Microbe-mediated adaptation in plants

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September 11, 2020

Abstract

Interactions with microbial symbionts have yielded great macroevolutionary innovations across the tree of life, like the origins of chloroplasts and the mitochondrial powerhouses of eukaryotic cells. There is also increasing evidence that host-associated microbiomes influence patterns of microevolutionary adaptation in plants and animals. Here we describe how microbes can facilitate adaptation in plants and how to test for and differentiate between the two main mechanisms by which microbes can produce adaptive responses in higher organisms: microbe-mediated local adaptation and microbe-mediated adaptive plasticity. Microbe-mediated local adaptation is when local plant genotypes have higher fitness than foreign genotypes because of a genotype-specific affiliation with locally important microbes. Microbe-mediated adaptive plasticity occurs when local plant phenotypes have higher fitness than foreign phenotypes as a result of interactions with locally important microbes. These microbial effects on adaptation can be difficult to differentiate from traditional modes of adaptation but may be prevalent. Ignoring microbial effects may lead to erroneous conclusions about the traits and mechanisms underlying adaptation, hindering management decisions in conservation, restoration, and agriculture.

Introduction

Major evolutionary innovations and events that span the tree of life are the result of host interactions with microorganisms. Perhaps the most dramatic example of microbe-mediated evolutionary events occurred when symbioses with the bacterial predecessors of chloroplasts and mitochondria were incorporated as part of the host cell (Sagan 1967). In more recent evolutionary history, the gut microbiome has been implicated in the rapid diversification of herbivorous mammals (Price *et al.* 2012) and wasps (Brucker & Bordenstein 2013). In these cases, interactions with microbiota transformed the macroevolutionary trajectory of their hosts. There is accumulating evidence that microorganisms are also affecting patterns of host adaptation on microevolutionary timescales.

The human microbiome project as well as work with other vertebrates, insects, and plants has illuminated a complex feedback loop of host affecting microbial form and function and microbial form and function feeding back to affect host phenotype (Fig. 1A; Kohl *et al.* 2014; Mueller & Sachs 2015; Sanders *et al.* 2015; Weese *et al.* 2015; Gehring *et al.* 2017; Moeller *et al.* 2019; Petipas *et al.* 2020a). Host genotypes enrich for specific microbiome components. For example, the mycorrhizal communities associated with Pinyon pines are almost entirely determined by pine morphotype (Gehring *et al.* 2017), soil microbiomes differ across *Arabidopsis thaliana* genotypes (Bulgarelli *et al.* 2012; Lundberg *et al.* 2012), and both mouse (Benson *et al.* 2010) and human (Goodrich *et al.* 2014) genotype shape their respective microbiomes. Reciprocally, this variation in microbiome composition can affect host phenotypes and performance. Across host organisms, the microbiome

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can affect nutrient acquisition (Krajmalnik-Brown *et al.* 2012; Newell & Douglas 2014), for example, access to phosphorus is often mediated by the unique enzymatic capabilities of arbuscular mycorrhizal fungi (Smith & Read 2008). Microbes also affect stress tolerance (Bang *et al.* 2018), immune phenotype (Foster *et al.* 2017), and pathogen susceptibility (King *et al.* 2016). For example, microbial symbionts of aphids provide their insect hosts with enhanced heat tolerance (Russell & Moran 2006), and *Clostridium* in the human gut produces butyrate, a compound essential to host immune homeostasis (Velasquez-Manoff 2015). Often these changes are assumed to be adaptive (i.e. increasing host fitness, Kohl & Carey 2016) but this assumption is rarely tested. If these microbial effects increase host fitness then they can lead to microbe-mediated adaptation , defined as enhanced host fitness in a particular environment that is partially or entirely the result of interacting with microorganisms.

Adaptive responses can occur through local adaptation or adaptive plasticity, two non-mutually-exclusive responses to the heterogeneous selection pressures species experience in nature. Local adaptation is the result of genetic differentiation in response to local conditions and is manifest when local genotypes have higher fitness in their home habitat compared with foreign genotypes (Kawecki & Ebert 2004). Adaptive plasticity is a form of phenotypic plasticity and is manifest when the environment affects organismal traits in ways that increase fitness in that particular environment (i.e., local *phenotypes* have higher fitness in their home habitat compared with foreign *phenotypes* ; Dudley & Schmitt 1996). Both local adaptation and phenotypic plasticity might be influenced by microbes, where in the absence of microbes you might observe low fitness and no pattern of local adaptation (Fig. 1B). We propose that microbe-mediated adaptive responses are the result of **microbe-mediated local adaptation** when local host genotypes have higher fitness than foreign genotypes because of a genotype-specific affiliation with locally important microbes (Fig. 1C), or **microbe-mediated adaptive plasticity** when local host phenotypes have higher fitness than foreign phenotypes as a result of interactions with locally important microbes (Fig. 1D).

Although microbe-mediated adaptation (including both microbe-mediated local adaptation and microbe-mediated phenotypic plasticity) may occur for many taxa (Alberdi *et al.* 2016; Sharpton 2018; Trevelline *et al.* 2019; Moeller & Sanders 2020), here we focus primarily on plants for three reasons: 1) The foundation for investigations into microbe-mediated adaptation have been laid through decades of avid interest in plant-microbe interactions. 2) Plants are tractable experimental systems amenable to classic experimental designs for testing local adaptation and adaptive plasticity, and 3) as sessile organisms, plants cannot move to escape stress and therefore may be even more dependent on microbes for adaptive responses. Additionally, while many interactions with microorganisms may be antagonistic (reducing plant fitness), here we focus on local adaptation to beneficial microorganisms as they have the potential to affect host adaptive responses, thus providing a unique avenue to adaptation. The population level consequences of antagonistic interactions have been extensively discussed elsewhere (e.g. Thompson 2005).

There are a growing number of examples showing that microbes *can* affect adaptive plant responses (Chanway *et al.* 1989; Schultz *et al.* 2001; Johnson *et al.* 2010, 2013; Smith *et al.* 2012; Lau & Lennon 2012; Lankau & Nodurft 2013; Wagner *et al.* 2014; Pickles *et al.* 2015; Barrett *et al.* 2016; Rúa *et al.* 2016; Van Nuland *et al.* 2016; Revillini *et al.* 2016; Gehring *et al.* 2017; Porter *et al.* 2020). Here we provide a framework that first identifies and defines the potential patterns and processes underlying microbe-mediated effects on adaptation (i.e., microbe-mediated local adaptation and microbe-mediated adaptive plasticity) and then propose empirical approaches to identify and differentiate between these two modes of microbe-mediated adaptation. Finally, we discuss implications and propose future research directions in the study of microbe-mediated adaptation.

Microbe-mediated local adaptation

Microbe-mediated local adaptation results when natural selection operates on plant traits that attract, retain, and regulate locally important microbes (hereafter "microbe facing traits"). The outcome is microbe-mediated changes to plant functional traits (hereafter "environment facing traits") that lead to higher fitness of local plant genotypes compared with foreign plant genotypes that do not associate as well with local microbes (Box 1). Microbe-mediated local adaptation can occur when plant genotype and *microbial geno-*

*types/phenotypes*interact to determine plant fitness in response to environmental conditions, or when plant genotype affects *microbial community composition and/or function* in ways that determine plant fitness in response to environmental conditions. These possibilities are not easily differentiated and also result in similar plant fitness responses so here we have included these processes together, but in Box 2 we further discuss the complications of identifying microbes driving host fitness effects.

The studies that have provided evidence for microbe-mediated local adaptation generally involve classic, widespread resource mutualisms and adaptation to soil nutrient availability, likely because plant ecologists focusing on these mutualisms are well aware of the importance of such mutualisms to population (Bennett *et al.* 2017), community (van der Heijden *et al.* 1998), and ecosystem processes (Vitousek & Walker 1989), and because extensive mutualism theory predicts how nutrient availability should influence the evolution of these associations (West *et al.* 2002; Akçay & Simms 2011). Given the prevalence of these interactions (80% of plant families are mycorrhizal and a number of others engage in tight symbiotic relationships with N-fixing bacteria like rhizobia), the following examples may represent common phenomena rather than rare exceptions.

In one of the most complete tests of microbe-mediated local adaptation, Johnson and co-authors (2010) used a fully factorial greenhouse experiment manipulating seed source, soil source, microbial source, and the presence/absence of microbes, to show that genotypes of*Andropogon gerardii* were coadapted/coevolved with local arbuscular mycorrhizal communities to access the most limiting nutrient at each site. They found accessing limiting nutrients was predicated not only on plant genotype but also on being paired with coevolved/coadapted microbes and growing in home soils. However, this enhanced nutrient uptake only translated to increased fitness and patterns of local adaptation for two out of three plant populations, indicating context dependency in microbe-mediated effects (Johnson *et al.* 2010).

In another example, microbial symbionts acted as a heritable component of the plant phenotype, a necessary condition for evolution by natural selection (Box 1), and these heritable microbial communities affected plant phenotypes in ways that led to adaptive responses. Ectomycorrhizal fungal (EcM) communities of drought-adapted pinyon pine were nearly entirely determined by plant genotype (drought tolerant vs. drought intolerant), and microbes associated with drought-tolerant trees reduced mortality and enhanced plant growth of drought tolerant plant genotypes by 25% under drought conditions (Gehring *et al.* 2017). Authors identified an Ascomycete fungus in the genus, *Geopora* , that was associated predominantly with drought tolerant trees, and whose abundance was correlated with plant drought tolerance even in the drought intolerant tree genotypes. In this case, a combination of greenhouse, field, and molecular data support the case that microbial communities, which are determined by plant genotype, underlie plant adaptation to drought (i.e., microbe-mediated local adaptation).

Despite the examples highlighted above, there are few empirical examples of microbe-mediated local adaptation; however, we suspect that this reflects the fact that microbially-mediated effects are often cryptic. The gold standard in testing for local adaptation is the reciprocal transplant experiment where seeds are transplanted into their natal (home) habitat and also moved to a novel (foreign) habitat. In these designs it is unusual for researchers to manipulate the biotic environment (including mutualists and pathogens; Cheplick 2015) even though adaptation to biotic effects may be important (Benning & Moeller 2019). In a review of the literature, we found that the potential for microbe-mediated local adaptation is very high; in 94% of studies where local adaptation was detected authors transplanted plants into soil in the presence of natural microbial communities and measured traits that could be microbially-mediated (Petipas 2018). In contrast, although sample size is small (6 studies), half of studies that failed to detect local adaptation transplanted plants in the absence of natural microbial communities (e.g. autoclaved soils). These results suggest that microbe-mediated local adaptation may be common, but the vast majority of classic plant local adaptation studies cannot differentiate between microbe-mediated effects and non-microbe-mediated effects. For example, Roy Turkington's classic work suggested patterns of local adaptation were the result of fine-scale interactions between plant competitors (Turkington & Harper 1979; Aarssen & Turkington 1985). Authors demonstrated that clover, *Trifolium repens* , was adapted to neighboring pasture plants (Turkington and Harper 1979),

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and this effect was primarily driven by the presence of a grass neighbor, *Lolium perenne* . Clover genotypes had greater biomass when grown in association with grass genotypes that they had previously coexisted with (Aarssen & Turkington 1985). However, in experiments designed to explicitly consider soil microbes, authors found biotic specialization between the plant species was only evident when *Rhizobium* isolated from parental *Trifolium* was included (Chanway *et al.* 1989), demonstrating the pattern of local adaptation was microbe-mediated.

Microbe-mediated adaptive plasticity

Phenotypic plasticity is when the environment affects the expression of an organism's traits (Richards *et al.* 2006), and adaptive plasticity occurs when these environmentally-induced changes in phenotype increase fitness in that environment (Dudley & Schmitt 1996). Microbe-mediated adaptive plasticity is when local plant phenotypes have higher fitness than foreign phenotypes as a result of interactions with locally import-ant microbes and could occur in two ways. First, plants might have higher fitness because they demonstrate plasticity in traits that attract, retain, and regulate important microbes (microbe facing traits), and associa-ting with these important microbes subsequently affects plant functional traits (environment facing traits) in ways that enhance fitness. The commonly observed autoregulation response of legumes (Wang *et al.* 2018) may exemplify this process; in low nitrogen environments plants form many rhizobium-housing nodules and are rewarded with fixed nitrogen, while in high nitrogen environments where biologically-fixed nitrogen is less useful, plants plastically reduce nodulation, therefore reducing the costs of supporting bacterial symbionts. Plastic shifts in investment in key microbes likely maintain fitness across a range of nutrient conditions.

Second, local environmental conditions can affect the abundance and composition of microbial communities, and this variation in microbial communities can induce plastic changes in plant phenotypes. A number of studies have now demonstrated that foliar and root endophytes, diverse soil microbial communities, and individual bacterial or fungal taxa affect expression of plant functional traits (e.g., Wagner *et al.*2014; Giau-que *et al.* 2019). For example, Giauque and Hawkes (2019) measured trait plasticity in *Panicum virgatum* exposed to low or high water conditions (3% or 15% gravimetric soil moisture), and inoculated with one of 35 different fungal isolates. Plasticity was calculated for six traits (whole plant water loss, relative growth rate, tiller number, and number of wilt free days, and root biomass). Average plasticity (mean plasticity of all six traits) was almost double in plants infected with endophytes compared to uninfected plants, presuma-bly because endophytes influence the expression of plant traits that influence subsequent physiological and growth responses to soil moisture. Authors also demonstrated that endophytes isolated from hotter drier environments increase plant survival under dry conditions, likely because endophyte communities from dif-ferent environments differ in their effects on the expression of plant traits associated with drought tolerance. However, the relationship between traits and fitness was not empirically tested.

Work by Lau and Lennon (2012) also is consistent with microbe-mediated adaptive plasticity. They mani-pulated soil moisture for replicated plant populations and their associated microbial communities over the course of multiple plant generations. There was minimal plant evolutionary response to soil moisture across multiple generations, but microbial communities that had experienced ˜16 months of drought buffered plants against contemporary exposure to drought. Plants experienced a 58% reduction in fitness during drought when grown in association with wet-adapted microbes, but only a 20% reduction in fitness when grown in association with drought-adapted microbes. Likewise, plants grown with wet-adapted microbes had higher fitness under higher soil moisture conditions. The authors postulate that microbial community effects on flowering phenology, a trait that commonly exhibits plasticity to drought, may underlie the observed fitness effects (Lau & Lennon 2012), although the link between flowering time and fitness responses to drought also was not explicitly demonstrated.

The two pathways through which microbes can elicit adaptive plasticity are not independent and are likely to feedback to affect the evolution of each pathway. For example, plant plastic responses to abiotic environmental variation can affect the abundance and diversity of microbes attracted to the rhizosphere (Jones *et al.* 2019), and this variation in microbial community composition can in turn cause plastic shifts in plant traits that increase fitness. Ultimately, the same forces that favor the evolution of adaptive plasticity, like temporal

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or spatial environmental heterogeneity, are also expected to select for microbe-mediated adaptive plasticity. Additionally, plasticity in microbe-facing traits might be expected to evolve when different microbes promote plant fitness in different environments, and microbe-induced plasticity in environment-facing traits might be expected to evolve when microbes are better predictors of environmental conditions than other environmental cues (Metcalf *et al.* 2019) or when microbes elicit larger changes in plant phenotypes than genetic changes within the plant itself (Hawkes *et al.* 2020).

The most convincing studies of adaptive plasticity explicitly link phenotypes (traits) to fitness (Schmitt *et al.* 2003). In the case of microbe-mediated adaptive plasticity, the traits underlying these adaptive responses are often unknown, hard to measure, and/or cryptic, particularly for microbe-facing traits. Although there is substantial evidence that microbes mediate plant phenotype, few studies explicitly link plasticity with fitness to definitively demonstrate microbe-mediated adaptive plasticity (vs. microbe-mediated plasticity, Goh *et al.* 2013).

Testing for microbe-mediated adaptation

Reciprocal transplant experiments can be used to understand how microbes affect patterns of plant adaptation and to distinguish between microbe-mediated local adaptation and microbe-mediated adaptive plasticity. Reciprocal transplants can be employed exactly as in the traditional design to study local adaptation and plasticity (Clausen *et al.* 1948) with the added element of manipulating microbes (Fig. 2).

In classical reciprocal transplant experiments, seeds from one population are transplanted both into a different habitat and replanted into their natal habitat. Populations are often chosen because one or more environmental variable is demonstrably different between populations (*e.g.* serpentine vs. non-serpentine soils, Wright *et al.* 2006 or presence or absence of a competing species, Lau 2006). A reciprocal transplant to test for microbe-mediated adaptation would involve transplanting seeds into different habitats and providing those plants with local or foreign microbes. Although it can be difficult or impossible to completely exclude microorganisms, sterilized control plants should be included to definitively attribute effects to microbes (*e.g.* , if microbe-mediated local adaptation is strong, then patterns of local adaptation will be much weaker or not evident when microbes are absent).

A statistical model to understand interactive contributions of plant and microbes to plant local adaptation includes some measure of plant fitness (e.g. germination,

survival, fecundity, or ideally an integrated fitness metric encompassing all life history stages [ASTER models] Geyer *et al.* 2007; Shaw *et al.* 2008) as a response variable and habitat type, plant source, microbial source, and sterilization as fixed effects (Table 1). While this design potentially results in a four-way interaction, with large enough sample sizes and patience, the complexity of this and other interactions in the models can be understood, especially through hypothesis testing with planned comparisons. The figures presented here assume that patterns of adaptation are largely attributable to habitat-specific microbes (Fig. 1B, C, D), however in the text we also discuss the importance of significant sterilization terms.

A three-way interaction between plant source, microbial source, and habitat (G_pxG_mxE) would indicate microbe-mediated local adaptation, if home genotypes have higher fitness with their home microbes in their home habitat (Fig. 3A). Whereas, an interaction between plant source and microbial source (G_pxG_m) may indicate that plant genotypes do best with their own microbes, regardless of habitat and are locally adapted to their natal microbes (Fig. 3B). For example, Petipas *et al.* (2020) found that germination of *Hypericum perforatum* sourced from limestone barrens was highest when transplanted with microbes from limestone barrens into limestone habitats, a pattern consistent with microbe-mediated local adaptation (G_pxG_mxE). However, survival of *H. perforatum* from limestone barrens was highest when transplanted with microbes from limestone barrens regardless of transplant site, a pattern suggesting that plants are locally adapted to their natal microbial communities (G_p x G_m), rather than indicating a pattern of microbe-mediated local adaptation.

An interaction between habitat and microbial source may be indicative of microbe-mediated adaptive plasti-

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city. In this case, local microbes are responsible for locally adapted plant phenotypes; however, plant source populations may be equally adept at utilizing local microbes (*i.e.* they exhibit plasticity in microbe facing traits, allowing them to attract, retain, regulate relevant local microbes; Fig. 3C). An example of a trait underlying these adaptive patterns is microbe-mediated flowering time, where plants only achieve the optimal flowering time phenotype in association with local microbes (Fig. 3D).

Significant sterilization terms can also indicate microbe-mediated local adaptation and microbe-mediated adaptive plasticity. For example, where hosts strongly control microbiome composition (e.g. Gehring et al. 2017), microbe-mediated local adaptation would be evident as a G_p x E x sterilization effect (rather than G_pxG_mxE), where plant local adaptation is only observed in the presence of microbes, but the microbial effects are primarily driven by plant genotype specific recruitment from a common soil source. This may still be considered microbe-mediated local adaptation because the relative importance of different microbes varies across context and the observed plant adaptation is still due to interactions with relevant microbes. However, in this case, the plant nearly entirely controls the composition of those microbes. Similarly, sterilization could indicate microbe-mediated adaptive plasticity when the environment elicits plastic shifts in plant phenotypes that in turn influence microbial communities in ways that feedback to affect plant fitness. Because the sterilization effect could also simply indicate the benefits or costs of association with microbial communities, inferring microbe-mediated adaptive plasticity from a significant sterilization effect is challenging and would require: 1) showing that shifts in plant phenotypes are associated with changes to the microbial community, and 2) these changes to the microbial community are associated with shifts in plant traits and/or fitness. The potential for this type of interaction is exemplified by host-mediated microbiome engineering approaches that use plant phenotypes to select adaptive microbiomes (Swenson *et al.* 2000; Panke-Buisse *et al.* 2015, 2017). In one example, wheat was cyclically exposed to drought and in each cycle the microbiomes associated with the most drought tolerant seedlings were moved to the next cycle. After six cycles, a plant phenotype mediated drought microbiome was selected that deferred drought symptoms in wheat seedlings by five days (Jochum *et al.* 2019).

Additionally, microbe-mediated adaptation necessarily involves a complicated experimental design and multiple players each with the capacity of becoming locally adapted and exhibiting trait plasticity. Consequently, there are many patterns that can emerge from these interactions, which requires careful experimental planning (including of experimental tests) and careful interpretation of the resulting data. Finally, although we have discussed microbe-mediated local adaptation and microbe-mediated adaptive plasticity largely independently, they are not mutually exclusive.

The same caveats and considerations that apply to typical reciprocal transplant experiments also apply to microbe-mediated adaptation transplant experiments, including the relevant spatial scale, which aspects of fitness to measure, and how long the experiment should be monitored (Cheplick 2015). The latter is especially important given that contamination in the field is likely to erode microbial treatments over time. For tests of microbe-mediated adaptation, additional care should be taken to determine relevant microbes, proper controls, and the feasibility of different microbial transplant designs (Box 3, Fig. 4).

Implications and Future Research Directions

Increasingly researchers, using a wide range of experimental systems, are exploring microbe-mediated adaptation (Kohl *et al.* 2014; Weigel & Erwin 2017; Sison-Mangus *et al.* 2018; Moeller *et al.* 2019). If microbes commonly influence adaptation through microbe-mediated local adaptation or microbe-mediated adaptive plasticity, then our understanding of the traits and mechanisms underlying adaptation may be incomplete, therefore making our responses to environmental and agricultural challenges potentially inappropriate.

Just as Clausen, Keck, and Heisey (1948) realized 70 plus years ago, plants are excellent study systems to perform reciprocal transplants and partition the variation of plant phenotypes into genetic and environmental components (Núñez-Farfán & Schlichting 2001). However, early pioneers were unaware that microbes can be heavily influenced by their host's genotype (Bulgarelli *et al.* 2012; Lundberg *et al.* 2012; Gehring *et al.* 2017) and have large effects on plant phenotypes (Friesen *et al.* 2011; Friesen 2013; Wagner *et al.* 2014; Giauque

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et al. 2019). Consequently, in early studies many microbial effects may have been erroneously attributed to plant genotype and at other times erroneously attributed to environment effects (e.g. Chanway *et al.* 1989). In many cases, such bookkeeping may not fundamentally change outcomes. For example, in the Gehring *et al.* (2017) case study the mycorrhizal community is so tightly under plant control, it functions as an extended phenotype of the plant host. However, even in this case, one must wonder what will happen to those plant genotypes enriching for drought tolerance promoting mycorrhizae if the ectomycorrhizal community is eroded by other forces? Below we discuss how explicitly considering microbial effects may substantially alter our views of the spatial scales, pace, and consequences of plant adaptation.

Microbe-mediated adaptation and the spatial scale of adaptation

Selection can vary over exceptionally small spatial scales (Turkington & Harper 1979; Kalisz 1986), posing challenges to the evolution of local adaption by plants if the scale of gene flow through pollen and/or seeds exceeds the strength of selection (Richardson *et al.* 2014). However, microbe-mediated local adaptation and adaptive plasticity can affect the spatial scale of adaptation. Microbes can facilitate adaptation via microbe-mediated local adaptation by acting as a selective barrier, where the fitness of migrants (relative to resident populations) is reduced before they are incorporated into the gene pool (Richardson *et al.* 2014). An example of this is microbe-mediated germination or survival (Petipas *et al.* 2020b), where local microbes facilitate germination and survival of local plant genotypes potentially allowing them to outcompete or exclude foreign plant genotypes.

Microbe-mediated adaptive plasticity may be another hidden solution to dealing with heterogenous environments. Small-scale adaptive responses could be facilitated by microbes if plants exhibit a high degree of plasticity for traits related to interactions with microorganism, if microbes differentiate across fine-scale spatial variation (Nacke*et al.* 2016) and elicit adaptive phenotypic changes in host plants, or even if microbial communities vary little spatially but their effects on plant phenotypes are highly context-dependent and influenced by other aspects of the abiotic or biotic environment. For example, microbe-mediated adaptive plasticity may affect the drought phenotype of*Themeda triandra,* a native Kenyan grass, over small spatial scales. The presence of termite mounds dramatically increases nutrient availability, but reduces variability in water availability over small spatial scales (>50m), posing an adaptive challenge for wind pollinated *Themeda triandra* , which is unlikely to genetically differentiate in the on versus off-mound environments. However, microbes from on vs. off termite mounds differentially affect *T. triandra's* response to drought. Plants inoculated with arbuscular mycorrhizal communities collected off mounds closed stomata quickly when exposed to drought and halted biomass accumulation, whereas plants inoculated with on-mound fungi kept stomata open longer under drought conditions and continued to acquire biomass (Petipas *et al.* 2017). In this case, fine-scale variation in microbial community composition on vs. off termite mounds led to the production of plant phenotypes that are potentially adaptive in those two different environments. Future work, should include looking at the fitness consequences of these plant trait responses especially over a more realistic time scale for a long-lived perennial grass.

Microbe-mediated adaptation and temporal scale of adaptation

Just as microbes mediate plant adaptation to spatially heterogeneous environments, microbes may also promote adaptive plant responses to temporally varying environments, and these effects may allow for more rapid adaptation to changing environmental conditions than evolution through genetic differentiation (Hawkes *et al.* 2020). Specifically, microbes might respond more rapidly to environmental change (both in terms of shifts in community composition and evolutionary changes within key microbial populations) because of incredibly diverse metabolic strategies, high mutation rates, large population sizes, diverse communities, short generation times, and the potential for lateral gene transfer (Gillings & Stokes 2012). These shifts in microbial form or function can introduce novel pathways of adaptation (e.g., endophytes producing novel chemical defenses in the face of increased herbivory) or change/enhance existing traits (Friesen 2013). If only plant genotypes that are locally adapted to those microbial symbionts can take advantage of these innovations, then microbe-mediated local adaption would result. If any plant genotype can benefit from these innovations, then microbe-mediated adaptive plasticity is possible. For example, in Lau & Lennon's 2012 experiment,

plant responses to drought were almost entirely mediated by microbes and occurred over very short times scales (~16 months; Lau & Lennon 2012), indicating the potential for rapid responses mediated by microbes to climate change. Microbes affecting response to climate change might be a wide-spread phenomenon, at least for fungi. Kivlin and coauthors (2013) found that fungal symbionts widely affect plant responses to global change, and fungal symbionts particularly benefited plants exposed to drought.

Microbe-mediated adaptation and speciation

Perhaps the most extreme potential outcome of microbe-mediated adaptation is speciation of plant hosts. Although there are examples in animals of microbes leading to speciation (Shropshire & Bordenstein 2016), there is minimal evidence in plants. Given that microbes can influence plant reproductive traits including plant phenology (Lau & Lennon 2012; Wagner *et al.* 2014, 2020; Panke-Buisse *et al.*2017; Metcalf *et al.* 2019) and pollinator preferences (Vannette & Fukami 2016; Rebolleda-Gómez *et al.* 2019), reproductive isolation and eventually speciation could potentially be affected by microbes. In one recent example, arbuscular mycorrhizal fungi may have facilitated sympatric speciation in two species of *Howea* palms in Australia (Osborne *et al.* 2018). The authors propose that colonization of a new habitat by ancestral *Howea* was facilitated by developing a mutualistic relationship with endemic mycorrhizal communities in the novel calcareous soils. This led to evolutionary divergence into two sister species, *H. forsteriana* and *H. belmoreana.* *Howea forsteriana* lost the ability to affiliate with ancestral mycorrhizal communities and therefore to survive on the ancestral volcanic soils, therefore leading to reproductive isolation. Microbes also may be correlated with host speciation at deeper evolutionary time scales. For example, plants in the coffee family (*Rubiaceae*) form symbiotic associations with leaf fungal endophytes. These endophytes produce secondary metabolites that protect hosts from pathogens and herbivores. Verstraete *et al.* (2017) proposed endophyte associations affect macro-evolutionary patterns in the host, observing that plant lineages hosting leaf endophytes have higher rates of speciation compared with those without endophytes.

Applied implications

The research program outlined above serves the ultimate goal of unraveling how plant genotypes, microbial genotypes (or communities), and the environment interact to determine adaptive outcomes. This is important for understanding first principles of evolutionary ecology but also is a shared goal with agronomists interested in using microbial technologies for enhanced crop production (Busby *et al.* 2017; Toju *et al.* 2018). Microbes have the potential to make crops more productive, less susceptible to disease, and more drought tolerant (Bakker *et al.* 2012; Reid & Greene 2012). Additionally, microbes are increasingly recognized as an important factor in plant restoration and conservation (Ji *et al.* 2010; Middleton *et al.* 2015; Cheeke *et al.* 2019). In one example, Douglas-fir trees, transplanted in a provenance trial, decreased in height as much as 15% as ectomycorrhizal communities diverged from communities at their home sites (Kranabetter *et al.* 2015), indicating that assisted migration will be inhibited when coevolved/coadapted plant microbe interactions are disrupted. Despite a growing appreciation of the importance of microbes in applied contexts, microbial technologies have been hampered by the complexity of the plant microbiome, the context dependency of plant-microbe interactions, and the difficulties of successfully establishing introduced microbial communities.

To better understand the effects of microbes so that we can harness them as technologies, we need to be able to identify and isolate important members of the microbial community and determine whether their effects are generalized vs. host-genotype specific. The age of informatics has allowed us a glimpse into the diversity and complexity of the microbiome, but much work remains to disentangle single species from multi-species effects and how relevant microbial genotypic diversity is for adaptive benefits. For example, we know relatively little about intra-specific diversity in many microbial taxa, including important groups such as arbuscular mycorrhizal fungi (Johnson *et al.* 2012) or how diversity (whether intra- or interspecific) maps onto microbial function and influences the likelihood of microbe-mediated local adaptation or microbe-mediated adaptive plasticity in plants.

A further challenge is understanding context dependency in plant-microbe interactions. Plants do not universally benefit from interactions with microbes (even those generally referred to as mutualists), but rather

these interactions, like many species' interactions, are highly context dependent (Johnson 1993; Bronstein 1994; Chamberlain *et al.*2014). One of the top research priorities into microbe-mediated adaptation should be efforts to delineate this context dependency. Factors that could drive context-dependency in plant-microbe relationships include plant characteristics such as mating system, invasive status, life-history strategy, as well as microbial characteristics such as vertical/horizontal transmission, obligate/facultative, microbial species interactions, and priority effects, and habitat characteristics such as aridity, nutrient availability, and competition. In short, although the body of data suggests that microbial communities commonly shift in response to environmental change (Allison & Martiny 2008) and that microbes can influence plant phenotypes and plant fitness (Goh *et al.* 2013; Hawkes *et al.* 2020; Kolodny & Schulenburg 2020; Petipas *et al.* 2020b), we still have little understanding about how and when microbial communities are likely to change in ways that influence microbe-mediated local adaptation or microbe-mediated adaptive plasticity in plants.

Conclusions

Darwin described the complexity of nature as a tangled bank, where organisms depend on each other in "so complex a manner" (Darwin 1859). Burgeoning understanding of the largely cryptic role microbes play in host ecology and evolution further tangles an already tangled bank, challenging our classic views on adaptation, and potentially calling into question many of our long-standing assumptions about the causes of natural selection and traits underlying adaptation. Although microbial effects on adaptation complicate our ability to disentangle genotype vs. environment effects on phenotypes, it is an important field of study because microbial communities may also provide novel avenues of adaptation. As a result, understanding the role microbes play in adaptive responses has the potential to provide new approaches for increasing population resilience to environmental change in both natural and managed systems (Mueller *et al.* 2019). Ultimately, to harness the potential adaptive power of microbes requires carefully delineating microbe-mediated adaptation, testing it adequately, and eventually identifying the conditions favoring it. We hope this article stimulates discussion around these topics and provides a framework to accomplish these goals.

Box 1: The tenets of natural selection applied to microbe-mediated adaptation

The phenotype of an organism is fundamental to local adaptation and adaptive plasticity because natural selection acts upon traits (or selection acts upon plasticity in traits). Evolution by natural selection will occur when the following criteria are met (Lewontin 1970):

1. Variation exists in host traits that mediate or respond to interactions with specific microbes or microbial communities, which leads to different host genotypes associating with or responding to different microbes.
2. Host traits that mediate interactions with microbes are heritable
3. Traits mediating interactions with specific microbes or microbial communities affect fitness.

Box 2: What's up with the microbes?

Microbe can affect host phenotypes: 1. by affecting important plant functional traits(Friesen *et al.* 2011), 2. acting as an extended phenotype (i.e. acquiring resources otherwise not available, Koskella & Bergelson 2020) or 3. by serving as an environmental cue (Metcalf*et al.* 2019). However, understanding the characteristics/identity of the microbes underlying these effects can be complicated. Demonstrating reciprocal evolutionary change in microbes is complicated when in many cases fitness is hard to quantify and relevant traits are unknown, although new methods are making this seem possible(Burghardt*et al.* 2018). Documenting differences in community composition is more tractable given the explosion of sequencing-based technologies (Hugerth & Andersson 2017). However, detecting changes in microbial richness or shifts in community composition does not exclude the possibility of simultaneous (and relevant) genetic change to microbial communities or microbial plasticity. Additionally, microbial diversity may not be a relevant reflection of microbial function. At this point, understanding and differentiating ecological from evolutionary effects on microbial communities is probably not possible given current technologies but will be an important research goal of the future. Given the issues with understanding the microbial end of the interaction, researchers more commonly explore the population biology of the plants. For example, in one study(Johnson *et al.* 2010) highlighted in

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the main text, reciprocal transplants of *Andropogon gerardii* genotypes collected from areas that either had limiting phosphorus or limiting nitrogen demonstrated that local mycorrhizal communities were locally adapted to local nutrient conditions, such that home communities of AMF provided optimal benefits to plants when transplanted into home environments. AMF also had higher fitness when matched with their sympatric plant genotype. What is unclear from this study is which microbial players were responsible for the observed effect. Some options include: 1) AMF genotype(s) that were particularly good at accessing nutrients locally, 2) AMF species that were particularly good at accessing nutrients locally, or 3) synergistic effects of multiple AMF mutualists, such that the effects on plants could not be predicted based on the presence or abundance of a particular species or genotype.

Box 3: Designing a successful experiment

Manipulating microbes can be challenging, but microbial treatments can be created by isolating some fraction of the plant microbiome, for example isolates of individual taxa, microbial washes, or whole soil inoculum (for a discussion of efficacy see Howard *et al.* 2017, Fig. 4A). Defining proper controls is also critical. For example, researchers should isolate soil microbial effects without confounding them with soil abiotic effects by using a sterilized soil mixture (either a sterilized field soil mixture; e.g. Petipas *et al.* 2020 or sterilized potting mix; Howard *et al.* 2020, Fig. 4B) with a small amount (1-5% soil volume) of live or sterilized inoculum added back. The choice of soil sterilization methods can be taxon specific (e.g. fungicide) or general (autoclaving or gamma irradiation). Each method has advantages and disadvantages, and each method often has effects beyond direct removal of microbes (Trevors 1996). In addition, given that even sterilized treatments are rapidly colonized by weedy microbes, they should be more likely considered low diversity microbial treatments rather than no or low microbial abundance communities (Lau & Lennon 2012).

Transplantation can occur directly in the soil using a mesh (0.45μm) barrier to keep in microbial inoculum and exclude microbial taxa (fungi and some bacterial taxa) from the surrounding soil environment (Mcguire 2007). Alternatively, plants can be transplanted into pots with microbial treatments and placed in aboveground arrays in the two habitats, although aboveground conditions in pots are often warmer and subject to more rapid drying than in ground transplants (Fig. 4C). When transplanting into the field (and even the greenhouse), especially if plants are surrounded by the soil environment, contamination by non-target microbes will always be an issue as mentioned above. For this reason, the duration of the experiment will need to be planned carefully. Since microbe-mediated adaptation experiments are simultaneously manipulating multiple factors, proper planning of sample sizes is important to have the power to detect significant interactive effects.

Works cited

Aarssen, L.W. & Turkington, R. (1985). Biotic specialization between neighbouring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. *J. Ecol.* , 73, 605–614.

Akçay, E. & Simms, E.L. (2011). Negotiation, sanctions, and context dependency in the legume-rhizobium mutualism. *Am. Nat.* , 178, 1–14.

Alberdi, A., Aizpurua, O., Bohmann, K., Zepeda-Mendoza, M.L. & Gilbert, M.T.P. (2016). Do vertebrate gut metagenomes confer rapid ecological adaptation? *Trends Ecol. Evol.* , 31, 689–699.

Allison, S.D. & Martiny, J.B.H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci.* , 105, 11512–11519.

Bakker, M.G., Manter, D.K., Sheflin, A.M., Weir, T.L. & Vivanco, J.M. (2012). Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant Soil* , 360, 1–13.

Bang, C., Dagan, T., Deines, P., Dubilier, N., Duschl, W.J., Fraune, S., *et al.* (2018). Metaorganisms in extreme environments: do microbes play a role in organismal adaptation? *Zoology* , 127, 1–19.

Barrett, L.G., Zee, P.C., Bever, J.D., Miller, J.T. & Thrall, P.H. (2016). Evolutionary history shapes patterns of mutualistic benefit in Acacia-rhizobial interactions. *Evolution* , 70, 1473–1485.

Bennett, J.A., Maherali, H., Reinhart, K.O., Lekberg, Y., Hart, M.M. & Klironomos, J.N. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* , 355, 181–184.

Benning, J.W. & Moeller, D.A. (2019). Maladaptation beyond a geographic range limit driven by antagonistic and mutualistic biotic interactions across an abiotic gradient. *Evolution* , 73, 2044–2059.

Benson, A.K., Kelly, S.A., Legge, R., Ma, F., Low, S.J., Kim, J.,*et al.* (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc. Natl. Acad. Sci.* , 107, 18933–18938.

Bronstein, J.L. (1994). Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.* , 9, 214–217.

Brucker, R.M. & Bordenstein, S.R. (2013). The hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus*Nasonia* . *Science* , 341, 667–669.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., *et al.* (2012). Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* , 488, 91–95.

Burghardt, L.T., Epstein, B., Guhlin, J., Nelson, M.S., Taylor, M.R., Young, N.D., *et al.* (2018). Select and resequence reveals relative fitness of bacteria in symbiotic and free-living environments.*Proc. Natl. Acad. Sci.* , 115, 2425–2430.

Busby, P.E., Soman, C., Wagner, M.R., Friesen, M.L., Kremer, J., Bennett, A., *et al.* (2017). Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biol.* , 15, 1-14.

Chamberlain, S.A., Bronstein, J.L. & Rudgers, J.A. (2014). How context dependent are species interactions? *Ecol. Lett.* , 17, 881–890.

Chanway, C.P., Holl, F.B. & Turkington, R. (1989). Effect of*Rhizobium leguminosarum* biovar Trifolii genotype on specificity between *Trifolium repens* and *Lolium perenne* . *J. Ecol.* , 77, 1150–1160.

Cheeke, T.E., Zheng, C., Koziol, L., Gurholt, C.R. & Bever, J.D. (2019). Sensitivity to AMF species is greater in late-successional than early-successional native or nonnative grassland plants. *Ecology* , 100, 1–14.

Cheplick, G.P. (2015). *Approaches to plant evolutionary ecology* . Oxford University Press, New York, New York, USA.

Clausen, J., Keck, D.D. & Heisey, W. (1948). Experimental studies on the nature of species. III. Enivronmental responses of climate races of*Achillea* . Publication 581, Carnegie Institution of Washington, Washington, D.C.

Darwin, C. (1859). *The Origin of Species.* Penguin Books, New York, New York, USA.

Dudley, S.A. & Schmitt, J. (1996). Testing the adaptive plasticity hypothesis : density-dependent delection on manipulated stem length in*Impatiens capensis* . *Am. Nat.* , 147, 445–465.

Foster, K.R., Schluter, J., Coyte, K.Z. & Rakoff-Nahoum, S. (2017). The evolution of the host microbiome as an ecosystem on a leash.*Nature* , 548, 43–51.

Friesen, M.L. (2013). Microbially Mediated Plant Functional Traits. In: Molecular Microbial Ecology of the Rhizosphere, Second edition.John Wiley and Sons, Hoboken, NJ, USA. pp. 87–102.

Friesen, M.L., Porter, S.S., Stark, S.C., Wettberg, E.J. Von, Sachs, J.L. & Martinez-Romero, E. (2011). Microbially Mediated Plant Functional Traits. *Annu. Rev. Ecol. Evol. Syst.* , 42, 23–46.

Gehring, C.A., Sthultz, C.M., Flores-Renteria, L., Whipple, A. V & Whitham, T.G. (2017). Tree genetics defines fungal partner communities that may confer drought tolerance. *Proc. Natl. Acad. Sci.* , 114, 11169–11174.

Geyer, C.J., Wagenius, S. & Shaw, R.G. (2007). Aster models for life history analysis. *Biometrika* , 94, 415–426.

Giauque, H., Connor, E.W. & Hawkes, C.V. (2019). Endophyte traits relevant to stress tolerance, resource use and habitat of origin predict effects on host plants. *New Phytol.* , 221, 2239–2249.

Gillings, M.R. & Stokes, H.W. (2012). Are humans increasing bacterial evolvability? *Trends Ecol. Evol.,* 27, 346-352.

Goh, C.H., Veliz Vallejos, D.F., Nicotra, A.B. & Mathesius, U. (2013). The impact of beneficial plant-associated microbes on plant phenotypic plasticity. *J. Chem. Ecol.* , 39, 826–839.

Goodrich, J.K., Waters, J.L., Poole, A.C., Sutter, J.L., Koren, O., Blekhman, R., *et al.* (2014). Human genetics shape the gut microbiome. *Cell* , 159, 789–799.

Hawkes, C. V., Bull, J.J. & Lau, J.A. (2020). Symbiosis and stress: how plant microbiomes affect host evolution. *Philos. Trans. R. Soc. B Biol. Sci.* , 375, 1–10.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity, 396, 69–72.

Howard, M.M., Bell, T.H. & Kao-kniffin, J. (2017). Soil microbiome transfer method affects microbiome composition, including dominant microorganisms, in a novel environment. *FEMS Microbiol. Lett.* , 364, 1–8.

Howard, M.M., Munoz, C.A., Kao-Kniffin, J. & Kessler, A. (2020). Soil microbiomes from fallow fields have species-specific effects on crop growth and pest resistance. *Front. Plant Sci.* , 11, 1–12.

Hugerth, L.W. & Andersson, A.F. (2017). Analysing microbial community composition through amplicon sequencing: from sampling to hypothesis testing. *Front. Microbiol.* , 8, 1–22.

Ji, B., Bentivenga, S. & Casper, B. (2010). Evidence for ecological matching of whole AM fungal communities to the local plant-soil environment. *Ecology* , 91, 100319061621033.

Jochum, M.D., McWilliams, K.L., Pierson, E.A. & Jo, Y.K. (2019). Host-mediated microbiome engineering (HMME) of drought tolerance in the wheat rhizosphere. *PLoS One* , 14, 1–15.

Johnson, D., Martin, F., Cairney, J.W.G. & Anderson, I.C. (2012). The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. *New Phytol.* , 194, 614–628.

Johnson, N.C. (1993). Can fertilization of soil select less mutualistic mycorrhizae? *Ecol. Appl.* , 3, 749–757.

Johnson, N.C., Angelard, C., Sanders, I.R. & Kiers, E.T. (2013). Predicting community and ecosystem outcomes of mycorrhizal responses to global change. *Ecol. Lett.* , 16, 140–153.

Johnson, N.C.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A. & Miller, R.M. (2010). Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc. Natl. Acad. Sci.* , 107, 2093–2098.

Jones, P., Garcia, B.J., Furches, A., Tuskan, G.A. & Jacobson, D. (2019). Plant host-associated mechanisms for microbial selection.*Front. Plant Sci.* , 10, 1–14.

Kalisz, S. (1986). Variable Selection on the Timing of Germination in*Collinsia verna* . *Evolution* , 40, 479–491.

Kawecki, T.J. & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecol. Lett.* , 7, 1225–1241.

King, K.C., Brockhurst, M.A., Vasieva, O., Paterson, S., Betts, A., Ford, S.A., *et al.* (2016). Rapid evolution of microbe-mediated protection against pathogens in a worm host. *ISME J.* , 10, 1915–1924.

Kivlin, S.N., Emery, S.M. & Rudgers, J.A. (2013). Fungal symbionts alter plant responses to global change. *Am. J. Bot.* , 100, 1445–1457.

Kohl, K.D. & Carey, H. V. (2016). A place for host-microbe symbiosis in the comparative physiologist's toolbox. *J. Exp. Biol.* , 219, 3496–3504.

Kohl, K.D., Weiss, R.B., Cox, J., Dale, C. & Dearing, M.D. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins.*Ecol. Lett.* , 17, 1238–1246.

Kolodny, O. & Schulenburg, H. (2020). Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Philos. Trans. R. Soc. B Biol. Sci.* , 375, 1–11.

Koskella, B. & Bergelson, J. (2020). The study of host-microbiome (co)evolution across levels of selection. *Philos. Trans. R. Soc. B Biol. Sci.* , 375, 1–8.

Krajmalnik-Brown, R., Ilhan, Z.E., Kang, D.W. & DiBaise, J.K. (2012). Effects of gut microbes on nutrient absorption and energy regulation.*Nutr. Clin. Pract.* , 27, 201–214.

Kranabetter, J.M., Stoehr, M. & O'Neill, G.A. (2015). Ectomycorrhizal fungal maladaptation and growth reductions associated with assisted migration of Douglas-fir. *New Phytol.* , 206, 1135–1144.

Lankau, R.A. & Nodurft, R.N. (2013). An exotic invader drives the evolution of plant traits that determine mycorrhizal fungal diversity in a native competitor. *Mol. Ecol.* , 22, 5472–5485.

Lau, J.A. (2006). Evolutionary Responses of Native Plants To Novel Community Members. *Evolution* , 60, 56–63.

Lau, J.A. & Lennon, J.T. (2012). Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proc. Natl. Acad. Sci.* , 109, 14058–14062.

Lewontin, R.C. (1970). The Units of Selection Author. *Annu. Rev. Ecol. Evol. Syst.* , 1, 1–18.

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

Mcguire, K.L. (2007). Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. *Ecology* , 88, 567–574.

Metcalf, C.J.E., Henry, L.P., Rebolleda-Gomez, M. & Koskella, B. (2019). Why evolve reliance on the microbiome for timing of ontogeny?*MBio* , 10, 1–10.

Middleton, E.L., Richardson, S., Koziol, L., Palmer, C.E., Yermakov, Z., Henning, J.A., *et al.* (2015). Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere* , 6, 1-16.

Moeller, A.H., Gomes-Neto, J.C., Mantz, S., Kittana, H., Segura Munoz, R.R., Schmaltz, R.J., *et al.* (2019). Experimental evidence for adaptation to species-specific gut microbiota in house mice.*mSphere* , 4, 1–10.

Moeller, A.H. & Sanders, J.G. (2020). Roles of the gut microbiota in the adaptive evolution of mammalian species. *Philos. Trans. R. Soc. B Biol. Sci.* , 375, 1–10.

Mueller, E.A., Wisnoski, N.I., Peralta, A.L. & Lennon, J.T. (2019). Microbial rescue effects: How microbiomes can save hosts from extinction. *Funct. Ecol.* , 00, 1–10.

Mueller, U.G. & Sachs, J.L. (2015). Engineering Microbiomes to Improve Plant and Animal Health. *Trends Microbiol.,* 23, 606-617.

Nacke, H., Goldmann, K., Schoning, I., Pfeiffer, B., Kaiser, K., Villamizar, G.A.C., *et al.* (2016). Fine spatial scale variation of soil microbial communities under European beech and Norway spruce.*Front. Microbiol.* , 7, 1–14.

Newell, P.D. & Douglas, A.E. (2014). Interspecies interactions determine the impact of the gut microbiota on nutrient allocation in *Drosophila melanogaster* . *Appl. Environ. Microbiol.* , 80, 788–796.

Van Nuland, M.E., Wooliver, R.C., Pfennigwerth, A.A., Read, Q.D., Ware, I.M., Mueller, L., *et al.* (2016). Plant–soil feedbacks: connecting ecosystem ecology and evolution. *Funct. Ecol.* , 30, 1032–1042.

Nunez-Farfan, J. & Schlichting, C.D. (2001). Evolution in changing environments : the synthetic work of Clausen, Keck, and Hiesey. *Q. Rev. Biol.* , 76, 433–457.

Osborne, O.G., De-Kayne, R., Bidartondo, M.I., Hutton, I., Baker, W.J., Turnbull, C.G.N., *et al.* (2018). Arbuscular mycorrhizal fungi promote coexistence and niche divergence of sympatric palm species on a remote oceanic island. *New Phytol.* , 217, 1254–1266.

Panke-Buisse, K., Lee, S. & Kao-Kniffin, J. (2017). Cultivated sub-populations of soil microbiomes retain early fowering plant trait. *Microb. Ecol.* , 73, 394–403.

Panke-Buisse, K., Poole, A.C., Goodrich, J.K., Ley, R.E. & Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J.* , 9, 980–989.

Petipas, R.H. (2018). Microbe-mediated adaptation in plants. PhD Dissertation. Cornell University.

Petipas, R.H., Bowsher, A.W., Bekkering, C.S., Jack, C.N., McLachlan, E.E., White, R.A., *et al.* (2020a). Interactive effects of microbes and nitrogen on *Panicum virgatum* root functional traits and patterns of phenotypic selection. *Int. J. Plant Sci.* , 181, 20–32.

Petipas, R.H., Gonzalez, J.B., Palmer, T.M. & Brody, A.K. (2017). Habitat-specific AMF symbioses enhance drought tolerance of a native Kenyan grass. *Acta Oecologica* , 78, 71–78.

Petipas, R.H., Wruck, A.C. & Geber, M.A. (2020b). Microbe-mediated local adaptation to limestone barrens is context dependent. *Ecology* , 101, 1–12.

Pickles, B.J., Twieg, B.D., O'Neill, G.A., Mohn, W.W. & Simard, S.W. (2015). Local adaptation in migrated interior Douglas-fir seedlings is mediated by ectomycorrhizas and other soil factors. *New Phytol.* , 207, 858–871.

Porter, S.S., Bantay, R., Friel, C.A., Garoutte, A., Gdanetz, K., Ibarreta, K., *et al.* (2020). Beneficial microbes ameliorate abiotic and biotic sources of stress on plants. *Funct. Ecol.* , 00, 1–12.

Price, S.A., Hopkins, S.S.B., Smith, K.K. & Roth, V.L. (2012). Tempo of trophic evolution and its impact on mammalian diversification. *Proc. Natl. Acad. Sci.* , 109, 7008–7012.

Rebolleda-Gomez, M., Forrester, N.J., Russell, A.L., Wei, N., Fetters, A.M., Stephens, J.D., *et al.* (2019). Gazing into the anthosphere: considering how microbes influence floral evolution. *New Phytol.* , 224, 1012–1020.

Reid, A. & Greene, S.E. (2012). *How microbes can help feed the world* . American Academy of Microbiology. Washington D.C., USA.

Revillini, D., Gehring, C.A. & Johnson, N.C. (2016). The role of locally adapted mycorrhizas and rhizobacteria in plant – soil feedback systems. *Funct. Ecol.* , 30, 1086–1098.

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* , 9, 981–993.

Richardson, J.L., Urban, M.C., Bolnick, D.I. & Skelly, D.K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends Ecol. Evol.* , 29, 165–176.

Rua, M.A., Antoninka, A., Antunes, P.M., Chaudhary, V.B., Gehring, C., Lamit, L.J., *et al.* (2016). Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. *BMC Evol. Biol.* , 16, 1–15.

14

Russell, J.A. & Moran, N.A. (2006). Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures.*Proc. R. Soc. B Biol. Sci.* , 273, 603–610.

Sagan, L. (1967). On the origin of mitosing cells. *J. Theor. Biol.* , 14, 225–274.

Sanders, J.G., Beichman, A.C., Roman, J., Scott, J.J., Emerson, D., McCarthy, J.J., *et al.* (2015). Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivores.*Nat. Commun.* , 6, 1–8.

Schmitt, J., Stinchcombe, J.R., Shane Heschel, M. & Huber, H. (2003). The adaptive evolution of plasticity: phytochrome-mediated shade avoidance responses. *Integr. Comp. Biol.* , 43, 459–469.

Schultz, P.A., Miller, M.R., Jastrow, J.D., Rivetta, C.V. & Bever, J.D. (2001). Evidence of a mycorrhizal mechanism for the adaptation of*Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies.*Am. J. Bot.* , 88, 1650–1656.

Sharpton, T.J. (2018). Role of the gut microbiome in vertebrate evolution. *mSystems* , 3, 1–5.

Shaw, R.G., Geyer, C.J., Wagenius, S., Hangelbroek, H.H. & Etterson, J.R. (2008). Unifying life-history analyses for inference of fitness and population growth. *Am. Nat.* , 172, 35–47.

Shropshire, J.D. & Bordenstein, S.R. (2016). Speciation by symbiosis: the microbiome and behavior. *MBio* , 7, 1–11.

Sison-Mangus, M.P., Metzger, C.M.J.A. & Ebert, D. (2018). Host genotype-specific microbiota do not influence the susceptibility of*D. magna* to a bacterial pathogen. *Sci. Rep.* , 8, 1–10.

Smith, D.S., Schweitzer, J.A., Turk, P., Bailey, J.K., Hart, S.C., Shuster, S.M., *et al.* (2012). Soil-mediated local adaptation alters seedling survival and performance. *Plant Soil* , 352, 243–251.

Smith, S.E. & Read, D.J. (2008). *Mycorrhizal Symbiosis* . Third edition. Academic Press, Boston, Massachusetts, USA.

Swenson, W., Wilson, D.S. & Elias, R. (2000). Artificial ecosystem selection. *Proc. Natl. Acad. Sci.* , 97, 9110–9114.

Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution* . University of Chicago Press, Chicago, IL, USA.

Toju, H., Peay, K.G., Yamamichi, M., Narisawa, K. & Hiruma, K. (2018). Core microbiomes for sustainable agroecosystems. *Nat. Plants* , 4, 247-257.

Trevelline, B.K., Fontaine, S.S., Hartup, B.K. & Kohl, K.D. (2019). Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proc. R. Soc. B Biol. Sci.* , 286, 1–9.

Trevors, J.T. (1996). Sterilization and inhibition of microbial activity in soil. *J. Microbiol. Methods* , 26, 53–59.

Turkington, J. & Harper, J.L. (1979). The growth , distribution and neighbour relationships of *Trifolium repens* in a permanent pasture . vi . Conditioning effects by neighbours. *J. Ecol.* , 77, 734–746.

Vannette, R.L. & Fukami, T. (2016). Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar consumption by pollinators. *Ecology* , 97, 1410–1419.

Velasquez-Manoff, M. (2015). Gut microbiome: The peace-keepers.*Nature* , 518, 33–39.

Verstraete, B., Janssens, S. & Ronsted, N. (2017). Non-nodulated bacterial leaf symbiosis promotes the evolutionary success of its host plants in the coffee family (*Rubiaceae*). *Mol. Phylogenet. Evol.* , 113, 161–168.

Vitousek, P. & Walker, L.R. (1989). Biological invasions by *Myrica faya* in Hawai'i: Plant demography, nitrogen fixation, ecosystem effects. *Ecol. Monogr.*, 59, 247–265.

Wagner, M.R., Lundberg, D.S., Coleman-Derr, D., Tringe, S.G., Dangl, J.L. & Mitchell-Olds, T. (2014). Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild *Arabidopsis* relative. *Ecol. Lett.*, 17, 717–726.

Wagner, M.R., Tang, C., Salvato, F., Clouse, K.M., Bartlett, A., Sermons, S., *et al.* (2020). Microbe-dependent heterosis in maize. *bioRxiv*. bioRxiv preprint doi: https://doi.org/10.1101/2020.05.05.078766.

Wang, C., Reid, J.B. & Foo, E. (2018). The art of self-control –autoregulation of plant–microbe symbioses. *Front. Plant Sci.*, 9, 1–8.

Weese, D.J., Heath, K.D., Dentinger, B.T.M. & Lau, J.A. (2015). Long-term nitrogen addition causes the evolution of less-cooperative mutualists. *Evolution*, 69, 631–642.

Weigel, B.L. & Erwin, P.M. (2017). Effects of reciprocal transplantation on the microbiome and putative nitrogen cycling functions of the intertidal sponge, *Hymeniacidon heliophila*. *Sci. Rep.*, 7, 1–12.

West, S.A., Toby Kiers, E., Pen, I. & Denison, R.F. (2002). Sanctions and mutualism stability: when should less beneficial mutualists be tolerated? *J. Evol. Biol.*, 15, 830–837.

Wright, J.W., Stanton, M.L. & Scherson, R. (2006). Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. *Evol. Ecol. Res.*, 8, 1–21.

Table 1: Hypothetical linear model terms used to understand microbe-mediated adaptation reciprocal transplant experiments. We also include potential interpretations when main effects or interactions are found to be significant and how that interaction type might be interpreted in a quantitative genetics framework.

Model term	Interpretation
Habitat	Habitat quality differs
Plant Genotype	Plant genotypes differs
Microbial Source	Microbes from one source are more beneficial to plants than microbes from a
Habitat*Plant Genotype	Plant local adaptation (G_pxE)
Habitat *Microbial Source	Microbe-mediated adaptive plasticity (G_mxE)
Plant Genotype * Microbial Source	Plants are adapted to their home microbes (G_pxG_m)
Habitat * Plant Genotype *Microbial Source	Microbe-mediated local adaptation (G_pxG_mXE)

Figure legends:

Figure 1: Host-microbiome interactions are governed by the complex interplay between host genotype, microbial genotype/community composition, and the environment (A). The environment directly affects both host and microbial genotypes/communities and microbes and host reciprocally affect each other. Reciprocal transplants of seeds into natal and non-natal environments, with and without microbes can reveal when microbes are responsible for host adaptive responses. For example, without microbes there is no pattern of adaptation (B), but when microbes are manipulated in reciprocal transplant experiments you may see either microbe-mediated local adaptation (C) when local plant genotypes have higher fitness than foreign genotypes because of a genotype-specific affiliation with locally important microbe(s) or microbe-mediated adaptive plasticity (D) when local plant phenotypes have higher fitness than foreign phenotypes as a result of interactions with locally important microbes. Squares represent plants collected from population 1 (P1), and circles represent plants collected from population 2 (P2). Population 1 plants can either be transplanted into their natal habitat (P1, represented by a square) or into a foreign habitat (P2, represented by a circle), both with (C,D) and without (B) microbes.

Figure 2: A fully factorial reciprocal transplant design can be used to transplant seeds and microbes into

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home and away habitats to investigate how microbes affect patterns of local adaptation. To definitively attribute effects to microbes you need to include sterilized controls (depicted here as a red line crossing out the microbial communities). Squares represent plants and microbes collected from population 1 (P1), and circles represent plants and microbes collected from population 2 (P2). Population 1 plants and microbes can either be transplanted into their natal habitat (P1, represented by a square) or into a foreign habitat (P2, represented by a circle).

Figure 3: The additional manipulation of moving microbes and plant genotypes between sites can unravel if G_pxG_mxE interactions dominate (A), in this case positive effects of microbial symbionts would only be evident in their natal habitat. Alternatively, if G_pxG_m interactions dominate (B), plants would still benefit from microbial symbionts when both were moved into novel habitats. Microbe-mediated adaptive plasticity could be the result of either microbes specialized for their particular habitat (C) or the result of either direct or indirect (plant mediated) changes to microbial function or shifts in community composition that affect plant fitness. Microbe-mediated plasticity in important plant functional traits, such as flowering time (D) might underlie microbe-mediated adaptive plasticity, where microbes push flowering time phenotype into an optimal range (depicted by the shading) for a given habitat. Squares represent plants and microbes collected from population 1 (P1), and circles represent plants and microbes collected from population 2 (P2). Population 1 plants and microbes can either be transplanted into their natal habitat (P1, represented by a square) or into a foreign habitat (P2, represented by a circle) and vice versa.

Figure 4: Experiments to identify microbe-mediated adaptation will require identifying a source of microbial inoculum (A) and determining proper controls (B) for the experimental design. Another major consideration is where the experiment will take place, evolutionary ecology experiments with plants are typically performed in common gardens (greenhouses or outdoors) or as reciprocal transplant experiments.

1. Create soil inoculum (A)　　　2. Identify controls (B)　　　3. Select transplant location (C)

●Individual taxa
●Bacterial washes
●Whole soil inoculum

Control soil inoculum

Live soil inoculum

●Fungicide
●Autoclaving
●Gamma irradiation

Greenhouse

Common garden

Reciprocal transplant

Habitat ●　　Habitat □

Increasing biological complexity