

MINI REVIEW

Where less may be more: how the rare biosphere pulls ecosystems strings

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Rare species are increasingly recognized as crucial, yet vulnerable components of Earth's ecosystems. This is also true for microbial communities, which are typically composed of a high number of relatively rare species. Recent studies have demonstrated that rare species can have an over-proportional role in biogeochemical cycles and may be a hidden driver of microbiome function. In this review, we provide an ecological overview of the rare microbial biosphere, including causes of rarity and the impacts of rare species on ecosystem functioning. We discuss how rare species can have a preponderant role for local biodiversity and species turnover with rarity potentially bound to phylogenetically conserved features. Rare microbes may therefore be overlooked keystone species regulating the functioning of host-associated, terrestrial and aquatic environments. We conclude this review with recommendations to guide scientists interested in investigating this rapidly emerging research area.

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Introduction

Microbial communities typically show a skewed species abundance distribution, with relatively few dominant species co-existing alongside a high number

of rare species (Nemergut *et al.*, 2011). Although the importance of rare species is increasingly being recognized for macroorganisms (Lyons *et al.*, 2005; Mouillot *et al.*, 2013; Soliveres *et al.*, 2016), many rare microbial taxa (for example, singletons) are routinely removed from data sets, thereby systemically overlooking a substantial part of the biosphere. Most of our knowledge is still based on dominant species, despite the increasing attention for the rare microbial biosphere (Pedrós-Alió, 2007; Reid and Buckley, 2011; Lynch and Neufeld, 2015). In this review, we place rare microbes in

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an interdisciplinary spotlight and discuss their relevance across a range of current hot topics in microbial ecology, including microbiome assembly and function, and biogeochemical cycling. We present patterns and consequences of rarity and link these to existing and emerging theoretical frameworks.

Microscopic organisms, both eukaryotic and prokaryotic, display a huge biodiversity (Locey and Lennon, 2016). Microbial communities drive Earth's biogeochemical cycles (Falkowski *et al.*, 2008) and are essential for animal and plant survival (Russell *et al.*, 2014). An estimated 1.5–28% of all microbes are 'conditionally rare taxa', which are rare in most conditions but become dominant occasionally (Shade *et al.*, 2014). These often-overlooked taxa may be key to understanding community assembly and function (Allan *et al.*, 2011).

The increasing resolution provided by high-throughput sequencing technologies has shed new light on microbial diversity and revealed a diverse collection of microbes occurring at low densities: the 'rare biosphere' (Sogin *et al.*, 2006). As such, the rare biosphere is currently one of the new frontiers of microbial ecology. Recent studies have shown that low-abundance species should be considered full members of microbial communities and not treated as analytical annoyances (Nipperess and Matsen, 2013; McMurdie and Holmes, 2014; Lynch and Neufeld, 2015).

We provide an overview of theories on why species are rare (including community assembly, evolutionary processes and biogeographic patterns), and how low-abundance species can drive ecosystem processes. We also provide evidence for the importance of rare microbes across different habitats, and present

approaches to examine the most pressing questions surrounding them.

Drivers of rarity

Rarity can be defined in several ways, including for instance local abundance, habitat specificity and geographical spread (Rabinowitz, 1981). These concepts are routinely applied to plants and animals, and can be easily transferred to microbial communities. Local abundance is the easiest and the most common index used to quantify species' rarity in microbial ecology. However, microbial rarity may also be expressed as a restriction to a low number of habitats, thereby reflecting habitat specificity (Barberán *et al.*, 2014) and geographic range (Tedersoo *et al.*, 2014).

Rarity can result from stochastic processes, inherent trade-offs in life-history strategies, and biotic and abiotic interactions (Figure 1). Species rarity can for instance emerge simply by stochastic population fluctuation (Ai *et al.*, 2013), without implying any specific physiological characteristics. Rarity is also an element of community assembly processes as a recently immigrated species is necessarily rare when it first enters a new community. External abiotic and biotic factors can have pivotal roles in species rarity. For instance, a highly specialized species showing a very narrow environmental niche may be abundant in a few habitats but remain rare in most others (Figure 1a). Local rarity may also be the result of fitness trade-offs, for instance, when stress resistance comes at the cost of a lower growth rate. Slow-growing species might not reach a high density, but may persist well under stressful conditions (Gudelj *et al.*, 2010). An extreme case is dormancy, in which microbes

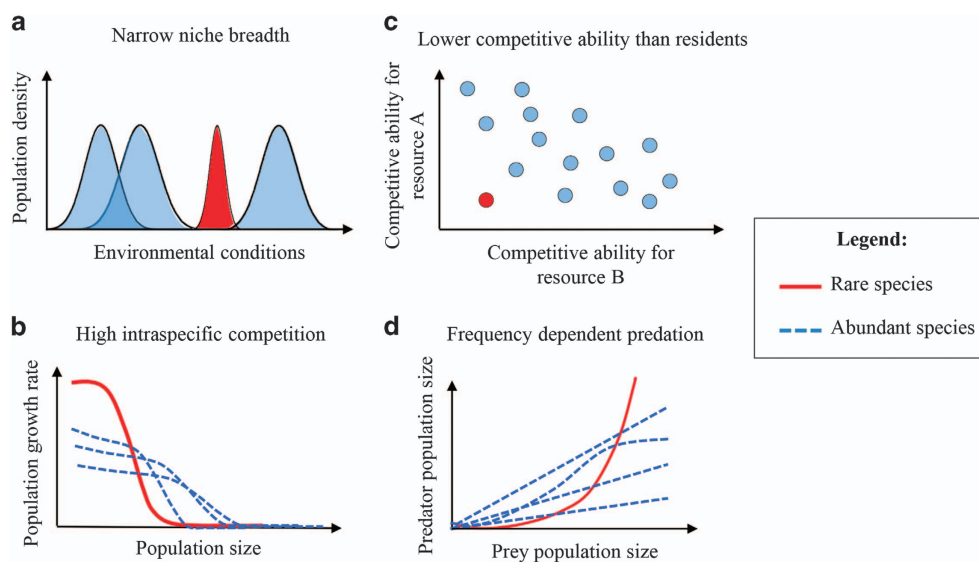


Figure 1 Potential mechanisms that can drive local rarity. Rarity can be linked to two types of mechanisms: (1) mechanisms related to species characteristics (a, b), and (2) mechanisms related to local biotic and abiotic conditions (c, d). Drivers of rarity include (a) narrow niche breadth, (b) high intraspecific competition, (c) low competitive abilities and (d) frequency-dependent predation. In all panels, rare species are indicated in red, while abundant species are indicated in blue.

completely stop growth but gain greatly in stress resistance (Gobet *et al.*, 2012). Many microbes can remain generally inactive and at low density most of the time, only becoming dominant when more favorable conditions arise (Aanderud *et al.*, 2015). Biotic interactions also have an important role in explaining rarity. An uncompetitive species may often remain rare rather than going extinct. Rare persistence has been demonstrated for microbes that are sensitive to antibiotics produced by competitors (Narisawa *et al.*, 2008), unable to build protective structures such as biofilms (Schluter *et al.*, 2015) or incapable of using crucial resources (García-Fernández *et al.*, 2004). The dependance of one species on another may lead to negative frequency dependency, where the fitness of the dependent phenotype decreases when it becomes more abundant. A well described example involves social cheaters depending on public goods produced by other species (Kummerli and Brown, 2010; Jousset *et al.*, 2013). Cheats may be highly successful while rare, but their competitive advantage rapidly dwindles as they spread (Figure 1b). The strength of frequency-dependent selection may vary with environmental factors such as predators (Meyer and Kassen, 2007): the steeper the slope of the frequency-dependent fitness, the more persistent a rare species will be, but at the cost of remaining rare (Yenni *et al.*, 2012).

Bacteriophages and protists often show frequency-dependent predation and tend to over-consume abundant prey species (Jousset *et al.*, 2009; Winter *et al.*, 2010), preventing them from dominating the community and creating space for rare species (Rodríguez-Valera *et al.*, 2009; Figure 1d). Finally, rarity may be driven by changes in abiotic conditions, as rare species are more sensitive to environmental fluctuations and more prone to extinction (Gaston, 2008). Current abrupt changes linked to human activity, such as global change and intensive land use, may thus have a deep impact on the rare biosphere and associated ecosystem functions (Rodrigues *et al.*, 2013). Monitoring changes in relative abundances or activities of rare microbes might then serve as an early warning system of environmental change (Chambers *et al.*, 2016).

Ecological relevance of rare species

Rare species are increasingly recognized as drivers of key functions in terrestrial and aquatic ecosystems, as well as host-associated microbiomes (Figure 2; Table 1). Their functional importance may be due to effects that are disproportionately large given their abundance or via the provision of insurance effects. This may seem counter-intuitive given the expected high functional redundancy in microbial communities (Rousk *et al.*, 2009): with many species sharing similar functions, rare species should not be necessary for maintaining function. Species that are considered functionally non-relevant under a given environmental condition may become important

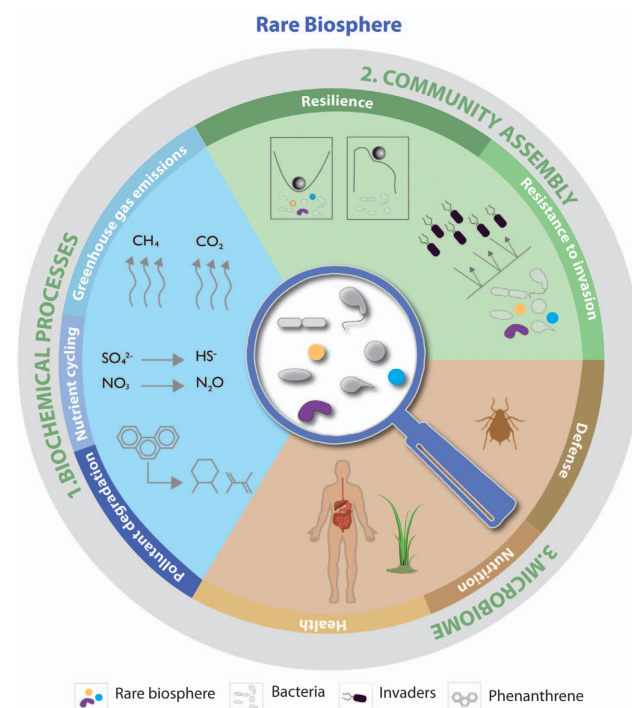


Figure 2 Overview of ecosystem functions supported by rare species. Rare species can drive key processes in geochemical cycles and are major players in nutrient cycling greenhouse gas emissions and pollutant degradation. They can affect community assembly by preventing the invasion of new species, and stabilize community function in fluctuating environments. Further, rare microbes are essential players of host-associated microbiomes in plants and animals by preventing pathogen establishment and stimulating host immunity. References are given in Table 1.

under changing conditions by providing necessary traits or acting as partners in new interspecific interactions (Shade *et al.*, 2014; Fetzer *et al.*, 2015). We propose that rare species offer a pool of genetic resources that may be activated under the appropriate conditions. This may provide insurance effects (Yachi and Loreau, 1999), as at least one species will perform a given process under a given environmental condition. Different processes vary in their sensitivity to the loss of rare species (Peter *et al.*, 2011) with specialized functions, like pollutant degradation (Dell'Anno *et al.*, 2012), particularly affected.

Research on the ecological importance of rare species is still in its infancy, but empirical evidence that the rare biosphere is involved in many microbial-driven processes is accumulating (Figure 2). Below, we highlight three areas in which rare microbes could be particularly influential.

Biochemical processes—nutrient cycling and pollutant degradation

Some nutrient cycling processes provide illustrative typical examples of the disproportionate effects of rare microbes. Low-abundance green and purple sulfur bacteria were found to be highly active

Table 1 References for processes or functions which are supported by rare microbes

Process or function	References
<i>Biochemical processes</i>	
Sulfate reduction	Pester <i>et al.</i> , 2010 Loy and Pester, 2010 Steger <i>et al.</i> , 2011
Methane consumption	Bodelier <i>et al.</i> , 2013
Carbon cycling	
Substrate use	Franklin <i>et al.</i> , 2001 Matos <i>et al.</i> , 2005 Hernandez-Raquet <i>et al.</i> , 2013 Mallon <i>et al.</i> , 2015
Straw decomposition	Griffiths <i>et al.</i> , 2001 Baumann <i>et al.</i> , 2013
Nitrogen cycling	
Nitrification	Griffiths <i>et al.</i> , 2004
Nitrogen uptake	Musat <i>et al.</i> , 2008
Denitrification	Philippot <i>et al.</i> , 2013
Fixation	Hua <i>et al.</i> , 2015
Pollutant degradation	
2,4,6-trichlorophenol	Sanchez <i>et al.</i> , 2004 Dell'Anno <i>et al.</i> , 2012
Petroleum hydrocarbons	Giebler <i>et al.</i> , 2013
Phenanthrene mineralization	Hernandez-Raquet <i>et al.</i> , 2013
Triclosan, microcystin-LR	Delgado-Baquerizo <i>et al.</i> , 2016
<i>Community assembly</i>	
Resistance to invasion	Yan <i>et al.</i> , 2015 Garland <i>et al.</i> , 1999 Matos <i>et al.</i> , 2005 Van Elsas <i>et al.</i> , 2012 Vivant <i>et al.</i> , 2013 Mallon <i>et al.</i> , 2015
Resilience	Griffiths <i>et al.</i> , 2004 Tardy <i>et al.</i> , 2014
<i>Microbiome</i>	
Human health	
Lung infection	Van der Gast <i>et al.</i> , 2011
Periodontal disease	Hajishengallis <i>et al.</i> , 2011
Gut microbiota	Stecher <i>et al.</i> , 2013
Plant health	
Plant biomass, herbivores	Hol <i>et al.</i> , 2010
Plant biomass	Hol <i>et al.</i> , 2015a
Plant pathogens	Hol <i>et al.</i> , 2015b

Background information for Figure 2.

keystone species in freshwater and crucial for nitrogen and carbon uptake (Musat *et al.*, 2008). Similarly, Pester *et al.* (2010) found that the most important sulfate reducer in peatland was a rare bacterium with only 0.006% relative 16 S rRNA gene abundance. Denitrification also likely relies on rare species. A decrease of 75% of the measured species richness reduced soil denitrifying activity by a factor of 4–5 fold (Philippot *et al.*, 2013), suggesting that dominant species cannot carry out this process alone.

Microbial communities play a key role in the degradation of organic compounds, including pollutants and the insurance provided by rare species can therefore contribute to ecosystem resilience to anthropogenic pollution. Organic pollutant degradation involves complex metabolic pathways shared across different species (Fuentes *et al.*, 2014).

Removal of rare microbes in activated sludge and freshwater greatly reduced the capacity to degrade pollutants and toxins (Dell'Anno *et al.*, 2012; Hernandez-Raquet *et al.*, 2013; Delgado-Baquerizo *et al.*, 2016). Rare species probably offer the required gene pool to catalyze complex degradation processes, a hypothesis supported by observations that pollutants are often degraded by species falling below the detection limit in pristine samples (Giebler *et al.*, 2013).

The role of rare species in the breakdown of organic matter is far less clear. Some studies found no consistent relationship between bacterial diversity and litter decomposition, concluding that a low number of species sufficed for efficient decomposition (Griffiths *et al.*, 2001; Franklin and Mills, 2006). Other studies, however, highlighted that rare species may speed up decomposition (Salonius, 1981), especially the degradation of recalcitrant organic matter such as chitin and cellulose (Peter *et al.*, 2011; Jimenez *et al.*, 2014).

Community assembly

Experimental removal of rare species resulted in an increased establishment of new species (Van Elsas *et al.*, 2012; Vivant *et al.*, 2013), suggesting that rare species occupy a key niche and slow down invasive species establishment. A recent study demonstrated that rare species are vital in controlling invasions into soil communities by unwanted (for example, pathogenic) microbes (Mallon *et al.*, 2015), suggesting that rare microbes may play an important role in biotechnological applications such as hygiene or crop protection.

Microbiome—host health

Rare species have been detected within the microbiomes of diverse hosts, from the rhizosphere (Nuccio *et al.*, 2016) to human lungs (Guss *et al.*, 2011), and evidence suggests that they can be critical to microbiome functionality and thus host health. Low-abundance plant-associated microbes are for instance involved in the production of antagonistic volatile compounds that protect the host plant against pathogens (Hol *et al.*, 2015b). Further, removing rare species from an agricultural soil led to a higher plant biomass, albeit at the cost of a reduction of defense compounds against above-ground herbivores (Hol *et al.*, 2010), suggesting that rare microbes can fine-tune the balance between host growth and defense.

In the human lung, a high diversity of low-abundance bacteria is associated with a reduced severity of bacterial infection in individuals with cystic fibrosis (van der Gast *et al.*, 2011). Conversely, rare species may contribute to pathogenesis, as shown by the initiation of periodontal disease by low-abundance species that trigger changes in the oral microflora (Hajishengallis *et al.*, 2011). Several

studies have further identified low-abundance pathogens in clinically relevant samples (Guss *et al.*, 2011; Bittinger *et al.*, 2014).

Whilst rare species have traditionally received little attention from a clinical or biotechnological perspective, a better understanding of their ecology, and impact on microbiome function, may provide new tools to predict, and enhance, health effects of host-associated microbiomes.

Mechanisms behind disproportional effects of rare microbes

Here, we discuss three, non-mutually exclusive, mechanisms that may explain the high importance of rare microbes for ecosystem functioning: enhanced species activity, increased community functional diversity and community-wide species interactions.

Rare microbes can be more active than the abundant ones

In marine ecosystems, conditionally rare bacterial taxa tend to be more active when rare (Campbell *et al.*, 2011). In fluctuating conditions, a rare taxon could be disproportionately active due to continuous regrowth. However, growth may not necessarily be responsible for this increased activity; *Desulfosporosinus* species, for instance, remain at low abundance despite increasing their ribosome content under activating conditions (Hausmann *et al.*, 2016). A similar observation was reported for protists in lakes, where some species were always rare and yet highly active (Debroas *et al.*, 2015). These examples utilized rRNA:rDNA ratios as a proxy for activity. Similar results have been observed using respiration as a proxy for bacterial activity. Rare species appear to sustain community activity; similar sized communities with rare microbes were shown to have higher respiration than communities with fewer rare microbes (Dimitriu *et al.*, 2010). Thus, rare yet highly active microbes can contribute more to ecosystem functioning than expected based on their abundance, akin to some macroorganisms, such as rare but active predators or pollinators (Herrera, 1989).

Rare species represent a vast functional gene pool

Collectively, rare microbes represent a huge genetic pool and, as such, contribute to the metabolic potential of the community. Many microbial species are auxotrophs, that is, they need to obtain certain vitamins or amino acids from other organisms in their environment (Helliwell *et al.*, 2013). Secretion of such compounds as metabolic waste by other microbes can greatly improve overall community function (Mee *et al.*, 2014). Further, metabolism of complex products requires a large set of enzymes, which are often not present in one single organism. Potentially toxic by-products may thus accumulate if

not further degraded by other organisms (Haruta *et al.*, 2009). Dimitriu *et al.* (2010) measured activities of several enzymes involved in the degradation of organic material and concluded that more diverse bacterial communities harbor a greater set of functions due to the functional differences between species. They suggest that rare species are likely to be functionally dissimilar from abundant ones and therefore are likely to offer complementary, or unique, metabolic pathways to support community function. This high functional diversity of rare species can also be found in macroorganisms, for example, Amazonian fish, rainforest trees and tropical birds (Leitão *et al.*, 2016).

Rare microbes enhance functionality of abundant microbes

Microbial physiology can be strongly influenced by the presence of other microbial species (Garbeva *et al.*, 2011). Accordingly, the presence of rare microbes could induce metabolic responses in more abundant microbes, implying that rare microbes have indirect effects on ecosystem functioning. A comparison of communities containing and not containing rare species showed that bacterial communities including rare species strongly reduced fungal growth via production of antifungal volatiles (Hol *et al.*, 2015b), suggesting that rare bacteria either produce or trigger dominant bacteria to synthesize these compounds. Similarly, for mammals the presence of a single predator can impact the behavior and physiology of a herd of prey. Another example of rare microbes influencing abundant microbes stems from the work of Low-Décarie *et al.* (2015), who showed that rare microbes rescued whole communities from lethal stress, possibly via horizontal gene transfer. Horizontal transfer of genetic material can also be found in animals and plants (Panaud, 2016), but occurs more frequently between microbes and probably has a larger role in microbial communities.

Experimental and technological approaches

Rare microbe research is a rapidly emerging field. Given the obstacles to exploring rare microbes and the rapid advances in experimental approaches, we provide a condensed summary of methods and strategies that can be employed to address the most pressing questions in this field (Table 2, see also Reid and Buckley, 2011; Lynch and Neufeld, 2015).

Synthetic communities are a powerful approach to test potential causes of rarity, such as order of arrival (Fukami and Morin, 2003), or density-dependent microbial interactions. Studies that manipulate species' abundances can directly examine the impact of rare microbial species on community functionality. The use of flow cytometry, selecting for small cells before cultivation, could increase the likelihood

Table 2 Overview of the research questions and general approaches in the study of rare microbes

Research questions	Method	Critical issues	Options	References
<i>Synthetic communities</i>				
Order of arrival	Vary order of arrival to test priority effects	Cultivation dependent	Selective/spatially structured media	Fukami and Morin, 2003
Density-dependent effects	Vary abundances to test effects of rare species	Cultivation dependent	Cell separation via microfluidic or flow cytometry	Zhang <i>et al.</i> , 2009
<i>Manipulation of natural communities</i>				
Removal	Dilution-to-extinction	Equal biomass in all treatments	Incubation period for recovery of biomass	Philippot <i>et al.</i> , 2013; Mallon <i>et al.</i> , 2015; Hol <i>et al.</i> , 2015a,b; Delgado-Baquerizo <i>et al.</i> , 2016
Enrichment	Salinity, dry-rewet, predation, pollution, nutrient amendments	Molecular methods for composition (DNA) and activity (RNA)	DNA normalization; improve coverage rare biosphere via single-cell genomics	Giebler <i>et al.</i> , 2013; Aanderud <i>et al.</i> , 2015
<i>In situ</i>				
Microbial population dynamics	Time series	Availability of data sets	Increase sampling	Shade <i>et al.</i> , 2014
Genome recovery of rare species; predict metabolic pathways	Single-cell genomics	Selection of target	Labeling via FISH	Podar <i>et al.</i> , 2007; Freilich <i>et al.</i> , 2011
Function of rare species	SIP; Nano-SIMS	Low throughput	Combine with single-cell analysis	Musat <i>et al.</i> , 2008; Pester <i>et al.</i> , 2010

Abbreviations: FISH, fluorescence *in situ* hybridization; SIMS, secondary ion mass spectrometry; SIP, stable isotope probing.

of obtaining rare phyla and candidate divisions (Portillo *et al.*, 2013; Brown *et al.*, 2015), essential to examine their role in the community.

The relevance of rare microbes can also be assessed by removal and enrichment experiments. Manipulating environmental conditions can enrich specific groups and help reveal the role of rare taxa. For example, soil spiked with alkanes harbor several previously undetectable alkane-degrading taxa (Giebler *et al.*, 2013), suggesting that temporarily rare species have important, specific functions.

Both removal and enrichment studies can be combined with molecular methods to assess community composition and relative activity of each taxon. Although molecular methods have undergone tremendous improvement in the recent years, one should still keep in mind that estimates of relative abundances can be inaccurate due to primer bias and limited genome coverage. Overcoming primer bias (Schadt and Rosling, 2015) through DNA normalization methods (Gagic *et al.*, 2015) and increasing coverage of genomes from candidate phyla (Brown *et al.*, 2015) will improve our insight into the rare biosphere. To date genome reconstructions from metagenomics studies have been limited to only the most dominant microbes, but with increasing coverage and sequencing power, we are starting to make roads into recovery of genomic information from rarer members of microbial communities. Single-cell genomics (Hedlund *et al.*, 2014) and reconstructed genomes (Brown *et al.*, 2015; Youssef *et al.*, 2015) can provide valuable insights regarding the metabolic capabilities, physiological preferences, genomic architecture and ecological roles of candidate phyla. This will enable predictions of metabolic pathways and inference of competitive interactions (Freilich *et al.*, 2011) and reveal whether rare species play a role as waste product consumers or as facilitators of growth for the remaining community. Such predictions can subsequently be tested using *in situ* studies, for instance via stable isotope probing to identify microbes actively processing labeled compounds (Chen and Murrell, 2010). This approach may help explore the role of rare microbes for important ecosystem processes (Pester *et al.*, 2010; Aanderud *et al.*, 2015). Coupling of stable isotope probing with high-resolution analytic methods, for example, nano-scale secondary ion mass spectrometry facilitates analysis of the metabolic products of individual cells from cultures or complex microbial communities. Nano-scale secondary ion mass spectrometry can also be combined with *in situ* hybridization to link phylogeny and metabolic function in single cells (Li *et al.*, 2008; Musat *et al.*, 2008). This technology may offer unique insights into the ecological functions of rare microbes in both experimental set-ups and natural environments (Musat *et al.*, 2008; Hua *et al.*, 2015).

Conclusion

Rare microbes may represent the hidden backbone of microbial communities, and our aim was therefore to increase awareness of and stimulate research on these rare ecosystem players. Rare taxa may vary in their ecological relevance and methods are becoming increasingly available to (1) assess relatively active taxa, (2) isolate them and (3) empirically determine their influence on community functioning. We summarized how rare microbes are important for several ecosystem processes and discussed the mechanisms by which these disproportional effects may become manifest. Future studies should further investigate the life-history traits that enable such widespread yet low-abundance existence. It may be important to determine the fraction of rare microbes that are active and dormant in order to predict functional consequences of rare species loss. We propose that including rare microbes in future investigations will improve our understanding of microbial community functioning, and help explain the buffering capacities of ecosystem against environmental change.

Conflict of Interest

The authors declare no conflict of interest.

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Author contributions

AJ and WHGH initiated the workshop where the ideas for this synthesis were developed. All participants of the workshop contributed ideas and text. AJ and WHGH compiled the first draft and all authors contributed substantially to revisions.

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