

## CHAPTER TWENTY

# Microbial mutualists and biodiversity in ecosystems

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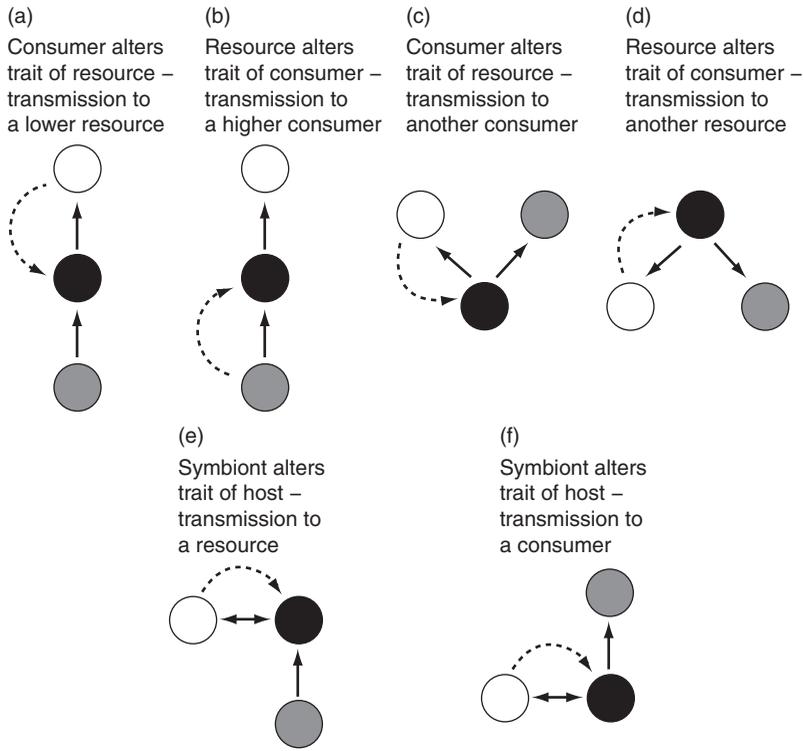
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### **Defining and detecting microbial trait-mediated indirect interactions**

Microbial trait-mediated indirect interactions (TMIs) occur when a microbe (species A) changes a trait of its host species (species B) that consequently affects another species in the community (species C, or the enemy of the host in a protection mutualism). This trait-mediated effect is distinguished from a density-mediated effect, in which the impact of the microbe would spread to other species exclusively through changes in the density of the host organism (e.g., via increased host mortality caused by a pathogen, density-mediated indirect interactions, DMII). Importantly, models reveal that TMIs have different dynamical consequences than DMIIs (Chapter 1), thus it is important to distinguish between these effects.

Food web diagrams can bring clarity to the characterization of TMIs. In contrast to typical predator–prey interactions, microbially-mediated protection mutualisms can involve a microbial partner at the same, or very similar, trophic level as the host organism (Fig. 20.1). Furthermore, symbioses involve the exchange of resources, for example photosynthetic carbon exchanged for soil nutrients in the symbiosis between plants and mycorrhizal fungi; this exchange is depicted explicitly in our diagrams, which include trait-mediated effects that are transmitted to a responding resource species (Fig. 20.1e) or to a consumer species (Fig. 20.1f).

In microbial TMIs, the presence of the symbiont acts as an agent of phenotypic plasticity within the host, whereby hosts that acquire symbionts experience a shift in trait expression. This shift is classified as constitutive if the microbe changes a host trait with no additional flexibility in trait expression. Alternatively, microbial symbiosis may alter trait plasticity. We include in our definition of microbial TMIs cases where the trait is microbial in origin as



**Figure 20.1** Examples of microbial and non-microbial agents of TMIs using the notation of Werner and Peacor (2003). Open circles indicate agents of TMIs, and filled circles indicate the reacting species. Solid arrows indicate energy transfer, through consumption or resource exchange. Dotted arrows indicate trait change and point to the species undergoing trait change (shown as black fill). Note that microbial TMIs have the microbe and host species exchanging resources (double arrows, e.g., carbon traded for physically protected growing space) often at the same trophic level.

well as cases where the microbe induces a change in a trait of the host because the trait is expressed as part of the host phenotype.

In evaluating examples of microbial TMIs, it is useful to consider the question: How does one separate trait-mediated effects from density-mediated effects? In some cases, the symbiont will have no direct effect on host density and will only change traits that are expressed through the host. The simplest experiment would thus compare interactions for symbiotic versus symbiont-free host populations. However, because symbiotic host lineages may differ genetically from symbiont-free lineages, it is also important to manipulate symbiont presence in order to decouple microbial effects from host genotype. Ideally, these manipulations would occur in both symbiotic host genotypes (removal of the symbiont) and in symbiont-free host genotypes (addition of the symbiont). To determine that the interaction is

indirect, experiments need to compare the performance of hosts in both the presence and the absence of natural enemies in order to detect any direct benefits of the microbial symbiont to the host that are independent of the indirect protection against natural enemies.

It is less clear how the appropriate manipulations can be achieved if the microbe affects both host traits and host density. TMII experiments with predators typically expose prey to predator cues (e.g., volatiles or chemically modified water) or eliminate predation ability (e.g., gluing spider mouthparts) to separate trait-mediated effects of the predator (e.g., predator-avoidance morphologies or behaviours) from density-mediated effects. A similar approach may be applied when specific traits are known to be altered by microbial interactions. For example, in symbioses between fungal endophytes and grasses, the fungi produce a well-characterized suite of alkaloids, which have defensive properties. Synthetic alkaloids could be added to plant leaves in quantities mimicking natural levels to manipulate the trait directly (Clay and Cheplick 1989). Another approach, particularly for relatively immobile macro-organisms, involves press experiments that repeatedly adjust host densities, such that only host traits differ between treatments. For example, in experiments manipulating endosymbionts in aphids, aphid densities could be adjusted by repeatedly adding new individuals to maintain equivalent densities in symbiotic and symbiont-free populations.

### **Examples of TMIIIs: protection mutualisms via microbial symbiosis**

Here, we briefly review an array of examples involving microbes that may be unfamiliar to many researchers working on macro-organisms (Table 20.1). We focus on studies that have experimentally manipulated symbiont presence and that describe trait changes associated with symbiosis. Our goal is to consider these examples in light of the TMII framework and to illustrate where additional TMIIIs might occur in nature. Several microbial systems that have been explored in great detail with experimental tests of microbial effects clearly represent TMIIIs (e.g., endophytic fungi in grasses, bacteria in bryozoans). A common theme is that host traits are often chemical and of microbial origin, reflecting the considerable biochemical capabilities of microorganisms. However, some of the microbial interactions described here have yet to be fully characterized, and in particular, key trait(s) altered by microbial symbiosis have yet to be identified (Table 20.1).

Advances in current understanding of microbial TMIIIs are most likely to come from studies that utilize manipulative experiments in natural environments that allow for a diversity of indirect interactions with other species. Vertically transmitted symbionts provide especially tractable opportunities for experimental investigations because contagious spread is often absent or rare. If the symbionts can be eliminated, it is unlikely that they would be

**Table 20.1** Examples and reviews of microbially mediated TMIs in aquatic and terrestrial ecosystems. Included are cases where a community member has been documented to respond to the presence of a microbial symbiont. In some examples, traits of the microbe have been identified, in other cases traits have been suggested, but not documented, to mediate the interaction

Host	Microbial symbiont	Trait	Responding species	Reference
<b>Aquatic/marine</b> ciliates ( <i>Euplotidium</i> spp.)	epibiotic bacteria ( <i>Verrucomicrobia</i> )	a subset of bacteria make a ribbon-like apparatus extruded in response to predators	predatory ciliate ( <i>Amphileptus marina</i> )	Görtz et al. (2009)
corals (e.g., <i>Oculina patagonica</i> , <i>Acropora palmata</i> )	diverse bacterial community, particularly in surface mucus	suggested: antibiotic compounds	pathogenic bacteria ( <i>Vibrio</i> spp.)	Reshef et al. (2006); Ritchie (2006); Rosenberg et al. (2007)
bryozoan larvae ( <i>Bugula neritina</i> )	bacteria ( <i>Endobugula sertula</i> )	cytotoxic compounds (bryostatins)	predatory fish ( <i>Lagodon rhomboides</i> , <i>Monacanthus ciliatus</i> )	Lindquist and Hay (1996); Davidson et al. (2001); Lopanik et al. (2004)
amphipod ( <i>Gammarus roeselii</i> )	microsporidian ( <i>Dictyocoela</i> sp. <i>roeselium</i> )	reduced susceptibility to behavioural manipulation by the worm	parasitic worm ( <i>Polymorphus minutus</i> )	Haine et al. (2005)
marine isopods ( <i>Santia</i> spp.)	episymbiotic cyanobacteria ( <i>Synechococcus</i> -type)	red colouration, suggested: chemical defence	predatory reef fish ( <i>Chromis</i> , <i>Amblyglyphidodon</i> , <i>Dascyllus</i> , <i>Pomacentrus</i> )	Lindquist et al. (2005)
brine shrimp ( <i>Artemia</i> spp.)	bacteria	not identified	pathogenic bacteria ( <i>Vibrio proteolyticus</i> CW8T2)	Verschuere et al. (2000)
lobster ( <i>Homarus americanus</i> )	bacteria (Gram negative)	antifungal compound (tyrosol)	pathogenic fungus ( <i>Lagenidium callinectes</i> )	Gil-Turnes and Fenical (1992)

shrimp ( <i>Palaemon macrodactylus</i> )	bacteria ( <i>Alteromonas</i> sp.)	antifungal compound (isatin)	pathogenic fungus ( <i>Lagenidium callinectes</i> )	Gil-Turnes et al. (1989)
squid ( <i>Euprymna scolopes</i> )	bacteria in light organ (Vibrionales)	production of light to eliminate shadow	predatory fish and seals ( <i>not well studied</i> )	Ruby (1996); Stabb and Millikan (2009)
<b>Terrestrial - animal</b>				
entomopathogenic nematodes	bacteria	anti-microbial compounds (bacteriocins)	microbes and invertebrates	Reviewed by Koppenhofer and Gaugler (2009)
locust ( <i>Schistocerca gregaria</i> )	diversity of bacteria in gut	suggested: toxic phenols	pathogenic fungus ( <i>Metarhizium anisopliae</i> ) pathogenic bacteria ( <i>Serratia marcescens</i> )	Dillon and Chamley (1988); Dillon et al. (2005)
pea aphid ( <i>Acyrtosiphon pisum</i> )	'secondary' endosymbiont ( <i>Regiella insecticola</i> )	not identified	pathogenic fungus ( <i>Pandora neoaphidis</i> )	Scarborough et al. (2005)
pea aphid ( <i>Acyrtosiphon pisum</i> )	'secondary' endosymbiont ( <i>Hamiltonella defensa</i> )	toxin-producing genes present in a bacteriophage	parasitoid wasp ( <i>Aphidius ervi</i> )	Oliver et al. (2003); Moran et al. (2005)
fruit fly ( <i>Drosophila hydei</i> )	bacteria ( <i>Spiroplasma</i> )	not identified	parasitoid wasp ( <i>Leptopilina heterotoma</i> )	Xie et al. (2010)
fruit fly ( <i>Drosophila neotestacea</i> )	bacteria ( <i>Spiroplasma</i> )	not identified	parasitic nematode ( <i>Howardula aoronymphium</i> )	Jaenike et al. (2010)
rove beetles ( <i>Paederus</i> spp.)	bacteria ( <i>Pseudomonas</i> relative)	chemical (pederin)	predatory spiders ( <i>Pardosa, Pirata, Evarcha</i> )	Kellner and Dettner (1996); Piel (2002)

Table 20.1 (cont.)

Host	Microbial symbiont	Trait	Responding species	Reference
fruit flies ( <i>Drosophila melanogaster</i> Culex), mosquito ( <i>Culex quinquefasciatus</i> )	bacteria ( <i>Wolbachia pipientis</i> )	not identified	West Nile virus	Osborne et al. (2009); Glaser and Meola (2010)
common dog tick ( <i>Dermacentor variabilis</i> )	endosymbiotic fungus ( <i>Scopulariopsis brevicaulis</i> )	not identified	pathogenic fungus ( <i>Metarhizium anisopliae</i> )	Yoder et al. (2008)
leaf-cutter (attine) ants	actinobacteria ( <i>Pseudocardinia</i> )	antibiotic protection of fungal garden	parasitic fungus ( <i>Escovopsis</i> )	Currie et al. (1999a); Currie et al. (1999b); Currie et al. (2003)
digger wasps (Crabronidae)	actinobacteria in antennal glands ( <i>Streptomyces</i> sp.)	antibiotics	pathogenic fungi in brood cells	Kaltenpoth et al. (2005); Kaltenpoth et al. (2010)
hoopoe bird ( <i>Upupa epops</i> )	bacteria in uropygial gland used for preening ( <i>Enterococcus faecalis</i> )	bacteriocins	feather decomposing bacteria ( <i>Bacillus licheniformis</i> )	Ruiz-Rodriguez et al. (2009)
lab mouse ( <i>Mus musculus</i> )	latent herpes virus	up-regulation of host innate immune response	pathogenic bacteria ( <i>Listeria monocytogenes</i> , <i>Yersinia pestis</i> )	Barton et al. (2007)
lab mouse ( <i>Mus musculus</i> )	bacteria ( <i>Bacteroides fragilis</i> )	not identified	pathogenic bacteria ( <i>Helicobacter hepaticus</i> )	Mazmanian et al. (2008)

lab mouse ( <i>Mus musculus</i> )	bacteria ( <i>Lactobacillus reuteri</i> )	not identified	pathogenic bacteria ( <i>Helicobacter hepaticus</i> )	Pena <i>et al.</i> (2005)
<b>Terrestrial – plant</b>				
tall fescue grass ( <i>Lolium arundinaceum</i> )	endophytic fungus ( <i>Neotyphodium coenophialum</i> )	alkaloids	herbivorous insects, arthropods, plant diversity, plant succession	Clay and Holah (1999); Clay <i>et al.</i> (2005); Rudgers and Clay (2007); Rudgers <i>et al.</i> (2007); Rudgers and Clay (2008)
several grasses (Poaceae)	endophytic fungi (epichloae)	alkaloids	herbivorous insects, pathogenic fungi	Clay and Schardl (2002); Koh and Hik (2007); Crawford <i>et al.</i> (2010)
sedges ( <i>Cyperus virens</i> , <i>Cyperus pseudovegetus</i> , Cyperaceae)	epiphytic fungus ( <i>Balansia cyperi</i> )	suggested: alkaloids	herbivorous insects, pathogenic fungi	Clay <i>et al.</i> (1985); Stovall and Clay (1991)
legumes ( <i>Astragalus</i> , <i>Oxytropis</i> spp., Fabaceae)	endophytic fungi ( <i>Erbellisia</i> spp.)	indolizine swainsonine alkaloids	herbivorous mammals	Ralps <i>et al.</i> (2008); Cook <i>et al.</i> (2009)
morning glory ( <i>Ipomoea asarifolia</i> , Convolvulaceae)	endophytic fungi ( <i>Periglandula</i> sp.)	ergot alkaloids	suggested: herbivores	Steiner <i>et al.</i> (2006)
shrub ( <i>Baccharis cordifolia</i> , Asteraceae)	epiphytic fungus (Hypocreales)	mycotoxins (trichothecenes)	suggested: pathogenic fungi	Bertoni <i>et al.</i> (1997); Rosso <i>et al.</i> (2000)

Table 20.1 (cont.)

Host	Microbial symbiont	Trait	Responding species	Reference
clover ( <i>Trifolium repens</i> , Fabaceae)	rhizobia bacteria	suggested: cyanogenic defences	herbivorous insect ( <i>Spodoptera littoralis</i> )	Kempel <i>et al.</i> (2009)
soybean ( <i>Glycine max</i> , Fabaceae)	rhizobia bacteria (naturally occurring strains)	not identified	herbivorous insect ( <i>Aphis glycines</i> )	Dean <i>et al.</i> (2009)
legumes	rhizobia bacteria	several traits	herbivorous insects	Reviewed by Hartley and Gange (2009)
plants	mycorrhizal fungi	several traits	herbivorous insects	Reviewed by Hartley and Gange (2009); Koricheva <i>et al.</i> (2009); Vannette and Hunter (2009)
plants	mycorrhizal fungi	several traits	pathogenic fungi, bacteria	Reviewed by Garrido (2009)

re-acquired horizontally. However, because most macro-organisms support diverse microbial communities, understanding how multiple symbionts interact within hosts will be important to deciphering their community consequences. For example, when pea aphids were co-infected by the secondary bacterial symbionts *Hamiltonella defensa* and *Serratia symbiotica*, the aphids showed enhanced resistance to parasitoid wasps relative to infection by *H. defensa* alone; however, titres of *S. symbiotica* increased 20-fold in co-infections, which may be detrimental to host aphids (Oliver *et al.* 2006). A central issue for microbial TMIs is connecting microbial community composition to function for hosts with diverse microbial symbiota. In systems lacking a single, dominant microbial symbiont, such as in sponges or mammalian guts, metagenomic approaches may prove useful for elucidating both microbial diversity and the diversity of genes controlling key traits, such as secondary chemistry. Terrestrial systems are better characterized at this point, but ultimately aquatic systems, especially marine organisms, may prove to be a richer source of microbial TMIs given the prevalence of symbiotic interactions in the sea.

### **Aquatic ecosystems**

Symbiotic interactions are common in aquatic environments where they provide hosts with an array of services including nutrition, dispersal and protection. Marine systems are particularly diverse in symbioses involving invertebrates, emphasizing that symbiosis is evolutionary ancient and ecologically significant. Examples of TMIs from aquatic systems illustrate symbiont-mediated protection via multiple pathways including chemical defence, morphological changes and behavioural alterations.

Symbiont-mediated protection has been documented for both macroscopic and microscopic hosts. For example, experiments have shown that epibiotic bacteria of both shrimp and lobster embryos produced anti-fungal compounds that significantly reduced attack by fungal pathogens (Gil-Turnes *et al.* 1989; Gil-Turnes and Fenical 1992). Similarly, unicellular *Euplotidium* ciliates host epibiotic bacteria that differentiate into a ribbon-like apparatus that is extruded in response to predators, disrupting prey recognition (Görtz *et al.* 2009). In perhaps the best experimentally documented example, larvae of the bryozoan *Bugula neritina* often host the bacterium *Endobugula sertula*, which produces the macrocyclic polyketides, bryostatins (Davidson *et al.* 2001). Interestingly, extracts from larval *Bugula*, but not from adults, were unpalatable to fish, and bryostatin concentrations in adults (which are less vulnerable to predators than larvae) were very low (Lindquist and Hay 1996; Lopanik *et al.* 2004). The role of symbiont-produced bryostatins as a TMII was demonstrated by experimental elimination of *Endobugula* with antibiotics where the loss of bryostatins in treated larvae increased their palatability to fish (Lopanik *et al.* 2004).

In addition to the few experimental studies (Table 20.1), anti-fouling and anti-biotic compounds have been documented in several corals, tunicates, seaweeds and sponges (Piel 2004; Ritchie 2006). Corals, in particular, have been proposed to gain microbially mediated protection against pathogens from microbes present in the surface mucus layer (the coral probiotic hypothesis: Reshef *et al.* 2006; Rosenberg *et al.* 2007). A number of other marine organisms have a high probability of microbial TMIs, ranging from toxin-producing dinoflagellates (Görtz *et al.* 2009) to squid with light-generating bacteria that may camouflage their shadows (Stabb and Millikan 2009). For example, marine sponges host a wide variety of archaea, bacteria, fungi and algae, and bacteria alone can constitute as much as 40–60% of sponge biomass (Schmitt *et al.* 2007). In aquatic organisms with complex morphologies, gut-associated bacteria likely play protective roles and may have applications for managing disease resistance. For example, probiotic bacteria are commonly added to cultures to protect shrimp, oysters, fish and scallops (reviewed by Tinh *et al.* 2008). However, for the majority of cases, whether compounds are produced by the host, by a symbiont or by the host in response to a symbiont has yet to be determined. If symbionts commonly alter the defensive traits of foundational species, such as corals, there could be large impacts on a diverse community of associated macrobiota.

### **Terrestrial ecosystems**

Terrestrial systems differ from aquatic habitats not only in the particular species involved but also in more general characteristics such as the localization and movement of nutrients in the soil versus the water column, the greater dominance of insects and angiosperms on land versus clonal invertebrates and algae in the water and the higher prevalence of fungi in terrestrial than aquatic habitats. Despite these fundamental differences, a diversity of TMIs can be found in both habitat types that illustrate similar underlying microbial mechanisms.

#### *Animals: protective symbioses in invertebrates*

Invertebrates present many examples of microbial TMIs - many of them involving chemical traits (Table 20.1). Invertebrate examples illustrate that not only the presence, but also the diversity, of microbes can play a protective function for hosts. For example, an experiment manipulating the diversity of gut bacteria in *Schistocerca* locusts significantly reduced the density of a pathogenic bacterium (Dillon *et al.* 2005). Some of these protective effects may result from competitive interactions between microbes colonizing the shared host, rather than from TMIs. Thus, further work will be needed to determine whether these interactions are in fact trait-mediated.

The two best-documented examples in terrestrial invertebrates are arguably leaf-cutter ants with protected fungal gardens and aphids with endosymbiotic

bacteria that defend against natural enemies. Currie and colleagues (1999a, 1999b, 2003) have reported that leaf-cutter ants harbour antibiotic-producing actinobacteria (*Pseudocardinia*) in specialized structures, and that bacterial compounds are inhibitory to the parasites of their fungal gardens. While experimental manipulations of leaf-cutter ant nests are difficult to conduct in nature, laboratory bioassays clearly indicate this is a TMII in which antibiotics produced by symbiotic bacteria protect fungal gardens from their pathogens (Currie *et al.* 2003). Distinct from extracellular antibiotic-producing bacteria, bacterial symbionts occur internally in aphids and include ‘primary’ endosymbionts, which are obligate and nutritional, as well as ‘secondary’ endosymbionts, which are facultative and sometimes protective. Secondary symbionts of the pea aphid, *Acyrtosiphon pisum*, have received the most study (reviewed by Oliver and Moran 2009). For example, *Regiella insecticola* improved aphid survival when challenged with an entomopathogenic fungus (Scarborough *et al.* 2005), *Hamiltonella defensa* protected aphids against the parasitoid wasp *Aphidius ervi* (Oliver *et al.* 2003), and a *Rickettsiella* changed aphid body colour from red to green – which may provide protection as well (Tsuchida *et al.* 2010). Interestingly, the protection conferred by *H. defensa* appears to arise from toxin-producing genes present in a bacteriophage, adding a fourth player to the interaction (Moran *et al.* 2005). Complete genome sequencing has revealed that pea aphids lack many of the genes for microbial recognition and immune response that are present in other insect groups, suggesting that symbionts may have co-opted these defensive traits in aphids (Gerardo *et al.* 2010). Clearly, bacterial symbionts can alter aphid traits in ways that affect aphids’ natural enemies – whether there are consequences for host plants and the broader food web remains to be investigated.

Putative TMIIIs involving symbiont-mediated protection of arthropod vectors against pathogens of animals and humans are being reported with increasing frequency and may have significant biocontrol value. For example, a transcriptional profile of malaria-transmitting *Anopheles gambiae* mosquitoes suggested that the gut microbiota caused an up-regulation of mosquito immune responses, including anti-*Plasmodium* factors, resulting in a decreased capacity to sustain *Plasmodium* infection (Dong *et al.* 2009). Thus, the microbiota of vectors could potentially reduce disease levels in human and wildlife populations by altering the vector traits and immunities.

#### *Animals: protective symbioses in vertebrates*

As in invertebrates, gut bacteria likely play protective roles against disease in many vertebrate taxa. Laboratory studies on rodents have been numerous. For example, latent herpes virus defended laboratory mice against the bacteria *Listeria monocytogenes* (a food-borne pathogen causing listeriosis) and *Yersinia pestis* (agent of plague) by up-regulating the host’s innate immune responses

(Barton *et al.* 2007), highlighting that this particular effect was due to a change in the host traits. Similar effects on innate immune responses have been documented for probiotic bacteria, such as *Bifidobacterium* (e.g., Sonnenburg *et al.* 2006). Experimental studies on model vertebrate systems combined with observational data from the Human Microbiome Project (Turnbaugh *et al.* 2007) have high potential to inform our understanding of microbial TMIs in humans.

Protective symbioses in vertebrates are not limited to gut microbiota. For example, in birds, bacteria present in secretions used during preening are known to protect against feather degradation, thereby suppressing decomposers in laboratory experiments (Rodriguez *et al.* 2009). While it seems unlikely that the cascading effects to the broader community are strong in avian systems (feathers comprising a small fraction of animal detritus), this case raises the question, how many other symbioses may alter decomposer communities only after the host organism has died (or excreted or shed)?

#### *Plants: aboveground symbioses*

Over the past several decades, toxin production by a number of well-known poisonous plants has been shown to result from microbial infection. Clay (1988) suggested that many of these toxic associations represent a defensive mutualism in which microbes produce physiologically active compounds that protect host plants against herbivory. The best documented of these TMIs involve endophytic fungi in the family Clavicipitaceae, which grow systemically in aboveground plant tissues, are vertically transmitted through seeds, and form symbioses with grasses, sedges and morning glories (Clay and Schardl 2002; Steiner *et al.* 2006). Protection of plants from herbivores mediated by toxin-producing fungal symbionts is not limited to fungal endophytes in the Clavicipitaceae, and includes Asteraceae and Fabaceae as well as lichens and conifers (Table 20.1). Fungal and bacterial endophyte symbioses appear to be ubiquitous in plants, and many of these endophytes may also play a defensive role (Arnold *et al.* 2003; Rodriguez *et al.* 2009).

The TMII mediated by fungal endophyte symbiosis is clearly documented by experimental elimination of the fungus, resulting in the loss of both alkaloids and herbivore resistance. For example, endophyte-infected tall fescue grass (*Lolium arundinaceum*), which has been especially well studied given its economic importance, exhibited increased resistance to herbivores compared to uninfected fescue (Rudgers and Clay 2005) due to the production of alkaloids by the endophyte (Bush *et al.* 1997). Similar increases in herbivore resistance have been reported in native grasses (Table 20.1). In the case of tall fescue, endophyte symbiosis also has cascading consequences – reducing plant community diversity (Clay and Holah 1999; Rudgers *et al.* 2010b), suppressing plant succession (Rudgers and Clay 2007) and altering

food web interactions (Rudgers and Clay 2008) and ecosystem processes, including invasion resistance and decomposition (Lemons *et al.* 2005; Rudgers *et al.* 2005).

#### *Plants: belowground symbioses*

In addition to hosting aboveground endophytes, plants associate with a number of bacterial and fungal symbionts present in the soil; these organisms also have potential to interact with plant enemies in TMIs (Table 20.1). Symbioses with mycorrhizal fungi are present in >80% of flowering plant species (Brundrett 2002), and while mycorrhizal fungi are generally considered nutritional mutualists, they have long been known to protect host plants against pathogens and have more recently been implicated in herbivore resistance (Table 20.1). The degree to which these effects are trait mediated remains unresolved, as another mechanism of protection may simply be competition for colonization sites on the roots. However, some plant traits have been documented to be affected by mycorrhizal symbioses, including defensive chemistry, tolerance to herbivory and plant signalling pathways. Thus far, studies suggest stronger negative impacts of mycorrhizal associations on generalist, chewing insect herbivores relative to specialists and sucking insects (Koricheva *et al.* 2009). In addition, a few reports highlight effects on the third trophic level, including increased attraction of parasitoids of herbivores (Guerrieri *et al.* 2004) as well as reduced production of extrafloral nectaries (Laird and Addicott 2007), demonstrating the potential of these TMIs to influence additional community members. While far fewer studies have investigated TMIs for rhizobia than for mycorrhizal fungi (Table 20.1), in both symbioses it appears that effects on herbivores, pathogens and even the enemies of herbivores can vary considerably. Understanding variation caused by plant identity (e.g., Sikes *et al.* 2009), symbiont identity (e.g., Maherali and Klironomos 2007) and the type and degree of specialization of the plant's natural enemy (Koricheva *et al.* 2009) may improve the ability to predict the strength and direction of these TMIs.

#### *Other terrestrial examples*

While we have reviewed the widespread distribution of TMIs involving plants associated with microorganisms (bacteria and fungi), recent work suggests that these microbes themselves may form symbiotic associations for the production of secondary metabolites for protection or pathogenesis. For example, Partida-Martinez *et al.* (2007) demonstrated that 'mycotoxin' production by the fungus *Rhizopus microsporus* was actually the result of infection by bacteria in the genus *Burkholderia*. In general, mycotoxins are thought to be secondary compounds produced for defence or for competition with other microbes or animals. A wide range of fungi are associated with bacterial

endosymbionts (Bertaux *et al.* 2005), raising the question of which partner is producing the defensive chemistry.

## Hypotheses for community and ecosystem impacts

### Pairwise species interactions

The simplest microbial TMIs consist of changes to pairwise interactions with the host organism, and these constitute the majority of examples that we reviewed above. While we have focused on microbes altering host–consumer interactions (Fig. 20.1f), TMIs can also occur in other types of pairwise species interactions. First, the presence of one microbial symbiont could alter host traits in ways that influence host interactions with a mutualist. For example, studies on grasses have shown that plants with aboveground fungal endophytes have reduced colonization of roots by mycorrhizal fungi, relative to endophyte-free plants (Omacini *et al.* 2006). Such antagonisms between mutualistic symbionts could be trait mediated, for instance, if the endophyte altered host root exudates in ways that disrupted mycorrhizas. Conversely, pairwise mutualisms could be enhanced by microbial TMIs. For example, mycorrhizal fungi could increase plant attractiveness to pollinators by altering plant traits, such as floral size or nectar production (Cahill *et al.* 2008). Second, the presence of a microbial symbiont could alter host competitive interactions. Indeed, the roles of soil mutualists, such as arbuscular mycorrhizal fungi, are well investigated in this context (e.g., Hoeksema *et al.* 2010). Similarly, experiments have demonstrated that the outcome of competition between two *Steinernema* nematode species depended on the presence and identity of their bacterial symbionts (Sicard *et al.* 2006). Third, some microbial symbionts can improve the host’s ability to capture prey, thereby altering host–resource interactions (Fig. 20.1e). For example, the deep-sea anglerfishes host bioluminescent bacteria in lures to attract prey (Haygood and Distel 1993). Altogether, these examples serve to highlight a number of pathways through which microbes may generate TMIs by altering pairwise species interactions.

### Community structure and assembly

TMIs are likely to have consequences that cascade to other members of the community, particularly when one (or more) of the organisms involved is a keystone species, a foundation species (e.g., corals) or a dominant member of the community (e.g., grasses in a grassland). In such cases, microbial TMIs may alter community diversity. For example, our work with tall fescue grass and its *Neotyphodium* endophyte has shown reduced plant and arthropod diversity in the presence of the symbiosis (Clay and Holah 1999; Rudgers and Clay 2008). In order to classify a community response as a TMII, it is important to show (1) that the interaction is indirect and therefore does not

occur (or is substantially weaker) in the absence of the intermediary species (here, host natural enemies) and (2) that the interaction is trait mediated, such that direct manipulation of the trait alone would produce a similar community response. For example, in the tall fescue system, we have documented that the benefit of *Neotyphodium* to the host grass is indirect: experimental exclusion(s) of natural enemies (insects and mammals) resulted in weaker increases in symbiotic tall fescue relative to plots where herbivores were present (Clay *et al.* 2005). Furthermore, although experiments to manipulate directly the traits (alkaloids) would be technically difficult on a large, community-wide scale, we have shown that a fungal genotype lacking the full complement of alkaloid toxins had weaker effects on the community than the wild-type fungal genotype (Rudgers *et al.* 2010b) and in laboratory trials, adding alkaloids to endophyte-free leaves reduced consumption by herbivores (Clay and Cheplick 1989). Depending on the system, the effects of a microbially altered host trait could attenuate or accumulate (e.g., biomagnification) at higher trophic levels, and more work will be needed to elucidate when attenuation versus accumulation may be most likely to occur. We hypothesize that effects could accumulate when particular species are resistant to microbial toxins and sequester them for their own defence, similar to insect herbivores that sequester plant secondary compounds. In contrast, attenuating effects may be more common for non-resistant, generalist natural enemies that lack sequestration abilities. Furthermore, TMIs that cascade to a large number of community members have the potential to alter the temporal and spatial patterns of community assembly. For example, the tall fescue endophyte, by increasing dominance of the host grass, slowed community succession from grassland to forest (Rudgers *et al.* 2007). For hosts that commonly persist as mosaics of symbiotic and symbiont-free individuals or populations, the presence/absence of the symbiont could produce alternative stable states in the system, one associated with the presence of the symbiont and one with the absence of the symbiont. For example, Koh and Hik (2007) demonstrated that grassland patches close to predator refuges had high mammalian herbivory and high levels of endophyte symbiosis, whereas patches subject to low herbivory had lower levels of endophyte symbiosis. Alternatively, symbiont-free populations may be transitory and subject to rapid extinction.

### **Ecosystem-level consequences**

Microbial TMIs could have ecosystem-level consequences, particularly in cases where the host organism is a foundation or a dominant species in the ecosystem. These effects may include direct impacts on ecosystems due to microbially mediated changes in host traits, such as the presence of fungal alkaloids altering the rate of litter decomposition (Lemons *et al.* 2005). In addition, indirect effects could occur due to microbially-mediated shifts in

community structure. For example, in tall fescue grass, the strong reductions in plant diversity in plots with the endophyte (Clay and Holah 1999) could subsequently alter pools of C, N and other nutrients in the soil. Leaf-cutter ants, whose fungal farms can be protected from pathogens by symbiotic, antibiotic-producing bacteria (Currie *et al.* 1999b), may have significant effects on forest ecosystems. For example, Folgarait (1998) showed that leaf-cutter ants can reduce 17% of the annual leaf production in a tropical forest. These ecosystem impacts could be significantly reduced if more ant nests were lost to pathogens. Nutritional mutualists, such as plant-associated rhizobia and mycorrhizal fungi, have obvious ecosystem consequences by making N or P available to host plants, which in turn may form the basis of the TMII. Herbivores, such as aphids, could also have ecosystem-level impacts by differentially feeding on plant species. Those impacts would be stronger when aphids are protected from predation and parasitism by their bacterial endosymbionts (Oliver and Moran 2009). In marine environments, foundation species such as corals may affect ecosystem properties, such as productivity and nutrient dynamics.

### Predicting the direction and strength of microbial TMII

In general, we expect stronger effect sizes of microbial TMII that cause greater magnitudes of change in host traits. Importantly, the rate of change in traits may be much faster than the rate of change in the densities of host organisms, thus resulting in stronger immediate impacts on communities than density effects alone (see also Werner and Peacor 2003). We suggest that several additional factors may also influence the strength and direction of microbial TMII.

First, the *ecological dominance of the symbiosis* should increase the strength of its impacts on the community and ecosystem. For example, if traits are modified in a keystone species, foundation species or ecosystem engineer (Gribben *et al.* 2009), these changes are more likely to have strong impacts at the community and ecosystem level than changes in species with a smaller ecosystem footprint. We are not aware of any published studies comparing the impacts of microbial TMII for species that vary in their ecological dominance.

Second, we predict that the *specificity of the host-microbe association* will influence the direction of its impact on the surrounding community and ecosystem. If the microbial symbiont only alters traits of a single host, this should promote host dominance and thereby reduce the biodiversity of the system. While we are unaware of any direct tests of this idea, explorations of pairwise species interaction networks show very different structural patterns for networks of specialized and symbiotic mutualists compared to generalist, non-symbiotic mutualists (Guimaraes *et al.* 2007). Furthermore, an example of the influence of specificity comes from symbioses between grasses and fungal

endophytes, which are highly host specific. In tall fescue grass, the endophyte promotes host dominance to the exclusion of other species in the community, with effects not only on competing plants (at the same trophic level as the host, Clay and Holah 1999; Rudgers *et al.* 2007) but also on consumers and on their consumers (Rudgers and Clay 2008). Under this scenario, the host becomes more of a keystone species in its community and ecosystem impacts relative to other species in the community as a result of the microbial symbiont. It is also possible that tightly coupled host-microbe associations could select for an arms race where all species need a symbiont (or a better symbiont) in order to compete within the community. Such an arms race has been suggested for leaf-cutter ants where fungal gardens are attacked by pathogens and protected by symbiotic bacterial antibiotics (Poulsen *et al.* 2010). In contrast, if microbial symbionts simultaneously alter the traits of several co-occurring hosts, for example during the formation of a mycorrhizal network connecting plant roots belowground, this interaction may level the playing field, reducing the competitive dominance of any one host species, and thereby, increasing biodiversity (van der Heijden *et al.* 1998).

Third, the *evolutionary novelty of the symbiosis with respect to the surrounding community* is additionally likely to influence the strength, and possibly the direction of community and ecosystem impacts. In a community of highly coevolved species, there will have been time for co-occurring species to adapt to any dramatic, symbiont-mediated changes in host traits, whereas the introduction of a novel, non-native host-symbiont association to a community may have stronger immediate impacts on resident, native species (e.g., a novel weapon, Callaway and Ridenour 2004). Evolutionary novelty has been proposed as an explanation for the strong community impacts of the tall fescue-*Neotyphodium* symbiosis (Saikkonen *et al.* 2006), although the data required to test this hypothesis, including responses in replicated non-native and native symbiotic systems, are currently lacking.

Fourth, as has been suggested for non-microbial TMIs (Werner and Peacor 2003), the *duration and timing of change in the trait* likely play a role in the strength of impacts. Host traits that microbes alter constitutively are expected to have longer lasting and stronger impacts than more ephemeral or plastic changes. In assessing the host response, we suggest that adopting a population dynamics approach, such as projection matrix models, is likely to provide the most comprehensive assessment of the consequences of the TMII for the host species because changes in host traits are likely to affect multiple aspects of host demographic rates (Damiani 2005; Rudgers *et al.* 2010a).

Finally, taking a *community genetics perspective* (Whitham *et al.* 2003), it is likely that even the genotype of the species involved in symbiosis could alter community composition. We have found that two fungal endophyte genotypes differing in alkaloid chemistries had differential impacts on the plant

community (Rudgers *et al.* 2010b). Similarly, recent work in a barley-aphid-parasitoid system showed that > 10% of the variation in the size of parasitic wasps could be explained by complex interactions among aphid genotypes, plant genotypes and the presence of rhizobacteria (Zytynska *et al.* 2010).

## Conclusions

In summary, new studies are required better to characterize the diversity and function of microbial TMIs in a wider array of systems. The research reviewed here emphasizes the need to consider whether ecologically important plant and animal traits may actually be of direct microbial origin or are indirectly affected by microbial symbiosis. Molecular tools such as pyrosequencing and metagenomics provide new opportunities for characterizing cryptic host-associated microbial communities and their functional capabilities. Combined with experimental manipulations of microbe presence/absence or genotypic variation, the ecological impacts of microbial TMIs can be better elucidated. While several studies have successfully characterized the ecological impacts of single microbial symbionts, a major challenge for future research will be to understand the functional significance of the diverse microbial communities within hosts. In addition, the wholesale movement of plants, animals and microbes around the world, the potential constitution of new host-microbial associations, and the widespread use of antibiotics in agriculture and medicine all suggest that microbial TMIs will be dynamic and have unexpected consequences. While certain systems have been well characterized (e.g., endophytes of grasses, bacterial endosymbionts of aphids, leaf-cutter ants and their associated communities), a wider taxonomic and habitat diversity needs to be explored. Finally, relatively few systems have been subject to experimental manipulation and measurement of consequences, especially in real-world environments. These gaps in our knowledge provide many opportunities for enhancing our understanding of TMIs and the complex interactions between microbial symbionts and their hosts.

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