Research

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Dynamic plant–soil microbe interactions: the neglected effect of soil conditioning time

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Summary

- Plant–soil feedback (PSF) may change in strength over the life of plant individuals as plants continue to modify the soil microbial community. However, the temporal variation in PSF is rarely quantified and its impacts on plant communities remain unknown.
- Using a chronosequence reconstructed from annual aerial photographs of a coastal dune ecosystem, we characterized > 20-yr changes in soil microbial communities associated with individuals of the four dominant perennial species, one legume and three nonlegume. We also quantified the effects of soil biota on conspecific and heterospecific seedling performance in a glasshouse experiment that preserved soil properties of these individual plants. Additionally, we used a general individual-based model to explore the potential consequences of temporally varying PSF on plant community assembly.
- In all plant species, microbial communities changed with plant age. However, responses of plants to the turnover in microbial composition depended on the identity of the seedling species: only the soil biota effect experienced by the nonlegume species became increasingly negative with longer soil conditioning. Model simulation suggested that temporal changes in PSF could affect the transient dynamics of plant community assembly.
- These results suggest that temporal variation in PSF over the life of individual plants should be considered to understand how PSF structures plant communities.

Introduction

Plants often cause changes in the composition of the soil microbial community, which can then feed back to affect the growth of neighboring plants or plant individuals that colonize the soil subsequently, the process known as plant–soil feedback (PSF) (Bever *et al.*, 1997; Bever, 2003). Because plant species vary in their effects on, and responses to, soil microbes, PSF can modify interspecific differences in plant performance, thereby affecting plant community composition (Klironomos, 2002; Mangan *et al.*, 2010; Eppinga *et al.*, 2018). The strengths of these feedbacks are commonly assumed to be constant through time. Under this assumption, most empirical studies quantify feedback strengths via short-term glasshouse experiments that are terminated at the same time for all species (Kulmatiski & Kardol, 2008; Kardol *et al.*, 2013a). However, given that plant individuals often arrive at different times and die at different ages in the field, understanding how feedback strengths vary over time can be critical for predicting the consequences of PSF for plant community assembly.

The strength of plant–soil microbe interactions can vary temporally because of changes in the soil microbial community composition with increasing time of soil conditioning (Lepinay *et al.*, 2018). This mechanism differs from previous studies, which typically focused on ontogenetic changes in plant responses to soil microbes (Hawkes *et al.*, 2012; Bezemer *et al.*, 2018; Dudenhöffer *et al.*, 2018). Studies have shown that changes in the microbial community can proceed at different rates depending on host plant identity (Knelman *et al.*, 2012; Chen *et al.*, 2019; Hannula *et al.*, 2019). As a result, seedlings of the same species could face different microbial communities and experience different PSF strengths, depending on the species identity of conditioning plants and for how long they have modified the soil (Kardol *et al.*, 2013b; Peay, 2018). Moreover, plant responses to temporal changes in the microbial community may depend on the plant functional group. For example, studies have shown that legumes can be less sensitive to changes in soil properties by forming effective symbiotic relationships with a range of ubiquitous rhizobia (Birnbaum *et al.*, 2018; Png *et al.*, 2019; but see Yang *et al.*, 2020). However, few studies have quantified the changes in plant–soil microbe interactions with increasing soil conditioning length.

Two logistical challenges may explain the current paucity of relevant empirical work. First, preparing soils with different conditioning lengths for an experiment is labor-intensive and often infeasible (Kardol *et al.*, 2013a; Kulmatiski, 2018). Second, the soil conditioning length in the field can only be quantified with coarse resolution due to uncertainty about the ages of individual plants (e.g. Day *et al.*, 2015; Speek *et al.*, 2015). Moreover, although the plausible consequences of time-varying PSF for

plant communities can be studied theoretically, most models treat feedback strengths as time-independent parameters (e.g. Fukami & Nakajima, 2013; Teste *et al.*, 2017; Ke & Wan, 2020). In this study, we overcome these challenges by using high-resolution aerial photographs of a coastal dune ecosystem that were taken annually from 1990 through 2017. These photographs allowed us to estimate the age of individual plants and use it as a proxy for soil conditioning length. By sampling soils from individual plants of different ages, we apply a chronosequence approach to test the hypothesis that soil microbial communities would vary across plant species and conditioning time. We then evaluate how changes in the soil community influenced the strength of their effects on plant performance in a glasshouse experiment that preserved plant age-specific soil properties. Specifically, we test the hypothesis that the observed soil biota effects would vary with soil conditioning length and that the temporal patterns would differ among different plant species or functional groups (i.e. legume or not). Finally, to investigate potential general consequences of time-varying PSF beyond our specific study system, we use an individual-based model to examine how temporal patterns of PSF may affect plant community assembly.

Materials and Methods

Study system

We conducted our study at the coastal foredunes of Bodega Bay, California, USA (38°19′N, 123°3′W), located within the UC Davis Bodega Marine Reserve and the Sonoma Coast State Beaches. This region experiences a Mediterranean climate, with an average annual temperature of 15.8°C and annual precipitation of 760 mm, mostly occurring between October to April (Barbour *et al.*, 1973). The soils in our 400 m × 500 m study area are predominantly sand, with a negligible amount of silt and clay (Kleinhesselink *et al.*, 2014). The sandy top soils generally contain little organic matter and are fast draining, nitrogen-poor, and strongly alkaline (Barbour *et al.*, 1973; McNeil & Cushman, 2005; Lortie & Cushman, 2007). We focused on the four dominant species of the foredune plant community, including the introduced grass *Ammophila arenaria* (Poaceae), the introduced succulent dwarf-shrub *Carpobrotus edulis* (Aizoaceae), the native shrub *Baccharis pilularis* (Asteraceae), and the native nitrogen-fixing leguminous shrub *Lupinus arboreus* (Fabaceae). The first two species have fibrous root systems, with *C. edulis* having a shallower root system concentrated in the upper 50 cm of the soil (D'Antonio & Mahall, 1991); the latter two species have well-developed taproot systems.

Soil sampling

We used a series of aerial photographs that were taken annually by Delta Geomatics Corporation and curated by the Bodega Marine Reserve since 1990 (Danin *et al.*, 1998). Since the foredune vegetation has little vertical structure, we were able to identify plant individuals to the species level and estimate their age (i.e. identify the first year the individual appeared in the photographs) by comparing photographs across multiple years. Age estimates were used as proxies for soil conditioning length as the foredune undergoes primary succession starting from unconditioned bare sand. For the four dominant species, in 2016 we selected individuals of different ages. In total, we selected 30 individuals of *A. arenaria*, 30 individuals of *B. pilularis*, 33 individuals of *C. edulis*, and 43 individuals of *L. arboreus*. All individuals were selected to sample evenly along the plant's age span provided by the aerial photographs, which ranged between 2 to 12 yr for *L. arboreus* and between 3 to 26 yr for the other three species. No spatial autocorrelation was evident for the age of selected individuals (Moran's I, $P = 0.24$; Mantel test, $P = 0.39$). See Fig. 1 for a representative aerial photograph, the spatial distribution of selected individuals, and representative examples of different age classes for each species.

To study how soil microbial communities varied with plant age, in July 2016 we collected three soil samples beneath each plant individual (i.e. at azimuth angles 0°, 120° and 240°; half way between the plant's center and edge) in separate sterile 50-ml Falcon tubes. We also collected soil samples from three randomly selected juveniles (i.e. one sample per juvenile, which are individuals that germinated within 1 yr and were too small to be visible on the aerial photographs from the previous year) for three of the four species (i.e. all but *A. arenaria*). Finally, a total of 13 soil samples from randomly selected bare sand areas (i.e. no vegetation in a *c.* 3 m radius throughout the entire length of time of the aerial photographs) were collected across our field site (Fig. 1). All soil samples were stored at 4°C up to a week before being processed in the laboratory. Each soil sample was passed through a disposable sieve made out of 2-mm iron mesh (sterilized by soaking in 5% bleach for 30 s and then 95% ethanol for 30 s), and further homogenized thoroughly in separate sterile plastic bags. The fungal and bacterial communities of the resulting 430 soil samples (i.e. 136 individuals × 3 samples + 3 species × 3 juveniles + 13 bare sand samples) were characterized with DNA sequencing.

DNA sequencing of fungal and bacterial communities

For each processed soil sample collected in July 2016, we extracted microbial DNA from 0.25 g of subsampled soil with the PowerSoil DNA Isolation Kit. We then PCR-amplified the bacterial 16S ribosomal DNA region and the fungal internal transcribed spacer 1 region (ITS1) with specific primer pairs. Amplicon libraries were then normalized, pooled based on DNA concentration, sequenced by the Illumina MiSeq sequencer, and processed through a bioinformatic pipeline to obtain a rarefied sample × operational taxonomic units (OTUs) matrix (see Supporting Information Methods S1 for detailed description of primer design and bioinformatic pipeline).

Glasshouse experiment

To examine how changes in the soil communities affect plant performance, we conducted a glasshouse experiment assessing

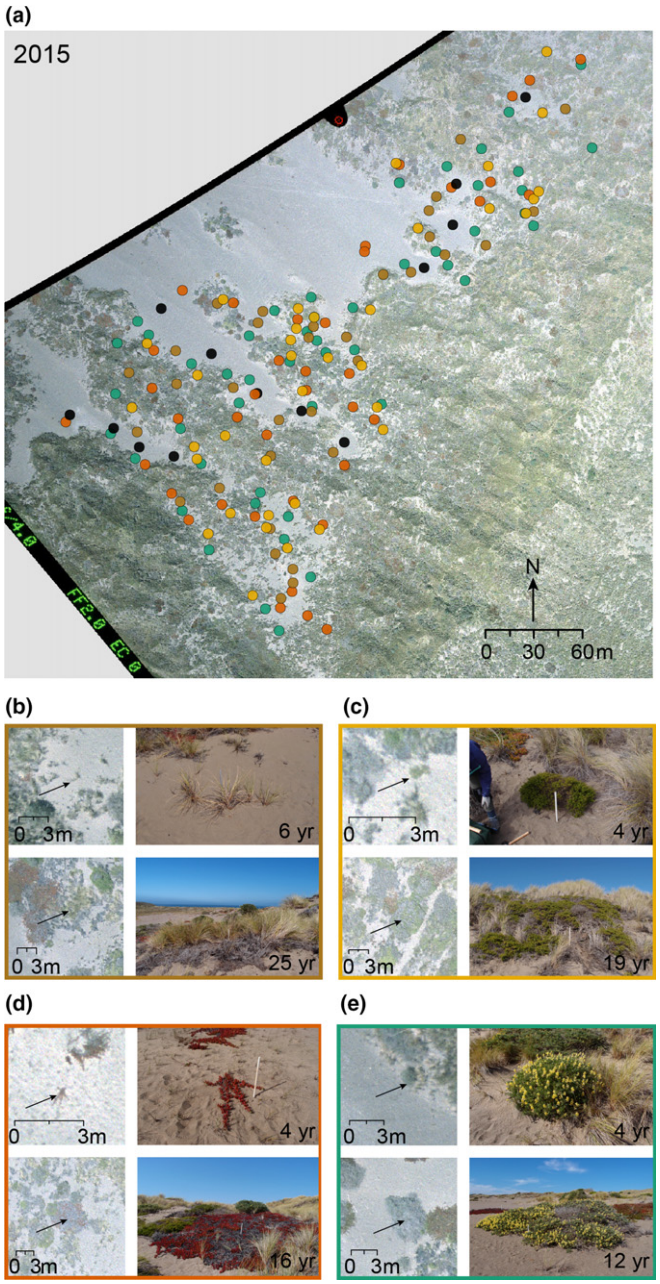

Fig. 1 Study area at Bodega Bay. (a) Aerial photograph taken in 2015, labeled with the location of selected plant individuals and bare sand sampling locations. The four species are represented by different colors, following the color scheme in panels (b–e); bare sand sampling location are in black. (b–e) Examples of young (upper row) and old (lower row) individuals of the four dominant species. (b) *Ammophila arenaria* (brown); (c) *Baccharis pilularis* (yellow); (d) *Carpobrotus edulis* (orange); (e) *Lupinus arboreus* (green). Left column of panels (b–e) are taken from the same 2015 aerial photograph, zoomed in on different selected plant individuals (indicated with arrows); right columns of panels (b–e) are photographs of the focal individuals in the field in 2016. As individuals arrived at different years in the past, they reach different ages at the year of sampling (indicated at the bottom-right corner).

seedling performance in soils that differed in their host plant species and conditioning length. In July 2017, we revisited the same plant individuals and collected soils for our experiment. For all four species, we collected soils from 27 individuals (i.e. a random subset of the previously sampled individuals) and three new randomly selected juveniles. For each individual, we used a sterilized soil core sampler to collect 300 ml of soil from the top 15 cm, which were pooled together from three sampling positions adjacent to the original sampling position in 2016 (100 ml from each position). Soils collected from all 120 individuals (i.e. (27 individuals + 3 juveniles) × 4 species) were processed with the same method as earlier and stored at 4°C before the glasshouse experiment.

Our glasshouse experiment aimed at transplanting seedlings of all four species in soils collected from the 120 plant individuals. To this end, soils collected from different plant individuals were kept separated throughout the experiment so that each soil maintained its age-specific properties (Rinella & Reinhart, 2018; Peacher & Meiners, 2020). We performed the glasshouse experiment in two separate rounds, which started in late August and September 2017. The range and variance of soil conditioning length were kept similar among the two experiment rounds, which was achieved by sorting soil source individuals based on their age and assigning every other individual along the age axis to different rounds. Half of the soil volume (150 ml) collected from each individual was autoclaved to create a sterilized treatment (120°C for 60 min, sit overnight for 24 h, and another 120°C for 60 min), allowing us to assess the potential effects of soil communities. Autoclaving was used as other approaches (e.g. gamma irradiation) were not accessible, although autoclaving might have changed soil physico-chemical properties and might not have completely eliminated soil biota (Dietrich *et al.*, 2020). It would have been desirable to have soils collected from 60 individuals in each experimental round (i.e. 15 individuals for each of the four species), but we had to discard soils collected from nine individuals due to handling mistakes during the sterilization process: six in the first round (i.e. soils from one individual of *A. arenaria*, three individuals of *C. edulis*, and two individuals of *L. arboreus*) and three in the second round (i.e. soils from two individuals of *B. pilularis* and one individual of *C. edulis*). With the two experiment rounds combined, our soil preparation step created 222 unique soil environments: 108 for the first round (i.e. (14 *A. arenaria* + 15 *B. pilularis* + 12 *C. edulis* + 13 *L. arboreus*) individuals × 2 sterilization treatments) and 114 for the second round (i.e. (15 *A. arenaria* + 13 *B. pilularis* + 14 *C. edulis* + 15 *L. arboreus*) individuals × 2 sterilization treatments). Live and sterilized soil environments collected from the same plant individual were always tested within the same experimental round.

Seeds of the four species were surface-sterilized by soaking in 5% bleach for 30 s, 95% ethanol for 30 s, and rinsing them with deionized water for 1 min. The sterilized seeds were spread evenly onto germination trays filled with sterilized sand (1 : 1 mixing of sterilized play sand and Lapis Lustre #2/12 sand (Cemex) to mimic the soil particle distribution in the field), and placed in a growth chamber (16 h : 8 h, light : dark, and temperature held at 16°C). After 2 wk, we transplanted the seedlings individually into 107-ml 'cone-tainers' pots (i.e. one seedling per pot) filled with 80 ml of sterilized sand (prepared with the same

method as for germination) and added 20 ml of either live or sterilized soil inoculum to the top. A relatively small volume of soil inoculum was added to minimize the potential side effects of autoclaving on soil abiotic properties (Brinkman *et al.*, 2010). Three of the four species were transplanted in both experimental rounds, except *B. pilularis*, the germination rate of which was too low for the second round. The final number of pots therefore deviated from our original full factorial design, with a total of 774 pots: 432 from the first round (i.e. 54 soil source individuals × 2 sterilization treatments × 4 species) and 342 from the second round (i.e. 57 soil source individuals × 2 sterilization treatments × 3 species).

Transplanted pots were randomly placed onto every other cell of 98 well trays (to avoid crowding) and were grown in the glasshouse for 12 wk (14 h : 10 h, light : dark with ambient temperature). To mimic precipitation regimes in the field, which was mainly fog during the summers, 30-s brief water spray with automatic misting nozzles were applied every hour. Seedlings that died within the first 10 d were replanted, and for seedlings that died afterwards their live–sterilized soil pair were discarded. With two rounds combined, data for 20 live–sterilized soil pairs were discarded due to seedling mortality: three seedlings of *A. arenaria* (i.e. one in *A. arenaria* soil, one in *B. pilularis* soil, one in *L. arboreus* soil), five seedlings of *B. pilularis* (i.e. two in *A. arenaria* soil, one in *B. pilularis* soil, one in *C. edulis* soil, one in *L. arboreus* soil), nine seedlings of *C. edulis* (i.e. two in *A. arenaria* soil, three in *B. pilularis* soil, two in *C. edulis* soil, two in *L. arboreus* soil), and three seedlings of *L. arboreus* (i.e. one in *B. pilularis* soil, one in *C. edulis* soil, one in *L. arboreus* soil). No effect of soil conditioning length on seedling survival was found (logistic regression, $P = 0.39$). After 12 wk, we harvested and oven-dried all plant tissues from each pot at 70°C for 96 h. The resulting total dry biomass was weighed to assess the effects of soil communities on plant performance.

Data analysis

We analyzed fungal and bacterial communities separately. To better match our microbial community data from soil samples to the soils used in our glasshouse experiment, we summed the OTU reads of the three samples that belonged to the same plant individual. As a result, the following statistical analyses were performed by viewing plant individuals as the unit of replication. To examine how species richness of the microbial community varied with plant age, we fitted linear, quadratic, and Monod functions with R package 'NLME' (Pinheiro *et al.*, 2019) to model observed OTU richness as a function of plant age. Models were fitted for each plant species separately, and the best model was selected based on their Akaike information criterion (AIC) with sample correction (AICc) values. To visualize compositional differences among microbial communities, we used nonmetric multidimensional scaling (NMDS) to ordinate microbial communities based on Bray–Curtis dissimilarity matrices with R packages 'VEGAN' (Oksanen *et al.*, 2019) and 'PHYLOSEQ' (McMurdie & Holmes, 2013). Effects of soil host species identity (i.e. the species that conditioned the soil) and plant age on microbial community composition were tested with permutational multivariate analysis of variance (PERMANOVA with 999 permutations, Anderson, 2001). To identify the microbial taxonomic groups that drove the observed community pattern, we aggregated the microbial communities to the family level and performed another NMDS ordination. For the fungal community, we further assigned OTUs to functional groups based on the FUNGuild database (Nguyen *et al.*, 2016) and performed linear regression to see how the abundance of different functional groups changed with plant age. The earlier-mentioned statistics with plant age as a predictor were performed for each soil host species separately.

In our glasshouse experiment, seedlings of the same plant species were paired based on the plant individual where field soils were collected, with one seedling inoculated with live soil and the other with sterilized soil from the same plant individual. As soils from different individuals were not mixed (Rinella & Reinhart, 2018), we were able to evaluate the effects that the soil community from a k-yr-old individual of species j had on the seedling of species i as:

$$\text{soil biota effect}_{i,j^k} = \log_{10}\left(\frac{B_{i,j^k,\text{live}}}{B_{i,j^k,\text{sterilized}}}\right),$$

where $B_{i,j^k,\text{live}}$ and $B_{i,j^k,\text{sterilized}}$ represent seedling biomass of species i when grown in pots inoculated with either live or sterilized soil from a k-yr-old individual of species j, respectively. Since autoclaving attempts to remove the whole soil community instead of targeting soil microbes, we will refer to this metric as soil biota effect (i.e. biotic feedback, *sensu* Semchenko *et al.*, 2018). A positive (or negative) value means that the soil biota associated with the k-yr-old individual of species j had a net beneficial (or detrimental) effect on the seedling of species i. The metric represents conspecific soil biota effects when seedlings were grown in soils collected from the same species (i.e. $i = j$), and it represents heterospecific soil biota effects when soils collected from different species were used (i.e. $i \neq j$). Since the inocula used in the two biomass measurements were collected from the same plant individual in the field, we obtained an age-specific biota effect associated with the individual that conditioned the soil.

We used two approaches to analyze the age-specific soil biota effects. First, we took the time-averaged value for each plant × soil host species combination (i.e. ignored the age information by taking the temporal mean), which is the common approach when field-conditioned soils were used but information of soil conditioning length not being available. For each of the four plant species, the effects of soil host species on the time-averaged soil biota effect were tested by fitting generalized linear mixed models (GLMMs, using R package 'LME4'; Bates *et al.*, 2015). We included the identity of soil host species as a fixed effect and glasshouse round, when present, as a random effect (note that a separate model including glasshouse round as a fixed effect was not significant, $P = 0.478$). An additional GLMM with the 16 plant × soil host species combinations as fixed effect and glasshouse round as random effect was fitted to assess whether time-averaged soil biota effects were significantly different from zero (i.e. by offsetting the intercept). *Post hoc* group comparisons

and compact letter display of pairwise comparisons were performed with R package 'MULTCOMP' (Hothorn *et al.*, 2008).

The second approach took advantage of the age information provided by the aerial photographs. Specifically, we visualized the age-specific soil biota effect on the temporal axis (i.e. soil conditioning length) and quantified its temporal trends by fitting GLMMs. The model included the age-specific biota effects as the response variable, and plant species identity, soil host species identity, soil conditioning length, and their pairwise interactions as fixed effects; glasshouse round was included as a random effect. We did not include the three-way interaction term as preliminary analysis suggested that it is insignificant (Table S1). We also fitted models using plant functional group (i.e. a dummy variable indicating whether the plant species is a legume) instead of plant species identity as a predictor, and compared model performance based on AIC values. In our model, a significant interaction between plant species (or plant functional group) and soil conditioning length would indicate that the plant species identity (or being a legume) affects how soil biota effects varied through time. If a significant temporal trend was found, we dug further into the pattern by fitting GLMMs with plant total biomass as the response variable, and soil host species identity, soil conditioning length, sterilization treatment, and their interactions as fixed effects. To ease interpretation, this three-way interaction model was fitted separately for different plant species or different plant functional groups (i.e. legume or not). A significant interaction between sterilization treatment and soil conditioning length would indicate that plant performance in live vs sterilized soils followed different temporal trend, therefore creating time-varying soil biota effects.

Simulation model

Our empirical study measures temporal changes in plant–soil interactions in the Bodega Bay dune system, but we were also interested in exploring potential consequences of temporally varying PSF more broadly across plant communities. As a first step towards general understanding of how the temporal changes in PSF strengths may affect plant community assembly, we used an individual-based model modified from Fukami & Nakajima (2011) (see also Fukami & Nakajima, 2013; Zee & Fukami, 2015; Fukami *et al.*, 2017). The purpose of this simulation exercise was not to predict what may happen specifically in the Bodega Bay dune system, but to investigate general possibilities of how different temporal changes in PSF strengths affect the transient and steady states of plant community assembly.

The model consisted of species pools containing 50 plant species (each with a different trait value) and patches consisting of 1024 local sites (each with a different habitat condition). We simulated immigration, reproduction, arrival, competition for establishment, and death of plant individuals. Competition for establishment at empty sites is determined not only by the match between species' trait values and local habitat conditions (i.e. environmental filtering) but also by the soil microbial legacy effects (i.e. PSF) created by the previously established plant species. Following empirical evidence (e.g. Semchenko *et al.*, 2018; Chen *et al.*, 2019), we allowed the microbial legacy effects to be either positive or negative. The key distinction between our model and previous studies is that microbial legacy effects in our model are age-dependent, i.e. the strength depends on the age of death of the previous established individual. See Methods S2 for full details of the simulation model.

For our simulation, we generated 10 patches for the regional species pool to colonize independently and one set of baseline microbial legacy effects, which represent the microbial effects created by the previous individual if it died a year immediately after colonization. The microbial effects experienced by a new arriving species depended on the previous individual's age of death and the temporal development pattern of microbial effects. We considered five feedback scenarios: (1) no interaction, where plants do not create microbial effects; (2) constant, where microbial effects remain unchanged despite individuals becoming older; (3) magnifying, where both positive and negative microbial effects intensify in strength as individuals become older (i.e. the longer the previous individual lived before it died, the stronger its impact on the new individual); (4) decaying, where both positive and negative microbial effects attenuate in strength because mature individuals support less pathogens and rely less on mutualists; and (5) bidirectionally varying, where both intensifying and attenuating are possible. For each scenario, we simulated 20 replicated runs of community assembly, where 20 independently created sets of species pool (each with 50 species) were allowed to colonize the same set of 10 patches, using the same set of baseline microbial effects. We quantified beta diversity among the 10 patches for each replicated run, which was measured as gamma diversity divided by mean alpha diversity, and compared temporal patterns of beta diversity among different scenarios. All analyses and simulations were performed in *R* v.3.3.1 (R Core Team, 2016).

Results

Temporal patterns of microbial communities

Fungal community composition differed among plant species (Fig. S1a, PERMANOVA, $R^2 = 0.148$, $P < 0.001$). Within each plant species, fungal composition varied with plant age (Fig. 2a–d, age effect for *A. arenaria*: $R^2 = 0.096$; *B. pilularis*: $R^2 = 0.083$; *C. edulis*: $R^2 = 0.100$; *L. arboreus*: $R^2 = 0.077$; all $P < 0.001$), becoming progressively different from bare sand communities with increasing conditioning time (fungal richness ceased to increase further after a few years following plant colonization, Fig. S2). Similar results were obtained for bacterial communities (Figs 2e–h, S1b, S3; species effect: $R^2 = 0.126$; age effect for *A. arenaria*: $R^2 = 0.115$; *B. pilularis*: $R^2 = 0.112$; *C. edulis*: $R^2 = 0.120$; *L. arboreus*: $R^2 = 0.116$; all $P < 0.001$). Figure S4 shows qualitatively how different fungal families change over time. For example, the contribution of fungal families Trichocomaceae and Mycosphaerellaceae decreased towards the left-hand side of Fig. S4a, indicating that their relative abundance decreased with longer soil conditioning length. Moreover, the fungal family Lasiosphaeriaceae increased in soils associated with *L. arboreus* (i.e. towards lower-left of Fig. S4a) whereas

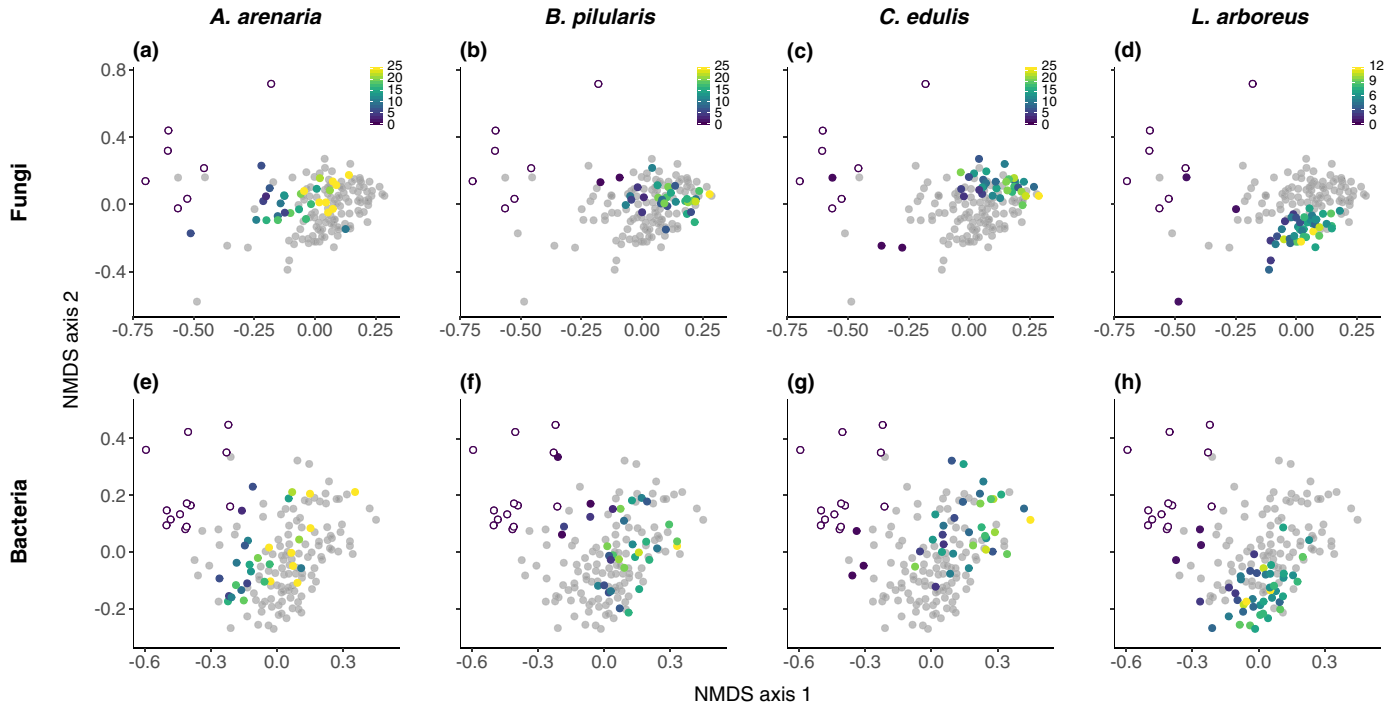

Fig. 2 Microbial community composition as a function of plant species and the age of plant individuals, visualized on a nonmetric multidimensional scaling (NMDS) ordination plot. Fungal communities are shown in (a–d) and bacterial communities in (e–h). Each point represents the fungal or bacterial community of one plant individual; open points that appeared in all panels represent fungal or bacterial communities associated with bare sand samples. In each panel, samples from one of the four plant species are highlighted while those from the other three plant species are in gray. Fungal and bacterial communities associated with the focal species are color-coded by individual plant age; purple to yellow represent the age gradient from young to old, with species-specific minimum and maximum age. (a, e) *Ammophila arenaria*; (b, f) *Baccharis pilularis*; (c, g) *Carpobrotus edulis*; (d, h) *Lupinus arboreus* associated fungal and bacterial communities, respectively. The color gradient are shared among panels that highlight the same focal species (i.e. each column). See the same ordination plot but color-coded with species identity in Supporting Information Fig. S1.

Teratosphaeriaceae increased in the soils associated with the other three species (i.e. towards upper-left of Fig. S4a; see also Fig. S5 for bacterial family patterns).

Temporal patterns of soil biota effects on plant performance

By quantifying the time-averaged soil biota effects that each plant species experienced when grown in soils conditioned by different soil host species, we found that the soil biota effects were positive for *L. arboreus* but negative for the other three plants (Figs 3, S6). Most soil biota effects were significantly different from zero (except when *B. pilularis* grown in soils from *A. arenaria* and *L. arboreus*; Fig. 3b), but the identity of the soil host species had no effect on the soil biota effects that plants experienced (soil host species effect insignificant for *A. arenaria*: $F_{3,101.06} = 0.354$, $P = 0.786$; *B. pilularis*: $F_{3,44} = 1.190$, $P = 0.324$; marginally significant for *C. edulis*: $F_{3,95.216} = 2.203$, $P = 0.092$; Fig. 3a–c). The only exception was *L. arboreus*, which grew best in soils from *C. edulis* individuals and worst in soils from *A. arenaria* individuals (soil host species effect: $F_{3,101.08} = 3.492$, $P = 0.018$; Fig. 3d; see also Table 1 for significant interactions between plant species (or plant functional group) and soil host species identity).

Soil conditioning length significantly influenced the strength of soil biota effects when using either plant species or plant functional group (i.e. legume or not) as the predictor (Table 1). The model that best described temporal changes in soil biota effects included legume, soil host species identity, soil conditioning length, and their two-way interactions (Table S2). The temporal pattern of soil biota effects experienced by *L. arboreus* differed from that experienced by the three other plants (i.e. there was a significant interaction between soil conditioning length and plant functional group, but not with soil host species nor plant species; Tables 1, S3). Based on these statistical results, in Fig. 4 we visualized the temporal pattern of soil biota effects by separating *L. arboreus* from the other three plant species. The soil biota effects experienced by *A. arenaria*, *B. pilularis* and *C. edulis* became more negative with longer conditioning time (Fig. 4a; see also Fig. S7), with the ratio of plant performance in live soils to that in sterilized soils decreasing 2.6% yr^{-1} (i.e. $1 - 10^{-0.0113}$; Table S3). In contrast, the soil biota effects experienced by *L. arboreus* showed little temporal change (Fig. 4b; Table S3).

The temporal pattern for *A. arenaria*, *B. pilularis*, and *C. edulis* arose because plant performance slightly increased when grown in sterilized soils with longer conditioning history but decreased when grown in live soil with longer conditioning history (Fig. 4c; see also Tables S4 and S5 for significant interaction terms

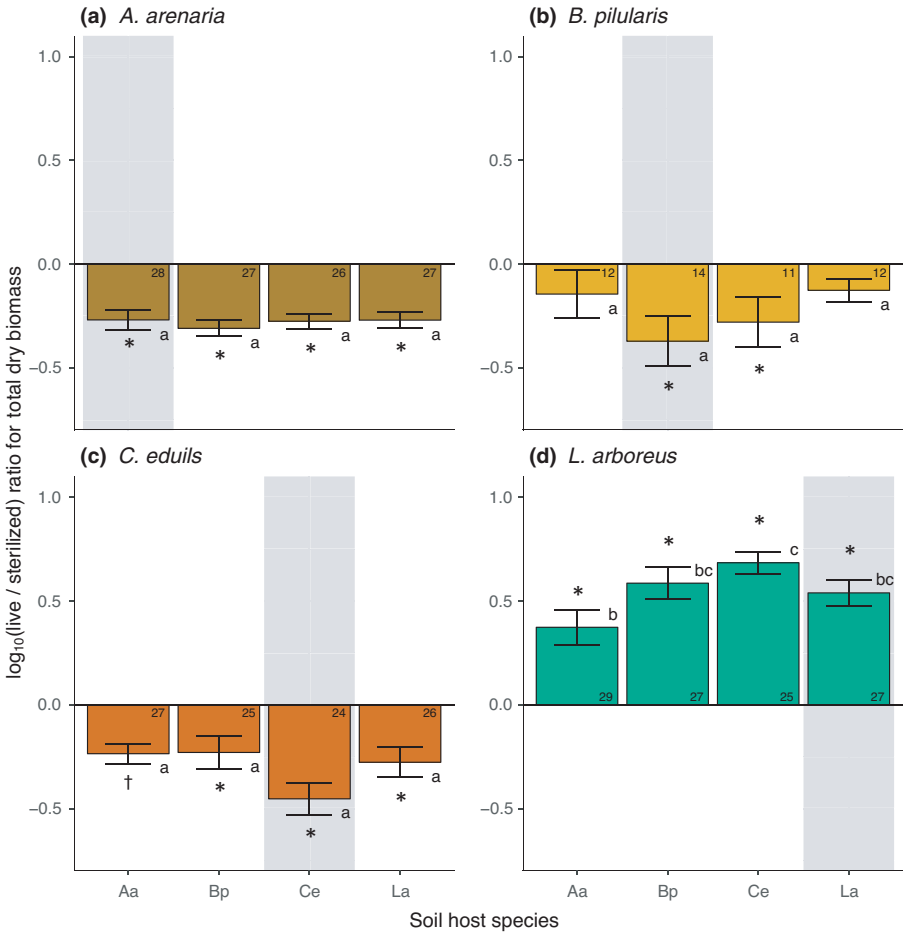

Fig. 3 Mean (±SE) soil biota effects for each plant species in soils conditioned by different plants, neglecting the effect of soil conditioning length. (a) *Ammophila arenaria* (brown); (b) *Baccharis pilularis* (yellow); (c) *Carpobrotus edulis* (orange); (d) *Lupinus arboreus* (green). The *x*-axis represents the plant species that conditioned the soil: *A. arenaria* (Aa), *B. pilularis* (Bp), *C. edulis* (Ce), and *L. arboreus* (La). The *y*-axis represents the soil biota effects, defined as the log-ratio of plant total biomass in live soil and sterilized soil, imposed by the soil host species. Shaded bars represent conspecific soil biota effects. Asterisks indicate soil biota effects that are significantly different from zero; daggers indicate marginal significance ($P < 0.1$); different letters represent significant difference among the plant × soil host species combinations. The numbers in the right corner of each bar indicate the number of data points included in the bar plot.

between soil conditioning length and sterilization treatment for the three plant species). In contrast, the performance of *L. arboreus* did not show different temporal patterns between live and sterilized soil (Fig. 4d; see also insignificant interaction terms in Tables S4 and S5 for *L. arboreus*).

Effects of temporally varying soil microbial effects on plant community assembly

Simulation results showed that plant communities converged in all scenarios, i.e. beta diversity declined through time, as communities became dominated by a subset of species. Despite eventually reaching similar beta diversity values, simulations ran under different temporal development scenarios converged with different rates (Fig. 5a). Similar to a previous study (Fukami & Nakajima, 2013), beta diversity declined most rapidly when plants did not create microbial legacies (Fig. 5b; light gray line in Fig. 5a), but was maintained at high levels and declined at slower rates if plants created microbial legacies that maintained a constant strength as individuals became older (Fig. 5c; black line in Fig. 5a). When the strength of microbial legacies varied depending on the previously established individual's age of death, communities converged most rapidly in the decaying scenario (Fig. 5e; blue line in Fig. 5a), followed by the magnifying scenario (Fig. 5d; orange line in Fig. 5a), and slowest in the bidirectionally varying scenario (Fig. 5f; green line in Fig. 5a).

Discussion

Our data provide evidence that the strength of plant–soil microbe interactions can depend on the duration of soil conditioning (Table 1). We showed that microbial community composition became more and more different from that in bare sand (Figs 2, S4, S5), indicating a potential change in their functional composition and effects on plants (Fig. S8). However, plant response to changes in the soil microbial community depended on the species identity of the transplanted seedling (i.e. whether or not it was the legume, *L. arboreus*). In particular, only the soil biota effect experienced by nonlegume plants varied with soil conditioning length (Fig. 4a): the performance of nonlegume plants decreased when grown in live soils collected from older soil source individuals, but slightly increased when grown in corresponding sterilized soils (Fig. 4c; Tables S4, S5). The former may result from accumulation of multiple generalist pathogens, whereas the latter may result from accumulation of organic matter and nutrients in soils with longer conditioning length (Conser & Connor, 2009; although we cannot rule out the possibility of a larger autoclave side effect on soil abiotic properties in such soils). In contrast, it

Table 1 ANOVA table summarizing the effects of plant species (or plant functional group, i.e. legume or not), soil host species identity, soil conditioning length, and their two-way interactions on the soil biota effects experienced by plants.

	Sum Sq.	Mean Sq.	Num df	Den df	*F*	*P*
Two-way interaction model with plant species as predictor						
Plant species	9.3167	3.1056	3	336.43	31.706	**< 0.0001**
Soil host species	0.2415	0.0805	3	336.12	0.822	0.4825
Soil conditioning length	0.4475	0.4475	1	336.43	4.569	**0.0333**
Plant species × soil host species	2.7169	0.3019	9	336.02	3.082	**0.0014**
Plant species × soil conditioning length	0.6570	0.2190	3	336.05	2.236	**0.0839**[a]
Soil host species × soil conditioning length	0.1937	0.0646	3	336.11	0.659	0.5776
Two-way interaction model with legume as predictor						
Legume	9.2414	9.2414	1	346.18	94.248	**< 0.0001**
Soil host species	0.3032	0.1011	3	346.1	1.031	0.3790
Soil conditioning length	0.6848	0.6848	1	346.49	6.984	**0.0086**
Legume × soil host species	1.9100	0.6367	3	346.01	6.493	**0.0003**
Legume × soil conditioning length	0.4823	0.4823	1	346	4.919	**0.0272**
Soil host species × soil conditioning length	0.1824	0.0608	3	346.12	0.620	0.6025

In the legume model, adding model terms with soil conditioning length increased the conditional R^2 from 0.605 to 0.620. Bold typeface in the last column indicates statistically significant terms ($P < 0.05$).
[a]Marginally significant ($P < 0.1$).

appeared that only the presence/absence of soil biota mattered for *L. arboreus* (Fig. 4d; Kandlikar *et al.*, 2021). We speculate that this is because legumes can form effective symbiotic relationships with a range of rhizobia (Birnbaum *et al.*, 2018; Png *et al.*, 2019), or the relative abundance of their microbial partners did not vary significantly through time. Future studies can more formally test the effects of plant functional groups on the temporal pattern of plant–soil microbe interactions by including multiple species in each functional group.

We focused on the temporal changes in plant–soil microbe interactions during the conditioning phase. Previous studies on the temporal dynamics of PSF have mostly focused on changes during the response phase (i.e. monitoring its strengths across different plant ontogenetic stages; Hawkes *et al.*, 2012; Bezemer *et al.*, 2018; Dudenhöffer *et al.*, 2018). For example, Hawkes *et al.* (2012) conducted a 19-month-long experiment and quantified PSF at four different time steps as the planted seedling matures. Their result suggested that the effects of soil microbes on native plants became more negative through time (see also Bezemer *et al.*, 2018; Dudenhöffer *et al.*, 2018). As seedlings mature, they not only drive continuous turnover in the microbial composition (Husband *et al.*, 2002; Meaden *et al.*, 2016; Dinnage *et al.*, 2019), but their sensitivity to soil microbes may also change (Reinhart *et al.*, 2010; Ke *et al.*, 2015). Experiments like ours can disentangle the underlying mechanisms and quantify the effects of microbial turnover during the conditioning phase.

The relationship between soil conditioning length and feedback strength has been investigated in the context of plant invasion. These studies have quantified how the PSF strength experienced by the invading species changes after multiple generations, focusing on how the benefit of escaping host-specific soil pathogens in their native range attenuates with their resident time (Diaz *et al.*, 2010; Dostál *et al.*, 2013; Day *et al.*, 2015; Speek *et al.*, 2015). For example, Diaz *et al.* (2010) found that nonnative plant species that became established in New Zealand for a longer time (e.g. hundreds of years) experienced stronger negative PSF (but see Day *et al.*, 2015; Speek *et al.*, 2015). The importance of soil conditioning length has also been studied in the context of successive planting in agricultural systems, which demonstrated intensifying negative microbial effects with increasing rounds of planting (Mazzola, 1999; Packer & Clay, 2004). Recent studies have generalized the traditional focus of single species to consider multiple rounds of soil conditioning by different species, showing that the order of species conditioning the soil explained a large part of plant performance variability (Wubs & Bezemer, 2017). Our results show that the negative soil biota effects experienced by the two invasive plants may aggravate within a few years in the field (Fig. 4a; Table S3), which may indicate a decreasing degree of enemy release as shown in other studies (Beckstead & Parker, 2003; de la Peña *et al.*, 2010).

Recognizing that plant–soil microbe interactions are more dynamic than generally assumed can be useful when studying their effects on plant community recovery after disturbance. Some disturbance, such as severe wildfire, kills all individuals, whereas other forms of disturbance cause higher mortality for specific age classes (Sousa, 1984). For example, insect herbivore outbreak may cause plant juveniles to suffer higher mortality, whereas windthrow may have a more significant direct impact on large adults (Sousa, 1984). Different types of disturbances thus terminate soil conditioning at various stages, leaving behind different microbial legacies that could alter recovery trajectories. Other studies have also shown that PSF affects restoration (Wubs *et al.*, 2016, 2019) and information on how plant–soil microbe interactions change through time can also help design restoration projects. At our field site, we found that the two invasive species performed worse in soils with longer conditioning history. This result suggests that removing old individuals may be an effective restoration strategy since the soil legacies that they leave behind are more detrimental for propagules from nearby nonnative individuals to regenerate.

Our study was conducted in a sand dune ecosystem where individual plants are often spatially separated from one another, providing the unique opportunity to demonstrate the importance

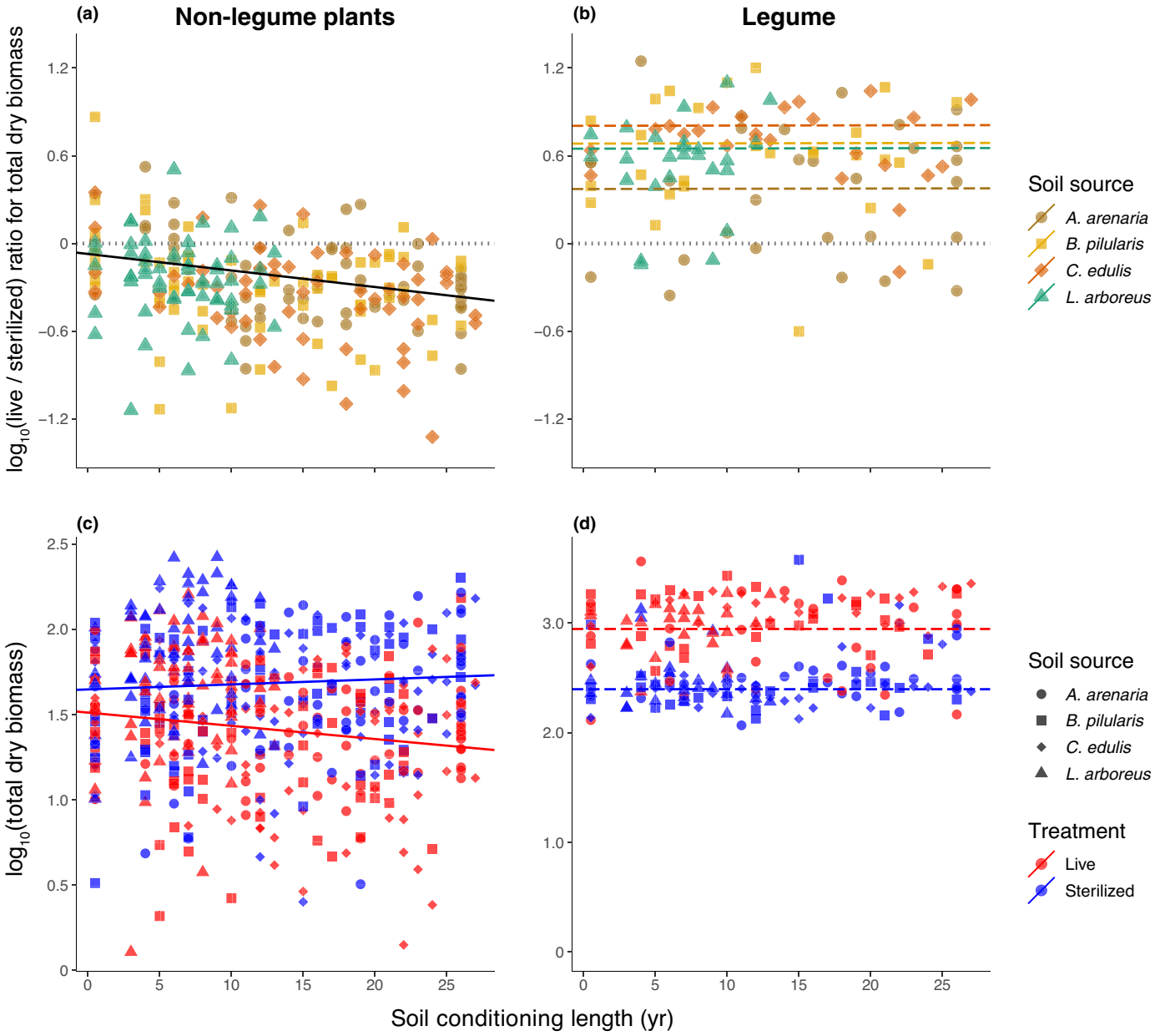

Fig. 4 Temporal trends of the effects of soil biota on plant seedling performance. Temporal trend of soil biota effects, defined as the $\log_{10}$-ratio of plant total biomass in live soil and sterilized soil, for (a) nonlegume plants (i.e. *Ammophila arenaria*, *Baccharis pilularis*, and *Carpobrotus edulis*) and (b) legume (i.e. *Lupinus arboreus*). For these two panels, each point represents the soil biota effects generated by soils collected from one plant individual, with the soil host's species identity indicated by the color and shape: *A. arenaria* (brown circles), *B. pilularis* (yellow squares), *C. edulis* (orange diamonds), and *L. arboreus* (green triangles); the gray dotted line indicates no soil biota effect (see also Supporting Information Fig. S7 where the species identity of the transplanted seedling is indicated by the inner color of each point). The black line in (a) represents how the soil biota effects experienced by nonlegume plants vary with soil conditioning length (*x*-axis), and the colored horizontal lines in (b) represents how the soil biota effects experienced by *L. arboreus* vary with the species identity of soil source individuals (see Tables 1, S3 for statistical results). Panels (c) and (d) show temporal trends of plant total dry biomass for nonlegume plants and legume, respectively. For these two panels, the *x*-axis shows the soil conditioning length and the *y*-axis shows the $\log_{10}$-transformed total dry biomass. Each point represents the growth performance of one plant seedling, growing in either live (red) or sterilized (blue) soils, and point shapes represent the species identity of soil source individuals. For visualization purpose, the fitted lines here are from a statistical model that uses $\log_{10}$ total biomass as the response variable, and soil conditioning length, sterilization treatment, and their interaction as predictors. In (c), we show the significant interaction between soil conditioning length and the sterilization treatment, whereas in (d) we only show the effects of the sterilization treatment as the soil conditioning length term was not significant (Table S5; see also Table S4). Note the different scales on the *y*-axis in (c, d). In (b, d), dashed lines were used to indicate insignificant interaction term between soil conditioning length and sterilization treatment for *L. arboreus* (Table S5).

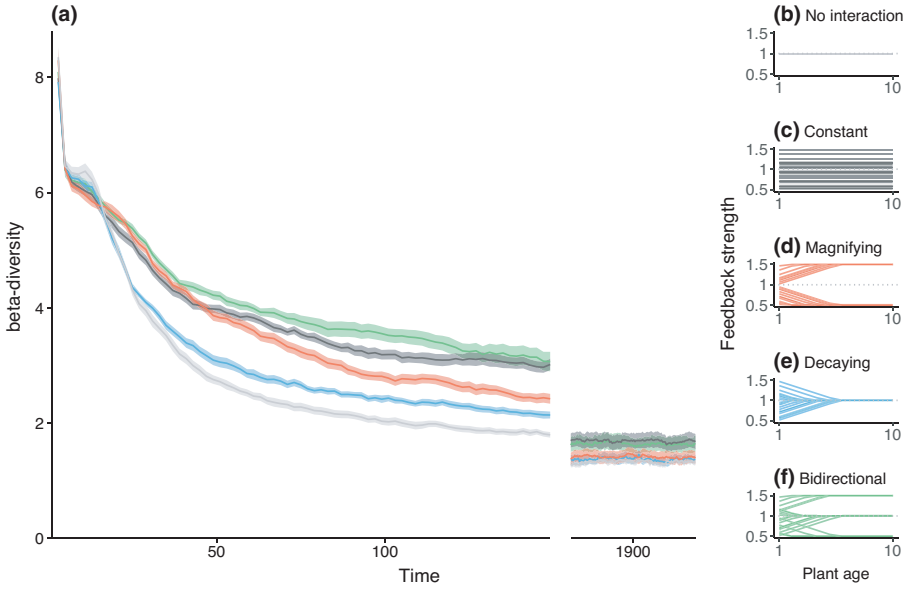

Fig. 5 Simulated community convergence patterns under different temporal development scenarios of the underlying plant–soil microbe interactions. (a) Temporal trends of beta diversity (mean ± SE, $n = 20$) among the 10 simulated patches for five different plant–soil microbe interaction scenarios. (b–f) Schematic diagrams of the five different scenarios in (a), demonstrating how the interaction strength changes with the age of the conditioning individual. (b) No plant–soil microbe interactions (light gray); (c) plant–soil microbe interactions that are independent to plant age (black); (d) magnifying interaction strengths that intensify to their biological extremes with increasing plant age (orange); (e) decaying interaction strengths that attenuate to one with increasing plant age (blue); (f) bidirectionally varying interaction strengths that either intensify or attenuate with increasing plant age (green). See Supporting Information Methods S2 for detailed model description.

of soil conditioning length for different species. In many other systems, however, plant roots may be more intermingled and different plant species co-culture the local soil community (Wubs & Bezemer, 2018). Understanding the temporal development of PSF in these systems is of importance because plants may easily encounter patches with different conditioning length as their roots explore nearby soil (Hendriks *et al.*, 2015a). One can use glasshouse experiments with various conditioning duration to isolate the effects of soil conditioning length for different plant–soil pairs (Lepinay *et al.*, 2018; though we note that glasshouse conditioning may occur at a shorter timescale due to more confined soil volume). Future studies can then develop methods to calculate the overall soil biota effects on a focal individual based on its root system distribution (Hendriks *et al.*, 2015b).

Our general individual-based model provides an opportunity to broaden our perspective of temporally dynamic PSF beyond the specific system. The simulation exercise suggested that plant communities assembled under different temporal development patterns of PSF could exhibit various transient dynamics and converge at different rates (Fig. 5). Without soil microbes, species' competitiveness in our model solely depends on environmental filtering (i.e. the match between species' trait value and local habitat condition). However, species' competitiveness is modified when plants create microbial legacies, and a more heterogeneous PSF scenario (e.g. more complicated temporal changes) can delay community convergence by preventing the immediate dominance of the species with the best fit trait. Previous studies suggested that a positive correlation between plant successional stage and PSF strength can facilitate plant species turnover (Kardol *et al.*, 2006; Middleton & Bever, 2012; Bauer *et al.*, 2015). In nature, local soils may vary spatially in how long they have been conditioned as plant individuals often arrive at different timings and die at different ages (Kardol *et al.*, 2013b; Peay, 2018). If the duration of soil conditioning affects PSF strength, as shown in our study, heterogeneity in soil conditioning length may weaken the correlation between PSF strength and plant successional stage and create complex PSF that delay community convergence (Fig. 5; Fukami & Nakajima, 2013). These implications suggest that future theoretical models should incorporate the different temporal aspects of PSF when studying their effects on plant community assembly (Kardol *et al.*, 2013a; Ke & Miki, 2015; Ke & Levine, 2021).

Conclusion

We have shown here that plant–soil microbe interaction strengths can vary depending on how long the previous plant individual has conditioned the soil, illustrating the importance of interaction timing in determining species interaction strength (Kardol *et al.*, 2013b; Peay, 2018). Our results indicate that the length of soil conditioning can influence the estimated strength of plant–soil interactions for a majority of dominant plant species in a system, highlighting the long-term time dependency of plant–soil interactions. By treating the temporal dynamics of plant and microbial communities as a crucial component of PSF (e.g. Chung *et al.*, 2019; in 't Zandt *et al.*, 2021), it should be possible

to more properly place experimental results in a natural context to better predict how soil microbes influence plant community assembly.

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Author contributions

P-JK and TF conceived the study; P-JK led the field, laboratory, and glasshouse work; P-JK and PCZ performed the simulation; P-JK wrote the first draft of the manuscript and all authors contributed to editing the manuscript.

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Data availability

All primary data and computer scripts for the individual-based model are deposited on Dryad, https://doi.org/10.5061/dryad.tmpg4f4zd.

References

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32–46.

Barbour MG, Craig RB, Drysdale FR, Ghiselin MT. 1973. *Coastal ecology: Bodega Head*. Berkeley, CA, USA: University of California Press.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Bauer JT, Mack KML, Bever JD. 2015. Plant–soil feedbacks as drivers of succession: evidence from remnant and restored tallgrass prairies. *Ecosphere* **6**: art158.

Beckstead J, Parker IM. 2003. Invasiveness of *Ammophila arenaria*: release from soil-borne pathogens? *Ecology* **84**: 2824–2831.

Bever JD. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist* **157**: 465–473.

Bever JD, Westover KM, Antonovics J. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* **85**: 561–573.

Bezemer TM, Jing J, Bakx-Schotman JMT, Bijleveld EJ. 2018. Plant competition alters the temporal dynamics of plant–soil feedbacks. *Journal of Ecology* **106**: 2287–2300.

Birnbaum C, Bissett A, Teste FP, Laliberté E. 2018. Symbiotic N_2-fixer community composition, but not diversity, shifts in nodules of a single host legume across a 2-million-year dune chronosequence. *Microbial Ecology* **76**: 1009–1020.

Brinkman EP, van der Putten WH, Ej B, Verhoeven KJF. 2010. Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology* **98**: 1063–1073.

Chen L, Swenson NG, Ji N, Mi X, Ren H, Guo L, Ma K. 2019. Differential soil fungus accumulation and density dependence of trees in a subtropical forest. *Science* **366**: 124–128.

Chung YA, Collins SL, Rudgers JA. 2019. Connecting plant–soil feedbacks to long-term stability in a desert grassland. *Ecology* **100**: e02756.

Conser C, Connor EF. 2009. Assessing the residual effects of *Carpobrotus edulis* invasion, implications for restoration. *Biological Invasions* **11**: 349–358.

Danin A, Rae S, Barbour M, Jurjavcic N, Connors P, Uhlinger E. 1998. Early primary succession on dunes at Bodega Head. *Madrono* **45**: 101–109.

D'Antonio CM, Mahall BE. 1991. Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. *American Journal of Botany* **78**: 885–894.

Day NJ, Dunfield KE, Antunes PM. 2015. Temporal dynamics of plant–soil feedback and root-associated fungal communities over 100 years of invasion by a non-native plant. *Journal of Ecology* **103**: 1557–1569.

Dietrich P, Cesarz S, Eisenhauer N, Roscher C. 2020. Effects of steam sterilization on soil abiotic and biotic properties. *Soil Organisms* **92**: 99–108.

Diez JM, Dickie I, Edwards G, Hulme PE, Sullivan JJ, Duncan RP 2010. Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters* **13**: 803–809.

Dinnage R, Simonsen AK, Barrett LG, Cardillo M, Raisbeck-Brown N, Thrall PH, Prober SM. 2019. Larger plants promote a greater diversity of symbiotic nitrogen-fixing soil bacteria associated with an Australian endemic legume. *Journal of Ecology* **107**: 977–991.

Dostál P, Müllerová J, Pyšek P, Pergl J, Klinerová T. 2013. The impact of an invasive plant changes over time. *Ecology Letters* **16**: 1277–1284.

Dudenhöffer J, Ebeling A, Klein A, Wagg C, Farrer E. 2018. Beyond biomass: soil feedbacks are transient over plant life stages and alter fitness. *Journal of Ecology* **106**: 230–241.

Eppinga MB, Baudena M, Johnson DJ, Jiang J, Mack KML, Strand AE, Bever JD. 2018. Frequency-dependent feedback constrains plant community coexistence. *Nature Ecology & Evolution* **2**: 1403–1407.

Fukami T, Nakajima M. 2011. Community assembly: alternative stable states or alternative transient states? *Ecology Letters* **14**: 973–984.

Fukami T, Nakajima M. 2013. Complex plant–soil interactions enhance plant species diversity by delaying community convergence. *Journal of Ecology* **101**: 316–324.

Fukami T, Nakajima M, Fortunel C, Fine PVA, Baraloto C, Russo SE, Peay KG. 2017. Geographical variation in community divergence: insights from tropical forest monodominance by ectomycorrhizal trees. *The American Naturalist* **190**: S105–S122.

Hannula SE, Kielak AM, Steinauer K, Huberty M, Jongen R, De Long JR, Heinen R, Bezemer TM. 2019. Time after time: temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. *mBio* **10**: e02635-19.

Hawkes CV, Kivlin SN, Du J, Eviner VT. 2012. The temporal development and additivity of plant–soil feedback in perennial grasses. *Plant and Soil* **369**: 141–150.

Hendriks M, Visser EJW, Visschers IGS, Aarts BHJ, de Caluwe H, Smit-Tiekstra AE, van der Putten WH, de Kroon H, Mommer L. 2015a. Root responses of grassland species to spatial heterogeneity of plant–soil feedback. *Functional Ecology* **29**: 177–186.

Hendriks M, Ravenek JM, Smit-Tiekstra AE, van der Paauw JW, de Caluwe H, van der Putten WH, de Kroon H, Mommer L. 2015b. Spatial heterogeneity of plant–soil feedback affects root interactions and interspecific competition. *New Phytologist* **207**: 830–840.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* **50**: 346–363.

Husband R, Herre EA, Young JPW. 2002. Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. *FEMS Microbiology Ecology* **42**: 131–136.

Kandlikar G, Yan X, Levine JM, Kraft NJB. 2021. Soil microbes generate stronger fitness differences than stabilization among California annual plants. *The American Naturalist* **197**: E30–E39.

Kardol P, Bezemer MT, van der Putten WH. 2006. Temporal variation in plant–soil feedback controls succession. *Ecology Letters* **9**: 1080–1088.

Kardol P, De Deyn GB, Laliberté E, Mariotte P, Hawkes CV. 2013a. Biotic plant–soil feedbacks across temporal scales. *Journal of Ecology* **101**: 309–315.

Kardol P, Souza L, Classen AT. 2013b. Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. *Oikos* **122**: 84–94.

Ke PJ, Levine JM. 2021. The temporal dimension of plant–soil microbe interactions: mechanisms promoting feedback between generations. *The American Naturalist*. doi: 10.1086/715577.

Ke PJ, Miki T. 2015. Incorporating the soil environment and microbial community into plant competition theory. *Frontiers in Microbiology* **6**: 1066.

Ke PJ, Miki T, Ding T. 2015. The soil microbial community predicts the importance of plant traits in plant–soil feedback. *New Phytologist* **206**: 329–341.

Ke PJ, Wan J. 2020. Effects of soil microbes on plant competition: a perspective from modern coexistence theory. *Ecological Monographs* **90**: e01391.

Kleinhesselink AR, Magnoli SM, Cushman JH. 2014. Shrubs as ecosystem engineers across an environmental gradient: effects on species richness and exotic plant invasion. *Oecologia* **175**: 1277–1290.

Klironomos JN. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* **417**: 67–70.

Knelman JE, Legg TM, O'Neill SP, Washenberger CL, González A, Cleveland CC, Nemergut DR. 2012. Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield. *Soil Biology and Biochemistry* **46**: 172–180.

Kulmatiski A. 2018. Community-level plant–soil feedbacks explain landscape distribution of native and non-native plants. *Ecology and Evolution* **8**: 2041–2049.

Kulmatiski A, Kardol P. 2008. Getting plant–soil feedbacks out of the greenhouse: experimental and conceptual approaches. In: Lüttge U, Beyschlag W, Murata J, eds. *Progress in botany*. Berlin/Heidelberg, Germany: Springer, 449–472.

Lepinay C, Vondráková Z, Dostálek T, Münzbergová Z. 2018. Duration of the conditioning phase affects the results of plant–soil feedback experiments via soil chemical properties. *Oecologia* **186**: 459–470.

Lortie CJ, Cushman JH. 2007. Effects of a directional abiotic gradient on plant community dynamics and invasion in a coastal dune system. *Journal of Ecology* **95**: 468–481.

Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD. 2010. Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**: 752–755.

Mazzola M. 1999. Transformation of soil microbial community structure and Rhizoctonia-suppressive potential in response to apple roots. *Phytopathology* **89**: 920–927.

McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**: e61217.

McNeil SG, Cushman JH. 2005. Indirect effects of deer herbivory on local nitrogen availability in a coastal dune ecosystem. *Oikos* **110**: 124–132.

Meaden S, Metcalf C, Koskella B. 2016. The effects of host age and spatial location on bacterial community composition in the English Oak tree (*Quercus robur*). *Environmental Microbiology Reports* **8**: 649–658.

Middleton EL, Bever JD. 2012. Inoculation with a native soil community advances succession in a grassland restoration. *Restoration Ecology* **20**: 218–226.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. Funguild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P *et al.* 2019. *vegan: community ecology package*. R package v.2.5-6. [WWW document] URL https://CRAN.R-project.org/package=vegan [accessed 2 September 2019].

Packer A, Clay K. 2004. Development of negative feedback during successive growth cycles of black cherry. *Proceedings of the Royal Society: Biological Sciences* **271**: 317–324.

Peacher MD, Meiners SJ. 2020. Inoculum handling alters the strength and direction of plant–microbe interactions. *Ecology* **101**: e02994.

Peay KG. 2018. Timing of mutualist arrival has a greater effect on *Pinus muricata* seedling growth than interspecific competition. *Journal of Ecology* **106**: 514–523.

de la Peña E, de Clercq N, Bonte D, Roiloa S, Rodríguez-Echeverría S, Freitas H. 2010. Plant–soil feedback as a mechanism of invasion by *Carpobrotus edulis*. *Biological Invasions* **12**: 3637–3648.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2019. *nlme: linear and nonlinear mixed effects models*. R package v.3.1-143. [WWW document] URL https://CRAN.R-project.org/package=nlme [accessed 11 December 2019].

Png GK, Lambers H, Kardol P, Turner BL, Wardle DA, Laliberté E. 2019. Biotic and abiotic plant–soil feedback depends on nitrogen-acquisition strategy and shifts during long-term ecosystem development. *Journal of Ecology* **107**: 142–153.

R Core Team. 2016. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Reinhart KO, Royo AA, Kageyama SA, Clay K. 2010. Canopy gaps decrease microbial densities and disease risk for a shade-intolerant tree species. *Acta Oecologica* **36**: 530–536.

Rinella MJ, Reinhart KO. 2018. Toward more robust plant–soil feedback research. *Ecology* **99**: 550–556.

Semchenko M, Leff JW, Lozano YM, Saar S, Davison J, Wilkinson A, Jackson BG, Pritchard WJ, Jonathan R, Oakley S *et al.* 2018. Fungal diversity regulates plant-soil feedbacks in temperate grassland. *Science Advances* **4**: eaau4578.

Sousa WP. 1984. The role of disturbance in natural communities. *Annual Review of Ecology and Systematics* **15**: 353–391.

Speek TA, Schaminée JH, Stam JM, Lotz LA, Ozinga WA, van der Putten WH. 2015. Local dominance of exotic plants declines with residence time: a role for plant–soil feedback? *AoB Plants* **7**: plv021.

Teste FP, Kardol P, Turner BL, Wardle DA, Zemunik G, Renton M, Laliberté E. 2017. Plant–soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science* **355**: 173–176.

Wubs ERJ, Bezemer TM. 2017. Temporal carry-over effects in sequential plant–soil feedbacks. *Oikos* **127**: 220–229.

Wubs ERJ, Bezemer TM. 2018. Plant community evenness responds to spatial plant–soil feedback heterogeneity primarily through the diversity of soil conditioning. *Functional Ecology* **32**: 509–521.

Wubs ERJ, van der Putten WH, Bosch M, Bezemer TM. 2016. Soil inoculation steers restoration of terrestrial ecosystems. *Nature Plants* **2**: 16107.

Wubs EJ, van der Putten WH, Mortimer SR, Korthals GW, Duyts H, Wagenaar R, Bezemer TM. 2019. Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. *Ecology Letters* **22**: 1145–1151.

Yang G, Roy J, Veresoglo SD, Rillig MC. 2020. Soil biodiversity enhances the persistence of legumes under climate change. *New Phytologist* **229**: 2945–2956.

in 't Zandt D, Herben T, van den Brink A, Visser EJW, de Kroon H. 2021. Species abundance fluctuations over 31 years are associated with plant–soil feedback in a species-rich mountain meadow. *Journal of Ecology* **109**: 1511–1523.

Zee PC, Fukami T. 2015. Complex organism–environment feedbacks buffer species diversity against habitat fragmentation. *Ecography* **38**: 370–379.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Ordination of fungal and bacterial communities sampled from different plant species.

Fig. S2 Fungal community richness as a function of plant age.

Fig. S3 Bacterial community richness as a function of plant age.

Fig. S4 NMDS based on fungal family composition with the loading of abundant families.

Fig. S5 NMDS based on bacterial family composition with the loading of abundant families.

Fig. S6 Total dry biomass of each plant species in soils conditioned by different plants.

Fig. S7 Temporal trends of the effects of soil microbes on plant seedling performance.

Fig. S8 Temporal pattern of the abundance of different fungal functional group.

Methods S1 Supporting methods for soil microbial community characterization.

Methods S2 Detailed description of the individual-based model examining the effects of age-dependent plant–soil microbe interactions.

Table S1 ANOVA table for the effects of plant species (or plant functional group), soil host species, conditioning length, and their interactions on microbial effects.

Table S2 AIC value comparison across models with different interaction terms and predictors.

Table S3 Model results for the effects of plant functional group, soil host species, conditioning length, and their two-way interactions on microbial effects.

Table S4 ANOVA table for the effects of soil host species, conditioning length, sterilization treatment, and their interactions on plant biomass of nonlegume plants (i.e. *Ammophila arenaria*, *Baccharis pilularis*, and *Carpobrotus edulis*) and legume (i.e. *Lupinus arboreus*).

Table S5 Model results for the effects of conditioning length, sterilization treatment, and their interaction on plant biomass of nonlegume plants (i.e. *Ammophila arenaria*, *Baccharis pilularis*, and *Carpobrotus edulis*) and legume (i.e. *Lupinus arboreus*).

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