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Phytobiomes Journal A Transdisciplinary Journal of Sustainable Plant Productivity

PERSPECTIVE

Exploring Diverse Approaches to Iterative Microbiome Passaging in Soil and Plant Systems

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Accepted for publication 12 February 2025.

Abstract

Efficient use of managed land depends on our ability to optimize relevant processes (e.g., crop growth) in that space. Microbial activities are critical to this goal, given their enormous contributions to biogeochemical flux and organismal health. Unfortunately, we still cannot predictably harness their potential in the same way that we can introduce nutrients or manipulate plant composition, for example. In recent years, iterative microbiome passaging has been investigated as an approach for capturing and optimizing groups of microorganisms that contribute additively to functions of interest, such as plant growth promotion or litter decomposition. Early trials show that this approach can alter microbiome function, but functional gains can seem almost stochastic, unlike archetypes of breeding within individual lineages. In this Perspective, we highlight the importance of continuing to explore diverse approaches to iterative microbiome passaging in soil and plant systems, given our limited knowledge about how this process works. There is no single "best" approach, but experimental design choices can have large impacts on outcomes. Ultimately, we believe that a better understanding of different forms of iterative microbiome passaging will allow us to (i) leverage the power of uncultivated microbes, additive/synergistic microbial contributions, and intermicrobial interactions and (ii) understand how land use choices will shape the functional trajectories of microbiomes through time.

Keywords: agriculture, microbiome selection, probiotics, soil

Intentional microbial management in soil and plant systems (e.g., to enhance crop growth) has been ongoing for over a century but faces consistent challenges (Kaminsky et al. 2019). Among those is our general inability to tap the functional potential of "wild" microbiomes, from which most microorganisms cannot yet be cultivated. Processes shaped by microbiomes often result from the combined activities of many microbial types, whose relative contributions shift across diverse environmental landscapes. Understanding how even one microbe would interact with all its potential neighbors and environments is a lofty goal, and perhaps an unrealistic one.

Fortunately, we may be able to harness microbiomes by building our understanding from the top down rather than the bottom up (Faller et al. 2024). Although the term phenotype is typically applied to individuals, we can also identify emergent group "phenotypes" from microbiomes, referred to by Shuster et al. (2006) as "community phenotypes." By screening microbiomes based on emergent phenotypes, observing and capturing what microbes do in combination, we can focus on collective performance in various environments of interest. Microbiomes displaying target phenotypes can then be transplanted to alter function in new environments; for instance, inoculating soils with microbiomes has dramatically shifted key functions, such as plant survival and nitrogen cycling (Allsup et al. 2023; King et al. 2023).

However, just as the cob of corn and its wild relative teosinte have little in common, microbiome traits that are ideal from a management perspective may differ from what we find in nature. Thus, it may be of interest to passage and screen iteratively for beneficial microbiome phenotypes, as has been done through the breeding of individual organisms for many years. The end products of this passaging process can then be applied as probiotics for open agricul-

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Funding: Support for this work was provided by the Natural Sciences and Engineering Research Council of Canada (a Discovery Grant to T. H. Bell, RGPIN-2023-03411, and a USRA to S. Percy), the University of Toronto (UTEA to R. Ye and CRESS award to H. Ahmadpanah), the "Bio-inoculants for the promotion of nutrient use efficiency and crop resiliency in Canadian agriculture" project funded by the Government of Canada through Genome Canada and Genome Prairie (CSAFS-ICT 19308), and the Government of Ontario through an Ontario Research Fund-Interdisciplinary Challenge Teams (ORF-ICT) – Climate Action Genomics Initiative grant (File ICT 19308).

The author(s) declare no conflict of interest.

tural systems, additives in controlled environments, amendments to defined growth substrates (e.g., potting mixes, soil plugs), or used for other applications, such as bioremediation (e.g., Arias-Sánchez et al. 2024) or to facilitate litter decomposition. Such an approach does not only apply to the development of probiotics, as it can also be used to more quickly investigate how microbiome function shifts due to human land use choices (Bell and Tylianakis 2016), essentially, our inadvertent management of microbes in soil and plant systems.

Going by many names, iterative microbiome passaging has received increased attention in the past decade. Coincidentally or not, this follows large advances in high-throughput sequencing and early successes in using whole microbiomes for human benefit, such as in fecal microbiome transplantation (Fuentes et al. 2017). In this approach, microbiomes are bottlenecked at a set point in time and passaged forward to seed a new microbiome "generation" (we will refer to this by the term *mb*Generation, to avoid confusion with true organismal generations). Often, the goal is to generate groups of microbes that are increasingly effective at producing a target phenotype. Swenson et al. (2000) initially showed that some community traits could be targeted in this way. The forward passaging of microbiomes at each *mb*Generation can be untargeted (i.e., repeated passaging in a set condition, without screening of microbiome phenotypes) or directed by quantified group traits to select for increasing strength of phenotype (e.g., highest plant growth promotion), the latter of which is sometimes referred to as microbiome "breeding." Iterative microbiome passaging experiments do not necessarily need to be used to enhance any sort of phenotype. Instead, to model environmental change or land use choices, we might apply consistent or oscillating environments to shape microbiome trajectories and then assess the functional consequences.

Iterative microbiome passaging has led to impressive changes in microbiome-affected phenotypes, such as plant phenology and CO_2 output (e.g., Blouin et al. 2015; Panke-Buisse et al. 2015), but unlike the predictable shifts seen in single-organism breeding, phenotypes can oscillate wildly from one *mb*Generation to the next. For instance, Swenson et al. (2000) roughly tripled plant biomass in one *mb*Generation of a "high plant biomass" passaging treatment, with this difference vanishing in the next *mb*Generation, but then reappearing. Although the broad approach and goals of single-organism breeding and iterative microbiome passaging can be similar, these processes differ in critical ways. In single-organism breeding, we can (i) select discrete individuals after each replication event, (ii) know that all genes responsible for a phenotype are present within the same individual, and (iii) track factors such as the developmental stage and physiological status of the organisms at the time of selection. In iterative microbiome passaging, this will be much more challenging, if possible at all.

It is challenging to apply a single ecological or evolutionary concept to explain what iterative microbiome passaging actually does. Although some researchers directly explore the concept of community selection (e.g., Lean et al. 2022) or holobiosis (Doolittle and Booth 2017), natural or artificial selection is mostly understood at the level of genes and individual lineages, not communities. Yet few would argue that changing a community cannot have profound functional consequences. The gain or loss of keystone species or impactful invasives can reshape the biogeochemistry, productivity, and biodiversity of ecosystems (Wardle et al. 2011), and the evolution of organisms within a new species landscape can alter community-influenced processes (Lawrence et al. 2012). Even without fully understanding the processes involved, we can see that iterative microbiome passaging changes microbiome function, often in the target direction (e.g., Panke-Buisse et al. 2015; Swenson et al. 2000), and composition, with that change potentially plateauing after enough *mb*Generations (King et al. 2022; Morella et al. 2020).

Iterative microbiome passaging often follows the essence of traditional plant breeding, screening phenotypes of interest and transferring forward microbiomes from which those are most pronounced, such as microbiomes associated with early or late plant flowering (as in Panke-Buisse et al. 2015). In other cases, a phenotype-independent approach is applied, repeatedly passaging microbiomes in a set environment, without screening for traits of interest to guide the passaging process (as in Lawrence et al. 2016). This latter approach can also provide insights into potential microbiome trajectories in the field, in response to changing environments or agricultural treatments, for example. Overall, few such studies have been conducted in soil and plant systems (Table 1). In those that have, the methods used are in some ways quite narrow (e.g., *mb*Generation length is often 4 to 6 weeks), but with little consensus between studies on the details of the approach, including methods of microbiome transfer, which have been shown to have substantial effects on microbial composition (Howard et al. 2017). In this Perspective, we do not suggest a "best" method for iterative microbiome passaging but highlight some of the potential consequences and artifacts of different experimental choices (Table 2). We believe that there are many valid approaches and advocate for further exploring a wide range of experimental design conditions to better define the potential of this emerging biotechnology in soil and plant systems.

Recent Perspectives on Iterative Microbiome Passaging

Ideally, the use of iterative microbiome passaging for probiotic design will complement existing approaches for managing land and organisms. For instance, although plant breeding has led to huge gains in crop productivity, microbiomes are (i) a genetic reservoir that has barely been leveraged, from a management perspective; (ii) quick to adapt, meaning that new microbiome variants may be quicker to develop than new crop genotypes; and (iii) able to influence non-host features of cropping systems, including soil fertility and decomposition. Unsurprisingly, this has led to substantial recent interest in exploring iterative microbiome passaging in many scientific fields.

Several recent perspectives explore the processes that may drive iterative microbiome passaging and ways to better achieve target outcomes (e.g., Arias-Sánchez et al. 2019; Lawson et al. 2019; Mueller and Linksvayer 2022; Sánchez et al. 2021; Silverstein et al. 2023), although often focused heavily on scenarios in which initial composition is more easily controlled (e.g., communities in liquid culture). Sánchez et al. (2021) described two main ways to subset communities to create variation during microbiome passaging, subsampling from either a single parent pool (propagule method) or a mixed parent pool (migrant pool method). They also describe several mechanisms for creating such variation, including microbiome mixing (through active merging or allowing migrants), reducing microbiome diversity through bottlenecking, shifting environmental conditions to a new state, or directly adding or removing individual microbes. Lawson et al. (2019) highlighted ways to conceive of iterative microbiome passaging from an engineering perspective (e.g., top-down versus bottom-up design, the latter depending on initial control over strain composition), and Mueller and Linksvayer (2022) highlighted mechanisms and approaches to microbiome passaging, with specific emphasis on passaging microbiomes associated with eukaryotic hosts. Mueller et al. (2021)

TABLE 1
Studies that have used iterative microbiome passaging in plant and soil systems and some of the key experimental choices in those studies

Reference	Number of *mb*Generations	*mb*Generation length	Conditioning environment(s)	Passaging approach	Trait(s) targeted
Transfer of microbes in soil, potting mix, or soil-like substrate					
Swenson et al. 2000	16	35 days	*Arabidopsis*	Transfer of top/bottom performers	Plant biomass
Lau and Lennon 2012	3[a]	At plant senescence (likely >30 days)	*Brassica rapa*; wet and dry soil	Phenotype-independent transfer	Flowering/seeding traits; soil nitrogen
Panke-Buisse et al. 2015	10	Variable: ∼20 to 55 days	*Arabidopsis*	Transfer of top performers	Flowering time; EEA[b]
Tkacz et al. 2015	3	4 weeks	Multiple plants (*Arabidopsis*, *Medicago*, *Brachypodium*, *Brassica*, *Pisum*, and *Triticum*); varied nutrient conditions	Phenotype-independent transfer	Segregation of root-associated microbiome composition
Kaminsky et al. 2018	4[a]	3 weeks	*Medicago sativa*; varied nutrient conditions	Transfer of top performers	EEA, biomass, plant tissue nutrients, nodule counts
Lu et al. 2018	3	40 to 50 days	Acclimated to WT or pgr5 *Arabidopsis*	Phenotype-independent transfer	Flowering time + associated plant expression, microbial functional genes, soil nutrients, root exudates
Mueller et al. 2021	10[a]	20 to 32 days + 68 days for final *mb*Generation	Ramping salt stress	Transfer of top performers	Plant biomass; seed mass
Yin et al. 2021	9	4 weeks	*Triticum aestivum* cultivar Alpowa; *Rhizoctonia solani* inoculation	Transfer of top/bottom performers	Plant resistance to *R. solani*
Jacquiod et al. 2022, 2025	10	4 weeks	*Brachypodium distachyon*	Transfer of top/bottom performers + random	Leaf greenness; microbiome transferability across plants and soils
King et al. 2022	6	32 days	*Brassica rapa*; ± NaCl	Transfer of top performers + random	Plant biomass
Kalachova et al. 2023	11	5 weeks	*Arabidopsis*; *Pseudomonas syringae* DC3000	Phenotype-independent transfer	Leaf area postinoculation; plant expression
Morris and Bohannan 2023	5	4 weeks	Elevated CH_4 in closed jars	Transfer of top performers	CH_4 oxidation rate
Enders et al. 2024	6 to 10	3 to 4 weeks	*Solanum pimpinellifolium*; insect herbivory	Transfer of top/bottom performers + random	Insect herbivory
Transfer of soil-sourced microbes for hydroponic plants					
Faller et al. 2024	19 to 22	3 days	Hydroponic-like media; low/high phosphorus availability	Phenotype-independent transfer + transfer of top performers + random	Phosphorus solubilization
Transfer of microbes on leaf surfaces					
Morella et al. 2020	4[c]	6 weeks + 3 days; inoculation was repeated multiple times in each	Five *Solanum* genotypes	Phenotype-independent transfer	Host specificity of microbiome
Meyer et al. 2023	6	3 weeks; inoculation repeated multiple times in each	Multiple plants (*Solanum lycopersicum*, *Solanum pimpinellifolium*, *Capsicum annuum*, *Phaseolus vulgaris*); consistent vs. plant switching	Phenotype-independent transfer	Host specificity of microbiome
Ehau-Taumaunu and Hockett 2023	9	7 days	Inoculated with *Pseudomonas syringae* pv. *tomato*; tomato	Transfer of top performers + random	Disease severity on leaves

[a] Passaging was then followed by an additional reciprocal transplant *mb*Generation.
[b] EEA, extracellular enzyme activity.
[c] Passaging was then followed by an additional microbiome coalescence *mb*Generation with a sample subset.

suggested several interesting approaches for varying microbiome passaging using plant hosts, including focusing on different plant compartments, varying host traits, varying soil nutrient conditions for passaging, and manipulating microbiome diversity at the outset of iterative passaging.

These perspectives provide an excellent conceptual framework for thinking about how iterative microbiome passaging might work and some ways to approach it moving forward. As Sánchez et al. (2021) pointed out, the outcomes of early studies in iterative microbiome passaging have been mixed, particularly those using undefined starting inocula. Using an in silico modeling study, Chang et al. (2021) suggested that previous attempts at iterative microbiome passaging tended to be inefficient at optimizing target functions due to the fact that only one or a few initial microbiomes are the targets of iterative passaging. They instead suggested screening a variety of microbiomes that are first stabilized to a target environment. Although we generally agree with the approach proposed by Chang et al. (2021), they made some key assumptions in their work, including that generational stability in a microbiome (i.e., minimal change from one *mb*Generation to the next) is critical and that the initial function of a microbiome is predictive of its potential function (i.e., that higher-performing initial microbiomes will yield higher-performing offspring). They also simulated changes in species composition alone, rather than both composition and evolution. We know, however, that short-term evolution can have large impacts on microbial functioning, without changes in taxonomy. For instance, with no changes in species inclusion, Lawrence et al. (2012) showed large changes in cell growth rate and resource use when synthetic bacterial communities were evolved over 8 weeks, including an approximately 16% boost in supplied carbon use when isolates were evolved together rather than alone. Such large changes show that the potential function of a microbiome may far exceed what we would observe after taxonomic filtering alone due to the evolution of species and species interactions.

Prior perspectives have generally focused on maximizing microbiome performance within a controlled environment or, interestingly, optimization of a microbiome's environment to maximize microbiome performance (Sánchez et al. 2024). Where environments can be controlled and standardized en masse, this should be the goal. However, for many applications in plant and soil systems, environmental diversity and variability are not easily controlled or avoided. Different applications may thus require different approaches to iterative microbiome passaging. A few examples are provided below:

- To more efficiently break down plant material in batch culture, the best approach likely follows the principles outlined by Sánchez et al. (2021), Chang et al. (2021), and others, using diverse starting inocula and following their rational design principles to optimize function under these conditions. It would still be important to consider the extensive environmental changes that can occur in such systems (e.g., pH shifts) and how to capture microbiomes that are robust to those changes.
- Sometimes, environmental compatibility may be the function of interest, perhaps because establishing a diverse microbiome helps counter disease agents. For instance, Morella et al. (2020) aimed to generate microbiomes that established on leaf surfaces better than their ancestors did. Here, the most important consideration for the passaging process will be how efficiently microbiomes can establish in the target environment(s) and remain resilient across the range of conditions they are likely to encounter. One should also ask whether it would be more effec-

TABLE 2
Hypothesized barriers to optimizing iterative microbiome passaging, potential consequences of those barriers, and areas worthy of experimental investigation to understand the true impact of these proposed barriers

Barriers	Consequence	Areas for investigation
• Substantial taxonomic filtering in earliest *mb*Generations	• Passaging favors microbes adapted to experimental system rather than those contributing most to target phenotypes	• Introduce a low-stakes initial conditioning phase to filter for microbes that are system-compatible prior to passaging targeted at enhancing microbiome traits
• Growth rate gaps • Dominance by microbial "weeds"	• Exclusion of important taxa and/or variability/fluctuation in microbiome composition at each *mb*Generation transfer event	• Assess how varying *mb*Generation length impacts inclusion/exclusion of taxa, as well as predictability in microbiome composition at the time of transfer • Explore how introduction of cues (e.g., substrate additions; plant inclusion) impacts predictable microbiome succession within each *mb*Generation • Attempt to include/exclude/reintroduce potential viral and eukaryotic antagonists using differential filtering (e.g., by size)
• Later successional taxa depend on the presence or prior activity of early successional taxa • Persistence of a taxon depends on frequent outside propagule pressure • Antagonistic microbes that interfere with those producing target traits	• Key taxa fail to establish in conditions that could support their growth	• Introduce immigration events from relevant microbial sources • Assess how varying *mb*Generation length impacts diversity of taxa included and benchmark against systems in which immigration events are introduced • Passage microbiomes along multiple tracks to target complementary functions (e.g., capture microbes that metabolize carbon source A separate from those that metabolize carbon source B, but combine at application)
• Key microbial traits are not highly conserved phylogenetically	• Phenotype enhancement depends on selecting for broad microbial traits, not specific taxa	• Repeat passaging across many environments to assess consistency (e.g., across pH gradient) • Assess a variety of microbiome traits beyond taxonomy (e.g., biofilm formation) to identify possible links between traits and outcomes
• Iterative microbiome passaging systems are far more homogeneous than natural systems	• Generated phenotypes are not replicable when iteratively passaged microbiomes are introduced to new environments	• Perform passaging under heterogeneous conditions, particularly those that mirror expected environmental variability

tive to generate a single microbiome with a wide habitat breadth or a range of habitat specialist microbiomes.

- It may be important to assess the predictability with which different environmental factors shape diverse starting microbiomes. This could help us to understand how agroecosystems shape microbiomes through time or guide the process of iterative microbiome passaging for applications in complex environments. For instance, Jacquiod et al. (2022, 2025) showed that iterative passaging of microbiomes within the roots of a specific plant led to a microbiome phenotype that was transferable across different soils but not different plants. This then raises questions about how varying the passaging environment might control the breadth, resilience, or robustness of microbiome phenotypes.
- Many farmers seek to produce local enrichments of beneficial microbes for their soils, through approaches such as compost tea production (Scheuerell and Mahaffee 2002). In such cases, many of the principles for optimizing microbiome function in a batch culture will be impractical, especially because the practices, conditions, and microbes of each farm are unique. Instead, we should seek to understand how simple approaches to iterative passaging can enhance microbial function across a wide range of microbiomes and soil conditions to better advise the process of this practice.

Of course, there are many other potential applications in plant and soil systems. Thus, iterative microbiome passaging should be seen not just as a path for creating silver bullet probiotics but also as a diverse biotechnological tool and a useful experimental model. As a result, experimental approaches must reflect the uniqueness of different questions and applications. Because this is still a very new field, the designed microbiome passaging systems and approaches have been quite diverse (Table 1). In some cases, this reflects the variety of goals and concepts that researchers have had. In others, it reflects a lack of benchmarks for decision-making, ranging from *mb*Generation length to number of *mb*Generations required to the microbiome transfer approach. In the absence of other guidance, this is likely to reflect practical considerations; for instance, the total iterative passaging process (i.e., all *mb*Generations) in most studies has spanned a few months to half a year. However, much more work is required to understand how different choices impact the compositional and functional trajectories of microbiomes. In the next sections, we explore some high-value targets for near-future research to improve our understanding of the controls on this process.

The Relative Importance of Species Sorting and the Evolution of Lineages

Iterative microbiome passaging drives at least two major processes that will both shape microbiome function: (i) species sorting (i.e., changes in the composition and abundance of organisms within an environment) and (ii) the evolution of individual lineages. Mueller and Linksvayer (2022) also argued for a third process, which is evolution of the actual microbiome as a unit. Although we would agree that microbes will evolve in response to both the passaging environment and co-occurring microbes (and that in some cases these microbes may become interdependent), the long-term cohesion of a repeatedly passaged microbiome is unknown and likely depends on the specific passaging process and environment. We will instead focus on the former two processes here while acknowledging the potential for a higher level of group selection that should be considered in a fundamentally different way.

Although both species sorting and the evolution of lineages will occur throughout iterative microbiome passaging, species sorting should be most prominent in the earliest *mb*Generations. Microbes that are best adapted to the experimental system will persist, as others become less abundant or are filtered out entirely (Fig. 1A), and several studies have shown dramatic changes in microbiome composition in the first *mb*Generations (e.g., King et al. 2022; Morella et al. 2020; Yin et al. 2021). During this stage, the ability to intentionally select upon positive or negative microbiome phenotypes may be dwarfed by the large initial taxonomic filtering effect of the experimental system itself, as well as the passaging process. For instance, in a previous study, we intended to select from the outset for soil microbes linked to high *Brassica rapa* growth. We found, however, that we could influence *B. rapa* biomass through

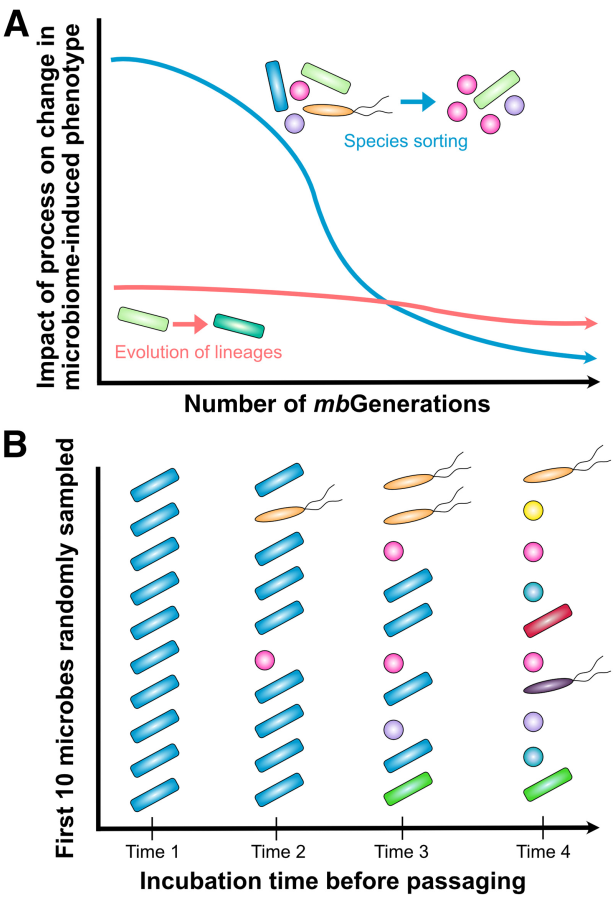

Fig. 1. Here, we visualize how the impact of iterative microbiome passaging may differ with time. **A,** Hypothetical impact of two concurrent processes (species sorting, i.e., changes in the composition and abundance of organisms within an environment, and evolution of lineages) on changes in microbiome-induced phenotypes across *mb*Generations through the iterative microbiome passaging process. This does not make assumptions about the direction of those changes, simply that the initial phase of iterative microbiome passaging will have a disproportionate impact on species sorting, as those organisms that survive best under the experimental conditions are retained. As has been observed in experimental evolution studies in culture, we expect evolution of lineages to persist as an important force indefinitely. We expect evolution will eventually become the dominant process influencing microbiome phenotypes, as species turnover between *mb*Generations lessens over time. **B,** Within a given *mb*Generation, the organisms that we are most likely to transfer should shift with time. If a microbiome transfer event occurs shortly after a prior transfer, the fastest-growing microbes will be dominant. With more time between transfers, there are opportunities for community succession events and potentially codominance by numerous taxa.

iterative microbiome passaging in certain *mb*Generations but that our impact on microbiome divergence and plant growth was roughly equal when microbiomes were passaged forward from high biomass plants or randomly selected plants (King et al. 2022). Thus, during the time we expected to select for phenotype-inducing microbes, we likely observed the overwhelming impact of taxonomic filtering imposed by the experimental system, which still enhanced the target phenotype in certain *mb*Generations.

Interestingly, we also saw that turnover in bacterial composition was leveling off after six *mb*Generations in one treatment (+NaCl) but that otherwise, substantial bacterial and fungal turnover continued through the end of the experiment. Other data also suggest that bacterial turnover can level out after a few *mb*Generations, at least in some systems (e.g., Morella et al. 2020), but we have minimal understanding of the turnover dynamics of other microbial types, such as fungi and phages. Iterative microbiome passaging typically begins with a diverse inoculum collected from the environment, so studies that end after only a few *mb*Generations will largely focus on this initial sorting phase (assuming no prefiltering step), in which microbial composition and interactions are in constant flux. Of the plant- and soil-based studies that we identified, nearly all used 10 or fewer *mb*Generations (Table 1). Co-evolution of strains in a microbiome can lead to more synergistic interactions over time (Lawrence et al. 2012), but the compositional flux at the outset of iterative microbiome passaging may limit the opportunities for this to occur between microbes that will ultimately survive the initial sorting phase. Overall, initial species sorting may overwhelm any impacts of lineage-level evolution on microbiome phenotypes, which may become more apparent when composition stabilizes later in the passaging process. Intriguingly, Swenson et al. (2000) observed, in some cases, more consistent phenotype differentiation between their microbiome passaging lines later in their passaging process; however, this has not been tested extensively.

Challenges to moving beyond the initial species sorting phase include the short project timelines available to many hands-on researchers in academic environments and the time and resources required to iteratively passage microbiomes across many generations with sufficient replication and appropriate controls. This is especially challenging in plant and soil systems, as *mb*Generation times may need to be far longer than is possible in culture-based systems, especially when plant growth and time to maturity are considerations. In addition, the longer such experiments run, the higher the risk of experimental catastrophes such as system contamination, errors in passaging/sample tracking, or equipment failure (one of our studies was cut short by a drying oven fire!). One way to combat this is to prefilter the starting microbiome within an experimental system such as the one to be used for microbiome passaging, which could then be stored and used as a starting point for downstream studies. This idea was proposed by Chang et al. (2021) in their in silico study, which they pitched as a means for better prescreening of microbiome potential. This approach was employed by Faller et al. (2024) in passaging for P-solubilizing microbiomes for possible hydroponic applications, although the relative success of phenotype-guided passaging through time remains to be tested in soil and plant systems.

As an example, if we wanted to generate a wheat growth-promoting soil microbiome, we could establish one or more microbiome conditioning pots of wheat growing within the soil/substrate to be used in the study. The system could either be closed or open to microbial influx, and the establishing microbiome could either be bottlenecked at intervals or not. The goal would be to bias the starting microbiome toward microbes that could thrive within the system's constraints, especially (i) in the growth substrate, (ii) in the presence of wheat roots, and (iii) despite forced bottlenecking of the microbiome at intervals. This prefiltering can be low in both stakes (e.g., no attempt to exclude microbial immigrants) and time investment (e.g., few managed pots), potentially helping to leap beyond the initial taxonomic filtering phase of iterative microbiome passaging.

Complications from Growth Rate Gaps

A unique challenge for iterative microbiome passaging is that microbes grow at different rates. This includes substantial within-group differences (e.g., fast- and slow-growing bacteria) and between-group differences (e.g., bacteria versus fungi versus phage). Unlike traditional breeding, in which the gene landscape remains relatively consistent even as allele frequencies change, the actual ratios of genes and organisms present at the end of each *mb*Generation can differ due to these growth rate differences. Furthermore, the growth rates of organisms can change through iterative passaging, and some organisms, such as fungi, may even cycle through different life stages from one *mb*Generation to the next, which will not be easily detected through sequencing data. Fungi are already noted to display heterogeneity in growth and function in highly controlled batch systems (Wösten et al. 2013), before adding in the complexity inherent to community passaging, especially in intricate environments such as soil. Although approaches could be taken to specifically omit organisms such as fungi, this would remove a large proportion of the metabolic potential in soils; in fact, fungal transplantation to enhance plant growth is the basis of new startups, such as Funga (https://www.funga.earth/).

Microbial growth rates can vary by orders of magnitude (Caro et al. 2023) and can be conserved within specific taxa or altered by features of the environment and/or interactions with surrounding organisms (Ernebjerg and Kishony 2012). Such differences will lead to a clear bias toward faster-growing microbes during iterative microbiome passaging (Arias-Sánchez et al. 2019), dependent on the length of each *mb*Generation. In many studies, microbiomes are diluted at least tenfold into a sterilized substrate at the end of each *mb*Generation (dilution rates vary widely; for instance, the inoculum represented from $>15\%$ to $<1\%$ of total volume in one study; Swenson et al. 2000), so there is potential to lose rarer taxa. This process actually mirrors the dilution-to-extinction strategy that is used to experimentally reduce biodiversity in microbiomes (e.g., Wagg et al. 2014), which has itself been noted to suffer from a bias toward faster-growing microbes (Mao et al. 2023). Whereas microbes have essentially no time to grow back during the setup of a dilution-to-extinction experiment, microbial regrowth can be manipulated in iterative microbiome passaging studies by varying the time of transfer (i.e., the length of time in which they are allowed to grow before a subsequent passage). Such decisions should have large impacts on which microbes are abundant at the time of dilution and thus retained (Fig. 1B), given that compositional turnover in microbiomes can be substantial over the course of weeks (e.g., Kaminsky et al. 2021). How the available microbial pool is reduced through iterative passaging will then affect the extent to which we can optimize various functions of interest (Hagan et al. 2021).

Perhaps a more insidious issue is that, even among the microbes that grow quickly enough to remain in the available taxonomic pool, within-community dynamics may cause an organism that is high in abundance at the end of one *mb*Generation to be low in abundance at the end of the next. Seasonal fluctuations in microbial composition are common (e.g., Gilbert et al. 2012), and changes in relative abundance could result from many processes, such as predator-prey cycles (including predation by bacteriophages), intermicrobial competition, and shifts in resource availability. A key issue is that if composition shifts asynchronously with the timing of *mb*Generation transfer, it could obscure cumulative change; for instance, if a microbe that is abundant in one *mb*Generation contributes disproportionately to function but then declines in abundance the next, the strength of the phenotype may similarly rise and then drop. We may also see dynamics that mirror ecological succession within each *mb*Generation, as microbiomes are transferred from a mid- or late-successional community state to seed a new sterile environment that favors pioneer-type taxa.

Modeling has suggested that repeatedly standardizing the biomass of each microbial type in simple two-species communities could improve community selection outcomes (Xie et al. 2019), but this is entirely impractical for diverse microbiomes, especially when some microbes resist straightforward cultivation. Instead, we recommend approaches that embrace growth differences and target predictable microbiome trajectories within each *mb*Generation. First, we can assess the impact of different cycling periods (e.g., 3 days versus 4 weeks) on the predictability of composition at the end of each *mb*Generation; it may be, for instance, that the endpoint composition is more deterministic with a longer incubation after transfer. Second, we can attempt to control environmental cues that either guide microbiome succession or prevent sporadic shifts in composition (e.g., sudden change in soil moisture in one *mb*Generation could stimulate desiccation responses or promote drought-resistant taxa). Alternatively, we might introduce consistent cues to influence community succession and activity, through substrate addition or plant growth, for example, the latter of which has been shown to lead to more predictable microbial networks as plants develop (Shi et al. 2016).

Some have suggested that a key trait of a selected microbiome is that it be "generationally stable," meaning that the composition will be near-identical in parent and offspring *mb*Generations, as well as subsequent *mb*Generations (Chang et al. 2021; Sánchez et al. 2021). If true, this would encourage us to omit organisms that could disrupt this stability, due to generation times that are offset from *mb*Generations, for instance. Indeed, this will certainly be true for some applications; for instance, we might value a stable microbiome within a batch reactor to ensure consistent process rates over a prolonged period. However, there is not clear evidence that this is universally required. In fact, we can see that direct transplants of microbiomes from different origins (in the absence of an iterative microbiome passaging process) can have profound and reproducible effects on plant growth (Allsup et al. 2023). Furthermore, the microbiomes associated with plant hosts can change rapidly through plant development (Chaparro et al. 2014). Rather than targeting microbiome stability, it may be advantageous to select for a diverse collection of microbes that are well adapted to varied environmental and plant conditions. Again, there is no single "best" way to generate microbiomes with transferable functions (nor do we suggest that this end goal is trivial at the present time), but it is important to ensure that goals and approaches are well aligned.

Interference from Microbial "Weeds"

A related but distinct issue is the microbial "weed." We assume that initial *mb*Generations will filter for system-adapted taxa, with some contributing to the target phenotype and others having neutral or negative impacts. When microbes that are of minimal, neutral, or negative benefit to the desired phenotype (the "weeds") grow more quickly, occupy more resources, or otherwise outcompete highly beneficial microbes, it may interfere with our ability to optimize microbiomes through iterative passaging.

Cray et al. (2013) analogized microbial weeds to weeds found in plant communities, citing characteristics such as vigorous growth (especially in uncolonized habitats), versatility in resource use, and superior competitive abilities. Such characteristics do not preclude a microbe from contributing productively to a target phenotype, but they do define microbes that are more likely to survive in an iterative microbiome passaging system, regardless of whether they are functionally of interest. Passaging microbiomes alongside growing plant roots does not ensure that we will filter for microbes that are plant-beneficial, nor does passaging microbiomes in an environment spiked with a target contaminant (e.g., phenanthrene) ensure that each taxon will contribute to contaminant biodegradation, because in both cases, other carbon sources may be available. For instance, we saw that bacterial composition on agar containing tryptic soy and crude oil was impacted more by the presence of tryptic soy than crude oil (Bell et al. 2016). Unless a passaging process is designed to make the function of interest obligate for survival (e.g., a contaminant targeted for biodegradation is the sole available carbon source), the presence of microbial weeds is a near certainty. In traditional breeding, metabolic pathways that divert energy and resources from a target phenotype (e.g., big corn cobs) are also unavoidable, and it is more about minimizing than eliminating interference with phenotypic goals.

There is likely no perfect way to exclude weeds without dramatically limiting diversity in the microbial pool. For instance, to iteratively passage for microbiomes optimized for biodegradation, we could initially bulk culture on media that contained the contaminant as a sole carbon source; however, this would exclude microbes that are not easily captured by traditional cultivation. Instead, we could weight the passaging system toward the function of interest. If, for example, we aim to select specifically for microbes that associate with a given plant, we could passage microbiomes into gnotobiotic systems that already contain adult plants with highly developed root systems rather than introducing them alongside seeds or seedlings, at which point root-provided resources may be sparse. Additionally, introducing certain nutrients to the system (e.g., N- and P-based fertilizers) can favor some taxonomic groups over others, so pairing amendment trials with sequencing-based analysis may help identify and limit really problematic weeds. The use of targeted antimicrobials early in passaging might be helpful in specific cases, as could CRISPR editing of microbiomes in the future (Ramachandran and Bikard 2019).

Weeds might be particularly prominent soon after passaging microbiomes from one *mb*Generation to the next if they exhibit vigorous growth in uncolonized environments (Cray et al. 2013). If so, extending the length of each *mb*Generation could enrich for less weedy species (assuming succession by slower-growing taxa) while diluting out weedy early growers through time. Identifying whether weeds are more prominent among early growers could involve a combination of sequencing-based analysis, time-repeated functional assays (of consortia and/or specific taxa), and comparisons in group phenotype change with variations in *mb*Generation length. In addition, phenotype-guided screening, either through iterative microbiome passaging or screening of initial source microbiomes, may inherently point to microbiomes within which weeds are less problematic.

Is Microbial Immigration Important?

As suggested by Sánchez et al. (2021), connecting a microbiome with an immigrant pool is one mechanism for generating compositional variation. During iterative microbiome passaging, the initial species pool is winnowed to a subset that thrives in the experimental system and ideally contributes to a desired phenotype. Through this process, however, potential phenotype-contributing microbes may be excluded for various reasons, such as interference from microbial weeds early in the passaging process. In addition, more transient microbes (i.e., those less able to persist in a system) might be important to microbiome function but rely on periodic immigration to sustain their populations. Thus, there may be value in reintroducing microbial diversity later in the passaging process to maximize inclusion of beneficial taxa.

Interestingly, introducing immigration to closed experimental microbiomes has had divergent effects on microbiome function. Lawrence et al. (2016) iteratively passaged microbiomes under both ambient and elevated temperatures and found that (i) repeated immigration often increased ambient-passaged microbiome function and growth, whereas (ii) the reverse was true in microbiomes passaged at elevated temperatures. The authors' interpretation was that because the immigrant pool was also ambient-adapted, immigrants were, in general, maladapted for elevated temperature and so did not contribute positively to community function. This highlights that (i) microbial immigration can enhance the performance of bred microbiomes but that (ii) considering the source and environmental history of immigrants (e.g., similarity between recent environmental history and current environment) is critical.

Many aspects of immigration could be manipulated in iterative microbiome passaging experiments, including the timing/frequency of immigration events, quantity of immigrants, microbial source material (e.g., introduce microbes from multiple wild soils or plants), and conditioning of source material (e.g., salt amendment to immigrant source to select for microbes adapted to a high salt environment). In fact, a comparative passaging approach could take place in both the field, embedded in wild soil, and in closed growth chambers to assess the influence of real-world microbial immigration. We currently know little about the temporal variability in the abundance and composition of microbial immigrants in the wild, which should also be assessed and considered in the context of iterative microbiome passaging.

Diversifying the Function and Environmental Range of Microbiomes

A potential advantage of selecting for microbiomes rather than individual organisms is that we might more easily co-select for multiple beneficial functions and/or survival of beneficial microbes across diverse environmental conditions. Most previous attempts at iterative microbiome passaging have involved passaging in a relatively consistent environment (King et al. 2022; Lawrence et al. 2016; Morella et al. 2020; Panke-Buisse et al. 2015; e.g., Swenson et al. 2000) rather than under intentionally variable environmental pressures.

If attempting to select for multiple microbiome functions, we might either target multiple endpoints (e.g., plant growth promotion AND decomposition of organic residues) or the same endpoint from multiple angles (e.g., plant growth promotion through nitrogen fixation AND ethylene reduction). Diversifying the beneficial contributions of a microbiome could potentially be used as a hedging strategy to increase the predictability of its benefits when applied to real-world environments (i.e., the loss of individual taxa/functions is compensated for by others that thrive at the location) and could reduce the extent to which any one trait needs to deviate from the ancestral microbiome. To achieve diversification, several models from true organismal breeding could be followed. Tandem selection (Young 1961) could be used to target one trait and then another, and an independent culling approach (Young 1961) could be used to ensure that multiple traits exceed target thresholds within the same microbiome. Such approaches could, respectively, lead to exclusion of taxa not responsible for the targeted trait or less dramatic selection for each target phenotype, so we could instead try to iteratively passage for each microbiome phenotype in parallel and then combine the resulting microbiomes into a single inoculum. An advantage of microbiome passaging is that traits of interest are not necessarily linked to one organism, creating a unique opportunity for parallel passaging paths that can be combined into a single inoculum; however, interactions between differentially passaged microbiomes would need to be assessed.

One of the biggest limitations to the efficacy of any microbial product is inconsistency in survival and performance across real-world environments (Kaminsky et al. 2019). Thus, generating microbiomes with a broad environmental range (e.g., functional at both low and high pH) could increase the range of environments in which the microbiome could conceivably establish or could make them more robust to pulsed changes that shift microbial composition and function, such as nutrient influx (Yan et al. 2021). The passaging approaches described above could also be applied to microbiome function in diverse environments and might follow approaches used for multi-environment trials in plants (Piepho et al. 2012). Indeed, Meyer et al. (2023) applied an interesting approach to selecting for host breadth in the plant phyllosphere, transferring microbiomes between the same plant host or across different species, showing increased host specialism in microbiomes selected within a single plant type. A key point of interest will be to assess whether it is more effective to generate "generalist" microbiomes, in which each organism is forced to survive under condition A and also B, or to combine multiple "specialist" microbiomes, allowing the receiving environment to select for the best-adapted organisms. Such possibilities may be unique opportunities in selecting microbiomes rather than individuals.

Finally, it is important to consider that we do not know how including or excluding different elements of the environment in passaging will shape the transferability of microbiome phenotypes. For instance, Jacquiod et al. (2025) showed that their passaging process led to microbiomes with robust effects across soil types but not plant types; different choices in the passaging system may have impacted phenotypic transferability.

Are We Selecting for Composition or Something Else?

In iterative microbiome passaging studies, we often assume we are selecting for combinations of taxa and genotypes that are responsible for the phenotypes we observe. However, we may also be selecting for other community properties with important functional effects. For instance, if our passaging process alters microbial biomass or turnover rates, this in itself could have big impacts on processes of interest, such as nutrient cycling (Prommer et al. 2020). Arias-Sánchez et al. (2019) also noted microbial biomass as a confounding factor that makes it difficult to separate overall process rates from per-cell process rates. Culture-based experimental evolution studies have shown that even basic experimental choices, such as dilution factor, can dramatically impact selection for traits such as growth rate (Lin et al. 2020). Similarly, optimizing a range of other traits could be influenced by how we design iterative microbiome passaging systems, such as biofilm production, motility, or exoenzyme production, leading to a decoupling between composition and observed phenotypes. For instance, if a passaging system broadly promotes increased production of extracellular polymeric secretions (e.g., due to low-water conditions) and this has an impact on our target microbiome phenotype, we may not see clear links between phenotype and taxonomic composition. Rather than filtering for taxa that prolifically produce extracellular polymeric secretions, the system may broadly promote increased extracellular polymeric secretion production across many taxa. The level of phylogenetic conservation of relevant microbial traits should influence the extent to which we can draw a line between microbiome phenotypes and taxonomic composition. If relevant traits are highly conserved (e.g., ability to photosynthesize), we would expect stronger phenotype-taxonomy links.

We may not be able to avoid generalized trait selection, but assessing community parameters such as biomass, turnover rate, or motility may help in explaining phenotypic trends. Also, if generalized trait selection leads to target outcomes, it is not necessarily a problem. In fact, many farmers aim to enrich for beneficial local microorganisms (Scheuerell and Mahaffee 2002), using processes that sort of resemble iterative microbiome passaging experiments (e.g., compost tea production), so identifying repeatable processes that enrich beneficial microbial traits, irrespective of composition, could be highly desirable. In general, we advocate for broader investigation into the connection between iterative microbiome passaging, microbial taxonomy, and phenotypic change.

Conclusions and Future Directions

Ultimately, we strongly believe that there is no single "best" approach to iterative microbiome passaging, but we do believe that experimental design choices can have substantial impacts on outcomes. What we should take from initial studies of iterative microbiome passaging is that, regardless of which underlying processes are responsible, we can manipulate microbiome-induced phenotypes, sometimes dramatically. However, in the absence of greater understanding, we will continue to accumulate example studies that make this point, more or less, without a clear path to improving or optimizing the approach for different goals. We highlight here a number of factors that we expect will influence the outcomes of iterative microbiome passaging, as well as recommended areas for future study (Table 2). Broadly, future studies should address three key challenges: (i) the link between experimental approach and the predictability/magnitude of outcomes, (ii) the link between microbial composition and functional outputs, and (iii) whether elements of the passaging process or microbiome features can be used to predict the emergent properties of bred microbiomes. Predictably linking the approach to outcomes can help guide industrial pipelines but could also empower independent land managers, such as farmers, to create effective local microbial enrichments. Given the wide heterogeneity in conditions across the global agricultural landscape, this could be an impactful way to leverage local microbial contributions at scale. Whether microbial management can impact the function of systems such as agricultural soils is not in question, nor is our ability to manipulate microbiomes. However, to fully leverage the diversity and adaptability of microbiomes, we need a better handle on the ways we are changing them and the consequences of those changes.

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