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RESEARCH ARTICLE

Zooming in on the temporal dimensions of plant–soil feedback: Plant sensitivity and microbial dynamics

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Abstract

1. The magnitude of plant–soil feedback (PSF) can depend on the time of conditioning as well as the length of feedback. Understanding the temporal variation in PSF requires insight in the response of both soil characteristics and the plant.
2. We examined how conspecific PSF varies with the length of conditioning and the size of the response plant using *Jacobaea vulgaris*, a species known to experience negative conspecific PSF. Together with reanalysis of an existing microbial sequence dataset, we tested whether the temporal variation in PSF is due to size-dependent plant sensitivity to conditioned soil or due to compositional changes in microbial communities of conditioned soil. Further, by reanalysing another existing dataset, we examined temporal dynamics of the relative growth rates (RGR) of *J. vulgaris* during the feedback phase.
3. Testing varying conditioning lengths, uncovered that *J. vulgaris* exhibited the strongest negative PSF at 5 weeks of conditioning, after which PSF gradually attenuated. Plant sensitivity to conditioned soil decreased with increasing plant age/size of the response plant. In the feedback phase, the RGR of *J. vulgaris* was first higher, then lower and at the end similar in 'away' soil compared to 'home' soil.
4. The dissimilarity in bacterial and fungal communities in 'home' and 'away' soil significantly decreased during the feedback phase. When *J. vulgaris* grew in 'away' soil, the relative abundance of 10 (out of 80) bacterial OTUs that positively correlated with plant growth decreased over time, while 5 (out of 86) OTUs that negatively correlated with plant growth the relative abundance increased over time. Additionally, only one (out of 10) fungal OTU that negatively associated with plant growth increased over time in 'away' soil.
5. *Synthesis*. Our findings illustrate that PSF varies with the duration of soil conditioning and of the feedback phase. During the feedback phase, changes in PSF can be attributed to both the size-dependent plant sensitivity to conditioned soil and temporal changes in the microbial community of conditioned soil. This highlights the importance of considering the reconditioning of soil microbial communities by the test plants during the feedback phase for understanding temporal variation in PSF.

KEYWORDS
conditioning period, microbial community, plant sensitivity, plant size/age, plant–soil feedback, relative growth rate, temporal variation

1 | INTRODUCTION

Plant–soil feedback (PSF) describes the process that a plant first alters soil abiotic and biotic characteristics, and that these changes in the soil then influence the performance of succeeding plants that grow in the same soil (Bever, 1994; Bever et al., 1997; van der Putten et al., 2013). The classic experimental design of PSF studies contains two phases, the conditioning phase and the response phase. Experimental studies typically employ a fixed duration for both phases (Kardol et al., 2013). However, both the effects of plants on soil biotic and abiotic characteristics as well as the response of plants to changes in soil characteristics can vary over time and may depend on the age and thus also size of the responding plant.

Conspecific plant–soil feedbacks can become more negative with an increasing duration of conditioning (Chung, 2023). At long timescales (multiple generations), there is evidence for this from invasive alien plants where PSFs (home-away comparison) become more negative with longer residence time of aliens (Diez et al., 2010). At short time scales, there is also evidence that conditioning time or the duration of the growth period that the plant influences the soil influences the size of the feedback effect (live-sterile comparison in Ke et al., 2021; home-unconditioned comparison in Lepinay et al., 2018). This is because the impact of a plant on the microbial community or other characteristics of the soil depends on the duration of growth of this plant in the soil (Ke et al., 2021; Lepinay et al., 2018; Steinauer et al., 2023). Plants vary greatly in nutrient uptake, in the release of organic compounds in the soil via rhizodeposits and in their influence on soil properties such as moisture and pH (Bennett & Klironomos, 2019; Nannipieri et al., 2023). The scale of these effects of a plant on the soil are likely to be determined by the size of the influencer (i.e. plant size) as well as the duration of the influence and hence the duration of growth of the plant in the soil. For example, in a greenhouse experiment, the duration of soil conditioning affected the magnitude of PSF, in which the negative conspecific PSF of *Rorippa austriaca* became stronger with an increase in soil conditioning duration, but this effect reached a maximum after 6 weeks of conditioning (Lepinay et al., 2018). As both plant size and the duration of influence increase with increasing periods of soil conditioning, we expect that the magnitude of conspecific PSFs that are created by conditioning the soil will increase with an increase in the duration of soil conditioning.

In the feedback phase, plant responses to conditioned soil also change over time. Previous studies have examined the temporal dynamics of plant growth during the feedback phase by harvesting plants after different growth periods (Bezemer et al., 2018; Steinauer et al., 2023). The conclusion from these studies is that young or smaller plants appear more sensitive to soil conditioning effects than older/larger plants, and that as a result the PSF gradually becomes neutral when plants get older (Case I in Figure 1a, both positive and negative PSFs can decline over time, i.e. *Jacobaea vulgaris* generally exhibited a declining negative conspecific PSF during the feedback phase in Bezemer et al., 2018). However, we cannot distinguish whether this decrease in the strength of PSF is because the plant becomes less sensitive as it grows larger (plant effects, Figure 1b) or because the plant gradually modifies the 'away' soil so that it becomes 'home' soil over time. Consequently, older plants may perceive the heterospecific conditioned soil as their 'home' soil (soil effects, Figure 1c). Clearly, plants not only respond to the conditioned soil but also modify the conditioned soil gradually during the feedback phase (Bezemer et al., 2018; Hannula et al., 2021; Steinauer et al., 2023). Theoretically, it is also possible that during the feedback phase, the response of the plant to the soil does not change (Case II in Figure 1a) or that the response becomes stronger over time (Case III in Figure 1a, i.e. PSF of *Panicum virgatum* in Hawkes et al., 2013).

The turnover of soil microbial communities occurs at the timescale of hours to days for bacteria and months to years for fungi (Hannula et al., 2021; Rousk & Bååth, 2011). In the feedback phase, plants re-condition the heterospecific soil microbial community they are exposed to and steer it towards their 'home' soil microbial community. For example, the explained variation by succeeding plants on bacterial and fungal communities both increased over time in a longer-term plant–soil feedback study (Hannula et al., 2021). Soil microbes are crucial for plant performance and health and some microbiome members are even actively recruited by the host plant (Berendsen et al., 2012; Stopnisek & Shade, 2021). Therefore, changes in the relative abundance of potentially important bacterial and fungal taxa in the feedback phase could explain observed PSF outcomes. Specifically, if the relative abundance of microorganisms that are positively associated with plant growth decrease and/or the relative abundance of microbes that are negatively associated with plant growth accumulate in 'away' soil over time (Figure 1e, Case I and II), we expect that the magnitude of the negative PSF will become smaller over time. Alternatively, if the relative abundance of microbes that are negatively associated with plant growth in 'home' soil declines and/or the relative abundance of microbes that are positively associated with plant growth increase (Figure 1e, Case III and IV), the magnitude of the negative PSF is also expected to decline. Hence, besides the general focus of many studies on changes in the (entire) composition of the microbial community in plant–soil feedback studies, it is important also to examine the linkage between the relative abundance of important bacterial and fungal taxa and PSF effects during the feedback phase (Figure S1). Furthermore, a limitation of many typical PSF studies is that they use a fixed duration of plant growth during the feedback phase, and hence this prohibits us from examining when and how long the conditioned soil influences the response plant (Bezemer et al., 2018; Zhang et al., 2022). If the

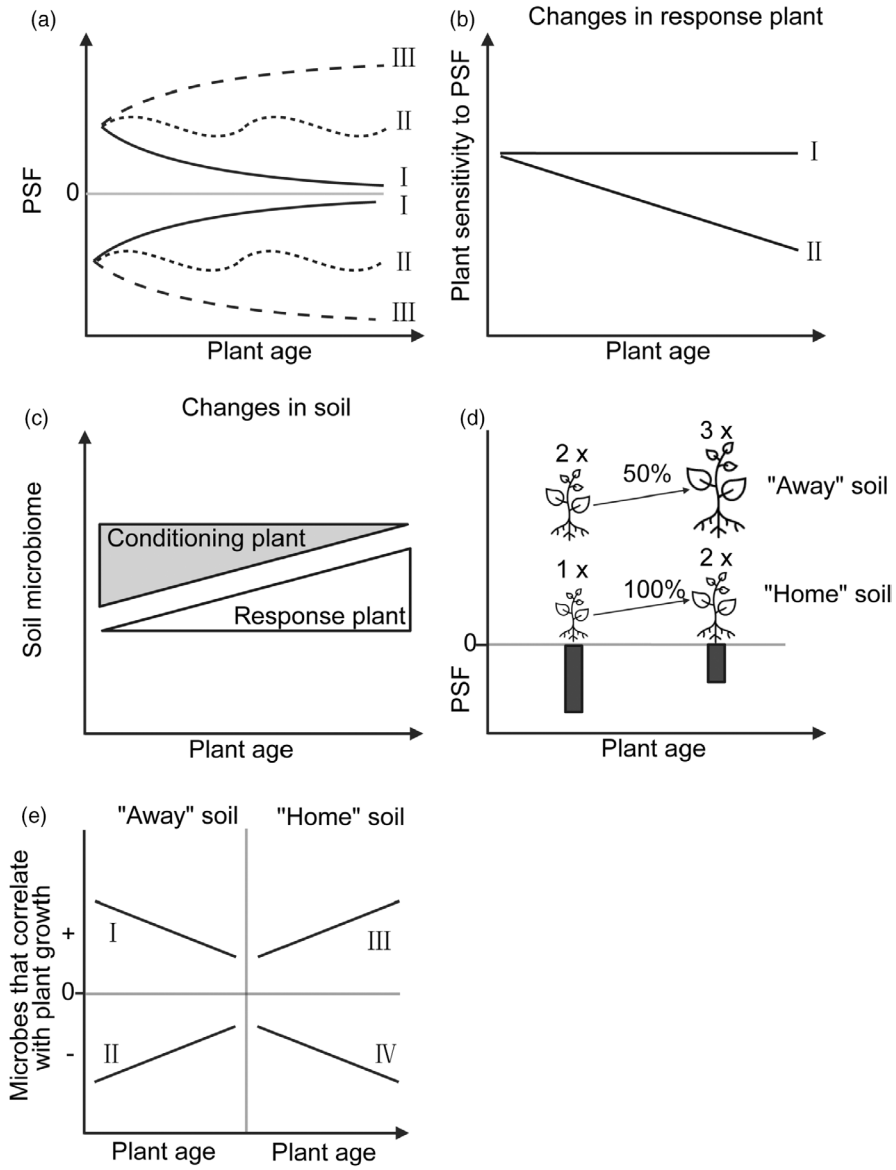


FIGURE 1 Conceptualized patterns that can explain temporal changes in plant–soil feedback. (a) Changes in the relative strength and direction of plant–soil feedbacks. (b) Temporal changes in plant sensitivity to soil conditioning effects due to plant size or age. (c) The proportion of the soil microbiome that can be allocated to the conditioning plant and response plant. (d) Inconsistency between relative growth rates of response plants and PSF. (e) Temporal changes in microbes that significantly correlate with plant growth. In (a) both positive and negative plant–soil feedbacks can become weaker (I), fluctuate (II) or become stronger (III) over time (adapted from Kardol et al., 2013; to simplify, our conceptual models do not include the cases where PSF changes direction during the feedback phase). In (b) the sensitivity of the response plant to soil conditioning effects can be relative constant (I) or decline over time (II). In (c) the soil microbiome that is associated to the response plant (current plant) is expected to increase and the one that belongs to the conditioning plant (previous plant) is expected to decrease over time. In (d) due to a negative feedback experienced at the early growth phase, relative plant size is 1× in 'home' soil and 2× in 'away' soil. After an additional period of growth, plant size has become 2× in 'home' soil and 3× in 'away' soil and thus relative growth is 100% in 'home' and 50% in 'away' soil. Hence plant relative growth is higher in 'home' soil, but, the resulting PSF is negative during both stages. In (e) microbes that positively correlate with plant growth decrease in 'away' soil (I) and/or increase in 'home' soil (II), and microbes that negatively correlate with plant growth increase in 'away' soil (III) and/or decrease in 'home' soil (IV) can explain that negative PSF becomes less strong over time. Figure created using BioRender (https://biorender.com/).

magnitude of the negative PSF decreases over time, as reported in several studies (e.g. Bezemer et al., 2018), and hence, that plants growing in 'home' soil catch up with plants growing in 'away' soil, this can only be the case, when the relative growth rate in 'home' soil at a certain stage exceeds that in the 'away' soil (Figure 1d).

In this study, we focus on the temporal dynamics of conspecific PSF both in the conditioning phase and in the feedback phase using the perennial forb *J. vulgaris*. This plant species has been reported to suffer from negative conspecific PSF, and hence it grows better in the heterospecific than in conspecific conditioned soil (Bezemer

et al., 2018; van de Voorde et al., 2011). To examine the response of plants of different ages in the feedback phase, rather than harvesting the plants after different growth periods in the feedback phase, as done in several other studies (but where one cannot disentangle the effects of plant age and changes in the conditioned soil caused by the duration of growth of the response plant), we measure the response of plants of different sizes to soil conditioning. In the first phase, we grew *J. vulgaris* and the grass, *Holcus lanatus*, in monocultures to condition the soil but we varied the duration of conditioning (2, 5 and 8 weeks) by initiating the monocultures at different times. Meanwhile, we grew *J. vulgaris* individually for 2-, 5- and 8- weeks in sterilized soil. We removed the soil from the roots of the 2-, 5- and 8-week-old plants grown in sterilized soil and then transplanted these plants of different sizes as well as seedlings in the 'home' and 'away' soil that had been conditioned for 2, 5 and 8 weeks. We determined their response as the difference in plant size before planting and after 4 weeks of growth in conditioned soil. We hypothesized that (1) the magnitude of PSFs will increase with increasing conditioning time; (2) young plants will be more sensitive to soil conditioning effects than older plants; we also used data from a previous study that used the same two plant species, and where plants were harvested repeatedly for 10 weeks during the feedback phase (Bezemer et al., 2018). We calculated relative growth rates to examine (3) whether the relative growth rates of plants are consistently lower in 'home' soil than in 'away' soil, or whether growth rates are only reduced during the first weeks of the feedback phase, as this can also lead to longer-term reduction in biomass (even though the growth rates no longer differ). Finally, we re-analysed data from a third PSF study with the same plant species where during the feedback phase bacterial and fungi communities in the rhizosphere soil of *J. vulgaris* grown in 'home' and 'away' soil were collected weekly and sequenced (Steinauer et al., 2023). We examine whether the dissimilarity in microbial communities in 'home' and 'away' soil decreases over time and whether changes in the relative abundance of bacteria and fungi that correlate with plant biomass are related to changes in conspecific PSFs.

2 | MATERIALS AND METHODS

2.1 | Plant species

Holcus lanatus L. (Poaceae) is a fast-growing biennial grass native to Europe and western Asia (Beddows, 1961). *J. vulgaris* Geartn. subs. *vulgaris* (syn. *Senecio jacobaea* L.; Asteracaea) is a monocarpic perennial forb that forms a rosette in the first year (Harper & Wood, 1957). Both species are common in (semi)natural grasslands and along road sides in The Netherlands and the two species frequently co-occur. *J. vulgaris* seeds were collected from plants growing in a coastal dune grassland in Meijendel, Wassenaar, The Netherlands. Seeds of *H. lanatus* were purchased from Cruydt-Hoeck (Nijeberkoop, The Netherlands), a supplier of seeds obtained from wild plants.

2.2 | Experiment 1: Duration of conditioning and response plant size

2.2.1 | Phase 1

A large amount of soil was collected from a grassland near Driebergen, The Netherlands and stored on a concrete base and covered with plastic sheeting for several years at the Netherlands Institute of Ecology, Wageningen, The Netherlands. The soil is characterized as holtpodzol sandy loam with a particle size distribution: 2% < 0.002 mm, 11% 0.002–0.063 mm, 84% > 0.063 mm, with ~3% organic matter, 1150 mg kg^{-1} N, 61 mg P_2O_5 100 g^{-1}, 2.4 mmol K kg^{-1} and pH 5.9. The soil was sieved (1 cm mesh size) to remove stones and large root fragments. Seeds from both species were sterilized for 20 min in 5% sodium hypochlorite solution and rinsed with sterilized MillQ water afterwards. Seeds of both species were germinated on sterile glass beads in a climate room with 400 W high pressure lamps at a 16/8 h light–dark regime and a 20/15°C temperature regime. Seedlings were grown on glass beads for 1 week after germination and then stored at 4°C until further use.

In the first phase, soil was conditioned by growing either *J. vulgaris* or *H. lanatus* in monocultures (five plants per pot) in pots (13 cm × 13 cm × 13 cm) filled with 1.45 L homogenized and sieved soil. A microbial soil inoculum was added to each pot as the live soil originated from the pile where no plants were grown and had been stored in 10 kg bags for several weeks before the setup of the first phase. The soil for the inoculum was collected from a grassland area in Leiden, The Netherlands. The soil was sieved through a 1 cm mesh to remove stones and large root fragments and mixed with water (1 soil: 2 water) and stirred. The soil slurry was then sieved through a 500 μm sieve and sieved liquid was stored at 4°C. We added 50 mL soil inoculum and 50 mL Steiner nutrient solution to each pot. A third set of pots was kept unplanted. All planted seedlings were similar in size. Seedlings that emerged from the soil seedbank were removed. The preparation was initiated at different times, so that plants were grown for 8, 5 and 2 weeks to create soils that were conditioned for different periods of time (Figure S2). In total, there were 117 pots (8 weeks conditioning: three treatments [*J. vulgaris* monocultures, *H. lanatus* monocultures or 'unconditioned' soil] × 15 replicates + 5 weeks conditioning: three treatments × 12 replicates + 2 weeks conditioning: three treatments × 12 replicates). All pots were watered once every 2 days when needed. After plants had grown for the designated period, all above-ground biomass was harvested from each pot and large root fragments were removed from the soil using a 4 mm sieve. All pots were harvested on the same day. Soil from different pots that belonged to the same treatment was homogenized and mixed. Subsequently, there were five batches of soil conditioned for 8 weeks (each batch was a mixture of soil from three pots), six batches of soil conditioned for 5 weeks and six batches of soil conditioned for 2 weeks (each batch was a mixture of soil from two pots). To arrive at five soil batches, for the 5- and 2-weeks conditioned soil, the sixth batch was divided equally and mixed with the other five batches of soil. In total, there were

45 batches of soil (three conditioning treatments, three conditioning durations and five replicates).

2.2.2 | Preparation of plants of different ages

Prior to starting the feedback phase, we grew *J. vulgaris* alone in the gamma-irradiated sterilized soil (>25 kGray; Synergy Health, Ede, The Netherlands). The soil was the same as described above. Plants were grown for 8, 5 and 2 weeks. Seedlings of *J. vulgaris* were prepared as described above. Eight weeks before starting the feedback phase, a single seedling was planted in each of 17 pots (8 cm × 8 cm × 9 cm) filled with 500 g sterilized soil. Five weeks and 2 weeks before starting the feedback phase, a single seedling was planted in 50 pots (8 cm × 8 cm × 9 cm) filled with 500 g sterilized soil. Ten days before starting the feedback phase, seeds were germinated as described before. The seeds germinated after 5 days. Therefore, plants were 8, 5 or 2 weeks old and seedlings were 1 week old when planted in the feedback phase (Figure S2). Two extra 8-week-old and five extra 5- and 2-week-old plants were harvested, weighed and oven-dried at 70°C for at least 72 h to determine the fresh weight/dry weight ratio and water content.

2.2.3 | Phase 2

A total of 150 pots (8 cm × 8 cm × 9 cm) were filled with 500 g soil from the conditioning phase. To test whether PSF is influenced by the age (size) of the response plant, we carefully washed the roots of the 8-, 5- and 2-week-old plants from soil and transplanted them in pots with three types of soils. Water was absorbed from the roots using tissue paper and total fresh biomass of each plant was determined before planting. Seedlings grown on sterile glass beads were also planted in the soils. A separate set of seedlings was weighed to determine fresh weight. For soil conditioned for 8 weeks, there were 60 pots: four different response plant ages × three types of soil (conditioned by *H. lanatus*, conditioned by *J. vulgaris*, no plant previously) × five replicates. For 5- and 2-week conditioning soils, there were not enough 8-week-old plants and hence there were 45 pots for those sets (three response plant ages [without 8-week-old plants] × three types of soil [conditioned by *H. lanatus*, conditioned by *J. vulgaris*, no plant previously] × five replicates). To improve the probability of survival after transplanting, plants were watered daily when needed. The climate conditions in the plant growth room were as described above. Pots were randomly placed in the growth room. After 4 weeks of growth, the soil was carefully rinsed and the roots were cleaned in water. Hereafter, we removed adherent water from the plant with tissue paper and recorded total fresh biomass. Root and shoot material of each plant was then separated and oven-dried at 70°C for at least 72 h and weighed.

Besides the experiment described above, we reanalysed data from two published experiments on the response of *J. vulgaris* to soil conditioned by *H. lanatus* and *J. vulgaris* (Bezemer et al., 2018; Steinauer et al., 2023). The experimental details and methods of these two experiments are described in the original publications. A summary of experimental conditions of all three PSF experiments and the total dry mass of *J. vulgaris* in 'home' and 'away' soil over the overlapping 7 weeks in these two existing PSF experiments are presented in the Supporting Information (Table S1, Figure S3).

2.2.4 | Soil properties

Soil samples collected from each batch (45 in total) at the end of the Phase 1 were analysed. Soil samples were oven-dried at 40°C and sieved through a 0.5 mm mesh. Ten gram of dried soil was shaken for 3 h in 25 mL 0.1 M calcium chloride ($CaCl_2$) solution. Hereafter, soil pH was measured in the resulting soil: calcium chloride ($CaCl_2$) (1: 2.5) suspension with a pH meter. Another 3 g of dried soil was shaken for 3 h in 25 mL 0.1 M calcium chloride ($CaCl_2$) solution and the filtrated suspension was used to measure orthophosphate (PO_4^{3-}) following the ascorbic acid method (Clesceri et al., 1998). Five gram of soil was shaken for 3 h with 25 mL 1 M potassium chloride (KCl) solution, and soil ammonium (NH_4^+) and nitrate (NO_3^-) were determined using the vanadium chloride method (Doane & Horwath, 2003). The absorbance was measured using a Tecan Spark 10 M Microplate Reader (Männedorf, Switzerland) and the corresponding phosphorus, ammonium and nitrate concentrations were calculated based on the standard curves (details described in the Supplementary Methods). Soil organic matter content was estimated by loss-on-ignition (LOI) analysis (Ball, 1964). Approximately 28 g dried soil samples were dried at 105°C for 16 h and weighted, then samples were heated at 550°C for 5 h and reweighed again. Soil organic matter was calculated as the percentage weight loss.

2.3 | Experiment 2: Changes in relative growth rates during the feedback phase

To examine the influence of soil conditioning effects on the relative growth rates of *J. vulgaris*, we used plants that were grown individually from this experiment. Briefly, one seedling of *J. vulgaris* was planted in pots (10 cm × 10 cm × 11 cm) filled with 1 kg of soil conditioned for 8 weeks by monocultures of *J. vulgaris* or *H. lanatus*. Here we used data from 114 pots (two soil conditioning treatments × 57 pots). Nineteen times (twice a week), during the feedback phase three plants from both treatments were harvested (week 2–11 after transplanting). Total plant dry mass after oven drying at 70°C was then determined.

2.4 | Experiment 3: Changes in rhizosphere bacterial and fungal communities during the feedback phase

Eighty pots (10 cm × 10 cm × 11 cm) were filled with 1 kg homogenized live soil (from same source and pile as described above),

and 40 *J. vulgaris* and 40 *H. lanatus* plants were grown individually in these pots for 10 weeks. After 10 weeks of soil conditioning, plants were removed from the soil. In the feedback phase, 54 pots (10 cm × 10 cm × 11 cm) were filled with a homogenized mixture of 1.62 kg of sterilized soil from the same field (gamma-irradiated >25 kGray; Synergy Health, Ede, The Netherlands) and 0.18 kg of conditioned soil (10% inoculum). For the feedback phase, rooted *J. vulgaris* plantlets from a tissue culture were used. We used the data from 54 pots (two soil treatments × 27 pots). Plants were harvested weekly for nine times during the feedback phase (week 2–10). During each harvest, rhizosphere samples were collected for molecular identification of soil microbial community (Illumina sequencing of bacteria and fungi) and shoot and root biomass of each plant was determined. A detailed description of the DNA extraction and information about sequencing and primers are presented in the original paper (Steinauer et al., 2023).

2.5 | Data analysis

2.5.1 | Experiment 1

The effects of soil treatment ('home' soil, 'away' soil or unconditioned soil), the duration of soil conditioning and the age of the response plant on total dry mass of *J. vulgaris* were tested with three-way ANCOVA with soil treatment, duration of conditioning and age of the response plant as main factors and with plant total fresh biomass before transplanting as covariate. A Tukey's post hoc test was used for pair-wise comparisons of soil treatments.

The total dry mass of each plant before transplanting was calculated from fresh weights using mean water content of different ages of the response plant. For each response plant age, a two-way ANOVA with soil treatment, duration of conditioning and its interaction was performed on the increment in total dry mass. Total dry mass and the increment in total dry mass were square-root transformed to fulfil requirements of normality in residuals.

PSF was calculated as: ln (plant biomass in 'home' soil)—ln (plant biomass in 'away' soil), and a negative value indicates worse growth in 'home' than in 'away' soil (Bezemer et al., 2018; Pernilla Brinkman et al., 2010; Petermann et al., 2008). The increment in total dry mass, and the final shoot and root dry mass were used to calculate the feedback effect. The feedback effect was calculated separately for each replicate (i.e. ln ('home soil' replicate 1)—ln ('away soil' replicate 1), etc.). Afterwards, we used two-way ANOVA to test the effects of duration of condition, the age of response plant and its interaction on feedback effects. A Tukey's post hoc test was used for pair-wise comparisons of soil treatments and of duration of conditioning.

Soil properties at the end of Phase 1 were analysed using two-way ANOVA with conditioning treatment (*J. vulgaris*, *H. lanatus* or without plants) and duration of conditioning as main factors. A Tukey's post hoc test was used for pair-wise comparisons. Linear regression was then used to analyse the relationship between each soil property in home and away soil and the increment of total dry mass for seedlings, 2-week-old and 5-week-old plants, respectively. ANOVA was carried out with the 'aov' function and linear regression was done by 'lm' function in R. The post hoc test was performed using the 'glht' function with the 'multcomp' package (Hothorn et al., 2008).

2.5.2 | Experiment 2

ln-transformed total dry mass of *J. vulgaris* was plotted against time (19 time points × three replicates per time point). Then we determined the slope from a linear regression over three consecutive time points (i.e. a 1-week interval) in 'home' and in 'away' soil, and hence for three replicates of nine measurements, to calculate the relative growth rate (Zhang et al., 2022). As overlap of time points in separate regressions was allowed, there were 17-time intervals in total. A pair-wise t-test was applied to compare the difference in slopes between 'home' soil and 'away' soil over all time intervals (17 pair-wise slopes) and then for each time interval separately. Additionally, slopes between 'home' and 'away' soil were compared using a pair-wise t-test in three combined intervals of the feedback phase. The pair-wise t-test was carried out with the 'pairwise.t.test' function in R. We also calculated the PSF at each time point using the formula described above, and the PSF was tested against zero using a one-sample *t*-test for each time point.

2.5.3 | Experiment 3

To examine whether the rhizosphere microbial communities in 'away' soil gradually change to 'home' soil over time in the feedback phase, we analysed the Bray–Curtis dissimilarity in rhizosphere bacterial and fungal communities between 'home' soil and 'away' soil. There were three samples per time point, per soil, potentially resulting in nine comparisons. A linear mixed effect model was used to analyse the relationship between dissimilarity and time with the identity of home soil and the identity of away soil as two random effects. We also checked the estimate variance of random effects and compared models with and without random effects. The estimate variance of random effects was 0 and there was no difference between models with and without random effects. This indicates that the random effects were not necessary, and linear regression was then used to analyse the relationship between dissimilarity and time. Bray–Curtis dissimilarity was assessed with the 'vegdist' function from the package 'vegan' (Oksanen et al., 2024). For analyses of bacterial OTUs, we followed bioinformatic analyses done in the original paper and hence samples with less than 4964 reads or more than 80,000 reads were removed, which resulted in removal of two samples (Steinauer et al., 2023). For analyses of fungal OTUs, samples with less than 1000 reads or more than 80,000 reads were removed which resulted in removal of one sample (Steinauer et al., 2023). OTU tables were rarefied to 4964 and 3125 sequences/samples

for bacteria and fungi reads, respectively. OTUs present in less than three samples or with a relative abundance of less than 0.05% were removed from the dataset.

To examine the linkage between the relative abundance of important bacterial and fungal taxa and the outcomes of PSF, bacterial and fungal taxa associated with plant growth were identified. We selected bacterial and fungal OTUs, based on Spearman's rank correlation coefficient between the relative abundance of each OTU and shoot dry mass at each time point separately. Significance of the correlation was set at $p < 0.01$. We then selected the bacterial and fungal OTUs that were significantly correlated with shoot dry mass. For this subset of bacterial and fungal OTUs that were significantly correlated with plant growth, for each OTU separately the relationship over time in 'home' and 'away' soil was then determined using linear regression. Because we tested all OTUs that significantly correlated with shoot dry mass independently, the Benjamini-Hochberg method, which controls the false discovery rate (FDR) was used to adjust p values of the linear regression (Benjamini & Hochberg, 1995). The *p* value adjustment was carried out with the 'p.adjust' function in R with the false discovery rate set at 5%.

All analyses were performed using the R statistical language, version 4.2.2 (R Core Team, 2022).

3 | RESULTS

3.1 | Experiment 1

Jacobaea vulgaris plants exhibited highest growth in unconditioned soil and lowest in 'home' soil, both in terms of the increment in total dry mass and final total dry mass (Table 1, Figure 2, Figures S4 and S5). The increment in total dry mass was significantly higher in 'away' soil than in 'home' soil except for 8-week-old plants (Table 1 and Table S2, Figure 2). The negative PSF of *J. vulgaris* varied with the duration of soil conditioning, with the lowest PSF in soil conditioned for 2 weeks, the highest PSF in soil conditioned for 5 weeks and intermediate PSF in soil conditioned for 8 weeks (Table 1, Tables S2 and S3, Figure 2). Older (and larger) plants tended to be less sensitive to soil conditioning than younger plants (Table 1 and Table S3, Figure 2). Seedlings and 2-week-old plants experienced highest negative soil conditioning effects and 5-week-old plants exhibited lowest negative soil conditioning effects (Table S3, Figure S4).

Soil pH and nitrate at the end of Phase 1 varied significantly among different conditioning durations (Figure 3, Table S4). After 5 weeks of conditioning, soil pH was significantly higher in away soil than in home soil (Figure 3a, Table S4). Moreover, the concentration of soil nitrate was significantly lower after 5 and 8 weeks of conditioning compared to 2 weeks of conditioning (Figure 3d, Table S4). In the feedback phase, there was a significant positive relationship between the increment in total dry mass and soil nitrate concentrations (Table S5).

3.2 | Experiment 2

Analysed over the entire growth period, the relative growth rate in 'home' soil and 'away' soil was similar (Figure 4, t-value = 0.61, $p = 0.55$), but it was higher in 'home' soil than in 'away' soil at week 5.5 (t-value = 2.59, $p = 0.04$) when analysed for each time point separately. Relative growth rates were initially higher in 'away' soil during the first 2 weeks (t-value = −18.03, $p < 0.001$), then lower from week 4 to 7 (t-value = 3.73, $p = 0.02$) and eventually became similar to those in 'home' soil from week 7 to 10 (t-value = 0.89, $p = 0.40$; Figure 4a). The negative PSF of *J. vulgaris* tended to become less negative over time, and PSF became neutral (did not differ significantly from 0) after 7 weeks of the starting of the feedback phase (Figure 4b).

TABLE 1 Results of two-way ANOVA testing the effects of soil treatment (two levels: Either conditioned by *Jacobaea vulgaris* or by *Holcus lanatus*), duration of conditioning and the interaction on the increment in total dry mass of different ages of *J. vulgaris* plants during the feedback phase.

		Increment in total dry mass (g)		
		df	*F*	*p*
Seedlings	Soil treatment	1, 24	9.19	<0.01
	Duration of conditioning	2, 24	64.88	<0.001
	Soil treatment × duration of conditioning	2, 24	0.40	0.68
2-week-old	Soil treatment	1, 24	9.27	<0.01
	Duration of conditioning	2, 24	26.95	<0.001
	Soil treatment × duration of conditioning	2, 24	1.10	0.35
5-week-old	Soil treatment	1, 24	16.40	<0.001
	Duration of conditioning	2, 24	25.58	<0.001
	Soil treatment × duration of conditioning	2, 24	0.02	0.98
8-week-old	Soil treatment	1, 8	3.71	0.09

Note: Presented are treatment and error degrees of freedom (df), *F*-values and *p* values.

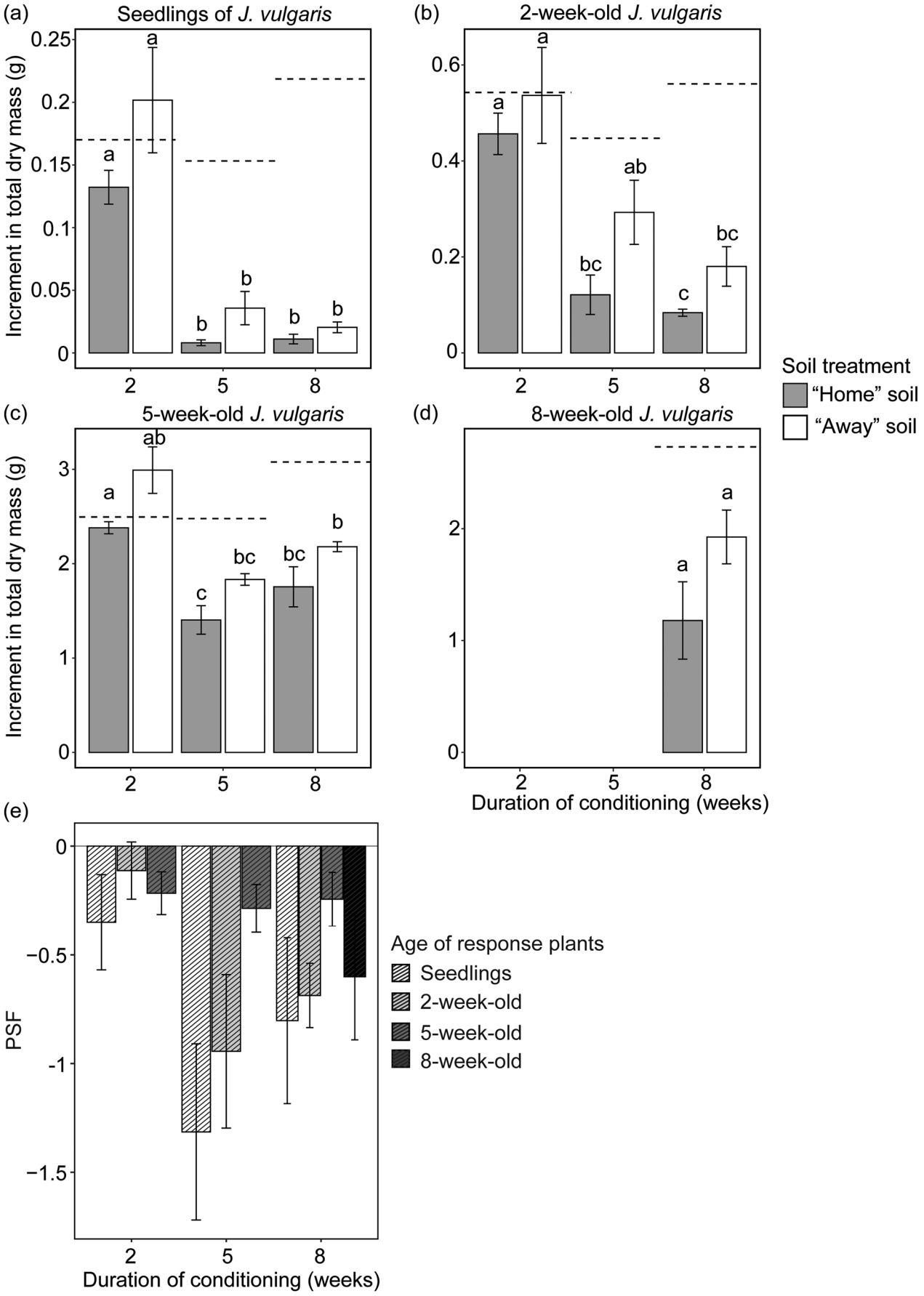
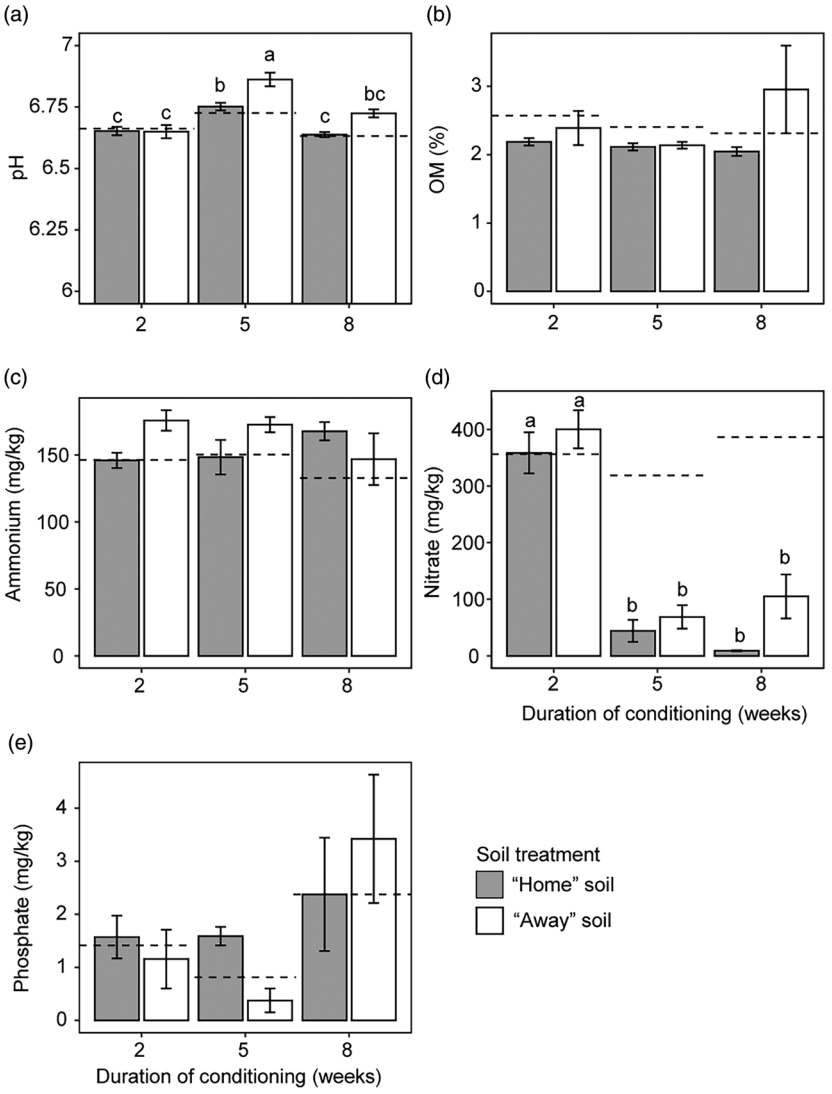


FIGURE 2 Effects of soil treatments and duration of soil conditioning (weeks) on the increment in total dry mass of seedlings (A), 2-week-old (B), 5-week-old (C) and 8-week-old (D) *Jacobaea vulgaris* plants and PSF (E). Values are mean ± SE. Eight-week-old plants were tested only in the soil that was conditioned for 8 weeks. In (A–D) the dashed lines above each set of bars represent the average increment in total dry mass in unconditioned soil. In (A–D) different letters indicate significant differences among conditioning time and conditioning treatment in a Tukey post hoc comparison. In (E) PSF was calculated as: ln (increment in total dry mass in 'home' soil/in 'away' soil).

FIGURE 3 Mean (±SE) soil pH (A), organic matter (B), ammonium (NH_4^+) (C), nitrate (NO_3^-) (D) and phosphate (PO_4^{3-}) (E) at the end of phase 1. In (A) and (D) different letters indicate significant differences among conditioning time and conditioning treatment in a Tukey post hoc comparison.



3.3 | Experiment 3

Both the Bray–Curtis dissimilarity in rhizosphere bacterial and fungal communities in 'home' and 'away' soil decreased over time (Figure 5b). A total of 80 bacterial OTUs exhibited positive and 86 negatively correlated with shoot dry mass of *J. vulgaris* (Figure 6). The relative abundance of five (out of 86, 5.8%) bacterial OTUs that negatively correlated with shoot dry mass increased in 'away' soil, and the relative abundance of 10 (out of 80, 12.5%) bacterial OTUs that positively associated with shoot dry mass decreased in 'away' soil (Table S6, Figure 6). In 'home' soil, there were three bacterial OTUs that negatively associated with shoot dry mass that decreased and only one bacterial OTU that positively associated with shoot dry mass that increased over time (Table S6, Figure 6). There were 10 fungal OTUs that were significantly associated with shoot dry mass of *J. vulgaris*. The relative abundance of one fungal OTU that was negatively associated with shoot dry mass increased over time in 'away' soil (Table S7).

4 | DISCUSSION

The aim of this study was to dissect multiple contributors of the temporal dynamics of conspecific PSF using the plant *J. vulgaris*. We first examined the effects of duration of conditioning and the age (and size) of response plants on conspecific PSF of *J. vulgaris*. Further, we carried out new analyses on two datasets

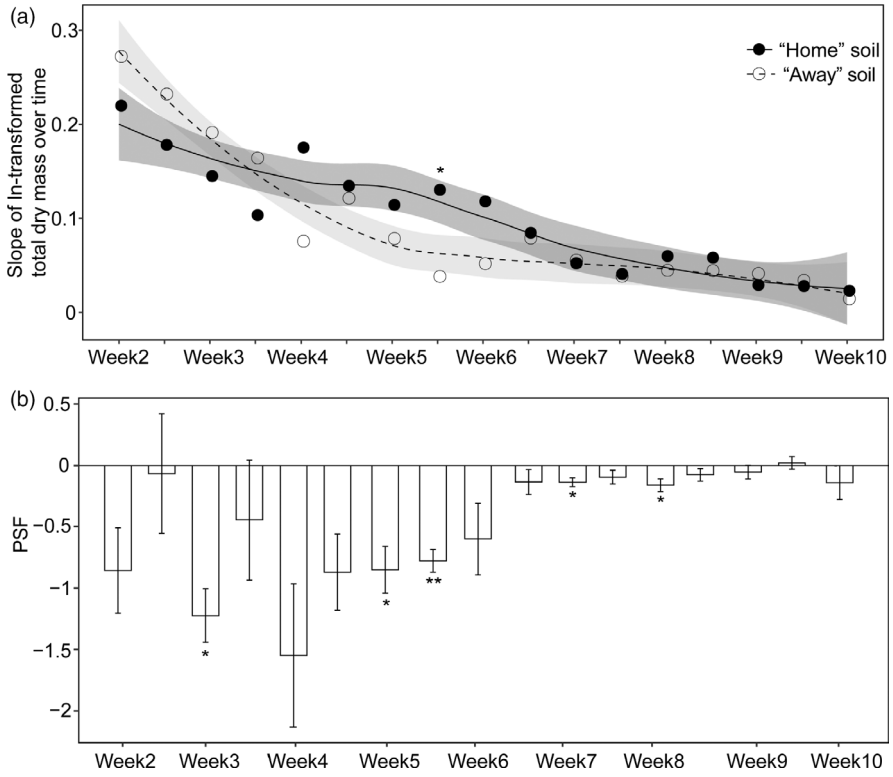


FIGURE 4 Linear regression slopes of ln-transformed total plant dry mass of *Jacobaea vulgaris* in the 'home' and 'away' soils against time (a) and PSF (mean ± SE) of *J. vulgaris* (b) over time. In (a) the slope was determined per 7-day time interval and overlap of time points was allowed. In (a) the 95% confidence intervals calculated using a locally weighted scatterplot smoothing (LOESS) regression model are also presented. The difference in slopes in 'home' and 'away' soil was tested using a *t*-test for each time point separately. In (b) the strength of PSF was calculated as: ln (total dry mass in 'home' soil / in 'away' soil) and it was tested against zero using a one-sample t-test for each time point separately. In (a) an asterisk indicates the slope differs significantly between 'home' and 'away' soil. In (b) an asterisk indicates that the feedback effect differs significantly from zero. ** indicates significant difference at $p < 0.01$, * indicates significant difference at $p < 0.05$.

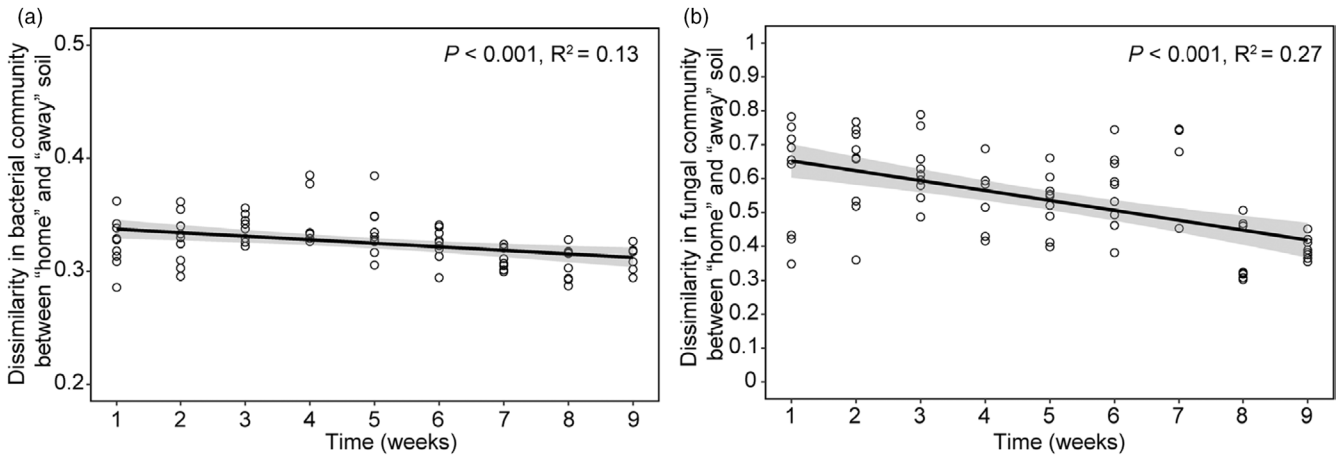


FIGURE 5 Bray–Curtis dissimilarity between the 'home' soil and the 'away' soil over time for bacterial (a) and fungal (b) soil communities of *Jacobaea vulgaris*. R^2, *p*-values and the 95% confidence interval from a linear regression analysis are also presented.

of PSF studies of *J. vulgaris* that used the same conditioning soil (home-away comparison) to examine temporal changes in relative growth rates of the test plants and temporal changes in microbial communities in 'home' soil and 'away' soil. Lastly, for bacterial and fungal taxa that significantly positively or negatively associated with plant growth, we explored whether their relative abundance increased or decreased over time in 'home' and 'away' soil as a potential explanation of temporal changes in plant–soil feedback. Four main findings arise from our study. First, the negative PSF of *J. vulagris* was strongest for younger plants, and the magnitude of the negative PSF rapidly increased to a maximum at 5 weeks and then gradually attenuated. This variation in PSF

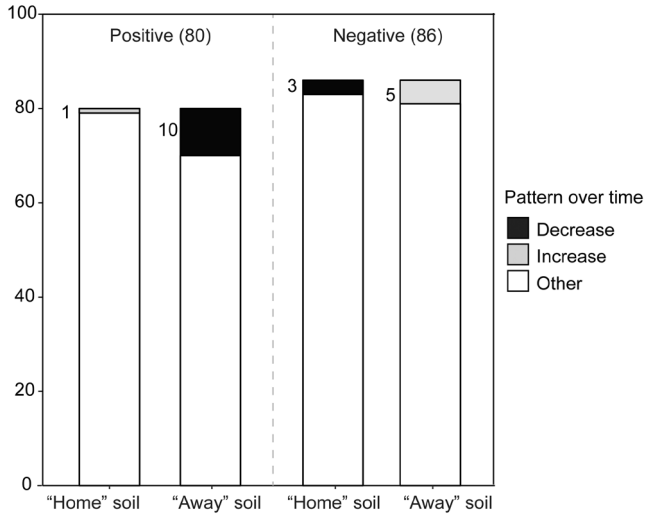


FIGURE 6 Number of bacterial OTUs that significantly positively or negatively correlated with plant shoot dry mass. The total number of positive and negative ones is shown and the number of bacterial OTUs that significantly decrease or increase in relative abundance over time in 'home' soil and in 'away' soil.

across conditioning durations is strongly influenced by soil nitrate. Second, during the feedback phase, plant sensitivity to soil conditioning decreased when plants became older. Third, even though the PSF was negative, the relative growth of *J. vulgaris* was higher in 'home' than in 'away' soil after an initial negative effect on the relative growth rate in 'home' soil. Lastly, based on the putative positive and negative effects of rhizosphere microbes on plants, the pattern of declining negative PSF of *J. vulgaris* over time, appear to be due to the decrease in relative abundance of beneficial and the increase in relative abundance of detrimental bacteria in 'away' soil, rather than due to the decline of detrimental or the increase of beneficial bacteria in 'home' soil. Hence, our study illustrates that in the feedback phase, both plant sensitivity to soil conditioning and changes in the microbial communities, in particular the changes in relative abundances of important bacterial OTUs, can act as important drivers of temporal changes in PSF.

Previous studies have generally observed an increase in the magnitude of PSF with longer conditioning durations, although varying definitions of PSF were used (home-away comparison in Diez et al., 2010; home-unconditioned comparison in Lepinay et al., 2018; live-sterile comparison in Ke et al., 2021). In our study, using the home-away comparison, we found that conspecific PSF initially increased with longer conditioning times and then gradually attenuated. A similar pattern was observed for the negative conspecific PSF of *R. austriaca* (Lepinay et al., 2018). Furthermore, our results confirm that the negative PSF is strongly linked to soil nitrate depletion, which is an important element for plant growth (LeBauer & Treseder, 2008; Lepinay et al., 2018).

In addition, PSF was observed to vary with the response time by harvesting plants at different time points (Bezemer et al., 2018; Dudenhöffer et al., 2018; Hawkes et al., 2013). However, this approach does not allow us to disentangle whether temporal changes of PSFs are due to the size-dependent sensitivity of the responding plant to soil conditioning, or due to the subsequent changes caused by the responding plants in the conditioned soil. We show that *J. vulgaris* suffered from a negative conspecific PSF across different ages, even though the effect diminished when the plants were older. It is important to note that our conclusion, which suggests that plant sensitivity to soil conditioning decreases when plants are order, is based on the assumption that plants of different ages experienced similar transplant shocks. Although the increment in total dry mass of 8-week-old *J. vulgaris* plants did not depend significantly on when the plant was grown in 'home' or 'away' soil, we did observe that leaves were more yellow in 'home' soil than in 'away' soil (Figure S6). This may indicate that 8-week-old *J. vulgaris* plants suffered more from transplant shock. Unfortunately, we did not grow extra plants in parallel in the pots without transplanting them. This would have enabled us to compare the relative biomass difference with and without transplantation to indicate the influence of transplant shock on plants of different ages. It is also important to emphasize that larger plants grew less than smaller plants and hence there was less room for PSF effects based on biomass differences. The increased yellowness indicates that the larger plants still suffered in freshly conditioned 'home' soil compared to 'away' soil, even though there was no significant difference in soil properties between home and away soil after 8 weeks of conditioning. This also indirectly suggests that changes in the soil microbiome during the feedback phase are responsible for the decline in the negative PSF over time for *J. vulgaris*.

Recent studies have found evidence that *J. vulgaris* plants during growth alter the composition of differentially conditioned microbial communities in the feedback phase (Hannula et al., 2021; Steinauer et al., 2023). The dissimilarity in rhizosphere bacterial and fungal communities between 'home' and 'away' soil decreased over time in our study. This partly confirms that the rhizosphere microbial communities exhibit strong temporal dynamics during the feedback phase and that this highly depends on the identity of the response plant (Hannula et al., 2021). Notably, already at the beginning of the feedback phase, the dissimilarity in bacterial communities was relatively low (around 0.3) and the dissimilarity in fungal communities was relatively high. A possible explanation could be that in this study (experiment 3) bacterial communities developed from the inoculum (10% of live soil) that was introduced in a sterile substrate (Steinauer et al., 2023). Further, we explored the association between the bacterial and fungal OTUs and plant growth during the feedback phase to identify important bacterial and fungal taxa in the microbiome (Liu et al., 2023; Wang et al., 2023). Interestingly, even though the dissimilarity patterns were clearer for fungi than for bacteria, many bacteria taxa and few fungal taxa significantly correlated with plant shoot dry mass in feedback phase. Previous studies emphasized that the turnover of bacteria and the changes in bacterial communities are much faster than those in fungal communities (Hannula et al., 2021; Rousk & Bååth, 2011; Sun et al., 2017). We detected that the relative abundance of 12.5% of the bacterial taxa that

positively associated with growth of *J. vulgaris* decreased, and that 5.8% of the bacterial taxa that negatively associated with plant growth increased in 'away' soil over time. We propose that the temporal dynamics of important bacterial OTUs in the feedback phase can be an important driver of temporal changes in the variation of PSFs. Interestingly, our study shows that these changes mainly occur in the away soil and hence that due to the current influence of the focal plant, the microbiome composition of the 'away' soil is steered towards the composition in the 'home' soil. Fewer changes occur in the 'home' soil, where the same plant was growing previously and is growing currently. In our pot experiment (experiment 3), we identified significant microbial taxa that associated with plant growth during the feedback phase. However, it is important to note that the subset of microbial taxa we selected may not fully represent the community that was responsible for the conspecific PSF. A recent study has highlighted that 'many weak' plant-microbe interactions play important roles in shaping PSF (Aaronson et al., 2023). Further studies are needed to examine whether PSF can be attributed to a specific group of microbial taxa or if it arise from numerous weak plant-microbe interactions.

Generally, PSF is calculated using plant biomass data collected at the end of the feedback phase, which refers the overall impact of interactions between plants and conditioned soil (the net effect). However, even though the net effect of conspecific PSF of *J. vulgaris* is known to be negative, our results on the relative growth rates suggest that plants experienced the negative feedback mainly at the earlier growth phase (Zhang et al., 2022). This also aligns with our results that the negative PSF of *J. vulagris* was strongest for younger plants. The size-dependent sensitivity of plants to conditioned soil and their reconditioning abilities on modifying soil microbial communities may explain the varying net effects of PSF.

In conclusion, our study shows that both the length of conditioning and the age (size) of the response plant can affect PSFs, and that plant sensitivity to soil conditioning declines with the increasing in plant age. We also highlight that a better understanding of the temporal dynamics and abundance of important microbial taxa as well as the temporal changes in the influence of plants on the microbiome are important if we aim to better understand plant–soil feedback responses.

AUTHOR CONTRIBUTIONS

Xiangyu Liu and Thiemo Martijn Bezemer conceived and designed the study and ideas. Xiangyu Liu and Karin van der Veen-van Wijk carried out the experiment 1. Katja Steinauer carried out experiment 3. Xiangyu Liu analysed all data. Xiangyu Liu and Thiemo Martijn Bezemer wrote the manuscript. All authors approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14435.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.41ns1rnq6 (Liu et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Experimental conditions of the three PSF experiments with *J. vulgaris* growing in conspecific and *H. lanatus* conditoned soil.

Table S2. Results of a two-way ANOVA testing the effects of conditioning time, age of response plants and the interaction on PSF effects calculated by the increment in total dry mass, and final shoot and root dry mass. Presented are degrees of freedom (df), *F*-values and *p* values.

Table S3. Results of a three-way ANCOVA with the initial total fresh biomass of plant species as a covariate testing soil treatment, duration of conditioning, age of response plants and their interactions on shoot and root dry mass of *J. vulgaris*. Presented are degrees of freedom (df), *F*-values and *p* values.

Table S4. Results of a two-way ANOVA testing conditioning treatment (2 levels: *J. vulgaris* or *H. lanatus*), duration of conditioning and their interaction on soil pH, organic matter (%), ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^-) at the end of phase 1. Presented are degrees of freedom (df), *F*-values and *p* values.

Table S5. Relationship between soil pH, organic matter (%), ammonium (NH_4^+), nitrate (NO_3^-) and phosphate (PO_4^-) and total dry mass increment of seedlings, 2-week-old and 5-week-old *J. vulgaris* plants. Presented are slopes, *p* values and adjusted R^2 from linear regressions.

Table S6. Information about 10 positively correlated bacterial OTUs that decreased and 5 negatively correlated bacterial OTUs that increased over time in "away" soil, and of 1 positively correlated bacterial OTU that increased and 3 negatively correlated bacterial OTUs that decreased over time in "home" soil.

Table S7. Number of fungal OTUs that significantly correlated with shoot dry mass of *J. vulgaris* for which the relative abundance increased or decreased over time in "home" soil and in "away" soil.

Figure S1. Conceptualized patterns of the relative abundance of important bacterial or fungal OTUs in "home" and in "away" soil during the feedback phase.

Figure S2. Experimental design of both conditioning and feedback phases and preparation of responding plants of different ages for experiment 1.

Figure S3. Mean (±SE) total dry mass of *J. vulgaris* in "home" and "away" soil over the overlapped seven weeks in two existing PSF experiments.

Figure S4. Mean (±SE) shoot (a) and root dry mass (b) of *J. vulgaris* after 4 weeks of growth during the feedback phase, and plant-soil feedbacks were calculated based on shoot dry mass (c) and root dry mass (d) of *J. vulgaris*.

Figure S5. Effects of the soil treatment and duration of soil conditioning (weeks) on the increase in total dry mass of seedlings (a), 2-week-old (b), 5-week-old (c) and 8-week-old *J. vulgaris* plants (d), and PSF (e).

Figure S6. Representative pictures of seedlings (a), 2-week-old (b), 5-week-old (c) and 8-week-old *J. vulgaris* (d) grown for 4 weeks in soil conditioned for 8 weeks. In (a–d), pots from top to bottom are "unconditioned" soil, "away" soil and "home" soil.

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