Video Article

Experimental Protocol for Manipulating Plant-induced Soil Heterogeneity

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Abstract

Coexistence theory has often treated environmental heterogeneity as being independent of the community composition; however biotic feedbacks such as plant-soil feedbacks (PSF) have large effects on plant performance, and create environmental heterogeneity that depends on the community composition. Understanding the importance of PSF for plant community assembly necessitates understanding of the role of heterogeneity in PSF, in addition to mean PSF effects. Here, we describe a protocol for manipulating plant-induced soil heterogeneity. Two example experiments are presented: (1) a field experiment with a 6-patch grid of soils to measure plant population responses and (2) a greenhouse experiment with 2-patch soils to measure individual plant responses. Soils can be collected from the zone of root influence (soils from the rhizosphere and directly adjacent to the rhizosphere) of plants in the field from conspecific and heterospecific plant species. Replicate collections are used to avoid pseudoreplicating soil samples. These soils are then placed into separate patches for heterogeneous treatments or mixed for a homogenized treatment. Care should be taken to ensure that heterogeneous and homogenized treatments experience the same degree of soil disturbance. Plants can then be placed in these soil treatments to determine the effect of plant-induced soil heterogeneity on plant performance. We demonstrate that plant-induced heterogeneity results in different outcomes than predicted by traditional coexistence models, perhaps because of the dynamic nature of these feedbacks. Theory that incorporates environmental heterogeneity influenced by the assembling community and additional empirical work is needed to determine when heterogeneity intrinsic to the assembling community will result in different assembly outcomes compared with heterogeneity extrinsic to the community composition.

Video Link

The video component of this article can be found at https://www.jove.com/video/51580/

Introduction

One of the primary goals of community ecology is to explain and predict the processes governing community assembly. However, plant communities are frequently more diverse than predicted by coexistence theory[1], and restoration ecologists need to understand coexistence mechanisms to successfully restore diverse native communities[2]. Environmental heterogeneity is a theoretically important mechanism that can help explain high levels of community diversity, but experimental manipulations of heterogeneity are infrequent[3] and focus on abiotic heterogeneity (*e.g.* reviewed in Lundholm[4]). Theory that incorporates heterogeneity typically assumes that heterogeneity is *extrinsic* to the assembling community. Extrinsic heterogeneity is governed by factors such as landscape typology, which are independent of the community composition. Extrinsic heterogeneity can result in coexistence through niche partitioning (reviewed in Melbourne *et al.*[3], *e.g.* Pacala and Tilman[5] and Chesson[6]). However, much of the environmental heterogeneity relevant to plant communities may be *intrinsic* to the community, developing as the community assembles and depending on the identity of the species in the community. Intrinsic heterogeneity can result from biotic feedbacks, which can lead to coexistence through negative frequency-dependence (*e.g.* Bever *et al.*[7]). Here, we describe a novel method for manipulating plant-induced soil heterogeneity, a type of soil heterogeneity that is intrinsic to the community and arises from plant-soil feedbacks.

Plant-soil feedbacks (PSF) occur when plants influence the soil structure, chemistry, or biota in a manner that affects subsequent plant performance in that soil, and PSF have large mean effects on plant performance in native plant communities[8]. Studies of PSF have typically either collected soils from the field or conditioned soils experimentally, then asked how plants perform in conspecific soil relative to heterospecific or sterilized soil[9]. If plants perform better in conspecific soil relative to reference soils, then PSF are positive, while if plants perform better in reference soils, PSF are negative. Reciprocal negative PSF can lead to frequency-dependent coexistence between species[7]. While the mean effects of PSF are well-characterized[8], the effects of spatial heterogeneity in PSF are poorly understood[10].

Because PSF occur on the scale of individual plants[7] and because plants are often nonrandomly distributed in space and time, PSF are likely to lead to soil heterogeneity, which we call **plant-induced soil heterogeneity**. Unlike many other forms of heterogeneity (*e.g.* landscape topology), this heterogeneity is intrinsic to the assembling community and may thus influence community assembly differently than more extrinsic forms of heterogeneity. In order to understand the influence of this form of heterogeneity on plant performance and coexistence, we need experimental methods that manipulate plant-induced soil heterogeneity. Here, we demonstrate such a method, which uses soils conditioned by two species to create a heterogeneous treatment with separate patches from two soil origins and a homogeneous treatment, which is a mixture of the two soil

origins. This soil mixing could represent at least two plausible scenarios in the field: (1) disturbance (*e.g.* rodent, agriculture) which mixes soils of different origins or (2) plants of two species growing in close proximity, such that their zones of root influence intermingle and homogenize.

We present two example experiments that use plant-induced soil heterogeneity to answer key questions at different levels of ecological organization: (1) Do plant populations respond to plant-induced soil heterogeneity? and (2) Do individual plants respond to plant-induced soil heterogeneity? We describe a field experiment using 6 soil patches to address the first question and a greenhouse experiment using 2 soil patches to address the second question. Quantifying both population and individual plant responses to soil heterogeneity is essential to understanding how heterogeneity influences community assembly.

Protocol

1. Collect Field Soils to Produce Heterogeneous and Homogeneous Soil Treatments

1. Identify two cooccurring plant species in similar habitats and soil types for study (*e.g.* **Figure 1a**). Determine amount of soil needed based on soil patch size to use in the experiment (*e.g.* one-third to one-half of the focal species' primary root zone, or volume encompassed by the root ball) and the number of patches needed for the experiment (see Protocol 2 and 3, below).
    1. For *Rumex* spp. (**Figure 1a**), use soil patches of 10 cm x 10 cm x 18 cm (length x width x depth) for approximate root zone of 10 cm radius from base of plant to 18 cm depth (5,655 cm^3 soil from this defined zone of root influence for one individual plant yields 3 soil patches of 1,800 cm^3).

2. Survey field site to locate adult individuals of the two focal species. Avoid individuals whose root zone likely overlaps with the other focal species (*e.g. Rumex* individuals located within 0.25 m of the other species). Mark the number of individuals needed to obtain the required amount of soil with pin flags.
    1. Mark 20 randomly chosen individuals of each *Rumex* spp. for 20 experimental units (2 species x 2 soil treatments x 5 replicates) that each require 3 soil patches from each focal species (as in Protocol 2, below).

3. Sterilize soil collecting equipment (shovels, gloves, transporting containers) with an approximately 1:10 mixture of bleach (5-10% NaClO) and water to remove all soil particles. Dry equipment before use to avoid bleach effects on soil biota. Label each piece of equipment with laboratory tape to indicate the focal species whose soil it will collect or transport to avoid cross-contamination (*e.g.* label one shovel for species A and a second shovel for species B).
4. Check weather and soil conditions before beginning soil collection for recent precipitation events. Avoid collecting soils that are too wet (muddy or fully saturated) or collecting soil during a precipitation event to minimize soil compaction. Avoid collecting soils that are too dry (very hard and difficult to insert a shovel into) to aid in separating soil from roots.
5. Collect field soil from marked individuals of the two focal species in replicate batches to avoid pseudoreplication (*e.g.* a single soil sample containing a rare pathogen being subsequently mixed across experimental replicates).
    1. Dig up the complete root zone of an individual of species A (as defined in step 1.1) with the appropriately-labeled sterile shovel, remove visible coarse roots from the soil with the appropriately-labeled sterile gloves, and place soil in the appropriately-labeled sterile transport container (*e.g.* bucket). For an experiment with 4 experimental units in each block and 1 individual plant's root zone providing enough soil for 1 experimental unit (as in Section 1.2.1), repeat this procedure for 3 additional individuals of species A.
    2. Repeat Section 1.5.1 for species B.
    3. Transport the replicate soil collection from both species to the experimental site. Place soil in experimental units for block 1 of the experiment (as in Protocol 2 or 3, below), where a block contains an experimental unit from each treatment combination (*i.e.* each species x each soil treatment).
    4. Repeat Section 1.5.3.
    5. Repeat Sections 1.5.1-1.5.4 for the remaining blocks in the experiment. Collect soil from groups of individuals at least 1 m distant from previous collecting locations for each replicate collection to minimize pseudoreplication.

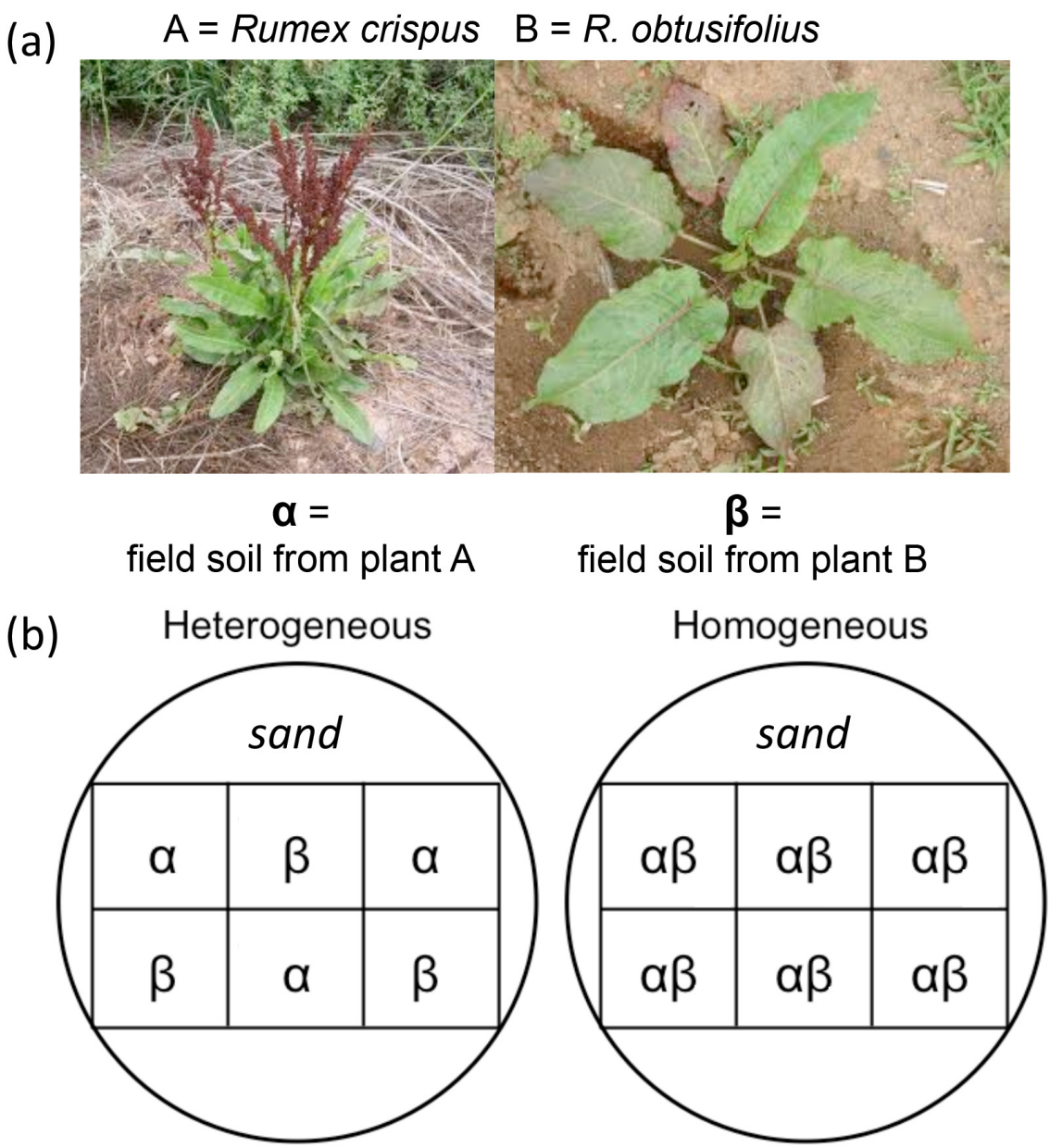

Figure 1. Example field experimental design manipulating plant-induced soil heterogeneity. (**a**) Soils are collected from the zone of root influence of conspecifics (α) and heterospecifics (β) in the field, following standard protocols for studying the effects of plant-soil feedbacks[9]. (**b**) Experimental treatments with heterogeneous soils composed of soils from plant A (α soils) and soils from plant B ('β' soils) are arranged in a grid, and homogenized soil treatments created with an equal mixture of soils from these two origins. In this example, grids of field soils are inserted into large-diameter pots sunken into the ground and the area around each grid is filled with coarse, sterilized sand. This figure has been modified from Brandt *et al.*[10]

2. An Example Field Experiment, Creating Grids of Heterogeneous and Homogeneous Soil to Measure Plant Population Responses

1. Use soils from a replicate field soil collection (Protocol 1) to produce a heterogeneous treatment (*i.e.* alternating grid cells contain soil collected from species A or species B) and a homogeneous treatment (*i.e.* each grid cell contains a 1:1 mixture of soil from species A and B) (**Figure 1b**) for block 1 of the experiment in the following steps.
    1. Sterilize soil handling equipment (trowels, gloves, plastic grid) with an approximately 1:10 mixture of bleach (5-10% NaClO) and water to remove all soil particles. Dry equipment before use to avoid bleach effects on soil biota. Label each piece of equipment with laboratory tape to indicate the focal species whose soil it will handle to avoid cross-contamination (*e.g.* label one trowel for species A and a second trowel for species B) and the plastic grid to indicate whether it will be used for the heterogeneous or homogeneous soil treatment.

2. Place removable plastic grid into a pot sunken into the ground [or other plot or container large enough to accommodate the grid] to be filled with field collected soil (*e.g.* 1,800 cm^3/grid cell as in step 1.1.1). Use a custom-constructed grid of the desired dimensions, *e.g.* a 2 x 3 grid with 6 cells of the size determined in step 1.1 (**Figure 1b**).
    1. For the heterogeneous treatment of alternating soil patch types, place spadefuls of soil from species A into grid cells for α soil and soils from species B into cells for β soil (**Figure 1b**).
    2. For the homogeneous treatment, place alternating spadefuls of soils a and b into **each** grid cell, taking care to evenly distribute the two soil types and avoid the formation of layers of each soil type (**Figure 1b**). For the example soil patch of 1,800 cm^3 (Section 1.1.1), place 900 cm^3 of each soil type in each grid cell.
    3. Be sure to produce equal amounts of disturbance (*i.e.* break up soil clods to the same degree) when creating heterogeneous and homogeneous soil treatments to avoid confounding soil disturbance with soil heterogeneity.
3. If any empty area remains around the outside of the grid within the large pot used (as in **Figure 1b**), fill this area with sterile sand. Sterilize sand using any standard method (*e.g.* autoclaving, gamma-irradiation).
4. Lift the plastic grid vertically out of the pot, leaving the soil patches intact. This allows roots from different individuals to interact in the soil and each plant to experience multiple patches, which is essential for allowing plant roots to grow into multiple soil patches (*i.e.* grid cells).
5. Repeat Sections 2.1.2-2.1.4 for a second pair of experimental units to produce a complete experimental block (*i.e.* a grid of heterogeneous soil into which to plant each focal species and a grid of homogeneous soil into which to plant each focal species, for a total of 4 experimental units).

2. Repeat step 2.1 for remaining blocks in the experiment with the remaining soil replicate collections (Protocol 1). Randomize blocks, and plots within blocks, throughout the experimental area, using landscape fabric around the plots if desired, to keep shading from outside vegetation at a minimum (not shown).
3. Plant seeds of focal species into each grid cell of each experimental pot (2 focal species x 2 soil treatments). Use pooled seed to avoid confounding plant genotypes or seed-surface microbial communities with the soil treatments. For example, plant seeds of each *Rumex* spp. into the pot for that species (**Figure 1a**) individually glued to plastic toothpicks with water-soluble glue in 12 planting positions (2 positions per grid cell) to clearly identify the planted individuals.
4. Measure population responses of the experimental units, such as germination and survival, through a regular census of individual marked planting locations of seeds (*e.g.* using toothpicks as in step 2.3). Minimize disturbance of the soil for any response measured to avoid mixing soil among patches.
    1. Determine the appropriate census intervals based on the expected rate of population responses for the focal species. For example, conduct weekly censuses for *Rumex* spp. that can germinate quickly.
    2. Continue the experiment for an appropriate duration based on the life history of the focal species. For example, continue the experiment for at least 2 years for short-lived perennial *Rumex* spp. to obtain data on all life stages.

3. An Example Greenhouse Experiment, with Heterogeneous and Homogenized Soil in Pots to Measure Individual Plant Responses

1. Use soils from a replicate field soil collection (Protocol 1) to produce a heterogeneous treatment (*i.e.* each half of a pot filled with soil collected from species A or species B) and a homogeneous treatment (*i.e.* pot contains a 1:1 mixture of soil from species A and B) (**Figure 2a**) for block 1 of the experiment in the following steps.
    1. In the transport container (*e.g.* bucket) and before potting, mix the field collected soil from each species with sterile sand to produce a 1:1 mixture. Do this to mitigate soil compaction and improve drainage in the pots, which is especially helpful for washing roots, facilitating the separation of roots from soil. Sterilize sand using any standard method (*e.g.* autoclaving, gamma-irradiation).
    2. Be sure to produce equal amounts of disturbance (*i.e.* break up soil clods to the same degree) when mixing each batch of field soil with sand.

2. Sterilize soil handling equipment (trowels, gloves, plastic sheet) with an approximately 1:10 mixture of bleach (5-10% NaClO) and water to remove all soil particles. Dry equipment before use to avoid bleach effects on soil biota. Label each piece of equipment with laboratory tape to indicate the focal species whose soil it will handle to avoid cross-contamination (*e.g.* label one trowel for species A and a second trowel for species B) and each side of a stiff plastic sheet to indicate which soil type will be potted on each side of it in the heterogeneous soil treatment.
3. Create a heterogeneous soil treatment using the field soil-sand mixtures from the two focal species (from Section 3.1.1).
    1. Place the stiff plastic sheet in the center of a pot to divide it in half (**Figure 2a**). Use a pot sized such that half of it is equivalent in volume to the appropriate soil patch size (step 1.1). For example, use a pot with a 15 cm diameter and 18 cm depth for the *Rumex* spp. in **Figure 1a** (total volume of 3,181 cm^3).
    2. Have two researchers simultaneously add the field collected soil from each species to the appropriate side of the pot (*e.g.* α soil from species A) with appropriately-labeled trowels, to keep the sides even as the pot is filled.
    3. Remove the plastic divider by lifting it vertically out of the soil, leaving the soil patches intact. This allows the plants to experience both patches of soil within a pot, essential for the plants to experience heterogeneity.
    4. Repeat Sections 3.3.1-3.3.3 for 3 additional experimental units to produce a complete block of the heterogeneous soil treatment (2 species x 2 soil patch types in the heterogeneous treatment; **Figure 2**).

4. Create a homogeneous soil treatment using the field soil-sand mixtures from the two focal species (from Section 3.1.1).
    1. Repeat Section 3.3.1. Have two researchers simultaneously add both α and β soils to both sides of the pot with appropriately-labeled trowels, evenly distributing both soil types within each patch and avoiding the creation of layers. Repeat Section 3.3.3.
    2. Repeat Section 3.4.1 for a second experimental unit to produce a complete block of the homogeneous soil treatment (one pot for each of the 2 focal species).

5. Repeat steps 3.2-3.4 for remaining blocks in the experiment with the remaining soil replicate collections (Protocol 1). Randomize blocks, and plots within blocks, across the greenhouse bench.

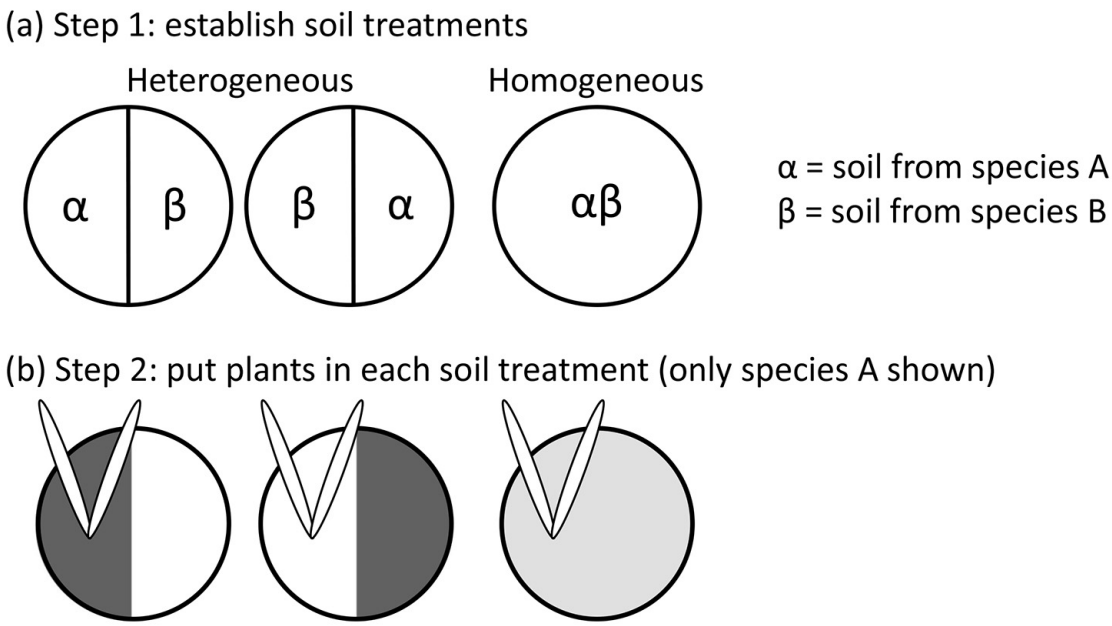

Figure 2. Example greenhouse experimental design manipulating plant-induced soil heterogeneity. (**a**) Soils collected from the zone of root influence of species A (α soils) and species B (β soils) in the field are placed in each half of a pot (heterogeneous treatment) or mixed throughout the pot (homogeneous treatment). (**b**) Plants of species A are then planted into the experiment in each soil patch type in the heterogeneous treatment and on one side of the homogeneous treatment. Here, only one species (A) is shown planted into this design. A fully reciprocal design would include plants of the second focal species (B) planted into each soil treatment and patch type within the heterogeneous treatment.

6. Plant seedlings of focal species A and B into each soil patch type in a factorial design (2 species x 3 soil patch types [α soil, β soil, and a homogeneous mixture of α and β soil]; **Figure 2b**).
7. Measure any individual plant responses that indicate plant performance, such as plant size, biomass production, or functional traits. Appropriate traits to measure will depend on the focal species and the scientific questions of interest.
    1. Continue the experiment until the plants appear large relative to soil patches (*e.g.* approximately 1.5x the width of the soil patch for rosette-forming forbs) to ensure the plant roots are growing into both soil patches. Experimental duration will thus depend on the growth rate of focal species.
    2. Harvest plants and/or measure responses before plants become pot-bound (*i.e.* root growth is limited by the confines of the pot and roots circle the bottom of the pot) if realistic responses to the soil environment are desired. For example, roots of pot-bound plants are unlikely to forage throughout the soil column in a similar manner to plants in the field.

Representative Results

Species responded to plant-induced soil heterogeneity in diverse ways at both the population and individual level (**Figures 3** and **4**), with implications for community assembly. To determine whether plant populations respond to plant-induced soil heterogeneity, a field experiment was established as in Protocol 2 using three congeneric pairs of species. Plant populations were censused weekly for three months and the total proportion of planted seeds that germinated and the total proportion of those seedlings that died in the first growing season (where mortality represents the inverse of survival) were calculated (**Figure 3**). We found significant plant population responses to plant-induced soil heterogeneity, suggesting that this experiment was successful in manipulating heterogeneity that influenced plant populations. Some species exhibited nonadditive responses to soil mixing, such that the response observed in the homogeneous treatment (αβ soil patches) was not an intermediate response to the two soil types that were mixed to create that treatment (α, or conspecific, soil patches and β, or congener, soil patches). For example, *Solanum carolinense* had lower germination and *Rumex crispus* had greater mortality in a homogenized mixture of conspecific and congener's soils than in either soil type alone (**Figure 3**)[10]. These results provide potential mechanisms by which homogeneous soil environments might facilitate coexistence, where reduced germination or increased mortality of one species could provide open patches for other species to colonize.

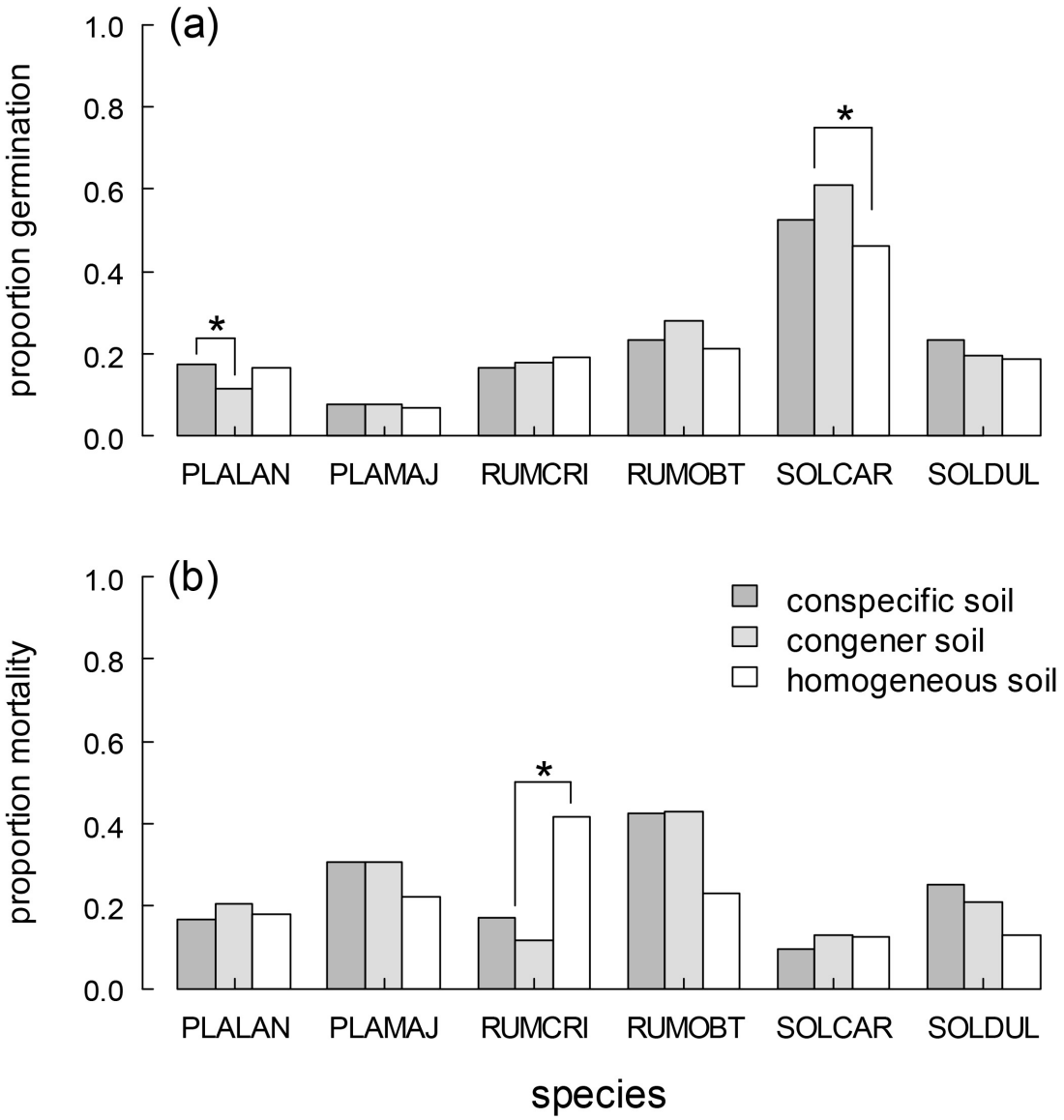

Figure 3. Example results from a field experiment with heterogeneous and homogeneous soils. (**a**) Proportion germination and (**b**) proportion mortality of 3 pairs of congeneric species planted into patches of conspecific soil, congener soil, or a homogeneous mixture of the two soil types. Some species responded to soil mixing in a nonadditive manner; for example, *Rumex crispus* had greater mortality in the homogeneous treatment than in either conspecific or congener (*R. obtusifolius*) soil patches in the heterogeneous treatment. PLALAN = *Plantago lanceolata*, PLAMAJ = *P. major*, RUMCRI = *R. crispus*, RUMOBT = *R. obtusifolius*, SOLCAR = *Solanum carolinense*, and SOLDUL = *S. dulcamara*. *P<0.05 from orthogonal contrasts of homogeneous vs. unmixed soil and conspecific vs. congener soil within each species in a mixed effects model with a binomial error distribution in the R Statistical Environment[11]. Mean proportions are presented (total count divided by total sample, for consistency with statistical methods[12]). This figure has been modified from Brandt *et al.*[10] See Brandt *et al.*[10] for detailed analysis methods.

To determine whether individual plants respond to plant-induced soil heterogeneity, a greenhouse experiment was established as in Protocol 3 using two congeneric pairs of species. Because we expected plants to respond to the soil patch type in which they were grown in addition to the pot-level soil heterogeneity, seedlings were planted into each patch type within the heterogeneous treatment (as in **Figure 2b**). The plants were grown for 2 months and then harvested to measure performance (total biomass) and functional traits (including specific leaf area (SLA), as shown in **Figure 4**). Significant plant responses to soil heterogeneity were observed, suggesting that this experiment was successful in manipulating a form of soil heterogeneity that influences plant performance. We observed a nonadditive response to soil mixing, in which species tended to have lower SLA, or thicker leaves, in a homogenized mixture of conspecific and congener's soil than in either soil type alone (P = 0.031 from an orthogonal contrast of homogeneous vs. unmixed soil in a mixed effects model with plant biomass as a covariate and block as a random effect; **Figure 4**). Specific leaf area was also lower in conspecific compared to congener soil patches (P = 0.004 from an orthogonal contrast of soil patch types in the heterogeneous soil treatment; **Figure 4**). These results suggest that plant resource acquisition strategies respond to plant-induced soil heterogeneity, which has implications for the effect of such heterogeneity on plant growth, fecundity, and species interactions.

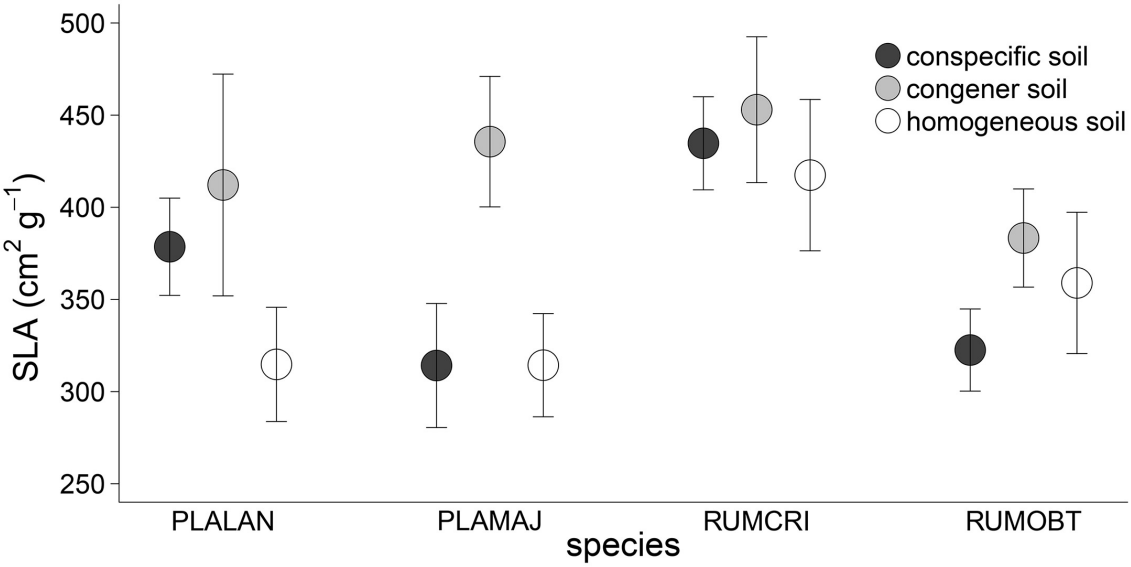

Figure 4. Example results from a greenhouse experiment with heterogeneous and homogeneous soils. Specific leaf area (SLA), calculated as leaf area divided by dry mass, of 2 pairs of congeneric species grown in patches of conspecific soil, congener soil, or a homogeneous mixture of the two soil types. Overall, species responded to soil mixing in a nonadditive manner, where SLA was lower in the homogeneous treatment than in either conspecific or congener soil patches in the heterogeneous treatment. Mean traits ± 1 SE. PLALAN = *Plantago lanceolata*, PLAMAJ = *P. major*, RUMCRI = *R. crispus*, RUMOBT = *R. obtusifolius*.

Discussion

Plant-induced soil heterogeneity is highly likely in natural communities because plants have large and often species-specific effects on their soil environment and the subsequent plants that experience that soil (*e.g.* Petermann *et al.*[13]). However, our understanding of the role of this type of heterogeneity on plant communities is minimal[10,14]. Here, we present a method for manipulating plant-induced soil heterogeneity, using soil from different origins (*i.e.* zones of root influence of different species) in the field. The critical steps within the protocol to avoid confounding plant-induced soil heterogeneity with other variables that affect plant responses are: (1) avoiding pseudoreplication of soil samples collected from the field and placed into experimental units and (2) equalizing the amounts of soil disturbance for field soils placed into heterogeneous and homogeneous soil treatments. Pseudoreplication of soil samples could occur if heterogeneous and homogeneous treatments receive soil collected from different individual plants or collecting locations in the field, which can be avoided by collecting soil in replicate batches from well-spaced locations (step 1.5). Pseudoreplication can affect the study's results if, for example, a rare pathogen occurs in the root zone of one plant in the field and that plant's soil is only placed in one of the two soil treatments. Additionally, collecting locations in the field may differ in management, vegetation history, or soil properties; thus soil from each collecting location should be used in both experimental treatments, randomizing across potential confounding factors. Disturbance to field soils (*i.e.* breaking up of soil clods) increases soil compaction and can affect the interpretation of results. For example, if the homogeneous soil treatment receives greater disturbance than the heterogeneous treatment, then the effect of soil heterogeneity would be an effect of soil disturbance. Readers should also consider statistical power in their design of these experiments, which would affect the total amount of soil required (step 1.1) and the number of individuals of each focal species from which to collect soil in the field (step 1.2). Preliminary experiments could be used to determine the effect size and variance expected, and this information could then be used to design an experiment with sufficient replication[15].

This protocol could be easily modified to accommodate other methods for studying plant-soil feedbacks (*e.g.* by using soils conditioned in the greenhouse[16]) and alternative experimental designs (*e.g.* different spatial or temporal scales, interspecific competition[17]). Additional experiments could also use alternative focal species. The selection of focal species has implications for both the study's logistics and interpretation of results, but above all should be done to best address the investigator's questions of interest. We show Representative Results for closely related pairs of species, which allowed us to control to some extent for growth form[18] and an effect of phylogeny on plant-soil feedback responses (*e.g.* Burns and Strauss[19]). The size of focal species determines the scale of soil patches used and their life history may affect the duration of the experiment. Two focal species of different size might respond differently to the same scale of plant-induced soil heterogeneity if the smaller species cannot integrate across different soil patch types to the same degree as the larger species (*e.g.* the smaller species' root zone is restricted to a single soil patch). Additionally, using species with a clonal growth form is likely to produce different responses to soil heterogeneity than species that reproduce solely from seed (as reviewed in Reynolds and Haubensak[14]).

An important limitation of this method is that the mechanisms governing responses to soil heterogeneity (*e.g.* abiotic factors such as soil chemistry, biotic factors such as soil microbial communities) are not identified without further work. For example, our results suggest that homogeneous soil (*i.e.* mixed soil) can result in nonadditive effects at both the population and individual plant level, where plant responses in the homogeneous treatment were not intermediate relative to plant responses in the two soil types that composed the mixture. Soil mixing could occur in natural communities as a result of disturbances, such as rodents' digging, or as a result of overlapping zones of root influence as plants of different species grow into each other's rooting zones. One possible mechanism for these findings is an effect of soil biota, which could contribute to nonadditive responses if communities of bacteria, fungi, or other organisms interact upon soil mixing, changing the biotic composition of the soil and thus the plant responses to that soil. Abiotic mechanisms could also result in nonadditive responses, if plant functional responses to abiotic drivers (*e.g.* nutrient levels) are nonlinear[20]. Additional experiments and sampling are thus necessary to identify the drivers of plant responses to these soil treatments, with implications for their application to natural communities. For example, the role of soil

biotic factors in governing plant responses to plant-induced soil heterogeneity could be tested by using soils conditioned by focal species in the greenhouse compared with sterilized soils, which provide a negative control for testing the role of soil biota.

We demonstrate significant plant responses to plant-induced soil heterogeneity, suggesting that plants themselves influence the environmental heterogeneity that affects plant community assembly (**Figure 5**). Heterogeneity can thus be *intrinsic* to the community composition, as opposed to the more *extrinsic* types of heterogeneity that studies of plant community assembly have more frequently considered[3]. The novel method presented here has potential to reconcile conflicting predictions from theory and results from experiments about the relationship between environmental heterogeneity and species coexistence, or diversity[4,14]. Theory predicts that environmental heterogeneity leads to increased species diversity (reviewed in Melbourne *et al.*[3]), however previous experiments directly manipulating soil nutrient heterogeneity have often found the opposite trend (reviewed in Lundholm[4] and Reynolds and Haubensak[14]), suggesting that other types of heterogeneity, such as those resulting from plant-soil feedbacks, should be examined. This method of manipulating plant-induced soil heterogeneity indicates specific mechanisms by which coexistence may be facilitated in homogeneous environments. For example, if resident plants have lower germination or higher mortality in homogeneous soils, as was found for some species (**Figure 3**), then these more homogeneous environments may be more invasible, counter to the traditional predictions of theory. Further, coexistence predictions for each species pair in heterogeneous soils can be calculated[7] using species responses to each soil patch type within the heterogeneous treatment, allowing researchers to determine for which species they might expect plant-induced soil heterogeneity to facilitate coexistence[10].

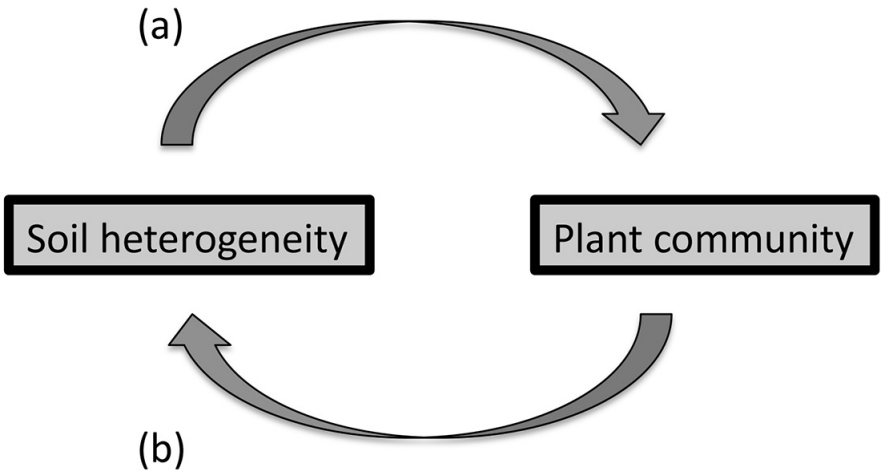

Figure 5. Conceptual overview of the value of addressing plant-induced soil heterogeneity. (**a**) Traditional conceptualization of the influence of heterogeneity on coexistence and community assembly has focused on the effect of heterogeneity *extrinsic* to the community composition (*e.g.* landscape topology) on community assembly. (**b**) The new methods proposed here include the effects of plants on soil heterogeneity driven by plant-soil feedbacks, where plants influence the biota, chemistry, or structure of the soil in a manner that influences subsequent plant performance in that soil. This perspective acknowledges that heterogeneity *intrinsic* to community composition, including both biotic and abiotic effects, also influences assembly.

Manipulating heterogeneity that is intrinsic to the community allows rigorous testing of the potential for frequency-dependent coexistence that could result from biotic feedbacks, such as plant-soil feedbacks, and integrates our understanding of this mechanism with the greater body of coexistence theory[9]. Because this type of heterogeneity is dynamic over time, its influence on community assembly will depend on the length of time it takes for plant-soil feedbacks to develop and the length of plant-soil feedback legacy effects in communities. Thus, further empirical work is needed on the temporal scale over which these effects develop and degrade. Future applications that could emerge from this work thus include informing new theory that incorporates heterogeneity driven by intrinsic feedback processes (*e.g.* Fukami and Nakajima[20]) and further empirical tests of the role of these intrinsic processes in assembly (*e.g.* Brandt *et al.*[10]).

Disclosures

The authors have nothing to disclose.

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