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Review

Can a microbial community become an evolutionary individual?

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Microbial communities provide crucial services for human well-being, driving an interest in designing and controlling them towards optimised or novel functions. Unfortunately, promising strategies such as community breeding — sometimes referred to as 'directed evolution' or 'artificial community selection' — have shown limited success. A key issue is that microbial communities do not reliably exhibit heritable variation, limiting their capacity for adaptive evolution. In other words, microbial communities are not evolutionary individuals. Here, we provide an overview of the literature on evolutionary transitions in individuality and, with insights from paradigmatic organisms, build a multidimensional space in which the individuality of a multispecies community is characterised by three ecological traits: positive interactions, functional integration, and entrenchment. We then place microbial communities within this individuality space, explore how they can be directed toward increased individuality, and discuss how this perspective can help improve our approach to community breeding.

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Introduction

For centuries, humans have successfully used microbes to produce goods. From beer and bread to pharmaceuticals, most biotechnological applications involving microbes use single species. However, in nature, microbial functions are performed primarily by the collaboration of multiple species within communities [4]. This observation has generated an interest in manipulating whole communities towards optimised or novel functions.

Community breeding, directed evolution, or artificial community selection is a promising approach for steering communities to desired states [21]. In community breeding experiments, communities compete against each other for several cycles of selection. During each cycle, the best performing communities are assigned a score that allows them to preferentially reproduce in the following cycle [58]. Compared to bottom-up strategies, in which communities are designed from a mechanistic understanding of the interactions among its members, community breeding methods do not require knowledge of how community functions arise, allowing experimentalists to focus on a wider range of functions [63].

Unfortunately, community breeding experiments have shown limited success [10,2,69]. The reasons are varied. First, community functions in the laboratory often exhibit low heredity [1,63]. Second, due to the limited number of communities and their low species richness, laboratory settings are likely to accentuate the decrease in variability over time inherent to the process of directed selection. In addition, competition within communities is pervasive, and as a consequence, selection within the community can override selection at the community level [3]. In other words, these microbial communities are not evolutionary individuals.

The limited capacity of microbial communities for adaptive evolution raises questions about how evolutionary individuality emerges, what limits it, and whether we can control it. To explore these questions, here we provide an overview of the literature on the evolutionary individuality and, with the insights from paradigmatic organisms, propose an organising framework to explore the individuality of multispecies collectives such as microbial communities. The premise is that for community breeding in the laboratory to be successful, microbial communities must first increase their evolutionary individuality. Leveraging insights from the literature, we argue that the potential of microbial communities to respond to selection can be increased before breeding them. To guide this process, we construct an empirically tractable multidimensional space in which the individuality of a multispecies community is defined by three ecological traits: positive interactions, functional integration, and entrenchment. Our primary goal is to bring across ideas from the evolutionary individuality literature to propose strategies to increase the individuality of microbial communities used in community breeding experiments.

Evolutionary transitions in individuality

The framework of the *Evolutionary Transitions in Individuality* aims to explain how evolutionary individuals arise throughout evolution [9,51]. Generally, an evolutionary transition in individuality starts with the formation of a collective in which each member is an individual on its own. Then, during the transition, adaptations that favour the collective are gradually acquired, increasingly constraining the evolutionary potential of its members. By the end of a transition, the group has transformed: the individuals previously existing have lost their individuality, becoming parts of a higher-level individual and giving rise to a novel echelon of the biological hierarchy [7,9].

Evolutionary transitions in individuality can be classified into two types: fraternal and egalitarian [55]. Fraternal transitions are the transitions that occur among individuals of the same species, such as in the case of complex multicellularity [27]. Conversely, egalitarian transitions occur among distantly related individuals, such as in the rise of the eukaryotic cell through endosymbiosis [17,57]. Fraternal transitions from unicellular prokaryotes to large multicellular organisms with differentiated cell types have occurred several times throughout the history of life [27]. In contrast, egalitarian transitions leading to the emergence of complex multicellularity have not yet been observed. This does not mean that egalitarian transitions towards multicellularity have never taken place. Rather, they have likely been transient and minor [59]. Perhaps because of this, the theory of evolutionary transitions has focused on fraternal transitions, leading to a historical bias and a gap in theoretical work that limits the generalisation of the transitions in individuality.

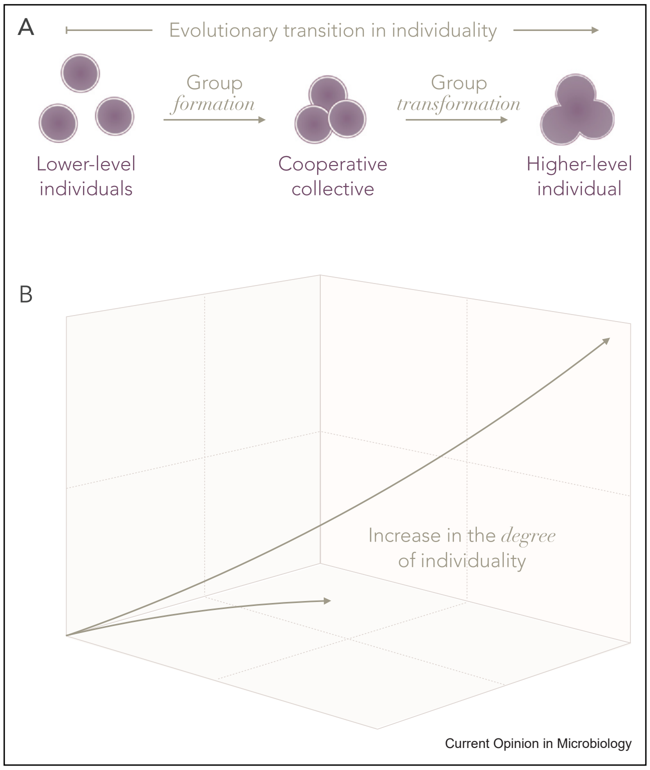
Descriptions of the evolutionary transitions in individuality often include the formation of a collaborative group and its transformation into a higher-level individual through higher-level adaptations [7,67] (Figure 1a). More recently, Godfrey-Smith developed the concept of Darwinian space, a multidimensional trait space that illustrates to what extent a collective has transitioned into a paradigmatic individual. That is, how much a collective resembles our intuitions of what an organism is [19] (Figure 1b). This space highlights the continuous character of individuality and allows to place both paradigmatic and nonparadigmatic cases in the same ground, facilitating thinking about their intermediate stages. In the following, we use the Darwinian space as an organising tool to describe the degree of individuality of microbial communities. Microbial communities are what Godfrey-Smith calls marginal individuals: organism-like entities, but not all the way there [19]. With this framework, we take a step toward expanding on the theory applicable to fraternal transitions in individuality.

Reshaping the Darwinian space to capture the individuality of multispecies collectives

To describe the evolutionary individuality of multispecies collectives such as microbial communities, we make use of Godfrey-Smith's concept of the Darwinian space but replace the axes he uses to describe organisms that undergo single-cell bottlenecks, with three axes that we propose are more appropriate for describing multispecies collectives: *positive interactions*, *functional integration* and *entrenchment* (Figure 1b). It is important to note that these axes are *not* independent, neither is our list of traits exhaustive. Our goal here is to introduce the Darwinian space as a complementary way of thinking about the individuality of multispecies collectives beyond variability and heredity (as in Lee et al. [35], e.g.), as we expect variability and heredity to emerge from traits such as those we use for our Darwinian space. Moreover, each trait provides an opportunity for intervention, helping us design strategies that build on the collective understanding acquired in different areas of microbial ecology. We illustrate the importance of each of our three axes through their ubiquity in paradigmatic individuals such as higher metazoans and provide a roadmap for potential ways to define them when it comes to nonparadigmatic cases.

Our first axis measures *positive interactions*, or cooperation, among community members. Mutual cooperation is a fundamental criterion for defining evolutionary individuals because it creates a positive correlation between the fitness of partners so that the benefits provided to a partner are returned to the actor [46,55]. Yet, not all cooperative collectives become individuals. The emergence of so-called cheaters, noncooperating members of a community that benefit from cooperative partners, poses a threat to the maintenance of cooperation [67]. All else being equal, cheaters have a fitness advantage over cooperators because they do not pay the cost of cooperation while benefiting from it. In paradigmatic organisms, cheating is tightly controlled, ensuring that conflict within the organism is low [11,19]. For example, although cancerous cells within paradigmatic organisms have a fitness advantage over other cells, their evolutionary fate is tied to the evolutionary success of the organism they inhabit [30]. Hence, cooperation, while important, is not sufficient to describe individuality; additional mechanisms must be in place to ensure its evolutionary stability.

Figure 1



Two ways of thinking about evolutionary individuality. **(a)** Classical description of a transition in individuality. First, lower-level individuals come together to form a cooperative group. Then, the group transitions to a higher-level individual by the acquisition of higher-level adaptations. Here, evolutionary individuals lie both at the beginning and at the end of a transition. **(b)** Evolutionary individuality described as a point in a multidimensional space. In this space, there are no discrete transitions in individuality, rather, collectives exhibit greater degrees of individuality the further they find themselves from the origin (arrows). Dimensions in the space describe mechanisms and thus vary across systems of inquiry. To represent this flexibility, we leave the axes free of labels. (a) Adapted from Ref. [66]. (b) Adapted from Ref. [19].

Our second axis, *functional integration*, measures how much members of a collective specialise to carry out different tasks. Functional integration is the characteristic that defines a true collective function, thus creating interdependencies among community members. Notice that our definition of functional integration does not make assumptions about the evolutionary origins of the benefit. For example, functional integration can emerge as a consequence of metabolic loss [48]. What is important is that once these interdependencies are established, selection can act on them [28], opening the possibility of acquiring collective-level adaptations. In paradigmatic individuals, one of the most salient examples of functional integration is germ-soma separation. Separation of germinal and somatic lines ensures that selfish somatic mutations are not transmitted from parents to offspring, reducing the potential for conflict within the organism [9,11]. At its most extreme, the germinal line will undergo a single-cell bottleneck, eliminating all variation within the organism at the beginning of development. The capacity of undergoing a single-cell bottleneck is absent in multispecies individuals, limiting their chances of becoming paradigmatic individuals. But multispecies collectives need not become paradigmatic cases. Instead, we can strive to drive them towards greater degrees of individuality.

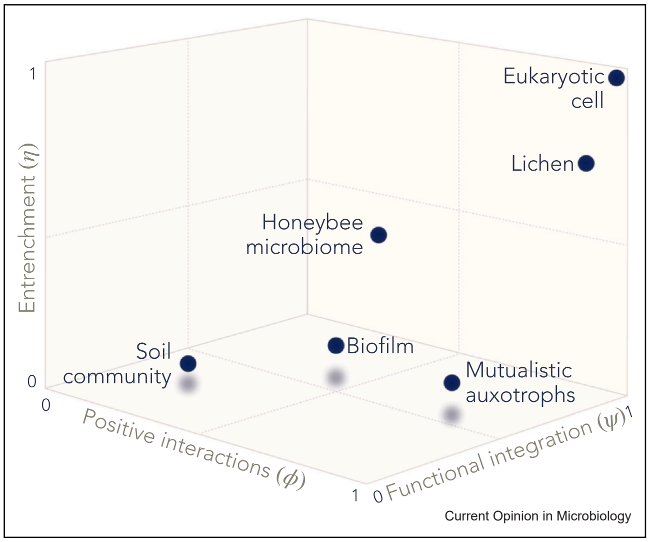
Our third axis, *entrenchment*, refers to the robustness of a collective's degree of individuality. The central idea of entrenchment is based on the observation that paradigmatic individuals are cohesive entities whose structure is internally, rather than externally, defined. Humans and other higher metazoans are highly entrenched, regardless of their surrounding environment. No parts of us, whether our cells or organs, could become autonomous if the context in which we find ourselves changes.

Entrenchment is a much less studied aspect of individuality, and its definition has thus far been contingent on the specific mechanisms that bring it about. For example, in Ref. [38], entrenchment is defined as the probability that the cells of a multicellular individual reverse to their ancestral independent state [12]. This definition requires discrete individuality states and cannot accommodate a continuous definition of individuality. An alternative approach is to define entrenchment as an analogue of structural stability [50,60], focusing on the capacity of a multispecies collective to preserve its identity in different environments. Entrenchment is then defined as the parameter space where the degree of positive interactions and functional integration of a multispecies individual remain above a given threshold.

The individuality of microbial communities

Microbes exhibit a wide range of social behaviours [47], from competition that drives the evolution of weapons [22] to highly altruistic behaviours such as altruistic suicide [43,56]. Amidst the vast diversity of microbial lifestyles, some have captured the imagination of researchers because of their resemblance to evolutionary individuals (Figure 2). Biofilms, for example, have been considered individuals because they often interact with their surrounding environment as cohesive wholes [13] using the extracellular matrix as a medium for metabolic exchange and protection against environmental stress [8,14]. Similarly, auxotrophic bacteria engaging in mutualistic cross-feeding are used as a model system for the evolution of cooperative division of labour [18]. In contrast, to our knowledge, soil communities are not thought of as individuals. Neither do we know of any considerations of host-associated microbiomes as individuals on their own. However, the individuality of

Figure 2



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The individuality of multispecies collectives. All microbial collectives are placed according to intuition. Our intuitions are guided by three questions: What is expected sign of the interactions dominating the collective? What are the main functions of the collective and how is labour divided among members? How often are the members found outside of the collective? The Eukaryotic cell, a paradigmatic case of an egalitarian transition from a symbiotic association of prokaryotes, is placed at the highest point of the individuality space. To describe the Eukaryotic cell as a collective, we consider the relationship between the symbiotic partners as if they would exist outside the association. Because the symbiotic partners can only reproduce within the relationship, the Eukaryotic cell lies high in the axes of positive interactions and entrenchment. Similarly, we imagine perfect, or near-perfect, functional integration, as one of the partners became an organelle of the other [39,57]. Lichen, our only example of an egalitarian multicellular collective, lies high in the individuality space because free-living populations of symbiotic partners are rare, and lichen fungi require the presence of a phototroph partner to complete their sexual cycle [62]. Similarly, the honey bee microbiome lies high compared to other microbial communities because it is vertically transmitted and some of its members are rarely found outside the association [40].

microbiomes has been discussed in the context of the holobiont, which considers the host and its microbiome as one individual [6]. What is the difference between these communities, where would they score in our Darwinian space, and how would one go about measuring the three traits that we propose to describe their individuality?

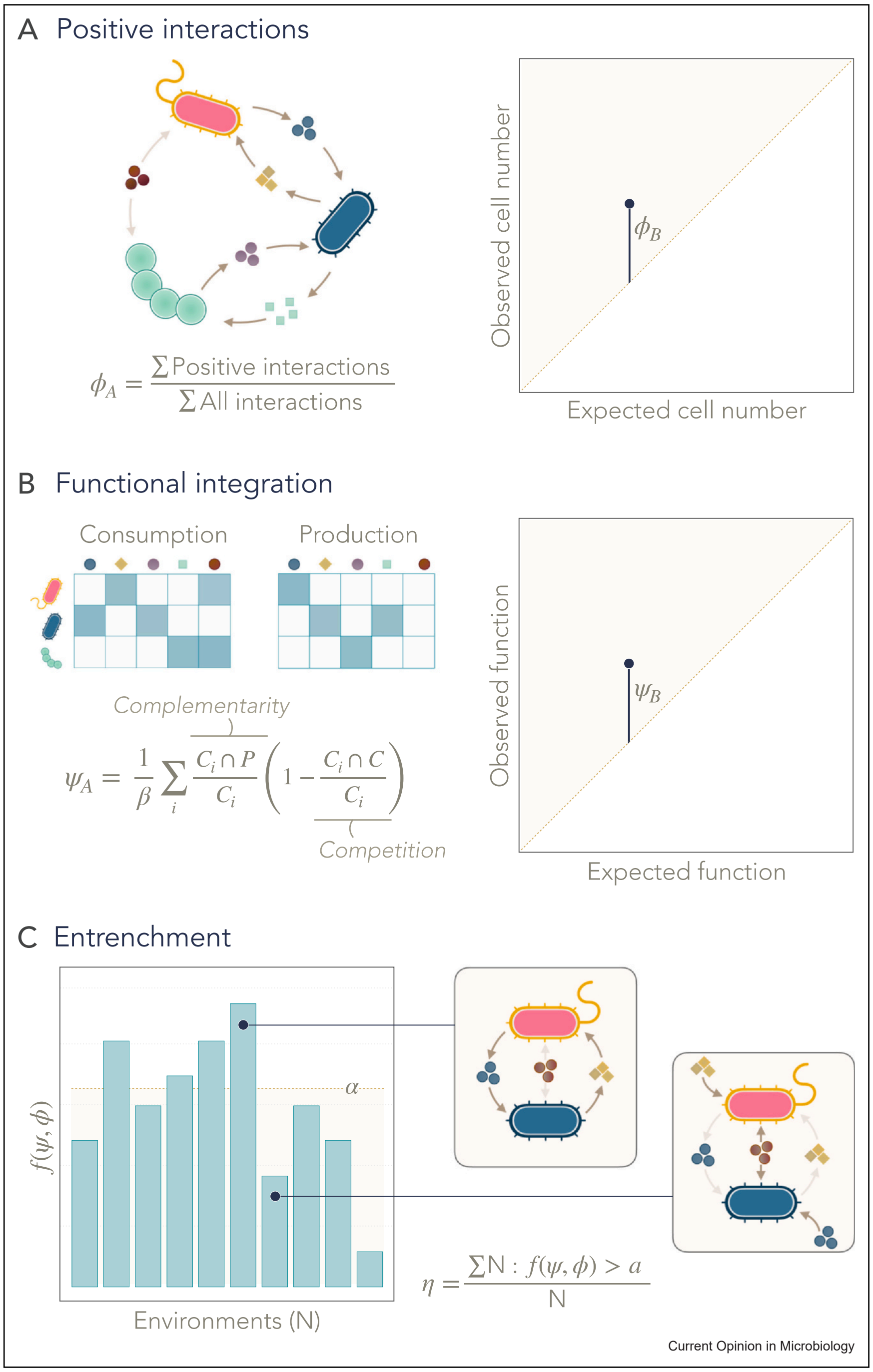
Measuring individuality as three ecological traits

To measure the first trait, positive interactions, within microbial communities, studies often focus on pairwise interactions, which are then used to build interaction networks of the entire community [45]. Measuring pairwise interactions has often proven successful, as many communities, at least in the laboratory, exhibit transitive linear interactions [16,20]. In such systems, the degree of positive interactions at the community level, ϕ_A, can be quantified as the ratio of positive interactions relative to all possible interactions within the community (Figure 3a, left). For example, a recent article measured all pairwise interactions between 20 soil bacteria across 40 environments, finding that around 20% of the 180 408 measured one-way interactions were positive (i.e. $\phi_A = 0.2$) [33]. However, microbial communities often exhibit higher-order interactions, which cannot be captured through pairwise interactions [44]. In such cases, there are several alternative ways to measure ecological interactions (for a recent review, see Ref. [45]). One alternative, for example, is to grow each member of the community on its own (monocultures) and in the entire community (cocultures) and compare them [15,32] (Figure 3a, right). Using biomass as a proxy of fitness, the sum of the biomass of the community members in monocultures serves as a neutral model of interactions. The difference between the observed growth and the neutral expectation is their degree of positive interactions, ϕ_B. If the observed growth in cocultures is greater than the neutral expectation, the members of the community are likely to exhibit positive interactions. One limitation of this approach is that a higher overall community biomass does not guarantee that all interactions are positive — a single member could greatly benefit from the community while still decreasing the biomass of other members [47]. These methods describe a phenomenological understanding of positive interactions. For a mechanistic understanding of interactions within communities, we can use consumer-resource and metabolic models [45]. However, such descriptions are more relevant to measure functional integration.

Measuring functional integration, ψ, requires an understanding of how the community function of interest is divided among community members. Community functions can be very varied, for example, how much biofilm a community produces [26], to what extent a community enhances the health of its host [25,31], or how much of a metabolic product of interest it produces [34]. Here, we will use this latter category of metabolic functions to demonstrate how to measure functional integration, but our approach can be extended to other types of functions.

To characterise the metabolism of a community, the capabilities of each member can be assessed from monoculture growth data on individual metabolites combined with metabolomics analyses on the resulting spent media [54,65]. For species that cannot be cultured alone, the metabolism can be inferred using genetic data in combination with metabolic models [42,61,70,41]. Once the metabolism of each member is described, we propose to divide it into two general categories: consumption and production. The consumption and production profiles of a community member are then, respectively, the set of compounds that it acquires from

Figure 3



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Measuring individuality in microbial communities. **(a)** Positive interactions can be measured in two ways: as the ratio of positive pairwise interactions (ϕ_A) or the difference in abundance of a species when grown in monoculture versus in community (ϕ_B). In this example, $\phi_A = 4/5$. Note ϕ_A and ϕ_B are in different units and not directly comparable. **(b)** The metabolites that each species of the community illustrated in panel (a) consume and produce are shadowed in blue. Functional integration can be measured as the average of the product of complementarity times competition for each of the β species in the community, where C_i is the set of all metabolites that species i consumes, and C and P are, respectively, the set of metabolites consumed and produced by the entire community. In this example, $\beta = 3$ and $\psi_A = 4/9$. Alternatively, functional integration can also be measured as the difference in function when species are grown in monocultures versus in community (ψ_B). **(c)** Entrenchment is measured as the fraction of tested environments in which $f(\phi, \psi)$ is larger than a threshold a. In this example, a mutualistic pair of species is shown in two different environments. Left: only one metabolite is supplied, resulting in observed cross-feeding and high $f(\phi, \psi)$. Right: the cross-fed metabolites are supplied, resulting in observed competition and lower $f(\phi, \psi)$. In this figure, $\eta = 4/10$.

the environment and secretes into the environment. These profiles can be used to measure competition and complementarity [36]. Competition measures how much of what a species i consumes is consumed by any other member of the community. In contrast, complementarity measures how much of what a species i consumes is produced by any other member of the community. Together, competition and complementarity describe functional integration, ψ_A, in microbial communities (Figure 3b, left). In cases where trait information is not available, an alternative is to measure and compare community function in mono- and co-cultures. The general idea is analogous to that of positive interactions: the measured function in monocultures provides a neutral model that can be compared with the function in cocultures. Synergies in function can be interpreted as the degree of functional integration, ψ_B (Figure 3b, right). Here, special care should be taken to ensure that the function of interest is a true community function, rather than a function that members of the community can do on their own. Otherwise, if each member of the community can perform the function on its own, and the function correlates with cell number, then it is not functional integration that is being measured, but positive interactions.

To quantify entrenchment, η, we propose exposing microbial communities to a wide range of environments and measuring positive interactions ϕ and functional integration ψ in each. Entrenchment is then the fraction of environments in which a given minimal threshold a of a function of the two measures ($f(\phi, \psi)$, e.g. $\phi \times \psi$) is achieved (Figure 3c). These experiments can be time-consuming and expensive. Thus, it is sensible to complement experiments with models when possible and to exploit the knowledge about the systems of interest. For example, looking back at Figure 2, mutualistic auxotrophs are hypothesised to exhibit low entrenchment despite their strong interdependence because such dependence can easily be reversed if the growth medium contains metabolites for which they are auxotrophic. But perhaps a more relevant question is whether natural environments are likely to contain these metabolites. A recent paper has compiled data on vitamin concentrations in the ocean, finding that vitamins are limiting and auxotrophic growth is likely by cross-feeding [23].

Increasing individuality for community selection experiments

Experiments of community selection have largely ignored selection occurring within communities, perhaps assuming that it would be overridden by selection at the higher level (but see Refs. [63,2,49]). This intuition is further reinforced because in a selection experiment, some properties of individuality are scaffolded on communities [5,24]. For example, separating communities in test tubes allows for the emergence of parent–offspring relationships, providing the context in which heredity can emerge. Generally, scaffolds have been hypothesised to provide collectives with a structure to acquire collective-level adaptations, helping them transition to a higher level of individuality. These adaptations are expected to lock the collective at the higher level of individuality in a process known as scaffold endogenisation [5,51].

However, previous studies suggest that microbial communities could have limited capacity for scaffold endogenisation. In Ref. [64], for instance, the authors tested a novel selection algorithm to increase pollutant degradation in small bacterial communities *in silico*. Over the course of 50 rounds of selection, the average pollutant degradation significantly improved, compared to the best ancestral communities. Nonetheless, as soon as the communities were no longer under selection, pollutant degradation decreased as important community members went extinct, suggesting that selecting for increased function does not necessarily increase the degree of individuality of microbial communities.

How can we then increase the individuality of microbial communities? We envision a selection experiment in which communities subjected to artificial selection are not randomly assembled but are designed such that evolutionary individuality is maximised at the beginning of the experiment. We hypothesise that the response to selection should correlate positively with the average degree of individuality, as measured by our three axes. Furthermore, even though selection for improved function might reverse the transition in individuality, we hypothesise that communities whose transition is irreversible will outcompete those in which within-community competition reemerges. Testing these hypotheses would help to improve and refine the definitions of the three axes.

Our approach can be summarised under the notion *individuality first, selection second*. Given a pool of microbial species, we argue that functional integration can be increased by assembling combinations that maximise metabolic complementarity while minimising competition. Positive interactions, in turn, can be increased by making the environment more challenging to grow in. For example, environmental toxicity [53], low concentration and/or diversity of nutrients [29,33], and lack of oxygen [52] have been shown to increase positive interactions between species. Incorporating these ideas into the implementation of selection experiments should increase their chances of success. Although it is yet unclear how to increase entrenchment without using scaffolds, increasing other dimensions could in turn increase entrenchment. Indeed, within nascent multicellular individuals, functional integration has been found to decrease the relative fitness of unicellular revertants, thereby increasing the evolutionary stability of multicellularity [12]. This suggests that simultaneous manipulation of multiple dimensions of our individuality space is possible.

Conclusions

Microbial communities are not evolutionary individuals, limiting the success of community breeding experiments. To address this issue, we propose an organising framework where evolutionary individuality is defined by how much a collective exhibits positive interactions, functional integration, and entrenchment. Our framework is intended to provide a complementary perspective to discussions on selection and individuality of microbial communities, creating testable predictions, and helping experimentalists design more effective community breeding strategies. More specifically, it allows us to place multispecies collectives, such as microbial communities, in an individuality, or Darwinian, space and propose strategies to increase their degree of individuality, thereby increasing their response to selection.

One of the major challenges of our multidimensional space is that it does not tell us how much individuality is enough to exhibit a desired evolutionary response. Nor can we directly map our individuality space to the classic definition of evolutionary individuality of heritable variation. More theoretical work should also aim to better bring across concepts like cooperation and reproduction to multispecies collectives. Addressing these challenges should help to bridge our understanding of the ecological dynamics of multispecies collectives to evolutionary individuality, from which we expect to learn how to drive microbial communities to become more like individuals, allowing us to harness evolution as an engineering tool, and ultimately to more effectively use microbial communities — and not just individual microbial species — in biotechnological applications.

CRediT authorship contribution statement

Afra Salazar: Conceptualization; Visualization; Writing – original draft; Writing – review & editing. **Sara Mitri:** Conceptualization; Supervision; Writing – review & editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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- • of special interest
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Glossary

Community reproduction: The transmission of a sample of an adult parent community to one or more sterile environments or offspring communities.

Evolutionary individual: The objects on which selection acts. That is, entities whose populations exhibit phenotypic variation, differential fitness and phenotypic heritability [37].

Heredity: Parent-offspring similarity. When measured as the correlation between parents' and the average of offspring's trait values, it is known as heritability.

Paradigmatic individual: The end point of an evolutionary transition in individuality. Examples include eukaryotic cells and higher metazoans. The term is closely related to that of an evolutionary individual.

Community function: Community-level activities that can only be achieved through the collaboration of multiple community members.

Interaction: The phenomenological description of the fitness effects of one biological entity on another.

Ecological scaffolding: An external structure that imposes individuality criteria on biological entities such that variation, reproduction, and heredity emerge [5].

Cooperation: A behaviour which provides a benefit to another individual (recipient), and which is selected, at least partially, because of its beneficial effect on the recipient [68].