground evolutionary consequences of belowground variation

While several studies have shown the effects of plant phenotypic variation on soil communities and emergent ecosystem properties over the last decade, far fewer have shown how soil communities may shape plant phenotypes, although that is beginning to change (Friesen et al. 2011; Revillini et al. 2016, Herrera Paredes & Lebeis 2016; Table 1.2). For example, using an invasive tree *Ailanthus altissima*, Felker-Quinn et al. (2011) found that genetic variation and among-population variation for plant biomass was only expressed when the soil microbial community was living (relative to sterilized controls). These results indicate that (i) genes associated with certain phenotypes may only be expressed given particular microbial interactions; and (ii) the interpretation of evolution on the landscape is specific to the microbial context. Similarly, soil microbes alter the strength and direction of selection on plant phenology by altering plasticity in flowering time and impacting fecundity (Wagner et al. 2014). Moreover, the reciprocal interactions that drive ecological changes to soil microbes can drive plant evolution in novel environments (Lau & Lennon 2011, 2012), and the evolutionary response by plants may shift belowground community composition (Bailey et al 2012; Schweitzer et al. 2014; terHorst et al. 2014) resulting in genetically based differences in ecosystem processes (sensu Fitzpatrick et al. 2015).

Widespread soil variation is an important selective pressure on plant phenotypes, and recent studies demonstrate plant adaptation and divergence along soil gradients (Table 1.2). Perhaps the earliest and clearest example of plant evolutionary responses to soils comes from decades of work studying serpentine plant populations (reviewed in Anacker 2014). Soil-mediated selection has repeatedly been shown to result in plant evolution under these harsh conditions using reciprocal transplants (Brady et al. 2005) and gene sequencing approaches (Turner et al. 2010) to examine local adaptation. In these studies, plant populations evolve increased resilience to soil toxicity by adapting to high metal concentrations that are characteristic of serpentine soils (e.g., phenotypes with an enhanced ability to sequester, transport, or selectively uptake Mg and Al). Plant phenotypes that condition the soil microbial community to suppress pathogen activity or to facilitate resource uptake may also be favoured (Kinkel, Bakker & Schlatter 2011, Revillini et al. 2016). For example, when *Andropogon gerardii* ecotypes collected from phosphorus (P)-limited and N-limited grasslands were grown

Table 1.2. Examples of soil factors that impact plant phenotypes and drive evolutionary responses

Abiotic

Soil Factor	**Plant phenotype response** (*species*)	**Evo implication**	**Method**
Serpentine	Flowering time (*Collinsia sparsiflora*)	Local adaptation	Wright, Stanton & Scherson 2006
Serpentine	Growth (*Achillea borealis*)	Local adaptation	Kruckeberg 1954
Serpentine	Metal detox a transport (Arabidopsis lyrata)	Local adaptation	Turner et al. 2010
Aluminum	Al tolerance	Local adaptation	Gould, McCouch & Geber 2014
Nutrients	Growth (*Populus angustifolia)*	Local adaptation	Van Nuland et al. *in review*

Biotic

Soil Factor	**Plant phenotype response** (*species*)	**Evo implication**	**Method**
Bacteria & fungi	Productivity (*Ailanthus altissima)*	Divergence	Felker-Quinn et al. 2011
Microbes	Spring bud break (*Populus angustifolia)*	Divergence	Van Nuland et al. *in review*
Bacteria & fungi	Flowering time (*Arabidopsis* spp.)	Selection intensity	Wagner et al. 2014
Microbes	Growth and Phenology (*Brassica rapa*)	Selection intensity	Lau and Lennon 2011
Fungi	Survival, Growth (*Pseudotsuga menzeisii*)	Local adaptation	Pickles et al. 2015
Microbes	Biomass (*Populus angustifolia)*	Local adaptation	Pregitzer et al. 2010, Smith et al. 2012
Microbes	Survival, growth (*Populus angustifolia)*	Local adaptation	Van Nuland et al. *in review*
Rhizobia	Specific Leaf Area (*Glycine max)*	N/A	Harris, Pacovsky & Paul 1985
AM fungi	Clonality (*Prunella vulgaris)*	N/A	Streitwolf-Engel et al. 2001
Rhizobia	Leaf frost sensitivity (*Medicago sativa)*	N/A	Bertrand et al. 2007
Rhizobia	Height (*Oryza sativa)*	N/A	Perrine-Walker et al. 2007
AM fungi	Specific root length (*Zea mays*)	N/A	Kothari, Marschner & George 1990
EM fungi	Fine root diameter (*Pinus taeda)*	N/A	Rousseau, Sylvia & Fox 1994
Rhizobia	Root distribution (*Trifolium subterraneum*)	N/A	Morris & Djordjevic 2006
AM fungi	95% rooting depth (*Triticum aestribum)*	N/A	Ellis, Larsen & Boosalis 1985
Bacteria	Stress recovery (*Capsicum annuum*)	N/A	Marasco 2012

Microbes: non-specified collection of bacteria, fungi, archae, and viruses. EM fungi: ectomycorrhizae; AM fungi: arbuscular mycorrhiza. N/A implies no direct test of plant evolutionary response or implication to the soil factor in the respective study.

with all possible 'home' and 'away' combinations of soils and mycorrhizal communities, soil fertility was a key driver of locally adaptated symbioses such that mycorrhizal exchange of the most limiting soil nutrient resource for each ecotype was maximized (Johnson et al. 2010). Reciprocal transplant experiments such as those used in Johnson et al. (2010) consistently indicate that varying selective pressures from soil microbes or nutrients lead to patterns of local adaptation and geographic mosaics of plant-microbe interactions that vary in strength (Pregitzer et al. 2010; Smith et al. 2012; Andonian et al. 2012). Such variation in plant-microbe interactions have the potential to create PSF differences along environmental gradients that may lead to genetic divergence on the landscape through the evolution of feedbacks (Schweitzer et al. 2014; Evans et al. 2016). Because plant-microbial interactions also modify soils, the selective gradients that underlie plant-microbial interactions may be reinforced, ultimately affecting the direction and pace of evolution.

The ability of plants to alter their surrounding soil creates environmental variation that can lead to and maintain genetic divergence. This occurs when soil gradients drive phenotypic evolution, thus changing the distribution of plant traits that control ecosystem processes and structure soil communities (Vitousek 2004; Pregitzer et al. 2013; Felker-Quinn et al. 2011; Smith et al. 2012). Variation in soil parent materials and underlying chemistry may result in fitness differences among individual as plants differ in their ability to tolerate specific soil conditions (Ellis & Weis 2006; Alvarez et al. 2009). These soil gradients influence plant phenotypes indirectly by affecting soil microbial communities and altering GxG interactions. For example, natural gradients in soil pH have been shown to influence the community composition of bacteria, fungi, and arbuscular mycorrhizal communities (Fierer & Jackson 2006; Dumbrell et al. 2010). A classic example for understanding how soils may impact plant phenotypic evolution is the long-term soil age gradient of Hawai'i. Gradients of substrate age and soil development influence nutrient availability (Vitousek 2004). With low soil nutrient availability, symbiotic mutualists and a slow cycling microbial community generally occur. Low nutrient availability may also select for small leaves, high nutrient use efficiency, slow growth rates, long foliar life span, high nutrient resorption, and recalcitrant plant tissues that decay slowly. Such recalcitrant plant tissues may lead to continued low soil nutrient availability and thus a consistent selective pressure on plant traits that convey fitness advantages under these environmental conditions. With high nutrient availability, the opposite patterns have been demonstrated. Reinforcement of these patterns may ultimately lead to local adaptation due to variation in soil nutrient availability and divergence in functional phenotypes (sensu Bertness & Callaway 1994; Wooliver et al. 2016).

Synthesis and conclusions

The relative importance and extent of reciprocal interactions between the ecology of populations, communities, and ecosystems, and their evolutionary dynamics remains an open issue (Fussman, Loreau & Abrams 2007; Post & Palkovacs 2009; Schoener 2011; Matthews et al. 2014). While other examples in aquatic and plant-herbivore realms are emerging (Reznick 2013; Fitzpatrick et al. 2015), recent empirical and theoretical work suggests that genetically based plant-soil-microbe interactions are a model arena to demonstrate these eco-evo linkages (Lau & Lennon 2011, 2012; terHorst, Lennon & Lau 2014; Schweitzer et al. 2014; Evans et al. 2016; Revillini et al. 2016; terHorst & Zee 2016). Here, we outlined how plant genetic effects can change soil communities and nutrient cycling (Table 1.1), and how soil biotic and abiotic selective pressures drive plant phenotypic evolution (Table 1.2). Although both are key components of eco-

evolutionary feedbacks that emerge from PSF, such research has developed largely independent of one another. Below, we outline a conceptual synthesis of these ideas using a genes-to-ecosystems approach, highlighting the opportunity of PSF research to address the links between evolution, community dynamics, and ecosystem function.

Three levels of eco-evolutionary feedback in plant-soil systems

Feedbacks shape the strength and direction of natural selection on the landscape. To date, feedbacks have generally been regarded as a population or coevolutionary phenomenon (Thompson 2005). However, ecosystem-level feedbacks can affect evolutionary dynamics in at least three ways (Fig. 1.1). First, feedbacks can be coevolutionary and driven by GxG interactions between plants and soil microbes (FB1). Here, they are largely a localized phenomenon within populations and communities (Thompson 1994). Depending upon the geographic distribution and underlying differences in phenotypic variation of the interacting species, these interactions may drive among-population level patterns of genetic divergence on the landscape, irrespective of other abiotic environmental factors (Thompson 2005, 2013). Second, feedbacks can result from ecosystem-level effects of past GxG interactions that vary independently of geographic variation in abiotic selective gradients (FB2). These are ecosystem-level feedbacks that are a consequence of local plant-microbe interactions. Similar to coevolutionary feedbacks, ecosystem level feedbacks may vary based upon the geographic distribution and phenotypic variation of the interacting species. This holds evolutionary significance because ecosystem-level feedbacks may interact with coevolutionary feedbacks to reinforce or disrupt among-population patterns of genetic divergence. Third, feedbacks can result from ecosystem level effects of past plant-microbe interactions that co-vary with geographic variation in abiotic selective gradients (FB3). Abiotic selective gradients such as climate, parent material, and mineral nutrient availability commonly impact plant phenotypes that are expressed by organisms and drive evolutionary dynamics of those species (Read et al. 2014; Wooliver et al. 2016). The energy and nutrients that emerge from plant-microbe interactions reinforcing selective gradients, as is the case with PSF (Kylafis & Loreau 2008; Schweitzer et al. 2014), are the drivers of ecosystem level evolutionary dynamics. While these types of eco-evolutionary feedbacks remain difficult to identify and test in nature (Reznick 2013), terHorst & Zee (2016) describe how GxG interactions between plants and soil microbes offer a promising direction for future research because of short generation times that allow for rapid evolution, strong selection pressures, and tight coevolutionary dynamics. As an empirical test, Evans et al. (2016) identify components of an eco-evolutionary feedback operating in the invasive garlic mustard, where heritable phenotypic variation of an allelopathic compound drives plant-soil interactions that feedback to alter demographic rates. If eco-evolutionary feedbacks are common and explain both above- and belowground community structure, then the interaction of plant-soil and eco-evolutionary dynamics could be important for generating spatial and temporal variation in PSF.

Research frontiers

As highlighted in this and other papers throughout the special feature, understanding how the strength and direction of PSF relates to genetic interactions between plants, soils, and microbes is an important new step for identifying how systems respond to increasing stress caused by human activity.

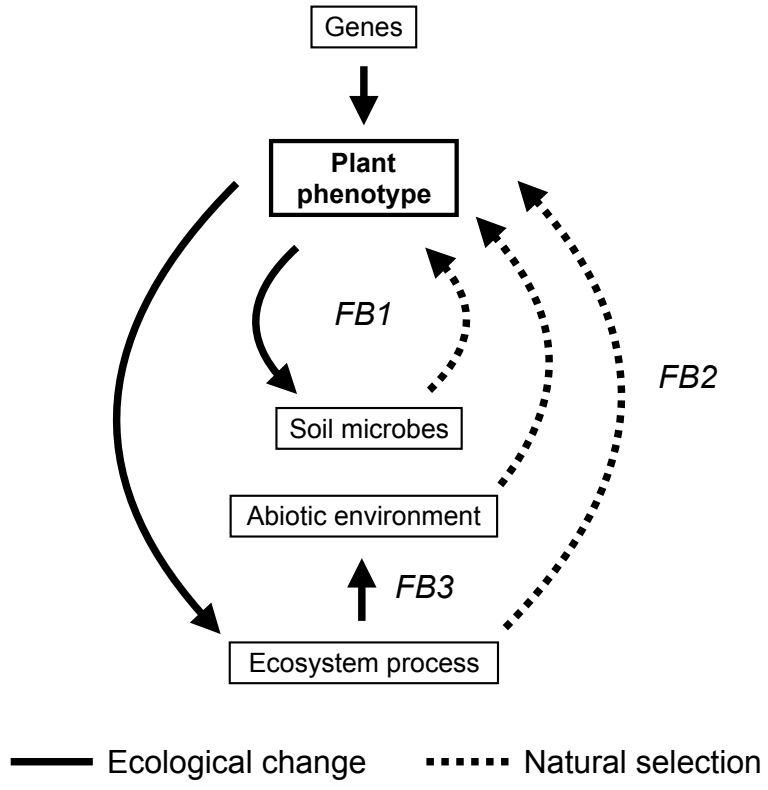

Figure 1.1. Genes-to-ecosystems conceptual model of ecological and evolutionary dynamics that show three possible pathways for feedback in plant-soil systems. Genetically based plant phenotypes structure soil communities and influence ecosystem processes. These represent ecological changes to the environment that are based on underlying plant genetics. In addition, changes to the soil community may result in ecosystem-level changes that persist into the future (e.g., plants that decrease the relative abundance of decomposers might indirectly lower nutrient pools). Plants can evolve irrespective of changes to soils from other abiotic environmental factors, but when they evolve in response to past soil changes this creates three levels of eco-evolutionary feedbacks: they can be the result of co-evolution and driven by GxG interactions between plants and soil microbes (FB1); they can result from ecosystem-level effects of past GxG interactions that vary independently of geographic variation in abiotic selective gradients (FB2); or they can result from ecosystem-level effects of past GxG interactions that co-vary with geographic variation in abiotic selective gradients (FB3). This is most easily seen with PSF where plant species evolve phenotypes to use soil resources differently (e.g., soil nitrogen), which then feedbacks to alter variation in soil resources (Wooliver et al. 2016). Plant evolution may shape how their phenotypes interact with soil microbial communities (ter Horst et al. 2016, Evans et al. 2016) driving shifts in the composition and evolution of soil microbes (Revillini et al. 2016) and how soil microbial communities can become locally adapted to abiotic soil factors and drive the expression of plant phenotypes (Revillini et al. 2016, Herrera Paredes & Lebeis 2016). Such plant-soil-microbe interactions affect ecosystem processes by altering nutrient cycles and pools relative to the background environmental gradient. In the conceptual diagram, solid lines depict ecological changes (or the effect of genes on phenotypes), and dashed lines depict selective forces.

As a result, the eco-evolutionary dynamics of PSF could offer a unique and underused resource to empirically test the response of populations, communities, and ecosystems to environmental change. To achieve this, we have identified research frontiers covering local and landscape scales that form an integrated set of challenges critical to advance PSF research across disciplines and tackle serious global change issues.

A major frontier for ecological research is a more precise understanding of how plants and microbes interact (a significant component of the "black box"; Kardol et al. 2015; Fig. 1.2). For instance, there has been a great deal of work on the genetic and molecular mechanisms that underlie plant-pathogen signaling in crops (Baker et al. 1997) and the signal exchanges between plants and *Rhizobium* that form root nodules (Long et al. 1996). However, this level of detail has been largely absent from PSF studies despite its obvious importance for understanding the basis and consequences of plant-microbe interactions vary on the landscape. As Herrera Paredes and Lebeis (2016) review, new methods and statistical approaches are making it easier to test the microbial mechanisms of PSF, such as sequencing and bioinformatic tools that link plant and microbial phenotypes with functional gene content. In addition, Revillini et al. (2016) use a novel approach with optimal resource allocation models to characterize these interactions and predict PSF outcomes across resource gradients. Characterizing plant-microbe and microbe-microbe signaling pathways alongside plant and soil manipulation experiments will allow for a more mechanistic understanding of how plants condition different soil microbial communities and microbes that mediate plant phenotypic expression. Importantly, this frontier can only be addressed through greater collaboration between ecologists and cellular and molecular biologists working to understand plant-soil linkages from molecular to ecosystem scales (Herrera Paredes and Lebeis 2016).

A significant goal for eco-evolutionary PSF studies is to examine how abiotic stress gradients influence plant genetics, soil microbial structure and function, and GxG interactions between plants and microbes that alter ecosystem processes (Fig. 1.2). Evans et al. (2016) provide one of the first empirical examples of an eco-evolutionary feedback that is driven by plant-soil linkages and could be important for understanding garlic mustard invasion in the Southeastern US. Moreover, terHorst and Zee (2016) outline why feedbacks between ecological and evolution dynamics might be common and interact with PSF as soil microbes influence ecosystem processes and the expression of specific plant phenotypes that may be critical to plant responses and resilience to global change through feedbacks. Using a meta-analysis, Wooliver et al. (2016) examine how phylogenetic relationships relate to plant strategic responses to elevated N, demonstrating that past evolution towards greater growth responses to N are associated with certain plant functional types and suggesting that N enrichment could lower functional diversity. There was no evidence, however, for divergent selection across biomes and along climatic gradients, indicating that evolutionary consequences of PSF vary independently of environment. Future studies that examine how environmental selection pressures alter plant-soil GxG interactions and their ecosystem consequences will generate important information for predicting if populations can adapt, respond plastically, or migrate towards more favorable sites under environmental change.

Feedbacks may interact at different levels, but it remains unclear what conditions cause local-scale feedbacks to disrupt or accelerate ecosystem-level feedbacks, and vice versa (Fig. 1.2).

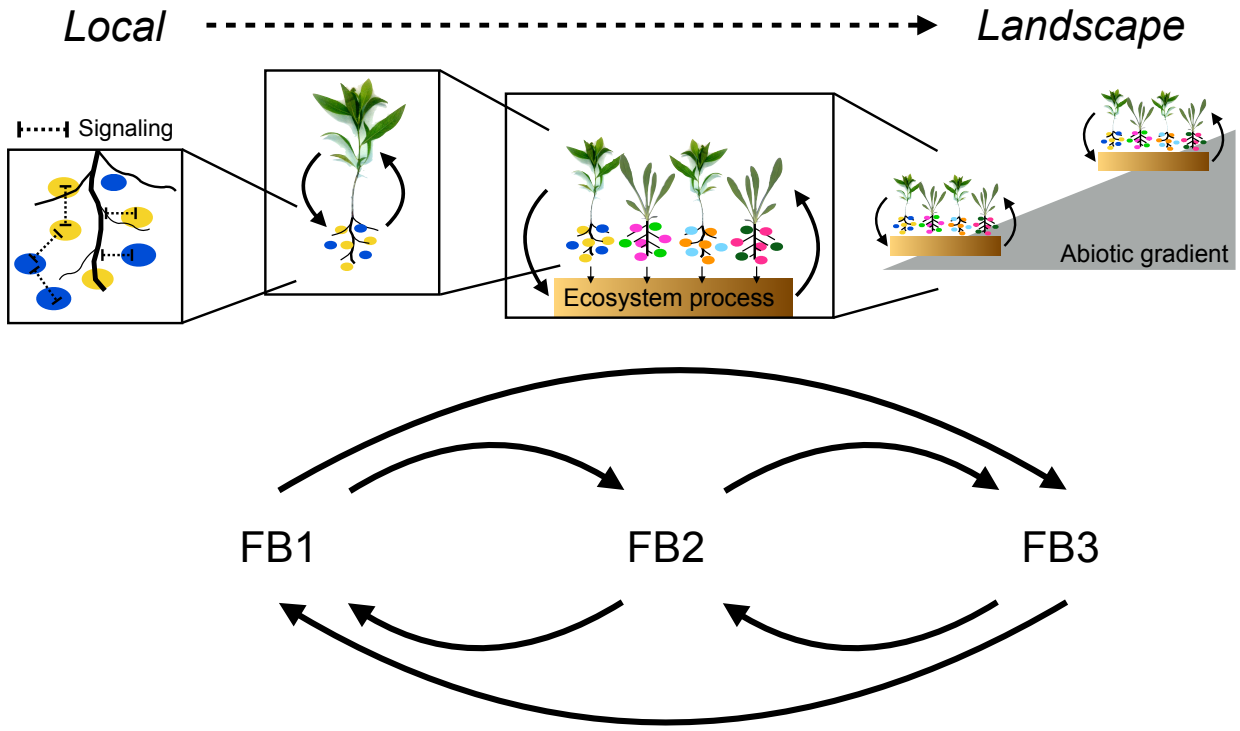

Figure 1.2. Research frontiers for eco-evolutionary dynamics of plant-soil feedback (PSF). Based on papers in this special feature, we emphasize a suite of integrated challenges spanning local to landscape scales that represent the frontiers of eco-evolutionary PSF research. First, a deeper examination of plant-microbe and microbe-microbe signaling pathways will improve our mechanistic understanding of how plants condition soil communities (colored ovals), the role of soil microbes in plant phenotypic expression, and how plant-microbe interactions change ecosystem processes that reciprocally influence plant traits. Second, as the scope and severity of global change increases, investigating how abiotic stress gradients impact plant genetic diversity and selection on functional traits, soil microbial community structure and function, and genetically based plant-microbe interactions that control ecosystem processes will offer novel insights towards population responses (e.g., adaptation, plasticity, and/or migration). Third, it is critical to study how feedbacks interact across scales, such as with models that investigate conditions where local, coevolutionary feedbacks (FB1) disrupt or enhance community- and ecosystem-level feedbacks (FB2 and FB3). Finally, a significant challenge remains in moving eco-evolutionary and plant-soil feedback experiments from highly controlled settings to field environments where we can begin to determine their effects in natural systems.

However, recent mathematical models indicate the possibility of an overarching link between heritable plant traits, selection, and plant-soil-microbial feedbacks (Kylafis & Loreau 2008; Jiang & DeAngelis 2013, Schweitzer et al. 2014). For instance, Schweitzer et al. (2014) used a spatially explicit individual-based model that incorporated genetically based plant-microbe interactions, belowground consequences of aboveground plant genetic variation, and abiotic selective pressures to examine the links between PSF and plant evolution. Their findings demonstrate that feedbacks from the ecosystem level drive phenotypic trait change as well as the evolution of the feedback itself through plant-soil conditioning. Moreover, both positive and negative feedbacks evolved along a gradient of gene flow, highlighting how processes at broader spatial scales influence local-scale feedbacks.

Highly controlled experiments unequivocally show the importance of PSF for structuring biodiversity and ecosystem processes (van der Putten et al. 2013), as well as the possibility of reciprocal dynamics between ecological and evolutionary processes (Post and Palkovacs 2009, Turcotte et al. 2013). The next great challenge will be testing real-world scenarios of where, when, and how such feedbacks occur in natural settings and dictate plant and soil responses to ongoing environmental change. To help in this effort, van der Putten et al. (2016) present a PSF triangle that balances symbiont, decomposer, and enemy contributions to net PSF values under human-induced global changes. In general, they indicate that climate change (i.e., CO_2, temperature, and precipitation changes) might lead to increasingly negative PSF based on strong enemy effects, although the proportional contribution of symbionts and decomposers would become more important under certain conditions. Beyond changing climates, land use transformation is one of the most dramatic and devastating ways that human activity affects Earth's systems by destroying or homogenizing habitat and reducing biodiversity both above- and belowground (Zuppinger-Dingley 2014; Veresoglou, Halley & Rillig 2015). Conversion of agricultural land back to semi-natural ecosystems continues and de la Peña et al. (2016) show how the legacy of soil changes due to plant-soil interactions impacts plant community assembly and could be considered to improve the success of restoration efforts.

Conclusions

Theoretical and empirical advances have revolutionized our view of how phenotypes are determined based on the causes and consequences of GxG interactions, whereby individuals make up the biotic environment of other individuals. In the field of PSF, this provides an opportunity to unify ecosystem ecology in an evolutionary framework at local and landscape scales. Such an effort is important as feedbacks from the ecosystem level can reinforce or disrupt genetically based species interactions that affect the strength of natural selection and the rate of evolutionary change. Examining the role of genetic interactions at the ecosystem level could begin a paradigm shift that places ecosystems within an evolutionary framework and evolution within an ecosystems framework, transforming our understanding of factors that affect the strength and direction of natural selection and population divergence. The papers in this special feature represent the frontier of PSF research, linking ecosystems and evolution like never before which will open many novel empirical and theoretical research directions for years to come.

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CHAPTER II
PLANT-SOIL FEEDBACKS CONTRIBUTE TO THE GENETIC DIVERGENCE OF *POPULUS* GROWTH TRAITS ACROSS A SOIL NITROGEN GRADIENT

Abstract

Variation in soil nitrogen (N) influences plant distributions, fitness, and functional traits, but there is little experimental evidence that phenotypic responses to soil N are adaptive. An evolutionary response to soil nutrient variation may impact plant traits that, in turn, alter soil N conditions. This could lead to large-scale nutrient-mediated feedbacks that change the quantitative genetic differences among populations, but few studies have simultaneously explored the scale at which soil nutrient gradients may influence plant evolutionary processes or how genetic variation then influences local soil processes. Using a combined approach of field observations, an experimental common garden, and a small local adaptation experiment with *Populus*, we hypothesized and found that: 1) a soil N gradient across the western US positively correlates with genetic divergence in plant growth traits (determined from field observations and experimental common garden); 2) high N populations are locally adapted to their soil communities across the N gradient (determined from local adaptation experiment); and 3) plants and soil communities from high N populations experimentally increase soil N more than plants and soil biota from low N populations (determined from experimental common garden). Collectively, this suggests that a positive nutrient feedback loop contributes to patterns of genetic divergence throughout the species' range and is reinforced by plant genetics and soil community function. These results expand our understanding of the role of soil nutrients in plant evolutionary processes and how ongoing anthropogenic N pollution could have unintended consequences for the genetic underpinnings of plant-soil linkages.

Introduction

The balance of elements in nature structures biological activity and interactions (Elser et al. 2000), such as how soil nitrogen (N) limits plant growth in many terrestrial ecosystems. As such, it is well documented that plant growth traits respond to N gradients with evidence from fertilization experiments (Tilman 1986, Silvertown et al. 2006), soil chronosequences (Vitousek et al. 2004, Wardle et al. 2004, Laliberté et al. 2013), and succession gradients (Zak et al. 1990). Much of this work has focused on the ecological links between plants and N, such as how plant biomass and nutrient content (often, but not always) positively respond to increasing N levels (Tilman 1986, LeBauer and Treseder 2008, Wooliver et al. 2016), or how nutrient availability affects plant performance and resource competition, thereby structuring communities (i.e., conifer-broadleaf coexistence; Frelich et al. 1993, Enright 2001, Ushio et al. 2017) and biogeochemical processes within an ecosystem (Hobbie 1992, 2015). However, there are few direct tests of soil nutrients as a selective force or if certain phenotypic responses to N are adaptive. This limits our ability to know whether the evolutionary consequences of soil N variation are as equally important to consider as their ecological implications.

Plant evolutionary responses to soil N gradients (genetic divergence or adaptation) could arise through nutrient-mediated feedbacks between above- and belowground systems. Plants modify soil nutrient pools through functional traits that have ecosystem consequences (Hobbie 1992, 2015); reciprocally, the same plant traits are shaped (in part) by the soil properties they change (Vitousek 2004). For instance, field observations suggest that soil N availability constrains productivity and the rate of *Populus grandidentata* biomass accumulation along an 87-year-old chronosequence (Pastor et al. 1984, White et al. 2004), and that the amount of available N depends on litter decomposition and N mineralization (Zak et al. 1989). A nutrient feedback might exist as higher plant biomass accumulation produces more leaf and root litter that

enters the soil food web providing greater resources for decomposition and mineralization, thus releasing more N available for plant uptake and biomass accumulation. Recent modeling work shows that nutrient cycling in plant-soil interactions can create evolutionary feedbacks where plant fitness is enhanced by improving their soil nutrient environment (Kylafis and Loreau 2008). Specifically, the model predicts that increasing the direct benefit of soil nutrient conditioning to plants leads to selection for higher values of soil conditioning traits (i.e., plants can adaptively regulate their local soil nutrient pool). Implicit in this model is a genetic link between plants and soils (genetic variation in a heritable soil conditioning trait being acted upon by a soil selective gradient), and a recent model showed how genetically based plant-soil feedbacks can also evolve along soil gradients (Schweitzer et al. 2014). Together, these models outline the possibility that nutrient feedbacks might contribute to plant evolution and vice versa, but little experimental work has been done to test this prediction in natural settings.

Over a decade of empirical work with *Populus* tree species provides a foundation for understanding how nutrient feedbacks may correspond to plant evolution on the landscape. Plant-soil feedback studies have shown how species- and genotype-level variation in soil conditioning influences both the survival and performance of *Populus* seedlings (Pregitzer et al. 2010; Smith et al. 2012). In addition, experimental work has found that elevated soil N increased growth and survival in *Populus* spp. (Walters and Reich 2000). Together, these studies point towards the possibility that soil nutrients may act as a selective gradient on *Populus* growth traits. Moreover, genetic variation in *Populus* growth and chemical phenotypes influences soil communities and the ecosystem processes that they mediate (Schweitzer et al. 2004, 2012; Lojewski et al. 2009). For instance, in experimental common gardens with clonal replicates, *Populus* genotypes have been shown to condition different soil microbial communities (Schweitzer et al. 2008a) and soil nutrient pools (Schweitzer et al. 2008b, 2012, Pregitzer et al. 2013). These conditioning effects may be widespread as genetic variation in *Populus* spp. is linked to belowground ecosystem processes at regional and landscape scales (Fischer et al. 2010, Madritch et al. 2014).

Building on the substantial body of work in *Populus spp.* (and other species) that have demonstrated important genetic links between plant functional traits and soil processes, we investigated the possibility that a nutrient-mediated feedback might contribute to plant phenotypic evolution. Specifically, we used a combination of field observations, a common garden greenhouse experiment, and a local adaptation experiment with populations across more than 80% of the total distribution of *Populus angustifolia* James in the field to test whether nutrient-mediated feedbacks can contribute to the genetic divergence of plant growth traits at the landscape scale (Fig. 2.1). We hypothesize that: (1) *P. angustifolia* populations have genetically diverged in growth traits, (2) consistent with patterns of a soil N selective gradient, a genetic cline in growth positively correlates with soil nutrients and populations are locally adapted to their soil communities across the N gradient, and (3) plants and soil communities from high versus low N populations condition greater soil nutrient levels. Support for these three related hypotheses would suggest that a landscape-scale nutrient feedback between *P. angustifolia* and its soil nutrient environment contributes to population genetic divergence across the western US.

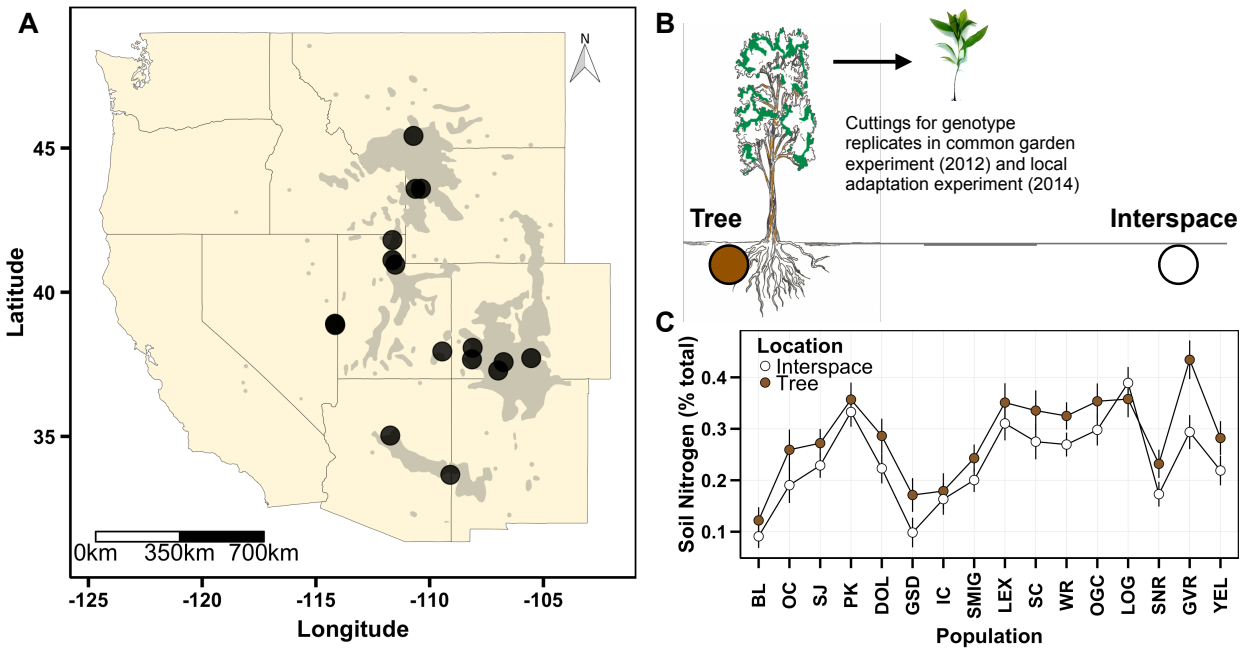

Figure 2.1. **Species range map of *Populus angustifolia* with locations of sampled populations, plant and soil sampling design, and the soil nitrogen (N) gradient**. (**A**) The sixteen populations sampled in this study across the western US. These populations span a range of approximately 8 °C mean annual temperature, 40 cm annual precipitation, and cover 980-2900 meters in elevation. (**B**) We collected cuttings from *P. angustifolia* genotypes in the field (verified with microsatellites; Ware et al. *in review*) for common garden and local adaptation experiments. In addition, we sampled soils in a pairwise design from beneath trees (Tree) and associated interspaces that were beyond the influence of tree conditioning (Interspace). This allowed us to test separate hypotheses related to soil gradient effects on plant traits (Interspace soils) versus plant-soil conditioning (Tree soils). (**C**) There is a large N gradient for tree and interspace soils, revealing more than a 3-fold difference in soil N between the lowest (BL) and highest (LOG) populations. Populations are arranged from lowest to highest latitude along the x-axis, and points represent population means ± 1 standard error.

Methods

Study species and sampling gradients

Populus angustifolia is a high elevation, dominant tree species in riparian ecosystems throughout the intermountain western United States (Little 1976; Braatne *et al*. 1996; Fig. 2.1a). Building on previous work with *Populus*, we identified and sampled plant and soil traits across sixteen river systems (hereafter 'populations') with naturally occurring riparian stands of *P. angustifolia* along a ~1,350 km latitudinal gradient from southern Arizona to southern Montana. These populations included: Blue River (BL) and Oak Creek (OC), AZ; Park Creek (PK), San Juan River (SJ), Dolores River (DOL), and San Miguel River (SMIG), CO; Lexington Creek (LEX) and Snake Creek (SC), NV; Indian Creek (IC), Ogden River (OGC), Logan River (LOG), and Weber River (WR), UT; Snake River (SNR) and Gros Vente River (GVR), WY; and Yellowstone River (YEL), MT. Each population was sampled along an elevation gradient (average ~450 m) to capture a wide range of within-population variation. Specifically, we identified and sampled the highest and lowest elevation sites that comprised the sixteen distributions, in addition to sampling 1-3 sites (separated by at least 50 m in elevation) that were located between the uppermost and lowermost sampling locations. We use individual river systems as distinct populations in our analyses because gene flow is largely restricted within drainages owing to the obligate riparian life history strategy of *P. angustifolia* (Braatne et al. 1996). Moreover, temperature and precipitation are important environmental gradients that vary widely across the distribution of *P. angustifolia* and have been shown to drive plant trait responses in many systems. We therefore also gathered mean annual temperature (°C, MAT) and annual precipitation (mm, AP) from the WorldClim database, using QGIS version 2.0.1-Dufour (QGIS Development Team), for field-marked GPS points (Oregon® 550t, Garmin, Germany) at each sampling location to quantify environmental conditions at each sampled site (Fig. 2.1a).

Plant collection and field growth measurements

To examine the role of genetic based functional traits in plant-soil interactions at the landscape level, we sampled ten mature *P. angustifolia* genotypes (verified with microsatellites; Ware et al. in review) from the 3-5 sites across each population's elevation gradient (n = 526; Fig. 2.1b). Because *P. angustifolia* reproduces sexually and vegetatively, genotypes were identified in the field using morphological indicators (Schweitzer et al. 2002) and by separating sampled trees over one tree-height distance away to avoid confounding clones as distinct genotypes (Everitt 1968; Rood et al. 1994). After taking these spatial precautions, adult trees were then haphazardly chosen and sampled at each site within populations by harvesting twenty branch-tip cuttings (~20 cm) collected from each genotype for planting in the common garden experiment.

Individual tree sizes were recorded by measuring their diameter at breast height (DBH) in the field. DBH measurements reflect a combination of tree age (Beschta et al. 2003) and growth in a given environment (Pliura et al. 2007). The smallest trees measured were <5 cm and the largest were >100 cm, meaning that sampled *P. angustifolia* trees in this study trees ranged from approximately 3 – 300 years old (Beschta et al. 2003). However, DBH is not a strict measure of tree age. For instance, DBH measurements of 10-year-old hybrid poplar clones across four clonal trial sites ranged from 4.5 to 33.8 cm, illustrating the importance of both genetic and environmental effects that influence this growth trait. Therefore, we refer to DBH as a *P. angustifolia* growth trait because it simultaneously represents (i) the total growth of trees over their lives (even if they are different ages), and (ii) the growth response to specific environmental conditions.

Soil collection and analysis

We examined plant-soil linkages for each genotype in all sixteen populations to identify whether: 1) genetically based growth responses in plants were correlated with soil gradients; 2) plant-soil conditioning in *P. angustifolia* was related to among population level differences in growth traits (Fig. 2.1b). For each plant genotype collected above, soil samples were collected from underneath the drip-line of the tree (hereafter referred to as "tree") and from a randomly sampled paired interspace location (~5 m from the base of the tree trunk) beyond the direct influence of trees (hereafter referred to as "interspace"). Sampling at these two locations allowed for a pairwise comparison to separate the effects of the tree conditioning from the natural drainage environment (i.e., the effect of tree conditioning on soils; Madritch *et al*. 2009). Because of the random nature of the paired interspace samples, this approach is a strong method for understanding the relationship between plants and soils as it allows us to compare soil properties when associated with tree litter, roots and exudates relative to areas not directly influenced by trees. Soil samples were collected with a 2 cm diameter Oakfield soil sampler to a vertical depth of 10 cm and stored in a cooler during transportation to the University of Tennessee where they were stored at 4°C before analysis. Each sample was sieved to 2 mm and a 2:1 slurry of soil to deionized water (10 g to 20 mL) was created for pH measurements (Denver Instruments, New York). A separate oven-dry soil subsample was measured for total percent soil carbon (C) and N using chromatography (FlashEA® 1112 Elemental Analyzer, Thermo Electron S.p.A, Italy). Measures of total soil N (both organic and inorganic forms) in this study are likely accurate representations of the complete N spectrum available since *P. angustifolia* roots associate with both ecto- and arbuscular mycorrhizae (Pregitzer and Friend 1996), and increasing evidence suggests plants use diverse N attainment strategies to create unique plant-litter-soil feedbacks (Chapman *et al*. 2006; Ke *et al*. 2015).

We collected soils in June 2014 from a subset of *P. angustifolia* populations across the N gradient examine differences in soil microbial communities and how they may relate to plant and soil responses to the local adaptation experiment (see below). Specifically, five genotypes were sampled from each of the BL, SJ, SNR, and LOG populations. Soils were collected directly from the rhizosphere of genotypes in the field; as a result, microbial diversity should directly reflect conditioning and we did not collect a paired unconditioned soil away from the tree. Soils were stored in a cooler during transport to the University of Tennessee, where they were subsequently stored at -40 °C before processing. We extracted DNA using MoBio PowerSoil DNA Isolation kits (MoBio Laboratories, Carlsbad, CA, USA). Please see *Amplicon sequencing and bioinformatic processing* in Chapter IV Supplementary Material for a full description of sequencing and workflow steps. Briefly, sequencing was carried out on a MiSeq Desktop Sequencer (Illumina Inc, San Diego, CA) running in paired end 2x250 mode. We processed 16S amplicon data using akutils (https://github.com/alk224/akutils-v1.2) that includes modifications to a QIIME 1.9.1 workflow because default QIIME settings can significantly overestimate microbial diversity (Krohn et al. 2016).

Common garden experiment

We created a common garden greenhouse experiment using replicate genotypes to measure genetic variation and test whether populations have genetically diverged throughout the *P. angustifolia* range (Conner and Hartl 2004, Dutkowski and Potts 2012; Blanquart *et al*. 2013). Tree cuttings collected in the field were transported in a cooler to the Northern Arizona

University greenhouse where each cutting was scored at the base with pruning shears, treated with a rooting hormone growth regulator (indole-3-butyric acid [IBA]; Hormodin® 2, OHP Inc., Pennsylvania), and potted in general potting mix. After three months, individual cuttings were transferred to 6.4 x 36 cm Deepots™ (D60, Stuewe and Sons Inc, Oregon) and randomized in the common greenhouse environment, allowing further root growth and reduced competition. After 12 months, the cuttings were transported to the University of Tennessee greenhouse.

Trees in the common garden were surveyed four times from 2013-2016 to examine an alternative interpretation of genetic divergence versus phenotypic plasticity and maternal effects. We measured the diameter of annual growth on cuttings in the common garden because cuttings taken from trees in the field were initially different sizes and we sought to avoid confounding the effect of initial size differences as genetic differences in growth. Importantly, we found no relationship between basal stem diameter traits and annual growth diameter (see Supplementary Material; Fig. S1 and Fig. S2), indicating that any differences in annual growth diameter among populations in the common garden reflect genetic differences that are not influenced by the initial size of cuttings collected from the field (i.e., maternal effects; see Supplementary Material *Analysis of maternal effects* for further information). This is consistent with previous work that has found little evidence for maternal effects on *P. angustifolia* growth traits (Holeski et al. 2013).

The diameter of annual growth for trees in the greenhouse was measured for four randomly selected individuals of each surviving genotype in May 2013 (n = 1,353) and May 2014 (n = 1,050). A representative subset of seven populations (BL, OC, OGC, SJ, SMIG, SNR, and WR) were measured in May 2015 (n = 690), and 5-7 replicate genotypes from each population were measured again in June 2016 (n = 375). We acknowledge that tree DBH in the field and annual growth diameter of trees in the common garden are not the same trait and, as a result, may be linked but still evolve independently from one another. However, since both traits offer some measure of *P. angustifolia* growth, we examined whether field traits (DBH) were correlated with common garden traits (annual growth diameter). We created a restricted estimated maximum likelihood (REML) model with DBH, year, and DBH x year as fixed effect, annual growth diameter as the response variable, and genotype nested within population and population as random effects. We find that DBH predicts annual growth diameter consistently across 2013-2016 measurement time points (Fig. S3), indicating these two traits are relatable for the purposes of understanding how growth traits respond to and influence soil environments in this study.

Local adaptation experiment

Based on our prediction that soil N is related to genetic variation in *P. angustifolia* growth, we established a reciprocal transplant experiment to test whether populations are locally adapted to their soil communities. We sampled plants from three populations (SNR, SJ, and LOG) and soils from four populations (BL, SNR, SJ, and LOG) that span a range of soil nutrient levels across the species range (approximately 0.1 – 0.4 % mean total soil N; Fig. 2.1c). Cuttings and soils were recollected from 10 previously sampled mature *P. angustifolia* genotypes within each of the populations in June 2014 using the same collection and storage procedures as 2012 samples. Cuttings were allowed to establish and grow in standard potting mix for four months before they were transferred to 350 ml pots (Tinus rootrainers, Stuewe and Sons, Inc, Oregon) to receive soil treatments in October 2014. Plant mortality during the establishment and growth phase resulted in a total sample size of 99 plants: SNR (n = 74), SJ (n = 11), and LOG (n = 14).

Soils for the experimental treatments were pooled at the population level by combining samples from the 10 genotypes and applied as inoculum to cuttings in a 3:1 ratio of standard potting mix to field soil. Cuttings were grown in a reciprocal design with soil inoculum from their "home" population and with inoculum from the three other "away" populations from October 2014 to November 2015. This resulted in replicate genotypes from each population being grown across a soil biotic gradient corresponding to the natural variation in soil nutrients throughout the *P. angustifolia* range. We hereafter refer to "inoculum source" as a way to reflect soil community variation and its influence on plant and soil responses in the experiment. Plant height and diameter traits were measured at the beginning and throughout the experiment. We used an allometric equation that incorporates these traits and explains more than 90% variation in biomass of 12 to 36-month-old *P. angustifolia* cuttings (Biomass = [0.005 * plant volume] – 0.11) to calculate the initial biomass of cuttings in the experiment (Van Nuland et al. *in review*). At the end of the experiment, aboveground biomass was measured after shoots were clipped at the soil surface and dried for 48 hrs at 74° C; additionally, soil N (%) was measured in a subset of soils (total n = 75; SNR = 52, SJ = 10, LOG = 13) using the same procedure as described above to experimentally assess plant-soil nutrient conditioning (see below). We calculated plant growth as the difference between final versus initial aboveground biomass. Because cuttings were sourced from populations that vary in soil N, larger growth in "home" versus "away" inoculum from across the N gradient would be consistent with patterns of local adaptation to soil communities that might contribute to population genetic divergence in *P. angustifolia* growth traits.

Statistical Analyses

Plant growth divergence

To test whether *P. angustifolia* populations have genetically diverged in growth traits (hypothesis 1) we examined whether the size of growth traits in the field and common garden differs among populations. To test whether plant growth in the field (DBH) varies among populations, we constructed a generalized linear model (GLM) with population as a fixed effect and DBH as the response variable. For common garden measurements of plant growth (annual growth diameter) that includes genotype replicates and four years of measurements (2013-2016), we constructed a restricted estimate maximum likelihood (REML) model with population and measurement year as fixed effects, genotype nested within population as a random effect (Read et al. 2016), and annual growth diameter as the response. We do not include the interaction of population x measurement year in the common garden model since not all populations received the same sampling effort across years. Additionally, we created similar REMLs for each measurement year to gather estimates of broad sense heritability of annual growth diameter (the percent variation explained by genotype nested within population in each respective model). For all common garden models, we use Satterthwaite approximation of degrees of freedom. Residuals of log-transformed traits were tested for and met assumptions of homoscedasticity using Levene's Test. We interpret consistent patterns between field and common garden traits as evidence of genetic divergence and support for hypothesis 1 (Dutkowski and Potts 2012; Blanquart *et al*. 2013).

Plant growth responses to soil nutrient and microbial gradients

We tested whether soil N acts as a selective gradient on plant growth and populations are locally adapted to their soil communities (hypothesis 2) using model selection to compare environmental

factors to genetic variation and a reciprocal transplant greenhouse experiment. First, we examined whether selection might impact genetic variation in *P. angustifolia* growth through backward model selection as a means to identify the most important environmental factors that predict 2013 measurements of annual growth diameter. We limit our model selection analysis to 2013 measurements because all populations received equal sampling effort and the most replicate genotypes were measured at this time point (449 total replicate genotypes sampled in 2013 compared to 376 genotypes sampled in 2014, 161 genotypes sampled in 2015, and 214 genotypes sampled in 2016). This, in combination with little evidence of maternal effects (see Supplementary Material) means that 2013 average growth values for populations in the common garden are more likely to reflect the true quantitative genetic variation of this trait in *P. angustifolia.* Using replicated genotypes in the common garden, environmental factors that predict quantitative genetic variation reflect a correlation between environmental gradients and a genetic cline. Environment-genetic cline relationships are strongly indicative of selective gradients that might lead to genetic divergence among populations (Primack and Kang 1989). Interspace soil factors (C, N, and pH), MAT, AP, elevation, and latitude were incorporated in backward model selection where minimum AIC scores were used to identify the most parsimonious set of predictors accounting for genetic variation in annual growth diameter in the common garden. We then used multiple regression analysis to test how multiple factors retained by model selection relate to patterns of growth differences among populations. If only one factor was retained in the model, we used GLM with annual growth diameter as the response, the environmental factor, measurement year, and their interaction as fixed effects. We predict that interspace soil N in the field is positively related to annual growth diameter in the common garden, which would suggest that soil nutrients may be acting as a selective gradient on *P. angustifolia* growth (support for hypothesis 2).

We examined how soil prokaryotic composition and the relative abundance of dominant and rare phyla vary across the N gradient to (1) verify that soil biota differed among inoculum treatments based on the underlying N gradient, and (2) compare how differences in population performance in the experiment may or may not correspond to differences in soil community diversity. We created a GLM with inoculum source as a fixed effect and the first axis from an unweighted Unifrac principal component analysis (PCA) as the response. Similarly, we created a GLM with inoculum source as the fixed effect and the relative abundance of archaea and bacteria phyla as the response. We then tested whether *P. angustifolia* populations are locally adapted to their respective soil communities by comparing how their growth responses differed across soil inoculum treatments that were sourced from the N gradient in the field. Specifically, we created a REML model with population, inoculum source (% soil N), and population X inoculum source as fixed effects, genotype as a random effect, and aboveground biomass growth as the response variable. If adaptation to soil biota helps shape genetic differences in growth among *P. angustifolia* populations, then we expect populations to vary in their response to soil N treatments (e.g., a significant population X inoculum source interaction). Specifically, populations performing best when matched with their respective soil inoculum sources relative to other inoculum treatments would suggest that populations are locally adapted to their soil communities across the N gradient (additional support for hypothesis 2). We used mixed effects models to examine each population's response to the inoculum source treatments with aboveground biomass growth as the response, inoculum source as a fixed effect, and genotype as a random effect.

Plant-soil nutrient conditioning

We tested whether *P. angustifolia* populations and growth traits under selection were positively related to soil nutrient conditioning in two ways (hypothesis 3). First, we analyzed plant-soil conditioning in the field as the difference between *P. angustifolia* tree soil environments relative to the paired interspace soil (Madritch *et al*. 2009). We used a two-sample t-test approach to quantify the effect of plant-soil conditioning as the difference between tree and interspace soil N. Higher N levels beneath trees compared to interspace locations would be consistent with plant-soil conditioning (trees changing their soil nutrient pools relative to the surrounding soil environment; Zinke 1962), but could also reflect tree spatial arrangements that are determined by local resource heterogeneity (environmental filtering; Tilman 1982). As a result, we tested the alternative hypothesis that spatial differences between tree and interspace soil traits might be observed due to environmental filtering rather than plant-soil conditioning by randomly shuffling tree-interspace pairs and testing whether our results are different from this null distribution (i.e., results that would be consistent with plant-soil conditioning rather than filtering; see Supplementary Material).

Second, to experimentally test plant-soil conditioning, we examined whether soil N levels were different in pots at the end of the local adaptation due to plant populations, soil communities (i.e., soil inoculum source), or the interaction of plants populations and soil communities. We created a REML with soil N as the response, plant population, inoculum source, and population X inoculum source as fixed effects, and genotype nested within population as a random effect. Results that plants from higher N populations have greater amounts of soil nutrients relative to lower N populations would support our hypothesis that genetic divergence along the N gradient could be reinforced by population level differences in soil nutrient conditioning. All analyses were conducted in R version 3.3.1 (R Core Development Team 2013).

Results

Plant growth divergence

Consistent patterns between field observations (DBH) and common garden measurements (annual growth diameter) indicate genetic divergence in growth among *P. angustifolia* populations. Observational results show that plant growth in the field differs among populations ($F_{15,510} = 4.3$, $p < 0.001$; Fig. 2.2a), with the general trend that DBH increases from the southern to northern populations. Having established that some aspect of *P. angustifolia* growth varies geographically and that maternal effects have little impact on annual growth diameter (see Supplementary Material; Fig. S1 and S2), we tested whether underlying genetic differences among populations could be influencing the observed variation in growth. Using a conservative analysis of genetic variation whereby replicate genotypes were nested within population, plant growth in the common garden significantly differed among populations and follows the same trend as plant diameter in the field (Fig. 2.2b).

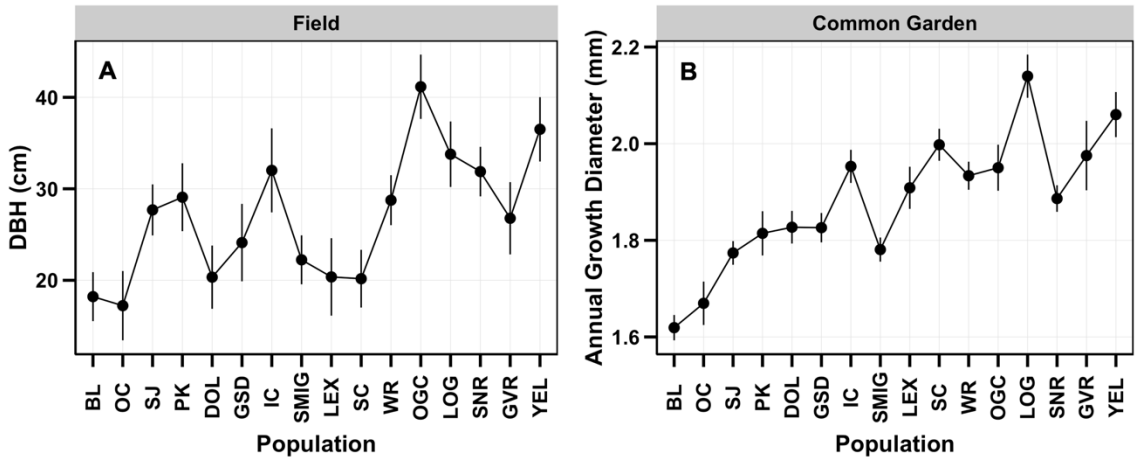

Figure 2.2. Comparisons of field and common garden patterns show genetic divergence in *P. angustifolia* growth traits. (**A**) DBH varies among *P. angustifolia* populations in the field, reflecting a combination of genetic and environmental effects on phenotypic expression. (**B**) By isolating genetic effects using replicate genotypes in the common garden, we find evidence that supports genetic divergence as annual growth diameter varies among populations. Moreover, there are consistent patterns of DBH and annual growth diameter variation, such that plant growth generally trends upwards from southern to northern populations. Populations are arranged from lowest to highest latitude along the x-axis. Points represent population means (averages of all four measurement years for annual growth diameter) ± 1 standard error.

The diameter of annual growth for trees in the common garden differed among populations ($F_{15,3119} = 14.6$, $p < 0.001$), again with the general trend that growth increases from southern to northern populations. In addition, the average annual growth diameter was different across measurement years in the pattern of 2014 > 2015 > 2016 > 2013 ($F_{3,3119} = 440.4$, $p < 0.001$). When analyzed by each year individually, populations significantly differed in growth every year except 2016 (2013: $p < 0.001$; 2014: $p < 0.001$; 2015: $p = 0.001$; 2016: $p = 0.3$). In addition, we found that annual growth diameter exhibits an average $H^2_B = 14.2\% \pm 2.8$ (range = 7.3 - 20.4%) across all measurement years. Overall, these common garden results are consistent with field observations illustrating a genetic cline where *P. angustifolia* populations have diverged in growth from south to north.

Plant growth responses to soil nutrient and microbial gradients

The *P. angustifolia* genetic cline revealed by the common garden is associated with a soil nutrient gradient in the field. Following backward model selection, interspace soil N from field locations was the most important predictor of population level genetic variation in plant diameter (Supplementary Table 1). Specifically, interspace soil N positively predicted annual growth diameter overall ($F_{1,54} = 7.3$, $p = 0.01$), and consistently across four years of measurement (interaction of interspace soil N x year: $F_{3,54} = 50.4$, $p < 0.001$; Fig. 2.3). Although growth traits generally increased from south to north, latitude was not retained in the model as an important predictor of the genetic cline, suggesting that the 3.2-fold total difference of interspace soil N across populations (0.09 - 0.38% mean interspace soil N) may be a stronger (or more specific) selective force on these growth traits. Additional soil factors (C and pH), climate (AP and MAT), or unspecified environmental gradients (elevation) were not retained under model selection, indicating these factors did not explain significant levels of genetic variation and may not be exerting strong selective pressures on *P. angustifolia* growth. The relationship between quantitative genetic variation in plant diameter and soil nutrients suggests that selection from soil N may play some role in the observed genetic divergence of *P. angustifolia* growth.

By sequencing 16S amplicons from field-collected soils that were used as the experimental inoculum in the local adaptation study, we find that soil community composition (Fig. 2.4a) and the relative abundance of certain dominant (Fig. 2.4b) and rare (Fig. 2.4c) prokaryotic phyla vary across soil inoculum source N gradient. The first axis of the unweighted Unifrac PCA analysis (explaining 27% variation in community composition) and the relative abundance of *Acidobacteria* ($r^2 = 0.39$, $p < 0.01$), *Plantomycetes* ($r^2 = 0.21$, $p = 0.04$), *Proteobacteria* ($r^2 = 0.25$, $p = 0.03$), *BRC1* ($r^2 = 0.28$, $p = 0.02$), *Chloroflexi* ($r^2 = 0.46$, $p = 0.001$), and *Nitrospirae* ($r^2 = 0.23$, $p = 0.03$) all declined with inoculum source N levels. In contrast, *Crenarchaeota* (Archaea) ($r^2 = 0.29$, $p = 0.02$), *Bacteroidetes* ($r^2 = 0.35$, $p = 0.03$), and *Elusimicrobia* ($r^2 = 0.15$, $p = 0.1$) showed modest increases with inoculum sourced from higher N populations.

Populations from different N environments vary in their growth responses to experimental soil inoculum treatments sourced from across the N gradient; a pattern consistent with local adaptation to soil communities.

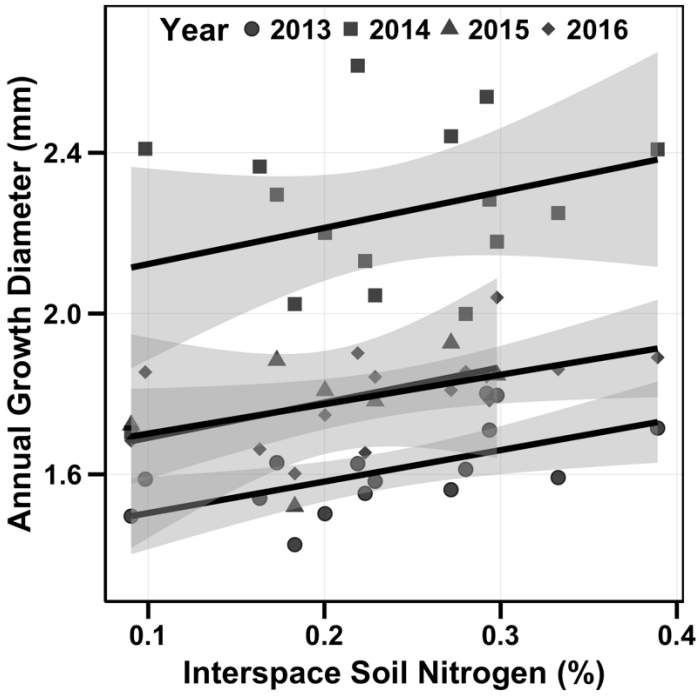

Figure 2.3. Interspace soil nitrogen (N) correlates with genetic variation in plant growth. Interspace soil N from field samples was the main factor retained under backward model selection and positively predicts genetic variation *P. angustifolia* growth (measured by the diameter of annual growth across four years in the experimental common garden). Even though annual growth diameter differed between measurement years, interspace soil N was positively related to growth in the common garden for each year. Symbols depict different years for each mean population value, and shaded areas represent 95% confidence intervals.

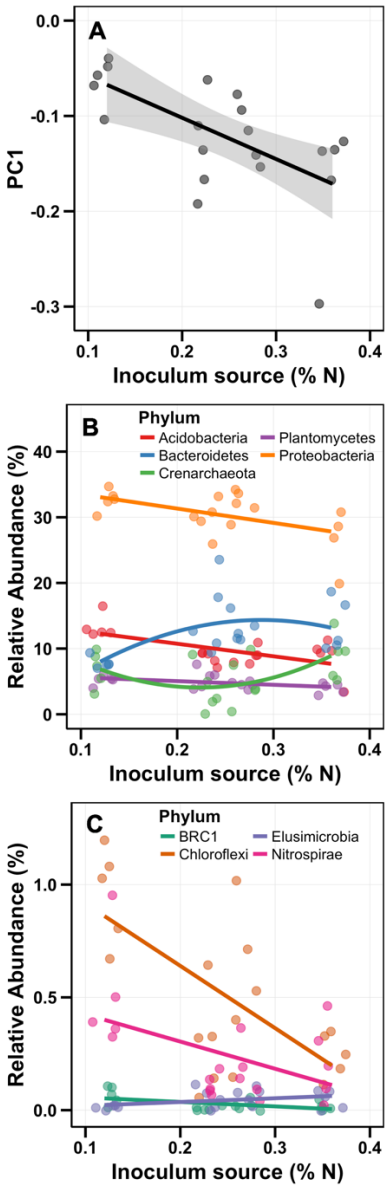

Figure 2.4. Soil microbial communities vary across experimental soil inoculum treatments that were sourced from across the nitrogen (N) gradient and applied in the local adaptation study. (**A**) The first axis of the unweighted Unifrac principal component analysis (PCA1) negative corresponds with increasing soil inoculum source from the N gradient, indicating an overall shift in community composition with soil nutrients in the field. (**B**) For dominant phyla, the relative abundance of *Acidobacteria*, *Plantomycetes*, and *Proteobacteria* gradually declines from low to high N inoculum sources. In contrast, *Bacteroidetes*, and *Crenarchaeota* (Archaea) have greater relative abundance in higher N inoculum sources. (**C**) For rare phyla, the relative abundance of *BRC1*, *Chloroflexi*, and *Nitrospirae* decline from low to high N inoculum sources; *Elusimicrobia* gradually increased at higher versus lower N inoculum sources.

Plants from lower nutrient populations had little or no response to soil inoculum source compared to plants from higher nutrient populations, shown by the significant interaction between population and soil inoculum source ($F_{3,96} = 3.3$, $p = 0.03$). Genotypes from the SNR population did not grow larger or smaller with different soil inoculum that was sourced from across the N gradient ($r^2 = 0.003$, $p = 0.7$; Fig. 2.5a). In contrast, genotypes from the SJ population showed a marginally positive response to soil inoculum source ($r^2 = 0.31$; $p = 0.08$; Fig 2.5b), and genotypes from the LOG population ($r^2 = 0.54$; $p = 0.003$; Fig. 2.5c) showed the most positive growth response to soil inoculum source (i.e., highest growth when LOG plants were matched with LOG soil communities). Additionally, we did not detect a relationship between *P. anugstifolia* growth and the quantity of soil N in each sample (see Supplementary Material; $F_{1,55} = 0.6$, $p = 0.4$), suggesting that inoculum source effects result from soil community differences as opposed to soil nutrient differences.

Plant-soil nutrient conditioning

The combination of field patterns and experimental results indicate that plant-soil nutrient conditioning occurs and is greater with plants and soil communities from higher versus lower N populations (Fig. 2.5d-f). Pairwise comparisons of tree and interspace soil N in the field revealed a significant effect of trees on belowground nutrients (see Supplementary Fig. S4), with tree soils (mean = 0.26% ± 0.01 SE) containing 21% greater total N than the adjacent interspaces ($t_{1,1069} = -5.2$, $p < 0.001$). Importantly, we found no evidence to support an alternative interpretation of environmental filtering (see Supplementary Material *Analysis of environmental filtering*; Fig. S5 and S6), suggesting that the difference between soil locations relates to plant-soil nutrient conditioning. In the local adaptation study, we found soil N at the conclusion of the experiment was affected by plant populations ($F_{2,75} = 3.0$, $p = 0.06$) and soil inoculum sources ($F_{1,75} = 17.2$, $p < 0.001$), but not the interaction of population x inoculum source ($F_{1,75} = 0.4$, $p = 0.7$). Specifically, LOG plants from the highest N population (mean soil N = 0.52 ± 0.03 SE) had 19% and 14% more soil N at the end of the experiment than those from lower N populations (SNR and SJ, respectively; Fig. 2.5f), and inoculum source (increasing along the N gradient from the field) positively predicted greater levels of soil N ($r^2 = 0.26$). The highest N levels recorded at the end of the experiment were from LOG plants matched with LOG soil inoculum (mean = 0.59 ± 0.03 SE), and the lowest N amounts were found when SNR and SJ plants were grown with SNR soil inoculum (mean = 0.39 ± 0.2 SE and 0.37 ± 0.05, respectively). Overall, these results suggest that *P. angustifolia* trees condition soil nutrient environments across the western US, with plants and soil communities from higher N populations leading to greater amounts of soil N.

Discussion

Taking a combined approach with field observations, four years of experimental common garden data, and reciprocal transplants across soil communities, we provide evidence that is consistent with a genetically based plant-soil nutrient feedback. Our results are among the first to illustrate: 1) population level genetic divergence in response to variation in the soil microbiome associated with nutrient gradients; and 2) population level differences in soil nutrient conditioning that reinforce a widespread soil N selective gradient. Specifically, these results expand previous work and suggest that soil selective agents may not be limited to unusual geologies with steep ecological gradients (e.g., serpentine soils or heavy metal polluted sites) or relatively small scales, but might be widespread in common soil gradients at large scales.

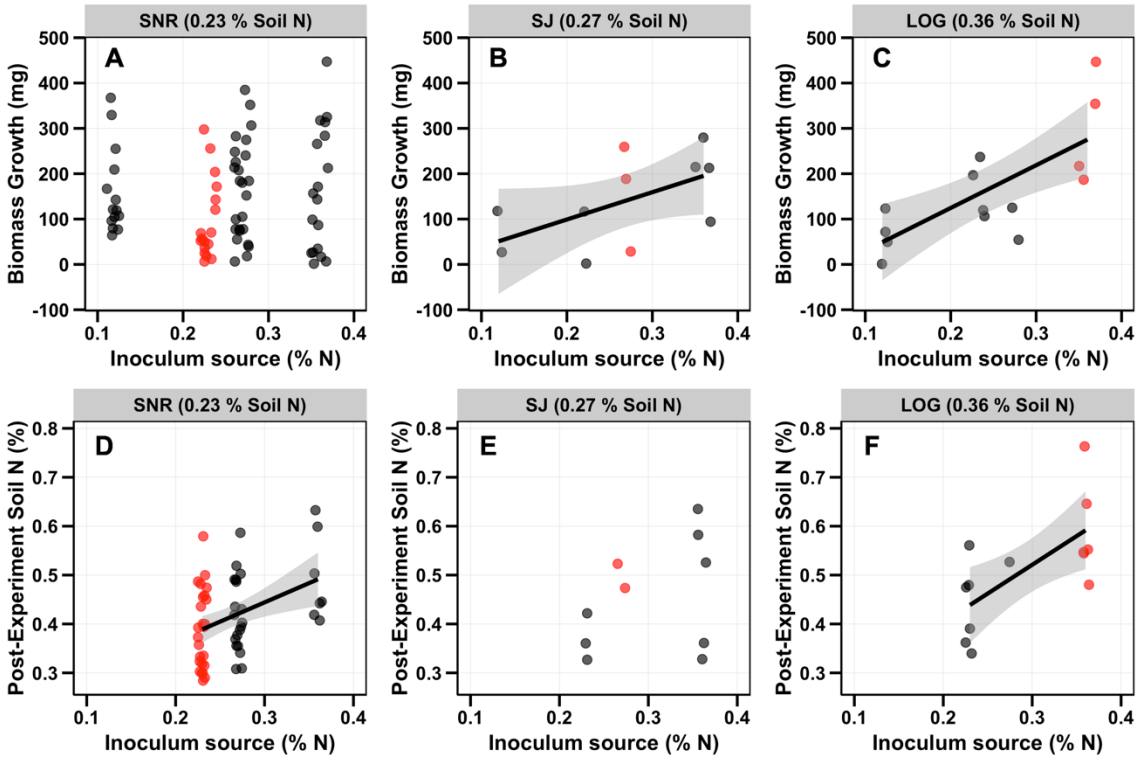

Figure 2.5. Populations vary in growth responses and condition different soil nutrient levels with experimental soil inoculum that was sourced from across the nitrogen (N) gradient. (**A**) Plants from the SNR population, coming from relatively low N levels in the field (0.23% N), showed no biomass growth response to variation in soil inoculum, even when SNR plants were grown with SNR soil inoculum. (**B**) SJ plants (0.27% N) showed a marginally positive response to soil inoculum sourced from increasing N populations. (**C**) LOG plants (0.36% N) had the largest growth response to increasing soil N inoculum sources and had the highest growth with LOG soil inoculum. Biomass growth was measured as the difference between final and initial aboveground biomass. (**D**) Increasing soil inoculum source led to greater soil nutrient levels at the end of the experiment with SNR (low N) plants. (**E**) There was no relationship between soil inoculum source and post-experiment soil N with SJ (med N) plants. (**F**) Increasing soil inoculum source positively predicted greater soil N levels at the end of the experiment with LOG (high N) plants. In addition, plants from the highest N field levels (LOG) consistently had greater soil N at the end of the experiment than plants from lower N levels (SJ and SNR), regardless of soil inoculum source. Shaded areas represent 95% confidence intervals, red points indicate treatments where plant populations were matched with their "home" soil communities.

In addition, we find that plant populations and soil communities may work together to maintain the soil fertility selection gradient, thereby creating a feedback loop between ecological processes (plant-soil interactions that have ecosystem consequences) and evolutionary dynamics (genetic divergence and adaptation). Together, our study highlights the reciprocal nature of evolution and ecosystem ecology as revealed by investigating intraspecific plant-soil linkages across the *P. angustifolia* species range.

Evolutionary divergence and adaptation of *P. angustifolia* growth is associated with gradients of soil nutrients and microbial communities. Much work has focused on the ecological response of *Populus* to soil N or clonal variation in abiotic stress tolerance, and our study shows that the relationship between soil nutrients and biotic communities likely contributes to evolutionary changes in *P. angustifolia* growth. Not surprisingly, most studies find that *Populus* growth and survival respond positively to higher N (Walters and Reich 2000), including studies where *P. tremuloides* aboveground biomass increased 62% (King et al. 1999) and 272% (Zak et al. 2000) between low and high soil N availability treatments. Our results show that genetic variation in annual growth diameter positively correlates with soil N from the field, suggesting that soil nutrient selective pressures act on growth and may ultimately contribute to the quantitative genetic differences observed among populations in this study (Primack and Kang 1989). If *P. angustifolia* populations in this study are N-limited (LeBauer and Treseder 2008), then belowground communities might also be important mediators of plant adaptation to a soil N selective gradient (Johnson et al. 2010). However, we find that only the highest N population (LOG) performs best when matched with their high N soil communities relative to other communities across the N gradient. This is surprising because lower N populations might be expected to adapt to greater soil N selective pressures by forming tight co-evolutionary feedbacks with their soil community, similar to what was found when arbusucular mycorrhizae improved the heavy metal stress tolerance of *Populus alba* grown with copper and zinc polluted soils (Cicatelli et al 2010). One possible explanation for why adaptation was not consistent among the three populations tested in the reciprocal transplant is that inoculated soil communities were grown with plants in standard potting mix that had ~60% higher soil N levels than natural field conditions. Although soil N levels in the experiment were unrelated to plant growth responses, this difference may have altered how soil communities interacted with plants in unexpected ways. Further work that incorporates more plant-soil community reciprocal transplants and characterizes the activity of belowground organisms is key for identifying where the influence of soil N selection and adaptation to soil communities are mismatched on the landscape.

P. angustifolia populations and soil communities reinforce the landscape-level N gradient by conditioning different soil nutrient levels. We find that, in general, trees have more N in the soil directly beneath them compared to their surrounding environment, and that this is more likely caused by plant-soil conditioning as opposed to a strong environmental filter where trees only survive and grow in relatively high soil nutrient locations. Through our local adaptation experiment, we find that plants from the highest N population have greater soil N levels at the end of the experiment compared to other populations and regardless of which soil community they were grown with. Although we cannot determine whether this is caused by greater N inputs or lower N uptake, this demonstrates a genetic basis by which *P. angustifolia* trees alter soil nutrient environments. The underlying mechanism that describes how tree growth impacts soil N likely comes from the relationship between organic matter input and soil nutrients cycling (Bardgett 2005; Bardgett and Wardle 2010). Larger trees produce more biomass and greater

quantities of organic matter that are delivered to the soil (through litterfall, root turnover, and root exudates), providing enhanced resource levels for decomposition and N mineralization by soil organisms and thus increasing soil N concentrations (Aber et al. 1989, 1998). This is supported by evidence that *Populus* biomass accumulation in an 87-year-old chronosequence is most likely limited by the amount of soil N made available from litter decomposition and N mineralization (White et al. 2004), implying that large-scale feedback mechanisms can exist between tree growth and soil N conditioning (Hobbie 1992, Vitousek et al. 2004). In addition, patterns of dominant and rare prokaryotic groups declining in relative abundance with increasing N are generally consistent with global summaries of soil microbial responses to N fertilization (Ramirez et al. 2010, Ramirez et al. 2012) and might be caused by phylogenetic differences in growth strategies (e.g., copiotrophic versus heterotrophic; Leff et al. 2015). Although the effect of plant genetic variation on belowground processes and communities has previously been shown in *Populus* (Whitham *et al*. 2003; Schweitzer *et al*. 2004, 2008a, 2008b, 2011, 2012; Madritch *et al*. 2009; Madritch and Lindroth 2011; Pregitzer *et al*. 2013), the scale at which the genetic effect of plant-soil conditioning occurred remained relatively unknown (Madritch *et al*. 2014).

Ecological interactions are central to evolution in natural systems, while at the same time evolution and selection impact the ecology of organisms (Thompson 2005, 2013; Wade 2007). Understanding the extent of reciprocal interactions between the ecology of populations, communities, and ecosystems, and evolutionary dynamics is an important frontier in evolutionary ecology (Schoener 2011), yet little evidence exists beyond tightly controlled experiments due to the difficulty of teasing apart ecological and evolutionary effects that occur simultaneously (Yoshida et al. 2003, Reznick 2013; Turcotte *et al*. 2013). However, recent modeling and empirical approaches indicate that plant-soil feedbacks may commonly result in eco-evolutionary interactions (Kylafis and Loreau 2008, terHorst et al. 2014, Van Nuland et al. 2016), allowing for greater potential to experimentally separate and combine different ecological interactions (plant-soil conditioning) with evolutionary processes (soil selective agents). Using data from *Populus* as a foundation, it has also been shown that plant-soil feedbacks can evolve if soil conditioning leads to trait divergence, and the response to soil conditioning affects phenotypic selection (Schweitzer *et al*. 2014). Our results are compatible with these model predictions as we find *P. angustifolia* trees consistently condition their soils across environmental gradients, relative to adjacent interspaces, and this conditioning is influenced by populations that have evolved in response to selection by soil N gradients. Importantly, our work also parallels evidence of a positive plant-soil-microbe feedback in the Hawaiian *M. polymorpha* system, whereby soil fertility determines plant functional traits that impact subsequent nutrient cycling and reinforces the underlying soil nutrient gradient (Vitousek 2004). Thus, although experimental evidence is rare for plant-soil linkages, reciprocal ecological and evolutionary interactions in natural systems may have widespread consequences for understanding evolutionary processes and the linkage between genes and ecosystems (Pelletier *et al*. 2009; Post and Palkovacs 2009; Reznick 2013; Bailey *et al*. 2014; Schweitzer *et al*. 2014).

In summary, these field, common garden, and experimental data represent an empirical case demonstrating the reciprocal interactions of soil selective agents and genetically based plant-soil conditioning. This work is congruent with experimental and model-based results indicating that soil gradients can influence the genetic divergence of traits that condition soils and lead to positive plant-soil-nutrient feedbacks (Treseder and Vitousek 2001, Vitousek 2004, Schweitzer *et al*. 2014). Our study also has implications related to the economic and agricultural

importance of *Populus* for identifying and selecting genotypes with the most positive nutrient feedback loops, greatest performance boosts from specific soil biotic additions, and that adapt quickest to ongoing N pollution or progressive N limitation under elevated CO_2 conditions. Overall, our results show that interactions between soil selective gradients and plant-soil conditioning may be widespread throughout natural systems, significantly advancing the scale and scope linking ecological to evolutionary processes.

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CHAPTER III
INTRASPECIFIC *POPULUS ANGUSTIFOLIA* FUNCTIONAL TRAIT VARIATION PREDICTS SPECIES CLIMATE AND SOIL RANGE LIMITS

Abstract

Soil gradients can influence the spatial distribution of plants at large scales, yet soil data is rarely included in ecological niche models that predict suitable habitats for species and identify important abiotic factors in the model. Moreover, few niche models are designed to test mechanisms for how environmental values past a certain point lead to population declines (i.e., how environments determine range limits). Here, plant functional traits are an important link between inherently correlational niche models and a mechanistic understanding of range limits. This is because traits related to the growth and fitness of plants are formed, in part, by abiotic environments that are used to parameterize niche models. The expression of functional trait variation at regional and global scales, therefore, reflects the underlying physical constraints applied by abiotic environments (before biotic interactions mediate phenotypic expression at local scales). As a result, plant trait variation should be able to predict the environmental range of a species, identifying range limits where climate and soil environments occur that, beyond which, are unsuitable for sustained population growth. In this study, we use two complimentary approaches (niche models and double quantile regressions) to examine how dominant abiotic gradients and intraspecific trait variation shape the niche of a widespread *Populus* tree species.

Introduction

Plant range limits reflect the energy and nutrient constraints that determine where populations are no longer self-sustaining (i.e., the realized niche). Species distribution models (SDMs) visually describe where certain environmental factors shape a species' realized niche, often using only climate data. However, biogeochemical gradients can influence the spatial distribution of plant diversity and species turnover at large scales (Vitousek et al. 2004, Paoli et al. 2006, Laliberté et al. 2013, Zemunik et al. 2016), and recent work shows how SDM accuracy is improved with soil information. For instance, soil pH is a more useful predictor of the current distribution of field maple (*Acer campestre*) in France than numerous climate factors (Coudon et al. 2006), and improves estimate of future plant ranges when incorporates in SDMs (Beauregard and de Blois 2014). As the accuracy and availability of soil information grows, models that incorporate soil gradients will continue to improve predictions of plant distributions since the ecological and evolutionary dynamics that define ranges are influenced by more than climate gradients.

While SDMs are useful for visualizing correlations between current or future environments and species occurrences, they are not designed to test mechanisms of those relationships. For instance, a plant species' distribution may fall between a certain range of precipitation values (thus, precipitation may be an important factor for the accuracy and precision of an SDM), but few niche models are designed to test why or how precipitation values outside this range lead to population declines. Plant functional traits offer an important bridge between inherently correlational SDMs and a mechanistic understanding of range limits. This is because plant traits related to their growth and fitness are formed, in part, by abiotic environments that can be used to parameterize niche models. The expression of functional trait variation at regional and global scales, therefore, reflects the underlying physical constraints applied by abiotic environments (before biotic interactions mediate phenotypic expression at local scales) (Stahl et al. 2014). As a result, plant trait variation should be able to predict the environmental range of a species, identifying range limits where climate and soil environments occur that, beyond which, are unsuitable for sustained population growth.

A unique approach using double quantile regressions was recently used to examine the mechanistic relationship between plant functional traits and climatic range limits (Stahl et al. 2014). In general, this method incorporates functional traits associated with a species' climate niche to predict species' range limits (upper, median, and lower climate extremes). The resulting response patterns shed light on potential filtering mechanisms and trait adaptations to climatic range limits. In their original trait-based approach, Stahl et al. (2014) use species-specific means for 250 North American tree species and ignore intraspecific variation. However, mechanisms causing inter-versus intraspecific variation are likely to vary among species, and population and genetic structure are not homogenous across species ranges (Hampe and Petit 2005). For instance, within-species variation might improve SDMs forecasting range shifts when applied to the presence/absence of genetic clusters (Gotelli and Stanton-Geddes 2015). As recognition that within-species variation can impact ecological responses to global change continues to grow, it is imperative that ecological models begin incorporating intraspecific trait information (Moran et al. 2016).

In this study, we use two complimentary approaches to examine how dominant abiotic gradients and intraspecific trait variation relate to the ecological niche of a widespread *Populus* tree species (*Populus angustifolia* James). In Approach 1, we create SDMs with Maxent using geo-references specimen records to compare how climate, soil, and climate + soil models predict habitat suitability for the tree species. In Approach 2, we apply the double quantile regression method from Stahl et al. (2014) to a single tree species (using population-specific means) test whether intraspecific trait variation can predict climate and soil range limits. Here, plant growth and leaf trait data from the field and a common garden experiment were included to separate environmental and genetic effects on trait variation. We hypothesize that: (1) soil gradients are more important than climate variables for predicting the *P. angustifolia* ecological niche across Western North America, (2) variation in plant growth and leaf traits from field observations will predict some, but not all, climate and soil range limits, and (3) consistent with evidence that plant traits are adapted to the abiotic extremes at the species' range limits, there will be consistent trait-environment response patterns such that trait variation in the field and common garden predict the same climate and soil limit.

Methods

Study species, occurrence data, and environmental characterization

P. angustifolia is a high elevation, dominant tree species in riparian ecosystems throughout the Rocky Mountains (Little 1976; Braatne et al. 1996; Fig. 3.1a). This is an excellent species to investigate the relationship between range limits and intraspecific trait variation: (i) the species' range spans large environmental gradients, including ~10 °C Annual Mean Temperature (AMT), ~100 mm Annual Precipitation (AP), and 0.5 percent total soil nitrogen (Braatne et al. 1996, Van Nuland et al. *in review*, Ware et al. *in review*), (ii) previous work has revealed the importance of intraspecific genetic and phenotypic effects on ecosystem properties (Schweitzer et al. 2008, Schweitzer et al. 2012, Van Nuland et al. 2016), and (iii) these habitats are predicted to experience significant climate changes (e.g., 2-6 °C AMT increases) in the next 50 years (Van Nuland et al. *in review*, Capon et al. 2013). As a result, suitable habitat and trait adaptations to climatic range limits identified in this study may have important consequences for understanding the species' ecological and evolutionary responses to ongoing environmental change.

Figure 3.1. Distribution map for *P. angustifolia* and a summary of the two approaches used to examine the species' climate and soil niche. (**A**) Geo-referenced herbarium samples (closed circles) were gathered from the Intermountain Region Herbarium Network. Locations of 16 field sites (distinct populations) across the *P. angustifolia* range that were sampled for plants and soils in 2012 are depicted (blue triangles). (**B**) Approach 1 consists of species distribution models constructed with Maxent using climate data (bioclim data from the Worldclim database) and soil data (physical and chemical properties from SoilGrids.org). (**C**) Approach 2 utilizes double quantile regression (adapted from Stahl et al. 2014) to examine how intraspecific variation predicts the climate and soil limits of *P. angustifolia*. Briefly, populations within the species' range exist across environmental gradients, encompassing habitats that shape plant traits at the upper and lower range limits. Within-species functional trait variation is then used to predict the upper limits (95^{th} quantile), median niche (50^{th} quantile), and lower limits (5^{th} quantile) of the environmental range (solid lines represent significant correlations, dashed lines represent non-significant correlations). The trait-environmental relationships also identify "no-go areas" (i.e., areas where no trait value occurs that might allow for that habitat to be occupied by *P. angustifolia*). Three types of response patterns can emerge where outer regression lines (blue and red) surround the environmental range of the species, and the median regression line (black) represents the average realized environmental niche as predicted by traits. Functional trait variation from the field and the common garden that predict the same environmental limits would suggest trait adaptation, whereas different patterns between field and common garden traits would suggest plasticity of traits. Overall, the double quantile approach compliments the distribution models (Approach 1) by adding mechanistic information as to why certain habitats are predicted to be more suitable than others (i.e., how climate and soil environments impact plant functional traits related to growth and performance).

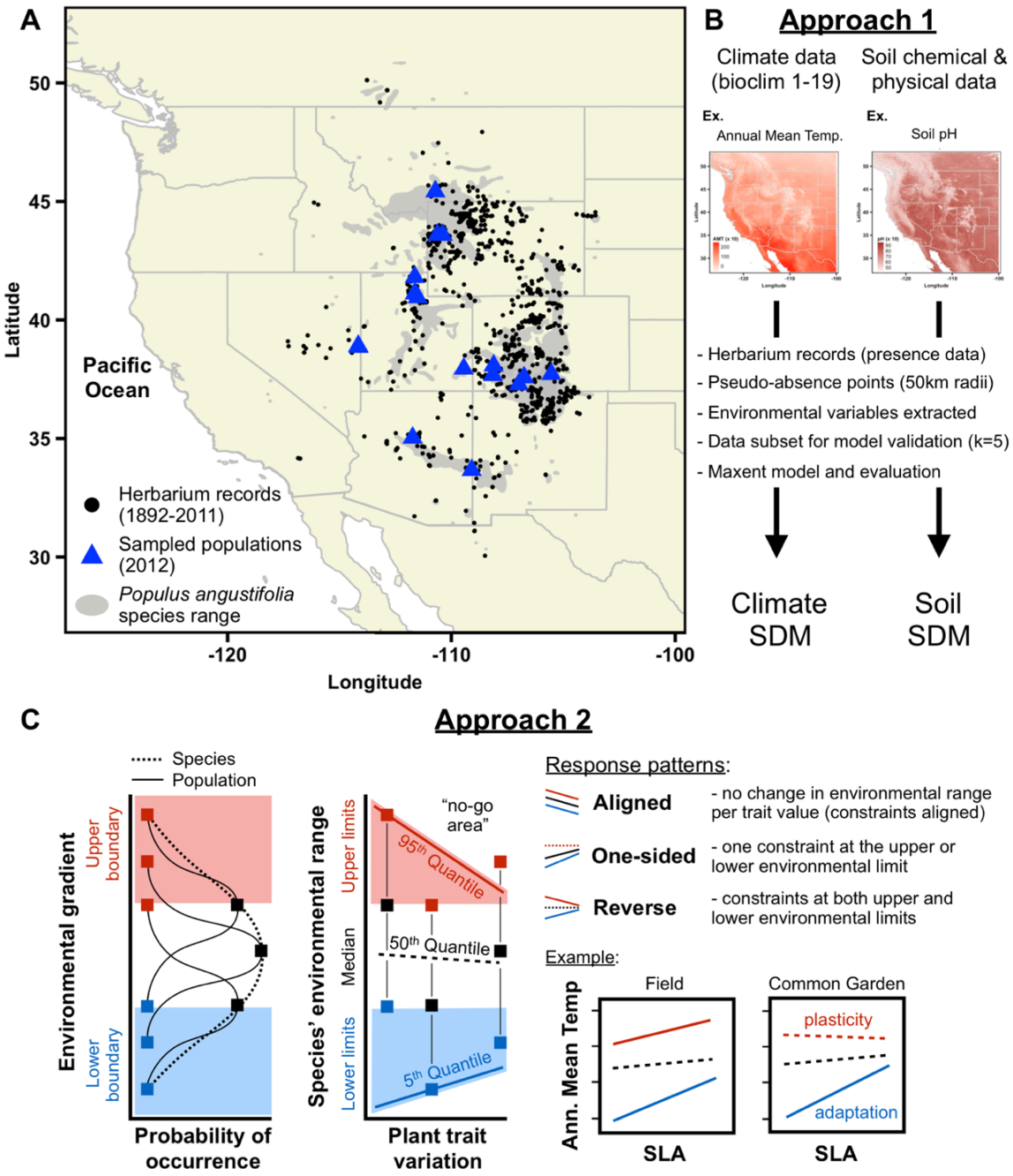

Figure 3.1 (continued)

We gathered geo-referenced occurence data for *P. angustifolia* in two ways. First, we gathered all *P. angustifolia* occurrence records from the Intermountain Region Herbarium Network (http://intermountainbiota.org/portal/index.php). Second, we performed an extensive field survey in June of 2012 where individual *P. angustifolia* genotypes (n = 557) were marked with GPS points (Oregon® 550t, Garmin, Germany) (see *Field sampling and common garden experiment* below). In total, our dataset includes 1713 records of *P. angustifolia* occurrence data spanning more than a decade across western North America. Visual inspection of the dataset confirms that it likely captures most of the geographic variation of *P. angustifolia* occurrence (including the possibility of isolated populations outside of the known species range; see Fig. 3.1a).

Abiotic variables related to temperature, water, and soil form important gradients that vary widely across the distribution of *P. angustifolia* and have been shown to drive plant trait responses in many systems. We therefore gathered 19 bioclim variables from the WorldClim database at 2.5 minutes spatial resolution (http://www.worldclim.org/). These variables cover annual and monthly trends of temperature and precipitation mean values and seasonal variation. In addition to climate data, we gathered four soil variables from ISRIC global soil datasets (https://www.soilgrids.org; Hengl et al. 2014). Soil data include estimates of bulk density, cation exchange capacity, percent organic carbon, and pH at 0-15 cm depth. Together, these environmental data were used in Approach 1 to build separate climate and soil SDMs (see *Climate and soil SDMs* below; Fig. 3.1b), and in Approach 2 to quantify the abiotic conditions at each field-sampled site for trait-environment relationships (see *Predicting environmental limits with functional traits* below; Fig. 3.1c). We examined collinearity among environmental variables with the 'vif' function in the usdm package in R (Babak 2015). Of the 19 total climate variables, 10 were identified as having collinearity problems. As a result, we retained the following climate variables that had variance inflation factors with values of 8 or below: bio1 = Annual Mean Temperature (°C), bio2 = Mean Diurnal Range (°C), bio4 = Temperature Seasonality, bio8 = Mean Temperature Wettest Quarter (°C), bio9 = Mean Temperature Driest Quarter (°C), bio13 = Precipitation Wettest Month (mm), bio14 = Precipitation Driest Month (mm), bio15 = Precipitation Seasonality, bio18 = Precipitation Warmest Quarter (mm). There were no soil variables identified having collinearity problems (i.e., variance inflation factors > 10), and no variables in the combined climate + soil model that had collinearity problems using the same procedure.

Field sampling and common garden experiment

Field sampling and common garden experiments are the same as described in Chapter Two above, but are re-stated here for clarity as applied to the specific hypotheses in the current chapter. We sampled plant and soil traits across sixteen river systems (hereafter 'populations') with naturally occurring riparian stands of *P. angustifolia* along a ~1,350 km latitudinal gradient from southern Arizona to southern Montana. These populations included: Blue River and Oak Creek, AZ; Medano Creek, Park Creek, San Juan River, Dolores River, and San Miguel River, CO; Lexington Creek and Snake Creek, NV; Indian Creek, Ogden River, Logan River, and Weber River, UT; Snake River, Gros Vente River, and Yellowstone River, MT. Each population was sampled along an elevational gradient (average ~450 m) to capture a wide range of within-population variation. Specifically, we identified and sampled the highest (coolest) and lowest (warmest) elevation sites that comprised the sixteen distributions, in addition to sampling 1-3

sites (separated by at least 50 m in elevation) that were located between the uppermost and lowermost sampling locations.

Ten mature *P. angustifolia* genotypes were sampled from the 3-5 sites within each population along the sixteen elevation gradients (n = 557). Genotypes were identified using morphological indicators and by separating sampled trees over one tree-height distance away to avoid sampling clones as distinct genotypes (Everitt 1968; Rood et al. 1994; verified by microsatellite analysis in Ware et al. *in review*). After taking these spatial precautions, adult trees were then haphazardly chosen and sampled at each site within populations. Sizes of individual trees in the field were recorded by measuring the diameter at breast height (DBH). Leaf morphology was assessed for specific leaf area (SLA) by scanning with WinFolia software, then dried at 60 °C for 72 hrs before mass measurements (Cornelissen et al. 2003). Each SLA measurement represents the average of five individual leaves from the same individual.

Soil samples were also collected from beneath the drip-line of the tree to "ground-truth" two of eight soil variables used in constructing the soil SDM (soil pH and organic C content). Here, soil samples were collected with a 2 cm diameter Oakfield soil sampler to a vertical depth of 10 cm and stored in a cooler (0 °C) during transportation to the University of Tennessee where they were stored at 0°C before analysis. Each sample was sieved to 2 mm and a 2:1 slurry of soil to deionized water (10 g to 20 mL) was created for pH measurements (Denver Instruments, New York). A separate oven-dry soil subsample was measured for percent total soil carbon (C) using chromatography (FlashEA® 1112 Elemental Analyzer, Thermo Electron S.p.A, Italy). We find a strong positive relationship between observed soil pH (field) and predicted soil pH (SoilGrid) ($r^2 = 0.47$, $p < 0.001$; see Supplementary Material, Fig. S1a), and a moderately positive correlation between observed total soil C (%) and predicted organic C content ($r^2 = 0.10$, $p = 0.03$; see Supplementary Material, Fig. S1b). These positive "ground-truth" results indicate the general usefulness of incorporating SoilGrid data in this study.

We created a common garden greenhouse experiment in June 2012 with replicate genotypes to explicitly examine the effect of genetic variation on plant traits that may relate to the species' environmental range. Twenty branch-tip cuttings (~20 cm in length) were collected from each genotype in the field for planting in the common garden. Cuttings collected in the field were transported in a cooler (0°C) to the Northern Arizona University greenhouse where each cutting was scored at the base with pruning shears, treated with a rooting hormone growth regulator (indole-3-butyric acid [IBA]; Hormodin® 2, OHP Inc., Pennsylvania), and potted in general potting mix. After three months, individual cuttings were transferred to 6.4 x 36 cm pots (D60 Deepots™ Stuewe and Sons Inc, Oregon) and randomized in the common greenhouse environment, allowing further root growth and reduced competition. In June of 2013, we measured the diameter of annual growth for replicate cuttings in the common garden (total n = 1514). In addition, leaves were collected from sampled cuttings for SLA measurements (identical methods as above). We compare measurements of annual growth diameter and SLA in the common garden to DBH and SLA in the field to separate patterns of plasticity versus adaptation (Connor and Hartl 2004; Fig. 3.1c).

Analysis

Climate and soil SDMs

To address our first hypothesis (that climate and soil gradients differ in predicting the *P. angustifolia* niche), we use geo-referenced occurrence records (herbarium and field survey data) with climate and soil layers to construct SDMs using the dismo package in R (Hijmans et al.

2017). First, we created pseudo-absence data by drawing random points from 50km radius circles surrounding each occurrence record. Next, we cropped each environmental layer to match the extent of the *P. angustifolia* range (27 to 53 degrees latitude, -127 to -100 degrees longitude), and matched the extent, resolutions (~4.5 km^2), and dimensions of climate and soil layers using the projectRaster function in the raster package in R (Hijmans et al. 2016). Next, we created a raster stack of all climate and soil layers using the 'stack' function. We then used the 'kfold' function to separate the dataset into five groups (k = 5) of equal size to train and test the model (i.e., constructing the model with a portion of the dataset and then validating the model with the remaining data not used during model construction). We created a Maxent model from the training presence and absence data with the 'maxent' function to estimate habitat suitability using climate, soil, and climate + soil environmental data.

To evaluate the predictive accuracy of the models (how good they are at identifying a data point as presence or absence), we used Area Under the Receiver Operator Curve (AUC) with the 'evaluate' function. The AUC statistic comes from the curve of sensitivity versus 1 - specificity for a range of probability threshold values that ranking points as presence or absence (i.e., the receiver operating characteristic curve). We consider AUC values >0.8 to be good, 0.6-0.8 as moderate, and <0.6 to be poor (similar evaluations as Beauregard and de Blois 2014, Wang et al. 2012). An AUC = 0.5 indicates the model is no better than random at correctly identifying presence and absence points. Because there are known issues in using AUC statistics for model evaluation, we first use point-based distance sampling to remove spatial sorting bias (the difference in distance between test-presence to train-presence and test-absence to train-presence points; Hijmans et al. 2012). We report mean AUC values for the five training/testing data subsets used in Maxent model construction and evaluation. There has been much criticism over the use of Maxent in distribution models, particularly related to potential biases and misinterpretation of model output (Gotelli and Stanton-Geddes 2015). Our method takes steps to avoid these known problems, including our correction for spatial sorting bias (resulting in more conservative AUC) and not confusing habitat suitability as a direct measure of occurrence probability. With approach 1, we test whether there are geographic similarities and differences in how climate and soil factors shape the ecological niche of *P. angustifolia* across western North American.

Predicting environmental limits with functional traits

To test our second hypothesis (that field traits will predict climate and soil range limits), we explored relationships between DBH and SLA in the field and environmental variables using double quantile regression (Fig. 3.1c). Whereas Stahl et al. (2014) used multiple species ranges that varied in occurrence probability (i.e., some relatively dominant and others relative rare), we use a single species range with populations occupying spots along the overall species-level occurrence probability distribution. We then performed regressions on population-mean values for 5^{th}, 10^{th}, 45^{th}, 50^{th}, 90^{th}, and 95^{th} quantile for each trait-climate and trait-soil combination using the 'rq' function in the quantreg package in R (Koenker 2016).

To test our third hypothesis (that genetic clines revealed by the common garden experiment will predict climate and soil range limits, consistent with patterns of adaptation at these environmental extremes), we used a similar double quantile approach as above but substitute field traits for analogous common garden traits (annual growth diameter and SLA). Similar constraint patterns between the same trait-environment relationships in the field and common garden would be consistent with patterns of adaptations to specific climate or soil range

limits (Fig. 3.1c). Oppositely, different trait-environment constraint patterns between field and common garden locations would suggest traits may be plastic to environmental range limits. All data manipulation and analysis was done in R version 3.3.2 (R Core Team 2016).

Results

Climate and soil SDMs

The distribution models show clear geographic similarities and differences in *P. angustifolia* climate and soil habitat suitability across western North America (Approach 1). All models performed moderately better than random at correctly identifying presence and pseudo-absence points after accounting for spatial sorting bias (Climate AUC = 0.64 ± 0.02 SE (range = 0.60-0.70); Soil AUC = 0.65 ± 0.03 (range = 0.56-0.70); Climate + Soil AUC = 0.68 ± 0.01 (range = 0.64-0.71)). In the southern portion of the range, climate and soil habitats appear equally suitable along the Mogollon rim in central Arizona. The central portion of the range through Utah and Colorado also have similarly suitable climate and soil properties, with the exception that soil habitats were more suitability in eastern Utah than climates. The models also predicted larger areas of suitable soil habitat than climate habitat in the north, particularly in southern Idaho and Canada. Diurnal range and precipitation seasonality were the two most important temperature and precipitation variables in the Climate model (Fig. 3.2a). Soil organic carbon was the most important variable in the Soil model (Fig. 3.2b). In the combined Climate + Soil model, soil organic C (mean permutation importance = 24.1 ± 0.7) was 77% more important than the closest precipitation factor (precipitation seasonality) and 90% more important than the nearest temperature factor (annual mean temperature) (Fig. 3.2c). Together, these results show that while both climate and soil properties are important predictors of suitable habitat for *P. angustifolia* (i.e., climate and soil model AUCs were comparable), soil organic C is more important than temperature and precipitation variables for defining the species' ecological niche.

Predicting environmental limits with functional traits

Using double quantile regressions, we found that *P. angustifolia* functional traits predicted the climate and soil range occupied by *P. angustifolia* (Approach 2). For diameter traits, 4 of the 9 climate variables and 2 of the 4 soil variables were unrelated to DBH or annual growth diameter (Fig. 3.3a-b). The second most common response type was one-sided, with reversed and aligned patterns the least common. There were three instances where one-side responses in the common garden were matched by the respective trait-environment correlations in the field to suggest adaptation in plant growth has occurred at these range limits. These include diameter predictions of temperature seasonality (lower limit; field slope = 0.51 ± 0.09, $p < 0.001$; common garden slope = 39.5 ± 6.4, $p < 0.001$; Fig. 3.4a-b), precipitation in the warmest quarter (upper limit; field slope = -0.72 ± 0.25, $p = 0.01$; common garden slope = -62.4 ± 20.1, $p < 0.01$; Fig. 3.4c-d), and precipitation in the warmest quarter (upper limit; field slope = -1.6 ± 0.6, $p = 0.01$; common garden slope = -165.3 ± 50, $p < 0.01$; Fig. 3.4e-f). Surprisingly, given the importance of soil organic C and bulk density in the Climate + Soil SDM, diameter traits did not predict these soil variables.

Figure 3.2. Species distribution models (SDMs) perform moderately well at predicting suitable climate and soil habitat for *P. angustifolia*. Maxent SDMs constructed with (**A**) climate, (**B**) soil, and (**C**) climate + soil variables. Distributions of suitable habitat show large areas of overlap through the southern and south-central locations of the species range. In contrast, the soil SDM predicts greater areas of suitable habitat in northern locations than the climate SDM. The most important variable for the climate SDM is mean temperature in the wettest quarter. The most important variable for the soil SDM is organic carbon (C) content. In the combined climate + soil SDM, soil organic C and bulk density were more important than all other climate and soil variables for predicting suitable habitat. Soil variables are shown in brown, temperature variables are shown in red, and precipitation variables are shown in blue.

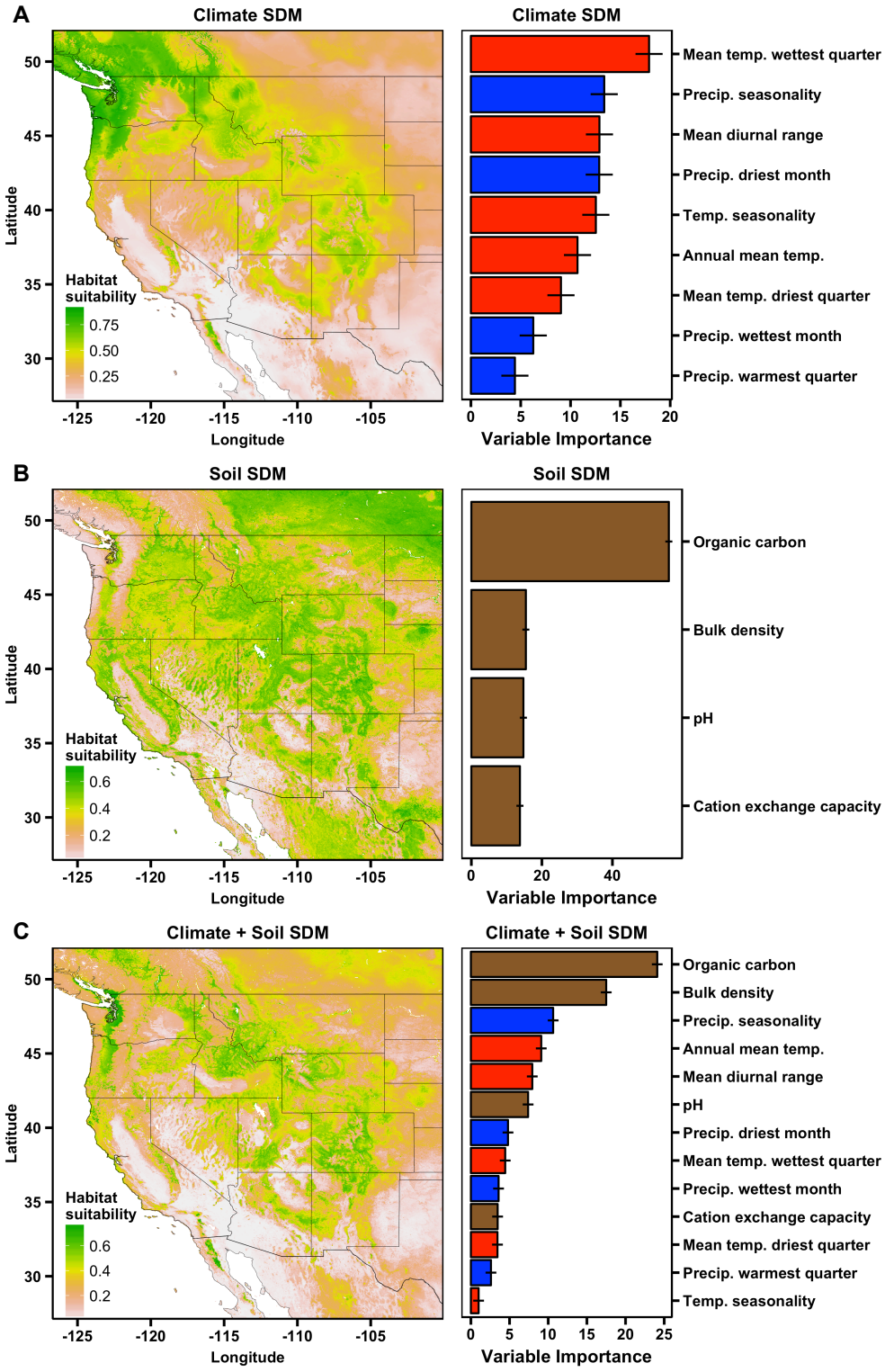

Figure 3.2 (continued)

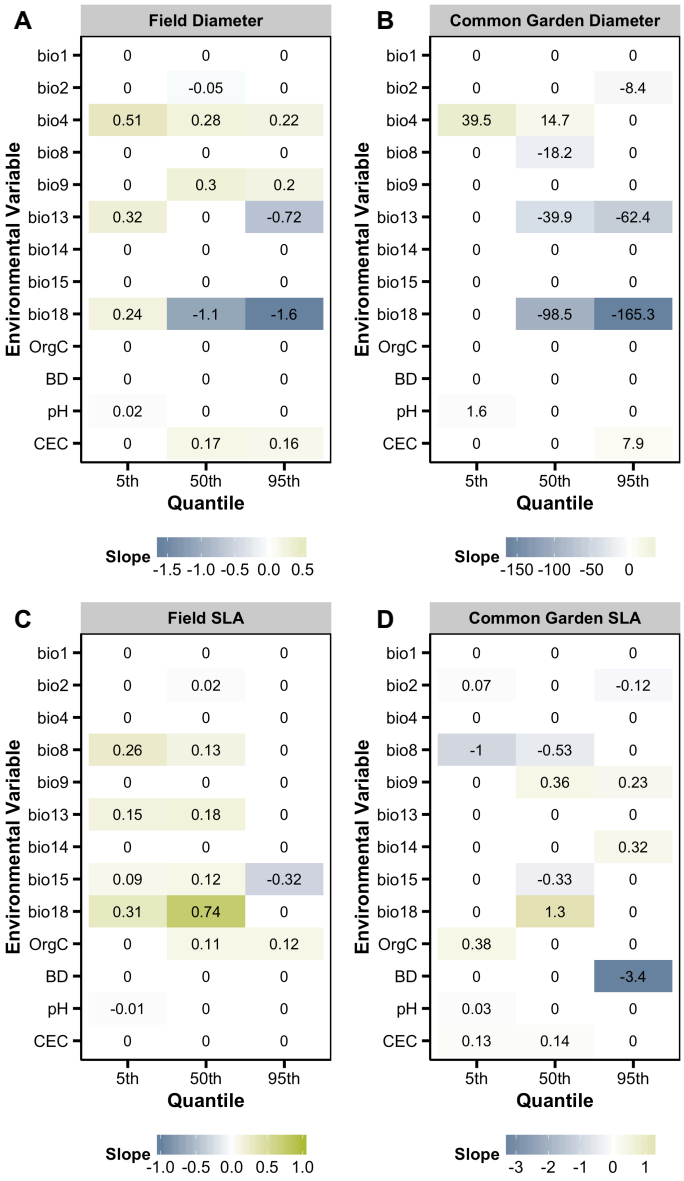

Figure 3.3. Summary of beta coefficients for trait-environment relationships show examples of adaptation and plasticity to climate and soil range limits. Non-significant regressions are depicted with zero beta coefficient values. Positive slopes are shown in green, negative slopes are shown in blue. Field diameter = DBH (cm), common garden diameter = annual growth diameter (mm). Climate variables: **bio1** = Annual Mean Temperature (°C), **bio2** = Mean Diurnal Range (°C), **bio4** = Temperature Seasonality (standard deviation * 100), **bio8** = Mean Temperature Wettest Quarter (°C), **bio9** = Mean Temperature Driest Quarter (°C), **bio13** = Precipitation of Wettest Month (mm), **bio14** = Precipitation of Driest Month (mm), **bio15** = Precipitation Seasonality, **bio18** = Precipitation of Warmest Quarter (mm). Soil variables: **OrgC** = Organic Carbon (g/kg), **BD** = Bulk Density (kg/m^3), **pH** = pH, and **CEC** = Cation Exchange Capacity (cmolc/kg).

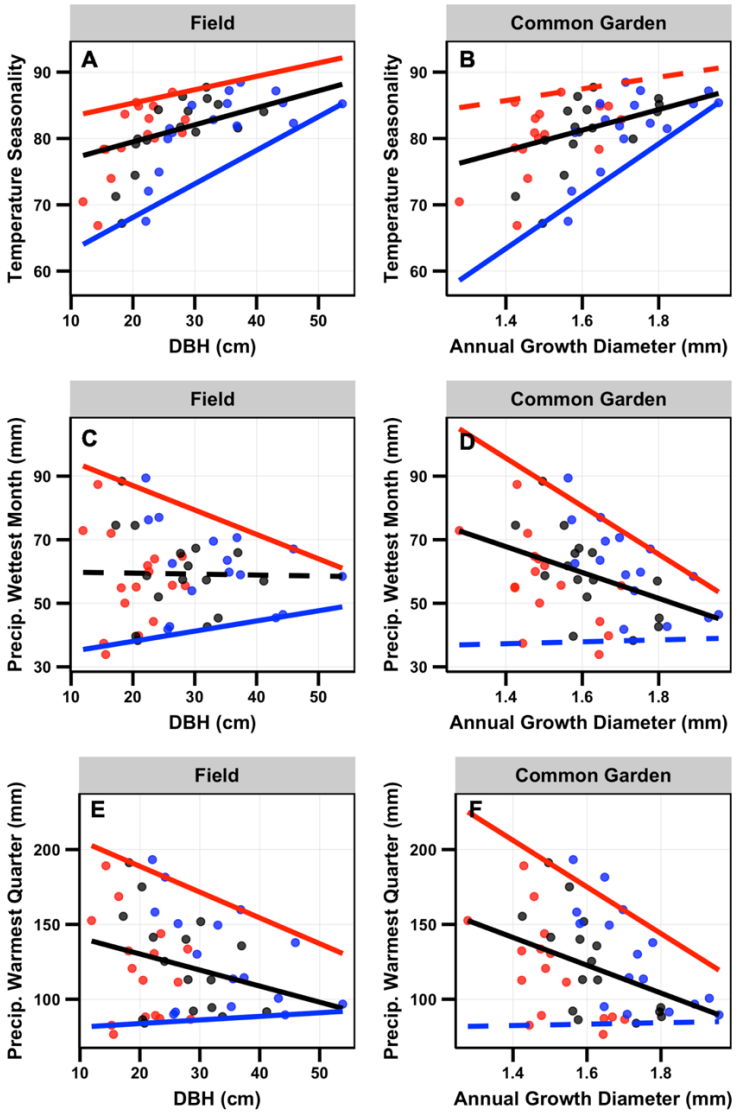

Figure 3.4. Trait-climate relationships show how growth traits are likely adapted to climatic limits. The median niche and lower limits of temperature seasonality were positively correlated with (**A**) diameter at breast height (DBH) and (**B**) annual growth diameter, with the response pattern changing from aligned (field) to one-sided constraint (common garden). DBH and annual growth diameter both negatively predicted the upper limit of precipitation of wettest month, with a reverse trait-environment response pattern in the field (**C**) and a one-sided constraint in the common garden (**D**). Similarly, both diameter traits negatively predicted the upper limits of precipitation of warmest quarter, with a reverse response pattern in the field (**E**), and one-sided constraint pattern in common garden (**F**). The area between the outermost lines reflects the total environmental range predicted by trait variation, whereas locations outside the upper and lower lines represent "no-go areas" for the tree species. Red = 95th quantile, black = 50th quantile, and blue = 5th quantile. Solid lines represent significant slopes, dashed lines represent non-significant slopes.

Instead, we found consistent one-sided responses across field and common garden measures for diameter-pH (lower limit; field slope = 0.02 ± 0.01, p = 0.01; common garden slope = 1.6 ± 0.54, p < 0.01) and diameter-cation exchange capacity (upper limit: field slope = 0.17 ± 0.04, p < 0.001; common garden slope = 5.0 ± 1.4, p = 0.04) relationships (Fig. 3.3a-b), indicating that these traits are adapted to the lower limits of soil pH and upper limits of soil cation exchange capacities.

SLA was a better predictor than plant diameter of soil organic C, the most important environmental factor identified by the Climate + Soil SDM for predicting *P. angustifolia* habitat suitability. Both field and common garden SLA-organic C relationships showed one-sided responses, but at opposite range limits. Specifically, field SLA positively predicted the median niche (slope = 0.10 ± 0.04, p = 0.02) and upper limits (slope = 0.12 ± 0.03, p < 0.001) of soil organic C (Fig. 3.5a), while common garden SLA only positively predicted the lower limits of soil organic C (slope = 0.38 ± 0.05, p < 0.001) (Fig. 3.5b). This appeared to be a common trend for SLA-environment relationships; although we recorded 5 and 7 one-sided responses patterns in the field and common garden, respectively, there were no cases where significant patterns matched at the same environmental limit (Fig. 3.3c-d). Overall, we found patterns that support plasticity and adaptation of traits to climate and soil range limits, and from this we can begin to understand the ecological and evolutionary mechanisms by which abiotic environments influence the ecological niche of *P. angustifolia*.

Discussion

Our study combines SDMs and functional trait-environment relationships to improve mechanistic understanding of how climate and soil range limits are structured. We found soil gradients (organic C and bulk density) were more important than temperature and precipitation factors for predicting *P. angustifolia* suitable habitats across the Rocky Mountains. As far as we know, this is the first niche model created for this environmentally and economically important riparian tree species, and one of few that directly compares the importance of soil versus climate factors for species distributions. Using double quantile regressions, we found that intraspecific variation in *P. angustifolia* functional traits in the field predicts the upper and lower climate and soil range limits of the species, as well as the species' median niche in certain cases. By relating functional trait variation in the common garden to field environments, we found patterns that support both trait adaptation and plasticity to the species' climate and soil range limits. Our focus on intraspecific trait variation and multiple environmental gradient types advances previous work (and specifically Stahl et al. 2014) by (1) incorporating a common garden experiment to provide a more accurate assessment of adaptation versus plasticity at range limits than field-based trait measurements, and (2) including soils as an important environmental force that works together and against climate variables to impact phenotypic expression and genetic variation. These findings add to the growing amount of work that highlights the role soil gradients play in defining species ecological niches. Because climates, plants, and soils interact in ways that influence plant fitness and soil organic C pools, further work is required to identify environmental conditions favorable for plants to actively construct suitable soil habitat under range shift scenarios and where currently suitable soil habitat is threatened to change with climate warming.

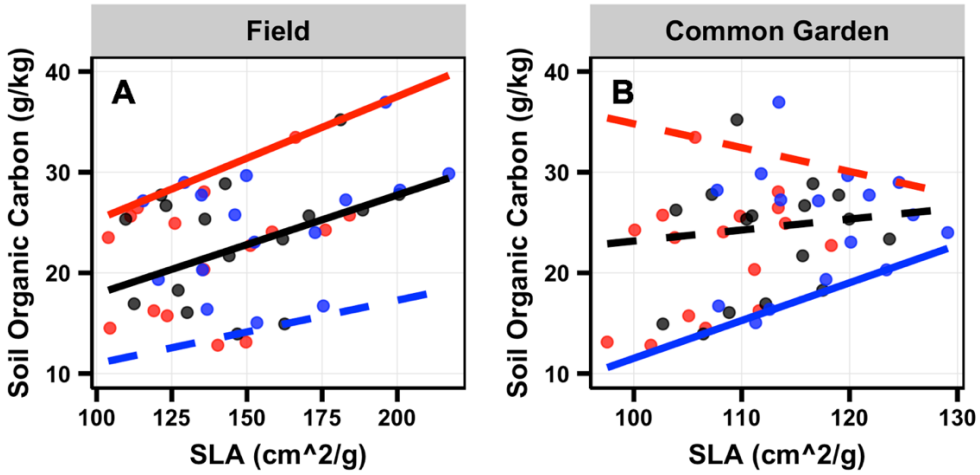

Figure 3.5. **SLA-environment relationships showing how plant leaf functional traits predict the soil organic carbon (C) range occupied by *P. angustifolia***. (**A**) Standard leaf area (SLA) variation in the field was positively related to the median and upper limits of soil organic carbon (C) content. (**B**) In contrast, SLA variation in the common garden showed a positively relationship with the lower limits of soil organic C, and no correlation with the median and upper soil C limits. The area between the outermost lines reflects the total environmental range predicted by trait variation, whereas locations outside the upper and lower lines represent "no-go areas" for the tree species. Red = 95th quantile, black = 50th quantile, and blue = 5th quantile. Solid lines represent significant slopes, dashed lines represent non-significant slopes.

A central issue with ecological niche modeling is the disconnect between observational correlations (comparing species occurrence data to environmental variation) and the mechanisms that underlie those correlations (how populations respond to environmental gradients that define species range limits). For instance, the Climate + Soil SDM shows that soil organic C is the most important variable in predicting suitable habitat for *P. angustifolia* – but that information alone does not tell us how soil nutrients define the species' range limits. The double quantile regression approach showed that intraspecific variation in field SLA (presumably linked to plant fitness and demography) positively predicted the median and upper limits of soil C. This patterns supports previous work showing that SLA positively scales with soil fertility at the global scale (Ordonéz et al. 2009), and our findings advance this by showing how the link between soil nutrients and SLA may contribute to the niche requirements and range limits of *P. angustifolia*. This provides clear direction for future work to experimentally test the relationships of functional trait variation and population dynamics at the limits of soil organic C (e.g., with reciprocal transplants across soil C gradients) to better understand how soils act as a range-defining force.

Species range limits do not always directly reflect their niche limits for a variety of reasons (reviewed in Hargreaves et al. 2014), and here we cannot be certain that the edges of *P. angustifolia*'s distribution are defined by some inherent niche constraint (i.e., whether the finite rate of population growth declines in areas past range boundaries). With an increased attention towards transplant experiments across species range boundaries, replication across sites or different gradient types (elevation versus latitude) remains an issue that causes uncertainty over the identity and importance of ecological and evolutions forces that influence range dynamics and functional trait expression (Pfennigwerth et al. *submitted*, Hargreaves et al. 2014). However, our results show how the double quantile approach could present an opportunity to "scale-up" and test how broad patterns may that are identified from small experiments. This is relevant because (1) populations are expected to vary in phenotypic and genetic structure due to environmental pressures that vary across the species' distribution (Eckert et al. 2010, Valladares et al. 2014), (2) traits can be constrained by adaptive responses to environmental pressures at range limits, signifying 'no go' areas for species' distributions under certain environmental conditions (Bridle and Vines 2007), and (3) both transplant experiments and the double quantile approach (as applied here with field and common garden functional trait variation) test for patterns that are consistent with local adaptation. Therefore, our study suggests that the double quantile regression approach may not only be useful to test mechanisms associated with SDMs, but also could be used in combination with transplant experiments to examine how general patterns of trait adaptation or plasticity are to certain environmental range limits. This could directly benefit species range shift predictions that, at the present, scarcely account for the effects of local adaptation and phenotypic plasticity (Valladares et al. 2014).

Understanding future suitable habitat requires the consideration of how intraspecific variation interacts with surrounding environments. Plant traits not only reflect the abiotic stressors of a certain location, but may also play a role in shaping the local environment in ways that impacts their performance and fitness (Odling-Smee 2003). This can be seen by interactions between plant traits and soils where variation in chemical, morphological, and physiological plant phenotypes change soil biota and biogeochemical processes belowground (Bardgett and Wardle 2010). Such plant-soil interactions are likely present in our study as decades of previous work have examined the influence of phenotypic and genetic variation of *Populus* on soil microbial communities (Schweitzer et al. 2008), nutrient pools (Pregitzer et al. 2010, Van Nuland et al. 2016) and ecosystem processes (Fischer et al. 2010, Schweitzer et al. 2012).

Building on this work, our study illustrates the potential consequences of these linkages for species range dynamics, and particularly at soil range limits where there may arguably be greater effects of genetically based plant-soil interactions driving plant adaptation and soil ecosystem dynamics due to the increased environmental stress. Moreover, we identify organic carbon content as a key predictor of suitable habitat. This is important because many plants (including *P. angustifolia*) interact with and regulate the soil carbon pool through litter inputs and root exudates that become resources for decomposer and microbial communities (Bardgett 2005, Pregitzer et al. 2013. What remains unclear is the importance of such plant-soil interactions for creating and maintaining suitable habitat as species ranges and environments change. For instance, if *P. angustifolia* migrates northward (as it most likely did following the last glacial maximum as historical climates warmed; Evans et al. 2015), to what extent will its success depend on the current soil C conditions of a site versus its ability to improve soil organic C pools and create favorable soil environments over time? In addition, plant populations that are locally adapted to specific soil environments may further complicate predictions of range shifts (Bailey et al. 2014, Valladares et al. 2014, Van Nuland et al. *in review*). Further work investigating how plant-soil interactions vary across species distributions will be critical for understanding how suitable habitat is maintained and created at environmental limits and in novel habitats.

In conclusion, this study represents a unique combination of ecological niche modeling paired with double quantile regression within a single tree species using field observations and experimental common garden measures of functional trait variation. Through these complimentary approaches, we identified environmental gradients that were most important for predicting suitable habitat and found that functional trait variation predicted the environmental range occupied by the species in a manner consistent with patterns of adaptation or plasticity. Because plant ranges are not determined by climate factors alone, our study includes soil information and shows how other SDM models with a climate-centric approach may be missing key environmental gradients that contribute to species ecological niches. Specifically, our findings specifically point towards the importance of soil gradients as evolutionary mechanisms that may cause range limits to occur in *P. angustifolia*. Overall, combining SDMs with double quantile regressions may serve as a useful guide for future research by identifying how certain populations may adapt (or not) in response to increasing climatic and soil stress.

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CHAPTER IV
DIVERGENT PLANT-SOIL FEEDBACKS DRIVE ELEVATION RANGE DYNAMICS AND ECOSYSTEM PROCESSES

This chapter by Michael E. Van Nuland, Joseph K. Bailey, and Jennifer A. Schweitzer is currently in press:

Van Nuland, M. E., Bailey, J. K. and Schweitzer, J. A. "Divergent plant-soil feedbacks drive elevation range dynamics and ecosystem processes." *Nature Ecology and Evolution*, in press.

Abstract

Plant-soil feedbacks (PSF) are important interactions that may influence range dynamics in a changing world. What remains largely unknown is the generality of plant-soil-biotic interactions across populations and the potential role of specific soil biota, both of which are key for understanding how PSF might change future communities and ecosystems. We combined landscape-level field observations and experimental soil treatments to test whether a dominant tree alters soil environments to impact their performance and range shifts towards higher elevations. We show: 1) soil conditioning by trees varies with elevation, 2) soil biota relate to PSF, 3) under simulated conditions, biotic PSF constrain range shifts at lower elevations but allow for expansions at higher elevations, and 4) differences in soil conditioning predict feedback outcomes in specific range shift scenarios. These results imply that variable plant-soil-biotic interactions may influence the migration and fragmentation of tree species, and that models incorporating soil parameters will more accurately predict future ranges.

Introduction

Climate broadly determines where plants occur on a global scale, but the local influence of soils ultimately affects their distribution on the landscape (Coudon et al. 2006, Beauregard and de Blois 2014). Understanding how soil variation impacts plant fitness and performance traits is a frontier for predicting how species ranges will respond to climate change (van der Putten 2012, van der Putten et al. 2013, Bailey et al. 2014, Van Nuland et al. 2016). For example, plant ranges may be limited to chemically unique soil environments, as is the case with ecotypes that occur on granite and serpentine outcrops. Over half a century of work on serpentine plants shows that adaptive phenotypes for drought and heavy metal tolerance are distributed based on steep gradients of soil chemistry and texture (Anacker 2014). Plants also adapt to stressful soil environments through biotic interactions such as associations with mycorrhizal fungi (Johnson et al. 2010) to alleviate soil nutrient limitations. However, these important root symbionts are not always consistent across species' ranges, and it was recently shown that ectomycorrhizal fungal richness declines from the center to the edge of two temperate tree species ranges (Lankau and Keymer 2016), which may affect how certain populations adapt or migrate as climates warm. Here, successful plant range expansion might depend on the co-expansion of their mycorrhizal symbionts, which appears to be the case for exotic *Pinus* spp. in Africa (Richardson et al. 1994) and South America (Nuñez et al. 2009). Similarly, the current geographic range of a California perennial differs based on the presence of a fungal endophyte that may improve drought tolerance, thus increasing the species' habitable area size to include drier areas (Afkhami et al. 2014). Although species distributions and range limits are shaped by a complex set of abiotic, biotic, and evolutionary forces (Pearson and Dawson 2003, Sexton et al. 2009), evidence has begun to emerge that plant-soil-biotic interactions could play a significant role as they encompass many aspects of these range-defining factors (van der Putten 2012, Bailey et al. 2014, Classen et al. 2015, Van Nuland et al. 2016).

Soil biota will affect plant range shift responses to climate warming if plant-biota linkages

drive variation in plant fitness across environmental gradients. As plants move to new soil environments, their fitness will vary depending on the net impact of previously conditioned or determined soil communities on plant survival and performance (i.e., plant-soil feedbacks [PSF] Bever et al. 1997, van der Putten et al. 2013, Van Nuland et al. 2016). The degree to which plants modify the diversity and activity of soil communities could influence range shifts through such feedback effects. For instance, expansion beyond range limits could be promoted by a release from belowground enemies when soil pathogens accumulate in the native range and decrease plant fitness compared to plants in the expanded range that have accumulated fewer soil pathogens. This has been shown by comparing soil communities of range-expanded versus congeneric native plants, where natives had higher abundance of *Fusarium* spp. pathogens (Morriën and van der Putten 2013) and experienced more overall belowground attack (Engelkes et al. 2008) than range-expanders. However, most examples describing how PSF relate to plant range shifts use latitude gradients and make comparisons among species (Engelkes et al. 2008, Van Grunsven et al. 2010, McCarthy-Neumann and Ibañez 2012, Morriën and van der Putten 2013, Gundale et al. 2014), even though plants generally have much shorter distances to travel in elevation (167 m) relative to latitude (145,000 m) to track the same 1 °C temperature change (Jump et al. 2009), and that within-species variation can have important consequences for understanding the ecological and evolutionary consequences of feedbacks in plant-soil systems (Bailey et al. 2014, Schweitzer et al. 2014, terHorst and Zee 2016). Consequently, the importance of intraspecific PSF in determining range shift responses to climate warming could be greater and more immediate along elevation gradients relative to latitude gradients.

Variation in plant-soil interactions between the interior and edge of plant elevation ranges could determine how PSF affect range shifts. In response to rising global temperatures, plants are widely predicted to move upwards in elevation (Jump et al. 2009, Chen et al. 2011). Interactions between plants and soil communities will likely change along elevation gradients because i) patterns of gene flow and adaptation create genetic and phenotypic differences between the interior and edge of plant ranges (Bridle and Vines 2007, Angert 2009), and ii) soil microbial diversity is highly variable even at small scales (Ettema and Wardle 2002), including along elevation gradients (Yang et al. 2014). For example, using an elevation gradient in the Canadian Rocky Mountains, soil microbial communities from only the highest elevation site (1800 m compared to 1400 m and 800 m) increased *Pinus contorta* and *Picea glauca* x *engelmannii* growth rates relative to sterile controls (Wagg et al. 2010), showing how plant-soil-biotic interactions can influence plant performance differently across elevation ranges. However, movement towards higher elevations will disrupt these above- and belowground linkages (Bailey et al. 2014, Classen et al. 2015), causing geographic variation in plant-soil interactions that might affect the likelihood of range shifts (van der Putten 2012). For instance, mountain-slope transects in Switzerland showed no evidence that variation in soil biota affected *Salix herbacea* growth under experimental shifts into foreign soils at higher elevations, but migration beyond their current range reduced growth to negatively impact future expansion (Sedlacek et al. 2014).

Based on previous work that highlights the importance of plant-soil interactions near geographic limits (Wagg et al. 2010, Sedlacek et al. 2014), we examined intraspecific variation in landscape level patterns of biotic PSF across elevation ranges by comparing patterns from range interiors (lower elevations; henceforth "interior") to range edges (higher elevations; henceforth "edge") and beyond current range limits (henceforth "beyond"). We used a combination of field observations (Fig. 4.1a-c) and experimental soil treatments (Fig. 4.1d-e) collected from seven sites covering 980-2900 m in elevation, across ~1500 km of latitude, and a

large range of temperature and precipitation levels to measure plant-soil conditioning and feedbacks of a widespread native *Populus* spp. (*Populus angustifolia* James) (Table 4.1 and see Supplementary Table 1). Similar to previous methods of measuring plant-soil conditioning by *Populus* in the field (Madritch et al. 2009), our sampling design (Fig. 4.1c) led to comparisons of soil characteristics directly beneath trees (henceforth "conditioned") relative to randomly chosen locations beyond tree driplines (~5 m away; henceforth "unconditioned"). Although an alternative hypothesis could describe that trees selectively colonized sites with certain characteristics, past work using *P. angustifolia* common gardens consistently shows that abiotic and biotic soil properties vary from conditioning by different genotypes (Schweitzer et al. 2008, Pregitzer et al. 2010, Schweitzer et al. 2012, Pregitzer et al. 2013); therefore, we consider soil measurements directly beneath trees in the field to reflect plant-soil conditioning. Additionally, our feedback experimental designs (Fig. 4.1d-e) used soil inoculum treatments from field-collected soils (rather than a multistage approach with separate conditioning and feedback phases that is typical for studies using short-lived grass and shrub species; Bever et al. 2010), since a field approach provides both larger inference (i.e., more realism) and has successfully been used in experiments that measure how PSF relate to northern range shifts in long-lived temperate tree species (McCarthy-Neumann and Ibañez 2012) (see *Soil conditioning and feedbacks* for additional information).

We examined whether *P. angustifolia* alters the soil environment to impact future plant performance and simulated range shifts towards higher elevations. Specifically, we test the following related hypotheses:

(1) Plant-soil conditioning across *P. angustifolia* elevation gradients in the field will be greater within the range interior than at the range edge by comparing soil qualities measured from beneath trees ("conditioned") versus random locations in the interspace away from trees ("unconditioned").

(2) Tree growth in the greenhouse will increase with soil communities from conditioned versus unconditioned soil locations (i.e., positive PSF) using paired live and sterile soil inoculum treatments collected from the two soil locations. Moreover, greater plant-soil conditioning (i.e., larger differences between conditioned versus unconditioned soil qualities) will positively correspond with PSF such that trees exerting greater changes to their local soil environment show more positive feedback responses.

(3) If conditioned soil communities differ across elevation ranges, the performance of *P. angustifolia* trees will depend on whether they are grown with their current soil community or a soil community from the next elevation. Specifically, feedbacks from simulated upward range shifts ("range shift PSF") will change with increasing elevation from positive (opposing range shifts) to negative (favoring range shifts).

(4) Feedbacks will be more positive when plant-soil conditioning improves soil nutrient levels relative to the surrounding environment. Additionally, plant performance under range shifts should be negatively affected when soil properties are "mismatched" between sites. Therefore, greater differences in conditioned soil properties between elevation sites will correspond to more positive range shift feedback effects.

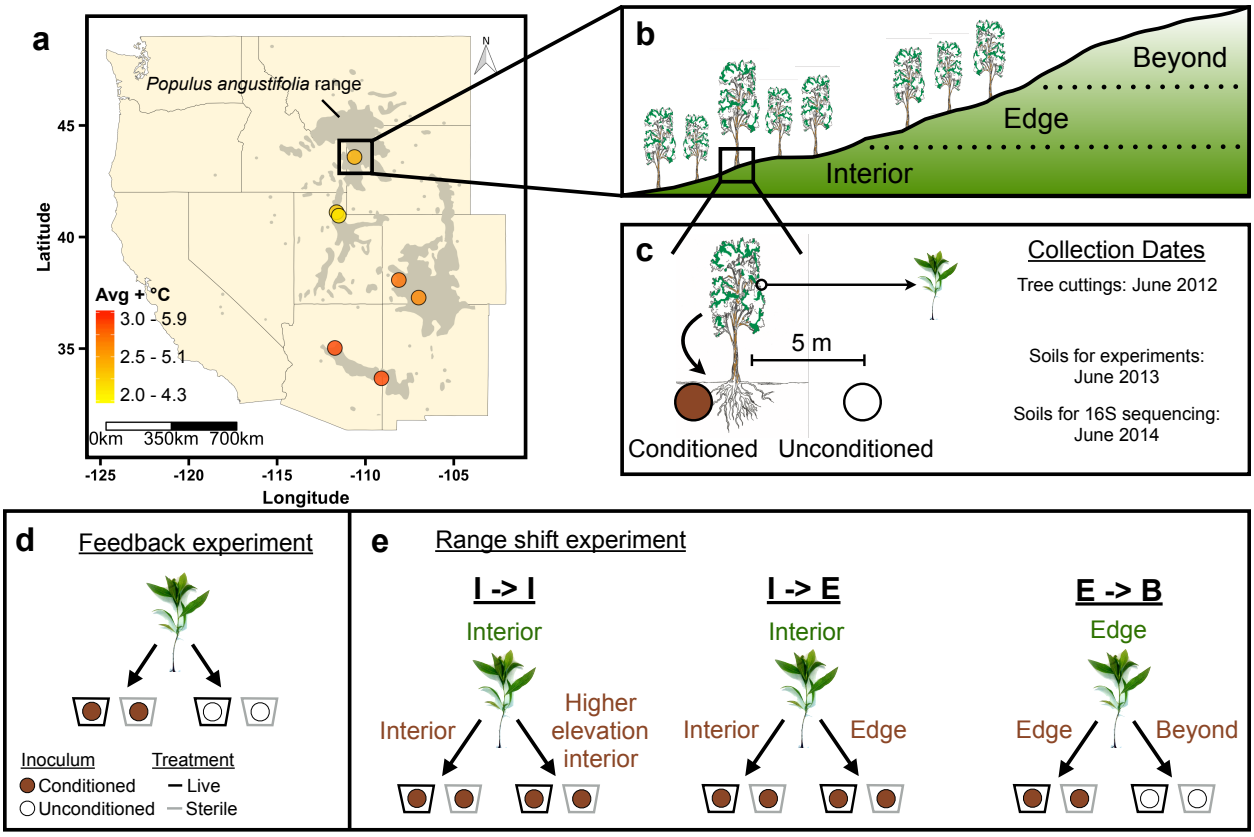

Figure 4.1. Field sampling and experimental design to test how plant-soil feedbacks (PSF) may contribute to *Populus angustifolia* range shifts. (**a**) We identified and sampled plants and soils from seven populations across the *P. angustifolia* range that are predicted to have approximately 2-6°C higher mean annual temperatures by 2070. (**b**) Tree cuttings were collected across 3-5 elevation sites (comprising the interior and edge of their elevation ranges) within each population and allowed to establish and mature for one year in the greenhouse. (**c**) Soils were collected from two locations to quantify the effects of plant-soil conditioning by *P. angustifolia* in the field[36,43]. (**d**) To test if conditioning affects plant performance leading to feedback effects, trees were grown in soil having been inoculated with conditioned or unconditioned soils in a paired 'live' or 'sterile' (gamma irradiated) treatment design that measures the effect of soil communities on tree growth. (**e**) For the range shift PSF experiment, trees were inoculated with conditioned soil communities from their elevation site or from the next higher elevation site. As a result, we used three range shift categories[32]: lower interior trees (I) grown with higher elevation interior communities (I->I), higher interior trees grown with edge (E) communities (I->E), or edge trees grown with communities beyond (B) current range limits (E->B). Note that edge trees grown with soils beyond current range limits are represented as unconditioned since there is no *P. angustifolia*-specific conditioning happening at those sites, but are influenced instead by a suite of heterospecific plant species at higher elevations. Feedback and range shift experiments occurred concurrently from September 2013 (initial biomass measurements) to May 2015 (final biomass measurements).

Table 4.1. Summary of sampling locations, climate, and tree diameter at each field site[a]

Site[b]	Elevation (m)	MAT (C)[c]	AP (mm)[c]	DBH (cm)[d]
BL (33.68, -109.09)				
Interior (4)	1792-2084	9.1 + 3.0-5.5	467	17.4 ± 3.1
Edge	2238	7.4 + 1.8-4.3	474	22.2 ± 7.5
Beyond	2323	7.4 + 1.8-4.3	474	-
OC (35.02, -111.73)				
Interior (2)	1683-1782	10.0 + 3.8-6.2	574	15.7 ± 6.1
Edge	1982	8.8 + 2.6-5.1	572	17.3 ± 7.5
Beyond	2023	8.8 + 2.5-5.0	572	-
SJ (37.30, -106.92)				
Interior (3)	2178-2464	4.8 + 2.4-5.1	496	31.6 ± 3.7
Edge	2663	2.1 + 3.1-5.7	680	24.6 ± 5.5
Beyond	2707	2.1 + 1.9-4.6	680	-
SMIG (38.00, -107.98)				
Interior (4)	1961-2515	4.3 + 2.8-5.5	578	19.9 ± 3.2
Edge	2749	1.5 + 2.5-5.1	681	19.8 ± 6.5
Beyond	2925	-0.7 + 2.1-4.8	825	-
OGC (41.37, -111.60)				
Interior (3)	1625-2085	4.5 + 2.4-5.3	531	44.2 ± 5.1
Edge	2325	3.0 + 1.8-4.7	544	27.4 ± 9.2
Beyond	2608	2.2 + 1.4-4.2	592	-
WR (40.91, -111.39)				
Interior (3)	1413-1958	6.1 + 3.3-6.3	542	30.9 ± 4.2
Edge	2167	3.0 + 2.6-5.6	573	27.4 ± 6.5
Beyond	2333	2.1 + 2.1-5.0	643	-
SNR (43.59, -110.60)				
Interior (4)	1695-2026	1.9 + 2.4-5.2	513	35.7 ± 3.4
Edge	2209	0.6 + 2.2-5.0	537	14.3 ± 6.9
Beyond	2488	-0.6 + 1.7-4.5	556	-

[a] Sites are from the following *Populus angustifolia* populations: BL = Blue River, AZ; OC = Oak Creek, AZ; SJ = San Juan River, CO; SMIG = San Miguel River, CO; OGC = Ogden Canyon, UT; WR = Weber River, UT; and Snake River, WY.

[b] Mean latitude and longitude are reported for each population in decimal degrees. Numbers in parentheses refer to how many lower elevation interior sites were sampled for each population.

[c] Data refers to current mean annual temperature (MAT) and projected MAT in 2070 using the average of two global circulation models (GISS-E2-R and HadGEM2-ES). Data is reported as current values (averaged for multiple interior sites) plus the mean range of 2.6 to 8.5 relative concentration pathways that predict MAT increases. AP = annual precipitation. All climate data were gathered from the WorldClim database.

[d] DBH = diameter at breast height.

Methods

Study species and sampled populations

High elevation riparian ecosystems of the Rocky Mountains are the predominant habitat for *P. angustifolia* (Braatne et al. 1996). These systems are predicted to experience significant climatic changes due to anthropogenic activities (Capon et al. 2013) and are critical areas to examine plant-soil responses to increasing temperatures (Fischer et al. 2013). Moreover, *P. angustifolia* populations span natural elevation gradients that occur along river drainages (Braatne et al. 1996), creating the opportunity to investigate variation in PSF across replicated gradients on the landscape. Since *P. angustifolia* is a riparian obligate species, this allowed for better identification of suitable habitat beyond current range limits (i.e., higher elevation sites within riparian zones), thus minimizing the risk of choosing inappropriate sites for experimental soil treatments (Hargreaves et al. 2014).

Seven populations of *P. angustifolia*, spanning a ~1500 km latitude gradient that covers a wide range of environmental variation (Fig. 4.1a, Table 4.1), were sampled across 980-2900 m in elevation. Total elevation changes (i.e., difference from highest to lowest sampling points) across these gradients ranged from 350 to 1000 m. All sampling points were marked via GPS (Oregon series 550, Garmin), and mean annual temperature (MAT) and annual precipitation (AP) were collected for these coordinates (WorldClim database). The populations span an average of 7 °C in MAT and 100 mm in AP from the southernmost to northernmost sites and include: Blue River and Oak Creek, AZ; San Juan River and San Miguel River, CO; Ogden River and Weber River, UT; and Snake River, WY. To quantify the predicted intensity of global warming on these populations, we calculated the MAT predicted for these sites in 2070 based on the average of two global circulation models (GISS-E2-R and HadGEM2-ES) for mild (RCP2.6) and extreme (RCP8.5) representative concentration pathways of greenhouse gases (WorldClim database). Approximately, a 2-6 °C increase in MAT is expected to occur over the next 50 years (Fig. 4.1a, Table 4.1), indicating that these *P. angustifolia* populations will experience similar temperature changes that have been recorded under periods of major species distribution shifts (Ordonez and Williams 2013). Moreover, recent work shows that *P. angustifolia* migrated northward as temperatures warmed after the last glacial maximum (Evans et al. 2015), illustrating that this species has the potential to shift geographic ranges in response to rising temperatures.

Plant collection and elevation ranges

A total of 310 genotypes (verified with microsatellite data; unpublished data) of *P. angustifolia* were sampled across elevation gradients from distinct populations in June 2012 (Blue River = 50, Oak Creek = 25, San Juan River = 56, San Miguel River = 50, Ogden River = 30, Weber River = 49, Snake River = 50). Each population comprised an elevation gradient containing 2-4 sites within the range interior that were separated by ~100 m in elevation, and one site existing at the uppermost range edge where current population boundaries exist based on visual surveys (Fig. 4.1b). Ten mature genotypes were sampled at each site and were identified in the field using morphological indicators and by separating sampled trees over one tree-height distance away to avoid measuring clones (which was also verified by the microsatellite data). Twenty branch tip cuttings (15 cm) were collected from *P. angustifolia* trees and stored in a cooler during transport to the greenhouse. In the greenhouse, the bottom 3 cm of each cutting was scored with clippers and treated with rooting hormone to promote establishment (Hormodin 2 with 0.3% Indole-3-butyric Acid); ten cuttings per genotype were grown together in 3.8 L pots containing general potting mix composed of equal parts peat, perlite, and vermiculite. After four

months, to reduce crowding and allow further growth under equivalent environmental conditions, trees were transplanted to 6.4 x 35.5 cm individual pots (D60 Deepots, Spencer Lemaire, Edmonton, Alberta, Canada) and grown for an additional 10 months with equal water, fertilizer, and light regimes to enable root establishment before growth trait measurements. Importantly, we found no relationship between tree size in the field (DBH) and growth traits in the greenhouse experiment (see *Analysis of maternal effects on tree growth traits* in Supplementary Information, Supplementary Fig. 2). This supports past work that has also found limited maternal effects for *P. angustifolia* growth in common environments (Holeski et al. 2013), and suggests that maternal effects likely play a limited role in determining *P. angustifolia* growth variation in our greenhouse experiments.

Soil collection and chemical analysis

Soils were collected from the same populations and field sites in June 2013 to quantify plant-soil conditioning in the field (hypothesis 1) and to use as soil biotic treatments in the feedback experiment (hypotheses 2 and 3). Specifically, soils were sampled in a paired design for all *P. angustifolia* genotypes in which conditioned soils were collected directly beneath trees and unconditioned soils were sampled from randomly chosen locations ~5 m from the base of trees (Fig. 4.1c). This sampling design has previously been used to examine patterns of soil conditioning by *Populus* under field conditions (Madritch et al. 2009). We pooled conditioned and unconditioned soils at the elevation site level because 1) we wanted to remove possible genotype-specific conditioning effects; 2) recent work shows that site is important for structuring *P. angustifolia* soil fungal communities along an elevation gradient (Gehring et al. 2006); and 3) elevation site is the level at which soil treatments are applied in the feedback experiment (see below). In order to identify suitable habitat at sites beyond current *P. angustifolia* range limits, we walked at least 50 m in elevation above edge populations while remaining within the riparian zone (Braatne et al. 1996, Hargreaves et al. 2014). Soil was then collected from 5-7 random locations and pooled for each population to characterize an average soil environment where *P. angustifolia* might expand beyond current range limits (Fig. 4.1b). All field-collected soils were sampled to a depth of 10 cm and stored in a cooler during transport to the University of Tennessee, where they were subsequently stored at 4° C before analysis. We measured total soil carbon (C) and total soil nitrogen (N) using chromotagraphy (FlashEA 1112 Elemental Analyzer, Thermo Electron S.p.A, Italy) after soils had been sieved through 2 mm mesh, ground, and oven-dried at 105° C. Soil pH was measured using a 2:1 slurry of 10 g soil to 20 ml 0.2M $CaCl_2$ (Denver Instruments, New York). We used a subset of populations (BL, SJ, SMIG, and SNR) where soils from the same elevation sites were sampled in 2012, 2013, and 2014 (see *Soil microbial analysis* below) to test whether soil chemistry varies across elevation (a continuous variable), between populations, and by year. We constructed a generalized linear model (GLM) with soil traits (C, N, and pH) as the response, elevation, population, and year, as fixed effects, and all possible interactions as fixed effects. We found no significant three-way interaction between elevation × population × year, indicating that soil traits remained relatively consistent within each population's elevation gradient across sampling years. Importantly, this result suggests that interannual variation is small and that soils sampled and analyzed across years are comparable in this study (see Supplementary Table 9).

Soil microbial analysis

We collected soils in June 2014 from a subset of *P. angustifolia* genotypes across the same elevation sites as 2012 and 2013 sampling to examine differences in soil microbial communities and how they may relate to feedback effects. Specifically, interior genotypes (n = 3) and edge genotype (n = 1) were sampled from each of the BL, SJ, and SNR populations (see Supplementary Table 1). Soils were collected directly from the rhizosphere of genotypes in the field; as a result, microbial diversity should directly reflect conditioning and we did not collect a paired unconditioned soil away from the tree. Soils were stored in a cooler during transport to the University of Tennessee, where they were subsampled for C, N, and pH analyses (identical protocols as above) to compare how microbial communities relate to soil chemical factors. Soils were then subsequently stored at -40° C before DNA extraction using MoBio PowerSoil DNA Isolation kits (MoBio Laboratories, Carlsbad, CA, USA). See *Amplicon sequencing and bioinformatic processing* in Supplementary Material for a full description of sequencing and workflow steps. Briefly, sequencing was carried out on a MiSeq Desktop Sequencer (Illumina Inc, San Diego, CA) running in paired end 2x250 mode. We processed 16S amplicon data using akutils (https://github.com/alk224/akutils-v1.2) that includes modifications to a QIIME 1.9.1 workflow (Caporaso et al. 2010) since it has been shown that the default settings can significantly overestimate microbial diversity (Krohn et al. 2016). Although it is possible that rare taxa could have substantial effects on plants, we focus on the five most abundant bacteria taxa identified by class (representing 58% of the total abundance) across the 12 field samples in this study: *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Synergistia*. These taxonomic groups were also among the most abundant in soil samples collected in 2012 from the same elevation sites (unpublished data), indicating that the identity of dominant taxa in these sites are generally consistent across years (i.e., soils collected for experimental inoculum in 2013 should be comparable to the microbial diversity uncovered by sequencing 2014 soils that were collected from the same sites during the same time of year).

Analysis

Soil conditioning and feedbacks

To address hypothesis 1 (that plants vary in their effect on soil environments; Fig. 4.1c) we compared conditioned and unconditioned soil C, N, and pH to investigate this initial premise of PSF (Bever et al. 1997). Previous work has shown the effectiveness of conditioned-unconditioned comparisons to measure *Populus* soil conditioning in the field (Madritch et al. 2009), and common garden experiments have shown *P. angustifolia* affects soil nutrient pools, nutrient cycling, and belowground microbial communities (Van Nuland et al. 2016). Conditioned and unconditioned soils (N = 60) were analyzed across range locations based on our hypothesis that PSF might impact range shifts if interior and edge genotypes differ in soil conditioning. We constructed a Restricted Maximum Likelihood (REML) model using the lme4 package with soil C, N, or pH, as the response variable, soil location, range location, and soil location × range location as fixed effects, and population as a random effect. We used Tukey HSD for post-hoc analyses using the multcomp package in R. In addition, we examined whether the relative abundance of dominant soil bacteria in the *P. angustifolia* rhizosphere differ between the range interior and edge of three populations. We constructed a GLM with the relative abundance of microbes as the response, and range location, population, and range × population as fixed effects. Differences in the effect of conditioned versus unconditioned soils (or rhizosphere communities between range locations) were considered to be consistent with the hypothesis that *P.*

angustifolia influences its soil abiotic and biotic environment, which may result in PSF. Finally, we tested whether conditioning effects could be related to differences in the amount of time trees in the field have had to change soil environments by using DBH as a proxy for tree age. We created a GLM with DBH as the response, population, range location, and population × range as fixed effects to examine whether younger (smaller) trees exist at range edges while older (larger) trees occur within range interiors consistently across all populations.

We established a large feedback experiment (initial N = 858) from September 2013 to May 2015 to address hypotheses 2 and 3: whether conditioned soil communities reciprocally affect plant performance, and if such biotic PSF vary with simulated range shifts (see *Range Shift PSF* below). To address these hypotheses, ~12-month-old trees were grown in soils that received different soil inoculum and placed in a complete randomized design in the University of Tennessee greenhouse (trees were also given random tag IDs during the experiment to mask their genetic identity, range location, and soil treatment). For each site within populations, 3-5 genotypes were selected at random from the pool of the ten possible genotypes that were collected in 2012 (number of unique genotypes: BL = 41, OC = 15, OGC = 17, SJ = 35, SMIG = 40, SNR = 36, and WR = 27). Each tree was removed from their individual pots and the soil was gently removed from their roots. Trees were then placed back into pots that were filled with 70% (~200 g) general potting mix before receiving soil inoculum treatments. To examine the role of biotic communities in determining feedback effects, one-half of all soils collected underwent gamma irradiation (48 kGy, Steris Isomedix, Spartanburg, SC) for subsequent 'live' and 'sterile' treatments. The comparison of live versus sterile soil inoculum provides a direct measure of the effect of soil communities on the growth of *P. angustifolia* trees in the experimental treatments. Approximately 20 g of either live or sterile soil inoculum was added in direct contact with the top of tree root systems, and a layer of general potting mix was added above to avoid cross-contamination between pots. Each live soil inoculation treatment had an equivalent, sterilized counterpart for a paired statistical design that directly tests the role of soil communities in affecting plant responses. All trees were measured for initial height and basal diameter as a baseline to quantify plant growth over the course of the experiment that had over 80% survival (N = 704). We used height and stem diameter traits from separate *P. angustifolia* trees of similar age and size to create an allometric equation using these growth traits that predicted more than 98% variation in aboveground biomass (see Supplementary Information and Supplementary Fig. 1).

Our experimental design using field-collected soils as inoculum is different from the common approach of creating multistage feedbacks (i.e., distinct conditioning and response phases; Bever et al. 2010, Brinkman et al. 2010), but has been effective for identifying variation in PSF on the landscape as it relates to tree species range shifts (McCarthy-Neumann and Ibañez 2012). Specifically, we use this design because: (1) we sought to simulate biotic plant-soil interactions using soils that most accurately reflected the diversity of soil communities near *P. angustifolia* trees (Sykorova et al. 2007, Brinkman et al. 2010); (2) unlike most studies of PSF where the focal species are short-lived grasses or forbs, mature trees in the field have clear zones of influence beneath their crowns where plant-soil dynamics are formed and attributable to a single individual (e.g., conditioned versus unconditioned soil locations; Zinke 1962, Madritch et al. 2009); and (3) feedbacks created in the field are not necessarily changed by further conditioning in the greenhouse since trees are long-lived organisms that have already made specific changes to soil environments (McCarthy-Neumann and Kobe 2010). Although the length of time needed to destroy these conditioning effects in the greenhouse is typically

unknown, if trees respond differently to the various experimental soil treatments (conditioned versus unconditioned soil locations, or range shift treatments into soil communities that were conditioned at higher elevations) then we presume these effects are a result of field-based soil changes that are transferred through the inoculum approach. Inoculated soil communities may have also developed or interacted with existing soil biota in the greenhouse or potting mix soil in ways that do not necessarily reflect plant-soil-biotic interactions in the field, but we are unable to determine whether this occurred or had any effect on our experimental design besides comparing live versus sterile treatments. In addition, soil sterilization procedures may result in nutrient pulses from the release of immobilized N within the biotic community or changes in physicochemical structure (Sykorova et al. 2007), but this effect is likely to be substantially reduced with our use of gamma irradiation versus autoclaving to sterilize and an experiment whereby ~10:1 ratio of standard potting mix to sterile soil inoculum would create an exceptionally small nutrient addition or change in soil structure that would affect tree growth and performance during the experiment.

We addressed hypothesis 2 (that soil communities drive feedback differences across elevation ranges and that feedbacks are related to soil conditioning; Fig. 4.1d) using two approaches: by measuring how trees perform when grown with conditioned soil inoculum (beneath trees) and unconditioned inoculum (away from trees), and ii) by comparing the relative abundance of dominant microbial taxa from genotypes where conditioned soil inoculum was collected to the mean aboveground growth of trees in those corresponding soil treatments. This is a main principle of PSF: that plant-driven changes to the soil (i.e., the difference between conditioned and unconditioned soil locations) reciprocally affect plant performance. In the first approach, we used paired live and sterile soil treatments to examine whether soil biota contribute to PSF in *P. angustifolia*. For genotypes at each elevation site, one replicate was grown in soil having been inoculated with live or sterile soil inoculum treatments from the pooled conditioned or unconditioned soil locations at their respective elevations. This led to a total of 215 comparisons of trees grown in soil with live or sterile inoculum from conditioned or unconditioned soil locations across seven *P. angustifolia* elevation ranges (populations). We created a mixed effects model with aboveground biomass growth (the difference between final and initial biomass estimates from the allometric equation) as the response, genotype and population as a random effects, and soil location, soil treatment, and soil location × soil treatment as fixed effects. An interaction between soil location and soil treatment would be evidence that trees in the greenhouse grow differently with conditioned versus unconditioned soil inoculum based on soil biotic effects. We created similar models separated by interior and edge range locations to test if growth responses to soil locations and treatments vary across elevation gradients. In the second approach, we constructed a GLM with mean aboveground biomass of trees within the same conditioned soil treatment (i.e., pooled at the elevation site level) as the response, the relative abundance of dominant microbial taxa (*Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Synergistia*) from the corresponding elevation site, range location, and microbial abundance × range location as fixed effects. Here, correlations between microbial abundance in field soil (collected in 2014) and tree growth with the corresponding experimental soil inoculum (collected in 2013) would suggest that *P. angustifolia* conditioning of their soil biotic environment is related to variation in growth across soil treatments.

Range shift PSF

We tested hypothesis 3 (that plant-soil conditioning of soil biota feeds back to alter the performance of *P. angustifolia* under predicted range shift scenarios) by experimentally shifting trees to higher elevation soils in the greenhouse (Fig. 4.1e). Specifically, trees from replicate genotypes were grown in soil inoculated with live or sterile soil communities that were conditioned at the next highest elevation site by *P. angustifolia* genotypes. Trees from the edge of elevation ranges were grown in soil inoculated with live or sterile communities from soils that were collected beyond current range limits but still within the dispersal distance of *P. angustifolia* (Braatne et al. 1996). As a result, we categorized range shift feedbacks using the following delineations that represent this basic scenario by which *P. angustifolia* range shifts might occur (Hargreaves et al. 2014; Fig. 4.1e): Interior-Interior, Interior-Edge, and Edge-Beyond. Specifically, lower elevation interior genotypes shifted to higher elevation soils while remaining within the interior of the range (I->I), highest elevation interior genotypes shifted to edge soils (I->E), and edge genotypes expanded beyond current range limits (E->B). This design allowed for both 'home'-'away' and 'local'-'foreign' approaches to compared plant performance (Blanquart et al. 2013). The first is soil-centric: 'home' refers to conditioned soils from a given elevation site while 'away' refers to conditioned soils from the next highest elevation site (or unconditioned beyond soils) within each population; the second is plant-centric: 'local' refers to trees from a certain elevation site while 'foreign' refers to trees from the next lowest elevation site.

We assessed range shift PSF in the greenhouse using the 'home'-'away' approach where the growth of trees with soil communities conditioned at their current elevation sites ('home') was compared to the average growth of trees with soil communities conditioned at the next highest elevation site that was within or beyond range limits ('away'). Range shift PSF were also assessed by comparing the performance of 'local' genotypes with their conditioned soil community to the average growth of 'foreign' genotypes from the next lowest elevation site in the same soil community. We quantified PSF as the log response ratio between aboveground biomass growth using 'home'-'away' comparisons (N = 219) and 'local'-'foreign' comparisons (N = 169), where larger plant growth in 'home' versus 'away' soil or 'local' versus 'foreign' genotypes would be evidence of positive feedbacks (i.e., positive response ratio). Based on these calculations, neutral feedbacks would arise with similar tree growth between the two categories being compared (which could be a result of simply no feedbacks or a similarity of soil communities and their reciprocal effects on plant growth between soil inoculum treatments). We created mixed effects models with range shift PSF as the response, range shift category, soil treatment, and range shift × treatment as fixed effects, and genotype and population as random effects. To follow, we analyzed whether feedback effects differed from zero using one-sample t-tests (Brinkman et al. 2010). Different patterns of PSF across range shift categories and soil treatments would suggest that future *P. angustifolia* migratory responses to climate change could be influenced by the legacy of biotic plant-soil interactions. Range shift PSF might also occur for trees grown with soil communities collected from ~150 m away from the riparian zone within the same elevation band (i.e., equivalent to the distance between edge trees and beyond soils). Although we did not explore this possibility because our experimental approach focused on upward range shifts within the riparian zone, the obligate riparian life history of *Populus* spp. suggests this is an improbable type of range shift (Braatne et al. 1996).

Feedback mechanisms

We compared soil variation in the field to plant performance across soil treatments in the greenhouse to test hypothesis 4 (that feedback effects should be related to variation in soil conditioning). The strength and direction of feedbacks should be related to variation in soils because feedbacks result from changes to the soil environment (Bever et al. 1997, van der Putten et al. 2013, Ke et al. 2015). We measured variation in soil conditioning two ways: differences between conditioned and unconditioned soil traits at the same elevation, and differences in conditioned and unconditioned soil traits between elevation sites. This allowed us to test how soil conditioning relates to feedback effects between conditioned-unconditioned soil locations (i.e., at the same elevation site), as well as how conditioned soil differences versus natural soil variation across elevation gradients relates to range shift PSF.

We tested whether the level of soil conditioning relates to the strength and direction of PSF between interior and edge range locations. We calculated soil conditioning effects as the log response ratio between conditioned and unconditioned soil traits (C, N, and pH) for each elevation site. Similarly, we calculated feedback effects as the log response ratio between aboveground biomass growth in live conditioned soils versus mean growth in live unconditioned soils for each respective elevation site within populations (Brinkman et al. 2010). We then constructed a Restricted Estimates Maximum Likelihood (REML) model with PSF as the response, soil conditioning, range location, soil conditioning × range location as fixed effects, and population as a random effect. If soil conditioning effects positively correspond to feedback effects, then we infer that trees which increase the average conditioned soil C, N, or pH levels relative to unconditioned soils at that site might directly or indirectly affect soil communities that generate positive feedback effects.

For range shift PSF, we examined whether differences in soil properties across elevation sites might explain the strength and direction of soil biotic feedbacks when plants were growth with lower ('home') versus higher ('away') conditioned soil communities. Differences in conditioned soil communities across elevation sites could arise from variation in plant-soil conditioning, natural soil changes that occur with elevation, or a combination of these factors, As a result, we calculated standard differences in both conditioned and unconditioned soils across elevation gradients as the log response ratio between lower versus higher elevation soil C, N, and pH values (corresponding to each interior-interior, interior-edge, and edge-beyond range shifts within populations). If conditioned and unconditioned soil C, N, and pH differences across elevations co-vary, then we calculated the residual variation of the linear model between conditioned and unconditioned soil differences to account for variation in conditioned soil differences that may be due to natural differences in soil environments across elevation (i.e, isolate how soil conditioning differences are related to range shift PSF). We then constructed a REML using 'home'-'away' PSF from live soil inoculum treatments as the response, residual conditioned soil differences, range shift category, and residual conditioned soil differences × range shift category as fixed effects, and population as a random effect. If residual differences in conditioned soil environments from the field predict range shift PSF in the greenhouse experiment, then we infer that differences in plant-soil conditioning change soil communities that reciprocally affects *P. angustifolia* performance. All analyses were conducted using R version 3.3.1 (R Core Team 2013), where data met assumptions of normality and variance among groups met assumptions of homoscedasticity using Levene's Test.

Results

Soil conditioning and feedbacks

In support of hypothesis 1, we found the effect of *P. angustifolia* soil conditioning in the field (based on the paired comparisons between conditioned versus unconditioned soil locations; Fig. 4.1c) is greater for interior versus edge trees across current elevation ranges. Measures of total soil C, total soil N, and pH had significant soil location effects (conditioned versus unconditioned), but little or no effects of range location (interior versus edge) or soil location × range location interactions (see Supplementary Table 2). Conditioned soils that were collected beneath trees had higher amounts of C ($F_{1,50} = 9.0$; $p < 0.01$), N ($F_{1,50} = 4.8$; $p = 0.03$), and more basic pH ($F_{1,50} = 6.4$; $p = 0.02$) compared to unconditioned soils (Fig. 4.2a-c). However, post-hoc analyses reveal that interior trees are responsible for creating these effects. Within range interiors, conditioned soil C was 52% higher (mean = 5.7 ± 0.7 standard error [SE]; $z = 3.5$, $p < 0.01$), conditioned soil N was 40% higher (mean = 0.33 ± 0.04 SE; $z = 2.8$, $p = 0.03$), and conditioned soil pH was 5% higher (mean = 5.6 ± 0.04 SE; $z = 4.5$, $p < 0.001$) relative to paired unconditioned soils. By comparison, edge trees had no conditioning effect on soil C, N, or pH, suggesting there may be less plant-soil conditioning at range limits. In surveying the diversity of dominant rhizosphere bacteria (using next-generation 16S amplicon sequencing) associated with a representative subset of *P. angustifolia* trees in the field, only the relative abundance of *Betaproteobacteria* (7.5% overall) showed a difference in conditioned soils between interior and edge range locations (Fig. 4.2d). *Betaproteobacteria* abundance was 63% higher in edge (mean = 0.09 ± 0.01 SE) versus interior tree-conditioned soils ($F_{1,11} = 6.4$, $p = 0.05$). This observation could be due to differences in plant-soil conditioning or to site differences between range interiors and edges. No other dominant bacterial class (including *Actinobacteria* [9%], *Alphaproteobacteria* [19%], *Gammaproteobacteria* [18%], or *Synergistia* [4%]) differed by range location (see Supplementary Table 3). Moreover, differences in conditioning between range locations could be related to tree age since there is a marginal effect of interior trees being 20.7% larger (and presumably older) than edge trees ($F_{1,210} = 3.7$, $p = 0.06$), and this pattern is consistent across populations (population × range location: $F_{6,210} = 1.4$, $p = 0.2$). Overall, these results show that the conditioning of soil nutrients, pH, and possibly soil biota by *P. angustifolia* trees in the field is greater at range interiors than range edges and may depend on tree age.

In support of hypothesis 2, we found in our greenhouse experiment that tree-conditioned soil communities (collected from the field), created significant positive feedback effects for *P. angustifolia* trees. We examined feedback effects by comparing aboveground biomass growth (final-initial biomass) in the greenhouse in response to experimental soil biotic treatments and in relation to conditioned soil communities from the field (initial tree biomass was calculated from an allometric equation that explained more than 98% variation in biomass; see Supplementary Information, Supplementary Fig. 1, and Supplementary Fig. 2). There was no effect of soil location ($F_{1,359} = 0.6$, $p = 0.4$) or soil treatment (live versus sterile soil inoculum; $F_{1,391} = 0.2$, $p = 0.6$) on aboveground biomass growth, indicating that these factors alone did not improve or reduce tree performance in the greenhouse (see Supplementary Table 4, Supplementary Fig. 3).

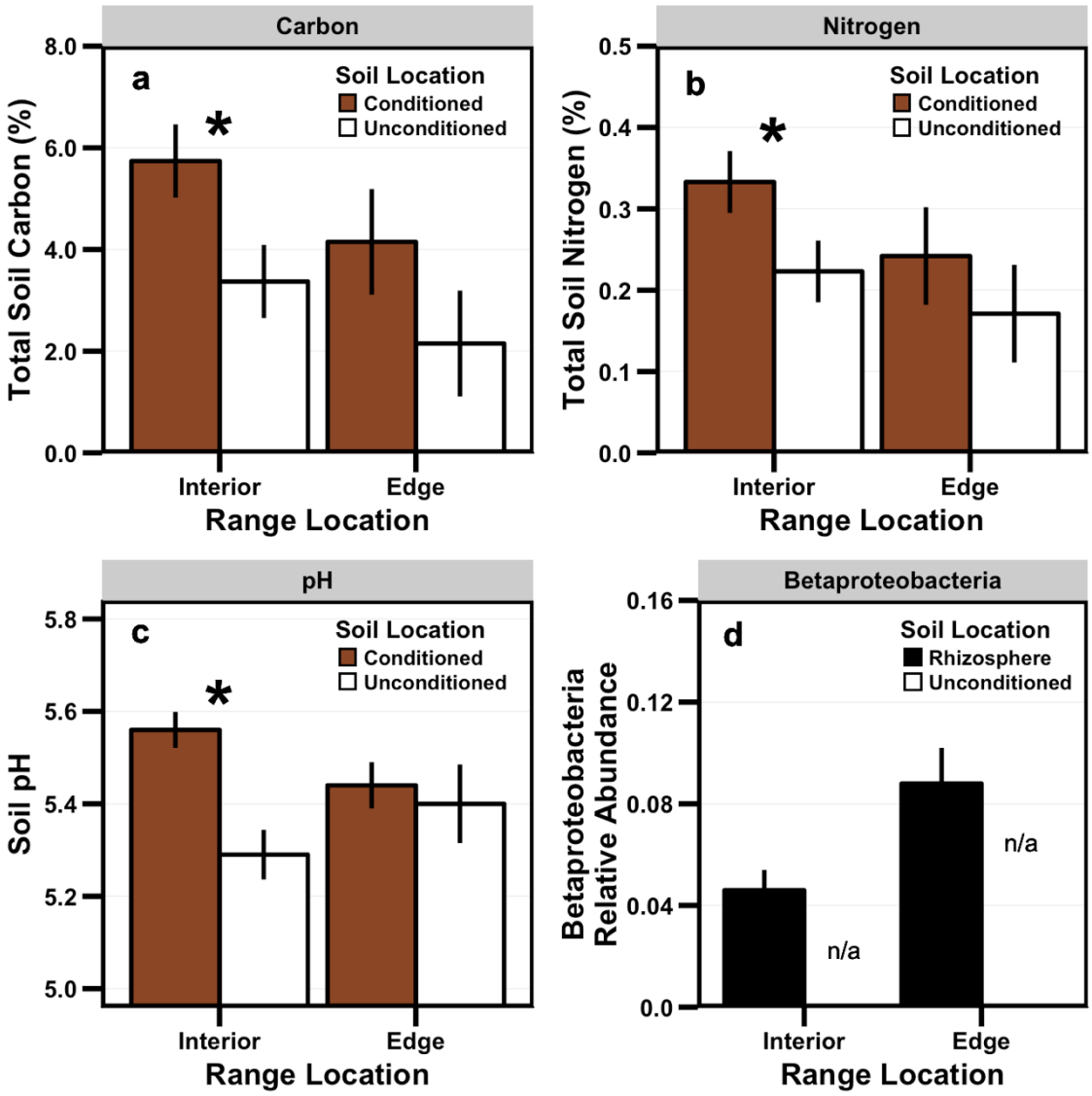

Figure 4.2. Plant-soil conditioning in the field varies by range location. Soils conditioned beneath interior *P. angustifolia* trees have higher soil carbon (**a**), soil nitrogen (**b**), and soil pH (**c**) compared to unconditioned interspace locations away from trees. In contrast, no conditioning effects were found at the range edge. The relative abundance of *Betaproteobacteria* (**d**) in soils collected beneath *P. angustifolia* trees was found to be lower within the range interior compared to the range edge (unconditioned soils outside of the influence of trees were not sequenced). It should be noted that although these soils were collected directly from the rhizosphere and range location differences may be caused by variation in conditioning, it is also possible that *Betaproteobacteria* are more abundant at higher elevations regardless of *P. angustifolia* conditioning effects. Bars depict least square means ± 1 standard error.

However, there was a significant soil location × soil treatment interaction ($F_{1, 368} = 4.0$, $p = 0.05$), whereby trees grew an average of 24% larger with live conditioned soil relative to live unconditioned soil (Fig. 4.3a). Removing field soil biota in sterile gamma irradiated inoculum also removed the effect of soil location on *P. angustifolia* growth, suggesting that biotic variation in live soil inoculum from the two soil locations (plant conditioned vs. un-conditioned, interspace soils) led to growth differences. We found no interaction between soil location and soil treatment when interior trees (i.e., trees collected from the interior of their population) and edge trees (i.e., trees collected from the edge of their population) were analyzed individually (see Supplementary Table 4). In other words, positive feedbacks were not limited to plant-soil community interactions from any particular range location, but instead only appeared as a range-wide phenomenon. This also means that while there was little evidence that edge trees condition their soil nutrient or pH environments in the field, they do alter soil communities in a way that affects their performance.

By examining how specific microbial taxa relate to tree performance, we found that higher *Betaproteobacteria* abundance in conditioned field soils was positively correlated with greater aboveground biomass growth of trees in the corresponding live soil inoculum treatment ($r^2 = 0.5$, $F_{1,11} = 11.1$, $p = 0.01$; Fig. 4.3b). Additionally, there was no effect of range location ($F_{1,11} = 1.7$, $p = 0.2$) or interaction between *Betaproteobacteria* and range location ($F_{1,11} = 0$, $p = 0.9$), which suggests that variation in how strongly *P. angustifolia* trees influence the abundance of *Betaproteobacteria* community members may lead to differences in future plant performance regardless of range location. No other dominant microbial taxon was correlated with tree performance in the greenhouse experiment, and there were no interaction effects of microbial abundance and range location on tree growth (see Supplementary Table 5). These results show that plant-soil conditioning of soil communities (specifically *Betaproteobacteria*) relates to the overall positive feedback effect on *P. angustifolia* performance across elevation ranges.

Range shift PSF

To test hypothesis 3, we compared range shift PSF across three categories (Fig. 4.1e): lower elevation interior trees grown with higher elevation soil communities but still within the interior of the range (I->I), highest elevation interior trees grown with edge soil communities (I->E), and edge trees grown with soil communities from beyond current range limits (E->B). This allowed us to compare range shift PSF using 'home'-'away' and 'local'-'foreign' approaches (i.e., soil-centric versus plant-centric approaches for analyzing range shift responses). Specifically, we calculated the log response ratio between either: i) trees grown with soil communities conditioned at their current elevation sites ('home') versus trees grown with soil communities conditioned at the next highest elevation site that was within or beyond range limits ('away'), or ii) trees grown with soil communities conditioned at their current elevation sites ('local) versus lower elevation trees grown with the same soil community as 'local' trees (foreign). In support of hypothesis 3, we found that variation in plant-soil conditioning creates feedback effects by simulating *P. angustifolia* shifts upslope with positive range shift PSF at lower elevations and negative range shift PSF at higher elevations (see Supplementary Fig. 4). For 'home-away' comparisons (Fig. 4.4a), we found significant effects of range shift category ($F_{1,214} = 13.1$, $p < 0.001$) and a marginal interaction of range shift category × soil treatment on range shift PSF ($F_{1,212} = 2.3$, $p = 0.10$).

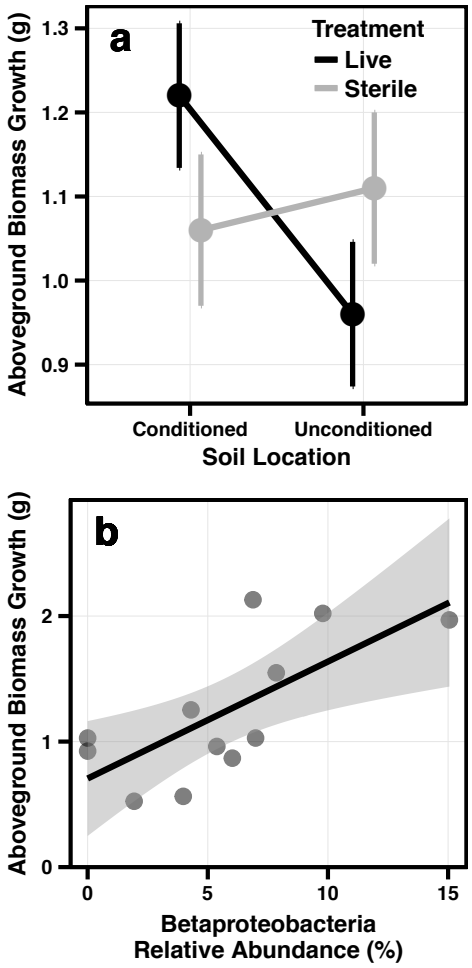

Figure 4.3. Indicative of a positive biotic plant-soil-biotic feedback, tree growth relates to conditioned soil communities and the relative abundance of *Betaproteobacteria*. (**a**) Higher aboveground biomass growth with conditioned versus unconditioned, soil biotic communities at the same elevation (i.e., positive plant-soil feedbacks) was found for *P. angustifolia* trees in live soil treatments (black). No growth differences were found when soil inoculum was sterilized with gamma irradiation (grey). Symbols depict means ± 1 standard error. Growth refers to the difference in total aboveground biomass between the beginning and end of the experiment. (**b**) Aboveground biomass growth in conditioned soils is positively related to the relative abundance of *Betaproteobacteria* from field soils. Points represent biomass growth in the experiment averaged at the level where soil inoculum treatments were pooled (i.e., elevation site); microbial data is from field soils collected beneath a subset of genotypes (from Blue River, AZ, San Juan River, CO, and Snake River, WY populations) corresponding to the same elevation sites.

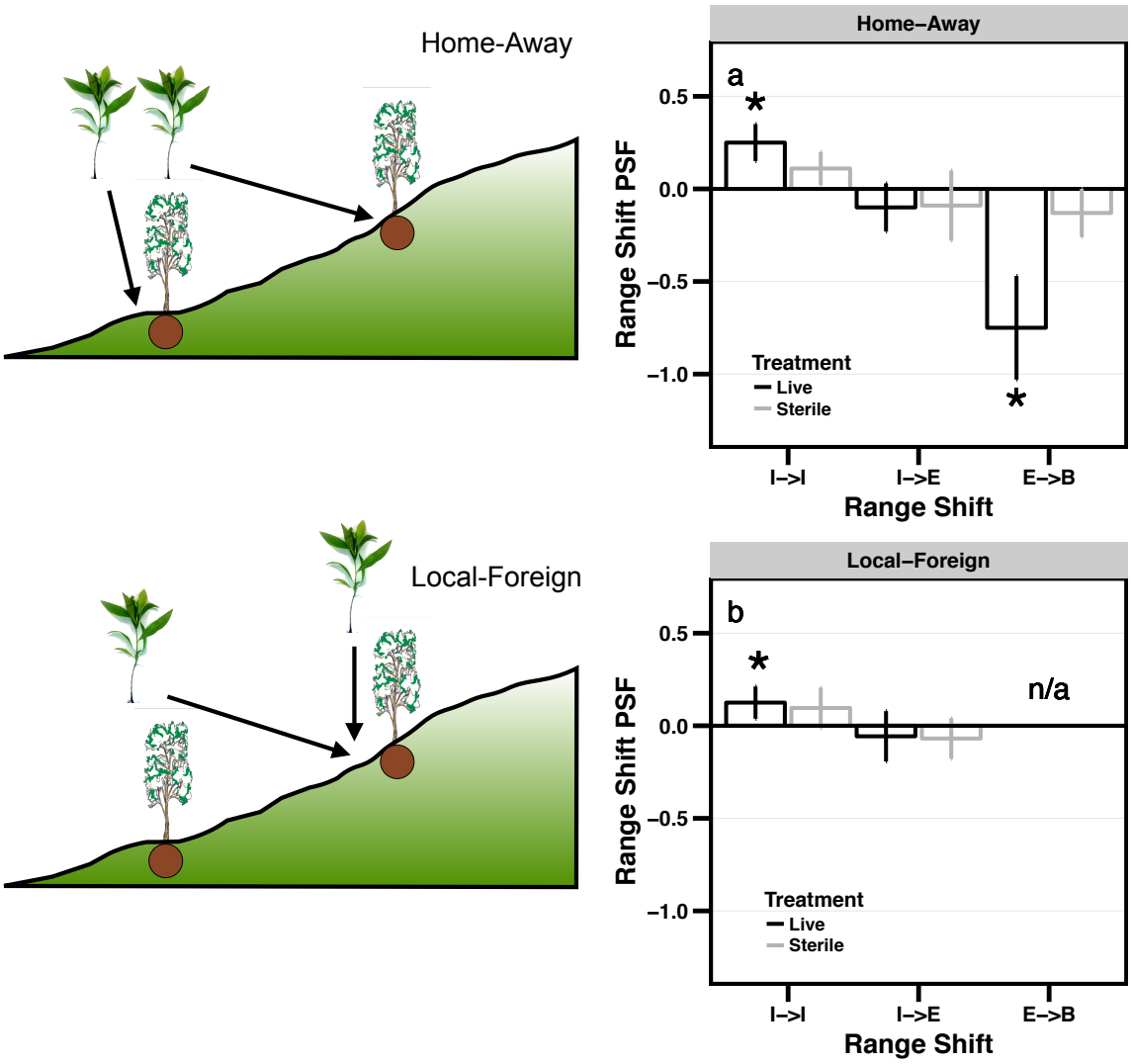

Figure 4.4. Range shift plant-soil feedbacks (PSF) change from positive to negative as trees are simulated to move upwards in elevation by interacting with soil communities from higher sites. (**a**) 'Home'-'away' comparisons of plant growth with live soil communities (black bars) at home elevations versus higher elevations showed that interior trees with higher elevation interior soil communities (I->I) had positive plant-soil feedbacks (PSF), interior trees with edge communities (I->E) had neutral PSF, and edge trees with communities from beyond range limits (E->B) had negative PSF. (**b**) Using 'local'-'foreign' comparisons between higher versus lower elevation tree growth with the same soil community, positive PSF were found for comparisons of interior shifts within the interior I->I, and neutral PSF for interior shifts moving to the range edge (I->E). Neutral feedbacks were found for all sterile treatments, suggesting that any non-zero PSF are driven by soil communities. Sterilizing soil communities (grey bars) led to neutral PSF effects across all range shift categories and comparison types, indicating the importance of the soil biotic effects on tree growth. Bars depict means ± 1 standard error.

Although these effects were not significant for 'local'-'foreign' comparisons (see Supplementary Table 6), the general patterns of range shift PSF for 'local'-'foreign' comparisons matched those from using the 'home'-'away' approach (Fig 4.4b).

Interior trees from lower elevations created positive range shift PSF. Trees from the range interior had larger growth with live soil inoculum from 'home' sites than with live inoculum from higher elevation 'away' sites within the interior (I->I; Fig. 4.4a), which resulted in a positive average feedback effect of 0.31 ± 0.07 SE ($t_{1,57} = 2.4$, $p < 0.01$). The 'local'-'foreign' comparison for I->I range shifts also showed a positive feedback (mean = 0.13 ± 0.09). Specifically, 'local' interior trees had higher growth than 'foreign' interior trees when grown with the same live soil communities (Fig. 4.4b), which trended towards positive feedback ($t_{1,63} = 1.4$, $p = 0.08$). Sterilizing the same soil inoculum resulted in neutral feedback effects for both 'home'-'away' ($t_{1,50} = 1.2$, $p = 0.12$) and 'local'-'foreign' comparisons ($t_{1,22} = 0.1$, $p = 0.9$), suggesting that soil communities play a role in shaping the positive feedback effects from live soil inoculum treatments.

Trees at the transition from interior to edge of *P. angustifolia* ranges (I->E) performed equally with soil communities from either range location. Interior trees did not grow significantly better or worse with live 'home' soil communities compared to higher elevation 'away' communities from the range edge ($t_{1,28} = 0.8$, $p = 0.2$; Fig. 4.4a). In addition, soil communities conditioned by 'local' edge trees in the field did not enhance the growth of edge trees more than 'foreign' interior trees ($t_{1,26} = 0.4$, $p = 0.3$; Fig. 4.4b). There were no differences for I->E range shift PSF between live and sterile treatments ('home'-'away': $t_{1,26} = 0.5$, $p = 0.3$; 'local'-'foreign': $t_{1,26} = 1.0$, $p = 0.2$), indicating that higher elevation interior and edge tree growth may be less dependent on feedbacks from soil communities with which they interact. Together, this shows that the highest elevation trees within the interior performed equally well as edge trees regardless of whether different soil communities are present.

Edge trees experienced negative range shift PSF when simulating migration beyond current range limits. Edge trees had reduced growth in soils containing live 'home' communities compared to live soils from beyond current range limits (E->B), resulting in a negative mean range shift feedback of -0.75 ± 0.28 SE ($t_{1,26} = 2.6$, $p = 0.01$; Fig. 4.4a). Consistent with our previous findings, soil communities appear to create this feedback effect since an overall neutral feedback was found using sterile inoculum treatments ($t_{1,26} = 1.0$, $p = 0.2$).

Feedback mechanisms

In support of hypothesis 4, we found that plant-soil conditioning effects were related to the strength and direction of PSF across the elevation range (see Supplementary Table 7). When predicting feedback effects generated from soil conditioning (quantified as the log response ratio of soil traits between conditioned versus unconditioned soil locations at the same elevation site in the field; Fig. 4.1c and Fig. 4.1d), there was a significant interaction between soil C conditioning and range location ($F_{1,76} = 5.2$, $p = 0.03$; see Supplementary Fig. 5a), and soil N conditioning and range location ($F_{1,73} = 4.8$, $p = 0.03$; see Supplementary Fig. 5b). Feedback effects in the greenhouse were positively correlated with soil C conditioning ($r^2 = 0.1$, $p = 0.01$) and soil N conditioning ($r^2 = 0.1$, $p = 0.01$) for interior trees. As a result, the most positive feedback effects for interior trees relate to the highest levels of soil C and N conditioning by interior trees in the field. Soil C and N conditioning by edge trees did not predict PSF (see Supplementary Table 7), which is likely related to the overall lack of soil conditioning detected for edge trees in the field. Although interior trees have a large impact on soil acidity in the field, pH conditioning did not

predict PSF variation regardless of range location (see Supplementary Fig. 5c; Supplementary Table 7). Moreover, *Betaproteobacteria* abundance was negatively correlated with conditioned soil total C ($r^2 = 0.44$, $p = 0.02$) and N ($r^2 = 0.38$, $p = 0.03$), but not pH ($r^2 = 0.05$, $p = 0.5$), further illustrating a link between soil nutrient conditioning and the abundance of specific biota that relate to plant performance (see Supplementary Fig. 6a-c).

Range shift PSF were predicted by conditioned soil differences across elevation gradients (quantified as the log response ratio of lower versus higher conditioned soil traits in the field). Conditioned and unconditioned soil differences across elevation positively co-varied (C: $r^2 = 0.30$, $p < 0.001$, N: $r^2 = 0.45$, $p < 0.001$; pH: $r^2=0.15$, $p < 0.001$), so we first removed variation attributed to natural soil changes across elevation (unconditioned soil differences) and used the residuals of conditioned soil differences (i.e., soil differences across elevation that result from plant-soil conditioning). Residuals of conditioned soil C and N differences did not predict range shift PSF responses overall (see Supplementary Table 8), and there were no soil difference × range shift category interactions. Residual conditioned soil pH differences were negatively correlated with range shift PSF overall ($F_{1,113} = 15.7$, $r^2 = 0.17$, $p < 0.001$), and we found a significant soil pH difference × range shift category interaction ($F_{1,112} = 3.6$, $p = 0.03$). Examination of range shift categories individually showed non-linear relationships between I->E feedbacks and residuals of conditioned soil C and N (Fig. 4.5d-e). Here, range shift PSF were most negative (i.e., most favorable for upward shifts) when conditioned soil differences were closest to zero, meaning tree conditioning was relatively "matched" between elevation sites for soil C ($r^2 = 0.30$, $p = 0.002$) and N ($r^2 = 0.26$, $p = 0.02$). Residuals of conditioned soil pH differences also showed a negative correlation with I->E feedbacks (Fig. 4.5f), where increasingly dissimilar pH environments led to more negative range shift PSF ($r^2 = 0.21$, $p = 0.01$). For edge trees shifted to soil communities beyond range limits (E->B), there was a non-linear relationship between residual conditioned N differences and range shift feedbacks ($r^2 = 0.20$, $p = 0.05$) where more "matched" soil N environments had more positive feedback effects (i.e., unfavorable for range shifts) (Fig. 4.5h). In contrast, greater differences in residual conditioned soil pH was related to more negative range shift PSF ($r^2 = 0.20$, $p = 0.02$) that would favor upwards range expansion (Fig. 4.5i). Finally, aboveground biomass growth in the experiment was unrelated to the different soil inoculum treatments C, N, or pH levels from the field (Supplementary Table 9), which shows that feedback effects predicted by soil conditioning were not dependent on any growth correlations with field soil characteristics associated with the various treatments.

Discussion

This study demonstrates that plant-soil interactions affect the performance of a widespread tree species along elevation gradients and how these dynamics may change under predicted range shifts. We show that plant-soil conditioning in the field varies between the interior and edge of *P. angustifolia* elevation ranges, with interior trees exerting much greater changes to soil nutrient pools and pH levels than edge trees, but edge trees containing potentially more bacterial taxa (e.g., *Betaproteobacteria)* within their rhizosphere than interior trees. These conditioning differences relate to soil communities that feed back and positively affect the growth of trees in the greenhouse experimental manipulations.

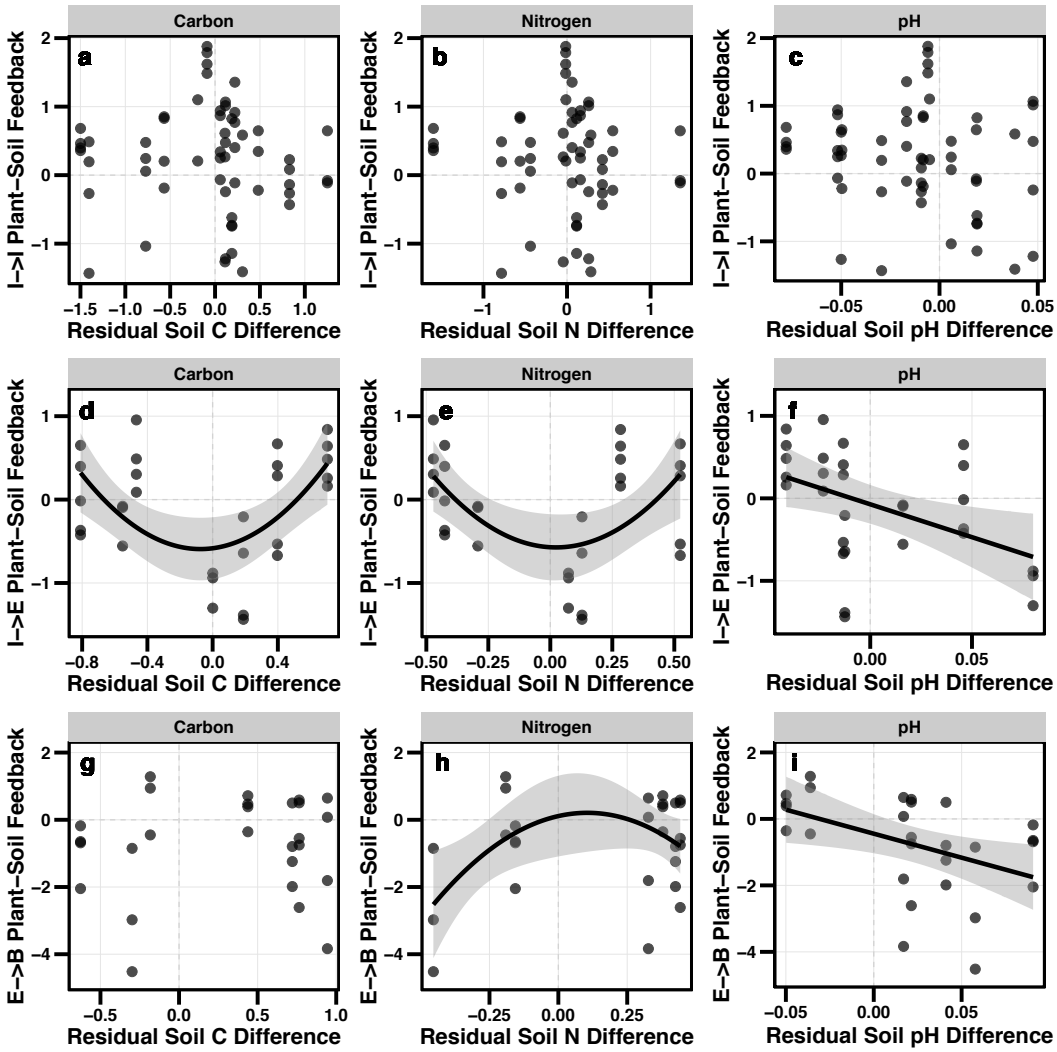

Figure 4.5. Range shift plant-soil feedbacks (PSF) are related to residual variation in conditioned soil differences between elevation sites after accounting for natural soil variation (unconditioned soils) across elevation gradients. Range shift PSF variation for interior trees grown with higher elevation soil communities (I->I) were not predicted by differences in residual conditioned soil carbon (C), nitrogen (N), or pH across elevation (**a-c**). For interior trees experimentally shifted to edge soil communities (I->E), residual C and N in conditioned soils had non-linear patters where the most negative biotic PSF corresponded to the least amount of residual soil conditioning (**d-e**). Residual pH differences in conditioned soil was negatively related to I->E range shift PSF (**f**). Residual conditioned soil C differences were unrelated to feedbacks at the highest range shift category (**g**) where edge trees were grown with soil communities from beyond elevation range limits. However, residual soil N showed a non-linear response with lowest E->B range shift PSF with the most and least amount of conditioned N differences across elevation (**h**), and residual soil pH differences negatively predicted E->B feedbacks. Regression lines depict significant trends with shaded area representing 95% confidence intervals. Residual conditioned soil differences that differ from zero reflect mismatches in conditioned soil environments across elevation.

In simulating *P. angustifolia* range shifts by manipulating plant-soil community interactions in the greenhouse, we show that range shift PSF change from positive to negative as trees are grown with soil communities from the next highest elevation site, and higher elevation interior trees outperform lower elevation interior trees with the same soil community. Moreover, we show novel empirical evidence that the amount of plant-soil conditioning and soil "matching" across elevation sites predicts feedback responses and range shift PSF, illustrating an important connection between abiotic and biotic soil environments modified by trees that occur simultaneously to determine PSF. Collectively, our results are among the first that illustrate the potential consequences of intraspecific plant-soil feedback variation across elevation gradients. Specifically, these patterns suggest that plant-soil biotic interactions could impede range shifts at lower elevations by reducing the performance of plants that shift upwards in elevation relative to plants that remain at lower sites. In contrast, interactions at upper range limits might propel expansions past current range boundaries if abiotic conditions are not limiting and increased growth at these higher sites accelerates upslope movement. Although plant interactions with soil communities are just one factor among many that could alter the pace and direction of plant range shifts, examining the biotic mechanisms that drive plant-soil linkage and feedback differences across elevation ranges will shed light on the relative importance of PSF in shaping plant responses to warming environments.

Plants modify their local soil environment by regulating the quantity and quality of resources that are delivered belowground (Bardgett and Wardle 2010), and our study provides rare empirical evidence that variation in the amount of soil conditioning directly corresponds to PSF. We find that *P. angustifolia* trees in the field condition their soils more at range interiors than range edges, and these conditioning differences may be related to tree age. Moreover, our study shows that soil conditioning by *P. angustifolia* trees in the field creates an overall positive feedback effect on tree growth in the greenhouse, and that this effect likely comes from soil biotic variation. These positive soil biota effects on plant performance are similar to past experiments using *P. angustifolia* plants of comparable size which found larger growth and reduced mortality with soil communities conditioned by *P. angustifolia* relative to different *Populus* spp. (Pregitzer et al. 2010) and the same versus different *P. angustifolia* genotypes (Smith et al. 2011), even when their own soils were less fertile overall. Soil community members related to these results were not identified. Our findings advance this work by illustrating a central but rarely shown principle of plant-soil research: that the amount by which plants change soil environments relates to the strength and direction of feedback effects (Ke et al. 2015). In our case, greater soil C and N conditioning within the interior positively corresponded to feedback effects between conditioned and unconditioned soil locations in live treatments. In other words, trees from interior elevation sites that improved the average soil nutrient environments in the field relative to adjacent unconditioned soil grew better with live soil communities from conditioned rather than unconditioned soil locations. We also found that quantifying the difference in conditioned soil properties across elevation gradients predicted the outcomes of plant-soil community interactions with simulated range shifts. Greater standardized differences in soil pH (and likely microbial composition; Fierer and Jackson 2006) between lower and higher sites increased tree growth in the greenhouse with higher elevation biota than with their lower elevation 'home' biota (i.e., negative range shift PSF). In certain cases, greater soil C and N "matching" between range shift sites, shown with standardized soil differences near zero, were related to the most negative range shift PSF (favorable for upward shifts), but also the most positive range shift feedbacks (unfavorable for upward shifts). These non-linear relationships

provide some of the first empirical evidence to support theoretical models that predict the importance of soil "matching" and "mismatching" for determining plant performance and the conditions under which feedbacks can evolve (Schweitzer et al. 2014). Our results also suggest that ecological niche models that incorporate soil factors may capture part of plant-soil-biotic interactions that could be important for defining future plant ranges (Coudon et al. 2006, Beauregard and de Blois 2014).

We identify *Betaproteobacteria* as a soil community member related to plant-soil nutrient conditioning and biotic PSF observed in our study while acknowledging that further work is required to determine causality for this plant-soil-microbe interaction. Members of *Betaproteobacteria* (including nitrifiers and diazotrophs; Hawkes et al. 2007) are involved in key steps of the N-cycle and are dominant in the rhizosphere of *Populus* (Gotell et al. 2011) and many other plant species (Hawkes et al. 2007), though they are unlikely to have the same ecological function across all populations or even within the taxonomic level of class in this study. Nonetheless, we do find that their relative abundance increases from range interiors to range edges (which may be due to conditioning or site differences), they are positively correlated with *P. angustifolia* tree growth, and they are negatively related to conditioned soil C and N levels. Moreover, prokaryotes are just one component of belowground diversity that impacts plant performance, and root-mycorrhizal interactions could modify these patterns by directly affecting plant growth through the economy of plant photosynthate and soil mineral nutrient exchange, or indirectly through competition with saprotrophic organisms for N resources (Johnson et al. 2010, Bardgett and Wardle 2010). However, even if some plant-associated microbes keep pace with their host range shifts (Peay et al. 2016), it is possible that plants and microbes will move asynchronously in response to warming as systems gain and lose species (e.g., plant migration success/failure and soil microbe migration success/failure may occur in all combinations). An important future direction is to experimentally test how different aspects of soil diversity influence *P. angustifolia* performance and range dynamics under experimental global change conditions and determine which C and N sources (e.g., litter traits and root exudates) are important for structuring soil communities in these systems.

Our study offers an important new direction linking PSF with plant and ecosystem responses to climate change by building on previous work using latitude transects and testing whether similar plant-soil trends emerge along elevation gradients. The majority of evidence to date shows that negative range shift PSF allow for plant migrations past range boundaries (van der Putten et al. 2013), such as how seedlings from eight temperate tree species in southern regions grew better with soil communities from higher latitude conspecifics compared to lower latitude heterospecifics (McCarthy-Neumann and Ibańez 2012). Similarly, we found negative range shift PSF as edge plants were simulated to expand beyond their current elevation range limits (increased performance with soil communities from beyond range limits relative to range edge soil communities). Since plant communities in 'beyond' sites vary across *P. angustifolia* populations in this study (southern populations transition to pinyon pine and juniper forests while northern populations transition to aspen and spruce forests past their respective elevation range limits), the overwhelmingly negative range shift PSF of edge trees grown with beyond soils (E->B) is more likely due to pathogen accumulation at most edge sites because it is unlikely that beneficial soil biotic communities are present across the different 'beyond' soils in this study. Interestingly, even though *Betaproteobacteria* were more abundant at edge versus interior sites and positively related to aboveground biomass growth, edge trees still performed better with 'beyond' soil biota than their home edge soil communities. More work is needed to test the

generality of negative PSF at elevation range limits and whether similar mechanisms from latitude studies can explain these effects (i.e., pathogen build-up and enemy escape). At the opposite end of species' ranges, warmer populations at the trailing edge are predicted to be locally adapted to harsher environments than populations from cooler conditions near the species' distribution center (Woolbright et al. 2014), and these trailing edge populations may rely more heavily on positive plant-soil-biotic interactions (e.g., mycorrhizal associations that alleviate water stress with increasing drought) in order to adapt and persist (Jump et al. 2009). Our results support these predictions by showing that lower elevation trees (at or near the trailing edge of *P. angustifolia* elevation ranges) have positive range shift PSF mediated by soil communities (possibly related to *Betaproteobacteria*, but also other taxa not identified in this study). Further experiments comparing soil diversity between these lower elevation sites to trailing edge populations and tests of whether similar community members improve plant performance under harsh conditions are paramount to understand if soil biota can mitigate increasing environmental stress for *P. angustifolia*.

Elevation transects could be a valuable but rarely used tool for plant-soil feedback research, and our results are broadly consistent with patterns from biotic PSF studies across spatial and temporal gradients (Kardol et al. 2006). For example, it is unclear whether growth patterns of 2-year-old trees correspond to performance differences of longer-lived *P. angustifolia* in the field (e.g., whether conditioning effects or feedback responses change with plant ontogeny or environmental changes across the sites; Kardol et al. 2013). Because nutrient conditioning positively relates to feedback responses in our study, perhaps continual C/N conditioning by edge trees might gradually shift the range shift feedback from negative to positive if a decomposition-related feedback becomes more important for plant growth over time. In addition, we know little about whether potting soil impacted how inoculated soil communities developed or plant-soil biotic interactions were formed under greenhouse conditions. However, our manipulation with live and sterile inoculum shows that soil biotic effects remain worthwhile to consider and test under field conditions, and our results predict that i) negative range shift PSF might permit *P. angustifolia* expansions past their current range limits as they move away from their conditioned soil communities that may harbor less mutualistic taxa or higher pathogen loads, and ii) lower elevation interior trees might be inhibited from shifting upwards to track changing temperatures (due soil biota that reduce tree growth relative to lower elevation sites and outperformance by higher elevation interior trees in the same soil community). What remains unknown is the influence of biotic PSF on plant range dynamics relative to the strength and duration of other environmental forces in the field. It could be that plant-soil interactions are short-term regulators of plant performance and will not play a significant role determining the long-term success or failure of plant range shifts; in contrast, it may be that PSF accumulate and work synergistically with other factors to impede or improve plant range shifts. In this way, investigating plant-soil linkages across elevation gradients may serve as a similarly helpful space-for-time tool as is widely used by climate change studies (Sundqvist et al. 2013), and future studies conducted in the field will continue to benefit our understanding and predictions of how PSF combine with other environmental factors to influence plant range dynamics.

In conclusion, these field observations and experimental data represent an empirical case demonstrating the ecological and potentially evolutionary consequences of PSF and their influence on plant range shift responses to global climate change. Examining intraspecific plant-soil linkages across multiple elevation ranges of a foundation tree species' provided a strong test for how reciprocal effects between plants, soils, and soil communities vary on the landscape. Our

findings illustrate how plant-soil conditioning is greater within the *P. angustifolia* range interior than edge, and that conditioning of soil communities created an overall positive feedback effect on plant growth. Moreover, we show that simulating upward shifts to higher soil communities led to positive feedbacks at lower elevations (adverse range shift outcomes) and negative feedbacks past current range limits (favorable range shift outcomes). These results are congruent with previous work along plant succession and latitude gradients, and advance understanding on the role of PSF in plant range shifts (van der Putten 2012) and their ecological significance when examined across environmental gradients (Van Nuland et al. 2016, Schweitzer et al. 2014). Overall, soil communities that link plant and ecosystem processes will be increasingly important for mediating plant range dynamics in the future as they influence and are influenced by the combination of abiotic, biotic, and evolutionary pressures that shape geographic distributions and range limits of many species.

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CONCLUSION

Ecosystems provide the energy and nutrient constraints - the environmental context - within which species evolve, while genetically based species interactions drive many ecosystem processes. Despite the fact that phenotypic variation is at the heart of both ecology and evolution, ecosystem ecology and evolutionary biology remain largely disconnected. My dissertation pushes these important research frontiers by exploring ecosystems ecology in an evolutionary framework, using plant-soil linkages and feedbacks from the local to landscape scale. Using plant-soil interactions to bridge these seemingly disjointed research areas, my work reveals four major insights that have informed both my understanding and broadened the scientific understanding of eco-evolutionary concepts: (i) there is an ecosystem level plant-soil feedback mechanism that was previously undiscovered but may be acting in nature to influence the pace and direction of plant evolution; (ii) widespread, foundation tree species may evolve along common soil gradients (i.e., soil fertility); *Populus* genetics and their soil microbial communities act to reinforce a landscape scale soil N selective gradient; (iii) soil nutrients are more important than numerous climate variables in defining the ecological niche and species range of *P. angustifolia*, with evidence that certain functional traits are adapted to soil range limits; and (iv) climate-driven range shifts along elevation gradients could be shaped by plant-soil feedbacks and changes in ecosystem function.

These results show that plant evolutionary responses to soils may be much more common and important than currently recognized. My findings show this directly with strong evidence that soil N acts as a selective gradient on *Populus* growth phenotypes, and indirectly by showing that soil nutrients are the most important environmental component for predicting the potential future range of *P. angustifolia*. This shows that unusually steep soil gradients (e.g., serpentine/non-serpentine soils) are not the only instances where soils drive plant evolutionary changes. They may also be especially prevalent when soil nutrients vary widely across plant geographic distributions, in support of the anecdotal evidence from soil chronosequences in Hawaii and elsewhere.

The importance of the soil microbiome for plant and ecosystem functioning is apparent across all aspects of my dissertation. Not only do whole communities and specific taxa (i.e., *Betaproteobacteria*) appear to mediate the expression of growth phenotypes, but the combination of plant genetics and soil microbial function reinforce the soil nutrient differences observed between populations on the landscape. Moreover, variation in belowground communities across elevation could be critical for predicting how range dynamics and soil nutrient processes respond to the expected 2-4° C increase in temperature expected to occur throughout the *P. angustifolia* range by 2070. My dissertation research is some of the first to show how local, co-evolutionary feedbacks between plants and microbes can impact community- and ecosystem-level feedbacks involved in shaping plant genetic structure and nutrient dynamics across latitude and elevation gradients. In addition, with such wide sampling of soil microbial communities across the species distributions, it may be possible to begin defining the core microbiome of this important *Populus* tree. This represents an important step for understanding how the mechanisms of plant-microbe interactions change in different environmental contexts, thus improving our potential ability to design microbiomes engineered for particular plant phenotypes and soil community functions in a given environmental context.

From a wide-range of approaches, I investigated the genetic basis and evolutionary outcomes of plant-soil interactions in *P. angustifolia* across large environmental gradients. Overall, my work identified novel pathways by which ecosystems and evolution are connected

through plant-soil relationships, showed how reciprocal dynamics can occur between soil selective gradients and plant-driven reinforcement of those gradients, and highlighted the role of plant-soil interactions in shaping the species' current and future range. Together, my findings significantly advance the scale and scope by which ecosystems and evolution are linked in natural systems, and shows clear benefits of using an eco-evolutionary approach to understand and predict how natural ecosystems respond to our changing world.

VITA

Michael E. Van Nuland was born in Seattle, WA. He attended Seattle University where he received a Bannan Science and Engineering Scholarship and the Seattle University Biology Award. He graduated in 2011 with a Bachelor of Science degree in biology.

Michael began his PhD training at the University of Tennessee in 2012 with Dr. Jennifer Schweitzer. He has had multiple grants that were successfully funded during this time, including an NSF Graduate Research Fellowship, NSF Doctoral Dissertation Improvement Grant, and Soil Science Society of America Francis and Evelyn Clark Biology Scholarship. To date, he has authored and co-authored 7 peer-reviewed articles, of which one was included as an Editor's Choice and another nominated for the British Ecological Society Haldane Award. He also has received awards from multiple professional societies and the University of Tennessee, including the Soil Ecology Society Conference Best Oral Presentation (honorable mention), a University of Tennessee Outstanding Research Award, and the Ecology and Evolutionary Biology Department award for "Best Progress Towards Dissertation". Michael also served as the student representative of the British Ecological Society Plant-Soils-Ecosystems special interest group for more than one year. He has mentored more than 12 undergraduate students in scientific research, writing, and presenting, and has peer-reviewed manuscripts for many ecology journal.