CHAPTER 9
Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild
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9.1 Introduction

Microbial communities have been and continue to be used widely to test basic ideas in ecology, and studies of the relationship between biodiversity and ecosystem functioning are no exception. Although microbial microcosm studies have been successful at shedding light on debates in biodiversity-ecosystem functioning research (Petchey et al. 2002), for many community ecologists, their raison d’être is limited to testing hypotheses with a degree of abstraction just slightly below that of mathematical models. The underlying idea is that simple microcosms, like theoretical models, are surrogates for what might be occurring in more complicated and, it is often implied, more interesting communities composed of larger organisms. In this context, the aim of most microbial microcosm studies is to create a highly simplified system to ensure unequivocal identification of patterns and causal mechanisms. In biodiversity-ecosystem functioning research, this has required experimental assembly of communities that are much less species-rich than the microbial communities in natural ecosystems.

Apart from the small coterie of ‘microcosmologists’ (Carpenter 1996), there is a separate fraternity of environmental microbiologists who typically publish in a different suite of journals and attend different conferences than ecologists interested in larger organisms, even though many of the research questions are similar. The focus of environmental microbiologists is to understand naturally occurring microbial populations and communities, so a familiar refrain is that microcosm experiments with constructed communities are much too simplified to have a bearing on what occurs in nature. In addition, many environmental microbiologists have argued that experiments using artificially assembled microbial communities are irrelevant at best (misleading at worst) because the experiments are conducted at inappropriate temporal and spatial scales. Although the dichotomy we describe between environmental microbiologists and microcosm microbial ecologists is a caricature, there is nevertheless a clear need for increased communication between these groups of researchers.

Largely due to the development of powerful and accessible molecular techniques, it is increasingly possible to ask ecological and evolutionary questions of natural microbial communities, even though it remains difficult to manipulate microbes in the field the way we can manipulate larger organisms. Without the ability to manipulate microbes in the field, causal mechanisms continue to be difficult to pin down.

The first purpose of this review is to give a glimpse of the considerable advances in microbial ecology, and to outline how microbial biodiversity affects the functioning of ecosystems in what is a rapidly expanding field of study. The second purpose is to contrast the results reported primarily in microbiological journals with those of ecologists who use microbes as model systems. We concentrate on non-pathogenic prokaryotic and eukaryotic microbial communities, leaving discussions of pathogenic
microbial communities to Chapter 15. We do not discuss viruses in the current review, in part because of a lack of biodiversity-ecosystem functioning studies explicitly using viruses. However, given that many viruses appear to be host-specific and that they are important in controlling microbial populations and hence community structure in many ecosystems (Suttle 2007), viral diversity is very likely to have considerable repercussions for ecosystem functioning.

9.2 Microbial biodiversity and ecosystem functioning

9.2.1 Microbial biodiversity

One of the principal difficulties in analyzing the relationship between microbial diversity and ecosystem functioning is that it is notoriously complicated to describe the diversity of microbial communities. Most microbial groups lack sufficient morphological features to permit identification to the species level using conventional taxonomy or classical microbiological techniques (e.g. staining cell walls or using selective media). In addition, most of the microbial world eludes investigation under controlled conditions because of difficulties in culturing most microbial strains in the laboratory (Rappé and Giovannoni 2002). DNA-based molecular techniques have provided highly resolved descriptions of microbial communities (e.g. Venter et al. 2004, Sogin et al. 2006), but even current high-throughput sequencing approaches have not comprehensively surveyed a single ecosystem, and all techniques suffer from both sampling error and sampling bias (Fig. 9.1).

The first difficulty in assessing microbial diversity is that the number of individuals to identify is too large for any reasonable ecological survey (Hughes et al. 2001), although new sequencing technologies have the potential to provide reasonably good estimates of diversity in some species-poor microbial habitats in the foreseeable future (Quince et al. 2008). Even extremely unproductive environments such as drinking water will have thousands of microbial cells per cubic centimetre, and typical communities contain more cells than could possibly be identified individually using current technology. Techniques to assess microbial diversity therefore proceed by taking a number of sub-samples: first by taking only a subset of the cells in the community, then a subset of the DNA from those cells, and then, for example, amplifying a particular locus from the extracted DNA before sequencing a subset of the DNA that has been amplified (Fig. 9.1). Although it is true that surveys of non-microbial organisms do not identify all of the individuals in the examined community, such ecological surveys of larger organisms generally record a significant percentage. Surveys of microbial communities, in contrast, typically identify many orders of magnitude fewer individuals than are actually present. Currently, a clone library (i.e. a collection of clones of chromosomal DNA that can be used to quantify microbial diversity; Fig. 9.2) might comprise a few hundred individuals (perhaps hundreds of thousands of individuals in the near future), whereas the community would be likely to contain at least $10^5$ cells per cubic centimetre (of soil or water), often several orders of magnitude more, and the community contains many thousands of cubic centimetres. Much less than 1 per cent of a community is therefore recorded for even the most extensive surveys of microbial communities. This creates a problem because many richness estimators rely on guessing at the distribution of abundance in the community (i.e. how many rare species have not been observed in the survey). Since such a small portion of the community is sampled, the distribution of abundance in microbial communities is still unknown. While it is possible to make educated guesses at a distribution of abundance and extrapolate numbers of species (Curtis and Sloan 2004, Quince et al. 2008), such estimates remain speculative. The new generation of sequencing technologies will curtail this problem to some extent, but even sequencing hundreds of thousands of individuals in a microbial community will still represent only a minuscule portion of the total community.

The second difficulty in assessing microbial biodiversity is that the sample is not a random subset of the larger community. In Fig. 9.1, each arrow represents a situation in which a sub-sample is taken. Each sub-sample is biased (i.e. non-random); for example, some sequences amplify more readily than others during a polymerase chain reaction
Metagenomics. Extracted DNA is sequenced and assembled into putative genomes. The number of genomes is an estimate of species richness.

Cells. A sample from the ecosystem containing living microbial cells.

Culture independent techniques. DNA is extracted and purified from the cells.

Culture dependent techniques. Cells are cultured on agar plates and characterized by morphology or using selective media.

Reassociation kinetics. Double stranded DNA is heated until the strands separate. The kinetics describing the rate at which strands re-associate gives a measure of diversity.

Polymerase chain reaction. One locus is amplified.

Microarrays. The PCR product is washed over a slide printed with oligonucleotides. Complementary DNA binds to the oligonucleotides and produces a fluorescent signal. The number of positive signals gives an estimate of diversity.

Community fingerprints. Amplified DNA is separated according to some sequence-specific property. For example, denaturing-gradient gel electrophoresis (DGGE) separates DNA sequences according to their propensity to denature at a particular denaturant concentration. Denatured DNA stops moving through an agarose gel leading to different banding patterns depending on the composition of the community.

Clone libraries. Amplified DNA is sequenced. The rate of accumulation of novel sequences provides an estimate of diversity.

Figure 9.1 Popular methods for estimating microbial diversity. Microbial cells are too abundant and morphologically similar to survey using conventional ecological techniques. Rather, a series of subsamples are taken, where each arrow in the figure represents a subsample. Difficulties arise in estimating microbial diversity because the degree to which these are random sub-samples is poorly understood.

(PCR) (Muyzer and Smalla 1998), which biases the final DNA copy number of each species compared to the original species mixture. Different initial copy numbers in different species can also significantly affect the final mixture of PCR products (Farrelly et al. 1995). Although the same issues of sample bias exist for larger organisms, the degree to which the sub-samples constitute non-random samples of the community remains largely unknown and an area of active study. It is only after these problems have been resolved that it will be possible to accurately assess microbial diversity.
The final difficulty with estimating microbial diversity is that there is ongoing controversy in defining what constitutes a microbial species (Achtman and Wagner 2008). As with larger organisms, species identity is relatively clear among microbial species that reproduce sexually. When reproduction is asexual, as is generally the case, the main alternative is to infer whether two individuals are of the same species if they share an arbitrary degree of DNA sequence similarity. Often, 97 percent sequence similarity is taken as the threshold, although different cut-offs are used for different loci or for different taxonomic groups. In addition, many microbes frequently transfer DNA directly from an individual of one species to an individual of a different species, a process referred to as horizontal gene transfer. Because the biological species concept does not extend to the typical reproductive systems of most microbes, measures of ‘biodiversity’ in the microbial literature can have vastly different interpretations depending on the particular species definition that is employed. Although we use the term ‘species’ in the current chapter, it is evident that microbial biodiversity-ecosystem functioning studies will have to be interpreted in light of the species definition used, and conflicting results might simply result from differences in species definitions.

### 9.2.2 Microbial functions

Microbial communities are pivotal for the functioning of the world’s ecosystems (Falkowski et al. 2008, van der Heijden et al. 2008). In aquatic environments, microbes often set the level of primary production and decomposition. In terrestrial ecosystems, microbial communities are important drivers of decomposition, thus converting organic matter into forms that are available for uptake by plants. As a result, microorganisms account for a major portion of ecosystem metabolism and biomass, on a global scale, accounting for a reported 50 percent of the total protoplasm on Earth (Whitman et al. 1998). Microbes contribute to the regulation of Earth’s climate by mediating fluxes of carbon dioxide, nitrous oxide, methane, and other greenhouse gases (Bardgett et al. 2008). The ability of microbial communities to metabolize even the most recalcitrant molecules is a testament to their enormous metabolic repertoire.

Microbes are also important in an applied context. For example, they are a key consideration for agricultural practices, particularly when agriculture relies on renewable resources and is employed to minimize environmental degradation. Microbes have been exploited as ‘microbial pesticides’ (Qaim and Zilberman 2003), including both naturally occurring and genetically modified pathogens that directly or indirectly reduce pest populations. Perhaps the best-known example is Bacillus thuringiensis, which produces a toxin that deteriorates insect mid-gut epithelial cells, thus halting larval insect foraging. There is enormous functional variation even within microbial species. In B. thuringiensis, for example, the variation in pathogenicity and host specificity is attributed in part to which
plasmids they carry (Rasko et al. 2005), but plasmid diversity in the context of microbial biodiversity-ecosystem functioning studies has been largely ignored. There is also the possibility that horizontal gene transfer is important in maintaining ecosystem functioning, which also is a process that is largely absent in larger organisms.

There are numerous other examples in which microbial diversity affects managed ecosystems and human life, the intimate involvement of microbes in so many processes making it difficult to put a monetary value on microbial diversity. Despite the growing recognition of the importance of microbial biodiversity, the significance of this biodiversity component is only beginning to be acknowledged in biodiversity policy debates. While most are not worried about microbial species going extinct, even small changes in the functioning of microbial communities have the potential for significant impacts on economy, welfare and other aspects of human life (Table 9.1). Perhaps as a consequence, all signatories to the World Trade Agreement now must adopt and implement patent laws for microorganisms and for biotechnology processes applied to living organisms, based on international tariff and trade rules (FAO 1998). This will undoubtedly have implications for the use and management of microbial diversity, as it becomes the legal property not only of the patent depositor, but also of the country of origin, regardless of where the organisms were collected. For microbial communities, it might not be imperative to conserve specific species or strains, but rather to conserve specific metabolic pathways, which may be the result of millions of years of evolution and might be lost due to chance conditions (Falkowski et al. 2008).

In summary, the importance of microbial communities in determining flows of energy and matter in ecosystems makes them of particular importance both for basic ecological investigations and in an applied context. Unlike with larger organisms, research on microbial diversity is not driven by conservation worries, but by the enormous benefits (and harm) to humans that could arise once the factors affecting microbial functions are well understood in terms of harnessing beneficial services and preventing harmful impacts. For example, even marginal increases in the ability of a microbial community to metabolize harmful constituents in sewage might have significant economic ramifications (Wagner and Loy 2002); algal communities have promise as biofuels or as food (Gross 2008); and altering microbial gut functioning might prevent obesity and therefore reduce medical costs (Backhed et al. 2004). In many of these areas, the key is to understand how differences in the structure and performance of microbial communities translate into differences in functioning, which is precisely the question being addressed by ecologists investigating the relationship between biodiversity and ecosystem functioning.

### 9.3 Functioning of microbial communities under controlled conditions

#### 9.3.1 Model systems for biodiversity research

Microbial communities have a venerable history as model systems for studying questions in community ecology (Jessup et al. 2005). Their popularity stems in part from the ease with which it is possible to conduct experiments over many generations under controlled laboratory conditions. Such rapid generation times allow the communities to arrive at the equilibrium conditions upon which many mathematical models are based. In addition, controlled laboratory microcosm experiments allow for communities to be experimentally assembled according to a prescribed design by drawing different combinations of species from laboratory stocks of pure cultures. In contrast, studies of natural communities lack this opportunity, in part because the levels of diversity are too high to be tractable for this type of approach. The combination of short generation times and fine control of community membership has resulted in microbial diversity evaluations of some important microbial services. These data were compiled by FAO in 1998 and have not been adjusted to current market value.

<table>
<thead>
<tr>
<th>Ecosystem service</th>
<th>Value (billion US$ per year)</th>
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<tbody>
<tr>
<td>Soil formation on agricultural land</td>
<td>500</td>
</tr>
<tr>
<td>Nitrogen fixation for agriculture</td>
<td>90</td>
</tr>
<tr>
<td>Pharmaceuticals of microbial origin</td>
<td>42.5</td>
</tr>
<tr>
<td>Synthesis of industrial enzymes</td>
<td>1.3</td>
</tr>
</tbody>
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Table 9.1
microcosms becoming a reasonable ‘halfway house between mathematical models and the full complexity of the field’ (Lawton 1995).

Critics have argued that microcosm experiments lack generality, rely on overly simplified communities, or are experiments that are conducted at an inappropriate scale (Carpenter 1996, Jessup et al. 2004). Clearly, if the question under investigation is to understand how a specific ecosystem operates then there is no substitute for the real thing. But if the purpose is to understand more generally the processes that are important for how biodiversity affects ecosystem functioning, there is no intrinsic reason to choose one system over another, and it would seem sensible to use systems in which it is easiest to conduct the necessary experiments. With this line of reasoning, microbial communities appear to be the ideal model system for biodiversity-ecosystem functioning studies. Since much of this research was reviewed previously (Petchey et al. 2002), we limit the following sections to subsequent advances in the field.

9.3.2 Microcosm biodiversity-functioning experiments

9.3.2.1 Protistan food webs

Since the last review of studies of biodiversity and ecosystem functioning in eukaryotic microbes (Petchey et al. 2002) there have been a number of new developments. Having established that positive diversity-functioning relationships often exist in these microcosms (Petchey et al. 2002), the field has begun investigating how this relationship is modified by important ecological drivers, for example how systems of different diversity respond to experimental perturbations, to alterations in the amount of available resources, and to manipulating other factors which indirectly manipulate diversity, such as predator abundance.

Steiner and colleagues explored patterns of temporal variability in the presence or absence of perturbations in multitrophic food webs of differing diversity (Steiner 2005a, Steiner et al. 2006). Theoretical studies predict that increased productivity destabilizes populations (Rosenzweig 1971) but can stabilize total community biomass. In unperturbed food webs, greater diversity was correlated with reduced temporal variability in total community biomass, especially in more productive environments. Temporal variability in the biomass of individual populations was not affected by diversity, nor by productivity. Differences in the species composition among diversity treatments had no influence on the variability of community-level biomass, while composition did affect the variability of biomass at the level of individual populations. The effect of diversity, but not the destabilizing effects of productivity predicted by the paradox of enrichment, predominated in these microcosms.

In a separate experiment, diversity was manipulated and community biomass was predicted based on allometric relationships between organism size and abundance (Long et al. 2006). Microcosms with greater diversity tended to yield more total biomass than systems of lower diversity, and organism size played only a transient role in determining biomass. This result is consistent with positive effects of diversity on overyielding noted in other, very different systems (Loreau and Hector 2001, Loreau et al. 2001). Krumins et al. (2006) further explored the indirect impact of bacterivore diversity on rates of bacterially mediated decomposition of wheat seeds, and they found that bacterivores altered both the taxonomic composition and overall metabolic activity of the bacterial community.

Other studies have explored the impacts of various factors, including assembly history, productivity, and consumers on patterns of diversity. Fukami and Morin (2003) showed that the specific temporal sequence of species arrival had a profound effect on the form of relationships between microbial diversity and productivity, estimated as biomass accumulation (Fukami and Morin 2003). Hump-shaped, concave, or monotonically increasing patterns can all result simply from differences in the history of community assembly. Other work shows that different forms of predation, specifically that imposed by specialist versus generalist consumers, can either promote or eliminate positive relations between productivity and protist diversity (Jiang and Morin 2005).

Finally, a reanalysis of an earlier biodiversity-ecosystem functioning study (McGrady-Steed et al. 1997) in response to critiques of the design of
early biodiversity experiments (Fukami et al. 2001, Loreau et al. 2001) confirmed that elevated biodiversity can reduce variability in some aspects of ecosystem functioning (Morin and McGrady-Steed 2004). The effect is not related to a reduction in the temporal variation in ecosystem functioning, but rather reflects reduced variation among replicate communities of similar diversity at any point in time. This is perhaps best interpreted as reduced spatial variation in functioning in more diverse systems.

9.3.2.2 Aquatic bacteria

Bacteria have provided some important advances in understanding biodiversity-ecosystem functioning relationships because of the relative ease with which it is possible to assemble communities and store strains for prolonged periods. For example, in the most species-rich microbial biodiversity-ecosystem functioning experiments to date, Bell et al. (2005b) used 72 naturally co-occurring bacterial species and found a positive relationship between species richness and community respiration in aquatic microcosms (Bell et al. 2005b). Both diversity and species identity were important in determining functioning in this study. A similar conclusion was reached when investigating the relationship between genotype diversity and ecosystem functioning using a single species of bacteria, *Pseudomonas fluorescens* (Hodgson et al. 2002), where evidence was found for a significant effect of functional (ecotype) and genotypic diversity on both productivity and invasion resistance as measured by the degree to which mixture yields exceeded the maximum monoculture yields of the constituent strains. There is therefore the opportunity for significant diversity effects even within species. In contrast, experiments using four bacterial species found little evidence that species richness affected ecosystem functioning (wheat seed mass loss) because species that contributed little to seed mass loss tended to become dominant (Jiang 2007). Similar experiments have suggested that a significant relationship between bacterial diversity and functioning might only become apparent in the context of the larger food web (Naem and Li 1997, Naem et al. 2000).

These outcomes might be determined by the method used to isolate bacteria from the environment. One recent study looked at mixtures of up to eight bacterial strains selected for their ability to grow on cellulose in monoculture (Wohl et al. 2004). By selecting only species that utilized a particular substrate (cellulose), and then offering those species only that substrate in spatially homogenous (shaken) microcosms, the study was designed to prevent resource use complementarity from being important. It was therefore not surprising that the observed influence of diversity was partly attributable to a selection effect. Interestingly, despite being selected on a single substrate, species richness appeared to be important in addition to the effects of selection, possibly due to facilitation among species. In contrast, facilitation is unlikely to have been driving the overall pattern of increase in functioning with increasing biodiversity in the Bell et al. (2005b) study or else an accelerating biodiversity-ecosystem functioning relationship (i.e. where the slope of the relationship increases with increasing biodiversity) would have been observed, although such an observation would have been hidden if facilitation was rare. This does not exclude the possibility that facilitation is common in microbial communities, but it might often be masked by the increasing degree of redundancy as species are added to the community.

One of the principle advantages of using bacteria in biodiversity-ecosystem functioning experiments is that short generation times allow simultaneous investigations of evolutionary and ecological processes. For example, a single generalist genotype of *Pseudomonas fluorescens* diverges into a number of distinct specialist ecotypes that inhabit the microcosm sides, air water interface, and open water (Buckling et al. 2000). This niche differentiation leads to increased levels of functioning (Hodgson et al. 2002), so any processes that disrupt the extent to which diversification occurs (Brockhurst et al. 2006, Brockhurst et al. 2007) will also affect the level of ecosystem functioning. Thus, unlike with larger, longer-lived organisms, in microbial systems it is possible to examine the evolutionary processes directly and so too make hypotheses about which ecological mechanisms will be important in determining functioning (Fukami et al. 2007, Venail et al. 2008). If, as many have suggested, there is widespread functional redundancy in bacterial
communities, bacterial diversity must be maintained in nature in the absence of niche differences, which is contrary to many theories of species coexistence. For the microcosms used by Bell et al. (2005b), there is evidence that the communities were neutrally assembled (Woodcock et al. 2007), leading to an independent prediction that any positive relationship between diversity and functioning in this system should be a consequence of the selection effect. The next generation of microbial experiments in this field is therefore likely to include studies that simultaneously investigate the causes and consequences of biodiversity.

9.3.2.3 Fungi

Fungal communities are important drivers of litter decomposition with potential for influencing carbon sequestration in soils and, for aquatic fungi, in downstream lakes, reservoirs and oceans. Consequently, there is great interest in understanding the degree to which the diversity and structure of fungal communities affect rates of decomposition. Varying the species richness of leaf-colonizing stream fungi (aquatic hyphomycetes) in microcosms had no effect on average leaf decomposition rates in communities with up to eight fungal species (Dang et al. 2005, Duarte et al. 2006), which implies a high degree of functional redundancy among these fungi. However, in a separate experiment, cultures containing two early fungal colonizers of leaves enhanced decomposition by 73 per cent compared to values expected from decomposition rates of single-species cultures (Treton et al. 2004). This outcome, in contrast to results from experiments using communities of several species (Dang et al. 2005, Duarte et al. 2006), is strong evidence of complementarity resulting in faster litter decomposition. In a similar vein, Bärlacher and Corkum (2003) reported a tendency towards faster decomposition with increasing fungal richness (1 to 5 species), although mixed communities never caused greater mass loss than the most effective species alone (Bärlacher and Corkum 2003). Raviraja et al. (2006) also found that both species richness and identities affected leaf mass loss in microcosms, although again the most effective fungal species degraded leaves faster than species mixtures. To date, all of these studies have been phenomenological in nature, so that the mechanisms behind and circumstances under which fungal diversity effects on litter decomposition emerge in aquatic microcosms are currently unknown.

There is also evidence that richness of aquatic hyphomycete communities can indirectly enhance decomposition through a positive effect on resource quality for invertebrate detritivores (Lecerf et al. 2005). Further more ecosystem processes other than litter decomposition (e.g. fungal biomass production) may be enhanced by diverse communities (Duarte et al. 2006). Lastly, even when average rates of decomposition are independent of species richness, variability of rates has been found to decline strongly with increasing fungal richness (Dang et al. 2005), as predicted from theoretical models (Doak et al. 1998) and observed in other types of systems (Tilman et al. 2006b, Lecerf et al. 2007). All else being equal, this should lead to higher predictability of litter decomposition rates when fungal communities in streams are diverse.

In microcosm experiments with culturable saprotrophic soil fungi, increased richness resulted in faster decomposition rates on grass litter (Deacon et al. 2006), forest soil (Wardle et al. 2004a, Tiunov and Scheu 2005) and powdered cellulose (Tiunov and Scheu 2005). Although species richness in these experiments was much lower than the number of species in the ecosystems from where the fungi were isolated, positive diversity effects on decomposition also emerged in an experiment that involved a rather high number of fungal species (43 taxa) (Setälä and McLean 2004). Nevertheless, communities with low species richness (six species) were as effective as the most diverse community in maintaining ecosystem functioning (Setälä and McLean 2004), indicating that fungal diversity effects on decomposition saturate at low levels, as has been found in many other circumstances.

9.3.2.4 Mutualistic microbe plant interactions

Mycorrhizal fungi and nitrogen-fixing bacteria form symbiotic associations with approximately 80 per cent of all terrestrial plants, and therefore constitute an important pathway by which nutrients (e.g. inorganic nitrogen and phosphorus) are taken up by primary producers. Several studies have manipulated mycorrhizal diversity and measured
plant performance. Results indicate that plant diversity, productivity and invasion success are responsive to either mycorrhizal diversity or mycorrhizal identity (van der Heijden et al. 1998, Stampe and Daehler 2003, Vogelsang et al. 2006). Similarly, manipulation of rhizobia in experimental dune grassland showed that presence of these nitrogen-fixing bacteria enhanced nitrogen capture (+85 per cent), plant productivity (+35 per cent) and plant evenness (+34 per cent) (van der Heijden et al. 2006). The diversity of bacterial symbionts was partly responsible for these effects because several of the legume species present in the microcosms formed host-specific associations with specific rhizobia. There is evidence that some of the effects of mycorrhizal diversity depend on the environmental context, depending on the species of plant that is infected and fertility of the soil (Jonsson et al. 2001). Recently, there have been some clues of how complementarity among mycorrhizal strains operates. In particular, some mycorrhizal families appear to protect against fungal pathogens, while other families enhance phosphorus uptake. As a consequence, plant growth is enhanced when both types of family are present (Maherali and Klironomos 2007).

9.4 Functioning of microbial communities in the wild

9.4.1 Diversity and functioning in the microbial wilderness

Since most microbes cannot be cultured, it is generally impossible to experimentally assemble communities from a library of constituent species to reflect the makeup of communities in natural ecosystems. The alternative to the culture-dependent approach is to conduct experiments or perform comparative analyses using exclusively culture-independent techniques to manipulate or describe microbial diversity and simultaneously measure ecosystem process rates in situ (Cavigelli and Robertson 2000) or in artificially created ecosystems (Bonkowski et al. 2001, Griffiths et al. 2004, Girvan et al. 2005). In both cases, differences in the microbial communities among replicates can be identified or created, and quantified. The advantage of this approach is that the results apply to whole microbial communities and not only to the subsets that can be cultured (Fig. 9.1).

In general, the difficulty with current culture-independent studies is that any genuine biodiversity effect tends to be confounded because of differences among species in their susceptibility to elimination during the biodiversity manipulation procedure. This is evident from the titles of the articles that use this kind of approach, where some studies claim to examine the impact of diversity (Cavigelli and Robertson 2000, Bonkowski et al. 2001, Muller et al. 2003), others investigate the impact of community structure (Franklin et al. 2001, van der Gast et al. 2003, Griffiths et al. 2004), and others focus on the impact of species composition (Cavigelli and Robertson 2000). Yet all of these studies performed precisely the same kind of manipulation; rare or susceptible species were eliminated. What these studies can demonstrate is whether either reductions in biodiversity or the elimination of particular species are correlated with changes in the magnitude or stability of functioning, but unambiguous attribution of effects to diversity changes is not possible.

9.4.2 Field experiments and observations

9.4.2.1 Soil microbes

Soil microbial communities play a pivotal role in determining rates of litter decomposition (Wardle 2002). One of the major hurdles is that microbial diversity in soils is extraordinarily high, with as many as tens of thousands of bacterial and fungal species in a single gram of soil. With so many species apparently competing for a limited set of resources, the predominant view is that many soil microbes are functionally redundant (Chapin et al. 1997, Wardle et al. 2004a, Deacon et al. 2006). There are opportunities for, resource partitioning especially on the most recalcitrant or exotic substrates. Lignin, for example, is most efficiently degraded by some basidiomycetous fungi commonly referred to as white-rot fungi. Although there may be pronounced differences in enzymatic capacities among microbial species to degrade other plant polymers, it appears that most have the metabolic machinery to break down the majority of the common substrates they encounter. Hutchinson’s
‘paradox of the plankton’ (which asks how a large number of species can be maintained in a given community when they appear to be occupying similar niches) (Hutchinson 1961) remains unresolved for soil microbes. While the high levels of diversity might be explained in part by the intricate physical structure of the soil environment (Crawford et al. 2005) as well as the rich set of interactions among substrate availability, abiotic conditions (e.g. pH, temperature) and the specific species that are in the vicinity (Treton et al. 2004, Wardle et al. 2004a), it is difficult to envision how thousands or, for bacteria, many more species can occupy finely differentiated niches. The prediction is therefore that many soil species are functionally redundant.

There have been several experiments in which soil microbial diversity has been reduced through either fumigation or dilution. When a fumigation treatment was applied, Griffiths (2000) found that ‘general’ soil functions (such as total community respiration) were inversely correlated with biodiversity, while the relationship was positive for ‘specific’ functions such as nitrification (Griffiths et al. 2000). In contrast, Degens (1998) found that the reductions in diversity caused by fumigation caused a decrease in decomposition rate of simple organic compounds, although this relationship disappeared at high levels of soil moisture. A similar decrease in decomposition rate was found in soil microbial communities with reduced diversity achieved by dilution, although differences were only found between the highest and lowest dilution levels (Griffiths et al. 2001). Wertz et al. (2006) created differences in soil microbial diversity among replicate communities by diluting soil microbial suspensions before inoculation, after which they allowed soil microbial biomass to recover (Wertz et al. 2006). Their results suggest considerable functional redundancy, even for the specific processes they measured, such as ammonium transformation, which are carried out by relatively limited numbers of taxa (ammonia oxidizers, which carry out the first step of nitrification). Comparative studies that used natural differences in soil nitrifier communities found that the identity of the species in the soil affected rates of ammonia oxidation, and therefore concluded that there is potential for important effects of nitrifier community structure and diversity on nitrification rates (Cavigelli and Robertson 2000).

Manipulating the diversity of higher trophic levels in soil communities can also influence decomposition rate indirectly through their effect on bacterial and fungal populations (Cragg and Bardgett 2001, Hättenschwiler et al. 2005). For example, there was no increase in decomposition with increasing bactivorous nematode richness, even though some nematode species suppressed bacterial activity and diversity (De Mesel et al. 2006). However, species composition appeared to play a role in controlling decomposition when richness had no effect in collembola (Cragg and Bardgett 2001) or microbivorous nematodes (Mikola and Setälä 1998).

Overall, artificially reduced levels of diversity have not resulted in a clear effect on soil functioning. It is certainly the case that that functioning is sometimes decreased when biodiversity is reduced, but the reasons why this occurs remains equivocal (see 9.4.1 above). The predominant theme in the soil literature is that it is difficult to draw generalizations about the relationship between soil microbial diversity and processes (Raffaelli et al. 2002, Hättenschwiler et al. 2005). Relationships often appear to be idiosyncratic (Raffaelli et al. 2002, Hättenschwiler et al. 2005) although aspects of redundancy and keystone species effects are also evident (Setälä and McLean 2004). Clearly, these kinds of experiment remain in their infancy, and further investigations are necessary to construct an image of how the diversity and composition of soil microbial communities influence soil processes.

9.4.2.2 Microbe plant interactions
Soil microbial communities interact directly and indirectly with plant roots as pathogens and symbionts (direct interactions) and decomposers and antagonists (indirect interactions) (Wardle et al. 2004a). In turn, plants manipulate the chemical environment surrounding their roots by secreting chemicals that favour particular microbial consortia, thereby altering the composition and diversity of the root-associated microbial

There is some evidence to suggest that plant diversity plays a role in determining microbial diversity and ecosystem functioning (Hooper et al. 2005), but the importance of plant diversity compared with processes within soil microbial food webs remains uncertain (Kowalchuk et al. 2002). Although there are examples of effects of plant species richness on soil functioning (Zak et al. 2003), for the most part the effect of plant diversity appears to be plant species-specific (Cleland et al. 2004, De Deyn et al. 2004, Wardle et al. 2004a, Viketoft et al. 2005), and is often complicated because plant and microbial communities operate at different time-scales (Korthals et al. 2001, Hedlund et al. 2003) and there are complex interactions among soil microbial species (Milcu et al. 2006).

Influences of soil microbial diversity on plant performance or plant communities have been rarely examined, mostly owing to the difficulty of manipulating soil microbial diversity. Selectively excluding part of the soil microbial community requires severe manipulations (e.g. via fumigation, see above) before an effect on ecosystem processes can be observed (Bonkowski and Roy 2005). There have been some initiatives, such as Bradford et al. (2002), who manipulated size classes of soil organisms and concluded that effects of soil organisms from different size classes on plant productivity and plant community composition are relatively minor and that effects of larger organisms supersede those of the smaller organisms (Bradford et al. 2002).

Results from studies that have included soil microbes and soil invertebrates indicate that soil microbial effects on plant community performance may be overruled by effects of the invertebrates (Bonkowski et al. 2001, Bradford et al. 2002, Bezemer et al. 2005, Wurst et al. 2008). Overall, the effects of the decomposer community appear to depend more on community composition and relative species abundance than on diversity (Wardle et al. 2004a, Milcu et al. 2006).

### 9.4.2.2 Aquatic fungi

The relationship between fungal diversity and ecosystem functioning has also received some attention in aquatic ecosystems because of the importance of fungi in litter decomposition. Results from two surveys in streams suggest that the species-poor fungal communities presumably affected by forestry practices or water pollution do not result in altered leaf decomposition rates (Raviraja et al. 1998, Bärlocher and Graça 2002). However, the general paucity of such field data and the problem of drawing inferences about cause and effect from correlational data currently impede conclusive answers about the significance of aquatic fungal diversity for litter decomposition.

### 9.5 Synthesis

The experiments to date that have assembled microbial communities in microcosms point toward a positive relationship between diversity and ecosystem functioning in most cases, although this outcome is by no means universal. While much more research is required before firm conclusions can be drawn, the positive relationships observed primarily in microcosm experiments appear to be driven by a variety of mechanisms, including complementary resource use and selection effects. As with studies involving larger organisms, however, the task of identifying causal mechanisms is only just beginning.

While the experiments reviewed above have demonstrated that there is potential for positive biodiversity-ecosystem functioning relationships in microbial communities, they have not shown whether such a relationship is common in species-rich natural communities. In contrast with the results of manipulative microcosm experiments, non-manipulative surveys (which compare measures of ecosystem functioning associated with natural variation in microbial communities) and experiments (in which natural microbial communities are manipulated) have found inconsistent effects of diversity on ecosystem functioning. This raises the principal question in microbial
biodiversity-ecosystem functioning studies of why apparent discrepancies exist between these two approaches.

There are real difficulties in attempting to bring together the two lines of enquiry that assemble species from their constituent species versus experiments or observations using natural communities. Currently unculturable bacteria cannot be directly manipulated, making it impossible in practice to conduct well-designed manipulative biodiversity experiments with truly natural microbial communities. Even though the ability to describe the diversity of these communities is rapidly increasing as new DNA sequencing technologies are becoming widespread, the approach with natural communities will remain comparative unless new means are devised to manipulate microbial species directly without the need for culturing. Such comparative studies can give clues to causal mechanisms, but they inevitably suffer from the fundamental problem of relating cause and effect in an unambiguous manner. Even if it were possible to manipulate natural communities directly, attempting to manipulate communities with thousands of species poses enormous practical challenges because of the exorbitant number of possible species combinations.

There are a number of possible reasons as to why discrepancies commonly occur between small-scale manipulations of species-poor cultivable communities and large-scale observations of natural microbial communities. Perhaps the most obvious possibility is that the two approaches focus on different parts of the same relationship. Manipulative experiments with assembled communities consider levels of species richness from $10^0$ to at most $10^2$ species, whereas field manipulations reduce levels of diversity from much greater than $10^2$ down to $10^2$ species (Wertz et al. 2006), and observational studies are comparing communities with $> 10^3$ species (Fig. 9.2). Since microbial studies have typically found a saturating relationship between diversity and ecosystem functioning, even large reductions of diversity in species-rich communities are expected to have little effect on ecosystem functioning. For example, Bell et al. (2005b) found that a generic measure of ecosystem functioning brought about by bacteria (community respiration) scaled with the logarithm of bacterial species richness. Consequently, the addition of the first 100 species to the community is expected to increase the level of community respiration by 200 per cent, whereas adding an additional 100 species would increase respiration rate by $< 10$ per cent. Thus, extrapolating curves from microcosm experiments such as those by Bell et al. (2005b) would predict that even very large reductions in the diversity of natural communities have negligible effects on ecosystem functioning.

A second possibility is that those species that can be cultured may be poorly representative of the larger community, in which case we should not expect the results of manipulative experiments to apply to microbial communities as a whole even if their diversity were as low as in the experiments. While it is certainly the case that libraries of cultivable microbes are unrepresentative of the larger community, and also that only an extremely small portion of the total microbial diversity has been studied in this manner, biodiversity-ecosystem functioning experiments have now been conducted with an array of cultivable organisms. In addition, it might be important to bear in mind that there is a flip-side to the ‘cultivability’ debate, which is that culture-independent techniques might be picking up primarily metabolically inactive cells. Depending on how community membership is defined, these inactive cells might not be community members of interest in the context of assessing effects on ecosystem functioning. If indeed diversity in field experiments is mostly reduced by affecting species in a resting state, it would not be surprising that even large declines in diversity fail to curtail ecosystem functioning, at least at a gross level and in the short-term scales. Evidently, metabolically inactive cells might play a key role in fluctuating or shifting environmental conditions, but it is unclear to what extent inactive cells become metabolically active. Thus, the question of how microbial diversity is measured or defined remains pivotal to discussions about the effects of microbial biodiversity on ecosystem functioning.

A third possible reason is that while microcosm studies favour fast-growing r-strategists, experimental manipulations of natural microbial
communities are likely to eliminate preferentially rare species. Even when rare species have the potential to play a significant role they are least likely to contribute notably to ecosystem functioning (compared to common species) due simply to their rarity. Therefore, to some extent such experimental manipulations may lead to conservative estimates of microbial diversity effects on ecosystem functioning.

Advances in molecular techniques have revealed that there are many similarities in the community ecology of microbes and larger organisms, suggesting that there might also be similarities in the relationship between diversity and ecosystem functioning. These technological advances have not only increased our ability to do experiments, but also to survey the microbial wilderness the way we have explored wild communities of larger organisms for centuries. Currently, several studies have revealed that prokaryotes have been on Earth billions of years before eukaryotes and multicellular organisms, that microbes constitute enormous biomass, exhibit unique and extraordinarily varied biochemistry that drives biogeochemistry, have phylogenies and taxonomies that remain beyond our ability to resolve, and exhibit lateral gene transfer that is almost unheard of elsewhere in nature. Nevertheless, it appears that many of the patterns of diversity in microbial communities are broadly similar to those of larger organisms (Green et al. 2004, Horner-Devine et al. 2004, Bell et al. 2005a, Fuhrman et al. 2006, Martiny et al. 2006).

Establishing the generality of this conclusion in relation to microbial biodiversity-ecosystem functioning research is imperative. There is a great deal of excitement that findings in microbial biodiversity-ecosystem functioning research can produce both considerable academic advances in understanding how ecosystems operate, and practical advances in harnessing microbial communities or preventing microbial disease. However, the inconsistencies between the various approaches that we highlight in the current review underline the real need for a better understanding of the causes and consequences of changing microbial biodiversity before the potential of microbial biodiversity-ecosystem functioning studies can be fully realized.