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Review

Engineering Microbiomes to Improve Plant and Animal Health

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Animal and plant microbiomes encompass diverse microbial communities that colonize every accessible host tissue. These microbiomes enhance host functions, contributing to host health and fitness. A novel approach to improve animal and plant fitness is to artificially select upon microbiomes, thus engineering evolved microbiomes with specific effects on host fitness. We call this engineering approach host-mediated microbiome selection, because this method selects upon microbial communities indirectly through the host and leverages host traits that evolved to influence microbiomes. In essence, host phenotypes are used as probes to gauge and manipulate those microbiome functions that impact host fitness. To facilitate research on host-mediated microbiome engineering, we explain and compare the principal methods to impose artificial selection on microbiomes; discuss advantages and potential challenges of each method; offer a skeptical appraisal of each method in light of these potential challenges; and outline experimental strategies to optimize microbiome engineering. Finally, we develop a predictive framework for microbiome engineering that organizes research around principles of artificial selection, quantitative genetics, and microbial community-ecology.

Microbiome Engineering

Animals and plants are universally and persistently inhabited by microbes. These host-associated microbial communities (microbiomes) thrive on host surfaces, inhabit multiple tissue types, and colonize both inter- and intracellular host habitats [1,2]. Microbiomes of animals and plants are often dominated by eubacteria, but fungi, protozoa, archaea, and viruses also can play important roles in these communities [1–5]. Microbiomes are not passive players [6,7]; rather, microbes can alter host development, physiology, and systemic defenses [2,8,9], enable toxin production and disease resistance [10,11], increase host tolerance to stress and drought [12–14], modulate niche breadth [15], and change fitness outcomes in host interactions with competitors, predators, and pathogens [6]. Because microbiomes can encompass a hundred-fold more genes than host genomes [16], and because this ‘hologenome’ of a host-microbiome association can vary over space and time [17,18], microbiomes can function as a phenotypically plastic buffer between the host-genotype’s effects and the environmental effects that interact to shape host phenotypes. Expression of virtually any host phenotype thus depends to some extent on the presence and taxonomic makeup of host-associated microbes.

A primary research goal in microbiome research is to elucidate microbiome functions that alter host performance. Several complementary approaches (Box 1) have emerged to differentiate between beneficial, neutral, and detrimental effects on host fitness [19,20]. A common preliminary method is to conduct a microbial phylotyping survey to define a host’s core microbiome.

Trends

The microbiome’s evolutionary potential is often ignored in medical and agricultural research.

Evolutionary engineering protocols can shape microbiomes that improve animal and plant health.

Microbiome engineering leverages host traits that evolved to control associated microbes.

Microbiome engineering employs basic principles of quantitative genetics and community ecology.

Optimized microbiome engineering could revolutionize research on agriculturally and medically important microbiomes.

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Microbiome functions can be deduced by (i) correlating the presence and abundance of microbial phytophylotypes with measures of host performance, and (ii) defining a core microbiome associated with healthy hosts [21,22]. **Advantages:** Correlational analyses are straightforward with next-gen methods that utilize conserved barcoding loci (e.g., 16S rDNA for bacteria; ITS region for fungi). **Disadvantages:** Phytype abundances are subject to PCR-biases [81] and must be interpreted cautiously. Although some bacterial metabolic functions can be inferred from 16S phytypes [82], closely related bacteria can differ significantly at genomic regions that influence function [79,83,84].

### Single-cell Genomics, Whole-Community Metagenomics and Metatranscriptomics

Genomic, transcriptomic, or proteomic data can inform biochemical and metabolic analyses of microbiome-host interactions [2,24,85,86]. **Advantages:** Network interactions between microbiome components and their potential effects on the host can be elucidated [87]. **Disadvantages:** Analyses can be costly and time-consuming for whole-community metagenomics because deep sequencing is needed to capture contributions of rare community members, which can have important effects on microbiome function [88]. Reconstruction of individual genomes from metagenomic information is challenging (e.g., for bacteria with similar genomes; for genetic elements that are horizontally transferred between community members). Analyses can also be complicated by genetic or protein contaminations stemming from the host.

### Experimental Manipulation

Microbiomes can be manipulated experimentally to test their contributions to host fitness, for example by inoculating gnotobiotic hosts with specific microbial strains, synthetic communities, or natural communities (e.g., experimental gnotobiotic hosts). **Advantages:** Network interactions between microbiome components and their potential effects on the host can be elucidated [87]. **Disadvantages:** Analyses can be costly and time-consuming for whole-community metagenomics because deep sequencing is needed to capture contributions of rare community members, which can have important effects on microbiome function [88]. Reconstruction of individual genomes from metagenomic information is challenging (e.g., for bacteria with similar genomes; for genetic elements that are horizontally transferred between community members). Analyses can also be complicated by genetic or protein contaminations stemming from the host.

### Synthetic Microbiomes

Microbial strains with candidate functions can be combined into simple synthetic microbiomes (containing few to several dozen species) as clinical tools to promote host health or as streamlined models of microbiomes in nature [27,89]. **Advantages:** Synthetic microbiomes allow increased control over microbiome composition, potentially testing antagonistic versus synergistic effects among strains in host performance [90], uncovering host loci that mediate microbiome metabolic function [89], or to reverse effects of dysbiosis, for instance in cases of Clostridium infections in humans [26]. **Disadvantages:** Only cultivable or easily transferable microbes can be used to construct synthetic microbiomes. Microbial combinations and concentrations that can be tested increase exponentially with the number of microbial types per synthetic community; there exists presently no clear strategy to reduce the combinations that need to be tested to explore all regions of the combinatorial ‘hyperspace’. The spatial structure within synthetic microbiomes is likely different compared to natural microbiomes.

### Microbiome Engineering by Artificial Selection on Host–Microbiome Associations

Artificial selection can be used to engineer microbiomes using methods detailed in Boxes 2 and 3. **Advantages:** Unlike synthetic microbiomes (see above), a community comprised of both culturable and unculturable microbes can be engineered. Because microbiomes can be engineered to optimize different functions (e.g., enhancing versus degrading host health), microbiome contributions can be deduced in experimental contrasts that compare taxonomic and genetic makeup of diverged microbiomes that received different selection treatments. **Disadvantages:** Selection experiments can be time-consuming. (i.e., microbial taxa consistently present in a healthy host; see **Glossary** [21,22]) and to correlate microbial taxa with specific measures of host performance (e.g., host health [23]). A second approach is to employ metagenomics, metatranscriptomics, or metaproteomics to infer functional properties of the whole microbial community or of focal microbial taxa within it [2,24]. Third, the taxonomic makeup of microbiomes can be experimentally manipulated to test hypotheses about microbiome function. For example, gnotobiotic hosts can be maintained with a defined set of microorganisms, and microbiomes can be manipulated with antibiotic treatments or transfer of microbiomes between hosts [25-29]. With any of these approaches, it remains challenging to elucidate specific functional roles of the microbiome in shaping host performance traits (e.g., growth, health, enemy deterrence, mate attraction, fertility, and overall fitness). Central to this challenge is the complexity of microbiome properties, which can be driven by interactions among taxa within the microbiome community and which can vary with both the host genotype and the environment [30].
Box 2. Methods of Host-Mediated Microbiome Engineering in Plants and Animals

Microbiome-engineering methods differ in key elements of the selection regime (Table I). The experimenter can focus selection only on the microbiome by keeping the host genotype constant (the microbiome is selected in a specific host-genotype background, such as an inbred or clonal host; Method 1). Alternatively, the experimenter can select only on the host without experimental selection on associated microbiomes (Method 2; the experimenter permits ecological changes in the microbiomes paralleling selection on the host, but does not select specific microbiomes for propagation). These approaches involve One-Sided Host–Microbiome Selection because only the microbiome, or only the host, is shaped through artificial selection. Third, artificial selection can be applied concurrently on both the microbiome and host, such that both the host and the microbiome are shaped by parallel processes of artificial selection (Two-Sided Host–Microbiome Selection; Method 3). Two-sided selection experiments are more complicated, because hosts have to be grown long enough to be propagated to the next generation (i.e., plants have to flower, seeds have to ripen). Two-sided selection offers the potential advantage of yielding host–microbiome associations shaped by co-evolution, whereas reciprocal co-evolutionary processes are impossible under one-sided host–microbiome selection (however, one-sided selection can yield co-adapted host–microbiome associations). For example, two-sided selection could lead to co-evolutionary modifications in both host and microbes that increase host–microbe fidelity between generations (e.g., modifications that reduce dissociation and turnover of beneficial microbes, or improve host control and capture of beneficial microbes).

Method 1a: Host-Mediated Selection on Microbiome

This method has been used successfully to engineer rhizosphere plant microbiomes [33,34] and can be adapted to animal hosts (Figure 1). Swenson et al. [33] first used this method, selecting upon increased plant-shoot biomass (High-Line selection lines) or decreased biomass (Low-Line selection lines) of Arabidopsis thaliana host plants, and Panke-Buisse et al. [34] used a similar approach to select on early versus late flowering of A. thaliana. After initial soil sterilization, plants were inoculated with a starter soil-community. At the end of each growth cycle (generation), a host trait was measured for each replicate (e.g., plant-shoot biomass, or flowering time); then soils of the best-performing (or poorest-performing) replicates were chosen to inoculate the next generation of sterilized soils of the respective High- and Low-Line. This scheme propagated all viable organisms (e.g., viruses, bacteria, fungi, nematodes, and mites) from a parental community to the next generation, whereas the host plants did not evolve between generations because all seeds were taken from stock of an inbred Arabidopsis line. In this scheme, the propagated soil communities can change through ecological processes (e.g., change in abundance of community members) and evolutionary processes (e.g., extinction of community members; allele frequency changes within species; horizontal gene transfer between bacteria). The experiment by Swenson et al. [33] has been frequently criticized because of methodological problems (e.g., pseudoreplication because only a single High- and a single Low-Line was used per experiment) and because the microbial changes between generations were not analysed [31,40,41]. Panke-Buisse et al. [34] also used a low number of selection lines (see our recommendation on the Number of Selection Lines in Box 3), but elucidated microbiome changes with next-gen sequencing. In both studies [33,34], ten rounds of selection on soil-microbiomes produced significantly different plant phenotypes between the microbiome-selection lines.

Method 1b. Host-Mediated Selection on Submicrobiomes

A variant of Method 1a is to propagate only a portion of the host-associated community, for example by filtering out community members with larger cells (e.g., fungi, nematodes, and arthropods) while retaining for co-propagation only smaller community members (e.g., bacteria and viruses). Despite the time-consuming filtering step, elucidating changes in the co-propagated microbiome is simplified because only small-celled microbes (e.g., bacterial communities) have to be analysed. Sub-microbiome selection will be more useful to engineer some microbiomes (e.g., gut microbiomes dominated by bacteria; rhizosphere microbiomes of root-nodulating plants), but less so for microbiomes with strongly interacting fungal and bacterial components (e.g., endophyte microbiomes of leaves).

Method 2: Selection upon Hosts in Diverging Microbiomes

Lau and Lennon [92] artificially selected on the mustard plant Brassica rapa in the background of two changing soil communities that adapted to wet soil and dry soil conditions. Plants were selected by propagating seeds from the best-performing plants. In contrast, the soil microbiomes were not experimentally selected on (unlike in Method 1, no specific microbiomes were chosen to be propagated; instead, all soil communities were perpetuated between plant generations). Microbiomes therefore changed within plant generations as they adapted to their respective moisture condition, and microbiome changes that accumulated within a plant generation were perpetuated between generations by first removing half of the soil of the previous generation from a mesocosm, then mixing old soil (including line roots) with sterilized fresh soil. All soil organisms surviving the soil-mixing step (including mites, arthropods, nematodes, fungi, bacteria, etc.) were potentially co-propagated between plant generations. After three rounds of such direct selection on plant populations in the background of experimentally unselected (but changing) soil communities, a reciprocal transplant experiment assessed responses to selection in plants, crossing wet/dry-selected plants with wet/dry-adapted soils. This 2 x 2 factorial experiment showed that response to selection (increased drought tolerance) was weak in the plant populations, but the evolved plants performed best if the final test condition (wet vs. dry soil) matched the historical conditions (wet vs. dry soil) of their associated soil community. Such a final cross-factoring experiment of microbiomes that were artificially selected under different treatments (see Experimental Contrasts, Box 3) can also be a powerful approach to test whether microbiomes evolved with Method 1 have treatment-specific effects on a host.
A new research horizon in medicine and agriculture aims to improve animal and plant performance by altering their microbiomes [28,31,32]. Towards this end, a novel and underutilized approach employs artificial selection on a host–microbial association to engineer microbiome function [33,34], a process that we term host-mediated microbiome selection, or more simply microbiome engineering (Boxes 2 and 3). The aim of microbiome selection is to improve host performance via artificial selection on the microbiome. Host performance can include any trait that is biologically, medically, or economically important (e.g., growth rate or disease resistance). The artificial selection on microbiomes is applied over multiple generations and in an indirect manner, meaning that the host traits are used to direct whether the host’s microbiome gets to ‘reproduce’ via experimental passage to the next generation of hosts (Figure 1, Key Figure). Typically, only the microbiome is selected on, but not the host (i.e., the host can be kept genetically invariable, and thus cannot evolve).

Artificial selection on a microbiome can be efficient because (i) many important traits in animals and plants are directly influenced by interactions with microbes, and (ii) hosts can mediate microbiome assembly and relative abundance of microbial components [6,35–38]. Host-mediated microbiome selection therefore leverages host traits that have evolved to manipulate microbiomes in ways to enhance host fitness. Microbiome functions that have been artificially selected can then be analysed by comparing taxonomic makeup and genomic properties among diverged communities that evolved under different selection regimes (e.g., selection for microbiomes promoting early versus late flowering [34]), to quantify types and diversity of microbiome taxa that diverged under different selection treatment, to resolve candidate drivers of altered functions, and to identify microbial taxa for focal experiments (Box 3).

To our knowledge, only a handful of studies have used such an experimental-evolutionary approach to shape microbiomes or to elucidate microbiome function (Box 2), yet both theoretical modeling and empirical work suggest that host-mediated artificial selection can generate diverged microbial communities with significantly improved effects on hosts [33,34,39–41]. The next research frontier is to optimize microbiome engineering for key host traits (e.g., drought tolerance, immune defense, rapid growth, or fecundity) and to accumulate vital information for elucidating the nature of microbial communities that can modify these host phenotypes. Towards these goals, host-mediated microbiome selection is a novel tool that is complementary to prevailing research approaches (Box 1).
**Box 3. Designing Experiments to Engineer Microbiomes**

**Experimental Contrasts**
To elucidate the roles of microbiomes in host performance, selection can be applied via contrasting treatments or selection regimes, either in addition to controls (below) or without any formal control treatment. For example, selection contrasts can aim to enhance (High-Lines) or attenuate (Low-Lines) host performance in the same experiment, or treatment contrasts can involve different stresses (e.g., different diseases; different temperature challenges). Emerging differences in microbiomes between the contrasted treatments serve as clues for candidate microbes that impact host fitness. To infer microbiome function, the analytical power of experimental contrasts is one of the main advantages of microbiome-engineering experiments over nonexperimental surveys of microbial abundances in microbiomes.

**Number of Selection Lines, Replicates per Line, and Statistical Power**
The total number of samples in any experiment is limited, and the number of independent selection lines and the number of replicate samples per selection line therefore should be adjusted to optimize statistical power [74]. A minimum of six independent selection lines is typically recommended (six selection lines all responding in the same direction compared to controls will meet the significance criteria of a sign test). More than six selection lines will increase statistical power, but in many experimental systems even six selection lines per treatment are unfeasible. Typical number of replicates per selection lines are 10–20 [74], but replicate numbers required per selection line will depend on the extent of uncontrolled within-line variation, and more replicates (or fewer) may be needed.

**Control Treatments**
Typical controls in experimental evolution are random-selection lines. In the case of microbiome engineering, a microbiome is chosen randomly from among the replicates in a random-selection line [39]. Random-selection lines greatly increase experimental effort, and several alternatives can be used. One option is constant inoculation, where a preserved, nondeveloping microbiome is inoculated from a stored source (e.g., bacterial community frozen in glycerol storage; fungal spores in dry storage). Another time-efficient experimental control is null inoculation, in which controls are inoculated with sterilized water or soil. Lastly, propagation of microbiomes typically involves not only transfer of live microbes, but also associated solutes (e.g., nutrients that may be harvested together with a microbiome); such solutes can also impact host performance, and it may be necessary to include a control condition where all living components are removed from an inoculant (e.g., by filtering or autoclaving) to use the remaining solutes as a control treatment.

**Mixed versus Unmixed Propagation of Microbiomes**
In some experimental systems, it is possible to mix microbiomes harvested from different hosts before propagating to new hosts. Propagation of mixed or unmixed microbiomes are therefore two principal propagation schemes [33,39]. The respective advantages of mixed versus unmixed propagation have yet to be tested, but may include the same kind of consequences that apply also to recombination at the genetic level, for example generating (i) novel combinations of microbes with novel synergistic effects on a host, or (ii) generating novel competitive interactions that degrade or improve overall microbiome function.

**Ramped versus Unramped Selection**
Selection pressures can be applied as constant stresses throughout all selection cycles (e.g., constant pesticide concentration), or pressures can be ramped to gradually increase the selection intensity (e.g., gradual increase in pesticide during or between selection cycles). Constant selection pressures may lead to a decelerating response to selection as the host-microbiome associations adapt to the constant environment during several selection cycles. Ramped selection pressures can potentially generate adaptations to more extreme conditions and increase host fitness differences between treatments.

**Subcommunity (Incomplete) Selection versus Whole-Community (Complete) Selection**
Experimental propagation of a microbiome between host generations can be complete (all microbiome members are propagated) or incomplete, for example by excluding specific microbial components (e.g., exclusion of fungi by passing a microbiome through a size-selecting filter before propagation; suppression of fungi with antibiotics; see Method 1b, Box 2). In the latter case, only a subcommunity will be shaped by selection (e.g., only bacterial and viral components). Subcommunity selection can simplify analyses of the microbial responses to indirect selection (e.g., because only bacterial communities have to be analysed), but it might slow the response to selection because fungal components that could respond to indirect selection are excluded. Importantly, any process that is used to exclude a subset of the community can drive its own response to selection. For instance, when using a size-selecting filter to exclude fungal cells from microbiomes, the filtered bacterial taxa can evolve reduced size to maximize passage through the filter. Alternatively, if antibiotics are used to exclude taxa, resistance to antibiotics can evolve. These responses to subcommunity filtering occur independently of the intended evolutionary changes resulting from microbiome selection.

**Closed, Semi-open, and Open Experimental Systems**
Selection on host-microbiome associations can be applied in closed (sealed) mesocosms, which can be expensive and challenging; or in open mesocosms, which allow some degree of microbial recruitment from external sources. Recruitment from external sources can be minimized through permeable barriers (semi-open system), or recruitment can be uncontrolled (open system). Recruitment of novel microbial types into a host-microbiome association can
destabilize a host–microbiome association because of microbial turnover, but occasional recruitment of novel microbes could enhance microbiome function [51,54]. Alternatively, openness will lead to little or no microbial turnover if a resident community is resistant to invasion or is resilient to disturbance [68]. The advantages of relative openness of an experimental system therefore depends on the resistance to invasion of resident communities, on the invasion pressure from external sources (i.e., likelihood of contamination), and on the need for microbial diversity through recruitment of novel community members from external sources.

**Sources of Starter Microbiomes**

Any microbiome can potentially be used as starter inoculum, but microbiomes will undergo less drastic ecological changes during the first selection cycle if inoculated with typical microbiomes (e.g., gut microbiomes are not used to inoculate rhizospheres). Any microbiome will likely show some initial changes in response to the new experimental conditions. Response to selection may therefore become more apparent in later selection cycles, after microbiomes have become adapted to general laboratory conditions.

**Selection on Microbiomes on a Host Surface/Integument versus Selection on Microbiomes inside a Host**

The efficiency of microbiome engineering depends on the degree to which the host can control community membership (i.e., on the extent of host control and symbiont choice; [50,51]). Hosts likely have more control over internal, sequestered microbiomes (e.g., microbiomes in gut, caeca, or integumental pockets; endophytic microbiomes) compared to exposed, surface-dwelling microbiomes. Thus microbiome engineering should be easier for internal microbiomes (e.g., endophytic root bacteria), and more difficult for surface-dwelling microbiome (e.g., rhizoplane bacteria).

**Domesticated versus Wild Hosts**

Domesticated hosts such as crop plants were selected during domestication for yield in microbially varying environments, such that beneficial microbial symbionts were not consistently present during domestication. Compared to wild ancestors, therefore, domesticated plants may have lost the full capacity for host control and symbiont choice to shape their acquired microbiomes [43]. Consequently, host-mediated engineering of microbiomes may be more successful in undomesticated than in domesticated hosts when selecting on such environmentally acquired microbiomes. In contrast, some domesticated animals such as the honeybee have gut microbes with both vertically transmitted and environmentally acquired components [62], and both wild and domesticated honeybees may therefore be suitable models for microbiome engineering of the vertically transmitted components.

**How Does Host-Mediated Microbiome Engineering Work?**

The efficacy of microbiome engineering derives from host control, the suites of traits that animals and plants have evolved to selectively recruit beneficial microbes into symbiosis, reward beneficial genotypes, and exclude or sanction ineffective symbionts [18,36,38,42–49]. At the initiation of a host–microbe interaction, host control occurs via partner choice or screening, in which the host selectively alters the subset of microbes that are allowed to colonize or persist in association with the host (e.g., via resistance, immunity, and genotypic specificity [50,51]). After colonization of the host, control can also occur via sanction mechanisms in which the host regulates microbial proliferation and disfavors ineffective microbes [18,36–38,42,48]. Finally, some hosts captured a subset of microbes by transmitting them vertically to their offspring, as occurs in bacterial and fungal symbionts that are co-propagated from parent to offspring within animal and plant lineages [49,52–54]. Vertical transmission combined with host control strongly ties the fitness interests of microbes to their host [55] and allows the host to guarantee that their offspring acquire specific genotypes or microbiomes.

Using microbiome engineering, novel and improved microbiome functions can be selected upon without any knowledge of the microbiome composition, or of their synergistic interactions (Box 2). Synergy can emerge, for example, from interactions between microbiome members to enable the microbiome to metabolize toxins, or to ameliorate stresses (e.g., salinity or ultraviolet light) that would kill each individual community member in isolation [56]. Synergy can also emerge from host–microbe interactions, for instance in root–nodule symbioses where the trait of nitrogen-fixation occurs only in compatible pairings between plant and bacterial genotypes, and where neither partner expresses nitrogen-fixation in the absence of the other [57]. The important feature of these selected microbiome traits is that they must affect a host phenotype. Consequently, selection can target the host phenotype to distinguish indirectly among microbiomes that affect host phenotype in different ways. In essence, the host is used as a probe to evaluate microbiome functions that impact host fitness.
Key Figure

Host-Mediated Engineering of Microbiomes in Animal and Plant Hosts.

Figure 1. One-sided artificial selection on microbiomes in the plant rhizosphere (top), honeybee gut (left), and mouse gut (right). Different microbiomes are shown in different colors. In one-sided selection experiments, the host is kept genetically homogeneous and cannot evolve between selection cycles because uninfected hosts are taken each generation from nonreverting stock (bottom left in each panel). At the start of each experiment (top left in each panel), microbiomes differ in community composition between hosts; host–microbiome associations are allowed to mature (Step 1), then are phenotyped for the trait used as a direct target for indirect selection on microbiomes (Step 2), then microbiomes are chosen for transmission to the next generation of hosts (Steps 3 and 4). The selection regimes in the plant and honeybee panels are identical in that the most extreme host phenotypes are chosen to identify microbiomes for propagation (microbiomes from the largest plant or the healthiest bee are propagated), but the regime in the mouse panel propagates microbiomes from hosts of intermediate host phenotypes (i.e., selecting against gut microbiomes from extreme lean and extreme obese mice). Drawings by C.-C. Fang.
Microbiome functions are most often expressed as continuously-varying phenotypes of the host. A quantitative-genetic view of microbiome engineering therefore is that artificial selection occurs directly upon the host (because the host phenotype is measured directly), whereas the microbiomes are indirectly selected through their effects on the host phenotype. Such indirect selection can be more efficient and more cost-effective than direct selection [58]. Indirect selection is particularly useful when the indirectly selected trait is difficult or costly to measure [58], as is typically the case for microbiome properties, compared to the ease of measuring host traits. Moreover, host-mediated selection can be designed to simplify analyses, for example, the host genotype can be kept constant to focus artificial selection only on microbiomes (i.e., microbiomes are engineered in a specific host background; Box 2). Multiple experimental approaches are possible that differ in complexity of the selection regime and experimental power to engineer functional changes in microbiomes (Boxes 2 and 3).

**How Does Host-Mediated Selection Alter the Makeup of the Microbiome?**

Microbiome engineering can alter microbiomes through both ecological and evolutionary processes. The ecological processes include changes in community diversity and evenness, relative species abundances, and the structure of host-microbe and microbe-microbe interaction networks. The evolutionary processes include extinction of microbial types in the community, changes in allele frequencies, mutation, and horizontal gene transfer that restructure microbial genomes. Both ecological and evolutionary changes can be tracked with high-throughput DNA sequencing methods that infer taxon presence–absence and abundance, active microbial functions that are being expressed, and permit mechanistic inferences of microbiome functions (Box 1). Host-mediated microbiome engineering is thus a powerful technique to both manipulate microbiomes and understand their functions.

Many animal and plant hosts have traits that enable the perpetuation of microbiomes to other hosts, either through transfer of microbes among individuals of the same generation (horizontal transmission) or through inheritance of microbiomes from parent to offspring between host generations (vertical transmission) [52]. Horizontal transmission can occur via infectious, contact-transmission among hosts, or expulsion of microbial partners into an environmental pool that become available to new hosts [20]. Vertical transmission is readily apparent in simple host-microbe associations, such as bacterial endosymbionts in insects [59]. Vertical transmission can occur via a diversity of pathways, including transovarial transmission, or via behavioral mechanisms such as a mother coating an egg casing with bacteria which the offspring then acquires upon hatching [49,54]. Likewise, components of the human microbiome are inherited from a mother during or even before birth [53,60], and the honeybee gut microbiome of eight bacterial species clusters is inherited by newborn bees from sibling workers or the hive environment [61,62]. Importantly, vertical transmission of microbiomes can occur with different degrees of fidelity, measured as the likelihood with which an individual microbial genotype or whole community is passed successfully from mother to offspring. This fidelity ranges from nearly 100% in the case of transovarial transmission (all maternal symbionts are passed on to the offspring, and their relative abundances of inherited symbionts may change little between generations) to lower fidelities in the case of gut or surface microbiomes transmitted less faithfully from mother to offspring. However, even moderate inter-individual perpetuation of a microbiome (or microbial components thereof) across host generations can generate microbiome-dependent phenotypic variation in host phenotypes that natural selection can act on [63]. A host’s capacity to selectively transmit a beneficial microbiome to subsequent generations or experimentally enforced high-fidelity transmission is thus a key feature to optimizing microbiome function.

Whereas vertical transmission can help stabilize a microbiome community over time, rapid turnover in microbiome community composition can limit the efficiency of host-mediated microbiome engineering. Specifically, stochastic loss of microbial genotypes, or recruitment of new
genotypes, can erode microbiome properties that are shaped by artificial selection. However, two key mechanisms can stabilize a microbiome across host generations, help preserve changes in microbiome composition between generations, and consequently increase heritability of the microbiome [64, 65]. First, co-dependency of microbial partners reduces the chance that one of the partners is lost from the microbiome [66, 67]. Second, symbiont choice exerted by a host can differentially acquire, amplify and retain specific microbial types with beneficial effects on host fitness [36, 37, 42–50], again reducing turnover. Microbiomes can also sometimes be inherently stable, because they quickly reassemble to an original state if disturbed (so-called ‘community resilience’) and because they are difficult to invade once established (so-called ‘community resistance’) [68]. Because of such turnover-reducing mechanisms, one might be more likely to obtain a response to indirect selection on microbiomes that have inherent co-depends (or on microbiomes for which co-dependency can be experimentally enforced), or on microbiomes that are naturally vertically transmitted (e.g., honeybee gut microbiome) rather than environmentally acquired (e.g., rhizosphere microbiomes). As a consequence, environmentally restructured microbiomes are more likely to require continuous selection to maintain the beneficial properties of the engineered microbiomes (e.g., rhizosphere microbiomes engineered in greenhouse agriculture), whereas vertically transmitted, engineered microbiomes such as the honeybee gut microbiome are more likely to persist across several bee generations even in the absence of continuous selection maintaining microbiome functions.

Quantitative-genetic and community-ecology approaches have recently converged to model the ecology and evolution of communities (here, microbiomes) that co-propagate with host lineages over generations [64, 69, 70]. In a quantitative-genetic framework, a host’s phenotype is an emergent synergistic property of the genotypes of multiple interacting partners (i.e., host and associated symbionts). These interactions can change host evolution by contributing to phenotypic variation of the host, possibly shifting response to selection on the host [70–73]. Host-mediated indirect selection on microbiomes is predicated on such community heritability (here, microbiome heritability), specifically that a microbiome property existing in one generation can be perpetuated to the next generation across a selection cycle, as shown by experiments selecting artificially on host-associated microbiomes (Box 2).

Methods of Host-Mediated Microbiome Engineering

Microbiome engineering can be imposed by selecting on a microbiome in a specific host-genotype background (e.g., in an inbred host line and in a constant environment; through one-sided host-microbiome selection), or by selecting simultaneously on both the microbiome and the host (two-sided host-microbiome selection) (Boxes 2 and 3). No study has compared the efficiency of these selection approaches, but we predict more efficient selection on a microbiome in a specific host-genotype background, for instance in an inbred or clonal host and in a constant environment, rather than in a genetically heterogeneous host environment that introduces genetic variation underlying host phenotype that is uncorrelated to microbiome properties. When developing a new host-microbiome system for microbiome engineering, it will be prudent to rigorously control such environmental noise that could obscure any signal, and keep experimental design simple and time-efficient [74]. Box 3 summarizes some salient features of microbiome engineering experiments.

The most important part of protocol design for microbiome engineering is to choose a host trait (phenotype) to act as the direct target of selection. The chosen host trait must be significantly affected by the microbiome, thus allowing indirect selection for microbiome function. The host trait should ideally be easy to measure, so that it can be assessed readily during an experiment shortly before the microbiome is transferred among host generations. Finally, the host trait should be biologically, clinically, or economically important, for example encompassing proxies...
of host health, growth, stress tolerance, desiccation resistance, metabolism, or any such phenotypically plastic trait with known microbiome influences.

Native microbiomes are likely best suited to generate diverse and functionally variable host–microbiome associations to select on initially. For instance, using microbiomes from wild hosts (e.g., soils that surround native plant roots), rather than from random microbiomes (e.g., bulk soils not surrounding a plant) should expedite the response to selection, because a randomly selected microbiome will first have to adapt to the new host environment during the first selection cycles. Selection in closed axenic systems (Box 3) will also likely generate a faster response to selection, but even the experimentally simpler, open systems appear adequate [33,34]. Lastly, we predict that host-mediated microbiome engineering will often be more efficient using wild hosts rather than hosts that have experienced domestication, or adaptation to microbially depauperate laboratory environments. This is because genes that enable hosts to control interactions with microbes may have been lost during domestication [31,43], and agricultural soil microbiomes likely varied greatly between successive plant generations in the absence of host–microbiome co-propagation. In contrast to domesticated plants, however, both honeybees and their vertically-transmitted gut microbes may have been shaped by artificial selection during domestication, and both wild and domesticated honeybees may therefore be suitable models for microbiome engineering. Overall, the large number of design criteria (Box 3) for host-mediated microbiome engineering suggests manifold possibilities for variation in experimental design. Optimal choice of selection regime will depend on the experimental system (e.g., animal or plant), experimental control to minimize within-treatment variation, and available resources to conduct a long-term engineering experiment.

Concluding Remarks and Future Research Directions
Host-mediated microbiome engineering has diverse applications, particularly in agricultural research aiming to enhance plant productivity, including drought and salt tolerance, and disease resistance. Microbiome engineering could also improve health, growth, and productivity of domesticated animals, and aid in research on model systems (mice, Drosophila; Figure 1) relevant to understanding and manipulating the human microbiome. We predict that the ‘generalized’ host–microbiome co-propagation regimes outlined in Boxes 2 and 3 and in Figure 1 will help to stimulate such research on diverse host systems and develop efficient protocols to shape microbiomes through host-mediated artificial microbiome selection (see Outstanding Questions). Future research should optimize selection regimes by varying experimental parameters summarized in Box 3; combine optimized selection regimes with advanced methods to infer genetic and metabolic properties of the engineered microbiomes (e.g., microbiome-wide association studies [75] and with methods to quantify the microbiome changes resulting from artificial microbiome selection (e.g., metagenomic time-series analyses [76]); and build on the principal methods summarized in Box 2 to develop the full potential of host-mediated microbiome engineering.

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References

Outstanding Questions
What methods of microbiome engineering are most efficient (Box 3)? Does mixing of evolving microbiomes between hosts accelerate or decelerate the response to selection? Are undomesticated hosts better suited for microbiome engineering than domesticated hosts? Are semi-open systems of microbiome engineering more efficient than more closed systems because novel microbial components can be regularly recruited into a microbiome? Which methods maximize microbiome heritability?

Does host-mediated artificial selection generate microbiomes with beneficial effects specific to the host genotype in which they were evolved, or generate generalized effects benefiting multiple host genotypes or multiple species? Are there keystone microbiome members that can enhance host performance traits in multiple hosts?

For key traits (e.g., immune defense, fecundity, longevity, or climate-change tolerance), what proportion of host phenotypic variance can be explained by a microbiome that was shaped by host-mediated engineering?

When significant host phenotypic variance can be explained by microbiome content, does one bacterial taxon dominate this effect, or are there emergent effects depending on multiple, interacting microbial partners?

Can microbiome engineering enhance animal and plant performance traits beyond plateaus that limit traditional animal and plant breeding and genetic modification?


64. Shuster, S.M. et al. (2006) Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. Evolution 60, 991–1003
73. Webster, J.B. et al. (2014) Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME J. 4, 642–647
75. Funke, K. et al. (2015) Genome sequences of Lactobacillus sp. strains wkB8 and wkB10, members of the Firm-5 clade, from honey bee guts. Genome Announc. 2, e01176-e1214
76. Li, B. et al. (2014) Genome sequences of Lactobacillus sp. strain wkB8 and wkB10, members of the Firm-5 clade, from honey bee guts. Genome Announc. 2, e01176-e1214
79. Kwong, W.K. et al. (2014) Genome sequences of Lactobacillus sp. strains wkB8 and wkB10, members of the Firm-5 clade, from honey bee guts. Genome Announc. 2, e01176-e1214
81. Engblom, C. et al. (2013) Associations with rhizosphere bacteria can confer an adaptive advantage to plants. Nat. Plants 1, 15001
86. Devanna, S.M. et al. (2014) Horizontal gene transfer from symbiotic microbes may extend the phenotype of marine sessile hosts. Front. Microbiol. 5, 638