

## PERSPECTIVE



## Transitivity and intransitivity in soil bacterial networks

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Competition can lead to the exclusion of bacterial taxa when there is a transitive relationship among competitors with a hierarchy of competitive success. However, competition may not prevent bacterial coexistence if competitors form intransitive loops, in which none is able to outcompete all the rest. Both transitive and intransitive competition have been demonstrated in bacterial model systems. However, in natural soil microbial assemblages competition is typically understood as a dominance relationship leading to the exclusion of weak competitors. Here, we argue that transitive and intransitive interactions concurrently determine the structure of soil microbial communities. We explain why pairwise interactions cannot depict competition correctly in complex communities, and propose an alternative through the detection of strongly connected components (SCCs) in microbial networks. We finally analyse the existence of SCCs in soil bacterial communities in two Mediterranean ecosystems, for illustrative purposes only (rather than with the aim of providing a methodological tool) due to current limitations, and discuss future avenues to experimentally test the existence of SCCs in nature.

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**TRANSITIVITY AND INTRANSITIVITY IN BACTERIAL COMMUNITIES**

Microorganisms display an enormous array of competitive interactions for space and resources. Competition has been demonstrated in the laboratory including mechanisms of interference (e.g., production of toxins and antibiotics, cell lysis using nanoneedles, or disruption of communication) and resource exploitation (e.g., phosphorus sequestration, iron scavenging, space encroachment, or stimulation of competitors' dispersal) [1, 2].

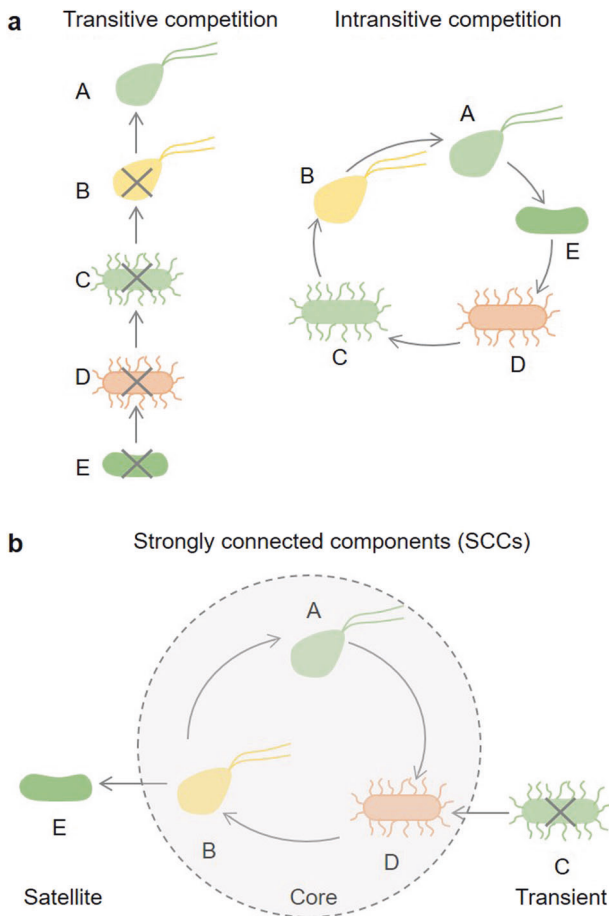
Competition has been traditionally thought of as a dominance relationship, in which species A dominates species B ( $A > B$ ). In sets of multiple species with a hierarchy of dominance and competitive success, this can be depicted as a chain of interactions (Fig. 1a left). This is the basic representation of transitive relationships, in which a set of species compete for a single resource, and only one species (the top competitor) is able to outcompete all other species, the next species is able to outcompete all but the top competitor, the third species outcompetes all but the first two species, and so on. Contrarily, intransitive assemblages lack such a strict hierarchy of competitive success, and no species is able to outcompete all the rest (Fig. 1a right). In mathematical terms, a transitive set is one where whenever  $A > B$  and  $B > C$ , then  $A > C$ . If the latter condition is not satisfied, then the set is intransitive in such a way that  $A > B$ ,  $B > C$ , and  $C > A$ , forming a cycle or loop. The competitive exclusion principle determines that only the top competitor can persist in a transitive set of species, while theoretically all species can coexist in an intransitive group. Intransitivity is a property of the relationships among elements, in this case the elements being the species and the relationship among them being competition. As such relational property, intransitivity can leave a signal in

networks. Indeed, it is one of the structural properties of ecological networks that has a clearer potential to explain the coexistence of competing species [3, 4].

Both competition by interference and resource exploitation can generate transitive patterns when interactions are asymmetric in terms of competitive success. However, in bacterial model systems it has been shown that the introduction of a third species can modulate the asymmetry of paired interactions, generating a cyclic dominance which involves both interference and resource exploitation. The classic example of such an intransitive network is that involving toxin-producing, sensitive, and resistant *Escherichia coli* strains [5, 6]. A toxin-producing strain (K, killer) outcompetes a sensitive strain ( $K > S$ ) that can exclude a third resistant strain ( $S > R$ ) since it does not incur in the metabolic cost of resistance. In turn, the resistant strain is a superior competitor than the killer ( $R > K$ ) since it does not incur in the cost of toxin production. This cyclic dominance has been suggested as a mechanism begetting biodiversity based on game theory models [7] and subsequent experimental validation [5, 8]. However, experimental evidence also supports the role of transitivity on competitive interactions in soil bacteria in the laboratory, for instance, among several species of Bacillales, *Arthobacter*, and *Cupriavidus* [9]. These apparently conflicting results may be reconciled by showing that both transitivity and intransitivity are simultaneously operating in experimental microbial communities. Indeed, Fiegna & Vellicer [10] demonstrated that different genotypes of *Myxococcus xanthus* show a predominance of hierarchical competition but also cases of circular competition.

In natural soil bacterial communities, competition is typically understood as a transitive process and used more and more often to discuss the patterns of mutual exclusion between pairs of taxa

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**Fig. 1 Transitive and intransitive competition.** **a** Transitive competition is hierarchical and leads to the exclusion of weak competitors, while intransitive competition forms a loop allowing the coexistence of competitors. **b** In complex soil bacterial communities, it is expected that both transitivity and intransitivity act simultaneously, a situation that can be properly described through the analysis of the strongly connected components (SCCs). Arrows represent asymmetric competition pointing from the loser to the winner species (e.g., A outcompetes B under transitive competition), which results in competitive exclusion (crossed out taxa).

(reviewed in [11]). However, a scenario of simultaneous transitive and intransitive competition is more consistent with our current knowledge on soil bacterial communities, where some phyla (i.e., Proteobacteria and Actinobacteria) outcompete other groups. However, their dominance does not compromise species richness because environmental heterogeneity in resource availability promotes coexistence within the dominant phyla [12]. Compared to experimental systems, soils shelter extremely complex assemblages of multiple species with differential competitive abilities on the multiple limiting resources, where not all species may compete with all others due to spatial or temporal segregation. A graphical example of spatial fragmentation reducing competition between kin comes from the human skin microbiome, where closely related genotypes of the same species coexist at the centimetre scale, each genotype colonizing a single skin pore [13]. This situation is very likely in soil microorganisms given the extremely heterogeneous nature and dynamism of the system, where pores of various shapes and diameters configure a tridimensional labyrinth [14]. Environmental heterogeneity, together with niche and life history differences between species (i.e., differential tolerance to predators or pathogens), are likely to

prevent any single species from outcompeting all others everywhere and every time they co-occur. Under complete segregation, for instance in highly structured environments, species will simply avoid competition. However, under less extreme scenarios, environmental heterogeneity, niche or life history differences may modulate the importance or access to limiting resources, and thus continuously shift the rules of competition. In this case, a complex network may contain groups of species that interact intransitively between them, possibly forming loops of different lengths, and that interact also transitively with other species. The immediate question is how to predict which subsets of species will be able to coexist.

### CLASSIC ANALYSIS OF PAIRWISE INTERACTIONS DOES NOT SUFFICE TO UNDERSTAND COMPETITION

The outcome of the interaction between two competing species can change depending on the presence of other species in the community. The presence of third species may induce the emergence of interaction chains (i.e., pairwise interactions connected to other pairwise interactions forming a complex network) and higher-order interactions (i.e., when the density of other species determines the impact of one competitor on another) [15]. As a consequence, the dynamics of species coexistence in systems with more than two species cannot be simply extrapolated from the analysis of isolated pairwise interactions. For example, for three species competing under Lotka-Volterra dynamics, it is possible to find sets of model coefficients so that coexistence of each pair in isolation is possible but the three species cannot coexist when simultaneously present; the other way around, one can find sets of coefficients so that none of the pairs would coexist in isolation but the three species could coexist when they co-occur [16]. With more than two species competing, the structure of the interaction network enters into play to determine coexistence [3, 17]. In hyperdiverse systems, like soil bacterial communities, the complex network of competitive relationships established among all the taxa will necessarily determine the competitive dynamics of the system beyond pairwise interactions. For this reason, the analysis of pairwise competition experiments often fails in predicting the outcome of experiments involving multiple bacterial species. Indeed, Friedman et al. [18] conducted competition experiments involving 8 soil bacteria cultured in all possible pairs, all trios, all 7-species combinations, and the 8 species. Using the observed outcomes of pairwise interactions, they could correctly predict the set of species persisting in 40 out of 56 possible trios, but in none of the 8 combinations of 7 species. Still, when using the observed outcome of trios, they could predict the persisting species in two of the 7-species experiments. Finally, they could not fully predict the set of species persisting in the 8-species competition experiment.

Experimental considerations of the structure of interactions in multispecies systems typically use arrangements of interactions between small groups of species [18, 19], falling short from the diversity of most ecological communities. The ecological networks perspective [20, 21] seeks to address this challenge by exploring a relevant set of the pairwise interactions that occur within communities. This increase in the scale of complexity is made at the expense of detail on the properties (e.g., strength, frequency, linearity) of each pairwise interaction. Nevertheless, some dynamic or stability properties of the community can be qualitatively determined from the structure of the interaction network.

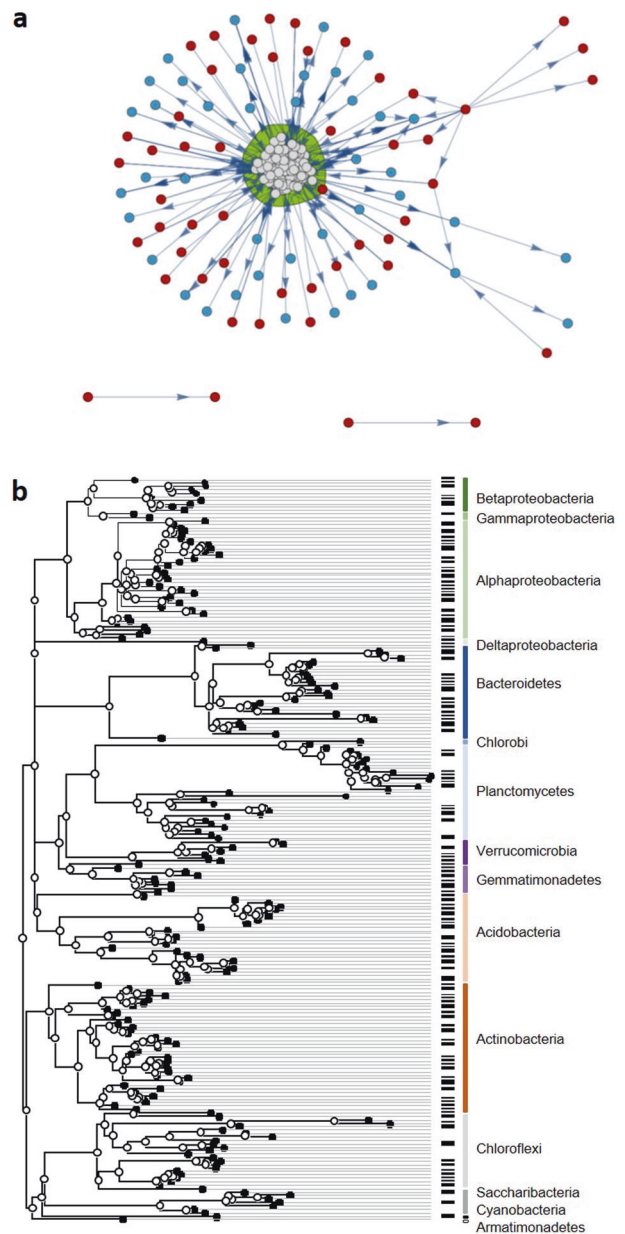
### DISENTANGLING TRANSITIVE AND INTRANSITIVE GROUPS: STRONGLY CONNECTED COMPONENTS

A way to disentangle complex networks in terms of transitive and intransitive groups is the detection of strongly connected

components (SCCs), which are key structural components of directed ecological networks (i.e., those containing information on the directionality of the interactions) that explain stability properties [3, 22]. The SCCs of a network are the largest non-overlapping subgroups of nodes such that all nodes in each subgroup are connected through at least one closed directed walk, where each node can be reached from any other node following the direction of the arrows (Fig. 1b). A SCC can comprise any number of nodes, from just one (so-called trivial SCC) to all the nodes of the network. Accordingly, a network of  $n$  nodes can contain between 1 and  $n$  SCCs, and may contain several non-trivial SCCs of different sizes. The relevant point here is that a non-trivial SCC is an intransitive group of competing species, and that different trivial and non-trivial SCCs are connected forming transitive groups (Fig. 1b). The set of potentially coexisting species is determined by how the different SCCs are connected and by which one dominates the dynamics. This last aspect is not possible to discern without knowledge of the details of the interactions, but under most circumstances one can expect that the largest SCC will dominate [3, 22]. Even though the arrangement of number and size of SCCs within a network can potentially be enormous, directed networks can be described in three parts as follows: the largest SCC that forms the intransitive core of the network, a set of transient trivial and/or small non-trivial SCCs that are outcompeted by some species from the core, and a set of satellite trivial and/or small non-trivial SCCs that can outcompete some core species. Species in the core and satellite SCCs have the largest probability of coexisting. Although both satellite and transient taxa are involved in transitive competitive interactions, the former are not necessarily doomed to local extinction because they can survive by outcompeting some species of the core.

In order to evaluate the simultaneous existence of transitive and intransitive interactions in natural soil bacterial communities, we analysed the structure of SCCs in two ecosystems in southeastern Spain. We followed a four-step workflow, which is described in detail in Appendix S1. Importantly, we used this procedure for illustration purposes only, rather than with the aim of proposing a methodological tool, given the current limitations associated with our approach that includes the use of co-occurrence networks. In brief, we computed SCCs from a putative competition network based on the detection of OTU (operational taxonomic unit) pairs that show mutually excluding patterns, and whose distribution cannot be explained by chance, spatial or environmental parameters. In our putative competition network, the links are directed from the loser to the winner OTU, indicating that the winner receives a benefit (e.g., access to more resources). In this way, the network can be interpreted in terms of the flow of benefits between OTUs, just like food webs can be interpreted in terms of flows of biomass between species. We considered that the largest SCC (with more OTUs) was the core of the network, which most likely dominates the dynamics of the community. We then found whether each non-core SCC was connected to the core in the direction of the arrows or reversely. Those connected with the core in the direction of the arrows are considered as satellite SCCs. The OTUs in satellite SCCs directly or indirectly benefit from some core OTU. In turn, SCCs connected with the core in the opposite direction, or disconnected from the core, are considered transient SCCs, which do not receive any benefit from the core. Finally, we compared the observed number of core, satellite and transient OTUs against their frequency distribution in a set of 10,000 random networks.

Analysing these two independent datasets we reach the conclusion that transitivity and intransitivity simultaneously shape the coexistence of soil bacterial taxa, determining the formation of strongly connected components. Both ecosystems are semi-arid Mediterranean, but are located 100 km apart with soils originating from different parent materials (metal polluted mine tailings vs. gypsum outcrops) and sustaining distinct plant communities



**Fig. 2 Strongly connected components in a soil bacterial competition network.** **a** Strongly connected components in the soil bacterial competition network in mine tailings (Cartagena-La Unión, Spain). Each OTU is represented by a node. The arrows indicate significant mutual exclusion interactions, pointing from the loser to the winner OTU. The core SCC is shaded in green with its nodes indicated in grey. Blue nodes are satellite OTUs and red nodes are transient OTUs. **b** Taxa within the core are widespread across the phylogenetic tree of the bacteria.

(Appendix S1). In both cases, the topology of the competition networks is composed of just one intransitive structure (the core), a common feature in other ecological networks [22]. For a given network, coexistence is more likely when most taxa belong to a single non-trivial SCC than when the same number of taxa are distributed across multiple intransitive structures [3]. The core of the bacterial network included OTUs widespread through several phyla that intransitively compete among them (Fig. 2; Appendix S1). Although these represent a significant proportion of OTUs in the network, they were fewer (mine tailings) or equal (gypsum outcrops) than expected in random networks of the same number

of nodes and links. The observed ratio of satellite to transient species was well within the range found in random networks. With the observed network structure, the majority of the OTUs could coexist in the long term, either by participating in the intransitive structure of the core or by benefiting (in)directly from core OTUs. This high level of persistence was nevertheless either lower or equal than expected in random networks. The other way around, the percentage of transient species in the empirical network was larger or equal than expected in random networks. The large proportion of transient species in metal-polluted mine tailings fits well with the idea that the soil bacterial community is away from equilibrium, as could be expected given the large disturbance in this system [23]. In brief, data taken from two independent ecosystems indicate that the soil bacterial network is simultaneously structured by both transitive and intransitive competitive interactions. Although the proportion of species involved in intransitive competition is lower or equal than expected by chance, it suffices to maintain the huge bacterial diversity found in the soil. These results reconcile the apparent contradiction between experiments showing that most taxa pairs outcompete each other when isolated from the rest of their community members but coexist when living in the context of a multispecies community [24].

#### CURRENT LIMITATIONS AND FUTURE RESEARCH

The necessary step forward is to validate the inferences on coexistence based on the analysis of SCCs in microbial networks. However, both experimental and observational approaches seem impracticable in the short run for different reasons. Experimental validation requires setting up complex competition experiments that include both pairwise and multiple species combinations to account for the emergence of higher-order interactions. If coexistence is an emergent property of the interactions network, it will be inherently difficult (if not impossible) to predict accurately such property from knowledge of the basic units of the network in isolation (i.e., from the outcome of interactions between pairs of species isolated from the rest of the community). As the study of Friedman et al. [18] suggests, community-level coexistence will be more accurately predicted from experiments involving larger subsets of the community, not just pairs. It will be interesting in future experiments to (1) observe the outcome of the interaction between a given pair of species by placing it within a larger subset of the community (trios, quartets, dozens, or more complex associations), (2) build the interaction network from the outcomes of these subsets, (3) use the derived network to predict sets of species that could coexist in different mixtures, and (4) validate such predictions experimentally.

Future experiments should ideally include a wide diversity of taxa, given the opposing results obtained in the literature when dealing with different lineages as reviewed above. However, these experiments are necessarily run with cultivable organisms, thus overlooking the vast majority of microorganisms that cannot be cultured in standard media because of their dependence on public goods provided by other organisms, their low growth rates or simply the lack of basic information on their biology. In order to reduce the mismatch in the outcomes of experimental and observational approaches (e.g., [19]), validation experiments should also account for the variety of factors that are known to modulate competitive hierarchies, including environmental heterogeneity and harshness, as well as the availability of multiple limiting resources. Factors such as resource concentration can change the strength of ecological interactions, and others such as the genetic distance [10] or environmental harshness [25] can even shift the sign of interactions from cooperation to competition. To add complexity, rapid evolutionary changes might alter the outcome of competition experiments [18].

Observational field studies aiming to disentangle the ecological mechanisms behind microbial exclusion patterns in soils also suffer from several limitations. Among them, there are 1. the mismatch between the spatial scale at which microbial interactions occur and the scale of sampling, 2. the difficulty to discern the relative influence of factors other than ecological interactions (e.g., stochastic, climatic, or soil factors) in the generation of exclusion patterns, 3. the need to technically solve the possibility to include rare taxa in network reconstruction, or 4. to address the reproducibility of soil microbial networks (reviewed in [11]). Assuming that such limitations are overcome, one way to verify the analysis of SCCs under field conditions would consist in (1) building the interaction network for a given local ecosystem, (2) determine the communities present in a set of validation samples from the same ecosystem, and (3) assess to which extent the communities and species present in validation samples are more likely to persist according to the network as compared with a sample of the same number of nodes taken at random from the network. In addition, repeated samplings of the same community with time would help to validate the predictions emerging from the transitive or intransitive structure of the soil bacterial network in terms of richness, diversity and stability. Combining controlled experiments, observations and modelling approaches with accurate reconstructions of interaction networks should pave the road to a more complete understanding of the mechanisms behind the generation of the astonishing levels of microbial diversity found in soils.

#### REFERENCES

- Ghoul M, Mitri S. The ecology and evolution of microbial competition. *Trends Microbiol.* 2016;24:833–45.
- Hibbing ME, Fuqua C, Parsek MR, Peterson SB. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol.* 2010;8:15–25.
- Alcántara JM, Pulgar M, Rey PJ. Dissecting the role of transitivity and intransitivity on coexistence in competing species networks. *Theor Ecol.* 2017;10(2):207–15.
- Laird RA, Schamp BS. Competitive intransitivity promotes species coexistence. *Am Nat.* 2006;168:182–93.
- Kerr B, Riley MA, Feldman MW, Bohannan BJ. Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature.* 2002;418:171–174.
- Riley MA, Gordon DM. The ecological role of bacteriocins in bacterial competition. *Trends Microbiol.* 1999;7:129–33.
- Czárán TL, Hoekstra RF, Pagie L. Chemical warfare between microbes promotes biodiversity. *Proc Natl Acad Sci USA.* 2002;99:786–90.
- Kirkup BC, Riley MA. Antibiotic-mediated antagonism leads to a bacterial game of rock-paper-scissors in vivo. *Nature.* 2004;428:412–14.
- Higgins LM, Friedman J, Shen H, Gore J. Co-occurring soil bacteria exhibit a robust competitive hierarchy and lack of non-transitive interactions. *BioRxiv* (2017) 2017.08.16.175737. <https://doi.org/10.1101/175737>
- Fiegna F, Velicer GJ. Exploitative and hierarchical antagonism in a cooperative bacterium. *PLoS Biol.* 2005;3:1980–7.
- Goberna M, Verdú M. Cautionary notes on the use of co-occurrence networks in soil ecology. *Soil Biol Biochem.* 2022;166:108534.
- Goberna M, Verdú M. Phylogenetic-scale disparities in the soil microbial diversity-ecosystem functioning relationship. *ISME J.* 2018;12:2152–62.
- Conwill A, Kuan AC, Damerla R, Poret AJ, Baker JS, Tripp AD, et al. Anatomy promotes neutral coexistence of strains in the human skin microbiome. *Cell Host Microbe.* 2022;30:171–82.
- Erktan A, Or D, Scheu S. The physical structure of soil: determinant and consequence of trophic interactions. *Soil Biol Biochem.* 2020;148:107876.
- Levine JM, Bascompte J, Adler PB, Allesina S. Beyond pairwise mechanisms of species coexistence in complex communities. *Nature.* 2017;546:56–64.
- Hallam TG, Svoboda LJ, Gard TC. Persistence and extinction in three species Lotka-Volterra competitive systems. *Math Biosci.* 1979;46:117–24.
- Barabás G, Michalska-Smith MJ, Allesina S. The effect of intra- and interspecific competition on coexistence in multispecies communities. *Am Nat.* 2016;188:E1–12.
- Friedman J, Higgins LM, Gore J. Community structure follows simple assembly rules in microbial microcosms. *Nat Ecol Evol.* 2017;1:0109.
- Godoy O, Stouffer DB, Kraft NJ, Levine JM. Intransitivity is infrequent and fails to promote annual plant coexistence without pairwise niche differences. *Ecology.* 2017;98:1193–1200.

20. Allesina S, Levine JM. A competitive network theory of species diversity. *Proc Natl Acad Sci USA*. 2011;108:5638–42.
21. May RM. Will a large complex system be stable? *Nature*. 1972;238:413–14.
22. Alcántara JM, Rey PJ. Linking topological structure and dynamics in ecological networks. *Am Nat*. 2012;180:186–99.
23. Colin Y, Goberna M, Verdú M, Navarro-Cano JA. Successional trajectories of soil bacterial communities in mine tailings: the role of plant functional traits. *J Environ Manag*. 2019;241:284–92.
24. Chang C-Y, Bajic D, Vila J, Estrela S, Sanchez A. Emergent coexistence in multi-species microbial communities. *bioRxiv* (2022) 2022.05.20.492860; <https://doi.org/10.1101/2022.05.20.492860>
25. Piccardi P, Alberti G, Alexander JM, Mitri S. Microbial invasion of a toxic medium is facilitated by a resident community but inhibited as the community co-evolves. *ISME J*. 2022;16:2644–52.

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## AUTHOR CONTRIBUTIONS

MV, JMA and MG conceived the idea of the manuscript. JNC collected the data, and all authors contributed to data analyses. MV, JMA and MG wrote the first draft of the manuscript, which all authors reviewed.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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