



# New Phytologist

## Time will tell: the temporal and demographic contexts of plant-soil microbe interactions

Journal:	<i>New Phytologist</i>
Manuscript ID	Draft
Manuscript Type:	Tansley Review
Date Submitted by the Author:	n/a
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Key Words:	conspecific negative density dependence, demographic models, Janzen-Connell hypothesis, microbial community, patch occupancy model, plant-soil feedback

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# Time will tell: the temporal and demographic contexts of plant–soil microbe interactions

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March 31, 2023

**Total word count for main body of text: 7198**

**Number of color figures: 6**

**Number of Boxes: 3**

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# Summary

Soil microorganisms can have profound impacts on plant community dynamics and have received increasing attention in the context of plant–soil feedback. The interactions between plants and soil microbes are classically evaluated with a two-phase experimental design that consists of a conditioning phase, during which plants modify the soil microbial community, and a response phase, during which the biomass performance of plants is measured as their response to the soil modification. This experimental design assumes that plant–soil microbe interactions follow a simple temporal trajectory. However, a growing body of research highlights that microbial effects vary with the conditioning duration, plant development, and time since host plant death. These experimental designs also carry implicit assumptions that measuring plant biomass sufficiently captures the consequences of microbial impacts on plant population dynamics, neglecting that soil microbes also govern other key demographic processes over the plant life cycle. Here, we discuss the relevance of these temporal and demographic dimensions of plant–soil microbe interactions when extrapolating experimental results and propose modeling frameworks that can incorporate the new empirical evidence. By integrating empirical and theoretical approaches, we provide a roadmap for more nuanced predictions of the long-term consequences of plant–soil microbe interactions in nature.

# Keywords

conspecific negative density dependence, demographic models, Janzen–Connell hypothesis, microbial community, patch occupancy model, plant–soil feedback

## I. Introduction

Plants interact with a diverse array of soil microbes, including mutualists, decomposers, and pathogens. These interactions are bidirectional, with plants conditioning the soil microbial community, and the resulting changes in microbial community composition impacting subsequent plant performance in the conditioned soil (Bever *et al.*, 1997, Bever, 2003). The study of plant–soil microbe interactions has its origin in agricultural science (Huang *et al.*, 2013, van der Putten *et al.*, 2013) and has been integrated into community ecology under the framework of plant–soil feedback (PSF). Since its introduction by Bever *et al.* (1997), studies have extensively discussed how plant–soil microbe interactions influence plant coexistence (Bever *et al.*, 2010, Ke & Miki, 2015). The PSF framework has also been used to explore how soil microbes affect patterns in the relative abundance of plant communities (Mangan *et al.*, 2010, Reinhart *et al.*, 2021), restoration success (Wubs *et al.*, 2016, Koziol *et al.*, 2018), plant invasion (Callaway *et al.*, 2004, Suding *et al.*, 2013), and the biodiversity–productivity relationship (Kulmatiski *et al.*, 2012, Forero *et al.*, 2021).

To characterize the direction and strength of plant–soil microbe interactions, most studies follow a two-phase experimental design aimed at capturing the two-way interactions between plants and soil microbes (Bever *et al.*, 1997). The classic greenhouse experiment consists of a conditioning phase during which plants modify the soil microbial community, immediately followed by a response phase during which plants of the same or other species respond to the modified soil community (Fig. 1a; Bever *et al.*, 2010, Brinkman *et al.*, 2010). While the distinct two-phase design of such experiments contradicts the conceptualization of PSF as a continuous process during which plants are simultaneously conditioning and responding to the soil microbial community, the design elegantly captures the necessary information for parameterizing the key terms in the classic plant–soil feedback model (Bever *et al.*, 1997, 2012, see also Yan *et al.*, 2022). Although the experimental design has enabled a strong empirical foundation of PSF research across ecosystems (Crawford *et al.*, 2019), this approach implies a number of assumptions about the nature of plant–soil microbe interactions that fail to comport with our contemporary understanding of their dynamics. In particular, a growing number of studies have highlighted the importance of accounting for different temporal and demographic dimensions of plant–soil microbe interactions (Kardol

*et al.*, 2013, Gundale & Kardol, 2021, Chung, 2023; see also other studies in this review). Such evidence shapes both the design of experiments (e.g., how long should the conditioning phase last?) and the interpretation of their results (e.g., how do microbial effects on early-life stage plant performance translate to population-level consequences?). Explicitly considering these unspoken assumptions about time and demography is thus essential when the goal is to infer the role that soil microbes play in shaping plant communities in nature.

The temporal duration of the conditioning and response phases in typical two-phase experiments is generally short (e.g., a few months), with the same time frame applied across all species despite potential life history differences between the focal species (e.g., annual vs. perennial plants; Box 1). Field-based studies may also source conditioned soil microbial communities by collecting soil from individuals growing in the field, but the age of the conditioning plant is generally unknown. Both approaches implicitly assume that microbial effects develop instantaneously and maintain constant strength throughout different plant developmental stages (Fig. 2a). This assumption is at odds with growing evidence that within a single plant generation, microbial communities undergo a continuous turnover (e.g., Edwards *et al.*, 2018, Gao *et al.*, 2019), and that their resulting effects on plant performance can vary based on the duration of plant conditioning and response phases (e.g., Hawkes *et al.*, 2013, Bezemer *et al.*, 2018, Lepinay *et al.*, 2018; Fig. 2b). Moreover, experiments in which the conditioning phase is immediately followed by the response phase neglect the temporal delays that often happen in nature (e.g., when plant growing seasons are separated by long periods of dormancy). Extrapolating from such results assumes that microbial effects remain as long-lasting legacies after plant senescence to impact the next generation. Therefore, while experimental designs are understandably constrained by feasibility, explicit examination of the system's temporal context is critical to better predict the influence of soil microbes.

The short-term nature of most experiments also necessitates that researchers measure a plant demographic response that presumably reflects all critical impacts of the microbial community (Ke & Wan, 2022). The most frequently measured performance proxy is plant biomass, which is then used for calculating theoretically-derived metrics to infer how soil microbes influence plant coexistence. For instance, the biomass of plants in conspecific- and heterospecific-conditioned soils can

be used to calculate the pairwise feedback metric that quantifies the frequency-dependent feedback loops generated by plant–soil microbe interactions (Bever *et al.*, 1997). Negative frequency-dependence arises when both plants condition their soil microbes in a way that favors heterospecifics over conspecifics, thereby promoting plant coexistence (depicted in Fig. 1a; Crawford *et al.*, 2019). Recently, multi-species extensions of the feedback metric have been proposed (Eppinga *et al.*, 2018, Mack *et al.*, 2019), as well as metrics that capture the frequency-independent microbial effects (Kandlikar *et al.*, 2019, 2021, Yan *et al.*, 2022). In the context of the classic PSF model, where soil microbes are the main driver of plant community dynamics by changing plants' intrinsic growth rates (Bever *et al.*, 1997), these metrics are well suited to extrapolate from biomass measurements to predict plant coexistence. However, soil microbes can also affect other demographic processes across the plant life cycle (e.g., changing survival rates or the nature of density-dependent interactions; Chung & Rudgers, 2016), potentially with opposing effects at different plant ontogenetic stages (Dudenhöffer *et al.*, 2018, Dostálek *et al.*, 2022). Integrating these different impacts, instead of making predictions based on any one life stage, is another challenge when predicting the long-term demographic consequences of soil microbes.

Here, we discuss why the temporal and demographic aspects represent two critical knowledge gaps when one studies the role of soil microbes in a natural context. We humbly recognize that a complete review of the plant–soil microbe interaction literature is beyond the scope of a single review. Instead, we aim to highlight evidence of the two missing components, discuss their relevance when extrapolating experimental results, and outline future avenues to incorporate them. In particular, we advocate for a shift from using biomass-based performance indices to parameterizing plant demographic models with microbial effects, which can integrate the temporal and demographic details to better predict the outcome of plant–soil microbe interactions in their natural context.

## II. Dissecting different temporal dimensions of microbial effects

Studies on the temporal patterns of plant–soil microbe interactions have classically focused on its variation along plant succession, which typically involves plants with different traits or shifts in

the external environment (Kardol *et al.*, 2006, 2013, Bauer *et al.*, 2015). However, temporal variation also occurs across shorter time scales within a single plant generation because the conditioned soil microbial community and plant response both vary over time (Fig. 2b). The strength and direction of plant–soil microbe interactions thereby depends on the timing of interactions. Recognizing this type of temporal variation directly influences the experimental design and how we interpret experimental results. In this section, we review evidence of temporal variability and discuss mechanisms of how the impact of microbial communities on plant biomass performance varies with the duration of soil conditioning (subsection II.1), the time lag between consecutive generations (subsection II.2), and the ontogeny of responding plants (subsection II.3). We then discuss how to design experiments that tackle the temporal complexities observed in nature (subsection II.4). Note that for this section we focus on studies that measure plant biomass as the key performance proxy; we will discuss other demographic responses in section III.

## II.1 Temporal development during the conditioning phase

Understanding the temporal dimensions begins by examining the soil conditioning phase. By growing plants in soils that were conditioned for different durations (red vertical arrow (i) in Fig. 1b), studies have shown that the relative impact of conspecific- and heterospecific-conditioned soil on the responding individual can vary with the duration of soil conditioning. For example, Lepinay *et al.* (2018) found that after a brief conditioning period of two weeks, heterospecific soil had a more negative impact on *Rorippa austriaca* performance than its conspecific soil. However, a longer duration of soil conditioning resulted in the opposite relationship: conspecific soil had an increasingly stronger negative impact peaking at six weeks of conditioning, whereas the negative effect of heterospecific soils diminished after four to eight weeks of conditioning. The temporal dynamics of the soil microbial community likely contribute to such variation in conditioned soil effects with conditioning duration, as the taxonomic composition of the soil microbial community often changes as plants mature or enter different developmental stages (Dombrowski *et al.*, 2016, Edwards *et al.*, 2018, Hannula *et al.*, 2019; but see Lundberg *et al.*, 2012). The influence of conditioning time effects on plant–soil microbe interactions may also arise due to temporal shifts in the functionality of the microbial community. For example, metatranscriptomics revealed that



the expression of mRNA associated with nitrogen fixation increased during the growth phase of *Arabidopsis*, while antibiotic defense activity increased during bolting and flowering (Chaparro *et al.*, 2013). Importantly, these temporal dynamics of the soil microbial community have crucial consequences on the results of two-phase experiments: arresting soil conditioning at different time points causes the responding plant to encounter microbial communities with different compositions and functions, thereby experiencing different plant–soil microbe interactions.

Current literature discussing mechanisms of conditioned microbial community assembly has distinguished between plant- and microbe-centered mechanisms, although we note that both forces often act simultaneously. Plant-centered mechanisms often result from physiological changes in nutrient allocation or root exudation during different plant developmental stages (Chaparro *et al.*, 2013, Zhalnina *et al.*, 2018). For example, plants with different life histories may prioritize shoot and root growth differently, therefore recruiting different mutualistic microbial communities through exudates at different times according to their nutritional needs (Kulmatiski *et al.*, 2017, Huberty *et al.*, 2022). In addition to shifts in resource allocation, increases in immunity against pathogens as plants mature can also affect the structure of soil microbial communities (Bulgarelli *et al.*, 2013). On the other hand, microbial-centered mechanisms focus on microbe–microbe interactions such as competition and facilitation, as well as other processes affecting microbial community assembly such as stochastic drift and evolutionary response (Barret *et al.*, 2015, Herrera Paredes & Lebeis, 2016, Bittleston *et al.*, 2021). Importantly, these mechanisms underlying changes in the microbial community are interconnected: changes in microbial interactions often occur due to plant-induced changes in soil chemistry (Lepinay *et al.*, 2018) and physical properties (Edwards *et al.*, 2018), and outcomes of microbial interactions can trigger further plant physiological responses.

The significance of temporal development during the soil conditioning phase is exemplified when one considers plants with different life histories. Short- and long-lived plants may structure microbial communities at different rates, and the same duration of soil conditioning may correspond to different developmental stages and microbial effects (Kulmatiski *et al.*, 2017): the species-specific microbiome of a short-lived annual plant may be fully mature, whereas that of a long-lived perennial may require a longer conditioning time. As shown in Box 1, a substantial fraction of studies (18%; 19 out of 106) have included annual–perennial pairs in their experiment

while implementing the same (usually short) conditioning time, raising the critical issue of how to interpret the results in the context of the focal system's natural history. In addition to life history strategies, plants belonging to different functional groups may respond differently to the same temporal turnover in microbial communities. In Ke *et al.* (2021), the microbial effect experienced by the legume species *Lupinus arboreus* remained constant despite temporal changes in the microbial community, whereas that experienced by the other three non-legume species strengthened with longer conditioning time. This result potentially indicates functional redundancy within the soil microbial community (Birnbaum *et al.*, 2018, Huberty *et al.*, 2022). We return to the logistical challenges when trying to detect microbial functionality in section V.

## II.2 Alterations of microbial effects after plant death

Greenhouse experiments typically initiate the response phase immediately after soil conditioning (i.e., immediate transplant). This neglects the fact that in nature, there may be a temporal lag between the senescence of the conditioning individual and the growth of the next responding individual, potentially due to discrete growing seasons or dispersal limitation. During this lag phase when the conditioned soil is left empty for an extended period of time, processes such as litter decomposition, abiotic filtering, and stochastic drift may continue to change the microbial community. Studies growing seedlings in soils collected from dead individuals (red vertical arrow (ii) in Fig. 1b) suggests that such lags can have distinct effects across different systems. For example, Bennett *et al.* (2022) showed that microbial communities from soils collected under dead and live adult *Populus tremuloides* trees had similar effects on conspecific seedlings. Conversely, Esch & Kobe (2021) showed that the negative effects of soil from live *Prunus serotina* on the survival of conspecific seedlings faded away within one year after tree removal. Below, we discuss the mechanisms that could either maintain or alter microbial effects when a temporal lag exists between consecutive generations.

Microbial effects could persist after active plant conditioning ceases due to the continued survival and functioning of the conditioned microbial community in the soil (Pepe *et al.*, 2018, Esch *et al.*, 2021, Hannula *et al.*, 2021). For example, Esch *et al.* (2021) found that the persisting

pathogenic oomycetes collected from live versus dead tree stumps have similar negative effects on conspecific seedling survival. Similarly, Pepe *et al.* (2018) showed that arbuscular mycorrhizal fungi remain active and can spread from roots after host shoot removal. The maintenance of microbial activity can occur if root systems remain active after the removal of aboveground tissues or if the release of nutrients from dead belowground tissues mirrors exudates from living plants (Johansen & Jensen, 1996, Müller *et al.*, 2013). Additionally, trophic flexibility (e.g., saprotrophic ability of certain pathogens; Bonanomi *et al.*, 2010) and dormancy of soil microbes can allow the microbial communities to persist after the death of their host, enabling microbes to wait for the arrival of a new host (Lennon & Jones, 2011, Shade *et al.*, 2012, Shemesh *et al.*, 2023). In these cases, the succeeding (response) individual will experience a similar microbial effect despite the temporal lag in arrival timing, and predictions from immediate transplant experiments are relevant to natural systems.

However, various processes can cause the conditioning effect to change after plant senescence, such that subsequent responding individuals encounter a different soil microbial community than that obtained in an immediate transplant scenario (Grove *et al.*, 2015, Veen *et al.*, 2019). The process of litter decomposition can introduce phyllosphere microbes to the soil (Fanin *et al.*, 2021, Minás *et al.*, 2021) and release chemicals and nutrients that shift microbial communities (Veen *et al.*, 2021). Additionally, different causes of plant death (e.g., herbivory, fire, and disease) are often associated with further changes in abiotic factors, with potential effects on the composition and function of microbial communities. For example, canopy gaps caused by wind disturbances modify nearby light and moisture levels in a way that suppresses pathogens (Augspurger, 1984, Reinhart *et al.*, 2010, Nagendra & Peterson, 2016). Finally, stochastic drift could decouple microbial community from plant conditioning influence if the soil remains uncolonized over an extended period of time due to plant propagule limitation. In these scenarios, immediate transplant experiments fail to capture the microbial effects experienced by the responding plant in nature.

### II.3 Temporal development during the response phase

The final temporal dimension of plant–soil microbe interactions involves the duration of the response phase, i.e., how the strength and direction of the microbial effect can change over the lifetime of the responding plant (Kardol *et al.*, 2013). Studying such dynamics has been the typical focus when past studies examine the temporal development of plant–soil feedback (Gundale & Kardol, 2021). By sequentially harvesting the responding plant individuals at different time intervals, studies have found that the relative impact of conspecific- and heterospecific-conditioned soil can vary as the responding plant matures. Moreover, the specific temporal pattern depends on nutrient availability (Dostál, 2021), the origin of the responding species (i.e., native or invasive; Hawkes *et al.*, 2013), and whether individuals face co-occurring competitors (Dostál, 2021, Bezemer *et al.*, 2018). This empirical evidence provides a strong impetus to consider the temporal variability in the response phase since harvesting an experiment at different endpoints can alter our understanding of the microbial effect.

While studies distinguish between conditioning and response phases in their experimental designs, it is important to note that the two processes operate simultaneously in nature and represent two sides of the same coin. Therefore, the plant- and microbe-centered mechanisms that drive changes in the soil microbial community during the conditioning phase as discussed in II.1 also apply to explain patterns in the response phase. For example, strengthening of immunity as plants mature can reduce pathogen abundance and alter the soil microbial community as the conditioning phase progresses (Bulgarelli *et al.*, 2013); it can also reduce plant susceptibility to pathogens and alleviate negative microbial effects experienced by the plant as the responding individual matures. Similarly, mechanisms that reduce the abundance of beneficial microbes after soil conditioning (e.g., mature plants becoming less reliant on mutualistic partners) also act upon the responding individual to diminish the observed positive microbial effect. Moreover, since the conditioning and responding processes act simultaneously, responding individuals will also impose their own conditioning impact, the extent of which can depend on plant and microbial functional group (Hannula *et al.*, 2021). We will elaborate on necessary experiments that can tease apart different temporal dimensions in the next subsection.

## II.4 Implications for experimental designs

While the current lack of empirical information limits our understanding of the temporal dimensions of plant–soil microbe interactions, we see a path forward. First, the temporal settings of the experiment should guide our interpretation of the results. For instance, in Mediterranean annual plant communities where the growing season only lasts a few months, traditional experiments in which a short-term conditioning phase is immediately followed by the response phase may adequately reflect potential microbial effects that unfold within a growing season. However, such a design may not be adequate to project microbial effects on long-term population dynamics because it overlooks the clear seasonality of plant growth in nature (Kandlikar *et al.*, 2021). Second, we encourage experiments that are explicitly designed to reflect the temporal aspects of a focal plant–soil system in nature (Fig. 1b). For Mediterranean annual plant communities, mirroring the temporal dynamics of the natural system by incorporating a decay phase during which the conditioned soils are exposed to a prolonged drought with no vegetative growth (red vertical arrow (ii) in Fig. 1b) may provide a better understanding of how soil microbes shape plant community dynamics across years. Moreover, researchers can build on natural experiments, long-term monitoring plots, or historical information to account for variation in conditioning duration, host plant age, or time since host tree death. This approach may be especially applicable in studies that focus on plant–soil microbe interactions in long-lived plants, which often source field-conditioned soils for greenhouse experiments (44%; 47 out of 106 studies in Box 1). For example, Ke *et al.* (2021) used historical aerial photos to track plant age and employed a chronosequence approach to study how plant–soil microbe interactions vary with the duration of soil conditioning. Other examples include using host tree size as a proxy of conditioning time (Chen *et al.*, 2019) and utilizing chronosequences of abandoned fields or agricultural harvest times to study the persistence of microbial effects (van de Voorde *et al.*, 2012, Esch & Kobe, 2021).

One can also design experiments that isolate a particular facet of temporal variability, potentially disentangling the mechanisms behind observed temporal patterns. Current studies on the temporal development of microbial effects typically employ sequential harvesting (e.g., Hawkes *et al.*, 2013, Bezemer *et al.*, 2018). Since soil conditioning and plant response in fact unfold simultaneously in nature (Chung, 2023), the observed temporal changes in microbial effects result from

the combination of varying plant physiological responses and any changes to the soil community that are due to the effects of the responding plant itself (red diagonal arrow (iii) in Fig. 1b). To quantify the effects associated with changing soil microbial communities during soil conditioning, studies could plant seedlings of the same age in soils with different conditioning duration (red vertical arrow (i) in Fig. 1b). Alternatively, if the goal is to isolate the effects caused by changing plant physiology, an experiment could instead grow plants of different ages (kept in a relatively sterilized environment such as a Magenta box before transplanting) in soils with identical conditioning duration (red horizontal arrow (iv) in Fig. 1b). In addition, mutants or cultivars with different developmental rates can also be used to separate the effects of plant developmental stage (e.g., vegetative growth or flowering) and age *per se* (Dombrowski *et al.*, 2016). While the above scenarios are deliberately artificial, these experiments can provide a mechanistic understanding underlying the observed temporal patterns of plant–soil microbe interactions.

While we have focused on changes happening over the course of a single plant-to-plant replacement, these dynamics are closely related to other temporal patterns. One direction of research is how microbial effects build up over generations through multiple tiers of conditioning and response. A wealth of literature has explored the microbial underpinning of reduced crop yield following repeated planting (i.e., soil sickness; reviewed in Huang *et al.*, 2013) and the strengthening of conspecific microbial effects experienced by non-native plants after their introduction (Diez *et al.*, 2010, Dostál *et al.*, 2013; but see Day *et al.*, 2015). While this temporal pattern has been demonstrated by experiments using soils with conditioning histories that span multiple generations, few studies have generalized the traditional focus of single species to multiple species. In a unique greenhouse experiment consisting of two rounds of soil conditioning by different combinations of six plant species, Wubs & Bezemer (2018) demonstrated the interactive effects arising from multiple rounds of soil conditioning. Future work can expand upon Wubs & Bezemer (2018) to study how the unique sequences of soil conditioning result in different plant–soil microbe interactions. Another tightly interconnected aspect is the demographic facet of plant–soil microbial interactions: as the responding individual matures, soil microbes can influence various demographic processes in addition to varying biomass responses. We elaborate on this in the next section.

### III. Assessing multiple demographic consequences of soil microbes

Most two-phased studies of plant–soil microbe interactions are designed to evaluate how different soil microbial contexts influence plant biomass performance. Experimentally, the implicit assumption is that individual biomass at the end of the experiment integrates all impacts of the microbial community across all plant life stages, and that biomass is a good indicator of plant fitness at the population level. This design corresponds well with the classic feedback model of Bever *et al.* (1997), where microbes control plant species' intrinsic growth rates. However, such an assumption may not hold for real plant communities because soil microbes can alter not only biomass performance but also other key demographic processes across the plant life cycle, often in opposite directions (Dudenhöffer *et al.*, 2018). These effects are not directly captured by studies that only focus on biomass, and integrating these different impacts to predict the long-term consequences of soil microbes on plant dynamics remains a gap (Dostálek *et al.*, 2022). Here, we highlight key studies that provide insights into microbial control over non-biomass plant demographic processes, with a particular focus on early life stage transitions.

#### III.1 Microbial regulation of seed-to-seedling transition

Soil microbes can have drastic consequences on the early life stages of plants. While these effects can arise from microbial effects on distinct life history processes (i.e., seed survival, germination, and early seedling survival; Fig. 3), they are often grouped together given the logistical challenges of separating these effects in field settings. For example, for long-lived plants such as forest trees, repeated demographic censuses are often used to monitor seed-to-seedling transitions (e.g., Harms *et al.*, 2000, Swamy *et al.*, 2011). A large body of evidence for microbial effects on plant early life stages comes from field studies finding that fungicide applications altered patterns of seed and seedling demography (e.g., Bell *et al.*, 2006, Bagchi *et al.*, 2014, Krishnadas *et al.*, 2018). Many of these studies are conducted in the context of evaluating microbes as potential drivers of conspecific negative density-dependence (CNDD; Comita & Stump, 2020, Song & Corlett, 2022), a demographic signature of the Janzen–Connell hypothesis (Janzen, 1970, Connell, 1971; see also Box 2). While evaluating the integrative microbial effect across multiple early life stages can



yield critical insights, studies that isolate microbial effects on specific underlying demographic transitions, i.e., seed survival, seed germination, and early seedling performance, can enable a nuanced and mechanistic understanding of microbial effects on plant population dynamics (see Box 3).

Soil-borne pathogens can cause substantial mortality at the seed stage in nearly all terrestrial ecosystems (e.g., Kotanen, 2007, Sarmiento *et al.*, 2017, Li *et al.*, 2019). One system where the role of fungal seed pathogens in structuring plant community dynamics has been systematically dissected is that of pioneer tree species in neotropical forests, especially those in the genus *Cecropia*. As pioneer species whose seeds need to germinate quickly in response to new gap openings, these species produce seeds that can persist in the soil until the formation of nearby gaps. These seeds are vulnerable to pathogen attack during their time in the soil seed bank, and as a result, fungicide treatments can nearly double their survival and emergence (Dalling *et al.*, 1998, Gallery *et al.*, 2010). Moreover, Dalling *et al.* (1998) found that seeds were more susceptible to pathogen attack in soils close to conspecific adults than in soils far from conspecifics, potentially implicating soil pathogens as drivers of Janzen–Connell dynamics (Box 2). Recent advances have largely focused on employing molecular methods toward understanding longstanding questions about pathogen host-specificity. Zalamea *et al.* (2021) found that seeds of closely related *Cecropia* species harbor vastly distinct fungal communities, with species identity explaining substantially more variation than the seeds' location or their viability. Working with a more diverse group of pioneer tree species, Sarmiento *et al.* (2017) showed that while many fungi can grow on seeds of multiple plant species, their effects on seed mortality are highly species-specific. Together, this series of studies have highlighted soil-borne fungal seed pathogens as key microbial players in the dynamics of pioneer trees in tropical forests. While quantifying microbial effects on seed survival requires laborious methods (e.g., tetrazolium staining for testing seed viability; Sarmiento *et al.*, 2017), a better understanding of these effects is critical given that seed limitation can be a bottleneck on plant population dynamics (Harper, 1977, Clark *et al.*, 2007), especially in communities dominated by plant species that form persistent seed banks.

Soil microbes can also affect the rates and timing of germination. Such regulation primarily arises due to the production and/or metabolism of key germination-related phytohormones like



gibberellins (reviewed in Keswani *et al.*, 2022 and Bottini *et al.*, 2004) or ethylene (reviewed in Ravanbakhsh *et al.*, 2018 and Ishaq, 2017). While studies of how soil microbes regulate germination have historically focused on managed settings, evidence that microbes affect germination is now accumulating in ecological settings. In one of the few two-phase experiments focused on pairwise feedback effects on germination, Miller *et al.* (2019) found species-specific effects of conditioned microbes on germination. Specifically, the legume *Desmodium illinoense* achieved lower germination rates in conspecific-conditioned soils than in sterilized or heterospecific-conditioned soils, while germination of *Bromus inermis* and *Solidago canadensis* was unaffected by soil microbes. Across a large-scale microcosm experiment, Eldridge *et al.* (2021) found that soil bacterial and fungal communities help explain substantial variation in patterns of seed germination across nine plant species, suggesting a relationship between soil microbes and plant germination that is not explained simply by their shared responses to abiotic soil properties. Even when soil microbes do not affect overall rates of germination, they can alter the phenology of germination (Keeler & Rafferty, 2022) and could either harm (e.g., if later germination reduces seedlings' performance due to competition; Orrock & Christopher, 2010) or benefit (e.g., if later germinants escape severe competition at the seedling stage or avoid abiotic stress; Leverett *et al.*, 2018) population growth. However, current studies often neglect germination effects due to the common practice of transplanting pre-germinated seeds into conditioned soils. Accordingly, a first step in enhancing our understanding of this phenomenon is for two-phase studies to directly plant ungerminated seeds and report germination rates along with biomass performance of germinated seedlings (e.g., Dudenhöffer *et al.*, 2022).

Soil microbes also play a key role in determining the survival of seedlings after germination. The widespread role of mycorrhizal symbioses in promoting seedling survival and the potential for soil-borne pathogens to cause mortality among seedlings has been studied for decades and reviewed elsewhere (e.g., Horton & van der Heijden, 2008, Gilbert, 2002). Recent advances have focused on elucidating the relative role of harmful and beneficial soil microbes in driving seedling survival and establishment across different environmental contexts, including abiotic conditions (Bingham & Simard, 2011), the relative abundance of conspecific and heterospecific adults (Teste *et al.*, 2017), and the functional groups of mycorrhizal fungi (Liang *et al.*, 2016, Bennett *et al.*,

2017). In addition to studies that directly track the fate of newly germinated seedlings in specific microbial contexts, studies that monitor the fate of older plant individuals also often speculate soil microbes as the underlying mechanism (e.g., CNDD studies on the survival of larger individuals; Comita *et al.*, 2010). Although microbial controls over seedling survival are known to be common in the field, such effects are poorly studied in the context of greenhouse experiments given their common biomass focus. However, for some systems such as forest trees, individual survival can be size-dependent, and therefore measuring microbe-mediated changes in plant biomass may still provide insights into this demographic transition. Evidence comes from data-driven demographic models showing that the survival rate of seedlings remains high once they achieve a certain size (Chang-Yang *et al.*, 2021). Therefore, soil microbes that decrease seedling biomass performance can manifest in higher mortality among forest seedlings, thereby having a clear demographic consequence on plant populations.

### III.2 Microbial effects beyond early life stages

As seedlings establish and grow into reproductive adults, the soil microbial community continues to affect their performance in various ways not captured by experiments that focus only on plant biomass. While an exhaustive review of all such effects of soil microbes is beyond the scope of this study, we briefly highlight soil microbial regulation of flowering phenology and susceptibility to herbivores. Over the past decade, evidence of microbial regulation of flowering phenology across systems has become widespread (Lau & Lennon, 2012, Wagner *et al.*, 2014, Igwe *et al.*, 2021). Although the consequences of such phenological shifts at the population level are seldom quantified, the few-day differences reported in these studies can have drastic consequences for plant fitness, especially under abiotic stress when earlier flowering can be critical to reproductive success and fitness (reviewed in Kazan & Lyons, 2016, O'Brien *et al.*, 2021). The soil community can also regulate plant susceptibility to herbivores (e.g., Howard *et al.*, 2020, Pineda *et al.*, 2020, Kalske *et al.*, 2022), with such effects likely arising due to changes in leaf metabolomes or volatile organics induced by soil microbes (Kalske *et al.*, 2022, Huberty *et al.*, 2022). The consequences of microbial-induced shifts in plant–herbivore interactions on insect and population dynamics are becoming increasingly well-studied (reviewed in Shikano *et al.*, 2017), but whether these changes

scale up to affect plant population dynamics is less well established. We do not see a universal path for integrating all such microbial effects into a single mathematical model or experiment, but propose that an ideal way forward is to pair experiments with system-specific models that propagate their consequences on long-term plant population dynamics.

## IV. Incorporating plant–soil microbe interactions into ecological models

As reviewed in the above sections, the strength and direction of plant–soil microbe interactions vary along different temporal axes and can influence various demographic processes. While empirical studies are essential for growing our understanding of these aspects, predicting their long-term consequences requires an integration of data with models of plant population dynamics. Therefore, we encourage studies to move from biomass-based performance metrics to demographic models that directly incorporate microbial effects. Developing suitable theoretical models for the focal plant–soil system and connecting them with empirical data remains a critical research direction. Below, we discuss two theoretical frameworks that are especially well-suited to incorporate the temporal and demographic components of plant–soil microbe interactions and highlight studies that have parameterized them with empirical data.

### IV.1 Patch occupancy models

Patch occupancy models represent a relatively straightforward framework for studying plant–soil microbe interactions (Pacala & Tilman, 1994, Mouquet *et al.*, 2002). In this group of models, plants compete for unoccupied sites (patches) and the probability that a particular plant species establishes in a local site depends on the site’s microbial legacy (Stump & Comita, 2018, Miller & Allesina, 2021, Ke & Levine, 2021). Such models can either be spatially implicit, which assumes that the landscape can be divided into an infinite number of patches and tracks the proportion of different plant–soil microbe states (e.g., Miller & Allesina, 2021, Ke & Levine, 2021), or spatially explicit, which considers a fixed-size arena and allows one to consider spatial proximity when modeling

microbial impact (e.g., the diffusion of microbial effects from live individuals nearby; Bever *et al.*, 1997, Mack & Bever, 2014, Bauer *et al.*, 2015). Detailed formulation aside, a common assumption in such models is that plant modification of soil microbial legacies is the only source for plant–plant interactions, an assumption also made in Bever *et al.* (1997) where plants grow exponentially with an intrinsic growth rate affected by soil microbes. This assumption aligns well with two-phase experiments that grow individual plants in soils with different conditioning histories, and as such, patch occupancy models can be readily parameterized with biomass measurements from pot experiments (e.g., by assuming colonization probability scales with the relative biomass performance of different plants in the soil). Alternatively, patch occupancy models can also be parameterized with recruitment data from repeated censuses, thereby incorporating microbial effects on early life stages (e.g., seed survival, germination, and seedling survival in Fig. 3; Krishnadas & Stump, 2021). Due to this connection with empirical data, patch occupancy models are commonly used in the PSF literature when studies wish to extrapolate predictions based on pairwise biomass-based metrics to multi-species communities (e.g., Mangan *et al.*, 2010, Teste *et al.*, 2017, Dudenhöffer *et al.*, 2022).

The patch occupancy framework offers a pathway to effectively incorporate various temporal aspects of plant–soil microbe interactions (Fig 4a). This is because such models can treat different developmental stages of the soil microbial community as distinct states, so that the transitions between states reflect the conditioning and decay rates of soil microbes. The explicit inclusion of microbial legacies in the form of an unoccupied but conditioned patch differs from previous feedback models, which usually assume tight coupling between plants and microbes via timescale separation (Eppinga *et al.*, 2018, Mack *et al.*, 2019). For example, Ke *et al.* (2021) modified a previous model (Fukami & Nakajima, 2013) by making microbial effects vary with the duration of soil conditioning, which in turn influences the transient trajectory of community assembly. In another example, Ke & Levine (2021) used a spatially-implicit model to show that the strength of stabilization driven by host-specific pathogens depends on how quickly the conditioning effects of plants erode. The above models directly track the changes of microbial impact on plants through time, thereby these models can be parameterized with the type of experiments mentioned in subsection II.4. Alternatively, one can build simulation-based models that explicitly track the

population size of microbes at each local site, allowing the temporal development and decay of microbial effects to emerge naturally (Schroeder *et al.*, 2020). However, such models are harder to parameterize with empirical data since they require detailed knowledge of microbial traits and population dynamics (Jiang *et al.*, 2020).

## IV.2 Models incorporating multiple demographic processes

In contrast to patch occupancy models, which usually assume that microbes only impact the colonization process, other studies have formulated models that directly consider distinct microbial impacts on distinct plant demographic processes. Such an approach, which can be difficult to implement due to the extensive amount of work required to obtain all parameters, may be particularly fruitful in demographically complex systems. Demonstrating the power of this approach, a series of studies (Mordecai, 2013a,b, 2015, Uricchio *et al.*, 2019) integrated models and empirical observations to investigate how pathogens affect competition between native perennials and invasive annual grasses. The plant demography components of these models begin with an approach often used for annual plants: they track the yearly population of each species' seeds, which persist in the soil seed bank from previous years or are produced by reproductive-stage individuals, and capture the effect of plant competition through density-dependent decreases in seed production (Fig. 4b; see also Box 3). The authors then incorporated perennial demography by additionally tracking the number of adult perennials, reflecting successful seed germination and recruitment, as well as adult survival from the previous year. This model structure can flexibly incorporate the effect of microbes by allowing them to modify various demographic transitions; in particular, the authors focused on a soil-borne pathogen that reduces seed persistence and germination (Mordecai, 2013a). With a plant competition experiment and manipulations of pathogen densities, Mordecai (2013b) parameterized a model with density-dependent microbial effects and concluded that pathogen spillover promotes the persistence of perennial bunchgrasses. Subsequent work further demonstrated the adaptability of this framework: Mordecai (2015) showed that the plant life stage attacked by pathogens (i.e., seedlings or dormant seeds) and environmental variation jointly determined the coexistence of competing annual plants. In another application, Uricchio *et al.* (2019) combined field observations and experiments to parameterize an even more realis-

tic model, considering multiple annual and perennial species and incorporating two additional microbial effects (i.e., the impacts of foliar pathogens on seedling survival and adult perennial fecundity).

In addition to integrating multiple microbial effects, a demographically explicit model can help identify the most critical microbial effect via simulations. For instance, in the annual–perennial plant model in Uricchio *et al.* (2019), foliar pathogens have little impact but seed pathogens can have a more significant effect on perennial competitors in the system. Such a sensitivity analysis is particularly useful when models include many mechanistic parameters for microbial dynamics (e.g., Ke *et al.*, 2015, Schroeder *et al.*, 2020) and represents another reason why isolating microbial effects on specific demographic transitions can be enlightening. Even for models that do not explicitly incorporate microbial dynamics, identifying the bottleneck for population growth can provide insights for future studies and guide more targeted experiments. With an integral projection model parameterized with long-term demographic data, Chu & Adler (2015) showed that feedback loops during the recruitment stage contributed most to plant coexistence compared to that during the growth and survival stages. The authors speculated this is due to the recruitment stage involving many demographic transitions that are susceptible to soil pathogens (Chu & Adler, 2015). In Box 3, with an annual plant model incorporating microbial effects as qualitative switches in parameter values, we also demonstrate how sensitivity analysis can help identify the relative importance of different microbial effects. In conclusion, we believe that formulating demographic models not only allows smooth integration of the temporal and demographic dimensions of plant–soil microbe interactions but also provides an opportunity to explore their consequences in multi-species communities.

## V. Moving forward with an empirical-theoretical feedback loop

Since its introduction to community ecology, the field of plant–soil microbe interactions has long been shaped by a tight link between empirical and theoretical approaches. By showing how empirically tractable greenhouse experiments can yield data to calculate theory-derived metrics, the approach from Bever *et al.* (1997) has motivated more than two decades of research to predict the

long-term consequences of soil microbes (Crawford *et al.*, 2019). To date, new studies continue to follow this integration, proposing new theories to capture different impacts of soil microbes as well as new experimental designs to quantify them (e.g., Kandlikar *et al.*, 2019, 2021, Yan *et al.*, 2022). Two key assumptions of this approach are that plant–soil microbe interactions follow a simplified temporal trajectory, and that measuring microbial impact on plant biomass is sufficient to capture the population dynamic consequences of soil microbes. While such abstractions have helped make models generalizable, increasing evidence has proven the relevance of the two knowledge gaps when predicting the role of soil microbes in natural communities (Chung, 2023). Explicit consideration of the temporal and demographic aspects not only leads to new research questions but also allows researchers to draw conclusions grounded on relevant experimental settings. As such, we see tremendous value in future efforts that aim to (1) develop theoretical models that can explicitly incorporate the temporal and demographic components of plant–soil microbe interactions, and (2) parameterize such models with corresponding observational data or experiments aimed at quantifying these past-missing components.

New modeling frameworks should be developed in order to incorporate the aforementioned temporal and demographic components. Here, we identify two paths moving forward (Fig 4). First, patch occupancy models can be used to study the temporal dimensions of plant–soil microbe interactions by tracking the transition between different soil microbial states, each with a different impact on future plant recolonization. This framework also echoes recent theoretical studies suggesting that competition for limited colonization sites generates more interpretable frequency-based dynamics for multi-species communities (Miller *et al.*, 2022). Second, instead of tracking species' occupancy frequency, one can also build demographic models that explicitly track plant population densities; this approach offers the opportunity to easily incorporate microbial effects on multiple plant demographic stages. We note that in practice, these modeling approaches are both flexible and can be used to answer more than one research question (e.g., decay dynamics can also be built into a demographically explicit model; Senthilnathan & D'Andrea, 2023). Ultimately, the choice depends on the research question and the focal plant–soil system. For example, in systems with disturbances that may truncate soil conditioning at different timings (Nagendra & Peterson, 2016), or those with low propagule pressure such that conditioned soils are not immediately



recolonized, investigating the temporal dimension can provide great insights into the role of soil microbes in nature. On the other hand, when different soil microbes are known to impact different parts of the plant life cycle, integrating multiple microbial effects into a single model may be more important.

With assumptions reflecting the ecological processes underlying common experimental setup, patch occupancy models can be parameterized with either common biomass measurements from two-phase experiments or census data from CNDD studies (Mangan *et al.*, 2010, Teste *et al.*, 2017, Dudenhöffer *et al.*, 2022). However, we caution that the model itself is agnostic to the details of plant–soil microbe interactions and will encompass different microbial effects depending on the empirical data used for parameterization (Fig. 3). For instance, Stump & Comita (2018) parameterized their patch occupancy model with CNDD patterns based on 5-year seedling survival (Comita *et al.*, 2010), which correspond to microbial effects on the performance and size-dependent survival of older seedlings. On the other hand, Krishnadas & Stump (2021) parameterized a similar model with CNDD patterns based on the seed-to-seedling transition, thereby representing microbial effects on earlier life stages. Moreover, using different data to parameterize the model implies different assumptions on how microbial effects operate. In particular, using biomass measurements from single-individual pot experiments with greenhouse-conditioned soil may imply that soil microbes affect the intrinsic growth rate of plant populations, whereas using CNDD patterns from observational data might imply that soil microbes modify the nature of density dependence among plants.

Designing new experiments that provide the necessary information to parameterize the new plant demographic models of plant–soil microbe interactions is another frontier of research. Some models require experiments that are similar to current two-phase experiments. For instance, to depict temporal development patterns, one can repeat an experiment along naturally occurring variation in the duration of soil conditioning; or to track multiple early life stage microbial effects, one can directly plant ungerminated seeds into cultivated soils (see sections II and III). However, some microbial effects cannot be reliably estimated by classic two-phased experiments with a single-growing plant individual. For example, in models that allow direct plant–plant interactions (e.g., an annual plant model or a Lotka–Volterra model), it is often assumed that soil microbes



can modify the density-dependency of plant population growth (e.g., competitive coefficients  $\alpha_{ij}$  in Box 3; see also Bever, 2003). Recent studies linking plant–soil microbe interactions and coexistence theory specifically highlight this scenario where soil microbes influence the model’s density-dependent parameters (Kandlikar *et al.*, 2019, Ke & Wan, 2020), which require employing experiments that directly manipulate plant density and soil origin (Chung & Rudgers, 2016, Cardinaux *et al.*, 2018). An empirical–theoretical feedback loop is also central to the design of such theory-driven experiments. For example, a proposed design based on the premise that plant–plant interactions are competitive (Ke & Wan, 2020) was challenged by the observation that facilitation is common, leading to a revised density gradient design with greater flexibility (Ke & Wan, 2022). Again, the optimal approach depends on feasibility and which research question can provide a critical understanding of the focal plant–soil system.

One of the remaining challenges is a better understanding of the dynamics and functionality of soil microbial communities (Jiang *et al.*, 2020). With a plant-centered viewpoint, theoretical models often assume simplified microbial dynamics (e.g., separation of timescales) or treat soil microbes as a qualitative modifier of plant parameters. Incorporating microbial community assembly processes, as outlined in section II, can help inform which processes need to be prioritized when building mechanistic models of microbial community dynamics (e.g., Schroeder *et al.*, 2020). Moreover, experiments that establish the causal relationship between measured microbial dynamics and plant demographic responses can help feed theory with realistically-parameterized temporal patterns. To this end, a starting point is to simultaneously measure shifts in both plant response and microbial community composition within studies that vary the temporal components (Esch & Kobe, 2021, Ke *et al.*, 2021, Hannula *et al.*, 2021). However, some technical challenges remain when detecting the dynamics of the microbial community. For example, DNA from dead microbes may still be detected in sequencing time series (Carini *et al.*, 2016), which can lead to erroneous conclusions about how conditioning time, time lags, or other temporal considerations affect plant–soil microbe interactions. Given that taxonomic information may not reliably reflect the function of microbial communities due to their functional plasticities and redundancies, there is also a need for future developments in the detection of microbial functionality. Explicit quantification of microbial activity, such as measurements through multi-omics or metabolite outputs, can allow

for better modeling of functional microbial dynamics. Future studies taking a microbe-centered perspective can further facilitate the empirical–theoretical feedback loop when studying the two missing components of plant–soil microbe interactions.

In summary, we conclude that studying the temporal dimension and the multiple demographic consequences of plant–soil microbe interactions provides a better understanding of their role in the natural context. In addition to the maintenance of plant coexistence and diversity, the two knowledge gaps can also be important for other ecological processes (e.g., recovery trajectory following disturbance and gap dynamics). Recognizing that soil conditioning and plant response are not instantaneous processes also provides insights into studying the context-dependency of plant–soil microbe interactions: shifts in the abiotic environment can occur throughout a plant's lifetime and the timing of which can alter the temporal trajectory differently. Ultimately, knowledge of the system's natural history should guide researchers to recognize which aspects of the temporal and demographic components are important for the focal system and the research question. With the most critical aspect being identified, we believe that parameterizing new demographic models provides an avenue to predict the long-term consequences of plant–soil microbe interactions against a backdrop of real-world conditions in which these interactions play out.

## Boxes

### Box 1: Quantitative summary of the temporal aspects of PSF experiments

We compiled information on the length of soil conditioning and response phases of experiments included in Crawford *et al.* (2019) and Yan *et al.* (2022), two prominent meta-analysis data sets of plant–soil feedback studies. Since the above two studies focused on the pairwise stabilization metric of Bever *et al.* (1997), we compiled information on plant life history and categorized each pairwise comparison as either annual (both plants are annuals; orange in Fig. 1), perennial (both plants are perennials; green), or annual–perennial (match of an annual versus a perennial; blue). While the majority of the plant pairs were perennials, 19 out of 106 studies ( $\approx 18\%$ ) compared microbe-mediated performance change of annuals to that of perennials (see the central panel where each pie chart represents a study and the color proportions indicate the percentage of different “pair types” within the study). However, this comprises only 107 out of 1519 experimental pairs ( $\approx 7\%$ ; see the upper and right stacked histograms based on the number of experimental pairs). When positioning studies based on the duration of their conditioning (x-axis; field-conditioned soil as a separate category) and response phase (y-axis), we see that most studies employed short-term conditioning (mean: 6.5 months, after excluding studies with field-conditioned soils;  $n = 59$  studies) and 47 out of 106 studies comparing perennial pairs relied on field-collected soils ( $\approx 44\%$ ). On average, the length of the response phase (mean: 4.5 months;  $n = 106$  studies) is shorter than that of the conditioning phase. Note that one study with a conditioning length of 48 months and a response length of 32 months (Kulmatiski, 2019) was excluded from the figure to improve visualization (see supplementary data).

[Box Figure 1 about here]

### Box 2: Linking soil microbes to Janzen–Connell patterns in plant demographic transitions

The Janzen–Connell (J–C) hypothesis states that seeds and seedlings farther from conspecific adults have higher per-capita survival because distance helps to escape potential host-specific enemies that aggregate around focal adults (Janzen, 1970, Connell, 1971). By extension, more abundant species are more likely to encounter their enemies and individual survival decreases at higher conspecific densities (i.e., negative conspecific density dependence, CNDD). Biocide experiments have implicated soil microbes as key drivers of J–C effects in seed and seedling mortality (Bell *et al.*, 2006, Bagchi *et al.*, 2010, Song & Corlett, 2022, Krishnadas & Comita, 2018). While the processes that drive J–C effects mirror the conditioning and response processes captured by the classic greenhouse experimental design (Fig. 1a), surprisingly, few studies integrate insights from these two types of assessments. Part of the challenge in connecting different perspectives is that greenhouse experiments of plant–soil microbe interactions tend to estimate microbial effects on plant biomass, while J–C studies (using in-situ field experiments and observational data) usually estimate conspecific effects on survival (Comita *et al.*, 2010, Swamy *et al.*, 2011, Zhu *et al.*, 2018). In addition, J–C studies often lack heterospecific treatments, whereas a reciprocal design is much more common in greenhouse experiments.

Some changes in study design can facilitate the connection between the microbial impacts on demographic transitions estimated from field census and the microbial effects estimated from biomass performance in pot experiments (McCarthy-Neumann & Ibáñez, 2013). For field-based J–C studies, laying seeds and seedlings at both conspecific and heterospecific trees in a factorial design, especially when combined with biocides, will provide the metrics to assess the potential for coexistence. Furthermore, adding a reference treatment in randomly located field soil (in addition to a distance treatment) allows one to estimate microbial impacts on frequency-independent survival, which echos recent studies that situate plant–soil microbe interactions more squarely in the domain of modern coexistence theory (Kand-

**Box 2 (continued)**

likar *et al.*, 2019). For biomass-focused greenhouse experiments, sourcing field-conditioned soil from locations with different local adult densities, or performing a pot experiment with varying densities of ungerminated seed, can capture density-dependent microbial effects implicit in J–C studies (Ke & Wan, 2022). Moreover, microbial impact on biomass performance can be used to parameterize demographic models and further simulate stage transition probabilities (Chang-Yang *et al.*, 2021).

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### Box 3: Detecting the most critical microbial effect with a demographic model

Here, we demonstrate how situating microbial effects within a demographic model of plant population dynamics can help integrate multiple microbial effects and identify the most critical one. We consider two annual plant species,  $N_1$  and  $N_2$ , with dynamics described by the classic Beverton–Holt annual plant model (see also Fig. 4b):

$$N_{i,t+1} = s_i (1 - g_i) N_{i,t} + \frac{\lambda_i g_i N_{i,t}}{1 + \alpha_{ii} g_i N_{i,t} + \alpha_{ij} g_j N_{j,t}}.$$

The first term represents the survival of ungerminated seeds, with  $g_i$  and  $s_i$  representing seed germination and survival rate, respectively (circular loop in Fig. 4b). The second term represents seed production and density-dependent interactions among germinated seeds, with  $\lambda_i$ ,  $\alpha_{ii}$  and  $\alpha_{ij}$  representing intrinsic plant fecundity, intraspecific and interspecific competitive impact experienced by  $N_i$ , respectively (rightward arrows in Fig. 4b).

For each plant species, there are five demographic parameters that can be affected by soil microbes ( $g_i$ ,  $s_i$ ,  $\lambda_i$ ,  $\alpha_{ii}$ , and  $\alpha_{ij}$ ); researchers can design experiments to quantify each of them (e.g., by comparing the demographic process in conditioned versus sterilized soil). To illustrate the potential value of such an experiment, we consider a case where the soil microbes affect the population dynamics of the inferior species (i.e.,  $N_2$ , with a low relative abundance indicated by the grey dashed line in Box Figure 2). The first strength of a demographic model, as opposed to biomass-based metrics, is that it can integrate multiple microbial effects. For example, if soil microbes increase all parameters of  $N_2$  by 40%, the model suggests that the relative abundance of  $N_2$  will nearly double (i.e., from grey to blue dashed line). Only quantifying microbial effects on intrinsic fecundity ( $\lambda_2$ ), as is commonly done in studies that grow individual plants in conditioned soils, would substantially overestimate microbial effects on  $N_2$ 's equilibrium relative abundance. The second strength of a demographic model is that it helps identify the most critical microbial effect. For example, sensitivity analysis (see Box figure legend for details) revealed that

**Box 3 (continued)**

microbe-mediated increase in seed survival rates ( $s_2$ ) had negligible effects on the  $N_2$  population, suggesting experiments might be justified in omitting this effect.

[Box Figure 2 about here]

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**Acknowledgments**

We thank Xinyi Yan for contributing to the dataset used for Box Figure 1 and for insightful comments that improved the manuscript. We thank Chia-Hao Chang-Yang, Y. Anny Chung, Yu-Pei Tseng, Yi Sun, and Shuo Wei for their discussions. P.-J. Ke is funded by Taiwan MOE Yushan scholar program 111V1010-2 and MOST 111-2621-B-002-001-MY3. G.S. Kandlikar, M. Krishnadas, and P.-J. Ke acknowledge support from sDiv, the Synthesis Centre of iDiv (DFG FZT 118, 202548816).

**Author Contributions**

P.-J. Ke, S.X. Ou, and G.S. Kandlikar conceived the study and took the lead in writing the first draft. All authors contributed critically to developing the ideas and finalizing the manuscript. P.-J. Ke and S.X. Ou contributed equally.

**Data Availability**

The dataset and code used to generate figures in Box 1 and Box 3 will be made available on Dryad upon publication.



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## Figure legends

**Figure 1.** Experiments for studying plant–soil microbe interactions. (a) The classic two-phase experimental design, consisting of a conditioning phase during which plants modify the soil microbial community and a response phase during which plants respond to the soil modification. Depicted here in the response phase is the case of negative frequency-dependent feedback where conditioned soils favor the performance of heterospecifics over conspecifics. (b) Proposed experimental designs to study the various temporal dimensions in the main text (measuring the orange plant’s performance in soils conditioned by the blue plant as an example): (i) isolating changes in the soil microbial community by varying the duration of soil conditioning, (ii) isolating the decay process by incorporating a time lag after soil conditioning, (iii) sequential harvesting with both conditioning effect and plant age advancing simultaneously, and (iv) isolating changes in plant physiology by transplanting individuals of different age in the same conditioned soil.

**Figure 2.** Temporal dimensions of plant–soil microbe interactions throughout the repeated process of plant establishment, growth, death, and recolonization by another individual. (a) The common assumptions of plant–soil microbe interactions implied by the design of classic experiments: microbial communities develop instantaneously, with resulting microbial effects that are constant throughout different plant life stages and remain as long-lasting legacies after plant senescence to impact the next generation. (b) The dynamic plant–soil microbe interaction perspective highlighted in our review: microbial communities change continuously throughout the conditioning process, with impacts on plant performance that depend on both the duration of plant conditioning (subsection II.1) and response (subsection II.3). Moreover, microbial communities and their impacts on plant performance may diminish with time after the senescence of the previous conditioning individual (subsection II.2) or undergo different trajectories depending on the previous rounds of conditioning (mentioned as a future direction in subsection II.4).

**Figure 3.** Conceptual diagram depicting multiple demographic consequences of soil microbes, with a particular focus on early plant life stages following most empirical studies. The inner circle (black arrows) indicates the distinct demographic processes that can be affected by soil microbes; in



the main text, we highlight empirical evidence on seed mortality, germination, and early seedling survival. The outer circle (grey dashed arrows) indicates the life stages included in different studies on conspecific negative density dependence (CNDD). Operationally, CNDD studies may include multiple demographic processes due to the logistical challenges of repetitive censuses.

**Figure 4.** Two modeling frameworks that can incorporate the missing temporal and demographic components of plant–soil microbe interactions. In both panels, demographic processes influenced by soil microbes are highlighted in red. (a) An example from Ke & Levine (2021) demonstrating how the temporal decay of microbial effects can be studied with a patch occupancy model. The model tracks the frequency of different plant-soil microbe states (here depicted as various combinations of plants and soil) and the transitions among them. State transitions may occur due to plant colonization and death (solid arrows) or the conditioning and decay of microbial effects (dashed arrows). In this example, soil microbes affect the ability of plants to recolonize conditioned soils. (b) An example demonstrating how microbial effects on multiple demographic processes can be incorporated in the annual plant model in Mordecai (2015) (see also Godoy & Levine, 2014), which tracks the density of seeds prior to germination. Here, soil microbes influence the seed survival in the soil seed bank, the germination rate of seeds, the fecundity of germinated plants, and the density-dependency of plant population growth.

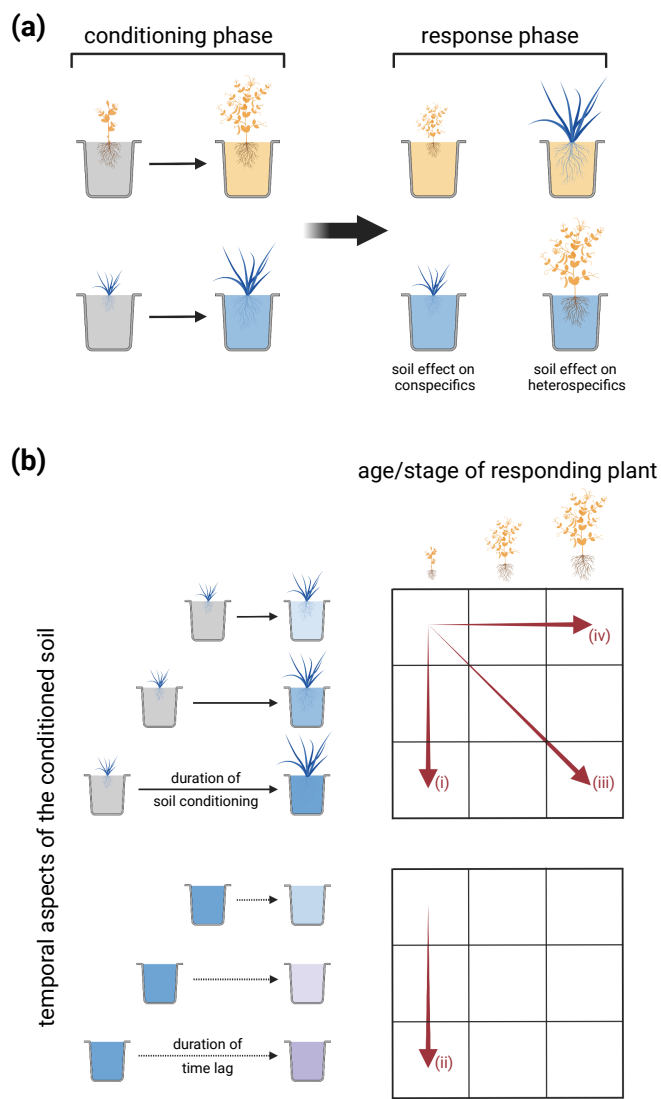
**Box Figure 1.** A summary of the experimental duration and life history information of the study species in the Crawford *et al.* (2019) and Yan *et al.* (2022) data sets. See text in Box 1 for more details.

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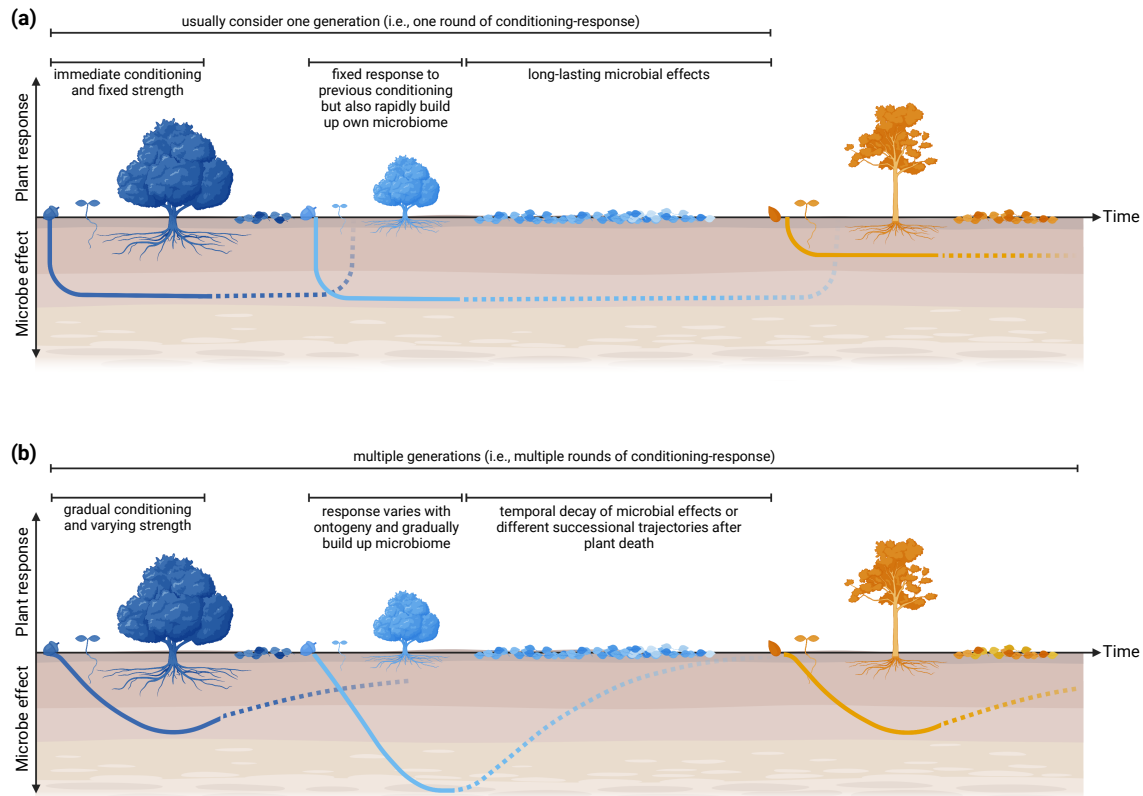


1051 impact); this process was repeated 100 times for each focal parameter. Each grey point represents a  
1052 random simulation (100 time steps) for the focal parameter (red points and error bars represent the  
1053 means and standard deviations). The grey dashed line represents the original relative abundance  
1054 of  $N_2$  and the blue dashed line represents its relative abundance when soil microbes increased all  
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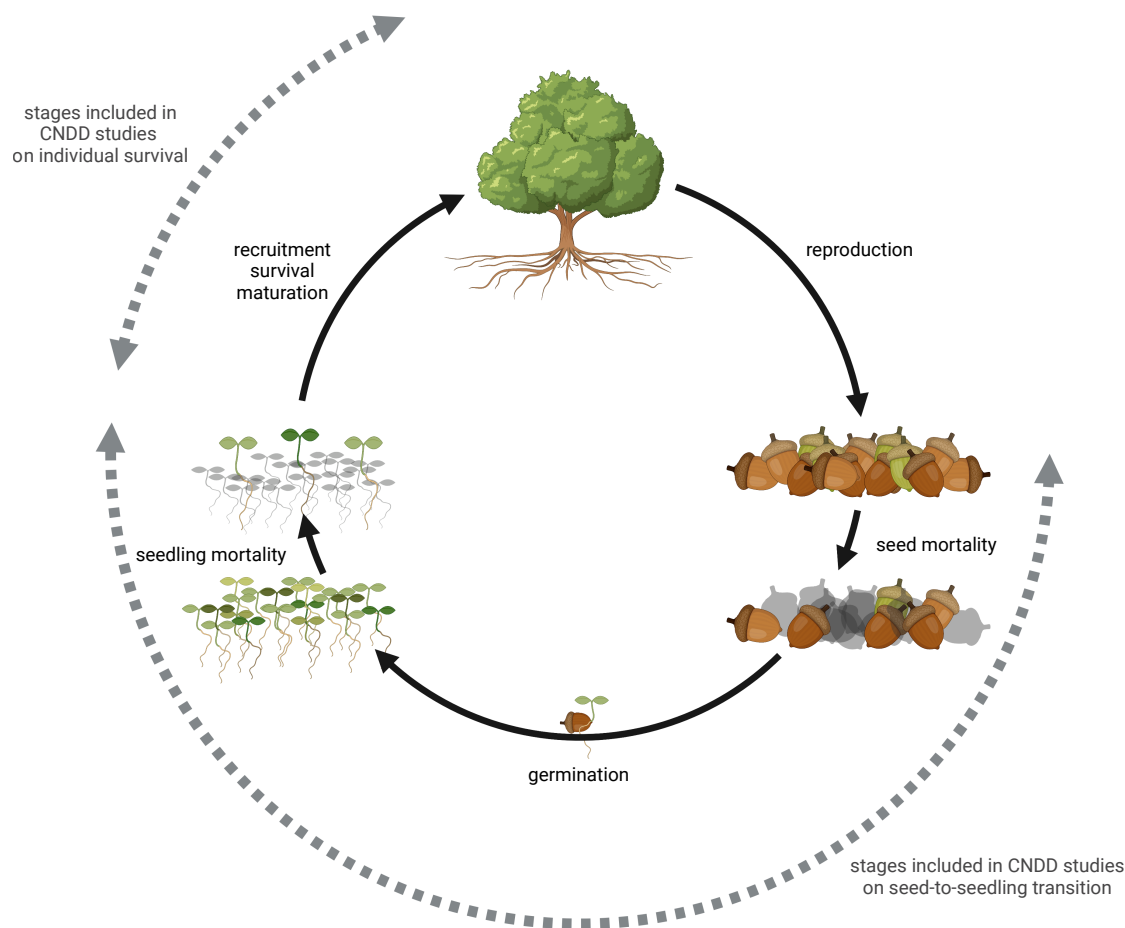
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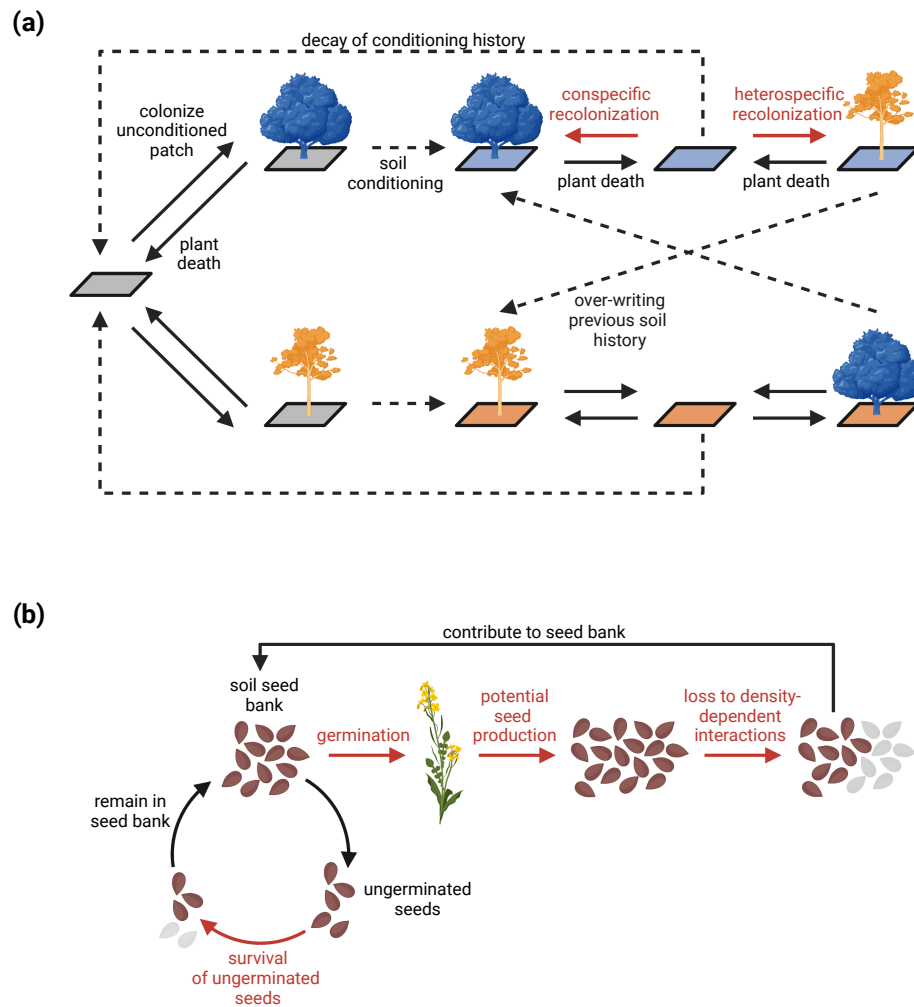
**Figure 1** Experiments for studying plant–soil microbe interactions. (a) The classic two-phase experimental design, consisting of a conditioning phase during which plants modify the soil microbial community and a response phase during which plants respond to the soil modification. Depicted here in the response phase is the case of negative frequency-dependent feedback where conditioned soils favor the performance of heterospecifics over conspecifics. (b) Proposed experimental designs to study the various temporal dimensions in the main text (measuring the orange plant’s performance in soils conditioned by the blue plant as an example): (i) isolating changes in the soil microbial community by varying the duration of soil conditioning, (ii) isolating the decay process by incorporating a time lag after soil conditioning, (iii) sequential harvesting with both conditioning effect and plant age advancing simultaneously, and (iv) isolating changes in plant physiology by transplanting individuals of different age in the same conditioned soil.



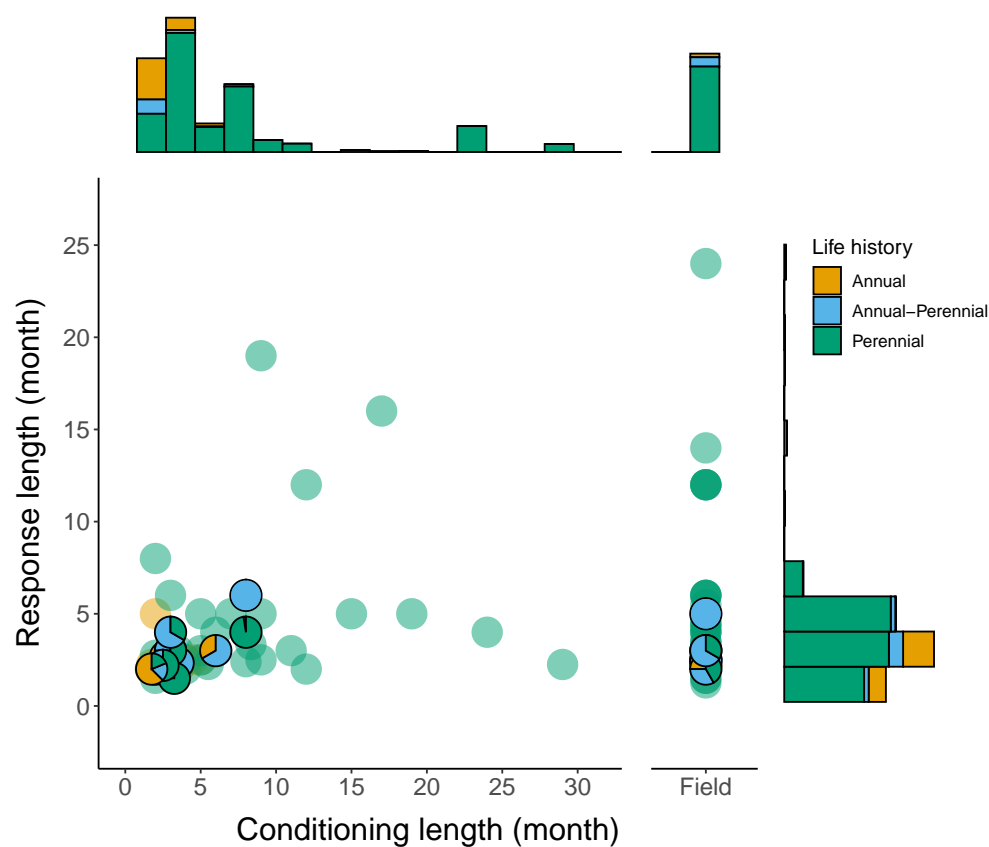
**Figure 2** Temporal dimensions of plant–soil microbe interactions throughout the repeated process of plant establishment, growth, death, and recolonization by another individual. (a) The common assumptions of plant–soil microbe interactions implied by the design of classic experiments: microbial communities develop instantaneously, with resulting microbial effects that are constant throughout different plant life stages and remain as long-lasting legacies after plant senescence to impact the next generation. (b) The dynamic plant–soil microbe interaction perspective highlighted in our review: microbial communities change continuously throughout the conditioning process, with impacts on plant performance that depend on both the duration of plant conditioning (subsection II.1) and response (subsection II.3). Moreover, microbial communities and their impacts on plant performance may diminish with time after the senescence of the previous conditioning individual (subsection II.2) or undergo different trajectories depending on the previous rounds of conditioning (mentioned as a future direction in subsection II.4).



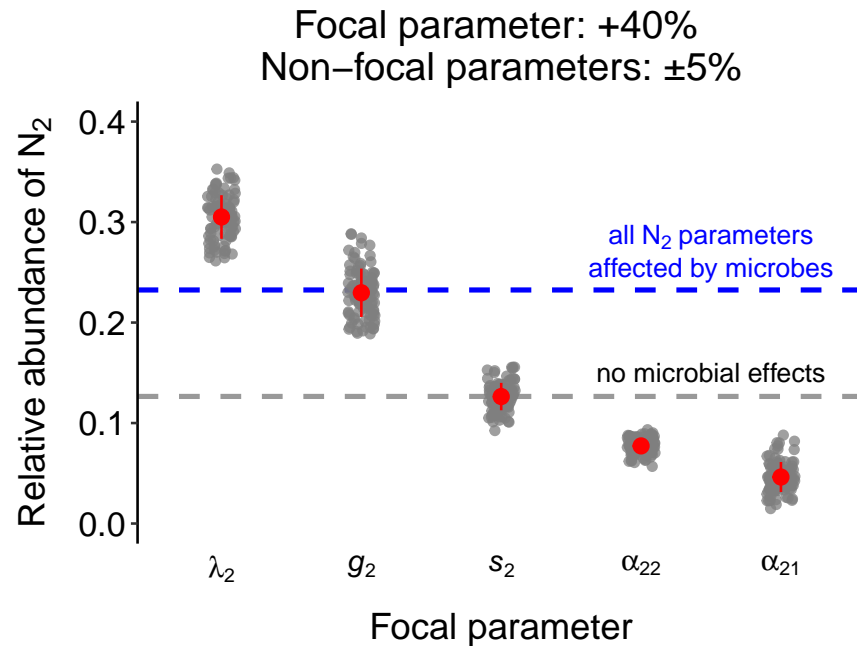
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