Chapter 8

Microbially Mediated Plant Functional Traits

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8.1 INTRODUCTION

Interactions between plants and their associated microbial communities have profound implications for managed and natural ecosystems. All plant surfaces and tissues—including roots, root nodules, stems, leaves, and seeds—can be colonized by fungi, bacteria, and archaea (Rosenblueth and Martinez-Romero, 2006). For example, endophytic bacteria can be found at population densities of 10^7–10^{10} cells per gram of tissue (Hardoim et al., 2008; see Section 5), while bacteria occur at 10^4–10^6 cells per gram of rhizosphere soil (von Felten et al., 2010; Rafi and Charyulu, 2010) and in the rhizoplane at 10^8–10^{14} CFU/g of root (Cavaglieri et al., 2007). Plant-associated microbes provide up to 20% of nitrogen in grasslands and 80% nitrogen in forests, as well as 75% of plant phosphorus (van der Heijden et al., 2008). With molecular techniques revolutionizing our ability to quantify microbial populations, classify them taxonomically, and characterize them functionally, we are entering a phase of rapid knowledge growth. Although they may comprise only a small amount of biomass, these microbes could play major roles in ecosystems through their interactions with plants. In addition to the direct negative and positive controls that pathogens and mutualists can have through their impacts on host survival, biomass production, and reproduction, these multitudinous microbes can impact plants' interactions with the abiotic environment and ecological communities by affecting plant functional traits. Plant-associated microbes can fundamentally alter ecological processes, leading to an increase or decrease in community level diversity both of plants and the higher levels of the food webs they support. These effects may then feed back upon the evolution of the microbes involved by altering transmission patterns through host demographic processes or connections between host traits and microbial transmission. Finally, hosts may directly impose selection on microbial trait mediation through partner choice or sanctions, evolutionary mechanisms that are predicted to be needed to maintain cooperative microbial behavior.

Plant functional traits are part of a growing paradigm that seeks to explicitly link the phenotypes of individual plants to ecosystem processes and services (Römermann et al., 2008; Westoby and Wright, 2006). A functional trait is a measurable morphological, physiological, or phenological property that is correlated to individual fitness (e.g., biomass, survival, and/or reproductive output) (Violle et al., 2007). These fitness components are not typically considered functional traits. Defining functional traits at the level of individual plants is important as it enables trait variation to be scaled through eco-physiological and demographic processes to the ecosystem level (Moorcroft and Hurtt, 2001; Díaz and Cabido, 2001; Díaz et al., 2007; see Chapter 7), including impacts on ecosystem services (de Bello et al., 2010). In addition to the well-known "leaf economics spectrum," wherein leaves with higher nitrogen content tend to be thinner and less defended (Wright et al., 2004), other suites of correlated traits have been recognized that may indicate insurmountable physical or physiological

trade-offs (Westoby and Wright, 2006). These include trade-offs between wood density and both relative growth and survival (Chave et al., 2009), and several root traits including root mass versus specific root area, root area versus nitrogen uptake ability, and nitrate versus ammonium uptake ability (Maire et al., 2009). These trade-offs can be used to predict relationships between environmental variables and ecosystem services, such as grasslands in the French Alps that span a soil-fertility-based gradient and whose functional traits have been used in a structural equation modeling framework to predict agronomic and cultural value (Lavorel and Grigulis, 2011). The distributions of leaf and root functional traits change in response to experimental disturbance and predict biomass productivity and soil carbon sequestration, highlighting the importance of these traits in responding to environmental change and in turn influencing ecosystem properties (Klumpp and Soussana, 2009; see Chapter 7). However, commonly measured functional traits may not capture all relevant information about a community, as a recent study found that phylogenetic diversity explained variation in the relationship between plant communities and ecosystem function that functional trait variation was not able to explain (Flynn et al., 2011). While methods have been proposed by which the most informative functional traits can be identified in large data-sets (Bernhardt-Römermann et al., 2008), it remains an open challenge to mechanistically understand the basis of these trade-offs. In addition to the examples mentioned earlier in which abiotic factors can alter functional trait distributions in communities, biotic interactions can alter the expression of functional traits within and/or between species. The remainder of this chapter focuses on the patterns, mechanisms, and implications of plant functional trait alteration through associations with soil-transmitted microbes.

8.2   MICROBIAL EFFECTS ON PLANT FUNCTIONAL TRAITS

In a recent review, Friesen et al. (2011) asked to what extent the values of plant functional traits are determined, in part or in whole, by interactions with microbes. Out of thirty functional traits that are commonly measured in plant ecological research (Cornelissen et al., 2003), fourteen have evidence in the literature of microbial mediation (Table 8.1), including "nutrient uptake strategy" which is primarily concerned with which microbial symbionts a plant associates with (i.e., nitrogen-fixing rhizobium bacteria, arbuscular mycorrhizal fungi (AMF), and/or ectomycorrhizal fungi). These examples are all of commensal or mutualistic microbes, but there are also examples of pathogens impacting host traits, such

as *Phytophthora cinnamomi* infestations that alter the distribution of functional traits in Southwest Australian woodlands (Bishop et al., 2011). Of relevance to this volume, all five root functional traits show substantial evidence of microbial effects; potential mechanisms for these effects will be discussed in the following sections. Five out of eight leaf functional traits have examples of microbial effects, in two of these cases it is below-ground associations with rhizobia that alter leaf traits: *Bradyrhizobium japonicum* inoculation increases *Glycine max* specific leaf area by 10% and increases carbon uptake rate by 23% (Harris et al., 1985), and *Medicago sativa* freezing tolerance depends on the rhizobium strain it is inoculated with (Bertrand et al., 2006). Five plant functional traits are not likely to be under the control of microbes, such as photosynthetic pathway, dispersal mode, and growth form (Table 8.1). An intriguing example in *Prunella vulgaris* shows that the identify of mycorrhizal fungus can cause 1.8-fold variation in the degree of clonal reproduction, with both fungal strain and plant genotype significantly influencing clonality (Streitwolf-Engel et al., 2001). For 11 functional traits, we lack studies in which the presence or identity of microbial associates is manipulated; this is a ripe avenue for future research. The functional trait framework has been extended to microbes, with the proposal of a trait-based classification of arbuscular mycorrhizal fungi that should enable links to ecosystem properties despite taxonomic difficulties; these fungal functional traits include hyphal length and lifespan (van der Heijden and Scheublin, 2007).

In general, we lack systematic estimates of the relative contributions of plant genetic variation and microbial community variation to the values of plant functional traits (but see Chapter 7). In one study of *M. sativa* wherein 24 plant cultivars were factorially inoculated with 11 strains of rhizobia, the rhizobium strain explained as much or more variation as plant genotype for shoot biomass, root biomass, and plant height; there were also substantial amounts of cross-species epistasis for these traits (Tan and Tan, 1986) (Fig. 8.1). These effects could be due to variation in nitrogen fixation as well as potential interactions involving phytohormone pathways. The prevalence of microbial mediation of plant functional traits has not yet been estimated, but two studies that focused on particular plant pathways suggest that these effects are common. Across 13 strains of rhizobia, 38% of strains produced ACC deaminase under lab conditions (Ma et al., 2003)—this enzyme alters plant ethylene levels and could interact with root architecture as well as pathogen resistance and abiotic stress tolerance, as discussed in greater detail later. Across 300 cultivated bacteria isolated from the rhizosphere of sugarcane, 33% were antagonistic to *Colletotrichum falcatum*, a fungal

Table 8.1 Examples of microbial alteration of plant functional traits[a]

Category	Trait	Plant	Microbe	Reference
Leaf	Specific leaf area	Soy (*Glycine max*)	Rhizobia *Bradyrhizobium japonicum*	(Harris et al., 1985)
Leaf	Leaf size	Grasses (*Agrostis perennans, Poa autumnalis*)	Fungal endophyte *Clavicipitaceae*	(Davitt et al., 2010)
Leaf	Leaf dry matter content			
Leaf	Leaf N concentration	*Welfia georgii*	Cyanobacteria	(Bentley & Carpenter, 1984)
Leaf	Leaf P concentration		AMF	(Smith et al., 2009)
Leaf	Leaf physical strength			
Leaf	Photosynthetic pathway			
Leaf	Leaf frost sensitivity	Alfalfa (*Medicago sativa*)	Rhizobia *Sinorhizobium meliloti*	(Bertrand et al., 2006)
Regenerative	Dispersal mode			
Regenerative	Dispersal shape			
Regenerative	Dispersal size/mass			
Regenerative	Seed mass	Grasses (*Schedonorus phoenix, Schedonorus pratensis*)	Fungal endophyte *Neotyphodium*	(Saari et al., 2010)
Regenerative	Resprouting capacity			
Stem	Stem-specific density			
Stem	Twig dry matter content			
Stem	Twig drying time			
Stem	Bark thickness			
Vegetative	Growth form			
Vegetative	Life form			
Vegetative	Plant height	Rice (*Oryza sativa*)	Rhizobia *Rhizobium leguminosarum bv. trifolii*	(Perrine-Walker et al., 2007)
Vegetative	Clonality	*Prunella vulgaris*	AMF *Glomus sp.*	(Streitwolf-Engel et al., 2001)
Vegetative	Spinescence			
Vegetative	Flammability			
Vegetative	Leaf life-span	Cacao (*Theobroma cacao*)	Leaf endophytic fungi	(Arnold et al., 2003)
Vegetative	Leaf phenology			
Root	Specific root length	Maize (*Zea mays*)	AMF *Glomus mosseae*	(Kothari et al., 1990)
Root	Fine root diameter	Pine (*Pinus taeda*)	Ectomycorrhizae: *Pisolithus tinctorius, Cenococcum geophilum*	(Rousseau et al., 1994)
Root	Root depth distribution	Clover (*Trifolium repens*)	AMF *Glomus mosseae*	(Joner & Leyval, 2001)
Root	95% rooting depth	Wheat (*Triticum aestivum*)	AMF *Glomus fasciculatum, Glomus deserticola*	(Ellis et al., 1985)
Root	Nutrient uptake strategy		Association with microbes	

[a]From Cornelissen et al. (2003).

▓ implausible that microbes could alter; ▓ major impacts of microbes; ▓ some examples of microbial influence; ▓ no empirical tests found.
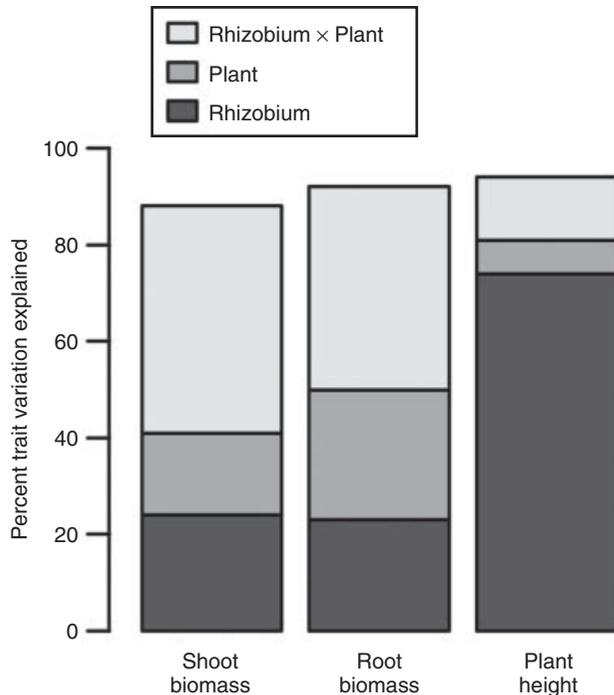
Figure 8.1 The percentage of plant trait variation explained by *Medicago sativa* cultivar, rhizobium strain, and the interaction between plant and rhizobium genotype for three traits. Data from (Tan and Tan, 1986).

pathogen, and 49% of these antagonistic strains produced the phytohormone indole-3-acetic acid (IAA), that is, auxin (Hassan et al., 2010). These studies highlight the value of understanding the molecular basis of microbial effects on plant traits, since these lab-based screens using chemical or genetic tests are much more high-throughput than characterizing phenotypic effects *in planta*. While more studies are clearly needed, these examples demonstrate that microbes can have substantial impact on plant traits and that these abilities are widespread among the microbes that associate with plants.

Microbes are known to play important roles in plant nutrition, defense against pathogens and herbivores (see Section 7), and abiotic stress tolerance (Rodriguez et al., 2008). Many bacteria and archaea can fix atmospheric nitrogen to biologically available ammonium (Martinez-Romero, 2006); those that do so in root nodules are termed rhizobia and on average cause 16% growth enhancement in field studies and 59% in pot experiments (Kaschuk et al., 2010). Nitrogen fixation also occurs in symbiotic interactions with actinorhizal bacteria, and many rhizospheric and endophytic bacteria can fix nitrogen (see Chapter 44). While in many cases these associates have been shown to provide growth benefits, we still await confirmation on whether the atmospheric nitrogen fixed in symbiosis by

these bacteria is provided to the plant and is responsible for this growth increase or whether other mechanisms underlie this phenomenon (Bhattacharjee et al., 2008). The provision of fixed nitrogen can alter the maximum photosynthetic rate by enabling increased rates of protein production; nitrogen availability via symbiosis may also interact with developmental pathways controlling root architecture (see below). Mycorrhizal fungi (see Chapter 43) can extract phosphorus and nitrogen from soil and transfer these resources to host plants (Smith et al., 2009; Lambers et al., 2008); on average, inoculation with AMF increases plant growth by 45% in pot experiments though there is not an overall significant difference between mycorrhizal inoculated plants and non-inoculated plants under field conditions, perhaps due to the difficulty of excluding mycorrhizae from controls (Kaschuk et al., 2010). Rhizobia and mycorrhizae can reduce plant herbivory (Dean et al., 2009; K Saikkonen et al., 1998) and many plant-associated microbes can defend against pathogens directly by producing antimicrobial compounds (Fravel, 1988) or interfering with quorum sensing that is required for virulence by pathogens (Qian et al., 2010). Microbes can also enhance pathogen resistance indirectly through the induction of plant resistance pathways, such as *Trichoderma*'s induction of systemic as well as localized resistance (Harman et al., 2004) and rhizospheric *Pseudomonas aeruginosa*'s production of salicylic acid, which induces systemic acquired resistance (Maurhofer et al., 1998). Abiotic stress tolerance, discussed in more detail later, can be strongly influenced by microbes. Across grasses, some taxa exhibit genetically based tolerance to soil salinity while others require infection by endophytic fungi for salt tolerance (Rodriguez and Redman, 2008). In some cases, epistatic interactions between plant and microbial genomes determine responses to abiotic stress. For example, in a study of six *Medicago truncatula* genotypes inoculated with one of two strains of *Sinorhizobium*, stem length showed a three-way interaction between plant genotype, rhizobium strain, and salt concentration (Kahen and Friesen, unpublished data). Greater discussion of these microbially mediated traits can be found in (Friesen et al., 2011). In the spirit of this collection, the following section focuses on the molecular mechanisms by which microbes alter root traits and the pathways that may constrain how roots interact with microbes.

8.3   ROOT ARCHITECTURAL TRAITS AND PATHWAYS

Root architecture plays a major role in plant performance and abiotic stress adaptation (Lynch, 1995; de Dorlodot

et al., 2007). This generic term includes properties such as root diameter, lateral root branching patterns, and root lifespan as well as the more commonly measured traits consisting of fine root diameter, specific root length (related to root tissue density), and rooting depth (Table 8.1). Selection on root traits has been one of the key determinants of increased yield in crop plants, enabling higher planting densities in crops such as maize (Hammer et al., 2009); recent work in *M. truncatula* populations shows that root diameter and root length experience significant total natural selection (Friesen et al., in press). These traits can be integrated with aboveground traits: root tissue density (specific root length) is significantly correlated with relative growth rate and maximum plant height across 14 Mediterranean herbaceous species (Hummel et al., 2006); tissue nitrogen content is a good predictor of root and leaf respiration, and both nitrogen and respiration predict root and leaf longevity (Tjoelker et al., 2005).

Microbes can alter these traits by improving plant nutrition as discussed earlier; they can also interact directly with plant pathways to modify root architecture. Roots are complex tissues and much has been learned in *Arabidopsis* by transcriptional analysis using fluorescence-based cell-sorting of individual cell types (Birnbaum and Benfey, 2004). These data, in conjunction with whole-root and root-tip transcriptional studies, have elucidated multiple components of the environment sensing and developmental patterning gene regulatory networks, which include nutrient-sensing pathways with phytohormone signaling (Krouk et al., 2011), often involving small RNA regulation (Sunkar et al., 2007; Khan et al., 2011). Root traits are highly plastic in response to the environment, particularly nutrient and stressor levels. One of the best understood processes is lateral root initiation and growth, which is promoted by both nitrate and glutamate, with nitrate also upregulating genes involved in nitrate uptake and transport (Forder and Walch-Liu, 2009). The phytohormones auxin (IAA), ethylene, cytokinin, and abscisic acid have all been implicated in lateral root development (Castaings et al., 2011; Kiba et al., 2011; Yoo et al., 2009). For auxin and ethylene in particular, there is evidence (presented above) for microbes commonly interacting directly with these plant pathways by producing phytohormones, as in the case of IAA (discussed in greater detail later; see also Chapter 29), or altering phytohormone levels, as in the case of ACC deaminase.

Other microbially produced small molecules can act through phytohormone pathways, such as three diketopiperazines produced by *P. aeruginosa* that alter root architecture and gene expression similar to exogenously supplied auxin (Ortíz-Castro et al., 2011). The production of these molecules is under the control of a quorum-sensing system (Ortíz-Castro et al., 2011). Intriguingly, quorum-sensing molecules that microbes use to interact with one another (see Section 9) have been demonstrated to impact root architecture directly. Primary root length, lateral root formation, and root hair growth of *Arabidopsis thaliana* respond to the application of bacterial acyl homoserine lactones (AHLs) (Ortíz-Castro et al., 2008), well-studied quorum-sensing molecules. In *M. truncatula*, 7% of root protein levels responded to bacterial AHLs (Mathesius, 2003). In the case of rhizobia, mutations in quorum-sensing genes often cause reduced symbiotic ability (reviewed in Sanchez-Contreras et al., 2007; see also Chapter 50). Furthermore, plants can stimulate or inhibit quorum-sensing genes of specific bacteria (Sanchez-Contreras et al., 2007), suggesting the potential for dynamic coevolution of these signaling systems.

8.3.1 Auxin

The plant growth promoting rhizobacterium *Phyllobacterium* alters the response of root architecture to nitrate—inoculated *Arabidopsis* maintain constant root fresh weight and lateral root number across a nitrate gradient while uninoculated plants have fewer lateral roots and lower overall root weight under increasing nitrate concentrations (Mantelin et al., 2005). *Phyllobacterium* inoculated plants have constant root and shoot nitrate pools, possibly resulting from altered expression of nitrate transporters (Mantelin et al., 2005). This effect was found to depend on auxin signaling, with auxin transport and signaling mutants failing to respond to bacterial inoculation (Contesto et al., 2010). However, *Phyllobacterium* does not produce high amounts of indole-acetic acid and inoculated roots do not have increased IAA levels relative to control; they do show increased shoot expression of IAA biosynthesis genes (Contesto et al., 2010). This indirect alteration of plant auxin pathways is in contrast to the estimated 80% of plant-associated microbes that can produce IAA directly through a variety of biosynthetic pathways (Spaepen et al., 2007). A clear example of the importance of this pathway comes from *Azospirillum*, with mutation in indole-3-pyruvic acid decarboxylase (*ipdC*) reducing auxin production 10-fold and preventing bacteria from causing shorter roots with denser root hairs in wheat (Dobbelaere et al., 1999; see Chapters 27 and 29). A similar phenotype in *Arabidopsis* is observed when the small RNA miR393 is overexpressed; this small RNA is upregulated in response to nitrate and acts through the auxin receptor AFB3 (Vidal et al., 2010).

8.3.2   Overlap Between Root Architecture and Nodulation Pathways

The nitrogen-fixing symbiosis between legumes and rhizobium, perhaps the best studied beneficial plant–microbe interaction, highlights potential overlap between microbial associations and root development pathways. This interaction also shows how plant regulation of symbiosis interacts with nutrient and hormonal signaling. The development of legume nodules shares many features of lateral root formation and there are multiple observations supporting genetic overlap/crosstalk between these processes. The initial Nod factor (NF) (see Chapter 45) signal increases lateral root formation in *M. truncatula*, and this requires early components of the NF signal transduction pathway (Oláh et al., 2005). Nodules typically do not form on mature roots, except for near the site of lateral root emergence, and both types of organ contain the expression of ENOD40 and ENOD12A (Mathesius et al., 2000). The LATD/NIP gene, which is stimulated by cytokinin but repressed by ABA and auxin, is a relative of the *A. thaliana* nitrate transporter/receptor NRT1.1 and is required for both lateral root formation and nodule development, with an allelic series showing a progression of nodulation defects (Yendrek et al., 2010; reviewed in Harris and Dickstein, 2010). Furthermore, a *M. truncatula* cytokinin receptor MtCRE1 has been shown to positively regulate lateral roots but negatively regulate nodule formation (Gonzalez-Rizzo et al., 2006); homologous histidine kinase cytokinin receptors in *A. thaliana* are involved in maintaining meristem activity in the root as well as the shoot (Nishimura et al., 2004). The *M. truncatula* "super numeric nodules" (*sunn*) mutant (Penmetsa et al., 2003) also shows decreased root length, exhibits defects in auxin transport, and acts upstream of CLE peptides which repress nodulation (reviewed in Mathesius (2008) and Saur et al. (2011)). Furthermore, MtCLE12 overexpression abolishes nodulation and decreases root growth, along with an alteration of auxin response in roots which could be due to increased transport or sensitivity (Saur et al., 2011). Another over-nodulating *M. truncatula* mutant, *sickle* (Penmetsa and Cook, 1997), is ethylene-insensitive and also shows altered auxin transport (Prayitno et al., 2006). The *Lotus japonicus* homolog to *sunn*, *Har1*, shows uncontrolled nodulation, increased lateral root formation, decreased primary root length, and decreased root diameter (Wopereis et al., 2000). These pleiotropic effects of genes on nodulation by rhizobia and other aspects of root architecture suggest that plants may face trade-offs between symbiosis and other functional traits, as well as indicating genetic mechanisms by which microbial interactions may influence functional trait values.

In contrast to these observations, other mutants indicate a degree of independence between root architecture and nodulation and evolutionary flexibility in the underlying pathways. For example, the "compact root architecture" (*cra1*) mutant in *M. truncatula* shows roots with increased diameter and decreased length without changes in lateral root or nodule formation (Laffont et al., 2010). Transcriptional analysis showed reduced lignin biosynthesis and additional experiments suggest that polar auxin transport may also be reduced. Cross-species comparisons show divergence in the genetic mechanisms underlying root architecture. The *A. thaliana* transcription factor NAC1 influences lateral root formation (Xie et al., 2000) and is under several forms of regulation including by the small RNA miR164 (Guo et al., 2005). However, while its closest homolog MtNAC1 in *M. truncatula* does not alter lateral root formation or nodulation, overexpression of miR164 causes decreased nodulation, likely acting through other targets (D'haeseleer et al., 2011). This is a clear example of divergence in function between *A. thaliana* and *M. truncatula* pathways influencing root architecture. A related transcription factor, MtNAC969, when knocked down with RNAi leads to increased lateral root formation and higher root biomass and root length under salt stress, but does not cause changes in nodule number (de Zélicourt et al., 2012). This same TF is induced in nodules upon nitrate treatment, but repressed in this tissue by salt treatment; overexpression leads to premature nodule senescence (de Zélicourt et al., 2012).

While much progress continues to be made elucidating the molecular basis of microbial interactions with root functional traits, it is clear that there are multiple pathways involved. These include many of the known phytohormones as well as nutrient signaling and developmental regulation. Thus, there are multiple potential points at which microbes could exert influence over plant traits and pleiotropic effects might limit their plant host's ability to manage these interactions. Although more work remains to be done at the molecular level, studies at the ecological and evolutionary scales serve to provide guidance as to the processes that demand increased attention.

8.4   ECOLOGICAL IMPLICATIONS

Microbial associations can play major roles in all ecological aspects of a plant's existence in nature through their alteration of functional traits. A species' ecological niche, that is, the multidimensional range of conditions over which it is found in nature, can be reshaped through microbes' effects on how plants interact with abiotic factors and other species. These effects can impact plant

diversity at the community level and scale up to influence ecosystem level processes.

8.4.1 Microbial Limitation or Expansion of Plant Ranges

Microbial symbionts can limit or can expand a plant's range, depending on the pattern of specificity and interactions with abiotic environmental variation. The introduction of *Pinus patula* to Zambia succeeded only when the compatible ectomycorrhizal fungi were co-introduced (Richardson et al., 1994). Similarly, *Cytisus scoparius* seedlings were symbiont limited when planted beyond their home range, though they were able to nodulate at low levels with rhizobia found on *Desmodium canadense* that occurred naturally at these novel sites (Parker et al., 2006). In addition to these specific microbes, plant–soil feedbacks involving the microbial community in general are widely recognized to play a major role in species range expansions and invasions (Bever et al., 2010; Mitchell et al., 2006). Microbial biogeography demonstrates that most microbes face dispersal limitation at some scale (Horner-Devine and Bohannan, 2006; Martiny et al., 2006). Symbiont limitation in novel habitats can be overcome through (i) reduced dependence on symbionts, (ii) acquisition of novel symbionts, or (iii) co-invasion with symbionts (Pringle et al., 2009). Intriguingly, recent work with the ectomycorrhizal fungus *Amanita phalloides* shows that this symbiont can invade novel habitats by switching hosts (Wolfe and Pringle, 2011), suggesting that microbial invasions may in some cases precede invasions by their original hosts. Furthermore, invasive symbionts can displace native symbionts and alter the community structure of mutualisms. For example, where Australian *Acacia* species have invaded Europe, native European legumes nodulate with rhizobia bearing Australian symbiotic genes (Rodríguez-Echeverría, 2010).

8.4.2 Niche Expansion Through Abiotic Stress Tolerance

In addition to participating in the colonization of novel ranges as described earlier, microbial associates can in many instances expand the abiotic range of conditions a plant can tolerate. Abiotic stress tolerance is a key plant functional trait (see Table 8.1), but requires experimental manipulation of replicated genotypes across environments to accurately assess. A dramatic example of microbial mediation of abiotic stress tolerance comes from the grass *Dichanthelium lanuginosum* that occurs on soils near thermal vents in Yellowstone park; when cured of its fungal endophyte *Curvularia*, the grass is no longer able to tolerate high temperatures (Redman et al., 2002).

Intriguingly, follow-up work demonstrated that the ability of the fungus to confer thermal tolerance to the grass was dependent upon the presence of a virus within the fungal genome (Marquez et al., 2007). This is strikingly reminiscent of the presence of symbiosis islands within rhizobium that modulate the formation and functioning of symbiosis with legumes. Tolerance to heavy metals can be conferred by multiple bacteria and fungi (reviewed in Gamalero et al. (2009)). For example, the ectomycorrhizal fungus *Suillus luteus* shows local adaptation to copper and nickel when grown in isolation and copper-tolerant isolates pass along this advantage to *Pinus sylvestris* seedlings grown under copper treatment (Adriaensen et al., 2005). Rhizobial bacteria are also able to confer multiple forms of abiotic stress tolerance. Alfalfa (*M. sativa*) inoculated with contrasting strains of rhizobia differ in their freezing tolerance (Bertrand et al., 2006). Rhizobium strains engineered to produce IAA cause *M. truncatula* to exhibit increased salt tolerance (Bianco and Defez, 2009). Another observation pointing to connections between salt tolerance and nodulation comes from a calcium-dependent protein kinase that is induced both during nodulation and salt stress in *M. truncatula*; this gene plays a regulatory role in nodulation, with RNAi-transformed roots showing increased nodule number (Gargantini et al., 2006).

8.4.3 Microbial Alteration of Biotic Interactions and Plant Communities

Many soil microbes can influence the relationships that plant have with both herbivorous and pollinating insects; plant–insect interactions can in turn alter the degree of colonization by mycorrhizal fungi and the composition of plant-associated microbial communities (Pineda et al., 2010). Both endophytic and mycorrhizal fungi can confer protection against herbivores, but these effects depend on the type of herbivore, the species and genotypes involved, and the abiotic context (Hartley and Gange, 2009). In fact, some endophytes can increase herbivore abundance and alter their diversity. In the North American native grass *Festuca arizonica*, infection by *Neotyphodium* increases herbivore abundance (Faeth, 2009). Similarly, *Neotyphodium* infection of *Achnatherum robustum* increased herbivore abundance and species richness, while decreasing the relative abundance of parasitoids (Jani et al., 2010). These results are in contrast to the defensive effects of *Neotyphodium* on two invasive cultivated grasses, summarized recently using meta-analysis (Saikkonen et al., 2010). Some microbes may alter higher trophic levels directly. For example, arbuscular mycorrhizae can cause a reduction in *Vicia faba* extrafloral nectaries, which is predicted to decrease plant protection by ants (Laird and Addicott,

2007). In another study, mycorrhizae increased nectary number, but this did not translate into an increase in protective ants (Laird and Addicott, 2009). Another beneficial insect interaction is pollination mutualists. Mycorrhizae can increase pollinator visitation rates and shift pollinator community composition (Gange and Smith, 2005; Cahill et al., 2008); in one study, increased pollinator visitation doubled the seed set of *Chamerion angustifolium* (Wolfe et al., 2005). An inoculation experiment of three annual plants found consistent increases in pollinator visitation with arbuscular mycorrhizal inoculation, but the plant traits responsible for this increase differed between the species—one species responded through increased flower size, another through flower number, and the third through the amount of floral nectar (Gange and Smith, 2005). In contrast, fungal removal in *Polemonium viscosum* population did not alter pollinator visitation and seed set was higher in the removal treatment (Becklin et al., 2011). This study also found evidence that mycorrhizal colonization correlated with a reduction in volatile organic compounds and their diversity (Becklin et al., 2011).

A key ecological process structuring plant communities is plant–plant competition. Arbuscular mycorrhizal fungi can alter plant competitiveness through their effect on host traits, such as lateral spread and phosphorus uptake (Gross et al., 2010). *Neotyphodium* endophytic fungi infected *Lolium arundinaceum* tissue can inhibit arbuscular mycorrhizal colonization of other plants (Antunes et al., 2008). Plants can compete with one another by inhibiting microbial associates, such as several instances of plant extracts and living plants inhibiting nodulation (Rice, 1964). Furthermore, garlic mustard (*Alliaria petiolata*) invading forest plots show lower ectomycorrhizal colonization without a change in root biomass; this effect was confirmed experimentally, with *Pinus strobus* colonization declining in response to *A. petiolata* growth as well as to the growth of native *Impatiens capensis* (Wolfe et al., 2008). The presence of particular microbes can alter which species are dominant within a community, as illustrated by the identity of arbuscular mycorrhizal fungi impacting *Brachypodium pinnatum* and *P. vulgaris* coexistence as well as total productivity and tissue nutrient content (van der Heijden et al., 2003). The effects of microbial associates on competition can reduce community level diversity, as in the case of *Neotyphodium* infection, which enhances dominance by *Festuca arundinacea* but does not alter community productivity (Clay and Holah, 1999). This same fungal endophyte can slow succession in North American grasslands, with endophyte infection causing a 64–82% reduction in tree abundance (Rudgers et al., 2007). Similarly, arbuscular mycorrhizal fungi cause a saturating species

richness–productivity relationship that contrasts with the linear relationship found in the absence of this microbe (Klironomos et al., 2000). Furthermore, the shape of this relationship differed between two different AMF species. In contrast to this increase in competition, rhizobial bacteria increased the productivity and nitrogen content of experimental communities, allowing legumes to coexist in these communities (van der Heijden et al., 2006). Soil feedbacks can enhance diversity when soil conditioned by other species increases a species' most beneficial microbes (Bever, 1999), as found in a study of tropical tree seedlings (Mangan et al., 2010b). However, this effect is likely not driven by AMF, since a follow-up study found that AMF communities conditioned by two species did not impact performance of one species and favored the other species with its home AMF (Mangan et al., 2010a). Taken as a whole, these examples illustrate that microbes can alter ecological interactions in a myriad of ways, with consequences ranging from decreased to increased community and ecosystem level properties such as species diversity and total productivity. The range of outcomes highlights a need to better understand the mechanistic underpinnings of these effects so that we can ultimately formulate predictions of how these systems will respond to environmental perturbations such as global climate change (see Section 13).

8.4.4   Scaling from Microbial Trait Mediation to Ecosystem Processes and Services

Ecosystem level community processes that have value to humans are referred to as *ecosystem services*, a concept that is increasingly used to foster sustainable interactions between human societies and ecological communities (see Chapter 7). Many ecosystem services depend on plant functional traits as well as on interactions between plants and their microbial communities and higher trophic levels (de Bello et al., 2010). Several of these connections link plant functional traits for which we have evidence of microbial mediation (Fig. 8.2). For example, root size and architecture (discussed above) are related to the ecosystem services water regulation, soil stability, and soil fertility (de Bello et al., 2010). Similarly, leaf nitrogen content, which is determined in large part by interactions with nitrogen-fixing microbes, is related to soil fertility and fodder production (de Bello et al., 2010). Microbial alteration of herbivory (see previous sections) and the importance of microbes to decomposition processes highlight additional important ecosystem services. Endophytic fungi can reduce litter decomposition rates of *L. arundinaceum* (Lemons et al., 2005). Similarly, ectomycorrhizal colonized roots of *Pinus edulis* showed slower rates of
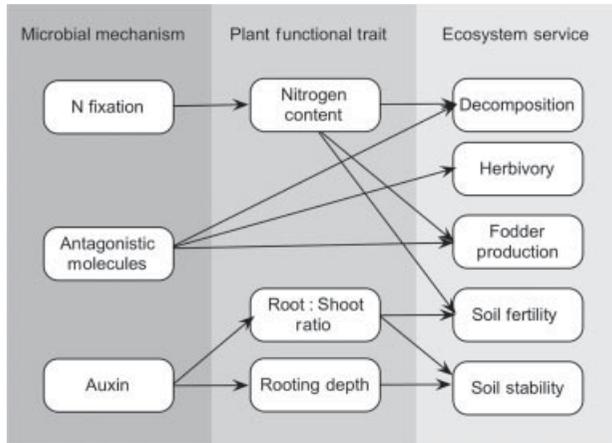
Figure 8.2 Ways that microbes can impact ecosystem services through plant functional traits.

decomposition than uncolonized roots (Langley and Hungate, 2003). In contrast, AMF inoculation did not alter the decomposition rate of roots, but increased shoot decomposition rate across four plant species (Urcelay et al., 2011).

Predicting the influence of global change on ecosystem fluxes requires models that scale up from individuals to entire regions. The majority of effort in this field has focused on aboveground processes, particularly the gas and water fluxes that occur during photosynthesis (de Pury and Farquhar, 1997). However, the uncertainties due to insufficient information about below-ground processes are often noted (e.g., Medvigy and Moorcroft, 2011). An important contribution of these models is the ability to integrate over environmental variation and take nonlinearities into account (Medvigy et al., 2010). Given knowledge in the literature regarding microbial alteration of leaf functional traits and covariance between environments and microbial interactions, leaf epiphytic and endophytic microbes are predicted to play a role in total production and evapotranspiration (Friesen et al., 2011). Belowground, root turnover is an important parameter for estimating the amount of carbon stored belowground. In two long-term elevated carbon dioxide experiments, net primary productivity is increased by 21% and 25% for sweetgum and pine, respectively, but only sweetgum shows a significant increase in allocation to roots under increased CO_2 (Matamala et al., 2003). Work in temperate forests shows that mycorrhizae increased the lifespan of first-order *Pinus palustris* roots by 45% in two out of three study years (Guo et al., 2008). There could be feedback between microbial alteration of fine root turnover and the composition of the microbial community that may introduce nonlinearities. For example, across 12 native species in the Bolivian Altoplana there is a positive relationship between fine root proportion and the ratio of arbuscular mycorrhizal fungi to dark septate endophytes,

but no correlation directly with the abundance of either associate (Urcelay et al., 2010). Since these differences are consistent between species, this relationship is likely driven by plant functional traits influencing colonization patterns rather than fungi altering the functional trait values although the previous example demonstrates that they can do so. Net primary productivity (including both fine root and litter production) increases with soil fertility across 10 sites in the Amazon, but the proportion of biomass allocated aboveground and belowground does not vary significantly (Aragão et al., 2009). Since plant associations with soil microbes vary in response to nutrient environment, the interaction rate and hence the effect of microbes on root traits such as turnover is predicted to vary across nutrient gradients. In these ways, soil microbes could play a role in shaping how root functional traits are scaled to regional scale ecosystem processes.

8.5 EVOLUTIONARY IMPLICATIONS: FITNESS CONFLICT AND ALIGNMENT

Since microbially mediated plant functional trait values depend on both the genotype of the plant and the genotypes of the microbes involved, their evolution will reflect selection on both partners. If plant and microbe fitnesses are optimized at different trait values, this could result in antagonistic coevolution and suboptimal traits from the perspective of both parties. Since rhizosphere communities colonize the plant from the soil, these microbes are not inherited from parent to offspring like the genome is. This "horizontal transmission" could result in fitness conflict, since microbes will be selected primarily for increased transmission (Sachs et al., 2004; Ebert and Bull, 2003). A part of this could include increases in abundance by enhancing host performance, but free-loaders that do not contribute to plant growth would have an evolutionary advantage if providing host benefits is costly (Bull and Rice, 1991; West et al., 2002; Friesen and Mathias, 2010). All else being equal, "vertical transmission" of microbes from parent to offspring via the seed is predicted to result in a higher degree of fitness alignment and more beneficial microbial associates (Sachs et al., 2004). The other way for fitness alignment to occur is for host plants to preferentially direct benefits to the microbes that provide the most benefit, which could occur through a variety of mechanisms including partner choice, in which plants interact more often with better strains, and sanctions, in which plants allocate resources to better strains.

Even though vertical transmission should lead to more beneficial microbes, there are relatively few

fungi (Clay and Schardl, 2002) and bacteria (López-López et al., 2010; Ran et al., 2010) that are inherited in this way (see Chapter 42). If dispersal is local for both partners, this can increase the correlation between parent and offspring microbial communities and in some cases might contribute to selection for microbial genes that benefit the host (for example Bever and Simms (2000)). Fungal endophytes of grasses include taxa that are vertically transmitted and those that are horizontally transmitted. Horizontally transmitted grass endophytes have higher frequency at higher host abundance, which could be due to increased transmission at higher host population densities (Rudgers et al., 2009). In invasive grasses, horizontally transmitted endophytes are often parasitic and can prevent hosts from reproducing while vertically transmitted endophytes can protect their hosts against drought and herbivores (Clay and Schardl, 2002). This pattern breaks down in native grasses, where endophytes increase herbivore population density by protecting them against parasitoids (Jani et al., 2010). Since insect herbivores are not affected by endophyte toxins, selection appears to have shifted to enhance transmission—endophytes cause hosts to flower earlier, which is predicted to reduce the rate of spontaneous loss (Faeth, 2009). This example illustrates the importance of transmission rate, rather than simply transmission mode, in the evolution of microbial effects on plant traits.

A much more potent form of selection on microbial genes is that which is imposed directly by the host through regulation of infection initiation or proliferation. This has been best studied in the legume–rhizobium symbiosis and two main processes appear to be in play. Plants form more nodules with strains that provide higher symbiotic benefit and this causes these strains to increase in frequency over successive generations (Heath and Tiffin, 2009). This "partner choice" mechanism (Noë and Hammerstein, 1994), since it occurs when infections are formed, requires that there be signals that correlate with the effect of the symbiont on the host; plant control may occur widely during the transition from epiphyte to endophyte (Hardoim et al., 2008). A significant positive pooled correlation between rhizobium competitive ability and legume aboveground biomass was found in a recent meta-analysis (Friesen, 2012); this lends further support to the suggestion that this interaction is structured to align host and symbiont fitness at the infection stage. Post-infection regulation, which has been termed "sanctions" or "partner fidelity feedback," occurs when hosts alter the benefits that symbionts receive in proportion to the benefits they provide (Sachs et al., 2004; Archetti et al., 2011). This mechanism may be most relevant for nutritional mutualisms, such as those involving nitrogen-fixing bacteria and resource-acquiring mycorrhizae. Legumes allocate more biomass, which typically translates into higher bacterial abundances, to nodules that fix more nitrogen (Kiers et al., 2003; Oono et al., 2009). Similarly, plants provide more carbon compounds to mycorrhizal fungi that provide more phosphorus (Kiers et al., 2011). Further experiments are needed with other potentially beneficial microbial associates to determine whether plants can reward them directly in mixed communities. If the benefits that microbes receive are due simply to increased host growth, this would not maintain cooperative behavior without other evolutionary mechanisms.

Quantifying fitness effects of plant–microbe interactions is a major challenge. A crucial point to bear in mind is that most interactions are context-dependent: the strength and even sign of the interaction (beneficial or harmful) can depend on abiotic and biotic factors (Bronstein, 1994). In many cases, the intermediary plant functional traits are not measured; one exception comes from a study of longleaf pine in which mycorrhizae altered root lifespan in contrasting ways across years (Guo et al., 2008). Performance can depend on the genotype of the plant and microbe (Klironomos et al., 2000; Parker, 1995) as well as the epistatic (G x G) interaction between them (Tan and Tan, 1986; Heath and Tiffin, 2007; Smith and Goodman, 1999). These cross-species epistatic interactions can further interact with environmental conditions to yield three-way G x G x E interactions; these have been observed in the legume–rhizobium interaction in response to nitrate (Heath et al., 2010) as well as salt stress (Kahen and Friesen, unpublished data). In some cases, these interactions signal local adaptation of one or both partners. For example, genotypes of the North American native legume *Amphicarpaea bracteata* performs best with strains of *Bradyrhizobium* collected from their home sites (Parker, 1995). No such adaptation occurs in French populations of *M. truncatula* and *Sinorhizobium medicae* (Heath, 2010). The ectomycorrhizal fungus *Rhizopogon* shows local adaptation to its *Pinus* host species, but the plant is not locally adapted to its symbiont (Hoeksema and Thompson, 2007). These asymmetries may arise for many reasons, including the potentially faster evolutionary rate of microbes relative to their hosts. Microbes' mutualistic ability can evolve on ecological timescales, as shown by the acquisition and loss of symbiotic ability within a population of *Bradyrhizobium* in California (Sachs et al., 2010) and underscored by the ability of *Mesorhizobium* strains to horizontally acquire a symbiosis gene island under both field and lab conditions (Sullivan and Ronson, 1998; Sullivan et al., 1995).

In addition to the effects of the abiotic environment on plant–microbe interactions, the presence of other microbes can alter bipartite interactions. In the mycorrhizal symbiosis, a meta-analysis shows that

inoculation with a soil bacterial pool enhances the amount of benefit that the plant obtains from the fungus (Hoeksema et al., 2010; see Chapter 4). Similarly, there are examples in which co-inoculation with *Agrobacterium* and *Sinorhizobium* leads to increased nodulation and, in some cases, plant growth relative to inoculation with only the rhizobium symbiont (Caetano-Anolles and Bauer, 1988; Salem et al., 2012). Since plants in natural settings are colonized by a diverse suite of microbes, it is in general difficult to extrapolate from lab or greenhouse studies to field conditions where the fitness effects ultimately matter for evolution. Field experiments that manipulate the relative abundance of particular genes or microbes and measure the effects of plant functional traits and performance as well as microbial performance are needed to systematically assess the importance of microbes for mediating ecosystem processes via plant traits. These challenging experiments will be facilitated by increasingly sophisticated microbial marking techniques that enable strains to be monitored *in situ* (see Section 11).

Several aspects of plant communities, including relative abundance and dispersal, will influence the pattern of microbial transmission. Furthermore, functional trait values can themselves alter patterns of association. The functional trait framework has been used in Californian grassland species to predict key epidemiological parameters of aphid-transmitted barley yellow dwarf virus (Cronin et al., 2010). A plant species' position on the leaf economics spectrum explained significant variation in susceptibility to infection, ability to transmit infection, and vector population size (Cronin et al., 2010). To the extent that functional trait values can be modified within species by the presence of microbial associates, microbes could influence their own transmission dynamics, which could then feed back onto their coevolution with plants.

8.6 CONCLUSION

Just as we humans can be profoundly impacted by the microbes that colonize our bodies (Turnbaugh et al., 2006; Foxman and Goldberg, 2010; Boerner and Sarvetnick, 2011), plants in large part rely upon microbes for nutrient uptake and pathogen defense, with approximately 20,000 species completely dependent upon microbes (van der Heijden et al., 2008). Plant-associated microbes are ubiquitous and can influence almost all plant functional traits, barring perhaps major switches in growth form or photosynthetic capability. In particular, all root functional traits show strong influences of microbial control, with multiple nutrient sensing and developmental pathways linking microbes to root architecture. These belowground partners could play major ecosystem roles by influencing,

for example, the rates of root turnover and hence the carbon balance of forests. Furthermore, many aboveground traits, including herbivore resistance, abiotic stress tolerance, and ecological interactions with pollinators can be influenced by belowground microbes. We have a broad range of examples wherein particular microbes impact plant traits, but we lack systematic estimates of the relative contributions of plant genetic- and species-level variation relative to variation in microbial strain or community composition. There are also several functional traits, especially stem traits, that are understudied with respect to microbial mediation. Furthermore, the majority of microbial manipulative studies are conducted under lab conditions in isolation from most of the community members that plants interact with. Field studies, though challenging, are essential to determine the evolutionarily relevant fitness effects of trait alteration by microbes. This is especially true given that the effects of these interactions are typically context-dependent, fluctuating according to abiotic conditions or the presence of other community members.

Very few plant-associated microbes are inherited with the rest of the genome, opening the door to potential fitness conflict. In particular, the plant functional trait values that are optimal from the perspective of the plant may differ from those that would maximize microbial fitness. Multiple evolutionary mechanisms could align plant and microbe fitness interests, falling broadly into pre-infection mechanisms such as partner choice and post-infection mechanisms such as sanctions or partner fidelity feedback. While we have evidence for both of these processes in the legume−rhizobium interaction and for post-infection regulation of the plant−mycorrhizal interaction, we lack estimates of the prevalence and strengths of these mechanisms across the multitude of plant-associated microbes. There is evidence that regulation of nodulation shares components of nitrogen signaling and exhibits overlap with the regulation of AMF infections, but we lack a detailed understanding of the mechanisms by which plants impose natural selection on their microbial partners.

In summary, as our knowledge grows with respect to the importance and molecular basis of plant−microbe interactions, there is a need for studies that focus on plant trait variation to consider the potential role of microbes. Tissue conserved from surveys and large experiments could in the future be screened for the presence of particular microbial taxa or functional genes. There is also a need for studies that manipulate the composition of microbial communities and measure ecosystem responses. An understanding of the ecological roles and evolutionary forces driving microbial mediation of plant functional traits will be important in predicting the response of ecosystems to global change, including

both species introductions and our changing climate. Similar to conservation efforts for macroscopic organisms, microorganisms need to be documented and their roles determined so that we can manage their evolution to promote ecosystem services.

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