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Model ecosystems could provide significant insight on the evolution and behavior of real ecosystems. We discuss the advantages and limitations of common approaches like mesocosms. In this context, we highlight recent breakthroughs that allow for the creation of networks of organisms with independently controlled environments and rates of chemical exchange.

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From Petri Dishes to Model Ecosystems

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Abstract

Model ecosystems could provide significant insight on the evolution and behavior of real ecosystems. We discuss the advantages and limitations of common approaches like mesocosms. In this context, we highlight recent breakthroughs that allow for the creation of networks of organisms with independently controlled environments and rates of chemical exchange.

We want to start with the following “food for thought”. Arguably, one of the greatest advances in chemistry was the development of glassware. It enabled the maturation of chemistry into a quantitative science by allowing to control the conditions of reactions (e.g., temperature and atmosphere composition) with modular, transparent setups that could be easily standardized and replicated. Similar statements could be made about the role of Petri dishes in the development of microbiology.

Here we convey this thought to plant science which could greatly benefit from the development of a “glassware” for biology: controlled, modular, scalable, transparent, connectable habitats for growing plants and microbes. We argue that such tools could enable the understanding of interactions in communities of organisms by providing a repeatable and quantitative experimental platform. A predictive understanding of these interactions and their collective effect on plants is essential for (i) breeding for specific environments/ecosystems (e.g., perennial cover crops[3]), (ii) understanding the genetic modulation of these interactions (e.g., quantitative trait loci mining[4]), and (iii) understanding how the environment controls the response to disease and stress (e.g., sudden death syndrome in soybean[5]).

Ecosystems – like the human organism – are complex systems whose behavior is often emergent, i.e., they are more than the sum of their parts. Therefore, holistic and reductionist approaches serve complementary roles in the study of complex systems. Holistic approaches (e.g., field work) capture behaviors and responses of real systems, but lack well defined control experiments, repeatability, and the knowledge of all variables. By contrast, reductionist approaches, do not usually seek to predict the behavior of the real system, but can provide insight on (i) cause-effect relationships (i.e., how the system responds to stimuli[6]), (ii) critical phenomena (i.e., how and when the state of the system can suddenly change[7]), (iii) feedback loops (i.e., what processes are interdependent[8]), and (iv) scaling behaviors (i.e., how the system’s behavior changes with its size and complexity[9]).

Creating Model Ecosystems

Model systems like tissue cultures and model organisms have advanced plant science greatly, but comparably little work has been done to create “model ecosystems” to understand plant communities and the rhizosphere. What is a model ecosystem? The development of an ecosystem depends on the environment and the chemical (e.g., signaling) and physical (e.g., shading) interactions between its members. Therefore, a model ecosystem should provide independent control of the environmental conditions of each organism (e.g., nutrients and water availability, humidity, light, and temperature) and of their mutual interactions (e.g., rates of flux exchange, distance of signaling).

Achieving independent control of these factors is technically difficult for two main reasons. (i) The environment of an organism is affected by its interactions (e.g., competition for nutrients). This raises the question, how to allow interactions between the organisms, if we are to control their environment? (ii) The characteristic signaling distances between organisms are often limited to the millimeter scale by the slow rate of diffusion in liquids (e.g., the rhizosphere is ~2-5 mm thick). Because the signaling distance is smaller than the size of the average root system, different parts of the same root system are at significantly different distances from their neighbors and therefore will experience different concentrations of exudates from them. This raises further questions: How to physically separate cm-scale organisms, such as plants (so that we can control their environments), if we have to keep them nearly in contact? And, importantly, how can we achieve the above for dozens of organisms independently, with a simple, robust, and frugal approach that can be widely adopted by the scientific community?

Available Tools

Current legacy tools (e.g., Petri dishes, Magenta boxes, rhizotrons) are exceptionally simple, standardized, inexpensive, and benefit from decades of protocol development, but were not designed for the creation of model ecosystems. They can easily produce micro- and mesocosms (Figure 1A-B) – simplified, smaller versions of real ecosystems – that do not control the interactions between organisms, nor their individual environments[10]. Engineered environments such as microfluidic circuits[11] physically confine the organisms and control mass transport at the micron scale, but are difficult to scale to the size of plants (especially crops).

Physical networks of independently controlled habitats are another approach to the creation of model ecosystems (Figure 1A-B). Every habitat physically confines an individual organism but allows it to chemically interact with the neighboring habitats.

In principle, the network approach has significant advantages over the microcosm approach: (i) the size and environment of each habitat can be controlled independently, (ii) the distance between the habitats (Figure 1C), and therefore the relative rate of chemical signaling (Figure 1D), can be determined (if the organisms are physically confined by semi-permeable membranes), (iii) organisms can be plugged in and out of the ecosystem to provide internal control experiments, (iv) each organism can be independently phenotyped, (v) large communities can be analyzed by network theory approaches to discover, quantify, and understand collective behaviors).

An apparently fundamental issue with the network approach is that the distances between habitats of cm-scale organisms (1-5 cm) are significantly larger than the diffusion-limited signaling distance (1-5 mm): the organisms confined to nodes of cm-scale networks would not be able to exchange signaling molecules by diffusion at a rate that is comparable to the one observed in their ecosystems, therefore rendering the model ecosystem useless.

A simple solution to this mass transport bottleneck was recently reported[2]. Oscillatory flows in these networks of habitats lead to a diffusion-like transport of chemicals whose rate can be controlled over several orders of magnitude (Figure 2A). Controlling the rate of diffusion between habitats is mathematically analogous to controlling their distance. In the conditions we reported, this effective distance could be made smaller than 1 mm. In other words, organisms could be made to signal to each other as if they would be only 1 mm apart (i.e., as if they would be in each other's rhizosphere) even though they were confined to independently controlled environments. This capability allowed us to quantify the effect of distance (1 mm, 2 mm and 10 cm) on the phenotypic response of *Brassica rapa* to *Bacillus megaterium* (cf. Figure 2B-C). Because this mass transport can be predicted by the laws of diffusion, the relative amounts of exudates exchanged at the different distances could be quantified and correlated with biomass.

An important side effect of these oscillatory flows is that they minimize biofouling of the semi-permeable membranes that we used to confine the colonies. In the presence of unidirectional flow, the filters clog in seconds, while our oscillatory flows persist for hours without clogging.

Concluding Remarks

At least for a time, model ecosystems in the form of physical networks of habitats are bound to be more complex, expensive, time consuming than some of the co-culture protocols commonly employed in laboratories (e.g., partitioned Petri dishes). They are likely to require simple programming of controllers, some understanding of mass transport, and entirely new standardized protocols. By contrast, we believe the fundamentally new possibilities offered by this network approach to model ecosystems will provide new opportunities for the communities of plant science, microbiology, and ecology. We suggest here five general examples for discussion. **(i) Quantifying interactions and microbiome evolution within the rhizosphere:** Habitats containing real soil microbiomes can be placed at different signaling distances from one or more plants. The evolution of the individual microbiomes can be then correlated with the distance from the root system, while observing the effect of the microbiome on the root phenotype. **(ii) Culturing unculturable bacteria:** Specific colonies can be exposed to exudates from established microbiomes in real soil while being physically confined from them, similarly to what was shown in work of Epstein and colleagues[12]. **(iii) Collective responses to stimuli.** A community of organisms can be stressed/stimulated in space (all organisms or a subset of the organisms) and/or in time (e.g., organisms can be plugged in or taken out, environmental variables can be changed for a short time, toxins can be flowed through the system for a determined time) and the local and global response can be phenotyped over time. Well-defined control experiments can quantify the role of the network's connectivity on the stress response. **(iv) Volatiles vs non-volatiles signaling.** The independent control of the above ground and below ground environment enables the distinction of signaling mechanisms. **(v) Interactions with pests.** Interactions with pests can be easily introduced in these communities thanks to the cm-scale dimensions.

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Figure 1. Different approaches to the creation of model ecosystems: microcosms vs physical networks. (A) Differences in the structure of the model ecosystems: microcosms share the same environment, while physical networks confine the organisms in separate but chemical connected environments. (B) Representative examples of microcosm[1] and physical network model systems[2]. (C) Qualitative plots describing the evolution over time of the distances between organisms in the two approaches: microcosms show a wide range of distances between different parts of the same organisms (see red arrows in panel A), while network distances are constant and determined a priori. (D) Qualitative plots of the concentrations of exudates at organism 1 as a function of time as a result of the distances between the organisms: microcosms show a wide range of interaction kinetics between different parts of the multiple organisms due to the wide range of distances, while networks should provide predictable diffusion kinetics due to the precisely defined distances.

Figure 2. Control of signaling distance in a model ecosystem[2]. (A) Time lapse of diffusion-like mass transport of a green dye in a series of three habitats connected in series and undergoing oscillatory flow (left). The rate of mass transport can be fit with the diffusion equation (right), using an effective (“signaling”) distance between habitats that is much smaller than the physical distance (2.4 mm instead of 10 cm). (B) Setup to quantify rhizosphere interactions as a function of distance between *B. megaterium* and *B. rapa* plants using a physical network approach and oscillatory flows to control the signaling distance. (C) Quantitative effect of the distance between colony and plant (1 mm and 2 mm) on the plant biomass, and its correlation with the relative amounts of exudates, calculated from the diffusion equation.