Plant Soil (2023) 485:57–70
https://doi.org/10.1007/s11104-022-05744-3

OPINION PAPER

A general approach for quantifying microbial effects on plant competition

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Received: 2 August 2022 / Accepted: 11 October 2022 / Published online: 26 October 2022

Abstract
Purpose A growing perspective in plant ecology highlights the ways that microbial interactions can promote or hinder plant coexistence. Towards this aim, recently-proposed approaches provide ways to empirically quantify how microbes affect the outcome of competition between plants. One such approach experimentally measures competition coefficients by comparing biomass performance of plants growing individually or with competitors, then quantifies microbial effects by comparing with a sterilization treatment. Nonetheless, the complexity of plant–microbe interactions presents general challenges when linking observations of microbial effects to underlying models.
Methods Building on insights from modern coexistence theory, we propose a comprehensive density gradient design to quantify the combined impact of plant–plant competition and plant–soil microbe interactions on plant coexistence patterns.
Results We show how the implementation of a density gradient design can help quantify potential nonlinearities and facilitative interactions in plant–soil microbe interactions, which are properties once thought to hinder the quantification of the microbial impact of plant competitive outcome. We provide guidelines for setting up the experiment and accurately interpreting the results.
Conclusion We argue that future studies should aim to parameterize suitable demographic models to characterize the contribution of soil microbes to plant coexistence.

Keywords Density gradient design · Fitness difference · Invasion analysis · Lotka–Volterra model · Niche difference · Plant–soil feedback

Introduction

Soil microbial communities play a central role in structuring plant communities (Bever et al. 2010; van der Putten et al. 2013). The interactions between plants and soil microbes are often characterized as bidirectional: plants condition the soil microbial community, causing changes in the prevalence of parasitic and mutualistic soil microbes, and these plant-specific compositional shifts in the soil microbial community feed back to affect plant performance (Bever

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Responsible Editor: Jonathan Richard De Long.

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et al. 1997; Bever 2003). Importantly, soil microbes can impact the growth of not only their host species but also individuals of other plant species, thereby altering plant competitive dynamics (Revilla et al. 2013; Ke and Miki 2015), community species richness (Johnson et al. 2012; Eppinga et al. 2018), and relative abundance patterns (Klironomos 2002; Mangan et al. 2010).

Many experimental designs and metrics have been proposed to quantify how soil microbes influence plant coexistence (Brinkman et al. 2010; Yan et al. 2022). The most common approach, inspired by the plant–soil feedback (PSF) model of Bever et al. (1997), entails growing seedlings of a plant species in soils conditioned by either conspecific or heterospecific plants. By examining how soil conditioning affects the performance of conspecific relative to heterospecific seedlings, Bever et al. (1997) developed a pairwise feedback metric to capture the frequency-dependent feedback loops generated by soil microbes. This approach has guided PSF studies for decades and meta-analysis suggests that plant modifications of soil microbes tend to generate negative feedbacks that stabilize plant coexistence (Crawford et al. 2019). Recently, studies have employed concepts from modern coexistence theory (Chesson 2000) to highlight that soil microbes can also affect plant coexistence by frequency-independent pathways (Stump and Comita 2018; Kandlikar et al. 2019; Ke and Wan 2020). These two impacts correspond to the niche differences (generating stabilization that favors coexistence) and fitness differences (generating competitive hierarchy that favors exclusion) in modern coexistence theory, which stresses that both components are critical when predicting species coexistence. Following the development of this new theoretical framework, experiments (Kandlikar et al. 2021) and meta-analysis (Yan et al. 2022) have started to quantify microbially mediated fitness differences, which require information on plant performance in an appropriate choice of reference soil.

However, since plants can affect the performance of their competitor via soil microbes, the community-level consequences of soil microbes should be characterized in the context of plant–plant competition (Casper and Castelli 2007; Shannon et al. 2012; Lekberg et al. 2018). For example, when soil microbes are known to confer fitness advantages to a specific plant, plant coexistence depends on whether the favored plant is competitively superior or inferior in the absence of microbial feedback (Yan et al. 2022). While pathogens may aggravate existing competitive asymmetry when they have a stronger impact on the inferior plant, they can also promote plant coexistence if physiological trade-offs cause the superior competitor to be more susceptible to pathogens (Laliberté et al. 2015; Lekberg et al. 2018). Previous plant–soil feedback experiments that grow single plant individuals alone, thereby strategically neglecting plant–plant competition, may therefore be insufficient to predict plant coexistence patterns. In this regard, recent studies have employed extensive experiments to capture both processes simultaneously (e.g., Burns and Brandt 2014; Chung and Rudgers 2016; Siefert et al. 2019; Huangfu et al. 2022, each with different designs). Based on modern coexistence theory, we (Ke and Wan 2020) recently proposed a minimal experimental design that can efficiently quantify both plant–plant competition and plant–soil interactions by manipulating plant density and soil inoculation. However, certain model assumptions that aim to simplify the complexity of plant–soil microbe interactions may limit the generality of this approach, a subject not extensively discussed in Ke and Wan (2020).

Here, we build upon our previous study by proposing a more comprehensive density gradient design to study how soil microbes alter plant competitive outcomes. This design originates from earlier experiments quantifying plant competition (Gibson et al. 1999; Inouye 2001) and has been often used in empirical studies of modern coexistence theory (e.g., Godoy et al. 2014; Kraft et al. 2015; Hart et al. 2019). However, its application for studying plant–soil microbe interactions remains to be thoroughly discussed (but see Cardinaux et al. 2018). Below, we first summarize how our experimental recommendations in Ke and Wan (2020) link to the niche and fitness differences of modern coexistence theory. To broaden empirical approaches beyond our original model, we then introduce the concept of invasion analysis from modern coexistence theory as a more general technique for predicting species coexistence. We discuss how the density gradient design connects to invasion analysis and thereby serves as a more general procedure for predicting coexistence, especially for systems with nonlinear and facilitative interactions. Finally, we elaborate on details to consider when setting up the experiment, including the choice of an appropriate performance proxy and reference soil, with the hope of helping future studies accurately interpret the effects of soil microbes on plant competitive outcomes.

Applying concepts from coexistence theory to study microbial effects

In complex real-world systems, it may be challenging to understand which of many interacting processes actually matter for coexistence. Modern coexistence theory (Chesson 2000) provides a general framework for predicting and understanding the outcome of species interactions. As a quantitative currency, this framework calculates the invasion growth rate, which determines whether a species can increase from rarity while its competitors are at their resident equilibrium (Grainger et al. 2019). Long-term coexistence thus requires factors that promote the invasion growth rate of all species, termed "stabilization" (Chesson 2003; Barabás et al. 2018). Thus, the theory serves as a unified framework for a variety of empirical and theoretical studies of community dynamics.

Quantifying microbial effects in systems with Lotka–Volterra type dynamics

In a previous application of modern coexistence theory to plant–soil interactions, we (Ke and Wan 2020) proposed to model the effects of soil microbes on plant–plant competition using a set of differential equations. Our model assumed that soil microbes grow toward a carrying capacity that increases linearly with the host plant population (due to the increasing supply of litter and root exudates) and that plant population growth also changes linearly with the density of soil microbes. The key takeaway is that this plant–soil microbe system can be simplified when microbial dynamics are faster than that of the plants, with the plant population N_i behaving as the following Lotka–Volterra model:

$$\frac{dN_i}{dt} = r_i N_i \left(1 + \alpha_{ii} N_i + \alpha_{ij} N_j\right). \tag{1}$$

Here, r_i is the intrinsic growth rate of the plant population and α_{ij} is the net per-capita effect of plant j on the population growth of plant i relative to the intrinsic rate; a negative α_{ij} indicates that net plant–plant interaction is competitive while a positive α_{ij} represents facilitative interaction. Importantly, since plants affect the population growth of each other via both plant–plant interactions and plant–soil interactions, α_{ij} in the model has two components and can be written as $\alpha_{ij} = c_{ij} + \sigma_{ij}\phi_j$. The first term represents plant–plant interaction, with c_{ij} capturing the effects of plant j on plant i that are unrelated to soil microbes, and the second term $\sigma_{ij}\phi_j$ represents plant–soil microbe interaction, with ϕ_j capturing the ability of plant j to condition its soil microbes and σ_{ij} capturing the impact of those microbes on plant i. A negative σ_{ij} indicates that the soil microbes are detrimental to the plant, whereas a positive σ_{ij} represents beneficial plant–soil microbe interactions. See also Kandlikar et al. (2019) for similar models with the same Lotka–Volterra dynamics.

To predict the outcome of competition in this model, our original study calculated whether stabilization was sufficient to overcome the competitive hierarchy between two competing species. Following a standard approach (Chesson and Kuang 2008; Chesson 2013), we predicted coexistence using niche overlap (ρ) and fitness difference $\left(\frac{f_j}{f_i}\right)$:

$$\rho = \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}} \tag{2}$$

$$\frac{f_j}{f_i} = \sqrt{\frac{\alpha_{ij}\alpha_{ii}}{\alpha_{ji}\alpha_{jj}}}, \tag{3}$$

where coexistence is achieved when $\rho < \frac{f_j}{f_i} < \frac{1}{\rho}$. This criterion mathematically ensures that each species has a positive invasion growth rate, and stabilization can be measured by niche difference, $1 - \rho$. Ecologically speaking, one species outcompetes the other if the two species have high niche overlap (i.e., small niche difference) and large fitness difference, whereas coexistence is possible when niche overlap is less than one (i.e., intraspecific competition is more negative than interspecific competition) and the fitness difference is not too extreme.

Ke and Wan (2020) suggested a minimal experimental design to quantify the impact of plant–plant competition and plant–soil interactions, as well as the impact of soil microbes on niche and fitness differences. This is achieved by growing a single individual of the focal species in a sterilized reference soil (i.e., no competition and microbial feedback) and comparing its performance to that when grown with competitors and competitor-conditioned soil microbes (Fig. 1a). Based on this experimental design, we have proposed an empirical calculation for the net interaction coefficient (i.e., combining both plant–plant competition and plant–soil interactions; $\alpha_{ij,\,\mathrm{soil}\,j}$) as:

$$\alpha_{ij,\,\mathrm{soil}\,j} = \frac{M_{i,j,\,\mathrm{soil}\,j} - M_{i,0,\,\mathrm{ref}}}{M_{i,0,\,\mathrm{ref}}} \cdot \frac{1}{\Delta N_j}, \tag{4}$$

where $M_{i,j,k}$ is the biomass of a plant i individual growing in competition with ΔN_j individuals of plant j in soil k; the subscript zero and *ref* in $M_{i,0,\,\mathrm{ref}}$ indicates that the plant i individual is growing alone without any competitors and in sterilized reference soil, respectively. This empirically-measured $\alpha_{ij,\,\mathrm{soil}\,j}$ quantifies the effect per competitor individual on

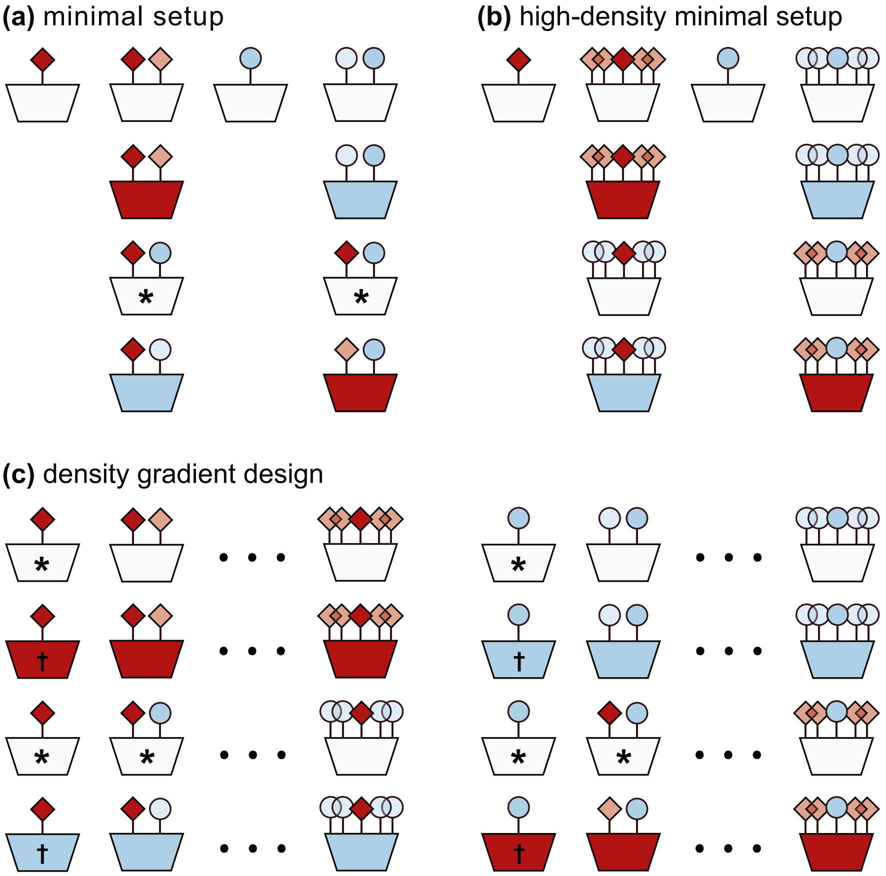


Fig. 1 Experimental designs for quantifying the effects of soil microbes on plant competition. In all panels, pots with different colors represent soils with different conditioning histories: no conditioning history or sterile soil (white), conditioned by the red diamond plant (dark red), conditioned by the blue circle plant (light blue). Focal plant individuals whose performance should be measured are highlighted with a saturated color, whereas background competitors have translucent colors. **a** Minimal experimental design proposed by Ke and Wan (2020); comparing the relative change in plant performance between the single growing individual (first and third column) and different competition treatments (second and fourth column) give corresponding competition coefficients. **b** A similar setup but with higher competitor density to ensure reduced performance compared to the minimal design with only one competitor individual. **c** A density gradient design that more accurately estimates the resident equilibrium and invasion growth performance, especially when nonlinear responses are expected. Pots with an asterisk (∗) represent treatments that are duplicated in the panel and therefore only need to be implemented once; pots with a dagger (†) indicate treatments with one individual growing in different conditioned soils, which are not necessary for our framework but can be used to quantify other plant–soil feedback metrics (e.g., Bever et al. 1997)

relative biomass suppression, treating it as a surrogate for the relative reduction of population growth rate. An important insight when quantifying microbial effects is that we only need to add soil inoculum that matches the identity of the competitor species (i.e., $k = \text{soil}\,j$ for $\alpha_{ij,\,\text{soil}\,j}$). By competing plant species with both conspecific and heterospecific individuals, we can obtain all parameters required to calculate niche and fitness differences in Eqs. 2 and 3. Moreover, by comparing $\alpha_{ij,\,\text{soil}\,j}$ and $\alpha_{ij,\,\text{ref}}$ (i.e., net plant–plant interaction without microbial feedback, calculated using $M_{i,j,\,\text{ref}}$ instead of $M_{i,j,\,\text{soil}\,j}$), we can estimate the impact of soil microbes on niche and fitness differences. The effectiveness of the above framework has been demonstrated in a recent study, which showed how nitrogen fertilization can modify niche and fitness differences between plants with different mycorrhizal strategies (Van Nuland et al. 2021).

An invasion analysis perspective for predicting plant competitive outcomes

While the conditions we provide above are specific to one particular model, modern coexistence theory actually provides a general technique for inferring competitive outcome known as invasion analysis (Turelli 1978; Barabás et al. 2018; Grainger et al. 2019). Thus, we suggest applying this perspective to broaden empirical approaches for predicting plant coexistence beyond the assumptions of the original plant–soil model of Ke and Wan (2020). To review how invasion analysis generalizes predictions of coexistence, we again consider a system where plant population dynamics can be approximated with the Lotka–Volterra model in Eq. 1. If net plant–plant interaction is competitive, the per capita growth rate of plant species i will decrease with slope $r_i\alpha_{ii}$ when growing with increasing density of conspecific competitors. This slope captures the sensitivity of plant i to intraspecific competition, with which we can calculate the monoculture equilibrium of plant i as $N_i^* = -\frac{1}{\alpha_{ii}}$ (i.e., the population density that results in zero population growth). For invasion analysis, this monoculture equilibrium should be viewed as the resident equilibrium density, at which the per capita growth rate of the invading plant j should be evaluated. We can then assess the invasion success of plant j using its sensitivity to interspecific competition imposed by the resident plant i. The invasion growth rate, IGR_j, for the Lotka–Volterra model is:

$$\text{IGR}_j = \lim_{N_j \to 0} \frac{1}{N_j}\frac{dN_j}{dt}\bigg|_{N_i = N_i^*} = r_j\left(1 + \alpha_{ji}N_i^*\right) = r_j\left(1 - \frac{\alpha_{ji}}{\alpha_{ii}}\right). \tag{5}$$

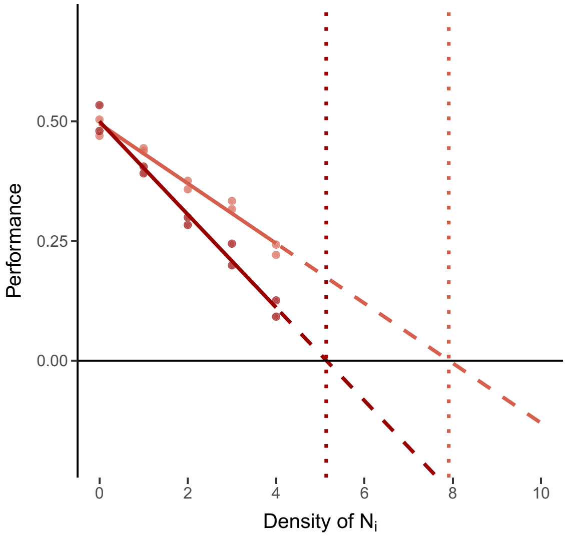
If IGR_j is positive (when $\frac{\alpha_{ji}}{\alpha_{ii}} < 1$), then plant j can invade the resident equilibrium of plant i; if IGR_j is negative (when $\frac{\alpha_{ji}}{\alpha_{ii}} > 1$), then we will predict that plant j cannot invade. We can then treat plant j as the resident plant and evaluate the invasion growth rate of plant i as $\text{IGR}_i = r_i\left(1 - \frac{\alpha_{ij}}{\alpha_{jj}}\right)$. We will predict that the two plants can coexist when mutual invasion is possible: that is, when both IGR_i and IGR_j are positive. This can be shown to be equivalent to the niche- and fitness-based criteria for coexistence above (i.e., $\frac{f_j}{f_i} > \rho$ and $\frac{f_j}{f_i} < \frac{1}{\rho}$, respectively), underscoring that invasion growth can be used as a common currency between models. Importantly, it can be clearly seen that coexistence requires that each plant species, when serving as the resident plant, has a stronger negative impact on itself than on the invader (i.e., α_{ii} being more negative than α_{ji} in IGR_j and α_{jj} being more negative than α_{ij} in IGR_i). In other words, coexistence is not assured when a species is itself more sensitive to the negative effect imposed by conspecifics compared to that imposed by heterospecifics (e.g., α_{ii} being more negative than α_{ij}; Broekman et al. 2019).

A competitor density gradient design

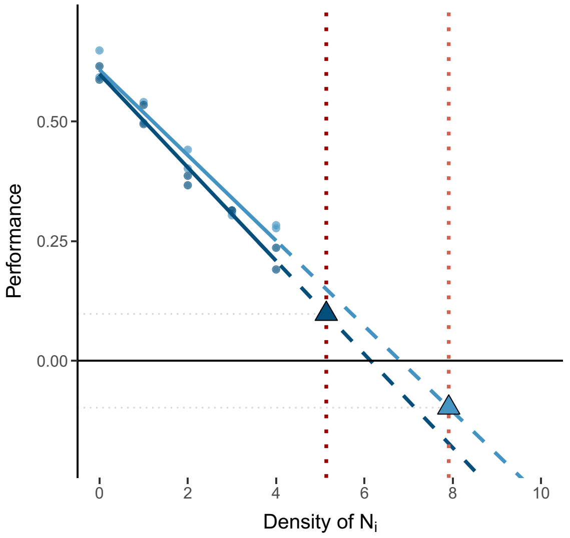
Applying invasion analysis requires (1) estimating the density at which the resident equilibrium of each species occurs, then (2) evaluating whether a heterospecific invader could attain positive population growth at that density. To do so, we advocate for a density gradient design (Fig. 1c). Such an experimental design has recently been applied to study the predictors of plant competitive outcome (e.g., Godoy et al. 2014; Kraft et al. 2015) and the influence of other trophic levels on plant coexistence (e.g., Petry et al. 2018; Cardinaux et al. 2018; Johnson et al. 2022). Below, we first introduce the essence of the density gradient design and then explain how it can be adapted to study plant–soil microbe interactions.

Fig. 2 An illustrative example of how results from the density gradient design can be used to make predictions of plant coexistence. **a** By measuring the performance of plant *i* when grown in a density gradient of conspecific competitors (points and solid lines), we quantify the sensitivity of plant *i* to intraspecific competition and obtain an estimation of plant *i*'s resident equilibrium (vertical dotted lines). Orange and red colors indicate that the density gradient was set up with sterilized reference soil and plant *i* conditioned soil, respectively. **b** We quantify the sensitivity of plant *j* to interspecific competition by measuring its performance when grown in the same density gradient of plant *i*, with light and dark blue colors indicating whether sterilized reference soil or plant *i* conditioned soil were used to set up the density gradient, respectively. From this we can evaluate the invasion success of plant *j* based on the sign of its invasion performance (triangles; where dashed extrapolated lines and corresponding vertical dotted lines cross). Dashed lines in all panels represent extrapolation of the fitted demographic model to the resident equilibrium density. See main text section *A competitor density gradient design* for more detail ▶

(a) N_i's sensitivity to intraspecific competition



(b) N_j's sensitivity to interspecific competition



To measure how a species' performance is influenced by competition, we can grow single focal individuals in density gradients of either conspecific (for estimating the resident equilibrium) or heterospecific individuals (for estimating invasion growth). Following the previous example, consider two plant species *i* and *j*, depicted by red diamonds and blue circles in Fig. 1c, respectively. The sensitivity of plant *i* to intraspecific competition can be quantified by growing a focal individual of plant *i* in pots with varying densities of competing plant *i* individuals (e.g., zero, one, two, four competing individuals, etc.; left-hand side upper two rows of Fig. 1c); by changing the competing individuals to plant *j*, we can quantify the sensitivity of plant *i* to interspecific competition (left-hand side lower two rows of Fig. 1c). The same experiment will also be set up using plant *j* as the focal individual, thereby quantifying the intra- and interspecific competition experienced by plant *j* (right-hand side rows of Fig. 1c). Again, here we suggest the inclusion of a treatment where one single focal individual is grown alone (i.e., with zero competitors). This treatment was essential for creating the necessary density difference in our minimal experimental design; here, it remains a cost-effective way to create the density treatments and is critical for estimating the species' maximum performance in the absence of any detrimental effect from plant–plant competition. As stated in Hart et al. (2018), this experimental setting is a modified additive design (*sensu* Gibson et al. 1999) where the number of focal individuals is set to one to prevent competition among them. At the end of the experiment, we can measure fecundity or other performance proxies (e.g., biomass growth; see section "Selecting appropriate proxies for competition and performance") to predict the competitive outcome or to quantify niche and fitness differences (see Section "A more flexible approach for calculating niche and fitness differences").

By setting up the above density gradient design with different soils, we can study how soil microbes modify plant–plant competition. To capture how a competitor affects focal plant performance via their soil microbes, we can inoculate the pots with soils conditioned by the competitor species (second and fourth row of Fig. 1c). These conditioned soils can be collected directly from individual plants in the field or conditioned separately in the greenhouse (Brinkman et al. 2010). Under the assumption of timescale separation, the implicit implication is that microbes from the inoculum will quickly grow to densities that correspond to the planted competitor density. As mentioned previously, insight from theory suggests that we need to quantify the plant's response to the soil microbes associated with the competitor species used to construct the density gradient (Ke and Wan 2020). Therefore, for focal species plant *i*, we would inoculate the pots with soils conditioned by plant *i* and plant *j* in the intraspecific and interspecific density gradients, respectively. To characterize the impact of soil microbes on plant competitive outcome, we can compare the predictions from a density gradient inoculated with competitor-conditioned soils to that inoculated with an appropriate reference soil (e.g., sterilized soil or live unconditioned soil; the first and third row of Fig. 1c). The choice of an appropriate reference soil has been discussed extensively (Kandlikar et al. 2019; Ke and Wan 2020; Abbott et al. 2021; Yan et al. 2022), a topic that we will elaborate on in a later section (see Section "Using a reference soil to understand microbial effects").

Figure 2 provides an illustrative example of how plant performance data from the density gradient design can be visualized and used to make inferences about the competitive outcome. Assume that we grew an individual of plant *i* along a conspecific density gradient ranging from zero to four plant *i* competitors (with two replicated pots for each density treatment), and furthermore, that we conducted this experiment with either sterilized soil or plant *i* conditioned soil, represented by orange and dark red colors in Fig. 2a, respectively. By fitting linear models to the data, we can observe that the performance of plant *i* decreases more rapidly when grown in self-conditioned soils (i.e., more negative slope for the dark red line), presumably due to the accumulation of soil pathogens. Such stronger self-limitation leads to a smaller resident equilibrium, which is obtained by extrapolating the fitted linear model (dotted vertical lines in Fig. 2a). When visualizing the performance of plant *j* along the same competitor density gradient of plant *i*, we would focus on its performance at the resident equilibrium (i.e., the crossing point of the vertical dotted lines and the extrapolated blue dashed lines, the latter indicating plant *j*'s sensitivity to interspecific competition imposed by plant *i*; Fig. 2b). In this particular example, plant *j* cannot invade the resident equilibrium of plant *i* when the two plants compete in sterilized reference soil (i.e., performance at the light blue triangle is negative), but it can invade if the soil has been conditioned by plant *i* (i.e., performance at the dark blue triangle is positive). Note that to fully predict plant competitive outcome we would also need to assess the invasibility of plant *i*, which requires the reciprocal experiment with a plant *j* density gradient and soil conditioned by plant *j* (not shown in Fig. 2, but in the Data Availability section we provide another mock data set and R script to demonstrate the full analysis). Nonetheless, Fig. 2 illustrates that, in addition to the commonly shown niche and fitness difference parameter space (Ke and Wan 2020; Kandlikar et al. 2021), presenting species' invasion growth rates provides a clear visualization of the impact of soil microbes on plant coexistence (e.g., see Chung and Rudgers 2016; Cardinaux et al. 2018; Siefert et al. 2019; Huangfu et al. 2022). Moreover, the visualization in Fig. 2 shows additional biological details regarding the microbial effects: the soil microbes conditioned by plant *i* had only a small impact on plant *j*, but rather allowed invasion because they strongly hamper the performance of their host.

Advantages of a competitor density gradient

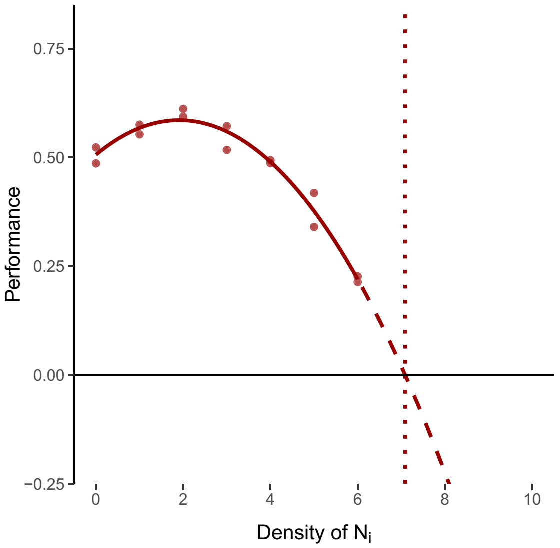
For the model proposed in Ke and Wan (2020), the number of competitor individuals implemented in the minimal design is inconsequential since the underlying model assumes linear negative density dependence. Therefore, the original minimal design with one competitor will suffice when the dynamics can be accurately described by a Lotka–Volterra model. However, when no such *a priori* knowledge is available, the density gradient design is a more general strategy for studying the effects of soil microbes on plant competition as it does not rely on a specific plant–soil model.

A more general strategy for predicting microbe-mediated plant competition

Nonlinear density dependence may not be uncommon when studying microbial effects (Vannette and Hunter 2011). Consider a scenario where mutualistic interactions with mycorrhizal fungi cause the focal individual to perform better when growing with a few neighboring plants (i.e., plant–plant facilitation). With competition eventually dominating when the density of neighboring plants is sufficiently high, the plant experiences nonlinear density dependence (red and blue lines in Fig. 3). If we were to apply the minimal design to the scenario using only one competitor, we would conclude that only plant–plant facilitation is operating in our experiment setup (i.e., positive α_{ii} and α_{ji}). Since a key element of predicting the outcome of competition is estimating the resident equilibrium, the inferred intraspecific facilitation would render invasion analysis unavailable as a finite resident equilibrium would no longer exist. While in principle we could still evaluate how the performance of the invader is influenced by increasing resident abundance, in this case the inferred interspecific facilitation would always result in positive invasion performance. As a result, our understanding of plant interaction would be limited by the insufficiently high competitor density in our setup, inaccurately representing their natural context. The density gradient design would be particularly useful under such a scenario as it allows better estimation of the resident equilibrium and thereby better prediction of invasion success.

A potential middle ground is to increase the number of competitors in the minimal design to a sufficiently high density, thereby capturing the competitive limb of the nonlinear density dependence curve (Fig. 1b). This is a slightly more complicated design than the original minimal design in Ke and Wan (2020) since interspecific competition among the two plants can no longer be estimated from the same pot (i.e., compare the third row between Fig. 1a & b). One potential caveat with this design is that using an exceedingly high competitor density may lead us to overestimate the resident equilibrium, particularly when final biomass is used as a performance proxy. This is because dead seedlings have a biomass close to zero. Thus, if we were to excessively overcrowd the pot such that the focal individual died, we would incorrectly infer that the resident equilibrium is close to the implemented density. Since we only have one single density treatment, we would not be able to validate the estimated resident equilibrium by assessing seedling performance at lower densities. As such, when logistical challenges prevent a full density gradient design, we still recommend a simplified

(a) N_i's sensitivity to intraspecific competition



(b) N_j & N_k's sensitivity to interspecific competition

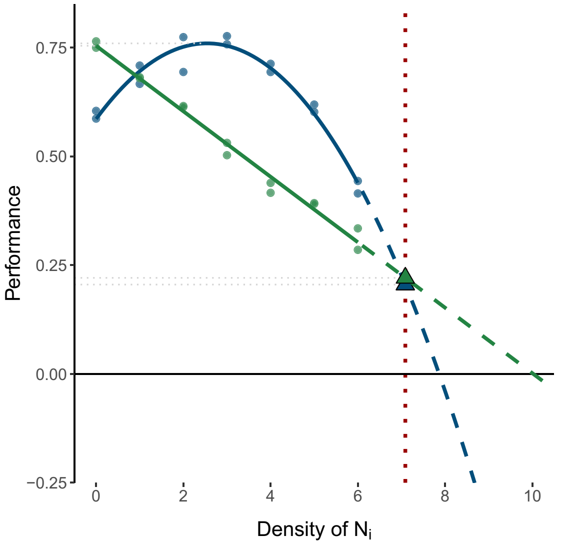


Fig. 3 An illustrative example of how the density gradient design can be useful when the system exhibits nonlinear density dependence. **a** The same density gradient of plant *i* as in Fig. 2a; the density gradient design reveals the nonlinear pattern (red points and solid line) but still allows the estimation of plant *i*'s resident equilibrium (red vertical dotted line). **b** Growing different plants (N_j in blue and N_k in green) in the same density gradient of plant *i* to characterize their sensitivity to interspecific competition and invasion performance (triangles; where the dashed extrapolated lines and the red vertical line cross). Note that while the two plants have different functional forms (N_j is nonlinear and N_k in linear) of density dependence, the two plants have similar maximum and invasion performance. Here, the nonlinear density dependence indicates that the Lotka–Volterra plant–soil model in Ke and Wan (2020) and the minimal experimental design may provide little understanding of the interactions.

gradient by combining the original minimal design with a high-density treatment (as depicted in Fig. 1c) to avoid the aforementioned issue.

A more flexible approach for calculating niche and fitness differences

In addition to studying coexistence via mutual invasion, one might still wish to calculate niche and fitness differences to further understand species coexistence. Here, we introduce how the concept of invasion analysis can be used to calculate niche and fitness differences in Eqs. 2 & 3. Specifically, one can calculate the relative reduction between a species' intrinsic growth rate and their invasion growth rate, S_j:

$$S_j = \frac{r_j - \mathrm{IGR}_j}{r_j}. \tag{6}$$

Carroll et al. (2011) named this term "sensitivity to competition", although here we refrain from this terminology to avoid confusion with the slopes in Fig. 2. For the Lotka–Volterra model, $S_j = \frac{\alpha_{ji}}{\alpha_{ii}}$, a quantity that can also be derived by setting the number of competitors to the resident equilibrium in Eq. 4. Since niche difference in modern coexistence theory evaluates the average tendency of species to recover from rarity (i.e., stabilization) whereas fitness difference measures the deviation of invasion growth from this average tendency (Chesson 2003), they can also be defined based on these re-scaled growth reductions (Carroll et al. 2011). For a two-species Lotka–Volterra model, one can recover Eqs. 2 & 3 by defining $\rho = \sqrt{S_i S_j}$ and $\frac{f_j}{f_i} = \frac{S_i}{\rho}$. This conceptualization also points out that while the outcome of competition can be directly predicted based on the sign of the invasion growth rates, calculations for niche and fitness differences require these growth rates to be standardized in order to consider differences in species' demographic time scale (Carroll et al. 2011).

For a system with nonlinearities in competitive and microbial responses, we recommend calculating niche and fitness differences based on the relative growth reduction in Eq. 6. As a more general procedure, we suggest defining $S_j = \frac{r_{\max,j} - \mathrm{IGR}_j}{r_{\max,\mathrm{j}}}$, which standardizes growth reduction with the maximum growth rate achieved by the species in the plant–soil system, $r_{\max,j}$. For a competitive Lotka–Volterra model with soil pathogens where the minimal design directly applies, the maximum growth rate is achieved when a single individual is growing in sterilized reference soil that minimizes the presence of pathogens (i.e., $r_{\max,j} = r_j$). For systems with nonlinearity due to mutualism, such as that in Fig. 3, $r_{\max,\mathrm{j}}$ used for standardization should be the plant performance achieved in live soil with a low density of neighboring plants (i.e., the hump of the nonlinear response curve). Since $r_{\max,\mathrm{j}}$ is the condition under which plants experience the least limitation, this calculation best matches the intuition of the competitive Lotka–Volterra model where r_j is the population growth in the absence of limitation. It is worth mentioning that according to the invasion perspective of modern coexistence theory, two systems can have the same niche and fitness differences if they have the same highest growth rate and invasion performance, despite different density dependent functional forms (e.g., blue and green lines in Fig. 3b). Finally, we note that modern coexistence theory is an expanding field and new approaches for calculating niche and fitness differences are continuously being developed (e.g., Spaak and De Laender 2020).

Designing realistic plant–soil interaction experiments

The approach we have outlined, based on invasion analysis, provides a theoretically justified approach to studying microbial effects on plant coexistence. However, applying it to a given system requires several empirical choices: researchers must choose which variables to measure, which soil treatments to apply, and whether or not to implement a full competition gradient. In each case, practical considerations may make the most realistic option infeasible. Fortunately, researchers should be able to select the most feasible option by considering the relationship between experiments and the processes they are meant to measure, and by considering how measurements are used in the underlying invasion analysis.

Selecting appropriate proxies for competition and performance

Though the limited size and duration of experiments may necessitate working at the scale of a few individuals, the ultimate goal when investigating coexistence is to understand dynamics at the scale of populations.

Thus, to estimate the results of an invasion analysis, we must select experimental variables to serve as proxies for population processes: a measure of competitor density, such as the number of individuals per experimental pot, and a proxy for population growth, such as the fecundity of a focal individual (Adler et al. 2018). As they measure different processes in the invasion analysis, these proxies do not need to be measured in the same units. Furthermore, since our approach is model-independent, statistical transformations of experimental variables may be used (e.g. square-root biomass) to improve statistical fitting.

There are many possibilities when quantifying plant performance, including plant survival, biomass growth, or seed set. Additionally, under the assumption that initial biomass is small and its variation insignificant, final plant biomass may be used instead of biomass growth. Choosing the best performance proxy requires considering the demography of the system at hand. For instance, in annual plant systems, researchers may be able to measure population growth by directly measuring plant fecundity (Godoy et al. 2014). Meanwhile, in perennial plant systems where clonal growth is more important, biomass change may be the most direct proxy for plant performance (Hart et al. 2018). Even when the complete plant life cycle cannot be studied in experiments, demographic knowledge may help choose the more realistic proxy: when working with long-lived plants, the choice between growth, survival, or a combination of the two should reflect whether population growth is more limited by biomass accumulation or seedling mortality (Chu and Adler 2015).

Likewise, competitor density can be measured in several ways, including the number of individuals (e.g., Hartnett et al. 1993) or biomass (e.g., Cardinaux et al. 2018). Here as well, the choice of measurement should reflect system-specific knowledge. For example, if competitor performance is expected to be highly variable during the experiment (due to, e.g., the use of unpooled soil inoculum or mortality from pathogens), measuring biomass may provide greater statistical power. Similarly, if competitors planted at high density experienced strong self-limitation, biomass may be more informative than density (Gibson et al. 1999). Differences in statistical power notwithstanding, since we expect that higher density and higher biomass of competitors generally correspond to stronger competition, we suggest that both can serve as consistent proxies for population density for invasion analysis.

Using a reference soil to understand microbial effects

Our approach studies the effect of microbes on plant coexistence by comparing plant performance in two kinds of soil: conditioned soil, in which a resident plant species has conditioned its specific microbial community, and reference soil, in which such conditioning has not been allowed to occur. Several possibilities exist when choosing this reference soil: broadly, plants may be inoculated with soil that has been sterilized to serve as a non-microbial control (i.e., *sterilized* reference soil), or soil from some field community where conditioning is assumed not to have taken place (i.e., *live unconditioned* reference soil). Further considerations apply within each category: in addition to a method of sterilization, researchers using a sterilized reference must also choose whether to sterilize unconditioned soil, species-specific soils, or mixtures of soils (Yan et al. 2022). When working with live soil, researchers must identify a suitably unconditioned field soil. In some systems, there may be a clear choice, such as bare ground or non-focal matrix vegetation; in other systems, however, there may be no obvious choice. Perhaps due to such ambiguities, most experiments choose sterilized reference soil: in a meta-analysis of soil microbial effects on plant fitness, Yan et al. (2022) calculated only 72 comparisons using data from live reference soil, versus 446 comparisons for sterilized soil.

While the live soil treatment is intended to reproduce natural dynamics, the reference soil is a conceptual and empirical tool used to identify the role of microbes. Thus, conclusions regarding the role of microbes depend on the choice of reference soil. Although live unconditioned soil may serve as a useful reference for certain questions (Abbott et al. 2021), artificially sterilized soil may be just as appropriate for other questions, even though it does not correspond to any natural condition. We support the notion that the choice of reference soil depends in part on the research question: researchers whose main interest is microbes cultivated during plant–soil feedback may benefit from using a live unconditioned reference, whereas those interested in the total effect of all microbes on coexistence may find a sterile reference soil to be a better conceptual fit (Kandlikar et al. 2019; Ke and Wan 2020). In fact, though few existing studies have used multiple reference soils (Yan et al. 2022), applying a combination of live unconditioned and sterilized reference soils may provide further insight into coexistence by disentangling total microbial effects and soil conditioning. A strength of the density gradient approach recommended here is that results from different soils are independently calculated and only compared at the final stage; thus, it can easily accommodate multiple reference soils.

Choosing between minimal and density gradient designs

While our previously-proposed minimal experimental design implements only one density treatment for each competitor–soil combination (e.g., Van Nuland et al. 2021), the density gradient design implements two or more different competitor densities. With N plant species, the total number of pots for the most comprehensive design would be $N^2 \times 2$ (i.e., conditioned versus reference) × number of density treatments × number of replicates. For example, our mock data (see Data Availability section) has two species and five density treatments in addition to the single-individual treatment; with two replicates per treatment, this results in 96 pots. For the purpose of estimating the resident equilibrium with a statistical model, we speculate it is more critical to have more density treatments to cover a wider density range (e.g., Cardinaux 2018 has only one replicate but eight density treatments). Although the total number of pots here is comparable to other experimental designs, it may be difficult to obtain that many background competitor individuals (i.e., 120 per competitor species for the mock data). This difficulty may limit the feasibility of the density gradient design to fewer replicates and fewer species pairs. Therefore, researchers must decide between the minimal design, which makes more assumptions about the system, or the density gradient design, which requires more experimental treatments but has a better chance of capturing the information needed for invasion analysis when nonlinearities are expected.

Another consideration is that the density gradient design allows more flexibility in the choice of reference soil. Measurements from the reference soil may enter in three calculation steps that make comparison with a reference performance value: (1) when estimating the response of plant performance to plant–plant competition (Eq. 4), (2) when calculating components of modern coexistence theory (Eq. 6 used for calculating niche and fitness differences), or finally (3) when comparing outcomes in conditioned and reference soils. Though separate considerations apply in each of these steps, calculations for our minimal experimental design (Fig. 1a) use the same reference soil measurement for all three. Furthermore, because the plant–soil model underlying the minimal design assumes that microbial effects are proportional to competitor density, we recommend the use of sterilized soil as the reference in this experimental design. On the other hand, the density gradient design (Fig. 1c) is less restrictive, and researchers with the resources to implement this approach can select the reference soil(s) most conceptually appropriate for the study question, as discussed above. Moreover, the procedure for calculating niche and fitness differences (Eq. 6) when employing the density gradient design allows more flexibility in standardizing the relative growth reduction, which gives more meaningful results in facilitative scenarios. Consequently, we recommend that the competition gradient design is most generally appropriate, and that researchers apply the minimal design only when working in systems where the assumptions of the plant–soil model hold (e.g., net pathogenic effects that are proportional to plant population) and where research questions are compatible with a sterilized reference soil.

Inferring coexistence at appropriate spatial and temporal scales

Like all investigations of community dynamics, researchers must consider the spatial and temporal scales most relevant to the research question at hand and design experiments accordingly. When applying invasion analysis to study plant coexistence, experiments should aim to recreate the resident equilibrium jointly reached by the plant and its soil community over the spatial and temporal scales of interest. Though direct invasion experiments may be impossible in some systems, this focus of the invasion analysis should allow researchers to prioritize the most realistic choices when working with their system.

The population dynamics and spatial distribution of microbes may be different from that of their host plants, a mismatch which complicates experimental design (Rudgers et al. 2020; Gundale and Kardol 2021). The invasion perspective highlights that invading plants experience only the resident equilibrium of plants and microbes. Since the soil community may continue to change over long periods of conditioning by plants (Ke et al. 2021), it is important to consider the time scale of interest: soil conditioned in greenhouses may be an appropriate treatment for systems with short-lived plants, whereas soil collected from long-established stands of each resident species is a more appropriate choice for studying dynamics of long-lived plants. Spatial mismatch due to different dispersal abilities between plants and microbes presents a related issue, and the soil treatment should reflect the case where the resident plant has, after sufficient time, found its specific community.

Moving forward

Recent theoretical studies have highlighted the diverse ways in which soil microbes can impact plant competition (Kandlikar et al. 2019; Ke and Wan 2020). To this end, empirical studies have utilized various experimental designs to quantify how these combine to influence community dynamics (e.g., invasion experiments in Burns and Brandt 2014, response surface in Chung and Rudgers 2016, replacement series in Siefert et al. 2019, different density manipulations in Huangfu et al. 2022). Using the invasion perspective from modern coexistence theory, we have provided an efficient experimental approach that predicts the effect of soil microbes on plant competition by checking whether each species can invade a resident equilibrium of its competitor. Since it relies only on the general principle of invasion analysis, the approach can predict the outcome of plant competition without being restricted to the assumptions of a specific plant–soil interaction model. Therefore, we hope it can serve as a general tool for understanding plant–soil interactions across systems.

Our invasion-based approach does not directly examine the situation where there is a mismatch between plant and microbe equilibrium in time and space. Nonetheless, extending the concept of invasion analysis may provide useful starting points for investigating such questions. For instance, researchers studying the temporal development of plant–soil feedback may repeat our approach with different lengths of soil conditioning (e.g., collected from field individuals of different ages; Ke et al. 2021) to investigate coexistence on different timescales (Diez et al. 2010). Meanwhile, researchers interested in the consequences of microbial dispersal limitation for plant communities may want to calculate the outcome of competition in specific soil scenarios (Hartnett et al. 1993; Cardinaux et al. 2018). Moreover, the model-agnostic invasion-based perspective applied here, while efficient in predicting coexistence, does not say much about the species' relative abundance when coexistence is achieved; accomplishing this goal would still require fitting a specific demographic model for the plant–soil system. Finally, our proposed design, similarly to other plant–soil feedback metrics, studies community dynamics by investigating plant–soil interactions among pairs of plant species. Theoretically, a multispecies invasion analysis can be performed by quantifying an invader's performance in the resident equilibrium reached by all other species. We look forward to future work that uses experiments to implement such multispecies invasion analysis (e.g., by transplanting seedlings of a focal species into a field where the non-focal species are presumed to be at a resident equilibrium, or by using soil co-conditioned in the greenhouse by multiple resident plants, e.g., Xue et al. 2018).

As with many empirical tests of modern coexistence theory, our proposed approach does not mechanistically account for the factors driving coexistence (Letten et al. 2017). However, comparing outcomes under multiple conditions, as in the reference–cultivated comparison at the core of our approach, can serve as a starting point for more mechanistic understanding. Researchers can gain greater insight by incorporating additional experimental conditions by varying the choice of reference (e.g., live unconditioned or sterilized) or conditioned soil (e.g., to include the effect of conditioning time) as we have recommended above, or by replicating the experiment under different environmental conditions (e.g., abiotic resource availability; van der Putten 2016; Dudenhöffer et al. 2022). Based on these insights, further progress in understanding plant–soil interactions may be able to explicitly model factors such as plant demography, resource dynamics, and microbial community processes (Schroeder et al. 2020; Jiang et al. 2020; Gundale and Kardol 2021). More broadly, as we have shown here, experimental designs informed by general ecological theory will continue to help elucidate how soil microbes structure plant communities.

Acknowledgements We thank Claire Willing, Kabir Peay, and Feng-Hsun Chang for discussions that helped crystallize our thoughts. P.-J. Ke is funded by Taiwan MOE Yushan scholar program NTU-110VV010 and MOST 111-2621-B-002-001-MY3.

Author Contributions P.-J. Ke and J. Wan conceived the study and wrote the manuscript with equal contributions.

Funding P.-J. Ke is funded by Taiwan MOE Yushan scholar program NTU-110VV010 and MOST 111-2621-B-002-001-MY3.

Data Availibility No new data was presented in this article. We provide simulated mock data and a R script to demonstrate the analysis at https://doi.org/10.5061/dryad.zgmsbccg0.

Declarations

Competing interests The authors declare no competing financial interests.

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