system, how long they linger if no rhythmic input follows, and other experimental parameters that might have been critical for the observed results (such as binaural noise) remain unknown, opening up exciting questions for future studies. These studies also need to clarify how much the observed alternation between ears (Figure 1B) [10] depends on the specific experimental protocol. Participants knew that targets would either appear in one ear or the other (but not in both). This is unlikely to happen in a more natural scenario, in which sounds in the two ears often differ in their intensity (inter-aural level difference), but only marginally in their timing (inter-aural time difference). It needs to be tested whether a similar sampling mechanism, alternating between the two ears, exists during binaural input. Modelling studies might be able to reveal whether such a mechanism is advantageous as compared to an in-phase sampling of both ears.

What exactly happens during phases of reduced sensory processing, when brain resources might be needed for other purposes? Where in the brain are different phases of the oscillatory cycle coordinated, and adjusted to both internal and external events? Although the answer to these questions is largely unknown, it has been suggested that phases of higher visual sensitivity alternate with phases of active exploration (for example, via saccades), controlled by fronto-parietal networks [3]. In the auditory system, equivalent phases of exploration, and the conductor of the oscillatory symphony, remain to be identified. Fully understanding the oscillatory cycle and the underlying neural circuits might be the key to understand how the brain can coordinate and optimize a complex pattern of interacting neural processes with such an apparent ease.

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Microbial Ecology: How to Fight the Establishment

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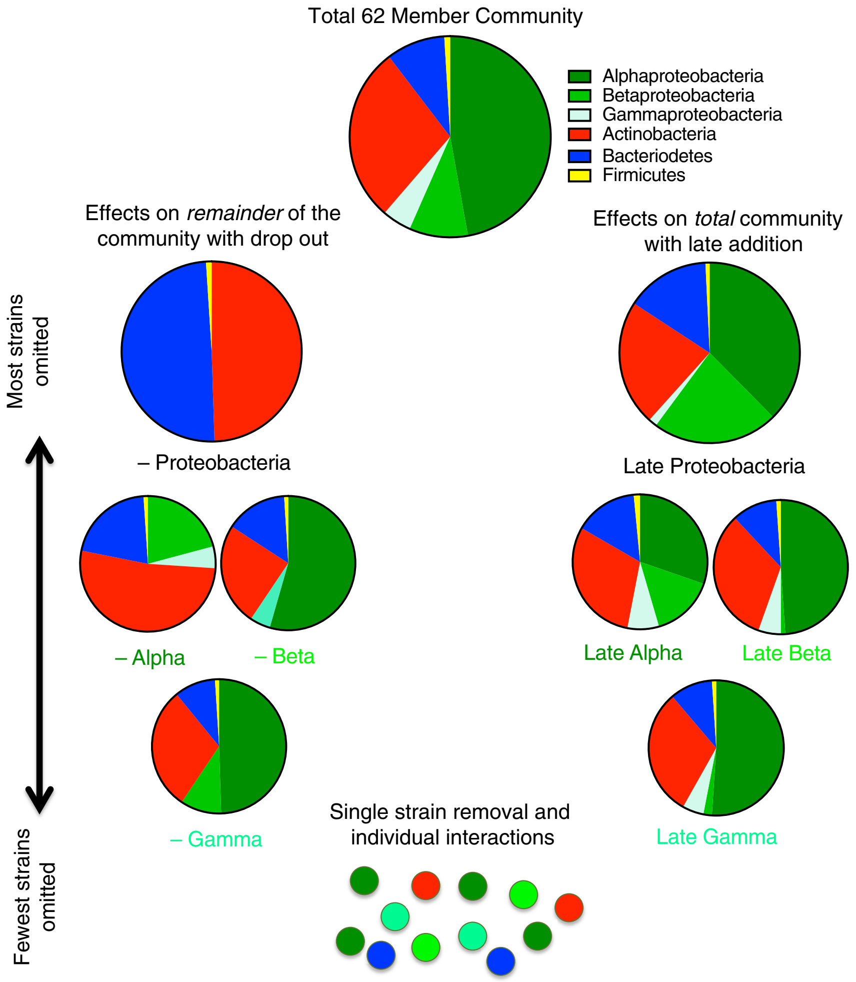
https://doi.org/10.1016/j.cub.2019.10.067

Creating microbial consortia capable of consistently producing desired qualities requires a detailed understanding of community interactions. A new paper demonstrates the role of historical contingency in *Arabidopsis thaliana* leaf-microbiome formation using an adaptable experimental approach, which could be applied to other host organisms.

Over the last decade, emerging sequencing technologies have been used with great success to reveal the microbial components of diverse hosts and environments. To predictably recreate and harness microbial communities, however, it is critical not only to identify the players involved, but also to define the rules of community assembly. The plant *Arabidopsis thaliana* is uniquely suited as a model in which to dissect the determinants of microbiome formation due to the extensive availability of host genetic resources and the high culturability of its microbiota in both above- and below-ground tissues — the phyllosphere and rhizosphere, respectively [1,2]. Constructing synthetic communities of cultured representatives of the microbiota is a useful approach to test the relevance of variables that modulate the *A. thaliana* microbiome. Whereas larger synthetic communities are predicted to capture a more robust representation of the genetic diversity, and therefore the potential functions, of a plant microbiome assembled in nature, smaller communities are more easily manipulated to reveal the importance of each member. For example, larger synthetic communities of 36–38 bacterial members were used to demonstrate the role of plant-root phosphate stress response and salicylic acid production in microbial community assembly [3,4]; however, these studies did not examine the impacts of individual strains and so the individual contributions of each member are unknown. In contrast, an eight-member bacterial synthetic community in *Zea mays* roots was used to reveal the influence of each organism on the overall bacterial community composition by methodically removing one member at a time [5]. The latter approach can be quite powerful, as it can identify keystone species — organisms whose presence is required to preserve the overall community structure [6]. A new study by Carlström *et al.* [7] published in *Nature Ecology and Evolution* moves the field significantly forward by using a large synthetic community comprised of 62 isolates and investigating class-level drop-out communities, as well as single-strain dropouts and community invasion. This ambitious experimental design allows for a more complete understanding of how microbes colonize in relation to each other from a high phylogenetic level to an individual strain perspective during phyllosphere colonization.



Current Biology

Figure 1. Robust phyllosphere assembly from a synthetic community of 62 bacterial strains.
The assembled phyllosphere community is largely unchanged except when Proteobacteria, specifically Alphaproteobacteria, are omitted from the initial inoculum (left side). The omission of Beta- or Gammaproteobacteria from the inoculum resulted in a remarkably similar assembled phyllosphere community. Individual strain omissions were used to determine the members of each bacterial class whose abundance was linked to the remaining synthetic community members. When these strains are later inoculated they are able to effectively invade the established community (right side). Among the bacterial classes, Alphaproteobacteria (dark green) were more successful in invading an established community.

By examining variable community assembly with reversed serial-inoculation orders Carlström *et al.* [7] were able to disentangle phyllosphere establishment from member persistence. Although the surrounding soil appears to be the major source of inoculum for rhizosphere and internal-root microbiotas, evidence suggests that the phyllosphere also possesses a consistent source of microbes, as microbial recolonization can be observed across generations of *A. thaliana* plants [8]. The three most commonly proposed sources for the phyllosphere inoculum are soil, air, and seeds, with the soil and seeds appearing to be the most frequently utilized [9–11]. Once colonized, however, microbial persistence in the microbiome is still understudied [12]. The experiments presented by Carlström *et al.* — using versions of their synthetic community that omit bacterial classes or individual strains — support the theory that the initial colonizers of the plant microbiome continue to persist [7] throughout its subsequent maturation. In their experiments, the authors omitted entire classes of Proteobacteria (for example, Alpha-, Beta-, or Gammaproteobacteria) and allowed the rest of the community to assemble, then added the omitted group back to the community three weeks later. They observed that once the initial community was established, community composition was not significantly altered by later introductions with the exception of the Alphaproteobacteria (Figure 1). Although some late additions are capable of invading the microbial community, their presence does not alter the structure of the established community. These results suggest that the founding members in early plant developmental stages have a large influence in determining the microbiome of the plant over time, confirming predictions made by *A. thaliana* greenhouse studies [13].

The use of drop-out communities in which entire bacterial classes, as well as single strains within those Proteobacteria classes, were omitted allowed Carlström *et al.* [7] to reveal novel roles that microbes play to impact each other during community formation. For example, they investigated how 25 of the 62 members of the synthetic community impacted the abundance of every other strain. 80% of the single-strain dropouts influenced the abundance of another community member, but in the add-back experiments, only 48% of the established community members were impacted by invading microbes. For the most part, the community members that were impacted by single-strain dropouts were only impacted either positively (27% of synthetic community members) or negatively (17% of synthetic community members). The one exception to these results, an *Arthobacter* strain, responded positively to one invading microbe, whereas all other interactions were negative. The reasons for this result require further study; however, the authors hypothesize that established microbiome members are resilient based on the niche they occupy due to critical resource usage. Keystone species, which they define here as ''organisms who have a reproducible, significant impact on the community'' were identified using this approach and were not influenced by any of the drop-out strains, further expanding our understanding of how microbes come to dominate communities.

The single-strain drop-out data also enabled Carlström *et al.* to generate a network to examine specific microbe–microbe interactions for the invading microbes. Of those interactions, approximately 75% were negative. This paints a picture of an intricate web of strain-specific interactions, which appear to be more supported by competition than collaboration, although a prior study reported positive correlations between plant-colonizing microbes within the same kingdom [14]. One hypothesis is that the microbial community members compete heavily for resources, with microbes that colonize generally overlapping for nutrients. The resources being competed for within the phyllosphere are not static and change with plant age, pathogen presence, temperature, and light [14–17]. The ability to examine the spatial location of microbial colonizers within the plant, along with micronutrient distribution, would help to explain the patterns observed in the new study.

Moving forward, the introduction of invading strains to newly formed environmental plant–microbe communities, such as those found in and on germinating seeds, may prove useful in field applications. For example, if early colonization events drive the microbial community, as Carlström *et al.*'s data suggest, then seed inoculation would have better success than inoculation of older plants. Although *A. thaliana* does not harbor seed endophytes, many other plants do, and it serves to reason that the seed endophytes are likely important reservoirs for the plant microbiome, as these organisms have earliest access to plant tissue. Therefore, it is critical to improve our understanding of seed endophytes in natural settings. The study presented by Carlström *et al.* also illustrates the predictive potential of a microbial community, as the clear patterns between the single-strain dropouts and microbe–microbe interactions showed consistent interactions. It further supports the idea that the members of a plant microbiome act in a predictable fashion that can be harnessed, as has been demonstrated previously in *A. thaliana* roots [18]. Expansion of this experimental approach to include other microbiota members, such as fungi, would provide a more complete view of the importance of early colonization patterns. The patterns observed in this new work provide ample ideas for future experiments in niche colonization of other host plants and could help with the generation of consistent and long-lasting microbial communities in agricultural settings.

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Metabolism: A Burning Opioid Issue in Obesity Therapeutics

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https://doi.org/10.1016/j.cub.2019.10.055

Food restriction triggers a lowering in body temperature. A new study now provides a mechanism for this process that relies on opioid signaling in the hypothalamus. These observations suggest potential new therapeutics for obesity.

Almost everybody who is overweight or obese has tried various diets to attempt to lose weight. Indeed, there is a clear correlation between eating less and losing weight. Besides the many pitfalls of various diets, our body also has an ‘unfortunate’ adaptation to decreased food intake, which is decreasing energy expenditure. The most effective way to decrease energy expenditure when there is no sufficient fuel uptake is to lower body temperature. This adaptive response of our body contributes to reaching a plateau even if we stick to a diet, and when that is combined with the various shortcomings of dieting (e.g., getting bored of eating the same foods, etc.) most people quit and rapidly regain the lost weight. If there was a way to prevent body temperature decline during dieting, a much more effective and sustainable weight loss could be accomplished. This very question was addressed by a paper published in this issue of *Current Biology* by Cintron-Colon *et al.* [1].

The hypothalamus is a critical part of the brain in the control of body temperature. This area controls sympathetic outflows affecting vascular constriction and brown adipose tissue-related heat production as well as shivering thermogenesis [2]. This central ‘sensor’ receives inputs from the periphery and visceral thermoreceptors. Nociceptors convey the cool and warm signals to primary neurons located in dorsal root ganglia, which will transmit the information to second-order sensory neurons in the dorsal horn of the spinal cord. The cold and warm signals from dorsal horn eventually reach the preoptic area of the hypothalamus. The preoptic area contains microcircuits that integrate the inputs coming from the periphery and coordinates the appropriate thermoregulatory response to control vascular constriction, shivering, and brown adipose tissue thermogenesis [3,4]. In addition to receiving information on ambient temperature, the preoptic area also receives signals from brain regions that can sense nutrients. These include the arcuate nucleus of the hypothalamus and the parabrachial nucleus [5].

Despite the wealth of information on central thermoregulation, the circuits responsible for food restriction-related decrease in core body temperature/energy expenditure are still not well defined. The paper by Cintron-Colon *et al.* provides a feasible mechanism for this interplay whereby dynorphin-producing neurons in the arcuate nucleus and the parabrachial nucleus modulate core body temperature during food restriction via κ-opioid receptor in the preoptic area of the hypothalamus [1] (Figure 1).

The opioid system of the brain is involved with systemic metabolism [6]. Three major classes of opioid receptors are expressed throughout the brain, peripheral nervous system and immune system: μ, δ, and κ-opioid receptors [7]. In their paper, Cintron-Colon *et al.* showed that animals exposed to caloric restriction presented a remarkable decrease in body temperature starting hours after the last meal and exhibited a 19% decrease in body weight. They also found that these