Microbial seed banks: the ecological and evolutionary implications of dormancy

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Abstract | Dormancy is a bet-hedging strategy used by a wide range of taxa, including microorganisms. It refers to an organism's ability to enter a reversible state of low metabolic activity when faced with unfavourable environmental conditions. Dormant microorganisms generate a seed bank, which comprises individuals that are capable of being resuscitated following environmental change. In this Review, we highlight mechanisms that have evolved in microorganisms to allow them to successfully enter and exit a dormant state, and discuss the implications of microbial seed banks for evolutionary dynamics, population persistence, maintenance of biodiversity, and the stability of ecosystem processes.

Storage effect
An ecological hypothesis stating that environmental fluctuations drive temporal variations in population growth that produce long-lived individual organisms, thus promoting multispecies coexistence.

In nature, most organisms live in unpredictable environments and typically experience conditions that are suboptimal for growth and reproduction. A very common response to environmental stress is for organisms to enter a reversible state of reduced metabolic activity, or dormancy[1]. By doing so, these organisms can drastically lower their energetic expenditures and evade unfavourable conditions that would otherwise reduce the fitness of the population. Dormancy is not a cost-free strategy, however. Organisms must invest resources into resting structures and the machinery that is needed for transitioning into and out of a dormant state[2,3]. In addition, dormant organisms must be able to interpret and respond to signals associated with favourable conditions, otherwise they will miss opportunities for growth and reproduction[4–6]. Despite these general trade-offs, microbial species from all domains of life have evolved the ability to use dormancy during periods of environmental stress[7–9] (FIG. 1).

Dormancy generates a seed bank, which we define as 'a reservoir of dormant individuals that can potentially be resuscitated in the future under different environmental conditions'. Seed banks can prolong the persistence of genotypes and populations, and also have important consequences for community- and ecosystem-level processes. Ecologists have developed theory and have empirically demonstrated the importance of seed banks for the diversity and functioning of communities of macroscopic organisms (that is, plants and animals). For example, the accumulation of long-lived organisms in a seed bank allows competing species to coexist via the storage effect, thus maintaining biodiversity[10]. Seed banks also determine how communities recover from disturbance[11] and potentially stabilize ecosystem processes under variable environmental conditions[12]. Because microbial dormancy is common in a range of ecosystems, it seems likely that the ecology and evolution of microbial communities are strongly influenced by seed bank dynamics.

Dormancy is a well-documented trait that helps microorganisms contend with environmental variability. Although it is common and phylogenetically widespread, dormancy is achieved through a diverse set of genetic and cellular mechanisms (FIG. 1). Dormancy has received considerable attention from microbiologists, in part because of its role in human diseases such as anthrax, cholera and tuberculosis[13]. As a result, microbiologists have unravelled the mechanistic underpinnings of dormancy in a handful of clinically and environmentally important strains of bacteria. Collectively, this information provides a strong foundation for exploring the prevalence and implications of dormancy in natural ecosystems, but we are still challenged by the complexity of environmental systems, which typically contain thousands of potentially interacting species. With recent innovations in molecular techniques and computational analyses, we are now poised to test theory about how dormancy influences the structure and function of microbial communities in complex natural settings.

In this Review, dormancy is broadly defined as "any rest period or reversible interruption of the phenotypic development of an organism" (REF. 14). From the outset,

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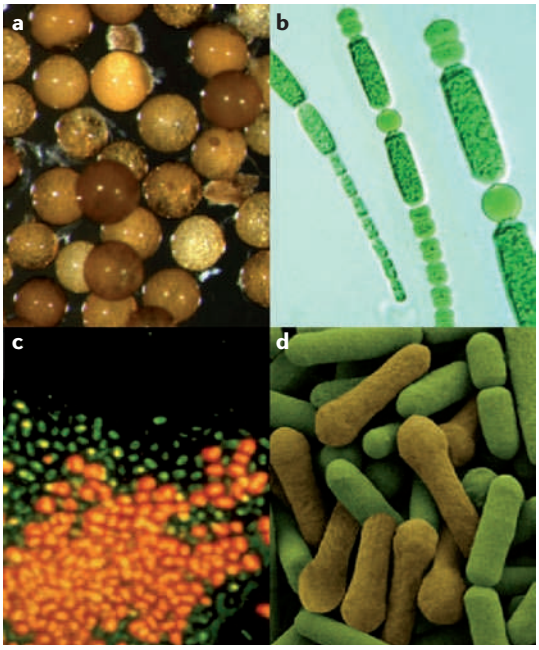


Figure 1 | **Examples of microbial dormancy. a** | Spores from an arbuscular mycorrhizal fungus (*Scutellospora castanea*) that forms mutualistic associations with plant roots. **b** | Akinetes (thick-walled resting structures) produced in filaments of a cyanobacterium (*Cylindrospermum* sp.). **c** | A cross section of a bacterial biofilm (formed of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) after a 1 hr exposure to a chemical stressor (chloramine). The biofilm was stained with 4′,6-diamido-2-phenylindole (DAPI) and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), distinguishing non-respiring cells (green) and respiring cells (orange). **d** | Dormant (yellow) and germinating (green) endospores of *Viridibacillus arvi*. Part **a** image is courtesy of A. Brennwald, University of Zurich, Switzerland. Part **b** image is courtesy of Y. Tsukii, Hosei University, Tokyo, Japan. Part **c** image is modified, with permission, from REF. 125 © (1995) American Society for Microbiology. Part **d** image © (2009) Dennis Kunkel Microscopy, Inc.

we acknowledge that there is a gradient of dormancy and a variety of ways by which microorganisms can reduce their metabolic activity[7,15]. Using this inclusive framework, we synthesize the evidence for dormancy in nature and present a metagenomic analysis of dormancy genes recovered from a range of natural and managed ecosystems. Next, we provide a general overview of some of the intracellular processes involved in microbial dormancy, and illustrate the potential for dormancy and seed bank dynamics to influence a number of key patterns and processes in environmental microbiology. We aim to bridge gaps between more traditional disciplines of microbiology and the growing interest in the ecological mechanisms that influence the diversity and functioning of microbial communities.

Evidence for dormancy in environmental systems

Microbiologists discovered dormancy more than a century ago, but the recognition of its ecological importance in natural systems emerged slowly. In the 1970s, the accumulating morphological and physiological evidence led to the controversial idea that a large fraction of the microorganisms in nature were metabolically inactive. For example, Stevenson formally hypothesized that dormancy is widespread and allows microorganisms in natural settings to contend with harsh environmental conditions, and he suggested that dormancy is responsible for the stable abundances and activities of aquatic microbial communities through time[16]. These ideas gained support when microbiologists recognized that most bacterial cells could not be cultured[17], even though they exhibited signs of viability[18]. Soon after this, various new techniques (such as microautoradiography, fluorescence *in situ* hybridization and the use of electron-accepting fluorescence dyes such as 5-cyano-2,3-ditolyl tetrazolium chloride (CTC)) allowed microbiologists to assess the metabolic status of individual bacterial cells from environmental samples[15]. Although these techniques generated debate about aspects of the methodology, they have ultimately provided consistent and robust estimates of microbial activity, especially when examined across broad environmental gradients[15]. Single-cell assays indicate that the activity of bacterial communities in environmental samples can be extremely variable (BOX 1). In freshwater and marine habitats, a large proportion of this variability in metabolic activity can be predicted based on the growth efficiency of the bacterial community[15] or on more direct indicators of ecosystem productivity (such as phosphorus availability or chlorophyll *a* concentrations)[19]. Currently, new molecular tools are used to assess how dormancy influences microbial community structure. For example, it is becoming more common for microbiologists to simultaneously characterize the active (ribosomal RNA-based) and total (ribosomal DNA-based) composition of microbial communities[20,21]. This combined rRNA–rDNA approach has been used to test theoretical predictions about microbial seed banks[22] and the dynamics of metacommunities[23]. Some of these studies suggest that the effects of dormancy on microbial composition may be determined by environmental factors that operate over broad scales, such as seasonal changes in temperature or photoperiod[22]. Genomic and metagenomic approaches provide additional opportunities to better understand the genetic basis of dormancy regulation in environmental systems (BOX 2), whereas expression-based techniques will almost certainly be fruitful for identifying novel loci involved in the dormancy of microbial communities[24,25].

Stages of microbial dormancy

In order to appreciate the impact of dormancy on the ecology and evolution of complex microbial communities, it is necessary to have a basic understanding of the cellular processes that underlie dormancy. Here, we describe three stages of dormancy: initiation, resting and resuscitation (FIG. 2).

Initiation of microbial dormancy — responsive and spontaneous switching. Dormancy is often initiated in response to unfavourable changes in an organism's environment. Microorganisms detect fluctuations in

Metacommunity
A collection of local communities within a heterogeneous landscape that are connected through the dispersal of potentially interacting species.

Box 1 | **When and where do we expect to observe microbial dormancy?**

As with other microbial traits, we expect ecosystem features to impose evolutionary and ecological constraints on microbial dormancy. In some systems, dormancy may be a beneficial trait that allows persistence in the face of environmental change; in other systems, dormancy may be maladaptive and purged by natural selection. Below, we outline system features that are likely to influence the prevalence of dormancy, and present data that lend support to our hypotheses.

Resource availability

Starvation is a well-documented cue that regulates dormancy in laboratory strains and natural communities[15,31]. In addition, theory predicts that the benefits of dormancy should be greater in environments with low resource availability[37].

Residence time

Because the reproductive rate of dormant individuals is zero, rapid dilution in a flow-through system (such as a chemostat) should quickly drive dormant populations to extinction. By contrast, systems with high residence times (or slow turnover rates) are likely to become resource limited, encouraging dormancy[7].

Perturbation regime

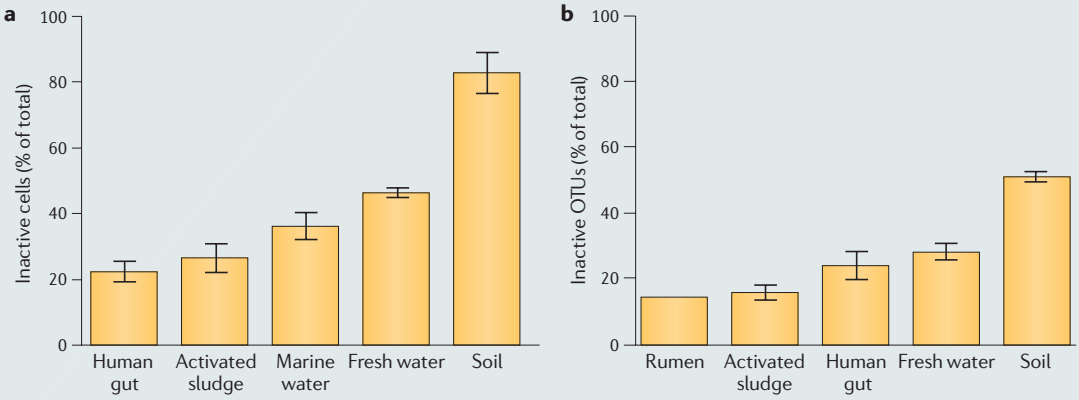
Because dormancy allows persistence during inhospitable periods, the fitness advantage conferred by dormancy increases with decreasing likelihood of 'good times' (REF. 110). This seems to be the case regardless of whether microorganisms rely on responsive or spontaneous switching mechanisms of dormancy (see main text for details).

Predation

Because dormant cells are not capable of cell division, the populations they belong to cannot compensate for losses due to predation, and are therefore at an increased risk of extinction. However, evidence suggests that predators preferentially feed on active cells[111], which may allow dormant populations to persist and possibly dominate microbial communities[12]. Similarly, dormant bacteria may experience less virus-induced mortality than active bacteria. For example, *Escherichia coli* is protected from prophage induction when it is in a dormant (persister) state, owing to suppression of lytic genes. However, when persisters switch to active growth, gene expression is resumed, leading to host lysis[112].

Evidence for dormancy in natural ecosystems

Large numbers of cells in different environments can be dormant. The figure shows the percentages of cells that were found to be dormant in various environments, as determined by fluorescence *in situ* hybridization (FISH) or staining with 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) (see the figure, part **a**), or by ribosomal RNA–ribosomal DNA terminal restriction fragment length polymorphism (TRFLP) (see the figure, part **b**). The data shown are for the mean ± the standard error of the mean (see Supplementary information S1 (table) for data).



OTU, operational taxonomic unit.

abiotic factors such as temperature, osmotic pressure, light and pH via membrane-bound histidine kinase sensors. Information about the external environment elicits an intracellular response that leads to changes in gene expression and protein synthesis[26] which can ultimately trigger dormancy[27]. This typically involves tightly controlled regulatory networks because the transition from an active state to a dormant state can require a substantial energetic investment. For example, although endospores allow *Bacillus subtilis* to resist harsh environmental conditions for prolonged periods, spore formation is costly, involving replication of the bacterial chromosome, differentiation of the cell, biosynthesis of protective layers for the spore and death of the mother cell[28]. Although comparisons among different dormancy strategies are rare or non-existent, it seems likely that there are trade-offs among the energetic investments (such as those for structural transformation), the degree of environmental resistance to stressful conditions and the responsiveness of a resting cell to changes in its environment.

One condition that commonly initiates dormancy is starvation or resource limitation. When intracellular concentrations of amino acids, fatty acids or other carbon compounds are depleted, many microorganisms will undergo a stringent response[29]. The stringent response is regulated by guanosine pentaphosphate (pppGpp) and guanosine tetraphosphate (ppGpp); these compounds

Histidine kinase sensor
A transmembrane protein that senses external stimuli and conveys signals that lead to changes in cell function.

Stringent response
The microbial stress response to starvation, leading to the reallocation of resources from growth to survival.

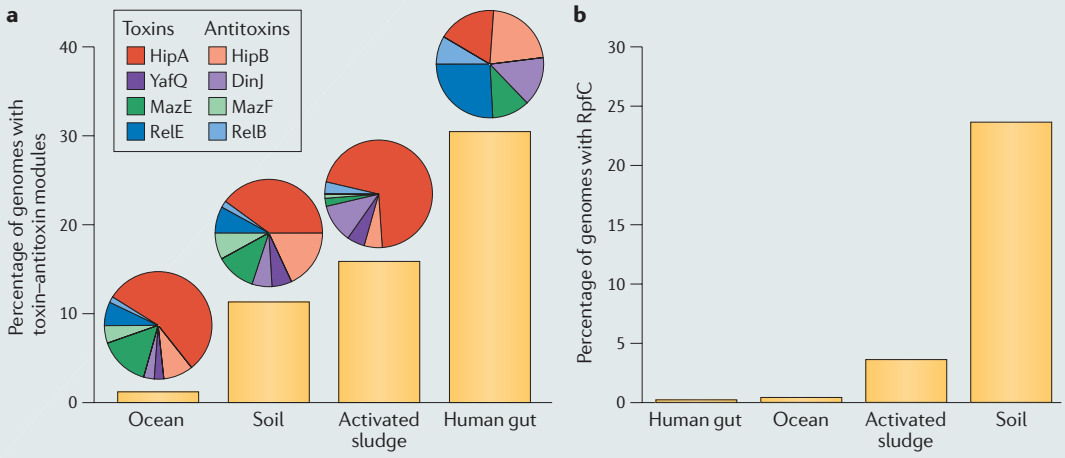
Box 2 | **Metagenomics of dormancy**

Metagenomics allows insight into the distribution and abundance of taxonomic and functional genes in complex microbial communities. Among other studies, metagenomics has been used to gain insight into antibiotic resistance in soils[113], cellulose degradation in termite guts[114] and phosphorus removal from sludge reactors[115]. We investigated the distribution of potential dormancy genes in a range of natural and managed ecosystems (oceans, activated sludge, soil and the human gut) by searching for orthologues of genes known or thought to be involved in dormancy.

Much of what we know regarding the mechanisms of microbial dormancy comes from the study of sporulation in *Bacillus subtilis*[28], persister cells of *Escherichia coli*[31,34] and latent properties of *Mycobacterium tuberculosis* and its relatives[116]. Therefore, we restricted our metagenomic analysis to key genes that have been associated with these model taxa and their contrasting dormancy strategies. We used tBLASTx (basic local alignment search tool using a translated nucleotide query to search a translated nucleotide database) to search for orthologues of: the *B. subtilis* sporulation proteins Spo0A and Spo0B, which encode proteins involved in the regulation of entry into sporulation; toxin–antitoxin systems (including RelB–RelE, DinJ–YafQ, MazF–MazE and HipA–HipB) that have been shown to be involved in the persistence of *E. coli*; and resuscitation-promoting factors (Rpfs) that have been shown to regulate resuscitation in *M. tuberculosis* and other taxa belonging to the Actinobacteria (see Supplementary information S2 (box) for additional information about the method of analysis).

We detected Spo0A in 15% of human distal gut genomes, but in general, sporulation genes were not common in the metagenomes that we investigated. Toxin–antitoxin systems were common in complex microbial communities (see the figure, part **a**), with the exception of communities from marine habitats; this is a somewhat surprising result, as marine microbial ecosystems are thought to have high levels of dormancy. However, genomic and proteomic studies of '*Candidatus* Pelagibacter ubique', a dominant marine microorganism, suggest that mechanisms other than toxin–antitoxin modules may be important for regulating dormancy in the marine environment[25]. Although RpfA and RpfB were only rarely detected in the sampled metagenomes, RpfC was much more common, especially in soils, in which the relative abundance of Actinobacteria can be high (see the figure, part **b**).

Our analyses show that dormancy may indeed be prevalent in various complex microbial communities. In addition, our results support the idea that there are various genetic mechanisms for entering and exiting dormancy, and suggest that some of these strategies may be more common in some ecosystems than in others.



also mediate other aspects of cellular activity, including the production of σ-factors (which are regulators of transcription) during stationary phase[29], the development of resistant myxospores[30], and possibly growth arrest cycles in environmentally stressed cells via toxin–antitoxin (TA) modules[31].

In some cases, dormant cells are spontaneously produced. Theory predicts that spontaneous switching from an active to a dormant state should be adaptive under stable environmental conditions, whereas responsive switching should be favoured in fluctuating environments[32]. One advantage of spontaneous switching is that organisms avoid the costs associated with the cellular machinery required for sensing their environment[32]. Another advantage is that spontaneous switching can generate subpopulations of individuals that can rapidly respond to stochastic perturbation events[32].

Persister cells represent one of the best examples of spontaneously initiated dormancy. Persister cells are a subpopulation that becomes apparent after a population is exposed to antibiotics, as persister cells survive this treatment[33,34]. When persister cells are revived from dormancy, they are again sensitive to antibiotics. Therefore, persistence cannot be attributed to mutations conferring antibiotic resistance. Instead, this tolerance to antibiotics is achieved through a form of dormancy that involves toxin–antitoxin modules (BOX 2). Persister cells can be produced spontaneously or in response to starvation during several stages of bacterial growth[35,36], and in many cases they do not seem to involve quorum sensing (cell–cell communication)[34]. Ultimately, persister cells may serve as an important reservoir, or seed bank, of cells that ensures long-term population viability. As persister cells can potentially rescue a population from

Quorum sensing
A process whereby gene expression in and/or growth of microorganisms are coordinated through the production and interpretation of signalling molecules.

extinction, it has been argued that the spontaneous production of dormant subpopulations is an example of kin selection[34,37]. In addition to being favoured in stable environments[32], theoretical explorations of dormancy strategies have revealed that spontaneous initiation of dormancy should be adaptive when there is a high degree of genetic relatedness among individual organisms within a focal population[37].

Microorganisms at rest — phenotypes, costs and duration of dormancy. Dormant microorganisms exhibit a wide range of phenotypes, which probably reflects the evolutionary diversity of microbial dormancy. One of the most obvious characteristics of some dormant microorganisms involves the differentiation of vegetative cells into resting structures such as spores, conidia, cysts or akinetes (FIG. 1; TABLE 1), all of which are easy to identify using basic microscopy techniques. Another obvious change is the reduction in cell size that occurs in many microorganisms when they enter dormancy. The finding that these 'dwarf' cells are common in nutrient-depleted marine environments supports the view that natural environments are dominated by dormant microorganisms[16,38]. Other phenotypes of dormant microorganisms can be more challenging to measure, but nevertheless have important implications for the functioning of microbial communities. For example, dormant cells often have reduced concentrations of nucleic acids, lipids, fatty acids and proteins (TABLE 1). By contrast, there may be an increase in the concentration of storage compounds or reserves that are necessary for meeting the energy requirements for survival during dormancy (TABLE 1). Last, there is evidence that the elemental composition of microorganisms changes during dormancy (TABLE 1). Various bacteria and fungi experience reductions in the cell quotas of carbon, nitrogen, phosphorus, sulphur and oxygen during stationary phase or resting stages[39,40]. In the case of *Escherichia coli*, growth-mediated changes in cell stoichiometry led to a 45% increase in the molar carbon/phosphorus ratio[39]. This observation is consistent with the theory of ecological stoichiometry, which links patterns of nutrient cycling in ecosystems to organism growth rates and their elemental demands[41].

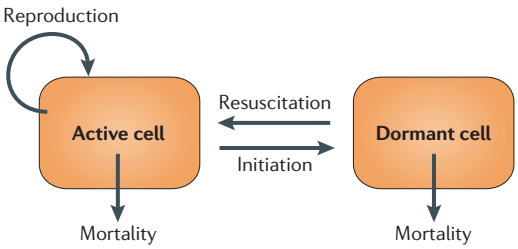


Figure 2 | **Dynamics of microbial dormancy.** The first stage of dormancy is the transition of metabolically active cells into the dormant state. Dormant cells can remain in an inactive state for prolonged periods, but must meet maintenance and survival energy costs, otherwise they will die. In order for dormancy to be a successful life history strategy, dormant cells must be resuscitated and return to the actively growing pool of individuals. Image is modified, with permission, from REF. 22 © (2010) US National Academy of Sciences.

Dormancy is not a cost-free state of existence. Energy demands approach zero in the special case of bacterial endospores, which are often considered to be metabolically inert[42], but in other scenarios microorganisms must allocate resources towards cell maintenance and survival[43]. Maintenance energy is used to support non-growth functions such as motility, turnover of macromolecules, regulation of osmotic pressure and intracellular pH, and maintenance of an energized

Table 1 | **Phenotypic characteristics of dormant microorganisms**

Phenotype	Examples	Refs
Physical differentiation to form a resting structure	Spores, conidia, cysts and akinetes	126
Reduced cell size	Ultramicrobacteria, microcolonies, dwarf cells and miniaturization	127,128
Reduced DNA and RNA content	Low-nucleic-acid (LNA) cells, ghost cells, non-nucleoid-containing cells (non-NuCC) and low RNA/DNA ratios	129–132
Altered quantity and composition of lipids and fatty acids	Commonly a reduction in total lipids, but increases in neutral lipids; changes in the ratios of saturated/unsaturated fatty acids; changes in the ratios of *trans*-/*cis*-monoenoic fatty acids; and changes in the ratios of cyclopropyl precursor/monoenoic precursor fatty acids	133,134
Altered quantity and composition of proteins	A decrease in protein content due to a reliance on internal reserves; reduced enzyme activity; and an increase in chromosomal-protection proteins (for example, Dps)	135,136
Ultrastructural features (changes in the internal cell structure)	Cell wall invagination, a thickened cell wall, synthesis of murein layers, a differentiated capsule and intramembrane particles	132,137
Accumulation of storage compounds (reserve molecules)	Increases in glycerol, polyhydroxylalkanoates (PHAs), poly-β-hydroxybutyric acid (PHB) and polyphosphates	48,138
Changes in the stoichiometry of biologically important elements	Changes in C, N, P, S, Ca^{2+}, K^+, Mg^{2+} and Na^+ levels, and changes in C/N, C/P, C/S, O/C, Ca^{2+}/K^+, P/S and K^+/Na^+ ratios	39,40
A less energized cell membrane	Accumulation of the dye rhodamine 123, and a change in the adenylate energy charge ratio	7,139

Kin selection
Evolutionary selection that occurs when a non-adaptive strategy of an individual improves the fitness of genetically related individuals.

Box 3 | **Dormancy and microbial evolution**

Just as dormancy can influence the persistence of populations and have implications for community dynamics, it can also influence the persistence of genotypes and thus have implications for evolutionary dynamics. The ability of an individual to exist in an active or dormant state depending on the environmental conditions is an example of phenotypic plasticity[117,118]. Phenotypic plasticity can weaken the effects of natural selection on individuals and their associated traits[119]. For this reason, dormancy should introduce time lags into the rates of co-evolution[120], which is critical for understanding the eco-evolutionary dynamics of microorganisms that commonly engage in antagonistic (bacteria and phages) or mutualistic (rhizobia and leguminous plants) interactions. Dormancy may also affect evolution by lengthening the generation time of microorganisms. This hypothesis was tested by comparing rates of amino acid substitutions among genomes of spore-forming and non-spore-forming bacteria belonging to the Firmicutes[118]. Although some *Bacillus* isolates have been shown to exist in a dormant state for prolonged periods of time[53], spore-forming bacteria do not seem to evolve more slowly than non-spore-forming bacteria. These comparable rates of molecular evolution may be explained by mutations that occur during dormancy[118].

Why, then, is dormancy not used by all microorganisms? It is likely that some species have never evolved the capacity of dormancy. Alternatively, some microorganisms may have lost the dormancy trait through regressive evolution. For example, theory predicts that there is a cost associated with phenotypically plastic traits[119,121]. Thus, if natural selection on phenotypic plasticity is relaxed, there is potential for microorganisms to lose the ability to successfully enter and exit dormancy[122]. In general, phenotypically plastic traits seem to be favoured in fluctuating environments[123], a topic which has been explored using simulation models of microbial dormancy[32,110]. This hypothesis was tested in long-term experimental evolution trials in which *Bacillus subtilis* was grown in conditions that favoured or did not favour sporulation[124]. After 6,000 generations, sporulation was either drastically reduced or lost in the absence of strong selection for sporulation. The loss of sporulation was most likely to be due to small insertions, small deletions or point mutations, as there was no evidence for large-scale deletions in the evolved genomes relative to the ancestral strain. Transcriptional patterns indicated that sporulation was affected by the misregulation of genes involved in the transition from exponential to stationary growth phase and during the initiation of sporulation[124]. Together, these results confirm that there are trade-offs associated with dormancy. Moreover, changes in the environmental cues that control dormancy may result in the rapid loss of dormancy as an adaptive trait, and thus explain the distribution of dormancy among different microbial lineages.

membrane for ATP synthesis[3]. These maintenance energy requirements can be drastically reduced or eliminated when microorganisms enter a dormant state. For example, the metabolic demands for maintenance are approximately three orders of magnitude higher than the demands for survival in a dormant state[44]. Survival energy is still required for repairing damage to macromolecules, however, and it is thought to be critical for the long-term viability of dormant microorganisms[44,45]. Without DNA repair, microbial genomes become degraded via hydrolysis and oxidation, and this damage can prevent the resuscitation of dormant cells[45]. Dormant microorganisms can meet survival and maintenance energy demands through cannibalism[46] or the consumption of endogenous reserves[47], including storage compounds such as glycogen, polyphosphates and polyhydroxyalkanoates[48] (TABLE 1). If microorganisms deplete their internal resource reserves before resuscitation, dormancy leads to death (FIG. 2).

Ultimately, the energy costs of maintenance and survival set limits on the amount of time that a microorganism remains dormant. Because some dormant populations can rapidly respond to sudden changes in their environment, in some cases dormancy may be an inexpensive and short-lived phenomenon[49]. However, there is also evidence that some microorganisms can manage cellular energy demands in ways that allow them to persist in a dormant state for prolonged periods. Reports of decade- or even century-old viable spores are not uncommon. In fact, viable microorganisms ranging from several thousand to several hundred million years old have been recovered from ancient materials, including amber[50], aquatic sediments[51], the deep subsurface[52], permafrost[45] and subterranean salt deposits[53]. The retrieval of these living fossils has attracted attention and generated discussion about a range of topics, including biological longevity, astrobiology and the interpretation of ancient DNA derived from non-microbial taxa[54–56]. In addition, the resurrection of dormant microorganisms from ancient samples provides an opportunity to explore the evolution of genomes and functional traits (BOX 3), and this may yield insight into the structure and function of contemporary communities.

Resuscitation from microbial dormancy — responsive and spontaneous switching. Dormancy can only be an adaptive strategy if dormant cells are resuscitated and able to reproduce. Therefore, the revival of dormant cells and spores has received considerable attention from microbiologists[57]. In many cases, microorganisms exit the dormant state in response to favourable changes in environmental conditions. For example, endospores in natural environments undergo germination in response to an increased availability of resources, typically low-molecular-mass compounds such as amino acids and sugars[58]. When these substrates bind to spore surface receptors, enzymatic activity hydrolyses the peptidoglycan content of the spore cortex, and this is followed by vegetative outgrowth of the cell[58]. Although different mechanisms are involved, non-spore-forming microorganisms are also revived from dormancy in response to changes in environmental conditions. For example, there are many reports that Gram-negative bacteria become dormant and enter a 'viable but non-culturable' (VBNC) state. Cells in the VBNC state experience a loss of culturability when exposed to unfavourable conditions, even though they retain signs of being alive and can be revived when favourable conditions return[18,49]. For example, *Vibrio vulnificus* enters the VBNC state at low temperatures (5 °C), but can resume growth within hours after returning to its optimum temperature (~37 °C)[59].

However, there has been debate about the existence of the VBNC state, and it is not recognized by all microbiologists as a legitimate form of dormancy. First, evidence that non-culturable cells are viable is often indirectly inferred through the use of labelling approaches (including redox indicators and membrane potential probes) that have caveats and limitations[60,61]. Second, it can be difficult to demonstrate that resuscitation from VBNC is not due to the recovery of injured cells or the regrowth of residual cells that never entered a dormant state[60]. Last, the genetic mechanisms underlying the VBNC state have not yet been identified. It seems likely that comparative transcriptional analyses of inactive and active cells will improve our understanding of the VBNC state and other less-well-studied forms of dormancy[24].

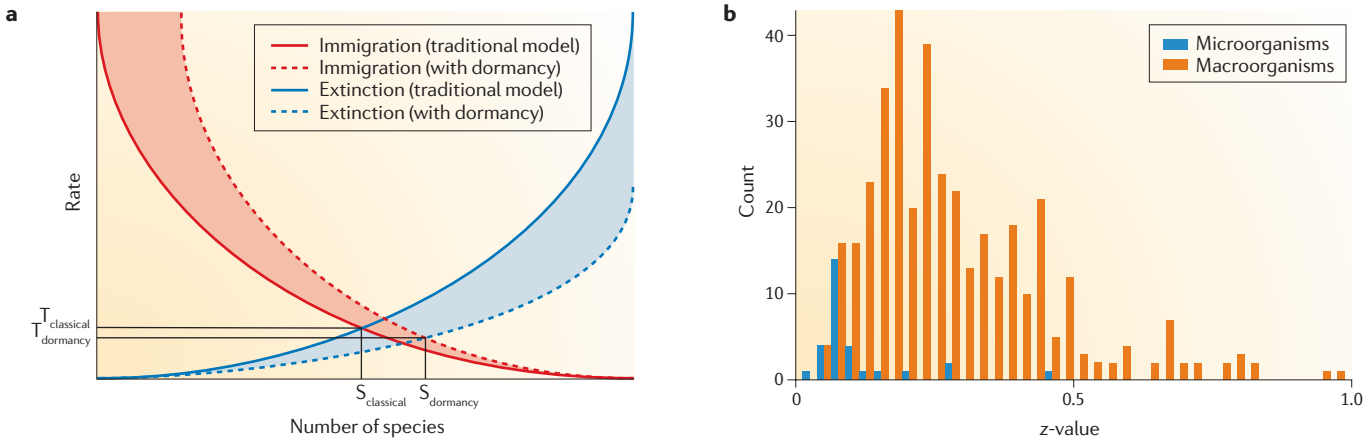


Figure 3 | **Predictions of species diversity and species turnover based on the Theory of Island Biogeography**[69]**.** **a** | Immigration rates increase (red shading) and extinction rates decrease (blue shading) when dormancy is introduced into the traditional model. This results in greater equilibrium species richness ($S_{classical}$ versus $S_{dormancy}$; this is referred to as α-diversity), but lower species turnover ($T_{classical}$ versus $T_{dormancy}$; referred to as β-diversity). **b** | The frequency distribution of *z*-values (the slopes of log(area) versus log(richness) plots describing the rate at which new species are encountered with increasing sampling area) for macroorganisms ($n = 432$) and microorganisms ($n = 21$). *z*-values for macroorganisms are significantly greater than those for microorganisms ($P < 0.0001$). See Supplementary information S3 (table) for additional resources. Part **a** image is modified, with permission, from REF. 74 © (2010) Wiley & Sons.

In some cases, resuscitation of microorganisms from dormancy involves quorum sensing. For example, dormant *Micrococcus luteus* cells were revived after 9 months of starvation by adding filter-sterilized supernatants from fresh cultures in logarithmic growth phase[62,63]. This phenomenon led to the discovery that active cells secrete a growth factor — or cytokine — that is necessary for resuscitating dormant cells[64]. This resuscitation-promoting factor (Rpf) is a 17 kDa protein with muralytic activity that is thought to remodel the cell envelope of dormant cells to facilitate cell division and regrowth[65]. The *rpf* genes from *Micrococcus luteus* have homologues in other Gram-positive bacteria[64,66], and we were able to detect *rpf* homologues in various ecosystems through the analysis of existing metagenomes (BOX 2). Signalling molecules such as Rpf may be produced after the spontaneous awakening of microorganisms from dormancy. The 'scout hypothesis' suggests that individual cells are randomly resuscitated and experience conditions that are either good or bad for growth. If a revived cell encounters suitable conditions, it may produce quorum sensing compounds (such as Rpf) that resuscitate their dormant kin[67].

Ecological patterns and processes of dormancy

Over the past decade, researchers have made major advances in understanding the structure and function of microbial communities in natural ecosystems. These advances have been achieved, in part, through the integration of new molecular technology and the testing of ecological theory[68]. Equipped with a basic understanding of the cellular mechanisms of dormancy and an appreciation for the state of the art in environmental microbial dormancy, we can now explore how seed banks are likely to influence important phenomena observed in the field of microbial ecology. Here, we highlight five areas of active research that are likely to benefit from the consideration of dormancy and seed bank theory.

Dormancy and biogeography. An important but unresolved issue in microbiology is the extent to which microbial communities are structured by biogeographical processes. It is well documented that the diversity of macroorganisms (such as plants and animals) is influenced by speciation, extinction and immigration[69,70]. It is less clear whether these fundamental biogeographical processes are strong drivers of microbial diversity[71]. Until recently, when considering the spatial distributions of microorganisms, most microbiologists agreed that "everything is everywhere, but the environment selects" (REF. 72). This view stems from the assumption that microbial dispersal is generally unhindered. Because microbial species are small and attain high population densities, they have a high likelihood of being transported considerable distances via wind, water or mobile macroorganisms. However, evidence to the contrary has accumulated, suggesting that dispersal limitation may be important for explaining the spatial patterns of microbial community composition in some ecosystems[71].

As a general microbial trait, dormancy should influence the biogeographical processes that structure microbial communities. This hypothesis can be evaluated by integrating dormancy into the 'Theory of Island Biogeography' (REF. 69). This theory predicts that the equilibrium species richness — the number of species — in a given habitat, such as an island, is a balance between local extinction rates and the immigration rates of new species from distant habitats (FIG. 3a, solid lines). Dormancy should reduce the risk of local extinction through the recruitment of individuals from a microbial seed bank; likewise, dormancy should increase the probability of successful colonization by allowing

immigrants to avoid mortality from initially unfavourable conditions (FIG. 3a, dashed lines). An extreme example of how dormancy could potentially influence immigration success comes from the case of the 'misplaced microorganisms'. Thermophilic bacterial spores are constantly supplied to permanently cold marine sediments[73]; the spores are dormant under ambient conditions (–2 to +4 °C), but when transferred to optimal conditions (50–60 °C), they rapidly transition into a metabolically active state capable of sulphate reduction, organic matter hydrolysis and fermentation. This study lends support to the notion that dormancy may contribute to the apparent cosmopolitan distributions of some microbial taxa.

Taken together, the effects of dormancy on immigration and extinction should increase the expected number of species in a given habitat (that is, species richness, or α-diversity) but decrease the diversity that is observed when sampling across a landscape (that is, species turnover, or β-diversity)[74] (FIG. 3a). One way in which β-diversity can be assessed is through the construction of species–area relationships or, in the case of microorganisms, taxa–area relationships[75]. The relationship between species richness (S) and geographic area (A) is commonly described with the power function: $S = cA^z$, where c and z are constants. When S and A are plotted in log–log space, z is the slope, which is often used to quantify the rate at which new species are encountered with increasing sampling area. In a comparison of microorganisms and macroorganisms in salt marsh ecosystems, the z-values for plant species were 2.5 times those for bacteria[75]. As expected, bacterial z-values were larger when microbial taxa were defined using stricter species definitions (such as increasing the required gene sequence similarity from 97% to 99%), but this could not account for the observed differences in β-diversity between bacteria and plants within the salt marsh system[75]. A more extensive analysis confirms that microorganisms have lower z-values than macroorganisms (FIG. 3b). It has been hypothesized that such low levels of spatial β-diversity observed for microbial communities may be due to high dispersal, low habitat specificity or high rates of lateral gene transfer[75]. We suggest that dormancy-generated microbial seed banks should also be considered when examining the biogeography of microorganisms.

Outbreaks, blooms and succession. A major goal of microbiology is to understand and predict microbial population and community dynamics in complex environments such as the open ocean, wastewater treatment plants or host-associated ecosystems. Microbial growth in these habitats is characterized by boom and bust population cycles owing to fluctuations in environmental variables, rapid depletion of growth-limiting resources, and interspecific interactions such as competition and predation[76,77]. Dormancy should be advantageous under such variable conditions[32], but may also directly contribute to the population and community dynamics observed in natural systems[10]. For example, many disease-causing organisms (including *Mycobacterium tuberculosis*) maintain low population densities and avoid detection by host immune systems when they are dormant. These latent infections can become active infections through shifts in host conditions (such as nutrition or stress) that lead to microbial resuscitation and subsequent outgrowth[13]. Similar dynamics are common in environmental systems with species that are capable of dormancy. For example, blooms of dinoflagellate species that are responsible for toxic red tides in marine ecosystems have been linked to changes in environmental conditions that trigger germination of a microbial seed bank[78]. Dormancy is also important for understanding ecological succession. It has long been known that dormant seeds are critical for plant successional dynamics[79], and more recent studies have speculated that dormant bacteria may be important for understanding microbial succession[80]. In addition, it is well established that seed banks play a key role in the recovery of terrestrial plant communities following disturbance. Dormant seeds germinate when environmental conditions (for example, light availability) change following perturbation events (such as a fire), but these pioneer taxa are typically replaced over time by competitively dominant species[11,81]. Recent studies have demonstrated similar patterns of microbial succession in a diverse set of ecosystems[82,83]. These repeatable patterns of community assembly are consistent with predictions of seed bank theory[10], but direct evidence linking microbial community dynamics and dormancy is currently lacking.

Diversity maintenance and the rare biosphere. One of the most familiar patterns in biodiversity research is that only a few species are common, whereas most species are rare. This phenomenon can be visualized in a rank abundance curve (FIG. 4), which portrays the total number of species (richness) and their relative abundances (evenness) in a sample or community. Over the past decade, new molecular techniques have allowed microbiologists to construct rank abundance curves for a range of microbial communities. Not surprisingly, microorganisms exhibit rank abundance curves that are qualitatively similar to those of larger organisms, with one notable exception: rank abundance curves for bacteria, viruses, archaea and micro-eukaryotes tend to have extremely long tails[84–87]. The observation that microbial communities are dominated by low-abundance taxa has led to the rare-biosphere concept. These observations raise questions about the maintenance of rare taxa and how they contribute to the functioning of microbially dominated ecosystems. For example, theory predicts that rare species may be at risk of extinction[88]. It is possible, however, that rare microorganisms represent a reservoir of genetic diversity that is capable of responding rapidly to environmental change[85,89]. These contrasting ideas were recently tested by constructing rank abundance curves for bacteria from freshwater lakes, using 16S rDNA and rRNA sequences. Based on the relative recovery of rDNA and rRNA, rare taxa had a higher probability of being active than common taxa[22]. This pattern could arise for a number of reasons. For example, it is possible that rare taxa are disproportionately active relative to common taxa because rare taxa are comprised of populations that lack the capacity to enter and exit dormancy.

Succession
An ecological phenomenon characterized by predictable changes in community composition over time owing to variation in the colonization potentials and competitive abilities of species and in their responses to disturbances.

Rare biosphere
A concept describing the observation that a very large proportion of the taxa in microbial communities are extremely uncommon.

Alternatively, microbial rank abundance curves may be more dynamic than previously thought, possibly being characterized by transitions between active and dormant states that could ultimately influence the relative abundance of microbial taxa (FIG. 4). In either case, the rare biosphere seems to be metabolically active and potentially important when attempting to make links between the structure and function of microbial communities.

Cultivating the uncultured majority. A major obstacle in microbiology is our inability to cultivate the vast majority of microbial species found in environmental samples. This problem has been dubbed The Great Plate Count Anomaly and reflects the fact that traditional cultivation techniques underestimate microbial abundance and diversity by multiple orders of magnitude[17]. For example, we currently lack cultured representatives for ~70% of all recognized bacterial phyla[90]. A common explanation for this low cultivation success is that in the laboratory we often fail to recreate the environmental conditions that are required by most microbial taxa for growth[91]. For example, many oligotrophic, slow-growing bacteria are unable to grow on traditional media because substrate concentrations are often too high[92]. Novel cultivation strategies have shown that the recovery of microbial taxa can be enhanced by experimenting with enrichment conditions that more realistically simulate environmental conditions[93,94]. Dormancy is another, possibly related, factor that may explain why many microorganisms escape cultivation[17,67]. For example, some bacteria enter a dormant state during cultivation in response to the production of oxygen free radicals[95]. Evidence suggests that the reduction of environmental stress can lead to increased culturability of dormant taxa. Cryptic ciliates were resuscitated from the seed bank of a hypersaline lagoon by reducing the salt concentrations of the enrichment conditions[96]. As mentioned above, the resuscitation of microorganisms from dormancy often involves the production and recognition of signalling molecules. Therefore, it is not surprising that the culturability of diverse microbial taxa from environmental samples can be improved by adding compounds that are involved in quorum sensing and signal transduction, including *N*-acyl-homoserine lactones and cyclic AMP[91,94,97]. Thus, by explicitly considering the mechanisms that control dormancy, we suggest that microbiologists may be able to increase the culturability of under-represented taxa and gain a better appreciation for the metabolic potentials of complex microbial communities.

Stability of microbial communities and ecosystem services. Microorganisms have a crucial role in the regulation of ecosystem processes such as contaminant degradation, nutrient cycling and carbon sequestration. Therefore, a major goal of microbial ecology is to understand how microbial communities respond to natural and anthropogenic perturbations. Often, the stability of microbially mediated ecosystem processes is attributed to the high levels of biodiversity that are typical of microbial communities[98–101]. Ecological theory predicts that biodiversity promotes the stability of ecosystem processes via functional redundancy, a condition that arises when there is overlap in the metabolic capacities of different species[102–104]. In order for this stabilizing mechanism of biodiversity to operate, however, functionally redundant species must respond differently to at least some environmental conditions[105].

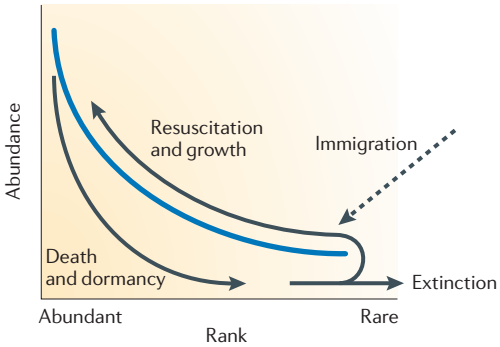


Figure 4 | **A dynamic rank abundance curve for a microbial community that is influenced by dormancy.** In most ecosystems, a small number of microbial species are dominant (abundant), and the remaining species are rare. The relationship between species abundance and the rank order of species in a community is shown by the blue line. Abundance can change over time owing to various factors (black lines). For example, species abundance can decrease because of predation and resource limitation, as well as dormancy, which prevents microorganisms from replicating. However, dormancy can also reduce the likelihood of extinction; after resuscitation, a population may return to a dominant position in the rank abundance curve. In the absence of dormancy, the persistence of a given species is more dependent on immigration (dashed arrow) and the species is more likely to be lost from the local community. Image is modified, with permission, from REF. 89 © (2006) Elsevier.

A recent meta-analysis found that the resistance and resilience of microbial communities to perturbations (including nutrient fertilization, carbon amendments, increased CO_2 levels and elevated temperature) are highly variable[106]. This variability may be attributed in part to the distribution and overlap of functional traits within microbial communities[106]. Dormancy is one trait that may have important implications for understanding the stability of microbial communities. Not only does dormancy allow for the coexistence of functionally redundant species[22], but it also generates seed banks, which should enable the rapid recruitment of species in response to environmental change[107]. Together, these features of dormancy are expected to contribute to compensatory dynamics and the stability of microbial communities[108]. However, because dormancy involves risks and costs, some microbial communities may not be dominated by taxa that are capable of dormancy (BOXES 1,3). When such communities are perturbed, functional redundancy will be determined by the immigration of metabolically similar populations that are able to contend with new environmental conditions. If this process of immigration or subsequent colonization is slow, ecosystem processes may be temporarily depressed and therefore less resilient[109] (FIG. 5). Therefore, differences in

The Great Plate Count Anomaly
The name given to the underestimation of microbial abundance and diversity, owing to the inability of microorganisms from environmental samples to form colonies on agar media under laboratory conditions.

Stability
In an ecological context: the extent to which populations, communities and ecosystems respond to natural and anthropogenic variability.

Compensatory dynamics
A process whereby a decrease in the abundance of one species results in the increase in the abundance of another species; this balancing can be due to competition or to differences in environmental optima, and can stabilize the functions of ecological communities.

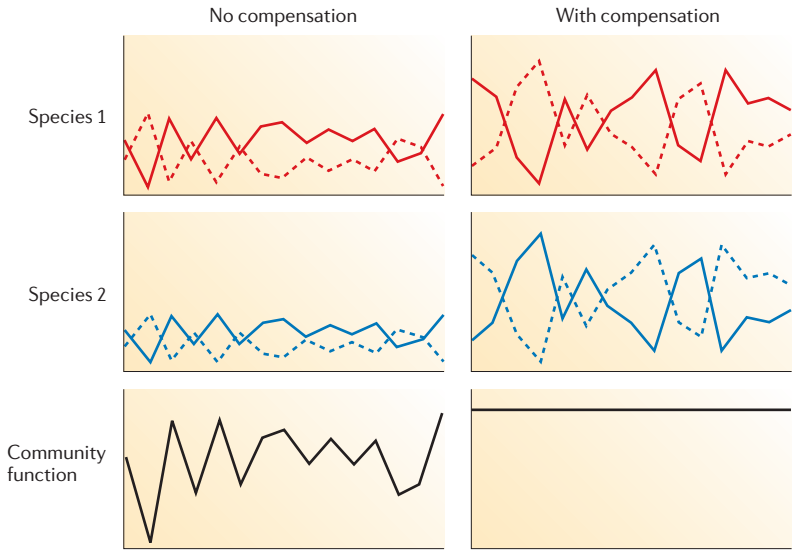


Figure 5 | **Microbial dormancy fosters stable community function in a simple two-species community via compensatory dynamics.** Graphs indicate the density of dormant (dashed) and active (solid) individuals for two species. Species in the left column possess similar responses to environmental cues, resulting in non-compensatory dynamics and unstable community function. The right column depicts species with contrasting environmental responses, driving compensatory dynamics and stable community function. Image is modified, with permission, from REF. 105 © (1999) Wiley & Sons.

the dormancy potential among ecosystems (BOXES 1,2) may help to better predict the stability of microbial communities.

Conclusion

Dormant cells are common, and sometimes dominant, in a range of ecosystems (BOX 1). The genetic underpinnings of dormancy are well characterized for a select group of microorganisms, and our metagenomic results suggest that some of these mechanisms are common in complex microbial communities (BOX 2). With recent advances in molecular biology and cultivation techniques, it is likely that new strategies of dormancy will be unveiled for many more microbial taxa. From an ecological perspective, existing theory and empirical data support the view that dormancy affects community composition and that this trait contributes to the maintenance of microbial diversity. In addition, dormancy may help explain various ecological phenomena that have been observed for microbial communities, including bloom dynamics, biogeographical patterns and dynamic responses to perturbations. Therefore, by considering the concept of dormancy and seed banks, we may be more successful in linking the structure and function of microbial communities in natural and managed ecosystems.

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Competing interests statement
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