OPINION

Keystone taxa as drivers of microbiome structure and functioning

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Abstract | Microorganisms have a pivotal role in the functioning of ecosystems. Recent studies have shown that microbial communities harbour keystone taxa, which drive community composition and function irrespective of their abundance. In this Opinion article, we propose a definition of keystone taxa in microbial ecology and summarize over 200 microbial keystone taxa that have been identified in soil, plant and marine ecosystems, as well as in the human microbiome. We explore the importance of keystone taxa and keystone guilds for microbiome structure and functioning and discuss the factors that determine their distribution and activities.

The role of microbial communities in ecosystem functioning is unequivocal[1,2], with microorganisms being key drivers of many ecosystem processes, including soil nutrient cycling, plant growth, marine biogeochemical processes and maintenance of human health[3–6]. In recent years, microbial network analysis has been used to visualize co-occurrence among members in communities[3,7–10]. Microbial network analysis enables testing of ecological theories, the assessment of which was once postulated to be a major impediment in microbial ecology[11,12]. The concept of co-occurrence and network thinking in ecology was proposed in 2005 (REF. 13), and since then, microbial ecologists have shown particular interest in network analysis[7,14–19], resulting in a large body of studies demonstrating microbial co-occurrence patterns in a diverse range of soil[7], plant[20] and marine[21] ecosystems, as well as in the human microbiome[22,23] (BOX 1). Reports are also available from the Antarctic ecosystem[24] and Arctic ecosystem[25,26]. In addition to co-occurrence patterns, microbial networks can be used to statistically identify keystone taxa[27].

The tenet of keystone taxa was originally proposed by ecologist Robert T. Paine in 1966. In a classic experiment, he demonstrated that the removal of sea stars (*Pisaster ochraceus*, which is a common predator of mussels) had a dramatic impact on the shoreline ecosystem community and local biodiversity at Makah Bay, Washington, USA[28]. Since the term was first coined, the definition of keystone taxa has followed different lines of thought[29–31]. The definition proposed by Paine in 1969 mainly suggests that keystone taxa are important for community structure and integrity, and their influence is non-redundant[32]. In 1996, a study defined keystone taxa by introducing the concept of 'community importance', which was calculated from proportional biomass and traits[31]. Subsequently, in 2012, other authors[30] presented the evolution of the term keystone taxa in ecology and how its overuse and misuse (for example, keystone mutualist, keystone modifier and reverse keystone) have resulted in considerable confusion about the actual meaning (readers are referred to their critical appraisal for further information on keystone taxa in ecology). Thus, there is no uniformly accepted operational definition of keystone taxa in ecology, especially in microbial ecology. Keystone taxa have also been frequently referred to as 'ecosystem engineers' owing to their large influence in the community[33]. On the basis of the available information, in addition to sea stars, other examples of keystone taxa include the Canadian beaver and African elephant in the animal kingdom and leguminous *Trifolium* in the plant kingdom. In microbial communities, examples of such taxa are now available from a diverse range of environments[24–26,34–61], and their reports are continuously increasing (BOX 1), including *Porphyromonas gingivalis* and *Bacteroides thetaiotaomicron* in the human microbiome[56,62–64]. *B. thetaiotaomicron*, an anaerobic symbiont found in the human intestine, is considered a keystone taxon based on empirical evidence[56,58]. Owing to the complexity of microbial communities, the importance of connectedness and the rapid turnover in both time and space, the definition of keystone taxa for microbiology needs to be adapted from the original concepts proposed in ecology[30–32]. In this Opinion article, we propose the following definition: microbial keystone taxa are highly connected taxa that individually or in a guild exert a considerable influence on microbiome structure and functioning irrespective of their abundance across space and time. These taxa have a unique and crucial role in microbial communities, and their removal can cause a dramatic shift in microbiome structure and functioning.

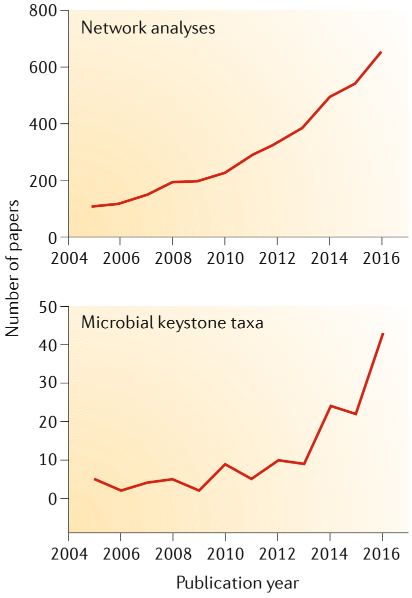
We briefly discuss how microbial network analysis can be used to identify keystone taxa and focus on recent evidence of keystone taxa based on computational inference and empirical evidence. We also discuss challenges in identifying keystone taxa, including the characterization and manipulation of such taxa, and explore their influence on microbiome functioning as well as the factors that may determine their distribution and efficacy.

Microbial networks and keystone taxa

With the advent of next-generation sequencing, millions of sequences are now available from various environments. Network analysis can help disentangle microbial co-abundance and provide comprehensive insight into the microbial community structure and assembly patterns[3,65]. Several algorithms are available to construct microbial networks, and

Box 1 | Microbial co-occurrence and network analysis

The number of papers (based on the Web of Science database) reporting microbial network analyses and keystone taxa is increasing exponentially (see the figure; data are based on the Web of Science database, time span 2004–2016, keywords ‘microbial network analysis’ and ‘microbial keystone’). A wide range of methods and algorithms are available to construct microbial networks. Starting with basic Pearson or Spearman rank correlation-based approaches[7,14], microbial networks quickly evolved to incorporate more robust methods. For example, the maximal information coefficient (MIC) relies on equitability and generality of relationships[15] and can yield a variety of linear and nonlinear associations among microorganisms that can be interpreted similarly to the coefficient of determination (denoted as r^2). Local similarity analysis (LSA) that detects change in abundance of operational taxonomic units (OTUs) over time in an environment is particularly useful for analysing microbial temporal variability data[16], whereas sparse correlation for compositional data (SparCC) is especially suited for compositionally diverse microbial data[17]. Moreover, the ensemble approach (CoNet) can use multiple measures (similarity, correlation and mutual information) with the generalized boosted linear model to generate comprehensive networks[18]. A recent study[19] reported that LSA, MIC and SparCC are well suited for both count and compositional data and are less sensitive to how data are distributed, whereas the CoNet ensemble approach performs better for data with high scatteredness or sparsity. However, it was also noted that different approaches may yield different results and significance levels for the same data set, and although the scores obtained from thousands of pairwise correlations are typically corrected for type I error using a Bonferroni correction or the false discovery rate, extremely rare OTUs or OTUs with a large number of zeros should be avoided for network construction.



these algorithms have been reviewed previously[19,65,66]; thus, for brevity, we only present a brief overview (BOX 1). Perhaps one of the most useful features of network analysis is that ‘hubs’ (also termed keystone operational taxonomic units (OTUs)), which are taxa that are highly associated in a microbiome (FIG. 1), can be identified. Unlike random networks with a Poisson distribution, scale-free or small-world networks with a power-law distribution comprise such hubs (also referred to as highly connected nodes; reviewed in REFS[67,68]). These hubs have been proposed as keystone taxa, as their removal has been computationally shown to cause a drastic shift in the composition and functioning of a microbiome[69,70]. Thus, network analysis can be a powerful tool for inferring keystone taxa from microbial communities. Although high betweenness centrality was previously used to identify keystone taxa statistically in several studies[16,39,40], it was recently shown that high mean degree, high closeness centrality and low betweenness centrality can be collectively used to identify keystone taxa with 85% accuracy[27]. Subsequently, these scores have been used to find putative keystone taxa in microbial networks in recent studies[12,41,42]. The importance of a quantifiable threshold for consistent identification and validation of keystone taxa has been highlighted[30], and we recommend that the combined score of high mean degree, high closeness centrality and low betweenness centrality[27] should be used as a threshold for defining keystone taxa in microbial communities.

Recent evidence of keystone taxa

Computational inference. Numerous studies have used network-based scores to identify putative keystone taxa in various environments (TABLE 1; see Supplementary Table 1). Hubs in microbial networks were identified in grassland soils, and the Pampa and Cerrado biomes in Brazil were shown to harbour different keystone taxa mostly belonging to the Actinobacteria and Proteobacteria phyla[35]. A network analysis at the continental scale showed that bacterial keystone taxa are members of the Alphaproteobacteria class and Actinobacteria phylum, and fungal keystone taxa belong to the Pezizomycotina subdivision[38]. Keystone taxa that were not numerically dominant in the communities have also been identified in the Arctic ecosystem[25,26,44,46] and Antarctic ecosystem[24]. Similar reports of numerically inconspicuous keystone taxa are also available for microbial communities in contaminated soils[47,48], roots[71] and aquatic systems[24,55,72]. Interestingly, our literature review revealed that various members of the Rhizobiales and Burkholderiales orders were consistently identified as keystone taxa in different studies and across different ecosystems (TABLE 1; see Supplementary Table 1). Rhizobiales comprises not only nitrogen-fixing bacteria, such as *Rhizobium* spp. and *Bradyrhizobium* spp., but also members of the genus *Methylobacterium*, which is known to be endosymbiotic and abundant in the phyllosphere[73]. By contrast, Burkholderiales includes important genera such as *Bordetella*, *Ralstonia* and *Oxalobacter*, which are well-known pathogens, as well as *Burkholderia*, which is one of the most versatile and diverse terrestrial microbial groups. The computational identification does not mean that all members of the Rhizobiales and Burholderiales can be considered keystone taxa (for example, many taxa in those orders are subordinate taxa in microbial communities and have no major influence on community composition or functioning). Computational inference of Rhizobiales and Burkholderiales as keystone taxa can also be due to their sheer abundance in various environments. Nonetheless, the likelihood of finding a keystone taxon within these two orders is high, and future studies are now needed to evaluate the role of putative keystone taxa in microbial functions.

Empirical evidence. Human microbiome studies have provided most of the empirical evidence of keystone taxa, linking keystone taxa to a range of processes, including inflammation, colon and gastric cancer, starch degradation and stabilization of the human-associated microbiota[22,23,56–60,64,74–76] (Supplementary Table 1). Perhaps one of the most prominent keystone taxa in humans is *Bacteroides fragilis*, which spurred the alpha-bug or keystone pathogen hypothesis[59,74]. Other examples of keystone taxa in humans include *P. gingivalis*[64], *B. thetaiotaomicron*[56], *Ruminococcus bromii*[60], *Methanobrevibacter smithii*[74] and *Helicobacter pylori*[57]. Both observational and manipulative studies of the gut microbiome show that these taxa can exert considerable control

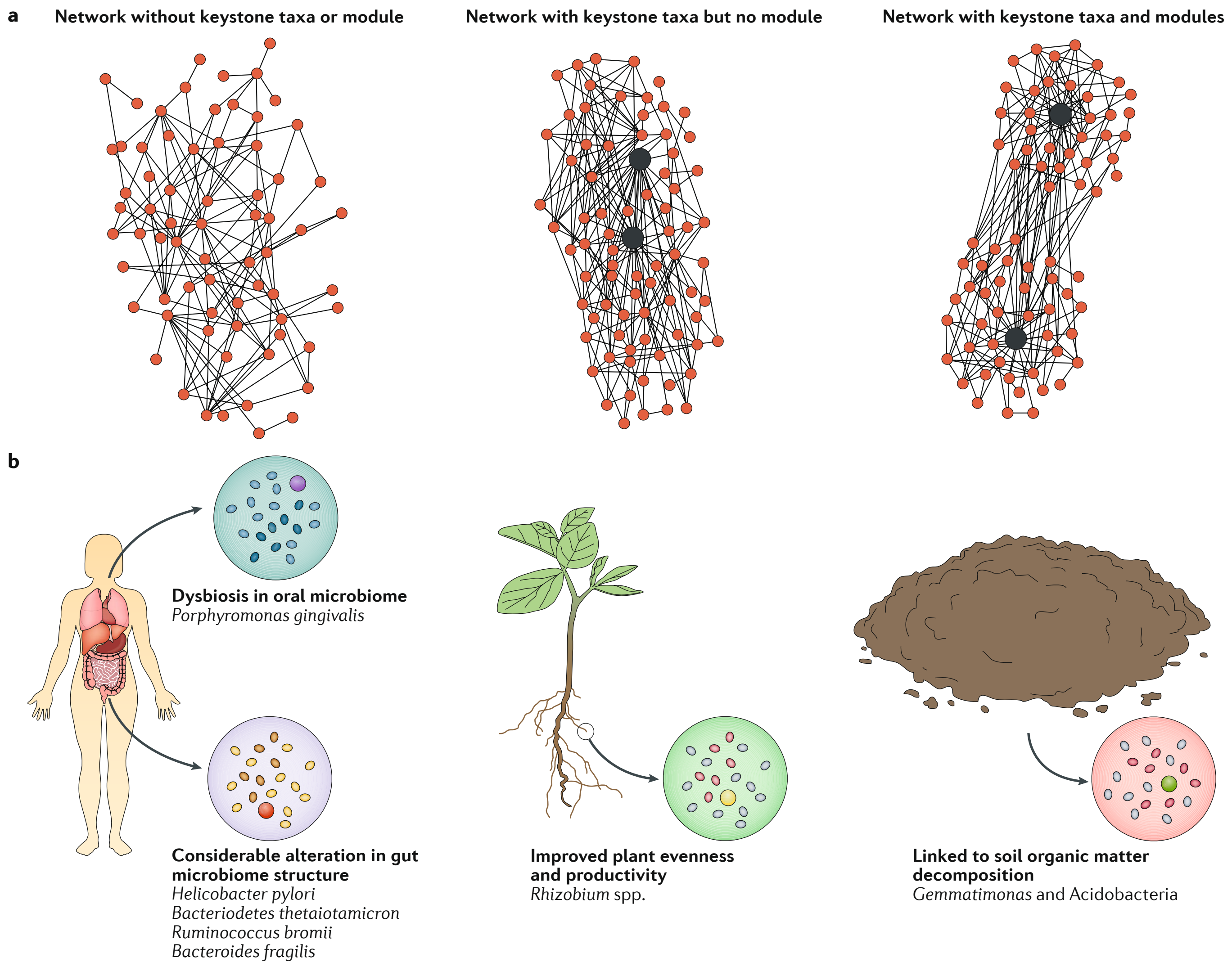


Fig. 1 | **Keystone taxa in the microbiome. a** | Modularity and keystone taxa in microbial networks. Nodes (small red dots) represent operational taxonomic units (OTUs), and solid lines represent edges, that is, relationships among nodes. A network consisting of many taxa (nodes), without any highly interacting keystone taxa (left panel), is similar to a random network that has a Poisson distribution of edges per node, that is, most nodes have similar number of edges and no highly connected nodes. A microbial network without any modules but with keystone taxa (large black dots) is shown in the middle panel. This is a scale-free network that has a power-law distribution of edges, that is, only two nodes are highly connected and holding the network together. A module is a cluster of highly interconnected nodes. A network with two highly connected keystone taxa (large black nodes) that are positioned in two distinct modules is shown in the right panel. These keystone taxa are holding the modules together. Thus, removal of such keystones may cause a dramatic shift in the composition[70]. **b** | Empirical evidence of keystone taxa in the human (left panel), plant (middle panel) and soil (right panel) microbiomes. Smaller dots and ovals represent general members, whereas larger dots represent keystone taxa in the community. In the oral microbiome, *Porphyromonas gingivalis* causes inflammatory tissue destruction and initiates imbalance or dysbiosis of the community that favours further growth of this keystone taxon[74]. Similarly, in the human gut microbiome, keystone taxa, such as *Bacteroides thetaiotaomicron*[56], *Bacteroides fragilis*[59], *Helicobacter pylori*[57] and *Ruminococcus bromii*[60], exert considerable control on microbiome structure and functioning. Nitrogen-fixing rhizobia have been proposed as keystone taxa as their abundance can improve plant productivity and community evenness[77]. In the soil microbiome, bacterial and fungal keystone taxa identified using network scores have been found to be linked to organic matter decomposition[39,41].

on the composition and functioning of the oral and gut microbiome. Examples are also available from plant and soil microbiomes, where keystone taxa have been identified through network-based scores and linked to microbiome functioning and ecosystem processes. The effect of abiotic factors (for example, sampling time and temperature) and host genotypes on phyllosphere microbial communities in *Arabidopsis thaliana* were shown to be mediated via microbial keystone taxa[20]. This not only supports the relevance of keystone taxa but also provides evidence of their importance for plant microbiome functioning. Nitrogen-fixing rhizobia have been proposed as keystone taxa, and their abundance has been shown to greatly improve plant productivity and community evenness[77]. Fungal and bacterial keystone taxa were recently identified that were linked to organic matter decomposition in an agricultural soil[39]. These taxa were also identified as keystones for soil organic matter transformation in another study[41], indicating the importance of similar keystone taxa for specific habitats and processes. Moreover, we predict that important plant symbionts, such as mycorrhizal fungi, function as keystone taxa in view of their role as ecosystem engineers and their impact on microbial

Table 1 | **Summary of studies reporting keystone taxa in different ecosystems**

Ecosystem or habitat	Keystone taxa[a]	Refs
Computational inference		
Grasslands	• Burkholderiales • Sphingobacteriales • Clostridiales • Actinomycetales • Acidobacteria GP4	34–36
Forest or woodlands	• Actinomycetales • Acidobacteria GP4 • Rhizobiales • Burkholderiales • Clostridiales • Sphingobacteriales • Rhodobacterales • Verrucomicrobia	8,35,37,38,61
Agricultural lands	• *Gemmatimonas* • Acidobacteria GP17 • Xanthomonadales • Rhizobiales • Burkholderiales • Solirubrobacterales • Verrucomicrobia	35,40,42,43
Arctic and Antarctic ecosystems	• Rhizobiales • Burkholderiales • Actinobacteria • Alphaproteobacteria	25,26,44,46
Contaminated soil	• Rhizobiales • *Nitrospira* • Pseudomonadales • Actinobacteria	47,48
Plant-associated microbiota	• Acidobacteria GP1, GP3 and GP6 • Rhizobiales • Burkholderiales • Pseudomonadales • Bacteroidetes • Frankiales	40,49,50
Aquatic ecosystems	• *Pelagibacter* • Oceanospirillales • Flavobacteriaceae • *Nitrospira* • Rhodobacteradaceae • Alteromonadaceae • *Chromatium* • Rhizobiales • Burkholderiales • *Chlorobium* • Verrucomicrobia • *Chloracidobacterium* • Chloroflexi • *Candidatus* OP3	24,51–55,72
Empirical evidence		
Agricultural lands[b]	• *Gemmatimonas* • Acidobacteria	39,41
Phyllosphere	• *Albugo* • *Dioszegia*	20
Human oral microbiome	*Porphyromonas gingivalis*	64,74
Human gut microbiome	• *Helicobacter pylori* • *Methanobrevibacter smithii* • Actinobacteria • *Bacteroides fragilis* • *Bacteroides stercoris* • *Bacteroides thetaiotaomicron* • *Ruminococcus bromii* • *Klebsiella pneumoniae* • *Proteus mirabilis*	22,23,56–60,76

[a] Members of Rhizobiales and Burkholderiales are consistently present across ecosystem types, except for the human microbiome. Keystone taxa across individual studies and links between keystone taxa and specific microbial ecosystem processes as reported in relevant studies are shown in Supplementary Table 1. [b] Keystone taxa were initially identified using network-based scores and linked to organic matter decomposition.

communities, plant diversity and ecosystem functioning[78,79]. A recent study found that low abundance keystone taxa that are highly connected in the microbiome can explain microbiome compositional turnover better than all taxa combined[80]. Indeed, such encouraging reports highlight the relevance of keystone taxa for microbiome composition and functioning.

Challenges in identifying keystone taxa

Correlation does not imply causation.

Keystone taxa identified using network-based scores have been linked to ecological processes in many studies (Supplementary Table 1), indicating that this is a suitable approach. However, network-based scores need to be complemented with experimental evidence showing the impact of the keystone taxa on microbiome composition and function. The detection of keystone taxa using network-based scores alone can be biased by habitat filtering, and networks can display positive associations between non-interacting microbial members in environmental samples. Moreover, network scores and co-occurrence patterns are ultimately based on correlations, and they must be interpreted with caution, as correlation does not mean causation. Statistical analyses such as structural equation modelling (SEM) can be used to move beyond correlation analysis and explore causal relationships among keystone taxa and microbiome composition or function. SEM is an advanced multivariate statistical approach that identifies such causal relationships and generates strong and distinct links between theoretical and experimental ideas[81]. The strength of SEM lies in the fact that it is theory oriented and not null hypothesis based, and thus, it provides a framework to interpret complex networks involving numerous response and predictor variables. Upon assessing the univariate and multivariate normality, an initial model is generated on the basis of existing knowledge, site information and background data[82,83]. Subsequently, a χ^2 test is conducted to assess whether the covariance structure indicated by the model adequately fits the actual covariance structure of the data, with a nonsignificant χ^2 test result suggesting sufficient model fit. Importantly, SEM requires a minimum sample size of 50 (REF.[81]). Moreover, determining the relative influence of keystone taxa can also be a challenge[84]. A recent study used sparse linear regression with bootstrap aggregation in a discrete-time Lotka-Volterra model to identify *B. fragilis* and *Bacteroides stercoris* as keystone taxa with disproportionate influence on the gut microbiome structure[22]. Using a novel approach called Learning Interactions from MIcrobial Time Series (LIMITS) on metagenomic data, it was statistically shown that the moderately abundant *B. fragilis* and *B. stercosis* can exert significant influence on the microbiome, and any perturbations applied to these taxa have a large impact on microbial community structure. This is encouraging because the algorithm identified *B. fragilis* as a keystone taxon, thus agreeing with existing empirical data[74].

Characterization and manipulation. Although experimental manipulation (for example, removing a putative keystone taxon to assess the impact) is the popular choice among plant and animal ecologists, one of the fundamental challenges that microbiologists are confronted with is the characterization and manipulation of such taxa. Manipulating growth or co-culturing microorganisms on nutrient media or Petri dishes or in microcosms can be challenging owing to individual physiological requirements. In past years, novel approaches have been developed to overcome the uncultivability issue. For example, the isolation chip, which is composed of numerous diffusion chambers, enables in situ cultivation of previously uncultivated microbial species[85]. Similarly, a microbial trap has been developed to capture and culture filamentous Actinobacteria under in situ conditions[86]. Moreover, on-chip microbial culture coupled with surface plasmon resonance enables the in situ detection of novel and rare microorganisms[87]. Droplet-based microfluidic technology also offers the opportunity to mimic natural conditions and co-cultivate synergistic microbial communities[88], and the microbiome-on-a-chip approach enables the study of microbial networks and their associations with host plants[89]. Future studies may include such promising approaches to isolate and characterize keystone taxa from various environments and explore their functioning. Removal of keystone taxa may lead to an alternative stable state (sensu[90]) of the microbial network, which results in dysfunction or even renewed functioning if the removed keystone had a negative impact. Future studies may also enable the experimental manipulation of microbial network structure in synthetic communities to assess whether the removal of keystone taxa disrupts microbiome functioning.

Influence on the microbiome

Influence, irrespective of abundance, distinguishes keystone taxa from dominant taxa. A dominant species often affects ecosystem functioning or a specific process exclusively by virtue of sheer abundance (FIG. 2a), whereas keystone taxa might exert their influence on microbiome functioning irrespective of abundance. The importance of keystone taxa may also be related to the broadness of a process, that is, a process involving many steps as well as functionally and taxonomically diverse microbial groups[1,91]. For example, dominant taxa with large biomass or major energy transformations might influence broad processes, such as denitrification or organic matter decomposition. By contrast, the influence of rare keystone taxa might be more pronounced if a process is narrow, consisting of a single step (for example, nitrogen fixation or ammonia oxidation) and being carried out by a small group of specialized microorganisms[1,91]. We postulate that the influence of rare keystone taxa on an ecosystem process is inversely proportional to the broadness of the process. However, it should be noted that some keystone taxa, such as *B. thetaiotaomicron* in the human intestine, can be numerically dominant, and thus, the distinction between dominant taxa and less abundant keystone taxa is not always clear. Whether numerically inconspicuous keystone taxa are more influential on narrow processes is a hypothesis that needs further investigation.

Keystone taxa might use a range of strategies to exert an influence on a microbiome. For example, they might function via intermediate or effector groups, whose abundance can be selectively modulated to regulate community structure and functioning[23,74]. Such selective modulation might include promotion (commensalism) or suppression (amensalism) of effector groups by secreting metabolites, antibiotics or toxins. In humans, *P. gingivalis* affects the community by causing dysbiosis, which results in inflammation and periodontitis[64]. Here, effector groups are accessories used by keystone taxa to alter microbiome composition and manipulate their hosts[92], and dysbiosis is the imbalance in the composition of the microbiome[93]. In the case of chronic periodontitis, *P. gingivalis* functions as the keystone taxon, whereas *Streptococcus gordonii* functions as the accessory[94]. *P. gingivalis* impairs host defence and causes overgrowth of the oral commensal bacterium *S. gordonii*. The co-adhesion of this keystone–accessory pair causes inflammatory tissue destruction and the release of nutrient-rich exudates, initiating dysbiosis of the oral microbiota and favouring further growth of *P. gingivalis*[74,94]. Similarly, in the case of inflammatory bowel disease or Crohn's disease, dysbiosis results in reduced diversity of major phyla such as Bacteroidetes and Firmicutes and increased abundance of Enterobacteriaceae[93,95].

In the plant microbiome, certain strains of *Pseudomonas fluorescens* produce a secondary metabolite (2,4-diacetylphloroglucinol) that suppresses *Gaeumannomyces graminis* var. *tritici*, which causes the take-all disease in wheat[96]. Alternatively, keystone taxa might produce bacteriocins to selectively alter microbiota composition. For example, bacteriocin production by *Enterococcus faecalis* can induce niche competition in the gastrointestinal tract to change microbiota composition[78]. Keystone taxa might also engage in synergistic relationships and change the abundance of their partners, and this could have an effect on community structure and performance. Some members of the *Burkholderia* genus can function as an endosymbiont in arbuscular mycorrhizal fungi to change the abundance and community characteristics of this important fungi[79], which subsequently may alter plant community richness and productivity[97]. Although not considered yet, we hypothesize that mycorrhizal fungi function as keystone taxa because these plant symbionts have a major impact on soil microbial communities, plant diversity and ecosystem functioning[79]. Thus, keystone taxa can use different strategies to shape the microbiota in their favour, but the selection of a particular strategy would depend on the microenvironment. We speculate that many such strategies are aimed to gain direct benefits, such as replacing indigenous microflora (in case of *E. faecalis*), gaining competitive advantage in the community (in case of *P. gingivalis*) or promoting further growth (in case of *B. thetaiotaomicron*). However, it is possible that metabolites or by-products from keystone taxa may influence members of the microbiome with indirect or even no benefits to the keystones.

Putative drivers of keystone taxa

The presence of keystone taxa in a microbiome does not necessarily guarantee their influence because a number of factors may still determine their distribution and efficacy (FIG. 2b). For example, spatiotemporal heterogeneity can be a major driver of the abundance and distribution of keystone taxa[29,31,84]. This is particularly true for soil,

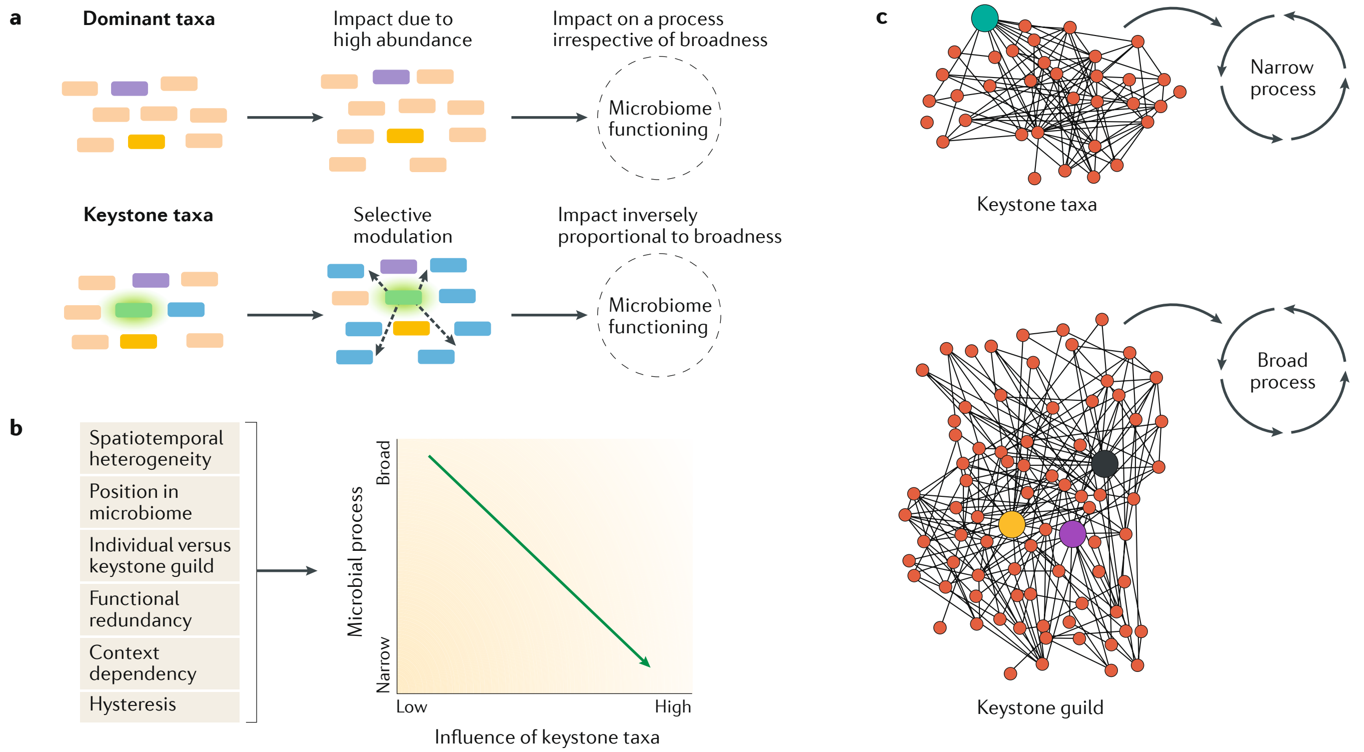


Fig. 2 | **Keystone taxa in microbial communities and the factors influencing their functioning in an environment. a** | The dominant taxa (light orange) affect microbiome functioning exclusively by virtue of sheer abundance, whereas keystone taxa (green) exert their influence irrespective of their abundance. As the impact of dominant species on a process is primarily due to greater abundance, the broadness of that process is less important. Here, broadness implies that a particular process consists of many steps and involves diverse microbial groups. By contrast, keystone taxa exert their influence by selectively modulating accessory microorganisms, and thus, they might have a greater influence on narrow processes (the processes that consist of a single step or a few steps and involve a select group of microorganisms). The accessory microorganisms whose abundance is selectively promoted by keystone taxa are shown in blue, whereas other community members are shown in dark orange and purple. **b** | Environmental and ecological factors that may determine the distribution and performance of keystone taxa in an environment. The influence of keystone taxa on a microbial process is inversely proportional to the broadness of that process. Spatiotemporal heterogeneity can drive the abundance and distribution of keystone taxa in any environment, especially in soil. The impact of a keystone taxon may be higher if it belongs to the core microbiome that is consistently present in an environment regardless of changes in environmental conditions. Keystone taxa may function alone, or a group of such taxa with similar functioning may form a keystone guild and alter the structure and dynamics of the ecosystem that they thrive in. In the microbial world, it is possible that keystone taxa may be functionally redundant or that they are only relevant in a particular context. Hysteresis suggests a time lag between the functioning of a keystone and its detectable outcome in the microbiome. **c** | Hypothetical diagram showing various modes of functioning of keystone taxa in an environment. Individually, keystone taxa (teal dot) might have greater influence on a narrow process (for example, biological nitrogen fixation performed by highly specific microorganisms). A keystone guild comprising multiple keystone taxa (yellow, black and purple dots) within a community might also be able to influence a broad process, such as organic matter decomposition and denitrification.

which is one of the most heterogeneous and multifaceted environments. Similarly, seasonal variability determines the structural and compositional properties of microbiomes in an environment, and as such, a keystone might be present only in a specific season or time period.

The occurrence and functioning of a keystone will also depend on its position in the microbiome. Recently, the tenet of core microbiomes and holobionts has been proposed for humans[6,98] and plants[92], and readers are referred to references[6] and[92] for the taxonomic and functional definitions of a core microbiome. Keystone taxa might be part of the core microbiome that is consistently present in an environment regardless of changes in environmental conditions[92,98]. A seminal paper presented the first evidence of a core gut microbiome in obese and lean twins[99]. A recent study reported an evolutionarily conserved core microbiome in plant roots, and, intriguingly, some of the well-known keystone taxa, such as *Rhizobium, Bradyrhizobium* and *Burkholderia*, are also part of the core root microbiome[100]. The contribution of keystone taxa will be higher if they are part of the core microbiome and consistently present in an environment, highlighting the importance of such taxa for microbiome functioning[23].

Microbiomes can also harbour keystone guilds (that is, groups of keystone taxa with similar functioning)[31] (FIG. 2c). Examples of such guilds that can alter the structure and dynamics of ecosystems are common in the animal world[101]. Perhaps the most famous example is the three species of kangaroo rat, which can be considered a keystone guild in the Chihuahuan desert, USA, and has a strong impact on local biodiversity and biogeochemical processes[101]. In the microbial world, keystone guilds may arise on the basis of a number of factors, including, for example, complementary resource acquiring strategies, resource sharing, niche partitioning and spatiotemporal coherence[62,84]. Whereas numerically inconspicuous keystone taxa might have a greater influence on narrow processes, a keystone guild consisting of diverse keystone taxa within a community might also influence a broad process. For example, certain keystone guilds of co-occurring denitrifiers can have an important role in denitrification, a broad process that involves heterogeneous groups of microorganisms[102]. We expect that

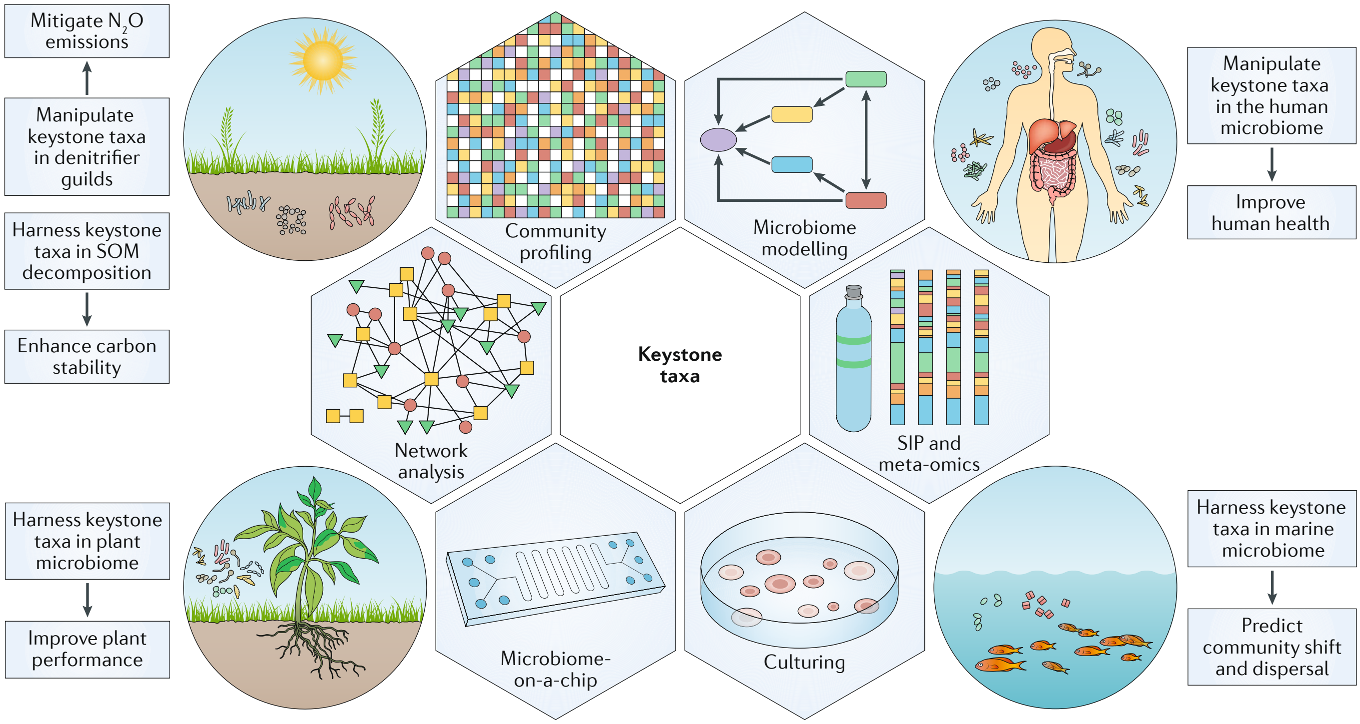


Fig. 3 | **Characterizing and harnessing keystone taxa.** Hypothetical diagram illustrating the tools (hexagons) for linking keystone taxa to ecosystem functioning and the research areas (circles) where keystone taxa can be used. Although network analysis can be used to statistically identify keystone taxa in microbial networks, it is important to link such taxa to ecosystem processes. With the advent of newer tools, such as chip or culture-based methods, keystone taxa can be isolated from environments and cultured or co-cultured. Functional profiling of such taxa can be performed using RNA-stable isotope probing (SIP) coupled with metatranscriptomics or metaproteomics. Upon functional profiling, the relative importance can be estimated through microbiome modelling. Such models involving causal relationships can be used to reveal the contribution of keystone taxa to ecosystem processes. There are several areas where keystone taxa or guilds have been identified and thus can be harnessed for improved ecosystem services. For example, emissions of nitrous oxide (N_2O), a potent greenhouse gas, can be mitigated by manipulating denitrifier guilds. Denitrification is a major contributor to nitrous oxide emissions. Similarly, keystone taxa that are linked to soil organic matter (SOM) decomposition can be manipulated to enhance carbon sequestration in soil. Harnessing keystone taxa in plant microbiomes can be valuable to enhance plant productivity in agricultural systems or to alter performance of invasive plants. Most of the empirical evidence on keystone taxa emerged from the human microbiome studies where keystone taxa such as *Porphyromonas gingivalis*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron* and *Ruminococcus bromii* have been identified. Targeted manipulation of these pathogens can facilitate medical interventions and improve human health. In aquatic systems, it has recently been shown that keystone taxa can explain microbiome compositional turnover better than all taxa combined[80]. Such keystone taxa can be harnessed to predict shifts in the community or to manipulate microbiome functioning.

examples of such keystone guilds in microbial communities will continue to be identified in the future. Indeed, such guilds may be particularly powerful if they belong to the core microbiome.

Keystone taxa or members of keystone guilds might be functionally redundant, or their effect might be context dependent. Such context dependency or conditionality may be more common in environments with turbulence or high spatiotemporal variability[31]. Thus, keystone taxa may not always be present in an environment or may not have the same impact on the community under changing conditions. A taxon should only be considered a keystone in the context or condition under which it has a large influence. A plausible challenge for assessing keystone taxa is also the fact that there might be a hysteresis effect, that is, a time lag between the change in the abundance of keystone taxa and their influence on microbiome functioning. With rapid microbial turnover, identifying such lags can be a daunting task. The above discussion of potential drivers of keystone taxa is not exhaustive, and there may be other factors influencing these taxa in the microbial world.

The rare species concept

Keystone taxa underline the importance of numerically inconspicuous taxa for microbiome functioning, which is also congruent with the rare taxa concept. Indeed, the fundamental premise of keystone taxa and rare taxa is the same: the abundance of a species is not the best determinant of its contribution to the community[103]. The importance of rare microorganisms has been documented for many biogeochemical processes, including nitrification, denitrification, methanogenesis, methanotrophy and sulfate reduction (reviewed in REFS[103,104]). For example, *Desulfosporosinus* spp., which only represent 0.06% of the total community, have a pivotal role in sulfate reduction and carbon flow in peatland soils[105]. The rare biosphere was also shown to be important in the human microbiome and even in depauperate ecosystems[104]. Evidence of such low abundant taxa with an overproportional influence obviously raises the possibility that members of rare biosphere can also be keystone taxa.

Outlook

Beyond the dominant taxa with large biomass and major energy transformations, keystone taxa can orchestrate microbial communities to perform ecosystem processes. This Opinion article highlights the relevance of keystone taxa as drivers of microbiome structure and functioning. Owing to current tautonyms and misconceptions surrounding keystone taxa, we proposed a definition of keystone taxa in microbiology. We also presented a summary of computational inference and empirical evidence of over 200 keystone taxa reported for soil, plant and marine ecosystems and the human microbiome. We explored various strategies of how keystone taxa exert their influence in microbial communities. We also discussed how keystone taxa may function individually or as part of a guild to influence narrow and broad processes. We noted the usefulness of correlation scores but emphasized the importance of causal relationships and experimental studies for identifying keystone taxa. To aid future studies, we summarized a number of approaches that can be used to characterize and harness keystone taxa in various ecosystems, and we identified uncharted territories where microbial keystone taxa have not been identified.

Network scores have been popular to statistically identify keystone taxa in recent years, and it is important to continue this momentum to strengthen the repertoire of keystone taxa. For a range of taxa, it has been shown that keystone taxa identified using statistical tools indeed have an impact on microbiome structure and performance[20,39,80]. However, for many other keystone taxa, such experimental evidence is still missing. Hence, it is a challenge to complement statistical evidence with empirical evidence for keystone taxa in microbial communities (some experimental tools for doing this are highlighted above). Moreover, information on keystone taxa from the desert, tropical forest or vadose zone is rare or not yet available. Similarly, knowledge of how keystone taxa respond to environmental disturbance, pathogen attack in plants or medical intervention in humans would be valuable. For example, determining whether keystone taxa help microbiome resilience against perturbations could be tested. The role of keystone taxa in plant invasion is an equally interesting area, especially in the light of observations that some invasive tree species cannot establish themselves without their microbial symbionts or that invasive species alter the soil microbiome[106]. Moreover, our knowledge of fungal, archaeal and protistan keystone taxa is limited, and only a few studies have considered fungal–bacteria or fungal–archaeal and bacterial–archaeal co-occurrence networks[38,39]. A cross-domain network may reveal how members of different taxonomic groups associate with each other, whether they share resources or whether there are keystone taxa important for inter-kingdom associations. Another intriguing question is whether keystone taxa in microbial communities follow similar ecological principles (for example, drift, dispersal, diversification and environmental selection; sensu[12]) as keystone taxa in plant or animal kingdoms.

Linking community structure to function is a central goal in microbial ecology[11], and it is necessary to extend microbial co-occurrence patterns and keystone taxa to ecosystem processes (FIG. 3). Studies investigating keystone taxa could include promising culturing approaches to explore complex ecological relationships, such as commensalism and amensalism, in natural conditions and assess the effect of keystones. The actual importance of keystone taxa to microbiome functioning and ecosystem processes can only be derived from robust functional profiling using the latest tools, such as RNA-stable isotope probing[107] coupled with metatranscriptomics or metaproteomics. Upon identifying keystone taxa in an environment, determining if there are structural keystones and functional keystones depending on whether they affect microbiome structure or functioning could be tested. As any change in microbiome structure may also have consequences for microbiome functioning, a clear distinction between structural and functional keystones in microbial communities is questionable. Nonetheless, the latest molecular tools have empowered microbiologists to test such theories and ideas. The contribution of microbial communities for ecosystem processes is often missing or insignificant in ecosystem models[5]. These models mostly consider the overall community characteristics (abundance, composition and diversity), which might blur the actual contribution of important microbial members. Keystone taxa observed across habitats and studies might be the missing pieces of the puzzle that could help microbial ecologists explain the unexplained variation in ecosystem processes.

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1. Fierer, N. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **15**, 579–590 (2017).
2. Bardgett, R. D. & van der Putten, W. H. Belowground biodiversity and ecosystem functioning. *Nature* **515**, 505–511 (2014).
3. Fuhrman, J. A. Microbial community structure and its functional implications. *Nature* **459**, 193–199 (2009).
4. van der Heijden, M. G. A., Bardgett, R. D. & Van Straalen, N. M. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* **11**, 296–310 (2008).
5. Graham, E. B. et al. Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Front. Microbiol.* **7**, 1–10 (2016).
6. Hamady, M. & Knight, R. Microbial community profiling for human microbiome projects: tools, techniques, and challenges. *Genome Res.* **19**, 1141–1152 (2009).
7. Barberán, A., Bates, S. T., Casamayor, E. O. & Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* **6**, 343–351 (2012).
8. Banerjee, S. et al. Determinants of bacterial communities in Canadian agroforestry systems. *Environ. Microbiol.* **18**, 1805–1816 (2016).
9. Zhang, Z. et al. Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota. *ISME J.* **8**, 881–893 (2014).
10. Fuhrman, J. A., Cram, J. A. & Needham, D. M. Marine microbial community dynamics and their ecological interpretation. *Nat. Rev. Microbiol.* **13**, 133–146 (2015).
11. Prosser, J. I. et al. The role of ecological theory in microbial ecology. *Nat. Rev. Microbiol.* **5**, 384–392 (2007).
12. Costello, E. K., Stagaman, K., Dethlefsen, L. & Bohannan, B. J. M. & Relman, D. A. The application of ecological theory toward an understanding of the human microbiome. *Science* **336**, 1255–1262 (2012).
13. Proulx, S. R., Promislow, D. E. L. & Phillips, P. C. Network thinking in ecology and evolution. *Trends Ecol. Evol.* **20**, 345–353 (2005).
14. Zhou, J. et al. Functional molecular ecological networks. *MBio* **1**, e00169–00110 (2010).
15. Reshef, D. N. et al. Detecting novel associations in large data sets. *Science* **334**, 1518–1524 (2011).
16. Ruan, Q. et al. Local similarity analysis reveals unique associations among marine bacterioplankton species and environmental factors. *Bioinformatics* **22**, 2532–2538 (2006).
17. Friedman, J. & Alm, E. J. Inferring correlation networks from genomic survey data. *PLoS Comput. Biol.* **8**, 1–11 (2012).
18. Faust, K. et al. Microbial co-occurrence relationships in the human microbiome. *PLoS Comput. Biol.* **8**, e1002606 (2012).
19. Weiss, S. et al. Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. *ISME J.* **10**, 1–13 (2016).
20. Agler, M. T. et al. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol.* **14**, 1–31 (2016).
21. Gilbert, J. a et al. Defining seasonal marine microbial community dynamics. *ISME J.* **6**, 298–308 (2012).
22. Fisher, C. K. & Mehta, P. Identifying keystone species in the human gut microbiome from metagenomic timeseries using sparse linear regression. *PLoS ONE* **9**, 1–10 (2014).
23. Shetty, S. A., Hugenholtz, F., Lahti, L., Smidt, H. & de Vos, W. M. Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies. *FEMS Microbiol. Rev.* **41**, 182–199 (2017).
24. Vick-Majors, T. J., Priscu, J. C. & Amaral-Zettler, L. A. Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. *ISME J.* **8**, 778–789 (2014).
25. Gokul, J. K. et al. Taxon interactions control the distributions of cryoconite bacteria colonizing a high Arctic ice cap. *Mol. Ecol.* **25**, 3752–3767 (2016).
26. Comte, J., Lovejoy, C., Crevecoeur, S. & Vincent, W. F. Co-occurrence patterns in aquatic bacterial communities across changing permafrost landscapes. *Biogeosciences* **13**, 175–190 (2016).
27. Berry, D. & Widder, S. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Front. Microbiol.* **5**, 1–14 (2014).

28. Paine, R. T. Food web complexity and species diversity. *Am. Nat.* **100**, 65–75 (1966).
29. Mills, L. S. & Doak, D. F. The keystone-species concept in ecology and conservation. *BioScience* **43**, 219–224 (1993).
30. Cottee-Jones, H. E. W. & Whittaker, R. J. The keystone species concept: a critical appraisal. *Front. Biogeogr.* **4**, 217–220 (2012).
31. Power, M. E. et al. Challenges in the quest for keystones. *Bioscience* **46**, 609–620 (1996).
32. Paine, R. T. A note on trophic complexity and community stability. *Am. Nat.* **103**, 91–93 (1969).
33. Dunne, J. A., Williams, R. J. & Martinez, N. D. Network structure and biodiversity loss in food webs: robustness increase with connectance. *Ecol. Lett.* **5**, 558–567 (2002).
34. Deng, Y. et al. Molecular ecological network analyses. *BMC Bioinformatics* **13**, 113 (2012).
35. Lupatini, M. et al. Network topology reveals high connectance levels and few key microbial genera within soils. *Front. Environ. Sci.* **2**, 1–11 (2014).
36. Zhou, J., Deng, Y., Luo, F., He, Z. & Yang, Y. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO2. *MBio* **2**, e00122–e00111 (2011).
37. Eldridge, D. J. et al. Soil-foraging animals alter the composition and co-occurrence of microbial communities in a desert shrubland. *ISME J.* **9**, 1–11 (2015).
38. Ma, B. et al. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *ISME J.* **10**, 1–11 (2016).
39. Banerjee, S. et al. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biol. Biochem.* **97**, 188–198 (2016).
40. Jiang, Y. et al. Plant cultivars imprint the rhizosphere bacterial community composition and association networks. *Soil Biol. Biochem.* **109**, 145–155 (2017).
41. Li, F., Chen, L., Zhang, J., Yin, J. & Huang, S. Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. *Front. Microbiol.* **8**, 187 (2017).
42. Liang, Y. et al. Long-term oil contamination alters the molecular ecological networks of soil microbial functional genes. *Front. Microbiol.* **7**, 1–13 (2016).
43. Wang, H. et al. Combined use of network inference tools identifies ecologically meaningful bacterial associations in a paddy soil. *Soil Biol. Biochem.* **105**, 227–235 (2017).
44. Hill, R. et al. Temporal and spatial influences incur reconfiguration of Arctic heathland soil bacterial community structure. *Environ. Microbiol.* **18**, 1942–1953 (2016).
45. Li, B. et al. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *ISME J.* **9**, 1–13 (2015).
46. Yang, S. et al. Hydrocarbon degraders establish at the costs of microbial richness, abundance and keystone taxa after crude oil contamination in permafrost environments. *Sci. Rep.* **6**, 37473 (2016).
47. Chao, Y. et al. Structure, variation, and co-occurrence of soil microbial communities in abandoned sites of a rare earth elements mine. *Environ. Sci. Technol.* **50**, 11481–11490 (2016).
48. Jiao, S. et al. Bacterial communities in oil contaminated soils: biogeography and co-occurrence patterns. *Soil Biol. Biochem.* **98**, 64–73 (2016).
49. Shi, S. et al. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. *Ecol. Lett.* **19**, 926–936 (2016).
50. Yan, Y., Kuramae, E. E., De Hollander, M., Klinkhamer, P. G. & Van Veen, J. A. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *ISME J.* **11**, 1–11 (2016).
51. Geng, H., Tran-Gyamfi, M. B., Lane, T. W., Sale, K. L. & Yu, E. T. Changes in the structure of the microbial community associated with Nannochloropsis salina following treatments with antibiotics and bioactive compounds. *Front. Microbiol.* **7**, 1–13 (2016).
52. Graham, E. B. et al. Deterministic influences exceed dispersal effects on hydrologically-connected microbiomes. *Environ. Microbiol.* **19**, 1552–1567 (2017).
53. Ji, Y. et al. Structure and function of methanogenic microbial communities in sediments of Amazonian lakes with different water types. *Environ. Microbiol.* **18**, 5082–5100 (2016).
54. Musat, N. et al. A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *Proc. Natl Acad. Sci. USA* **105**, 17861–17866 (2008).
55. Zhao, D. et al. Network analysis reveals seasonal variation of co-occurrence correlations between Cyanobacteria and other bacterioplankton. *Sci. Total Environ.* **573**, 817–825 (2016).
56. Curtis, M. M. et al. The gut commensal bacteroides thetaiotaomicron exacerbates enteric infection through modification of the metabolic landscape. *Cell Host Microbe* **16**, 759–769 (2014).
57. Maldonado-Contreras, A. et al. Structure of the human gastric bacterial community in relation to *Helicobacter pylori* status. *ISME J.* **5**, 574–579 (2011).
58. Trosvik, P. & de Muinck, E. J. Ecology of bacteria in the human gastrointestinal tract — identification of keystone and foundation taxa. *Microbiome* **3**, 44 (2015).
59. Wu, S. et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat. Med.* **15**, 1016–1022 (2009).
60. Ze, X., Duncan, S. H., Louis, P. & Flint, H. J. Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *ISME J.* **6**, 1535–1543 (2012).
61. Ding, J. et al. Soil organic matter quantity and quality shape microbial community compositions of subtropical broadleaved forests. *Mol. Ecol.* **24**, 5175–5185 (2015).
62. Nunez, M. A. & Dimarco, R. D. The encyclopedia of sustainability, vol. 5: ecosystem management and sustainability (eds Craig, R. K., Nagle, J. C., Pardy, B., Schmitz, O. J. & Smith, W. K.) 226–230 (Berkshire Publishing, 2012).
63. Hector, A. et al. Plant diversity and productivity experiments in European grasslands. *Science* **286**, 1123–1127 (1999).
64. Hajishengallis, G. et al. Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host Microbe* **10**, 497–506 (2011).
65. Faust, K. & Raes, J. Microbial interactions: from networks to models. *Nat. Rev. Microbiol.* **10**, 538–550 (2012).
66. Cardona, C., Weisenhorn, P., Henry, C. & Gilbert, J. A. Network-based metabolic analysis and microbial community modeling. *Curr. Opin. Microbiol.* **31**, 124–131 (2016).
67. Strogatz, S. H. Exploring complex networks. *Nature* **410**, 268–276 (2001).
68. Newman, M. E. J. The structure and function of complex networks. *Soc. Ind. Appl. Math. Rev.* **45**, 167–256 (2003).
69. van der Heijden, M. G. A. & Hartmann, M. Networking in the plant microbiome. *PLoS Biol.* **14**, 1–9 (2016).
70. Barabási, A. L., Gulbahce, N. & Loscalzo, J. Network medicine: a network-based approach to human disease. *Nat. Rev. Genet.* **12**, 56–68 (2011).
71. Hartman, K. et al. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* **6**, 14 (2018).
72. Steele, J. A. et al. Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *ISME J.* **5**, 1414–1425 (2011).
73. Andreote, F. D. et al. Culture-independent assessment of rhizobiales-related alphaproteobacteria and the diversity of Methylobacterium in the rhizosphere and rhizoplane of transgenic eucalyptus. *Microb. Ecol.* **57**, 82–93 (2009).
74. Hajishengallis, G., Darveau, R. P. & Curtis, M. A. The keystone-pathogen hypothesis. *Nat. Rev. Microbiol.* **10**, 717–725 (2012).
75. Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. & Gordon, J. I. Host-bacterial mutualism in the human intestine. *Science* **307**, 1915–1920 (2005).
76. Garrett, W. S. et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* **8**, 292–300 (2010).
77. Van Der Heijden, M. G. A. et al. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune grassland. *FEMS Microbiol. Ecol.* **56**, 178–187 (2006).
78. Kommineni, S. et al. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature* **526**, 719–722 (2015).
79. van der Heijden, M. G. A. et al. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**, 69–72 (1998).
80. Herren, C. M. & McMahon, K. D. Small subsets of highly connected taxa predict compositional change in microbial communities. bioRxiv https://doi.org/10.1101/159087 (2017).
81. Grace, J. B., Anderson, T., Olff, H. & Scheiner, S. On the specification of structural equation models for ecological systems. *Ecol. Monogr.* **80**, 67–87 (2010).
82. Lamb, E. G., Shirtliffe, S. J. & May, W. E. Structural equation modeling in the plant sciences: an example using yield components in oat. *Can. J. Plant Sci.* **91**, 603–619 (2011).
83. Banerjee, S., Bora, S., Thrall, P. H. & Richardson, A. E. Soil C and N as causal factors of spatial variation in extracellular enzyme activity across grassland-woodland ecotones. *Appl. Soil Ecol.* **105**, 1–8 (2016).
84. Mouquet, N., Gravel, D., Massol, F. & Calcagno, V. Extending the concept of keystone species to communities and ecosystems. *Ecol. Lett.* **16**, 1–8 (2013).
85. Nichols, D. et al. Use of ichip for high-throughput in situ cultivation of 'uncultivable' microbial species. *Appl. Environ. Microbiol.* **76**, 2445–2450 (2010).
86. Gavrish, E., Bollmann, A., Epstein, S. & Lewis, K. A trap for in situ cultivation of filamentous actinobacteria. *J. Microbiol. Methods* **72**, 257–262 (2008).
87. Bouguelia, S. et al. On-chip microbial culture for the specific detection of very low levels of bacteria. *Lab. Chip* **13**, 4024 (2013).
88. Park, J., Kerner, A., Burns, M. A. & Lin, X. N. Microdroplet-enabled highly parallel co-cultivation of microbial communities. *PLoS ONE* **6**, e17019 (2011).
89. Stanley, C. E. & van der Heijden, M. G. A. Microbiome-on-a-chip: new frontiers in plant–microbiota research. *Trends Microbiol.* **25**, 610–613 (2017).
90. Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B. & Jeppesen, E. Alternative equilibria in shallow lakes. *Trends Ecol. Evol.* **8**, 275–279 (1993).
91. Schimel, J. P. & Schaeffer, S. M. Microbial control over carbon cycling in soil. *Front. Microbiol.* **3**, 1–11 (2012).
92. Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A. & Dufresne, A. The importance of the microbiome of the plant holobiont. *New Phytol.* **206**, 1196–1206 (2015).
93. Maloy, K. J. & Powrie, F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* **474**, 298–306 (2011).
94. Hajishengallis, G. & Lamont, R. J. Dancing with the stars: how choreographed bacterial interactions dictate nososymbiocity and give rise to keystone pathogens, accessory pathogens, and pathobionts. *Trends Microbiol.* **24**, 477–489 (2016).
95. Hill, D. & Artis, D. Intestinal bacteria and the regulation of immune cell homeostasis. *Annu. Rev. Immunol.* **28**, 623–667 (2010).
96. Raaijmakers, J. M. & Weller, D. M. Natural plant protection by 2,4-diacetylphloroglucinol - producing Pseudomonas spp. in take-all decline soils. *APS.* **11**, 144–152 (1998).
97. van Der Heijden, M. G. A., Martin, F. M., Selosse, M. A. & Sanders, I. R. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol.* **205**, 1406–1423 (2015).
98. Shade, A. & Handelsman, J. Beyond the Venn diagram: the hunt for a core microbiome. *Environ. Microbiol.* **14**, 4–12 (2012).
99. Turnbaugh, P. J. et al. A core gut microbiom in obese and lean twins. *Nature* **457**, 480–484 (2009).
100. Yeoh, Y. K. et al. Evolutionary conservation of a core root microbiome across plant phyla along a tropical soil chronosequence. *Nat. Commun.* **8**, 215 (2017).
101. Brown, J. H. & Heske, E. J. Control of a desert-grassland transition by a keystone rodent guild. *Science* **250**, 1705–1707 (1990).
102. Jones, C. M. et al. Recently identified microbial guild mediates soil N2O sink capacity. *Nat. Clim. Chang.* **4**, 801–805 (2014).
103. Lynch, M. D. J. & Neufeld, J. D. Ecology and exploration of the rare biosphere. *Nat. Rev. Microbiol.* **13**, 217–229 (2015).
104. Jousset, A. et al. Where less may be more: how the rare biosphere pulls ecosystems strings. *ISME J.* **11**, 853–862 (2017).
105. Pester, M., Bittner, N., Deevong, P., Wagner, M. & Loy, A. A 'rare biosphere' microorganism contributes to sulfate reduction in a peatland. *ISME J.* **4**, 1591–1602 (2010).

106. Stinson, K. A. et al. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biol.* **4**, 727–731 (2006).
107. Manefield, M., Whiteley, A. S., Griffiths, R. I. & Bailey, M. J. RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Appl. Environ. Microbiol.* **68**, 5367–5373 (2002).

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S.B. researched data for the article. S.B. and M.G.A.v.d.H made substantial contributions to the discussion of content and writing of the article. S.B, K.S. and M.G.A.v.d.H. reviewed and edited the manuscript before submission.

Competing interests
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